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African Journal of Agricultural Research

*Full Length Research Paper*

# Management strategies for the noxious invasive parthenium weed (*Parthenium hysterophorus* L.) in Uganda

**Martha Ibore Natukunda[1,2*], Kevin Natukunda[1,3], Gerald Kyeyune[1], Sharon Mbabazi Tusiime[1,4], Isaac Agbemafle[5] and Jenipher Bisikwa[1]**

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**Parthenium weed is a noxious invasive species that has negative effects on agriculture and also causes allergic reactions in humans. The goal of this study was to evaluate several management strategies for parthenium weed and assess the suitability of each control measure for farmers, and other stakeholders in Uganda. Field experiments were conducted in a completely randomized design, and the quadrat sampling method used to assess the effect of mulching, foliar application of table salt solution, hand pulling, slashing, hand hoeing, foliar herbicide application, and integrated weed management on parthenium plant populations. All tested weed management strategies except foliar herbicide application significantly ($P \leq 0.05$) reduced parthenium plant populations, with parthenium weed counts for treated plots reducing on subsequent data collection days. The experimental data showed that parthenium plant populations increased for the untreated plot overtime. The authors recommend that a combination of multiple weed control measures (integrated weed management) are utilized for effective management of parthenium weed in Uganda to reduce limitations that result when one management strategy is used singly. This study informs farmers, the general public, and researchers how to effectively control parthenium weed, contributing to reduction of the numerous negative effects of parthenium weed on human livelihoods.**

**Key words:** Agriculture, invasive, management, noxious, parthenium weed, Uganda.

## INTRODUCTION

Weeds are undesirable plants that are growing out of place (Baucom and Holt, 2009; Monaco et al., 2002). Weeds reduce crop yields by competing for nutrients, water, carbon dioxide and sunlight (Monaco et al., 2002).

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Some weeds also exhibit allelopathy, a biological phenomenon in which weeds produce toxins that can influence germination, growth, survival, development, and reproduction of crops (Cheng and Cheng, 2015). In the livestock industry, weeds reduce the quality of forages, making them unpalatable or poisonous to livestock, ultimately lowering the quality of animal products (Bridges, 1994; Patel, 2011). Weeds can be alternative hosts for pathogens and insect pests of crops, aiding survival from one season to the next (Monaco et al., 2002; Onwueme and Sinha, 1991). Weeds negatively impact human health through allergies, and poisoning (Bridges, 1994). These negative attributes make weeds undesirable to humans. One of the noxious invasive weed species of public health concern in Uganda is parthenium weed (*Parthenium hysterophorus* L.), commonly known as Congress weed, or Lugono in the eastern region of Uganda. Parthenium weed originated from South America, and is one of the world's most noxious plants invading, Africa, Australia, and Asia (Joshi et al., 2016; McConnachie et al., 2011; Navie et al., 1996; Patel, 2011; Khari and Kumar, 2018). In Africa, Parthenium weed has successfully inhabited many areas in the east and southern regions (Abdulkerim-Ute and Legesse, 2016; Nigatu and Sharma, 2013; McConnachie et al., 2011; Seta et al., 2013; Wabuyele et al., 2014; Worku, 2010; Zuberi et al., 2014). A study conducted in East Africa (Uganda, Kenya and Tanzania) identified multiple locations inhabited by parthenium weed, and highlighted the need to devise control measures to prevent further spread (Wabuyele et al., 2014). Parthenium weed is well adapted to a wide range of growth conditions and soil types, is very prolific, and under favorable conditions, flowering can occur 28-42 days after seedling emergence (Chamberlain and Gittens, 2003; Abdulkerim-Ute and Legesse, 2016; Adkins and Shabbir, 2014; Navie et al., 1996). A single parthenium plant can produce more than 15,000 seeds which are spread by farm machinery, animals, pasture seed lots, stock feed, wind, and running water (Adkins et al., 2010). The observed widespread distribution of parthenium weed in various parts of Africa suggests the need for management strategies to control further spread.

Parthenium weed has negative effects on humans, both directly by impacting human health, and indirectly by affecting crop and livestock production (Adkins and Shabbir, 2014; Kaur et al., 2014). In humans, allergic reactions to parthenium weed can result in an acute form of contact dermatitis, bronchitis, asthma, and hay fever (Adkins and Shabbir, 2014; Kaur et al., 2014; Lakshmi and Srinivas, 2012; McConnachie et al., 2011). It is estimated that up to 73% of people are sensitive to parthenium weed with females being twice more sensitive than males (Khari and Kumar, 2018). In the livestock industry, parthenium weed reduces pasture carrying capacity by up to 90%, taints livestock products such as milk and meat reducing their value, and is toxic to livestock (Hundessa and Belachew, 2017). A significant amount (10-50%) of parthenium in pastures can kill cattle and buffalo (McConnachie et al., 2011; Tudor et al., 1982). In crop production, allelopathic effects of parthenium weed, mainly caused by the chemical allergen, parthenin can negatively impact agricultural crops (Belz et al., 2007; Wakjira et al., 2009). For instance, in Ethiopia, sorghum (*Sorghum bicolor*) grain yield was reduced by between 40-97% when parthenium weed was left uncontrolled throughout the season (Tamado et al., 2002).

In Uganda, parthenium weed has been reported in the central region (Kampala and Masaka districts), the eastern region (Jinja district), the western region (Mbarara and Kasese districts), and the northern region (Pader district) (Wabuyele et al., 2014). Although parthenium weed presents a major challenge to crop production, livestock rearing, and human health in Uganda, studies informing farmers, and other stakeholders (the public and researchers) on how to properly manage parthenium weed are limited. Therefore, farmers have inadequate knowledge on management strategies for this invasive weed. The goal of our study was to evaluate multiple control measures for parthenium weed and assess the suitability of each control measure for farmers in Uganda. Seven parthenium management strategies were tested including: mulching, foliar application of table salt solution, hand pulling, slashing, hand hoeing, foliar herbicide application, and integrated weed management (IWM) for their effect on parthenium weed populations in field experiments. Additionally, authors made recommendations for parthenium weed management based on their findings. The specific objective of this study was to generate knowledge on suitable measures for managing parthenium weed in Uganda for farmers, the general public, and researchers, reducing negative effects of parthenium weed on human livelihoods.

## MATERIALS AND METHODS

### Study area and experimental design

This study was conducted at Makerere University, Uganda (00°21'00''N 32°34'03''E) in a demarcated non-cropped site that was naturally infested with parthenium weed (approximately 80% parthenium weed) during the second rains of 2010 and first rains of 2011. A completely randomized design was utilized in this study, with seasons as replicates. To set up experiments, the demarcated area was divided into eight 3 m x 3 m (9 $m^2$) plots, with a spacing of 0.6 m between plots to prevent drift and inter-plot interference. At the start of the experiment, all plant species at the experimental site were recorded to determine the plants that co-existed with parthenium weed. This was followed by deep ploughing to remove all existing plants and allow different plant species to sprout on their own. Approximately three weeks later, when the parthenium weed was at the rosette growth stage (Kaur et al., 2014; Khari and Kumar, 2018) seven management practices (mulching, foliar

application of table salt solution, hand pulling, slashing, hand hoeing, foliar herbicide application and IWM) were randomly assigned to individual plots while the eighth plot was left untreated throughout the experiment. Prior to application of treatments, data on parthenium weed populations was collected for each plot. Other plant species were also recorded for each plot.

**Materials and equipment**

Materials used for respective treatments in assigned plots were; dry hay grass, table salt, and Gramaxone. Equipment used during treatments include: a watering can, a hand-held slasher, a hand hoe, a knapsack sprayer, a measuring cylinder, and all personal protective equipment (headgear, gloves, protective suits, aprons, respirators, foot, and eyewear).

**Treatments and application**

In total, this study had eight treatments, an untreated or control plot and seven treated plots. The seven parthenium management strategies used for our study were reviewed by Mekonnen (2017), Khari and Kumar (2018), Kaur et al. (2014), Isaac et al. (2013) and Swanton and Weise (1991).

***Control***

No weed management treatment was applied to the control plot and all the naturally inhabiting plants were allowed to grow throughout the experiment.

***Mulching***

Mulch is a layer of material (living or nonliving) placed over the surface of the soil to suppress weeds and protect the soil from erosion. In our study, dry hay grass, a nonliving mulching material (Isaac et al., 2013) was spread on the assigned plot at a thickness of approximately 3 cm. Mulching was done twice during the experiment, first at the start of the experiment and halfway through the experiment.

***Foliar application of table salt solution***

A 15% table salt solution was prepared and sprayed on all plants in the assigned plot using a watering can. This treatment was done thrice at two weeks intervals during the experiment. The use of a salt solution for parthenium weed management is also described by Mekonnen (2017) and Kaur et al. (2014).

***Hand pulling***

All weeds, including parthenium weed on this plot were plucked by hand and discarded in a designated area for decomposition. For our study, hand pulling was done thrice on the assigned plot at two weeks intervals during the experiment. During hand pulling, gloves were worn to prevent direct skin contact and avoid allergic reactions from parthenium weed. Mekonnen (2017), Kaur et al. (2014), and Khari and Kumar (2018) described the used of handpulling or manual uprooting to control parthenium weed.

***Slashing***

A hand-held slashing or mowing equipment (slasher) was used to manually cut down weeds on the assigned plot. Slashing was done thrice at two weeks intervals during the experiment. Mekonnen (2017), and Onwueme and Sinha (1991) described slashing as an effective weed management strategy.

***Hand hoeing***

All plants on the assigned plot were dug up with a hand hoe. Hand hoeing was done thrice at two weeks intervals during the experiment. The use of hand hoeing or ploughing to control parthenium weed is reviewed by Mekonnen (2017) and Kaur et al. (2014).

***Foliar herbicide application***

In our study, Gramaxone was sprayed using a properly calibrated knapsack sprayer on the assigned plot at a concentration of 100 ml per 15 L of water. During herbicide application, personal protective equipment was worn. Foliar herbicide application was done thrice at two weeks intervals during the experiment. Herbicide application to control parthenium weed is reviewed by Mekonnen (2017), Kaur et al. (2014), and Khari and Kumar (2018).

***Integrated weed management (IWM)***

Integrated weed management is the application of numerous alternative weed control measures which include cultural, genetic, mechanical, biological, and chemical methods of weed control (Swanton and Weise 1991). In our study, multiple selected treatments, that were also tested singly, were used for the IWM plot, with each treatment being applied two weeks after the previous treatment. Hand pulling was done first followed by slashing, then hand hoeing, and then foliar application of Gramaxone as previously described. Throughout the experiment, field hygiene was ensured by carefully disposing off all weeds in a designated area for decomposition.

**Data collection and sampling design**

At the start of the experiment, different plant species at the experimental site were recorded to determine the plants that co-existed with parthenium weed in spite of allelopathy. Identification of weeds was done with reference to photographs from previous literature (Ivens, 1971). Parthenium weed was identified by looking at photographs of the identification kit developed by the Integrated Pest Management Laboratory-Collaborative Research Support Project (IPM-CRSP) provided by the parthenium project coordination office at Virginia State University, Virginia USA. To determine the effects of the selected management strategies on growth and development of parthenium weed, data on parthenium plant populations on respective plots was collected using the quadrat sampling method. Sampling was done by randomly throwing a 0.25 $m^2$ quadrat to each plot and counting parthenium plants inside the quadrat. On each day of data collection, four throws of the quadrat were done for each plot, the number of plants in each throw counted and used to estimate the parthenium plant population for each plot. To determine the parthenium plant population on each plot based on the four throws of the quadrat, the four values were summed up and multiplied by the total area of the quadrat (9 $m^2$). The resulting number was divided by the unit area of the quadrat (sum x 9 $m^2$/0.25 $m^2$). Data was collected two weeks after each time of treatment applications. At each time of data collection, all weed species on each plot were recorded to identify weeds that inhabited the same area as parthenium weed. Photographs of plots were also taken at the start and end of the experiment.

**Data analysis**

Using SAS 9.4 statistical package (SAS Institute, 2012), one-way Analysis of Variance (ANOVA) was used to determine the main effect of each weed management treatment on parthenium plant populations. The Tukey's multiple comparison test was used to determine differences among treatments.

## RESULTS AND DISCUSSION

### Plant species in the study area

At the start of the experiment, the experimental site was inhabited by both annual and perennial plant species including: parthenium weed at different growth stages (constituted approximately 80% of the plant population), star grass (*Cynadon dactylon*), Wandering Jew (*Commelina benghalensis*), sodom apple (*Solanum incanum*), couch grass (*Digitaria scalarum*), black-jack (*Bidens pilosa*), milk weed (*Euphorbia hirta)*, pig weed (*Amaranthus hybridus)*, goat weed (*Ageratum conyzoides*), guinea grass (*Panicum maximum*), oxalis (*Oxalis latifolia*), nutsedge (*Cyperus rotundus*), *Acacia* species and wild *Malvaceae* species. Among these, parthenium weed, sodom apple, black-jack, milk weed, pig weed, goat weed, and guinea grass are annual while the rest are perennial.

### Statistical comparisons

There were significant differences ($P \leq 0.05$) in parthenium plant populations among treatments (Table 1). Parthenium plant populations were significantly fewer ($P \leq 0.05$) between the control and all treatments except herbicide (Table 2 and Figure 1). Therefore, all management strategies tested in our study with the exception of herbicide application effectively controlled parthenium weed. Mean parthenium plant populations ranged from 3762.0 to 56.2 per plot and populations were highest in the control plot, followed by the herbicide-treated, hand pulled, hand-hoed, IWM-treated, slashed, salt-treated, and mulched plots, respectively (Table 2 and Figure 1). The lowest parthenium plant populations observed for the mulched plot was not surprising because mulching smothers weeds, preventing emergence (Isaac et al., 2013). Parthenium plant populations were comparable between hand hoeing and IWM treatments, and also between slashing and salt treatments (Figure 1).

### Parthenium weed management strategies

An examination of trends of parthenium plant populations for each treatment across the four data collection days showed that all the seven tested weed management measures reduced weed populations (Figure 2). With the exception of the control plot, all weed management treatments reduced parthenium plant populations over time. In the untreated control plot, parthenium plant populations increased progressively for the four data collection days as expected (Figure 2). Out of the seven weed management strategies tested in our study, two treatments (salt application and IWM) completely eliminated parthenium weed at the end of the experiments. Variation within treatments measured by the standard error of the mean (SE) was highest in the control plot indicating rapid increases in parthenium plant populations on subsequent data collection days. The SE was lowest in the mulched plot, an observation that can be explained by suppression of weed growth by the dry hay grass. Figure 3 shows sample photographs of the experimental site at the start of the experiment before deep ploughing (Figure 3A), after deep ploughing but before weed control treatments (Figure 3B), and at the start of the treatments, that is, when parthenium weed was at the rosette growth stage (Figure 3C). Respective plots at the end of the experiment are shown in Figure 3, panels D-K.

At the end of the experiment, the untreated control plot was inhabited by parthenium weed at different growth stages (rosette growth stage to mature plants) (Figure 3D). This was because mature parthenium plants produced seeds which germinated. Other weed species in the untreated plot were milk weed, pig weed, star grass, *Malvaceae* species and sweet potatoes (*Ipomea batatas*). Observations from the control plot are consistent with previous reports of the prolific and invasive nature of parthenium weed (Adkins and Shabbir, 2014; Kaur et al., 2014). Therefore, weed control measures should be applied when parthenium weed is still at the rosette stage to prevent seed production and additional colonization by new plants (Kaur et al., 2014). Mulching applied after clearing the plot effectively reduced parthenium plant populations throughout the experiment and only a few plants had emerged in areas with thinner mulch on the last sampling day (Figure 3E). Therefore, mulching is suitable for controlling parthenium weed, but more mulch should be added regularly to maintain the thickness and avoid emergence of weeds on thinner areas after decomposition. Mulching suppresses weed growth by acting as a physical barrier against weed emergence and cutting off direct sunlight (Isaac et al., 2013). Additional benefits of mulching are conservation of soil moisture, soil fertilization, protection from soil erosion, and improved soil quality (Isaac et al., 2013). Mulching with dry grass also utilizes locally available plant materials that can be readily accessed by farmers. Although our study was not conducted in a cropped area, mulching for parthenium weed management, mulching is not suitable for large-scale crop cultivation because it is laborious and time consuming. In addition to parthenium weed, milk weed was also able to grow in thinner areas

**Table 1.** One-way ANOVA of main effect for parthenium plant population per plot.

| Treatment | Parthenium plant population per plot [z] |
|---|---|
| Parthenium weed control measures | 0.0018[y] |

[z] Plot sizes were 9 $m^2$.
[y] P-values of main effects were determined by Tukey's adjustment for multiple comparisons at P≤ 0.05.

**Table 2.** Mean parthenium plant population per plot, and pair-wise comparisons of each treatment to the control.

| Treatment [y] | Mean parthenium plant population per plot [z] |
|---|---|
| Control | 3762.0[a] |
| Herbicide | 1467.0[ab] |
| Hand pulling | 1008.0[b] |
| Hand hoeing | 900.0[b] |
| IWM | 882.0[b] |
| Slashing | 639.0[b] |
| Salt | 612.0[b] |
| Mulching | 56.2[b] |

[z] Plot sizes were 9 $m^2$.
[y] Pairwise difference between treatments were determined by Tukey's adjustment for multiple comparisons at P≤ 0.05.

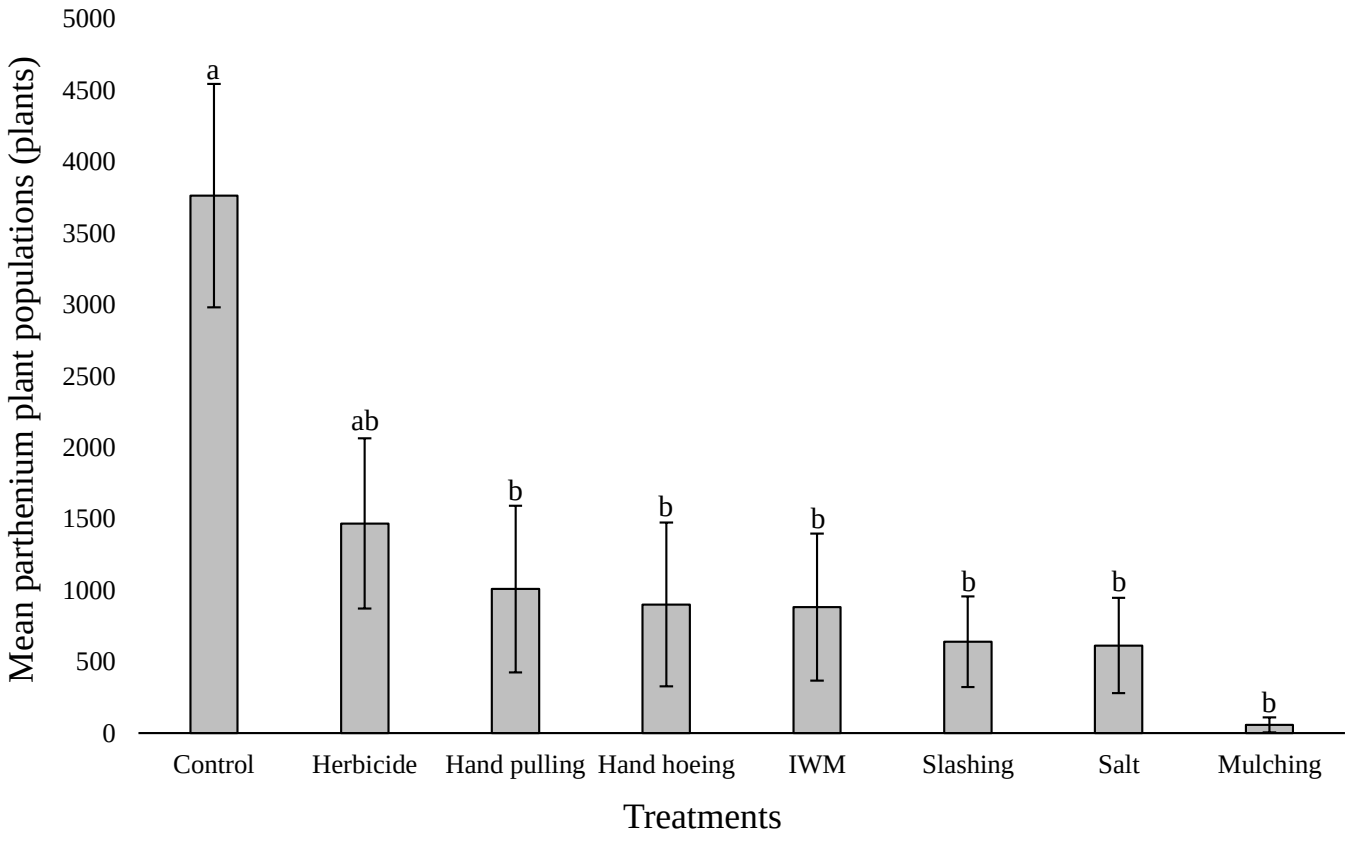


**Figure 1.** Mean parthenium weed populations for each treatment. Pairwise difference between treatments was determined by Tukey's adjustment for multiple comparisons at P≤ 0.05. Error bars represent the standard error of the mean.

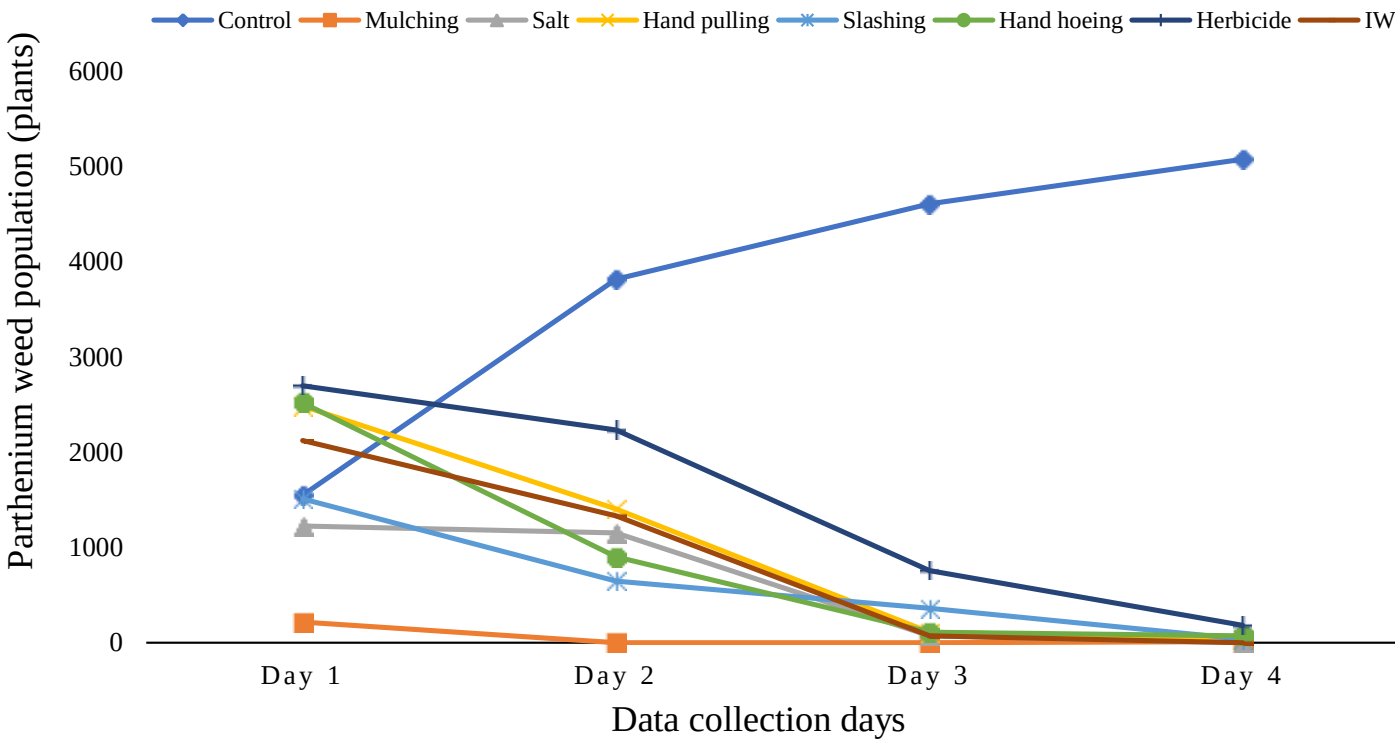


**Figure 2.** Trends of parthenium weed populations for each treatment on the four data collection days.


**Figure 3.** Photographs of the experiment. A) At the start of the experiment before deep ploughing; B) After deep ploughing but before treatments; C) Three weeks after deep ploughing (stage at which parthenium weed was at the rosette growth stage and weed control treatments were started); D-K) Plots at the end of experiment: Control (D), mulched (E), salt-treated (F), hand-pulled (G), slashed (H), hand-hoed (I), herbicide-treated (J), and (K) IWM-treated.

of the mulched plot.

Treatment of parthenium weed with a 15% table salt solution was effective and parthenium plants completely wilted and died after application of the salt solution (Figure 3F). Previous literature also reported that in non-cropped areas, open wasteland along railway tracks and roadsides, spraying of table salt solution at 15-20% concentration effectively controlled parthenium weed (Mekonnen, 2017). Application of table salt solution is suitable for controlling parthenium weed in Uganda because salt is affordable, does not pose health risks and application does not require any skill. However, other weed species such as star grass, Wandering Jew and milk weed were not killed by the salt solution which makes salt application a less suitable strategy for controlling parthenium weed in cropped areas.

In our study, hand pulling was effective for parthenium weed management (Figure 3G). Manual uprooting has been widely used for parthenium management by farmers. Hand pulling should be done before flowering and seed setting to prevent further spread. However, the downside of hand pulling is the risk of contact dermatitis (Kaur et al., 2014). Therefore, careful caution must be taken, and personal protective equipment worn when hand pulling parthenium weed to prevent allergic reactions. Additional constraints of hand pulling are its laborious nature and if the soil is dry, the entire root system may not be plucked out yet from these roots new plants can emerge. When plucking parthenium plants, all the root system should be removed to prevent re-growth (Abdulkerim-Ute and Legesse, 2016). Hand pulling is effective in areas where a minimum amount of disturbance is desired, parthenium plants are few, very close to the crop stand, or scattered to warrant use of other more costly methods (Onwueme and Sinha, 1991). Other weed species on this plot were milk weed, *Malvaceae* species and Wandering Jew.

Slashing was a suitable method for controlling parthenium weed and other weed species (Figure 3H). Our results are in agreement with Onwueme and Sinha (1991) who reported that slashing reduces growth of new plants by preventing seed production for most weeds and starving the underground parts. In our study, the roots left behind after slashing sprouted into new plants, which makes slashing not a very effective control measure if used on its own. Other weed species present in the slashed plot at the end of the experiment were oxalis, pig weed, and *Malvaceae* species.

Hand hoeing effectively controlled parthenium and other weed species (Figure 3I). Hand hoeing affects weed populations by harming weeds and also by greatly influencing the number, distribution, dormancy and viability of weed species (Ross and Lembi, 1985). For Ugandan farmers, hand hoeing is suitable for parthenium management because hoes are affordable, readily accessible and no skill is needed to use hoes. Similarly, Tamado and Milberg (2004), reported that hand hoeing was effective for controlling parthenium weed in smallholder farming systems in Eastern Ethiopia. Other weed species that co-existed with parthenium weed in the hand-hoed plot were black-jack, milk weed, sodom apple, Wandering Jew, star grass, oxalis, and *Malvaceae* species.

In our study, herbicide application did not significantly ($P>0.05$) control parthenium weed compared to the other management strategies but the trends showed reduction in parthenium plant populations (Table 2, Figures 2 and 3J). Our results suggest that parthenium weed could have some level of resistance to Gramaxone. Similar studies have also reported ineffectiveness of certain herbicides for parthenium control. For instance, Odero (2012) reported that parthenium weed was resistant to glyphosate. However, in contrast with these findings, herbicides such as Saflufenacil and Primextra gold effectively controlled parthenium weed (Khan et al., 2014; Odero, 2012). Therefore, when selecting herbicides for application, herbicides that are known to control parthenium weed should be used. Herbicide application is not very suitable for farmers in Uganda because herbicides are expensive, require skill for successful use, and can harm humans if not used as per manufacturer's recommendations (Aktar et al., 2009). For instance, glyphosate has been reported to be carcinogenic in humans and other organisms (Van Bruggen et al., 2018). Other weeds on the herbicide-treated plot were oxalis, milk weed, star grass, sodom apple, Wandering Jew, and sweet potatoes.

Integrated weed management was effective in controlling parthenium weed (Figure 3K). Combining multiple weed control measures overcomes limitations of other methods when used singly. For instance, roots left behind after hand pulling and slashing can be removed by hand hoeing. Mechanical methods of parthenium weed management can be utilized first and chemical control among the last options. In IWM, one should consider all available control measures, that is, cultural, mechanical, biocontrol, chemical, promoting competition from native plants, grazing, fire, and solarization (Adkins and Shabbir, 2014; Adkins et al., 2010; Tu et al., 2001). Together with parthenium weed, oxalis, black-jack, milk weed, nutsedge, and pig weed were present in the IWM-treated plot.

## CONCLUSIONS AND RECOMMENDATIONS

This study highlights weed management strategies that can be included in farmer education programs to control parthenium weed, while avoiding the negative effects of parthenium on humans and livestock. Therefore, extensionists in Uganda should train farmers and other stakeholders on specific management practices for parthenium weed and other noxious invasive weed species. Based on the findings of this study, the authors

recommend that a combination of multiple weed control measures should be utilized for effective management of parthenium weed in Uganda to reduce the limitations of specific methods when used singly. Control measures for parthenium weed should also be applied in before seed set to avoid further spread. In order to prevent harmful effects of parthenium weed on human health, personal protective equipment should be utilized when applying control measures.

## Strengths and future perspectives

Our study tested multiple control measures for parthenium weed, informing farmers, the general public, and researchers on ways to effectively manage this noxious weed, reducing its devastating effects on human health, and agriculture. Since our study was conducted on a non-cropped site, future studies should test effective strategies in cropped sites and pastures to determine location-specific control measures. Experimental studies should be done to examine mechanisms by which other weed species that co-existed parthenium weed reported in this study survived allelopathy. Additional experiments should also be conducted to examine allelopathic effects of parthenium weed on crop traits of economic importance such as plant growth rate and yield.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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*Full Length Research Paper*

# Evaluation of the pH of solid residues generated in the cellulose industry adequate to the sludge hygienization for use in agriculture

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**This paper looks into the possibility of using solid residues generated in the cellulose production process to hygienize sewage sludge for fertilization. During the cellulose fabrication process, dregs, grits and vegetable biomass ashes are generated. Seven experiments were conducted in the Hydraulic Research Institute´s laboratory at UFRGS, in order to determine the pH values of the three residues in analysis-ashes, dregs, grits and the combinations of sludge/ash, sludge/grits and sludge/dregs- at the beginning (IT) and at the end (after two hours-FT). The preparation of the dregs, grits and ash solutions consisted of the weighing of 20 g of each residue and its dissolution in 80 ml of water, thus obtaining the solutions of grits, dregs and ashes. The pH values of the samples were analyzed using bench pH-meter. The ashes, grits, the mixture sludge/ash and sludge/grits displayed an accentuated basic character due to their pH values which were ≥12. The dregs and the mixture sludge/dregs presented a less accentuated basic character than the ashes and the grits, with a pH equal to 9. The results show that the ashes and the grits can be used in the hygienization of sewage sludge.**

**Key words:** Sludge, ashes, grits, dregs, hygienization, pH.

## INTRODUCTION

In the paper and cellulose production process big quantities of residues are generated which need to be adequately disposed of. The main residues are dregs, grits, lime mud and biomass ashes. According to Almeida (2008), dregs are alkaline solid by-products composed of very small particles; their main constituents being carbonates, hydroxides and sulphides and, above all, Na and Ca. Grits are granular, yellow, odourless, alkaline solid residues that come from the lime and limestone mud calcinations process in the lime ovens (Nolasco et al., 2000). The biomass ashes are accumulated through the combustion of the vegetable biomass for the generation of heat and energy, containing a variety of macro and micronutrients resistant to incineration (Knapp

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and Insam, 2011). Among the various forms of final disposal of the sludge resulting from sewage treatment, the disposition on soil has been gaining popularity because of its richness in organic matter and nitrogen (Guerra et al., 2006). However, because the sludge comes from the sewers, it has some undesirable features such as the existence of pathogens, heavy metals and organic contaminants so it need to be stabilized/ hygienized before it is deposited on the soil (Prosab, 1999).

The destruction of pathogenic organisms is achieved through their exposure to conditions considered adverse to their survival, which include high temperatures, bactericidal effect of the Sun´s UV rays, aerobic conditions with high levels of $O_2$; reduction of the substrate, competition, predation by the zooplankton, high pH, presence of compounds toxic to certain bacteria or; combination of many of these factors (Andrade Neto, 1997).

Chemical stabilization consists of the addition of products that may inhibit biological activity or oxidise organic matter (Fernandes and Souza, 2001).

The most common chemical treatment is the alkaline in which a base, normally lime, is mixed with sludge, increasing its pH and destroying most of the pathogenic microorganisms. In order to achieve the intended goal, lime should be added to the sludge until its pH reaches 12 (Fernandes and Souza, 2001).

During the chemical stabilization, a series of reactions between sewage sludge and applied components occur. Thus, the chemical stabilization of sewage sludge is a process that converts sludge into a product appropriate for fertilization (Meurer, 2012). According to Von Sperling and Goncalves (2001), hygienization consists of the removal of pathogenic organisms.

The chemical features of wood ashes make them excellent material for the correction of the soil acidity, source of nutrients for tree plantations and, also constitutes a way of returning to nature what is collected as vegetable biomass, keeping or correcting the soil´s fertility and sustainability of the ecosystem (Bellote et al., 1994; Guerrini et al., 1994; Vance, 1996).

This experiment intends to evaluate the pH of three residues, namely, vegetable ash biomass, grit and dregs which are generated during the cellulose fabrication process, to hygienize sewage sludge for soil fertilization.

## Sludge characteristics

The sludge under analysis is the digested anaerobic, from the sewage treatment at Serraria Treatment Facility located on the southern region of Porto Alegre, Ipanema, which is operated by the Municipal Water and Sewage Department. (DMAE in Portuguese). The Sewage Treatment Facility, (ETE in Portuguese), uses centrifuges to dehydrate the sludge and, to facilitate the separation between solids and liquids, it uses the cationic polymer, polyacrimalide, (PAM). The samples were collected from the containers, after dehydration with 80% humidity between June and November 2017. ETE generates around 50 $m^3$ of sludge which is deposited at a sanitary landfill (DMAE, 2014).

## Location of the factory generating the residues

The three residues under analysis come from cellulose production facility at Veracel Company, operating in both forest and industrial areas, which is in Bahia, in the southern region of Brazil. Its plantations cover 5 municipalities, namely, Eunápolis, Canavieiros, Belmonte, Porto Seguro and Santa Cruz Cabrália. In the forestry area, the company´s cycle goes through the following stages: use of Technologies to produce eucalyptus clones, seedlings´ production, soil preparation, planting, fertilization, harvest and transportation to the factory. The processing begins with the reception of certified timber, harvested from the eucalyptus forests. After physical and chemical processes, the timber is transformed into cellulose.

## Cellulose production method

Veracel uses the Kraft method in its cellulose production process. The choice of this method is due to its efficiency and the fact that it is the most common in the cellulose industry. According to Amaral (2008), one of the main features of the Kraft method is the retrieval of chemical products and its stages are: cleaning, in order to achieve complete separation of mass from leach with the least dissolution possible; the evaporation of water from the leach until it reaches a combustible concentration; burning of the leach followed by the dissolution of the merged products; caustification- conversion of sodium carbonate into hydroxide.

According to the company, the facility generates about 1.960 tons/month of dregs/grits and 270 tons of ash. It is important to mention that, at Veracel, grits and dregs are mixed and considered a single type of residue. The process comprises the following stages.

## Timber preparation (debarking)

The timber comes to the mill in the form of logs from whence it is sent to the mechanic debarkers, if it is not debarked yet. The bark reduces the cellulose yields besides increasing the amount of impurities.

Figure 1 to 3 present simplified diagrams of a timber preparation yard, formation and treatment of wood chips and liquors´ retrieval, respectively.

## Chipping and chip classification

After debarking, the logs are sent to the chippers where

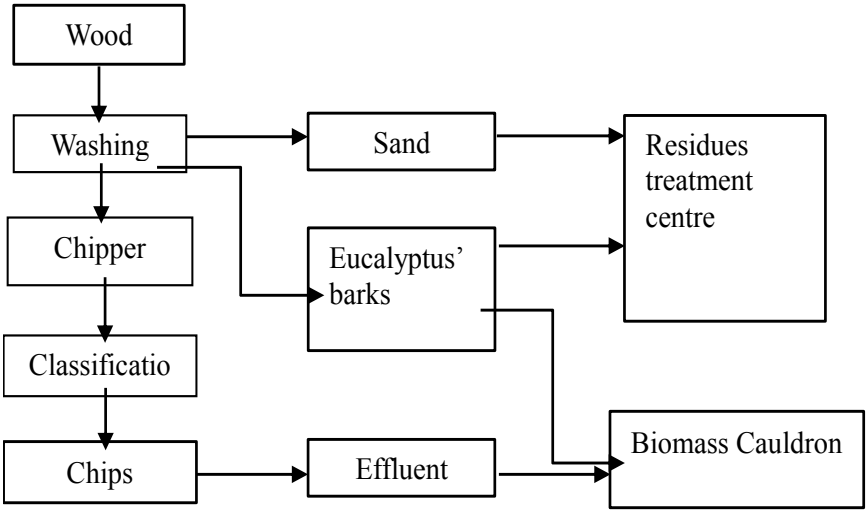



**Figure 1.** Simplified flow chart of the timber yard.

they are transformed into chips with appropriate dimensions for pulping (Guerra et al., 2006). This stage is meant to transform the logs into fragments small enough to allow the penetration of the pulping liquor used in the chemical processes. Figure 2 presents the chips´ formation and treatment process.

**Dark liquor retrieval**

The dark liquor's retrieval cycle and the alkaline´s energy processes reduce the loss of chemicals and minimize the production costs. The dark liquor´s heat generates enough energy to power the cellulose production plant. The main stages of the chemical retrieval process are: Evaporation of the dark liquor, incineration of the dark liquor in the retrieval cauldron, caustification and lime regeneration.

**Chemical composition of the residues**

As mentioned previously, in the cellulose and paper production process, large quantities of residues are generated which need to be adequately disposed of. The main residues are dregs, grits, lime mud and biomass. In this study, the pH of those residues was evaluated, except for lime mud. The residues are described in detail as follows.

**Dregs**

The limestone oxides originating from the process, organic matter, sulphur compounds, sodium and magnesium are the dregs' components. The metals present in the dregs may originate from wearing out of the equipment used in the process and the raw materials used for cellulose extraction (Maeda et al., 2010).

**Grits**

Calcium oxides, magnesium and potassium synthesized from lime mud are the main components of grits. Metallic content is greater than that of dregs. The next table presents the composition of grits.

**Ashes**

The ashes from the vegetable biomass are referred to as fertilizers due their significant basic cations content (Norstrom et al., 2012). However, they are primarily considered an acidity corrective material due to their high levels of oxides, hydroxides and calcium carbonates, although the magnesium hydroxides and carbonates, potassium and phosphorus are equally important (Haraldsen et al., 2011). Thus, the vegetable biomass ashes can be used as an acidity corrector, as well as sources of calcium, magnesium and potassium. Ferreiro et al. (2011) and Arshad et al. (2012) reported that soil acidity was the major limitation to food production worldwide and advocated the use of forest ashes as a corrective in acid soils. The main concern in relation to the use of forest biomass ashes as a fertilizer is the high concentration of potentially toxic elements, including heavy metals which may increase their concentration in the soil.

The composition of the ashes varies, according to the material used in and the intensity of the burning process. In the case of the forest biomass, the typical composition

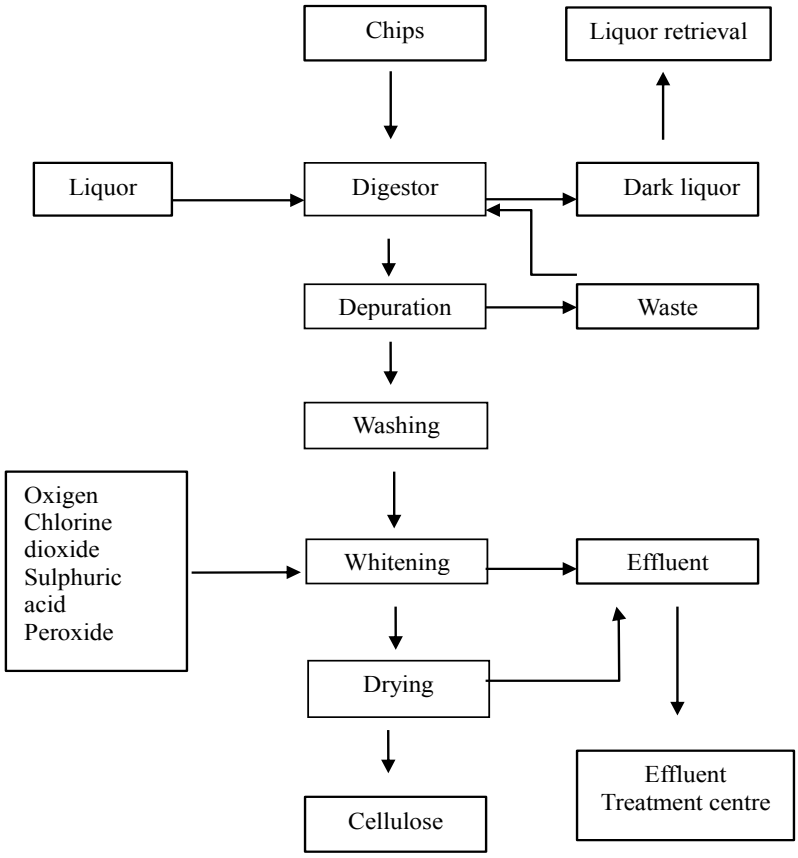



**Figure 2.** Chips´ formation and treatment flow chart.

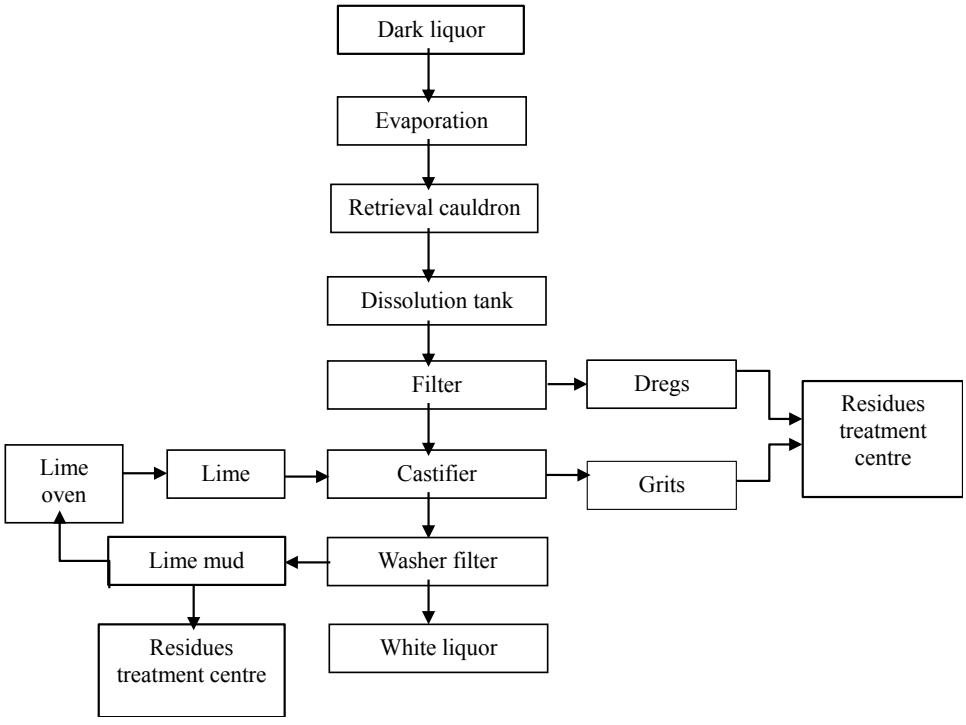



**Figure 3.** Simplified liquor retrieval flow chart.

is as shown in Table 3.

## MATERIALS AND METHODS

### Location of the study

The study was conducted in Porto Alegre, Brazil. The Municipality of Porto Alegre comprises 496.7 $km^2$ and a population of 1.476.867 inhabitants (IBGE, 2015). It is located at an altitude of 22 m; Latitude: 30° 1' 40" South; Longitude: 51° 13' 43" West.

The climate is subtropical humid; there are four seasons, though, owing to its being located along a transitional zone, weather tends to vary frequently.

The annual average temperature is 19.5°C; between 10 and 25°C in Autumn (March-June); Between 2 and 20ºC in winter (June to September); between 15 and 30°C in Spring (September to December) and varies between 25 and 35°C in Summer (December-March). (Porto Alegre Municipal Government-Tourism, available online through www2.portoalegre.rs.gov.br/turismo/default.php?p_ secao=260). Seven experiments were conducted at the Institute of Hydraulic.

Research, Federal University of Rio Grande do Sul, in order to determine the pH values of the three residues under analysis - ashes, dregs, grits and the mixtures sludge/ash, sludge/grits and sludge/dregs, at the beginning, (IT) and at the end (after two hours-FT) and the results are shown in Table 4. For the solutions of pure ash, dregs and grits, readings were taken only once, since their pH does not change over time. However, the readings of the combinations, were taken twice, at the beginning, (IT) and at the end, (FT), with a time lapse of 2 h.

Preparation of the dregs, grits and ashes solutions consists of the weighing of 20 g of each followed by its dissolution in 80 ml of water. Each experiment was conducted using sludge from a different batch; however, the three residues used for the pH monitoring came from the same batch.

After the preparation of the solutions, the following step consisted in the determination of the pH values for each one of them. For the mixtures of sludge/ash, sludge/grits and sludge/dregs, 150 g of sludge were weighed with 80% humidity, the equivalent to 30 g of dry sludge and the initial pH was determined. Afterwards, the 150 g of sludge with 80% humidity were mixed with100 ml of each previously prepared residue solutions, separately. Finally, the pH of each combination was determined.

Using a bench pH-meter, the pH values of the samples of digested sludge, ashes, dregs, grits and the combinations sludge/ash, sludge/dregs and sludge/grits, were determined.

## RESULTS AND DISCUSSION

Table 4 presents the pH values obtained from the three residues and the three combinations, in all the 7 experiments undertaken. Based on the pH results presented in Table 4, a second Table 5, was designed indicating the maximum, medium and minimum values for each parameter resulting from the seven experiments undertaken.

The graph bellow showing just the medium/average pH values is based on the results displayed in Table 5. From the data presented by Graph 1, the following can be noted: In relation to the digested sludge, (LD), the minimum, maxim and medium pH values show that it has a neutral pH and that no products with basic (chemical) properties were added during the dewatering process. These readings are considered excellent and typical for anaerobicaly digested sludge. A study by Fernandes and Silva (1999), that analyzed the efficiency of ETE Belém's sludge disinfection processes for agricultural use, came up with digested sludge with 7.0 pH.

In relation to the ashes, (C), the results displayed in Table 5, show that they have an accentuated basic character because their pH readings are ≥ 12. According to Table 3 data, the ashes have a high potassium content if compared to the grits and dregs. While analyzing the quality of vegetable ash for use as fertilizers in the Curitiba metropolitan area, Darolt and Osaki (1991) concluded that vegetable ashes, which were rarely used as soil fertilizer, contained calcium, magnesium, phosphorus and other elements. Diniz and Beig (1987) used ash in red/yelow latosoil, sandy phase, in doses oscillating between 1 and 4 t/ha, and obtained excellent results Maeda and Bognola (2013), while studying the chemical properties of soil treated with residues from the cellulose and paper industry, concluded that pH and Ca, Mg and P contents increased as the tested doses also increased while the AL content and its saturation decreased with application of all the tested materials, mainly wood ash. According to Erich and Ohno (1992), Etiegni and Campbell (1991) and Dahl et al. (2009), the forest ashes' ability to neutralize the soil's acidity depends mainly on the quantity of oxides, hydroxides and magnesium carbonates, potassium and limestone available.

Grits (G) - still based on the results of Table 5; it is noticeable that grits' pH values are similar to those of the ashes, which are ≥ 12. Such high pH values are due to their chemical composition, that is, the existence of compounds with accentuated alkaline behaviour, according to the data in Table 2. Chemically, the grits are 53% calcium oxide, which is an important ingredient for soil stabilization (Machado et al., 2003). Destefani et al. (2010) characterized and evaluated the grits form the cellulose industry and obtained the following composition. 96.80% CaO; 1.49% SO2; 1.37% $K_2O$; 0.22% SrO and 0.11% $Fe_2O_3$.

1. Dregs (D) - behaved differently from the ashes and the grits in respect to the minimum, medium and maximum pH values. Based on the results in Table, the dregs' medium pH value was 9.82. From this reading, it is evident that the dregs are residues with a basic behaviour, although less accentuated if compared to the ashes' and the grits'. The pH values' difference between dregs, dregs and ashes stems from their chemical compositions. As seen in Table 1, the dregs' calcium oxide content is 35%, less than the grits 53%. Medeiros (2008) analyzing the possibility of correcting the acidity of humid, aluminic cambisol soil, obtained the following chemical composition of dregs: Mg = 9 g/kg; Ca =354 g/kg; Na = 10 g/kg; pH= 10.7; neutralization capacity80%.

**Table 1.** Presents the chemical properties of the dregs

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| CaO-% | 35.7 | Pb- $mg.kg^{-1}$ | 50 |
| MgO-% | 3.62 | Cu- $mg.kg^{-1}$ | 100 |
| $SO_3^{-2}$-% | 1.6 | As- $mg.kg^{-1}$ | 3 |
| N-$NH_3$-% | <0.00 | Fe- $mg.kg^{-1}$ | 4.800 |
| N-total-% | <0.01 | Mg- $mg.kg^{-1}$ | 16.000 |
| C(fixo a 105°C)-% | 20.8 | Mn- $mg.kg^{-1}$ | 5.800 |
| Compost. de sódio-% | 4.7 | Ni- $mg.kg^{-1}$ | 100 |
| Metals-% | 3.2 | Ti- $mg.kg^{-1}$ | 500 |
| Silicatos-% | 30.4 | Zn- $mg.kg^{-1}$ | 40 |
| Sb- $mg.kg^{-1}$ | 20 | Al- $mg.kg^{-1}$ | 4.800 |
| Cd- $mg.kg^{-1}$ | 5 | PN-% | 72 |

**Table 2.** Chemical Properties of grits

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| CaO -% | 53 | Al- $mg.kg^{-1}$ | 3100 |
| MgO -% | 1.83 | Sb- $mg.kg^{-1}$ | 20 |
| $K_2O$-% | 1.2 | Cd- $mg.kg^{-1}$ | 50 |
| $SO_3^{-2-\%}$ | 0.7 | Pb- $mg.kg^{-1}$ | 50 |
| $OH^{-n}$-% | 0.4 | Cu-$mg.kg^{-1}$ | 20 |
| Sílica Solúvel-% | 0.4 | As- $mg.kg^{-1}$ | 2 |
| N-$NH_3$ % | <0.005 | Fe- $mg.kg^{-1}$ | 1600 |
| N-total | <0.01 | Mg- $mg.kg^{-1}$ | 2800 |
| Compost. de sódio % | 0.13 | Mn- $mg.kg^{-1}$ | 200 |
| Metais-% | 0.84 | Ni- $mg.kg^{-1}$ | 40 |
| Silicatos (balanço)-% | 41.5 | Ti- $mg.kg^{-1}$ | 500 |
| PN-% | 100 | Zn- $mg.kg^{-1}$ | 10 |

**Table 3.** Chemical properties of forest biomass ashes

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| $SiO_2$-% | 18-25 | Mn-% | 0.5 |
| CaO-% | 25-35 | $K_2O$-% | 10-15 |
| MgO-% | 6-7 | | |
| Fe-% | 3-5 | | |
| $Al_2O_3$-% | 2-4 | Densidade-$kg.m^{-3}$ | 193 |
| $P_2O_3$-% | 1.6-3.4 | PRNT-% | 25 |
| $Na_2O$-% | 0.5-0.9 | | |

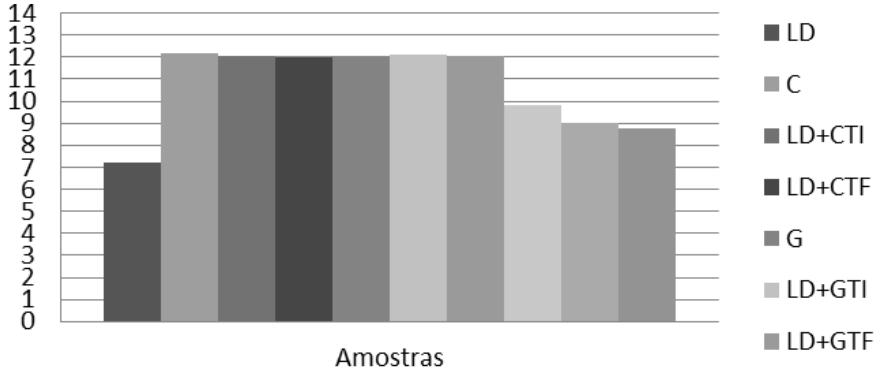
2. Sludge/ashes: This mixture's pH medium values were 12.05 at the beginning, (IT) and 11.98 at the end, (FT) as shown in Table 5. These readings showed that the mixture can obtain an excellent pH value for the sludge hygienization and stabilization process. According to Pinto (2001) chemical sludge hygienization mechanism uses an alkalinizing product to increase the sludge's pH to ≥ 12 values for, at least, 2 h.

3. Sludge/grits: Similarly to the sludge/ashes mixture, the sludge/grits mixture also presented excellent pH values, that is, ≥ 12. When the sewage sludge's pH increases, the cellular protoplasm's colloidal nature changes, resulting in an uninhabitable environment, lethal to the pathogens. Microbial activity takes place in environments

**Table 4.** pH values obtained during the experiments.

| Sample | Digested sludge | Ashes | Grit | Dreg | Sludge/ashes | | Sludge/grit | | Sludge/dreg | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TI | TF | TI | TF | TI | TF |
| E1 | 7.22 | 12.10 | 12.01 | 9.90 | 12.05 | 11.85 | 12.04 | 12.06 | 8.84 | 8.43 |
| E2 | 7.27 | 12.07 | 11.97 | 9.62 | 12.01 | 11.99 | 12.20 | 11.98 | 8.30 | 8.22 |
| E3 | 7.20 | 12.17 | 11.99 | 9.69 | 12.01 | 11.99 | 12.00 | 11.98 | 8.44 | 8.21 |
| E4 | 7.23 | 12.21 | 11.92 | 9.75 | 11.95 | 11.85 | 12.14 | 12.08 | 9.55 | 9.42 |
| E5 | 7.32 | 12.20 | 12.04 | 9.83 | 12.02 | 11.98 | 12.09 | 12.00 | 9.40 | 9.20 |
| E6 | 7.18 | 12.52 | 12.13 | 10.23 | 12.25 | 12.22 | 12.08 | 12.04 | 9.76 | 9.35 |
| E7 | 7.10 | 12.06 | 12.07 | 9.70 | 12.03 | 11.98 | 12.09 | 12.07 | 8.90 | 8.73 |

E-Experiment; IT-Initial time; FT-Final time.




**Graph 1.** Medium pH values. LD, digested sludge; C, ashes; G-grits, D-dregs; CTI, ashes initial time; CTF, ashes final time; GTI, grits initial time; GTF, grits final time; DTI, dregs initial time; DTF, dregs final time.

**Table 5.** Minimum, maximum and medium pH values from the 7 experiments.

| Simple | pH value | | |
|---|---|---|---|
| | Minimum | Maximum | Medium |
| Digested sludge | 7.10 | 7.32 | 7.22 |
| ashes | 12.06 | 12.52 | 12.19 |
| *Grits* | 11.92 | 12.13 | 12.02 |
| *Dregs* | 9.62 | 10.23 | 9.82 |

| Mixtures | TI | TF | TI | TF | TI | TF |
|---|---|---|---|---|---|---|
| sludge/ashes | 11.95 | 11.85 | 12.25 | 12.22 | 12.05 | 11.98 |
| sludge/*grits* | 12.00 | 11.98 | 12.20 | 12.08 | 12.09 | 12.03 |
| sludge/*dregs* | 8.30 | 8.21 | 9.76 | 9.76 | 9.03 | 8.79 |

between 6.5 and 9.0 pH, so pH is the primary issue in reducing microorganisms in sludge (Pinto, 2001). High pH does not just eliminate bacteria it also prevents the movement of heavy metals in the soil (Junior et al., 2001).

4. Sludge/dregs: This mixture's medium pH was 8.79, a lower figure if compared to the readings obtained from sludge/ash and sludge/grits mixtures. The dregs' low pH value might be because of the fact that in dregs, most of the cations are bound to the carbonate group.

## Conclusion

The solid residues generated in the cellulose industry– ashes, grits and dregs – are predominantly basic in their

chemical composition. The ashes and grits can be used to hygienize sewage sludge for use on agricultural soils. These two residues, ashes and grits, have a pH level equal or superior to 12 and when mixed with sludge they maintain this figure for two hours. Thus, the hygienization of sludge using vegetable biomass ashes is a viable alternative as, besides eliminating pathogens, there is an increase in potassium levels, which does not occur when sludge is hygienized using lime. The product resulting from the combination of sewage sludge, ashes and grits can be considered a biosolid as it contains the necessary nutrients to improve soil fertility.

## CONFLICT OF INTERESTS

The authors, Saidelamine Abibe Mahadal and Gino Roberto Gehling declare that there will be no conflict of interest within the scope of this article because it is their own and that all sources consulted were cited within the article.

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# Sources of productivity growth in Ethiopian agriculture

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**In Ethiopia, agricultural production and productivity are very low, and hence increase in production and productivity are vital to meet increasing food demand. This study identifies and quantifies the main sources of productivity growth in Ethiopian agriculture using the translog (TL) stochastic input distance function and the Ethiopian Rural Household Survey (ERHS) panel dataset. The true fixed effects (TFE) panel data estimator is used to separate inefficiency effects from observed and unobserved heterogeneity. The parametric Malmquist productivity index (MPI) is used to decompose total agricultural growth into three major sources. The average technical efficiency score was 0.875; this finding indicates that on average a farmer produces 87.5% of the value of the output that is produced by the most efficient farmer using the same technology and inputs. This implies that they can reduce the inputs required to produce the average output by 12.5% if their farming operation becomes technically efficient. MPI shows that the average annual productivity growth was 17.9% between 1994 and 2009. Further decomposition of the index shows that scale efficiency change is the most important source of this growth, and accounts for about 14.5%. Technological improvement accounts for approximately 4.8% while the contribution of technical efficiency change is negative, leading to an annual productivity decline of 1.3%. This finding suggests that increasing productivity is possible via improving these components by improving training to the farmers, extension services, research and development, and agronomic practices.**

**Key words:** Productivity growth, translog stochastic input distance function, Malmquist productivity index, Ethiopia.

## INTRODUCTION

Ethiopia is the second most populated country (109.2 million) in Africa, with the gross domestic product (GDP) of 84.4 billion USD, 7.6% GDP growth, 9.6% inflation, and 2.5% population growth as of 2018. Agriculture is a major economic activity in many developing countries. Ethiopia is no exception as it is predominantly an agrarian economy. Agriculture accounts for about 50% of GDP, 85% of employment, 70% of raw materials for

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**JEL codes**: D13; O12; O13; O33; O47; Q12; Q18.

industry, and 90% of foreign earnings. Agricultural production of crops and livestock are the main sources of income and employment for 70% of its rural population (World Bank, 2018a, b). The government aims to transform the economy of Ethiopia into a middle-income country by 2025. Thus, agriculture is part of this transformation with substantial growth in production and productivity. The population grows fast while the amount of cultivable land remains constant to produce food and fiber to the growing population. Thus, improving productivity in the agricultural sector is an important step forward to meet food supply challenges and to generate more income in rural areas. Total factor productivity (TFP) change is an important notion in developing countries because it measures the ability of households, firms, industries, and national economies to enhance the aggregate volume of outputs given the aggregate volume of inputs used (Balk et al., 2019).

Increasing agricultural productivity is one way to meet this growing demand. Improvements in agricultural productivity are also vital for economic development, especially in developing countries. In developing countries with low productivity, such as Ethiopia, there is limited surplus production over and above household consumption, which restricts market supply.

To my knowledge, there are no rigorous empirical studies that investigate sources of productivity growth in Ethiopia. To assess low agricultural productivity, we need to identify and quantify the main sources of productivity growth. In the literature, the main components of productivity growth include technical change and efficiency change. Efficiency change can be further decomposed into technical, mix and scale efficiency change (O'Donnell, 2012). O'Donnell (2016) states that the total factor productivity (TFP) index can be theoretically decomposed into measures of environmental change, technical change and other sources of efficiency change (technical, mix (input and/or output), and scale efficiency change). Kumbhakar et al. (2015) argue that most of a company's efficiency improvements come from technical efficiency improvements and technological improvements. Technical efficiency change means that the individual farmer moves closer to or further away from the boundary while technological improvement means that the set of feasible combinations expands or contracts (Balk, 2001). Scale efficiency measures the gap between constant and variable (increasing and decreasing) returns to scale. Therefore, scale efficiency change refers to the productivity growth that will arise because of a producer operating at a scale closer to the most productive scale size (MPSS) (Färe et al., 1994b). In comparison, mix efficiency is a measure of productivity change that arises when the input and/or output mix restrictions are relaxed, leading to an increase in the set of feasible input and/or output combinations (O'Donnell, 2012).

In agriculture, the three main sources of productivity growth are technological improvement, technical efficiency improvement, and scale efficiency change. Some nomenclatures are:

(1) Technical efficiency improvements essentially refer to increases in output-input ratios by reducing slack in the production process.
(2) Technological improvements usually refer to the expansion of a set of production possibilities that result from increased knowledge.
(3) Scale efficiency change refers to working at a scale level that is closer to the maximum productive scale size (Färe et al., 1994b).

Policies that are designed to improve agricultural productivity can target these different components. Such policies that are designed to increase productivity through improvements in technical efficiency include education, training and extension programs. Policies that seek to improve productivity through technical progress include government support for investment in scientific research and development. Policies that assist farmers to operate a scale closer to the most productive scale size include relaxing restrictions on land ownership and transfer, recommending proper input and/or output combination based on orientation and returns to scale. For example, if a producer is operating at decreasing returns to scale, then the scale of production can be optimized by a reduction of input(s).

There are limited empirical literatures measuring and decomposing productivity growth in Ethiopian agriculture. A summary of the various thematic strands of this empirical research are:

(1) Productivity comparisons between farmers who use an extension package program and those who do not (Ayele et al., 2006), or ways in which productivity can reduce the poverty of smallholder farmers (Abro et al., 2014).
(2) Assess the impact of sustainable agricultural practices (minimum tillage) (Kassie et al., 2011), or the effects of soil and water conservation (Adgo et al., 2013) on crop productivity.
(3) The effects of inefficiency as an explanatory variable on supply response using a profit function approach (Abrar and Morrissey 2006), and estimate and compare inefficiency from stochastic frontier analysis (SFA) with ordinary least square (OLS) during 1994 to 2004 (Bachewe, 2009). This paper covers a longer time (1994 -2009) than Bachewe (2009).

Methodologically, these studies employed the Tornqvist index (Ayele et al., 2006), propensity score matching methods and a switching regression model (Kassie et al., 2011), a macroeconomic approach to the growth accounting method (Bachewe, 2012), or stochastic

frontier analysis (SFA) (Abro et al., 2014; Bachewe, 2009). Most previous studies did not include risk and animal products and employed deterministic approaches. Besides, the model specifications in the parametric SFA approach used by these studies do not separate technical inefficiency and unobserved heterogeneity. Consequently, technical inefficiency might be over-estimated, and hence conclusions might be biased.

Moreover, these studies do not decompose productivity growth into its components. They neither disaggregate of crop and livestock products nor use multiple-input multiple-output (MIMO) approaches. Only a few of these studies use panel data, and when they do, the panels span short periods.

Finally, these few studies on productivity growth in Ethiopian agriculture are narrow in scope. Most of them only consider crop products, use cross-sectional data, shorter panel, and small sample sizes.

In comparison, this study employs modern methods on a large panel data set that contributes to the literature in the following ways. First, a comprehensive understanding of the main components of productivity growth can help to make Ethiopian agricultural policy more focused. This study contributes to this end by investigating the sources of agricultural productivity growth in Ethiopia into three major components. Second, it includes the risk preference behavior of households in the production function. Third, it takes into account livestock products separately from crop products by using a multi-out procedure using distance function techniques to give equal attention to crop and animal products. Fourth, it uses a true fixed effects (TFE) model that enables one to separate observed and unobserved heterogeneity from inefficiency.

Fifth, it simultaneously estimates production technology and inefficiency. Sixth, it employs a stochastic frontier approach with a longer panel. Last of all, it employs total agricultural productivity decomposition rather than the extant literature of partial agricultural productivity.

## METHODOLOGY

This part discusses Input Distance Function (IDF), Malmquist Productivity Index (MPI), econometric specification of IDF, and data sources and collection precisely.

### Input distance function

For a vector of inputs $\boldsymbol{X} = x_1, x_2, \ldots, x_k$ and a vector of outputs, $\boldsymbol{Y} = y_1, y_2, \ldots, y_m$, the multiple input-output production technology defined by *T*. The technology set *T* is defined as an input-output relationship given as follows:

$$T = \{(\xi, \psi): x \text{ can produce } \psi\}, \quad (1)$$

Where, $\xi \in \Re+^{K}$ is a vector of *K* inputs and $\psi \in \Re_{+}^{M}$ is a vector of *M* outputs of non-negative real numbers. This technology can be consistently represented using the input requirement set $\Lambda(\psi) = \{\xi: (\xi, \psi) \in T\}$. This input requirement set requires a technology that satisfies strong disposability[1] of inputs, and is closed and convex for all outputs (Coelli et al., 2005). The distance function measures the distance between a particular observation $(\xi, \psi) \in \Re+^{K} \times \Re+^{M}$ and the efficient technology boundary. Its value depends on a mapping rule, or a directional vector, which determines the direction in which the inputs are to be contracted and/or the outputs are to be expanded to reach this efficient boundary. The input distance function (IDF) $D_I(x, y)$ defined on the technology set given as:

$$D_I(x, y) = \max_{\lambda}\{\lambda \geq 1: \frac{\boldsymbol{X}}{\lambda} \boldsymbol{\epsilon}\, L(y)\}, \quad (2)$$

Where, λ is an input scaling factor by which the inputs can be contacted to make them technically efficient given the outputs. Concerning inputs, IDF $D_I(x, y)$ is non-decreasing, homogenous of degree one, and concave, whereas with respect to outputs, IDF $D_I(x, y)$ is non-increasing and quasi-concave (Färe and Primont, 1990). Further, IDF $D_I(x, y) \geq 1$ when the input mix is feasible or $\boldsymbol{X} \in L(y)$, whereas IDF $D_I(x, y) < 1$ when the input mix is infeasible or $\boldsymbol{X} \notin L(y)$. Therefore, for any feasible production mix, the technical efficiency (TE) is computed from IDF as:

$$\mathrm{TE} = \frac{1}{\mathrm{D_I(x,y)}} \quad (3)$$

Since, IDF $D_I(x, y) \geq 1$ for any data points, then $0 \leq TE \leq 1$ for any feasible observation. Both TE and IDF are equal to one when a household produces on the frontier for technically efficient households, and TE tends to zero as IDF tends to infinity for technically inefficient households.

The IDF, following input-output vector $(x_i^t, y_i^t)$, and the exogenous environmental variables, $z_i^t = z_{i,1}^t, \ldots, z_{i,q}^t$ is given as:

$$D_I^t(x_i^t, y_i^t) = F\left[\left(x_{i,1}^t, \ldots, x_{i,k}^t, y_{i,1}^t, \ldots, y_{i,m}^t\right), z_{i,q}^t, t\right] \quad (4)$$

$$\frac{1}{x_{i,r}^t} = F\left[\left(\frac{x_{i,1}^t}{x_{i,r}^t}, \ldots, 1, \ldots, \frac{x_{i,k}^t}{x_{i,r}^t}, y_{i,1}^t, \ldots, y_{i,m}^t\right), z_{i,q}^t, t\right] / D_I^t(x_i^t, y_i^t) \quad (5)$$

Following Orea (2002), *F* is a flexible translog (TL) technology proposed by Christensen et al. (1973). It is a more general and flexible functional form than Cobb-Douglas (CD) model. The TL approximation to the input based distance function is given as:

$$\begin{aligned} -lnx_{r,i,t}^t = \alpha_i \; + & \sum_{k=1}^{K-1} \beta_k \, lnx_{i,k}^{*t} \; + \frac{1}{2}\sum_{k=1}^{K-1}\sum_{j=1}^{K-1} \beta_{k,j} \, lnx_{i,k}^{*t} lnx_{i,j}^{*t} \\ & + \sum_{m=1}^{M} \theta_m lny_{i,m}^t + \frac{1}{2}\sum_{m=1}^{M}\sum_{j=1}^{M} \theta_{m,n} \, lny_{i,m}^t lny_{i,n}^t \\ & + \sum_{k=1}^{K-1}\sum_{m=1}^{M} \phi_{m,k} lny_{i,m}^t lnx_{i,k}^{*t} + \Upsilon_1 t + \frac{1}{2}\Upsilon_2 t^2 \\ & + \sum_{m=1}^{M} \psi_m t lny_{i,m}^t + \sum_{k=1}^{K-1} \eta_k t lnx_{i,k}^{*t} \\ & + \sum_{p=1}^{P} \xi_p z_{i,q}^t - u_{i,t} + v_{i,t} \end{aligned} \quad (6)$$

Where, $lnx_{i,k}^{*t} = lnx_{i,k}^t - lnx_{i,r}^t$, $v_{i,t}$ is the stochastic noise term, $\alpha_i$ is unobserved heterogeneity, and $u_{i,t} = lnD_I^t(x_i^t, y_i^t))$ is a non-negative error term capturing time-varying inefficiency. There are *k*

[1] The strong disposability assumption in inputs states that a proportional increase in inputs cannot decrease outputs (Färe et al., 1985; 1994a).

inputs and *m* outputs. Homogeneity of degree one in input quantities implies that:

$$\sum_{k=1}^{K}\beta_k = 1 \quad and \quad \sum_{K}^{K}\beta_{k,j} = \sum_{K}^{K}\phi_{m,k} = \sum_{K}^{K}\eta_k = 0 \tag{7}$$

Whereas, quadratic symmetry implies $\beta_{k,j} = \beta_{j,k}$ $and$ $\theta_{m,n} = \theta_{n,m}$. These restrictions are imposed before estimating IDF above by dividing the quantity of all inputs is divided by the quantity of oneof the inputs (Lovell et al., 1994). The method also allows the IDF to be estimated as it gives equation (6). Monotonicity requires all estimated IDF elasticities to satisfy the following conditions.

$$\frac{\partial lnD_I^t(.)}{\partial lny_m^t} = \theta_m + \sum_{m=1}^{M}\theta_{m,n}\, lny_n^t + \sum_{k=1}^{K-1}\phi_{m,k} lnx_{i,k}^{*t} + \psi_m t \le 0$$

and

$$\frac{\partial lnD_I^t(.)}{\partial lnx_k^{*t}} = \beta_k + \sum_{k=1}^{K-1}\beta_{k,j}\, lnx_{k,j}^{*t} + \sum_{m=1}^{M}\phi_{m,k} lny_m^t + \eta_k t \ge 0 \tag{8}$$

Saal et al. (2007) noted that there are two features that the above IDF differs from the standard translog approximation: (1) The addition of *q* exogenous operating characteristics, whose impact on input requirements is captured in the term $\sum_{p=1}^{p}\xi_p z_{i,q}^t$. (2) The additional household-specific intercept instead of the single intercept parameter that is the heterogeneous household-specific $\alpha_i$ parameters. These fixed effects allow controlling further for factors influencing input requirements that have not been specifically controlled for in the model.

**Malmquist productivity index**

Caves et al. (1982a; b) demonstrate that the Malmquist (1953) index can be used to measure the growth in productivity that occurred between two periods based on a given reference technology. The reference technology can be represented by the technology of one of the periods, as constructed from the observed input-output data or by some combination of technologies from both periods. For example, Färe et al. (1992) defined the input-oriented MPI, $M_I^{CCD}$, as the geometric mean of the Malmquist productivity indices for two adjacent periods, *t* and *t+1*, as:

$$M_I^{CCD}(x^t, x^{t+1}, y^t, y^{t+1}) = M_I^t(x^t, y^t, x^{t+1}, y^{t+1}) \times M_I^{t+1}(x^t, y^t, x^{t+1}, y^{t+1}) \tag{9}$$

$$= \left[\frac{D_I^t(x^t, y^t)}{D_I^t(x^{t+1}, y^{t+1})} \times \frac{D_I^{t+1}(x^t, y^t)}{D_I^{t+1}(x^{t+1}, y^{t+1})}\right]^{\frac{1}{2}}$$

Where, $(x^t, y^t)$ and $(x^{t+1}, y^{t+1})$ are input and output vectors that correspond to *t* and *t+1* , $D_I^t(.)$ and $D_I^{t+1}(.)$ are corresponding IDFs, and $M_I^t(.)$ and $M_I^{t+1}(.)$ are the respective Malmquist indices. It is possible to decompose the $M_I^{CCD}(.)$ index into technical efficiency change (that is catching up the best practice frontier), and technical change (that is a shift in the best practice frontier) (Saal et al., 2007; Fuentes et al., 2001; Färe et al., 1992). As indicated in Coelli et al. (2005), technical efficiency change between two periods can be expressed as:

$$M_I^I(x^t, x^{t+1}, y^t, y^{t+1}) = \frac{D_I^t(x^t, y^t)}{D_I^t(x^{t+1}, y^{t+1})} = \Delta\,\mathrm{TE}(x^t, x^{t+1}, y^t, y^{t+1}) = \frac{TE^{t+1}}{TE^t} \tag{10}$$

Given that $D_I^t(.) \geq 1$ for any feasibleinput-output mix, and $D_I^t(.) < 1$for any infeasible input-output mix,$M_I^{CCD}(.)$can be less than ,equal to, or greater than one to indicate productivity progress, stagnation, or decline, respectively.

This study follows that of Orea (2002), who suggested a parametric decomposition of the Malmquist productivity index that enables scale efficiency change to be introduced without computing scale efficiencies. For translog specifications, ODF (Orea, 2002) and IDF (Saal et al., 2007) defined the parametric MPI as the weighted difference between the average growth rates of output and inputs. Following Orea (2002) and Balk (2001) of an ODF, and Saal et al. (2007) and Pantzios et al. (2011) of an IDF, for a translog specification, the parametric MPI can be defined using distance elasticities with respect to inputs and outputs as weights. These weights are derived from estimated translog IDF elasticities with respect to outputs and inputs evaluated with data at time *t* and *t+1* as

$$lnM_I = -\frac{1}{2}M_I^t\sum_{m=1}^{M}[(\mathcal{E}_m^{t+1} + \mathcal{E}_m^t)(lny_m^{t+1} - lny_m^t)] - \frac{1}{2}M_I^t\sum_{j=1}^{J}\left[\left(\mathcal{E}_j^{t+1} + \mathcal{E}_j^t\right)\left(lnx_j^{t+1} - lnx_j^t\right)\right] \tag{11}$$

Where, $\mathcal{E}_m^t = \frac{\partial lnD_I^t(.)}{\partial lny_m}$ and $\mathcal{E}_j^t = \frac{\partial lnD_I^t(.)}{\partial lnx_j}$ indicate the output and input change weights evaluated at time *t* data, respectively, whereas $\mathcal{E}_m^{t+1} = \frac{\partial lnD_I^{t+1}(.)}{\partial lny_m}$ and $\mathcal{E}_j^{t+1} = \frac{\partial lnD_I^{t+1}(.)}{\partial lnx_j}$ are evaluated at time *t+1* data points. The negative sign before the output change and input change indices are to ensure positive weights.

The input weights sum to one because the IDF is homogenous of degree one in input quantities. However, the sum of the output weights does not equal one except under constant returns to scale. This implies that equation (11) violates the proportionality property that is required to satisfy to be a total factor productivity index (Orea, 2002). This is because the elasticity of scale, that is returns to scale (RTS), is measured for the IDF representation of technology by the negative of the inverse of the sum of the output elasticities (Färe and Primont, 1995).

$$RTS^t = -\left(1\Big/\sum_{m=1}^{M}\mathcal{E}_m^t\right) \tag{12}$$

To ensure that the proportionality property is satisfied, the study follows that of Orea (2002) and define the output weights as elasticity shares. From Orea (2002) and Saal et al. (2007), the generalized parametric MPI is given as:

$$lnG_I = -\frac{1}{2}\sum_{m=1}^{M}\left(\left(\mathcal{E}_{m=1}^{t+1}\Big/\sum_{m=1}^{M}\mathcal{E}_m^{t+1}\right) + \left(\mathcal{E}_{m=1}^{t}\Big/\sum_{m=1}^{M}\mathcal{E}_m^{t}\right)\right) ln(y_m^{t+1}/y_m^t) - \frac{1}{2}M_I^t\sum_{j=1}^{J}\left[\left(\mathcal{E}_j^{t+1} + \mathcal{E}_j^t\right)\left(lnx_j^{t+1} - lnx_j^t\right)\right] \tag{13}$$

By rearranging Equation (13), Saal et al. (2007) showed that it is possible to write the generalized parametric MPI as:

$$lnG_I = lnM_I + \frac{1}{2}M_I^t \sum_{m=1}^{M}\left((\mathcal{E}_m^{t+1}SF_I^{t+1}) + (\mathcal{E}_m^t SF_I^t)\right)ln(y_m^{t+1}/y_m^t) \quad (14)$$

Where, $SF_I^t = ((\sum_{m=1}^{M}\mathcal{E}_m^t + 1)/\sum_{m=1}^{M}\mathcal{E}_m^t) = 1 - RTS^t$ is an input distance scale factor and $RTS^t$ is the elasticity of scale at time *t*, as defined above. Thus, with constant returns to scale $RTS^t = 1$, $SF_I^t = 0$, and the generalized productivity index is equivalent to the Malmquist index. In contrast, with increasing (decreasing) returns to scale RTS>1 (RTS<1), and consequently$SF_I^t < 0 (SF_I^t > 0)$, and the generalized productivity index captures the positive (negative) impact of change in scale on productivity growth, which are not captured by MPI.

Orea (2002) used the quadratic identity (approximation) lemma of Diewert (1976) to decompose Equation 14 into the different components contributing to productivity growth. Following Diewert (ibid.), the quadratic identity lemma states that if F(s) is a quadratic function of its arguments, which is a vector of dimension R, then $F(S^1) - F(S^0) = \sum_{r=1}^{R}\frac{1}{2}[F_r(s^1) + F_r(s^0)][s^1 - s^0]$. In this equation, the superscripts on *s* represent certain data points (for example specific years), and $F_r = \frac{\partial F}{\partial S_r}$. In addition, $F(S^1)$ and $F(S^0)$ represent the evaluation of $F_r$ at two data points. Since the translog functional form is quadratic in the natural logarithms of its arguments, the difference between the evaluations of the IDF at two data points, which is a decomposition of total productivity growth, can be written as follows:

$$-ln\left(\frac{D_I^{t+1}(.)}{D_I^t(.)}\right) \equiv -\frac{1}{2}\sum_{m=1}^{M}\left(\mathcal{E}_j^{t+1} + \mathcal{E}_j^t\right)\ln(y_m^{t+1}/y_m^t) - \frac{1}{2}\sum_{j=1}^{J}\left(\mathcal{E}_j^{t+1} + \mathcal{E}_j^t\right)\ln(x_m^{t+1}/x_m^t) - \frac{1}{2}\left(\partial lnD_I^{t+1}(.)/_{\partial t} + \partial lnD_I^t(.)/_{\partial t}\right) \quad (15)$$

As the input distance is the negative inverse of the input based technical efficiency, i.e.,$-lnD_I^t = lnTE_I^t$, one can rewrite Equation 14 as:

$$lnG_I = [lnTE_I^{t+1} - lnTE_I^t] + \frac{1}{2}[(\partial lnD_I^{t+1}/\partial t) + (\partial lnD_I^t/\partial t)] + \frac{1}{2}\sum_{m=1}^{M}\left((\mathcal{E}_m^{t+1}SF_I^{t+1}) + (\mathcal{E}_m^t SF_I^t)\right)ln(y_m^{t+1}/y_m^t) \quad (16)$$

Therefore, one can decompose Equation 14 into three sources of productivity growth:

(1) Change in technical efficiency $\Delta TE = lnTE_I^{t+1} - lnTE_I^t$,
(2) technical change $\Delta TC = +\frac{1}{2}[(\partial lnD_I^{t+1}/\partial t) + (\partial lnD_I^t/\partial t)]$, and
(3) Scale change $\Delta SC = +\frac{1}{2}\sum_{m=1}^{M}\left((\mathcal{E}_m^{t+1}SF_I^{t+1}) + (\mathcal{E}_m^t SF_I^t)\right)ln(y_m^{t+1}/y_m^t)$.

Technical changes are the derivatives of the IDF with respect to the time trend evaluated with data at periods *t* and *t+1*. Thus, total factor productivity growth ($TFPG^{t,t+1}$) is the sum of technical efficiency change ($EC^{t,t+1}$), technical change ($TC^{t,t+1}$), and scale change($SC^{t,t+1}$) between *t* and *t+1* periods as:

$$TFPG^{t,t+1} = EC^{t,t+1} + TC^{t,t+1} + SC^{t,t+1} \quad (17)$$

**Econometric specification**

The translog IDF specified in Equation 6 was estimated using stochastic frontier analysis (SFA). The stochastic frontier production function framework independently introduced by Aigner et al. (1977), and Meeusen and Van den Broeck (1977) and latterly developed by Greene (2005a, b). The parametric SFA is employed to take account of the effect of measurement error and stochastic noise, and to test hypotheses on functional forms, parameters, and inefficiency (Coelli and Perelman, 1999; Pantzios et al., 2011). The Breusch-Pagan Lagrange multiplier (LM) was employed to test and check the presence of unobserved heterogeneity effects across households. Greene (2005a; b), the TFE and TRE models were chosen to separate time-varying technical inefficiency from unit-specific time-invariant unobserved heterogeneity. The Hausman specification test allows for checking if the true fixed effect (TFE) or true random effect (TRE) model specification is more preferred. The TRE model is more efficient, but its parameter can be biased if the Hausman test rejects the null hypothesis of no correlation between unobserved heterogeneity and the regressors and/or the model error term. The result of this test then decides the TFE estimator rather than the TRE panel estimator. Both models permit time varying-inefficiency, control for observed and unobserved heterogeneity in addition to separating inefficiency from unobserved heterogeneity. However, they differ concerning the assumption that the correlation between the unobserved heterogeneity and the regressors and/or error of the model. The TFE allows the correlation between them unlike the TRE model (Greene, 2005a, b). The test suggests that TFE is more appropriate then TRE. Therefore, the TFE model is used in the estimation.

Exogenous environmental variables $Z_{i,q}^t$ are included to account for observable factors affecting inefficiency beyond farmer's decision. These models allow $\alpha_i$ varying across households to control for the unobserved heterogeneity. Both the maximum likelihood estimator (MLE) of the TFE model and the TRE model can consistently estimate the unobserved effect models without dropping time-invariant variables (for example geographical variables) (Belotti et al., 2013b) though the latter drops it in the error term. Moreover, as noted by Wang and Schmidt (2002), the parameters of the technology and inefficiency are estimated using MLE in one-step to avoid biases associated with the two-step approach.

The decomposition of the residual random variable, $\varepsilon_{it}$, into $v_{it}$ and $u_{it}$ in the production function defines the stochastic production frontier, as first proposed by Aigner et al. (1977) and Meeusen and Van den Broeck (1977).

Battese and Coelli (1995) assume that $v_{it}$ are iid with mean zero and variance $\sigma_v^2$ ,i.e., $v_{it}$ ~N (0, $\sigma_v^2$). The $u_{it}$ are independently distributed non-negative truncations of a normally distributed random variable with mean $\mu_{it}$ and variance $\sigma_u^2$. The mean efficiency is $\mu_{it} = \alpha_i + \zeta_p Z_{iq}^t$

$\mu_{it} = \alpha_i + \zeta_p Z_{iq}^t$, where $Z_{iq}^t$ is a vector of observed exogenous variables like household characteristics, farm characteristics and geographic-specific variables that affect efficiency, while $\zeta_p$ is a vector of unknown parameters of the inefficiency equation, and $\alpha_i$

are a household-specific unobserved effect. The $v_{it}$ and $u_{it}$ are distributed independently of one another, and independently of the $X_{it}$ . Two additional parameters, $\lambda = \sigma_u^2/\sigma_v^2$ , and $\gamma = \sigma_u^2/(\sigma_u^2 + \sigma_v^2)$, are estimated to test the significance of inefficiency in the model. This shows the proportion of the inefficiency to noise variations in the variance of the estimated model.

**Data sources and collection**

This study used the Ethiopia Rural Household Survey (ERHS) dataset. The ERHS is a longitudinal dataset gathered from rural Ethiopia. Addis Ababa University (AAU), the Centre for the study of Africa Economics (CSAE) at Oxford, and the International Food Policy Research Institute (IFPRI) collaboratively collected the dataset in 1994, 1999, 2004 and 2009 from 4 major regions of the country: Amhara, Oromia, Southern Nation Nationalities and Peoples (SNNP), and Tigray. These four regions of the nine administrative regions in Ethiopia account for approximately 86% of the Ethiopian population. The ERHS dataset covers many villages in rural Ethiopia, including 18 farmers' associations (FAs), 15 of the 389 woredas[2] (districts), and 1,195 households. The surveys were conducted on a sample that is stratified over the country's three major farming systems across five agro-ecological zones (AEZs) (Dercon and Hoddinott, 2004). The three main sedentary farming systems are plough-based cereal farming, mixed plough/hoe cereal farming, and *enset* (false banana) farming systems. Finally, an unbalanced panel of 4,194 observations was created over four rounds.

The northern highland AEZ includes two villages in the Tigray region, Geblen and Harresaw, and one in the Amhara region, Shumsheha. The northern highlands are characterized by poor resource endowments, adverse climatic conditions, and frequent drought.

The central highland AEZ includes the villages of Dinki, Yetmen, and DebreBirhan, all located in the Amhara region, and Turufe Ketchema in the Oromia region.

The Arussi/Bale AEZ includes the villages of Koro Degaga and Sirbana Godeti, both located in Oromia. Adele Keke is the sole survey site locatedin the Hararghe AEZ of Oromia.

The remaining five villages of Imdibir, Aze Deboa, Gara Godo, Adado, and Doma are located in the enset-growing AEZ located in the SNNP region. Rainfall data from the National Meteorological Service Agency of Ethiopia are used.

For the variables defined above, all monetary terms are adjusted based on 1194 producer price index. The outputs of Ethiopian agriculture are crop and livestock. Crop output is represented by the value of about 60 types of crops (for example teff, maize, wheat, barley, sorghum, coffee, chat, *enset*, legumes, vegetables, etc.) which are annual and perennial crops produced in that specific production year. Livestock output is represented by the value of more than 10 types of livestock products ( for example meat, live animals, hides, skins, butter, cheese, milk, chicken, eggs, dung cakes, etc.) produced in the given production year. Soil fertility is an index from one to three indicating 1 for relatively bad and 3 for the relatively fertile land.

## RESULTS AND DISCUSSION

### Estimation and results

All variables were normalized by their geometric mean prior to transforming them into logarithmic form (Table 1). Hence, the first order parameters of the variables can be interpreted as distance elasticities at the geometric mean[3]. The maximum likelihood estimator (MLE), as implemented in the SFPANEL module of STATA 13.1 (Belotti et al., 2013a; b), is used to estimate the parameters in Equation 6. The translog production function is one of the flexible functional forms, but it is vulnerable to multicollinearity problems, is used. To proceed with a more parsimonious specification, I conducted various specification tests. As shown by test results reported in Table 2, restrictions for constant returns-to-scale technology, Cobb-Douglas technology specification, Hicks-neutral in input, joint restriction of Hicks neutral technology, no unobserved heterogeneity, no observed heterogeneity, time-invariant technical inefficiency, and truncated normal distribution for in efficiency are rejected at the 5%. However, scale neutral technology restriction cannot be rejected at the 5% level of significance. Hence, the non-restricted model of Equation 6 is estimated. The parameter estimates of the non-restricted model and truncated-normal distribution for technical inefficiency, which are estimated simultaneously, are reported in Table 3. The consequent computations of productivity growth (Tables 4 to 6) are based on this non-restricted model specification. As shown in Table 2, there is statistically significant decay over time captured by parameter*eta*. Hence, technical efficiency has declined over time.

### Parameter estimates

The estimated parametric stochastic frontier input distance function is presented. As depicted in Table 3, the input distance function parameters for inputs and outputs have the expected signs and are statistically significant at the 5% level of significance. The coefficients from the translog technology input distance function are distance elasticities at the geometric mean. The estimated input distance elasticities are 0.205, 0.003, 0.650, 0.006, 0.016 and 0.011 for labor, oxen, precipitation, seed, hoe and wealth, respectively. Modern inputs (for example seed), on average, contribute little and fertilizer does not lead to increased output. This reveals the extent to which Ethiopian agricultural production relies on conventional inputs (for example labor and precipitation) and explains why crop production in Ethiopia is sensitive to changes in the level of traditional and natural input use. The empirical evidence from the literature indicates that the probability of adopting fertilizer and improved seeds decreases as farm

[2] Woreda is a governmental administrative unit within zones of a given region, which is equivalent to the district designation elsewhere.

[3] All logged variables are divided by their geometric mean values before taking their logarithms. For the non-logged variable (that is the trend), the geometric mean value is subtracted from the observed values.

**Table 1.** Descriptive statistics of model variables.

| Variable | Symbol | Mean | Std. Dev. | Minimum | Maximum |
|---|---|---|---|---|---|
| **Output variables** | | | | | |
| Crop product (birr) | $y_1$ | 2950.54 | 4115.55 | 1.000 | 45821.48 |
| Livestock product (birr) | $y_2$ | 195.85 | 652.74 | 0.0001 | 14358.74 |
| **Input variable** | | | | | |
| Farm size (hectare) | $x_1$ | 1.56 | 1.29 | 0.01 | 10.9 |
| Labour (AE) | $x_2$ | 4.21 | 2.33 | 0.20 | 19.1 |
| Oxen (number) | $x_3$ | 0.81 | 1.10 | 0.00 | 11.00 |
| Precipitation (mm) | $x_5$ | 86.20 | 28.57 | 26.54 | 176.99 |
| Seed (birr) | $x_6$ | 321.93 | 835.27 | 0.00 | 13400 |
| Fertilizer (birr) | $x_7$ | 170.89 | 314.38 | 0.00 | 3782.37 |
| Hoe (number) | $x_8$ | 1.26 | 1.55 | 0.00 | 12.00 |
| Wealth (birr) | $x_9$ | 16506.78 | 39967.08 | 0.00 | 510947.90 |
| Soil fertility (index) | $x_{10}$ | 2.36 | 0.66 | 1.00 | 3.00 |
| **Environmental variable** | | | | | |
| Education (yes/no) | $x_{11}$ | 0.38 | 0.49 | 0.00 | 1.00 |
| Extension(yes/no) | $x_{12}$ | 0.32 | 0.46 | 0.00 | 1.00 |
| Market distance (minutes) | $x_{13}$ | 29.79 | 41.35 | 0.00 | 240.00 |
| Trend t(1=1994) | $t$ | 1.46 | 1.09 | 1.00 | 4.00 |

Source: by author's computation.

**Table 2**. Properties of the Ethiopian Farms' Household Technology.

| Restriction | Parametric Restriction | Wald test statistics | p-value |
|---|---|---|---|
| Constant returns-to-scale technology | $H_0: \sum_{m=1}^{M} \theta_m = -1, and \sum_{k=1}^{K} \phi_{mk} = \sum_{m=1}^{M} \theta_{mn} = 0$ | 41979.44 | 0.000 |
| Cobb-Douglas technology | $H_0$: All interaction terms are equal to zero | 4.3e+08 | 0.000 |
| Hicks neutral in inputs | $H_0: \eta_1 = \eta_2 = \cdots = \eta_{10} = 0$ | 644.81 | 0.000 |
| Hicks neutral in outputs | $H_0: \psi_1 = \psi_2 = 0$ | 5.23 | 0.073 |
| Hicks neutral in input and output/joint significance | $H_0: \eta_1 = \eta_2 = \cdots = \eta_{10} = \psi_1 = \psi_2 = 0$ | 14050.77 | 0.000 |
| No unobserved heterogeneity | $H_0: Var(u_{it}) = 0$ | 11.01 | 0.000 |
| No observed heterogeneity | $H_0: \xi_1 = \xi_2 = \cdots = \xi_7 = 0$ | 84.93 | 0.000 |
| Inefficiency is constant | $H_0$: *eta* = 0 | 31.86 | 0.000 |
| Truncated -normal distribution for technical inefficiency | $H_0: \mu = 0$ | 3.69 | 0.050 |

Source: by author's computation.

size declines (Croppenstedt et al., 1999; Amaha, 1999; Demeke, 1999). Endale (2010) indicated that the high price of fertilizer is the major constraint for about 47.6% of the farmers followed by a supply shortage and late arrival of fertilizer. In the study period, about 49 percent of smallholder farmers use fertilizer and 39 percent according to CSA survey (CSA (Central Statistics Agency of Ethiopia) of varies years).

As shown in Table 3, the input distance elasticity for wealth, which is a proxy variable for farmers' risk preference behavior (0.011), is positive and significant at the 5% level. This shows that as farmers become wealthier or become less risk-averse, they tend to use a greater quantity of inputs and hence the input distance

**Table 3.** Parameter estimates of the unrestricted Translog input distance function, 1994-2009.

| Variable | First-orders | $lnx_2$ | $lnx_3$ | $lnx_5$ | $lnx_6$ | $lnx_7$ | $lnx_8$ | $lnx_9$ | $lnx_{10}$ | $lny_1$ | $lny_2$ | t |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Constant | -1.171***(0.106) | | | | | | | | | | | |
| $lnx_2$ | 0.205***(0.008) | 0.167***(0.009) | | | | | | | | | | |
| $lnx_3$ | 0.003*(0.002) | -0.001 (0.003) | -0.010**(0.005) | | | | | | | | | |
| $lnx_5$ | 0.650***(0.013) | -0.126*** (0.016) | 0.007***(0.002) | 0.138***(0.020) | | | | | | | | |
| $lnx_6$ | 0.006***(0.002) | -0.000 (0.002) | -0.000 (0.000) | -0.002(0.002) | 0.001**(0.001) | | | | | | | |
| $lnx_7$ | -0.001(0.001) | -0.002 (0.003) | -0.000 (0.000) | 0.001 (0.002) | -0.000 (0.000) | -0.001(0.002) | | | | | | |
| $lnx_8$ | 0.016**(0.006) | -0.002 (0.001) | -0.000 (0.000) | -0.005* (0.003) | 0.000 (0.000) | 0.000 (0.000) | 0.007*(0.004) | | | | | |
| $lnx_9$ | 0.011*** 0.004) | -0.005 (0.009) | 0.000(0.001) | 0.004 (0.009) | -0.000 (0.000) | -0.000 (0.000) | 0.002***(0.001) | (0.001) | | | | |
| $lnx_{10}$ | -0.038(0.026) | -0.009 (0.034) | 0.002(0.005) | -0.004 (0.031) | -0.000 (0.006) | 0.002 (0.003) | -0.001 (0.006) | -0.001(0.003) | -0.044(0.097) | | | |
| $lny_1$ | -0.029***(0.011) | 0.003 (0.009) | -0.001(0.002) | 0.005 (0.011) | -0.000 (0.000) | 0.001 (0.001) | -0.000(0.001) | -0.000(0.004) | 0.021(0.028) | -0.005(0.009) | | |
| $lny_2$ | -0.001(0.001) | -0.001 (0.002) | 0.000 (0.000) | 0.003***(0.001) | -0.000 (0.000) | -0.000 (0.000) | 0.000 (0.000) | 0.001**(0.000) | -0.003 (0.002) | 0.001 (0.001) | 0.000(0.001) | |
| t | 0.038***(0.005) | 0.055***(0.012) | -0.002 (0.001) | -0.045*** (0.012) | 0.002 (0.001) | 0.002*(0.001) | 0.000(0.002) | 0.002(0.004) | -0.059***(0.017) | | | 0.032**(0.012) |
| Inefficiency determinants -Z-variables | | | | | | | | | | | | |
| $x_{11}$ | -0.104(0.079) | | | | | | | | | | | |
| $x_{12}$ | -0.035(0.090) | | | | | | | | | | | |
| $x_{13}$ | 0.001*(0.001) | | | | | | | | | | | |
| Sigma (u): $\delta_u^2$ | 0.460***(0.014) | | | | | | | | | | | |
| Sigma(v) : $\delta_v^2$ | 0.000***(0.000) | | | | | | | | | | | |
| Lambda: $\lambda = \frac{\delta_u^2}{\delta_v^2}$ | | 5545.03*** (0.014) | | Gamma:$\gamma = \frac{\delta_u^2}{\delta_u^2+\delta_v^2}$ | | =0.999 | | Log-likelihood function | | =3754.74 | | |

Significance codes: *** significant at the 1% level; **significant at the 5% level; * significant at the 10% level; robust standard errors reported in parentheses. Source: author's computations**.**

increases. Farmers' input allocation to each enterprise shows their risk preference behavior (Berbel, 1990). My basic premise is that farmers act rationally. Salimonu and Falusi (2007) argue that farmers' risk preference behavior affects enterprise selection, and thus input use and allocation pattern. Findings from the empirical literature suggest that absolute risk aversion decreases with wealth (Laffont, 1989; Arrow, 1965; Pratt, 1964), with income (Vickrey, 1945), and with endowment (Guiso and Paiella, 2008). The findings are in line with those of Collier and Gunning (1999), that is that farmers in developing countries tend to focus on low risk-low return activities. Since the pioneering work of Arrow (1965), who demonstrates the relationship between risk aversion and wealth, a growing body of literature has suggested individual risk attitudes are correlated with their wealth (Bucciol and Miniaci, 2011; Dohmen et al., 2011; Wik et al. 2004; Saha et al. 1994); with their constraint sets, such as access to credit, marketing, extension (Binswanger, 1980); with farm size, technology, wealth, or other personal traits (Lybbert and Just, 2007); and with fertilizer use (Holden and Westberg, 2016; McIntosh et al., 2013; Hagos and Holden, 2011).

The input distance elasticities for labor show approximately 20.5%, indicating that Ethiopian agricultural production technology is labor-intensive. This finding is not surprising given that 85% of the population depends on agriculture. As expected, the crop output elasticity (-0.029) is

**Table 4.** Technical efficiency and total factor productivity growth of Ethiopian Farm Households, 1994–2009.

| Year | Technical efficiency | Technical efficiency change | Technical change | Scale efficiency change | Total factor productivity growth |
|---|---|---|---|---|---|
| 1994 | 0.890 | - | - | - | - |
| 1999 | 0.891 | 0.004 | 0.017 | 0.765 | 0.785 |
| 2004 | 0.864 | -0.028 | 0.048 | -0.090 | -0.070 |
| 2009 | 0.855 | -0.015 | 0.080 | -0.253 | -0.190 |
| Average | 0.875 | -0.013 | 0.048 | 0.145 | 0.179 |
| Cumulative | | -0.039 | 0.145 | 0.422 | 0.525 |

Source: by author's computation.

**Table 5.** Technical Efficiency and total factor productivity Growth of Ethiopian farm households across agro-ecological zones (AEZs).

| AEZ | Technical efficiency | Efficiency change | Technical change | Scale efficiency change | Total factor productivity growth |
|---|---|---|---|---|---|
| Northern highlands | 0.884 | -0.009 | 0.045 | -0.060 | -0.024 |
| Enset, hoe | 0.872 | 0.017 | 0.048 | 0.648 | 0.713 |
| Hararghe, oxen | 0.853 | -0.052 | 0.048 | 0.191 | 0.187 |
| Arussi-Bale | 0.877 | -0.017 | 0.048 | -1.084 | -1.053 |
| Central highlands | 0.880 | -0.036 | 0.048 | 0.193 | 0.205 |
| Average | 0.875 | -0.013 | 0.048 | 0.145 | 0.179 |

Source: by author's computation.

**Table 6.** Technical Efficiency and total factor productivity growth of Ethiopian farm households across regions.

| Region | Technical efficiency | Efficiency change | Technical change | Scale efficiency change | Total factor productivity growth |
|---|---|---|---|---|---|
| Tigray | 0.863 | -0.023 | 0.040 | -0.293 | -0.275 |
| Amhara | 0.898 | -0.011 | 0.048 | 0.161 | 0.199 |
| Oromia | 0.857 | -0.050 | 0.048 | -0.379 | -0.381 |
| SNNP | 0.871 | 0.017 | 0.048 | 0.648 | 0.713 |
| Average | 0.875 | -0.013 | 0.048 | 0.145 | 0.179 |

Source: by author's computation.

negative and significantly different from zero at the 1% level. The negative value for output elasticity suggests that input distance decreases as output increases, that is the required input set to produce a given level of output decreases. The coefficient for trend variable (0.038) is positive and significant at the 1% level, indicating that the input requirement set of producing a given level of output expands if the trend variable increases. Additional factors that change over time, but that are not controlled for in the model are reflected by the trend variable.

As previously mentioned, the estimated input distance function is non-decreasing in input quantities and non-increasing in output quantities. Hence, it satisfies the monotonicity property of agricultural production technology at the point of normalization. However, as stated in Orea (2002), monotonicity with respect to the inputs of the output distance function has to be satisfied at all data points to avoid biased estimates of scale efficiency change. Likewise, the input distance function must be monotonous with respect to outputs at all data points. However, the translog function does not satisfy monotonicity globally (Orea, 2002). Hence, the estimated scale effects might be biased at those data points where monotonicity with respect to outputs is violated. In this

case, I find that the percentages of violations with respect to inputs range from 0 to 45% of the observations. The violations with respect to crop product and animal products are 21 and 52% of the observations, respectively [5]. To evaluate the effect of violations concerning crop and animal outputs on scale elasticity, I calculated the average scale elasticity (returns-to-scale/RTS) for all observations, and only for those observations that satisfy monotonicity with respect to both outputs. I find that the violations concerning crop output have a negligible impact on the scale elasticity estimate. Scale effects are also the most important source of agricultural productivity growth. The average scale elasticity (returns-to-scale) is -0.14, suggesting decreasing returns to scale. On average, the producers are operating at decreasing returns to scale. Hence, the scale of production can be optimized by the reduction of input(s) to produce a given level of production. The Eigen-value decomposition of the Hessian matrix supports this finding as that the curvature properties are violated at the geometric mean. Sauer et al. (2006) argue that violations of theoretical conditions are common in flexible functional forms, partly because of the tradeoff between flexibility and theoretical consistency.

## Technical efficiency

Given the estimated true fixed effects, time-varying technical efficiency scores of each farm household are obtained from the composite error term using the conditional expectation predictor of Jondrow et al. (1982). The inefficiency parameters are statistically significant with the expected signs. Again, the positive or negative signs of the parameters for these z-variables indicate that technical inefficiency has increased or decreased, respectively. Of the three z-variables controlled, the market distance measured in minutes affects inefficiency positively and significantly. The parameter estimates of gamma ($\gamma$= 0.99) in Table 3 indicates the share of technical inefficiency in the total error variance. The higher value is a measure of the suitability of the frontier approach compared with the least squares approach. Technical inefficiency accounts for approximately 99% of the total variability in output. The parameter estimates the lambda ($\lambda$= 5545.03), which measures the proportion of variance due to inefficiency as compared to statistical noise, which is many times fold than inefficiency, and it is statistically significant. The average technical efficiency score is 0.875, with a standard deviation of 0.13. This finding indicates that the average farmer produces 87.5% of the value of the output that is produced by the most efficient farmer using the same technology and inputs. This estimate is in line with the average agricultural efficiency score in China (88.4%), as reported by Yu et al. (2014) for the 1978 to 2010 period. Similar to Chinese farmers, Ethiopian farmers can improve their technical efficiency by fully utilizing existing inputs and technology. They can reduce the inputs required to produce the average output by 12.5% if their farming operation becomes technically efficient.

## Agricultural productivity growth and its sources

The total factor productivity growth rates (TFP) during the study period and its decomposition into three main components are presented in Table 4. The TFP is decomposed into technical efficiency change, technical change, and scale efficiency change. The results show that overall productivity growth is positive, and both scale efficiency change and technical improvement contribute positively while technical efficiency change contributes negatively.

As shown in Table 4, the average technical efficiency score drops from approximately 89.0% in 1994 to 85.5% at the end of the study period. The temporal decline in technical efficiency can partly be explained by the household decision on how to use limited inputs like fertilizers and improved seeds, risk preference behavior of the household, fragmented and small farm size, and the learning curve related to optimally using improved technologies.

In addition, the average technical efficiency was 89.0, 89.1, 86.4 and 85.5% in 1994, 1999, 2004 and 2009, respectively. These figures indicate that households have significant room for improvement in their farming practices over these periods compared with the best performers in the agricultural sector. I report the average and cumulative productivity growth during the 1994 to 2009 period.

The productivity decomposition in Table 4 shows that the productivity increase over time is mainly driven by scale efficiency change and technical change. Moreover, my results suggest that households were somehow approaching the most productive scale size in 1999. During the study period, productivity increased by 14.5% due to scale effects and 4.8% due to technical change. However, productivity decreased by 1.3% due to technical efficiency change, which was negative except in 1999. The scale efficiency change was 76.5% in 1999; it declined to -9.0% in 2004 and -25.3% in 2009. Unexpectedly, the scale effects show the inverse relationship between farm size and agricultural productivity. The cumulative productivity growth due to scale efficiency effect and technical change was 42.2% and 14.5%, respectively positive and far greater than that due to efficiency change.

[5]The percentages of violations with respect to inputs are 0.0% for precipitation, 0.6% for farm size, 5.7% for labor, 17.6% for wealth, 23.4% for seed, 34.6 for hoe, 45.2 for ox and 51.5 % for fertilizer.

The estimates are in line with the components of average productivity growth in agriculture reported for many other developing countries. Belloumi and Salah (2009) reported similar agricultural productivity growth rates (1%) from 1970-2000 in the Middle East and North African countries (Algeria, Egypt, Iran, Iraq, Israel, Jordan, Lebanon, Libya, Mauritania, Morocco, Saudi Arabia, Sudan, Syria, Tunisia, Turkey and Yemen). Similarly, Yu et al. (2014) reported an annual agricultural productivity growth of 2% for China from 1978-2010. Moreover, Belloumi and Salah (2009) concluded that technical change is the main source of productivity growth in the Middle East and North Africa. However, Fuglie and Wang (2012) reported that long-run TFP growth was below 1% per year in sub-Saharan Africa during the 1961 to 2009 period.

However, my results are much lower than the Fulginiti and Perrin (1998) findings for Turkey, Chile, Dominican Republic, Egypt, Portugal, Malaysia and Sri Lanka, during the 1961-1985 period; technical change ranges from 92.5% (Korea) to 100.9% (Egypt) and, efficiency change ranges from 97.3% (Thailand) to 103.3% (Dominican Republic) using output-based Malmquist index and a parametric Cobb-Douglas production functions).

With reasonable confidence, I thus conclude that scale efficiency and technical improvement contributes to productivity growth more than technical efficiency improvement. This result implies that there are many opportunities to increase production through technical efficiency, technological and scale efficiency improvements.

In Table 5, the further decomposition of productivity shows that technical inefficiency is somehow different across regions and AEZs. For example, the Central and the Northern highlands, and Arrusi/Bale AEZs have higher technical efficiency and above the overall national average. The enset-growing AEZ has technical efficiency slightly below the national average. The Hararghe AEZ has the lowest technical efficiency and it is below the national average. This is because precipitation is an important factor in this rain-fed agriculture, more specifically Hararghe relatively dry region and enset-growing AEZ is a relatively wet region of the country. Technical change is almost similar across AEZs and regions except for small variation in Tigray region and Northern highlands. This suggests that farmers use similar farming technologies.

Table 6 presents regional productivity decomposition, showing that the SNNP and Amhara regions are slightly more technically efficient and above the national average; and Oromia and Tigray are slightly less efficient and below the national average. Similarly, SNNP and Amhara regions show more productivity growth than the Oromia and Tigray regions. This is because scale efficiency and efficiency change contribute to SNNP, while only scale efficiency change contributes to Amhara region.

Agricultural production is not only a function of biophysical endowments but also a result of socio-economic conditions and the policy environment, which includes the availability of labor, the demand for food, the infrastructure between farms, and the presence of input and output markets (Chamberlin and Schmidt, 2011). The negative productivity growth in 2004 and 2009 is probably linked to the following factors. First, the Eritrean-Ethiopian war in 2000 increased the average military spending to GDP ratio from approximately 2.7 to 8.5%, spending that otherwise most likely would have been used in the agricultural sector to enhance input and credit supply during this period. Second, the Ethiopian government launched the National Extension Intensification Program (NEIP) in 1995, adopting methods originally introduced by Sasakawa Global 2000 (SG2000, 1995), with the intent of enhancing the availability of inputs and access to credit. Here, we note that the reach of and funding for the NEIP was subsequently reduced, and SG2000 abandoned its extension program in 2000. Finally, argumentative and unsettled national issues, such as land ownership and economic development; the institutional and constitutional structure of the Ethiopian state; and equality of ethnic and religious communities, were brought to the forefront more specifically during and after the 2005 election that have not been yet resolved.

## CONCLUSION AND POLICY IMPLICATIONS

Currently, the Ethiopian population is growing quickly, and natural resource stocks are depleting rapidly. Hence, improving agricultural productivity becomes increasingly important for several reasons: to increase agricultural production for home consumption and the market, to supply labor to other sectors, to conserve the environment, and to improve standards of living and thereby foster economic development. Future productivity growth in agriculture is also necessary to satisfy the increasing demand for food, fiber, fodder, and bio-energy, to contribute environmental conservation, and to bring Ethiopians to middle-income country in 2025.

Advances in agricultural productivity are also vital for economic development in developing countries. In developing countries with low productivity, such as Ethiopia, there is limited surplus production over and above household consumption, restricting the supply to the market. As a result, a large share of the population continues to participate in farming activities. For instance, in Ethiopia, 85% of the population farms, but these farmers cannot meet home consumption and domestic demands, mainly because of low agricultural

productivity. By contrast, in the developed countries with high agricultural productivity, a greater share of the produced quantity reaches formal markets. A small share of their population engages in farming, but these farmers manage to meet their domestic and export demands because of high productivity and efficiency. Because of this, more of the labor force is available for other sectors of the economy, such as services and manufacturing, which enables the overall economy to grow faster by increased demand for products from agriculture and other sectors.

Ethiopian farmers in particular and farmers in developing countries, in general, have an objective function of input contraction. Hence, the input distance function and decomposed TFP into scale efficiency change, technological change and efficiency change was estimated using the Malmquist productivity decomposition index.

The overall average technical efficiency score has been approximately 87.5%; this indicates that an average farmer produces 87.5% of the value of the output produced by the most efficient farmer using the same technology and inputs. In other words, farm households can reduce the inputs required to produce the average output by 12.5% if their farming operation becomes technically efficient. This implies that households have significant room to improve their farming efficiency and become close to the best performers in the agricultural sector.

On average, total factor agricultural productivity growth was estimated at about 17.9% during the study period. From this, agricultural productivity growth due to scale efficiency change, technical change, and efficiency change was approximately 14.5%, 4.8%, and -1.3, respectively. The first two components were the primary annual productivity growth during this period and unlikely there was a decline in efficiency change. Notably, according to my estimates, nearly all the agricultural productivity growth in Ethiopia is due to scale efficiency and technical change. This indicates that technical efficiency improvement becomes the main sources of productivity growth in the future.

Under the current production practices, and with such small and fragmented farm size, it is critical to fulfilling home consumption, domestic market demand, and allows people to leave agriculture for other jobs. However, narrowing the gap is possible by increasing productivity via technical change, scale efficiency change, and technical efficiency change through improving training to the farmer, extension services, research and development, and agronomic practices.

Scale efficiency effect and technological improvements are important sources of productivity growth in Ethiopian agriculture. The results suggest that scale efficiency change and technical change, not technical efficiency change, have been major sources of productivity growth in Ethiopian households during the study period. The average efficiency change decreases over the study period. There are also many opportunities to increase productivity by improving technical efficiency, which implies that there are many opportunities to increase production via efficiency improvements. First, smallholder farmers are technically inefficient. Technical efficiency improvement could be enhanced by improving farmer's education, training, and extension services that could reduce mistakes and encourage developing skills. The technical change could be enhanced through improved technologies (like an improved seed, fertilizer, irrigation, tractor, combiner, amongst other technologies) that must be introduced to improve the productivity of Ethiopian agriculture. Research and extension services should generate and promote appropriate technologies to boost the productivity of agricultural production systems, including improved farm implements, high-yielding varieties, better credit systems, better fertilizer application and usage, enhanced extension services, better irrigation facilities, and improved infrastructure. It is possible to increase the scale efficiency by scaling up best agronomic practices such as fertilizer application (amount, time and rate), seed rate, weeding, ploughing and pest, and disease management. These enable the farmer to produce at a scale closer to the maximum productive scale size. To sum up, my findings suggest that scale efficiency, technological and technical efficiency improvements are the three most important areas to consider in increasing productivity growth in Ethiopian agriculture.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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# Economic assessment of biological weed control using cover crop mixtures in maize production

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**The economics of using mixtures of a vegetable cowpea, Vigna unguiculata subsp. sesquipedalis L., known locally as 'Akidi' (A) in Eastern Nigeria, melon (M) and sweet potato (S) Ipomea batatas for weed management in maize were evaluated between 2007 and 2009 in Taraba State, College of Agriculture Teaching and Research Farm, Jalingo, Treatments include 20,000($_1$), 30,000($_2$) and 40,000($_3$) stands/ha of AM (AM$_1$, AM$_2$, AM$_3$), AS (AS$_1$, AS$_2$, AS$_3$), MS (MS$_1$, MS$_2$, MS$_3$) and AMS (AMS$_1$, AMS$_2$, AMS$_3$). Weeded (3+6 WAP) (C$_1$) and unweeded (C$_2$) checks served as control replicated three times in a randomized complete block design. Partial budget analysis was used to obtain the level of profitability. The cost of production in all the mixtures having sweet potato was slightly higher (₦61,740 .00-₦67,340.00) than the AM treated plots (₦51,460.00-₦52,880.00) in the three year production. The gross benefit of ₦205,490.00, ₦199,920.00, ₦164,940.00 and ₦130,270.00 was realized respectively from MS, AS, AMS and AM treated plots compared with ₦154,980 in the hand weeded plots. Over the three years, the net benefit was in the order MS> AS> AMS > C$_1$>AM , which resulted in 24.33, 23.22, 18.1, 17.57 and 13.67 times net profit when compared to the unweeded, respectively.**

**Key words:** Profitability, biological weed management, maize, cover crop mixtures.

## INTRODUCTION

The decision to use non-chemical weed management options including cover crops, either as sole or mixed by farmers is a business decision (SARE, 2019). The economics of weed control in maize has been reported for maize in many parts of Nigeria and elsewhere (Baba et al., 2015; Omovbude and Udensi, 2012; Maxwel et al., 2019). These studies emphazised use of non-food cover crops planted singly or with herbicdes expecially in forest-savannah transition zones. If any weed control option is not cutting cost or raising value, it is not lkely to be adopted (SARE, 2019). The food value of selected creepers, akidi, melon and sweet potato make them more adoptable to farmers across Nigeria and their weed suppression potentials have been established (Frick and Johnson, 2018; Kaur, 2017; Ahom et al., 2017). SARE (2019) observed adopting cover cropping, which is a conservative practice with high resilience may take several year period to have significant economic value if non- food cover crops are used. Therefore, the decision to adopt cover crop mixtures requires an economic

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outlook for sustainable usage. The profitability of weed management technologies for maize production (Saleh and Oyinbo, 2017) is indespensible to compliment the usual research focus on agronomic and productivity aspects of maize-cover crops intercrop system. The economic aspects of using cover crop mixtures has not been adequately addressed in the study area, thereby limiting research-based decisions of the farmers. Thus, this study compares the profitability of several mixed cover crops aimed primarily for weed suppression in maize production.

The impact of cover crops mixture in a production system is to reduce soil erosion and suppress weed. However, Maxwel et al. (2019) reported that in the US, planting and establishing a decent stand before winter are major challenges. This calls for a holistic perspective in appraising the benefit of such farms. The need for farmers to make several management changes that compliment the productivity of the target or primary crop of interest like maize and maximize the overall economic efficiency calls for the assessment of benefits derivable from the chosen weed control option. This is the essence of this study.

The potentials of vegetable cowpea (*Vigna unguiculata subsp. sesquipedalis* L), melon (*Citrullus lanatus*) and sweet potato (*Ipomea batatas*) to suppress weed in maize have been reported (Okpara, 2000; Ahom et al., 2017; Michael, 2015). Weed density reduction in maize field by sole planted akidi (A), melon (M) and sweetpotato (S) was 72-80, 55-63% and 60-71% in Northern Guinea Savanna of Nigeria, while the mixed cover AM, AS, MS, AMS reduced weed density by 61-66%, 67-71%, 56-65% and 59-66% more than the un-weeded (Michael, 2015). Herbicide free weed management practices could be cultural, mechanical and biological (Kaur, 2017) useful at initial emergence stage of weed or when weed population is below the economic threshold level. Some cover crop species also release chemicals from roots or decaying residue, which can inhibit weed seed germination (DeAnn and Anita, 2016).

The possibility of using cover crop mixtures for weed management has been suggested and used (Michael, 2015; Michael and Tijani-Eniola, 2013; Scott and Burt, 1985; Anuebunwa, 1991). Bunch (1995) suggested the potentials latent in new as yet untried species, like Akidi (*Vigna unguiculata subsp. sesquipedalis* L), including trees and non legumes, and the value to be derived from using combination of green manures and cover crops rather than individual species be harnessed (Frick and Johnson, 2018). Abdin et al. (2000) in their evaluation of cover crops for weed control in maize in Canada used a mixture of red clover and ryegrass, and white clover and ryegrass at 1:1 proportion. In their studies, the combination of cover crops and cultivation controlled 77-80% of weed. The red clover/ryegrass and white clover/ryegrass gave 21.3 and 32.4 $gm^{-2}$ of weed respectively, which significantly reduced weed weight when compared with weedy control (89.8 $gm^{-2}$).

In California, a mixture of melon and cowpea together with herbicide grow vigorously and out competed weed (Sullivan, 2003). Abdin et al. (2000) working on 12 cover crops in Canada observed that the mixtures ryegass, and clover at 1:1 plant population gave good ground cover, reduce weed weight, and have yield comparable to hand and chemically weeded treatment. The rationale for using cover crop mixture in weed suppression includes:

(i) Enhancement of biodiversity with consequent biological, physical, chemical input on the soil and environment.
(ii) Reduction in pest/disease attack
(iii) Reducing the yield depressive effect of some effective non leguminous cover crops like sweet potato and pumpkin in crops like cocoyam, plantain and maize. (Akinyemi, 1989; Nwagwu et al., 2000)
(iv) Possibility of regulating rate and duration of decomposition and subsequent nutrient supply.
(v) The nature of traditional farming culture, where farmers grow a number of such cover crops without observing their effects of weed control.

Gianessi and Reigner (2007) reported that though herbicides are used to control weed populations on 87 million ha of cropland in the United States; the major reason that organic crop hectarage totals only 565,600 ha is the difficulty of weed control without herbicides. Kyle et al. (2015) observed that enhanced agricultural productivity in developing country is hindered by the failure of farmers to adopt new technology and improved agricultural practices. Farmers cited financial constraints and need for specialized skills were some reasons for not adopting chemical weed control (Adedzwa and Ortese, 2004; Eni et al., 2013).

Organic crop growers cite weed control as their greatest difficulty in crop production because they are not permitted to use chemical herbicides. They substitute hand weeding and cultivation for herbicides at a greatly increased cost and with reduced effectiveness. The possibility of reducing herbicide use by roughly 50% by planting cover crops which enhances moisture retention and weed control (Winslow, 2018). The cover crop protected the soil from erosion and provided about a 50% reduction in weed biomass in the fall compared to bare fallow (Frick and Johnson, 2018).

Seeding a blend of cover crop species is often more effective than seeding a mono cropping system. Some species grow quickly and die during the winter, while others take longer to establish then living into the spring. A mix of species that collectively provides continuous living vegetation is often recommended – though not always critical – for increased weed suppression (Winslow, 2018).

Weed scientists must find cost-effective, ecologically based methods to manage undesirable plants. Economic

**Table 1.** Cover crop mixtures weed management treatments.

| S/N | Treatment | Plant population/ha |
|---|---|---|
| 1 | $AM_1$ | Akidi + Melon at 10,000 each (20,000) |
| 2 | $AM_2$ | Akidi + Melon at 15,000 each (30,000) |
| 3 | $AM_3$ | Akidi + Melon at 20,000 each ( 40,000) |
| 4 | $AS_1$ | Akidi + Sweet potato at 10,000 each (20,000) |
| 5 | $AS_2$ | Akidi + Sweet potato at 15,000 each (30,000) |
| 6 | $AS_3$ | Akidi + Sweet potato at 20,000 each ( 40,000) |
| 7 | $MS_1$ | Melon + Sweet potato at 10,000 each (20,000) |
| 8 | $MS_2$ | Melon + Sweet potato at 15,000 each (30,000) |
| 9 | $MS_3$ | Melon + Sweet potato at 20,000 each ( 40,000) |
| 10 | $AMS_1$ | Akidi + Melon + Sweet potato at 6,666 each (20,000) |
| 11 | $AMS_2$ | Akidi + Melon + Sweet potato at 10,000 each (30,000) |
| 12 | $AMS_3$ | Akidi +Melon + Sweet potato at 13,333 each ( 40,000) |
| 13 | $C_1$ | Hand weeded control (3+6 WAP) |
| 14 | $C_2$ | Unweeded control |

analyses are needed for management, policy making, and setting research priorities. The fundamental economic principle for weed management is to act only if the benefits exceed the costs. Therefore, the economics of using mixed cover of a vegetable cowpea "akidi" and melon (AM), akidi and sweet potato (AS), Melon and sweet potato (MS), akidi, melon and sweet potato (AMS) at three planting densities 20,000($_1$), 30,000($_2$) and 40,000($_3$) stands/ha for weed management in maize production were evaluated to ascertain their level of profitability.

## MATERIALS AND METHODS

### Experimental site

Field trials were conducted at the Teaching farm of Taraba State College of Agriculture (08° 50' N, 11° 50' E) in the Northern Guinea Savannah ecological zone. Jalingo has a wet and dry tropical climate with rainy season of about 150 days and an average annual rainfall of about 700- 1000 mm. Mean annual temperature of Jalingo is about 28°C with maximum temperatures ranges between 30 and 39.4°C. The minimum temperatures range between 15to 23°C. The rainy season is between May and October while the dry season is from November to April.

### Land preparation

The land used for the experiment was cleared manually using cutlass to reduce the few shrubs scattered on the field. Ploughing was done once using tractor.

### Experimental design and layout

The experiment was designed to study the influence of three planting densities of mixtures of akidi/melon (AM), akidi/sweet potato (AS), melon/sweet potato (MS) or akidi/melon/sweet potato (AMS) on weed suppression and performance of maize. The experimental design was a randomized complete block with three replications. There were 14 mixed cover treatments as in Table 1. Each plot measured 4m × 4m with 1m space between plots and 2 m border separating blocks. The total land area was (69 m × 16 m) 1104 $m^2$.

### Planting and trial management

Planting of maize was done on 16$^{th}$ June, 2007; 30$^{th}$ June, 2008 and 13$^{th}$ June, 2009. Cover crops were planted within 24 h. Maize was sown three seeds per hole at 25 cm × 100 cm spacing, to give a population of 40,000 plants/ha in all the plots and the seedlings were latter thinned to one plant per stand. The plot size was 4 m × 4 m. There were 64 stands of maize per plot (4 rows of 16 stands/ row).

Akidi and melon seeds were sown 4/hole, while 2-3 sweet potato vines/hole, spaced 50 cm × 100 cm and latter thinned to give the required population densities of 20,000 (One stand/hill); 30,000 (One and two stands in altenate hills) or 40,000 (two stands/hill) plants/ha. All cover crop treated plots were weeded once at 3 weeks after planting to enhance establishment and spread.

In each of the cover crop mixtures, cover crops were planted at 1:1 ratio in two way mixture and 1:1:1 in three way mixture. The cover crops were planted in alternate rows/hills. Field management was similar for all the treatments till harvesting.

### Data collection and analysis

Maize grain yield was estimated from 10 tagged plants sampled in the middle of each plot; cover crop yield was from 3 and 6 stands of each cover crop per plot in 2 way and 3 way mixtures respectively. Crop Enterprise Budget Technique (Wesley et al*.,* 1993) was used for the economic analysis of maize production under each of the mixed cover crops weed management treatments yearly. The cost of inputs, various farming operations and crop prices were the average prices prevailing in the study area during the experimental periods. The budget preparations included calculation of th e:

(i) Average yield of maize (t $ha^{-1}$)
(ii) Gross benefit (Naira/ha) based on prices of maize and or cover crop

**Table 2.** Cost of production and gross benefit of using cover crop mixtures to manage weed in maize production 2007 – 2009.

| Treatment | 2007 | | 2008 | | 2009 | | Average | |
|---|---|---|---|---|---|---|---|---|
| | CP (₦' 000) | GB (₦' 000) | CP (₦' 000) | GB (₦' 000) | CP (₦' 000) | GB (₦' 000) | CP (₦' 000) | GB (₦' 000) |
| $AM_1$ | 49.48 | 123.43 | 51.61 | 117.72 | 53.28 | 136.92 | 51.46 | 126.02 |
| $AM_2$ | 49.85 | 113.59 | 53.96 | 148.82 | 53.17 | 127.28 | 52.33 | 129.90 |
| $AM_3$ | 50.55 | 121.68 | 56.14 | 178.74 | 51.96 | 104.24 | 52.88 | 134.89 |
| AM | 49.96 | 119.57 | 53.91 | 148.43 | 52.80 | 122.81 | 52.22 | 130.27 |
| $AS_1$ | 63.11 | 200.50 | 65.08 | 141.45 | 64.56 | 157.47 | 64.25 | 166.47 |
| $AS_2$ | 66.96 | 209.50 | 69.47 | 159.05 | 65.88 | 243.10 | 67.44 | 203.88 |
| $AS_3$ | 69.75 | 322.42 | 71.99 | 153.28 | 69.26 | 212.53 | 70.33 | 229.41 |
| AS | 66.61 | 244.14 | 68.85 | 151.26 | 66.57 | 204.37 | 67.34 | 199.92 |
| $MS_1$ | 63.22 | 233.00 | 63.30 | 135.76 | 62.15 | 159.79 | 62.89 | 176.18 |
| $MS_2$ | 64.96 | 229.43 | 67.69 | 188.83 | 66.96 | 272.40 | 66.54 | 230.22 |
| $MS_3$ | 71.52 | 329.43 | 71.59 | 169.00 | 67.71 | 131.81 | 70.27 | 210.08 |
| MS | 66.56 | 263.95 | 67.53 | 164.53 | 65.61 | 188.00 | 66.57 | 205.49 |
| $AMS_1$ | 57.87 | 155.05 | 60.27 | 160.31 | 58.86 | 124.90 | 59.00 | 146.75 |
| $AMS_2$ | 59.63 | 151.61 | 62.41 | 155.12 | 62.76 | 167.85 | 61.60 | 158.19 |
| $AMS_3$ | 64.97 | 222.52 | 64.38 | 137.20 | 64.51 | 209.89 | 64.62 | 189.87 |
| AMS | 60.82 | 176.39 | 62.35 | 150.88 | 62.04 | 167.54 | 61.74 | 164.94 |
| $C_1$ | 52.44 | 118.05 | 55.17 | 163.55 | 56.36 | 183.35 | 54.66 | 154.98 |
| $C_2$ | 40.39 | 50.50 | 39.99 | 43.75 | 39.95 | 43.20 | 40.11 | 45.82 |

AM=Akidi + Melon, AS =Akidi + Sweet potato, MS = Melon + Sweet potato, AMS= Akidi + Melon + Sweet potato$C_1$=weeded control, $C_2$=unweeded control 1=20,000 stands/ha , 2=30,000 stands/ha, 3=40,000 stands/ha. CP: cost of production, GB: gross benefit.

Gross benefit (₦/ha) = (yield of maize x price) + (yield of cover crop x price)

(iii) Total variable cost (₦/ha) for each treatment comprising cost of land preparation, planting materials and labour (for planting, weeding, harvesting and processing).

(iv) Net benefit (NB) (₦/ha) under each treatment

Net benefit (₦/ha) = Gross benefit – Total variable cost.

(v) The marginal rate of return (%) that compared the extra (marginal) costs with the extra (marginal) net benefit

$$\text{Marginal Rate of Return (MRR)} = \frac{\text{Extra benefit from weed management}}{\text{Extra investment in the weed management}} \times \frac{100}{1}$$

(vi) Relative profitability was assessed with :

(a) Net benefit relative to hand-weeded control ($C_1$) ($NBRC_1$)

$$NBRC_1 = \frac{\text{Net benefit from a given weed management treatment}}{\text{Net benefit from hand-weeded control } (C_1)}$$

(b) Net benefit relative to the unweeded ($C_2$) ($NBRC_2$)

$$NBRC_2 = \frac{\text{Net benefit from a given weed management treatment}}{\text{Net benefit from the unweeded control } (C_2)}$$

(c) Percentage Net Benefit Gain (%NBG)

$$\%NBG = \frac{(\text{Net benefit from a given weed management treatment} - \text{Net benefit from unweeded}) \times 100}{\text{Net benefit from the unweeded control } (C_2)}$$

(vii) Weed control efficiency

$$WCE = \frac{\text{Weed population in unweeded control} - \text{Weed in treated plot} \times 100}{\text{Weed population on unweeded control}}$$

## RESULTS

The cost of production in all the mixtures having sweet potato were slightly higher (₦61,740 .00-₦67,340.00) than the AM treated plots (₦51,460.00-₦52,880.00) on the three year average (Table 2).The gross benefit fluctuated over time in most treatments. It generally declined between 2007 and 2008, and later appreciated in 2009. On the average ₦205,490.00, ₦199,920.00, ₦164,940.00 and ₦130,270.00 were realized respectively from MS, AS, AMS and AM treated plots compared with ₦154,980 from the hand weeded plots.

The net benefit followed the trend of the gross revenue. Averaged over the three years, net benefit increased with planting populations in all the covercrop mixture groups except in MS where $MS_2$ recorded the highest net profit of ₦163,680.00. The order MS> AS> AMS > $C_1$>AM was established, which resulted in 24.33, 23.22, 18.1, 17.57 and 13.67 times net profit when compared to unweeded respectively. The marginal rates of return of $AS_2$ (2.02), $AS_3$ (2.26), $MS_2$ (2.46),and $AMS_3$ (1.94) were higher than that of $C_1$ (1.82), while the rest were less. The

**Table 3.** Net benefit and marginal rate of return for using cover crop mixtures to manage weed in maize production 2007 - 2009.

| Treatment | 2007 | | 2008 | | 2009 | | Average | |
|---|---|---|---|---|---|---|---|---|
| | NB (₦' 000) | MRR | NB (₦' 000) | MRR | NB (₦' 000) | MRR | NB (₦' 000) | MRR |
| $AM_1$ | 73.96 | 1.49 | 66.11 | 1.28 | 83.64 | 1.57 | 74.57 | 1.45 |
| $AM_2$ | 63.74 | 1.28 | 94.86 | 1.76 | 74.11 | 1.39 | 77.57 | 1.48 |
| $AM_3$ | 71.13 | 1.41 | 122.60 | 2.18 | 52.28 | 1.01 | 82.00 | 1.53 |
| AM | 69.61 | 1.39 | 94.52 | 1.75 | 70.01 | 1.33 | 78.05 | 1.49 |
| $AS_1$ | 137.38 | 2.18 | 76.37 | 1.17 | 92.91 | 1.44 | 102.22 | 1.60 |
| $AS_2$ | 142.53 | 2.13 | 89.58 | 1.29 | 177.22 | 2.69 | 136.44 | 2.04 |
| $AS_3$ | 252.67 | 3.62 | 81.29 | 1.13 | 143.27 | 2.07 | 159.08 | 2.27 |
| AS | 177.53 | 2.67 | 82.41 | 1.20 | 137.80 | 2.07 | 132.58 | 1.98 |
| $MS_1$ | 169.78 | 2.69 | 72.47 | 1.14 | 97.64 | 1.57 | 113.30 | 1.80 |
| $MS_2$ | 164.47 | 2.53 | 121.14 | 1.79 | 205.44 | 3.07 | 163.68 | 2.46 |
| $MS_3$ | 257.92 | 3.61 | 97.41 | 1.36 | 64.10 | 0.95 | 139.81 | 1.97 |
| MS | 197.39 | 2.97 | 97.00 | 1.44 | 122.39 | 1.87 | 138.93 | 2.09 |
| $AMS_1$ | 97.18 | 1.68 | 100.05 | 1.66 | 66.04 | 1.12 | 87.75 | 1.49 |
| $AMS_2$ | 91.98 | 1.54 | 92.71 | 1.49 | 105.09 | 1.67 | 96.59 | 1.57 |
| $AMS_3$ | 157.55 | 2.42 | 72.82 | 1.13 | 145.37 | 2.25 | 125.25 | 1.94 |
| AMS | 115.57 | 1.90 | 88.53 | 1.42 | 105.50 | 1.70 | 103.20 | 1.67 |
| $C_1$ | 65.61 | 1.25 | 108.38 | 1.96 | 126.99 | 2.25 | 100.32 | 1.82 |
| $C_2$ | 10.11 | 0.25 | 3.77 | 0.09 | 3.25 | 0.08 | 5.71 | 0.14 |

AM=Akidi + Melon, AS =Akidi + Sweet potato,MS = Melon + Sweet potato, AMS= Akidi + Melon + Sweet potato$C_1$=weeded control, $C_2$=unweeded control 1=20,000 stands/ha , 2=30,000 stands/ha, 3=40,000 stands/ha. NB: Net benefit.

MRR increased with planting density in all group except in MS (Table 3).

The weed control efficiency and relative profitability of using various cover crop mixtures for weed management in maize is shown in Table 4. Though the weed control efficiency was highest of hand weeded (0.72), it was comparable with AS (0.70), AMS, AM (0.63 each), followed by MS (0.60). Profitability relative to the recommended hand weeding shows that the net benefit ratio of AS (1.02-1.59) and MS (1.13-1.63) treated plots was higher than the hand weeded while those of AM plots and AMS treated plots except at 40,000 stands/ha ($AMS_3$) were less. Relative to the unweeded, all the cover crop mixtures were more profitable, 13.06-28.67 times more profitable than the unweeded plots. Similar trend was observed for net benefit gain

## DISCUSSION

The economics of maize production under various cover crop mixture reflected the productivity of the component crops and not just the grain yield of maize (Michael, 2015). Though the main target crop was maize, the overall cost of production, gross return and net profits were higher in plots with sweet potato because appreciable tuber yields were harvested, while the absence of sweet potato in AM plot caused them to have the lowest economic value. The net profit of sole cover akidi, melon, or sweet potato reflected the impact of extra cash benefit derivable from the sale of cover crop in association with maize as well as the planting density. This observed efficacy of sole planted cover crops comparable with hand weeded plot is confirmed in this study (Michael and Tijani-Eniola, 2014). Among the cover crop mixture treated plots with melon had the least cost of production, gross revenue and net benefit when compared with mixtures having akidi and/or sweet potato because melon could not reach maturity and no harvestable yields were obtained throughout the experimental periods, simillar to the observation in melon/plantain system (Akinyemi and Tijani–Eniola 1997). Omovbude and Udensi (2012) reported that use of melon plus hoe weeding recorded the lowest financial return when compared with mulched or herbicides treated plots in a forest-savannah transition zone of Edo state, Nigeria. Anuebunwa (1991) reported that egusi melon (*Colocythis citrillus*. L) at 40,000 stands/ha in association with yam in Umudike grew vegetatively with no pod formation. This was the experience in this trial. Mixtures having sweet potato had higher economic value because of the yield obtained from the tuber. Before increasing seeding rates to enhance weed suppression, the economic benefit of higher seeding rates should be

**Table 4.** Weed control efficiency and relative profitability of using cover crop mixtures for weed management in maize.

| Treatment | WCE | NB (₦' 000) | $NBRC_1$ | $NBRC_2$ | %NBG (x100) |
|---|---|---|---|---|---|
| $AM_1$ | 0.62 | 74.57 | 0.74 | 13.06 | 12.06 |
| $AM_2$ | 0.61 | 77.57 | 0.77 | 13.58 | 12.58 |
| $AM_3$ | 0.66 | 82.00 | 0.82 | 14.36 | 13.36 |
| AM | 0.63 | 78.05 | 0.78 | 13.67 | 12.67 |
| $AS_1$ | 0.67 | 102.22 | 1.02 | 17.90 | 16.90 |
| $AS_2$ | 0.71 | 136.44 | 1.36 | 23.89 | 22.89 |
| $AS_3$ | 0.71 | 159.08 | 1.59 | 27.86 | 26.86 |
| AS | 0.70 | 132.58 | 1.32 | 23.22 | 22.22 |
| $MS_1$ | 0.56 | 113.30 | 1.13 | 19.84 | 18.84 |
| $MS_2$ | 0.58 | 163.68 | 1.63 | 28.67 | 27.67 |
| $MS_3$ | 0.65 | 139.81 | 1.39 | 24.49 | 23.49 |
| MS | 0.60 | 138.93 | 1.38 | 24.33 | 23.33 |
| $AMS_1$ | 0.63 | 87.75 | 0.87 | 15.37 | 14.37 |
| $AMS_2$ | 0.59 | 96.59 | 0.96 | 16.92 | 15.92 |
| $AMS_3$ | 0.65 | 125.25 | 1.25 | 21.94 | 20.94 |
| AMS | 0.63 | 103.20 | 1.03 | 18.07 | 17.07 |
| $C_1$ | 0.72 | 100.32 | 1.00 | 17.57 | 16.57 |
| $C_2$ | 0.00 | 5.71 | 0.06 | 1.00 | 0.00 |

considered because increased seed fee costs may exceed the benefits in weed suppression (Nice et al., 2001; Renner and Mickelson, 1997). Similarly, the substantial tuber yields of sweet potato obtained in the S plots resulted in higher gross and net benefit in which the yields from other plots having less stands of sweet potato could not offset. The high rainfall which favoured sweet potato growth and production was also to the disadvantage of akidi and melon, causing them to produce little or no harvestable yields.

## Conclusion

The net benefit was in the order MS (Melon/Sweetpotato) > AS (Akidi/Sweetpotato) > AMS (Akidi/Melon/ Sweetpotato) > $C_1$ (Hand weeding) > AM (Akidi/Melon), which resulted in 24.33, 23.22, 18.1, 17.57 and 13.67 times net profit when compared to the unweeded, respectively. Organic weed management using melon/ sweet potato, akidi/sweet potato or akidi/melon/sweet potato mixtures is more profitable than the recommended hand weeding.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Yield responses of bush bean varieties to different planting densities and rates of phosphorous fertilizer

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**Common bean is commonly grown by smallholder farmers under quite diverse farming systems and it is for both food security and income generation. The impact of plant density is important to bean growers for yield optimization and the inadequate source of phosphorus leads to low productivity due to its significant in growth and development. This study focused on determining optimal spacing of different bush bean varieties for enhancing productivity in relation to application rates of P-fertilizers. A field experiment was conducted at Selian Agricultural Research Institute during short and long rain cropping seasons of 2016/2017 and 2017/2018 respectively. Treatments comprised of three bush bean varieties (Lyamungu 90, JESCA and KATB1), three spacing options 50 cm x 20 cm; 40 cm x 20 cm and 30 cm x 20 cm and four levels of P-fertilizers of 0 kg ha$^{-1}$; 20 kg ha$^{-1}$; 40 kg ha$^{-1}$ and 60 kg ha$^{-1}$ replicated three times in randomized complete block factorial design. Both treatments and their interaction showed significant differences ($p \leq 0.05$). The combination for spacing of 30 cm x 20 cm with planting density of 333,333 plants ha$^{-1}$ and P-fertilizer rate of 60 kg ha$^{-1}$ with productivity of 2580 kg ha$^{-1}$ enhanced bean productivity compared to commonly used combinations with productivity of < 600 kg ha$^{-1}$.**

**Key words:** Productivity, interactions, food security, plant density and yield responses.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the leading leguminous crop, accounting for 78% of land under legumes in Tanzania (FAO, 2013). Annual consumption of beans in the world is as high as 66 kg per person (Grisley, 1990; Petry et al., 2015), while in Tanzania per capita bean consumption is 19.3 kg per person, contributing 16.9% protein and 7.3% calories in human nutrition and 71% of leguminous protein in diets

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(Rugambisa, 1990; Grisley, 1990; Katungi et al., 2009).

It is estimated that over 75% of rural households in Tanzania depend on beans for daily subsistence (Xavery et al., 2006; Kalyebara et al., 2008; Binagwa et al., 2018). The crop is grown by smallholder farmers under quite diverse farming systems and agro-climatic conditions; both for household food requirements and income generation (Allen et al., 1989; Allen and Edje, 1990; Musimu, 2018). Average bean yields in Tanzania are around 500 kg $ha^{-1}$ although the potential yield under reliable rain-fed conditions is 1500–3000 kg $ha^{-1}$, using improved bean varieties accompanied with good agricultural practices. The main reasons for the low yield obtained by most smallholders are; low soil fertility, inadequate use of improved bean varieties, poor crop managements and susceptibility to insect pests and diseases (Hillocks et al., 2006 and Saimon et al., 2016).

Under current management practices in Tanzania, beans are planted at a spacing of 50 cm between rows and 20 cm between planting hills, while maintaining two seeds per hill which results to 200,000 plants per ha (Kanyeka et al., 2007) without applying P-fertilizers. The impact of plant density on dry bean production is important to bean growers for optimizing yield. However, higher planting density will require higher application rates of fertilizers to compensate crop demands depending on the availability of such nutrients in the soil, hence the need to determine optimal spacing for planting high yielding with good market and culinary characteristics in relation to P-fertilizer application rates by considering the actual situation prevailing under local farmers conditions – little/no fertilizer use and financial constrain.

Loss of soil fertility may result from excessive nutrient mining through crop harvests, burning, leaching of nutrients, volatilization and de-nitrification (FAO, 2004; Drechsel et al., 2015), with no adequate nutrient replenishment particularly in areas where high yielding bean varieties are adopted by farmers (Stoorvogel et al., 1993). Most farmers in the northern zone are not using fertilizers on bean, hence low yield under farmers' condition. Mineral fertilizers may also be required to meet the crop nutrient requirement for optimum crop production (Breman, 1990; Drechsel et al., 2015) and in African region; farmlands are lost due to their poor management, causing average annual losses of topsoil in N, P and K at 22, 2.5 and 15 kg $ha^{-1}$ respectively.

Therefore, for better management of land resources, it is imperative to measure the initial soil fertility for its ability to provide essential nutrients for crop growth and development (Stoorvogel et al., 1993). Mineral fertilizers play important role in restoring soil fertility status. The purpose of this study was; i) to assess the effects of different planting densities for better yield of the bush bean varieties ii) evaluate the effects of different levels of phosphorous fertilizer on productivity of the bush bean varieties and iii) determine the interaction effects of different planting densities and levels of phosphorous fertilizer on productivity of the bush bean varieties.

## MATERIALS AND METHODS

### Description of the study site

The experiment was conducted at Selian Agricultural Research Institute (ARI-Selian) experimental site during the short rain cropping season of 2016/2017 and the long rain cropping season of 2017/2018. ARI-Selian is located at Arumeru district (Arusha region) in northern Tanzania which lies at 10° 22' S latitude and 40° 10' E longitude with an altitude of 1378 m above sea level with mean annual temperature and rainfall of 19.2°C and 1103 mm respectively.

### Soil types and its physical-chemical characteristics

The experimental site had the textural class of silty loam based on USDA textural class triangle (Brady and Weil, 2002) with pH value of 5.6, rated as medium acid, suitable for cultivation of most crops including bean (Landon, 1991). The soil has very low organic carbon (0.53%) corresponding to very low organic matter (0.92%), total nitrogen (0.079%), exchangeable potassium (0.17 cmol (+)/kg) and medium available phosphorous (8.0 mg/kg); this classify that, the soil fertility status is medium fertility which is moderately suitable for bean cultivation.

### Land preparation, treatments and experimental design

Land preparation was carried out by ploughing by tractor followed by harrowing. Treatments comprised of three bush bean varieties Lyamungu 90, JESCA and KATB1 sourced from SARI bean section; three plant densities of 200,000; 250,000 and 333,333 plants $ha^{-1}$ maintained at a spacing of 50 cm x 20 cm, 40 cm x 20 cm and 30 cm x 20 cm respectively and four levels 0, 20, 40 and 60 kg $ha^{-1}$ of di-ammonium sulphate (DAP) fertilizer as a source of phosphorous (applied prior to bean planting). The treatments were laid out in a randomized completely block design (RCBD) in a 3 x 3 x 4 factorial arrangement with three replications. The size of each experimental plot was 1.2 m x 3 m (3.6 $m^2$) and two seeds were planted per hole.

Other agronomic management practices including insect pest control and weeding were executed uniformly as recommended by Kanyeka et al. (2007) and national bean research program for bean production in Tanzania.

### Yield and yield components data

At harvesting stage (12 weeks after planting), five plants randomly selected from the net harvesting area of 3.0, 2.4 and 2.7 $m^2$ for 200,000, 250,000 and 333,333 planting densities respectively, were used for assessing the number of pods per plant and number of seeds per pod, while the biomass and grain yields were estimated from the plants within the entire net harvesting area. Number of pods was determined by detaching and counting the total number of pods from the collected parent plants of each plot while number of seeds was determined by counting the total seeds in each pod from the sampled plants per plot. The above ground biomass yield was determined by weighing the straw collected from each plot using weighing balance and the obtained weight was converted to kg $ha^{-1}$. Grain yield per plot was determined by weighing the grain harvested per plot using sensitive weighing balance and the

obtained weight was converted to kg ha$^{-1}$ after adjustment at 13% moisture content by using moisture tester.

**Statistical data analysis**

The data on the number of pods per plants, seeds per pod, biomass and grain yields collected were subjected to a combined analysis of variance (ANOVA) using Genstat (15$^{th}$ Edition) statistical software. Significant treatment means were separated by Duncan multiple range test (DMRT) at 5% level of probability.

## RESULTS AND DISCUSSION

### Yield performance of three bush bean varieties

There was significant difference ($p \leq 0.05$) among the three bush bean varieties with respect to number of pods per plant, number of seeds per pod, biomass and grain yields as illustrated in Table 1. Results showed that higher number of pods per plant (7.89) was observed in KAT-B1 variety followed by JESCA variety (6.96) with the least number of pods per plant (6.71) observed in Lyamungu 90 variety as shown in Table 1. Results from the study showed no significant differences ($p \leq 0.05$) between JESCA and KAT-B1 varieties with respect to number of seeds per pod while the lowest number of seeds per pod of 3.82 was given by Lyamungu 90 variety.

The highest biomass yields of 2767 kg ha$^{-1}$ was obtained in KAT-B1 variety while Lyamungu 90 and JESCA varieties did not show significant differences ($p \leq 0.05$) with respect to biomass yields. JESCA variety resulted higher grain yields of 1715 kg ha-1 followed by Lyamungu 90 variety (1441 kg ha-1) with the least grain yield of 1344 kg ha-1 in KAT-B1 variety as presented in Table 1. The trends of significant differences ($p \leq 0.05$) observed among the bush bean varieties in terms of yields and yield components may be ascribed to the differences in maturing characteristics among the tested varieties; JESCA and KAT-B1 being early maturing varieties while Lyamungu 90 variety being a late maturing variety. The findings of this study agree with the results of Eftekhar et al. (2012) who reported significant effects of different white bean cultivars on number of grains per plant and grain yields.

### Effects of planting density on number of pods per plant, number of seeds per pod, biomass and grain yields of the three bush bean varieties

There were no significant differences ($p \leq 0.05$) among the three planting densities (200,000, 250,000 and 333, 333 plants ha$^{-1}$) with respect to number of pods per plant and number of seeds per pod as shown in Table 2. This study is contrary with the results of Eftekhar et al. (2012) who reported significant effect of planting density on number of pods per plant of white bean cultivars.

Results revealed that the three planting densities significantly ($p \leq 0.05$) influenced increase in biomass and grain yields as illustrated in Table 2. Results also showed no significant difference ($p \leq 0.05$) between the plant densities of 250,000 and 333,333 plants ha$^{-1}$ in terms of in biomass and grain yields, however, these two planting densities gave the highest biomass and grain yields. The planting density of 200,000 plants ha$^{-1}$ gave lowest biomass and grain yields. The results support the findings of Eftekhar et al. (2012) who reported significant effect of planting density on biological and grain yield of white bean cultivars.

Similarly, the results of this study are in line with the findings by Moniruzzaman et al. (2009) who observed higher pod yield of French bush bean varieties in the highest plant density and the lowest pod yield with the lowest plant density. However, the study contradicts the findings of Hang et al. (1993) who reported no significant effects of grains per pods of adzuki beans and dry beans due to planting density.

### Effects of different rates of phosphorous fertilizer on number of pods per plant, number of seeds per pod, biomass and grain yields of the three bush bean varieties

Results from the study show that there were significant differences ($p \leq 0.05$) among the four rates of phosphorus fertilizer with respect to number of pods per plant except for number of seeds per pod, biomass and grain yields as shown in Table 2. The highest number of pods per plants of 7.944 was obtained when phosphorous fertilizer was applied at a rate of 60 kg ha$^{-1}$. However, the results show no significant differences ($p \leq 0.05$) with respect to number of pods per plant when phosphorous fertilizer at a rate of 0 and 40 kg ha$^{-1}$ was applied as presented in Table 2. The results of this study contradict the findings of Nkaa et al. (2014) who reported significant increase in biomass yields and seed yields of cowpea varieties due to application of phosphorous fertilizer at different rates in comparison to the control.

### Effects of the interaction of bush bean varieties and plant densities on number of pods per plant, number of seeds per pod, biomass and grain yields

Number of pods per plant, number of seeds per pod, biomass and grain yields was significantly different ($p \leq 0.05$) influenced by the interaction of bush bean varieties and planting densities as shown in Table 3. The maximum number of pods per plant of 8.52 was obtained in KAT-B1 variety by planting density of 250,000 plants ha$^{-1}$. The results show that the interaction of Lyamungu 90 variety by planting density of 250,000 plants ha$^{-1}$,

**Table1.** Performance three bush bean varieties on number of pods per plant, number of seeds per pod, biomass and grain yields.

| Treatment (variety) | No. of pod/plant | Seed/pod | Biomass (kg ha$^{-1}$) | Grain yields (kg ha$^{-1}$) |
|---|---|---|---|---|
| Lyamungu 90 | 6.71$^{a}$ | 3.82$^{a}$ | 2044$^{a}$ | 1441$^{ab}$ |
| JESCA | 6.96$^{ab}$ | 4.39$^{b}$ | 1936$^{a}$ | 1715$^{b}$ |
| KAT-B1 | 7.89$^{b}$ | 4.14$^{b}$ | 2767$^{b}$ | 1344$^{a}$ |
| CV (%) | 40.4 | 22.5 | 85.0 | 59.0 |

Means on with the same letter on the column indicates no significant difference (p ≤0.05) between the treatments using Duncan Multiple Range Test (DMRT).

**Table 2.** Effects of planting density and phosphorus on number of pods per plant, number of seeds per pod, biomass and grain yields.

| Treatment | | No. of pod/plant | Seed/pod | Biomass (kg/ha) | Grain yields (kg/ha) |
|---|---|---|---|---|---|
| Plant density (plants ha$^{-1}$) | 200,000 (50cm x 20cm) | 7.161$^{a}$ | 4.178$^{a}$ | 1341$^{a}$ | 878$^{a}$ |
| | 250,000 (40cm x 20cm) | 7.617$^{a}$ | 4.072$^{a}$ | 2613$^{b}$ | 1698$^{b}$ |
| | 333,333 (30cm x 20cm) | 6.781$^{a}$ | 4.099$^{a}$ | 2792$^{b}$ | 1923$^{b}$ |
| Phosphorous rate (kg ha$^{-1}$) | 60 | 7.944$^{b}$ | 4.163$^{a}$ | 2674$^{a}$ | 1582$^{a}$ |
| | 40 | 7.256$^{ab}$ | 3.974$^{a}$ | 2136$^{a}$ | 1471$^{a}$ |
| | 20 | 6.622$^{a}$ | 4.180$^{a}$ | 2197$^{a}$ | 1510$^{a}$ |
| | 0 | 6.922$^{ab}$ | 4.148$^{a}$ | 1988$^{a}$ | 1435$^{a}$ |
| CV (%) | | 40.4 | 22.5 | 85.0 | 59.0 |

Means on with the same letter on the column indicates no significant difference (p ≤0.05) between the treatments using Duncan Multiple Range Test (DMRT).

**Table 3.** The interaction effects of bush bean varieties and planting density on number of pods per plant, number of seeds per pod, biomass and grain yields.

| Treatment (variety x plant density) | No. of pod/plant | Seed/pod | Biomass (kg/ha) | Grain yields (kg/ha) |
|---|---|---|---|---|
| V1S1 | 7.10$^{ab}$ | 3.76$^{a}$ | 1428$^{ab}$ | 853$^{a}$ |
| V1S2 | 6.75$^{ab}$ | 3.75$^{a}$ | 1977$^{abc}$ | 1510$^{bc}$ |
| V1S3 | 6.28$^{a}$ | 3.94$^{ab}$ | 2725$^{cd}$ | 1958$^{c}$ |
| V2S1 | 6.50$^{a}$ | 4.43$^{b}$ | 1112$^{a}$ | 1058$^{ab}$ |
| V2S2 | 7.58$^{ab}$ | 4.50$^{b}$ | 2082$^{abc}$ | 2017$^{c}$ |
| V2S3 | 6.79$^{ab}$ | 4.23$^{ab}$ | 2614$^{bcd}$ | 2069$^{c}$ |
| V3S1 | 7.88$^{ab}$ | 4.34$^{ab}$ | 1483$^{ab}$ | 724$^{a}$ |
| V3S2 | 8.52$^{b}$ | 3.97$^{ab}$ | 3781$^{d}$ | 1566$^{bc}$ |
| V3S3 | 7.28$^{ab}$ | 4.13$^{ab}$ | 3037$^{cd}$ | 1744$^{c}$ |
| CV (%) | 40.4 | 22.5 | 85.0 | 59.0 |

Means on with the same letter on the column indicates no significant difference (p ≤0.05) between the treatments using Duncan multiple range test (DMRT). Varieties: V1= Lyamungu 90, V2= JESCA and V3= KAT-B1, planting density: S1=200,000 plants ha$^{-1}$, S2=250,000 plants ha$^{-1}$, S3= 333,333 plants ha$^{-1}$, phosphorous rates: P1= 60 kg ha$^{-1}$, P2= 40 kg ha$^{-1}$, P3= 20 kg ha$^{-1}$ and P4= 0 kg ha$^{-1}$.

JESCA variety by planting density of 250,000 and 333,333 plants ha$^{-1}$, KAT-B1 variety by planting density of 200,000 and 333,333 plants ha$^{-1}$ did not significantly ($p \leq 0.05$) influence increase in the number of pods per plant as shown in Table 3. Similarly, the results show no significant differences ($p \leq 0.05$) between Lyamungu 90 variety by planting density of 333,333 plants ha$^{-1}$ and JESCA variety by planting density of 200,000 plants ha$^{-1}$

in terms of number of pods per plant; however, these treatments gave the lowest number of pods per plants as compared to other treatment combinations as shown in Table 3.

Results showed no significant differences ($p \leq 0.05$) between JESCA variety by planting density of 250,000 and 200,000 plants ha$^{-1}$ respectively, in terms of number of seeds per pod; however, these treatments gave higher number of seeds per pod as compared to other treatment combinations. Similarly, Lyamungu 90 variety by planting density of 333,333 plants ha$^{-1}$, JESCA variety by planting density of 333,333 plants ha$^{-1}$, KAT-B1 variety by planting density of 250,000 and 333,333 plants ha$^{-1}$ did not show significant differences ($p \leq 0.05$) with respect to number of seeds per pod. The lowest number of seeds per pod was obtained in Lyamungu 90 variety by planting density of 333,333 plants ha$^{-1}$ and JESCA variety by planting density of 200,000 plants ha$^{-1}$, these treatments, however, did not show significant differences ($p \leq 0.05$) among themselves in terms of number of seeds per pod.

The highest biomass yields of 3781 kg ha$^{-1}$ was obtained from KAT-B1 variety by planting density of 250,000 plants ha$^{-1}$ followed by KAT-B1 variety by planting density of 333,333 plants ha$^{-1}$ and Lyamungu 90 variety by planting density of 333,333 plants ha$^{-1}$ which did not differ significantly ($p \leq 0.05$) among themselves in terms of biomass yields as shown in Table 3. Results also indicate that JESCA variety by planting density of 250,000 plants ha$^{-1}$ statistically gave similar biomass yields followed by treatment KAT-B1 and Lyamungu 90 varieties by planting density of 200,000 plants ha$^{-1}$ with the least biomass yields of 1112 kg/ha recorded in the JESCA variety by planting density of 200,000 plants ha$^{-1}$.

Results indicated that Lyamungu 90 variety by planting density of 333,333 plants ha$^{-1}$, JESCA variety by planting density of 250,000 and 333,333 plants ha$^{-1}$ and KAT-B1 variety by planting density of 333,333 plants ha$^{-1}$ did not show significant differences ($p \leq 0.05$) among themselves; however they gave higher grain yields as compared to other treatment combinations. Similarly, Lyamungu 90 and KAT-B1 varieties by planting density of 200,000 plants ha$^{-1}$ did not show significant differences ($p \leq 0.05$) between themselves and gave the lowest grain yields as compared to other treatment combinations as shown in Table 3.

The findings of significant increase in number of pods per plant, number of seeds per pod, biomass and grain yields was significantly ($p \leq 0.05$) due to the interaction of bush bean varieties and planting densities are in agreement with the results of Eftekhar et al. (2012) who also reported that the interaction between white bean cultivars and plant density was significant on number of branches, number of pods per plant, biological yield and grain yield. However, the findings of this study contradict the results of Pawar et al. (2007) who reported insignificant increase in yield components and grain yields of French bean due the interaction effect between the varieties and plant density.

**Effects of the interaction of bush bean varieties and rates of phosphorous fertilizer on number of pods per plant, number of seeds per pod, biomass and grain yields**

Results on the effects of the interaction of variety and rates of phosphorous fertilizer on yield attributes of bush bean varieties are presented in Table 4. Number of pods per plant, number of seeds per pod, biomass and grain yields were significantly ($p \leq 0.05$) influenced by the interaction of bush bean varieties and phosphorus fertilizer as presented in Table 4. Similarly, Chekanai et al. (2018) found that common bean dry biomass was significantly increased by application of N, P and NP in both degraded and non-degraded soils. On the other hand, Magelanga (2013) found significant effects of P application on yield and yield components on bean lines. Magelanga (2013) found that; plants grown without P fertilizer had the lowest pod yield and pod number caused by failure of fertilization due to production of non-viable pollen grains. Fageria et al. (2010) reported that, the contribution of yield components in increasing grain yield was in the order of number of pods per plant > seeds per pod > 100 grain weight. Similarly, Fageria and Santos (2008) observed that, number of grain per pod and weight of hundred grains are important yield components. Further, it has been documented that grain yield in beans as affected by the above mentioned seed yield components is usually affected by available P to the crop. Studies conducted by Hussain (1983) showed that application of P to legumes would improve seed yield considerably. Thus, any reduction in these yield components directly affects overall grain production. The reduction in yield is largely due to reduction in number of pods per plant as reported by Lopez et al. (1990). The maximum number of pods per plant of 8.41 was obtained from KAT-B1 variety was planted with phosphorous fertilizer at a rate of 60 kg ha$^{-1}$. Results indicate that all other treatment combinations did not show significant differences ($p \leq 0.05$) among themselves except when JESCA variety was planted with phosphorous fertilizer at a rate of 20 kg ha$^{-1}$ treatment that gave the lowest number of pods per plant. The highest number of seeds per pod of 4.64 was recorded when JESCA variety was planted with phosphorous fertilizer at a rate of 20 kg ha$^{-1}$ as compared to other treatments with the least number of pods per plant of 3.63 which was obtained from Lyamungu 90 variety, and was planted with phosphorous fertilizer at a rate of 60 kg ha$^{-1}$.

The highest biomass yields of 3586 kg ha$^{-1}$ was obtained when KAT-B1 variety was planted with phosphorous fertilizer at a rate of 60 kg ha$^{-1}$; followed by Lyamungu 90 variety which was planted with phosphorous fertilizer at a rate of 20 kg ha$^{-1}$; JESCA

**Table 4.** The interaction effects of bush bean varieties and rates of phosphorus on number of pods per plant, number of seeds per pod, biomass and grain yields.

| Treatment (variety x phosphorous) | No. of pod/plant | Seed/pod | Biomass (kg/ha) | Grain yields (kg/ha) |
|---|---|---|---|---|
| V1P1 | 7.24$^{ab}$ | 3.63$^{a}$ | 2107$^{a}$ | 1327$^{ab}$ |
| V1P2 | 6.42$^{ab}$ | 3.67$^{ab}$ | 2010$^{a}$ | 1459$^{ab}$ |
| V1P3 | 6.22$^{ab}$ | 3.86$^{abc}$ | 2248$^{ab}$ | 1494$^{ab}$ |
| V1P4 | 6.94$^{ab}$ | 4.10$^{abcd}$ | 1809$^{a}$ | 1483$^{ab}$ |
| V2P1 | 8.18$^{ab}$ | 4.41$^{cd}$ | 2329$^{ab}$ | 1991$^{b}$ |
| V2P2 | 7.26$^{ab}$ | 4.36$^{bcd}$ | 1876$^{a}$ | 1768$^{ab}$ |
| V2P3 | 6.09$^{a}$ | 4.64$^{d}$ | 1917$^{a}$ | 1700$^{ab}$ |
| V2P4 | 6.31$^{ab}$ | 4.14$^{abcd}$ | 1623$^{a}$ | 1400$^{ab}$ |
| V3P1 | 8.41$^{b}$ | 4.44$^{cd}$ | 3586$^{b}$ | 1430$^{ab}$ |
| V3P2 | 8.09$^{ab}$ | 3.90$^{abc}$ | 2523$^{ab}$ | 1187$^{a}$ |
| V3P3 | 7.56$^{ab}$ | 4.03$^{abcd}$ | 2427$^{ab}$ | 1337$^{ab}$ |
| V3P4 | 7.51$^{ab}$ | 4.20$^{abcd}$ | 2531$^{ab}$ | 1424$^{ab}$ |
| CV (%) | 40.4 | 22.5 | 85.0 | 59.0 |

Means on with the same letter on the column indicates no significant difference (p ≤0.05) between the treatments using Duncan multiple range test (DMRT). Varieties: V1= Lyamungu 90, V2= JESCA, V3= KAT-B1, phosphorous rates: P1= 60 kg ha$^{-1}$, P2= 40 kg ha$^{-1}$, P3= 20 kg ha$^{-1}$ and P4= 0 kg ha$^{-1}$.

variety JESCA variety was planted with phosphorous fertilizer at a rate of 60 kg ha$^{-1}$; and KAT-B1 variety was planted with phosphorous fertilizer at a rate of 40, 20 and 0 kg ha$^{-1}$. These, however, did not significantly (p ≤ 0.05) differ among themselves in terms of biomass yields with the lowest biomass yields obtained from the Lyamungu 90 variety which was planted with phosphorous fertilizer at a rate of 60 and 40 kg ha$^{-1}$, and JESCA variety which was planted with phosphorous fertilizer at a rate of 40, 20 and 0 kg ha-1.

Results show no significant differences (p ≤ 0.05) among Lyamungu 90 variety which was planted with phosphorous fertilizer at a rate of 60, 40, 20 and 0 kg ha-1; JESCA variety which was planted with phosphorous fertilizer at a rate of 40, 20 and 0 kg ha$^{-1}$; and KAT-B1 variety which was planted with phosphorous fertilizer at a rate of 40, 20 and 0 kg ha$^{-1}$ in terms of grain yields; except for JESCA variety which was planted with phosphorous fertilizer at a rate of 60 kg ha$^{-1}$ and that gave the highest grain yield of 1991 kg ha$^{-1}$.

The lowest grain yield of 1187 kg ha-1 was obtained from KAT-B1 variety which was planted with a phosphorous fertilizer at a rate of 40 kg ha$^{-1}$ as presented in Table 4.

**Effects of the interaction of planting densities and rates of phosphorus fertilizer on number of pods per plant, number of seeds per pod, biomass and grain yields of the three bush bean varieties**

Results on the effects of the interaction of different planting densities and rates of phosphorous fertilizer on grain yields and yield components of bush bean varieties are presented in Table 5. There were significant ($p \leq 0.05$) differences in yields components (number of pods per plant, seeds per pod and biomass) and grain yields of bush bean varieties due to the interaction effect of planting density and rates of phosphorous fertilizer.

The maximum number of pods per plant of 10.267 was recorded when KAT-B1 variety was planted at the lowest planting density of 200,000 plants ha$^{-1}$ and at a phosphorous rate of 40 kg ha$^{-1}$ followed by JESCA variety when planted at a lowest planting density of 200,000 plants ha$^{-1}$ and a phosphorous fertilizer at a highest rate of 60 kg ha$^{-1}$. The lowest number of pods per plant was recorded in JESCA variety when planted at a lowest planting density of 200,000 plants ha$^{-1}$ and a phosphorous fertilizer at a rate of 0 and 20 kg ha$^{-1}$ respectively. The decrease in number of pods per plant with increase in plant density could be due to increased intra specific competition which eventually caused reduction in number of pods per plant.

The findings support the results of Mulatu et al. (2017) who also reported that the interaction effect of plant density and level of phosphorous showed significant ($p \leq 0.05$) effect on number of pods per plant of haricot bean. Furthermore, the findings of the study contradict the results of Mulatu et al. (2017) who reported that the highest number pods per plant was recorded at the highest phosphorous level and the lowest plant population density, while the lowest number per plant recorded at the highest plant population density with the lowest phosphorous level which is not the case with the findings of the study. In agreement with this study, Abdel (2008) reported that faba bean developed more and vigorous leaves on low plant density, and helped to improve the photosynthetic efficiency of the crop and

**Table 5.** The interaction effects of bush bean varieties and rates of phosphorus fertilizer on number of pods per plant, number of seeds per pod, biomass and grain yields.

| Treatment | No. of pod/plant | | | Seed/pod | | | Biomass (kg/ha) | | | Grain yields (kg/ha) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant density x phosphorous** | **V1** | **V2** | **V3** | **V1** | **V2** | **V3** | **V1** | **V2** | **V3** | **V1** | **V2** | **V3** |
| S1P1 | 6.87$^{ab}$ | 9.07$^{ab}$ | 8.03$^{ab}$ | 3.20$^{a}$ | 4.33$^{abc}$ | 4.53$^{abc}$ | 1183$^{a}$ | 1489$^{a}$ | 1522$^{a}$ | 544$^{a}$ | 1156$^{abcdefgh}$ | 667$^{abc}$ |
| S1P2 | 7.57$^{ab}$ | 7.03$^{ab}$ | 10.27$^{b}$ | 3.60$^{abc}$ | 4.27$^{abc}$ | 4.30$^{abc}$ | 11111$^{a}$ | 944$^{a}$ | 1561$^{a}$ | 911$^{abcdef}$ | 1233$^{abcdefgh}$ | 789$^{abcd}$ |
| S1P3 | 5.80$^{a}$ | 4.9$^{a}$ | 6.77$^{ab}$ | 4.17$^{abc}$ | 4.60$^{bc}$ | 4.37$^{abc}$ | 1728$^{a}$ | 1211$^{a}$ | 1531$^{a}$ | 900$^{abcdef}$ | 933$^{abcdef}$ | 806$^{abcde}$ |
| S1P4 | 8.17$^{ab}$ | 4.93$^{a}$ | 6.47$^{ab}$ | 4.07$^{abc}$ | 4.53$^{abc}$ | 4.17$^{abc}$ | 1689$^{a}$ | 806$^{a}$ | 1317$^{a}$ | 1056$^{abcdefgh}$ | 911$^{abcdef}$ | 633$^{ab}$ |
| S2P1 | 7.97$^{ab}$ | 7.77$^{ab}$ | 8.67$^{ab}$ | 3.90$^{abc}$ | 4.47$^{abc}$ | 4.37$^{abc}$ | 2069$^{a}$ | 2361$^{a}$ | 6076$^{b}$ | 1361$^{abcdefghi}$ | 2236$^{ghi}$ | 1833$^{bcdefghi}$ |
| S2P2 | 6.17$^{a}$ | 7.70$^{ab}$ | 7.83$^{ab}$ | 3.33$^{ab}$ | 4.40$^{abc}$ | 3.37$^{ab}$ | 2479$^{a}$ | 1757$^{a}$ | 2736$^{a}$ | 1639$^{abcdefghi}$ | 1736$^{abcdefghi}$ | 1167$^{abcdefgh}$ |
| S2P3 | 6.00$^{a}$ | 7.30$^{ab}$ | 8.80$^{ab}$ | 3.53$^{abc}$ | 4.80$^{c}$ | 4.17$^{abc}$ | 2042$^{a}$ | 1847$^{a}$ | 3257$^{a}$ | 1514$^{abcdefghi}$ | 2167$^{fghi}$ | 1528$^{abcdefghi}$ |
| S2P4 | 6.87$^{ab}$ | 7.57$^{ab}$ | 8.77$^{ab}$ | 4.23$^{abc}$ | 4.33$^{abc}$ | 3.97$^{abc}$ | 1319$^{a}$ | 2361$^{a}$ | 3056$^{a}$ | 1528$^{abcdefghi}$ | 1931$^{cdefghi}$ | 1736$^{abcdefghi}$ |
| S3P1 | 6.90$^{ab}$ | 7.70$^{ab}$ | 8.53$^{ab}$ | 3.80$^{abc}$ | 4.43$^{abc}$ | 4.43$^{abc}$ | 3068$^{a}$ | 3136$^{a}$ | 3160$^{a}$ | 2074$^{efghi}$ | 2580$^{i}$ | 1790$^{abcdefghi}$ |
| S3P2 | 5.53$^{a}$ | 7.03$^{ab}$ | 6.1$^{ab}$ | 4.07$^{abc}$ | 4.40$^{abc}$ | 4.03$^{abc}$ | 2438$^{a}$ | 2926$^{a}$ | 3272$^{a}$ | 1827$^{bcdefghi}$ | 2333$^{hi}$ | 1605$^{abcdefghi}$ |
| S3P3 | 6.87$^{ab}$ | 6.00$^{a}$ | 7.10$^{ab}$ | 3.88$^{abc}$ | 4.53$^{abc}$ | 3.53$^{abc}$ | 2975$^{a}$ | 2691$^{a}$ | 2494$^{a}$ | 2068$^{efghi}$ | 2000$^{defghi}$ | 1679$^{abcdefghi}$ |
| S3P4 | 5.80$^{a}$ | 6.43$^{ab}$ | 7.30$^{ab}$ | 4.00$^{abc}$ | 3.57$^{abc}$ | 4.47$^{abc}$ | 2420$^{a}$ | 1704$^{a}$ | 3222$^{a}$ | 1864$^{bcdefghi}$ | 1358$^{abcdefghi}$ | 1901$^{bcdefghi}$ |
| CV (%) | | 40.4 | | | 22.5 | | | 85 | | | 59.0 | |

Means on with the same letter on the column indicates no significant difference ($p \leq 0.05$) between the treatments using Duncan multiple range test (DMRT). Varieties: V1= Lyamungu 90, V2= JESCA and V3= KAT-B1, planting density: S1=200,000 plants ha$^{-1}$, S2=250,000 plants ha$^{-1}$, S3= 333,333 plants ha$^{-1}$, phosphorous rates: P1= 60 kg ha$^{-1}$ , P2= 40 kg ha$^{-1}$ , P3= 20 kg ha$^{-1}$ and P4= 0 kg ha$^{-1}$.

supported large number of pods.

The maximum number of seeds per pod of 4.8 was recorded in JESCA variety when planted at a higher planting density 250,000 plants ha$^{-1}$ and a phosphorous fertilizer at a rate of 20 kg ha$^{-1}$. The lowest number of seeds per pod was recorded in Lyamungu 90 variety when planted at a lowest planting density of 200,000 plants ha$^{-1}$ and a phosphorous fertilizer at a highest rate of 60 kg ha$^{-1}$.

The findings of this study disagree with the results of Mulatu et al. (2017) who found that the highest mean number of seeds per pod was recorded at the lowest plant density of 125,000 plants ha$^{-1}$ with the highest phosphorous level, while the lowest mean number of seeds per pod was obtained at the highest plant density, 250,000 plants ha$^{-1}$ with no phosphorous application. Decreasing plant population density has increased number of seeds per pod across all the treatments. This variation might be due to low plant density encountered less intra-plant competition than high plant density and thus exhibited better growth that contributed to a greater number of seeds per pod. Similarly, these results disagree with Abdel (2008) who reported that number of seeds per pod increased with decreasing in plant density of faba bean. KAT-B1 variety resulted higher biomass yield of 6076 kg ha$^{-1}$ when planted at a higher planting density of 250,000 plants ha$^{-1}$ and a phosphorous fertilizer at a highest rate of 60 kg ha$^{-1}$. The lowest biomass yields of 806 kg ha$^{-1}$ was recorded in JESCA variety when planted at a lowest planting density of 200,000 plants ha$^{-1}$ and at a phosphorous fertilizer at a lowest rate of 0 kg ha$^{-1}$. The findings support the results of Mulatu et al. (2017) who found that higher biomass was recorded at the highest plant density, 250,000 plants ha$^{-1}$ and the highest phosphorous level 69 kg ha$^{-1}$ while the lowest biomass was recorded at the lowest plant density of 125000 plants ha$^{-1}$ and the lowest phosphorous level of 0 kg ha$^{-1}$.

The highest total dry biomass at the highest density and the highest level of phosphorous might be due to the greater number of plants per unit area, and more application of phosphorus fertilizer may have cushioned the competitive effects of haricot bean plants as population density was increased which might have led to efficient use of phosphorus fertilizer at higher plant population densities and improvement in fodder and grain yields ha$^{-1}$. In agreement with this study, Getachew et al., (2006) reported increased dry biomass of faba bean with increased plant density.

The maximum grain yields of 2580 kg ha$^{-1}$ was

recorded in JESCA variety when planted at a highest planting density of 333,333 plants $ha^{-1}$ and at a highest phosphorous fertilizer rate of 60 kg $ha^{-1}$, while the lowest grain yields of 544 kg $ha^{-1}$ was recorded in Lyamungu 90 variety when planted at a lowest planting density of 200,000 plants $ha^{-1}$ and at a highest phosphorous rate of 60 kg $ha^{-1}$. Application of phosphorus fertilizer may have cushioned the competitive effects of haricot bean plants as population density was increased which might have led to efficient use of phosphorus fertilizer at higher plant population densities and improvement in grain yields $ha^{-1}$.

The findings of the study disagree the results of Mulatu et al. (2017) who also recorded that the highest grain yield of haricot bean was obtained at a higher rate of phosphorous fertilizer and a higher planting density of 190478 plants $ha^{-1}$, while the lowest grain yield was recorded at the lowest plant density of 125,000 plants $ha^{-1}$ and no phosphorous fertilizer (0 kg $ha^{-1}$).

Similarly, the findings agree with result of Ball et al. (2000), who also reported that, increasing plant population reduced yield of individual plants but increased yield per unit area of common bean. Furthermore, Hamidi, et al. (2010) also reported combined effects of plant density and fertilizer rate were positive and the increased levels of both parameters led to the increase in grain yield.

## Conclusions

The findings of the study on yield response of bush bean varieties to different planting densities and rates of phosphorous fertilizer indicated significant improvement of yield productivity and other yield components of the three tested bush bean varieties. The tested bush bean varieties had varying yielding potentials with higher yields resulted by JECSA variety followed by Lyamungu 90 variety with KAT-B1 variety yielding lower than the other two varieties. The interaction of bush bean varieties and planting densities significantly increased the grain yields and other yield components. Also, the interaction of bush bean varieties and phosphorous fertilizer especially at higher rates only significantly increased the grain yields. The combination of bean variety, planting density and rates of phosphorous fertilizer on grain yield indicated that bean varieties planted at higher planting density of 333,333 plants $ha^{-1}$ and applied with 60 kg $ha^{-1}$ had the highest grain yields compared to the bush bean varieties planted at low density of 200,000 plants $ha^{-1}$ without application of phosphorous fertilizer. Therefore, the findings suggest to farmers to opt for this combination in order to have more yield for more income and food security from farmer to national level.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Expression profile of sex steroid hormone estrogen receptors (ERs) in the development of juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus polyphekadion* ♂)

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**Recognized for its traditional roles, estrogen is ever-present in all vertebrates, regulates reproduction by binding and activating estrogen receptors (ERs), and also controls several functions of vertebrates, including reproductive immune, and central nervous systems. In order to access any other possible functions of the estrogen receptors in the development of the juvenile Hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelu polyphekadion* ♂), full-length of ERs cDNA sequences were isolated and analyses were found to be 2391 bp for hgERα, 2626 bp for gERb1 and 2339 bp for hgERb2, respectively. The results of amino acid and phylogenetic analysis revealed that each hgER was grouped in consistent taxonomic groups of perciformes and demonstrated great evolutional conservation in functional domains. Real-time PCR examination discovered that the receptors expressed in all tissues examined, though, at a different level, the ERα mRNA level expressed higher than ERβ1, and ERβ2 in tissues examined. The ERα mRNA level of expression was found to be highest in the tissue of the heart, followed by muscle, and liver. The ERβ1 mRNA level was greatest in heart tissue, trailed by liver and muscle and ERβ2 was highest in the heart trailed by stomach and liver. The minimal expression was recorded in the kidney, the gill, and the brain for ERα, ERβ1, and ERβ2 respectively. These results put forward that steroid hormone estrogen receptors might be playing a significant part in the controlling of social behavior complexity, plasticity behavior, and the assessment of a gratifying inducement in Hybrid grouper.**

**Key words:** Estrogen receptors, Real-time POR, tissue expression, hybrid grouper.

## INTRODUCTION

Well known for its critical roles, Estrogen is known for regulation of reproduction through binding and activation

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of estrogen receptors (ERs) (Chen et al., 2011), some functions in vertebrates are also controlled by estrogen, for example; reproduction, the immune system, and the central nervous system (Bakker and Brock, 2010; McCarthy, 2010; Vasudevan and Pfaff, 2008). Several authors have researched the biological importance of estrogen in vertebrates including fish, mostly in regeneration (Hewitt and Korach, 2002; Wang, 2005). Two forms of estrogen receptors are reported in several vertebrates, ERα and ERβ, except, in teleost fish, where three models are detailed: ERα, ERβ1, and ERβ2. It seems that the ERαγ form in fish is genetically related to ERβ which might be due to gene duplication within the teleosts fish. Due to this result, ERβ and ERγ are named ERβ1 and ERβ2 (Katsu et al., 2011; Hawkins et al., 2000). According to Thornton (2001), the "ancestral condition for jawed vertebrates is considered to contain two forms of ER, corresponding to ERα and ERβ" (Katsu et al., 2010c). In mammals, two types of estrogen receptors are reported; examples are fishes, birds, reptiles, and amphibians. As well established, the estrogen is the key steroid hormone (Chen et al., 2011) that regulates, differentiate and plays essential roles in "growth of oocyte maturation for female reproduction" (Diotel et al., 2018; Ni et al., 2013; Lassiter et al., 2002; Pepe et al., 2002; Pelletier et al., 2000), and also play a precarious role in controlling the survival of spermatogonia and development of mature "spermatogenesis for male reproduction" in vertebrates (Ni et al., 2013; Makinen et al., 2001; Ebling et al., 2000).

The physiological functions of estrogens are reportedly mediated through the "specific cell surface receptors - the estrogen receptors" (Fu et al., 2014; Mermelstein and Micevych, 2008; Beyer et al., 2003). It is therefore necessary to critically look at the physiological role played by the estrogen receptors. Available literature has proven that the superfamily of nuclear hormone receptors (Perrotti, 2017) of which estrogen receptors belongs to (Blumberg and Evans, 1998) have many characteristics in common, in which proteins of this superfamily can be grouped into six distinct domains. "A/B domain which has a transactivation function, the C domain which consists of two zinc finger motifs" and consists of eight cysteine residues are essential for DNA binding. They also have a "D area which is the hinge region and enables the protein to change its conformation" (Fu et al., 2014); there is also the E domain which has the possibility of being the domain of ligand-binding and that of the F domain whose function is not clearly understood (Fu et al., 2014; Todo et al., 1996). Other authors have reported the third ER subtype of estrogen receptors (ER-b2) in many species of teleost such as the Atlantic croaker (Wang et al., 2005; Hawkins et al., 2000), goldfish (Ma et al., 2000), zebrafish (Bardet et al., 2002; Menuet et al., 2002) and largemouth bass (Sabo-Attwood et al., 2004), this has proven that at "least three subtypes" of estrogen receptors exist in teleosts (Guo et al., 2017; Wang et al., 2005).

To advance understanding of molecular endocrinology of phylogenetically hybrid grouper fish and also provide further data on the "evolution of teleosts estrogen receptors", estrogen receptors encoding cDNA sequences were sequestered and clones (Katsu et al., 2006) from juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂). Currently, little or no material information exist concerning estrogen receptors cloning and qPCR expression profile of hybrid grouper, hence, the objective of this study was to clone (Yu et al., 2008) the full length of juvenile hybrid grouper estrogen receptors and also studies their expression in the various tissues samples and analyzed the possible other roles played by the estrogen receptors in the development of the hybrid grouper fish. The data of this work could be helpful in researches intended at improving the production of hybrid grouper. Assessment of the resultant sequence data was done to determine their other possible role played by these receptors other than their traditional role of reproduction in vertebrates using COFACTOR server (Zhang et al., 2017) to analyze the Gene Ontology.

## MATERIALS AND METHODS

### Experimental fish

Groupers (*Epinephelus* spp*)* are teleosts, typical of them being "monandric protogynous hermaphrodites, meaning they mature as females" and can change sex after sexual maturity (Erisman et al., 2009; DeMartini et al., 2011). Hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus polyphekadion*) is an essential aquaculture fish recently developed through cross-breeding to provide a new aquaculture strain (James et al., 1999). Interestingly, "the hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus polyphekadion*♂) mature as a male meaning it grows from female to male and can change sex after sexual maturity between the ages of 3-5 years" (Amenyogbe et al., 2019). It must be noted that the fish developed gonads at its maturity which makes the study in gonad impossible at this stage of the hybrid grouper. A cumulative of 3 "female hybrid juvenile grouper (3 - 4 month) were used for the experiment with an average body weighing 82.3 ± 4.32 g, together with the length of 13.73 ± 0.13 cm obtained from Guangdong, Hengxing Group Co.LTD., Guangdong Province" (Amenyogbe et al., 2019). "Live fish procedures followed the guidelines by Institutional Animal Care and the Fisheries and Aquaculture College, Laboratory of Fish Breeding, Guangdong Ocean University, China" (Amenyogbe et al., 2019). Tissue samples of "brain, heart, intestine, muscle, head, kidney, liver, stomach, gill, and spleen were immediately dissected, iced up, and kept at -80°C with liquid nitrogen until use".

### RNA isolation

The "total RNA from the brain, gill, liver, muscle, intestine, spleen, stomach, head, kidney and heart tissues of the hybrid grouper was extracted by the use of MiniBEST Universal RNA extraction kit (TransGen Biotech, China) and using Trizol reagent (Invitrogen) following the manufacturer´s instructions". The "quality of total RNA was analyzed by the use of 1% agarose gel electrophoresis and UV spectrophotometry (Nandrop 2000, Thermo, USA)".

**Table 1.** The polymerase chain reaction primers used in this study of ERS.

| Primers Sequence | |
|---|---|
| M13 : CGCCAGGGTTTTCCCAGTCACGAC<br>RV : GAGCGGATAACAATTTCACACAGGA | Vector (Pmd-18) |
| UPM-long: CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT<br>UPM-short: CTAATACGACTCACTATAGGGC | Universal race primers |
| Al-F3: TACTCTGCTCCTCTGGAYGCMCACG<br>Al-R3: GCATCTCCAGCAGCAGGTCGTAYAG<br>B1- S1: CACTWCTGTGCTGTGTGYCACGACT<br>B1-R3: CTCACCCTGGAYGTGGCRGCTATCA<br>B2-S2: CGTCTACAAYGAACCCAGCCCACA<br>B2-A2: AGAGTCTGCTGCTGGGTCGWCA | Partial |
| 3B1-S1: TAACAGGACCAGCGTTGGGTTCATT | 3utr |
| 3B1-S2: GCCAAGAAGATTCCAGGATTCATAG | 3utr |
| 3A-S2: TCCTCCGATTCCTGTCAATAGTGC | 3utr |
| 3A-S3: ACTGGACCTGTAGACGGGTGTTGA | 3utr |
| 3B2-S1: CACAATGGACTACATCTGCCCCGC | 3utr |
| 3B2-S4: CTCCAGACTTCAAACTCAGCAGGG | 3utr |
| 5B1-A2: ACTTCACAGCATTTGCGGAGGCGAC | 5utr |
| 5B1-A3: CTTACGGCGGTTCTTGTCTATGGTG | 5ut |
| 5B2-A1: TTGATTAGTGGCGGGGCAGATGTA | 5utr |
| 5B2-A2: TTAGTGGCGGGGCAGATGTAGTCC | 5utr |
| 5A-A3: ATGATGAAAGGAGGTGTGCGTAAG | 5utr |
| 5A-A4: CTCATCTTTGCCCAGGACCTCATA | 5ut |
| GP-βactin(F) –TACGAGCTGCCTGACGGACA<br>GP-βactin(R)- GGCTGTGATCTCCTTTTGCA<br>EXPA-F2: ATGCCCACTTCGTAACAC<br>EXPA-R2: CACCTCAACACCCGTCTA<br>EXPβ1-F3: CTGGTCGTGTGAGGGGTGTA<br>EXPβ1-R3: GCCTTTGGTCTGTTGGTTCG<br>EXPβ2-F3: TCATTGCCTTCAGACAGAC<br>EXPβ2-R3: GCTCATCGACATCACCACC | RT-qPCR |

Where “M13, RV, UPM-Long and short are all universal primers, GP- βactin is Grouper βactin”, A1-F3, A1-R3, B1-S1, B1-R3, B2-S2, B2-A2 represent partials, and 3B1-S1, 3B1-S2, 3A-S2, 3A-S3, 3B2-S1, 3B2-S4 indicates 3UTR, while 5B1-A2, 5B1-A3, 5B2-A1, 5B2-A2, 5A-A3, 5A-A4 indicates 5UTR and EXPA-F2, EXPA-R2, EXPβ1-F3, EXPβ1-R3, EXPβ2-F3, and EXPβ2-R3 all indicates the primers used for gene expression of “estrogen receptor genes, and 3&5UTR stands for untranslated regions”.

**Cloning of estrogen receptors (ERs)**

To clone “a partial cDNA fragment of ERα, ERβ1, and ERβ2, primers, as shown in Table 1, were designed”. Primers were designed for amplification of hybrid grouper ERα, ERβ1, and ERβ2 gene coding sequences using the existing full-length sequences of *Epinephelus coioides-estrogen receptor alpha -(GU721076.1); Acanthopagrus schlegelii-estrogen receptor alpha-(AY074780.1); Sparus aurata-estrogen receptor alpha-(AF136979.2) and Acipenser schrenckii-estrogen receptor alpha1-(AB435631.1)* ; *Epinephelus coioides estrogen receptor beta 1-(GU721077.1); Sparus aurata-receptor type beta1-(AF136980.1); Acanthopagrus schlegelii –estrogen receptor beta1-(AY074779.1), and Acipenser schrenckii-estrogen receptor beta1-(AB435633.1)*; *Epinephelus coioides estrogen receptor beta2- (GU721078.1); Acanthopagrus schlegelii estrogen receptor beta 2-( EU346949.1) and Micropterus salmoides estrogen receptor beta2-( AY211021.1).*

Primer 5 software was used to design primers (Table 1). cDNA synthesis was performed using the TRANSgen First-strand cDNA synthesis kit with a total volume of 20 µl of reaction mixture following the manufacturer’s guidelines. The partial cDNA fragments of ERs were “amplified from the first-strand cDNA from” a mix of the liver, heart, and brain tissues. The PCR reaction was performed in 20 µl volumes of the reaction mixture. The amplification was performed following reaction conditions, “94°C for 5 min, followed by 35 cycles for 30 s at 94°C, for 30 s at 58°C, for

35 s at 72°C and 10 min at 72°C". The separation of PCR products was done by using electrophoresis, and the "DNA Bands were recycled and purified by the use of the SMART RACE cDNA purification Kit (Clontech, Palo Alto, CA)". The "purified DNA portions were then subcloned into the pMD18-T vector (Takara, Japan) and converted into competent *Escherichia coli* DH5a cells". A maximum of "three different positive clones each were selected and send to Sangon Biological engineering (Guangzhou) LTD". for sequencing (Amenyogbe et al., 2019). To "clone *3*′ and *5*′ untranslated region (UTR) end of ERα, ERβ1 and ERβ2, two sense primers were designed depending on the partial cDNA sequence of ERα, ERβ1 and ERβ2 as shown in Table 1 using the SMART RACE cDNA Synthesis Kit (Clontech, Palo Alto, CA) according to the manufacturer's guidelines". A "clear fragment was purified, cloned, and sequenced using the same procedure as described earlier".

3′ RACE was done in "both first and second amplification" using Amenyogbe et al. (2019) method. Additionally, "5′ RACE was carried out using the same procedures and methodologies that made use of reverse primers (Table 1) in place of sense primers". "PCR amplification was done in a 15-µl volume of the reaction mixture" following Amenyogbe et al. (2019) method.

#### Sequence analysis

The "sequences of partial, 3′ and 5′ UTR were assembled to form the full-length cDNA of the target gene by the use of LaserGene software" (*https://*dnaman.software*.informer.com)*. The "Gene translation, predictions of the amino acid sequence" was done using EXPASY web tools (http://expasy.org/tools). The conserved domains search was done using Marchler-Bauer et al. (2011) to ascertain conserved domain sequences among designated ER superfamily genes. To establish genetics relations, phylogenetic analysis was carried out, and a consensus tree was built using MEGA 6.0 software 6 (Tamura et al., 2013). We examined the physical and chemical properties of the protein sequences using the PROTEAN program (version 5.07; DNASTAR Inc.: Madison, WI, USA, 2003.) to ascertain their chemical compositions. COFACTOR server (Zhang et al., 2017) method was used for gene ontology GO terms predictions.

#### Tissue mRNA expression of ERs by qRT-PCR

The "mRNA levels of ERα, ERβ1, and ERβ2 in tissues were resolute by Real-time qRT-PCR" (Amenyogbe et al., 2019) in the liver, muscle, brain, stomach, spleen, intestine, gill, head kidney, kidney and heart using Transtar "Tip Green qPCR Supermix (TransGen Biotech, China)" the manufacturer´s instructions were followed, a Bio-Rad Connect ("Roche Light Cycler®96 SW1.1" Real-time Detection) in 10 ul reaction. We used *β-actin* as a control gene. The primers for ERα, ERβ1, and ERβ2 and *β-actin* were designed using primer5 designers (Table 1). The total mixture for the reaction for quantitative Real Time-PCR consisting of 5 ul Transtar "Tip Green qPCR Supermix (TransGen Biotech, China), and 0.4 ul of each sense and antisense primer, 3.6 ul of $H_2O$ and 0.6 ul of cDNA". A melting curve was performed to detect the specificity (Fu et al., 2014). The "reaction conditions of for the qRT-PCR were as follows: 30 s at 95°C; and was amplified for 40 cycles for 15 s at 95°C, annealing for 15 s at 59°C and extension for 15 s at 72°C". Each sample "verified in triplicate". The $2^{-\Delta\Delta Ct}$ methods (Livak, and Schmittgen, 2001) was used to calculate the results.

#### Statistical analysis

The data in this study were articulated as a means±SEM. The significant differences in the data between ERs are presented using one-way ANOVA followed by Duncan's posthoc test and a probability level less than 0.05 ($P < 0.05$) was used to indicate significance. Also, the Independent samples T-test was also used, and the significance level was set at $P < 0.05$. All statistical analysis were performed using SPSS 16.0 (SPSS, Chicago, IL, USA) (Cui et al., 2017).

## RESULTS

### Cloning and characterization of hybrid grouper ESRs

By the use of standard PCR procedures, the partial DNA fragments were augmented from Hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂) liver, heart, and brain RNA. The DNA fragment acquired, and sequence examination exhibited that the fragments had a resemblance to ERα, ERβ1, and ERβ2. The RACE technique was used "to clone full-length" of hybrid grouper ERα, ERβ1 and ERβ2 cDNAs with the following (GenBank accession no. MK575468, MK544841, and MK570511 for ERα, ERβ1, and ERβ2 respectively. The sequence analysis of cDNA for ERα show 621 amino acids and considered molecular mass 61.04 kDa and ERβ1 comprises 546 amino acids and a determined molecular mass 60.47 kDa, and ERβ2 show 244 amino acids and a determined molecular mass 27.49 kDa. The hybrid grouper ERs "sequence can be grouped into five domains based on its sequence identity to other species' ERs" (Figure 2) as suggested by Katsu et al. (2010c). Amino acid sequences of hybrid grouper hgERα shared the identity of 42.3 and 40.3% with hgERβ1 and hgERβ2 respectively while hgERβ1 show the identity of 56.8% with hgERβ2. Hybrid grouper sequences of ERs equated with different species (*Homo sapiens, Mus musculus, Epinephelus coioides, Dicentrarchus labrax, Oreochromis aureus, Sebastes schlegelii*, *Acanthopagrus latus* and *Sparus aurata*), Hybrid grouper ERα shared 81.9-98.8, and 57.4-958% identities in the C, and E domains, respectively (Figure 1A). In contrast, Hybrid grouper ERβ1 shared 63.3-97.5, and 66.1-98.7% identities in the C, and E domains, respectively (Figure 1B) and hybrid grouper. The clone protein sequence of ERβ2 shared 22.9-75.2% identities in the E domains. Therefore, "C domain or DNA-binding domain and E domain or the ligand-binding domains" were conserved in all vertebrate ERs considered. The general identities of hybrid grouper ERα with ERα in (*Homo sapiens, Mus musculus, E. coioides, D. labrax, and S. schlegelii*) were 46.1, 46.6, 89.1, 88.3, and 90.2%, respectively, the overall identities of hybrid grouper ERβ1 with ERβ1 from the (*Homo sapiens, Mus musculus, E. coioides, O. aureus, and S. schlegelii*,) were 48.2, 48.3, 91.5, 79.0, and 85.7%, respectively. The overall identities of hybrid grouper ERβ2 with ERβ2 from the (*Homo sapiens, Mus musculus, E. coioides, A. latus,* and *S. aurata*) species were 44.9, 44.4, 74.5, 69.1 and 70.0% respectively. The phylogenetic analysis showed that each of these ERs

A

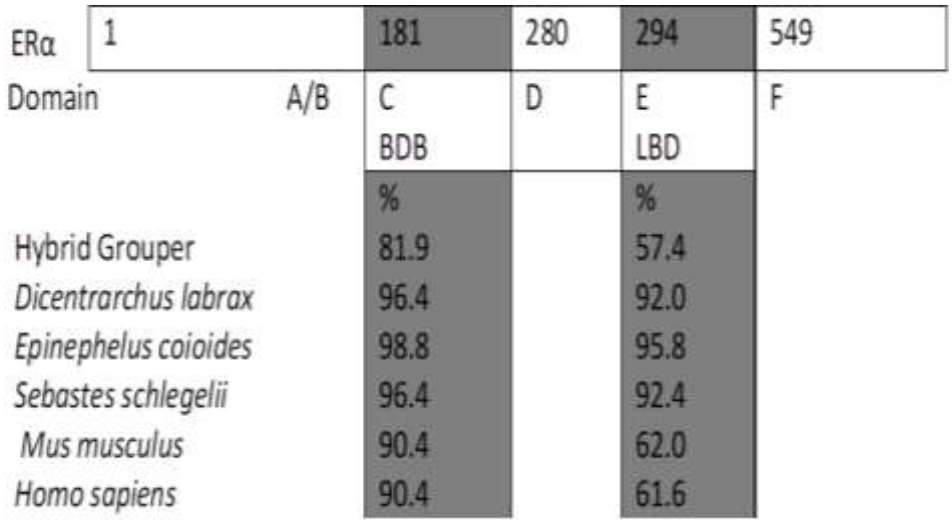



**Figure 1a.** The "DNA and ligand-binding domains" of Hybrid grouper ERα showing homology with some ERs. The figure presents the percentage "identities in the DNA and ligand-binding domains DBD and LBD" of the Hybrid grouper ERα in A with some sequences of the ER subfamily indicated. The Genbank accession numbers of ER sequences used are listed under the phylogenetic tree.

B

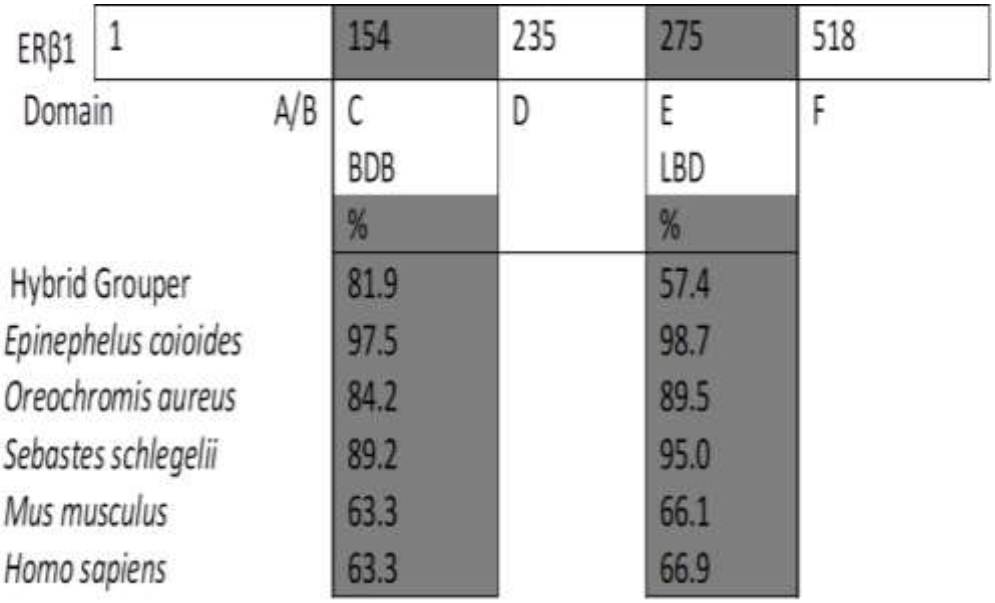



**Figure 1b.** The "DNA and ligand-binding domains" of hybrid grouper ERβ1 showing homology with some ERs. The figure presents the percentage identities in the "DNA and ligand-binding domains" of the hybrid grouper ERβ1 in B with some sequences from ER subfamily are indicated. The Genbank accession numbers of ER sequences used are listed under the phylogenetic tree.

belongs to ER superfamily (Figure 3).

The sequence alignments of ERs of different vertebrates including Hybrid grouper are demonstrated indicating the various domains. The Genbank accession numbers of ER sequences used are as same as ones used in the phylogenetic analysis. The MAPK, "P, and D-boxes from DBD", cAMP, PKC, AF-2 are indicated by Elbow Double-Arrow connector. The down arrow indicates the eight "cysteine residues of the zinc-finger motif". "A/B, B/C, C/D, D/E, and E/F" domains are marked with Double Arrows.

The phylogenetic examination was done using "Clustral W". The different ER sequences obtained from the Genebank database. Genebank accession numbers of

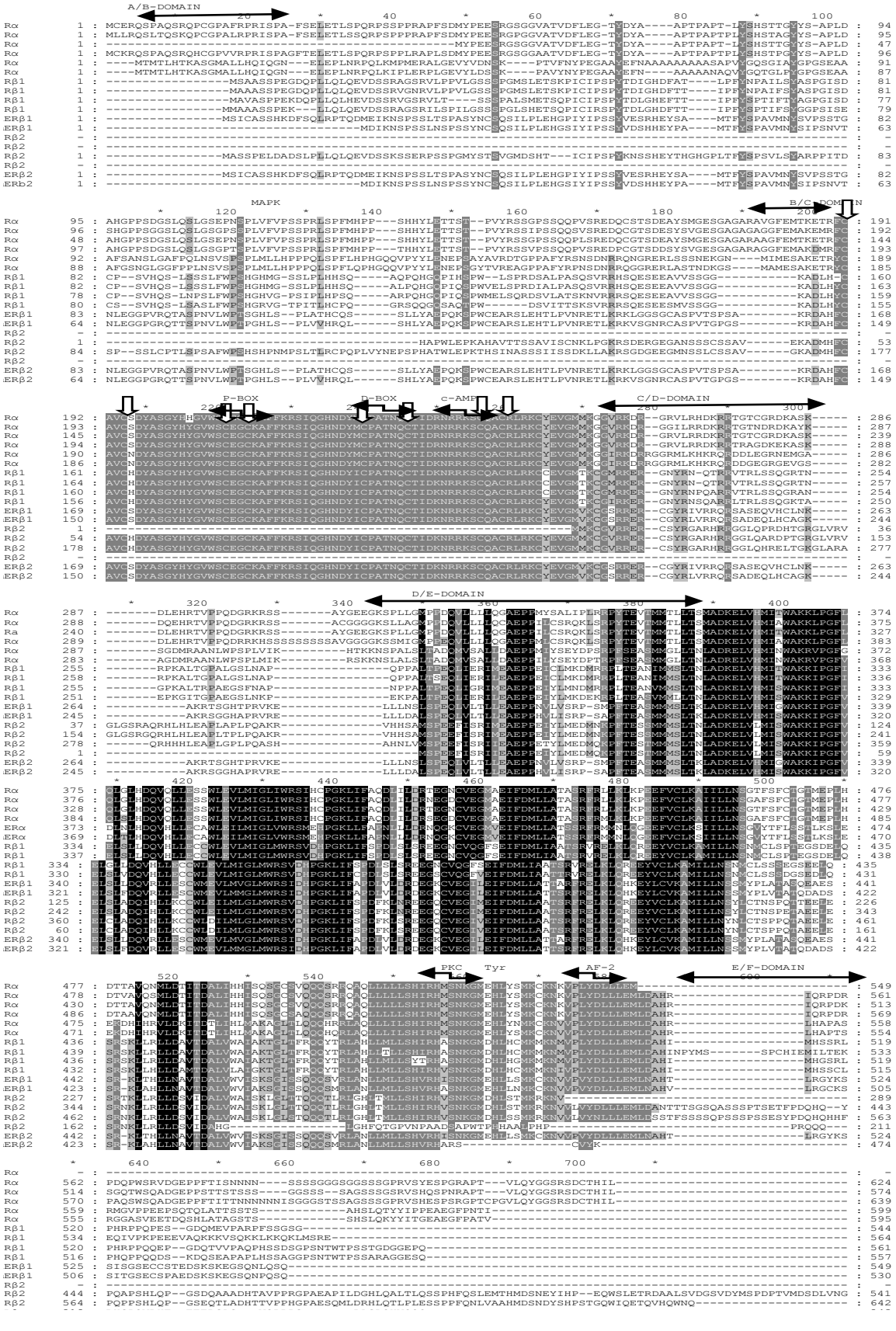



**Figure 2.** The essential amino acids in DNA and ligand-binding domains are highly conserved in hybrid grouper ERs.

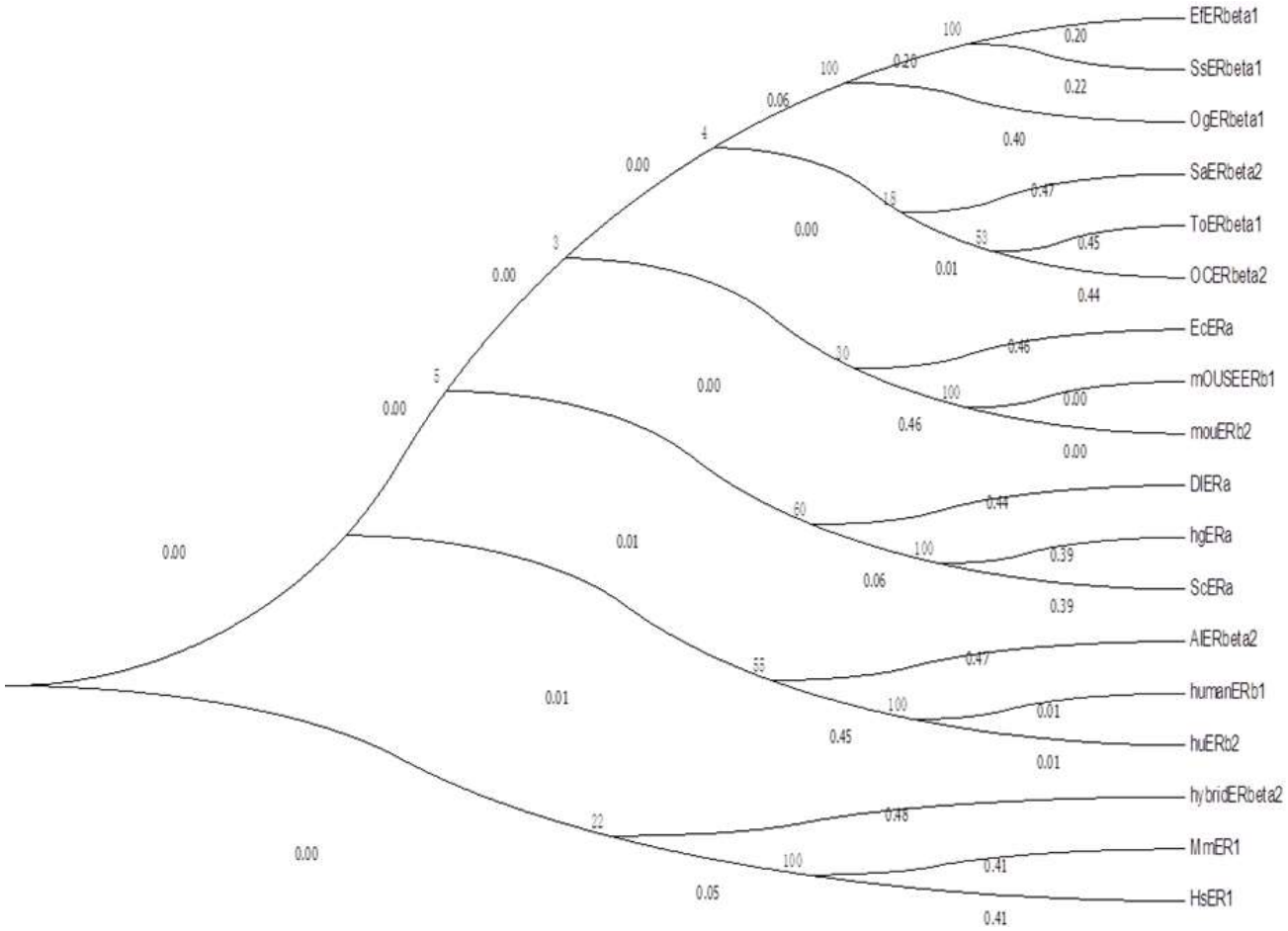



**Figure 3.** A phylogenetic tree is displaying the affiliations of the hgER with other ERs from other species.

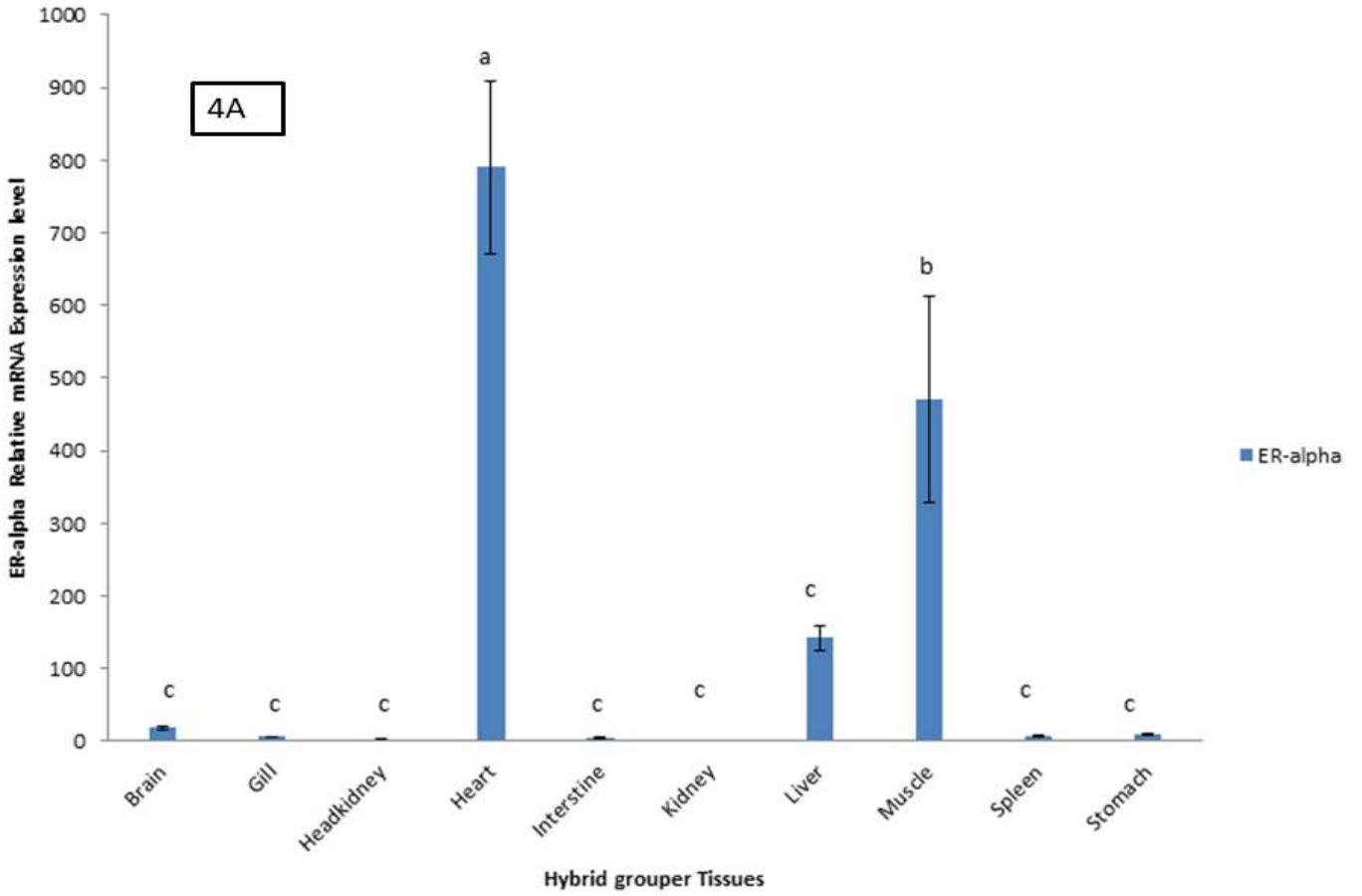
the sequences are: hybrid grouper ERα, MK575468; *E. coioides* ERα, ADK90033; *D. labrax* ERα, CAD43599; *S. schlegelii* ERα, ACN39246; *Mus musculus*-estrogen receptor isoform 1, NP_031982; *Homo sapiens*-estrogen receptor isoform 1, NP_000116; hybrid grouper ERβ1, MK544841; *E. coioides* ERβ1, ADK90034; *O. aureus* ERβ1, ACF75102; *S. schlegelii* ERβ1, ACN38898; *Mus musculus*-estrogen receptor beta isoform 2, NP_034287; *Homo sapiens*-estrogen receptor beta isoform 1,NP_001428 XP_495993; Hybrid grouper ERβ2, MK570511; *E. coioides* ERβ2, ADK90035; *A. latus* ERβ2, AEX68679; *S. aurata* ERβ2, CAE30471; *Mus musculus*, NP_034287 and *Homo sapiens,* NP_001258805. Where EFERbeta1- hybrid grouper ERβ1, SsERbeta1- *S. schlegelii* ERβ1, OgERbeta1- *E. coioides* ERβ1, mOUSEERb1- *Mus musculus*, ToERbeta1- *O. aureus*, SaERbeta2- *S. aurata* ERβ2, EcERa- *E. coioides* ERα, mouERb2- *Mus musculus*, DlERa- *D. labrax* ERα, HgERa-hybrid grouper ERα, SCERa- *S. schlegelii* ERα, AlERbeta2- *A. latus* ERβ2, humanERb1- *Homo sapiens*, HuERb2- *Homo sapiens*, HgERbeta2-hybrid grouper ERβ2, MmER1- *Mus musculus* and HsER1- *Homo sapiens.*

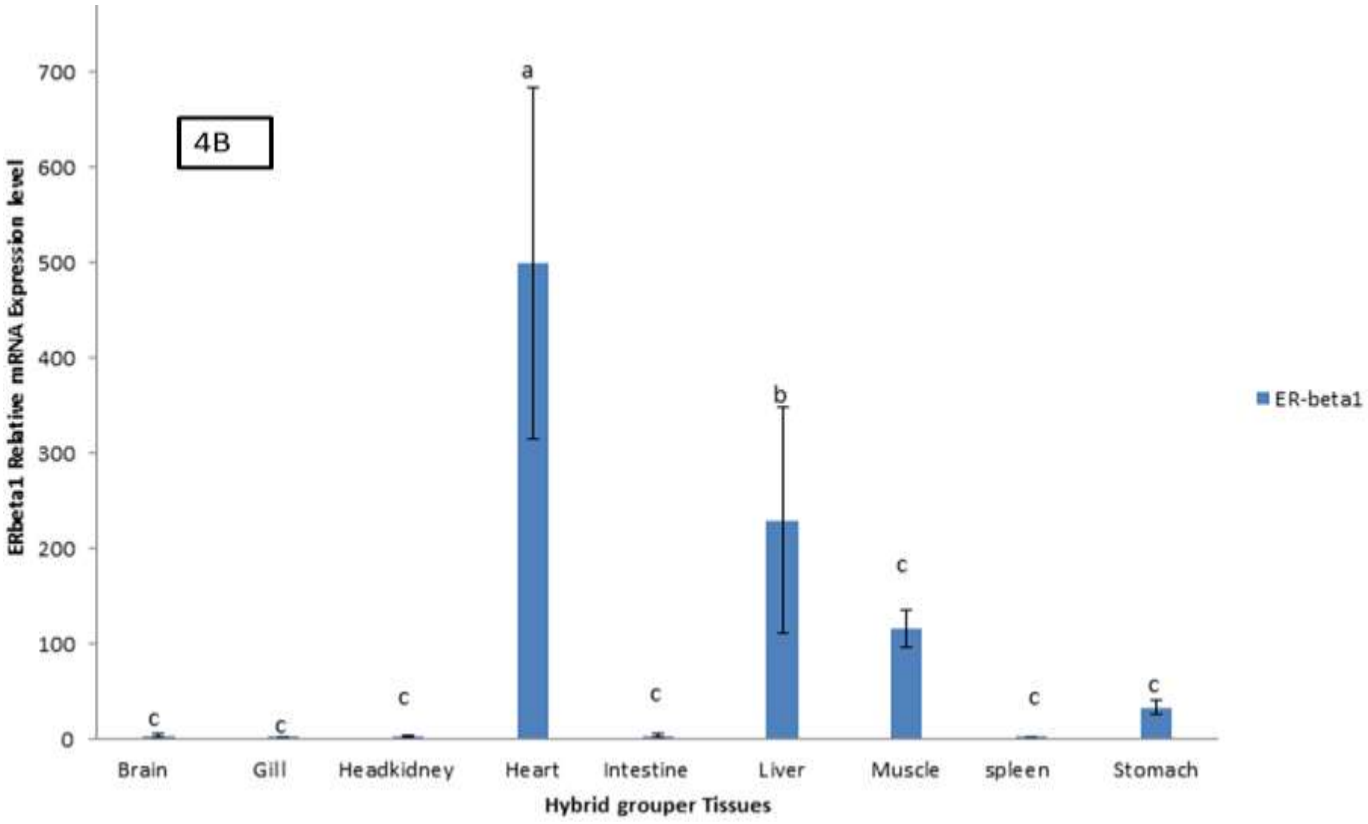
## Gene expression

While at a different level, hybrid grouper estrogen receptors (hgERs) were expressed in all tissues samples that were studied. The *hgER*α expression level was found to be highest in the heart among the ten tissues examined, followed by the muscle. The *hgER*β1 was noticeably paramount also in the heart, followed by the liver. The *hgER*β2 expressed most profoundly in the liver, followed by the stomach. Generally, the highest mRNA expression levels of hgERα, *hgERβ1* and *hgER*β2 were all found in the heart. There was a substantial difference between the expression in the heart and liver and other samples as shown in Figure 4a, b, and c. All hybrid grouper ERs studied were expressed in all tissues samples examined, although at different levels. Interestingly, *hgER*α expressed relatively higher in all tissues as compared to the *hgER*β1 and *hgER*β2.

## Physiochemical properties and sequence analysis

Scrutiny of the physical and chemical possessions of the


4A
ER-alpha Relative mRNA Expression level
1000
900
800
700
600
500
400
300
200
100
0
a
b
c
c
c
c
c
c
c
c
c
Brain
Gill
Headkidney
Heart
Interstine
Kidney
Liver
Muscle
Spleen
Stomach
Hybrid grouper Tissues
ER-alpha


4B
ERbeta1 Relative mRNA Expression level
700
600
500
400
300
200
100
0
a
b
c
c
c
c
c
c
c
Brain
Gill
Headkidney
Heart
Intestine
Liver
Muscle
spleen
Stomach
Hybrid grouper Tissues
ER-beta1

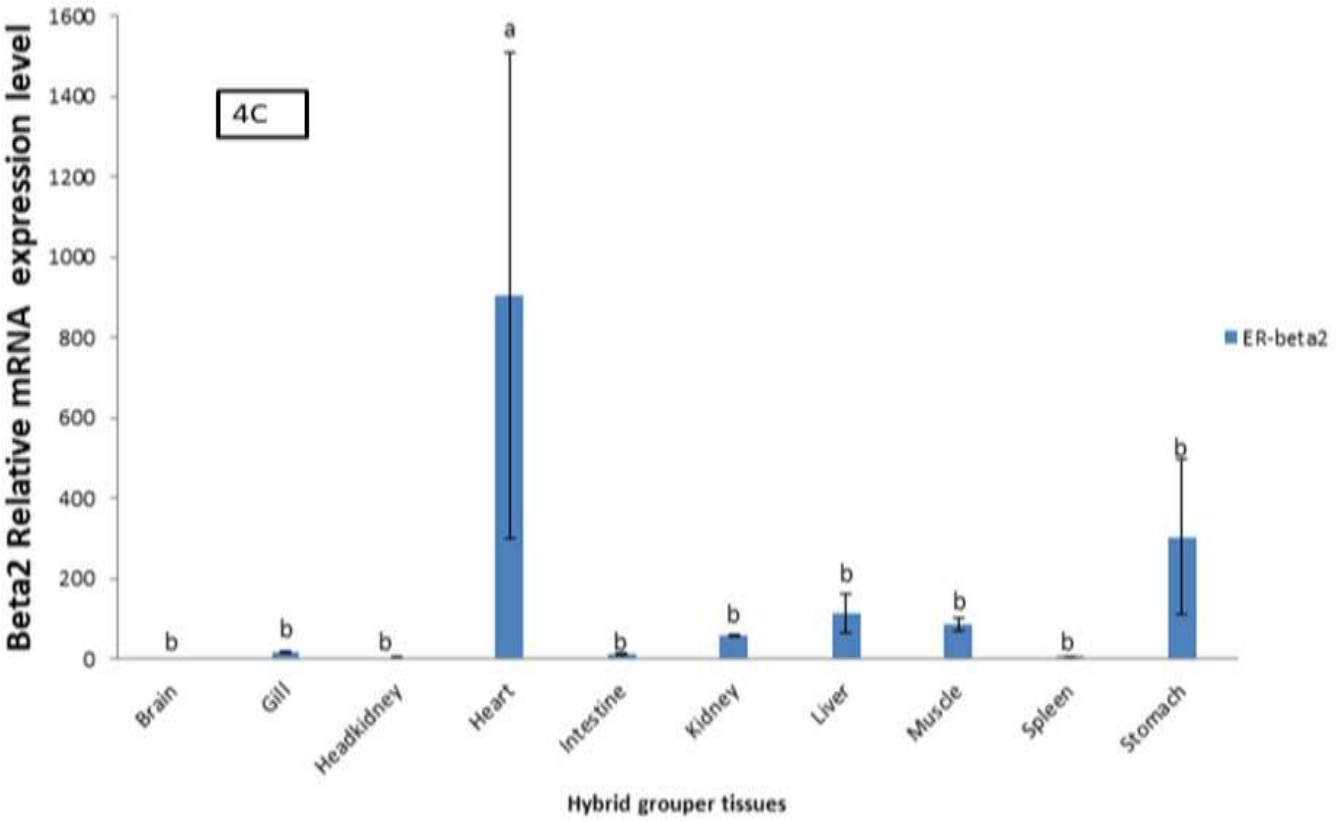



**Figure 4.** The mRNA expression profile of a) ERα, b) ERβ1, and c) ERβ2 respectively, in various tissues studies in Hybrid grouper. Statistics presented as the "mean ± SEM of triplicate experiments". Letters a, b, and c "indicate statistical differences at P < 0.05".

ERα sequence revealed the "molecular structural formula" of ERα to be $C_{2663}H_{4209}N_{759}O_{802}S_{42}$ "with a total atom number" of 8475. ERα has theoretically "predicted ion isoelectric value" of 8.19 and instability index of 65.10, classifying it as an unstable protein "with the molecular weight" of 68.40 kDa. While the scrutiny of the physical and chemical possessions of the ERβ1 sequence revealed the "molecular structural formula" of ERβ1 to be $C_{2632}H_{4203}N_{763}O_{783}S_{44}$ "with a total atom number" of 8425. ERβ1 has a "theoretical predicted ion isoelectric value" of 8.03 and instability index of 70.10, classifying it as an unstable protein with "molecular weight" of 60.47 kDa. On the other hand, the physical and chemical possessions of the ERβ2 sequence revealed the "molecular structural formula of ERβ1" to be $C_{1224}H_{1929}N_{329}O_{356}S_{17}$ with a "total atom number" of 3855. ERβ2 has "theoretically predicted ion isoelectric value" of 5.18 and instability index of 56.76, classifying it as an unstable protein "with the molecular weight" of 27.49 kDa.

## Amino acid composition and protein secondary structure

The "molecular examinations of the amino acid sequence of ERα showed that the protein contained 162 hydrophobic residues" (25.049%), 61 "acidic residues" (10.92%), 67 basic residues (14.27%) and 193 "polar amino acids" (30.06%). Aliphatic catalogue and "a grand average of the hydropath city (GRAVY) of growth was 70.73 and −0.403, correspondingly". The total quantity of "a negatively charged residue (Asp and Glu) was 56, and the total number of positively charged residues (Arg and Lys) was 60". The examination of "amino acid sequence of ERβ1 indicated that the protein contained 163 hydrophobic residues (29.02%), 53 acidic residues (10.78%), 56 basic residues (13.44%) and 152 polar amino acids (26.63%). Aliphatic catalogue and grand average of hydropath city (GRAVY) of growth were 81.25 and −0.309, correspondingly". The total quantity of "a negatively charged residue (Asp and Glu) was 53, and the total number of positively charged residues (Arg and Lys) was 56". Molecular "analysis of the amino acid sequence" of ERβ2 contained 84 hydrophobic residues (33.61%), 34 acidic residues (15.41%), 21 basic residues (10.91%) and 50 polar amino acids (20.01%). Aliphatic catalogue and grand average of hydropath city (GRAVY) of growth were 91.93 and −0.223, respectively. The total quantity of "a negatively charged residue (Asp and Glu) was 34, and the total number of positively charged

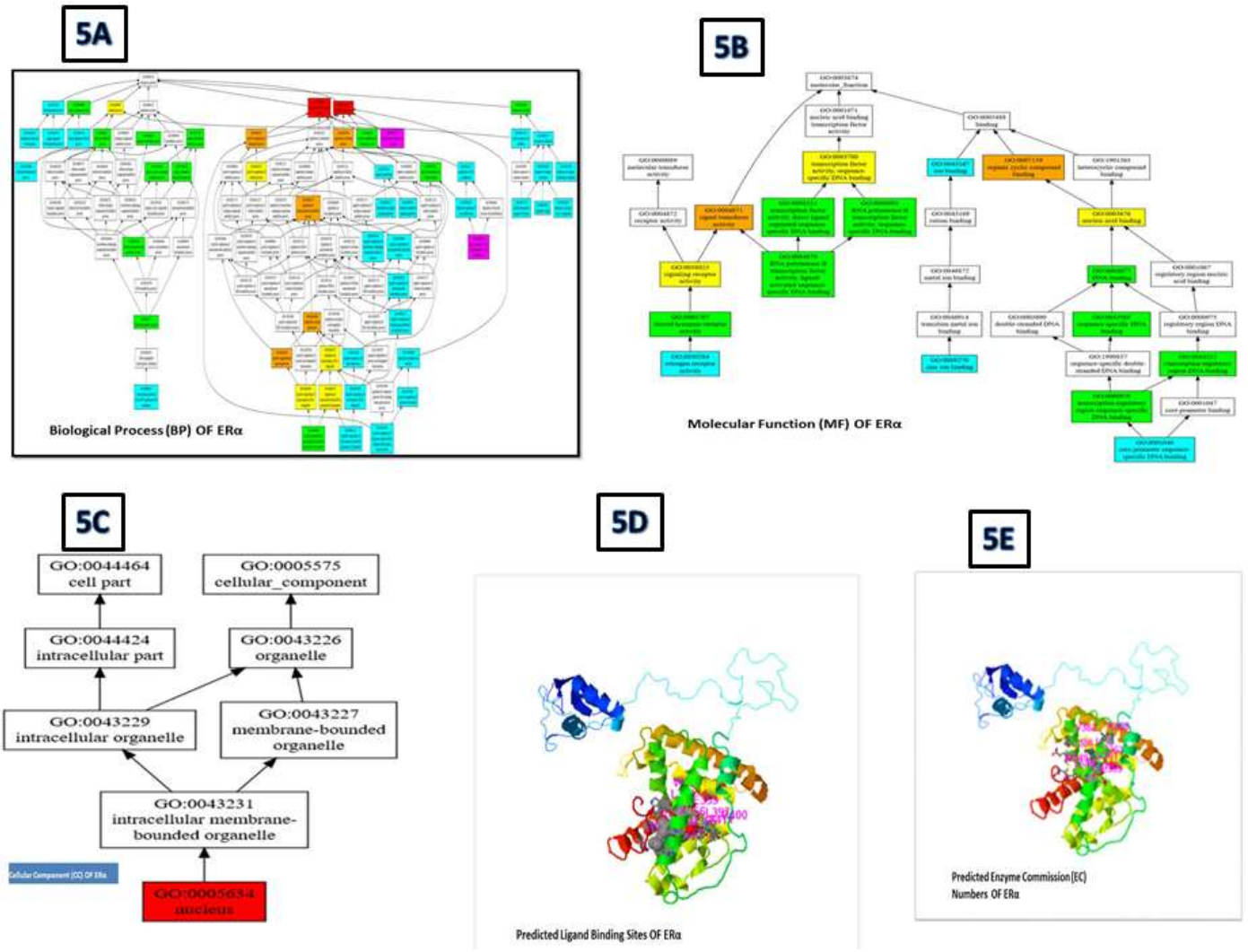



**Figure 5a, b, c, d and e.** The ERα's biological processes, molecular functions and cellular component.

residues (Arg and Lys) was 21".

## Predicted Gene Ontology analysis of hybrid grouper estrogen receptors using COFACTOR software

Using COFACTOR server to analyze Gene Ontology, two main functions were identified; namely, biological process and molecular function with Estrogen receptor mRNAs regulation relationships in the development of hybrid grouper by the GO. It has been found that the hybrid grouper "regulated by estrogen receptor alpha" is involved in many biological processes, including "biological regulation, regulation of biological process, regulation of macromolecule metabolic process, regulation of gene expression, positive regulation of biological process, regulation of cellular process, positive regulation of gene expression, regulation of transcription", DNA-templated, positive regulation of cellular process, positive regulation of transcription, DNA-templated, cellular process, regulation of transcription from RNA polymerase II promoter, positive regulation of transcription from RNA polymerase II promoter, organic substance metabolic process, response to stimulus, primary metabolic process, single-organism process, cellular metabolic process, organic substance biosynthetic process, developmental process etc (Figure 5a). Additionally, it also has molecular functions which includes, organic cyclic compound binding, signal transducer activity, transcription factor activity, sequence-specific DNA binding, signaling receptor activity, nucleic acid binding, DNA binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, transcription factor activity, direct ligand regulated sequence-specific DNA binding, sequence-specific DNA binding, RNA polymerase II transcription factor activity,

ligand-activated sequence-specific DNA binding, steroid hormone receptor activity, transcription regulatory region DNA binding, transcription regulatory region sequence-specific DNA binding, ion binding, estrogen receptor activity, zinc ion binding and core promoter sequence-specific DNA binding (Figure 5b). Added to this are the presence of cellular function (Figure 5c), predicted ligand binding sites (Figure 5d) and predicted Enzyme commissions (Figure 5e).

Furthermore, it has revealed that the hybrid grouper regulated by estrogen receptor beta1 is also involved in many biological processes that includes, regulation of biological process, regulation of macromolecule metabolic process, regulation of gene expression, positive regulation of biological process, regulation of cellular process, positive regulation of gene expression, regulation of transcription, DNA-templated, positive regulation of cellular process, positive regulation of transcription, DNA-templated, cellular process, regulation of transcription from RNA polymerase II promoter, positive regulation of transcription from RNA polymerase II promoter, metabolic process, organic substance metabolic process, primary metabolic process, cellular metabolic process, organic substance biosynthetic process, organic cyclic compound metabolic process etc (Figure 6a). In addition, it has molecular functions which include, organic cyclic compound binding, signal transducer activity, transcription factor activity, sequence-specific DNA binding, receptor activity, signaling receptor activity, RNA polymerase II transcription factor activity, sequence-specific DNA binding, nucleic acid binding and DNA binding (Figure 6b). Other functions such as cellular function (Figure 6c), predicted enzyme commissions (Figure 6d) and predicted ligand binding sites (Figure 6e).

In a similar functions, the study revealed that the hybrid grouper regulated by estrogen receptor beta2 in many biological processes, including regulation of biological process, organic cyclic compound biosynthetic process, primary metabolic process, cellular process, regulation of gene expression, regulation of cellular process, positive regulation of biological process, positive regulation of gene expression, RNA biosynthetic process, regulation of cellular metabolic process, regulation of transcription, DNA-templated, positive regulation of transcription, DNA-templated, single-organism process, regulation of transcription from RNA polymerase II promoter, positive regulation of transcription from RNA polymerase II promoter, response to stimulus, transcription initiation from RNA polymerase II promoter, signal transduction, developmental process, anatomical structure development, single-organism cellular process etc (Figure 7a). Additionally, it has molecular functions which includes, organic cyclic compound binding, signal transducer activity, heterocyclic compound binding, ion binding, metal ion binding, transition metal ion binding, transcription factor activity, sequence-specific DNA binding, receptor activity, signaling receptor activity, nucleic acid binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, DNA binding, steroid hormone receptor activity, transcription factor activity, direct ligand regulated sequence-specific DNA binding, zinc ion binding, and RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding (Figure 7b). Other functions such as cellular function (Figure 7c), predicted enzyme commissions (Figure 7d) and predicted ligand binding sites (Figure 7e).

## DISCUSSION

Estrogen receptors play very important traditional roles in the development of the reproductive system in vertebrates mostly in gonads and testis. Understanding and pinpointing the dissemination of ERs in hybrid grouper will help in understanding the possible other roles of estrogen in hybrid grouper development. Consequently, to our knowledge and understanding, there has been no study of the expression or the role of the ER in juvenile hybrid grouper a fish that has not developed gonads or testis yet. Estrogen is known traditionally to have a multiplicity of physiological functions and is tangled in regulating vertebrate metabolism, reproduction, cell proliferation, differentiation and inflammation through cellular machinery (estrogen receptors) required to warrant that estrogen executes these functions. Reproduction “activities in vertebrates, such as gonadal differentiation, maturation of the female reproductive tract, and procreative behaviors” are all associated with estrogen (Moore et al., 2005; Iguchi et al., 2001; McLachlan, 2001). In vertebrates, “estrogens seem to persuade both genomic and non-genomic cellular actions through the nuclear and perhaps G-coupled membrane receptors” (Moore et al., 2005; Iguchi et al., 2001; McLachlan, 2001). In 1990, the rainbow trout ER full-length sequence was reported in fish (Katsu et al., 2010c; Bjornstrom and Sjoberg, 2005). Ever since several other sequences have been recounted for teleost fishes, and three different types of ERs have been sequestered to date in a teleost (Katsu et al., 2010c; Hawkins et al., 2000).

In this study, full-length cDNA “sequences of distinctive ERα, ERβ1, and ERβ2” were cloned (Hu et al., 2018) from the juvenile hybrid grouper and characterized using PCR and the position of expressed ERs in the various tissues were examined. Hence, the prospective role of ERs in juvenile hybrid grouper development can be implicit. Upon aligning the amino acid sequence of the gene and sequences from different fish species, it has been found that the cDNA sequence of hybrid grouper estrogen receptors and their deduced amino acid sequence replicated a high degree of homology with the ERs homologs recognized from other animals and also the ERα and ERβ1 of juvenile hybrid grouper consist of

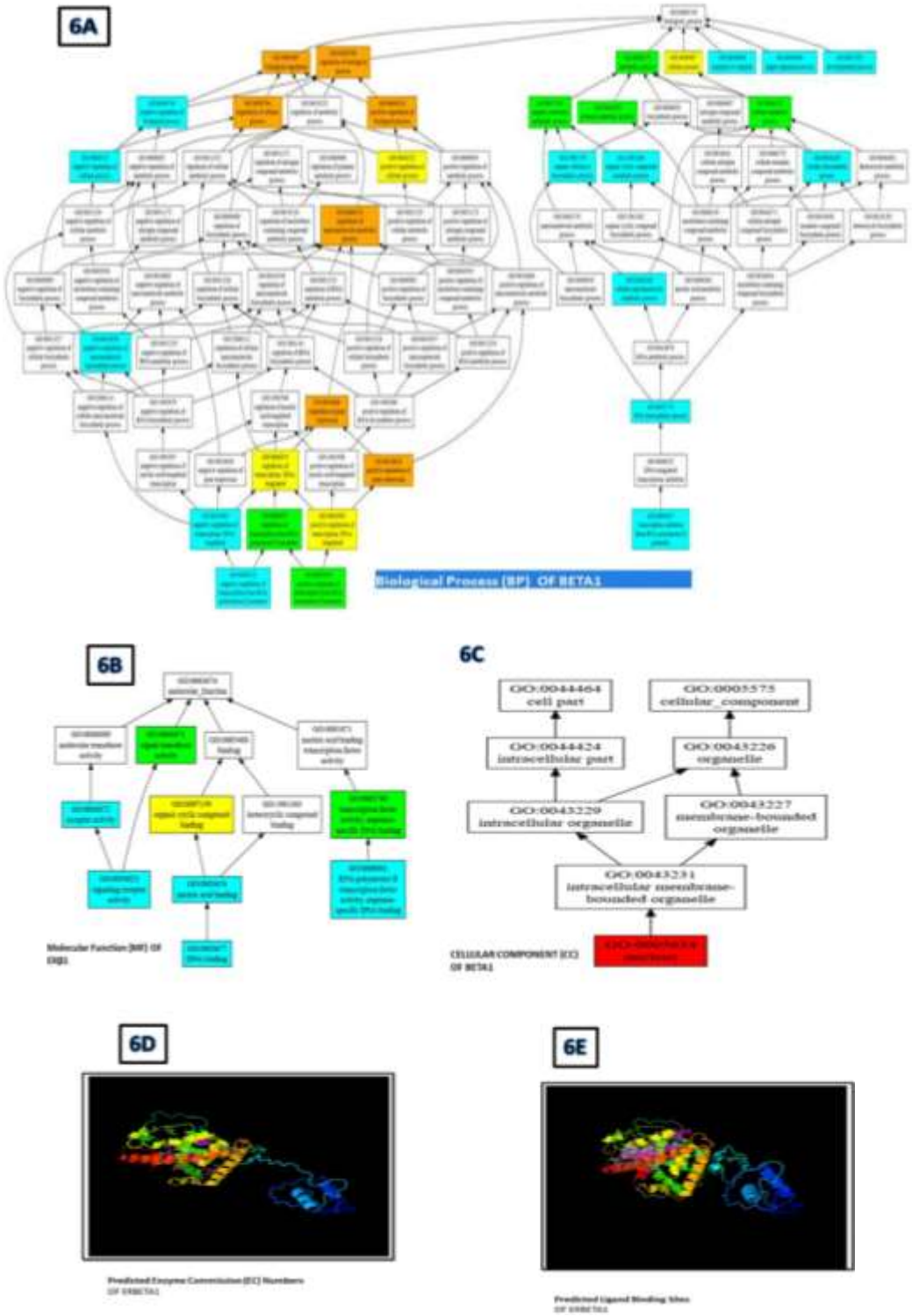



**Figure 6a, b, c, d and e.** The ERβ1's biological processes, molecular functions and cellular component.

well-known A/B, C, D, E and F molecular domains (Figure 2). This is an indication that this newly isolated cDNA encoded the hybrid grouper ERs protein.

Compared to other teleost fish, the A/B domain of the juvenile hybrid grouper ERα, and ERβ1, the C and E domains were less conserved (Ding et al., 2016). It was

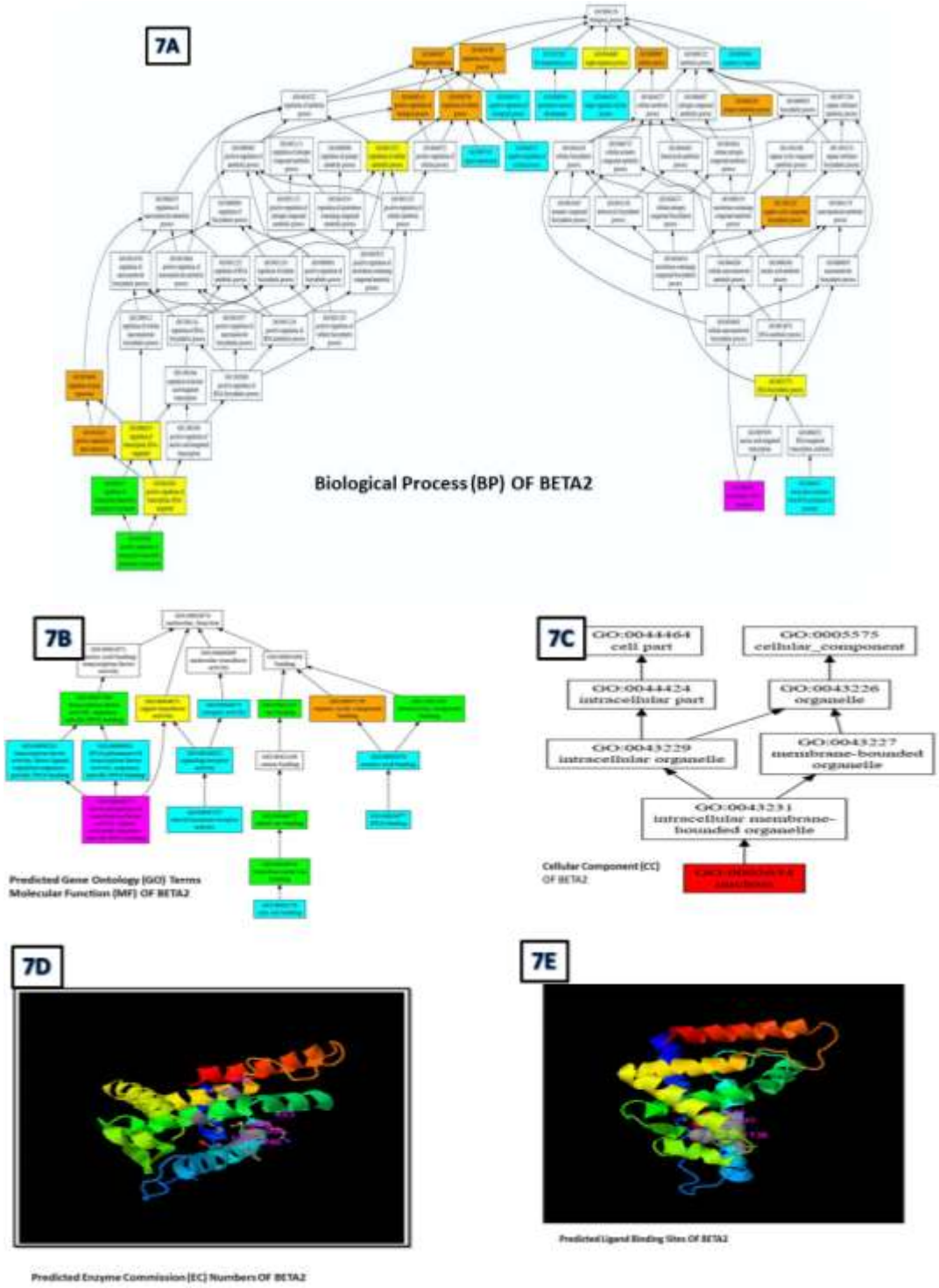



**Figure 7a, b, c, d and e.** The ERβ1's biological processes, molecular functions and cellular component.

also noted that the "phosphorylation sites known by mitogen-activated protein kinase (MAPK) were existent in the A/B domain of ERα and ERβ1", an indication that ERα and ERβ1 may influence the activation of MAPK pathway (Kato et al., 1995; Ding et al., 2016). It has also been noted that the AF-2 Activation domain (DLLLEML) occurred between amino acid residues 535 and 549 of the LBD domain, indicating that transcriptional activity was dependent on ligand binding (Ding et al., 2016; Kumar et al., 1987).

The hybrid grouper ERs genes shared high ranks of protein distinctiveness between the "DNA-binding domains" and contained the conserved motifs and elements believed to be essential for specific nuclear localization and command for target genes (Cui et al., 2017; Hall et al., 2002). There was also the reasonable uniqueness in the area called E/F domains or ligand-binding domains of the estrogen receptors which may be accountable "in part for the ligand-specificity and the different" answers to estrogen (Cui et al., 2017; Danielian et al., 1992; Kumar et al., 1987). The "ligand-binding domain", the AF-2 which is the "estrogen-dependent activation domains" were also observed to be conserved, indicating the similarity with other fishes, including *Scatophagus argus*, *E. coioides,* and *S. schlegelii* (Cui et al., 2017; Chen et al., 2011; Kim et al., 2003). Accepted functional sites of the protein for *hgER*s exhibited consistency with *ERs*. It is observed that the following domains "cysteine residues for two zinc fingers, P-box, D-box, and a cAMP site in the DBD domain, an AF-2 site, and a PKC phosphorylation site in the LBD domain" are conserved in *hgER*s (Figure 2). According to Mu et al. (2013), P- and D-boxes are shown to be crucial for DNA-binding. The importance of "PKC sites in all *ER*s" publicized by Härd and Gustafsson (1993) in which the initiation of PKC noticeably improves *"ER*-mediated transcriptional activation in a ligand-dependent manner" (Fu et al., 2008). The recognized A/B domain which contains the MAPK phosphorylation site was also observed to be conserved in the *ER*α subtype in sequence and position, as noted by others (Kato et al., 1995; Cho and Katzenellenbogen, 1993). Socorro et al. (2000) reported that the MAPK pathway could influence the "ligand-independent transcriptional activity of ER in both mammalian ERα and ERβ" (Fu et al., 2008).

While increasing literature exists on a phylogeny for numerous vertebrate steroid receptors (Howarth et al., 2008; Bury and Strum, 2007; Pakdel et al., 1990), scarce literature is available on hybrid species. Our study enhances essential material in this catalog. The analysis of sequences showed that two ERs protein sequences made up of the unique domain structures for NR superfamily while the hgERβ2 demonstrated the difference (Hu et al., 2006) slightly. A cautious examination of the phylogenetic tree discovered that this is in agreement with the case for ERs. At least "three sub-clusters of ERs were found in juvenile hybrid grouper even though maximally two ERs subtypes were isolated in many species" (Wang et al., 2005). The two ER-b subforms were reported in species of teleosts, such as the "Atlantic croaker, the Nile tilapia, and fugu, though not distributed in the same two sub-clusters as did the two ER-bs from zebrafish and goldfish" (Wang et al., 2005). An indication that "at least one of the two ER-b subtypes in the tilapia, Atlantic croaker, and fugu, tilapia-fugu ER-b2 clade has a diverse source from those of the zebrafish and goldfish, zebrafish ER-b1 clade" (Wang et al., 2005; Robinson-Rechavi et al., 2001b). It is possible that two consecutive lineage-specific replications might have transpired independently. Together they "took place after the divergence of teleosts". It is possible "the former took place only in zebrafish lineage, and the latter" transpired in the other teleosts deprived of the zebrafish lineage. The findings of the present study backed the proposition that most replicas of "fish genes arose more recently than the divergence of major fish groups" (Wang et al., 2005; Robinson-Rechavi et al., 2001b).

Examination of the tissue distribution of ERα, ERβ1, and ERβ2 offers comprehension into the prospective for targeting specific tissues. In the present research, we examined the expression configuration of ERs that is ERα, ERβ1 and ERβ2 mRNA in diverse tissues of juvenile hybrid grouper. The study revealed that ERs was expressed in all tissues of juvenile hybrid grouper examined. In goldfish, gilthead seabream and gilthead seabream the estrogen receptors were found to express mainly in gonads, but the overall expression in heart, liver, stomach, muscle intestine and head kidney showed largely corresponding expression patterns in hybrid grouper (Kato et al., 1995), the profiles of hgERs expression are similar to previously reported in tissue samples that were studied for all *ERs* in sea bass, with similar echelons among tissues (Lannigan, 2003) indicating possible similar function in hybrid grouper. The analysis of tissue expression in various tissue samples discovered all the three estrogen receptors expressed widely in hybrid grouper tissues which are in agreement with results from other studies (Cui et al., 2017; Cheng et al., 2015; Chen et al., 2011; Filby and Tyler, 2005; Halm et al., 2004; Kim et al., 2003) with the highest expression level in the heart contrary to study in goldfish which reported that ERα mRNA expression level was highest in the pituitary gland (Choi and Habibi, 2003). The expression of three estrogen receptors at differential expression configurations in tissues indicates that they might have diverse physiological roles. The hybrid grouper ERs gene was found to be highest in the heart but significantly lower in the kidney. Studies on *S. aurata* reported that ERα was expressed mainly in the liver and pituitary gland (Ding et al., 2016; Pinto et al., 2006), in partial agreement with the present study, whilst *Oreochromis mykiss* ERα and ERβ1 was found to expressed highest in the lever (Nagler et al., 2007). Altogether, the three estrogen receptors expressed highly in the heart, muscle, and liver, which suggests all of them, may be involved in growth and reproduction regulation in hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂). The ERs expression levels were comparatively high in the heart of ERα, ERβ1, and ERβ2. Even though ERs expressed in the liver, and the manifestation levels were low, this is a contradiction to other studies. This finding was contrary to the expression patterns of ERs in goldfish, *S. aurata*, and *O. mykiss*. The reason for these occurrences is not readily

known and further study is necessary to elucidate the implications.

## Conclusions

This study established the actuality of three estrogen receptors in juvenile hybrid grouper and demonstrated that ER-alpha, ER-beta1 and ER-beta2 are expressed throughout all tissues which implies that estrogen through these receptors may be responsible for the regulations of physiological and pathological functions in Hybrid grouper. The copious expression of hybrid grouper ERs advocates a broad expression pattern as in mammalian ERs. These results put forward that steroid hormone estrogen receptors might be playing a significant part in the controlling of social behavior complexity, plasticity behavior, and the assessment of a gratifying inducement in Hybrid grouper. Based on this study, further study is necessary to elucidate the effect of ERs in developmental stages.

There is also need for research of the spatial configurations of ER-transcript expression in adult hybrid grouper tissues and also further dichotomization of the part estrogen receptors might be playing in regulating the incredible malleability of social behavior within hybrid grouper.

## Funding

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## CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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# Evaluation of yield and yield components of low n maize (*zea mays* l.) varieties under low and high nitrogen conditions

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**Field studies were conducted at the teaching and research farm of the Osun State University Ejigbo Campus, Osun State, Nigeria, during the 2015 and 2016 cropping seasons to determine the agronomic performance of low N maize varieties under two nitrogen conditions (low and high). The aim of this experiment was to determine the response of low N maize to two levels of nitrogen fertilizer. Ten low N maize varieties were used in this study. Low and high-N conditions of the soil was induced by application of urea fertilizer at the rate of 30 and 90kg ha$^{-1}$ of nitrogen .The experiments were laid out in a randomized complete block design with three replications. High nitrogen application significantly improved maize vegetative growth, yield components and grain yield. The use of 90 kg ha$^{-1}$ of N gave the highest maize plant height, and number of leaves per plant, as well as grain yields of 3.50 and 3.58 tha$^{-1}$ was obtained with the application of 90 kg ha$^{-1}$ of N in 2015 and 2016 cropping seasons, respectively. The result indicated that application rates of nitrogen (kg ha$^{-1}$ of N) improved growth attributes of all the varieties with SINT MAR 20CA LARGA gave maximum grain yield that is not significantly different from 72PBPROLC$_3$SYN.**

**Key words:** Plant height, agronomic performance, low N maize, nitrogen levels.

## INTRODUCTION

Most Africans depend on maize as their stable food (Bänziger and Diallo, 2001) to feed both rural and urban dwellers. Maize cultivation in the tropics is seriously threatened by low nitrogen in the soil which causes low production in yield. Therefore, cultivation of low N tolerance cultivars is superior in the utilization of available N than other varieties.

Most soils in Nigeria are inherently low in N and its

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deficiency is due to the rapidity by which it is taken up or lost from the soil through erosion, volatilization or leaching nitrogen, and hence reducing maize yield by as much as 40% (Baenziger and Lafitte, 1997). Soil fertility problem constitutes a serious constraint to maize food production. Out of the essential soil nutrients, nitrogen is the most limiting factor (Fakorede et al., 2003).

Nitrogen deficiency is one of the most important stresses affecting maize production in tropical areas (Bänziger et al., 1999; Yara, 2009; Law-Ogbomo and Remison, 2008). The soils in Nigeria are low in organic matter and available. However, as a result of high cost of N fertilizer, poor distribution system and low purchasing power (Fakorede et al, 2003). The depletion has been attributed mainly to intensive and continuous cropping with little or no fertilizer application and thus culminating into imbalance between nutrients supply and extraction from the soil (Sanchez et al., 1997). The declining productivity of many tropical soils has been one of the major constraints limiting the realization of the genetic potentials of available improved crop varieties (Dudal and Deckers, 1993).

The need to take appropriate steps to check the declining soil productivity by improving the physico-chemical properties of the soil including its fertility in order to increase maize yield is urgent, because the rate of deterioration is increasing and it is bound to have serious implication on future food security in the region. Adequate inputs of nutrients as fertilizers as well as soil amendments to improve physico-chemical properties are required to overcome the constraints. Maize for example, has high demand for nitrogen thus nitrogen becomes the first limiting nutrient as land use intensifies. This explains why it is almost impossible to grow maize successfully on some soils in the Guinea savannah of Nigeria without nitrogen fertilizers.

In view of importance of maize in Nigeria economy, national and international bodies have developed interest in promoting maize production for households' food security and poverty alleviation. Some of these efforts have been channelled through biological and agronomic research into the development of high-yielding varieties along with best cultural practices. A considerable proportion of maize in the Nigeria is produced under N-stressed conditions (Bias et aI., 1997, Chantachume et al., 1997, Sallah et aI., 1997).

Farmers do not use much fertilizer because of the high price ratio between fertilizer and grain, limited availability of fertilizer, and the low purchasing power of farmers. Although organic N may be available to maize in some cases but the low yields (1.3 t/ha on average in farmers farm; (Afolabi et al., 2015) indicate that N from organic sources meets the demand of maize to a limited extent only. Several genotypes have been developed on the basis of anthesis-silking interval selection for drought tolerance, and a variety is being developed for soils with low fertility where N is the most limiting nutrient.

It was reported by Tilman et al. (2002) that the use of N fertilizer is not only adding to cost of production but also can negatively impact soil, water and air quality within a given ecosystem. For these reasons, reducing the amount of supplemental N used in maize production by developing low-N tolerance varieties will have positive economic and environmental benefits to world of agriculture. A possible approach to reduce N deficiency in soil is to lower crop demand for N through selection for low-N tolerance (Smith et al., 1995).

Most of the cultivated varieties, especially hybrids, require high doses of fertilizer to produce optimally. The use of these hybrid varieties under low-N condition can sometimes result in total crop failure. The declining use of fertilizer due to high cost and non-availability has limited the use of hybrid maize. A pragmatic strategy to boost productivity of maize is by the use of varieties that are tolerant to low-N soils. These varieties will be able to produce high yield reasonably well under low-N conditions.

In order to increase maize yield in environment with low soil N, Kogbe and Adediran (2003) gave two suggestions. One is the development of agronomic practices that efficiently utilized N from organic matter, N inputs from biological fixation and atmospheric deposition. Secondly, is by working with population that have reservoir of genes for low N tolerance. From where Low N varieties can be produced. CIMMYT research on low N tolerance began in 1985. Lafitte and Bänziger (2003) have led the work, which was done mostly at the Poza Rica station where long-term low nitrogen blocks have been established. It was reported that there are linkages between low N tolerance and drought tolerance.

Several research on maize has been developed to have genotypes that can tolerate low fertility where N is the most limiting nutrient but the breeding programme strategy has not been carried out on genetic dissection on this genotypes in order to have information on the nature of combining ability of parents, their behavior and performance of the hybrid combinations (Prasanna et al., 2001).

Employing an effective breeding procedure depends to a large extent on understanding of the genetic mechanisms controlling the characters to be improved (Malik et al., 2004). Various quantitative genetic approaches have been used for estimating the mode of gene action in controlling low N tolerance in maize. Most of the genetic design used to analyze mode of gene action assume absence of non-allelic interactions, however, there are contrary evidences to this assumption (Ashfa et al*.,* 2006). This will be a better and most cost efficient strategy that can produce reasonable grain yield under poor soil.

The objective of this study is to compare performance of ten (10) maize varieties under low N condition and investigate potential of ten (10) maize populations in Ejigbo, South-West Nigeria, Osun State.

**Table 1.** The weather conditions during the growing season of this study in 2015 and 2016.

| 2015 | Rainfall | Relative humidity (%) | | Temperature (°C) | |
|---|---|---|---|---|---|
| **Months** | **(mm)** | **0600 h** | **1800 h** | **Min** | **Max** |
| April | 112 | 72.3 | 62.1 | 25.4 | 32.5 |
| May | 212 | 70.1 | 61.3 | 25.3 | 31.7 |
| June | 401.32 | 69.6 | 60.4 | 26.5 | 30.3 |
| July | 500.32 | 70.45 | 60.7 | 25.2 | 29.57 |
| August | 525.6 | 70.3 | 60.45 | 25.5 | 28.7 |
| Mean | 350.248 | 70.55 | 60.99 | 25.58 | 30.55 |
| **2016** | | | | | |
| April | 112 | 70.23 | 60.1 | 26.4 | 32.52 |
| May | 212 | 69.91 | 59.3 | 25.53 | 31.73 |
| June | 401.32 | 70.6 | 60.4 | 26.5 | 30.43 |
| July | 500.32 | 71.45 | 61.7 | 25.32 | 29.67 |
| August | 525.6 | 71.3 | 59.45 | 25.45 | 28.57 |
| Mean | 350.25 | 70.70 | 60.19 | 25.84 | 30.58 |

## MATERIALS AND METHODS

### Experimental site and plot layout

Field studies were conducted during the 2015 and 2016 seasons at the Teaching and Research farm of the Department of Agronomy, Osun State University, Ejigbo Campus. The weather conditions during the growing season of this study in 2015 and 2016 are as shown in Table 1.

### Soil testing

At experimental site, eight soil core samples were taken from each plot using soil auger before planting. Cores for each plot were combined and the composite sample was air dried. The soil was passed through a 2 mm, and 0.5 mm sieve for chemical and physical analysis. Table 2 shows the physical and chemical properties of the soil.

### Description of experimental materials

The materials used for this study comprised of 10 open pollinated variety (OPV) of maize developed at International Institute of Tropical Agriculture (IITA), Ibadan for grain yield and adaptation to biotic and abiotic stress factors. The description of these materials was given in Table 3. The 10 maize varieties were evaluated during the planting season of 2015 and 2016 using randomized complete block design (RCBD). Low and high-N conditions of the soil were induced by application of urea fertilizer at the rate of 30 and 90kg $ha^{-1}$ of N. In 2015 and 2016 entries were made in a row plot of 5 m long, spacing was 75 cm inter-rows and 50 cm intra-rows. Three seeds were initially planted on a hill but were later thinned to two weeks after planting to give a planting density of 53,333 plants $ha^{-1}$.

**Table 2.** Physical and chemical characteristics of the soil.

| Physical characteristics | Amount |
|---|---|
| % Clay | 6.0 |
| % Silt | 19.0 |
| % Sand | 72.0 |
| % Organic matter | 8.5 |
| Texture | Sandy loam |
| **Chemical characteristics** | |
| % Organic Carbon | 8.7g/kg |
| % Nitrogen | 0.5 g/kg |
| pH | 6 |
| Potassium K+ | 0.29 cmol/kg |
| Sodium Na+ | 0.18 cmol/kg |
| Calcium Ca2+ | 1.5 cmol/kg |
| Magnesium Mg2+ | 1.3 cmol/kg |
| Available P | 6.2 cmol/kg |
| ECEC | 11.90 |
| Total Acidity | 1.1cmol/kg |

### Cultural practices

#### *Weeding*

Manual weeding was done throughout the experiment. Weeding was done regularly with hoes to keep the plots weed-free.

#### *Thinning*

This was done two weeks after planting. Hill population was reduced to two vigorously growing plant.

### Data collection

Data were collected for the following traits using maize descriptor:

(1) Plant height: This is the distance from the base of the plant to

**Table 3.** The description of experimental materials.

| Varieties | Names | Grain colour | Maturity group |
|---|---|---|---|
| V1 | SINT MAR 20CA LARGA | White | Late-intermediate |
| V2 | LN TP YC7 | Yellow | Late-intermediate |
| V3 | BR99 72L COMPI | White | Late-intermediate |
| V4 | 72PB PROL $C_4$ | White | Late-intermediate |
| V5 | LAPOSTA SEQUIA $C_6$ | White | Late-intermediate |
| V6 | 72L COMP $IC_6$ LNCI | White | Late-intermediate |
| V7 | DMR ESR W LN | White | Late-intermediate |
| V8 | 72PB PROL C3 SYN | White | Late-intermediate |
| V9 | LN TP YC6 SYN | Yellow | Late-intermediate |
| V10 | DMR ESR Y LN | Yellow | Late-intermediate |

the node bearing the tassel branch (measured in cm).
(2) Ear height: The distance from the base of the plant to the node bearing upper ear (measured in cm).
(3) Days to 50% anthesis: This was taken as the number of days from planting to the time (50%) when the plants shed their pollen grains.
(4) Days to 50% silking: This was taken as the number of days from planting to the time when 50% of the maize plants produce silk.
(5) Anthesis-silking interval: This is the interval between the first day plant shed their pollen grains to the first day plant brought out silk.
(6) Number of leaves per plant: The number of leaves per plant was determined by counting and the data from 10 plants from the middle rows was used to compute the score for each plot at 4, 8 and 16 WAP.
(7) Ear weight (g): The fresh weight of the peeled ear measured to the nearest gram and the mean weight of ears from 10 randomly selected plants from the middle row was used to compute the score for each plot.
(8) Grain yield (t $ha^{-1}$): Grain yield was computed from ear weight (EWT, kg/$m^2$), adjusted to 15% moisture content (MOIST) and 80% shelling percentage (Dhillon et al., 1976) using the formula:

Grain yield (t $ha^{-1}$) = EWT ×(100 – MOIST) / 85) ×(10000 x SHELL)

#### Data analysis

All data collected were subjected to statistical analysis of variance (ANOVA) using SAS Institute (1995). Significant means were separated using Duncans Multiple Range test at 5% propability level.

## RESULTS

The results of this study showed that the application of low and high Nitrogen was significantly improved the growth and yield of maize varieties. The performance of the maize during the 2015 and 2016 growing seasons was not statistically different in all the growth and yield traits evaluated (Tables 4 and 5).

Maize vegetative growth parameters assessed at flowering showed that plant height, number of leaves per plant was significantly higher in 90kgNha-1 than 30 kgNha-1 in both seasons (Table 4). This was associated with reduced nitrogen content in the soil. Considering the overall performance, SINT MAR 20CA LARGA and 72PB PROL C3 SYN were identified as the varieties with high number of leaf and leaf area.

In 90kgNha-1 kgN/ha, SINT MAR 20CA LARGA and 72PB PROL C3 SYN had the highest number of leaf in both season with 15.5, 14.5, 12.44 and 14.9 respectfully. Similarly, mean leaf area for 90kgNha-1 was significantly higher than 30kgN/ha in both seasons. It ranges between 662.59cm2 for SINT MAR 20CA LARGA and 573.44cm2 for DMRESRYLN 90kgNha-1 90kgN/ha for 2015 also for 2016, it ranges between 692.2 for SINT MAR 20CA LARGA and 579 for 72PB PROL C3 SYN.

Low average plant height was observed in 30kgNha-1 for both seasons. The plant height at 90kgNha-1 for 2015 ranges between 165.8 for 72PB PROL C3 SYN and 108 for LA POSTA SEQUIA C6. Likewise for 2016, plant height for 72L COMP IC6 LNCI and 127.40 for LN TP YC6 SYN (Table 5). The ear height and internode for 30 90kgNha-1 and 90kgNha-1 were not significantly different in both seasons.

Generally, average maize grain yield for low N tolerance maize varieties in 90kgN/ha was significantly higher compared with 30kgNha-1 in both seasons. This was associated with increase in plant height and decrease in anthesis-silking interval (Table 6). Considering the overall performance ,SINT MAR 20CA LARGA and 72PB PROL C3 SYN were identified as high yielders with average grain yield of 3.5t/ha and 3.00t/ha respectfully for 2015. This also had a similar trend in 2016 with average grain yield of 3.58t/ha and 3.24t/ha. The results further indicated that 90kgN/ha significantly enhanced maize yield in the two cropping season. The use of higher nitrogen (90kgN/ha) produced more vigorous maize planting having significantly bigger average ear weight, ear length than when 30kgN/ha was applied.

## DISCUSSION

Maize growth, yield and yield components for low N maize tolerant maize in 2015 and 2016 were significantly better with the use of 90kgN/ha than the use of30 kgN/ha. There was no significant differences in the effect of the

**Table 4.** Vegetative parameters of 10 maize varieties under low and high nitrogen.

| Varieties | Leaf number | | | | Leaf area ($cm^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2015 | | 2016 | | 2015 | | 2016 | |
| | low N | High N | Low N | High N | low N | High N | low n | High N |
| SINT MAR 20CA LARGA | 10.5 | 15.5 | 10.2 | 14.5 | 462.59 | 741.50 | 465.45 | 692.05 |
| LN TP YC7 | 9.78 | 11.08 | 10.74 | 12.08 | 548.64 | 648.64 | 550.04 | 645.64 |
| BR99 72L COMPI | 10.67 | 13.5 | 9.67 | 12.5 | 595.32 | 695.32 | 592.30 | 692.32 |
| 72PB PROL $C_4$ | 11.44 | 12.50 | 11.5 | 11.90 | 541.29 | 641.29 | 448.33 | 638.29 |
| LA POSTA SEQUIA $C_6$ | 8.56 | 10.56 | 9.50 | 10.53 | 496.34 | 596.34 | 487.44 | 593.34 |
| 72L COMP IC6 $LNC_I$ | 10.44 | 12.44 | 10.45 | 11.53 | 482.43 | 582.43 | 480.50 | 579.43 |
| DMR ESR W LN | 9.11 | 10.15 | 9.54 | 10.55 | 555.94 | 647.94 | 562.59 | 644.94 |
| 72PB PROL $C_3$ SYN | 10.33 | 13.53 | 10.00 | 12.53 | 542.30 | 634.3 | 548.64 | 631.3 |
| LN TP YC6 SYN | 8.67 | 12.44 | 9.60 | 14.49 | 498.33 | 590.33 | 595.32 | 587.33 |
| DMR ESR Y LN | 9.11 | 11.15 | 9.15 | 10.15 | 481.44 | 573.44 | 441.29 | 570.44 |
| Mean | 9.961 | 12.29 | 10.04 | 11.97 | 530.46 | 627.26 | 517.19 | 624.26 |
| LSD (0.05) | 0.95 | 2.1 | 1.05 | 1.95 | 11 | 14 | 10 | 14 |

**Table 5.** Mean of growth parameters of ten maize varieties under low and high nitrogen.

| Varieties | Plant height (cm) | | | | Ear height (cm) | | | | Internodes (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | | 2016 | | 2015 | | 2016 | | 2015 | | 2016 | |
| | Low N | High N | Low N | High N | low N | High N | low N | High N | Low N | High N | Low N | High N |
| SINT MAR 20CA LARGA | 146.74 | 184.58 | 143.73 | 174.58 | 106.75 | 121.75 | 114.25 | 123.75 | 5.45 | 5.67 | 5.47 | 6.07 |
| LN TP $YC_7$ | 130.45 | 159.17 | 127.40 | 156.17 | 89.53 | 104.53 | 88.67 | 106.53 | 6.41 | 6.91 | 6.51 | 6.75 |
| BR99 72L COMPI | 147.08 | 163.92 | 149.18 | 161.00 | 91.1 | 106.10 | 96.33 | 108.1 | 6.28 | 6.54 | 6.3 | 5.75 |
| 72PB PROL $C_4$ | 170.38 | 179.86 | 164.38 | 175.82 | 94.92 | 109.92 | 91 | 111.92 | 6.55 | 6.95 | 6.50 | 7.24 |
| LA POSTA SEQUIA $C_6$ | 108.55 | 157.78 | 121.05 | 155.68 | 86.33 | 101.33 | 66.78 | 103.33 | 5.67 | 6.07 | 5.75 | 6.56 |
| 72L COMP $IC_6$ LNCI | 163.09 | 191.87 | 173.19 | 192.87 | 91 | 106.00 | 94.25 | 107 | 5.99 | 6.05 | 6.00 | 7.45 |
| DMR ESR W LN | 131.19 | 160.22 | 135.19 | 163.00 | 66.78 | 81.78 | 88.67 | 82.78 | 5.48 | 5.75 | 5.5 | 5.67 |
| 72PB PROL $C_3$ SYN | 135.81 | 198.47 | 170.81 | 200.40 | 114.25 | 129.25 | 76.33 | 130.25 | 6.2 | 7.24 | 6.5 | 6.91 |
| LN TP YC6 SYN | 165.34 | 170.67 | 164.04 | 172.5 | 88.67 | 103.67 | 91 | 104.67 | 5.76 | 6.56 | 5.96 | 6.54 |
| DMR ESR Y LN | 137.67 | 156.33 | 147.67 | 158.02 | 76.33 | 91.33 | 76.78 | 92.33 | 6.41 | 7.45 | 6.55 | 6.95 |
| Mean | 146.63 | 172.29 | 149.66 | 171.00 | 90.57 | 105.57 | 88.41 | 107.07 | 6.02 | 6.52 | 6.55 | 6.5 |
| LSD (0.05) | 23.5 | 18.7. | 22.4 | 24.34 | 21.39 | 21.32 | 18.74 | 22.5 | 1.05 | 1.35 | 0.30 | 0.5 |

**Table 6.** Mean of yield and related parameters of ten maize varieties under low and high nitrogen.

| Varieties | Grain yield (T/Ha) | | | | Days to 50% silking (days) | | | | Anthesis silking interval | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | | 2016 | | 2015 | | 2016 | | 2015 | | 2016 | |
| | low N | High N | Low N | High N | low N | High N | low N | High N | Low N | High N | Low N | High N |
| SINT MAR 20CA LARGA | 2.43 | 3.50 | 2.5 | 3.58 | 55 | 56 | 54 | 56 | 3 | 2 | 2 | 2 |
| LN TP YC7 | 2.07 | 3.00 | 1.94 | 3.65 | 56 | 55 | 55 | 55 | 3 | 2 | 3 | 2 |
| BR99 72L COMPI | 1.52 | 2.32 | 1.53 | 2.59 | 56 | 57 | 54 | 57 | 3 | 2 | 2 | 2 |
| 72PB PROL C4 | 2.04 | 2.63 | 1.97 | 3.10 | 55 | 56 | 56 | 58 | 3 | 2 | 2 | 3 |
| LA POSTA SEQUIA $C_6$ | 1.38 | 2.50 | 1.40 | 2.52 | 54 | 55 | 54 | 55 | 3 | 3 | 2 | 3 |
| 72L COMP IC6 LNCI | 1.84 | 2.90 | 1.55 | 3.00 | 56 | 58 | 55 | 58 | 3 | 2 | 3 | 3 |
| DMR ESR W LN | 1.93 | 2.81 | 2.00 | 2.78 | 54 | 55 | 55 | 55 | 3 | 3 | 2 | 2 |
| 72PB PROL $C_3$ SYN | 2.88 | 3.00 | 2.03 | 3.24 | 55 | 57 | 55 | 57 | 3 | 2 | 3 | 3 |
| LN TP YC6 SYN | 1.91 | 2.89 | 1.73 | 3.06 | 55 | 53 | 54 | 53 | 3 | 3 | 2 | 2 |
| DMR ESR Y LN | 1.25 | 2.30 | 1.50 | 2.65 | 54 | 55 | 55 | 55 | 3 | 2 | 3 | 3 |
| Mean | 1.725 | 2.885 | 1.715 | 3.167 | 55 | 55.7 | 54.7 | 55.9 | 3 | 2.7 | 2.4 | 2.7 |
| LSD (0.05) | 0.15 | 0.75 | 0.23 | 0.44 | 0.95 | 1.2 | 1.1 | 1.3 | 0.01 | 0.33 | 0.20 | 0,23 |

seasons in the performances in all the yield and yield parameters between the two growing seasons. This may be likely be due to the fact that the same quality of urea fertilizer as well as similar field experimental conditions was used in both season. Also there were also non -significant differences in the weather conditions during the 2015 1nd 2016 growing seasons. The observed significant performance in growth and yield parameters with the application of 90 $kgNha^{-1}$ could be attributed to the sufficient percentage of nitrogen contained in 90 $kgNha^{-1}$ that are associated with increase in photosynthetic efficiencies (Bello et al., 2003). The greater number of leaves, plant height, and leaf area occur at higher rates of nitrogen content. This findings corroborates with the report of Jin et al. (1993), Uddin et al. (2009) and Agbowuro and Salami (2015) who observed significant increase in yield and yield components with the increase in nitrogen fertilizer. Increase in nitrogen fertilizer has the ability to promote vigorous growth, improve meristematic and physiological activities in the plants thereby resulting in the synthesis of increased photo-assimilates that enhanced maize yielding ability. The result also shows that with additional nitrogen to maize, it will help in boosting its vegetative parts which was contrary to the report of Islam 2004.

## Conclusion

From the aforementioned results, it could be concluded that yield advantages were gained by cultivating maize with the use of nitrogen fertilizer, albeit at high application rates (90kgN/ha). With the present scarcity of maize, increase in maize production can be attained with the use of more nitrogen fertilizer. Therefore, the yield potential of low N maize can be successfully maximized by application of fertilizer.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Molecular profiling of growth hormone in the juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂)

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**Many factors contribute to the underdevelopment of oceanic aquaculture. The most significant factor of the development of hybrid grouper culture is the supply of satisfactory quantities of fast growing juveniles to stock grow-out systems at a minimized cost. In order to evaluate the growth hormone status of hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂), cDNA encoding growth hormone from the liver of juvenile fish was cloned and characterized. qPCR method was used to determine the expression of the growth hormone in the tissues. Relative expression of growth hormone in the brain was found to be highest (290.91 folds) and lowest in the stomach (1.35 folds). Bioinformatics tools were used to analyze the growth hormone proteins and prediction of its physicochemical properties, and amino acid compositions, as well as Gene Ontology, GO term predictions. The cloned hybrid grouper growth hormone gene sequence was found to be clustered monophyletically with solid bootstrap backing. It was found to be close to the *Cromileptes altivelis* growth hormone gene sequence and is positioned in the identical clade and consequential from the identical family. The expression pattern is comparable to that comprehended in other fishes and provides extra data for molecular biological studies on hybrid grouper fish.**

**Key words:** Growth hormone, bioinformatics analysis, tissue expression, gene ontology, hybrid grouper

## INTRODUCTION

Growth rate is a factor of commercial significance in the field of aquaculture for directly impacting the production, and hence income. A number of studies have reported on the significance of the growth hormone playing primary roles in reproduction, growth, immunity, cellular differentiation, and metabolism (in liver and brain majorly), by means of its receptors (Brooks and Waters, 2010; Mingarro et al., 2002). Growth hormone activities are "initiated by binding to a specific receptor (GHR), and assumed as localized on the cell membrane of target tissues, which induces a phosphorylation cascade for signalling and gene expression" (Herrington and Carter-

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Su, 2001). Growth hormone plays a number of roles in cellular immune system directly or with the help of the insulin growth factor-1 and work through mediators for its actions and the induction of insulin-like growth factor 1 (Brooks and Waters, 2010; Li et al., 1998; Brooks et al., 2014). Growth hormone receptor activation often refers to an outcome of the effects of growth hormone. How be it, the malfunctioning of its receptor is likely to give rise to several consequences like retarded growth and undue delay in puberty (Brooks and Waters, 2010). Growth hormone has been reported as having "an anabolic effect on bone, cartilage, and muscle tissue in the course of binding to the growth hormone receptors in skeletal muscle" (Brahm et al., 1997). Both "Insulin-like growth factor-I (IGF-I) and II (IGF-II) are released during binding to the receptors in liver as reported by some other studies" (McArdle et al., 2010), which lead to the stimulation for the increase in bone growth, cell development inside the muscle tissue and protein synthesis (Casanueva, 1992; Godfrey et al., 2003).

Cultured hybrid grouper owns quite a high market value in China. Nevertheless, one of the key issues reported as inhibiting its commercial bases is the more retarded growth in comparison with the other grouper species (Sekar et al., 2014; James et al., 1999; Kohno, 1997; Wang, 1997; Amenyogbe et al., 2019). Interestingly, hybrid grouper grows as a female from the juvenile stages, meanwhile changing into a male at adulthood. This incident is conflicting to other grouper species that are well-known that sexually, they mature from male to female and capable of altering sex at the sexual maturity aged between 3 and 5 years as discussed by Amenyogbe et al. (2019).

A single possible tool for the evaluation of a growth status in hybrid grouper involves accessing the expression of a controlling hormone in the hybrid grouper somatic growth. Currently, there is little or no substantial information available regarding the growth hormone cloning and qPCR expression profile of hybrid grouper; accordingly, the current research work aimed at cloning the full length of the juvenile hybrid grouper growth hormone, in addition to also studying its expression in various tissues and predict the possible roles played by the growth hormone in the development of the *Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂ fish since the major problem reported to be associated with the hybrid grouper is the retarded growth. We hope that the data in the current study provide the bases for any future revisions, which are targeted at evolving the fast growth and development of hybrid grouper.

## MATERIALS AND METHODS

### Experimental fish

An aggregate of 3 female hybrid juvenile groupers (3-4 months) with the average body weights of 82.3 ± 4.32 g and length of 13.73 ± 0.13 cm obtained from Guangdong Hengxing Group Co. LTD., Guangdong Province, China was put to use for the purpose of experiment. They were kept in 500 L plastic that contained 400 L of water with the constant aeration for approximately 2 weeks with 288 h of day light and 288 h of darkness until the start of the experiment. The use of the live fishes for the experiment adhered to the guidelines of Institutional Animal Care and Fisheries and Aquaculture College, Laboratory of fish breeding, Guangdong Ocean University, China.

### Ethics approval and consent to participate

This experiment was carried out in accordance with the rules and regulation of Guangdong Ocean University Animal Care and Use Committee (Guangdong Province, China).

The only anaesthetic approved by the College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, China for the general use for fishes is MS-222. The fish was placed in a solution of MS222 dissolved in water concentration amounting to 250 mg/L until the death is attained. Verification was performed through the observation of the absence of the opercular movement for a period of approximately 3 min in order to ensure that the fish was dead prior to decapitation. This was performed for the purpose of alleviating the suffering of the fish put to use for the study.

The tissue samples from "brain, heart, intestine, muscle, head kidney, liver, stomach, gill, and spleen were dissected with the use of sterilized instruments, followed by getting immediately frozen in liquid nitrogen, and storing at the temperature of -80°C until use" (Edens and Talamantes, 1998).

### RNA isolation

The RNA isolation was done following (Amenyogbe et al., 2019) method. The procedure was carried out in accordance with the instructions of the manufacturer. The examination of the superiority of total RNA" was carried out using 1% agarose gel electrophoresis and UV spectrophotometry (NanoDrop, Thermo Scientific, USA).

### Cloning of growth hormone (GH)

Aimed at cloning a partial cDNA fragment of GH, primers, as presented in Table 1, there were designed on the basis of the growth hormone (GH) sequences of *Epinephelus bruenus* (*GU138644.1*), *Epinephelus altivelis* (*EU003991.1*), *and Coho_salmon* (*M24768.1*) from NCBI. "First-strand cDNA was synthesized with the use of TRANSgen First-strand cDNA synthesize kit in accordance with the manufacturer's instructions. The partial cDNA fragment of growth hormone (GH) was amplified from the first-strand cDNA from the liver and brain tissues. We performed the PCR amplification in a volume of 50 µl that included forward and reverse primers (Table 1) 2.5 µl each, cDNA 2.5 µl, Premix Taq (Takara Taq Version 2.0 plus dye) 25 µl, in addition to double distilled water 17.5 µl. The amplification was carried out with the use of the following reaction conditions: 94°C for 5 min, followed by 35 cycles for 30 s at 94°C, for 30 s at 58°C, for 35 s at 72°C and 10 min at 72°C. We segregated the PCR products through the use of electrophoresis, and the DNA Bands were recycled and purified by making use of the SMART RACE cDNA purification Kit (Clontech, Palo Alto, CA). Subsequent to that, the purified DNA portion was subcloned into the pMD18-T vector (Takara, Japan), followed by transforming into the competent *Escherichia coli* DH5a cells. Five different individual positive clones were picked, followed by sending to Sangon Biological engineering (Guangzhou) LTD. for the purpose of sequencing. Cloning of 3′ and 5′ untranslated region

**Table 1.** Polymerase chain reaction (PCR) primers used in this study.

| Primers Sequence | |
|---|---|
| M13 : CGCCAGGGTTTTCCCAGTCACGAC<br>RV : GAGCGGATAACAATTTCACACAGGA | Vector (Pmd-18) |
| UPM-long: CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT<br>UPM-short: CTAATACGACTCACTATAGGGC | Universal race primers |
| MGH-F1-CCATCGCCGTCAGCAGAGTTCAA<br>MGH-R3-GCCTCAGGAGAGAGTCGACATTT | Partial |
| KG-GH-F1-CTGCGACGAACCTACGAACTGCTGG | 3UTR |
| KG-GH-F2-TACGAACTGCTGGCGTGTTTCAAGA | 3UTR |
| KG-GH-R2-ACTCCCAGGACTCCACCAGCCGATA | 5UTR |
| KG-GH-R4-ATGTTGAACTCTGCTGACGGCGATG | 5UTR |
| GP-βactin(F) –TACGAGCTGCCTGACGGACA<br>GP-βactin(R)- GGCTGTGATCTCCTTTTGCA<br>KG-GH-F1-CGACAAGCACGAGACGCAG<br>KG-GH-R1-AGTTCCCATAAGGAGCCAA | RT-qPCR |

M13, RV, UPM-Long and short are all universal primers, GP- βactin is Grouper βactin, MGH and KGH indicate growth hormone gene, and 3&5UTR stands for untranslated regions.

(UTR) end of growth hormone (GH), was done following (Amenyogbe et al., 2019) method.

We carried out 3′ RACE in both the first and second amplification. First PCR amplification was carried out with a 20 µl volume of the reaction mixture, including 1 µl of UPM long primer, in addition to sense primer 1 (Table 1), 1 µl cDNA, 10 ul of premix Taq (Takara Taq Version 2.0 plus dye) and sterile distilled water 7 µl. We carried out second PCR amplification with a 50 µl volume of the reaction mixture, consisting 2.5 µl UPM short primer, and sense primer 2 (Table 1), 2.5 µl of the first reaction as template, 25 µl of premix Taq (Takara Taq Version 2.0 plus dye) and sterile distilled water 17.5 µl. In addition, 5′ RACE was carried out using Amenyogbe et al. (2019) procedures and methodologies.

### Gene analysis

We assembled the sequences of partial, 3′ and 5′ UTR to form the full-length cDNA of the target gene by the use of DNAman8 software (*https://*dnaman.software*.informer.com*). The "Gene translation, predictions of the amino acid sequence, and location of domains were done using EXPASY (http://expasy.org/tools) and SMART (htt://smart.emblheidelberg.de) web tool. "Cysteines and tyrosines residues site were predicted by using the http://cic.scu.edu.cn/bioinformatics/Predict_Cys.zip web tool". Multiple sequence analysis of amino acids was performed using ClustalX2 software (Larkin et al., 2007) and (https://www.softpedia.com/get/Science-CAD/GeneDoc.shtml to identify similarities. In order to establish genetic relationships, phylogenetic analysis was carried out, and a consensus tree builds using (https://www.megasoftware.net/). The physical and chemical possessions of the protein were analyzed using the PROTEAN program (DNASTAR Inc.: Madison, WI, USA, 200) to see their impact of the growth. The Subcellular localization was performed using PSORT ((http://psort.hgc.jp/form2.html). SoftBerry Psite software(http://linux1.softberry.com/berry.phtml?topic=psite&group=programs&subgroup=proloc) was also used to predict the potential phosphorylation sites and glycosylation sites. Karplus and Schulz Flexibility method (Karplus and Schulz, 1985), Kolaskar and Tongaonkar Antigenicity method (Kolaskar and Tongaonkar, 1990) and Parker Hydrophobicity Prediction method (Parker et al., 1986) were used to predict the flexibility, antigenicity, and hydrophobicity of GH respectively. The COFACTOR sever (Zhang et al., 2017) method was used for Gene Ontology (GO) terms predictions.

### Tissue mRNA expression of GH by qRT-PCR

Total RNA from the brain, gill, liver, muscle, intestine, spleen, stomach, head kidney and heart were used as the template for the first strand cDNA synthesis using TRANSgen First-strand cDNA synthesizes kit. The mRNA levels of GH in tissues were determined by Real-time qPCR using a Roche Light Cycler®96 SW1.1 with a 10 µl reaction consisting, 5 µl Transtart Tip Green qPCR Supermix (TransGen Biotech, China), and 0.4 µl of each sense and antisense primer, 3.6 µl of $H_2O$ and 0.6 µl of cDNA. *β-actin* was used as an internal control to normalize gene expressions (Table 1). A melting curve was performed to detect the specificity. Amenyogbe et al. (2019) procedures were followed for the reaction conditions for the Real-time qPCR. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to calculate the results.

### Statistical analysis

The data in this study were articulated as means±SD. We presented Significant differences in the data using one-way ANOVA followed by Duncan's post-hoc test and a probability level less than 0.05 ($P < 0.05$) was used to indicate significance. We performed all statistics using SPSS 16.0 (SPSS, Chicago, IL, USA).

## RESULTS

## Characterization of growth hormone

Sequence analysis of the cloned growth hormone (GH)


**Figure 1.** GH amino acid sequence was "compared with other similar sequences from other species including human and mouse. Conserved cysteine residues are indicated by Isosceles triangle. Down arrows indicate conserved tyrosine residues. Where *E. fuscogus* represent: *Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂ GH; *C. altiveli* represent: *Cromileptes altivelis* GH; E. coioides represent: *Epinephelus coioides* GH*; L. japonicus* represent: *Lateolabrax japonicas,* GH; *S. aurata* represent: *Sparus aurata* GH; *L. cyanellus* represent: *Lepomis cyanellus* GH; *H. sapiens* represent: *Homo sapien* GH and *M. musculus* represent: *Mus musculus* GH.

revealed it contained 899 base pair with an open reading frame of 588 base pair, 5′utr of 146 base pair and 3′ utr of 166 base pair. The GenBank accession number MK82763 was given after the sequence was submitted to NCBI. The sequence of deduced amino acid of GH was established to comprise of 156 amino acid residues and a putative Pfam Growth Hormone Binding Protein domain. Growth hormone comprises conserved cysteine residues of four and seven conserved tyrosine residues (Figure 1). The sequence of amino acid identities of hybrid grouper growth hormone (hgGH), results from the phylogenetic analysis consistent with growth hormone from other vertebrates (Table 2).

**Multiple sequence alignment**

The alignment of growth hormone (GH) amino acid sequence of hybrid grouper and similar sequences from other species was carried out using ClustalW online tool (Figure 1). The alignment results showed that the cysteines of growth hormone were conserved in all fish species which could be responsible for the stability of growth hormone (Figure 1). Growth hormone of hybrid grouper was found mainly to be single chain proteins. "A phylogenetic tree was built using the neighbor-joining method based on the deduced amino acid sequences of growth hormone (GH) protein sequence of the hybrid

**Table 2**. Percentage identity of the amino acid sequences.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | Species |
|---|---|---|---|---|---|---|---|---|---|
| | 86.0 | 86.0 | 85.4 | 84.5 | 84.3 | 34.1 | 6.7 | 1 | *E.fuscoguttatus*× *E.polyphekadion* |
| | | 99.4 | 98.5 | 97.7 | 97.1 | 36.7 | 7.9 | 2 | *C. altivelis* |
| | | | 98.8 | 98.3 | 97.7 | 36.7 | 7.9 | 3 | *E. coioides* |
| | | | | 97.7 | 97.4 | 37.0 | 8.2 | 4 | *L. japonicus* |
| | | | | | 96.8 | 36.2 | 8.2 | 5 | *L. cyanellus* |
| | | | | | | 36.2 | 8.2 | 6 | *S. aurata* |
| | | | | | | | 5.2 | 7 | *M. musculus* |

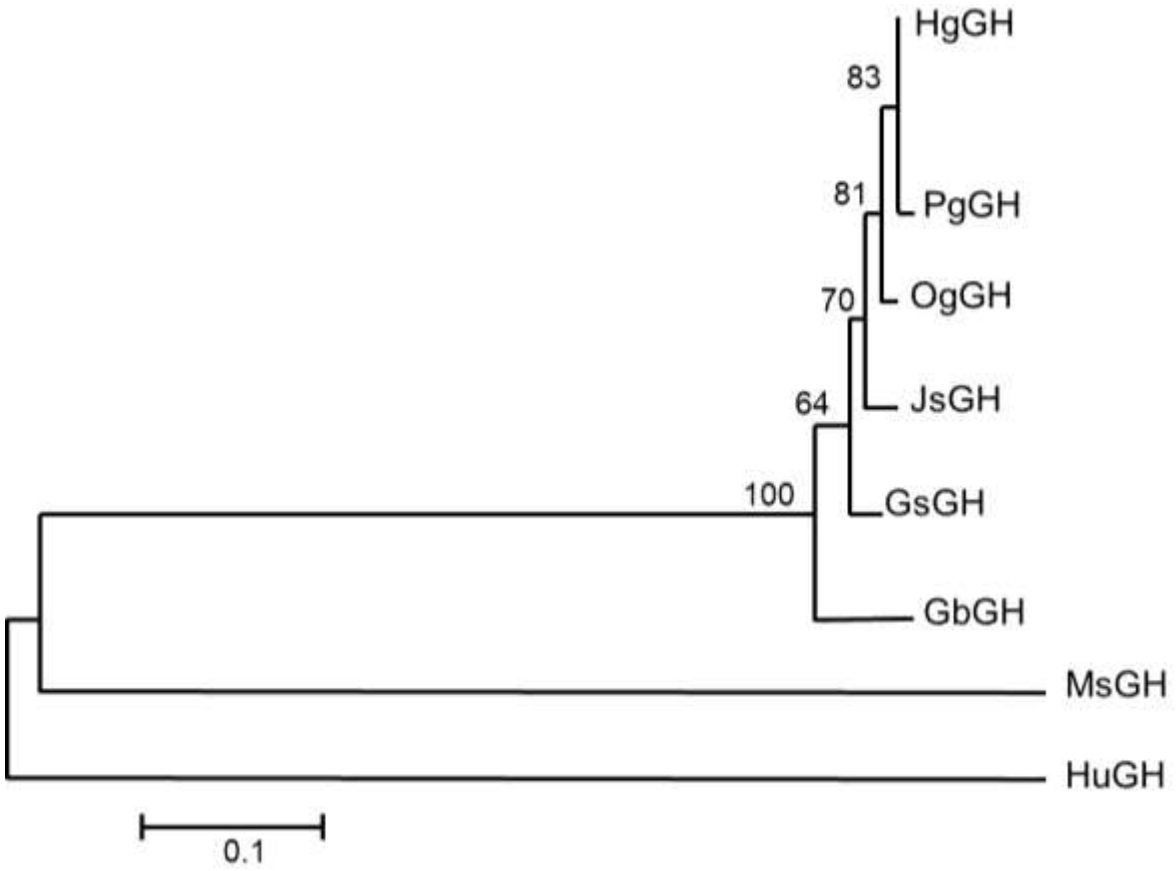



**Figure 2.** The neighbor-joining phylogenetic tree was built based on the deduced amino acid with growth hormone protein sequences of similar protein sequences of other species. The numbers at each branch designated the proportion of bootstrap values on 1000 duplicates. The phylogenetic space is 0.1 as untaken in the *scale bar.* The following are the species names and GenBank accession numbers; hybrid grouper (HgGH), *Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂ GH (*MK282763*); Panther grouper (PgGH), *Cromileptes altivelis* GH, (ABS19662); Orange-spotted grouper (OgGH), *Epinephelus coioides* GH (AAK57697)*;* Japanese seabass (JsGH), *Lateolabrax japonicas,* GH (AGD80842); Gilthead seabream (GbGH), *Sparus aurata* GH (AAA03329); Green Sunfish (GsGH), *Lepomis cyanellus* GH (AAS20461); Human (HuGH), *Homo sapien* GH (NM_014394); and Mouse (MsGH), *Mus musculus* GH (NM_028263).

grouper and other species, including human and mouse and the results showed that hybrid grouper" and *Cromileptes altivelis were* clustered together (Figure 2).

**Gene expression**

Even though at a different level, hybrid grouper growth

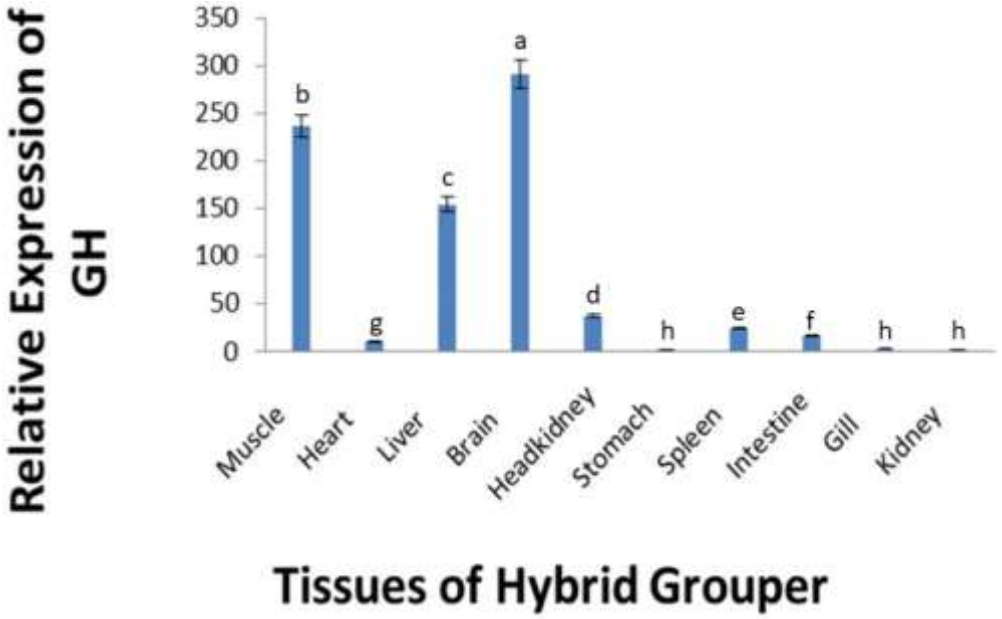



**Figure 3.** Expressions of hybrid grouper growth hormone were "detected by Real-time PCR from different tissues using β-actin as an internal control to normalize gene expressions. Relative expression of growth hormone in the brain was found to be highest, followed by the muscle, liver, head kidney, spleen, intestine, heart, gill, kidney and stomach respectively. Note： Statistics presented as the mean ± SD of triplicate experiments. Alphabets a, b,c,d,e,f,g,and h indicate statistical differences at $P < 0.05$.

hormone (HgGH) expressed in all the tissues that were examined, and relative expression of GH in the brain was found to be highest, followed by the muscle, liver, head kidney, spleen, intestine, heart, gill, kidney and stomach respectively as shown in Figure 3.

## Sequence analysis and physiochemical properties

Scrutiny of the physical and chemical possessions of the hybrid grouper growth hormone sequence revealed the molecular structural formula of hybrid grouper growth hormone to be $C_{782}H_{1246}N_{212}O_{244}S_5$ and an aggregate atom numeral of 2489. Growth hormone has "a theoretical predicted ion isoelectric value of 6.38 and instability index of 65.43 classifying it as an unstable protein with the molecular weight of 17.68 kDa. The amino acid sequence of GH potentially contains one casein kinase II phosphorylation sites, four protein kinase C phosphorylation sites, one N-myristoylation site, and two microbodies C-terminal targeting signal".

## Protein secondary structure and amino acid composition

The sequence examination of the amino acid of hybrid grouper growth hormone showed that "the protein contained 50 hydrophobic residues (30.91%), 19 acidic residues (13.32%), 18 basic residues (14.47%) and 53 polar amino acids (33.12%). Aliphatic catalogue and grand average of hydropath city (GRAVY) of growth were 90.71 and −0.4, respectively. The total quantity of negatively charged residues (Asp and Glu) was 19, and the total number of positively".

## Antigenic, hydrophobic structure and flexibility regions of hybrid grouper GH

The antigenicity, hydrophobic regions and flexibility regions of hybrid grouper growth hormone (hgGH) were predicted using Kolaskar and Tongaonkar, Parker Hydrophilicity and Karplus and Sechulz Flexibility methods respectively (Figure 4A, B, and C). The property of being able to promote a specific immune response was predicted (Table 3), and the antigenic sites are pronounced as surface domains evolved from side chains of amino acid which might be detached in sequence however close in space.

"Hydrophobic residues forming the binding interface were directed under the threshold value and represented by the residues" D24, I26, V40, E52, W54, F56, P57, S58, R59, P73, S76, E94, L95, D98, S99, L102, Q103, S113, T123, Y124, F130, T139, L141, T142, A144, K145 and C146. The transmembrane residues were directed

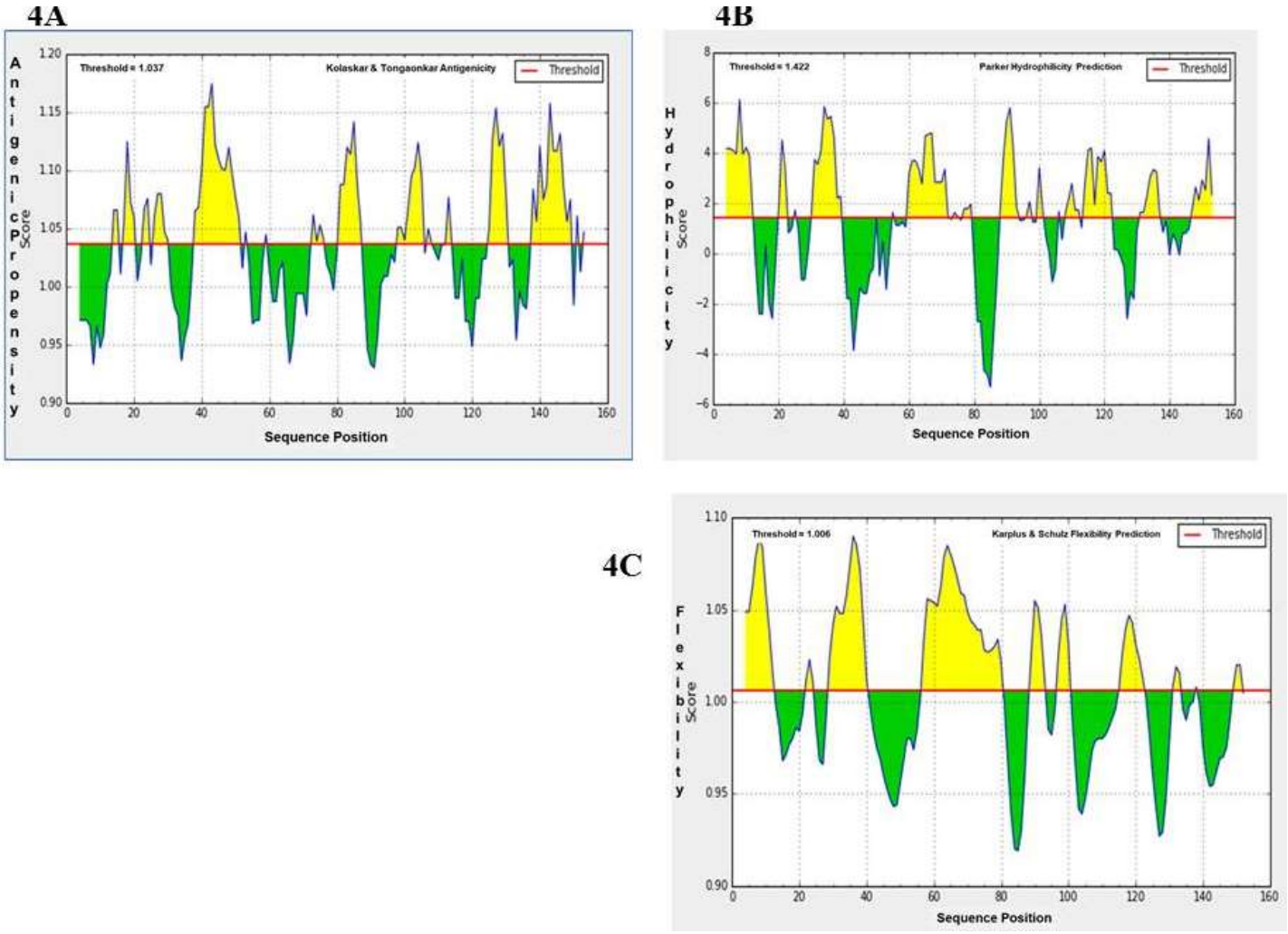



**Figure 4.** A) an antigenic property of GH was predicted using the Kolaskar and Tongaonkar method. Five "potential antigenic peptides have more than 1.0 antigenic propensity, and six or more amino acids in length were predicted". B) the hydrophobic regions of hybrid grouper GH and C) The flexibility regions of hybrid grouper GH.

**Table 3.** The antigenic amino acid sequence and its position.

| No. | Start Position | End Position | Peptide | Length |
|---|---|---|---|---|
| 1 | 38 | 51 | SSVLKLLSISYRLV | 14 |
| 2 | 81 | 87 | GILLLIR | 7 |
| 3 | 98 | 105 | DSSALQLA | 8 |
| 4 | 125 | 130 | ELLACF | 6 |
| 5 | 138 | 149 | ETYLTVAKCRLS | 12 |

below the threshold line and are represented as L104, A105, E125-C129, Y140 and V143 whilst Flexibility residues forming a flexibility loop were above the threshold value and are represented by the residues LQTEEQRQL (4-12), PIDKHETQRSSV (29-40), PSRSLSGGSAPRNQISPKLSEKT (57-80), NQDGS (97-100), PDSS (115-123), FKKD (130-133) E138 and SPEA (149-152).

**Predicted Gene Ontology (GO) TERM analysis of hybrid grouper growth hormone using COFACTOR software**

Two main functions were identified, namely biological process and molecular function with growth hormone mRNAs regulating relationships in the development of hybrid grouper by the GO using COFACTOR software. It

has been found that the growth hormone regulated by hybrid grouper is involved in many biological processes, including “Single-organism process, metabolic process, glucose metabolic process, biological regulation, regulation of biological process, regulation of cellular process, positive regulation of biological process, positive regulation of metabolic process, response to stimulus, regulation of biological quality, positive regulation of cellular process, regulation of cellular metabolic process, positive regulation of cellular metabolic process, regulation of transport, positive regulation of transport, response to abiotic stimulus, response to stress”, etc (Figure 5A). In addition, it also has molecular functions, including “signaling transducer activity, protein binding, signaling receptor activity, receptor binding, cytokine receptor binding, molecular transducer activity, receptor activity, transmembrane receptor activity, transmembrane signaling receptor activity and cytokine receptor activity” (Figure 5B). Also, it has cellular component, including extracellular region, extracellular space, membrane part, integral component of membrane (Figure 5C). Additionally, it also has predicted binding sites such as R10, L17, P22, P29, S26, Q36, T36, T139, T142, N22, N153, K132, K136, V136, V143, E135, E138, to nucleotides and are believed to be participating in transcriptional regulation (Figure 5D). Also present is the Predicted Enzyme Commission of growth hormone (Figure 5E).

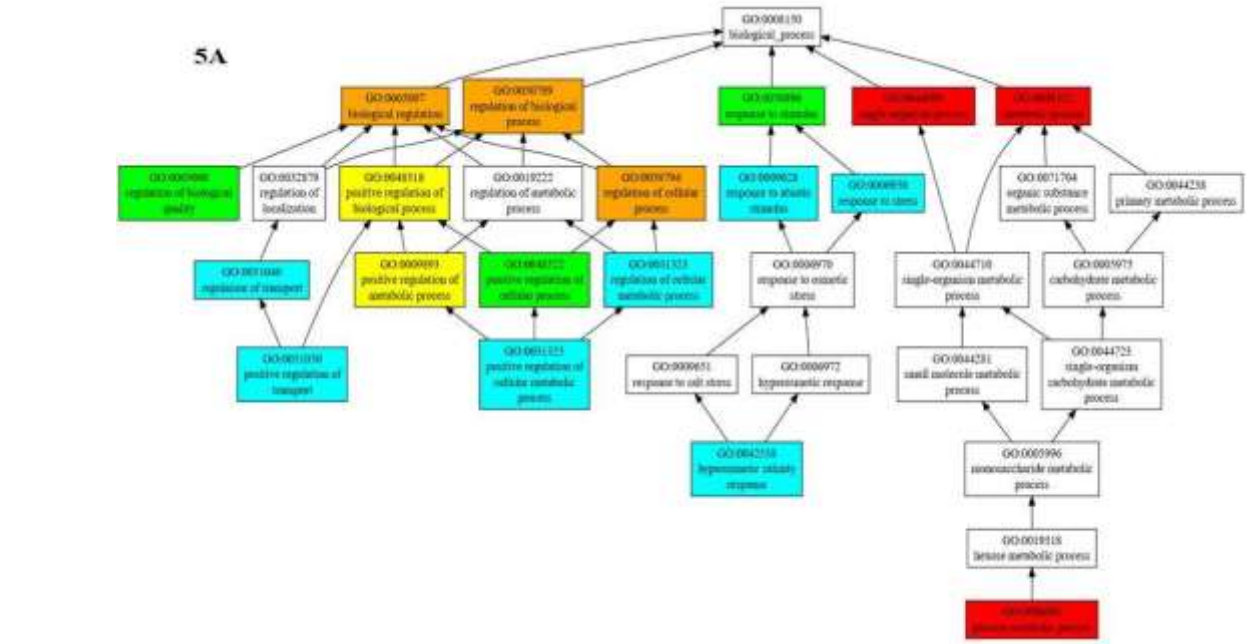
## DISCUSSION

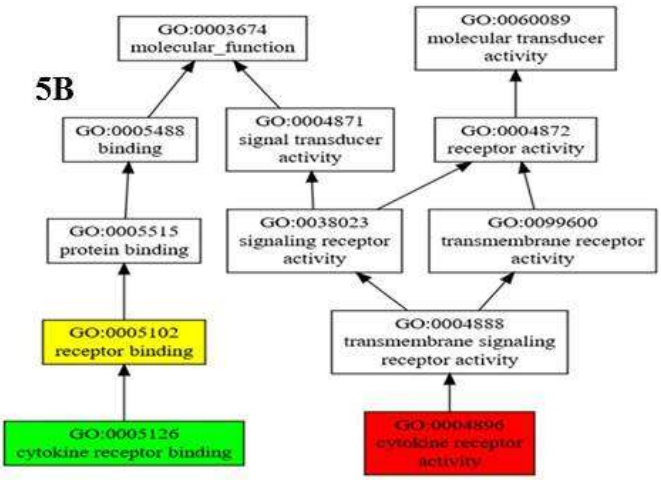
The development rate is of pivotal commercial significance in the farming of food faunas owing to the fact that the fast growth characteristically is associated with the fast-paced turnover of production. One of the potential tools for the assessment of nutritional status and growth in juvenile grouper deals with measuring the expression of a regulatory hormone, suggesting the somatic growth of the fish. The significance of growth hormone as a development-enhancing instrument has extensively acknowledged, as its possible implementation in aquaculture business (Li et al., 2005). In addition, the development of hybrid grouper fish by means of crossbreeding constitutes a practical means of yielding the grouper fish on a large scale. Nevertheless, the production of the hybrid grouper fish does not constitute a convenient strategy. The key challenge reported is slow growth. Moreover, the hybrid grouper has been effectively developed by means of the cultivation and genetic selection of hybrids of (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂). It is one of a number of groupers cultivated in China. It not only has a good taste but also huge size, making it an economically important marine water species. Growth hormone is primarily a single chain polypeptide that has two intramolecular disulphide bonds (Deng et al., 2014), known for its multifunction, for instance, regulations of several phases of growth, behaviour, immune function, metabolism, reproduction, and osmoregulation (Forsyth and Wallis, 2002; Björnsson et al., 2004; Norrelund, 2005; Norbeck et al., 2007; Møller and Jørgensen, 2009; Pérez-Sanchez, 2000; Very et al., 2005), which act through its receptor (Herrington and Carter-Su, 2001). The crucial role played by growth hormone in the promotion of growth has been reported in several vertebrates, which include fish as well (Ben-Atia et al., 1999; Acosta et al., 2008; Edens and Talamantes, 1998).

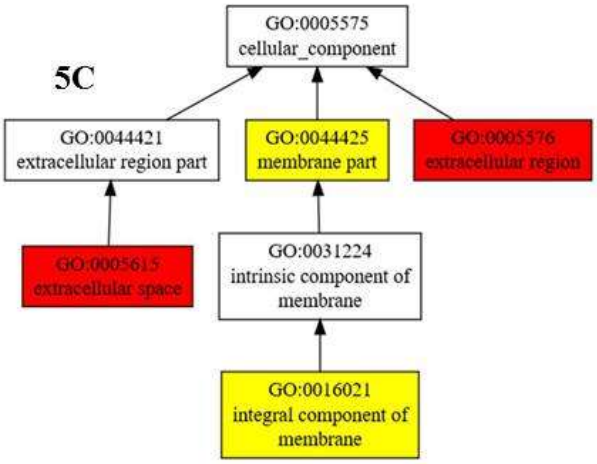
In the current research work, the juvenile grouper growth hormone was cloned for the first time, and its expression in diverse tissues was determined as well. In this research work, the sequence of deduced amino acid of growth hormone was detected as containing 156 amino acid residues as well as a putative Pfam Growth Hormone Binding Protein domain. It was discovered that the hybrid grouper growth hormone (HgGH) contained GHBP, besides being believed as prolonging the half-life of growth hormone among other functions (Chang et al., 1992). Nonetheless, it is deemed as quite essential to investigate further for the purpose of elucidating its specific role in the development of hybrid grouper.

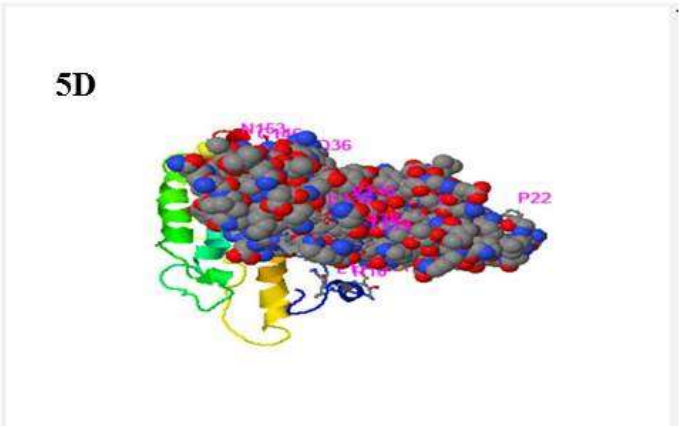
The hybrid grouper growth hormone contained the conserved cysteine residues of four and seven conserved tyrosine residues of which the four conserved cysteine residues formed two disulphide bonds. The findings of the present research work are in agreement with the findings in majority teleost growth hormones, except in goldfish and other cyprinids in which five cysteine residues were discovered (Law et al., 1996; Degani et al., 2006; Sciara et al., 2006), and might be playing a crucial role in the purpose of the hybrid grouper growth hormone (HgGH) biological activities and its stability as suggested by Deng et al. (2014) and Law et al. (1996). The four cysteine residues in hybrid grouper are found in the same locations as in virtually all of the growth hormone polypeptides, which consist of all of the studied fishes. The cysteine residues, which have the capability of establishing two disulphide bonds were anticipated to add to the tertiary structure of the hormone molecule. Owing to the fact that these residues are positioned at approximately undistinguishable positions in all of the growth hormones, “it can be predicted that these residues play an indispensable part in not only structural reliability but also sustaining the biologically energetic form of growth hormone.

Besides that, there is also a presence of double Asn-X-Thr/Ser motifs in the hybrid grouper growth hormone aa sequence and are believed to be the possible positions for N-linked glycosylation. This is in agreement with similar observation of the two comparable sequences in the tilapia, giant catfish, salmon and carp, while only one was observed in those of chicken, yellowtail, tuna, flounder, eel and sole growth hormone sequences (Pendon et al., 1994). Together with that, one tryptophan



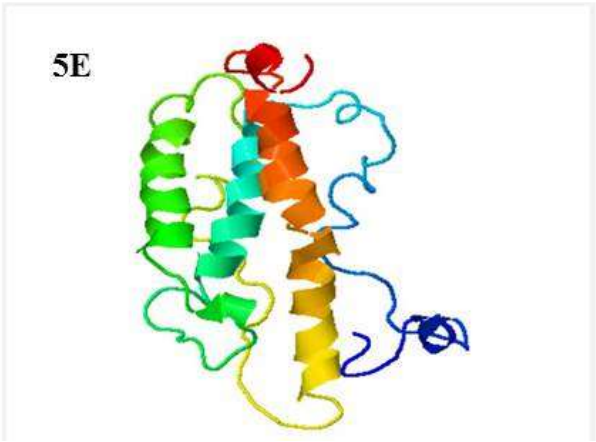




**Figure 5A, B, C, D and E.** Biological processes, molecular functions, cellular component, predicted ligand binding sites and predicted enzyme commission of growth hormone respectively.

| | |
|---|---|
| (a) | "Cscore$^{GO}$ is the confidence score of predicted GO terms. "Cscore$^{GO}$ values range in between [0-1]; where a higher value indicates a better confidence in predicting the function using the template". |
| (b) | The graph shows the predicted terms within the Gene Ontology hierachy for Molecular Function. Confidently predicted terms are color coded by CscoreGO: |
| | [0.4,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0] |
| (c) | The graph shows the predicted terms within the Gene Ontology hierachy for Biological Process. Confidently predicted terms are color coded by Cscore$^{GO}$: |
| | [0.4,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0] |
| (d) | The graph shows the predicted terms within the Gene Ontology hierachy for Cellular Component. Confidently predicted terms are color coded by Cscore$^{GO}$: |
| | [0.4,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0] |
| (a) | "Cscore$^{EC}$ is the confidence score for the Enzyme Commission (EC) number prediction. Cscore$^{EC}$ values range in between [0-1]; where a higher score indicates a more reliable EC number prediction". |
| (b) | "TM-score is a measure of global structural similarity between query and template protein". |
| (c) | "RMSD$^{a}$ is the RMSD between residues that are structurally aligned by TM-align". |
| (d) | "IDEN$^{a}$ is the percentage sequence identity in the structurally aligned region". |
| (e) | "Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein". |
| (a) | "CscoreLB is the confidence score of predicted binding site. Cscore LB values range in between [0-1]; where a higher score indicates a more reliable ligand-binding site prediction". |
| (b) | "BS-score is a measure of local similarity (sequence & structure) between template binding site and predicted binding site in the query structure". "Based on large scale benchmarking analysis, we have observed that a BS-score >1 reflects a significant local match between the predicted and template binding site". |
| (c) | "TM-score is a measure of global structural similarity between query and template protein". |
| (d) | "RMSDa the RMSD between residues that are structurally aligned by TM-align". |
| (e) | "IDENa is the percentage sequence identity in the structurally aligned region". |
| (f) | "Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein". |

residue (Trp) was observed in hybrid grouper as was figured out in the *Hemiramphus brasiliensis*, mullet, halfbeak, marine silverside fish, *Mugil platanus* and *Odenthesthes argentinensis*, to be one of the physiognomies of growth hormone (Marins et al., 2003; Meire et al., 2006).

The growth hormone protein sequences are termed as reflecting the molecular phylogeny of bony fishes (Bernardi et al., 1993; Schneider et al., 1992). The phylogenetic association of the designated fish species was assessed to bear in mind the growth hormone cDNA homology with the use of the neighbour-joining methodology. This methodology of examination proliferate the statistical significance of the data as well as similarity of the species. Figure 3 provides the perfect compassionate interpretation of the morphology grounded conventional taxonomy of fishes. The assemblages of fishes are linked, together with emanating beneath the same division, Teleostei. The freshly cloned hybrid grouper growth hormone gene sequence is observed as "clustered monophyletically with the solid bootstrap backing". In accordance with the expectation, it was close to the *C. altivelis* growth hormone gene sequence, besides being positioned in the identical clade and consequential from the identical family. That is why this examination illustrates the fact that the sequence cloned was the growth hormone gene with high resemblance with other teleost growth hormone genes in relations to both the structure and association.

Growth hormone is primarily produced in pituitary gland (Sciara et al., 2006); however, it is fully established that some of the other tissues express the growth hormone gene as well (Sciara et al., 2006; Yang et al., 1999). The expression of growth hormone (GH) mRNA was dominantly detected or highly expressed in the pituitary (Li et al., 2005; Sciara et al., 2006). In the present research exertion, the hybrid grouper growth hormone (HgGH) was observed as expressed in all of the tissue samples examined despite being at varying levels as well as the highest in the brain (Figure 3), which could be owing to the role it might be playing in the central nervous system. The distribution of tissue in this study is in line with the role of growth hormone in the key physiological mechanisms, for instance, metabolism and somatic growth (Reinecke et al., 2005). The expression of the hormone in various tissues proved its presence however, it is well known therefore that the malfunctioning of the growth hormone or its deficiency can results in retarded growth, therefore there is a need for further studies to elucidate the growth hormone's binding abilities to its receptors.

The flexibility residues, sequences that formed "the flexible loops" were observed as conserved in the fish species but not in other mammalians. These sites were

believed as being where the active sites are situated. The results shed light on the fact the “hydrophobic residues forming the binding interface were directed under the threshold value and represented by the residues” D24, I26, V40, E52, W54, F56, P57, S58, R59, P73, S76, E94, L95, D98, S99, L102, Q103, S113, T123, Y124, F130, T139, L141, T142, A144, K145 and C146. The transmembrane residues were directed under the threshold line, which are represented as L104, A105, E125-C129, Y140, and V143.

The predicted gene ontology analysis verifies the fact that proteins act together with other molecules for the execution of their biological functions in majority cellular mechanisms. These exchanges comprise the ligand bindings in “receptor sites, the antibodies binding to antigens, protein-DNA interactions of protein-DNA, and protein-protein interactions” (Katchalski-Katzir et al., 1992; Jones and Thornton, 1996; Berchmanski et al., 2002; Halperin et al., 2002).

## Conclusion

In the present research exertion, the hybrid grouper growth hormone (GH) has been effectively sequestered and cloned. The aa sequence, which is expected from the cDNA, conveys fresh statistics regarding the growth hormone structure of a teleost, a hybrid species. The isolation of growth hormone genes is still of significance; in addition, a number of investigations merely place emphasis on the other fish species but there are just a few research works addressing the hybrid fish species. To the best of our understanding, this constituted the first to report on the isolation of the growth hormone from the hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂), termed as a discovery believed as helping comprehend the molecular physiognomies of the hybrid grouper growth hormone gene, besides being likely to help construct a recombinant growth hormone protein, in addition to molecular exploration, aimed at enhancing the understanding of growth enhancement by means of the growth hormone regulation. This research work provides an understanding of molecular attributes of the development concept of a species acknowledged as having a retarded growth rate. We have high hopes that these findings are going to help researchers advance the growth enactment of the cultured hybrid grouper in future.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Screening of chickpea genotypes for resistance to Fusarium wilt (*Fusarium oxysporum* f.sp. *ciceris*) under field conditions in Ethiopia

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**Fusarium wilt is one of the economical important vascular root diseases affecting chickpea. A total of 427 chickpea germplasms were grouped into two types, desi type (385) and kabuli. 42 genotypes were evaluated to identify fusarium wilt resistant sources in Debre Zeit sick plot under natural infected field. The genotypes were grown in 2017/18 main cropping season and augmented design was used without replications; highly susceptible differential checks (JG-62) was replicated as indicator for disease appearance. The disease incidences were assessed three times at different growth stage and genotypes were graded as per ICRISAT rating scale. The fusarium wilt incidences revealed that five lines were resistant and ten had moderately resistant reaction in desi; five were resistant and 14 genotypes were moderately resistant in kabuli type of chickpea respectively. This implies that source of variability in desi type chickpea has low resistance to wilt/root rot and other major chickpea diseases. Most accession lines are early wilting type, which makes it difficult to identify slow wilting type of lines in chickpea. Thus, the promising genotypes indicate that it is most suitable for exploitation in breeding and its directly used in severely wilt affected areas as well as transfer of their gene to a commercial cultivar on the basis of resistance type.**

**Key word:** Chickpea, Fusarium wilt, incidence, inheritance, slow wilting.

## INTRODUCTION

Fusarium wilt (*Fusarium oxysporum* f. sp *ciceris*) is one of the most important root diseases that affects chickpea and is wide spread in chickpea growing areas such as Asia, Africa and Southern Europe where the chickpea-growing season is dry and warm (Asrat and Tolesa, 2018); inflicting accountable quantitative as well as qualitative losses (Thaware et al., 2016; Khilare et al., 2009). Attacks from Fusarium wilt pathogen can destroy a crop completely (Shivalingappa et al., 2018) or cause significant annual yield losses. The average yield reduction of chickpea due to Fusarium wilt globally varies from 10 to 15% and under severe conditions, the wilt infection can damage the crop completely and cause 100% yield loss in some countries (Navas-Cortés et al., 2000; Sharma et al., 2005). Early wilting is reported to cause 77-94% yield loss (Haware and Nene, 1980).

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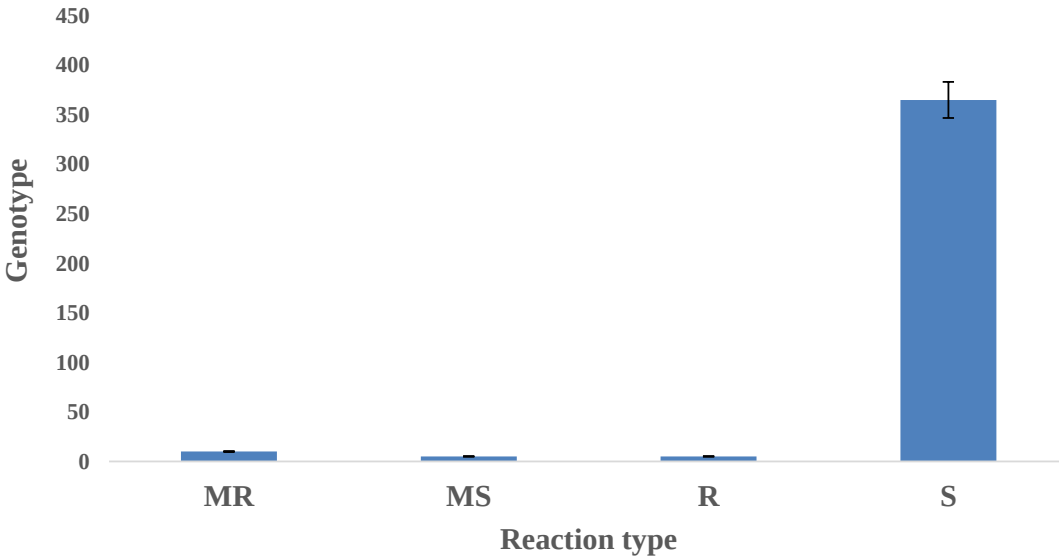


**Figure 1.** Reaction level of desi type of chickpea genotypes to fusarium wilt incidence. R= Resistant; MR= Moderately resistant; MS= Moderately susceptible; S= susceptible; HS= Highly susceptible.

In Ethiopia, about 30% yield loss of chickpea due to chickpea wilt has been reported (Meki et al., 2008). This pathogen can cause infection at all stages of plant growth with more incidences in flowering and podding stage (Maitlo et al., 2014). It is one of the major soil and seed borne disease of chickpea worldwide (Jalali and Chand, 1992). The most efficient method for the management of disease is using resistant cultivars (Karimi et al., 2012). To control these diseases, host plant resistance mechanism should be exploited and the sources of resistance in existing chickpea germplasm identified (Bakhsh et al., 2007; Duzdemir et al., 2014; Tariq et al., 2015). However, the problem is that the resistance mechanism is not stable, due to the introduction of new pathotypes/isolates. Considering the nature of damage and survival ability of the pathogen, use of resistant varieties is only economical and practical solution. Most of the resistant varieties have been found to be susceptible after some years, because of breakdown of their resistance due to evolution of variability in the pathogen (Arunodhayam et al., 2014). However, evolution of new races poses a serious threat to deployment of wilt resistance in chickpea. Wilt/root rot is more severe on sandy soil and less severe on clay loam soil. Therefore, there is continuous need to screen new source of germplasm and find further durable resistance source and slow wilting genotypes. The present study identifies the chickpea genetic source of resistance to fusarium wilt.

## MATERIALS AND METHODS

The experiment was conducted in Debre Zeit sick plot which is artificially infested field in 2017/2018 main cropping season. A wilt sick plot was prepared with a mixture of isolates representing different chickpea growing areas. A total of 427 chickpea germplasms were grouped in two chickpea type which is desi type (385) received from Ethiopian Bio-diversity Institute (EBI) and kabuli (42) genotypes introduced from International Center for Agricultural Research in the Dry Areas (ICARDA) for their reaction to Fusarium wilt disease. The design was an augmented design without replication. Each genotype was planted in a 2 m plot. Row to row and plant to plant distances were maintained at 30 cm and 10 cm, respectively. A highly wilt susceptible genotype, JG-62, was repeatedly planted after every two test entries. The disease incidences were assessed at different growth stage three times and the genotypes were graded as per ICRISAT rating scale that is Resistant (R) = 0-10% mortality; moderately resistant (MR) = 10.1-20% mortality; moderately susceptible (MS) = 20.1-30% mortality; Susceptible (S) = 30.1-50% mortality; and highly susceptible (HS) above 50% mortality.

## RESULTS AND DISCUSSION

In the present screening test, 385 lines were executed; five were resistant, ten were moderately resistant, five were moderately susceptible and three hundred and sixty five were susceptible to fusarium wilt. The resistant accessions were lines 41016, 41276, 41177, 41046 and 41227 (Figure 1). Govil and Rana (1984) evaluated 239 cultivars representing a range of variability among Indian and Iranian germplasm in wilt sick plot for years, which is consistent with the findings on desi type of chickpea accessions. None was found to be immune, but maximum resistance was shown by Indian cultivars such as P-597, P-621, P-3649, P4128 and P-4245. The resistance source of Fusarium wilt in chickpea germplasm is not uncommon and a number of other workers have also reported the occurrence against high

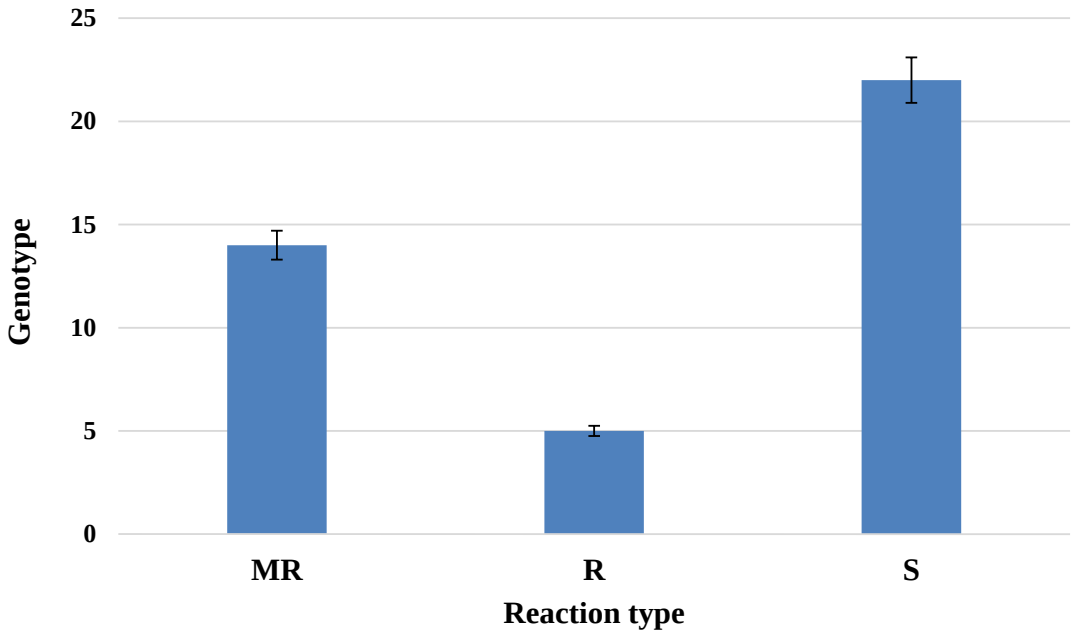


**Figure 2.** Reaction level of kabuli type of chickpea genotypes to fusarium wilt incidence. R= Resistant; MR= Moderately resistant; MS= Moderately susceptible; S= susceptible; HS= Highly susceptible.

level of resistance of Fusarium wilt (Iqbal et al., 1993; Iftikhar et al., 1997; Chaudhry et al., 2006, 2007). Iqbal et al. (2005) also report the sources of resistance against wilt/root rot in chickpea germplasm originating from national and international research institutes. The results are also consistent with those reported by Nazir et al. (2012) who screened 178 chickpea lines against fusarium wilt and observed that none of the test lines is immune.

The resistances were related to plant age and growth stage which is a vital source of parental materials to identify slow wilting type. Development of disease is slow in resistant lines and fast in susceptible lines. As the resistant lines at reproductive stage also became susceptible, field screening at reproductive stage seems to be more reliable (Muhammad et al., 2010).

Zote et al. (1986) reported that only five chickpea lines out of 15 tested for three successive years showed less than 10% wilt incidence. Similar earlier findings have also been reported by several workers from India (Nene et al., 1981; Haware et al., 1992; Reddy et al., 1991). Most of lines experienced early wilting than late wilting type.

Tullu (1996) reported variation in chickpea for wilting time. He also reported a genotype that was consistently and uniformly resistant, the use of these resistance genotypes as donors for disease resistance in breeding program and further study on their mode of inheritance.

However, in kabuli type of chickpea, it was observed that 5 genotypes were resistant, 14 genotypes were moderately resistant, while 22 were susceptible to the wilt disease (Figure 2). Among these resistant sources are FLIP-10-106C, FLIP-10-63C, FLIP-10-253C, FLIP-10-107C and FLIP-10-136C. According to Korde (2011), Mandhare et al. (2011) and Kumar et al. (2012) have screened a number of chickpea genotypes and identified promising genotypes, which are in line with these findings on chickpea kabuli types. Similarly, Iqbal et al. (2005) screened 145 chickpea genotypes against *F. oxysporum* f.sp *ciceris* and found that no one was resistant at reproductive stage, but 14 were resistant at seedling stage. Sarwar et al. (2012) evaluated 41 chickpea cultivars and observed that only 2 were highly resistant and 8 were resistant. The resistance source of Fusarium wilt in chickpea germplasm is not uncommon and a number of other workers have also reported the occurrence against high level of resistance of Fusarium wilt (Zote et al. 1983; Iqbal et al., 1993; Iftikhar et al., 1997; Chaudhry et al., 2006; Chaudhry et al., 2007). The findings of the research are in accordance with Bajwa et al. (2000) found that out of 32 genotypes, only one line was resistant, 4 lines were moderately resistant, and 27 were susceptible. Iqbal et al. (2005) also report the sources of resistance against wilt/root rot in chickpea germplasm originating from national and international research institutes. On the other hand, the genotypes that showed resistance are most suitable for exploitation in breeding programs or for direct sowing in wilt prone areas. Prior to such transfer of their resistance to a commercial cultivar, the genetic basis of resistance (vertical or horizontal) must be determined against the virulences of *F. oxysporum* f. sp. *ciceris*. The disease-free, resistant lines and moderately resistant can be utilized in resistant breeding programme towards

incorporations of resistant genes in releasing cultivars or hybrids.

## CONCLUSION AND RECOMMENDATION

Fusarium wilt is one the most destructive vascular disease of chickpea. In the present study, desi type of chickpea genotypes has low source of variability among different collections of land races and most lines experience early wilting type, which makes it difficult to identify slow wilting type of lines. The kabuli type of chickpea is more resistant to fusarium wilt and consistence in their reaction response. Results from the present study reveals that considerable variations were resistant in both desi and kabulit type of chickpea against fusarium wilt diseases. Kabuli germplasm proved to be a better source of resistance compared to the desi material. Besides cultivar variability, there is pathogen evolution that resulted in race variability in fusarium wilt and the resistant line will be evaluated further for their yield potential. On the other hand, the genotypes that showed resistance are most suitable for exploitation in breeding programs or for direct sowing in wilt prone areas. Thus, that consistently resistant line will be used as donors of disease resistance source in breeding programs. Resistance genotypes are used as donors of disease resistance in breeding program and further study on their mode of inheritance. Continuous mass screening genotypes under field and pot will be suggested as a result of break source resistance and phenotyping of major races in major chickpea growing regions. Although little information on the mechanism of resistance is available, a detailed research based on this material is needed to throw light on it.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

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*Full Length Research Paper*

# Productivity impact of growth enhancement support scheme on maize farm households in Kano State, Nigeria

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**In Nigeria, farmers depend on government support for farm inputs in form of subsidies in order to improve their livelihoods. In this article, the productivity impact of the Growth Enhancement Support Scheme (GESS) input subsidy support program implemented was examined in 2011. The study employs a two-stage probability design to collect household survey data from 390 households in Kano State. As an analytical approach, the study employed a propensity score matching and a Two-Stage Least Square (2SLS) regression estimator that corrects for selectivity and endogeneity problems respectively while Hedges "g" was used to estimate the effect size of GESS .Maize yield and total factor productivity index were used as indicators to estimate the productivity impact of GESS program. The result from two-stage least square estimator showed that GESS subsidy increased the yield of participants by 32.3% and the difference was statistically significant (P<0.05) while the result of total factor productivity index, showed that the participants were more productive) and had an average of 14.1% net gain from the cost incurred in production in the 2016 farming season. The size of the estimated treatment effect suggests an improvement in the productivity outcomes of participants. The study found that the results of the study are consistent with similar findings and therefore validate the hypothesis that the GESS subsidy programme improved the productivity of beneficiary households. The scheme obviously has enormous potentials and is also very promising for agricultural input procurement and distribution to resource-poor households in Nigeria. In addition, there is a need for capacity building of the farmers by local extension agents in the form of integrated crop management practices in order to sustain productivity gains. This study concludes that input use alone is not enough to increase maize production, improvement in input use efficiency through integrated crop management practices are also needed.**

**Key words:** Agricultural input subsidy, mobile phone, productivity outcomes, farming households.

## INTRODUCTION

In the first decade of the twenty-first century, a number of governments sponsored programs and schemes have been introduced in order to meet the socio-economic development objectives of inclusive growth in SSA. The

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most prominent among these programmes was the agricultural input subsidy support programmes. In the 1970s and 1980s, farm subsidies were the major drivers of agricultural development and growth. They were implemented as large scale subsidies spanning over a period of 5 to 10 years in Zambia, Malawi, Tanzania, Kenya, Ghana, Zimbabwe, Mali, Nigeria. However, there was a growing concern that the cost of subsidy program generally overweighs their benefits leading to welfare loss (Jayne and Rashid, 2013) and was also characterized by rent-seeking and diversion of the subsidized inputs to unintended beneficiaries. The subsidy programmes were later phased out during the liberalization programs of World Bank and IMF in the 90s on the premise that the private sector can provide farm input more efficiently through market-driven mechanisms (Ricker-Gilbert, 2014) and Tesfamicheal et al., 2017). This move led to a drastic fall in agricultural productivity. Ammani et al. (2010) observed that the liberalization period led to a decrease in maize yield and other cereals. Hassan et al. (2014) used time-series data between 1971 - 2010 to examine total factor productivity of maize production under various subsidy programs in Nigeria, with results revealing that the 40 years of subsidy programs produced a mean total factor productivity of 1.004, implying that a total factor productivity growth of 0.4% as well as a total factor productivity index of less than 1 shows that farmers are unproductive while fertilizer use stagnated at about 8 kg/ha compared to Sub-Saharan Africa average of 21 kg/ha.

Regional initiatives such as Maputo Declaration 2003 and the First African Fertilizer Summit 2006 led to the re-emergence of large-scale input subsidy programs across the continent (Jayne and Rashid, 2013; Jayne et al., 2013). These subsidies correspond to what is understood as a new model of pro-poor, targeted, and market-friendly smart subsidies (Chirwa and Dorward, 2013). They were said to overcome the challenges of past subsidy programmes by depending on institutions and innovative mechanisms to effectively target resource-poor households with farm inputs (Chirwa and Dorward, 2013). In 2011, the Federal Government of Nigeria launched the called “Growth Enhancement Support Scheme (GESS), designed and implemented with the broad objective of promoting agricultural productivity and food security and poverty reduction through increased use of fertilizer from the current 13 to 50 kg/ha (Adesina, 2012). Under the programme, farmers received messages via their mobile phone which entitled them to buy fertilizer and improved seed from accredited agro-dealers at a subsidized price. The e-voucher further specifies the total quantity of fertilizer and improved seed allocated to the farmer as well as the designated redemption center for collection. A registered farmer is entitled to 2 bags of 50 kg fertilizer and a 25 or 50 kg bag of improved maize seeds. A major policy stance underpinning the implementation of the scheme was the withdrawal of the federal government from the procurement and distribution of fertilizers, improved seeds and involvement of private agro-dealers in the procurement and distribution of subsidized fertilizer and improved seeds.

Impact evaluations are an important tool for the analysis of public policies and interventions and are increasingly being used by policymakers and practitioners for decision-making. Their main objective is to estimate the overall causal effect of an intervention or program, that is, identify whether there is a cause-and-effect relationship between the implementation of policy and the outcome(s) of interest by estimating the change that can be directly attributable to the intervention.

Empirical studies on the impact of targeted input subsidies suggested that subsidies increase production and productivity of beneficiary households.for example Malawian farm subsidy programme achieved the objective of increasing production and productivity (Dorward, et al., 2008). According to Ricker-Gilbert et al. (2011) also found that fertilizer use can be further increased productivity if rural poor are well targeted to receive fertilizer subsidy.

Understanding the impact of the GESS farm subsidy programme remains controversial because most of the studies did not apply rigorous impact evaluation methodology. For instance, farm households who participated in GESS subsidy programmes optimize the use of fertilizer and improved maize seeds and significantly improved their productivity (Liverpool-Tasie, 2013; Oguniyi and Kehinde, 2015; Kemisola et al., 2018; Ibrahim et al., 2018; Nwalieji et al., 2015). Most of these relied on single econometric models and did not properly control for potential differences between participants and non-participants, did not apply the widely acceptable impact assessment methodologies and are therefore subject to serious problems arising from selection bias and endogeneity. Further, the studies relied on propensity score matching (PSM) approach which only works if the difference between the two groups can be captured by using only observable variables. If there are unobservable characteristics, which can influence participation decisions and the outcome variable, the result from the PSM is likely to be biased (Ma and Abdulai, 2016).

Tesfamicheal et al. (2017) examined the productivity impact of GESS subsidy programme using a nationally representative household survey data, as well as statistically and econometrics approaches to control selectivity and endogeneity problems thereby establishing a clear causality between GESS subsidy programme and productivity. Under different circumstances, the current study validated the hypothesis and tested the consistency and generalization of findings by Tesfamicheal et al. (2017). When treatment effect is consistent from one study to the other, common effects can be identified and if there is variation, the reasons for such variation can be identified because decisions about the utility of an

intervention cannot be based on a single study (Obayelu, 2016). The study provided useful information that would guide policymakers and development makers to understand if the same subsidy gain can be delivered at the lowest cost. The information generated on the total factor productivity of the households is important for policymakers to know if efficiency should be addressed through research and development or improving the size of the subsidy.

Productivity is essentially focused on because agricultural productivity is a measure of the performance of the agricultural sector and thus provides a guide to the efficiency of the sector (Aloyce et al., 2014; Awotide et al, 2013; Lameck, 2016).

Kano State is the largest livelihood zone in Northern Nigeria which also represents a more densely populated area in Northwest Nigeria and one of the first states to join the scheme in Nigeria with over two million beneficiaries (Adesina, 2012). An evaluation of the scheme at the state level would provide the government with information relevant for identifying context-specific issues (gender roles and relations, climate variability and constraints) that are relevant to improving the effectiveness of the scheme at the state level.

In order to achieve the objectives of this study we intend to provide answers to these pertinent questions: does those access to subsidized inputs lead to higher productivity? How productive are the farmers in the use of these inputs? Meanwhile, from a policy perspective, we noted that answers to these questions are very important in addressing the dwindling agricultural productivity and attaining the objectives of poverty reduction and welfare improvement in Nigeria, particularly among the rural farming households.

## MATERIALS AND METHODS

### Study area

The study was conducted in Kano State, Northwest, Nigeria in 2017. Kano State is also located in the North-west geopolitical Zone of Nigeria between latitudes 130° N and 110° S and longitudes 80° N and 100° E with a landmass of 20,760 km$^2$. It is the largest state in Nigeria with 44 local governments. The state has a projected population of 11,206,688 million in 2012 based on NPC (2006). The average annual rainfall is 700 mm with 350 and 190°C as mean daily maximum and minimum temperature respectively. Major crops cultivated by farmers in the State include rice, maize, millet, cowpea, groundnut, and vegetables.

### Sampling procedure

The study employed a two-stage stratified probability sampling design to collect data from 390 farming households in Kano State between July and November 2017. In the first stage, 30 farming villages were selected from 44 local government areas based on probability proportional to size, whereas in the second stage, 400 respondents were randomly selected from a list of maize farmers association in the State. Data from 390 respondents was stratified into 170 respondents and 220 non-respondents. The survey questionnaire was designed to capture detailed information on socio-economic characteristics of the households, input use, allocation, crop output for maize and other notable cereals and participation in GESS. In addition, village-level data was collected on average district prices of key inputs and farm inputs among others. In terms of participation, relevant data was collected on the level of awareness about the Growth Enhancement Support Scheme (GESS) as well as other decisions to register for the GESS program. The same survey instrument was used to collect data from the same villages to avoid biases.

### Sample size determination

Arkin and Coulton (1963) was used to determine the population sample size of the study. It is given by:

$$n=\frac{NZ^2\ P(1-P)}{Nd^2+Z^2\ P(1-P)} \quad (8)$$

Where n = Sample size N = Total number of Households (3850) Z = Confidence level (at 95% level Z = 1.96) P = Estimated population proportion (0.5), this maximizes the sample size) d= error limit of 5% (0.05). Application of the above sample formula with values specified.The estimated population proportion of 50% is the power level that maximizes or increases the statistical power of the sample size , yielded a sample size of 333. Including a reserve of 20%, took the total sample size to 400.

### Methods of analysis

The data for this study were analyzed using descriptive statistics, propensity score matching, and instrumental variable approach to analyzed the impact of GESS subsidy program on productivity outcomes. The study adopted maize yield per hectare and total factor productivity index to examine the productivity impact of the subsidy program. The crops were aggregated into maize equivalent.

### Effect of GESS on maize productivity

#### *Propensity score matching (PSM)*

Household's decision to participate in the GESS subsidy scheme was based on each household's self-selection (non-randomized), hence GESS participants may be systematically different from non-participants. Propensity score matching adjusts for initial differences between the two groups by matching each participant to a non-participant based on similar observable characteristics (Rosenbaum and Rubin, 1983) before determining treatment effect. The first step in PSM is to predict the propensity scores for each observation using a logit model using characteristics that are not affected by the treatment variable. In order to get the most preferred propensity score equation, different model specifications were employed. The variables were selected based on economic theory and previous economic theory. The predicted propensity score indicated the probability of receiving treatment. After predicting the scores, imposing the common support region is the next step in the PSM framework. The common support region is the area within the minimum and maximum propensity scores of treated (participants) and comparison groups (non-participants). This is followed by the identification of an appropriate matching estimator. Dehejia and Wahba, 2002; Caliendo and Kopeinig (2008) and wooddrige, 2010 listed a number of matching estimators including the Nearest

Neighbor (an individual from a comparison group is chosen as a matching partner for a treated individual that is closest in terms of propensity score), Caliper (where an individual from the comparison group is chosen as a matching partner for a treated individual that lies within a given caliper) and Kernel (a non-parametric matching estimator uses weighted averages of all individuals in the control group to construct the counterfactual outcome). The final step was checking for matching quality whether the matching procedure has balanced the distribution of different variables or not. If the matching quality is satisfied, ATT was specified as the mean difference of maize yield of the participants matched with non-participants who are balanced on the propensity scores and fall within the region of common support (Mendola, 2007).

The PSM, which is the probability of assignment to treatment conditional on pre-treatment variables is given by:

$$P(X) = P[Z = 1/X] = [Z/X; F(h/X_i)] \quad (1)$$

F (.) is a logistic cumulative distribution and X is a vector of conditioning variables. Rosenbaum and Rubin (1983) defined the average treatment effect ($Z_i$) in a counterfactual framework as

$$ATE = Y_{1i} - Y_{0i} \quad (2)$$

where $Y_{1i}$and $Y_{0i}$ denote productivity outcomes of household i that participated in GESS and the household that did not participate in the program, respectively but either $Y_{1i}$or $Y_{0i}$ is normally observed, but not both of them for each household.

The average treatment effect on the treated (ATT), that is, the conditional mean effect is only defined within the region of common support. It is estimated as follows.

$$ATT = E[E\{Y_{1i} - Y_{0i}/Z_i = 1, P(X)\}] \quad (3)$$

$$ATT = E[E\{Y_{1i}/Z_i = 1, P(X)\} - \{Y_{0i}/Z_i = 0, P(X)/Z_i = 1\}] \quad (4)$$

PSM only adjust for selection bias that may come from observable factors. However, casual identification requires controlling for both observable and unobservable factors that influence participation in the GESS and productivity outcomes. Hence, the estimates of Equation 5 may give biased estimates of yield due to possible correlation arising from unobservable factors. An appropriate estimation approach was therefore necessary to minimize the bias in the error term, as well as to produce a consistent estimate of the impact of GESS subsidy program on the productivity outcomes of maize-producing households.

To deal with the non-random endogenous error term in Equation 5, the instrumental variable (IV) regression procedure which uses a two-stage least square estimator was used to estimate the impact of the GESS subsidy program on maize productivity. The procedure assumes the existence of a variable, $Z_i$, an instrument, that predicts participation in the program, but does not predict productivity (Cameron and Trivedi, 2005). Similar techniques have been used by (Nino-Zarazua, 2007; Ricker-Gilbert et al*.,* 2013; Ricker-Gilbert et al*.,* 2011; Tesfamicheal et al., 2017).

The study identified two instruments which correlate with farmer's decision to participate in GESS and had no direct effect on productivity except through its effect on farmers' decisions to participate in the GESS. The variables are the number of years that the household head has lived in the village and membership of the ruling party as instruments for GESS participation. Years of residence in the village is a measure of household political power that could influence GESS participation (Ricker-Gilbert et al*.,* 2011). Membership of ruling political measures a farmers' participation in social network and could also influence GESS participation. Jansen-Hargan test of over-identification was performed to verify the hypothesis that the instruments are exogenous and valid. The Wu-Hausman – F-test and Durbin-wu-Hausman –chi-square test was performed to test the hypothesis that GESS participation is exogenous. The study found that instruments were valid and GESS participation was endogenous hence instrumental variable estimator was found to be a consistent estimate of treatment effect.

In the first stage, GESS participation is treated as endogenous and is regressed on the identified instruments (years of residence in the village and membership of ruling party) other controls that can affect GESS participation were included; meanwhile, in the second stage the yield equation was specified in a log-linear functional form and predicted values of the residual were included along with other controls in the outcome equations. Given the IV model in a linear functional form as:

$$Y_{1ij} = \beta_0 + \beta_1 y_2 + \beta_2 z_1 + \beta_3 z_2 + \beta_i X_{ij} + \mu_i \quad (5)$$

Where $y_2$ represents the endogenous variable (GESS participation), $Y_1$ is the outcome (maize yield), Zs are the instrumental variables (years of residence in the village and membership of ruling party), $X_{ij}$ variables that influence both treatment and outcome, $\mu_i$ is the error that is assumed to correlate with $\mu_i$ and $X_i$. Assuming that the equation is over-identified with two instruments, the reduced form equation in the first stage would be

$$y_{2ij} = \pi_0 + \pi_1 z_1 + \pi_2 z_2 + \beta_i X_{ij} + v_i \quad (6)$$

In the second stage

$$Y_{1ij} = \pi_0 + \pi_1 y_2 + \pi_2 z_1 + \pi_2 z_2 + \beta_i X_{ij} + \gamma_i s_{ij} + \gamma_i C_{ij} + \delta_i w_{ij} + \vartheta \hat{v} \quad (7)$$

Where $Y_{ij}$ rrepresents the outcome (maize yield in kg/ha), $y_1$ is the endogenous variable (GESS participation), $X_i$ household demographic characteristics such as age, years of education, farming experience, gender, household size) which influence GESS participation, $s_i$ represents membership of commodity and cooperative association, $w_i$ represents household landholding and number of working population, $C_i$ represents community characteristic such as distance to redemption center, farm and access to credit while z represents the instruments (number of years household have lived in the village and, membership of ruling party). $\gamma_1$, $\pi_1$ and $\delta_i$ are population parameters, $\beta_i$ are a vector of the $X_i$ variables. $\vartheta$ is tested if its significantly different from Zero if Cov ($\mu_i$, $v_i$) =0.

**Estimation of total factor productivity of the households**

The total factor productivity analysis was used to estimate the total productivity of inputs used in maize productivity.

$$TFP = \frac{Y}{TVC} \quad (8)$$

Where TFP is the total factor productivity, Y, the yield of maize realized in Kg/ha (maize equivalent ) and TVC is the total variable cost in Naira. Equation 2 can further be stated as

$$TFP = \frac{Y}{\sum_{i=1}^{n} P_{X_i} X_I} \quad (9)$$

Where Xs are the inputs is the $P_{X_i}$ is the price of the ith input.

We also calculated the Hedges *g* (sample size correction) standardized mean difference. For studies using parallel-group or matching strategies, *g* and its standard error (Borenstein et al*.,* 2009) are computed as:

$$g=\frac{\bar{y}_{t-}\bar{y}_{c}}{sp} * [1-\frac{3}{4*(nt+nc-2)-1}] \quad (10)$$

$$SEg=\sqrt{[\frac{nt+nc}{nc+nt}+\frac{g^2}{2*(nc+nt)}]} \quad (11)$$

Where $\bar{y}_t$ is the mean outcome in the treatment group, $\bar{y}_c$ the mean outcome in the comparison group, nt and nc are the sample sizes of the treatment and comparison groups respectively, Sp is the pooled standard deviation and Sc and St are the standard deviations in treatment and comparison groups.

## RESULTS AND DISCUSSION

The results in Table 1 have shown the differences between participants and non-participants and have centered on mean differences in the outcome variable and farm and farmer characteristics. The results concerning the outcome variables suggest that GESS participation may have a role in improving farm productivity, but because participation is endogenous, a simple comparison of the productivity indicators of participants and non-participants has no causal interpretation. The above difference may not be the result of GESS participation but instead may be due to other factors, such as differences in household characteristics and farm characteristics as mentioned above so the outcome effect on individuals who participated in GESS might have been achieved even without participation, that is, the counterfactual effect. There is, therefore the need to further investigate these outcome effects by applying other rigorous analysis to test the impact of GESS participation on farmers' productivity. In consequence, we apply propensity score matching methods that control these observable characteristics to isolate the intrinsic impact of GESS and also an instrumental variable approach to a correct possible correlation between participation and unobservable characteristics.

### Descriptive statistics of the respondents

A chi-square test of independence on some categorical variables of interest between participants and non-participants is reported in Table 2. The data showed that GESS participant owns more land by inheritance, have more access to off-farm income, belong to ruling political party, own more phones, had more registered members, more risk advertised, was more informed of GESS and also use more fertilizer in the last farming season than non-participants. These are the variables that affect the household's decision to participate in the GESS program. The variable risk-aversion is measured by farmer's willingness to try new agricultural practices such as improved seed. We consider farmers as risk-averse if they are unwilling to ever try new improved varieties. The two groups did not differ in participation in terms of gender, access to credit and membership of commodity associations (Table 2). The difference in GESS participation between men and women reflects the fact that men in the area are more informed about agricultural technology than women. Doss (2001) also finds that male-headed farmers adopt new agricultural technologies faster than women farmers due to access to complementary inputs such as access to credit and access to extension services. Women often lack capacity (mobility and funds), education, self-confidence, and more limited opportunities to join in groups and organizations due to cultural and ethnoreligious differences, which often serve as platforms and avenues for consultations and information-sharing with other actors including policymakers, researchers, and technical experts. According to Aboh et al. (2006), access to and use of fertilizer tend to reflect a gender dimension reflecting the element of traditional roles in agriculture. While women constitute 60% of agricultural producers in Nigeria, they have less than commensurate access productive resources and inputs including fertilizers. Gender roles and power relations,therefore, have a critical influence on fertilizer access and use, just like fertilizer subsidy tend to impact differently.

With regards to productive variables, the result in Table 3 showed that the sampled average landholding is 3.5 ha, but landholding among participants was 3.6 ha and is not statistically different from non-participants. According to international standard judgment on farm sizes, farm size of fewer than 10 ha is considered as a small scale (Ozowa, 2005), which implies that most of the participants under the program were smallholders suggesting that access to farmland was not a constraint to maize production in the study area. Data also showed that GESS participants cultivated more plots for maize production during the farming season under consideration but there was no difference in the number of seeds used. The difference in yield was found to be 1660.92 kg/ha and statistically significant. Results in Table 3 also indicated a significant amount of heterogeneity in demand in the subsidy program seems to be associated with fertilizer used, with participants using an average of 197.53 kg/ha against 173 kg/ha for non-participant and the difference is statistically significant. Abdoulaye (2016) also found a statistically significant difference in fertilizer used among subsidy beneficiaries in Senegal, Liverpool-Tasie et al. (2017) found that maize farmers in the main cereal producing areas of Nigeria used about 211 kg/ha . It is likely that a reduction in the price of inputs as a result of subsidy would have encouraged farmers to purchase more fertilizer. However, this is subject to verification. From Table 3, data also reveals that both groups do not differ in total labour used and the total number of persons in the working population. The mean total factor productivity was found to be 0.87, meaning that if any of the sampled farmers are picked at random with equal probability, the expected TFP will be a factor of 0.87 meaning with about 13% increase in input the farmers would attend the production frontier.

**Table 1.** Socio-economic characteristics of respondents by participation status.

| Characteristics | Participants | | Non–participants | | Mean cliff | t- values | p- value | Total sample (N= 390) | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | | | | Mean | Standard deviation |
| Age of household head | 45.764 | 9.445 | 45.156 | 9.7024 | 0.3853 | 0.3934 | 0.6942 | 45.982 | 9.581 |
| Household years of education | 13.635 | 3.4533 | 12.895 | 3.6879 | 0.7398 | 2.6195 | 0.0441** | 13.2179 | 3.60179 |
| Distance to nearest redemption centre | 2.5647 | 1.5020 | 3.4522 | 0.9234 | 0.8875 | 7.1834 | 0.000*** | 3.66566 | 1.2863 |
| Household size | 13.3411 | 0.2814 | 17.03118 | 6.075 | 3.6906 | 0.5931 | 0.5535 | 15.42308 | 6.8893 |
| Extension visits per month | 2.21 | 1.146 | 12.58 | 11.520 | 0.371 | 2.656 | 0.008** | 1.8128 | 1.4045 |
| Number of off–farm livelihood activities | 1.6 | 0.6996 | 1.53 | 0.4986 | 0.0500 | 0.8233 | 0.4109 | 1.5718 | 0.5945 |
| Number of year of residence | 40 | 11.6051 | 37.350 | 9.9198 | 2.9323 | 2.6871 | 0.0075*** | 38.6282 | 1.0972 |
| Years of farming experience | 26.541 | 8.8646 | 27.000 | 9.8602 | 0.4588 | 0.4760 | 0.6343 | 26.8 | 9.4300 |
| Number of years in commodity association | 10.711 | 6.112 | 9.899 | 5.127 | 0.961 | 0.3661 | 0.562 | 11.120 | 6.900 |

The T-test was used to test for difference in socio-economic demographic characteristics between participants and non–participants; *, **, *** : Significant at 10, 5 and 1%, respectively.

**Table 2.** Mean difference in categorical variables between GESS participants variable and non – participants.

| Variable | Participant | Non-Participant | Mean difference | Chi-square |
|---|---|---|---|---|
| Gender (male=1) | 0.800 | 0.850 | 0.050 | 1.300 |
| Own phone (yes=1) | 0.847 | 0641 | 0.206 | 4.55*** |
| Membership of community association (yes=1) | 0.541 | 0.586 | 0.0452 | 0.890 |
| Access to credit (yes=1) | 0.600 | 0.641 | 0.4091 | 0.830 |
| Land ownership (yes=1) | 0.9471 | 0.759 | 0.1879 | 5.034*** |
| Access to Off-Farm Income (yes=1) | 0.971 | 0.759 | 0.1879 | 5.03*** |
| Risk Aversion (yes=1) | 0.7176 | 0.6591 | 0.0585 | 1.33 |
| Use of fertilizer in last farming season (yes=1) | 0.859 | 0.555 | 0.304 | 6.43*** |
| Register for GESS (yes=1) | 0.918 | 0.523 | 0.3949 | 8.40*** |
| Member of ruling party (yes=1) | 0.659 | 0.464 | 0.1952 | 3.84*** |
| Keep livestock (yes=1) | 0.6627 | 0.596 | 0.0672 | 1.36 |
| N= | 170 | | 220 | |

The T-test was used to test for difference in socio-economic demographic characteristics between participants and non-participants; *, **, *** : Significant at 10, 5 and 1%, respectively.

Results of the distribution of the propensity scores showed that propensity scores of participants range from 0.04 to 0.9 while among non-participants, the propensity scores range from 0.04 to 0.81. The probability of all households participating in GESS was 0.43 which means that the probability that households selected at random will participate in the scheme with respect to propensity scores is 43.5% (Table 5). The

**Table 3.** Mean difference in productivity variables by participation status.

| Productivity variable | Participants | | Non-participants | | Mean cliff | t-value | p-value | Whole sample | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | | | | Mean | Standard deviation |
| Total landholding (ha) | 4.60 | 1.26 | 3.52 | 1.12 | 0.14558 | 1.20 | 0.2292 | 3.59 | 1.18 |
| Area cultivated to maize | 4.32 | 1.64 | 3.12 | 1.25 | 1.2113 | 7.77 | 0.000*** | 3.64 | 1.7 |
| Quantity of fertilizer used (kg/ha) | 56.3 | 6.63 | 40.51 | 4.58 | 15.861 | 5.56 | 0.000*** | 48.60 | 4.02 |
| Total factor productivity index | 0.14 | 0.13 | 0.62 | 0.46 | 0.8558 | 8.34 | 0.000*** | 0.87 | 0.72 |
| Maize yield (kg/ha) | 860.90 | 103 | 643.30 | 130 | 217.6. | 6.97 | 0.000*** | 890.11 | 112.90 |
| N | | 170 | | 220 | | | | | |

The T-test was used to test for difference in productivity outcomes between participants and non-participants; *, **, *** : Significant at 10, 5 and 1%, respectively.

common support region lies between 0.04 and 0.81. In other words, households whose estimated propensity scores are less than 0.04 and larger than 0.81 are not considered for the matching exercise. As a result of this restriction, 36 participants were discarded from the analysis, (Dehejia and Wahba, 2002) noted that when the proportion of lost individuals is small, this poses a few problems. However, if the number is too large, there may be concerns about whether the estimated effect on the remaining individuals can be viewed as representative. Accordingly, the proportion of individuals lost in this case is very small and therefore there is no violation of the assumption of common support.

The common support condition was imposed and the balancing property was satisfied in the estimated regression model. The distribution of the propensity scores and the region of common support before and after matching are shown in Figure 2. The density distribution of the propensity scores shows a good overlap between GESS participants and non-participants (Figure 2).

The selection of matching techniques is based on three independent criteria; standardize mean biased a t-test (Rosenbaum and Rubin, 1985) and joint significance of covariates and pseudo $R^2$ (Sianesi, 2004). Our estimation results suggest that all the matching methods produce similar results but kernel matching was the best algorithm. Kernel matching estimator with a bandwidth of 0.01 satisfied the selection criterion and so was used to estimate average treatment effect (ATE), average treatment effect on the treated (ATET) and average treatment effect on the untreated (ATU).

The reliability of PSM results depends on the quality of matching. This can be seen from the overall covariate balanced and common support as presented in Figure 1 and Table 7 respectively. Table 7 shows the overall covariates' balanced test before and after matching. The result reveals that the standardized mean difference for all covariates used in the PSM is reduced from 28.9% before matching to 6.1% after matching. This result shows that matching reduced bias by 78.8%; in addition, the chi-square test of the joint significance of variables after matching (P-value=0.784) while the chi-square test for the joint significance of covariates was not rejected before matching (P-value=0.000). Moreover, the pseudo-$R^2$ declined from 20.3 to 2.3% after matching. As indicated in Table 5, the mean bias in the covariates X after matching lies below the 30% level of bias reduction suggested by Rosenbaum and Rubin. Therefore, the high total reduction, the insignificant p-value of the likelihood ratio test low pseudo-$R^2$ and significant reduction in mean standardized bias after matching are indicative of successful balancing of the distribution of covariates between participants and non-participants of GESS, hence we fail to reject the hypothesis that both groups have the same distribution in covariates after matching. The visual inspections of the distribution of the estimates of propensity scores reveal a substantial and sufficient overlap in density distribution of the estimated propensity scores between participants and non-participants suggesting that the common support condition was satisfied. Selection bias in GESS participation due to observed covariates have been eliminated. We can now attribute any change in maize yield and total factor productivity to GESS participation.

## Estimating treatment effect of GESS on productivity outcomes

The results of the treatment effects (ATE, ATT, and ATU) is presented in Table 9. The average

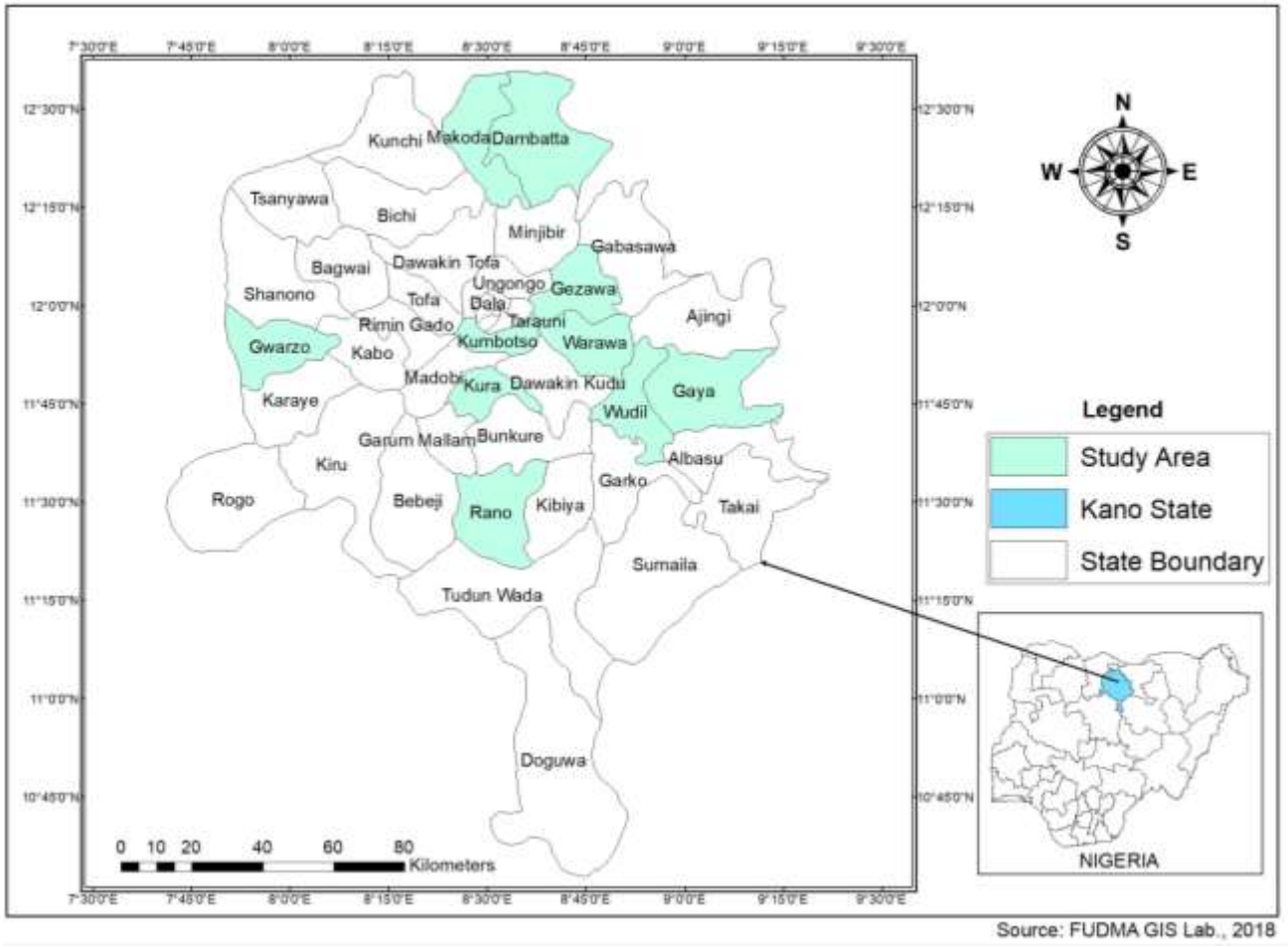


**Figure 1.** Sampled local government areas.

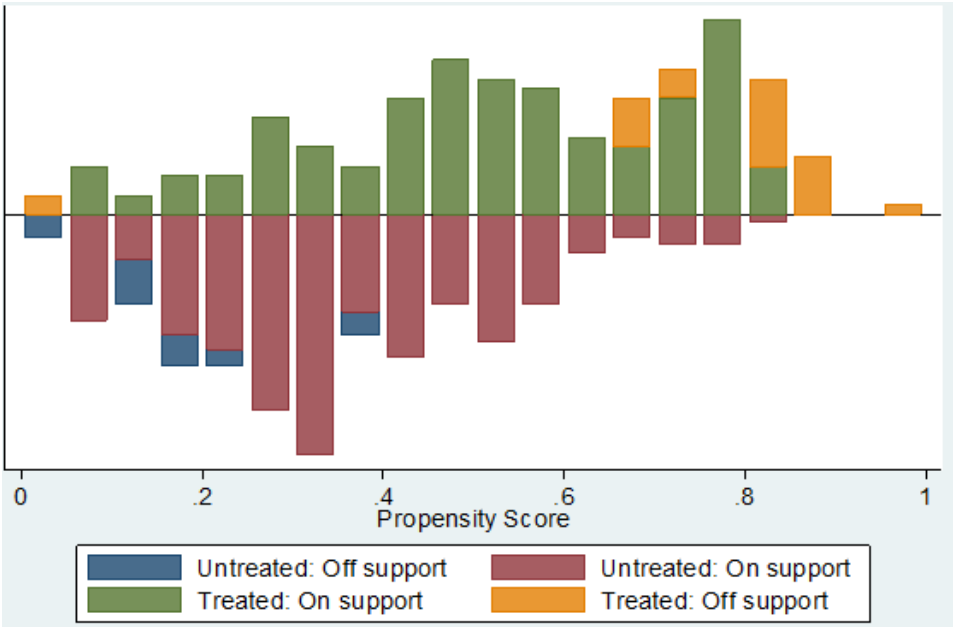


**Figure 2.** Propensity score distribution and common support for propensity score.

**Table 4.** Distribution of sampled households by estimated propensity scores and access to subsidized farm inputs.

| Group | Observation | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| Total household | 390 | 0.43 | 0.22 | 0.04 | 0.97 |
| Treatment household | 170 | 0.53 | 0.22 | 0.03 | 0.99 |
| Control household | 220 | 0.34 | 17 | 0.04 | 0.81 |

**Table 5.** Chi-square test for the joint significance of variables of propensity scores.

| Sample | Ps $R^2$ | LR $chi^2$ | p>$chi^2$ | Mean bias | Med bias | B | R | %Var |
|---|---|---|---|---|---|---|---|---|
| Unmatched | 0.203 | 108.56 | 0.000 | 28.9 | 13.2 | 117.5* | 1.42 | 38 |
| Matched | 0.023 | 8.88 | 0.782 | 6.1 | 6.0 | 35.6* | 0.59 | 25 |

**Table 6.** GESS participation effect on maize yield and total factor productivity**.**

| Participation | Productivity indicators | Kernel matching ATE, ATT, ATU |
|---|---|---|
| If a household is a participant | Maize yield (kg/ha) | 197.8. 212.6*** 200.6 |
| | Total factor productivity | 0.85 0.81*** 0.89 |
| N | | 390 |

*** P<0.01, ** P<0.05, *P<0.1.

treatment effect on the treated (ATT) value of production on the entire population of a participant was #48000.08/ha (P<0.001). The average effect of treatment (ATE) for a household drawn from the entire population at random was lower with a value of production #46800/ha compared to the treated category. The ATU is the counterfactual outcome of the treated indicating how much they would have lost if they were not treated. The results of the treatment effects on TFP was also indicated. The average treatment effect on the treated (ATT) was 0.87 (P<0.01). This means that if any of the participating farmers is picked at random with equal probability, the expected growth rate of TFP will be 0.8133; on the other hand, the average treatment effect (ATE) for a household drawn from overall population at random is somewhat greater with value of 0.86 compared to the treated category.

The total factor productivity of less than one means productivity is low though statistically significant. The low TFP could mean that there are some inefficiency factors in maize production. However, these differences in the value of production and total factor productivity cannot simply be attributed to GESS by looking at the mean differences between GESS participants and non-participants. In particular, these mean differences are only indicative of correlations and cannot be used to make causal inferences regarding the impacts of the GESS on maize yields and total factor productivity without controlling for another confounding factor.

The result from PSM is presented in Table 6. The result showed that ATT on maize yield was 212.6 kg/ha (P<0.001) and TFP was 0.81 (P<0.01). This result is robust and consistent with both models. However unobserved heterogeneity among smallholders could have caused potential endogeneity resulting in possible of the error term with the productivity outcomes.

To verify the claim that participation in GESS may be endogenous, we perform a post estimation test using “estat endogenous” to test the hypothesis that GESS participation is exogenous. The results of the test are as follows; Durbin (score) $Chi^2$ (1) = 4.41177 (P=0.02230); the robust regression-based test of Wu-Hausman F-statistics (1696) =4.7823 (P=0.03115) and $\chi 2$ and F-statistic (53.5). Besides, we fail to accept the null hypothesis and conclude that GESS participation is endogenous at 5% significant level and therefore OLS estimation might be considered inconsistent estimate of treatment effect. The result supports the choice of instrumental variable method of estimating the treatment effect. We tested the validity of instruments using the stata command ‘estat first’. We found that the minimum eigenvalue (53.2) is greater than the value of the nominal 5%, wald test at 5% bias tolerance and the joint significant test (F= 53.2, P=0.000) show that instruments are strong. Hansen–J test confirms that the model is correctly specified, thus, we fail to accept the null hypothesis that the instruments are weak and conclude that the instruments are valid and strong.

**Table 7.** IV-2SLS estimation of treatment effect on productivity outcomes.

| Variable | IV-2SLS | |
|---|---|---|
| | Yield | Total factor productivity |
| GESS | 0.217(0.058)*** | 0.141(0.0033)** |
| Phone ownership(1=yes) | -0.461 (0.336) | 0.346(0.240) |
| Marital status ( 1=married) | -0.154(0.131) | 0.029(0.0094)** |
| Gender(male headed=1) | 0.186(0.147) | -0.092(0.105) |
| Membership of commodity( 1=yes) | 0.124(0.130) | 0.0205(0.0928) |
| Household access to credit (1=yes) | 0.0300(0.0701) | 0.1904(0.0501) |
| Number of extension visits | 0.0757(0.068) | -0.205(0.049) |
| Distance to redemption centre(km) | -0.648(0.0250)** | -0.291(0.193) |
| Number of years of education of household head | -0.302(0.0203)** | 0.0667(0.146) |
| Household landholding | 0.307(0.0258)*** | 0.297(0.184) |
| Years of farming experience | -0.098(0.096) | 0.251(0.231) |
| Household size | 0.0838(0.0118)* | -0.141(0.125) |
| Age of household head | 0.187(0.0341)** | 0.154(0.153) |
| Joint significant all regression F- test | 3.68*** | 4.25*** |
| $R^2$ | 0.09133 | 0.01288 |

Observation: Durbin score $chi^2$= 3.41177 (P = 0.02230); Wu- HausmanF(1,379) = 4.40057 (0.0021); $Waldchi^2$ (9) = 58.97; $Prob>chi^2$ = 0.000; R-squared= 0.2888. *, **, *** : Significant at 10, 5 and 1%, respectively. Numbers in bracket are standard errors.

**Table 8.** Results of effect size on productivity outcomes base on mean comparison**.**

| Participants outcome | Hedges's | | |
|---|---|---|---|
| | Mean diff | Effect size | Decision |
| Value production (kg/ha) | 212.6 | 0.72 | Moderate effect |
| Total factor productivity | 0.87 | 0.75 | Moderate effect |

Source: Authors Calculation (2018).

The result of the IV-2SLS in Table 7 show that GESS participants got an average net gain of 14.1% from the cost incurred in maize production while maize yield gain was by 32.3%. We also found that the age of household head, household total landholding significantly improve yield per hectare while total factor productivity is influenced by gender of the household head, with male-headed households tending to be more productive. However, distance to redemption centres, a number of years of formal education negatively influenced yield per hectare and total factor productivity. The result of this study is consistent with the findings by Jayne et al. (2010) who found that increased maize production was positively associated with fertilizer subsidy in Malawi. World Bank (2010) also found 89% of the growth in output as a result of the subsidy program in Zambia. In another study, Tesfamicheal et al. (2017) also found that maize yield of GESS participants in Nigeria is increased by 26.1%. The findings of this study have shown that farmers who used subsidized maize seeds improved their productivity but contrary to Cesar et al. (2017) who found that input donation in Mexico did not improve the value of maize production. This tends to validate the argument that suggests that farm subsidy provides incentives for farmers to use inputs to improve farm-level productivity. The Hedges "g" test in Table 8 also suggested moderate improvement in the size of productivity outcomes . These results may be key ingredients in the renewed interest of subsidizing farm inputs across the continent.

## Conclusion

The impact of GESS subsidy program on maize of productivity farming households in Kano State, Nigeria was investigated to stimulate policymakers' commitment to the provision of assistance to farmers in the form of input subsidy. The study used propensity score matching analysis and IV-two stage least square method and Hedges 'g' effect size estimation to examine the size of outcomes. The matching method made a comparison between those who participated in the program and those

who did not and drew conclusions based only on those that participated in GESS farm subsidy programs. From the instrumental variable approach, we found that the yield per hectare of participants was (32.3%, P<0.01) and the TFP of participants was (14.1%, P<0.05). The result from hedges 'g' effect size of the estimation suggests a moderate improvement in productivity outcomes, meaning that there is considerable hope if the government can build on achievements to substantially raise program effectiveness, efficiency, and benefits to the farmers. We conclude that input use alone is not enough to increase maize production; improvement in input use efficiency through integrated crop management practices are also needed.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Screening of sesame genotypes for resistance against *Fusarium* wilt pathogen

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**Fusarium wilt is one of the most devastating diseases in sesame production in Uganda, caused by a fungus called *Fusarium oxysporum* f.sp*. sesami*. Its incidence ranges from 17.1 to 73.3%. Some sesame genotypes have been reported to resist Fusarium wilt; however, have not been precisely determined. In this study, 30 sesame genotypes that included released varieties, improved elite breeding lines and introductions were screened in the screenhouse under high pathogen pressure following artificial infection using five isolates of *F. oxysporum* f.sp *sesami*. The results revealed that sesame genotypes showed different response to the pathogen and thus disease development among the genotypes. The genotype effect was significant (P≤0.001) for disease incidence. Two genotypes (EM15-1-5 and Sesim 2) were identified and rated moderately resistant to Fusarium wilt (37.3 and 33.8%), respectively. Whereas, seven genotypes showed moderate susceptibility and 21 genotypes were susceptible for Fusarium wilt infection. No genotype was identified as being immune to the disease. It is noteworthy for sesame breeding programme in Uganda to continue evaluating other genotypes from existing germplasm which were not tested for resistance to Fusarium wilt in this study. Also more germplasm should be assembled and screened for resistance to the disease and other agronomic traits.**

**Key words:** *Fusarium oxysporum* f. sp. *sesame*, incidence, resistance.

## INTRODUCTION

Sesame is an important food and cash crop in Uganda, providing livelihoods for many households. However, its yield is very low, often below 1000 kg/ha. Low sesame yield in Uganda is due to some abiotic and biotic constraints. Abiotic constraints include fluctuations of rainfall and temperature, poor soil management while biotic factors include pests and diseases. Fusarium wilt (*Fusarium oxysporum* f. sp. *sesami*) is one of the

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**Figure 1.** Symptoms of planted sampled for isolating *Fusarium oxysporum* f.sp. *sesami*. Wilted sesame (left) and reddish-brown crown necrosis on stem (right).

diseases devastating sesame production in Uganda. Its incidence had been reported to range between 17.1 and 73.3% (Egonyu et al., 2005). Elsewhere the disease had been reported to cause yield loss ranging from 50 to 100% (El-Bramawy et al., 2009). *F. oxysporum* f. sp. *sesami* is a soil borne, parasitic pathogen which interacts with host plant (Bayoumi and EL-Bramawy, 2007). When inside the plant the pathogen feeds on plant's nutrients and colonizes the roots cells which then spread to other parts of the plants through the water transporting vessels (xylem). The pathogen grows and produce mycelia which leads to blockage of water supply to the plant consequently the plants develops wilt symptoms (Elewa et al., 2011; Joshi, 2018). Fusarium wilt in Uganda is managed by using cultural practices such as early planting, intercropping, crop rotation, and burning the crop residues. These cultural practices however are not efficient in managing the disease. Early planting is intended to enable the crop to escape from being affected by Fusarium wilt pathogen which is severe toward the end of the rainy season. This practice unfortunately exposes the crop to waterlogging and other diseases such as leaf spot (Egonyu et al., 2005). Intercropping and crop rotation are meant to reduce the pathogen population in soil and thus reduce disease incidence and severity. The effectiveness of these two methods is also reduced due to the effective survival strategies of the pathogen (Okungbowa and Shittu, 2012). Developing and growing disease resistant varieties is thus the most effective, environmental friendly, less costly and long term solution to disease management in crops (Shabana et al., 2014). There is therefore need to identify or develop sesame varieties with resistance to Fusarium wilt. Sesame genotypes such as Sesim 1 and Sesim 2 have been developed in Uganda and are reported to be resistant to Fusarium wilt (Anyanga-Okello et al., 2016b). Resistance levels in these genotypes however, have not been precisely determined. There is also need to increase the sesame germplasm base with resistance to Fusarium wilt to facilitate resistance breeding. This study therefore, was done to screen sesame genotypes for resistance to *F. oxysporum* f.sp. *sesami*.

## MATERIALS AND METHODS

### Pathogen isolation and identification of *Fusarium* species associated with sesame wilt

*Fusarium* spp. isolates were obtained from wilted sesame plants collected from a field at the National Semi-Arid Resources Research Institute (NaSARRI), Eastern Uganda. Fields at NaSARRI are known to be the hot spot of Fusarium wilt. Wilted plant samples (Figure 2) were uprooted gently; roots and the portion of the stems exhibiting reddish-brown necrosis (like crown rot), cut and placed in paper envelopes. The samples were taken to the Biotechnology Laboratory at Makerere University, Agriculture Research Institute, Kabanyolo (MUARIK) to isolate the disease causing organism (Figure 1).

### Isolation of the pathogen

Pathogen isolation was carried out according to Kavak and Boydak (2006) with some modifications. The roots and stem samples were washed under running tap water and air dried in a laminar flow hood. The air dried stems were cut in small pieces approximately 5 mm long and dissected longitudinally to observe internal vascular discoloration. Stem pieces that exhibited internal vascular discoloration and roots were further cleaned using distilled water and disinfected using 2.5% v/v commercial bleach (JIK) for 5 min followed by 70% ethyl alcohol for 1 min. Disinfected pieces were then rinsed three times in sterile distilled water and blotted dry using a sterile napkin in a Laminar flow hood.

The sterilized stems and roots were chopped into small pieces and placed on PDA media in separate Petri dishes and incubated at

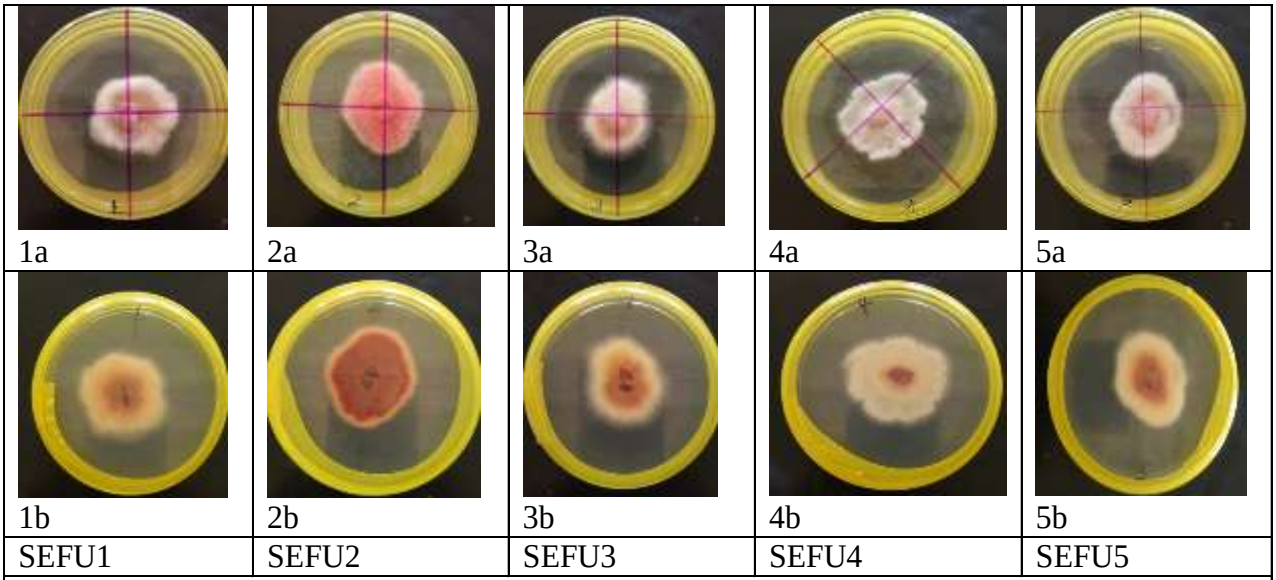


**Figure 2.** A colony characteristic of five *Fusarium oxysporum* f. sp. *sesami* isolates on PDA media at seven days old.

27°C under alternating 12 h light and dark conditions for seven days. Cultures were observed for conidia production. Those with conidia were transferred to fresh PDA media and incubated at same temperature and light conditions for another seven days. Pure cultures were then made from single hyphal tips, again on PDA. Mature cultures were observed macro and microscopically. Macroscopically cultures were observed for growth morphology and pigmentation of colonies (Burgess et al., 1994; Leslie and Summerell, 2006). The colony morphology, growth habit and pigmentation were observed and recorded.

For microscopic identification, mycelia from each isolates were teased on a microscope slide and mounted on a microscope to observe presence as well as the shape of macro and micro conidia and chlamydospores including their number of septa (Leslie and Summerell, 2006). Microscopically, many of the cultures conformed to *Fusarium* spp. five isolates (SEFU 1-SEFU 5) were consequently selected for use in this study.

**Molecular identification of *Fusarium* spp. associated with sesame wilt from cultured isolates**

Molecular identification of the fungal isolates was done based on DNA sequencing of the translation elongation factor-1 alpha (TEF-1 alpha) and Internal Transcribed Spencer regions (ITS). For this purpose, fungal DNA was extracted and subjected to polymerase chain reaction (PCR) as described by Namasaka et al. (2017). The TEF-1α was amplified using forward (TEF-Fu3f: Bioneer corporation 5'-GGT ATC GAC AAG CGA ACC AT-3) and reverse (TEF-Fu3r: Bioneer Corporation 5'- TAG TAG CGG GGA GTC TCG AA- 3') primers while the ITS was amplified using the forward and reverse primers ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG-3' and ITS4 KY0: 5'-TCCTCCGCTTWTTGWTWTGC-3'), respectively. DNA amplification was carried out in an ARKTIK Thermocycler (IngabaBiotech™, Model: 5020). From each of the amplified sample, 5 µl were subjected to gel electrophoresis on 1.5% agarose gel pre-stained with Gel-red fluorescent dye (Botium) in 1x TBE buffer at a 130 V for 30 min alongside 100 bp DNA ladder. Gel documentation was done using the Benchtop UV Trans-illuminator (BioDoc-It™ Imaging System, 8.0'' LCD/LM-20, PIN 97-0165-02, 230V-50Hz) and the bands of each sample scored by comparing its position with a specific band of the ladder in the gel to the ladder chart provided (Bioneer Corporation). Five isolates that conformed to *F. oxysporum* f. sp. *sesame* based on microscopic, macroscopic and DNA sequence analysis were selected and subjected to pathogenicity tests using 3 susceptible varieties before being used to screen germplasm.

**Screening of sesame germplasm against Fusarium wilt pathogen**

Thirty (30) sesame genotypes were sourced from NaSARRI (Table 1) and screened for Fusarium wilt resistance. The genotypes comprised local materials, improved lines and introductions from Tanzania (Table 1). These genotypes were screened two times (1st screening and 2rd screening). Screening was conducted in a screen house at MUARIK which is located in Central Uganda (32° 37'E, 0° 28'N) at an altitude of 1200 m above sea level. MUARIK receives an average annual rainfall of 1200 mm with a daily temperature ranging from 17 to 33°C (Namasaka et al., 2017).

**Experimental design, inoculum production and inoculation**

The test genotypes were grown in a chamber constructed inside a screenhouse. The chamber was constructed from a white transparent polythene sheet to increase temperature and humidity for pathogen multiplication (Namasaka et al., 2017). Five pathogen isolates were used to screen the thirty genotypes. The isolates were first cultured on sterilized sorghum in the laboratory for 21 days at room temperature. The fully colonized sorghum seed was then used to inoculate the sterilized soil in plastic pots. Inoculation was done using 75 g of inoculum for every 2 kg of soil. Inoculated

**Table 1.** List of genotypes, their attributes and origin used in the study.

| No. | Genotype | Attributes | Origin |
|---|---|---|---|
| 1 | Sesim 1 | Resistant | Ugandan variety |
| 2 | Sesim 2 | Resistant | Ugandan variety |
| 3 | Sesim 3 | Susceptible | Ugandan variety |
| 4 | Sesim1//Renner 1-3-1-16 | Unknown | Ugandan pure line |
| 5 | (Sesim2//5181)-2-2-1 | Resistant | Ugandan pure line |
| 6 | (Sesim 2//5181)//7029-1-2-1 | Unknown | Ugandan pure line |
| 7 | (Sesim 2//5181)//UCR3-1-19-2 | Unknown | Ugandan pure line |
| 8 | Ajimo A1-5//Renner 1-3-1-1 | Unknown | Ugandan pure line |
| 9 | Ajimo A1-6//7029-1-1 | Susceptible | Ugandan pure line |
| 10 | Adong 4-4//Renner 1-3-2-1-19 | Unknown | Ugandan pure line |
| 11 | Local 158//Renner 1-3-1-5-2 | Unknown | Ugandan pure line |
| 12 | (Local 158//6022)-1-2-1//4036-1-2-2-5 | Unknown | Ugandan pure line |
| 13 | (Local 158//6022)-1-2-1//Renner 1-3-1-3-3-5 | Unknown | Ugandan pure line |
| 14 | 4036-1-10-2 | Unknown | China |
| 15 | 4036-1-10-2//Local 158 | Unknown | Ugandan pure line |
| 16 | 4036-1-10-2//Renner 1-3-1-16-1 | Unknown | Ugandan pure line |
| 17 | 4036-1-10-2//Renner 1-3-1-16-2 | Unknown | Ugandan pure line |
| 18 | Renner 1-3-1-16-4 | Resistant | USA pure line |
| 19 | Renner 1-3-1-17 | Resistant | USA pure line |
| 20 | Renner 1-3-1-17-1 | Resistant | USA pure line |
| 21 | EM15-1-5 | Resistant | Ugandan pure line |
| 22 | Local 158//6022 | Unknown | Ugandan pure line |
| 23 | Local 158-5 | Medium resistant | Egyptian pure line |
| 24 | Sesim 2//Ajimo A1-5 | Unknown | Ugandan pure line |
| 25 | Ajimo A1-5//Renner 1-3-1-9 | Unknown | Ugandan pure line |
| 26 | Naliendele 92 | Unknown | Tanzanian variety |
| 27 | Ziada 94 | Unknown | Tanzanian variety |
| 28 | Lindi 2002 | Unknown | Tanzanian variety |
| 29 | Mtwara 2009 | Unknown | Tanzanian variety |
| 30 | Mtondo 2015 | Unknown | Tanzanian variety |

pots were then arranged in a split plot design with two replicates. Fungal isolates were the main plot treatment while genotypes were sub-plot treatments. Planting was done three days after inoculation. A total of 15 seeds per genotype were planted in each pot. Pots were watered regularly to ensure proper plant growth. Screening of this 30 genotypes was done two times (experiment 1 and 2) (Table 7) in the screenhouse.

**Data collection**

Phenotypic observations were made daily for Fusarium wilt symptoms from emergence to physiological maturity of the crop. The experimental germplasm was observed for necrotic lesions on the crown, yellowing of lower leaves, downward turning of growing plant (epinasty), wilting and death. Data were also collected for plant stand per pot and number of diseased plants per genotype. Plant stand was recorded two weeks after emergence and used to determine emergence percentage (EM %) and seed rotten%. Number of diseased plants per genotype was recorded at an interval of ten days from seedling to physiological maturity and used to compute disease incidence (DI %).

**Data analysis**

Data were subjected to Analysis of Variance (ANOVA) in Genstat 18th Edition Software. Fisher Protected Least Significant Difference (LSD) test at 5% probability level was used to compare treatment means. Genotypes were then grouped according to their resistance levels using the scale developed by Kavak and Boydak (2006) with some modifications (Table 2). The following are equations used to compute EM and DI%.

$$\text{EM } \% = \frac{\text{number of emerged plants}}{\text{total number of plant sown}} \times 100$$

$$\text{DI } \% = \frac{\text{number of diseased plants}}{\text{total number of plant emerged}} \times 100$$

The linear model used for analysis:

$$Y_{ijk} = \bar{\bar{Y}} + R_i + MP_J + (R * MP)_{ij} + SP_k + (SP * MP)_{kj} + E_{ijk}$$

**Table 2.** Scale for classification of the genotypes.

| Scale number | Infection (%) | Category of resistance |
|---|---|---|
| 1 | 0.00 | Immune (I) |
| 2 | 0.1 - 20 | Resistant (R) |
| 3 | 20.1 - 40 | Moderately resistant (MR) |
| 4 | 40.1 - 60 | Moderately susceptible (MS) |
| 5 | 60.1 - 80 | Susceptible (S) |
| 6 | 80.1 - 100 | Highly susceptible (HS) |

**Table 3.** Five Fusarium isolates compared to their most related database accessions based on TEF-1α gene and species associated.

| Isolate | Origin | Query cover (%) | Error value | %Similarity | Most related accession | Species identification |
|---|---|---|---|---|---|---|
| SEFU1 | NaSARRI | 92 | 1e180 | 99 | MF143099.1 | *Fusarium oxysporum* |
| SEFU2 | NaSARRI | 97 | 0.0 | 99 | KY798882.1 | *Fusarium oxysporum* |
| SEFU3 | NaSARRI | 97 | 1e198 | 99 | KY657521.1 | *Fusarium oxysporum* |
| SEFU4 | NaSARRI | 97 | 0.0 | 99 | KU872077.1 | *Fusarium oxysporum* |
| SEFU5 | NaSARRI | 97 | 2e178 | 99 | KU507175.1 | *Fusarium oxysporum* |

BLASTn comparison in NCBI, U.S.A National Library of Medicine.

where $Y_{ijk}$ is the observation value for genotype i$^{th}$, j$^{th}$ and k$^{th}$, $\bar{\bar{Y}}$ is mean, $R_i$ is the replication effect for i$^{th}$ , $MP_j$ is the isolate effect for j$^{th}$ , $(R*MP)_{ij}$ is the replication interacting genotype effect for the i$^{th}$ and j$^{th}$, $SP_k$ is the genotypes effect for k$^{th}$, $(SP*MP)_{kj}$ is the isolate interacting genotype for k$^{th}$ and j$^{th}$, and $E_{ijk}$ is the experimental error effect.

## RESULTS

### Isolation and identity of the *Fusarium* spp. associated with sesame wilt

Based on phenotypic characteristics and DNA sequence data, five isolates were identified and belonged to *F. oxysporum*. All five isolates produced white to magenta or magenta-pink cotton like mycelia on PDA (Figure 2) but in dark conditions the colour changed to and remained purple. The isolates produced macroconidia, microconidia and chlamydospores. Macroconidia were moon shaped and multiseptate (Figure 3). These features are associated with *Fusarium* spp. DNA sequences of the translation elongation factor-1 alpha (TEF-1 alpha) and Internal Transcribed Spencer regions (ITS) revealed 99% congruence with several *F. oxysporum* accessions deposited in data bases (Tables 3 and 4). All the five isolates resulted into a wilt on the susceptible genotypes, proving that they all were *F. oxysporium* f.sp. *sesami*.

### Response of 30 genotypes against five isolates of *F. oxysporum* f. sp. *sesami*

In Table 5, the isolate effect was non-significant in the first screening but significant (P≤0.05) during the second screening. The performances of genotypes were significant (P≤0.001) for all traits measured both in the first and second screening. Interaction between genotype and isolate was only significant (P≤0.001) for DI% during the first screening. A significant effect of the interaction of genotypes by isolates was noted for all traits during the second screening.

For genotypes response during the first and second screening, the analysis indicated that 22 and 25 genotypes, respectively had EM% above 70% (Tables 6 and 7). Across experiments, 24 genotypes had EM% above 70% (Table 8). During the first screening (Table 6), genotype (Sesim2//5181)-2-2-1 (86.0%) recorded the highest EM% and ranked first although the difference was not significant from 6 other genotypes. The genotype with the lowest EM% was Mtwara 2009 (31.4%). However, this was not significantly different from two genotypes Lindi 2002 (34.4%) and Naliendele 92 (44.3%). The highest seed rot percentage was recorded in the genotype Lindi 2002 (94.3%) and was significantly higher than values recorded on the other 29 genotypes. The lowest seeds rot percentage was recorded in genotype Ajimo A1-6//7029-1-1 (14.3%) and was significantly lower than seven other genotypes. For DI%,(Sesim 2//5181)//UCR3-1-19-2 (95.5%) recorded the highest value and it was significantly different from 24 other genotypes. Genotype EM15-1-5 (31.4%) recorded the

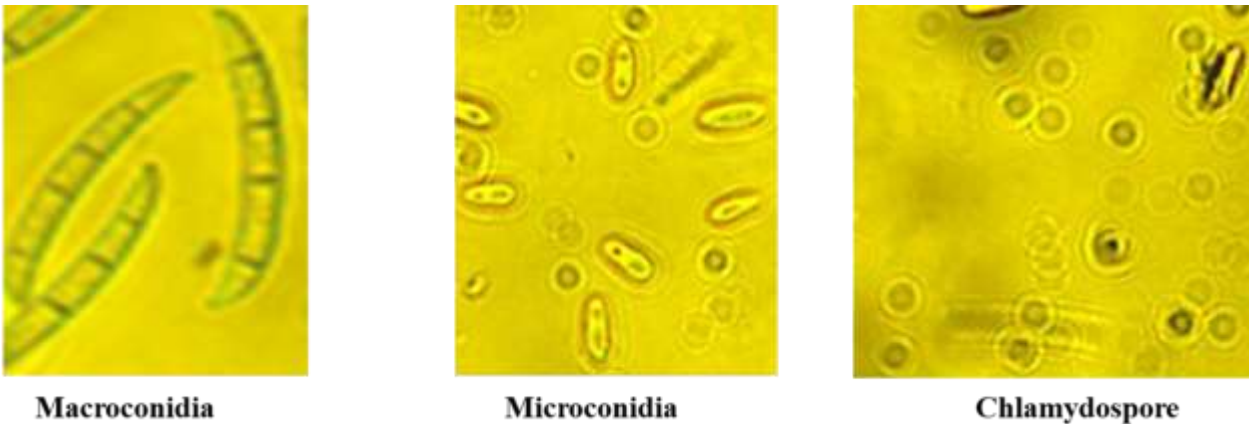


**Figure 3.** Microscopic characteristics of five *Fusarium oxysporum* f. sp. *sesami* isolates cultured on PDA media.

**Table 4.** Five *Fusarium* isolates compared to their most related database accessions based on ITS gene and species associated.

| Isolate | Origin | Query cover | Error Value | %Similarity | Most related accession | Species identification |
|---|---|---|---|---|---|---|
| SEFU1 | NaSARRI | 93 | 0.0 | 99 | KU872840.1 | *Fusarium oxysporum* |
| SEFU2 | NaSARRI | 99 | 0.0 | 99 | MG670445.1 | *Fusarium oxysporum* |
| SEFU3 | NaSARRI | 98 | 0.0 | 99 | KF278962.1 | *Fusarium oxysporum* |
| SEFU4 | NaSARRI | 96 | 0.0 | 99 | KU872826.1 | *Fusarium oxysporum* |
| SEFU5 | NaSARRI | 97 | 0.0 | 99 | HG423346.1 | *Fusarium oxysporum* |

BLASTn comparison in NCBI, U.S.A National Library of Medicine

**Table 5.** Mean squares of emergence (EM%), disease incidence (DI%) and seeds rotten% of 30 sesame genotypes at both first and second screening in screen house.

| SOV | df | 1st screening | | | 2nd screening | | |
|---|---|---|---|---|---|---|---|
| | | EM % | Seed rotten % | D I% | EM % | Seed rotten % | D I% |
| Rep | 1 | 2314.8$^{ns}$ | 3429.3$^{ns}$ | 3720.6* | 2733.1** | 3008.3* | 189.5$^{ns}$ |
| Isolate | 4 | 696.3$^{ns}$ | 1211.6$^{ns}$ | 1166.7$^{ns}$ | 1386.2* | 1441.2* | 7740.9* |
| Rep × Isolate | 4 | 1456.1** | 837.4$^{ns}$ | 281.6$^{ns}$ | 108.2$^{ns}$ | 169.2$^{ns}$ | 554.7$^{ns}$ |
| Genotype | 29 | 2040.5*** | 2905.2*** | 2785.2*** | 1370.5*** | 1533.4*** | 1381.6*** |
| Genotype × Isolate | 116 | 426.9$^{ns}$ | 536.7$^{ns}$ | 853.1*** | 504.4* | 563.8** | 920.4*** |
| Residual | 145 | 388.9 | 560.6 | 301.9 | 388.9 | 364.6 | 367.7 |
| CV% | | 27.6 | 74.3 | 25.7 | 23.1 | 90.8 | 32.8 |

Values with *, ** and *** represent significance at P≤0.05, P≤0.01 and P≤0.001, respectively while ns is non-significant.

lowest DI%. This was however not significantly different from two genotypes Renner 1-3-1-17 (31.5%) and Sesam2 (32.4%).

Contrary to the first screening, in the second screening (Table 7), the highest EM% was recorded in the genotype Mtondo 2015 (97.0%). This was significantly higher than 26 other genotypes. Genotype Renner 1-3-1-17 (48.0%) recorded the lowest EM% but differences were not significant from 18 other genotypes. The genotype with the highest seed rot percentage was Renner 1-3-1-17 (52.0%). However, this was not significantly different from nine other genotypes. Lowest seed rot percentage was recorded in the genotype Mtondo 2015 (3.0%). Genotype Mtwara 2009 (90.5%) recorded the highest DI%. The lowest DI% was recorded in a genotype Local 158-5 (34.3%). This figure was not significantly different from 28 other genotypes.

Genotypes were not consistent with respect to

**Table 6.** Means of emergence (EM %), disease incidence (DI %) Seeds rotten % and categories for resistance of 30 sesame genotypes in response to five isolates of *Fusarium* wilt pathogens at first screening in screen house.

| Genotype | EM% | Seed rot % | DI % | Categories for resistance |
|---|---|---|---|---|
| EM15-1-5 | 78.1 | 30.0 | 31.4 | MR |
| Renner 1-3-1-17 | 51.4 | 48.6 | 31.5 | MR |
| Sesim 2 | 82.9 | 17.1 | 32.4 | MR |
| 4036-1-10-2//Renner 1-3-1-16-1 | 82.9 | 17.1 | 48.0 | MS |
| Local 158//Renner 1-3-1-5-2 | 81.4 | 45.7 | 52.4 | MS |
| Lindi 2002 | 34.4 | 94.3 | 56.2 | MS |
| Sesim1//Renner 1-3-1-16 | 80.0 | 20.0 | 57.2 | MS |
| Mtwara 2009 | 31.4 | 68.6 | 60.4 | S |
| Ajimo A1-5//Renner 1-3-1-9 | 75.7 | 24.3 | 62.5 | S |
| Renner 1-3-1-16-4 | 84.3 | 15.7 | 63.5 | S |
| Ziada 94 | 71.4 | 28.6 | 63.8 | S |
| Local 158-5 | 81.9 | 18.6 | 67.3 | S |
| (Local 158//6022)-1-2-1//4036-1-2-2-5 | 75.6 | 31.4 | 67.4 | S |
| 4036-1-10-2//Local 158 | 78.6 | 28.6 | 67.9 | S |
| Ajimo A1-5//Renner 1-3-1-1 | 73.4 | 34.3 | 68.6 | S |
| (Local 158//6022)-1-2-1//Renner 1-3-1-3-3-5 | 70.0 | 30.0 | 69.3 | S |
| Sesim 2//Ajimo A1-5 | 70.0 | 30.0 | 70.3 | S |
| Ajimo A1-6//7029-1-1 | 85.7 | 14.3 | 71.0 | S |
| Mtondo 2015 | 68.6 | 31.4 | 73.0 | S |
| 4036-1-10-2 | 82.9 | 17.1 | 74.0 | S |
| Local 158//6022 | 60.0 | 28.6 | 75.1 | S |
| Sesim 1 | 76.2 | 31.4 | 76.1 | S |
| Renner 1-3-1-17-1 | 84.3 | 15.7 | 76.7 | S |
| Naliendele 92 | 44.3 | 55.7 | 78.3 | S |
| Sesim 3 | 64.3 | 35.7 | 83.1 | HS |
| (Sesim2//5181)-2-2-1 | 86.0 | 22.9 | 88.2 | HS |
| Adong 4-4//Renner 1-3-2-1-19 | 75.7 | 24.3 | 88.6 | HS |
| (Sesim 2//5181)//7029-1-2-1 | 71.9 | 38.6 | 88.7 | HS |
| 4036-1-10-2//Renner 1-3-1-16-2 | 68.6 | 31.4 | 90.2 | HS |
| (Sesim 2//5181)//UCR3-1-19-2 | 74.3 | 25.7 | 95.5 | HS |
| LSD (P≤0.05 ) | 17.5 | 20.9 | 15.4 | - |
| GM | 71.5 | 31.9 | 67.6 | - |

EM%-Emergence percentage, DI%-disease incidence, MR-moderate resistant, S-susceptible, MS-moderate susceptible HS-highly susceptible, LSD-least significant difference and GM-grand mean.

resistance to Fusarium wilt across the two experiments. During first screening, three genotypes (EM15-1-5, Renner 1-3-1-17 and Sesim 2) were categorized as moderately resistant, four genotypes were moderately susceptible, and 17 genotypes were susceptible while six genotypes were highly susceptible (Table 6). Results of the second screening showed that three genotypes (Local 158-5, Sesim 2 and Local 158//6022) were moderately resistant, 12 genotypes were moderately susceptible, 14 were susceptible and only one genotype (Mtwara 2009) was categorized as highly susceptible (Table 7). Across experiments, only two genotypes (Sesim 2 and EM15-1-5) were moderately resistant, seven were moderately susceptible while the majority were categorized as susceptible (Table 8).

While performance for isolates during the first screening showed that their effect was not significant for all traits recorded (Figure 4), in the second screening (Figure 5) the effect was significant. In the second screening, the highest EM% was recorded in isolate SEFU3 (85.1%) and it was not significant from other two isolate SEFU4 (81.5%) and SEFU2 (81.4%). Lowest EM% was recorded in isolate SEFU1 (72.6%) and was significantly different from three other isolates except SEFU5 (77.2%). Highest seed rot percentage was recorded from in isolate SEFU1 (28.5%) but was not significantly different from isolate SEFU5 (22.8%). The lowest seed rot percentage was in isolate SEFU5 (15.7%) and was not significantly different from three other isolates. Highest DI% was recorded from isolate SEFU5 (64.7%) although this was not significantly

**Table 7.** Means of emergence (EM%), disease incidence (DI%), seeds rotten% and categories for resistance of 30 sesame genotypes in response to five isolates of *Fusarium* wilt pathogens at second screening in screen house.

| Genotype | EM% | Seed rot% | DI% | Categories for resistance |
|---|---|---|---|---|
| Local 158-5 | 82.0 | 18.0 | 34.3 | MR |
| Sesim 2 | 76.0 | 24.0 | 35.3 | MR |
| Local 158//6022 | 57.0 | 43.0 | 39.8 | MR |
| Naliendele 92 | 62.0 | 38.0 | 42.2 | MS |
| EM15-1-5 | 61.0 | 39.0 | 43.3 | MS |
| 4036-1-10-2//Local 158 | 76.0 | 24.0 | 49.8 | MS |
| (Sesim 2//5181)//UCR3-1-19-2 | 87.7 | 17.0 | 52.0 | MS |
| 4036-1-10-2//Renner 1-3-1-16-2 | 91.0 | 9.0 | 54.0 | MS |
| Renner 1-3-1-17 | 48.0 | 52.0 | 54.3 | MS |
| 4036-1-10-2//Renner 1-3-1-16-1 | 84.0 | 16.0 | 55.4 | MS |
| Sesim 2//Ajimo A1-5 | 64.7 | 48.0 | 56.0 | MS |
| Ajimo A1-5//Renner 1-3-1-1 | 75.0 | 25.0 | 57.3 | MS |
| Ziada 94 | 86.0 | 14.0 | 57.4 | MS |
| Sesim 1 | 78.0 | 22.0 | 59.0 | MS |
| Ajimo A1-6//7029-1-1 | 76.0 | 24.0 | 59.3 | MS |
| Mtondo 2015 | 97.0 | 3.0 | 60.0 | S |
| Lindi 2002 | 96.0 | 4.0 | 60.2 | S |
| 4036-1-10-2 | 80.0 | 20.0 | 61.1 | S |
| Local 158//Renner 1-3-1-5-2 | 76.0 | 24.0 | 61.4 | S |
| Ajimo A1-5//Renner 1-3-1-9 | 81.0 | 19.0 | 63.2 | S |
| (Sesim 2//5181)//7029-1-2-1 | 94.0 | 6.0 | 63.9 | S |
| (Sesim2//5181)-2-2-1 | 91.0 | 9.0 | 64.0 | S |
| Renner 1-3-1-17-1 | 87.0 | 13.0 | 67.0 | S |
| (Local 158//6022)-1-2-1//Renner 1-3-1-3-3-5 | 78.0 | 22.0 | 67.5 | S |
| (Local 158//6022)-1-2-1//4036-1-2-2-5 | 78.0 | 22.0 | 68.2 | S |
| Sesim1//Renner 1-3-1-16 | 83.0 | 17.0 | 68.7 | S |
| Adong 4-4//Renner 1-3-2-1-19 | 92.0 | 8.0 | 68.7 | S |
| Sesim 3 | 76.0 | 24.0 | 70.7 | S |
| Renner 1-3-1-16-4 | 88.0 | 12.0 | 71.2 | S |
| Mtwara 2009 | 85.0 | 15.0 | 90.0 | HS |
| LSD (P≤0.05 ) | 35.9 | 37.4 | 38.2 | - |
| GM | 79.5 | 21.0 | 58.5 | - |

EM%-Emergence percentage, DI%-disease incidence, MR-moderate resistant, S-susceptible, MS-moderate susceptible HS-highly susceptible, LSD-least significant difference, and GM-grand mean.

different from isolate SEFU1 (61.8%), SEFU2 (64.6%) and SEFU4 (63.1%). Isolate SEFU3 resulted in the least in DI% (38.3%).

## DISCUSSION

Thirty sesame genotypes were screened for resistance to *F. oxysporum f.* sp. *sesame* in screenhouse conditions A range of symptoms were observed on the inoculated crop. These consisted of seed rot, reddish-browning of hypocotyls, damping off, reddish-brown necrotic lesions on stem and roots, wilting and plants death.

The findings demonstrated that the performance of all 30 sesame genotypes was significantly different in all the measured traits. This implies that genotypes were genetically diverse, presenting an opportunity for finding useful genotypes in the screened set. During the two screenings, most of the genotypes had percentage emergence values above 70% implying that the majority of the genotypes were able to germinate. This study has shown that *F. oxysporum. f.* sp*. sesami* does not result into serious seed rot that affects germination rate. This is not unusual since typical wilt pathogens do not cause much seed rots.

The study also showed that there was variation in disease incidence among the genotypes tested. During the first screening, three genotypes (EM15-1-5, Renner

**Table 8.** Means of emergence (EM%), disease incidence (DI%), seeds rotten% and categories for resistance of 30 sesame genotypes in response to five isolates of *Fusarium* wilt pathogens at both first and second screening.

| Genotype | EM% | seed rot% | DI% | Category for resistance |
|---|---|---|---|---|
| Sesim 2 | 79.4 | 20.6 | 33.8 | MR |
| EM15-1-5 | 70.1 | 34.5 | 37.3 | MR |
| Renner 1-3-1-17 | 49.7 | 50.3 | 42.9 | MS |
| Local 158-5 | 81.4 | 23.3 | 50.8 | MS |
| 4036-1-10-2//Renner 1-3-1-16-1 | 83.4 | 16.6 | 51.7 | MS |
| Lindi 2002 | 68.5 | 49.1 | 55.9 | MS |
| Local 158//Renner 1-3-1-5-2 | 78.7 | 21.3 | 56.9 | MS |
| Local 158//6022 | 58.2 | 44.4 | 57.5 | MS |
| 4036-1-10-2//Local 158 | 77.6 | 26.3 | 58.8 | MS |
| Naliendele 92 | 53.1 | 46.9 | 60.3 | S |
| Ziada 94 | 78.7 | 21.3 | 60.6 | S |
| Sesim 2//Ajimo A1-5 | 67.5 | 39.0 | 62.6 | S |
| Ajimo A1-5//Renner 1-3-1-9 | 78.4 | 21.6 | 62.9 | S |
| Ajimo A1-5//Renner 1-3-1-1 | 73.6 | 29.6 | 62.9 | S |
| Sesim1//Renner 1-3-1-16 | 81.5 | 18.5 | 63.0 | S |
| Ajimo A1-6//7029-1-1 | 80.9 | 19.1 | 65.1 | S |
| Mtondo 2015 | 82.8 | 17.2 | 66.5 | S |
| Renner 1-3-1-16-4 | 86.1 | 13.9 | 67.3 | S |
| (Local 158//6022)-1-2-1//4036-1-2-2-5 | 76.6 | 26.7 | 67.3 | S |
| 4036-1-10-2 | 81.4 | 18.6 | 67.6 | S |
| Sesim 1 | 76.6 | 26.7 | 67.6 | S |
| (Local 158//6022)-1-2-1//Renner 1-3-1-3-3-5 | 74.0 | 26.0 | 68.4 | S |
| Renner 1-3-1-17-1 | 85.6 | 14.4 | 71.9 | S |
| 4036-1-10-2//Renner 1-3-1-16-2 | 79.8 | 20.2 | 72.1 | S |
| (Sesim 2//5181)//UCR3-1-19-2 | 80.8 | 21.4 | 73.6 | S |
| Mtwara 2009 | 58.2 | 41.8 | 75.0 | S |
| (Sesim 2//5181)-2-2-1 | 88.8 | 15.9 | 75.9 | S |
| (Sesim 2//5181)//7029-1-2-1 | 82.3 | 22.3 | 76.4 | S |
| Sesim 3 | 70.1 | 29.9 | 76.9 | S |
| Adong 4-4//Renner 1-3-2-1-19 | 83.9 | 16.1 | 78.7 | S |
| LSD | 12.1 | 18.9 | 11.7 | - |
| GM | 76.3 | 26.5 | 62.9 | - |

EM%=Emergence percentage, DI%= disease incidence percentage, MR=moderate resistant, MS=moderate susceptible, S=susceptible, HS= highly susceptible, LSD=least significant difference and GM=grand mean.

1-3-1-17 and Sesim 2) had disease incidence below 40% of these genotypes; however, only Sesim 2 scored below 40% incidence in the second screening. Interestingly, two other genotypes (that is, Local 158-5 and Local 158//6022) also had disease incidence levels below 40%. This means that some genotypes were not consistent in their resistance level suggesting that resistance of Fusarium wilt in sesame may also be influenced by other factors in addition to genetic ones. These factors such as carbon dioxide, temperature, texture, soil pH, and moisture in most cases are environmental and have been reported to interact with soil borne pathogens in a way that influences disease occurrence. The more carbon dioxide or less pH in soil, the more multiplication of fungi (Anonymous, 1988; Stover, 1958; Tyagi and Paudel, 2014) unexpectedly the plant fail to fight against the infestation. Moreover, Agrios (2005) pointed out that when the temperature is not favorable for the pathogen may trigger the plants to escape.

The study showed a significant genotype isolate effect for disease incidence. This suggested that some pathogen isolates reacted differently with different sesame genotypes. This may be due to differences in then genetic make-up of either sesame genotypes or the pathogen isolates. Other environmental factors could also have contributed towards the observed differences. None of the genotype was immune or resistant to the disease, majority being susceptible. Other studies have also failed to find sesame genotypes immune to Fusarium wilt (El-Bramawy et al., 2001; El-Shazly et al., 1999; Kavak and

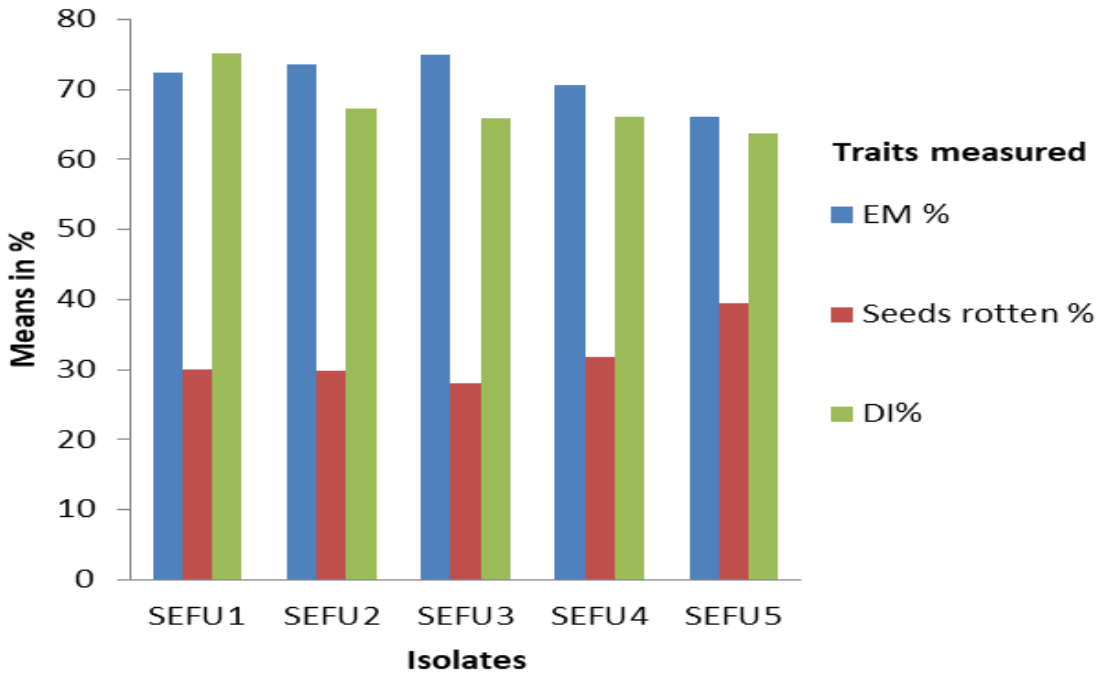


**Figure 4.** Mean performance of isolates in emergence (EM%), seed rot and disease incidence (DI%) against 30 sesame genotypes in the first screening. Least significant difference (LSD at P≤0.05) was 19.3 (EM%), 14.7 (Seed rotten%) and 8.5 (DI%).

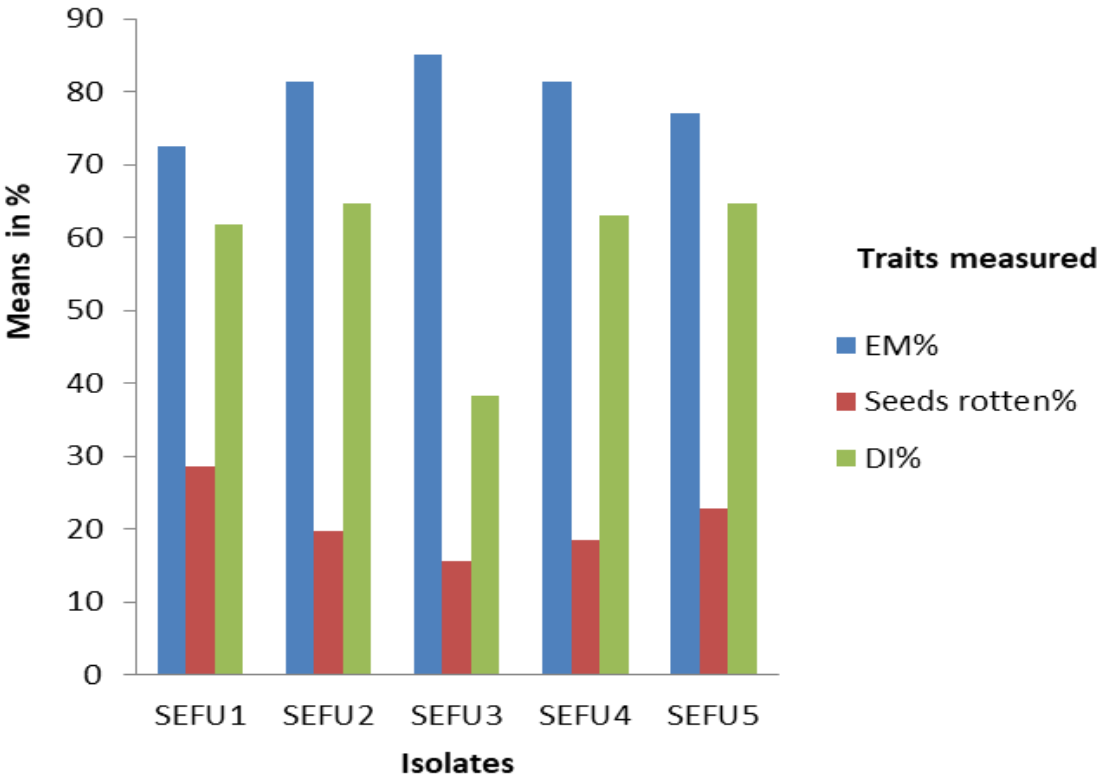


**Figure 5.** Mean performance of isolates in emergence (EM%), seed rot and disease incidence (DI%) against 30 genotypes in the second screening. Least significant difference (LSD at P≤0.05) was 5.3 (EM%), 6.6 (Seed rotten%) and 11.9 (DI%).

Boydak, 2006). Sesim 2 was moderately resistant across the two experiments. Anyanga et al. (2016a) had earlier reported that in addition to Sesim 1, Sesim 2 was resistant to Fusarium wilt. However, this study found Sesim 1 to be susceptible.

The isolate effect was significant for EM%, Seed rot% and DI%. This indicated that isolates were different in aggressiveness towards the genotypes tested. Isolate SEFU1, SEFU2, SEFU4 and SEFU5 were more aggressive compared to SEFU3. Therefore, any of these four isolates (SEFU1, SEFU2, SEFU4 and SEFU5) can be used to screen other sesame germplasm for wilt resistance. However, performance of these isolates differed in the two experiments probably due to differences in soil pathogen population. Several factors influence survival and saprophytic multiplication of fungi in the soil (Agrios, 2005; Stover, 1958; Tyagi and Paudel, 2014). Differences in these factors may have influenced performance of these isolates.

## CONCLUSION AND RECOMMENDATION

The findings of this study indicate that there are sesame genotypes that are moderately resistant to *F. oxysporum* f. sp. *sesami* in Uganda. Two genotypes (EM15-1-5 and Sesim 2) were moderately resistant to *F. oxysporum* f. sp. *sesami*. These genotypes can be used for commercial production as well as breeding activities. The majority of genotypes including Sesim 1 were susceptible to the pathogen however those with good attributes can be improved for resistance to wilt. It is significant for sesame breeding programme in Uganda to continue evaluating other genotypes from existing germplasm which were not tested for resistance to Fusarium wilt in this study. Also, for a long term solution to wilt, more germplasm should be assembled and screened for resistance to the disease, other agronomic traits and reaction to biotic stresses.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Management of the legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) with field applications of the entomopathogenic fungus, *Beauveria bassiana* and a mixed formulation of the baculovirus *Mavi*MNPV with emulsifiable neem oil

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**The present study assessed the effectiveness of the entomopathogen fungus *Beauveria bassiana* in comparison with neem oil combined with the baculovirus *Mavi*MNPV and a synthetic insecticide deltamethrin for the control of *Maruca vitrata* Fabricius under field conditions. The trial was conducted in six villages at Glazoue and Djakotomey, two districts in Benin. Four treatments consisting of (1) untreated control, (2) neem + *Mavi*MNPV, (3) *B. bassiana* and (4) deltamethrin were arranged in a complete randomized block design with three replicates. Reproductive organs of cowpea plants were sampled for estimating population of *M. vitrata*. In spite of the importance between-field variations observed, bio-pesticides significantly reduced the population density of *M. vitrata* as well as its damage level. Similarly, grain yields were improved in sprayed cowpea compared to the untreated control, regardless of the experimental zone. In Glazoue, yields ranged from 724.06 ± 5.04 kg/ha (deltamethrin) to 933.03 ± 8.7 kg/ha (*B. bassiana*). In Djakotomey, 649.1±4.7, 611.07±5.1, 583.19±4.04 and 217.11±3.9 kg/ha were recorded for deltamethrin, *B. bassiana*, neem + baculovirus, and untreated control, respectively. The possibilities of the using bio-pesticides as efficient IPM components in cowpea are discussed.**

**Key words:** Cowpea pests, bio-pesticides, *Beauveria bassiana*, neem, baculovirus MaviMNPV, deltamethrin.

## INTRODUCTION

Cowpea *Vigna unguiculata* L. (Walp) is one of the most important grain legumes in tropical and sub-tropical regions of the world (Singh et al., 2002). It is also a multipurpose crop grown as food and feed. Cowpea is

cultivated worldwide with an estimated annual production of 5.4 million tons with Africa producing nearly 5.2 million (FAOSTAT, 2015). Cowpea provides the cheapest source of protein for human consumption (Ajeigbe and Singh, 2006). Cowpea also is rich in vitamins and minerals. Its leaves are also consumed as fresh vegetables, while the plant after harvest is a valuable source of fodder for cattle. The plant tolerates drought, performs well in a wide variety of soils, and being a legume, it replenishes low fertility soils through deposited organic matter. Also, legumes play a key role in soil fertility improvement through biological nitrogen fixation (Ajeigbe and Singh, 2006). Cowpea is grown mainly by small-scale farmers in developing regions.

In Benin, cowpea is the most cultivated and consumed grain legume. In 2014, about 93488 tons of cowpeas were produced in Benin on 115,000 ha (FAOSTAT, 2015). It plays a role in human nutrition especially in rural areas characterized by unbalanced diet. However, the production is low and only 6.12 Kg of cowpea grain were supplied per capaiata per year (FAOSTAT, 2015). Indeed, various constraints seriously limit cowpea production. Of these, biotic constraints namely damage by insect pests remain the most important affecting both yield and quality of the harvested products (Egho, 2010). Of these, the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera, Crambidae) is reported to cause serious damage on cowpea. The incidence of larvae of the pest is all the more serious as each larvae attacks several organs of the same plant: Flowers, leaves, flower buds and green pods before migrating to another plant (Liao and Liu, 2000). The caterpillars destroy both cowpea flowers and pods causing yield losses up to 80% (Tamò et al., 2003). Indeed, its larvae, brown, yellow or light green at hatching, darken as they pass from one stage to another. They are hairy, blackheaded with dark brown spots on the body (Datinon, 2005). Several methods have been developed or tested against *M. vitrata* with chemical pesticides being the most used control means (Jackai, 1995). Chemicals application is no more attractive considering the so many side effects including environmental pollution and human health hazards, pest resistance and resurgence, secondary pest outbreaks and loss of biodiversity (Ekesi et al., 2002; Atachi et al., 2007). Alternative control methods such as host plant resistance and cultural control practices were not much effective to keep insect pests populations below economic thresholds. Investigation on cowpea selection did not yield in lines demonstrating a satisfactory level of resistance to the pod. Only *Vigna vexillata* (L.) A. Rich, a wild *Vigna* was identified as highly resistant to *M. vitrata.* Although *V. vexillata* is close to cowpea, it was not possible to transfer the desirable genes into cultivated cowpea varieties because of a strong cross-incompatibility at both pre and post-fertilization levels. Also, various cultural practices have been tested but required additional chemical application to achieve efficient control (Oso and Falade, 2010). Hence, the only alternative to overcome damage by the cowpea pod borer *M. vitrata* remains biological control. It consists of using various living organisms such as predators, parasitoids and entomopathogens to control pests while maintaining biodiversity and reducing production costs (Scholz et al., 1998; Girling, 1992). Among the entomopathogenic organisms identified for *M. vitrata,* the virus *Maruca vitrata* multiple nucleopolyhedrovirus (*Mavi*MNPV) was highly specific to *M.* vitrata (Lee et al., 2007). It was isolated from dead *M. vitrata* larvae in Taiwan, and was tested with success in West Africa against *M. vitrata* (Lee et al., 2007; Tamo et al., 2010; Sokame et al., 2015; Muhammad et al., 2017). Likewise the entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Hypocréales: Ophiocordycipitacea) are known for their ability to infect a wide range of insects including *M. vitrata* (Srinivasan et al., 2014). In fact, the *Beauveria* genus is mainly based on the special mode of conidial formation. Phialides carrying conidia are elongated sometimes spherical (Tanada and Kaya, 1993). In Africa and particularly in Benin, *B. bassiana* has been used to infect a wide range of insects in the field (Godonou et al., 2009; Douro Kpindou et al., 2011). Preliminary studies conducted in the laboratories of the International Institute of Tropical Agriculture (IITA-Benin) on the third and fourth instars of *M. vitrata* using the entomopathogenic fungi, *B. bassiana* (isolate Bb115) have shown that they are effective at various doses on the pest (Douro Kpindou et al., 2011; Toffa Mehinto et al., 2014a). The effect of *B. bassiana* was compared with that of Decis, a chemical pesticide commonly used in cowpea production in Benin and Neem + Virus already used in the biological control of cowpea pests (Sokame et al., 2015).

This study aims to assess the effectiveness of the entomopathogen fungus *B. bassiana* in comparison with the combination of neem oil and the virus *Mavi*MNPV as alternative to synthetic chemicals in the context of pests' management in cowpea.

## MATERIAL AND METHODS

### Experimental sites

The works described below were conducted at the laboratory

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of insect pathology of the International Institute of Tropical Agriculture (IITA), Benin Station (6°28N and 2°21E, 15 m altitude), near Cotonou, Benin, in climate chamber with a mean temperature of 26 ± 0.50°C and a relative humidity of 65.5 ± 5%.

The experiments were conducted also in two agro ecological zones of Central (Glazoue/Ouedeme) and Southern Benin (Djakotomey/Djakotomey 1). Sites that hosted the experiments were used to grow cowpea with different cropping systems. The climate at both sites is of Sudano-Guinean or subequatorial type, with two dry seasons alternating with two rainy seasons. The average rainfall ranged between 700 and 1100 mm per year at Djakotomey with temperature of 23 to 31.2°C. But at Glazoue, the annual rainfall varies between 960 and 1256 mm and the mean temperatures between 24 and 29°C (Capo-Chichi and Guidibi, 2006).

**Plant material**

The cowpea variety "Wankoun" and "Sakaouga", were used in the different experiments in the agro ecological zones of Central (Glazoue) and southern Benin (Djakotomey 1), respectively. The variety "Wankoun" is the common cultivated one at Galazoue while "Sakaouga" is the dominant variety at Djakotomey. Both varieties are semi-erect varieties with a development cycle lasting for 65 to 70 days.

**Bio-pesticides and synthetic pesticide**

The control agents used were the virus *Maruca vitrata* Multiple nucleopolyhedrovirus (*Mavi*MNPV) combined with the botanic pesticide “TopBio” (with neem oil (azadirachtin). The “TopBio” as main component and trace essential oil such as citronellal, citronellol, geraniol and nimbin from lemongrass); Topbio was purchased at BioPhyto-Collines, an artisanal plant extract factory in Benin. Also, the biopesticide of the entomopathogenic fungus *B. bassiana* isolate (Bb 115) and the chemical pesticide Deltamethrin, Decis (12.5 EC) was used. Colonies of *B. bassiana* was mass produced from dried conidia in a stock culture at IITA Benin. Conidia viability after 24 h incubation on Potato Dextrose Agar (PDA) was 95%. Chemical pesticide included in the present study was that applied by producers in the study area. The virus *Mavi*MNPV, a baculovirus was obtained from a stock culture at IITA Benin. The virus was introduced in Benin through a collaborative research between IITA-Benin and “World Vegetable Center (WorldVeg)”, in Taiwan. The virus was isolated from infected larvae of *M. vitrata* on “*Sesbania Pea* (*Sesbania cannabina*)” in Taiwan (Lee et al., 2007, Chen et al., 2008).

Indeed, authors such as Gouissi (2013) and Sokame et al. (2015) found that the formulation Neem + virus is more effective on *M. vitrata* than the two products used individually.

**Experimental set up**

Experiments were set up at the two experimental site:

1. In three villages of Glazoue/Ouedeme: Dogbo (08° 00 786’ N, 002° 08 198’E, 591m Alt), Gbaglavigo (08° 02 558’N, 002° 09 190’E, 682m Alt) and Allenoudji (08° 01 406’N, 002° 10 760’E, 674 m Alt) where the villages were considered as replications.
2. In three villages of Djakotomey/Djakotomey 1: Bitouhoue (N 06° 53 649’N, 001° 41 689’E, 153 m Altitude), Atchouhoue (06° 52 507’ N, 001° 41 919’E, 137m Altitude) and Hounkemey (06° 52 357’ N, 001°41 142’ E, 142 m Altitude) where the villages were considered as repetitions.

**Farmers' fields**

Six cowpea producers were selected from each of six sub-municipalities of Glazoue (Ouedeme) and Djakotomey (Djakotomey 1). The field experiments consisted of four treatments: Untreated control (no pesticide), cowpea treated with Topbio (neem) + virus (*Mavi*MNPV), cowpea treated with Deltamethrin and cowpea treated with *B. bassiana* (isolate Bb 115). Each treatment was repeated three times in a randomized complete block design (RCB) with experimental units of 100 $m^2$ (10 m × 10 m). Cowpea was sown at 75 cm × 25 cm. Plants were weeded twice. Maize was planted in alleys to avoid interference between treatments. Biopesticide was applied at a rate of 75 g conidia (active ingredient)/ha which corresponds to a quantity of $0{,}78 \times 10^{13}$ conidia/ha and the application volume of biopesticides was two litres per hectare. The dose of *Mavi*MNPV virus used was $1.6 \times 10^{11}$ OB of virus which is combined against one litre of neem (Topbio). The recommended application rate used in chemical pesticides plots was one litre per hectare for Decis. Plants were treated at the $32^{nd}$, $39^{th}$, $43^{th}$, $49^{st}$ and $30^{th}$, $37^{th}$, $41^{st}$ and $47^{th}$ days after planting (DAP) in Glazoue and Djakotomey, respectively. The different treatment dates are chosen according to the vegetative cycle of each cowpea variety used in each study area. Plant treatment started at the onset of flowering (flower buds stages), a period of *M. vitrata* infestation. All applications were done using a sprayer SWISSMEX-8l”. Temperatures and the mean relative humidity (± SE) recorded during applications were as follows: Tmin = 25.2 ± 0.2°C; Tmax = 31.7 ± 0.5°C; RHmin = 63.3 ± 3.1% and RHmax = 93.9 ± 2.1%.

**Assessment of *M. vitrata* population density**

Samples of 10 plants were weekly selected randomly per treatment to assess *M. vitrata* population. Flowers and pods were collected from each plant and stored in boxes containing 65% alcohol before treatment (Gouissi, 2013). So, *M. vitrata* were identified and dead or alive larvae were counted four days after treatment. Larvae were put individually in a 3.8 cm × 2.9 cm × 4.0 cm boxes with perforated cover for aeration. Cadavers were collected daily. They were put in Petri dishes (Ø = 9 cm) for 24 h to be dried out, and incubated in Petri dishes containing wet filter paper. The number of alive and dead larvae was recorded.

**Assessment of damage on the reproductive organs (flowers and pods)**

This parameter was determined weekly from the rows. Damage of reproductive organs (organs stung, rotten) was assessed weekly. The presence of *M. vitrata* was checked four times ($42^{nd}$ DAP for flowers and $48^{th}$ DAP for pods). Thus, ten flowers and/or 10 pods were randomly sampled per treatment.

Holes on flowers or pods and presence of *M. vitrata* larvae were recorded to estimate its damage index.

**Weather conditions**

The environment factors such as rainfall, temperature and relative humidity were recorded during the experiments. The rainfall was well distributed with minor variations. The months of May and June were the wettest with 108.2 and 94.2 mm, respectively in the central zone (Glazoue) and 192.2 and 101.7 mm, respectively in the southern zone (Djakotomey). In Glazoue, the maximum temperature averaging 28.9 ± 3.2°C was recorded in April (beginning of the experiments). It has decreased in July, the period of cowpea maturity.

**Cowpea yield assessment**

Yield was estimated at 65-70 days after planting (pods maturity period) by harvesting cowpea in three delimited areas (quadrants) similar to those used for *M. vitrata* population estimation. Quadrants consisted of 1 $m^2$ with 10 cowpea plants. Pods harvested on the 10 plants were kept in polyethylene bags and labelled according to treatments. Pods were dried by means of sunlight for 3 days and then shelled with hands. Grains in each treatment were weighed with triple beam balance (Mettler PJ 300).

**Statistical analysis**

Effects of synthetic pesticide (Decis), neem, *Mavi*MNPV virus and *Beauveria bassiana* suspension on the population and damage (%) by *M. vitrata* on different reproductive organs were compared by performing ANOVA using SAS software followed by the test of Student-Newman-Keuls. Percent data (larval mortality rate, sporulation rate of dead larvae) in the different treatments were log-transformed [log (x +1)] prior to the analysis.

## RESULTS

### Effect of *B. bassiana,* neem oil combined with MaviMNPV on *M. vitrata* population

At Glazoue, the different products namely *B. bassiana,* Decis and neem+MaviMNPV significantly reduced *M. vitrata* larval density compared to untreated control ($F$=10.61; $P$ < 0.0001). Indeed, shortly after the first application the average number of *M. vitrata* larvae was lower (0.13±0.6 larvae/plant) in plants treated with Deltamethrin than those treated with biopesticides. Then after the third application, this number slightly increased in Decis treatment (0.9±0.4 larvae/plant) at 46 days after planting (DAP). From this same DAP (46 DAP), the mean number recorded in biopesticide treatments decreased progressively. Despite their slow action, the bio products particularly *B. bassiana* significantly reduced the larval population over the period of this trial (Figure 1A).

At Djakotomey, the different products tested showed comparable efficacy over time. However, from 42 DAP, the number of *M. vitrata* larvae significantly increased for all tested products and reached at the end of the experiments, 1.5 ± 0.04; 2.3 ± 0.01 and 2.5 ± 0.02 larvae for Deltamethrin, *B. bassiana* and Neem + *Mavi*MNPV, respectively at 53 DAP (Figure 1B). The curve describing the fluctuation of *M. vitrata* larvae in untreated control was above those that describe the fluctuation in treated cowpea (Figure 1B).

In contrast to Glazoue (Ouedeme), the average number of *M. vitrata* larvae did not decrease at 46 DAP but showed a constant trend till the end of the experiment. However, significant differences were observed between the different products applied (F=2.31; P = 0. 1361).

There were significant interactions between the different pesticides used and the agro-ecological zones pointing at a sharp link between the number of alive *M. vitrata* larvae and the agro-ecological zone ($F$= 1.78; $P$ = 0.0363).

### Effect of synthetic chemical and bio-pesticides on *M. vitrata* population and damage to fruiting organs in cowpea producers' fields

At Glazoue, for all pesticides used, the damage was heavier on the pods than on the flower. These ranged from 9.2±3.5% (Neem+*Mavi*MNPV, flower buds) to 13.7±2.1% (Neem+*Mavi*MNPV, pods) (Table 1). However, statistical analysis reveals significant differences between treatment for the percent damage in the reproductive organs and controls at Glazoue ($F$ =13.16, P= 0.0018; $F$ =2.84, P=0.0051 for flowers and pods, respectively).

The presence of *M. vitrata* in reproductive organs was highly variable (Table 1). The average number of *M. vitrata* ranged from 0.08 ± 0.02 (Deltamethrin) to 0.97±0.08/plant (Neem+*Mavi*MNPV) for flowers and 0.02±0.01 (Deltamethrin) to 0.18±0.3/plant (Neem+*Mavi*MNPV) for the pods. Pesticides have better controlled the pest. Significant differences were observed between the different products applied and untreated control for the average number of *M. vitrata* larvae recorded on flowers and pods (Table 1).

At Djakotomey, the number of *M. vitrata* larvae recorded on flowers was lower in Decis-treated plants compared to untreated control. Furthermore, statistical analysis reveals significant differences between treatment for the percent damage in the reproductive organs and controls ($F$=6.38, $P$=0.0024; $F$=2.37, $P$=0.0374 for flowers and pods respectively) (Table 1). Thus, compared to untreated control, *B. bassiana* significantly reduced the damage level in pods while the combination of neem with *Mavi*MNPV treatment significantly reduced the damage to flowers (Table 1).

Moreover, the monitoring and incubation of dead larvae collected from field revealed that only larvae collected from plots treated with the fungus *B. bassiana* have sporulated.

### Yields in cowpea producers' field

The overall grain yield was improved in treated cowpea compared to the untreated control, regardless of the experimental zone (Figure 2). At Glazoue, yields ranged from 858.1 ± 5.04 kg/ha (Deltamethrin) to 1012.03 ± 8.7 kg/ha (*B. bassiana*). The performance of *B. bassiana* was significantly better than those of other treatments ($F$ = 13.62, $P$ = 0.0002). Unlike at Glazoue, yields ranged from 217.2±3.04 Kg/ha (untreated control) to 649.7±5.2 Kg/ha (Deltamethrin) at Djakotomey (Figure 2). So, the performance of Deltamethrin was better than those of other treatments. However, all treatments had significantly similar yields.

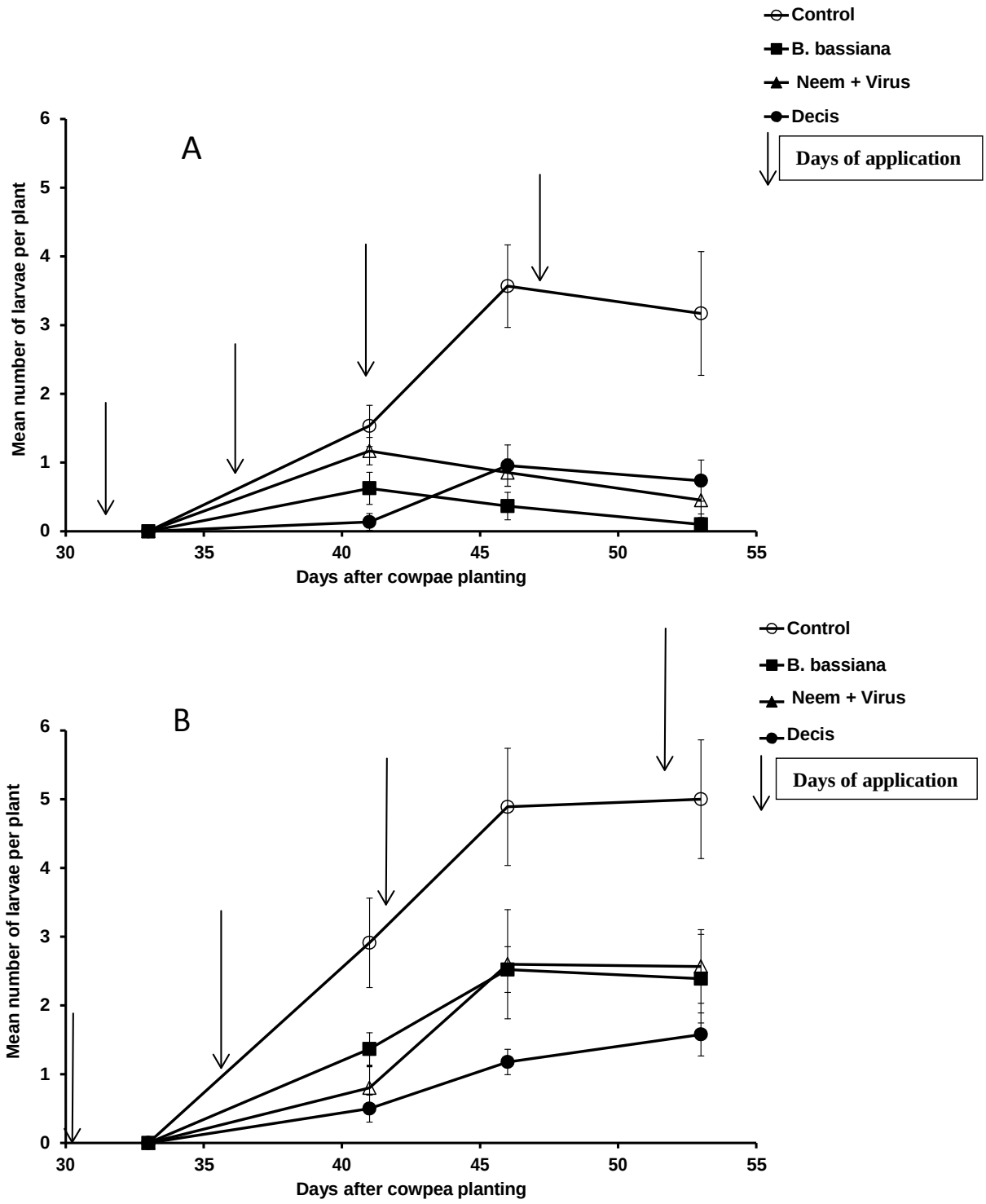


**Figure 1.** Fluctuation of *M. vitrata* population density under the influence of chemical and biological pesticides in central (A: Glazoue) and southern (B: Djakotomey) zones at Benin.

## DISCUSSION

Both the biopesticides and synthetic pesticide used in this study have significantly reduced the population of *M. vitrata* compared to untreated control. So, during the late cropping season, all the major insect pests were encountered in the study areas - an observation confirming, that reported by Jackai and Raulston (1988) according to which the major cowpea insect pests occur wherever the crop is cultivated.

**Table 1.** Effect of the synthetic pesticide (Decis (deltamethrin)), the combined formulation of a neem-based botanical pesticide Topbio with the baculovirus *Mavi*MNPV, and the formulation of the entomopatogenic fungus *Beauveria bassiana* on the population and damage (%) by the legume pod borer *Maruca vitrata* on different cowpea reproductive structures in central (Glazoue) and southern (Djakotomey) zones at Benin.

| Zone | Treatment | Flower | | Pods | |
|---|---|---|---|---|---|
| | | **Percent damage | * *M. vitrata* larvae | **Percent damage | * *M. vitrata* larvae |
| Central zone | Untreated control | 66.5±3.7$^{a}$ | 1.73±0.4$^{a}$ | 17.2±4.8$^{a}$ | 1.94±0.04$^{a}$ |
| | Decis | 12.±2.4$^{b}$ | 0.08±0.02c | 11.8±3.6$^{b}$ | 0.01$^{b}$ |
| | Neem+ | 9.2±3.5$^{b}$ | 0.97±0.08$^{b}$ | 13.7±2.1$^{b}$ | 0.18±0.3$^{b}$ |
| | *B. bassiana* | 11.2±4.1$^{b}$ | 0.12±0.02$^{b}$c | 8.3±2.7$^{b}$ | 0.02$^{b}$ |
| Southern zone | Untreated control | 69.5±6.1$^{a}$ | 3.04±0.6$^{a}$ | 33.5± 2.1$^{a}$ | 3.82±1.0$^{a}$ |
| | Decis | 12.6±2.2$^{b}$ | 0.16±0.07c | 11.8±1.1$^{b}$ | 1.44±0.1$^{b}$ |
| | Neem+ | 13.5±1.4$^{b}$ | 0.32±0.1$^{b}$ | 14.9±1.9$^{ab}$ | 2.11±0.7$^{ab}$ |
| | *B. bassiana* | 14.9±3.5$^{b}$ | 0.31±0.1$^{b}$ | 8.9±1.7$^{b}$ | 2.07± 0.9$^{ab}$ |

*Average number per plots ± SE (calculated on the basis of 3 replicates of 4 observations and on 10 organs); ** Average rates of flowers or damaged pods ± SE (calculated on the basis of 3 replicates of 4 observations and on 10 organs); Mv = *M. vitrata;* Means in the same column followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK).

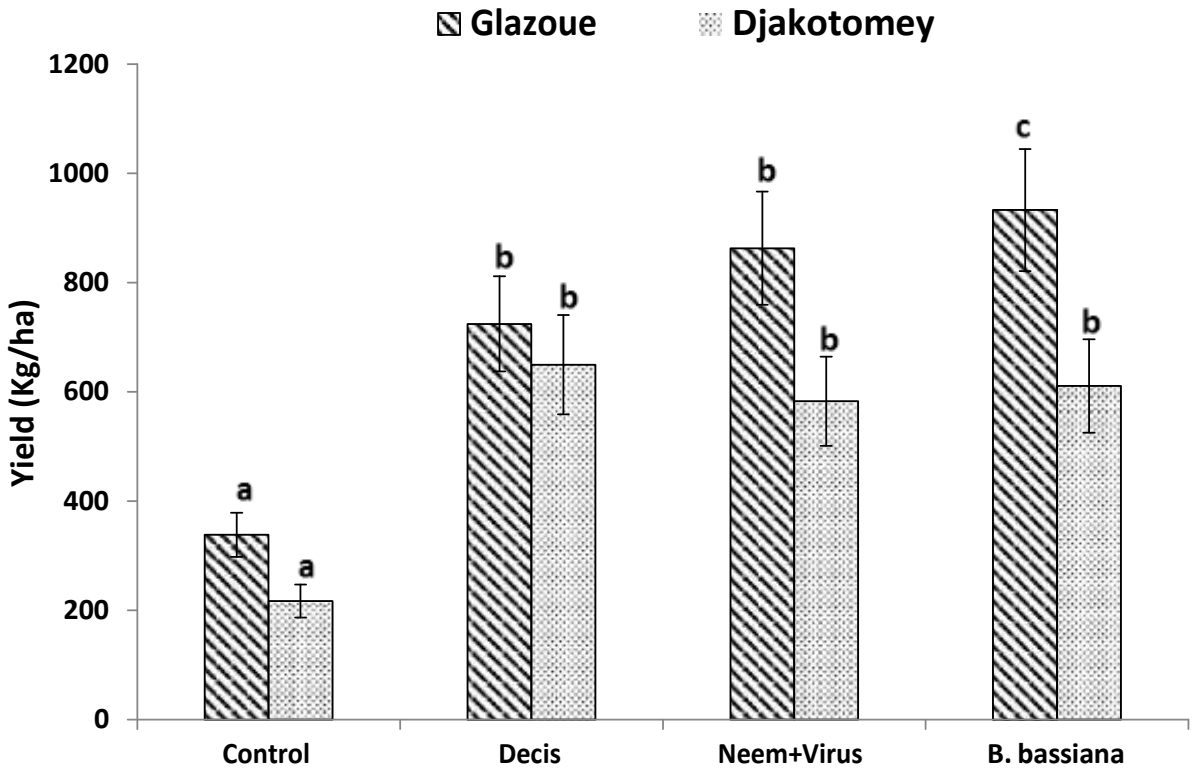


**Figure 2.** Cowpea yields (kg/ha) in central (Glazoue) and southern (Djakotomey) zones in Benin.

The degree of pest control results in a damage reduction on reproductive organs (flowers and pods). In addition, the synthetic insecticide rapidly reduced larval density than did bio-insecticides. The occurrence and distribution of insect species in this study in the two locations (central and southern zones) followed different trends. So, the chemical pesticide had reduced more rapidly larval density than the biopesticides, due to its broad-spectrum action. This insecticide protected old and new leaves including those that were not present during the application. Under the conditions of proper spraying, larval mortality was observed 2 days after application.

These results confirm studies by Atachi and Sourokou (1989) and Bognaho (1996) pointing at the effectiveness of Deltamethrin (Decis) on larval stages of cowpea pest. This can probably be explained by resistance of *M. vitrata* to Deltamethrin. Indeed, Ekesi (1999) reported the resistance to several chemical compounds including pyrethroids. However, this needs to be established during future investigations in Benin.

The dose and application volume should adjust to get similar results with the synthetic pesticide for large cowpea plots. The application of *B. bassiana* was slightly more effective than the combination Neem+ *Mavi*MNPV. Indeed, the positive action of *B. bassiana* 115 was confirmed by the sporulation observed in the dead larvae collected in fungus *B. bassiana* treated plots. Similar results were reported by Adanvè (2012) and Toffa Mehinto et al. (2014a, 2014b) who confirmed the pathogenicity and virulence of *B. bassiana* 115 to control *M. vitrata* in the laboratory with infection rates ranging from 43 to 98% between 4 to 10 days after treatment**).** Tumuhaise et al. (2015) also confirmed the effectiveness of both *Metarhizium anisopliae* and *Beauveria bassiana* against *M. vitrata*.

Several authors reported that the genus *Beauveria* attacks a variety of insects (Prior, 1992). The limited action of the combination Neem+MaviMNPV was not consistent with the observation of Gouissi (2013) who reported that Neem+MaviMNPV were effective against *M. vitrata.* This effectiveness was attributed to the repelling properties of azadirachtin, the active matter of Neem and the specificity of *Mavi*MNPV to *M. vitrata* (Honfoga, 2007). Similarly, Gahukar (1988) observed that the effect of the extract of Neem kernels is comparable to treatments with deltamethrin in the control of *H. armigera*'s larvae on groundnut plants. Sokame et al. (2015) found that the formulation Neem + MaviMNPV was more effective.

These differences could be explained not only by specificity in the entomopathogen species but also by the variation in environmental conditions of the study areas. Even both entomopathogenic diseases could be spread through dead larvae, the viral disease is specific to *M. vitrata* larvae, while that of *B. bassiana* would affect any insect species through sporulation of dead larvae. The viral disease may result from viral particles multiplication in infected larvae (Lee et al., 2007), while the fungus *B. bassiana* was reported to affect insects through active body entering (physical deterioration from germinating conidia) and physiological alteration from synthesized enzymes (Liu et al., 2003; Cho et al., 2006). Virulence of *B. bassiana* was reported to depend on its physiological characteristic and enzyme production (Liu et al., 2003). Enzymatic and mechanical pressure enables *B. bassiana* to destroy host cuticle proteins. However, the fungus also synthetizes toxic non-enzymatic compounds such as beauvericin and basianolide that speed the infestation process (Cito et al., 2016). Furthermore, other traits such as conidial viability, germination speed, hyphal growth rate and pathogenicity are influenced by environmental factors namely temperature and relative humidity and thereby the fungal efficacy (Liu et al., 2003). The agro-climatic conditions including rainfall would also explain differences in insects' activity at Glazoue (Central zone) and Djakotomey (southern zone) especially during the major cropping season. Differences between the two agro-ecological zones for the percent damage caused to the reproductive organs could also be explained by climatic factors such as the rainfall. Indeed, in the zone southern, high amount of rainfall was recorded, 48 h after spraying reducing then the efficacy of the bio-pesticides. Furthermore, yields were significantly higher in treated plots compared to the untreated ones at both Glazoue and Djakotomey. These results reflected the infestation and damage rates recorded at the two locations during the period of time covered by the present study. Similar observation has been done by Douro et al. (2013) who demonstrated the effectiveness of entomopathogenic fungi *M. anisopliae* and *B. bassiana* and Neem oil in the integrated management of *Helicoverva armigera* (Hübner) (Lepidoptera: Noctuidae) on cotton. In the absence of control measures, high losses were recorded (Atachi et al., 2007; Djiéto-Lordon et al., 2007). The current yield levels were a bit lower compared to that obtained (1.036 $kg.ha^{-1}$) by Affokpon et al. (2013). This is probably due to soil fertility depletion that characterized the experimental sites. Indeed, the sites were used for several years of cowpea monocropping, system in which the overall damage should be high on reproductive organs. A diagnosis of a mineral earth bar in southern Benin cowpea deficiency was also noted by Amadji and Glitho (2005). So, Balogoun (2012) confirms that the decline in soil fertility is a major problem and a major constraint to agricultural production in Benin. Moreover, Producers claimed higher insect infestations during the great cowpea season in southern zone.

## Conclusion

This study assessed the efficacy of entomopathogenic fungi *B. bassiana* (isolate Bb 115) and Neem + MaviMNPV on *M. vitrata*. Although the great variation in their effects, the biopesticides tested have significantly reduced the population density of *M. vitrata* as well as the damage level. The overall grain yield was improved in treated cowpea compared to the untreated control, regardless of the experimental zone. The current study revealed that biopesticides could be formulated from *B. bassiana* or neem + MaviMNPV as efficient alternative to synthetic insecticides for the management of major insect pests in cowpea.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Relationship between fruit fly (Diptera: Tephritidae) infestation and the physicochemical changes in fresh fruits

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**Infestation of fruit flies (Diptera: Tephritidae) causes physical and chemical changes in fresh fruit. Moreover, each species of fruit may react differently to the injuries caused by oviposition and larva feeding. In this study, we associated fruit fly infestation with physicochemical changes in five fruit species during six storage times. *Ceratitis capitata* (Wiedemann) infestation caused change in peel firmness (PEF), pulp firmness (PUF), pH, titratable acidity (TA) and total soluble solid (TSS) of star fruit (*Averrhoa carambola* L.). It led to changes in PEF, PUF, TA, TSS and weight loss (WL) of guava (*Psidium guajava* L.) and changes in PEF and TA of apple (*Malus domestica* Borkh). Infestation changed PEF, PUF, TA and WL in mango (*Mangifera indica* L.) and PEF, PUF, TA and TSS of tangerine (*Citrus reticulata* Blanco). *C. capitata* infestation caused significant physicochemical changes in fresh fruits. Our results demonstrated a marked loss of fresh fruit quality after four days of fruit fly infestation. This information can help assessment of fresh fruit quality for consumption and processing. We discuss how the relationship between fly/host fruit might influence physicochemical changes in fresh fruits and recommend applied studies to better understand these relationships.**

**Key words:** *Ceratitis capitata*, fruit damage, quality assessment, postharvest fruit

## INTRODUCTION

Fresh fruit have physical and chemical characteristics that best satisfy the sensorial expectations of the consumer. In addition, fresh fruit play an important role in the economy and human nutrition, mainly providing vitamins, fiber and energy (Altendorf, 2019). After harvesting, when the fruit are removed from the plant, the fruit undergo significant physiological changes that can compromise their sensorial quality for consumption and commercial sale (Ares et al., 2009).

The physicochemical characteristics of fresh fruit can be altered by internal and external factors. Internal factors involve changes in metabolic reactions and physiological systems (Chapman et al., 1991; Bashir and Abu-Goukh, 2003), while external factors refer to environmental conditions, such as temperature and relative humidity (Ueda et al., 2000; Plotto et al., 2017),

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diseases and insect attack (Aluja and Liedo, 1986; Umeh et al., 2004).

Infestation of fruit flies (Diptera: Tephritidae) is one of the factors that affects fruit health. Puncture and oviposition of fruit flies, as well as larvae feeding can lead to fruit drop (Keck, 1934; Umeh et al., 2004), accelerated ripening (Keck, 1934; Jayanthi et al., 2015), changes in fruit peel color (Jayanthi et al., 2015), changes in the nutritional composition of juices (Omoloye et al., 2016), pathogen proliferation in fruit peel (Selivon et al., 2002; Engelbrecht et al., 2004; Omoloye et al., 2016) and pulp deterioration (Zart et al., 2010; Jayanthi et al., 2015; Omoloye et al., 2016).

The injuries caused by infestation of fruit flies may impair fruit quality for consumption and commercial sale. However, due to the physicochemical differences of the fruit species (Gonçalves et al., 2012; Hafsi et al., 2016; Plotto et al., 2017) each host species may react differently to the injuries caused by oviposition and larval feeding. In this study, we associated infestation of fruit flies with physicochemical changes in five fruit species and evaluated these changes during the development of immature stages of insects in the laboratory. This information was used to evaluate the quality loss of fresh fruit for consumption and processing.

## MATERIALS AND METHODS

### Pre-test infestation

#### *Insects*

A colony of approximately 7,000 adults of *Ceratitis capitata* (Wiedemann) was developed by rearing them on an artificial diet (Raga et al., 1996). The adults were obtained from the Laboratory of Economic Entomology of the Advanced Research Centre in Plant Protection and Animal Health of the Biological Institute, in Campinas, São Paulo State, Brazil. Adults were kept in cages and fed with a normal diet and water (Raga et al., 2018). Females sexually mature at 8–10 days of age were used for the tests.

#### *Fruit*

Apple (*Malus domestica* Borkh, Rosaceae), guava (*Psidium guajava* L., Myrtaceae), mango (*Mangifera indica* L., Anacardiaceae), orange [*Citrus sinensis* (L.) Osbeck, Rutaceae], star fruit (*Averrhoa carambola* L., Oxalidaceae) and tangerine (*Citrus reticulata* Blanco, Rutaceae) were used. Guava and star fruit were collected directly from a fruit growing field (farm Maracujá, Campinas, SP, Brazil), while apple, mango, orange and tangerine fruit were purchased from a wholesale market (Food Supply Centre of Campinas, SP, Brazil). Fruit selection was based on *C. capitata* host preference (Raga et al., 2011) and on ranking of the most produced and commercialized fruit in the Brazilian market. Fruit with uniformity of maturity, weight, length and diameter were used for each species (Table 1).

#### *Infestation*

An infestation pre-test was performed to determine adequate exposure time of each fruit species to *C. capitata* infestation. This is because, in previous observations, fruits exposed to *C. capitata* infestation for 24 h or more resulted in infestation index greater than 100 puparia per fruit. This high index of infestation could compromise the study due to excessive stress, collapse and accelerated fruit rot. The relationship between the infestation level and acceleration of the maturation process has been described in another study (Díaz-Fleischer and Aluja, 2003).

Prior to infestation, the fruit were washed in sodium hypochlorite solution (0.5% v/v) for fungal and bacterial disinfection, rinsed with distilled water and dried naturally. For infestation, the fruit were individually arranged in 6-L glass cages with 9 cm at the top opening and 15 cm diameter at the bottom. A 500-mL glass jar was placed on the floor of each cage, upon which each piece of fruit was placed for infestation by ten mated females of *C. capitata* for periods of 3, 6, 12 and 24 h of exposure. The top opening of the cage was covered with a plastic cap with micro holes to allow air to pass through. One fruit per glass cage was considered a replica and we used 5 replicates for each fruit exposure time, totaling 20 replicates of each fruit species. The pre-test was performed in a room with conditions regulated to 25 ± 1ºC, 70 ± 10% relative humidity and no photoperiod.

#### *Storage of fruit*

After infestation, the fruit were individually packed in 1-L plastic pots, containing approximately 40 g of vermiculite at the base to allow pupation of *C. capitata*. The pots were covered with voile fabric fastened by a rubber band. The fruit were kept in a room at 25 ± 1°C, 75 ± 5% RH and 12 h of photoperiod. After 15 days, the vermiculite was sieved to count the puparia. The highest value of the infestation index based on number of puparia/fruit determined the ideal time of exposure of each fruit species for oviposition by *C. capitata*.

### Experimental design

The experiment comprised a description of the physical-chemical changes in fruit infested by the *C. capitata* fruit fly. The methods of selection, infestation and storage of fruit were the same as those described in the pre-test. The times chosen for the infestation test were 6 h for star fruit, guava and apple and 12 h for mango and tangerine, based on observations of the pre-test. Orange was excluded from the present experiment due to failure of larval development in the variety ‘Pera’ even after three successive attempts of infestation.

Thirty-two fruits of each species at similar and uniform sizes and maturation (Table 1) were chosen. Twenty fruits of each species were submitted to infestation by *C. capitata* but only 12 of them (those having the most obvious signs of punctures) were selected for evaluation. The 12 unexposed fruits formed the control group (non-infested). The evaluation was conducted in six replicates, being a replica with two fruits of each species. The following variables were evaluated at 48 h intervals for 12 days: peel firmness, pulp firmness, pH, titratable acidity, total soluble solids and weight loss.

Initially, peel firmness, pulp firmness and weight loss were evaluated. Thereafter, the fruits were ground with the peel and pulp (for tangerine, only pulp was ground), and the substrate obtained was used to evaluate pH, titratable acidity and total soluble solids.

#### *pH and titratable acidity*

A calibrated pH meter (DM-20, brand Digimed) was used with a buffer solution at 20°C. For the tests, 10 g of substrate was separated into a 250-mL beaker, and 90 mL of distilled water was

**Table 1.** Characteristics of fruits (N = 20) used in *Ceratitis capitata* (Tephritidae) infestation pre-test and in the experiment involving analysis of physicochemical changes in infested fruits.

| Fruit species | Variety | Length (cm) | Diameter (cm) | Weight (g) | Peel color |
|---|---|---|---|---|---|
| | | Average (Range) | | | |
| **Infestation pre-test** | | | | | |
| *Averrhoa carambola* L. | Malasiana | 11.6 (11.2-12.5) | 6.6 (6.1-7.4) | 120.6 (90-138) | Green |
| *Citrus reticulata* Blanco | Tangor Murcott | 6.9 (6.5-7.2) | 7.3 (7.0-7.7) | 201.8 (170-252) | Yellow and green |
| *Citrus sinensis* (L.) Osbeck | Pera | 7.1 (7.0-7.4) | 7.3 (7.0-7.8) | 175.9 (146-210) | Green and yellow |
| *Malus domestica* Borkh | Gala | 6.9 (6.7-7.1) | 7.4 (7.0-7.6) | 133.1 (124-142) | Red and yellow |
| *Mangifera indica* L. | Tommy Atkins | 12.0 (11.4-12.8) | 9.1 (8.9-9.5) | 548.7 (438-742) | Green |
| *Psidium guajava* L. | Tailandesa | 7.5 (6.9-8.2) | 7.3 (6.8-7.5) | 191.1 (142-240) | Green |
| **Infestation test** | | | | | |
| *Averrhoa carambola* L. | Malasiana | 10.6 (9.8-11.5) | 6.1 (5.1-6.7) | 107.3 (98-144) | Green |
| *Citrus reticulata* Blanco | Tangor Murcott | 6.1 (5.9-6.4) | 7.6 (7.4-8.1) | 202.0 (180-230) | Yellow and green |
| *Malus domestica* Borkh | Gala | 6.5 (5.9-7.5) | 7.2 (6.9-7.7) | 166.0 (150-182) | Red and yellow |
| *Mangifera indica* L. | Tommy Atkins | 10.6 (10.1-11.6) | 9.0 (8.6-9.4) | 437.0 (390-490) | Green and red |
| *Psidium guajava* L. | Tailandesa | 7.5 (7.0-8.3) | 7.2 (6.9-7.9) | 205.0 (174-232) | Green |

added. The beaker was placed on an agitator, and the electrode was immersed into the substrate solution in the beaker for pH measurement. Thereafter, it was titrated with NaOH 0.09772 to reach a pH of 8.1–8.2. The titratable acidity was expressed as a percentage of citric acid.

### *Peel and pulp firmness*

A texture analyser model TA-XT2i (Stable Micro Systems, Godalming, Surrey, England) was used with Texture Expert software for Windows system. Samples were evaluated by the drilling test using a 2-mm probe at a constant speed of 1 mm/s. Pre- and post-test speeds were 1 and 10 mm/s, respectively. The probe penetration distance was selected according to the fruit species analyzed; that is, 10 mm for star fruit, guava and apple and 15 mm for mango and tangerine. We performed ten perforations in each of 2 fruits per fruit species, totalling 20 holes per replica.

The results were expressed in terms of the maximum force (N) measured for peel rupture and pulp region penetration. We used the average force of peel rupture and pulp region penetration to represent the replica.

### *Total soluble solids*

A digital refractometer (Reichert r2i300 of Ametek) was calibrated with distilled water at 20°C. For the analysis, a small amount of milled fruit substrate was used, and was wrapped in cotton and pressed until one or two drops fell into the refractometer prism to perform the reading.

### *Weight loss*

Weight loss (WL) was assessed according to the calculation (Shahkoomahally et al., 2015):

$$\%WL = (W_0 - W_t) / W_0 \times 100$$

where $W_0$ = initial weight, and $W_t$ = fruit weight after six storage times.

### Statistical analysis

The physicochemical variables of infested and non-infested fruits were compared and the descriptive analysis was performed by infestation [infested fruit (yes) or non-infested fruit (no)], comparing values of the mean, standard deviation, minimum, maximum, median and quartiles for each fruit species. To compare the values of variables obtained for infestation (yes or no) and fruit storage time (2, 4, 6, 8, 10 and 12 days), the two-way analysis of variance (two-way ANOVA) was used with a test of the interaction effect between infestation and storage time, followed by the Tukey post-hoc test for multiple comparisons. The variables having non-normal distributions were transformed into ranks in the analyses.

Pearson's correlation was determined between peel firmness, pulp firmness, pH, total soluble solids, titratable acidity and weight loss with fruit storage time (2, 4, 6, 8, 10 and 12 days). The significance level adopted for the tests was 95% (Zhao et al., 2017). For all analyses, SAS System for Windows (Statistical Analysis System) software was used.

## RESULTS

### pH and titratable acidity

*C. capitata* infestation caused changes in pH only in star fruit (Table 2), increasing the values by 29 and 12% in relation to the control at 10 and 12 days after infestation (DAI), respectively. In mango and tangerine, the pH change occurred in the interaction of infestation and storage time. For guava, only storage time influenced the pH. In apple, the pH did not show interactions in any combination of variables.

Titratable acidity was altered by infestation in apple, guava, mango, star fruit and tangerine (Table 2). The

**Table 2.** Two-way ANOVA results for comparison of physicochemical parameters between fruit infested and non-infested and between infestation and storage time.

| Fruit species | Quality requirements | Fruit infested vs. non-infested | Storage time | Infestation vs. storage time |
|---|---|---|---|---|
| *Averrhoa carambola* | pH | $F_{(1, 24)} = 32.95$; $P < 0.001$ | $F_{(5, 24)} = 9.92$; $P < 0.001$ | $F_{(5, 24)} = 8.95$; $P < 0.001$ |
| | Peel firmness | $F_{(1, 228)} = 6.46$; $P = 0.012$ | $F_{(5, 228)} = 74.79$; $P < 0.001$ | $F_{(5, 228)} = 4.51$; $P < 0.001$ |
| | Pulp firmness | $F_{(1, 228)} = 9.47$; $P = 0.002$ | $F_{(5, 228)} = 57.35$; $P < 0.001$ | $F_{(5, 228)} = 8.35$; $P < 0.001$ |
| | Titratable acidity | $F_{(1, 24)} = 8.63$; $P = 0.007$ | $F_{(5, 24)} = 39.77$; $P < 0.001$ | $F_{(5, 24)} = 29.32$; $P < 0.001$ |
| | Total soluble solid | $F_{(1, 24)} = 40.17$; $P < 0.001$ | $F_{(5, 24)} = 55.30$; $P < 0.001$ | $F_{(5, 24)} = 17.60$; $P < 0.001$ |
| | Weight loss | $F_{(1, 12)} = 2.28$; $P = 0.157$ | $F_{(5, 12)} = 16.02$; $P < 0.001$ | $F_{(5, 12)} = 1.25$; $P = 0.346$ |
| *Citrus reticulata* | pH | $F_{(1, 24)} = 0.00$; $P = 0.962$ | $F_{(5, 24)} = 52.80$; $P < 0.001$ | $F_{(5, 24)} = 5.13$; $P = 0.002$ |
| | Peel firmness | $F_{(1, 228)} = 5.81$; $P = 0.017$ | $F_{(5, 228)} = 16.87$; $P < 0.001$ | $F_{(5, 228)} = 5.41$; $P < 0.001$ |
| | Pulp firmness | $F_{(1, 228)} = 16.14$; $P < 0.001$ | $F_{(5, 228)} = 4.31$; $P < 0.001$ | $F_{(5, 228)} = 4.67$; $P < 0.001$ |
| | Titratable acidity | $F_{(1, 24)} = 4.70$; $P = 0.040$ | $F_{(5, 24)} = 63.28$; $P < 0.001$ | $F_{(5, 24)} = 26.47$; $P < 0.001$ |
| | Total soluble solid | $F_{(1, 24)} = 8.11$; $P = 0.009$ | $F_{(5, 24)} = 40.39$; $P < 0.001$ | $F_{(5, 24)} = 27.63$; $P < 0.001$ |
| | Weight loss | $F_{(1, 12)} = 0.55$; $P = 0.474$ | $F_{(5, 12)} = 33.86$; $P < 0.001$ | $F_{(5, 12)} = 0.72$; $P = 0.619$ |
| *Malus domestica* | pH | $F_{(1, 24)} = 1.10$; $P = 0.306$ | $F_{(5, 24)} = 2.77$; $P = 0.051$ | $F_{(5, 24)} = 0.74$; $P = 0.600$ |
| | Peel firmness | $F_{(1, 228)} = 7.87$; $P = 0.006$ | $F_{(5, 228)} = 7.32$; $P < 0.001$ | $F_{(5, 228)} = 4.18$; $P = 0.001$ |
| | Pulp firmness | $F_{(1, 228)} = 1.45$; $P = 0.231$ | $F_{(5, 228)} = 7.80$; $P < 0.001$ | $F_{(5, 228)} = 7.26$; $P < 0.001$ |
| | Titratable acidity | $F_{(1, 24)} = 5.79$; $P = 0.024$ | $F_{(5, 24)} = 21.37$; $P < 0.001$ | $F_{(5, 24)} = 8.04$; $P < 0.001$ |
| | Total soluble solid | $F_{(1, 24)} = 0.63$; $P = 0.433$ | $F_{(5, 24)} = 29.37$; $P < 0.001$ | $F_{(5, 24)} = 5.49$; $P = 0.002$ |
| | Weight loss | $F_{(1, 12)} = 2.82$; $P = 0.119$ | $F_{(5, 12)} = 16.24$; $P < 0.001$ | $F_{(5, 12)} = 5.42$; $P = 0.008$ |
| *Mangifera indica* | pH | $F_{(1, 24)} = 1.71$; $P = 0.204$ | $F_{(5, 24)} = 22.49$; $P < 0.001$ | $F_{(5, 24)} = 10.29$; $P < 0.001$ |
| | Peel firmness | $F_{(1, 228)} = 227.13$; $P < 0.001$ | $F_{(5, 228)} = 138.39$; $P < 0.001$ | $F_{(5, 228)} = 15.84$; $P < 0.001$ |
| | Pulp firmness | $F_{(1, 228)} = 128.20$; $P < 0.001$ | $F_{(5, 228)} = 53.06$; $P < 0.001$ | $F_{(5, 228)} = 5.26$; $P < 0.001$ |
| | Titratable acidity | $F_{(1, 24)} = 219.86$; $P < 0.001$ | $F_{(5, 24)} = 200.86$; $P < 0.001$ | $F_{(5, 24)} = 101.74$; $P < 0.001$ |
| | Total soluble solid | $F_{(1, 24)} = 0.53$; $P = 0.476$ | $F_{(5, 24)} = 46.34$; $P < 0.001$ | $F_{(5, 24)} = 22.93$; $P < 0.001$ |
| | Weight loss | $F_{(1, 12)} = 28.58$; $P < 0.001$ | $F_{(5, 12)} = 56.12$; $P < 0.001$ | $F_{(5, 12)} = 1.36$; $P = 0.305$ |
| *Psidium guajava* | pH | $F_{(1, 24)} = 0.33$; $P = 0.572$ | $F_{(5, 24)} = 3.06$; $P = 0.028$ | $F_{(5, 24)} = 1.50$; $P = 0.227$ |
| | Peel firmness | $F_{(1, 228)} = 44.12$; $P < 0.001$ | $F_{(5, 228)} = 148.66$; $P < 0.001$ | $F_{(5, 228)} = 9.05$; $P < 0.001$ |
| | Pulp firmness | $F_{(1, 228)} = 63.41$; $P < 0.001$ | $F_{(5, 228)} = 65.29$; $P < 0.001$ | $F_{(5, 228)} = 17.75$; $P < 0.001$ |
| | Titratable acidity | $F_{(1, 24)} = 23.83$; $P < 0.001$ | $F_{(5, 24)} = 18.19$; $P < 0.001$ | $F_{(5, 24)} = 27.92$; $P < 0.001$ |
| | Total soluble solid | $F_{(1, 24)} = 16.33$; $P < 0.001$ | $F_{(5, 24)} = 39.07$; $P < 0.001$ | $F_{(5, 24)} = 14.34$; $P < 0.001$ |
| | Weight loss | $F_{(1, 12)} = 9.09$; $P = 0.011$ | $F_{(5, 12)} = 119.73$; $P < 0.001$ | $F_{(5, 12)} = 1.40$; $P = 0.292$ |

acidity decreased by 26% in star fruit, 22% in guava and 8% tangerine at 10 DAI, and 13% in apple at 8 DAI, besides increasing by 50% in mango at 10 DAI.

The behaviour of pH and acidity was different in each fruit species, following a trend of increase, decrease or strong variations. However, for the most fruit, the behaviour of pH (Figure 1) and titratable acidity (Figure 2) varied between infested and non-infested fruit. The pH of infested guava, star fruit, and tangerine increased proportionally during the storage time, whereas the titratable acidity decreased in infested apple, guava, mango, and star fruit over the time (Table 3).

**Peel firmness and pulp firmness**

Peel firmness of apple, guava, mango, star fruit and tangerine were altered due to *C. capitata* infestation (Table 2). Peel firmness decreased by 36, 52, 18, 46 and 20% at 10 DAI for star fruit, guava, apple, mango and tangerine, respectively.

Pulp firmness of guava, mango, star fruit and tangerine were also altered due to infestation (Table 2), whereas in apple, pulp firmness was not changed, although it demonstrated an interaction between infestation and storage time. Reduction of pulp firmness was 46% in star

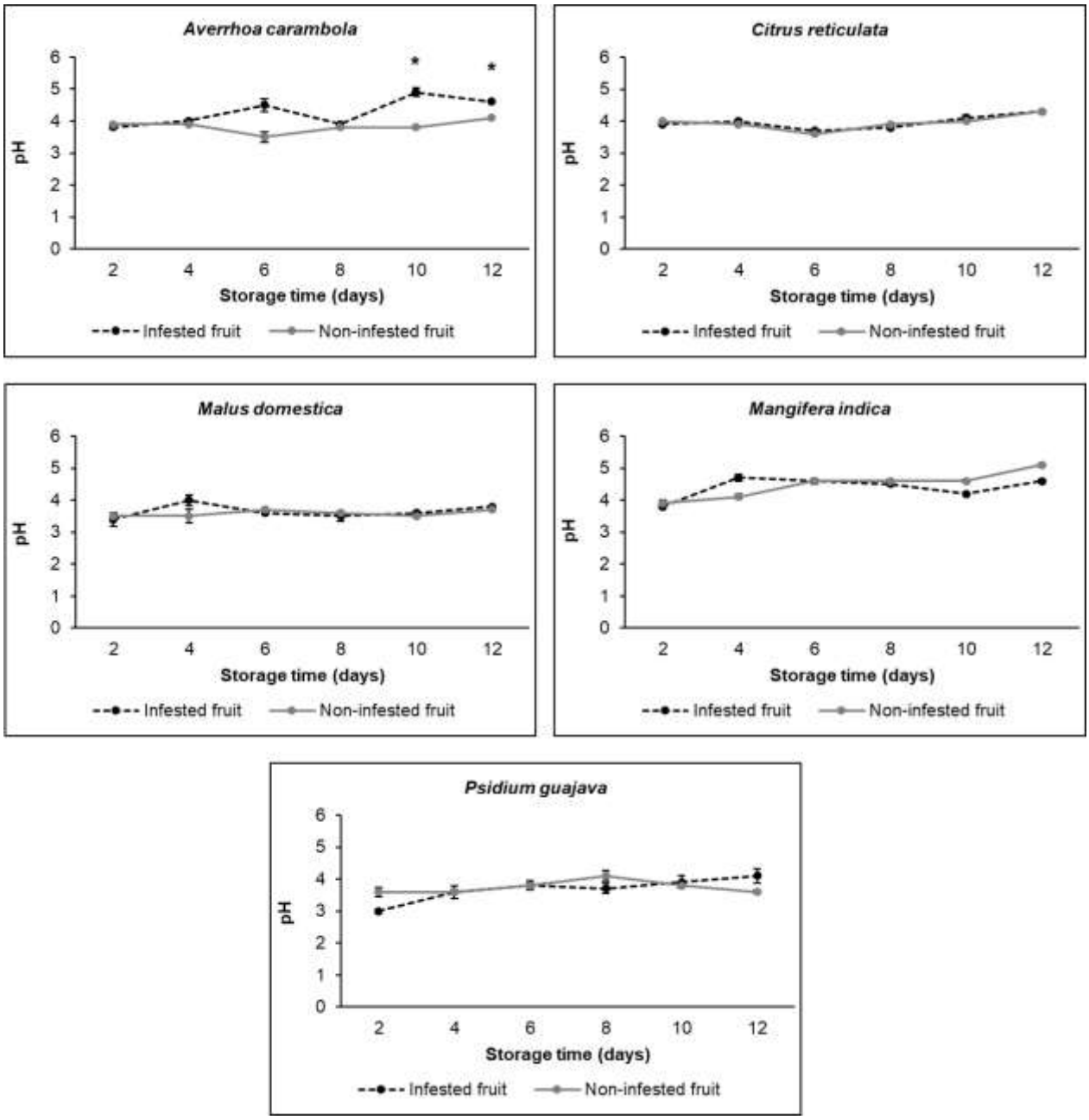


**Figure 1.** Mean values (± standard error) pH of fruit infested and non-infested by *Ceratitis capitata* (Tephritidae) during 12 days of storage. *Represents the storage time when infested and non-infested fruits presented statistical difference by Tukey test at 5%.

fruit, 5% in guava and 54% in mango at 10 DAI and 61% in tangerine at 12 DAI.

The behaviour of peel firmness (Figure 3) and pulp firmness (Figure 4) followed a trend of decreasing with storage time for guava, mango, star fruit and tangerine, but for apple this behaviour was observed only with respect to peel firmness (Table 3). Although peel and pulp firmness of infested and non-infested fruit followed a downward trend with storage time, firmness of infested fruit was lower in comparison with non-infested fruit.

## Total soluble solids

Total soluble solids in guava, star fruit and tangerine were altered due to *C. capitata* infestation (Table 2). Total soluble solids decreased by 30% in star fruit and 11% in guava at 10 DAI and by 8% in tangerine at 12 DAI. For

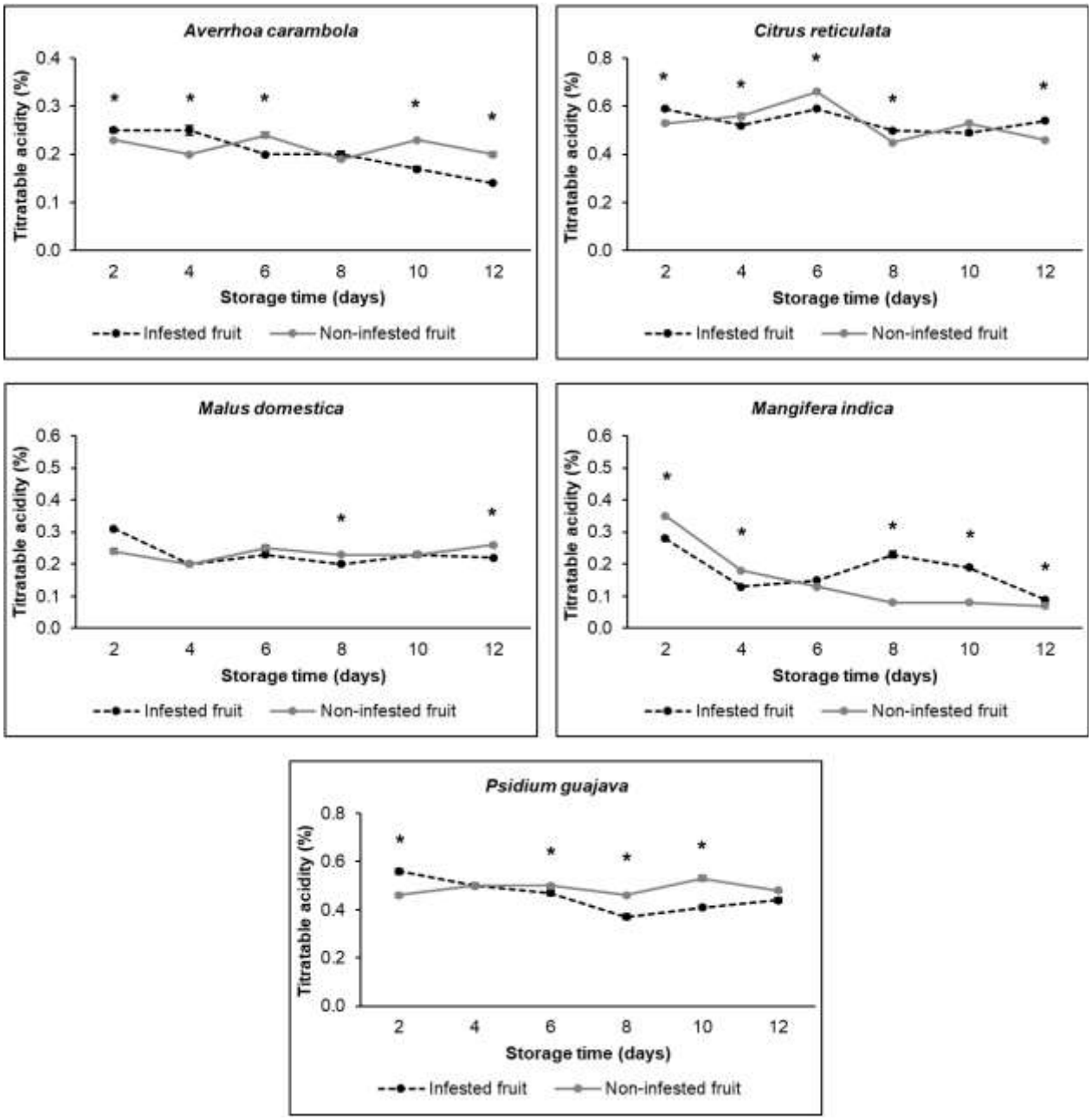


**Figure 2.** Mean values (± standard error) titratable acidity of fruit infested and non-infested by *Ceratitis capitata* (Tephritidae) during 12 days of storage. *Represents the storage time when infested and non-infested fruits presented statistical difference by Tukey test at 5%.

apple and mango, there was an interaction between infestation and storage time.

The behaviour of the total soluble solids was different for each fruit species, following a downward trend for guava, mango and star fruit (Table 3), while for apple and tangerine there was no increasing or decreasing trend, but instead variations among the samples (Figure 5). However, the total soluble solids of infested fruit had a different behaviour from that of non-infested fruit.

## Weight loss

*C. capitata* infestation caused changes in weight loss in guava and mango (Table 2). Weight loss reached 40 and 45% at 8 DAI and 36 and 40% at 10 DAI for guava and mango, respectively. In star fruit and tangerine, only storage time influenced weight loss, regardless of infestation. In apple, there was an interaction between infestation and storage time.

**Table 3.** Pearson's correlation results between storage time and physicochemical parameters of fruits infested and non-infested by *Ceratitis capitata* (Tephritidae).

| Dependent variable | Fruit infested (IN) or non-infested (NI) | *Averrhoa carambola* | | *Citrus reticulata* | | *Malus domestica* | | *Mangifera indica* | | *Psidium guajava* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *r* | *p*-value | *r* | *p*-value | *r* | *p*-value | *r* | *p*-value | *r* | *p*-value |
| pH | IN | 0.7373 | 0.0005 | 0.5849 | 0.0108 | 0.0276 | 0.9133 | 0.3845 | 0.1151 | 0.7568 | 0.0003 |
| | NI | 0.3306 | 0.1802 | 0.4339 | 0.0720 | 0.1107 | 0.6616 | 0.9203 | < 0.0001 | 0.1479 | 0.5578 |
| Peel firmness | IN | -0.7267 | < 0.0001 | -0.4485 | < 0.0001 | -0.2515 | 0.0056 | -0.7381 | < 0.0001 | -0.7547 | < 0.0001 |
| | NI | -0.7381 | < 0.0001 | -0.1571 | 0.0865 | 0.0095 | 0.9177 | -0.6654 | < 0.0001 | -0.7852 | < 0.0001 |
| Pulp firmness | IN | -0.6556 | < 0.0001 | -0.1655 | 0.0708 | -0.0838 | 0.3625 | -0.4942 | < 0.0001 | -0.6133 | < 0.0001 |
| | NI | -0.5541 | < 0.0001 | 0.1774 | 0.0525 | -0.3374 | 0.0002 | -0.5585 | < 0.0001 | -0.7580 | < 0.0001 |
| Titratable acidity | IN | -0.9442 | < 0.0001 | -0.4491 | 0.0615 | -0.5339 | 0.0225 | -0.4968 | 0.0359 | -0.6805 | 0.0019 |
| | NI | -0.3084 | 0.2130 | -0.4408 | 0.0671 | 0.4151 | 0.0867 | -0.8698 | < 0.0001 | 0.2414 | 0.3345 |
| Total soluble solids | IN | -0.5979 | 0.0088 | -0.2631 | 0.2913 | 0.1282 | 0.6121 | -0.7417 | 0.0004 | -0.4755 | 0.0461 |
| | NI | -0.6150 | 0.0066 | 0.2378 | 0.3420 | 0.3674 | 0.1336 | -0.2610 | 0.2955 | -0.1635 | 0.5167 |
| Weight loss | IN | 0.8160 | 0.0012 | 0.9267 | < 0.0001 | 0.8039 | 0.0016 | 0.9313 | < 0.0001 | 0.9424 | < 0.0001 |
| | NI | 0.8945 | < 0.0001 | 0.9588 | < 0.0001 | 0.7833 | 0.0026 | 0.9833 | < 0.0001 | 0.9458 | < 0.0001 |

In apple, guava, mango, star fruit and tangerine, weight loss presented an upward trend in relation to the storage time (Table 3). In infested fruit, weight loss was higher in relation to non-infested fruit (Figure 6). The highest percentages of weight loss were observed from the 6th day of storage onward, when the infested fruit showed 1st instar larvae of *C. capitata*.

## DISCUSSION

*C. capitata* infestation changed the physicochemical composition of apple, guava, mango, star fruit and tangerine. However, each fruit species presented different physicochemical changes and with different intensities between them. It is likely that the physical and chemical differences of the fruit species tested (Plotto et al., 2017), as well as, the interrelationship of *C. capitata* with host fruits may partially explain how each fruit exhibited distinct physicochemical changes during storage time.

The trophic fly/host fruit relationship may also be involved in the different responses of each fruit species because the nutritional quality of fruit for larval development is a factor that determines preference for hosts in polyphagous insects (Thompson, 1988; Danks, 2007), such as *C. capitata* (Liquido et al., 1990). The nutritional quality of fruit for feeding *C. capitata* larvae (Costa et al., 2011; Hafsi et al., 2016) may explain the susceptibility to physicochemical changes in guava, mango, star fruit and tangerine (Raga et al., 2011) and non-susceptibility of apple, which is not considered an appropriate feeding substrate for *C. capitata* larvae (Joachim-Bravo et al., 2001; Follett et al., 2019).

We observed that the behaviour of physicochemical changes caused by *C. capitata* infestation during storage was different in each fruit species. However, this behaviour was directly proportional to the development of immature stages of the fly; that is, small changes when the insect was at the egg stage, until 4 DAI, and significant changes when the larvae began to feed at 4 DAI. The physicochemical changes observed in the first evaluations, until 4 DAI, were caused by puncture and oviposition. When the female makes a puncture, a small opening occurs in the fruit peel. This opening may allow the release of volatiles and consequently promote enzymatic reactions that alter the fruit chemical composition (Plotto et al., 2017). Eggs deposited inside the fruit can cause cell stress, resulting in unexpected metabolic reactions (Omoloye et al., 2016). In addition, at oviposition, females release symbiotic bacteria (Selivon et al., 2002), which help the larvae feed and establish an environment conducive to their development (Díaz-Fleischer and Aluja, 2003). All these phenomena caused by puncture and oviposition of fruit flies are responsible for the first physicochemical changes in infested fruit.

The feeding of larvae, after 4 DAI, caused significant physicochemical changes. In a study on oranges infested by fruit flies in the field, the most significant damage to the enzymatic and metabolic structure of the fruit was also observed from larval feeding (Omoloye et al., 2016). In this study, physical changes caused by larval feeding significantly reduced firmness and accelerated the weight loss process in the fruit. Fruit fly infestation promotes premature fruit maturation (Keck, 1934; Jayanthi et al., 2015), and because the maturation level is directly related to fruit firmness (Messina and Jones, 1990; Plotto et al., 2017), changes caused by larval feeding that

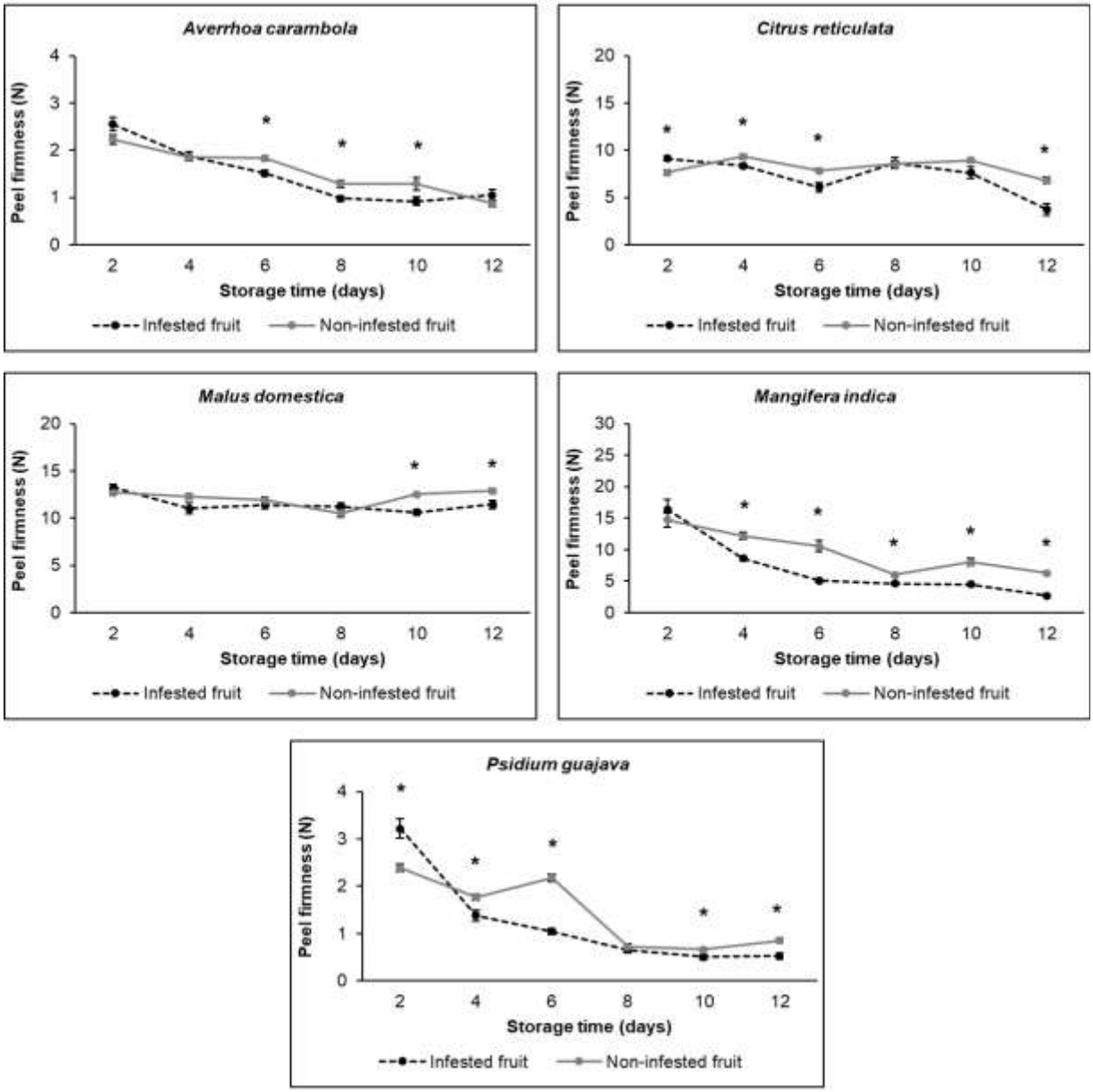


**Figure 3.** Mean values (± standard error) peel firmness of fruit infested and non-infested by *Ceratitis capitata* (Tephritidae) during 12 days of storage. *Represents the storage time when infested and non-infested fruits presented statistical difference by Tukey test at 5%.

promote premature maturation can also influence peel and pulp firmness of the fruit as observed in this study. Fruit weight loss may be related to reduced dry matter content in fruit and increased fluid production due to cell stress (Messina and Jones, 1990; Omoloye et al., 2016). Cell stress is attributed to larval feeding as well as digestive activities of bacteria associated with fruit fly infestation (Omoloye et al., 2016).

Changes in titratable acidity, pH and total soluble solids observed mainly in guava, mango and star fruit may also be related to cell stress caused by larval feeding and extracellular digestive activities of bacteria that degrade nutritional components of fruit (Omoloye et al., 2016). This was observed mainly from the 4th DAI, when the larvae began to feed, suggesting that puncture and oviposition of fruit flies do not alter the acidity, pH and total soluble solids of fruit.

Feeding of the *C. capitata* larvae reduced fruit quality for fresh consumption and caused processing limitations. Losses for fresh consumption are associated with firmness reduction and accelerated maturation (Keck, 1934; Jayanthi et al., 2015); consequently, reduction of

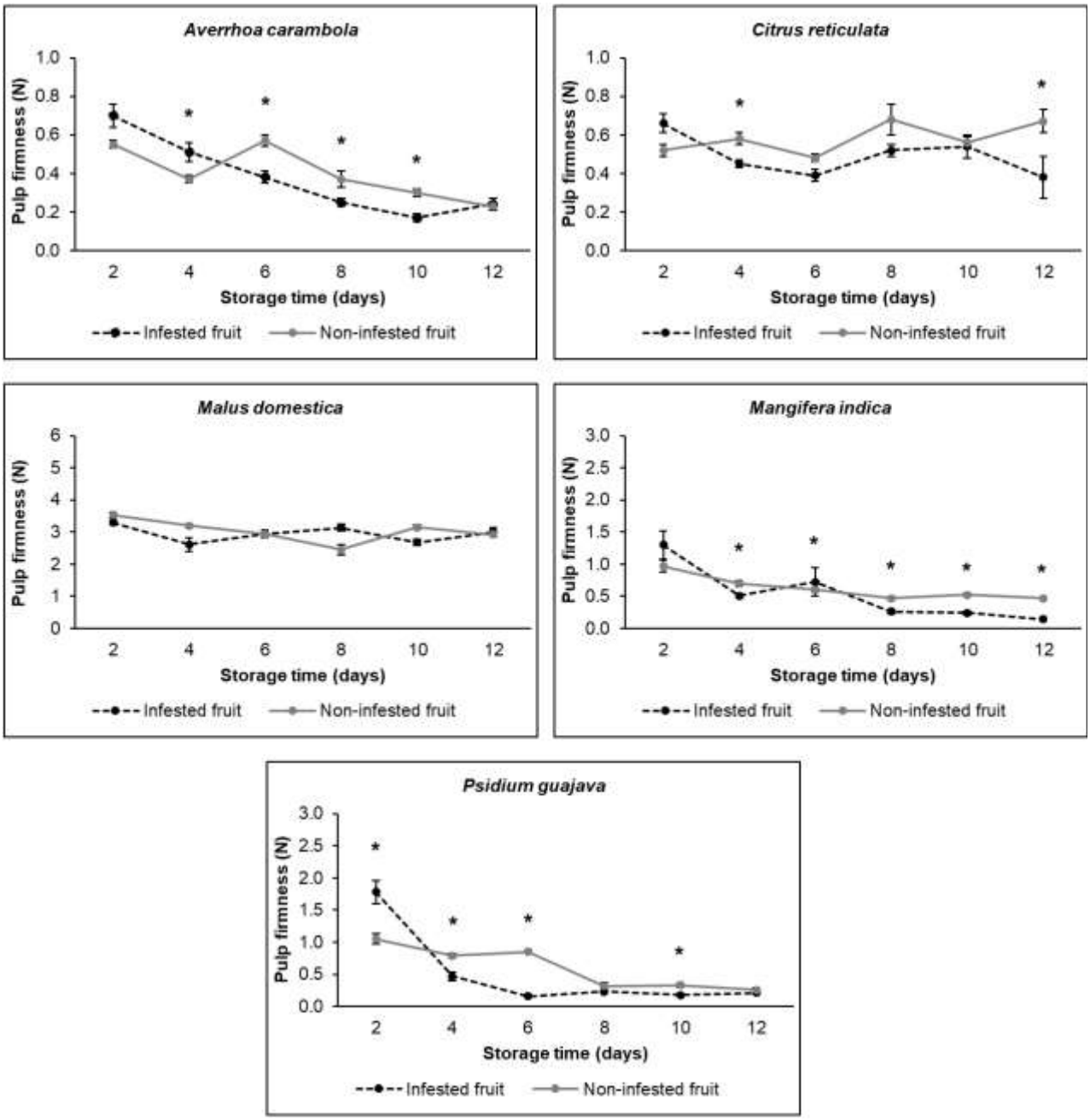


**Figure 4.** Mean values (± standard error) pulp firmness of fruit infested and non-infested by *Ceratitis capitata* (Tephritidae) during 12 days of storage. *Represents the storage time when infested and non-infested fruits presented statistical difference by Tukey test at 5%.

shelf life and changes in fruit flavour result, because infestation affects the ratio sweetness/acid (total soluble solids per titratable acidity) in addition to conferring a bitter taste to citrus juice (Omoloye et al., 2016). Losses in processing are associated with proliferation of microorganisms in the fruit peel and pulp. Moreover, chemical changes caused by *C. capitata* infestation compromised the processing of mango and star fruit to pulp, preserves, and sweets because the pH of these fruit does not comply with processing requirements and must be submitted to pasteurization treatment for preservation (Ministério da Agricultura Pecuária e Abastecimento, 2000, 2002).

## Conclusion

The infestation of medfly *C. capitata* causes physical and

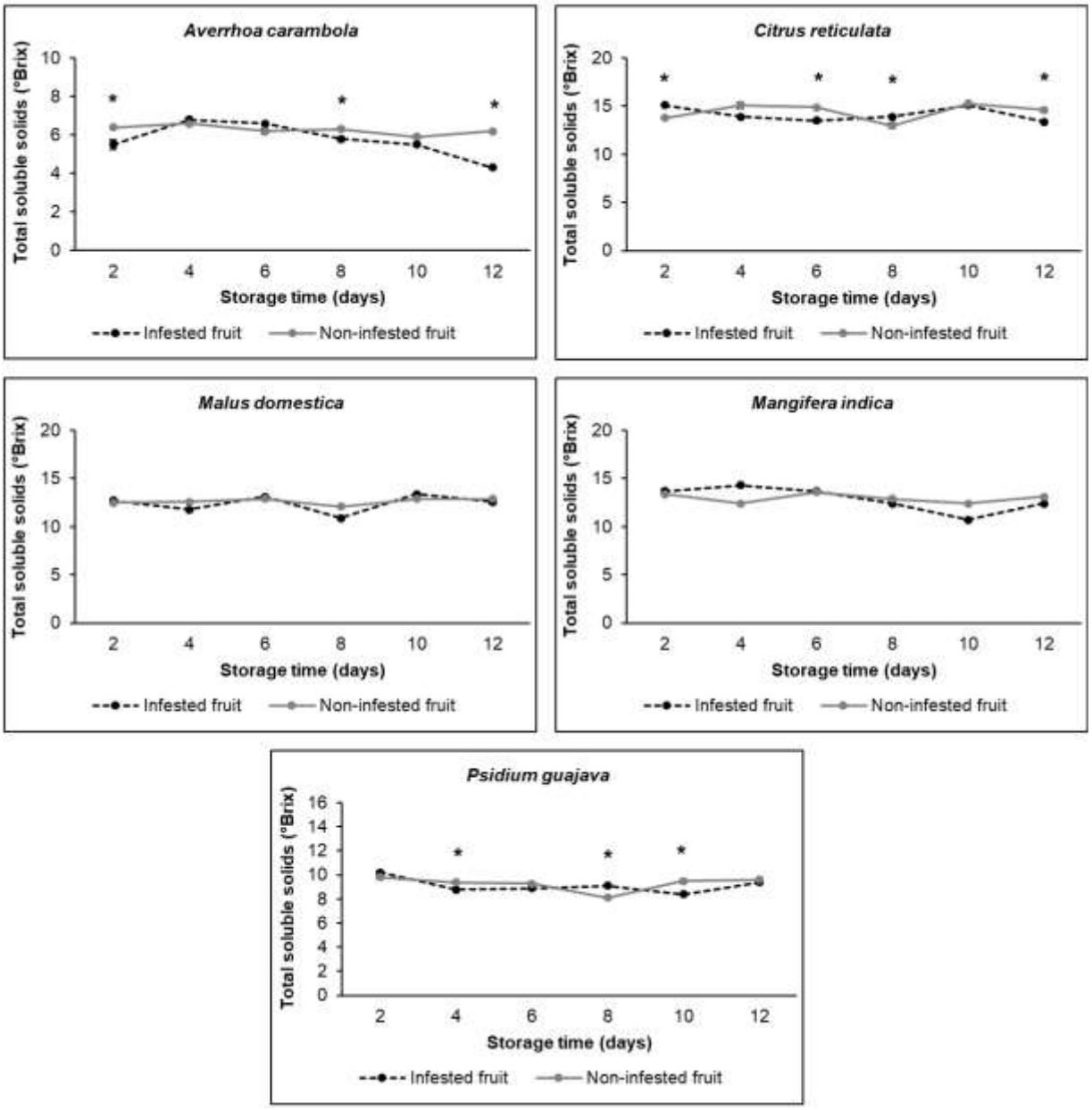


**Figure 5.** Mean values (± standard error) total soluble solids of fruit infested and non-infested by *Ceratitis capitata* (Tephritidae) during 12 days of storage. *Represents the storage time when infested and non-infested fruits presented statistical difference by Tukey test at 5%.

chemical changes in fresh fruit. All species of fruit undergo physicochemical changes caused by infestation of fruit flies. These changes considerably hamper the quality of fresh fruit for consumption and processing. Each fruit species presents distinct changes in peel firmness, pulp firmness, pH, titratable acidity, total soluble solids and weight loss as a specific response to stress caused by puncture, oviposition and feeding of *C. capitata* larvae. The fruits that suffered the most physicochemical changes were those with soft peel (< 4 N) and ratio total soluble solids/titratable acidity between 20 and 30, such as *A. carambola* and *P. guajava*. This can be explained by the intimate fly/host fruit relationship (Costa et al., 2011; Oliveira et al., 2014; Ruiz et al., 2015). Soft-skinned fruits allow for easy penetration of the aculeus, and fruits that provide a good balance of sweet/acid enhance larval development. *A. carambola*, *C. reticulata*, *M. indica* and *P. guajava* are more susceptible than *M. domestica* to the physicochemical changes caused by the infestation of fruit flies, most likely due to preference and adaptation of polyphagous insects to different larval feeding substrates (Thompson, 1988;

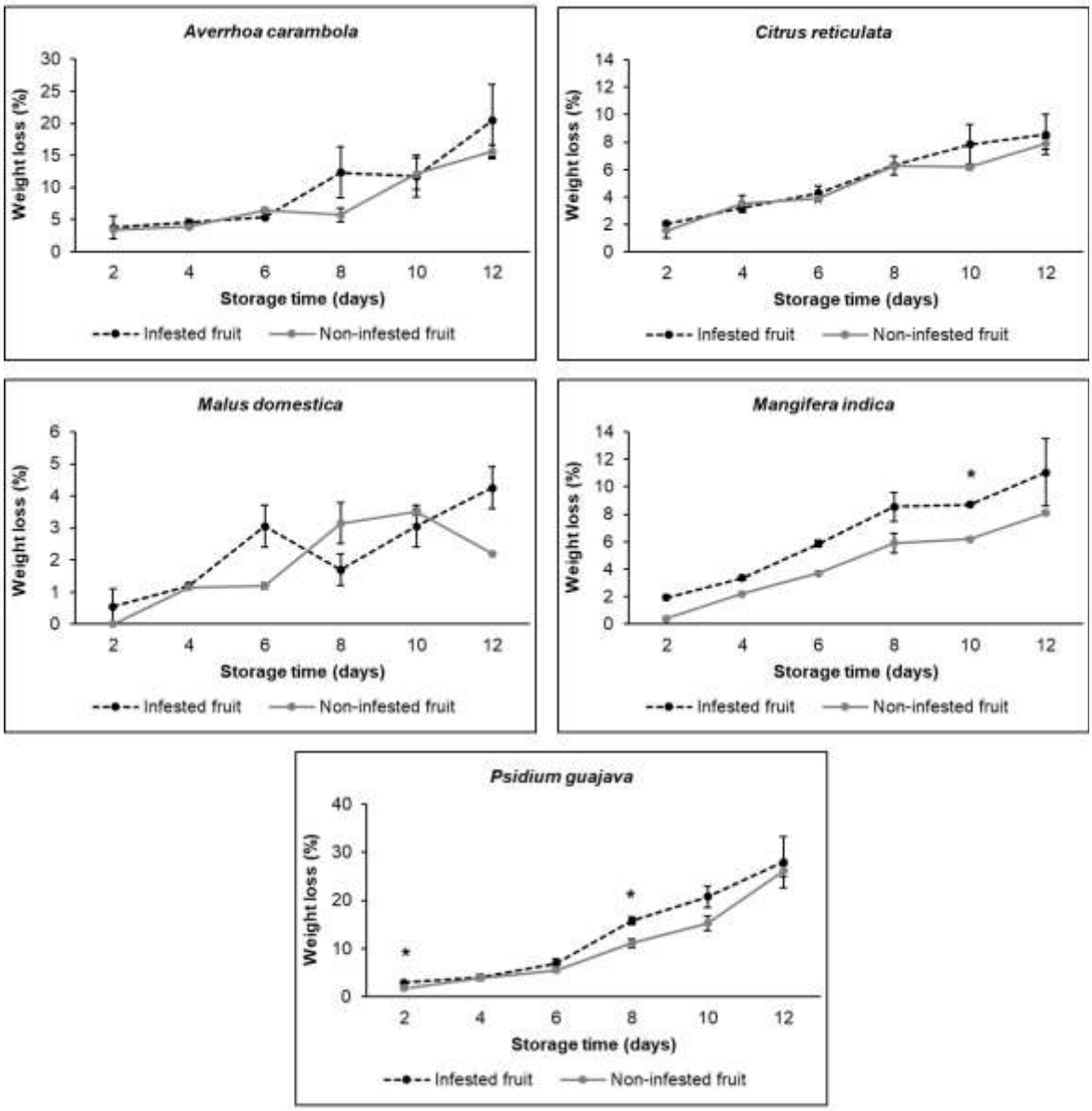


**Figure 6.** Mean values (± standard error) weight loss of fruit infested and non-infested by *Ceratitis capitata* (Tephritidae) during 12 days of storage. *Represents the storage time when infested and non-infested fruits presented statistical difference by Tukey test at 5%.

Danks, 2007). Thus, the preference of larval development substrate and diversity in the physicochemical composition of each fruit species probably influenced fruit status as susceptible or tolerant to changes caused by fruit fly infestation. However, basic studies should be developed to explain how host preference may be associated with the favorable substrate for fruit fly development and its physicochemical changes in fruits.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Inclusion of *Sargassum muticum* and *Parkia biglobosa* in diets for African Catfish (*Clarias gariepinus*) elevates feed utilization, growth and immune parameters

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**The use of antimicrobial agents and antibiotics as remedial measures against fish diseases has been questioned. The huge amounts of antibiotics used in animal husbandry has exerted a very strong selection pressure on the resistance among bacteria, which have adapted to this situation, mainly by a horizontal and philandering flow of resistance genes. Prebiotics, probiotics and symbiotics have been proposed as strategic means to enhance feed utilization, growth and immunity in fish production. In the present trial, a total of 180 fish was divided into 4 groups of 3 replicates each. Each replicate contained 15 fish. The formulated diets were supplemented with prebiotic (*Sargassum muticum*), probiotic (*Parkia biglobosa*) and symbiotics (a combination of *Parkia biglobosa* and *Sargassum muticum*). Efficiency of the inclusion of prebiotics, probiotics and symbiotics in formulated diets was evaluated in African catfish, *Clarias gariepinus* fingerlings (mean weight 2.53±0.05g). Formulated diet was fed 5% body weight to a group of 15 fish (in 3 replica) for 12 weeks, compared to fish fed control pellet containing similar ingredients but was not supplemented. Results showed on the skin of fish fed probiotics diet recorded improved GST and SOD activity and less CAT activity whereas in the liver fish fed prebiotic and symbiotic diet showed improved GST and CAT activity relative to the control. There were significant ($p<0.05$) differences between fish fed the control diet and all treatments (prebiotic, probiotic, symbiotics). It may be concluded based on the results recorded in this study that prebiotics, probiotics and symbiotics supplementation in diets has positive effect on antioxidant enzyme activity in African catfish, *Clarias gariepinus* to improve resistance against bacterial infections.**

**Key words:** African catfish, antioxidant enzyme, feed utilization, growth, diets.

## INTRODUCTION

African catfish is useful in diet of most populace, with optimum nutritional value with complete wide range of

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amino acids, vitamins and minerals (Akinrotimi et al., 2007), and it's a vital source of animal protein for both man and livestock in third world countries. Report from FAO (2014) shows that African catfish contributes above 60% of the world's supply of protein, especially in third world countries.

Though aquaculture is the fastest growing food sector, diseases most especially bacterial infection tends to hold back the expansion of aquaculture (Abd El-rhman et al., 2009; Pieters et al., 2008). Bacterial, viral and other types of diseases are usually treated with antimicrobials. Prevention and control of diseases have led to a substantial increase in the use of veterinary medicines in the recent years. However, the utility of antimicrobial agents and antibiotics as a remedial measure has been questioned. These huge amounts of antibiotics have exerted a very strong selection pressure on the resistance among bacteria which have adapted to this situation, mainly by a horizontal and philandering flow of resistance genes (Cabello et al., 2013; Yousefian and Amiri, 2009; Cabello, 2006). This is transmitted to the consumer and can cause allergy and diseases, cause an imbalance in the intestinal mucosa due to elimination of useful microbes in the gastro intestinal tract (Rombout et al., 2010).

WHO and EU regulations proscribing the use of antimicrobials as growth promoters in animal husbandry has led various researchers to seek alternative methods. Several researches have proposed alternatives methods which include Phytobiotics, Antimicrobial peptides (AMP), Inhibitors for bacterial Quorum sensing (QS), Feed enzymes, immunomodulatory agents, Bacteriophages and their Lysins, Biofilm and virulence, Antibacterial vaccines, Prebiotics, Probiotics and Symbiotics (Antache et al., 2013; Panigrahi and Azad, 2007).

Thus, the use of measures like prebiotics (*S. muticum*), probiotics (*P. biglobosa*) and symbiotics (a combination of *P. biglobosa* and *S. muticum*) has been of great value as alternative therapy in aquaculture, which appears to be strategic biological control and a necessary step for aquaculture practices for enhancing growth and disease resistance (Rombout et al., 2010). Widanarni and Tanbiyaskur (2015) and Fuller (1989) defined probiotic as live microbial feed supplements which are of benefits to the host by improving its intestinal microbial balance. Probiotica also strengthen the host immune system, improve the host's living environment and enhance nutritional value of the feed. Supplementation of probiotic has become successful based on the foundation of other concepts like prebiotic and symbiotic. Prebiotics are non-digestible food ingredients which selectively stimulates the growth and the physiology of health-promoting bacteria in the intestinal tract, thus improving an organism's gastrointestinal balance (Widanarni and Tanbiyas, 2015; Gibson and Roberfroid, 1995), whereas symbiotics are a combination of prebiotics and probiotics in a form of synergy.

Therefore, the objective of this trial is to mitigate these challenges by improving the aquaculture systems, the diet as well as the immunity state of the fish in order to arrest issues that may lead to their mortalities.

## MATERIALS AND METHODS

### Experimental diets

In the formulation used, fishmeal served as principal protein source whereas cornstarch was the energy source for all diets. Tapioca powder was used as binder. Other ingredients include vegetable oil as lipid source, mineral premix and vitamin C as sources of minerals and vitamins, respectively. After preparing the ingredients, it was weighed (OHAUS) and mixed in appropriate proportions to give the desired protein level required by the fish. Four experimental feeds were formulated at varying percentage inclusion of *S. muticum* (prebiotic 0.5%), *P. biglobosa* (probiotic 2%), symbiotic (prebiotic and probiotic 2.5%) and control with no inclusion.

The adopted feed formulation calculated for the trial showed that, for each 100g of feed contained approximately 46% cornstarch, 40% fishmeal, 7% vegetable oil, 4% mineral premix, 1% vitamin C and 2% tapioca(binder).

### Feeding, fish rearing conditions

The experimental tanks used for the research were twelve (12) 20L capacity plastic aquaria filled with water. The fingerlings of *C. gariepinus* were purchased at a Commercial fish farm (Mallam Farms) in Niger State; a total of 180 fingerlings was purchased and stocked at the rate of 15 fingerlings per tank using 20 L capacity plastic aquaria. The aquaria were kept in a complete randomized design (CRD), fish were acclimated to the experimental facility conditions and fed with control feed for one week. The fingerlings were fed at 5% body weight twice daily at 7.00 am and 5.00 pm except on sampling days. Feeding trial lasted for 12 weeks.

### Feed efficiency and growth parameters

Feed efficiency and growth parameters were calculated by applying the appropriate formulae where necessary, from the following:

#### *Feed efficiency*

Feed intake (FI) = total feed intake/number of fish
Feed conversion ratio (FCR) = total feed intake (g)/total wet weight gain (g)
Protein intake (PI) = feed intake (g) x percent protein in diet
Protein efficiency ratio (PER) = total wet weight gain (g)/ total feed intake (g)

#### *Growth parameters*

$$\text{Weight gain (WG)} = (W_f - W_i)/W_i$$

$$\text{Specific growth rate (SGR \%)} = [(\ln W_f - \ln W_i)/T] \times 100$$

**Table 1.** Growth parameters and feed efficiency of *C. gariepinus* (African catfish) fed formulated diets.

| Treatment | $W_i$ (g) | $W_f$ (g) | WG (g) | SGR | FI | FCR | PI | PER |
|---|---|---|---|---|---|---|---|---|
| Control | 2.54±0.05$^{a}$ | 17.31±0.02$^{b}$ | 14.77±0.01$^{b}$ | 0.16±0.01$^{ab}$ | 14.46±0.005$^{b}$ | 2.03±0.008$^{a}$ | 429.35±0.67$^{b}$ | 0.01±0.00$^{a}$ |
| Prebiotics | 2.27±0.05$^{a}$ | 15.54±0.05$^{a}$ | 13.26±0.01$^{a}$ | 0.16±0.00$^{a}$ | 13.33±0.01$^{a}$ | 1.94±0.01$^{a}$ | 371.29±0.84$^{a}$ | 0.02±0.00$^{a}$ |
| Probiotics | 2.52±0.03$^{a}$ | 18.14±0.01$^{c}$ | 15.60±0.01$^{c}$ | 0.16±0.01$^{ab}$ | 19.84±0.02$^{d}$ | 2.95±0.005$^{b}$ | 607.84±0.60$^{d}$ | 0.01±0.00$^{a}$ |
| Symbiotics | 2.31±0.06$^{a}$ | 16.88±0.01$^{b}$ | 14.55±0.03$^{b}$ | 0.17±0.01$^{b}$ | 17.95±0.00$^{c}$ | 3.17±0.05$^{b}$ | 470.36±0.67$^{c}$ | 0.05±0.00$^{a}$ |

Values were expressed as mean ± SEM. Columns with different superscripts were significantly different ($P < 0.05$).

where, $W_f$ refers to mean final weight, $W_i$ refers to mean initial weight, T is the feeding trial period in day and ln is natural log base.

***Immune parameters (antioxidant enzymes activities)***

Antioxidant enzymes activities catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione s-transferase (GST) of fish were determined using UV-3100PC spectrophotometer according to modified methods of Beers and Sizer (1952), Misra and Fridovich (1972), Mauk et al. (1998) and Habig et al. (1977), respectively.

**Statistical analysis**

Data were subjected to one-way analysis of variance (ANOVA). Results are presented as mean ± SEM of three replicate determinations. *P*-values of <0.05 were considered significant when compared by Turkey's test. All statistical analyses were carried out using statistical software package (SPSS v.21).

## RESULTS

Results for feed efficiency (FI, FCR, PI and PER), growth parameters (WG and SGR) and antioxidant enzymes activities monitored during the trial (CAT, SOD, GPx and GST) on the skin and liver of fish are represented in Table 1 to 3, respectively.

Table 1 reveals the growth parameters and feed efficiency of *C. gariepinus* fed formulated fish diet supplemented with *S. muticum, P. biglobosa* and a combination of the two. There was no significant differences noted in the initial weight, final weight significantly ($p<0.05$) differed with probiotic having the highest and the lowest was recorded in fish fed prebiotic diet. WG significantly ($p<0.05$) differed in the supplemented diets relative to the control, with the highest observed in fish fed probiotic diet and lowest in fish fed prebiotic diet. SGR was similar in fish fed supplemented diets relative to the control. FI, FCR, PI were all significantly ($p<0.05$) different relative to the control, but PER was similar across the diets.

CAT activity on the skin of fish fed supplemented diet was observed to be low relative to the control. There were significant ($p<0.05$) differences in the activity of CAT on the skin of fish fed supplemented diets relative to control.

Catalase activity was noted more in the liver of fish fed prebiotic diet relative to fish fed control diet. Fish fed probiotic and symbiotic diet were similar but recorded more activity relative to the control. CAT activity significantly ($p<0.05$) differed in the liver of fish fed supplemented diets relative to control.

Activity of SOD was similar on the skin of fish fed prebiotic and probiotic diets, more activity was noted relative to the control. However, SOD activity on the skin of fish fed symbiotic diet was similar to the control. SOD activity significantly ($p<0.05$) differed on the skin of fish fed prebiotic and probiotic diets relative to fish fed control diet. No significant difference was noted in SOD activity in fish fed symbiotic diet relative to control.

Activity of superoxide dismutase was noted to be more in the liver of fish fed probiotic diet relative to the liver of fish fed control diet. Prebiotic and symbiotic which were similar recorded more activity relative to the control. There were significant ($p<0.05$) differences in the activity of SOD in the liver of fish fed supplemented diets relative to the liver of fish fed control diet.

GPx activity was noted more on the skin of fish fed supplemented diets relative to the control. There were significant ($p<0.05$) differences in activity of GPx on the skin of fish fed supplemented diets relative to fish fed control diet.

Glutathione peroxidase was similar in the liver of fish fed prebiotic and probiotic diets, more activity was noted relative to the control. However, fish fed symbiotic diet was observed to have less activity relative to control. There were significant ($p<0.05$) differences in activity of CAT in the liver of fish fed supplemented diets relative to fish fed control diet.

On the other hand, activity of GST on the skin of fish fed supplemented diets were similar but, recorded more activity relative to fish fed control diet. There were significant ($p<0.05$) differences in GST activity on the skin of fish fed supplemented diets relative to fish fed control diet.

Glutathione s-transferase in liver significantly ($p<0.05$) differed among treatments. Fish fed prebiotic and symbiotic diets recorded more enzyme activity relative to fish fed control diet. However, no significant difference noted in fish fed probiotic diet relative to the control.

**Table 2.** Activities of antioxidant enzymes on the skin of African catfish fed supplemented diets.

| Treatment | Catalase (µ/l) | Superoxide dismutase (µ/l) | Glutathione peroxidase (µ/l) | Glutathione s-transferase (µmole/min/mg protein) |
|---|---|---|---|---|
| Control | 11.19±0.67$^{b}$ | 2.27±0.16$^{a}$ | 564.00±5.67$^{a}$ | 0.14±0.00 |
| Prebiotic | 9.90±0.22$^{ab}$ | 3.11±0.20$^{b}$ | 761.00±6.32$^{c}$ | 0.17±0.02$^{b}$ |
| Probiotic | 7.78±0.36$^{a}$ | 3.13±0.19$^{b}$ | 837.94±7.65$^{d}$ | 0.19±0.01$^{b}$ |
| Symbiotic | 7.99±0.43$^{a}$ | 2.35±0.21$^{a}$ | 627.44±5.67$^{b}$ | 0.18±0.02$^{b}$ |

Values were expressed as mean ± SEM of 3 determinations. Columns with different superscripts were significantly different ($P < 0.05$).

**Table 3.** Activities of antioxidant enzymes in the liver of African catfish fed supplemented diets.

| Treatment | Catalase (µ/l) | Superoxide dismutase (µ/l) | Glutathione peroxidase (µ/l) | Glutathione s-transferase (µmole/min/mg protein) |
|---|---|---|---|---|
| Control | 11.55±0.55$^{a}$ | 1.56±0.22$^{a}$ | 807.10±7.89$^{b}$ | 0.18±0.02$^{a}$ |
| Prebiotic | 19.26±0.56$^{b}$ | 2.49±0.27$^{b}$ | 874.14±8.90$^{c}$ | 0.20±0.01$^{b}$ |
| Probiotic | 13.27±0.57$^{a}$ | 4.78±0.35$^{c}$ | 885.75±11.34$^{c}$ | 0.17±0.01$^{a}$ |
| Symbiotic | 15.36±0.61$^{ab}$ | 2.98±0.26$^{b}$ | 658.74±7.89$^{a}$ | 0.20±0.01$^{b}$ |

Values were expressed as mean ± SEM of 3 determinations. Columns with different superscripts were significantly different ($P < 0.05$).

## DISCUSSION

Growth of an animal is described as a change in the animal, either through size (weight and length), tissues, internal chemical compositions or even reproductive abilities. Different factors are involved to achieve maximum growth potential. Dietary input with sufficient, high quality digestible nutrients and environmental input especially through oxygen and water are vital to drive the growth rate (Ahmad and Ibrahim, 2016; Bureau et al., 2000).

*C. gariepinus* has been described to be an omnivorous scavenger. Based on this, it should be expected to have the potential to efficiently utilize a wide range of feed ingredients of both plant and animal origin (Udo and Umorem, 2011; Clay, 1979). This contention was supported by the high numerical values of feed intake (FI) and protein intake (PI) in study of Udo and Umorem, (2011) which have shown catfish to consume more protein-rich diets.

The trial revealed no significant difference was noted in the initial weight of fish in this study. The final weight was significantly ($p<0.05$) different relative to the control, the significant different could be attributed to the inclusion of prebiotic, probiotic and symbiotic in the diets of the fish.

Weight gain significantly ($p<0.05$) differed, with the highest recorded in fish fed probiotic diet and lowest in fish fed prebiotic diet, however, fish fed symbiotic diet was similar to fish fed control. All the feeds performed well but probiotic diet performed better.

In this regards, the performance was an indication of positive contribution to growth. Orire and Muhammed, (2014) reported the best weight gain in fish fed diet supplemented with 100% *P. biglobosa* as protein source, the literature of Orire and Sadiku (2014) observed no significant differences in fish fed three levels of carbohydrate and protein. Nwanna et al. (2017) also reported fish fed with diets supplemented with probiotic had the better weight gain.

Specific growth rate in this trial differed insignificantly, in other words, they were similar. Specific growth rate expresses the growth over a certain period of time, and is the more popularly used formula (Strand et al., 2011). Oso et al. (2011) reported no significant differences in the specific growth rate of fish fed supplemented diets, insignificant differences was also reported in the literature of Orire and Sadiku (2014).

Feed intake significantly ($p<0.05$) differed in fish fed supplemented diets relative to the control. The highest feed intake was noted in probiotic diet and the lowest in prebiotic diet. The highest feed intake was recorded in the probiotic and symbiotic respectively. In this regards, the obtained result could be attributed to the inclusion of *P. biglobosa* in the diets which could lead to proliferation LAB which adhere to the gastrointestinal tract of *C. gariepinus* producing a wide range of relevant digestive enzymes (amylase, lipase and protease) which have the ability to denaturate the indigestible components in the diets, detoxify potentially harmful components of the diets and to produce a lot of essential vitamin B complex members particularly Biotin and vitamin B12, which

enhanced feed utilization and digestibility (Nwanna et al., 2017). The low feed intake in the prebiotic diet could be attributed to the low digestibility of *S. muticum* which could be as a result of high fibre (Orire and Abubakar, 2013).

Feed conversion ratio was significantly ($p<0.05$) different in fish fed supplemented diets relative to fish fed control. The literature of Orire and Sadiku, (2014) reported significant difference in fish, whereas Mahdavi et al. (2013) reported broilers fed with supplemented diet had lower feed conversion ratio but this might be possible because it's a different animal compared to the ones used in the current study and that of Orire and Sadiku (2014). However, this implies that as the fishes grow bigger the rate at which they convert feed to flesh decreases (Orire and Muhammed, 2014).

The trial revealed significant ($p<0.05$) differences in the fish fed the supplemented diets relative to the control. This could be attributed to the feed intake, the protein intake in decreasing order was probiotic, symbiotic, control and prebiotic which was similar with what was noted in the feed intake of the current study. The highest protein intake was observed in probiotic which suggests good growth performance in fish.

Protein efficiency ratio in this study noted symbiotic was significantly ($p<0.05$) different relative to the control but the prebiotic and probiotic were similar to the control. Protein efficiency ratio is an evaluation of the protein quality in the diet which contributes to growth of the fish rather than the overall diet (Kjorsvik et al., 2004). The highest protein efficiency ratio noted in symbiotic suggest fish fed the diet showed great feed efficiency which will equally translate to good growth performance in fish.

The repair enzymes that can regenerate some antioxidants are SOD, GPx, Glutathione Reductase (GR), CAT and the other metalloenzymes. CAT, SOD, and GPx constitute a mutually supportive team of defense against Reactive Oxygen Species (ROS). SOD lowers the steady-state level of oxygen, catalase and peroxidases do the same for peroxidase, hence making them the first line of antioxidant defense mechanism (Ighodaro and Akinloye, 2017; Lushchak, 2012; Wang et al., 2011). Glutathione S-transferases (GSTs), known as ligandins back in the days, comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification (Allocati et al.,2009).

In the present trial, fish fed prebiotic, probiotic and symbiotic expressed elevated level of antioxidant enzymes activities relative to the fish fed control diet. The expressed high level could be attributed to probiotic production of proteins in feed containing prebiotic, probiotic and symbiotic inclusion which is a precursor in the production of organic enzymes. Increased level of antioxidant enzymes activities is a sign of improved free radical scavenging in both the liver and skin of the fish.

Oxidative stress is a cellular condition which occurs as a result of physiological imbalance between levels of antioxidants and that of oxidants (ROS or free radicals), in which the imbalance is in favour of free radicals. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them. As a result of this, vital cellular structures and functions are lost and ultimately resulting in various pathological conditions. It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including diabetes, cancer, Alzheimer's disease, strokes, aging process, heart attacks and atherosclerosis (Abd El-rhman et al., 2009; Giustarini et al., 2009).

Hence, the significant increase in CAT, SOD and GPx activity in the liver indicates the first line of immune defence system plays an important role in the total defence mechanism in biological system (Ighodaro and Akinloye, 2017; Lushchak, 2012) where as high level of SOD and GST were noted on the skin, GST detoxifies endogenous compounds such as peroxidised lipids and enables the breakdown of xenobiotics and may also bind toxins and function as transport proteins (Oakley, 2011; Josephy, 2010).

It may be concluded based on the results obtained in this study that inclusion of prebiotic, probiotic and symbiotic in diets for *C. gariepinus* adversely affect feed efficiency and growth parameters and positively influenced activities of antioxidant enzymes which enhanced resistance against infection and metabolic status.

## CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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# Factors affecting adoption of dairy cattle milk production technologies in Mosop Sub County, Nandi County, Kenya

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**The study was carried out with the specific objective of analyzing the socio-economic factors affecting adoption of dairy cattle milk production technologies by smallholder dairy farmers in Mosop Sub County, Nandi County, Kenya. The study was guided by Innovation Diffusion Theory and descriptive research design was used. Cluster sampling and simple random sampling techniques were used to collect data from a sample of 198 smallholder dairy farmers, 70 large scale dairy farmers and 30 extension staff. To estimate the survey data, multivariate probit regression model was used. Descriptive statistics results revealed that 90% of smallholder dairy farmers were male-headed with 16.8 years of farming experience. Multivariate probit regression results showed that an increase in education level of the household head increased the marginal effect of adopting milking equipment by 7.5 percentage points. Results further revealed that a unit increase in the years of experience in dairy farming by the dairy farmers' household head resulted in a decrease in the marginal effect of adopting the vaccination regime by 24 percentage points, whereas gender of the household head increased the marginal probability of adopting milk equipment technologies by 56 percentage points. Male gender of the household head also increased the marginal effect probability of adopting dairy cattle vaccination regime technologies by 103 percentage points. Therefore, farmer to farmer exchange visits needs to be strengthened, introduction of farmers' mentorship programmes and revamping of extension service are paramount for technology adoption. Consequently, the county and national governments and their agencies should come up with strategies that would enhance the capacities of the dairy farmers so that they can continue appreciating new dairy cattle milk production technologies.**

**Key words:** Dairy cattle milk production technologies, smallholder dairy farmers, multivariate probit regression.

## INTRODUCTION

The Kenyan dairy sector is composed of over 625,000 smallholder dairy farmers who are distributed throughout the country. Smallholder dairy farmers produce over 56% of the total milk production produced in Kenya and 25% of the total marketed milk (Muriuki, 2001). Likewise, dairy cattle keeping helps in providing a year-round employment, diversifying production and spreading the risks. Whichever aspect that could increase expenses in the enterprise would be the genesis of risks in the efficiency of the dairy business (Bailey, 2001). The risks

that might affect milk production are hired labour, prices of milk, prices of animal feed, crop or production of forage among others.

Kenyan smallholder dairy farmers have always remained in the lead in embracing modern technologies in the region even though they have not reached the desired levels (Mekonnen et al., 2009). These technologies include growing of leguminous crops to supplement dairy cattle dietary requirements, artificial insemination, disease and pest control and commercial feed rations (Ouma et al., 2007). Some of examples of dairy cattle production technologies according to Mekonnen et al. (2009) are deworming, rotational grazing, better animal feed techniques and improved management, use of acaricides, crossbred animals, improved methods of detecting heat, vaccination, baling of hay, silage making and fodder beet.

In Nandi County, dairy milk production is a key foundation of livelihood and it impacts immensely on household income. Production of milk in the County is valued at Ksh. 7.44 Billion per year (County Integrated Development Plan 2018-2023, 2018). It is predicted that approximately 5% of milk produced within the County is consumed by calves, 10% on-farm, 5% spoiled/spillage and 80% is marketed (38.7% to formal and 41.3% to informal markets) (MOALF, 2013). The main dairy breeds that are kept are Friesians, Ayrshires and Crosses. There are a total of 33 milk chilling plants in the County that are owned and managed by New Kenya Cooperative Creameries (NKCC), farmer groups, Co-operatives and farmer companies (Department of Livestock Production Annual report, 2016). Nestlé Kenya, East Africa Dairy Development (EADD) and Kenya Dairy Board (KDB) in conjunction with the County Government of Nandi through the Department of Livestock supported farmers in Mosop Sub County on various dairy cattle milk production technologies which included the type of breeds and breed selection, forage establishment, balanced feeding, silage making, methods of milking, hygiene and health of the dairy cattle (Nestle, 2013).

The technologies that have been promoted all along in Mosop Sub County are the feeding regimes which incorporate two major components of feed establishment and feed conservation. Breeding systems of dairy animals are moving away from the use of bulls towards more advanced technologies like Embryo transfer (ET), Artificial Insemination (AI) and Sexed Semen (SS). There are also technologies that are utilized for dairy management, such as record keeping, paddocking, modern milking parlour and feeding areas; mobile platform and computer applications. However, there has been a mismatch between the technologies that have been promoted and the rate of adoption by the recipients (MOALF, 2013). There are 21,604 dairy farmers in Mosop Sub County, out of which 30% have adopted the dairy cattle milk production technologies while 70% have not adopted the technologies despite using the conventional methods of milk production (Nandi County, ASDSP Baseline Report, 2014). However, previous studies have focused on variables which are not specific to dairy cattle milk production technologies, and those studies have only focused on one technology adoption and its impact on production performance of dairy operations (Hisham and Mitchel, 2000). Conversely, as per the secondary review so far carried out by the researcher, there is scanty information in the previous studies on the analysis of factors affecting the adoption of dairy cattle milk production technologies by smallholder dairy farmers in Mosop Sub County, Nandi County. Thus, this current study endeavoured to breach the gap by analysing the factors affecting the adoption of dairy cattle milk production technologies. Therefore, the specific objective of the study was to analyse the socio-economic factors affecting the adoption of dairy cattle milk production technologies by smallholder dairy farmers in Mosop Sub-County, Nandi County, Kenya.

## MATERIALS AND METHODS

### Study area

The study was carried out in Mosop Sub County in Nandi County which covers area of 730.9 $Km^2$ of which 633.53 $Km^2$ is arable while 104.7 $Km^2$ is non-arable land. The population of the Sub County was projected to be 187,253 with 31,106 households by 2019 (KNBS, 2009). The Sub County has a cool and moderately wet climate and receives 1,200 to 2000 mm of rainfall per annum. The mean temperature ranges from 18 to 22°C during rainy seasons, while higher temperatures averaging 23°C are recorded during the drier months of December, January and February (Nandi County Development Profile, 2013). Mosop Sub County has a dairy farmer population of 21,604 owning about 67,843 dairy cattle that produce on average 248,208 L of milk per day (Nandi County Strategic Plan, 2018).

### Target population

The target population for this study was 21,604 smallholder dairy farmers, out of which 21,534 were smallholder dairy farmers with less than 10 dairy cattle while 70 smallholder dairy farmers were with more than 10 dairy cattle (Nandi County Strategic Plan, 2018).

### *Sample size*

To determine the '***n***' value, this study adopted Smithson (2015) size sampling methodology as shown in Equations 1.

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$$n = Z^2 \frac{P(1-P)}{D^2} \quad (1)$$

Where, *n* is the sample proportion, *P* is the true proportion of factor in the population, or expected frequency value, *D* is the maximum difference between the sample mean and the population mean (or the expected frequency value minus the worst acceptable value, that is: 11 - 7% = 4%, or 7 - 3% = 4%)), *Z* is the standard normal value of 1.96 significant at 5% confidence level,. Therefore, to compute the value of "***n***" (sample size), the values for the parameters were then substituted into Equation 1.

$$n = Z^2 \frac{P(1-P)}{D^2}$$

$$n = 1.96^2 \frac{0.07(1-0.07)}{0.04 * 0.04}$$

*n* = 200.9

Therefore, based on the above calculation, the sample size (number of dairy farmers) were calculated proportionately based on the number of dairy farmer households in each of the seven wards of the sub county and as a proportion of the total dairy farmers in the county against the desired sample size of 200.

**Sampling procedures**

Cluster sampling procedure was used to obtain the sample of smallholder dairy farmers in the seven administrative wards of the Sub County. Thereafter, in each ward, simple random sampling procedure was used to pick on the desired respondents for the study. A list of smallholder dairy farmers was obtained from Mosop Sub County Livestock Production Office and from the two major milk chilling plant companies namely, Kabiyet Dairies Company Limited and Tany Kina Dairies Company Limited respectively. The names of the farmers in the lists were serially numbered and randomly ordered in such a way that it gave each dairy farmer an equal opportunity of being selected. This would therefore, increase the chances of obtaining proportionate and representative sample size for the Sub county. Therefore, based on the aforementioned criteria, the random sample of dairy farmers were 36 from Kebulonik, 21 from Ndalat, 18 from Kabisagat, 31 from Chepterwai, 29 from Kabiyet, 33 from Kipkaren and 32 from Kurgung wards, respectively. After data cleaning, 198 observations remained for analysis.

**Data collection instruments**

A structured questionnaire and an interview schedule were utilized as instruments for data collection for this study. The questionnaire was administered to the respondents by the researcher with the assistance from seven trained enumerators through face to face interview. To obtain data from the key informants, an unstructured interview schedule was used. The tool was used to obtain technological information and opinions from experts whose information was crucial for the study.

**Data types**

The data types that were used in the study included a rrepresentative sample of sample of smallholder dairy farmer households, county extension staff, and extension staff of partner institutions, farm technicians and managers of chilling plants operating within the study area. In order to analyse the responsiveness of dairy farmers to technologies, the farmers were requested to state the level at which different socio-economic factors affect adoption of dairy cattle milk production technologies ($Y_j$) or otherwise ($Y_0$). Data collected included dairy farmers socio-economic characteristics (age, gender, and education level, farming experience), dairy cattle milk production technologies and socio-economic factors affecting adoption of dairy cattle milk production technologies.

**Operationalization of terms**

Continuous, ordinal, dummy and discrete variables for the study were identified and operationalized based on economic theories and econometric studies as follows:

1. Adoption of dairy milk production technologies: Adoption of milk production technologies is a dummy dependent variable that represents the probability of adoption of a dairy milk production technology by the dairy farmer household. The variable takes the value 1= if the farmer adopts the technology or 0 = (otherwise) if the household does not adopt the milk production technology.
2. Age: It is a continuous independent variable that is measured in terms of number of years of the household head. Age of the household head was hypothesized to increase or decrease the probability of adoption of dairy milk production technologies.
3. Gender: Gender is a dummy variable that takes the value 1 if the household head is a male and 0 otherwise. In dairy production, both male and female take part in dairy management.
4. Education level: An ordinal independent variable that is measured in terms of highest academic qualification (1= primary, 2 = secondary, 3=college, 4 = university). A positive relationship was hypothesized between educational level and adoption of dairy milk production technologies. Education plays an important role in adoption of new technologies and it is believed to improve the willingness of the household head to embrace new ideas and innovations.
5. Household size: Household size is a continuous independent variable that is measured in terms of the total number of people (related or not related) living together in the same household. Household size increases household consumption requirements and render the households more risk averse. Therefore, the variable is hypothesized to influence adoption of dairy milk production technologies
6. Farming experience: Continuous variable measured in terms of the number of years in dairy farming. A positive relationship was hypothesized between dairy farming experience of household head and adoption of dairy milk production technologies.

**Data analysis and presentation**

Parametric estimates of the probit model were used to give direction of the effects of independent variables on the dependent variable. These estimates represent neither the actual magnitude of change nor the probabilities. The coefficients had no direct interpretation. They were simply the values that maximized the likelihood function. The real expected change in probability was measured by use of marginal effects for the specific objective's dependent variable $(\mathbf{Y_j})$ with regard to a unit change in the independent variable from the mean (Green, 2002). Data for the objective was obtained by requesting the respondents to declare the level at which different socio-economic factors affected the adoption of dairy cattle milk production technologies. The data was then subjected to a multivariate probit regression analysis to determine the effect of socio-economic factors on the adoption of

**Table 1.** Gender of the household head

| Sex | Frequency | Percent (%) |
|---|---|---|
| Male | 178 | 89.9 |
| Female | 20 | 10.1 |
| **Total** | **198** | **100.0** |

**Table 2.** Age of dairy farmers' household head.

| Parameter | N | Minimum | Maximum | Mean | Std. deviation |
|---|---|---|---|---|---|
| HH head's age in years | 198 | 25 | 90 | 48.99 | 11.693 |

dairy cattle milk production equipment technologies. The analysis used 198 observations. However, two of the observations were with missing information and were therefore dropped from analysis. Descriptive and inferential statistics were analysed and the results presented in frequency tables. Econometric analysis of data used Multivariate probit model as shown in Equation 3 and as adopted from Greene (2012). It is based on the hypothesis that the errors are typically distributed and provides for joint determination and a framework for modelling in two or more common applications.

$$Y_j = \beta_0 + \beta_1 AG_j + \beta_2 GD_j + \beta_3 EL_j + \beta_4 FS_j + \beta_5 EX + \varepsilon_j \quad (2)$$

Where $Y_j$ is the response to positive adoption of technologies by the smallholder dairy farmer n, and 0 otherwise, $\beta_0$ is the intercept, $\beta_1$-$\beta_1$ are the coefficient for the socio-economic variables, *AG, GD, EL, FS* and *EX* are age, gender, education level, family size and years of farming experience respectively. $j$'s are the indexes for the adoption of dairy technologies, and $\varepsilon_j$ is the error term. It was also assumed that $E_{(\varepsilon j)} = 0$, $Var_{(\varepsilon j)} = \sigma^2$ and $Cov_{(\varepsilon j)} = 0, \forall \neq j$.

## RESULTS AND DISCUSSION

### Household socio-economic factors

As shown in Table 1, about 90% of the smallholder dairy farmer households were male headed while 10% were female headed. According to the findings by Oni et al. (2010), male and female-headed households have almost equal chance of participating in smallholder farming, which is in contrast and divergent with the current findings. Research findings by Ward et al. (2008) on factors affecting adoption of livestock production practices revealed that 89% of the respondents were males, which is in convergence with the current study findings. Therefore, these current study findings imply that male-headed households have greater chances of participating in up take of dairy cattle milk production technologies as compared to female-headed households.

Table 2 shows the age of the dairy farmer household head. The youngest and the oldest small-holder dairy farmer were aged 25 and 90 years, respectively. The mean age of the majority of smallholder dairy farmers in the study area was 49 years. Age of a dairy farmer household head is an important factor in the adoption of dairy cow milk production technologies. According to the study findings by He and CAO (2007) and Sidibe (2005), the probability of young household heads to adopt new technologies were high as compared to older household heads. These previuos study findings on age of household head are in convergence with the current study findings on age and adoption of agricultural technologies.

Results in Table 3 show that a large number of the smallholder dairy farmers in the study area had attained the pre-primary and primary levels of education which represented about 43% of the total respondents. About 31, 3 and 11% of the respondents had attained secondary, vocational training and post-secondary/college levels of education, respectively. Only two of the respondents had attained a university level of education. These results show that most of the smallholder dairy farmer household heads were fairly educated which could enable them to fairly adopt dairy cattle milk production technologies. Mishra (2010) found out in his study that higher education level leads to ease of access to knowledge and information on agricultural undertakings. This would lead to higher up take of technologies in agriculture. A study by Knowler and Bradshaw (2007) revealed that education level has a positive influence on dairy cattle milk production technology adoption because there is a correlation between education and knowledge. The findings of the two previous studies were in agreement with the current study findings. This means that dairy farmers with better education levels would easily adopt dairy cattle milk production technologies.

Table 4 of results shows the mean years of dairy farming experience of the dairy farmer household heads. From the results, dairy farmer household heads had on average 16.8 years of dairy farming experience. Farmers experience as put across by Ingabire et al. (2018) on the agricultural technology adoption found out that majority of none technology adopters had farm experience of

**Table 3.** Highest education level

| Education level | Frequency | Percent (%) |
|---|---|---|
| No formal education | 10 | 5.1 |
| Less than Primary | 13 | 6.6 |
| Pre-primary/Primary | 85 | 42.9 |
| Secondary | 61 | 30.8 |
| Vocational training | 5 | 2.5 |
| Post-sec/College | 22 | 11.1 |
| University | 2 | 1.0 |
| **Total** | **198** | **100.0** |

**Table 4.** Years of farming experience of the household head.

| Parameter | N | Mean | Standard deviation |
|---|---|---|---|
| Years of dairy farming experience | 198 | 16.7677 | 11.55022 |

**Table 5.** Multicollinearity test.

| Variable | VIF | 1/VIF |
|---|---|---|
| Gender | 3.91 | 0.255637 |
| Household head | 3.57 | 0.280337 |
| Age | 2.55 | 0.391419 |
| Farming years (experience) | 2.24 | 0.447239 |
| Education level | 1.20 | 0.830199 |
| Family size | 1.02 | 0.980780 |
| **Mean VIF** | **2.42** | |

between 1 and 4 years while adopters had experience of above 10 years, which is in convergence with the current study results.

## Diagnostic test

Table 5 presents the results of multicollinearity test. Multicollinearity was measured by use of the variance inflation factor (VIF) and contingency coefficient factor (CCF) among continuous and discrete variables for the analysed specific objective. Multicollinearity arises once two or more predictors in the model are correlated and provide redundant information about the response. According to Ringle et al. (2015) and Mile (2014), the maximum VIF values should be less than 5 and 10, respectively. Preliminary test results for the diagnostic test revealed that the output coefficient or collinearity statistics as shown by the VIF values ranged from 1.02 to 3.91. This shows that there were no potential multicollinearity symptoms among the predictors and hence found to have no potential influence on the estimates from the model. The small VIF values as shown in the table indicate that there was low correlation among the variables under consideration.

## Determinants of adoption of dairy cattle milk production technologies

A detailed econometric result of the multivariate probit regression model for socio-economic determinants of adoption of dairy cattle milk production technologies is presented here. The results of the analysis are as shown in Table 6. Results reveal that the likelihood chi-square ratio test of 43.63 with a p-value of 0.0000 means that the model as a whole was statistically significant, that is, it fits significantly better than a model with no predictors. Three predictor variables namely age, gender and education level are statistically significant. The probit regression coefficients give the change in the z-score or probit index for a one-unit change in the predictor. For a one-unit increase in age, the z-score increases by 0.039 and the z-score increases by 0.27 with a one-unit increase in the level of education.

Table 7 shows the results of the average marginal effects for the multivariate probit model estimates.

**Table 6.** Probit regression estimates of adoption of milk equipment and socio-economic factors.

| Milk equipment | dy/dx | Std. Err. | Z | P>z | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| Age | 0.0388243 | 0.0136529 | 2.84 | 0.004* | 0.0120651 | 0.065583 |
| Gender | 2.046087 | 0.4823852 | 4.24 | 0.000* | 1.100629 | 2.991544 |
| Education level | 0.2729715 | 0.1106942 | 2.47 | 0.014** | 0.0560149 | 0.489928 |
| Family size | -0.0165469 | 0.0198834 | -0.83 | 0.405 | -0.0555175 | 0.022424 |
| Farming years | -0.2550132 | 0.1312307 | -1.94 | 0.052 | -0.5122207 | 0.002194 |
| Constant | -3.426247 | 0.976716 | -3.51 | 0 | -5.340575 | -1.511919 |
| Number of Obs | 197 | | | | | |
| LR $ch^2$ (5) | 43.63 | | | | | |
| Prob > $ch^2$ | 0.0000 | | | | | |
| Log likelihood = | 96.693637 | | | Pseudo $R^2$ = | 0.1841 | |

*,** and ***= 1, 5 and 10% levels of significance.

**Table 7.** Marginal effects for socio-economic factors on the adoption of milk equipment.

| Parameter | dy/dx | Std. error | z | P>z | [95% Conf. | Interval |
|---|---|---|---|---|---|---|
| Age | 0.010631 | 0.0035388 | 3.00 | 0.003* | 0.0036951 | 0.0175668 |
| Gender | 0.5602664 | 0.1135051 | 4.94 | 0.000* | 0.3378004 | 0.7827323 |
| Family size | -0.0045309 | 0.0054283 | -0.83 | 0.404 | -0.0151702 | 0.0061084 |
| Highest education | 0.074746 | 0.0291629 | 2.56 | 0.010** | 0.0175877 | 0.1319042 |
| Farming experience | -0.0698286 | 0.03509 | -1.99 | 0.047** | -0.1386038 | 0.0010533 |

Average marginal effects: Number of obs = 197; Model VCE: OIM; Expression: Pr (Milk equipment), predict; dy/dx with respect to age, gender, family size, education level and farming experience; *,** and ***= 1, 5 and 10% levels of significance.

Results show that the signs of marginal effects variable are in line with the signs obtained from parameter estimates in Table 9. Output results reveal that the predicted probability for socio-economic factors on adoption of milk equipment technologies by dairy farmer household was significant with the following factors; level of education, age, years of farming experience and gender.

Results revealed that the age of the household head had a positive and significant marginal effect at 1% level of significance on the adoption of milk equipment technologies. For a unit increase in the age of the dairy farmer, the marginal probability of adopting milk equipment technology (z-score) increased by 1.1 percentage points, which means that as farmer's age increases, the adoption of the milk equipment increases or their willingness to adopt would be positive. This would be attributed to the generalized increase in experience. This finding could be in contrast with the finding of Tesfaw (2013), who reported that the age of the household head negatively influenced market participation decision since as the head gets older and older, they shift to production of lesser labour-intensive farming alternatives. But the current result is in convergence with the findings by Kafle and Shah (2012) who found out that the up take of potato superior varieties was popular amongst the adult farmers.

Gender of the dairy farmer household head had positive marginal effect and significantly related to the adoption of milk equipment at 1% level of significance. Results shows that when the gender of household head was male, the marginal effect of adopting dairy cattle milk equipment increased by 56 percentage points. The outcomes on the gender of head of the household as per the current study was in convergence with findings by Doss and Morris (2001) who found out that if the gender of head of the household was a male, then they would adopt new agricultural technologies easily compared to households headed by female. This is attributed to the easy access to resources by the male gender as compared to the female gender.

Results on the education level of the dairy farmer household head had positive marginal effect on the adoption of dairy cattle milk equipment and statistically significant at 5% level. From the results, a unit increase in the level of education of the dairy farmer household head increases the marginal effect of using the milk equipment by 7.5 percentage points. The findings of the household head on education were in convergence with the study findings by Caswell (2001) who found out that education facilitated a positive attitude to appreciating new technologies.

**Table 8.** Average marginal effect estimates for the adoption of AI technology.

| AI | dy/dx | Std. error | Z | P>z | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| Age | -0.0316774 | 0.0113199 | -2.80 | 0.005* | -0.053864 | -0.009491 |
| Gender | -0.2288996 | 0.348659 | -0.66 | 0.511 | -0.9122588 | 0.45446 |
| Education level | -0.0261317 | 0.0789844 | -0.33 | 0.741 | -0.1809384 | 0.128675 |
| Family size | 0.0083315 | 0.018395 | 0.45 | 0.651 | -0.0277219 | 0.044385 |
| Farming experience | -0.006034 | 0.1089846 | -0.06 | 0.956 | -0.2196399 | 0.207572 |
| _cons | 2.077987 | 0.7061331 | 2.94 | 0.003 | 0.6939913 | 3.461982 |
| Probit regression | | | | | | |
| Log likelihood = | 125.80161 | | | | | |
| Number of obs= | 198 | | | | | |
| LR chi² (5) = | 13.91 | | | | | |
| Prob> chi² = | 0.0162 | | | | | |
| Pseudo R² | 0.0524 | | | | | |

Further, results of the study revealed that farming experience of the dairy farmer household was statistically significant at 5% level with a negative marginal effect on the adoption of dairy cattle milk equipment. A one year increase in the farming experience of the dairy farmer household head reduces the adoption of dairy cattle milk equipment by 6.5 percentage points. The current study findings were in divergence with the one by Makokha et al. (2007), who found out that farmers with experience utilized their long term acquired knowledge and skills to reduce risks related with dairying and management of diseases. Further, a study was done by Kinambuga, (2010) revealed that experience assists in making decisions and allocation of resources which meant that the more experience one has, the wiser decisions are being made in terms of allocating resources to new technologies. Komolafe et al. (2014) confirmed that as the dairy farmers grow old, their level of output decline while Osanyinlusi and Adenegan (2016) found out that experience in farming was negatively related to production per unit area. Studies by Komolafe et al. (2014) and that of Osanyinlusi and Adenegan (2016) were in convergence with the current study which revealed that the adoption of milk equipments reduces as the household head's farming experiences increases by year.

Table 8 shows the results of the multivariate probit regression analysis to determine the effect of socio-economic factors on the adoption of AI technology. From the average marginal effect estimates for the adoption of AI technology results table, the likelihood ratio chi-square test of 13.91 with a *p*-value of 0.0162 shows that the model that was used as a whole was statistically significant and it fitted significantly better than a model with no predictors. Results shows that only age of the dairy farmer household head was statistically significant at 1% level even though with a negative marginal effect on the adoption of dairy cattle milk production technologies. The rest of the variables in the model namely gender, education level, farming experience and family size were not statistically significant.

The coefficient for age of the dairy farmer household head is -0.032 with a *p*-value of 0.005. This result shows that as the age of the dairy farmer household head increases by a year, the marginal probability of adopting AI technology decreased by 3.2 percentage points. This means that young dairy farmers as opposed to older farmers can easily adopt new AI technologies. They can also easily change to other technologies as compared to older dairy farmers who are reluctant to abandon old technologies for ones that are new. This result is in divergent with the study finding by Kaaya et al. (2005) who found out that age was positively connected to embracing and utilization of AI technology. The result is also in divergence with the study findings by Nzomoi et al. (2007) who found out that the age of the household head played an important role in the adoption of dairy technologies. Quddus (2013) found out that young farmers within the productive age are able to take up new technologies easily as compared to farmers who are old, which is in convergence with the current study findings. As a dairy farmer gets older, their experience notwithstanding, they tend to relax, lack long term planning and become a risk-averse and therefore adopting new technologies would be a challenge.

Table 9 presents the results of the analysed socio-economic factors that influenced the adoption of vaccination regime technologies by the dairy farmer households. Probit analysis was performed because the outcome of the predicted variables was binary. Results revealed that the likelihood ratio chi-square is 35.17 with a *p*-value of 0.0000. This shows that the model was statistically significant and it fitted significantly better than a model with no predictors. The *p*-values for years of farming experience and gender were statistically significance at 5% level with positive marginal effects on adoption of vaccination regime technologies. The rest of the socio-economic factors were insignificant to the

**Table 9.** Marginal effects estimate on use of vaccination regime technologies.

| Vaccination regime | dy/dx | Std. Err. | Z | P>z | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| Age | -0.0100701 | 0.0121704 | -0.83 | 0.408 | -0.0339236 | 0.013783 |
| Gender | 1.029128 | 0.4144704 | 2.48 | 0.013** | 0.2167806 | 1.841475 |
| Highest education | 0.0356271 | 0.082599 | 0.43 | 0.666 | -0.1262639 | 0.197518 |
| Family size | 0.0113402 | 0.0191948 | 0.59 | 0.555 | -0.026281 | 0.048961 |
| Farming experience | -0.2429342 | 0.1165877 | -2.08 | 0.037** | -0.4714418 | -0.014427 |
| _cons | 0.2435127 | 0.8036385 | 0.30 | 0.762 | -1.33159 | 1.818615 |
| Probit regression | | | | | | |
| Number of obs = | 196 | | | | | |
| LR chi² (5) = | 35.17 | | | | | |
| Prob> chi² = | 0.0000 | | | | | |
| Pseudo R² = | 0.1370 | | | | | |
| Log likelihood = | 110.73812 | | | | | |

*,** and ***= 1, 5 and 10% levels of significance.

adoption vaccination regime technologies by the dairy farmer households. Since gender was a dummy variable with values 1 for male respondents and 0 for non-male respondents, the coefficient of gender indicates that when the respondent was male, the z-score increased or the marginal probability of adopting vaccination regime by dairy farmer household increased by 103 percentage points. Similarly, a unit increase in the years of experience in dairy farming by the dairy farmers' household head results in a decrease in the marginal effect of adopting the vaccination regime by 24 percentage points. Experience in any venture cannot be overemphasized. In the dairy sector, experience is very important especially in improving the breeds and breeding. Farmers with fast experience are better placed to address the challenges related to dairy cow milk production. According to findings by Idrisa et al. (2012), farmers with more experience have enhanced skills, access to information and exposed to better technologies. The findings by Idrisa et al. (2012) are in convergence with current study finding.

The result for gender shows a positive marginal effect with a significant effect on the adoption of vaccination regime at 5% level. This shows that as gender of household head was male, the marginal effect of adopting dairy cattle vaccination regime technologies increased by 102 percentage points. According to study results by Adebiyi and Okulola (2013), households headed by females were less experienced in terms of dairy cow milk technologies as compared to male-headed households because the female was too engaged with home chores and family management as compared to male counterparts. Adesina and Chianu (2002) found out that the female head is less likely to adopt new technologies while according to findings by Baiyegunhi (2015), male farmers tend to accept new technologies as equated to female counterparts.

## CONCLUSION AND RECOMMENDATIONS

This article analysed the socio-economic factors affecting adoption of dairy cattle milk production technologies by smallholder dairy farmers in Mosop Sub County, Nandi County, Kenya using a multivariate probit regression model. Descriptive statistics results revealed that 90% of smallholder dairy farmers were male-headed with 16.8 years of farming experience. Results of the multivariate probit regression model revealed that age of the household head had a positive marginal effect on the adoption of dairy cattle milk equipment technologies. A unit increase in age of the dairy farmer household head increased the marginal probability of adopting milk equipment technology by 1.1 percentage points. A unit increase in the level of education of the household head increases the marginal effect of using the milk equipment by 7.5 percentage points. Gender of the head of household also had a positive marginal effect and increased the marginal probability of adopting milk equipment by 56 percentage points. Further, results shows that the gender of the household head had a significant positive marginal effect on the adoption of vaccination regime. As gender of household head was male, the marginal effect of adopting dairy cattle vaccination regime technologies increased by 103 percentage points. Similarly, a unit increase in the years of experience in dairy farming by the dairy farmers' household head resulted in a decrease in the marginal effect of adopting the vaccination regime by 24 percentage points. In conclusion, the present study contributes to our theoretical understanding by showing that the socio-economic factors, particularly age, gender, farming experience and education level affects positively the adoption of dairy cattle milk equipment technologies whereas, gender plays an important role in the adopting of vaccination regime by the dairy farmer household.

Therefore, the National and County governments should come up with helpful strategies and policies to reach out to the dairy farmer households at the various farming categories. The two levels of government should also come up with new extension approaches that would go towards enhancing the adoption of dairy cattle milk production technologies.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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