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Full Length Research Paper

Comparative study of yield performance and nutrient composition of the edible mushroom *Pleurotus pulmonarius*, cultivated on different substrates

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Sawdust from four types of woods (*Milicia excelsa, Gmelina arborea, Afzelia africana* and *Khaya senegalensis*) was used for cultivation of *Pleurotus pulmonarius*. Growth and yield of fruiting bodies were monitored and biological efficiency (BE) determined. Proximate composition of fruiting bodies was determined; total phenol content (TPC) and total flavonoid content (TFC) of the extract from the mushrooms were investigated. Results obtained show significant yield (P<0.05) of 361.30±4.55 g/kg substrate (BE = 36.13%) on *G. arborea* followed by *K. senegalensis*. The highest crude protein content (19.69±0.09%) was observed in mushrooms harvested on *A. africana*. Crude fibre was significantly (P<0.05) higher in mushrooms harvested from *A. africana*. Magnessium, sodium, potassium and calcium content of all the mushrooms were significantly different but all had significantly (P<0.05) higher potassium content (3.52±0.11 to 7.09±0.004 mg/kg). The highest TPC and TFC were recorded in mushrooms harvested from *M. excelsa* and the least TPC was observed for mushrooms harvested from *K. senegalensis* while the least TFC was recorded in *G. arborea* and *A. africana*. These findings indicate effective economic bioconversion of sawdust with varying effects on yield, nutritional composition, TPC and TFC of *P. pulmonarius*. *G. arborea* gave a better yield and can be further exploited in various capacities.

Key words: Sawdust, biological efficiency, *Pleurotus pulmonarius*, proximate composition.

INTRODUCTION

Sawdust is one of the major residues or waste produced during milling and re-sawing of wood and is one of the solid wastes available in large quantities in most developing countries. Its proper disposal has constituted a great challenge in most African countries. In Nigeria and most developing countries in Africa, residue generated from wood processing are regarded as waste and this has led to open burning practices, dumping in water bodies or dumping in an open area which constitutes environmental pollution (Aina, 2006; Aiyeloja

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et al., 2013). It has been reported that less than 5% of the total wood waste generated in Nigeria is being utilized (Ogunwusi, 2014). This leaves huge quantity of wood waste unutilized which also constitute health hazards if not properly disposed and they may also become breeding places for worms and insects (Dosunmu and Ajayi, 2002).

Mushroom cultivation is one of the economically viable biotechnological processes for the bioconversion of plant wastes and residues (Wood and Smith, 1987). Many agricultural and industrial by-products have been used in mushroom cultivation (Chinda and Chinda, 2007). Extracellular enzymes secreted during growth (Cohen et al., 2002) of Basidiomycetes have the capability of degrading lignin in plant cell walls (Yamakawa et al., 1992). This makes them able to decompose and mineralize plant cell components by converting the easily digestible carbohydrates into simpler sugars. Among these basidiomycetes are white rot fungi (WRF) including oyster mushrooms, *Pleurotus* species. Many species of the genus *Pleurotus* (Jacq.:Fr.) P. Kumm. are edible species and have been cultivated on a wide range of lignocellulosic materials (Guzman, 2000). Oyster mushrooms have been shown to have nutritive and medicinal properties such as antitumor, antiviral, antineoplastic and antioxidant potentials and properties (Akinyele et al., 2011; Weigand-Heller et al., 2012; Yashvant et al., 2012). Investigations on the use of agricultural wastes as substrate for cultivation of *Pleurotus* spp. have been reported by a number of researchers (Anyakorah and Olatunji, 2001; Yildiz et al., 2002; Oei, 2003; Peksen and Küçükomuzlu, 2004; Jonathan et al., 2012a).

Pleurotus pulmonarius is an edible fungus belonging to the Class: Agaricomycetes, Order: Agaricales, Family: Pleurotaceae, Genus: *Pleurotus* (Alexopolous et al., 1996; Jonathan et al., 2012b). The cultivation of *Pleurotus* spp. is common but not as much as the white button and shiitake in terms of global mushroom production, with versatility in terms of growth substrates and cultivation conditions (Gyorfi and Hajdu, 2007). The cultivation of mushroom serves as an alternative source of income for small holder farmers (Sánchez and Royse, 2001; Valencia and López, 2005).

In Nigeria, the commercial cultivation of mushrooms is still at the tender stage because most people still collect from the wild during rainy seasons for sale in the local market place. Among the cultivated edible mushrooms in the country, *Pleurotus* spp. ranks the first due to availability of suitable substrate, conducive growth condition and acceptability (Kues and Liu, 2000; Amuneke et al., 2011). Sawdust from different hard wood represents some of the solid waste in the country with the challenge of its proper disposal. The present study was undertaken to demonstrate economical bioconversion of sawdust and determine the most suitable wood sawdust substrate for the cultivation of *P. pulmonarius* and its effect on the nutrient composition of mushrooms harvested from the substrates.

MATERIALS AND METHODS

Collection of materials

Matured fruiting bodies of the mushroom (*P. pulmonarius*) used for this work were obtained from a commercial mushroom farm along Odo-ona Kekere road within Ibadan, Oyo State, Nigeria. Sawdust was obtained from freshly processed woods at a major sawmill located within Ogbomoso, Oyo State, Nigeria. The sawdusts used as substrates for mushroom cultivation were obtained from four different woods: *Milicia excelsa, Gmelina arborea, Afzelia africana* and *Khaya senegalensis.*

Tissue culture

Mycelia were obtained by tissue culture on freshly prepared PDA (Lab M, Neogen, Lancashire, UK) plate according to the method of Stanley (2010). Plates were incubated at 27°C±2 for 5 days when visible mycelia were observed. Subsequently, subcultures were made onto PDA slants and after incubation for visible mycelia growth; slants were stored for further analysis.

Preparation of spawn

Whole rice grains were used as substrates for the planting spawn. The grains was washed to remove dead seeds, shaft and dirt and boiled for 2 min to soften. Boiled grains were drained and spread out to air-dry on absorbent sheets. Two percent (w/w) of $CaCO_3$ and 4% (w/w) of rice bran were added to the grains mixed thoroughly and filled into clean empty jars till ¾ full, plugged with cotton wool and loosely covered with aluminium foil before autoclaving for 1 h. Autoclaved grains were inoculated with five 9.0 mm mycelia plugs from actively growing margins of 7-days old plate cultures of *P. pulmonarius* and incubated at 27°C±2 until full ramification.

Preparation and spawning of substrate

Sawdust (*M. excelsa, G. arborea, A. africana* and *K. senegalensis*) were spread separately on a flat surface in the shade to air-dry, sorted separately to remove wood chip and foreign particles. Substrates were then prepared by adding 2% (w/w) $CaCO_3$ and 4% rice bran to each of the 4 types of sawdusts and mixed thoroughly. Water was added to the mixture to moisten to about 60% moisture content. The indoor method of mushroom cultivation was used (Oei, 1996; Quimio, 2002) and carried out in the mushroom house. The substrate was packed separately in triplicates into heat resistant polythene bags (40×60 cm) with each bag containing 1 kg of the substrate. Polyvinyl chloride (PVC) tubes cut into sizes of 2 inches each were used as the bottle necks plugged with a piece of cotton wool and covered loosely with aluminium foil. Bags were steamed in 200 L capacity drum with tightened lid for 2½ h. The steamed substrates were allowed to cool overnight in the drum and inoculated under aseptic conditions with fully ramified grain spawn. Sterile pins were used to puncture bags randomly. After full ramification of substrates, the PVCs (used as bottle necks for the polythene bags) were removed and the polythene bags cut open for watering and aeration of ramified substrate in order to initiate ramification of substrates, the PVCs (used as bottle necks for the polythene bags) were removed and the polythene bags cut open for watering and aeration of ramified substrate in order to initiate

Table 1. Details of fructification of *P. pulmonarius* (in days) on different sawdust substrate.

Sawdust substrate	Full ramification	Primordia formation	Fruit body formation
ME	19.67±0.67^{ab}	25.33±0.33^{b}	29.67±0.33^{b}
GA	18.00±0.58^{a}	21.67±1.20^{a}	27.33±0.67^{a}
AA	21.33±0.88^{b}	27.67±0.58^{b}	34.00±0.58^{c}
KS	20.00±1.00^{ab}	28.69±0.33^{b}	35.00±0.00^{c}

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (ρ≤0.05) in columns. ME is sawdust from *M. excelsa*, GA is sawdust from *G. arborea*, AA is sawdust from *A. africana*, and KS is sawdust from *K. senegalensis*.

fruiting. Fully matured mushroom were harvested after about 5 to 7 weeks of spawning and weighed.

Yield of mushrooms and biological efficiency

Mushroom yield was determined by the division of total weight of fruiting bodies from all flushes (each bag) by the dry weight of the substrate. Biological efficiency (BE) which is defined as the ratio of fresh weight of mushrooms to dry weight of compost at spawning was expressed as a percentage using the following formula:

$$Biological\ Efficiency\ (BE) = \frac{Fresh\ weigh\ to\ f\ mushroom}{Dry\ weight\ of\ substrate} \times 100$$

Determination of proximate composition of fruiting bodies of mushrooms

Harvested fruiting bodies of mushroom were oven-dried at 60°C until constant weight, cooled in desiccators sand powdered in an electric blender. Moisture content was determined by the direct oven drying method. The weight loss after oven drying of each sample (1 g) at 105°C to constant weight was expressed as % moisture content (Sivrikaya et al., 2002). The nitrogen content was determined using the micro-Kjeldahl method and crude protein was calculated by multiplying the nitrogen content with a factor of 6.25 (Thimmaiah, 2004). Crude fibre was determined according to the standard method of AOAC (2005) where the sample was digested successively with acid and base. The difference in the weight of the residue obtained after digestion expressed in percentage gave the crude fibre content. Crude fat was determined by using the Soxhlet extraction method using petroleum ether as the solvent (AOAC, 2005). Ash content of 1 g powdered sample was determined as the residue of incineration at 550°C in a muffle furnace (AOAC, 2005). Mineral constituents (magnesium, sodium, potassium, calcium) were determined by atomic absorption spectrophotometry (AOAC, 2005). All proximate analyses of the mushroom powder were carried out in triplicate and reported in percentage.

Sample extraction with ethanol

Extraction was performed according to the method of Tibuhwa (2014). The residual solvent of ethanol extract was removed using a rotary evaporator to dryness. The obtained concentrated extracts were stored in dark at 4°C until further analysis.

Estimation of total phenol content (TPC) of fruiting body

Total phenol content in the mushroom extracts was determined using a Folin-Ciocalteu colorimetric assay following the methods of Ainsworth and Gillespie (2007) and Chai and Wong (2012) with modifications. A mixture of extract (0.1 mL) and 10% v/v Folin-Ciocalteu reagent from Sigma-Aldrich, St. Louis, USA (0.2 mL) was first incubated at room temperature for 3 min. A volume of 0.8 mL of 700 mM Na_2CO_3 was added and the mixture was incubated at room temperature for 2 h. Absorbance of the mixture was read at 765 nm. A standard curve was prepared from 0 to 42 mg/L gallic acid. Total phenolic content was expressed in mg gallic acid equivalents/g dry matter.

Estimation of total flavonoid content (TFC) of fruiting body

Total flavonoid content in the mushroom extracts was determined following the method of Chai and Wong (2012). Mushroom extract (0.2 mL) was added to 0.15 mL of $NaNO_2$ and the mixture was incubated at room temperature for 6 min. A volume of 0.15 mL of $AlCl_3.6H_2O$ (10 % w/v) was added to the mixture, which was then left at room temperature for 6 min. Exactly 0.8 mL of NaOH (10% w/v) was added and the absorbance of the mixture was read at 510 nm after standing at room temperature for 15 min. For the blank, the extracts were replaced with water. A standard curve was prepared from 0 to 500 μg/mL quercetin dissolved in 80% ethanol. Total flavonoid content was expressed in mg quercetin equivalents/g dry matter.

Statistical analysis

Each data value was presented as the mean standard error. Differences between the means of individual groups were assessed by one-way analysis of variance (ANOVA) with Duncan's multiple-range test using Statistical Package for the Social Science (SPSS 16.0).

RESULTS

The details of growth and fructification of *P. pulmonarius* from spawning of substrates to development of mature fruiting bodies is shown in Table 1. Time (in days) of full ramification of substrates by mycelia of *P. pulmonarius* and development of mature fruiting bodies was significantly (P≤0.05) different in the four types of sawdust used. Mycelia growth (spawn run) resulting in the full ramification of the substrates was fastest in *G. arborea* sawdust taking 18.00±0.58 days followed by *M. exelsa,* while *A. africana* took the highest number of days (21.33±0.88) to completely ramify. Formation of primordial was also fastest in *G. arborea* sawdust taking 21.67±1.20 days from spawning but there was no

Table 2. Yield and biological efficiency of *P. pulmonarius* cultivated on different sawdust substrate.

Sawdust substrate	First flush (g)	Second flush (g)	Yield g/kg of dry substrate	Biological efficiency (BE, %)
ME	165.57±2.98^{b}	135.80±2.49^{b}	301.37±3.67^{b}	30.14±0.60^{b}
GA	193.60±5.91^{c}	167.70±4.36^{c}	361.30±4.55^{d}	36.13±0.24^{d}
AA	140.33±4.88^{a}	115.28±5.87^{a}	255.61±2.05^{a}	25.56±0.60^{a}
KS	159.97±2.91^{b}	124.67±5.33^{ab}	284.64±2.42^{c}	28.46±2.42^{c}

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (ρ≤0.05) in columns.

Table 3. Proximate composition (%) of *P. pulmonarius* cultivated on different sawdust substrate.

Sawdust substrate	Moisture	Ash	Crude fibre	Crude protein	Fat
ME	2.17±0.09^{a}	7.75±0.05^{c}	5.54±0.27^{a}	18.70±0.40^{a}	1.85±0.05^{c}
GA	2.98±0.06^{b}	5.92±0.01^{a}	5.89±0.11^{a}	18.15±0.13^{a}	1.56±0.17^{b}
AA	3.72±0.04^{c}	6.91±0.05^{b}	6.73±0.12^{b}	19.69±0.09^{b}	1.19±0.06^{a}
KS	3.03±0.04^{b}	5.64±0.07^{a}	5.56±0.30^{a}	18.67±0.18^{a}	1.55±0.06^{b}

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (ρ≤0.05) in columns.

significant difference in the number of days for primordial formation in the other substrates. Number of days for fruiting body formation also followed a similar trend with *G. arborea* emerging as the fastest in fruiting body formation but there was no significant difference in the number of days for fruiting body formation on *A. africana* and *K. senegalensis*.

The weight of harvested mushroom in the first and second flushes for all the sawdust substrates used were significantly different at P≤0.05 and followed a similar trend in both flushes. The highest yield of 361.30±4.55 g/kg of substrate occurred in *G. arborea* sawdust, followed by *M. exelsa* while the least yield of 255.61±2.05 g/kg of substrate was recorded on *A. africana* sawdust (Table 2). The BE of mushrooms harvested from each of the substrates ranged from 25.56±0.60 to 36.13±0.24%. The BE of *G. arborea* was significantly (P≤0.05) higher than the BE of the other substrates with *A. africana* recording the least BE.

Proximate composition of *P. pulmonarius* harvested from the different substrates is shown in Table 3. The result shows significant differences (P≤0.05) in the proximate composition of mushrooms harvested from the different sawdust substrates. Moisture content of the dried and pulverized mushroom samples ranged from 2.17±0.09 to 3.71±0.04% with dried mushroom from *A. africana* showing the highest moisture content. Ash content was significantly higher in mushroom harvested from *M. excelsa* (7.75±0.05%) and least in mushroom harvested from *K. senegalensis* (5.64±0.07%). The crude fibre and crude protein content were significantly higher whereas there was no significant difference in the crude fibre and crude protein content of mushrooms harvested from the other substrates. Fat content was low for all mushroom harvested from all the substrate and ranged from 1.19±0.06 to 1.85±0.08% with *M. excelsa* having the highest fat content and *A. africana* having the least value. Fat content in *G. arborea* and *K. senegalensis* were not significantly different. Mineral content (Mg, Na, K and Ca in mg/kg) determined for fruiting bodies harvested from the different saw dust used showed that all the mushrooms had significantly higher potassium content followed by calcium (Table 4). All the mushrooms had low magnesium content compared to the other minerals investigated in this study. However, magnesium, sodium and calcium content for mushrooms harvested from *M. excelsa* were significantly higher than in mushrooms harvested from the other sawdust substrate. There was no significant difference in the magnesium and sodium content of mushrooms harvested from *G. arborea, A. africana* and *K. senegalensis.*

The TPC and TFC represented in Figure 1 for mushrooms harvested from *M. excelsa* were significantly higher (P<0.05), however, there was no significant difference between the TPC of mushrooms harvested form *K. senegalensis* and *G. arborea.* There were no significant differences (P<0.05) in the TFC of mushrooms harvested from *G. arborea, A. africana* and *K. senegalensis.*

DISCUSSION

The period for complete spawn run obtained in this study falls within the range reported by Liang et al. (2011) for *P. pulmonarius* cultivated on grass plants in Taiwan, but higher than what was reported by Shah et al. (2004). Sopit (2006) on the other hand reported as high as 34 days for completion of spawn run of *Pleurotus ostreatus* on saw dust while Akinmusire et al. (2011) reported 35

Table 4. Mineral composition (mg/kg) of *P. pulmonarius* cultivated on different sawdust substrate.

Sawdust substrate	Mg	Na	K	Ca
ME	0.41±0.007[b]	0.14±0.023[b]	6.51±0.012[d]	4.06±0.020[c]
GA	0.34±0.006[a]	0.12±0.011[a]	4.49±0.056[b]	2.77±0.031[a]
AA	0.36±0.007[a]	0.12±0.008[a]	7.09±0.004[c]	3.90±0.067[b]
KS	0.35±0.020[a]	0.10±0.007[a]	3.52±0.011[a]	2.65±0.033[a]

Each value is a mean of 3 replicates ± standard error, values accompanied by identical superscript letters are not significantly different (P≤0.05) in columns.

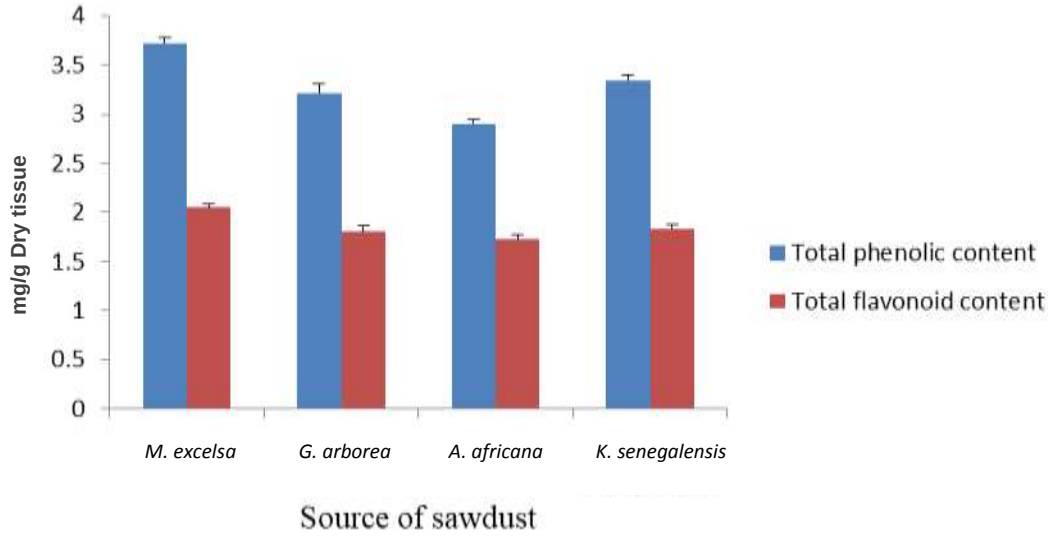

Figure 1. Effect of substrate on TPC and TFC of mushrooms

days for completion of spawn run of *P. pulmonarius* on saw dust which is higher than what is reported in this study.

The yield of *P. pulmonarius* recorded on all the saw dust substrates used in this work (expressed in BE) is lower than the yield obtained by some workers (Liang et al., 2011; de Siqueira et al., 2012), however, the yield from all the saw dust substrates was higher than what was reported earlier (Anyakorah and Dike, 2012; Adenipekun and Omolaso, 2015) for *P. pulmonarius*. The result obtained from the cultivation of oyster mushrooms on cassava waste showed that substrates containing up to 75% cassava peels and supplemented with rice bran have productions well comparable to yields obtained from the traditional saw dust based substrates (Anton et al., 2014). Jonathan et al. (2013) however reported greater yield of *P. pulmonarius* on rice straw and coir fibre than saw dust.

The variation in the rate of growth, spawn run and yield of *P. pulmonarius* on the different substrate in this present study corroborates the findings of Nasir et al. (2012) in the use of sawdust of different woods for the cultivation of oyster mushroom (*P. ostreatus*). These differences in growth and yield could be, in part, as a result of the variation in substrate type (Muhammad et al., 2007), substrate pre-treatment, additives, difference in strains of *P. pulmonarius* cultivated and the climatic condition at the time of cultivation.

Protein content reported in this present study falls within the range reported by earlier researchers (Ali et al., 2010; Khatun et al., 2012; Joshi and Shekhawat, 2014), but lower than that which was reported by Ashraf et al. (2013) for *P. ostreatus.* Protein and ash content was higher than that which was reported by Anyakorah and Dike (2012) for *P. pulmonarius* cultivated on saw dust, the fibre content was however lower. The lipid and protein content reported in the study fall within the range reported by Moni et al. (2004). The moisture content on the other hand for all the mushrooms harvested from all the saw dust substrate was lower than what was reported by Adenipekun and Omolaso (2015). The crude fibre content reported compares favourably with earlier finding (Anyakorah and Dike, 2012; Adenipekun and Omolaso, 2015). The high concentration of potassium compared to other nutrients in mushrooms may be due to a high content of potassium in agro-waste used for the

mushroom cultivation. The findings in this work are in agreement with earlier findings that substrates used in mushrooms cultivation have effect on chemical characteristics of mushrooms (Oyetayo and Ariyo, 2013) indicating a variation in the capability of such substrates to support mushroom growth (Kuhad and Singh, 1997).

The TPC reported in this study is higher than what was reported for both *Pleurotus eryngii* and *P. ostreatus* (Kim et al., 2008), falls within the range reported by Kumari and Acha (2008), but lower than what was reported by Abaaayah and Umi (2013). The TFC on the other hand, falls within the range reported by Abaaayah and Umi (2013). All mushroom harvested from the different sawdust substrates used showed varying TPC and TFC indicating that *P. pulmonarius* cultivated on different saw dust is able to synthesize phenolic compound thereby serving as a potential source of natural antioxidant for consumers. Further investigation is however necessary to determine ways of improving the TPC and TFC.

Conclusion

Based on the results obtained in this study, it can be concluded that most of the commonly available saw dust that constitute nuisance because of improper disposal are able to support growth and fructification of *P. pulmonarius* in varying degrees. And this indicates that the substrate recording the highest yield can be exploited for its cultivation thereby representing a bioconversion process of saw dust. Additionally, the fruiting bodies harvested from the substrates are nutritionally acceptable and posses antioxidant potential due to the TPC and TFC. Further investigations are necessary to determine influence of growth promoting additives so as to enhance higher yield of mushrooms on the different saw dust.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Evaluation of faba bean (*Vicia faba* L.) varieties for yield and reaction to chocolate spot disease at Chencha, Southern Ethiopia

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Field experiment was conducted at Chencha Woreda with the objective of evaluating faba bean varieties for better yield and chocolate spot disease resistance during 2015 main cropping season. Ten improved faba bean varieties and two local controls were evaluated in randomized complete block design (RCBD) with three replications. Field performances of varieties were evaluated for grain yield and yield parameters on sampled plant. Chocolate spot disease severity was recorded following 1 to 9 disease scale and used for area under disease progress curve (AUDPC) calculation. There was significant ($P < 0.05$) difference among the varieties for yield and resistance to chocolate spot disease. Higher yield was recorded in *Moti, Gora* and *Wolki* varieties while low yield was recorded from Hachalu and Degaga. Maximum chocolate spot severity and AUDPC were recorded on *Baela, Degaga, Gora* and *Gebelcho* varieties while minimum disease severity and AUDPC were recorded from *Tumsa, Moti, Hachalu* and *CS-20DK* varieties with a mean severity of 29.63, 24.69, 25.93 and 25.93%, respectively. *Moti, CS-20DK, Hachalu* and *Tumsa* varieties showed moderately resistant to chocolate spot disease severity while *Dosha* was moderately susceptible varieties. From the result it could be concluded and recommended that varieties *Moti*, *Gora* and *Tumsa* are promising at high lands of Chencha, and may used them in larger plot for identification of adaptable variety.

Key words: Faba bean, varieties, yield, disease severity, chocolate spot.

INTRODUCTION

Faba bean (*Vicia faba* L. 2n = 12), is a legume member belonging to the family Fabaceae. Species in genus *vicia* are genetically separated from each other according to differences in some of the seed characters such as weight, shape and size (Hawtin and Hebblethwaite, 1983). Genetic variability of faba bean is quite large. The great variability may be due to the presence of intermediate crossing system between autogamy and

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allogamy (Hanelt and Mettin, 1989). In fact, *V. faba* is partially pollinated by insects, so the pollinators can carry out both self-pollinations by the tripping process when they trip the flower and out crossing and when they visit other plants flowers (Nadal et al., 2003).

Crop diversification and low input agriculture are major drivers for sustainable agricultural policy. Pulse crops can contribute positively to these two policy goals because, as a result of biological nitrogen fixation, they require minimal inputs and provide a very effective break crop in a cereal dominated rotation. Pulse crops are also an efficient source of plant-derived protein for food and animal feed. In Ethiopia, three pulse crops (faba bean, field pea and fenugreek) occupied an area of 759,782.79 ha with a grain yield of 8.74 million quintals and its productivity is 10 to 45 quintal/ha across the country (CSA, 2013a).

Ethiopia is one of the largest producer of faba bean in the world second only to China (Hawtin and Hebblethwaite, 1983). The country is considered as the secondary center of diversity and also one of the nine major agro-geographical production regions of faba bean (Telaye et al., 1994). The production is mainly concentrated in the high-altitudes of Ethiopia ranging between altitudes 1800 and 3000 m.a.s.l with annual rainfall ranging from 700 to 1100 mm (Telaye, 1988). The study area has a total area of 37,360 ha land from this faba bean and field pea shares 2731 and 2285 ha of land annually, from these crops; faba bean ranks first in pulse crops and considered as the most important pulse crop in the area (Sankura, 2015 personal communication). Faba bean serves as a daily food and as cash crop in many parts of the country (Hawtin and Hebblethwaite, 1983). The pulses production and productivity is constrained by several biotic and abiotic stresses, of which lack of improved varieties, shortage of certified seeds, diseases such as rust, powdery mildew and root rot, insect pests such as aphids and low soil fertility are the major ones and becoming a major challenge to food security. In addition to this, its production in Ethiopia is limited and fails to face the increasing local consumption of seeds due to gradual decreases in its average yield. So, increasing crop production is the major target of the national agriculture policy and can be achieved by growing high yielding and stable cultivars under favorable environmental conditions (Graham and Vance, 2003).

This can be achieved by continuous highland pulse research to develop high yielding, pest resistance/tolerant, excellent in other agronomic traits, high quality, and widely adapted varieties that suit different cropping systems and farming conditions. Moreover, adequate seeds of released varieties should be made available to small scale farmers and commercial producers. The principal aim of variety trial research on highland pulses is generally to contribute to the general development policy of the nation by increasing production, productivity and thereby increasing income-generation capacity for the farmer through testing high-yielding varieties with stable performance and disease resistance. Agricultural office in the study area mentioned many problems in their extension system with the farmer associated with the production and productivity of faba bean. Among them lack of improved varieties, introduction of new varieties to the locality without conducting variety trial by only considering similar agro-ecological conditions and there are also insect pests affecting with the growth performance of this crop.

Besides these, the production of Faba bean is insufficient as a result of low crop yields because farmers grow varieties that are susceptible to diseases, insect pests, drought and high summer temperatures (ICARDA, 2008). FAOSTAT (2008) report showed that Faba bean production has declined from 4.8 million ha in 1961 to 2.4 in 2008 with 4.8 to 4.4 tons per hectare reduction in production. This reduction was due to susceptibility of faba bean varieties to biotic factors (Sillero et al., 2010) and abiotic stresses (Link et al., 2010). Chocolate spot (*Botrytis fabae*), Ascochyta blight (*Ascochyta fabae*) and faba bean rust (*Uromyces viciae-fabae*) are identified as the major diseases affecting faba bean in the country (ICARDA, 2008). In fact, chocolate spot and rust became the major threat worldwide in faba bean production.

In Ethiopia, the productivity of faba bean is far below its potential due to the aforementioned factors (Mussa et al., 2008). Winch (2006) reported that, the productivity of faba bean in Ethiopia is quite lower (15.2 qt/ha) (CSA, 2013b), as compared to that in UK, which is about 30 qt/ha). In Ethiopia, there are about 29 improved faba bean varieties which are adapted to different agro-ecology with varying reaction to diseases (Crop variety register issue No.17, 2014). Farmers in the Ethiopia commonly used to cultivate local varieties (Thijssen et al., 2008). In most cases local varieties are expected to be low yielding and susceptible to both biotic and abiotic stresses. Local landraces faba beans are highly susceptible to the disease and give low yield (Samuel et al., 2008). Chocolate spot disease is among the major diseases of faba bean which becomes the major threat in the study area. Therefore, growing of high yielding and disease resistance varieties of faba bean is crucial to ensure the sustainability of the crop and food security. Thus the experiment was conducted to evaluate improved faba bean varieties and local check for screening high yielding variety and assessing reaction to chocolate spot disease resistance in field condition to boost the productivity of the crop.

To intervene the aforementioned problems, this study was initiated with the general objectives specific in the highland pulse crops research strategy, the specific objectives to select and promote high yielding and well adapted faba bean varieties with desirable agronomic and quality traits for the highlands of Gamo Gofa area specifically in Chencha Woreda.

Objectives

The general objective is to evaluate faba bean genotypes for better yield and resistance to chocolate spot disease. The specific objectives include the following:

1. To identify promising faba bean varieties for seed yield and other important agronomic traits.
2. To assess the reaction of faba bean varieties to chocolate spot disease
3. To recommend promising varieties for further study in those important traits

MATERIALS AND METHODS

Experimental area description

The study was conducted at Chencha Woreda, Arba Minch University Gircha research Center, Chencha town. Chencha is found in the Gamo Gofa administrative zone of the Southrn Nation Nationalities People Republic of Ethiopia. The altitude is ranging between 1600 and 3200 m a.s.l. The mean annual temperature and rainfall of the study areas are 22.5°C and 1100 to 1600 mm/annum, respectively. The altitude of Gircha research is ranges up to 3007 m.a.s.l. The soil condition is characterized as 60, 30, 6 and 4% brown, black, grey and black in color, respectively and the topography of the Woreda is described as 65, 17, 13 and 3% mountainous, steeply, valley and flat, respectively (Sankura, 2015, personal communication). According to our observation the soil texturally looks like clay loam with brown color.

Plant material and planting

Faba bean cultivars namely Degaga (R-878-3), Gora (EK01024-1-2), Dosha (COLL 155/00-3), Gebelcho (EH96006-1), Hachalu (EH00102-4-1), Wolki (EH96049-2), Moti (EH95078-6), CS-20DK, Tumsa (EH99051-3), and Obse (EH95073-1) and local checks namely Baela and Orde Baela were used in this trial. The seeds of the cultivars were collected from legumes research coordinating centers (Kulumsa and Holeta Agricultural research centers) in Ethiopia. Recommended fertilizer rate was added during seed planting. Even though, the crop is nitrogen fixer in nature, recommended dose of nitrogen source fertilizer (Urea) was added to initiate nodulation process. Planting was done using seeds of faba bean at recommended rate per kilogram of seeds, in hills (two seeds/hill) to guarantee the germination of the seeds on the two sides of the ridges in row. Thinning was practiced after 21 days from sowing to secure one plants/hill. All the other recommended cultural practices for growing faba bean were followed like weed control, insect control, watering and others.

Experimental design

Ten faba bean cultivars advanced from pre varietal trial was tested along with two standard checks (local varieties) were planted on cropping season of June 2015 to December, 2015. Randomized Complete Block Design (RCBD) with three replications was used. The plots consisted of five rows of 4m length with inter- and intra-row spacing of 15 and 60 cm, respectively. Plot size was arranged as 4 m (length) × 2 m (width). The total area for this experiment was around 907.2 m^2. The experimental field was managed as per the standard field plot techniques and standard agronomic practice in the season.

Data collection

Plant heights (cm) at flowering at different interval were measured on 5 representative sample plants of faba bean cultivars. At harvesting time, field performance evaluations like seed yield (kg/m^2), 1000 seed weight in gram, number of pod per plant, number of seed per pod, The disease severity of chocolate spot was recorded from 65 to 86 days from sowing in 7 day intervals for 3 times to see disease severity at different growth stage using the scale of Bernier et al. (1993), where, 1 = no disease symptoms or very small specks (highly resistant), 3 = few small disease lesions (resistant), 5 = some coalesced lesions, with some defoliation (moderately resistant), 7 = large coalesced sporulating lesions, 50% defoliation some dead plants (susceptible), 9 = extensive, heavy spourlation, stem girdling, blackening and death of more than 80% of plants (heavily susceptible). The response of tested varieties were classified into six reaction groups according to Abo-Hegazy et al. (2012), where 0 to 2% is highly resistant (HR), > 2 to 15% is resistant (R), > 15 to 40% is moderately resistant (MR), > 40 to 60% is moderately susceptible (MS) and > 60 to 80% is susceptible (S) >80 to 100% is highly susceptible (HS) based on percent disease severity values.

Percent severity index (PSI%) was calculated using:

$$PSI\ (\%) = \frac{\sum(NPC*CR)*100}{NIP*MSC}$$

Where NPC = number of plants in each class rate, CR = class rate, NIP = number of infected plants and MSC = maximum severity class rate.

Area under disease progress curve (AUDPC) was calculated using the formula adapted from Cooke (2006) as followed by using the disease severity score of each plot in fixed interval of 7 day.

$$AUDPC = \sum_{i=1}^{n-1} [0.5\ (yi + 1\ + yi)(ti + 1 - ti)]$$

Where y_i = the average coefficient of infection of the i^{th} observation, y_{i+1} = the average coefficient of infection of the $i+1^{th}$ observation and; t_{i+1}-t_i = the number of days between the i^{th} observation and $i+1^{th}$ observation, and n = number of observations.

Data analysis

Analysis of variance (ANOVA) was conducted to see cultivar differences from the mean values of the sampled plant based on the experimental design used by statistical software (Gene Stat. 15^{th} edition). Fisher's LSD test at 5% level of significance was performed to determine whether there were significant differences among the cultivars for all measured traits. The coefficient of variation was calculated based on Burton (1952). Broad sense heritability ($h^2= S^2g/S^2p$ or $h^2= S^2g/S^2g+S^2e$) where, h^2 = heritability, S^2g = variance due to genotype, S^2p = variance due to phenotype = the sum of variance due to genotype and the experimental variance) was calculated according to (Johnson et al., 1955). The Pearson correlation coefficient between all measured trait means for twelve cultivars was determined.

RESULTS AND DISCUSSION

Growth and yield related traits

Faba bean varieties were significantly different ($P < 0.05$) for plant height (Figure 1A). The tallest plant heights were

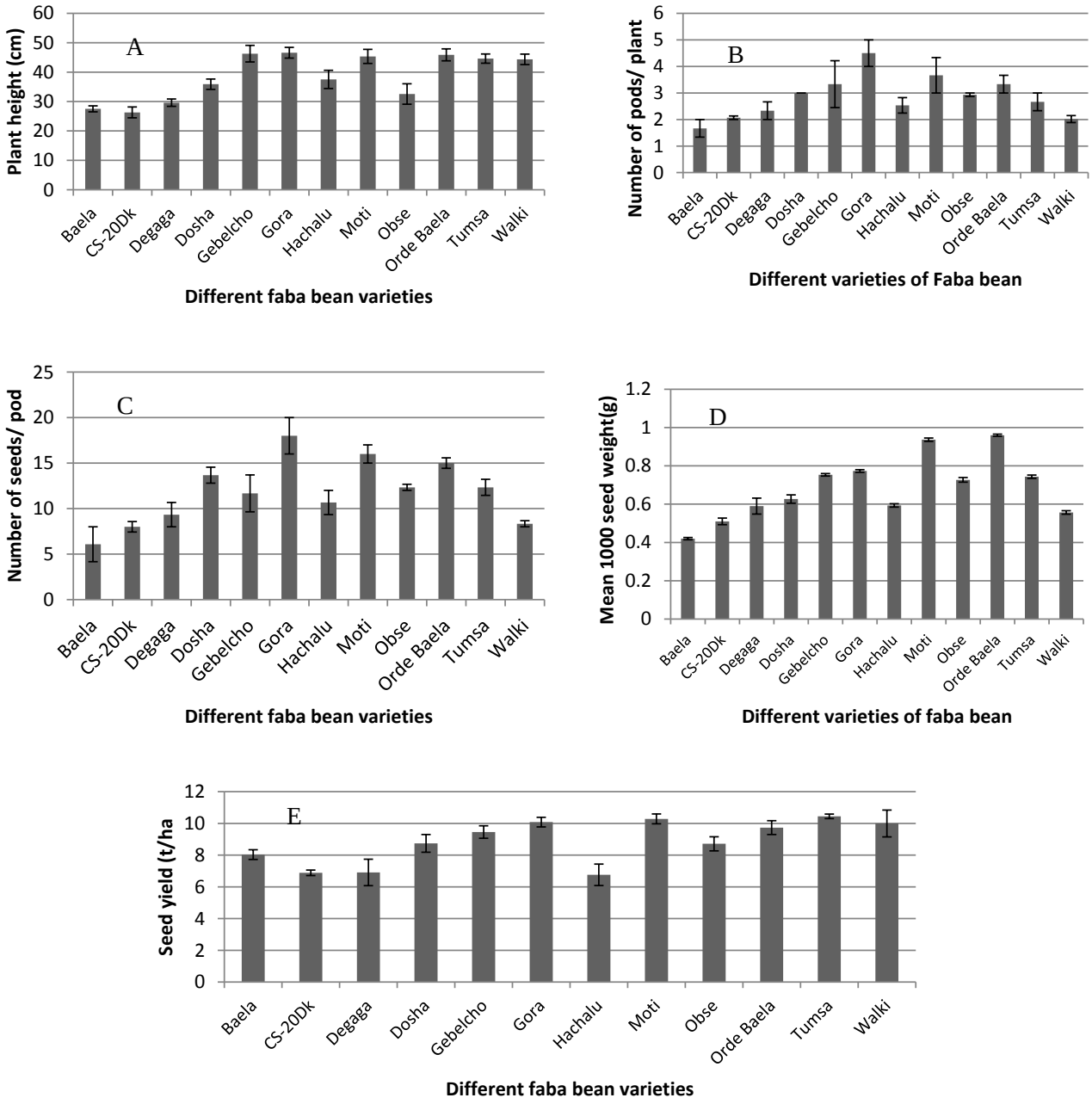

Figure 1. Effect of varieties on yield and yield components (A-E) of 10 improved and 2 local faba bean varieties during the main season of 2015/16 at Chencha Gircha research station Arba Minch, Ethiopia. Varieties were significantly ($P < 0.05$) different for all measured traits. Error bars are drawn from standard error of the mean.

recorded in Gora and Gebelcho with 46.6 and 46.27cm tall, respectively. This result disagreed with the result of Ashenafi and Mekuria (2015). They reported that Gebelcho had the shortest plant height at Sinana and Agarfa site, Ethiopia. This could be related to altitude difference of the study site. In most cases, the area becomes cooler as increasing altitude and plant metabolic activity becomes slower. On the other hand, the shortest plant heights were recorded in CS-20DK and local Baela varieties with a height of 26.3 and 27.53 cm, respectively. Thus, CS-20DK and local Baela varieties can be considered as dwarf varieties. Talal and Munqez (2013) reported that plant height differ significantly in faba bean accessions. Faba bean genotypes showed significantly different plant height of under rain fed conditions (Della, 1988).

Number of dry pods per plant was significantly different ($P < 0.05$) among all the tested varieties (Figure 1 B). *Gora*, *Moti* and *Gebelcho* ranked first to 3rd for number of pod per plant (4.5, 3.67 and 3.33). The result for

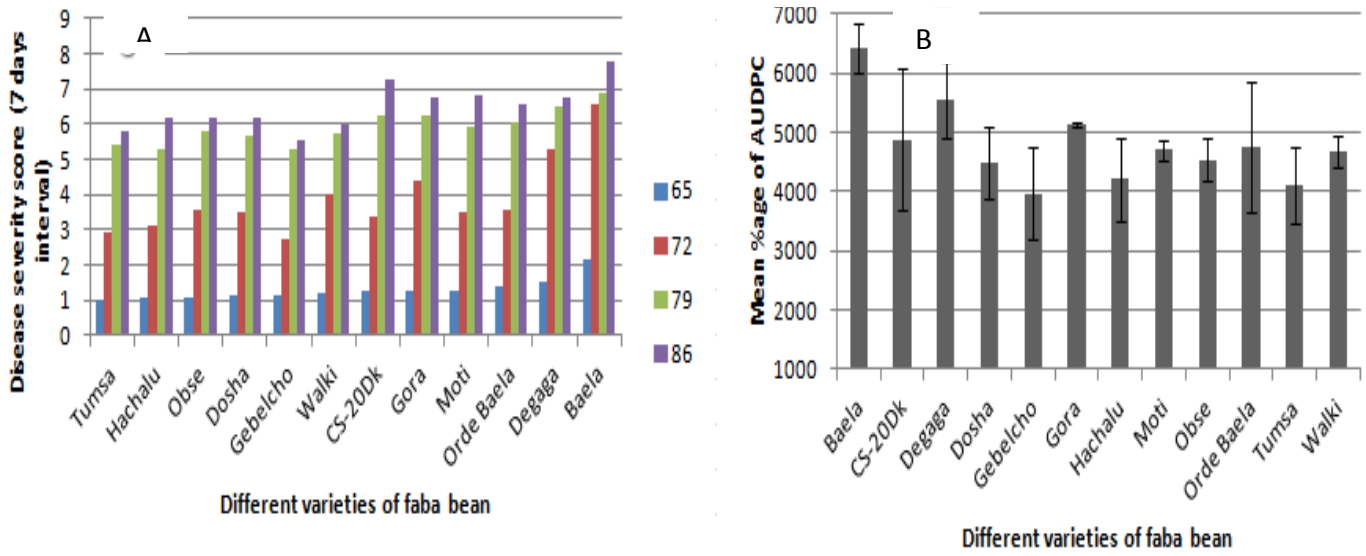

Figure 2. Disease severity score (A) from 65-86 DAS and the area under disease progress curve (AUDPC) (B) values on 10 improved and 2 local faba bean varieties 2015/2016 cropping season. Error bars are drawn from standard error of the mean.

Gebelcho was in line with the work of Ashenafi and Mekuria (2015) who reported that this variety had a higher number of pods per plant followed by variety Degaga at Agarfa experimental site. However, in the study of Tafere et al. (2012), Gebelcho and Moti varieties had the smallest number of pods per plant, which disagreed with our result. Whereas, Wolki from improved varieties and Baela from local check produced the lowest number of pods/plant. Faba bean genotypes significantly varied in production of pods per plant (Hassan and Ishaq, 1972; Pilbeam et al., 1992).

The analysis of variance revealed that there were significant ($P < 0.05$) differences between faba bean varieties in yield of dry seed (Figure 1E). The total grain yield recorded in the study was lower than the national average yield of faba bean; this could be due to strong acidic nature of the soil in the study area. In this study, *Moti* (1.028 t/ha) and *Gora* (1.008 t/ha) produced dry seed yield, which is the highest yield recorded. However, *Degaga* and *CS-20DK* produced lowest yield with 0.691 and 0.6887 t/ha, respectively. Following *Gora* and *Moti* varieties higher yield was obtained from *Wolki*, *Orde baela* and *Gebelcho* varieties at the study area with average seed yield of 0.999, 0.973 and 0.945 t/ha, respectively. Seed yield obtained from *Wolki* was in line with the result reported by Ashenafi and Mekuria (2015) at Sinana district even if there was differences the amount of yield obtained per hectare. However, *Moti* produced lowest grain yield in their study at this district. The report of ICARDA (2008) showed that shifting from traditional varieties to improved ones can bring an increment in yield of 18% in Egypt, 8% in Sudan and 42% in Ethiopia as reviewed by Ashenafi and Mekuria (2015). In this study, 30% increment of yield was found for using *Moti* variety (high yielder) as compared to Baela local variety (low yielder) at the study area.

There were significant variations in the values of 1000 seed weight shown by the faba bean varieties, confirming that the genetic variations among varieties. Of all tested varieties *Orde Baela* (969 g) produced heaviest seed weight followed by *Moti* (093 g), *Gora* (77 g) and *Gebelcho* (75 g) while, *Baela* resulted the lowest 1000 seed weight followed by CS-20DK, Wolki, Hachalu and Degaga (Figure 1D). This result indicated that *Orde Baela* local*, Moti, Gora* and *Gebelcho* varieties can be considered as large seeded while Baela local, CS-20DK, Wolki, Hachalu, and Degaga are small seeded. Seed size is one of the most important characters used for farmer to select variety. Hachalu was small seeded variety in this study which resulted in reduced seed yield. The result was in line with the work of Ashenafi and Mekuria (2015); they reported that *Moti* and *Gebelcho* varieties had the higher 100 grain weight while *Degaga* variety had the lowest grain weight. This result also coincides with the result of Tamane et al. (2014). The varieties evaluated in this study showed significant ($P < 0.05$) in number of seeds per pod. *Gora* had more number of seeds per pod followed by *Moti*. This had significant positive correlation with grain yield of faba bean varieties.

Disease severity

The intensities of disease occurrence varied at different intervals. Chocolate spot disease became sever as increasing time from the start of severity score (Figure 2). The result showed that there was a significant difference ($P < 0.05$) in disease severity score at 65 DAS among

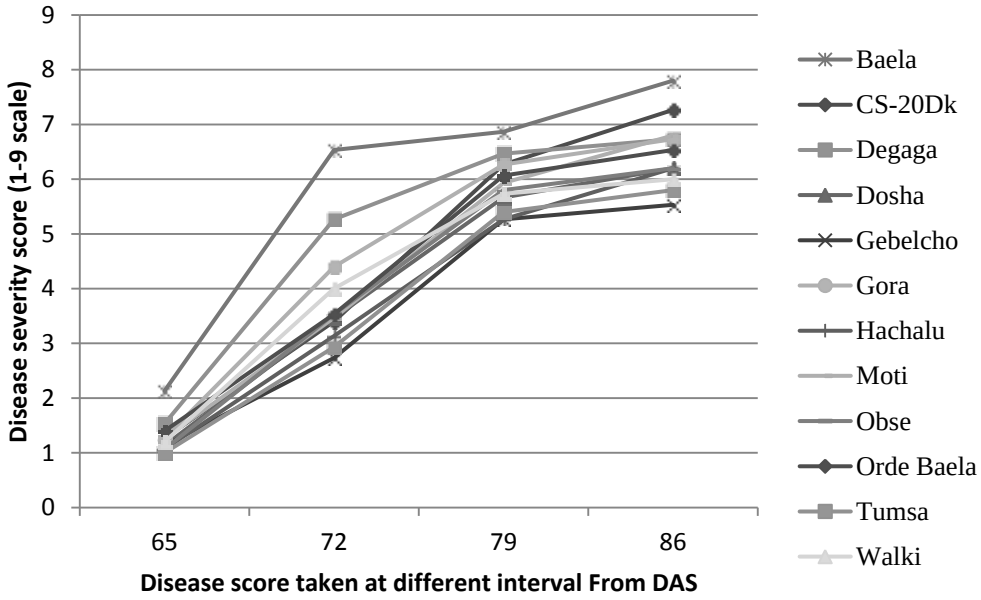

Figure 3. Disease Progress curve (DPC) for chocolate spot disease of faba bean varieties at Gircha research site Arba Minch, Ethiopia with different levels of resistance during the mainseason of 2015/16.

faba bean varieties. However, percent disease severity was not significantly different (*P > 0.05*). In our experimental location the lowest disease severity was recorded in variety *Tumsa, Moti, Hachalu* and *CS-20DK* varieties, that is, 29.63, 24.69, 25.93 and 25.93%, respectively, while maximum disease severity was recorded in *Degaga*, *Gebelcho*, *Dosha* and *Orde Baela* varieties, that was, 32.72, 33.95, 41.98 and 33.95% varieties at experimental site.

The result of this experiment indicated that the reaction of the individual varieties of faba bean for chocolate spot disease was more or less similar with the result of Mekuria and Ashenafi (2015). They reported that Tumsa as a resistant to chocolate spot disease. Similarly, Terefe et al (2012) and Tamene et al. (2015) reported that Tumsa variety was resistant to chocolate spot and Gebelcho variety was moderately resistant. The result was not inline to with the work of Mekuria and Ashenafi (2014). They reported that *Moti* and *Hachalu* had a moderate chocolate spot severity. This variation could be due to the difference in environmental conditions as the occurrence of disease chiefly depends on environmental condition.

There were Woreda differences among the faba bean varieties in the disease progression in the study area where the severity levels were more pronounced on all plants of the cultivar; *Dosha*, *Wolki* and *Degaga* during all the successive assessments 65 DAS as opposed to that of *Tumsa*, *Hachalu* and *Moti* (Figure 3). In general, the final chocolate spot severity was not significantly different (*P > 0.05*) among the varieties (Table 1). The highest chocolate spot infections on the local cultivars were 33.9%, as opposed to the lowest mean severity of 24.69% on *Moti* and 25.93% *CS-20DK* at the study site (3000 m a.s.l) (Table 1). Gora and Tumsa varieties had relatively lower infection values of 29.63 and 29.63% similar to the local check namely *Baela. Gebelcho*, *Obse* and *Degaga* had moderate infections that varied from 33.95, 32.41 and 32.72%, respectively. Accordingly, the four faba bean varieties namely *CS-20DK, Degaga, Moti, Tumsa* and *Hachalu* expressed relatively moderately resistance to chocolate spot at the study area natural condition. Thus, the uses of moderately resistant cultivars are recommended instead of depending solely on fungicides (Bouhassan et al., 2004; Josefina et al., 2010). In general, moderate resistance to chocolate spot with partial dominance has been reported in different researches obtained in this study.

Heritabilities of the tested varieties were found to be higher in all measured traits (Table 2). The heritabilities of most traits were above 0.5, indicating that more than 50% of the phenotypic variation observed for these traits was attributed to genetic factors. However, percent disease severity, and AUDPC showed low heritability. This could be due to severity of disease is mainly facilitated by environment.

Correlation between studied traits

Positive significant correlations were found between seed yield and plant height, number of pod per plant, number of seed per pod and 1000 seed weight (Table 3). The result was in agreement with (Silim and Saxena, 1992).

Table 1. Chocolate spot severity percent (%) on 10 improved and 2 local faba bean varieties at Chench Gircha research station, Arba Minch, Ethiopia during 2015/2016 main crop season.

Variety	Disease severity percent	Reaction group
Baela	29.63	MR
CS-20Dk	25.93	MR
Degaga	32.72	MR
Dosha	41.98	MS
Gebelcho	33.95	MR
Gora	29.63	MR
Hachalu	25.93	MR
Moti	24.69	MR
Obse	32.41	MR
Orde Baela	33.95	MR
Tumsa	29.63	MR
Wolki	33.95	MR
S.e.d	7	
LSD (?%)	14.5	
CV (%)	27.5	
P-value	0.539	

[a]DSI = mean disease severity percent assessed at 86 days after sowing (DAS). Means are not significantly different (P > 0.05) based on LSD value Test. Faba bean varieties with values >15 - 40 are considered moderately resistant while values >40 - 60 moderately susceptible (Abo-Hegazy et al., 2012).

Table 2. Heritabilities of different traits measured on 10 improved and 2 local faba bean varieties at Chench Gircha research station, Arba Minch, Ethiopia during 2015/2016 main crop season.

S/N	Measured traits	Heritability (%)
1	Mean of plant ht	85
2	Number of pod/plt	65
3	Number of seed/pod	81
4	1000 seed wt.	97
5	Yield (qt/ha	78
6	disease severity score	50
7	% disease severity	30
8	AUDPC%	32

Table 3. Correlation analysis among plant height, pod per plant, seed per pod, 1000 dry seed weight AUDPC, diseases severity score, percent diseases severity and grain yield of 10 improved and 2 local faba bean varieties at Chencha Gircha research station, Arba Minch, Ethiopia during 2015/2016 main crop season.

Parameter	Plant height (cm)	Pod/plant	Seed/pod	Yield (Kg/ha)	1000seed weight	AUDPC (%)	Disease severity score	Disease severity (%)
Plant height (cm)	-							
pod/plt	0.62*	-						
seed/pod	0.65*	0.90***	-					
yield (Kg/ha)	0.69**	0.45*	0.53*	-				
1000seed weight	0.68**	0.65*	0.7719**	0.58*	-			
AUDPC (%)	-0.49^{ns}	-0.44*	-0.44*	-0.27^{ns}	-0.34^{ns}	-		
Disease severity (score)	-0.30^{ns}	-0.17^{ns}	-0.254^{ns}	-0.04^{ns}	-0.25^{ns}	0.37^{ns}	-	
Dss sev (%)	0.07^{ns}	0.08^{ns}	0.20^{ns}	0.15^{ns}	-0.03^{ns}	0.06^{ns}	0.04^{ns}	-

*$P \leq 0.05$; ** $P \leq 0.01$; ***$P \leq 0.001$; ns = non-significant.

They reported that there is a positive relationship between seed yield and hundred seed weight, biological yield and seed per pod. Pod per plant, 1000 seed weight and number of seed per pod were positively significantly correlated with each other. These results suggested that for a high seed yield, fewer pods with large seed might be compensating for the low pod number per plant. The positive correlations observed between seed yield and 1000 seed weight might indicate the significance of the seed size in determining the final yield of faba bean varieties. Negative non-significant correlation between seed yield and AUDPC, disease severity score, and percent disease severity was recorded in this study. This indicates disease plays a vital role in reduction of yield.

CONCLUSION AND RECOMMENDATION

Ten improved and 2 local check faba bean varieties were evaluated for their yield, yield components and reaction to chocolate spot disease at Chencha Gircha research center Arba Minch, Ethiopia. There were variations between the varieties for most of the traits measured. The higher seed yield was recorded in variety Moti which was followed by variety Gora, Wolki and Gebelcho whereas varieties Hachalu and CS-20DK produced lower seed yield. Therefore, varieties *Moti*, *Gora* and *Wolki* are promising for grain yield at Chencha area. The varieties tested in this study produced lower yield compared to the national average yield of faba bean in Ethiopia, this could be associated with strong acidic characteristics of the soil. Regarding to faba bean varieties response to chocolate spot diseases under natural condition, It can be concluded from the current results that those faba bean varieties *Moti, CS-20DK*, *Gora* and *Tumsa* showed moderate resistance to *B. fabae* infection under field condition and superior yield especially in *Moti* and *Gora* are recommended to be adapted in chocolate spot prone areas of the study site and adjacent areas, of south Ethiopia. In addition, except variety Dosha, which is moderately susceptible, can also be grown in areas with contrasting environments within the faba bean production area. Using improved variety had an advantage of more than 30% increment of yield compared to Baela local variety at the study area. The varieties tested in this study produced lower yield compared to the national average yield of faba bean, this could be associated with strong acidic characteristics of the soil. This problem would lead to inefficient exploitation crops inherent capacity to use nutrient and produce potential yield. The promising varieties identified in the study may be further used at the same and other locations for confirmation of results on the performance of faba bean variety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Response of common bean (*Phaseolus vulgaris* L.) varieties to rates of blended NPS fertilizer in Adola district, Southern Ethiopia

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Common bean is one of the most economically important pulse crops cultivated in Ethiopia. However, its average yield reported at national level remains far below the potential yield to be attained. This is partly due to low soil fertility management, inappropriate agronomic packages and diseases and pest problems. Hence, this experiment was conducted to investigate the effect of blended NPS rates on growth, yield and yield components of common bean varieties and to identify economically feasible rates of blended NPS at Guji Zone Southern Ethiopia. The experiment was conducted in Adola sub-site of Bore Agricultural Research Center during 2016 to 2017 main cropping seasons. The factors studied were six rates of blended NPS (0, 50, 100, 150, 200 and 250 kg ha^{-1}) and three varieties of common bean (Angar, Ibado and Nasir). These were laid out in a factorial arrangement in randomized complete block design with three replications. Data on phonological, growth yield and yield related parameters were collected and analyzed using SAS software. The result showed that significantly the highest number of primary branches per plant (2.77) and the highest number of total pods (18.52) were recorded at the highest rate of 250 kg NPS ha^{-1} whereas the highest number of total nodules (80.47) and effective nodules per plant (35.54) were obtained from the application of 200 kg NPS ha^{-1}. Among the varieties, Angar gave significantly the highest number of primary branches per plant (2.55) and number of pods per plant (15.3). The interaction of variety and blended NPS had significant effect on almost all parameters except on the number of total and effective nodules per plant, number of primary branches per plant and number of pods per plant. Variety Nasir gave the highest plant height (99.72 cm) with application of 150 kg NPS ha^{-1} while Ibado with application rate of 200 kg blended NPS ha^{-1} had the highest hundred seed weight (54.33 g). The highest grain yield (3260 kg ha^{-1}) was recorded for variety Angar at 250 kg NPS ha^{-1}. However, the highest net benefit (29,825 Birr ha^{-1}) was obtained from combination of variety Ibado with application 200 kg ha^{-1} of blended NPS. Thus, it can be concluded that combined application of 200 kg ha^{-1} of blended NPS with variety Ibado proved to be superior with respect to economic advantage.

Key words: Blended fertilizer, nitrogen, phosphorus, sulphur.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.*)*, is herbaceous annual plant domesticated independently in ancient Mesoamerica and in the Andes, and now is grown worldwide for both dry seeds or as a green bean. Thousands of legume species exist but common bean in any form is the most eaten by human beings compared to

any other legumes (Broughton et al., 2003). When common bean is used for its unripe fruit, it is termed as green bean or snap bean. About 23.9 million tons of dry bean, 20.7 million tons of green bean, and 1.9 million tons of string or common bean were produced worldwide in 2012 (FAOSTAT, 2014). It is estimated that the crop meets more than 50% of dietary protein requirements of households in sub-Saharan Africa. The annual per capita consumption of common bean is higher among low-income people who cannot afford to buy nutritious food stuff, such as meats and fish (Broughton et al., 2003).

Common bean is highly preferred by Ethiopian farmers because of its fast maturing characteristics that enable households to get cash income required to purchase food and other household needs when other crops have not yet matured (Legesse et al., 2006). It is also an important food and cash crop in Guji zone with an area of 15,850.82 ha and average productivity of 1.52 tons/ha. Similarly, it contributed 39.49% for household consumption, 13.33% for seed, 44.1% for sale, 0.58% animal for feed and 2.05 other uses in the study zone (CSA, 2016).

Improved common bean production encompasses proper use of different agronomic practices which include improved variety, seed rate, spacing, fertilizer rate, and pesticide application as per recommendations. However, the current national average yield of common bean (1.48 tons) is far less than the attainable yield (2500 to 3000 kg ha^{-1}) under good management conditions for most improved varieties. This low yield of common bean in Ethiopia is attributed to several production constraints, which include lack of improved varieties for the different agro-ecological zones, poor agronomic practices such as low soil fertility management, untimely and inappropriate field operations (Alemitu, 2011).

A range of environmental factors, such as low soil nitrogen and phosphorus levels, and acidic soil conditions are important constraints for bean production in most areas where the crop is grown (Girma, 2009). Wortmann (2006) also reported that low soil fertility status especially low level of N and P to be the major constraints of common bean production responsible for the loss of grain yield up to 1.2 million tons in Africa. In general, an increase in grain yield and other agronomic parameters of common bean were observed as the rate of nitrogen and phosphorus increased till 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} (150 kg DAP ha^{-1}) (Girma, 2009). This fertilizer rate also gave yield advantages of 39% over the control.

Among the nutrients, nitrogen is the critical limiting element for growth of most plants including common beans due to its unavailability and poor fixation (Vance, 2001). Deficiency in N causes reduced growth, leaf yellowing, reduced branching and small trifoliate leaves in beans (CIAT, 1986). Previous surveys estimated that over 60% of the bean production areas in Central, Southern, and Eastern Africa were affected by N deficiency. This caused yield losses of up to 40% as compared to the N-fertilized areas (Singh, 1999). Besides, common bean is considered to be a poor fixer of atmospheric N when compared with other crop legumes and generally responds poorly to inoculation of rhizobia in the field. As a result, common bean is being generally considered as more responsive than other legumes to N fertilization (Graham, 1981).

Bean N fertilizer requirement depends on soil fertility levels; for low soil nitrogen levels (below 34 kg N ha^{-1}) N fertilizer is generally recommended in order for deficiency symptoms not to manifest and for full development up to production. Moreover, up to 60 kg N ha^{-1} also promotes increased nodule number, mass and size, giving highest yields ((Dwivedi et al., 1994). However, nitrogenous activity declines with applied nitrogen (Davis and Brick, 2009), decreasing the sink strength, and hence, reduce the quantity of photo-assimilate partitioned to nodules and grain. Early application may also result in excessive vegetative growth leading to delayed flowering, reduced pod set, lower seed yield and a greater risk of disease infestation (Setegne and Leggese, 2003).

The application of inorganic phosphorus fertilizer has positive effect on the yield and yield components of common bean. Rana and Singh (1998) revealed that grain weight per plant exhibited a pronounced response to phosphorus application, mean values of grain weight per plant records of 13.0, 17.4 and 20.7 g due to phosphorus fertilization of 0, 50 and 100 kg P_2O_5 ha^{-1}, respectively. Veeresh (2003) observed significant increase in grain weight per plant (8.65 g) due to increased P application up to 75 kg P_2O_5 ha^{-1}. Dwivedi et al. (1994) also reported linear increase in the number of grains per pod of common bean due to increase in phosphorus fertilization from 50 to 150 kg P_2O_5 ha^{-1}, but the differences were not significant beyond 100 kg P_2O_5 ha^{-1}. Saxena and Verma (1994) reported that the mean number of grains per pod linearly increased from 5.53 to 7.50 due to increased phosphorus fertilization from 0 to 120 kg P_2O_5 ha^{-1}.

Sulfur (S) is one of the essential nutrients for plant growth and it accumulates 0.2 to 0.5% in plant tissue on dry matter basis. It is required in similar amount as that of phosphorus (Ali et al., 2008). Sulphur plays a vital role in improving vegetative structure for nutrient absorption, strong sink strength through development of reproductive structure and production of assimilates to fill economically important sink. Sulphur nutrition of bean and other plants

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Table 1. Description of common bean varieties used for the study.

Characteristics	Varieties		
	Angar	Ibado	Nasir
Altitude (masl)	1300-2000	1400-2250	1200-1900
Annual Rainfall (mm)	1000-1300	500-850	500-800
Planting date	Mid-Late June	Mid-June-Early July	Mid June-Early July
Days of 50 flowering	41-52	43-58	40-55
Days to 95% maturity	85-96	90-120	86-88
Growth habit	Bushy	Bushy	Bushy
Seed colour	Dark red	Red	Red
Yield in research site (t ha^{-1})	2.0 - 3.2	2-2.9	2-3.2
Year of release	2005	2003	2003

Source: MoARD (2003, 2005).

is important since its application not only increases growth rate but also improves the quality of the seed (Clarkson et al., 1989). Total number of nodules and active nodules significantly increased with application of S up to 20 kg S ha^{-1} (Ganeshamurthy and Readly, 2000). Formation of nodules was increased due to sulphur application in blackgram (*Phaseolus mungo*) and is involved in the formation of nitrogenase enzyme known to promote nitrogen fixation in legumes (Scherer et al., 2006).

Soil fertility mapping project in Ethiopia recently reported the deficiency of K, S, Zn, B and Cu in addition to N and P in major Ethiopian soils and thus recommend application of customized and balanced fertilizers (EthioSIS, 2013). To address these nutrient deficiencies, farmers in Guji zone have been using uniform blanket application of 100 kg DAP ha^{-1} (18 kg N and 46 kg P_2O_5 ha^{-1}) for all legumes including common bean to increase crop yields for about five decades and this did not consider soil fertility status and crop requirement. This emphasizes the importance of developing an alternative means to meet the demand of nutrient in plants by using blended NPS that contains S in addition to the commonly used N and P fertilizers. However, no study has been done on response of common bean (*P. vulgaris* L.) varieties to the rates of blended NPS fertilizer in Adola district, Southern Ethiopia.

Thus, the objectives of this study were to investigate the effect of blended NPS rates on growth, yield and yield components of common bean varieties and to identify economically feasible rates of blended NPS at Guji Zone, Southern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Adola sub-site of Bore Agricultural Research Center (BOARC), Guji Zone, Oromia Regional State in Southern Ethiopia under rain-fed conditions during the 2016 cropping season (September-December). The site is located in Adola town in Dufa '*Kebele'* just on the west side of the main road to Negelle town. It is located at about 463 km south from Addis Ababa, capital city of the country. Geographically, the experimental site is situated at latitude of 55°36'31" North and longitude of 38°58'91"East at an altitude of 1721 masl.

The climatic condition of the area is a humid moisture condition, with a relatively shorter growing season. The area receives annual rainfall of 1084 mm with a bimodal pattern extending from April to November. The mean annual minimum and maximum temperature is 15.93 and 9.89°C, respectively. The type of the soil is red basaltic soil (Nitisols) and Orthic Aerosols ((Yazachew and Kasahun, 2011). The soil is clay in texture and moderately acidic with pH of around 5.88 (Table 3).

Experimental materials

Three common bean varieties, namely: Angar (medium-seeded); Ibado (large-seeded); and Nasir (medium seeded) were used (Table 1).

Variety Angar was released by Bako Agricultural Research Center in 2005. Ibado was released by Areka Agricultural Research Center in 2003 and Nasir by Melkasa Agricultural Research Center in 2003. Blended NPS (19% N, 38% P_2O_5, $_{and}$ 7% S) was used as sources of N, P and S, respectively, for the study.

Soil sampling and analysis

Pre-planting soil samples was taken randomly in a zigzag fashion from the experimental plots at the depth of 0 to 30 cm before planting. Twenty soil core samples were taken by an auger from the whole experimental field and combined to form a composited sample in a bucket. Then, the collected samples were air-dried at room temperature under shade and ground to pass through a 2 mm sieve for laboratory analysis of soil pH, and available phosphorus. Small quantity of this 2 mm sieved soil material allowed to passed through 0.2 mm sieve for soil organic carbon (OC) and total nitrogen. The composite soil samples were analyzed for selected physicochemical properties mainly textural analysis (sand silt and clay), soil pH, total nitrogen (N), available sulphur (S), organic carbon (OC), available phosphorus (P), cation exchange capacity (CEC) (c mol kg^{-1}), exchangeable potassium, magnesium and calcium using the appropriate laboratory procedures at Horticoop Ethiopia (Horticultural) PLC Soil and Water Analysis Laboratory.

Soil textural class was determined by Boycous Hydrometer

Table 2. Rate of fertilizer and their nutrient content (kg ha^{-1}) treatments for the experiment.

No.	Blended NPS Fertilizer rate (kg ha^{-1})	N	P_2O_5	S
1	0 kg NPS	0	0	0
2	50 kg NPS	9.5	19	3.5
3	100 kg NPS	19	38	7
4	150 kg NPS	28.5	57	10.5
5	200 kg NPS	38	76	14
6	250 kg NPS	47.5	95	17.5

Table 3. Physico-chemical properties of the experimental site soil before planting.

Character	Value	Rating
A. Soil texture		-
Sand (%)	30	-
Silt (%)	12	-
Clay (%)	58	-
Textural class	-	Clay
B. Chemical analysis		
Soil pH	5.88	Moderately acidic
Organic carbon (%)	2.3	High
Total N (%)	0.19	Low
Available P (mg kg^{-1})	5.61	Very low
Available S (mg kg^{-1})	14.50	Low
CEC [meq/100 g soil)	14.9	Low

Method (Aderson and Ingram, 1993). Organic carbon (OC) was estimated by wet digestion method (Walkey and Black, 1934) and organic matter was calculated by multiplying the OC% by a factor of 1.724. Total nitrogen was analyzed by Kjeldhal method (Jackson, 1962). The soil pH was measured potentiometrically in 1:2.5 soil-water suspensions with standard glass electrode pH meter (Van Reeuwijk, 1992). Cation exchangeable capacity (CEC) was determined by leaching the soil with neutral 1N ammonium acetate (FAO, 2008). Available phosphorus was determined by the Olsen's method using a spectrophotometer (Olsen, 1954). Available sulfur (S) was measured using turbidimetric method (EthioSIS, 2014). Exchangeable potassium, magnesium, and calcium were determined by Melich-3 methods (Mehlich, 1984).

Treatments and experimental design

The treatments were factorial combinations of six blended NPS fertilizer rates (0, 50, 100,150, 200 and 250 kg ha^{-1}) (Table 2) and three varieties (Angar, Ibado and Nasir). The experiment was laid out as Randomized Complete Block Design (RCBD) and replicated three times per treatment in factorial combination. The gross plot size was 3.0 m × 2.8 m = 8.4 m^2. The spacing between blocks and plots was 1.0 and 0.6 m, respectively. Each plot had 7 rows spaced 40 cm apart. One outer most row on each side of a plot and three plants (30 cm) on each end of rows were considered as border. One row next to the border rows on any side was used for destructive sampling. Thus, the net pot size was (1.6 m × 2.4 m = 3.84 m^2) having four rows each row with 24 plants.

Experimental procedure and crop management

The experimental field was prepared by using oxen-drawn implements (local plough maresha) according to farmers' conventional farming practices. The field was ploughed three times. The first plough was at the end of May 2016, the second in mid-July and the third during the middle of August before planting the crop to fine tilth. The plots were leveled manually. All the varieties were sown on 1 October. The dried seeds were planted by hand at a specified spacing (40 × 10 cm^2) by placing two seeds per hill and later thinned to one plant per hill after emergence. All the required amount of blended NPS was applied in band during planting. Furthermore, all necessary cultural and agronomic practices were carried out uniformly for all plots as per the recommendation for the crop at all stages of growth and development. The crop was harvested manually using a sickle when 90% of the leaves and pods turned yellow on 12 December, and dried under the sun for 4 days before threshing. Threshing was done separately for each treatment manually.

Data collected

An effect of blended NPS rate was investigated by measuring data on phonology, growth, yield and yield component parameters. Data on phonological parameters were measured through visual observation as the number of days from sowing to when 50% of plants in a net plot had reached flowering and 90% physiological maturity. Data on growth and yield component parameters were

taken in each plot from ten randomly selected plants at physiological maturity and at harvest time, respectively. For hundred seed weight and grain yield the whole plant from the net plot area was harvested and the yield per hectare was determined by converting the yield per plot (kg per plot) into kg per hectare

Statistical data analysis

All the measured parameters were subjected to analysis of variance (ANOVA) appropriate to factorial experiment in RCBD according to SAS software 9.1 versions. Significance difference (LSD) test at 5% probability level was used for mean comparison.

Economic analysis

Economic analysis was performed using partial budget analysis following the procedure described by CIMMYT (1988) in which prevailing market prices for inputs at planting and for outputs at harvesting were used. All costs and benefits were calculated on ha basis in Birr. The concepts used in the partial budget analysis were the mean grain yield of each treatment, the field price of common bean grain, and the gross field benefit (GFB) ha^{-1} (the product of field price and the mean yield for each treatment).

The net benefit (NB) was calculated as the difference between the gross benefit and the total cost. The average yield obtained from experimental plot was reduced by 10% to adjust with the expected farmers' yield by the same treatment. Prices of grain (Birr kg^{-1}) were obtained from local market for each variety: Ibado was 12 Birr kg^{-1} and Angar and Nasir were 8 Birr kg^{-1}, and total sale from 1 ha was computed using adjusted yield. Other costs such as cost of fertilizer (1400 Birr 100 kg^{-1} blended NPS) and its application cost (350 Birr ha^{-1}) were considered as the costs that vary for treatment to treatment.

RESULTS AND DISCUSSION

Physico-chemical properties of the experimental site soil

Soil texture is an important soil physical characteristic as it determines water intake rate (infiltration), water holding capacity of the soil, the ease of tilling, the amount of aeration, and also influences soil fertility (Gupta, 2000). It is one of the inherent soil properties less affected by management and determines nutrient status, organic matter content, air circulation and water holding capacity of a given soil. According to the soil textural class determination triangle, soil of the experimental site was found to be clay (Table 3). High clay content might indicate the better water and nutrient holding capacity of the soil of the experimental site.

According to the soil analysis test, the soil pH of the experimental site was 5.88 (Table 3). Thus, according to Landon's (1991) rating, the chemical reaction of the experimental site is moderately acidic. The available P level in the experimental site which is 5.61 mg kg^{-1} (Table 3) is very low according to the rating of EthioSIS (2014). This low available phosphorus could be due to fixation in such acidic soils.

The result of laboratory analysis showed that the total nitrogen percentage (0.19%) was low as per the rating of EthioSIS (2014). Cation exchange capacity is the capacity of the soil to hold and exchange cations. It provides buffering effect to changes in pH, available nutrients, calcium levels and soil structural changes. The result showed the CEC of the experimental soil to be 14.9 meq/100 g soils rated as moderate according to rating of Landon (1991). The total carbon content in the soil was 2.3% which was rated as high as per the classification of Hazelton and Murphy (2007). Thus, the OM content of the soil was optimum as rated by EthioSIS (2014). On the other hand, the available sulphur content in the soils has values of 14.50 mg kg^{-1} which was rated as low as per the classification of EthioSIS (2014).

Phenological and growth parameters of common bean

Days to flowering

Days to 50% flowering had significantly ($P<0.05$) influenced by interaction of blended NPS rate and varieties P rate, but the main effects of variety and blended NPS rate were found to be highly significant ($P<0.01$) on days to reach 50% flowering (Table 1). Significantly, the highest number of days (46.67 days) to reach flowering was recorded due to application of 200 kg ha^{-1} of blended NPS for variety Nasir and for variety Angar at NPS rate of 250 kg ha^{-1} while the earliest days to flowering (38.33 days) was recorded due to application of 50 kg ha^{-1} of blended NPS for variety Ibado (Table 4). Variety Ibado was found to be early maturing as compared to the other varieties across all NPS rates.

The result obtained from the current study revealed that the days to flowering were delayed with increment of application rate of blended NPS fertilizer which could be due to the delaying effect of nitrogen obtained from blended NPS fertilizer. This result was in line with the findings of Reta (2015) who reported that increasing the nitrogen rate from nil to 69 kg N ha^{-1} significantly prolonged the days to 50% flowering of linseed (*Linum usitatissimum* L). This might be due to the fact that excessive supply of N promotes luxuriant and succulent vegetative growth, dominating the reproductive phase. This result is corroborated by that of Ali and Raouf (2011) who reported that number of days from sowing to flowering increased significantly with increasing nitrogen application amount from 23 to 46 kg N ha^{-1} in chickpea.

However, Tesemma and Alemayehu (2015) reported that interaction of P with variety to be non-significant on common bean. This result is also in contrast to the finding of Nebret (2012) who reported non-significant interaction effects of nitrogen and sulphur on days to flowering of common bean.

Days to physiological maturity

Days to physiological maturity was highly significantly

Table 4. Mean number of days to flowering of common bean as affected by the interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	45.33^{abc}	45.33^{abc}	45.33^{abc}	45.33^{abc}	46.33^{ab}	46.67^{a}	45.72
Nasir	45.67^{abc}	45.00^{bc}	45.33^{abc}	45.67^{abc}	46.67^{a}	44.67^{c}	40.50
Ibado	41.67^{d}	38.33^{e}	39.67^{e}	39.67^{e}	42.00^{d}	42.00^{d}	45.50
Mean	44.22	42.89	43.44	43.56	45.00	44.33	-
LSD (0.05)	-	-	1.58	-	-	-	-
CV (%)	-	-	2.20	-	-	-	-

Means followed by the same letters are not significantly different as judged by LSD test at 5%. CV: Coefficient of variation.

Table 5. Mean number of days to physiological maturity of common bean as affected by the interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	96.00^{a-d}	93.33^{de}	98.00^{abc}	98.67^{ab}	94.00^{cde}	99.33^{a}	96.56
Nasir	96.33^{a-d}	95.67^{a-d}	94.00^{cde}	95.33^{a-d}	98.00^{abc}	93.33^{de}	95.44
Ibado	91.33^{e}	95.67^{a-d}	95.00^{b-e}	97.33^{a-d}	98.67^{ab}	98.00^{abc}	96.00
Mean	94.56	94.89	95.67	97.11	96.89	96.89	-
LSD (0.05)	-	-	3.48	-	-	-	-
CV (%)	-	-	2.2	-	-	-	-

Means followed by the same letters are not significantly different as judged by LSD test at 5%. CV: Coefficient of variation.

($p<0.01$) influenced by interaction of varieties with blended NPS application rate but not significantly influenced by main effect of variety (Table 5). Physiological maturity of common bean was delayed with increase in blended NPS rate. The highest number of days required to physiological maturity (99.33 days) was recorded for the highest rate of blended NPS application rate (250 kg ha^{-1}) for variety Angar while the shortest days to physiological maturity (91.33 days) was recorded without the NPS application for variety Ibado (Table 5).

The results indicated that days to maturity in most cases were prolonged in response to the increased levels of blended NPS which can be attributed to the role of nitrogen in the NPS that promoted vegetative growth. This is in line with the results of Gupta and Sharma (2000) who reported that nitrogen promoted vegetative and lush growth thereby delaying plant maturity of onion. This indicates that the nutrients taken up by plant roots from the soil were used for increased cell division and synthesis of carbohydrate, which will predominantly be partitioned to the vegetative sink of the plants, resulting in plants with a luxurious foliage growth (Marschner, 2012).

This result is further corroborated with the finding of Huerta et al. (1997) who reported delayed physiological maturity due to nitrogen fertilization of up to 80 kg ha^{-1} in common bean. In contrast, Nebret (2012) reported that the application of sulphur (0 to 60 kg ha^{-1}) had no significant effect on days to maturity on common bean.

Plant height

The analysis of variance showed highly significant ($P<0.01$) effect of varieties, blended NPS rates and their interaction on plant height at physiological maturity (Table 6). Variety Nasir showed the highest plant height (99.72 cm) with application of 150 kg NPS ha^{-1} whereas the shortest plants (31.08 cm) were seen for Ibado without NPS fertilizer (Table 6).

Plant height was significantly increased from 31.08 cm for variety Ibado with 0 kg NPS ha^{-1} to 99.72 cm for variety Nasir at 150 kg NPS ha^{-1}. The increase in plant height in response to the increased blended NPS application rate might be due to the maximum vegetative growth of the plants under higher N, P and S availability. Nitrogen helps in chlorophyll formation, phosphorus establishes strong root system and sulphur enhanced the formation of chlorophyll and encouraged vegetative growth (Halvin et al., 2003). In conformity with the current result, Moniruzzaman et al. (2008) found that plant height was significantly increased up to 160 kg N ha^{-1}. Also application of phosphorus at the highest level (120 kg

Table 6. Means of plant height (cm) of common bean as affected by the interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	56.30^{ef}	83.44^{bc}	75.12^{cd}	85.58^{abc}	89.71^{abc}	91.97^{ab}	80.35
Nasir	63.13^{de}	57.17^{efg}	88.94^{abc}	99.72^{a}	90.69^{abc}	90.52^{abc}	81.69
Ibado	31.08^{i}	38.57^{hi}	43.33^{ghi}	45.6f^{gh}	48.96^{fgh}	48.55^{fgh}	42.83
Mean	50.17	59.73	69.13	77.27	76.45	77.01	-
LSD (0.05)	-	-	13.69	-	-	-	-
CV (%)	-	-	12.00	-	-	-	-

Means followed by the same letters are not significantly different as judged by LSD test at 5%. CV: Coefficient of variation.

Table 7. Mean numbers of primary branches, total and effective nodules per plant of common bean as influenced by the main effects of variety and blended NPS fertilizer rates at Adola during 2016-2017 main cropping season.

Treatment	Number of primary branches per plant	Number of total nodules per plant	Number of effective nodule per plant
Variety			
Angar	2.55^{a}	63.01	32.88
Ibado	2.28^{ab}	68.09	32.56
Nasir	2.05^{b}	61.83	30.38
LSD (0.05)	0.28	NS	NS
NSP rate (kg ha^{-1})			
0	1.56^{d}	40.94^{c}	27.43^{c}
50	2.05^{c}	61.16^{b}	30.87^{bc}
100	2.25^{ab}	58.52^{b}	31.87^{b}
150	2.55^{a}	60.36^{b}	32.51^{ab}
200	2.58^{a}	80.47^{a}	35.54^{ab}
250	2.77^{a}	64.41^{b}	33.41^{ab}
LSD (0.05)	0.38	12.0	4.07
CV (%)	17.7	20.5	14.2

Means in the same column and treatment category followed by the same letters are not significantly different as judged by LSD at 5% level of significance. NS: Non-significant.

P_2O_5 ha^{-1}) increased plant height. The promotion effect of high P level on plant height of maize may be due to better development of the root system and nutrient absorption (Hussain et al., 2006). The increase in plant height might also be ascribed to better root formation due to sulphur, which in turn activated higher absorption of N, P, K and sulphur from soil and improved metabolic activity inside the plant. Similar results were r reported by Jawahar et al. (2017) where sulphur level of 40 kg ha^{-1} was found to increase the plant height, LAI, chlorophyll content and number of branches per plant of blackgram (*Vigna mungo).* In contrast to this result, Fisseha and Yayis (2015) reported no significant main and interaction effect of N and P levels on plant height of common bean. Similarly, Meseret and Amin (2014) also reported that P rate at 0 to 40 kg ha^{-1} had no significant effect on plant height in common bean.

Number of primary branches

The analysis of variance showed highly significant ($P<0.01$) main effect of variety and blended NPS fertilizer application rates on number of primary branches, while their interaction did not significantly influence the number of primary branches (Table 7). Variety Angar recorded the highest number of primary branches per plant (2.55) while the lowest number of primary branches (2.05) was recorded for variety Ibado. This difference might be due to genetic differences in production of number of primary branches among the varieties. This difference might be due to genetic differences in production of number of

primary branches among the varieties. The result was consistent with the finding of Addisu (2013) who reported that number of primary and secondary branches was highly significantly different among the chickpea varieties at Debre-Zeit with the Desi variety Natoli had significantly higher number of primary (3.21) and secondary branches (6.73) than the Kabuli variety Acos Dubie with 2.26 and 3.49, respectively.

The blended NPS rate had highly significant (P<0.01) effect on number of primary branches per plant. Increasing rates of blended NPS fertilizer from 0 to 250 kg ha^{-1} showed progressive increase in the number of primary branches per plant (Table 7). Thus, the highest number of primary branches per plant (2.77) was recorded at the highest rate of application of (250 kg NPS ha^{-1}) and it was statistically at par with NPS rates of 200, 150, and 100 kg NPS ha^{-1}, while the lowest number of primary branches per plant (1.56) was recorded for the control. The increase in number of primary branches per plant in response to the increased rate of blended NPS application rate indicates higher vegetative growth of the plants under higher N, P and S availability. In line with this result, Shubhashree (2007) reported significantly higher number of branches per plant of common bean with 75 kg P_2O_5 ha^{-1} over the control.

The increment in number of branches with increased rate of P might also be due to the importance of P for cell division, leading to the increase in plant height and number of branches (Tesfaye et al., 2007). In line with this result, Moniruzzaman et al. (2008) reported that the number of branches per plant increased significantly with the increase of N up to 120 kg ha^{-1} on common bean. The increased primary branches observed under blended fertilizer might be attributed to readily available form of S that enhanced uptake of nutrients even at the initial stage of crop growth. The result was also in agreement with the finding of Jawahar et al. (2017) who reported that application of 40 kg S ha^{-1} recorded highest number of branches per plant (7.75) in blackgram (*V. mungo*).

Total number of nodules

The main effect of variety and interaction of variety with blended NPS rate had no significant effect on total number of nodules, but the main effect of blended NPS rate had highly significant (P<0.01) effect on total number of nodules (Table 7). Thus, the highest number of total nodules per plant (80.47) was obtained from the application of blended NPS rate of 200 kg NPS ha^{-1} while the lowest number of total nodules (40.94) was recorded from nil application of blended NPS fertilizer.

Application of blended NPS fertilizers significantly increased the number of nodules up to 200 kg ha^{-1} which might be due to better root development with increasing levels of these nutrients. But the total nodule number decreased at 250 kg NPS ha^{-1}. The decrease in number of nodules per plant at highest rates of blended NPS might be due to increasing nitrogen application rates and thereby attributed to the negative effect of fertilizer-N on nodule formation and growth at the high rates. This result is in line with that of Chen et al. (1992) and Starling et al. (1998) who reported that high rate of nitrogen (56.58 kg N ha^{-1}), resulted in reduction of nodule number and nodule weight in soya bean.

The increase in number of total nodules at 200 kg NPS ha^{-1} might also be due to phosphorus which is needed in relatively large amounts by legumes for growth and to promote leaf area, biomass, yield, nodule number and nodule mass in different legumes. Consistent with this result, Amare et al. (2014) who reported that nodule number was significantly increased with increasing levels of phosphorus with the lowest (12.89) and the highest (31.85) numbers in common bean obtained from the control treatment and application of 20 kg P_2O_5 ha^{-1}, respectively. Yadav (2011) reported the synergistic effect of phosphorus and sulphur on number and weight of nodules per plant with the maximum number of nodules plant per plant was recorded at the highest level of phosphorus (40 kg P_2O_5 ha^{-1}) along with sulphur (20 kg S ha^{-1}) on clusterbean (*Cyamopsis tetrogonoloba*).

Number of effective nodules

Blended NPS fertilizer application had significant (P<0.05) effect on number of effective nodules per plant, but main effect of variety and interaction of variety with blended NPS had no significant effect (Table 7).

Number of effective nodules per plant increased with the increasing rate of blended NPS application rate. Increasing of blended NPS fertilizer from 0 to 200 kg ha^{-1} enhanced the number of effective nodules per plant (Table 7). The highest number of effective nodules per plant (35.54) was recorded at the rate of 200 kg NPS ha^{-1} while the lowest number of effective nodules per plant (27.43) was recorded at the rate of 0 kg NPS ha^{-1}. The increased number of effective nodules with the increase in NPS application up to 200 kg NPS ha^{-1} might be due to the vital role of phosphorus in increasing the number and size of nodule and the amount of nitrogen assimilated per unit of nodules. In agreement with this result, Bashir et al. (2011) reported that phosphorus plays a vital role in increasing plant tip and root growth, decreasing the time needed for developing nodules to become active (effective) for the benefit to the host legume. Similarly, Tsai et al. (1993) reported that application of nitrogen in the range of 22 to 33 kg ha^{-1} enhanced both nodulation and seed yield of French bean (*P. vulgaris*).

The increased number of effective nodules with the application of NPS over the control might also be from increased sulphur application which might be due to the high dose of sulphur and increasing its availability along with other major nutrients. This result is in line with the

Table 8. Mean stand count per plot at harvest of common bean as influenced by interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main cropping season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	88^{ab}	92.67^{a}	79.33^{cdef}	79.00^{c-f}	84.00^{bc}	72.33^{g}	82.56
Ibado	77.33^{efg}	89.33^{ab}	84.00^{bc}	73.33^{fg}	83.67^{bcd}	76.67^{efg}	80.72
Nasir	81.33^{cde}	88.67^{ab}	83.00^{bcde}	83.67^{bcd}	80.67^{cde}	83.67^{bcd}	83.50
Mean	82.22	90.22	82.11	78.67	80.67	83.67	-
LSD (0.05)	-	-	5.62	-	-	-	-
CV (%)	-	-	4.10	-	-	-	-

Means in rows and columns followed by the same letter are not significantly different judged by LSD test at 5% level of significance. CV: Coefficient of variation.

reported significant increase in the number of active nodules of soybean with the application of sulphur up to 20 kg ha^{-1}, at which point nodule production reached a plateau and did not increase further. Scherer et al. (2006) also reported that formation of nodule in blackgram was increased in response to sulphur application which is involved in the formation of nitrogenous enzyme known to promote nitrogen fixation in legumes.

Yield and yield components

Stand count at harvest

The main effect of NPS and the interaction of varieties and blended NPS rate had highly significant (P<0.01) effect on stand count at harvest. But varieties had no significant effect on stand count at harvest (Table 8). The highest stand count per plot at harvest (92.67) was obtained at applied blended NPS rate of 50 kg ha^{-1} for variety Angar, whereas the lowest stand count at harvest (72.33) was recorded for variety Angar at the highest rate of fertilizer application (250 kg NPS ha^{-1}). The reduction in final crop stand count at the highest NPS rate could be due to sufficient supply of nutrients which in turn favored vigorous vegetative growth, thereby resulting in higher intra-plant competition and crowding out of weaker plants by the vigorous ones.

Number of pods per plant

Highly significant (P<0.01) effects of blended NPS fertilizer application rate and varieties were observed on the number of total pods per plant while the interaction effect did not significantly influence the number of total pods (Table 9). The highest number of total pods per plant (18.52) was recorded at application rate of 250 kg NPS ha^{-1} whereas the lowest number of total pods (8.7) was obtained from the unfertilized plot (Table 9).

The increase in number of pods per plant with the increased NPS rates might possibly be due to adequate availability of N, P and S which might have facilitated the production of primary branches and plant height which might in turn have contributed for the production of higher number of total pods. In conformity with this result, Moniruzzaman et al. (2008) reported significant effect of N fertilizers on pod production per plant of French bean with the maximum number of pods per plant (25.49) obtained at 120-120-60-20-4-1 kg of N-P_2O_5-K_2O-S-Zn-B. The increment of number of pods per plant due to application of P fertilizer confirms the fact that P fertilizer promotes the formation of nodes and pods in legumes (Buttery, 1969). In agreement with this result, Dereje et al. (2015) also found that the number of pods per plant of common bean significantly increased in response to increasing rate of phosphorus up-to the highest rate (92 kg P_2O_5 ha^{-1}). On the other hand, Jawahar et al. (2017) reported that application of 40 kg S ha^{-1} recorded the highest number of seeds per pod of blackgram. This could be due to the increasing levels of sulphur application enhanced its availability to the crop and increase photosynthetic activity of crop

In this study, varieties also exhibited highly significant (P<0.01) difference in the number of pods per plant. Variety Angar produced the highest number of pods per plant (15.3) while the lowest number of pods per plant (10.24) was recorded for variety Ibado (Table 9). The variation in the number of pods per plant among the varieties might be related to the genotypic variation of the cultivars in producing pods. In line with the results of the present study, different authors reported significant variations in the number of pods per plant for common bean varieties (Fageria et al., 2010; Mourice and Tryphone, 2012).

Number of seeds per pod

The interaction effect of variety and blended NPS application rates and main effects of blended NPS application rates were not significant, but the main effects of varieties had highly significant (P<0.01) effect on the number of seeds per pod (Table 9). The highest number

Table 9. Mean number of pods per plant and seeds per pod of common bean as influenced by varieties and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Treatment	Number of pods per plant	Number of seeds per pod
Variety		
Angar	15.30[a]	5.35[a]
Ibado	10.24[c]	5.33[a]
Nasir	12.63[ab]	3.18[b]
LSD (0.05)	2.21	0.22
NSP rate (kg ha^{-1})		
0	8.70[c]	4.40
50	11.82[bc]	4.54
100	12.6[ab]	4.73
150	12.51[ab]	4.54
200	14.91[ab]	4.76
250	18.52[a]	4.75
LSD (0.05)	3.14	NS
CV (%)	25.1	6.2

Means in columns and rows followed by the same letter are not significantly different judged by LSD test at 5% level of significance. ns: Non-significant, CV: coefficient of variation.

Table 10. Means of hundred seed weight (g) of common bean as influenced by interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	23.33[e]	23.33[e]	38.33[c]	20.00[e]	21.67[e]	42.33[bc]	28.17
Ibado	38.33[c]	40.00[c]	38.33[c]	38.33[c]	54.33[a]	46.67[b]	42.67
Nasir	21.67[e]	20.00[e]	20.00[e]	20.00[e]	31.67[d]	30.00[d]	23.89
Mean	27.78	27.78	32.22	26.11	35.89	39.67	-
LSD (0.05)	-	-	5.58	-	-	-	-
CV (%)	-	-	10.6	-	-	-	-

Means in columns and rows followed by the same letters are not significantly different as judged by LSD test at 5% level of significance. CV: Coefficient of variation.

of seeds per pod (5.35) was recorded for variety Nasir followed by Angar (5.33) whereas the least number of seeds per pod (3.18) was recorded for variety Ibado (Table 9). This indicates that the trait is mainly controlled by genetic factors than the management. Consistent with the results of this study, Mourice and Tryphonne (2012) observed significant variations in number of seeds per pod among common bean genotypes. The variation in number of seeds per pod could be attributed to the variation in the size of seeds of the cultivars where variety Ibado with the highest seed size produced lower number of seeds per pod. In agreement with this result, Fageria and Santos (2008) also reported that the number of seeds per pod of different common bean genotypes varied in the range of 3.1 to 6 and attributed the difference due to the genetic variation of cultivars. However, the result of the present study was in contrast with the findings of Shubhashree (2007) who reported that the number of seeds per pod of French bean increased significantly with the levels of phosphorus added.

Hundred seed weight

Hundred seed weight was highly significantly ($p<0.01$) influenced by varieties, blended NPS rate and their interactions interaction (Table 10). Variety Ibado with application of 200 kg blended NPS ha^{-1} fertilizer scored significantly the highest hundred seed weight (54.33 g) while the lowest hundred seed weight (20 g) was for variety Nasir with 100 kg blended NPS ha^{-1} application rate (Table 10). This might be because nutrient use efficiency by crop was enhanced at optimum level of N, P and S since grain weight indicates the amount of

Table 11. Means of above-ground dry biomass yield (kg ha^{-1}) of common bean as influenced by interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	5794^{def}	5178e^{f}	9135^{ab}	5798^{def}	6191^{c-f}	10278^{a}	7062.33
Ibado	4129^{f}	4936e^{f}	5724^{def}	6527^{b-f}	8802^{abc}	7329^{b-e}	6241.17
Nasir	4045^{f}	6443^{b-f}	6640^{b-f}	5782^{def}	8073^{a-d}	9073^{ab}	6676
Mean	4656	5519	7166.33	6035.67	7688.67	8893.33	-
LSD (0.05)	-	-	2421.3	-	-	-	-
CV (%)	-	-	21.9	-	-	-	-

Means in columns and rows followed by the same letters are not significantly different as judged by LSD test at 5% level of significance. CV: Coefficient of variation.

resource utilized during critical growth periods. The increase in 100 seed weight with fertilizer application is in agreement with the finding of Shamim and Naimat (1987) who related the increment in 100-seed weight to the influence of cell division, phosphorus content in the seeds as well as the formation of fat and albumin. The increase in hundred seed weight as a result of increased P application might be attributed to important roles the nutrient plays in regenerative growth of the crop (Zafar et al., 2013), leading to increased seed size (Fageria, 2009), which in turn may improve hundred seed weight. Similarly, Amare et al. (2014) observed significant increase in thousand seed weights of common bean as a result of phosphorus application up to 40 kg ha^{-1}. In contrast to the results of this study, Fisseha and Yayis (2015) reported that the different levels of phosphorus (46, 69 and 92 kg P_2O_5 ha^{-1}) fertilizer used had not resulted in significant difference in 100 seed weight of common bean. Variation in hundred seed weight might have occurred due to the presence of difference in seed size among the common bean varieties as hundred seed weight increases with increase in the seed size. In line with this result, Tanaka and Fujita (1979) stated that the number of seeds per pod and weights of hundred seeds were strongly controlled genetically in field bean (*Pisum sativim*). The higher 100 seed weight for variety Ibado is associated with the size of the seed in accordance with Hawtin et al. (1980) who explained that the larger the seed, the higher its seed weight.

Above-ground dry biomass yield

The above-ground dry biomass yield was significantly (P<0.01) affected by the NPS fertilizer application and the interactions of fertilizer application with variety, however, the main effect of varieties had no significant effect on biomass yield (Table 11). The result generally showed an increase in biomass production with increase in the rate of blended NPS among the bean varieties. The highest recorded due to the application of highest rate of NPS fertilizer (250 kg NPS ha^{-1}) for variety Angar followed by variety Angar at 100 kg NPS ha^{-1}, whereas the lowest (4045 kg ha^{-1}) biomass yield was obtained for variety Nasir under the control NPS rate (Table 11). The increase in biomass yield of cultivars across blended NPS rates could be attributed to the fact that the enhanced availability of N significantly increased plant height, number of pods per plant and to the overall vegetative growth of the plants that contributed to higher aboveground dry biomass yield. This result was in line with that of Veeresh (2003) who reported that total dry matter production per plant increased significantly from 12.0 to 16.03 g due to increased nitrogen application from 40 to 120 kg N ha^{-1} on French bean (*P. vulgaris*).

The increment in dry matter yield with application of blended NPS fertilizer might also be due to the adequate supply of P from the NPS that could be attributed to an increase in number of branches per plant, which increased photosynthetic area and the number of pods per plant. The significant increase in the aboveground dry biomass yield in response to increasing rate of phosphorus application proves that the soil of the study area is in fact deficient in available soil P and requires external P fertilizer application for enhancing the yield of the crop. This result was in conformity with the findings of Getachew and Angaw (2006) who reported a significant linear response of above-ground dry biomass yield to phosphorus application in faba bean on acidic Nitisols. In contrast with this result, Nebret (2012) reported that application of sulphur up to 60 kg S ha^{-1} and interaction of nitrogen with sulphur did not result in significant effect on above-ground dry biomass of common bean.

Seed yield

Seed yield was significantly (P<0.05) affected by the above-ground dry biomass yield (10278 kg ha $^{-1}$) was main effect of variety and highly significantly (P<0.01) affected due to main effects of blended NPS fertilizer rate and the interaction of varieties with fertilizer combination (Table 12). The highest grain yield was recorded for variety Angar (3260 kg ha^{-1}) at 250 kg NPS ha^{-1} which

Table 12. Means of seed yield (kg ha^{-1}) of common bean as influenced by interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (kg ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	2485^{cde}	2360^{e}	2582^{b-e}	3044a^{bc}	2558^{b-e}	3260^{a}	2715
Ibado	1700^{g}	2249^{ef}	2389de	2521^{b-e}	3053^{abc}	3079^{ab}	2499
Nasir	1763^{fg}	2500^{b-e}	2747^{a-e}	2250^{ef}	2956^{a-d}	2505^{b-e}	2453
Mean	1983	2370	2573	2605	2856	2948	-
LSD (0.05)	-	-	-	497.4	-	-	-
CV (%)	-	-	-	11.7	-	-	-

Means within columns and rows followed by the same letter are not significantly different as judged by LSD test at 5% level of significance. CV: Coefficient of Variation.

was followed by Nasir (3079 kg ha^{-1}) at similar rate of blended NPS level while the lowest yield (1700 kg ha^{-1}) was observed for variety Ibado at control fertilizer treatment (Table 12).

Differences in seed yield among the common bean varieties might be related to the genotypic variations in P use efficiency. Hence, the cultivars which produced higher grain yield might have either better ability to absorb the applied P from the soil solution or translocate and use the absorbed P for grain formation than the low yielding cultivar. In agreement with the results of this study, Gobeze and Legese (2015) and Mourice and Tryphone (2012) observed significant variations in grain yield for common bean due to genotypic variations for P use efficiency which may arise from variation in P acquisition and translocation and use of absorbed P for grain formation in common bean. The result might be attributed to the fact that applying NPS fertilizer increases crop growth and yield on soils which are naturally low in NPS and in soils that have been depleted (Mullins, 2001). Similar results were reported by Gebre-Egziabher et al. (2014) that P application at the rate of 46 kg P_2O_5 ha^{-1} gave higher number of pods per plant and yield as compared to unfertilized plots in common bean. In line with this result, application of S with or without P recorded significantly higher seed yield up to 40 kg S ha^{-1} on chickpea (Shivakumar, 2001) and on blackgram (Jawahar et al., 2017). It might also be due to increased levels of S, its availability along with major nutrients and higher uptake of crop and influencing growth and yield components of the crop, which ultimately lead to effective, assimilate partitioning of photosynthates from source to sink in post-flowering stage and resulted in highest seed yield.

Differences in seed yield among the common bean cultivars might also be related to their response to applied N. In conformity to this result, Dwivedi et al. (1994) found increased yield of common bean due to increasing levels of nitrogen up to 100 kg ha^{-1} with the difference between 80 and 100 kg N ha^{-1} being not significant. Boroomanndan et al. (2009) also reported that seed yield of soybean increased significantly at 40 kg N ha^{-1} as compared to the control treatment. However, application of 80 kg N ha^{-1} decreased seed yield, indicating that there is a limit to the maximum level of nitrogen to be supplied to avoid its detrimental effect on the plant.

Harvest index

Harvest index was highly significantly ($P < 0.01$) affected by the interaction of variety with blended NPS rate (Table 13). The highest harvest index (0.53) and lowest harvest index (0.28) were recorded for variety Angar with application of blended NPS at 150 kg ha^{-1} and for Nasir at 250 kg ha^{-1}, respectively (Table 13). This might be that the higher NPS fertilizers rate had high influence on vegetative growth than nutrient translocation from plant biomass to seed. In line with this result, Singh and Kumar (1996) reported the highest harvest index of lentil was obtained when 45 kg P ha^{-1} and 30 kg S ha^{-1} were applied. The increment in harvest index with rates of fertilizer is in agreement with the findings of Dhanjal et al. (2001) who also reported improvement in harvest index values of 31.60, 31.99 and 33.86% due to increasing N level zero to 60 and 120 kg N ha^{-1}, respectively. However, Gifole et al. (2011) reported no significant response of harvest index of common bean to P application.

Economic analysis

The agronomic data upon which the recommendations are based must be relevant to the farmers' own agro-ecological conditions and the evaluation of those data must be consistent with the farmers' goals and socio-economic circumstances (CIMMYT, 1988).

The net benefit was computed due to common bean varieties, application of blended NPS fertilizer and interaction of varieties with application of blended NPS fertilizer. The economic analysis revealed that the highest net benefit (29825 Birr ha^{-1}) was obtained from combina-

Table 13. Means harvest index of common bean as influenced by interaction of variety and blended NPS fertilizer rates at Adola during 2016-2017 main season.

Variety	NPS rate (g ha^{-1})						Mean
	0	50	100	150	200	250	
Angar	0.43^{ab}	0.46^{ab}	0.28^{d}	0.53^{a}	0.41^{ab}	0.32^{d}	0.41
Ibado	0.41^{ab}	0.46^{ab}	0.42^{ab}	0.39^{abc}	0.35^{cd}	0.41^{ab}	0.41
Nasir	0.42^{ab}	0.39^{abc}	0.41^{ab}	0.39^{abc}	0.37^{cd}	0.28^{d}	0.38
Mean	0.42	0.44	0.37	0.44	0.38	0.34	-
LSD (0.05)	-	-	0.05	-	-	-	-
CV (%)	-	-	7.30	-	-	-	-

Means within columns and rows followed by the same letter are not significantly different as judged by LSD at 5% level of significance. CV: Coefficient of variation

Table 14. Result of economic analysis for response of common bean varieties to rates of blended NPS fertilizer rates at Adola 2016-2017 main season.

Treatment	Adjusted yield (kg ha^{-1})	NPS cost (Birr ha$^{-1)}$	NPS application cost (Birr ha^{-1})	Total cost (Birr ha^{-1})	Total revenue (Birr ha^{-1})	Net benefit (Birr ha^{-1})
Angar+0	2235.4	0	0	0	17883	17883
Ibado+0	1529.8	0	0	0	18358	18358
Nasir+0	1586.5	0	0	0	12692	12692
Angar+50	2123.6	700	350	1050	16989	15939
Ibado+50	2024.4	700	350	1050	24293	23243
Nasir+50	2250.1	700	350	1050	18001	16951
Angar+100	2324.0	1400	350	1750	18592	16842
Ibado+100	2150.3	1400	350	1750	25804	24054
Nasir+100	2471.9	1400	350	1750	19775	18025
Angar+150	2739.8	2100	350	2450	21918	19468
Ibado+150	2268.8	2100	350	2450	27226	24776
Nasir+150	2024.8	2100	350	2450	16198	13748
Angar+200	2301.9	2800	350	3150	18415	15265
Ibado+200	2747.9	2800	350	3150	32975	29825
Nasir+200	2660.3	2800	350	3150	21282	18132
Angar+250	2934.2	3500	350	3850	23474	19624
Ibado+250	2771.5	3500	350	3850	33258	29408
Nasir+250	2254.9	3500	350	3850	18039	14189

NPS cost=1400 Birr/100 kg, NPS application cost=350 Birr ha^{-1}, common bean grain price of Angar and Nasir = 8, Ibado=12 Birr kg^{-1}.

tion of variety Ibado with application of 200 kg NPS ha^{-1} while the lowest net benefit (12692 Birr ha^{-1}) was obtained from variety Nasir with no fertilizer application (Table 14).

Therefore, production of Ibado variety with the application of 200 kg NPS ha^{-1} was the most productive variety for economical production as compared to Angar and Nasir varieties and can be recommended for the study area. Dereje et al. (2015) reported that planting of the cultivar Nasir produced the highest net benefit (15903.1 Birr ha^{-1}) with acceptable marginal rate of return (3040%) compared to other cultivars at Areka. Fisseha and Yayis (2015) also reported net benefit of 21,070 ETB ha^{-1} with marginal rate of return of 80% by the application of 69 kg P_2O_5 ha^{-1} at Areka.

Conclusion

Response of common bean (*P. vulgaris* L.) varieties to rates of blended NPS fertilizer were investigated on Nitisols and Orthic Aerosols soils of Guji Zone, Southern Ethiopia. It was conducted during the main 2016 to 2017 cropping season with the objective to investigate the effect of blended NPS rates on growth, yield and yield components of common bean varieties and to identify

economically feasible rates of blended NPS at Guji Zone, Southern Ethiopia.

The result showed the main effects of NPS rate variety and their interaction had a significant effect on some growth and yield component parameters. The highest level of NPS rate (200 to 250 kg ha^{-1}) resulted in higher values of number of primary branches per plant, number of total nodules, number of effective nodules and total number of pods, number of total pods per plant, highest number of total nodules and effective nodules. Varieties exhibited variation on number of pods per plant, number of primary branches and number of seeds per pod. Variety Angar gave the highest number of primary branches per plant and number of pods per plant, whereas the highest number of seeds per pod was recorded for variety Nasir.

However, the interaction of variety and blended NPS had significant effect on almost all parameters except on the number of total and effective nodules per plant, number of primary branches per plant and number of pods per plant. The highest number of days to flowering and days to physiological maturity were recorded due to application of 200 and 250 kg ha^{-1} of blended NPS, respectively for variety Nasir. Variety Nasir gave the highest plant height with application of 150 kg NPS ha^{-1} whereas variety Ibado with application of rate of 200 kg blended NPS ha^{-1} had the highest hundred seed weight. The highest above-ground dry biomass yield was recorded due to the application of highest rate of fertilizer for variety Angar. The highest grain yieldwas recorded for variety Angar at 250 kg NPS ha^{-1} whereas the highest harvest index was recorded by variety Angar with application of blended NPS of 150 kg ha^{-1}.

Based on the partial budget analysis, the highest net benefit (29825 Birr ha^{-1}) was obtained from combination of variety Ibado with application of 200 kg NPS ha^{-1}, whereas the lowest was from variety Nasir (12692 Birr ha^{-1}) with no fertilizer application.

Thus, it can be concluded that application of 200 kg ha^{-1} with variety Ibado was found to be superior and can be used for common bean production in mid-land of Adola district, Southern Oromia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Protocol optimization for *in vitro* propagation of two Irish potato (*Solanum tuberosum* L.) varieties through lateral bud culture

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Potato is a versatile vegetable that is eaten all around the year. The objective of this study was to establish a protocol for *in vitro* micropropagation of Gudene and Belete popular varieties in Ethiopia from lateral bud explants. For shoot induction and multiplication, lateral bud explants were cultured on MS basal medium supplemented with different concentration levels of PGRS. Rooting was done on the same media type with different concentration levels of IBA and NAA in combination. The rooted plantlets were then acclimatized under poly-house conditions by transplanting the plantlets regenerated on moist soil mixture of loam soil, sand and compost in 2:1:1 ratio respectively. Results showed that shoot initiation, shoot multiplication and root formation responses were significant (P<0.05) at different hormone levels and combinations. 91.67 and 87.5% of explants survived and initiated for Gudenie and Belete varieties, respectively on shoot initiation MS basal medium supplemented with combination of 2.0 mg/l BAP and 1.0 mg/l IAA. In both varieties, number of nodes/explant, number of shoots/explant and shoot length/explant were significantly (P<0.05) higher at 0.5 mg/l BAP and 2 mg/l Kn. Number of days to shoot emergence was also found to be shorter at this level of hormonal combination than other treatments. Number of roots/shoot, root length/shoot, root fresh and dry weight were significantly affected due to growth regulators combination. The acclimatization experiment showed that plantlets of both varieties survived better on the sterilized soil mixture (loam red soil, san and compost) in a ratio of 2:1:1 with better Gudene performance as compared to the second variety.

Key words: Explants, auxin, cytokinin, *in vitro* propagation, MS media and lateral buds.

INTRODUCTION

Irish potato *(Solanum tuberosum* L.*)* is one of the world's most economically important tuber crops belonging to the family Solanaceae. It is a versatile vegetable that is eaten all around the year. It is considered to be the fourth major food crop of the world following rice, wheat and maize (Mustafa and Sarker, 2002). It contains about 79% water, 18% starch, 2% protein, 1% vitamins, minerals and many trace elements (Ahmad et al., 2011), though it is best

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known for its carbohydrate contents. The annual diet of an average global citizen in the first decade of the 21st century included about 33 kg of potato (Food and Agriculture Organization - FAO, 2008). Moreover, it is used as industrial raw material apart from its daily consumption by humans (Hoque, 2010).

In Ethiopia, potato production is expanding from highland areas to mid and lowland parts of the country due to its potential for production in a short period of time, high yield per unit area, source of food and cash crops to large number of food-insecure smallholder farmers and pastoralists in the country. It is the first root crop in production area coverage 70,131.32 ha with average productivity of 13.45 ton/ha (Central Statistical Agency - CSA, 2016) which is very low below world productivity (FAO, 2012). Nowadays, the main production season for potato represents only 22% (34,000 ha), while the off-season production is around 128,000 ha in northern and central Ethiopia (Haverkort et al., 2012).

Production of the crop could fill the gap in food supply during the hungry months of July to August before the grain crops are being harvested. Currently, in Ethiopia, potato grown on around 164,146 ha producing an estimated total tuber yield of 940,087 tons. This implies that average yield in the country reaches only 18.6 t/ha when the potential for small holder is around 25 t/ha (CSA, 2016). Among many, the major causes for the low productivity identified were as a result of many factors being identified as the causes for this low yield in Ethiopia and most of the East African countries, but the lack of high quality seed tuber is the major one which seems to explain most of the yield reduction. Moreover, unavailability of seed tubers, lack of well adapted cultivars, prevalence of diseases and insect pests, drought and frost at high altitude and extreme temperature at very low altitudes are some of the problems that contribute to the reduced yield of potato (Tsoka et al., 2012).

In vitro regeneration and micro propagation methods ar e now widely used for disease free plantlets regeneration and mass multiplication of plant material, germplasm conservation and for the improvement of crops through biotechnological methods. *In vitro* Irish potato production technology is a more advanced technology over traditional method of Irish potato production with respect to optimal yield uniformity, disease free planting material and true to type plants. Mass multiplication of tissue culture plants could also be done in a short time. The development of an optimal micro propagation protocol would have a major significance in the propagation of well adapted and better yielding varieties of good agronomic traits of Irish potato clones for its distribution to farmers (Rabbani et al., 2001).

So far, very limited efforts have been made for *in vitro* micro propagation of Irish potato in the country. In order to meet the objectives of mass propagation within short period of time and produce disease free seed tubers to benefit potato producers from tissue culture technique and contribute towards improvement of productivity, it is important to optimize efficient micro-propagation protocol as per facilities available which needs to be optimized. Few researchers have reported their research on Irish potato tissue culture. For example, Hussain et al. (2005) investigated micro propagation of potato from lateral bud explants on MS basal medium with plant growth regulators (auxins and cytokinin). Molla et al. (2011) also studied the effect of growth regulators (IAA, IBA, NAA, BAP and Kin) on direct regeneration of potato. However, only partial success and a low rate of multiplication was obtained with very limited work done on *in vitro* propagation of the two potato varieties (Belete and Gudene) from lateral meristem in Ethiopia. Therefore, this paper was prepared to share the information generated from the study conducted with the objective to optimize efficient micro propagation protocol for Irish potato varieties in Areka plant tissue culture laboratory conditions.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Plant Tissue Culture Laboratory of Areka Agricultural Research Center (AARC), in Southern Nations Nationalities and People's Regional State (SNNPRS), Wolaita Zone. It was located 300 km to the south of Addis Ababa and 3 km away from Areka town at an elevation of 1800 m.a.s.l. The annual rainfall of the area was 1520 mm and the average maximum and minimum temperatures are 26 and 14°C, respectively (AARC).

Explants source and surface sterilization

Two Irish potato varieties (Gudenie and Belete) sprouted tubers were collected from Areka Agricultural Research Center and were planted in pots in green house to be used as mother plants for explants source of the study. Young and healthy shoot explants (1.0 to 2.0 cm long) containing lateral buds were cut using sterilized surgical bloods and washed with double distilled water (DDW). Thereafter, explants were dipped in 70% alcohol for one minute and immediately washed with distilled water. Thereafter, they were sterilized in the laminar air flow cabinet with chloride in “Berekina” local bleach with 5% active chlorine diluted to 1.25% as replacement of Sodium hypochlorite for 8 min accompanied by continuous shaking. Surface sterilized segments were washed 4 to 5 times with sterilized distilled water.

Stock solution preparation

Murashige and Skoog (1962) basal medium supplemented with 30 g/l sucrose as a source of carbon and agar (6 g/l) as gelling agent was used throughout this research activity. Initially, full strength stock solutions of macronutrients, micronutrients and vitamins and other organic supplements were separately prepared. To do so, appropriate amount of each nutrient was weighted in grams per liter and dissolved in DDW consecutively in such a way that the next nutrient was added after the first one was completely dissolved. After all the components were completely dissolved using magnetic

stirrer, the solution was poured into plastic bottles and stored at +4°C until used, for maximum of four weeks.

Plant growth regulators stock solution preparation

Plant growth regulators (PGRs) were prepared in 1 mg/ml concentration. The PGRs used for the study were the cytokinins: 6-benzyl amimopurine (BAP) and Kinetin (KN), and the auxins: indol-3- butyric acid (IBA), indol acetic acid (IAA) and Naphthalene acetic acid (NAA). The powdered crystal of the PGRs was first weighed and dissolved in 3-4 drops of 1 N NaOH, and 1 N HCl based on the type of PGR (NaOH for auxins and HCl for cytokinins). Upon complete dissolution, the solution of each PGR was poured into labeled 50-ml plastic bottles and filled with double distilled water (DDW) to the required volume. Then gently stirred and stored at a temperature of +4°C for short term use (a week) and -5°C for long term use (up to a month) until used.

Culture media preparation

In this study, the culture medium used for shoot initiation, multiplication and root induction contained full strength of MS basal medium (Murashige and Skoog, 1962) composed of 100 ml macronutrient, 10 ml micronutrient, and 10 ml vitamin per liter, supplemented with 30 g/l sucrose as a source of carbon and agar (6 g/l) as gelling agent with or without (for control) PGRs, throughout the experiment. Finally, the pH of all media was adjusted to 5.8 using 1 N NaOH and/or 1 N HCL after addition of agar. Because the agar used has shown slight increase in pH after addition to the media, it was then autoclaved at 121°C for 20 min.

Experimental design

For all experiments from shoot initiation to rooting stage, completely randomized (CRD) with factorial arrangement replicated thrice was used.

Acclimatization

The *in vitro* well rooted Irish potato plantlets were taken out gently from each PGR treatments of the culture media jar and washed under running tap water to remove traces of agar that prevent the absorption of nutrients from the acclimatization culture substrates by roots. The plantlets were transplanted into acclimatization plastic cell tray filled with culture substrates of moist red soil, sand soil and compost soil or their mixture in 2:1:1 ratio, respectively. The plantlets were covered with white transparent polythene plastic bag to maintain high humidity, and the plantlets were acclimatized in an open greenhouse environmental condition within the same room by removing the polyethylene sheet and red cheese cloth and irrigated with tap water every day.

Plastic cover was partially removed after a week and completely removed after two weeks; thereafter regenerated plantlets were grown to maturity. Finally, after three weeks, percent of plantlets successfully hardened during growing and died were counted measured by the number of survived shoot lets and died shoot lets from the total transferred plantlets.

Data collection and analysis

Data for number of clean explants, average number of days for shoot emergence, mean number of bud per explants, mean shoot number, shoot length, days to root induction, root number, root length, and acclimatization percentage was carefully collected.

Data Analysis

Data collected at each stage was analyzed using SAS 9.2 version at probability level 0.05 in which means were separated by LSD test method.

RESULTS AND DISCUSSION

Lateral bud survival and shoot initiation

The highest percent number of explants shoot that initiated new shoots was recorded for Gudenie (91.67%) and Beletse (87.50%) varieties at PGR combination of 2 mg/l BAP with 1 mg/l IAA for both varieties (Table 1). Though the values were not significantly different, Gudene variety showed more response. Previously, some other researchers (Hussain et al., 2005; Molla et al., 2011) reported better shoot induction in other potato varieties cultured on MS medium supplemented with BAP and IAA in 2 mg/l to 0.5 mg/l combination and their results are somehow in agreement with the result of this study. With the rise of BAP to IAA concentrations up to 2 to 1 mg/l, shoot induction appeared to increase, but later decreased.

Effect of cytokinins on *in vitro* shoot multiplication

The result analysis showed significant difference at $P<0.05$ level test for number of nodes/explants, number of days to shoot emergence, number of shoot /explant, shoot fresh and dry weight between treatments in both varieties (Table 2). For Gudenie variety, number of nodes/explant, number of shoots/explant and shoot length/explant were significantly higher at 0.5 mg/l BAP and 2 mg/l Kn combination than at other treatments. Number of days to shoot emergence was also found to be shorter at this level of hormonal combination even if it is not significant statistically. Similar pattern had also been observed in Belete variety too, but the response of Gudenie (Figure 1) in terms of the mentioned parameters was better than that of Belete variety, suggesting that varietal difference evokes varying response to the same PGRs. In the case of Belete variety, 0 mg/l BAP to 2 mg/l Kn combination treatment had similar effects as that of 0.5 mg/l BAP to 2 mg/l Kn.

In many plants, multiple shoots can be obtained from the shoot tips or axillary buds by administering BAP or KIN (Bhat et al., 2010; Azar et al., 2011; Thiruvengadam et al., 2011). In line with this study, Mustafa and Sarker(2002) reported that some potato varieties treated with BAP showed better response in terms of shoot per explant, shoot length and number of nodes. The synergistic effect of BAP and Kn for increased shoot

Table 1. Percentage of initiated after 30 days of culturing.

Variety	Hormone combination		No. of explants/tree	No. of initiated explants	No. of explants that died	Shoot Initiation percentage
	BAP (mg/l)	IAA (mg/l)				
Gudenie	0	0	9	5.37	3.63	59.67^{cde}
	1	0.5	9	6.37	2.63	70.83^{c}
	2	1	9	8.25	0.75	91.67^{a}
	3	1.5	9	6.00	3.00	66.67^{cd}
	4	2	9	6.00	3.00	66.67^{cd}
Belete	0	0	9	5.00	4.00	55.56^{cde}
	1	0.5	9	6.75	2.25	75.00^{bc}
	2	1	9	7.87	1.13	87.50^{ab}
	3	1.5	9	6.37	2.63	70.83^{c}
	4	2	9	6.37	2.63	70.83^{c}
LSD						13.24
CV (%)						10.41

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different.

Figure 1. Well regenerated potato plantlets ready for acclimatization.

multiplication rate and proliferation was also reported on *B. tulda* and *M. baccifera* (Waikhom and Louis, 2014).

Effect of auxins on *in vitro* root induction

The development of healthy root system was required for the successful establishment of *in vitro* regenerated shoots to adapt to the external environments. For this, those shoots with 1 cm and more in height were taken and transferred onto rooting medium that contained full strength MS supplemented with different IBA and NAA in different concentrations solely or in combination. Compared to the control, both IBA and NAA alone or in

Table 2. Effect of different concentrations of cytokinins (BAP and Kin) combination on the morphogenetic *in vitro* responses of experimental varieties.

Variety	Hormone combination		No. of nodes per explant (n)	No. of days to shoot emergence (n)	No. of shoots per explant (n)	Shoot Length per explant (cm)	Shoot fresh weight per explant (g)	Shoot dry weight per explant (g)
	BAP (mg/l)	KN (mg/l)						
Gudenie	0	0	3.67^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.*05*^{j}	0.01^{f}
	0.5	0.5	6.67^{cdef}	3.33^{cd}	5.67^{ghij}	4.83^{efghi}	0.11^{efghi}	0.05^{cdef}
	1	1	8.33^{b}	3.17^{d}	7.67^{bcd}	7.50^{a}	0.21^{abcde}	0.07^{abcde}
	1.5	1.5	5.67^{fghi}	3.67^{bcd}	4.67^{jklm}	5.25^{def}	0.13^{fghi}	0.05^{cdef}
	2	2	4.33^{jkl}	3.33^{cd}	3.67^{m}	4.17^{hij}	0.15^{bcdefghi}	0.06^{cdef}
	0.5	0	7.33^{bcd}	3.67^{bcd}	7.33^{bcde}	4.83^{efghi}	0.17^{bcdefg}	0.08^{abcd}
	1	0	8.33^{b}	3.33^{cd}	8.33^{b}	5.50^{cde}	0.13^{efghi}	0.09^{abc}
	1.5	0	4.67^{ijkl}	3.83^{abcd}	6.00^{fghi}	4.50^{fghij}	0.14^{cdefghi}	0.06^{bcdef}
	2	0	3.67^{l}	4.00^{abcd}	5.33^{hijk}	4.08^{ij}	0.25^{ab}	0.08^{abcd}
	0	0.5	4.00^{kl}	4.67^{a}	4.33^{klm}	3.67^{j}	0.23^{abcd}	0.11^{ab}
	0	1	5.67^{fghi}	4.00^{abcd}	5.00^{ijkl}	4.67^{efghi}	0.15^{bcdefghi}	0.07^{bcde}
	0	1.5	6.33^{defg}	3.83^{abcd}	6.00^{fghi}	4.67^{efghi}	0.13^{fghi}	0.06^{bcdef}
	0	2	7.00^{cde}	3.33^{cd}	6.67^{defg}	5.83^{cd}	0.12^{efghi}	0.05^{cdef}
	0.5	2	9.67^{a}	3.17^{d}	10.67^{a}	7.167^{ab}	0.08^{ghi}	0.02^{ef}
	2	0.5	6.33^{defg}	3.33^{cd}	7.00^{cdef}	4.67^{efghi}	0.28^{a}	0.12^{a}
Belete	0	0	0.00^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.07^{hi}	0.02^{ef}
	0.5	0.5	6.33^{defg}	3.83^{abcd}	5.67^{ghij}	5.33def	0.11^{efghi}	0.06^{cdef}
	1	1	6.67^{cdef}	3.67^{bcd}	7.00^{cdef}	6.83^{ab}	0.17^{bcdefg}	0.08^{abcd}
	1.5	1.5	4.67^{ijkl}	4.00^{abcd}	4.67^{jklm}	5.17^{defg}	0.12^{efghi}	0.05^{cdef}
	2	2	3.67^{l}	4.67^{a}	4.00l^{m}	4.50^{fghij}	0.13^{efghi}	0.05^{cdef}
	0.5	0	6.67^{cdef}	4.33^{ab}	5.67^{ghij}	4.67^{efghi}	0.13^{defghi}	0.04^{def}
	1	0	7.67^{bc}	3.83^{abcd}	6.67^{defg}	5.00^{defgh}	0.18^{abcdefg}	0.05^{cdef}
	1.5	0	5.00^{hijk}	4.67^{a}	5.33^{hijk}	4.33^{ghij}	0.13^{defghi}	0.05^{cdef}
	2	0	5.33^{ghij}	4.17^{abc}	5.00^{ijkl}	4.17^{hij}	0.20^{abcdef}	0.08^{abcd}
	0	0.5	6.00^{efgh}	3.67^{bcd}	5.33^{hijk}	4.00^{ij}	0.18^{abcdefg}	0.08^{abcd}
	0	1	6.33^{defg}	4.67^{a}	5.67^{ghij}	4.67^{efghi}	0.15^{bcdefghi}	0.06^{bcdef}
	0	1.5	6.67^{cdef}	3.17^{d}	7.00^{cdef}	5.33^{def}	0.13^{efghi}	0.05^{cdef}
	0	2	7.33^{bcd}	3.50^{bcd}	8.33^{b}	7.33^{a}	0.14^{bcdefghi}	0.05^{cdef}
	0.5	2	7.33^{bcd}	3.33^{cd}	8.00^{bc}	6.33^{bc}	0.17^{bcdefgh}	0.07^{abcde}
	2	0.5	5.67^{fghi}	4.67^{a}	6.33^{efgh}	4.33^{ghij}	0.24^{abc}	0.10^{abc}
LSD			1.04	0.85	1.04	0.85	0.11	0.05
CV (%)			10.98	14.62	11.01	10.92	7.02	11.09

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, expt. = explants and wt = weight.

combination showed significant positive effect on root formation of shoots of both varieties of Irish potato at all concentration levels. The lowest number of day to rooting response per/shoot was 6.33 at 1 mg/l IBA with 0.25 mg/l NAA in Gudenie and 7.33 at 0.5 mg/l IBA with 0.5 mg/l NAA in Belete varieties; the highest root number per/shoot was 23.34 in Gudene and 17.33 in Belete at 1 mg/l IBA with 0.25 mg/l NAA concentration in both varieties; the highest length of root per/shoots was 8.33 cm in Gudenie and 5.33 cm in Belete at 1 mg/l IBA with 0.25 mg/l NAA concentration in both varieties; whereas the highest root fresh weight per/shoots was 0.19 at 0.5 mg/l IBA with 0.5 mg/l NAA in Gudenie and 0.24 at 1.0 mg/l IBA with 0.25 mg/l Kin in Belete varieties. Similar trend was also observed with regard to root dry weight. In both varieties, number of roots/shoot, root length/shoot and root fresh and dry weight had significantly higher values at 1 mg/l IBA to 0.25 mg/l NAA combination treatment than other treatments (Table 3). Though both varieties performed well at 1 mg/l IBA to 0.25 mg/l NAA

Table 2. Effect of different concentrations of cytokinins (BAP and Kin) combination on the morphogenetic *in vitro* responses of experimental varieties.

Variety	Hormone combination		No. of nodes per explant (n)	No. of days to shoot emergence (n)	No. of shoots per explant (n)	Shoot Length per explant (cm)	Shoot fresh weight per explant (g)	Shoot dry weight per explant (g)
	BAP (mg/l)	KN (mg/l)						
Gudenie	0	0	3.67^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.*05*^{j}	0.01^{f}
	0.5	0.5	6.67^{cdef}	3.33^{cd}	5.67^{ghij}	4.83^{efghi}	0.11^{efghi}	0.05^{cdef}
	1	1	8.33^{b}	3.17^{d}	7.67^{bcd}	7.50^{a}	0.21^{abcde}	0.07^{abcde}
	1.5	1.5	5.67^{fghi}	3.67^{bcd}	4.67^{jklm}	5.25^{def}	0.13^{fghi}	0.05^{cdef}
	2	2	4.33^{jkl}	3.33^{cd}	3.67^{m}	4.17^{hij}	0.15^{bcdefghi}	0.06^{cdef}
	0.5	0	7.33^{bcd}	3.67^{bcd}	7.33^{bcde}	4.83^{efghi}	0.17^{bcdefg}	0.08^{abcd}
	1	0	8.33^{b}	3.33^{cd}	8.33^{b}	5.50^{cde}	0.13^{efghi}	0.09^{abc}
	1.5	0	4.67^{ijkl}	3.83^{abcd}	6.00^{fghi}	4.50^{fghij}	0.14^{cdefghi}	0.06^{bcdef}
	2	0	3.67^{l}	4.00^{abcd}	5.33^{hijk}	4.08^{ij}	0.25^{ab}	0.08^{abcd}
	0	0.5	4.00^{kl}	4.67^{a}	4.33^{klm}	3.67^{j}	0.23^{abcd}	0.11^{ab}
	0	1	5.67^{fghi}	4.00^{abcd}	5.00^{ijkl}	4.67^{efghi}	0.15^{bcdefghi}	0.07^{bcde}
	0	1.5	6.33^{defg}	3.83^{abcd}	6.00^{fghi}	4.67^{efghi}	0.13^{fghi}	0.06^{bcdef}
	0	2	7.00^{cde}	3.33^{cd}	6.67^{defg}	5.83^{cd}	0.12^{efghi}	0.05^{cdef}
	0.5	2	9.67^{a}	3.17^{d}	10.67^{a}	7.167^{ab}	0.08^{ghi}	0.02^{ef}
	2	0.5	6.33^{defg}	3.33^{cd}	7.00^{cdef}	4.67^{efghi}	0.28^{a}	0.12^{a}
Belete	0	0	0.00^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.07^{hi}	0.02^{ef}
	0.5	0.5	6.33^{defg}	3.83^{abcd}	5.67^{ghij}	5.33def	0.11^{efghi}	0.06^{cdef}
	1	1	6.67^{cdef}	3.67^{bcd}	7.00^{cdef}	6.83^{ab}	0.17^{bcdefg}	0.08^{abcd}
	1.5	1.5	4.67^{ijkl}	4.00^{abcd}	4.67^{jklm}	5.17^{defg}	0.12^{efghi}	0.05^{cdef}
	2	2	3.67^{l}	4.67^{a}	4.00l^{m}	4.50^{fghij}	0.13^{efghi}	0.05^{cdef}
	0.5	0	6.67^{cdef}	4.33^{ab}	5.67^{ghij}	4.67^{efghi}	0.13^{defghi}	0.04^{def}
	1	0	7.67^{bc}	3.83^{abcd}	6.67^{defg}	5.00^{defgh}	0.18^{abcdefg}	0.05^{cdef}
	1.5	0	5.00^{hijk}	4.67^{a}	5.33^{hijk}	4.33^{ghij}	0.13^{defghi}	0.05^{cdef}
	2	0	5.33^{ghij}	4.17^{abc}	5.00^{ijkl}	4.17^{hij}	0.20^{abcdef}	0.08^{abcd}
	0	0.5	6.00^{efgh}	3.67^{bcd}	5.33^{hijk}	4.00^{ij}	0.18^{abcdefg}	0.08^{abcd}
	0	1	6.33^{defg}	4.67^{a}	5.67^{ghij}	4.67^{efghi}	0.15^{bcdefghi}	0.06^{bcdef}
	0	1.5	6.67^{cdef}	3.17^{d}	7.00^{cdef}	5.33^{def}	0.13^{efghi}	0.05^{cdef}
	0	2	7.33^{bcd}	3.50^{bcd}	8.33^{b}	7.33^{a}	0.14^{bcdefghi}	0.05^{cdef}
	0.5	2	7.33^{bcd}	3.33^{cd}	8.00^{bc}	6.33^{bc}	0.17^{bcdefgh}	0.07^{abcde}
	2	0.5	5.67^{fghi}	4.67^{a}	6.33^{efgh}	4.33^{ghij}	0.24^{abc}	0.10^{abc}
LSD			1.04	0.85	1.04	0.85	0.11	0.05
CV (%)			10.98	14.62	11.01	10.92	7.02	11.09

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, expt. = explants and wt = weight.

combination treatment, performance of Gudenie explants is better than Belete variety. This shows that as in the case of shooting response, varietal difference evokes varying rooting response to the same PGRs. Asma Rabbani (2001) reported that the best rooting response in plants such as *S. tuberosum* L., was observed when IBA concentrations is at higher proportion than NAA in a combination of the two. The work of Khadiga (2009) also showed that use of 2.5 mg/l of IBA is good to improve root initiation of potato plantlets which agrees with the present finding that rooting increases with high IBA concentration when treated with relatively higher amount of IBA in a combination of the two auxins.

Acclimatization

Results showed that plantlets grown on a mix of moist red soil: sand soil: and compost in 2:1:1 ratio hardened very well compared to other substrates used. Survival

Table 3. Effect of different concentrations of auxins (IBA and NAA) combination on the morphogenetic responses of Irish potato varieties on in *vitro* root induction.

Variety	Hormone combination		Days to rooting response (n)	Root no. per shoot (n)	Length of root per shoot (cm)	Root fresh weight per shoot (g)	Root dry weight per shoot (g)
	IBA (mg/l)	NAA (mg/l)					
Gudenie	0	0	12.33^{abc}	1.33^{lm}	1.67^{l}	0.09^{ef}	0.04^{cde}
	0.25	0.25	8.00^{hijkl}	16.67^{bc}	5.83^{c}	0.13^{bcdef}	0.04^{bcd}
	0.5	0.5	6.67^{kl}	19.00^{b}	7.17^{b}	0.19^{abc}	0.05^{abcd}
	0.75	0.75	9.33^{efghi}	10.00^{def}	5.17^{cde}	0.11^{ef}	0.06^{abcd}
	1	1	10.33^{def}	8.67^{efgh}	3.83^{fghij}	0.17^{bcde}	0.05^{abcd}
	0.25	0	11.33^{bcd}	6.33^{ghijk}	3.17^{hijk}	0.15^{bcdef}	0.06^{abcd}
	0.5	0	10.33^{def}	9.00^{efg}	3.83^{fghij}	0.12^{def}	0.05^{bcd}
	0.75	0	8.67^{fghij}	12.67^{d}	4.33^{defg}	0.14^{bcdef}	0.05^{abcd}
	1	0	6.67^{kl}	15.67^{c}	5.33^{cd}	0.12^{def}	0.05^{abcd}
	0	0.25	8.00^{hijkl}	12.67^{d}	3.50^{ghijk}	0.15^{bcdef}	0.06^{abcd}
	0	0.5	9.67^{defgh}	8.33^{efghi}	3.08^{ijk}	0.13^{bcdef}	0.05^{abcd}
	0	0.75	10.67^{cde}	7.33^{fghij}	2.83^{jk}	0.12^{cdef}	0.04^{bcd}
	0	1	13.33^{a}	4.67^{jk}	2.50^{kl}	0.13^{bcdef}	0.05^{abcd}
	0.25	1	8.33^{ghijk}	15.67^{c}	4.67^{def}	0.12^{cdef}	0.04^{cde}
	1	0.25	6.33^{l}	23.33^{a}	8.33^{a}	0.17^{abcde}	0.06^{abcd}
Belete	0	0	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{g}	0.00^{e}
	0.25	0.25	9.33^{efghi}	12.33^{d}	4.17^{efgh}	0.08^{f}	0.04^{cde}
	0.5	0.5	7.33^{jkl}	15.67^{c}	4.83^{cdef}	0.12^{cdef}	0.05^{abcd}
	0.75	0.75	10.00^{defg}	9.00^{efg}	3.50^{ghijk}	0.13^{bcdef}	0.06^{abcd}
	1	1	9.33^{efghi}	6.00^{hijk}	2.83^{jk}	0.13^{cdef}	0.05^{abcd}
	0.25	0	10.67^{cde}	4.67^{jk}	2.83^{jk}	0.12^{def}	0.04^{bcd}
	0.5	0	10.33^{def}	8.00$_{efghi}$	3.17^{hijk}	0.10^{ef}	0.04^{cde}
	0.75	0	9.67^{defgh}	9.00^{efg}	3.50^{ghijk}	0.09^{f}	0.03^{de}
	1	0	7.67^{ijkl}	12.67^{d}	4.50^{defg}	0.16^{bcdef}	0.06^{abcd}
	0	0.25	9.33^{efghi}	10.00^{def}	3.08^{ijk}	0.19^{abc}	0.07^{abcd}
	0	0.5	10.33^{def}	7.00^{ghij}	2.67^{kl}	0.14^{bcdef}	0.06^{abcd}
	0	0.75	11.33^{bcd}	5.67^{ijk}	2.50^{kl}	0.12^{cdef}	0.05^{abcd}
	0	1	13.00^{ab}	3.67^{kl}	1.67^{l}	0.19^{abcd}	0.08^{abc}
	0.25	1	9.67^{defgh}	10.66^{de}	4.00^{fghi}	0.21^{ab}	0.09a
	1	0.25	7.67^{ijkl}	17.33^{bc}	5.33^{cd}	0.24^{a}	0.08^{ab}
LCD			1.68	2.99	1.01	0.08	0.04
CV (%)			11.18	8.15	6.22	9.20	12.36

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, and wt = weight.

rates were 93.33% and 86.66% for Gudene and Belete varieties, respectively (Table: 4). However, for plantlets which acclimatized in unsterile soil mix the survival rates were 53.33 and 46.66% for Gudene and Belete varieties, respectively. This was severely affected by cutworms which cut down the stem from the bottom. Also, some leaves were dried up and consequently detached from the shoots. This may be due to unrestricted loss of water from their leaves or low hydraulic conductivity of roots and root-stem connections (Pospišilova et al., 1999).This result also revealed the highest survival rate on the Gudenie variety plantlets as compared to Belete Irish potato variety after 15 days in the open greenhouse environments. Further observation of survived individual plantlets in the greenhouse revealed no aberrant phenotypes (Table 4).

Conclusions

From the results of experiments conducted for different stages it is possible to conclude that the study provided optimal protocol for micro-propagation of two popular varieties of Irish potato through lateral bud culturing on

Table 4. Effect of different sterilized culture substrates on the survival rate of *in vitro* regenerated plantlets of Gudenie and Belete Irish potato varieties during acclimatization in greenhouse.

Variety	Type of culture substrates	Total no. of explants transferred	No. of survived explants	No. of explants that died	% of survived explants	% of explants that died
Gudenie	Loam soil alone	15	11.83^{bc}	4.25^{bc}	73.33^{bc}	26.67^{bc}
	Sand soil alone	15	9.00^{c}	6.70^{b}	60.00^{cd}	40.00^{ab}
	Compost soil alone	15	12.33^{bc}	3.66^{c}	80.00^{ab}	20.00^{cd}
	Mixture in 2:1:1 ratio	15	14.74^{a}	1.83^{d}	93.33^{a}	6.67^{d}
Belete	Loam soil alone	15	9.33^{c}	6.15^{b}	60.00^{cd}	40.00^{ab}
	Sand soil alone	15	7.83^{d}	8.00^{a}	46.66^{d}	53.34^{a}
	Compost soil alone	15	11.67^{bc}	4.33^{bc}	73.33^{bc}	26.67^{bc}
	Mixture in 2:1:1 ratio	15	13.01^{b}	2.67^{cd}	86.66^{ab}	13.34^{cd}
Significance			**	**	*	*
LSD			0.48	0.96	1.27	7.48
CV (%)			5.38	8.16	3.14	3.47

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05.

MS basal medium supplemented with appropriate concentrations of different PGRs in sole or combination. This protocol can thus be utilized to micro-propagate disease free and high quality planting materials of the two varieties to boost its production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Study on suitability of locally available substrates for cultivation of oyster mushroom *(Pleurotus ostreatus)* in Jimma zone, Oromia regional state, southwestern Ethiopia

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A mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above the ground on soil. The nutritional value of mushrooms is greater than one may think. Generally, it is a nature's hidden treasures of nutrition. The aim of this study was to access the suitability of available agro wastes of some lignocelluloses materials containing five different types of main substrates namely, sawdust (Sd), cow dung (Cd), teff straw (Tfs), corn cobs (CbZ) and chat left over (ChC). During this study, rate of mycelia invasion, cap diameters, stipe length, fresh weight per flush and total yield of *Pleurotus ostreatus* were recorded, accordingly. Results indicate that, CbZ alone, CbZ*Tfs, CbZ*Sd and Cd*CbZ showed highest biological efficiency of 83.62, 72.8 to 87.5, 62.6 to 7 and 63.4 to 63.8%, respectively, while the lowest yield was obtained from Cd*ChC and Tfs*ChC (46 to 50.16%). Moreover, CbZ alone as well as in combination with other agro wastes (Tfs*CbZ and CbZ*SdC, Cd*Tfs and Chat (*Catha edulis)) enhanced the yield of *P. ostreatus*. Thus, the currently used agro wastes, such as corn cobs, teff straw, sawdust and cow dung are promising substrate for domestic as well as industrial production of mushroom.

Key words: Agro wastes, oyster mushroom, spawn, substrate, yields.

INTRODUCTION

People in developing countries like Ethiopia often spend 60 to 80% of their income on food, but Americans spend less than 10%. Moreover, it have been reported that the amount of calories required by world countries keep rising from time to time (Choi et al., 2006). By 2030, global food demand is expected to rise by 35%. On the other hand, only one in seven people are expected to be consuming less than 2,500 kilo calories per day by 2030, but this circumstance seems uncertain in developing countries.

On the other hand, mushrooms production is getting attention globally to resolve the constraints of food

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insecurity, which is also technological and economically affordable. Recently, rapid growth of the industries is increasing the deposit of waste material into the environment (Yildiz et al., 2002). Interestingly, in addition to food source, mushrooms have a great role of decomposing environmental pollutants. In addition to nutritional value, the usefulness of mushrooms for medical purposes has been indicated, such as, antitumor, anticancer, immune modulator, cardiac diseases improve blood circulation, reduce cholesterol, and diabetes (Angeli et al*.,* 2006; Choi et al*.,* 2006; Grind et al*.,* 2006).

Besides mushrooms been endowed with vital nutrients, it has a good aroma and flavoring properties (Pathmashini et al*.,* 2008). It has been understood that mushrooms, such as, oyster and shitake have contributes largely in reducing poverty, by the substitution of plant origin and animal product food, and also a source of income (Masarirambi et al., 2011).

Cultivation of mushrooms is less expensive because it requires little space and inexpensive raw materials including agricultural and industrial waste (Chang, 2007). Thus, it is rational in expanding the mushroom production, particularly in Ethiopia where the mushroom production and consumption is scarce (Yenealem et al*.*, 2013). The mushrooms are considered as delicious and nutraceutical food for the human health but it is still not properly advertised in Ethiopia. To this effect, the present study was designed to evaluate the suitability of locally available substrates for cultivation of Oyster mushroom in Jimma zone, Oromia regional state.

MATERIALS AND METHODS

Spawn production

Pure cultures of *Pleurotus ostreatus* mushroom were obtained from Addis Ababa University. Impurity free sorghum grains had been soaked in tap water for 40 h. After the grain had absorbed water and reached 60% moisture, it was mixed with 1% $CaCO_3$ (Gume et al*.,* 2013). Then, grains supplemented with $CaCO_3$ were filled into glass jar up to ¾ of its volume, and autoclaved for 2 h. This was allowed to cool down, after which 2-pieces of agar block containing *P. ostreatus* culture were inoculated into glass jars containing sterile sorghum. These were then incubated at 25, 30, 35 and 40°C, until the grains were fully colonized by fungal mycelium.

Combinations of substrate

The substrate used for this study were, saw dust (from wood workshops), cow dung, teff straw, corncobs from local farmers around Jimma Zone, and chat gerba from chewing areas. Firstly, the substrates had been chopped into <1 cm pieces, and mixed in different ratios accordingly (1:0.75, 1:0.50, and 1: 0.25). These were then soaked in water for 12 h and the excess water drained off (Bonginkhosi et al*.,* 2012). Next, to the aerated substrate, 3% gypsum, 1% $CaCO_3$, and 5% maize bran was added (Mandeel et al., 2005). The substrates were transferred into rubber bags, and autoclaved at 121ºC for 2 h. Lastly, the polythene bags of the size 35 × 45 cm were filled with sterilized substrates and the top inoculation method was used with mushroom spawn. Both the control (only one substrate, that is, either cow dung or corn cobs or teff straw or chat gereba or sawdust) as well as polythene bags containing various combinations of substrates inoculated with fungal mycelium were arranged in randomized complete block design (RCBD) and incubated in a dark room. The experiment was performed in triplicates. The temperature and humidity of fungal cultivation room was maintained at 25ºC and 80 to 90%, respectively using a thermometer and humidity tester. When the mushroom pinhead emerged via prepared pin holes, sufficient light and air exchange was allowed by opening windows and door in the morning.

Yield measurements

The mushroom biomass, such as, number and weight of flushes per polythene bag, pileus diameter, stipe length, and maturation time were measured for four consecutive flushes (Iqbal et al*.,* 2005). Yield performance and biological efficiency of oyster mushrooms on the five kinds of substrates were calculated based on the following formula (Fan et al*.,* 2006).

$$\text{Biological Efficiency (BE)} = \frac{\text{Weight of fresh mushroom}}{\text{Dry weight of substrate}} \times 100\%$$

Statistical analysis

Results were presented as mean ± SD. Comparison of the yields among substrates was assessed using ANOVA. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of temperature on spawn production

The results indicate that upon inoculation of *P. ostreatus* mycelia, the spawns were fully colonized within 14 days at room temperature as compared to a temperature range of 30 to 40°C (Figure 1). Moreover, the mean of mycelial extension, such as, 0.62, 0.4, 0.31, and 0.12 cm were recorded at 25, 30, 35 and 40°C, respectively. In general as the temperature increases the rate of mycelial ramification was decrease.

Rate of mycelia invasion of substrates

The mycelia of *P. ostreatus* fully colonized the substrates in a range of 17 to 35d (Figure 2). The highest rate of mycelia ramification was recorded in ratio of CbZ*SdC 1:0.75 (0.70 cm/day) and 1:0.50 (0.69 cm/day), while the least in CbZ*Tfs 1:0.25 (0.37 cm/day) (Figure 2). There was no significant difference observed among most of the substrates for both invasion and pinning days of *P. ostreatus* mycelia.

In addition to the combination of CbZ, CbZ*SdC and Ts*CbZ, enhanced *P. ostreatus* mycelia ramification was within a short period of time; it also permitted the development of pin heads to mature mushroom. However, Cd*SdC, and Ts*Ch, were observed as least colonized by *P. ostreatus* mycelia, and also their pin

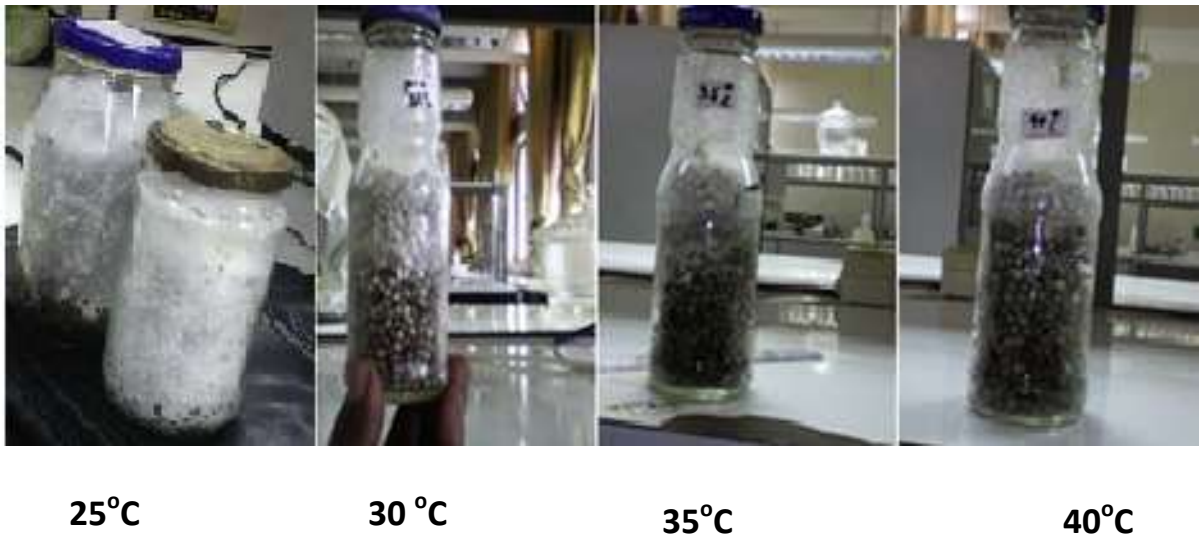

Figure 1. Spawn production at different temperature.

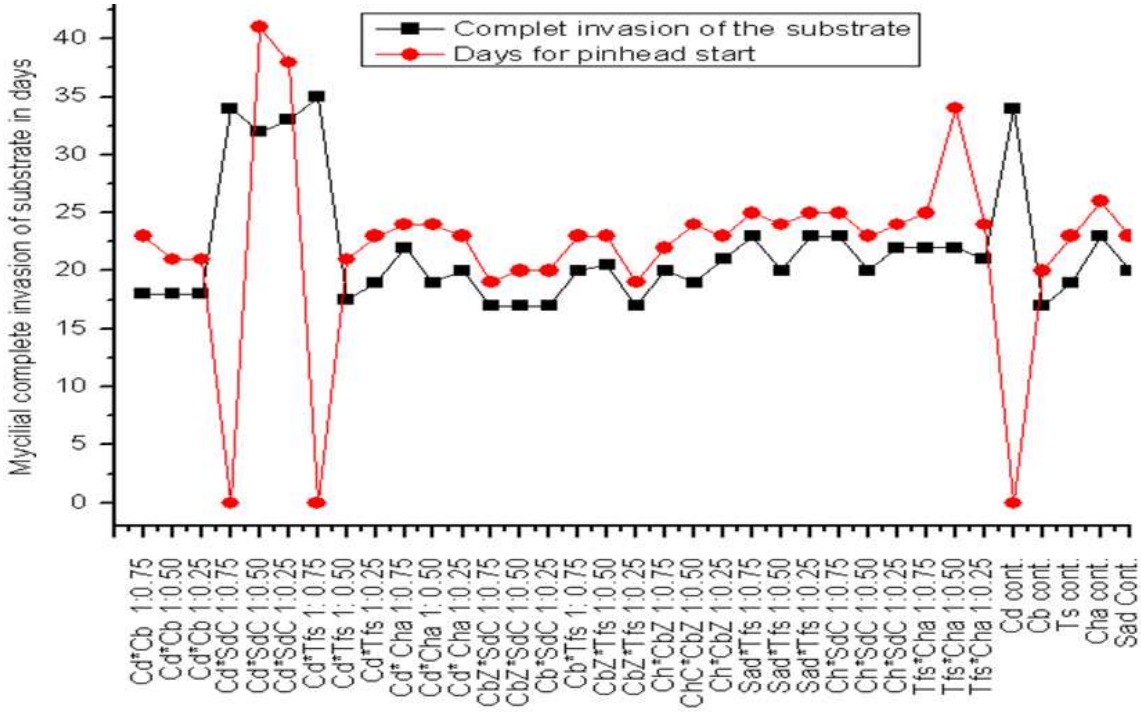

Figure 2. Days of complete invasion and pinning *P. ostreatus* across different substrate ratios. Cd*Cb (cow dung with corn cobs) = 1:0.75,1:0.50,1:0.25; Cd*SdC (cow dung with sawdust of *Cupressus lusitanica*) =1:0.75,1:0.50,1:0.25; Cd*Tfs (cow dung with teff straw) = 1:0.75,1:0.50,1;0.25); Cd*Cha (Cow dung with Chat or khat) = 1:0.75, 1:0.50,1:0.25; CbZ*SdC (corn cobs with sawdust of *C. lusitanica* = (1:0.75, 1:0.50, 1;0.25); CbZ*Tfs (corn cobs with teff straw by 1:0.75, 1:0.50,1;0.25); Cha*CbZ (chat with corn cobs = 1:0.75 1:0.50, 1;0.25); SdC*Tfs (sawdust of *C. lusitanica* with teff straw = 1:0.50, 1:0.25); Cha*SdC (chat with sawdust of *C. lusitanica* 1:0.75, 1:0.50, 1:0.25); Tfs*Cha (teff straw with chat by = (1:0.75, 1:0.50, 1:0.25) ration; cont.= control.

heads were aborted in most of the treatments. There was statistically significant difference among most of the substrates in both mycelia ramification and pinning days of *P. ostreatus* mushroom (Table 1).

Table 1. Ramification of mycelia in different combination of substrate.

Substrate	25%	50%	75%	100%
Cd*CbZ	8.2^{a}	7.2^{a}	8^{a}	5.2^{a}
Cd*SdC	7.3^{b}	7.4^{ab}	9^{b}	9.64^{b}
Cd*Ts	8.9^{c}	8.6^{c}	8.2^{ac}	9.64^{bc}
CbZ*Ts	9^{c}	8.7^{cd}	8.6^{cd}	9^{d}
Ch*CbZ	8.2^{ad}	8.4^{cd}	8.6^{cd}	8.6^{e}
ChC*Cd	8.4^{ad}	8.2^{cd}	7.4 ^{e}	8.4^{ef}
SdC*Ch	8.4^{ad}	9^{cde}	7.8^{ef}	8.4^{ef}
Cd*Ts	8.2^{ad}	8.3^{cdf}	8.5^{acg}	6.2^{g}
Sd*Ts	8.6^{acd}	8.4^{cdf}	7.9^{ef}	8.75^{efh}
Ts*Ch	9.6^{8e}	6.8^{ag}	7.1^{ef}	5.8^{ai}

Values are least significant difference (LSD). LSD=0.43996.

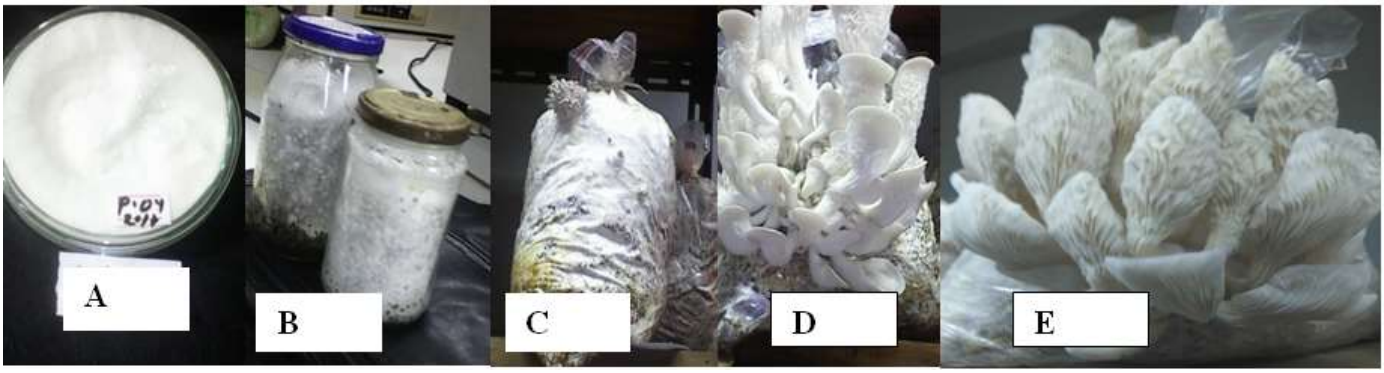

Figure 3. Mushroom cultivation. (A) Pure culture of *P. ostreatus*; (B) Mother oyster spawns; (C) pinheads emerging out at 2-3 day; (D) fruiting body of 3 days old after emerging out. (E) Mature oysters ready for harvest (5-6 days old).

Oyster mushroom maturation

After pinnate had appeared, mushroom maturation is taken 3 to 5 days in most treatment in case of 1st and 2nd flushes (Figure 3). However, Cd*SdC replicates did not provide any yield.

Yield parameters on *P. oysteretus* mushroom

During this study, comparatively, the longest stipe length, largest pileus diameter of mushroom and considerable number of pinning holes was observed in treatment with corn cobs combination as well as cobs alone.

Stipe length

In contrast to cow dung and chat mixtures, the longest stipe and highest bulk density of fruiting bodies of mushrooms were recorded from corn cobs mixtures or alone (Figure 4).

Pileus diameter

Highest pileus diameter were obtained from treatments of corn cob combinations (Cd*CbZ 1:0.50,CbZ*SdC 1:0.25, CbZ alone), and (Cd*Tfs 1:0.5), which were significantly different as compared to the control (Cd) and Cd*SdC (Figure 5).

Products per flush of substrates

The mean yield of mushroom in various substrates showed significant difference among the harvests (df = 10, 24; F = 505.372; P<0.001). Moreover, almost more than 75% of the total fruiting bodies were obtained from the first and second harvest, while the third and fourth harvests were relatively lower in quality as well as yield (df = 3, 72; F = 113.830; P<0.001) (Figure 6).

Biological efficiency of substrates

Biological efficiency was determined as the ratio of fresh

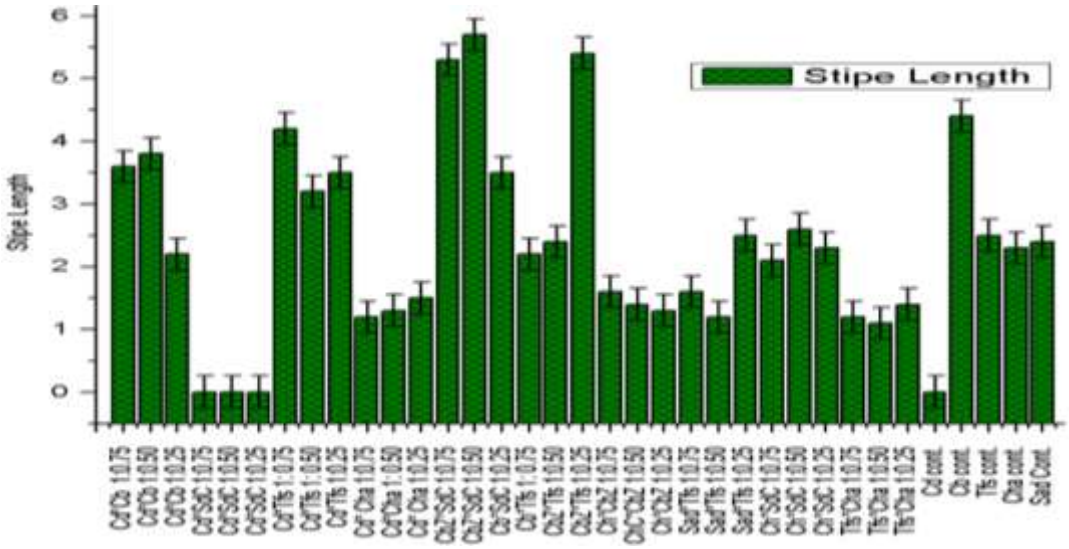

Figure 4. Stipe length (in cm) of *P. ostreatus* mushroom in different combination of substrate.

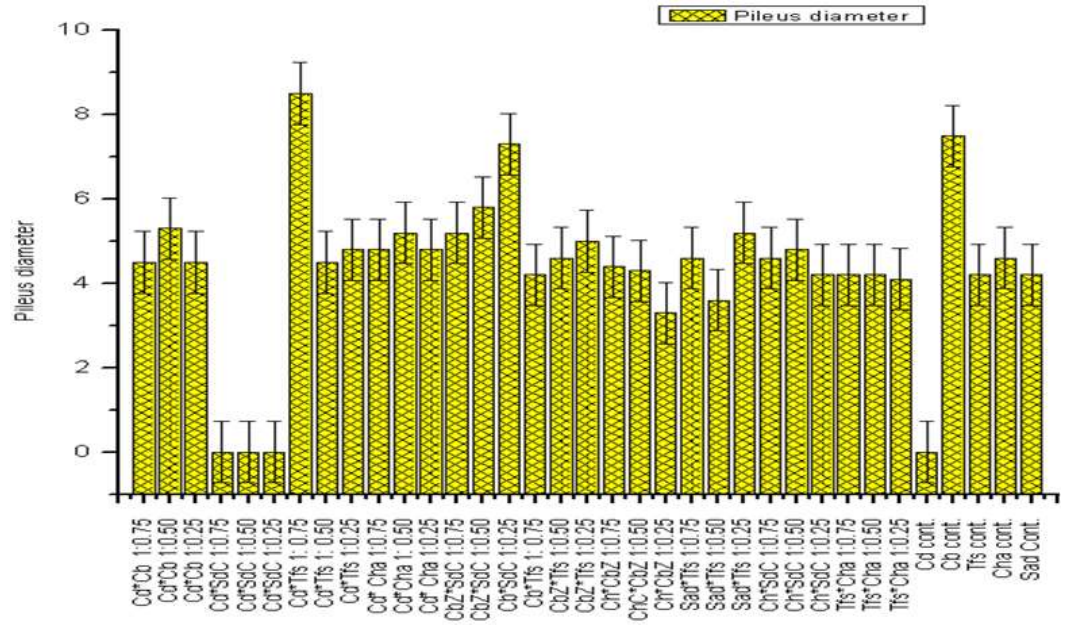

Figure 5. Pileus diameter of *P. ostreatus* mushroom across various combination of substrate.

mushrooms harvested (g) per gram of dry substrates and expressed as a percentage. Highest BE was recorded in combination of Tfs*CbZ, (1:0.75; 1:0.25 and 1:0.50), where BE, was 87.5, 79.5 and 72.8%, respectively. Moreover, the combination of Cd*Tfs (1:0.25) showed 79.9% BE (Figure 7)

DISCUSSION

In recent times, mushroom production has gained attention both globally and nationally because of its nutritional, industrial and medical value as well as its ecosystem sustainability or bioremediation. Generally, it have been understood that mushrooms such as *P. ostreatus* has a potential to turn over various agro-wastes. Thus, the substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at low cost and in huge amounts in southwestern part of Ethiopia.

Utilization of these agro-wastes for the production of *P. ostreatus* mushrooms could be significant to alleviate

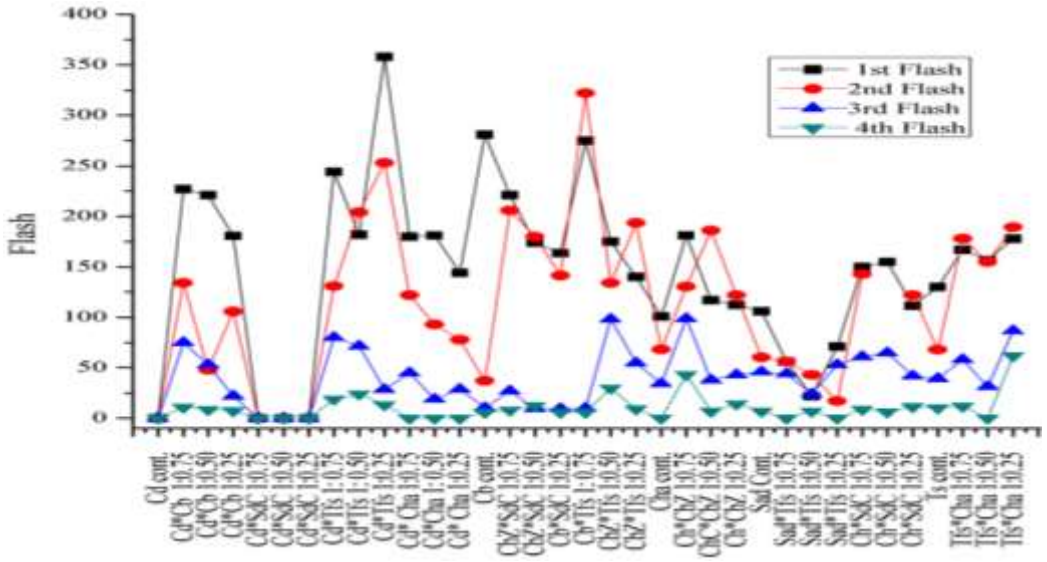

Figure 6. Mean yield per flush of dry weight substrates (400 g).

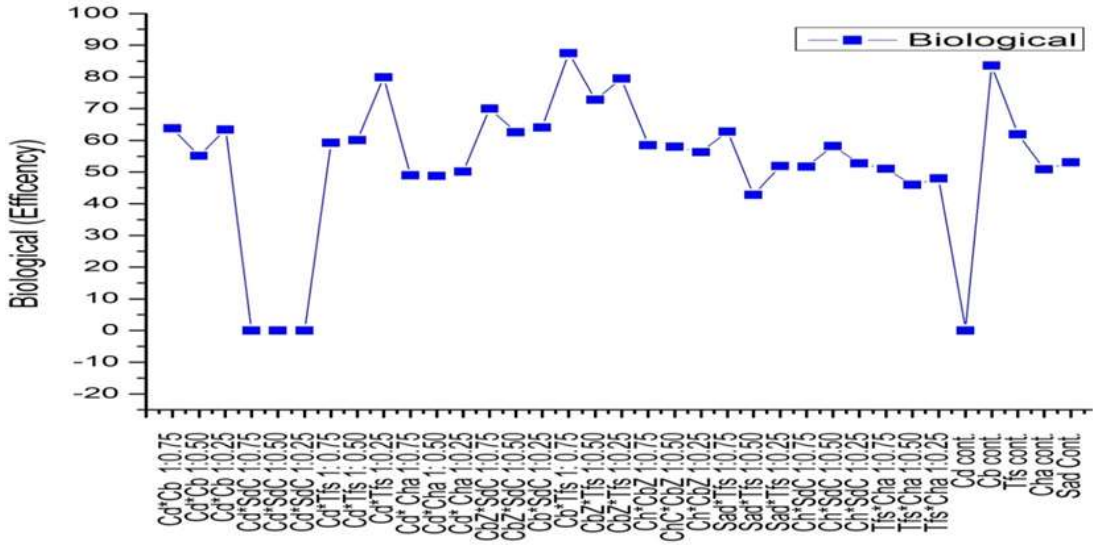

Figure 7. Biological efficiency of different substrates.

food security as well as environmental rehabilitation. This is the first report, in which the utilization of the combination of chat left over (*Catha edulis*) and sawdust (*C. lusitanica*) for *P. ostreatus* cultivation is been reported.

In current study, highest ramification of *P. ostreatus* mycelium was observed at room temperature during spawn production. This could be because room temperature is the optimal condition for *P. ostreatus* growth, as also reported by Siddhant et al. (2013).

Although the rate of substrates colonized by mycelium differ within treatments, invasion rate of substrate and pinning days were highly correlated. In contrast to the combination of CbZ*SdC and CbZ, emerging of pinnate in ChC*Cd and CbZ*Ts had taken long days and this could be because those substrates were not permissible for mycelia growth and pinning. This result is in agreement with the finding of Sher et al. (2010) as pinheads formation had spent 23 to 27 days. Nevertheless, the pinnate period was lower compared to the work of Pathmashini et al. (2008), where it spent 35 to 51 days. This could be because of variation in substrate composition, and environmental factors such as light, temperature, humidity and concentration of CO_2.

Generally, increasing CbZ ratio in the mixtures significantly enhanced mycelia ramification. Narain et al. (2009) also indicated that mushroom mycelia growth and primordial development relay on the composition of lignocellulosic materials, particularly on the C/N ratio.

During this study, paramount stipe length and pileus diameter were harvested from the combination of CbZ*SdC, particularly 1:0.50 ratio (5.7 cm), and CbZ alone. However, the biomass of *P. ostreatus* mushroom was obtained from the combination of ChaC*Cd, and chat alone were disregarded. This could be due to less turnover of chat or offensive chemical released during chat or Cd decomposition that affects mushroom growth. The less productivity of some agro-wastes substrate for mushroom production was also reported by Gume et al. (2013), in which 3.8 cm stipe length was recorded from combination of sawdust and coffee bean husks. Furthermore, considerable amount of mushroom biomass were harvested from 1st and 2nd flushes of the CbZ*Cd, Tfs*CbZ, CbZ*Sd combination as well as CbZ alone, but the least amount obtained from the 3rd and 4th flushes, this might be due to nutrient of substrate depletion, and retard the propagation of mushroom, or immobilization of nutrients as white rote fungus biomass saturated (Gume et al., 2013).

Despite the fact that Dawit (1998) obtained highest mushroom biomass from 2nd and 3rd flushes, others workers obtained significant yield from the 1st (Sher et al., 2010). In this work, the substrates was given up to four phases of flushes, and the duration of time taken for appearing succeeding flushes was statically insignificant among flushes. Interestingly, this study well addressed the suitability of the combination of corn cob, cow dung, saw dust, teff straw, and room temperature, and 80 to 90% of relative humidity for *P. ostreatus* mushroom production. Oei (2003, 2005), also indicated at least 90% of relative humidity is required for primordial formation with room temperature. Furthermore, we determined that the plastic holes size (15 mm^2) is the appropriateness for colonization of substrate by *P. ostreatus* mycelia, which is in accordance with previous work (Tesfaw et al., 2015). Significant amount of BE was recorded from the combination of Tfs*CbZ as well as was corn cob alone. This could be because of corn cob permissible for easily mycelia ramification. Thus, corn cob is a potential agro-waste for *P. ostreatus* cultivation, similarly to straw substrate as indicated by Mateus et al. (2012).

In general, the rate of mycelia invasion in most treatment group was highly related with completed invasion and pinning days. However, stipe length, pilus diameter and other parameters is not always indicator for higher yield obtained as also observed (Gume et al., 2013). Both pinhead maturation and abortion are affected by the type of substrates and environmental factors. During this experiment, in average 6 to 16.5% pinhead were aborted per treatment of bags in all of the substrates. But compared to previous study, we reduced lost of mushroom biomass by 25%, due to optimizing of the approach. It was also reported by Kimenju et al. (2009) in which more than 50% of pinheads emerged did not grow into marketable products. Over all, during this study the various combination of CbZ*SdC and Cd*Tfs were realized as best agro-wastes for *P. ostreatus* mushroom cultivation.

Conclusion

Based on the obtained results, the following conclusions were made. Room temperature, about 80 to 90% of relative humidity, substrates such as corn cob, saw dust, and teff straw are determined as the best promising agro-wastes for *P. ostreatus* mushroom production. Although this is the first work that attempted to use chat left over, and cow dung for mushroom cultivation, which is not suitable for mushroom cultivation. Thus, it needs further investigation for the chemical composition of chat left over. Moreover, high yield of *P*. ostreatus mushroom was obtained from 1st and 2nd flushes. On the other hand, ramification of substrate by mycelia, pinning days/ pinhead maturation, stipe length, and pilus diameter were determined by plastic holes size, substrate composition, temperature and relative humidifies. Finally, this work is a baseline for our institution to establish mushroom production at large scale.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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