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Full Length Research Paper

Behavior of the pollen tube of *Poincianella pyramidalis* (Tul.) L. P. Queiroz after compatible and incompatible crosses

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Aiming to analyze the pollen tube growth behavior in compatible and incompatible crosses in *Poincianella pyramidalis*, on-field crosses were performed using flowers of 30 randomly selected individuals, which were self- and cross-pollinated. In the cross-pollination, the pollen germinated and the pollen tube grew in the transmitting tissue of the pistil, followed by a succession of callose rings through the growth of the tubes towards the ovary; after 8 h, the ovules were fertilized. On the other hand, in self-pollination, the pollen grains germinated, but the pollen tubes were inhibited during growth at the stylar transmitting tissue, indicating that the self-incompatibility of the species is homomorphic and gametophytic.

Key words: Fabaceae, self-incompatibility, Caatinga, *Poincianella pyramidalis,* incompatible crosses, compatible crosses.

INTRODUCTION

The Caatinga biome is considered one of the largest geographic areas on the planet, distributed in Brazil across the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia and part of Minas Gerais (IBGE, 2011). It is a lush and thorny vegetation characterized mostly by its completely deciduous species, which are subjected to water shortage during most part of the year, due to the poor spatial and temporal distribution of rain, elevated evapotranspiration rate and low water-holding capacity of the soils, which are, in general, shallow and stony (Andrade Lima, 1989).

The family, Fabaceae consists of one of the most representative families of the Caatinga, it is the third largest family among the Angiosperms, having 727 genera and approximately 19,325 species (Lewis et al., 2005). The economic importance of the representatives of this family is indisputable, because many genera and

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species are utilized in human feeding (e.g. forages), in the recovery of poor soils, urban greening, and in the logging and chemical industries (Lewis et al., 2005).

Poincianella pyramidalis is an arboreal species (Monteiro et al., 2005) belonging to the family Fabaceae. It is endemic to the Caatinga that inhabits stony areas (Silva and Matos, 1998), and popularly known as *catingueira*, *pau-de-porco* and *catinga-de-porco* (Braga, 1960). It is one of the most widely dispersed species in the Brazilian northeast semiarid, and can be found in several plant associations. It is highly utilized by the communities for firewood and charcoal (Silva et al., 2009).

Regarding the family Fabaceae, its reproductive system has already been studied in at least 451 species, with 152 (33.7%) of them showing self-incompatibility (Kill and Drumond, 2001; Freitas and Oliveira, 2002; Costa et al., 2003; Leite and Machado, 2009). However, the mechanism of incompatibility (gametophytic, sporophytic or late-acting) has not been determined for most of the analyzed species. As to the Caesalpinioideae subfamily, there is still a large knowledge gap with regard to the reproductive system (Lewis et al., 2000; Leite and Machado, 2009).

Self-incompatibility (SI) is the incapacity of a fertile plant to generate seeds when fertilized by its own pollen. It is a gene-based physiological mechanism that promotes allogamy. As a reproductive barrier, prefertilization is one of the least costly mechanisms that impede self-pollination, regarding the allocation of maternal resources (Schifino-Wittmann and Dall'Agno, 2002). Many different forms of self-incompatibility are known, and in some cases, the molecular mechanisms involved have been at least partially elucidated (Franklin-Tong, 2008). However, information on the sites of inhibition or the genetics of the incompatibility, for many plant groups, remains incomplete (Bilinski and Kohn, 2012).

There are two main types of SI: gametophytic (GSI), in which the specificity of the pollen is generated by the S allele of the haploid genome of the pollen grain (gametophyte) and the sporophytic (SSI), in which specificity is determined by the diploid genotype of the adult plant, which originates the pollen grain. The sporophytic self-incompatibility can be homomorphic, when there are no floral modifications in the process, or heteromorphic, when, along with the SI process, there are floral modifications. The self-incompatibility reaction comprises the processes from the prevention of germination of the pollen until the rupture of the pollen tube (De Nettancourt, 2000).

Late-acting self-incompatibility (LSI) is characterized by the uniform abortion of the pistils shortly after self-pollination, even though the pollen tubes have traversed the style and penetrated most of the ovules (Seavey and Bawa, 1986; Sage et al., 1994; Bittencourt, 2008). Although, it is a widely distributed phenomenon that occurs in a clustered manner in some angiosperm families (Gibbs and Bianchi, 1999), the physiological and genetic mechanisms acting on LSI are not well known (Ladoux and Friar, 2006). Late-acting self-incompatibility can basically manifest in three different ways: (1) with inhibition of the pollen tubes, occurring before the ovules are penetrated; (2) with inhibition after the ovules are penetrated, but before their fecundation; or (3) with postzygotic rejection of the ovules (Bittencourt, 2008).

Self-incompatibility in *P. pyramidalis* has already been described by Leite and Machado (2009) as being late-acting, and so, the objective of the present study was to analyze the growth behavior of the pollen tube in compatible and incompatible crosses in *P. pyramidalis* so as to determine the location and time of inhibition for incompatible pollen tubes, in addition to observing the approximate fertilization time.

MATERIALS AND METHODS

Experimental area

The experiments were conducted at the Experimental Station Bacia Escola, at Federal University of Paraíba (UFPB), located in the municipality of João do Cariri/PB, Brazil. The geographic coordinates of the area are 7°23'30" S and 36°31'59"W, at an altitude of 458 m. According to the Köppen classification, the climate of the region is a hot semi-arid BSh-type, with rainy period from January to April, showing average annual temperatures of about 26°C, relative air humidity of approximately 68%, and annual average precipitation of 376.4 mm. The experimental area has 3.20 ha and is composed of a typical vegetation of Caatinga under regeneration. The area was fenced so as to prevent the access of animals.

The laboratory analyses were carried out at the laboratory of Plant Anatomy of the Department of Plant Biology (Department of Plant Biology, DBV) at Federal University of Viçosa (UFV), located in Viçosa/MG, Brazil.

Experimental material

All the crosses were performed from May to June 2010, during the flowering period of *P. pyramidalis*. The individuals utilized in the experiment were randomly selected among those showing leafy crown and good phytosanitary conditions (apparent absence of diseases or parasitic infestations).

For the open pollination (control), five individuals were tagged, and their tagged and uncovered flowers were monitored, under natural conditions of pollination, without manipulation. At cross-pollination (allogamy), five plants were used; their leaves were emasculated and the pollen from flowers of distinct plants which were at 100 m away from the recipient plant was deposited on the stigmas of its flowers. For manual self-pollination (geitonogamy), however, five individuals were tagged, and the same methodology for manual cross-pollination was adopted, but this time with pollen grains from flowers of the same plant.

Evaluation of the growth of the pollen tubes

The self-incompatibility reaction in the stigma and in the style was diagnosed according to the methodology proposed by Martin

Figure 1. *Poincianella pyramidalis*. a. Inflorescence; b. flower exhibiting nectar guide (arrow) in the center of the vexillum.

(1959), with a few alterations. For this purpose, the pistils of the flowers were collected at 10, 20, 30, 40, 50 and 60 min and at 8, 24 and 48 h, respectively, after the pollination, and fixed in ethanol: glacial acetic acid (3:1, v/v). For the observation of the germination of the pollen and growth of the pollen tube, the pistils were removed from the fixative solution, washed in phosphate buffer pH 7.5 and placed in a sodium hydroxide solution (NaOH) 1 M for approximately 8 h, at 60°C. This procedure was performed so as to soften and clarify the plant tissues, and at the end of this period the material was washed again three times in phosphate buffer (K_3PO_4) 7.5, and then placed on a slide. One drop of a blue-aniline solution was added, and it was left to sit for 30 min and then covered with a coverslip, which was slightly pressed upon to allow for the plant tissue to better spread. The observations were made with an Olympus BH2 photomicroscope (epifluorescence illumination using a BP-490 filter) and pollen tubes were identified by the fluorescence of the callose on the walls and on the plugs, and then photographed on a Kodak ISO 400 color negative film.

RESULTS AND DISCUSSION

External structures of the pistil of *P. pyramidalis*

The flowers of *P. pyramidalis* are arranged in a terminal or axillary-terminal inflorescence, in panicle (Figure 1a). Their bracts are ovate, apiculate, concave, slightly pilose, presenting small glandular spots on the back. The flowers are yellow, arranged in racemes (Maia, 2004), and this is the most common type of inflorescence in the family Fabaceae (Tucker, 2003). It presents zygomorphic symmetry, yellow color, a guide and high concentration of nectar, which, according to Faegri and Van der Pijl (1979), are typical attributes of the melittophily syndrome (Figure 1b).

With a fluorescence microscope, it was possible to view the external structure of the pistil of *P. pyramidalis* in detail (Figure 2). In the epidermis of this structure, there were numerous simple and secretory trichomes (Figure 2d), homogeneously distributed all over the epidermis, in addition to the presence of stomata (Figure 2e).

The cells from the stigmatic region could be characterized as elongated papillary cells, forming a hollow ring, which bent to the adaxial region (Figures 2a and 2b), and the same characteristic was observed in *Caesalpinia echinata* and *Caesalpinia peltophoroides* (Zaia, 2004). Dulberger et al. (1994) mention that most angiosperms have an exposed stigmatic surface that facilitates the process of capture and deposition of pollen, but in some species of the genera *Cassia*, *Senna* and *Chamaecrista* belonging to family Fabaceae, access to the stigmatic surface is protected by what was named the fringe of trichomes, in which the size and number of trichomes and the degree to which the stigmatic surface is exposed varies according to the species (Dulberger, 1981; Dulberger et al., 1994; Tucker, 1996). The pollen grains germinate in the sub-stigmatic chamber, or under the papillary cells; however, for the vast majority of species, there is still little information on their role in pollination. On *C. calycina*, Lewis and Gibbs (1999) stated that the fringe of trichomes of the stigma plays an important role in removing pollen from the body of the pollinator. In fact, during the visit of the pollinator, the abdomen of the insect comes in contact with these trichomes of the region of the stigma, and they scrape off the pollen present in its body, guiding its entry into the stigmatic region (Arceo-Gomez et al., 2011).

Behavior of the pollen tube in compatible crosses

The compatible crosses originated from cross-pollination, and their behavior occurred as can be seen in Figure 3. It

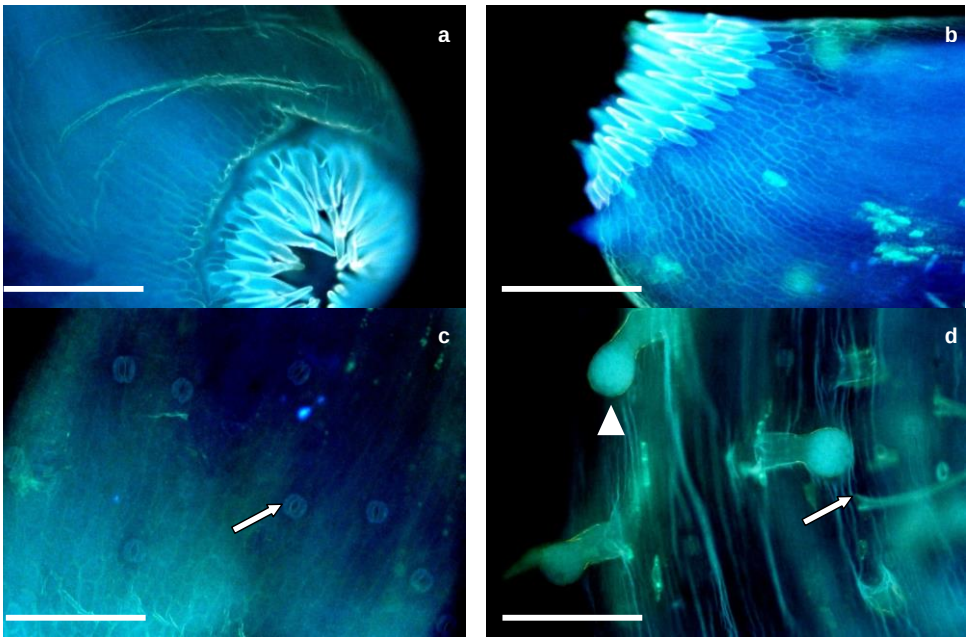

Figure 2. Structures on the pistil of the flower of *P. pyramidalis* Tul. a. Fringe of trichomes (front view); Fringe of trichomes (side view); c. Stomata in the style (arrow); d. Simple trichomes (arrow) and secretory trichomes (arrowhead). Bar = 10 µm.

is noteworthy that it is not possible to observe the germination of the pollen grain on the stigmatic surface, because it is located inside the stigmatic cavity, formed by the fringes of trichome, where the pollen grains fall and germinate, so it is only possible to visualize the several pollen tubes growing in the stylar transmitting tissue, until they reach the ovules.

Pollen tubes have a fluorescent green color when stained with clarified aniline blue solution and viewed under UV light on an epifluorescence microscope. The first evidence of the pollen germination was observed in general 20 min after pollination (Figure 3a). After 50 min the pollen tube growth was limited to the beginning of the first third of the pistil. By 60 min, the tube was found, on average, at the central third of the pistil (Figures 3b and c). Its growth was strictly guided in the transmitting tissue of the pistil and followed by a succession of callose rings along the growth of the tubes towards the ovary, resembling a flight of stairs (Figure 3c). After 4 h, the tubes began to approach the ovarian cavity (Figure 3d). The fertilization of the ovules (Figure 3e) started 8 h after pollination, and by 24 h, most of the ovules had already been fertilized (Figure 3f). In this sense, Leite and Machado (2009) observed, for the same species, ovules penetrated 24 and 48 h after cross-pollination.

Behavior of the pollen tube in incompatible crosses

In *P. pyramidalis*, pollen grains germinated on the surface of the stigma, within the stigmatic cavity, but this did not occur due to the presence of the trichomes fringes, which cover the surface, as previously mentioned (Figure 2). Characteristics of an incompatible gametophytic pollination were observed in these crosses. The pollen grains germinated approximately 20 min after pollination. In approximately 30 min, the pollen tube reached the beginning of the first third of the pistil, and at 50 min, the tube was found near the central region of the pistil (Figure 4). It is worth stressing that each tube was able to germinate and grow in the transmitting tissue of the stylus, and was inhibited within it, when the pollen tube expanded and burst. The callose synthesis is induced by the incompatible pollen, probably through proteins expressed by the S alleles, which were broadcast from the anther tapetum, a nutritive tissue (Sood et al., 1982) to the pores of the exine during the development of pollen; these are features found in species of gametophytic self-incompatibility. This is the most common system among plants, and it is also thought to be the most primitive (De Nettancourt, 2000; Schifino-Wittmann and Dall'Agnol, 2002).

The manual self-pollination showed self-incompatibility. In this case, the pollen grains germinated, penetrated the stigmatic tissue and were inhibited, in most cases, in the central region of the stylar transmitting tissue (Figure 4a). Two types of abnormalities were observed in the style: (a) narrowing of the pollen tube wall (Figure 4b) and (b) formation of bulb at the apex of the pollen tube (Figure 4c). The occurrence of these abnormalities and

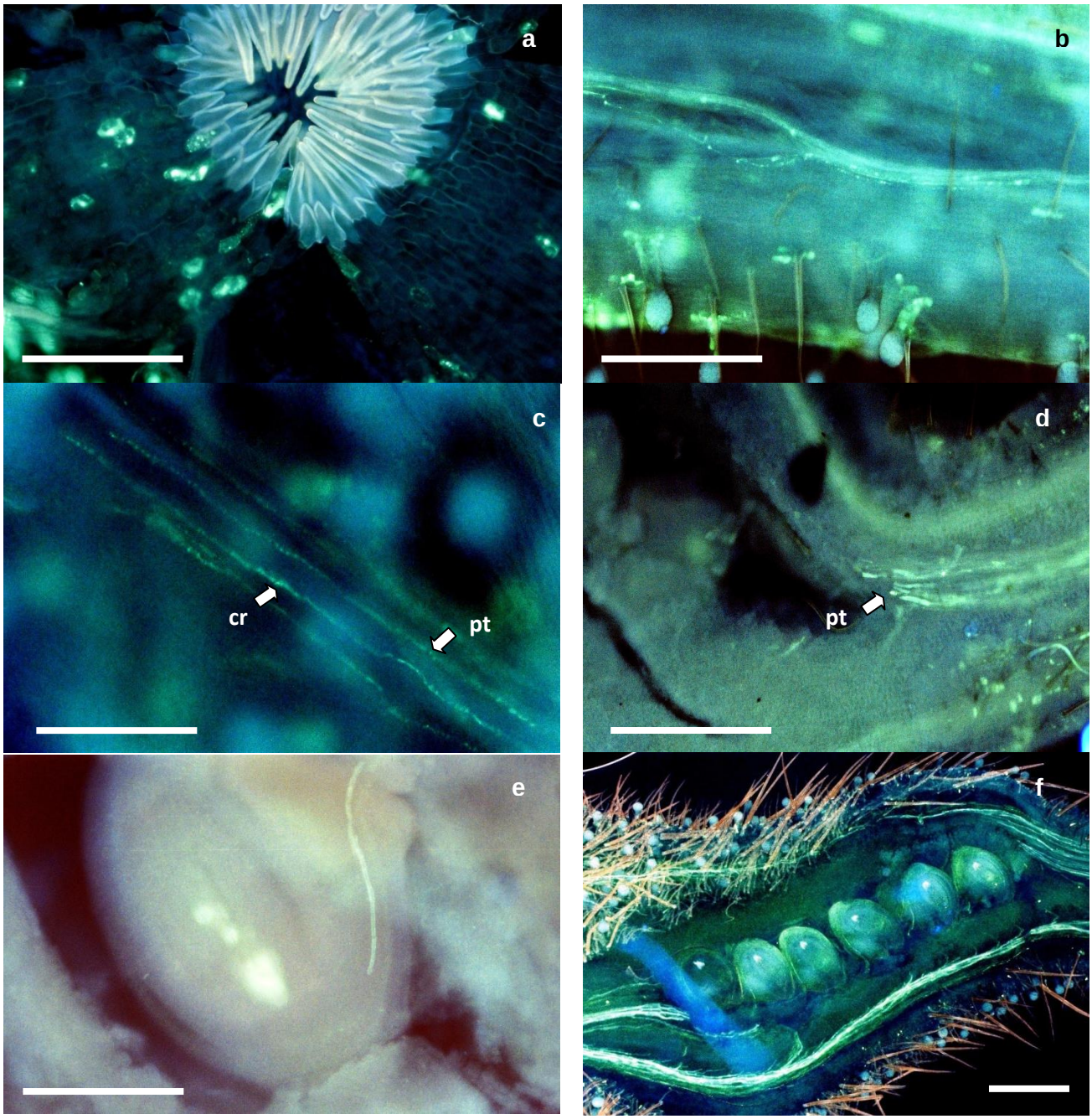

Figure 3. Behavior of the pollen tube (pt) in compatible crosses. a. Start of the elongation of the compatible pollen tubes after the passage through the chamber of trichomes, in the transmitting tissue, stained with clarified aniline blue and observed on a fluorescence microscope; b and c. Several pollen tubes developing in the style, with successive callose rings (cr); Lower part of the style and ovary, displaying several tubes reaching the ovary. e. Ovule being fertilized, displaying the entry of the pollen tube 8 h after pollination. f. Newly-fertilized ovules. Bar = 10 µm.

frequencies were constant among the slides of incompatible crosses analyzed. After formation of the bulb in the style, in some cases, the pollen tube wall is disrupted. These characteristics are usually found in species that express gametophytic self-incompatibility (De Nettancourt, 1977).

Based on the observations, *P. pyramidalis* appeared to be a species that has gametophytic self-incompatibility, and inhibition of the growth of the pollen tube occurred due to the callose deposition in the style, as verified in studies with many woody species (Oliveira and Gibbs, 2000). As opposed to the present study, Leite and

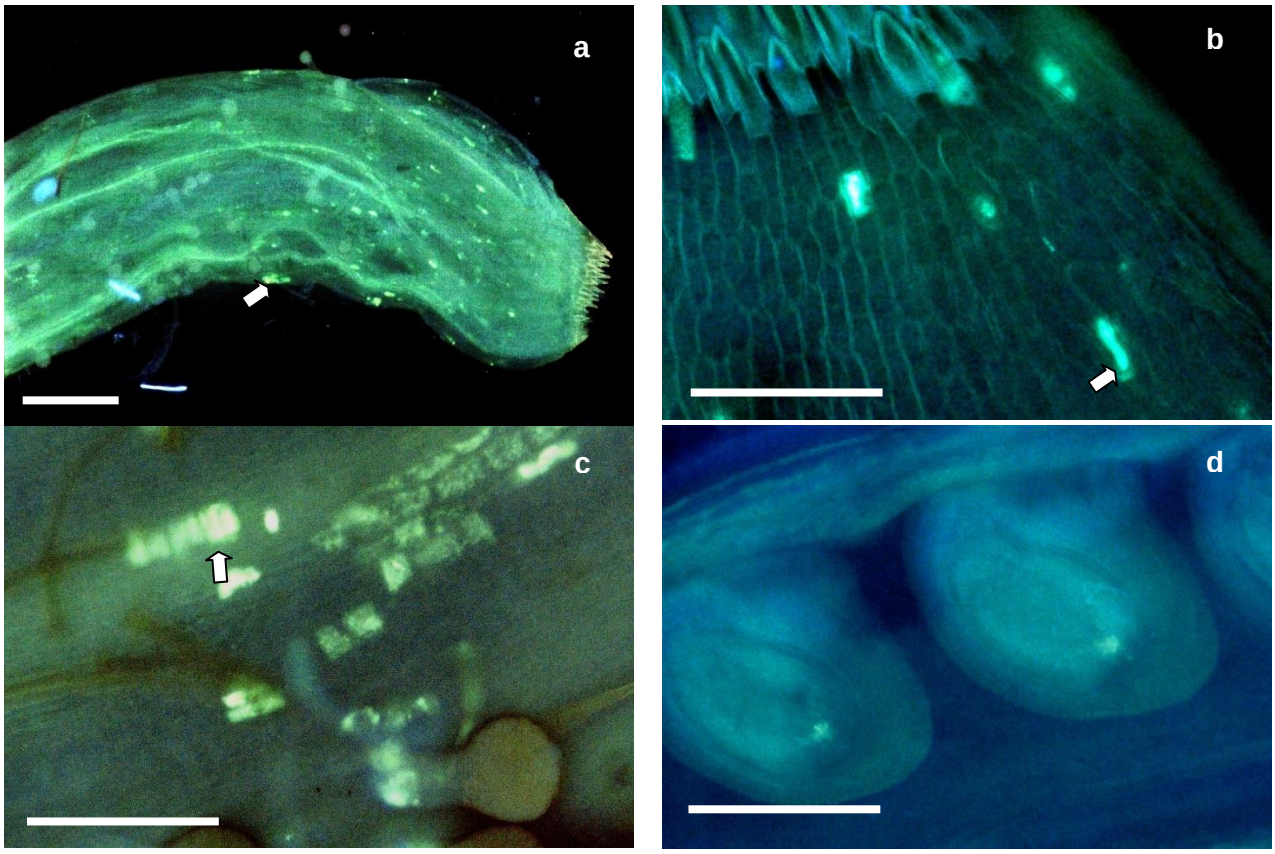

Figure 4. Development of the pollen tube in incompatible crosses. a. Inhibition of growth of pollen tubes in the style; b. Formation of bulb at the apex of the pollen tube; c. Narrowing of the pollen tube wall; d. Non-fertilized ovules. Bar = µm.

Machado (2009), working with *P. pyramidalis* in an area of the Caatinga, with observations on cross- and self-pollinations, verified growth of pollen tubes of manual self-pollination until the ovule and flowers fell off, from this treatment, between 24 and 72 h; these events were classified as late-acting self-compatibility (LSI). The gametophytic self-incompatibility is determined by the S locus, which codes two genes that determine the phenotype in the alleles of the pistil and of the pollen (Kao and Tsukamoto, 2004; Guerra et al., 2012).

Another point for discussion, to justify the fact that the studied species is shown to be gametophytic self-incompatible, contradicting the observations of Leite and Machado (2009), is the variation of the expression of self-incompatibility among populations of a species (Pailler and Thompson 1997; Sage et al 2001) expected particularly for some circumstances, e.g. in populations with different sizes and in the geographical limits of distribution of the species (Fausto et al., 2001; Stone et al., 2006). Thus, the fact that the results of Leite and Machado (2009) suggest that there is a mechanism of late-acting incompatibility (though inconclusive) does not exclude the possibility of existence of a mechanism of gametophytic incompatibility, provided it is partial, in which its expression varies according to both differences between the composition of the pollen load carried by the pollinators and differences between populations of the species (Wolowski and Freitas, 2010).

Conclusions

The site of inhibition of the pollen tube growth is the style. The inhibition of the pollen tube in the style takes place approximately 60 min after pollination. Fertilization occurred 8 h after pollination, in the crosses between compatible plants.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

The mechanism of seedlessness in watermelon generated using soft-X-ray irradiated pollen

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A new variety of seedless watermelon, with high sugar content, good taste, and easy storage has been developed using scanning electron microscopy (SEM) fluorescence staining, paraffin and 4',6-diamidino-2-phenylindole (DAPI) staining. The study investigated the mechanism behind the production of seedless watermelon after soft X-ray irradiation. The results showed that soft X-ray irradiation did not damage the cell walls of the watermelon pollen, and leading to normal pollination and fertilization. However, the chromosomal double helix of the watermelon pollen were damaged, thereby inhibiting embryotic developmental processes, leading to abortion of the embryo and degeneration of endosperm, which lead to the production of seedless watermelon. In conclusion, artificial pollination of pollen after soft X-ray irradiation can produce no seed watermelon, and the sugar content is higher than that of the seed watermelon.

Key word: Watermelon, X-ray, seedless.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a dicotyledonous flowering plant belonging to Cucurbitaceae with origins in Africa. As a new variety of *C. lanatus*, seedless watermelon is characterized by high sugar content, good taste, and easy storage. Producers and consumers find it attractive on the fruit market for its high quality, convenient eating, resistance against disease, waterlogging tolerance, high and stable yield, tolerance of storage and transportation, and other advantages (Akutsu and Sugiyama, 2008; Hassell and Schultheis, 2007). The cultivation area of Seedless watermelon was 230,000 hm^2 across China in 2009, with 80% being cultivated in the humid regions to the south of the Yangtze River and 90% in the regions to the South of the Yellow River (Liu, 2010). In many regions, the yield of *C. lanatus* with seeds is 2,000 to 2,500 Kg for every 667 m^2, while the yield of seedless watermelon can reach 3,000 to 5,000 Kg (Liu et al., 2006). The demand for seedless watermelon is rapidly increasing on the market of China and the United States (Liu, 2010; Schultheis et al., 2007; Yang et al., 2012), leading to the need for research into new methods for obtaining seedless watermelon.

Terada and Masuda (1943) could produce seedless watermelons by first generating tetraploid plant using colchicine, then hybridizing with diploid watermelon as the male parent, and finally obtaining triploid seedless

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watermelon by exploiting the high sterility of the triploid (Akutsu and Sugiyama, 2008; Eisho et al., 2002). However, compared with the diploid, triploid seedless watermelon had disadvantages such as high breeding cost, low germination rate, late ripeness, poor fruit quality, long production cycle (Akutsu and Sugiyama, 2008). Therefore, its cultivation area is gradually decreasing. In recent years, Sugiyama and Morishita, 2000 and Sugiyama et al. (2002) have discovered that seedless watermelon can be obtained by irradiating pollen from male flowers of diploids with 600 Gy soft X-rays and then fertilizing female flowers with the irradiated pollen. This technique can be used for producing seedless watermelon but has only been applied for a short time and its mechanism is still not clear. In this study, we research the mechanism of producing seedless watermelon in this way in order to provide a theoretical basis for the technique, and to facilitate further exploration of new methods for producing seedless watermelon.

MATERIALS AND METHODS

Huaian Academy of Agricultural Science bred new varieties of watermelon 'Huaime'. Non-irradiated pollen of diploid 'Huaime' and pollen irradiated by soft X-rays (600 Gy) were used in the experiment.

Observation of pollen by scanning electron microscope

Differences between irradiated pollen and control pollen exine were observed with a scanning electron microscope. Dried pollen were placed on the platform of a Philips SEM-505 scanning electron microscope that had been prepared with Mayer's albumn (50 ml glycerol + 50 ml egg white) (Of. 2009) and sprayed with an ion sputter coater. Pollens were then observed and photographed.

Pollen germination on stigma and growth in style

Pollen germination on the stigma and pollen tube growth were observed after pollinating diploid watermelon with irradiated and control pollen. Fresh styles and ovaries were picked at 2, 4, 8, and 24 h after pollination, and immediately placed in FAA fixative that is composed of 5 ml Formalin, 5 ml Acetic acid and 90 ml 70% Alcohol for 5 days. The styles and ovaries were then clarified for 24 h with transparent solution (alcohol mixed with an equal volume of xylene), and washed five times with distilled water, and stained for 24 h with aniline blue (0.1 g water-soluble aniline blue in 0.1 mol/L K_3PO_4). Photographs and observations of flattened styles and ovaries were taken with a fluorescence microscope (OLYMPUS BX43) to compare stigma germination and pollen tube growth behaviors.

The embryo sac and fertilization process were observed via a paraffin fixing method

Embryos of different days (days 1-7, 12, and 15) were washed with water and cut into 2 × 4 mm cuboids. These were section and examine using the paraffin method of Randolph (1935) (Randolph, 2009).

Differences in chromosomes were observed via DAPI staining

One mg of 4', 6-diamidino-2-phenylindole (DAPI) solution was dissolved in 3.6 ml 70% alcohol to obtain 1 M DAPI solution, and 50 ml of solution was prepared by adding 5 µL DAPI alcohol solution to PBS buffer solution. Pollen was cultured for 15 min in incubator at 22°C with 1/10 DAPI solution to pollen culture medium (Sucrose 15%, KNO_3 0.1%, $Ca(NO_3)_2$, H_3BO_3 0.01%, $MgSO_4$ 0.1%, pH 6.5). Cells were then washed twice with PBS buffer solution and chromosomes observed by fluorescence microscope (OLYMPUS BX43) with optical filter, with 360 mm excitation wavelength and 460 emission wavelength.

Data analysis

The software of Image J (Schneider et al., 2012) was used for measuring the fluorescence values of pollen chromosomes. Mean fluorescence values of chromosomes of *C. lanatus* pollen irradiated by soft X-rays and control pollen were compared and difference analysis of data (> 300 pollen was counted respectively) were calculated with Excel 2007.

RESULTS

Morphological comparison between irradiated pollen and control pollen

Under scanning electron microscope (Figure 1), ripe *C. lanatus* pollen is elliptical, with clearly defined irregular germination apertures, and a reticular texture on the exine with a tubercle-shaped substance in the grids. Side-by-side comparison between soft X-ray irradiated pollen and untreated pollen found no obvious differences, indicating that irradiation by soft X-rays does not directly influence pollen exine.

Pollen germination on stigma and growth in style

Soft X-rays were less detrimental to cell activity than hard X-rays but can still directly damage cells. In this study, pictures taken under fluorescence microscope show that both soft X-ray irradiated pollen and control pollen were able to germinate on stigma (Figure 2A and B) and enter the ovary (Figure 2C and D) through the style. Pollen tubes reached the ovule within 2 days, then entered embryo sac (Figure 2E and F), and released spermatoblasts. The results indicated that soft X-ray did not affect the viability of pollen.

Difference analysis of sac and fertilization process

Paraffin sectioning of the watermelon ovaries after pollination allowed observation of the embryo sac and fertilization 1 to 7 days after pollination. No readily apparent difference was observed between pollination by irradiated pollen and control pollen in embryo sac development and the fertilization process (Figure 3A and

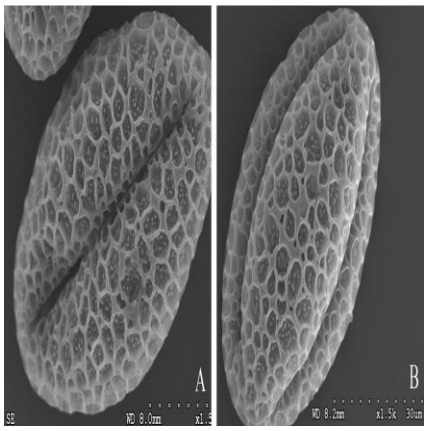

Figure 1. Photos of pollen by scanning electron microscopy. A. Control. B. Soft x-ray irradiated pollen treatment.

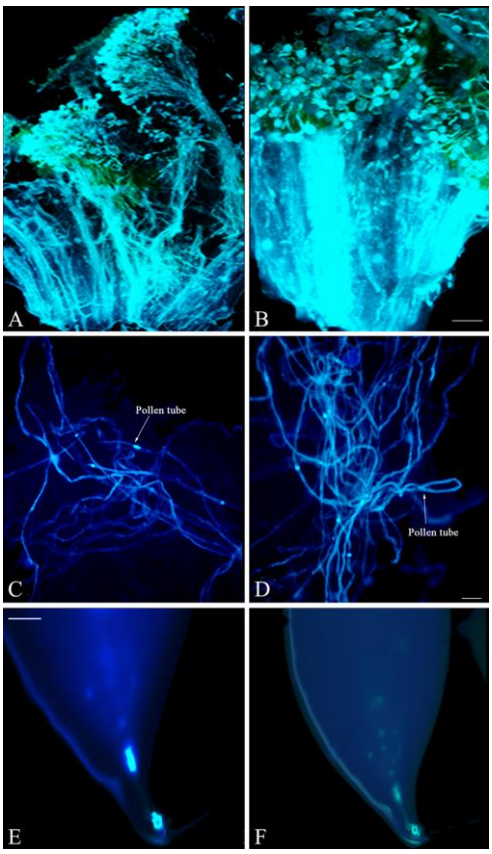

Figure 2. Pollen geminated and grew normally on stigma, entered into ovary, and then entered into embryo from micropyle. Controls are A, C, E. Soft x-ray irradiated pollen treatment is B, D, F. Scale bar is 1 mm in A, B, C, D and 200 µm in E, F.

B). Observation of embryo sac and fertilization 12 days after pollination showed many ovules in embryo sac, but with different levels of development. Formation of the embryo sac could not be observed in most *C. lanatus* pollinated by pollen. Amongst the few ovules that had formed in the embryo sac, the rate of abortion was very high. The embryo sacs were hollow and arrested growth of the ovule nucleolus affects the growth of the embryo sac. A black deteriorating structure was observed in the embryo sac but there was no fixed structure, then the embryo degrades and the ovule of the degrading embryo also does not develop. The endosperm degenerates (Figure 3A and B) and cannot develop into impervious seed of normal size as that of control *C. lanatus* (Figure 4A to C).

Difference comparison of *C. lanatus* pollen chromosome

DAPI can directly penetrate the cell membrane and bind to double-chain DNA in nuclei to show fluorescence over 20 times stronger than DAPI by itself. The fluorescence value of *C. lanatus* pollen chromosomes irradiated by soft X-rays is 107.05 while that of the control is 213.70. This indicates that pollen chromosome structure irradiated by soft X-rays and its DNA structure are damaged (Figure 5).

DISCUSSION

In this study, pollen irradiated by 600 Gy soft X-rays was used to fertilize diploid *C. lanatus* to produce seedless watermelon. No normal seeds were observed in the fruit but some abortive seeds were still present. Seedless watermelon produced by pollination with soft X-ray irradiated pollen were the same as the control with seeds in relation to size, shape, pulp color, skin thickness, and sugar content. In general, the quality is not influenced. Sugar content in some seedless watermelons even tends to be higher (Table 1). Sugiyama and Morishita (2000) fertilized normal diploid *C. lanatus* with irradiated pollen and abortive seeds were formed. This embryological research indicates that double fertilization still occurs after pollination and the embryo develops normally into a globular embryo, but then stops development and degenerates. Ovules of the degenerating embryos also do not develop, and so are unable to become impervious seeds of normal size but instead become abortive seeds that are smaller than normal seeds. Seedless mechanism of a mandarin cultivar 'Wuzishatangju' (*Citrus reticulata* Blanco) indicated that the activities of the male gamete and the fertility of the embryo sac were functioning normally with no embryo abortion during embryonic development. Gametophytic self-incompatibility (SI) caused seedlessness by blocking fertilization in the ovary (Ye et al., 2009). These are consistent with our result from observing *C. lanatus* pulp and the fertilization

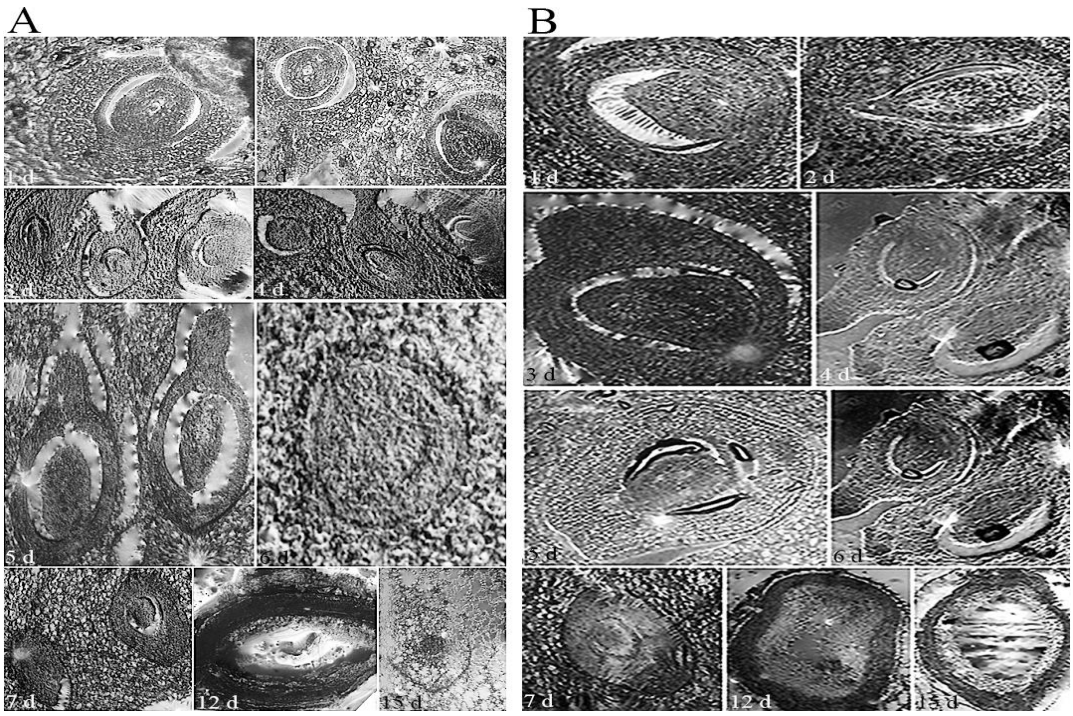

Figure 3. Paraffin sections of the watermelon ovary on days 1 to 15 after pollination. A. Control. B. Soft x-ray irradiated pollen treatment.

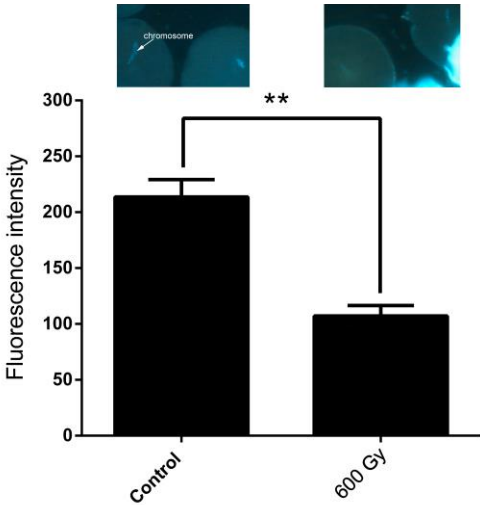

Figure 4. Watermelon which growing 25 days. The control and soft X-ray treatment of pollen at the comparison of the normal seeds of two varieties. Inner pictures were seeds. **, Highly significant (P <0.01).

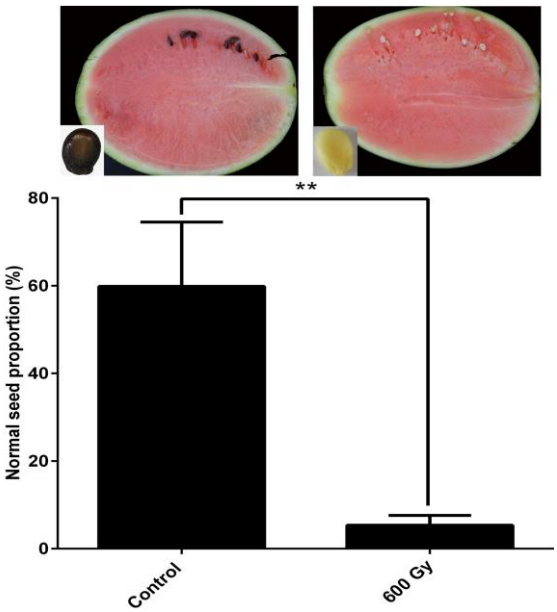

Figure 5. Pollen chromosome fluorescence value comparison. Inset pictures show watermelon pollen chromosomes after DAPI staining. **Highly significant (P <0.01).

process in the current study. Hu et al. (2007) found that *Citrus suavissima* Hort. Tanaka is seedless because pollen mutates during development and becomes inactive so that it cannot normally germinate on stigma. However, the research in this paper shows that *C. lanatus* does not become seedless due to pollen inactivity. Irradiation of *C. lanatus* pollen by soft X-rays damages the DNA double-helix structure of pollen, which can cause death of cells.

Table 1. Compared treatment with control on four main indicators.

Variety	Treatments	Central sugar concentration (%)	Edge sugar concentration (%)	Lycopene (μg/mg)	Citrulline (mg/100 g)
Huaimi	Control	9.93±0.29	8.87±0.33	1088.62±99.76	158.29±9.98
	600 Gy	11.43±0.23	10.07±0.54	1190.13±26.30	183.74±7.89

* All values are means +/- SD (n=18).

However, a low dosage of soft X-rays only slightly damages DNA structure and does not cause pollen death. Nevertheless, the change in DNA double-helix structure may influence chromosome pairing so that *C. lanatus* becomes seedless.

When researching changes in hormone content of ovule and sarcocarp of fertile and abortive Chinese dates, Luo et al. (2000) explored whether abortive embryos before and after stone hardening stage are directly related to hormone concentration in the embryo and sarcocarp. Chinese dates with abortive embryos contain clearly higher IAA, GA_3, and ZT, for example in sarcocarp than embryo in stone. Sarcocarp of seedless fruit contains more hormone than that of fruit with seeds. Thus, the sarcocarp is better able to grow and compete for nutrition than immature embryos, so embryos become abortive and fruits become seedless. According to research conducted by forerunners, the formation of seedless fruit is related to the development process of embryo sac. Nevertheless, it remains to be further researched whether seedless watermelon produced after soft X-ray irradiation is related to abortive embryos caused by differences of hormone content in the sarcocarp and immature embryo.

Conclusion

The research results of this paper show that irradiation of *C. lanatus* pollen by soft X-rays does not influence *C. lanatus* pollen exine, both soft X-rays irradiated pollen and control pollen can germinate on stigma, enter ovary through style, and the pollination and fertilization processes are also normal. However, irradiation of *C. lanatus* pollen by soft X-rays damages DNA double-helix structure of pollen chromosome so that *C. lanatus* embryo sac is blocked during development and the embryo gets aborted halfway. Then the ovules do not develop either, and are unable to become impervious seeds of normal size but become smaller abortive seeds. In this way, seedless watermelon is produced.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agricultural impact on environment and counter measures in Rwanda

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Rapid intensive agriculture often generates serious environmental concerns including soil erosion, water pollution and greenhouses gases. This paper assesses the impact of agriculture and its practices on environment in Rwanda from 1990 to 2012. Data provided by the World Bank were analyzed with Origin Pro 9 for statistical analysis. Also, a review on physical-chemical parameters and heavy metals of water resources home to or surrounded by cultivated mountains was adopted in this study. The results showed that agricultural records decreased from 1990 to 1994. However, after then, the short season cropland like cereals increased from 7.04 to 17.45%; roots and tubers increased from 13.17 to 21.69% in 1995 and 2012, respectively, whilst permanent cropland remained constant at 10.13%. As Rwandan soil is almost steep slope, this heavily exposes the soil to erosion, fertility loss and landslides as permanent crops to enhance fertility and erosion control are decreasing. Also, fertilizers increased from 2,149 to 27,748 tons, irrigation spaced from 4,000 to 10,000 ha which can be the reasons of rise of agricultural emissions. The reviewed studies estimated high concentration of the total nitrogen, total suspended solids, manganese, lead and iron exceeding the standards of the European Union and World Health Organization. From the above findings, it is suggested to regularly monitor water quality and promote its purification measures, to fertilize and irrigate timely and appropriately, expand areas under agroforestry and permanent crops, promote bench terraces practices for durable soil erosion control and water quality in Rwanda.

Key words: Agriculture, environment, Rwanda, soil erosion, water pollution.

INTRODUCTION

Emissions from agriculture, forestry and fishery worldwide, nearly doubled over the last 50 years and could increase at 30% in 2050 mainly being driven by population growth. Increasing size and usage of

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mechanized farm equipment and use of agrochemicals are negatively affecting the environment (Foley et al., 2011; Smith et al., 2008). Land use and land cover change through industrialization, urbanization and agricultural expansion are due to a strong dependence on natural resources (Ademiluyi et al., 2008; Brink and Eva, 2009; Foley et al., 2005). Agriculture is the backbone of Rwandan economy; it contributes 33% of the national GPD and 70% of the country' export revenues. The sector employs 80% of the population and can be subdivided into food crops (grown interchangeably in short period of time) namely cereals, root and tubers, leguminous and banana, cash crops also named permanent crops (coffee, tea and pyrethrum) and new crops introduced for cash/export reasons (fruits, vegetables, flowers and spices). Compared to the total cultivated land, more than 80% is occupied by food crops and approximately 6.3 and 1.6% by Coffee and Tea, respectively (Bizoza, 2014; Murenzi and Hughes, 2006; Ngabitsinze et al., 2011; REMA, 2014). Rwandan population is among the highest in Central and East Africa, as it grew from 2.996 million in 1961 to 11.4583 million in 2012 heading to 25.378 million in 2050 (Havugimana, 2009; NISR, 2012).

High demography with strong reliance on agriculture caused land scarcity, so that the per capita land decreased from 0.95 ha in 1960, 0.25 ha in 2010 leading to 0.10 ha by 2050. Forest cover decreased from 30% in 1930 to 8.9% in 2010 (Habiyaremye et al., 2011). In Rwanda, 16 to 40% of the land is steep slope easily exposed to soil erosion which causes approximately an annual loss of 1.4 million tons of fertile soil. This implies a high nutrients demand, (around 50% of all soils), due to the advanced level of erosion and acidity. Moreover, 63% of the irrigated area is on hillsides mostly depending on rainfall, while in dry season the productivity decreases, due to water insufficiency which finally leads to wetland degradation as the only productive area during dry periods (Giblin and Fuller, 2011; Kagabo et al., 2013; Kannan et al., 2011). All these facts accelerate the rate of fertilization and irrigation and natural resources degradation.

After the 1994 genocide against Tutsis, the country experienced a period of food insecurity due to high population growth rate (3.08 and 5.6% in 1996 and 2000, respectively), plus the war and genocide refugees coming back from neighboring countries. This led to consolidating policies for soil fertility enhancement toward higher agricultural production and caused cropland to increase from 14,850 Km^2 in 1995 to 18,567 Km^2 in 2012; the total arable land expanded from 7,000 to 11.817 Km^2 in 1995 and 2012, respectively. These changes were associated with the use of lime, organic manures, fertilizers and agroforestry, bench terraces and irrigation practices (Ansoms and Rostagno, 2012; Kathiresan, 2012; Rushemuka et al., 2014). Despite of the mechanisms consolidated, high population and its agricultural malpractices revealed natural resources degradation evidences. Previous studies have highlighted some of these efforts like increasing fertilizers application and crop land expansion on unprotected land, to be among the drivers to soil and water quality pollution through accelerating soil erosion and release of the phosphorus, nitrates and ammonia from agrochemicals applied, which in turn, cause water pollution and eutrophication (Hategekimana and Twarabamenya, 2007; Mupenzi et al., 2009; Wronski et al., 2015). These mechanisms merged may help in environmental protection, but on the other hand, under population growth and its rise in food demand, it can be predicted that, the magnitude is likely to increase negatively, if earlier interventions are not made. The objectives of this study are (1) to highlight the recent agricultural practices, (2) determine agricultural impact on land and water resources and (3) suggest future practices for agriculture and environmental sustainability in Rwanda.

MATERIALS AND METHODS

Description of the study area

Rwanda is located in East-central Africa and is bordered by Uganda to the north, Burundi to the south, Democratic Republic of Congo to the west and Tanzania to the east. Rwanda has two rainy seasons; the first starts from March to May and the last begins from October to November with an average rainfall of 110-200 mm per month. The first and short dry season starts from December to the end of February, while the longer one lasts from June to early September. Rwanda's average temperature ranges between 19 to 27°C (Giblin and Fuller, 2011) (Figures 1 and 2).

Data collection and analysis

This study used Statistical data from 1990 to 2012 by the World Bank Group (http://data.worldbank.org/country/rwanda) and encompasses data on the main crops grown (seasonal and permanent crops) and their appropriate land proportion compared to the total agricultural land, fertilizers and irrigation and agriculture and land use emissions. These data were analyzed by Origin Pro 9 for statistical analysis, to demonstrate changes on agriculture and its impact on environment with emphasis on land resources. To determine the impact on water resources, this study adopted the review methodology from previous studies conducted at Lake Muhazi, Akagera Transboundary River, Nyabugogo River, Rweru-Mugesera Wetland, Congo and Nile Basins (Rwandan Sub-catchments) and Kadahokwa Water Treatment Plant (Mupenzi et al., 2009; Nshimiyimana et al., 2010; REMA, 2014; RNRA, 2012; Sekomo et al., 2011; Usanzineza et al., 2011; Uwimana et al., 2010; Wali et al., 2011), where agriculture and human activities were attributed to the changes on physical-chemical parameters

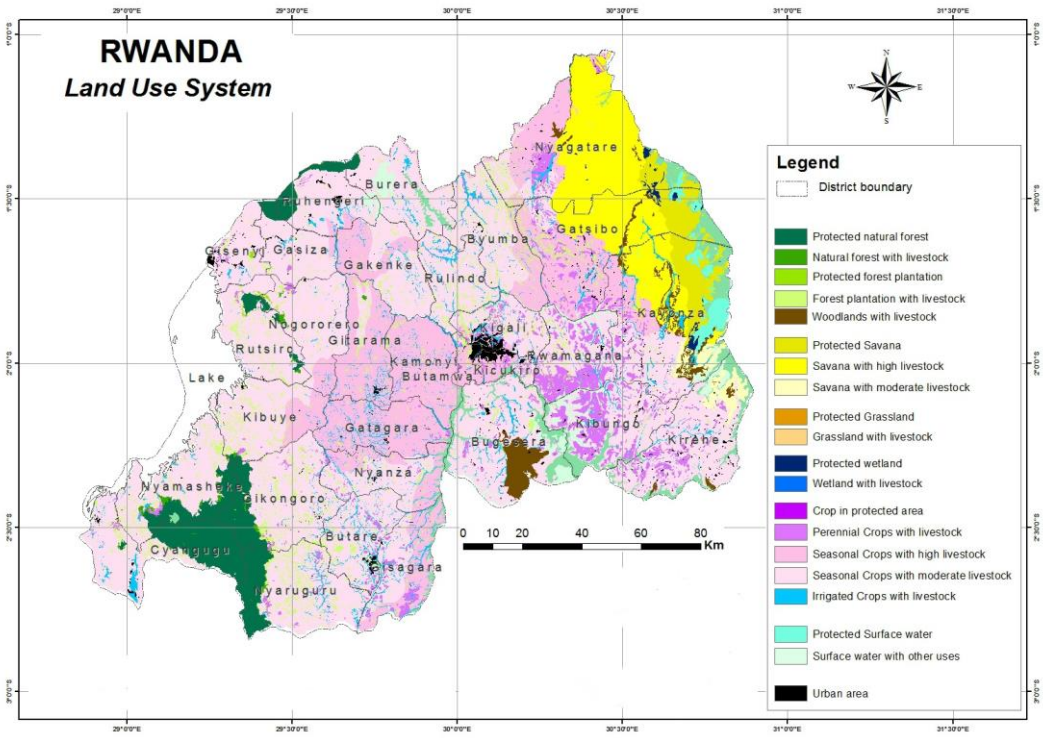

Figure 1. Land use system in Rwanda (Ernest et al., 2010).

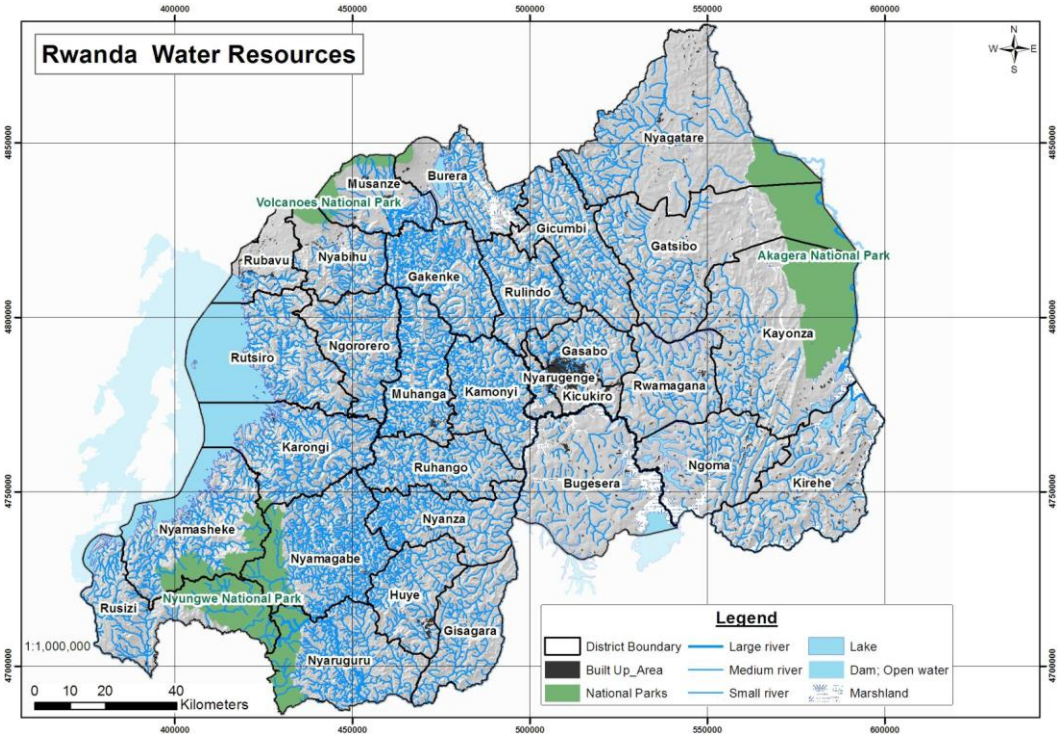

Figure 2. Rwanda's water resources network (REMA, 2014).

and heavy metals of these water resources considered (Tables 2 and 3). For these water quality studies reviewed, we only considered those conducted within the same time range as the present study, not later than 2012. Physical-chemical parameters like potential of hydrogen, total nitrogen, total phosphorus, total suspended solids, turbidity, ammonium-nitrogen, nitrate-nitrogen, dissolved oxygen and heavy metals like copper, zinc, lead, manganese, iron, chromium and cadmium were considered compared with the standards of the European Union (EU) (Wyness et al., 2003) and the World Health Organization (WHO) (WHO, 2004).

RESULTS

Agriculture, land uses and management

The study considered the main crops grown in Rwanda and their land proportion was compared to the total agricultural land as indicated in Figure 3.

Figure 3 shows that, after 1994, the percentage of permanent crops remains constant (10.13%) from 1997 to 2012. While the land area of cereals, roots and tubers, leguminous and other crops considerably increased. These cropland accounts indicate that land is not well used/managed due to lack of permanent crops.

Emissions from agriculture and other land uses in Rwanda

Figure 4 shows that the total land emission increased from 1990 to 2001, after then, the emission reduced until 2012. Contrarily, agricultural total emissions gradually keep on increasing with high marks in 2010 (3,059.01 Gg Co_2eq) (Figure 4).

This increase of agricultural emissions can be a result of expanding seasonal cropland than permanent crops (Figure 3), where, many fertilizers are applied and irrigation for high productivity in a short time, which in turn, can be the reasons for the rise of agricultural emissions. Detailed accounts of the size of Rwandan irrigated area and the use of fertilizers are illustrated in Table 1. It indicates that gradually both the use of fertilizers applied and irrigated area increased.

Agriculture and water quality in Rwanda

In this section, to show the evidences of agricultural impact on water resources, authors reviewed previous studies. In addition, the considered water resources as reported (methodology section), are home to agriculture or surrounded by cultivated mountains, with irrigation practices and fertilizers application which are not appropriately adapted to soil topography (Steep slope). As a consequence, this facilitates the transport of sediments and nutrients that pollute these water bodies (Tables 2 and 3).

DISCUSSSION

Agriculture in Rwanda encountered declined in production earlier and during 1994 Genocide against Tutsis. As a consequence of this instable situation, few people engaged in agriculture. However, five years after, population increased along with its food demand and policies like expanding the cropland area, increasing use of lime, organic manures and fertilizers, irrigation practices for the purpose of high productivity were applied (Booth and Golooba-Mutebi, 2014; Diao et al., 2010). These mechanisms can be the cause of expanding seasonal cropland (Figure 3) with high marked ups in the years of 2000 and 2010 along with increasing fertilizers applied and irrigated areas (Table 1). However, as stated by, Mulatu et al.(2014), Nabahungu, (2012) and Yeo et al. (2011), these land misuses lead to consequences like loss of ecological and socio-economic value of some species and lack of permanent crops to maintain those nutrients in the soil, which in turn, leads to soil infertility and facilitates erosion, which finally, reaches aquatic systems and result in associated pollution and eutrophication processes. It is possible to mention that, this is likely the expected results in Rwanda, if nothing is done to remove the gap between seasonal and permanent crops. The results of this study showed that agricultural emissions are continuously rising, while the total land use emission decreased (Figure 4), which can be a result of the efforts made by the Government of Rwanda on increasing the forest area from 12.89% to 18.62% in 1990 and 2012, respectively (REMA, 2011; WorldBank, 2015). Afforestation and reforestation helped in sequestering the gases emitted by soils, while agricultural land expansion gradually has been increasing its total emission.

However, as it has been reported, intensive agriculture, its increasing inputs (fertilizer and pesticides) and practices like enteric fermentation, irrigation and tillage and mechanization, crop residues burning, lead to emissions of N_2O, CH_4 and CO_2 which in turn, contribute to soil, air and water quality pollution with more effects on poor countries whose adaption measures are not sufficient (Barber and Quinn, 2012; Braune and Xu, 2010; O'Geen et al., 2010). By considering how faster agriculture is expanding in Rwanda, it is possible, that under the expansion of seasonal cropland (Figure 3), the increased forest area

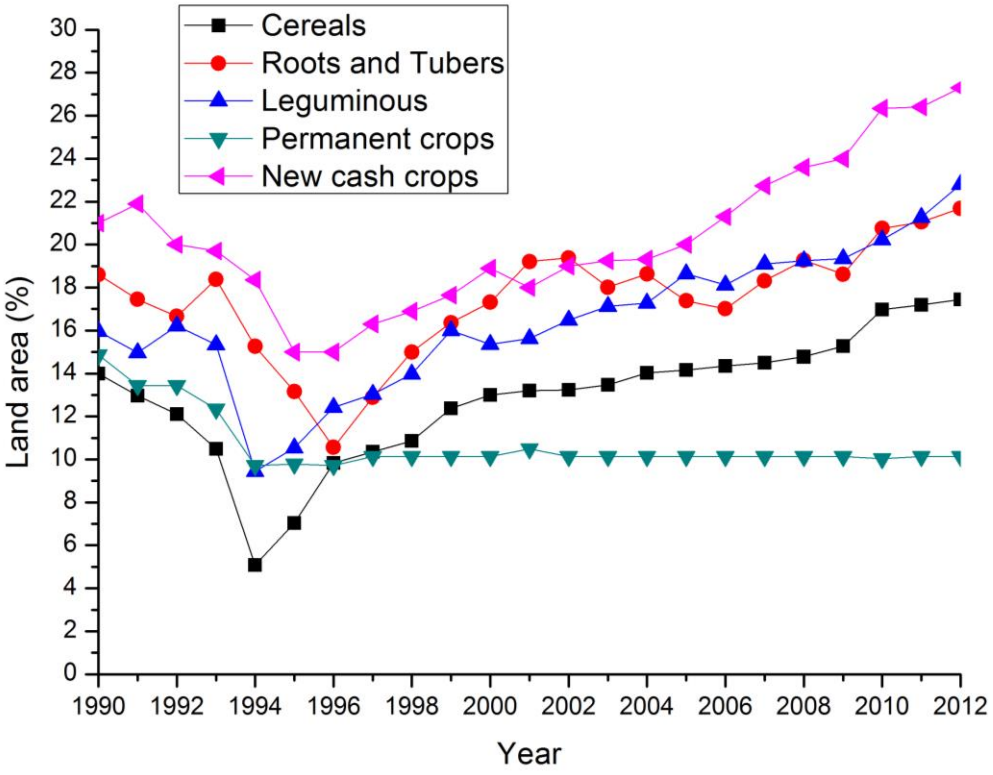

Figure 3. Land Proportion of different crops in Rwanda from 1990 to 2012.

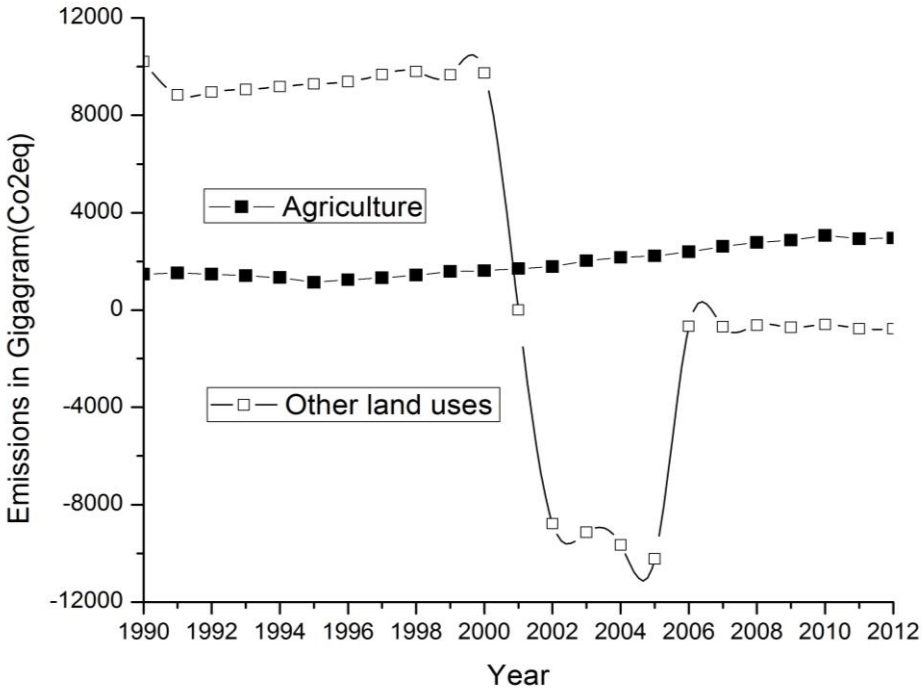

Figure 4. Total agriculture and other land use emissions in Rwanda (1990 to 2012).

Table 1. Irrigated area and use of agricultural fertilizers in Rwanda.

Year	1990	1992	1997	2002	2007	2012
Fertilizers (Tons)	2.149	6.143	3.858.8	5.835	10.989.8	27.748
Irrigated area (ha)	N/A	4.000	7.000	9.000	10.000	10.000

N/A: Not Available and ha: hectare Source: World Bank (2015).

Table 2. Estimated physical-chemical parameters of the reviewed studies.

Places	PH	TN	TP	DO	Turbidity	TSS	NO_3-N	NH_4^-N	Sources
Lake Muhazi	7.8	1.2	0.18	n/s	n/s	n/s	0.489	n/s	Usanzineza et al. (2011)
Rweru-Mugesera wetland	5	3.8	1.73	1.32	n/s	67.91	n/s	n/s	REMA (2014)
Nyabugogo River	5.9	0.14	0.12	4.3	2750	n/s	43	0.54	Sekomo et al. (2011)
Congo Basin (Rwanda)	7.6	n/s	0.38	4.8	4805	920.90	n/s	n/s	RNRA (2012)
Nile Basin (Rwanda)	7.7	0.93	0.20	4.21	780	162.86	n/s	n/s	RNRA (2012)
EU and WHO Standards	6- 8	<3 mg/l	<5 mg/l	5 mg/l	5NTU	<30 mg/l	50 mg/l	0.50 mg/l	WHO (2004) and Wyness et al. (2003)

n/s: not specified, TN: Total Nitrogen, TP: Total Phosphorus, DO: Dissolved Oxygen, TSS: Total Suspended Solids, NO_3-N: Nitrate-Nitrogen, NH_4-N: Ammonium Nitrogen, mg/l: milligram per liter, NTU: Nephelometric Turbidity Unity, EU: European Union and WHO: World Health Organization.

may be undermined for crop land reasons, being associated with increasing fertilizers application and expansion of irrigated areas (Table 1), which in turn, lead to increasing agricultural emissions. This, is in congruent with the reports of Kannan et al. (2011) and Rushemuka et al. (2014), that Rwanda is ahilly and rainy country, where fertilizers are applied and irrigation is practiced on unprotected soil, without great consideration of how much the soil is easily eroded due to agricultural malpractices and its natural topography (steep slope).

In addition, Fidèle et al. (2015) assigned subsistence agricultural malpractices, lack of timely updates and approaches to farmers to be the leading causes of soil erosion and water pollution in Rwanda, as evidenced by the reviewed water resources, where some physical-chemical parameters and heavy metals (Tables 2 and 3) were estimated to be higher than the standards of the European Union (EU) and the World Health Organization (WHO). It is good to reach farmers and invest more on soil erosion control as indicated by Fialho et al. (2013), Mupenzi et al. (2009), that terraces and agroforestry can be a good alternative , which also helps in water quality enhancement, since the rosion which transports sediments and nutrients into water is minimized. For agriculture and environmental sutainability in Rwanda, a developing country high demography with strong reliance on subsistence agriculture, expanding seasonal crops than permanent crops and more inputs, it can be predicted that, much is likely to happen in terms of environmental quality degradation, particularly land and water resources, if intervention policies are not well practiced and strengthened.

Conclusion

This study considered agricultural practices to assess its impact on environment. The results showed that seasonal cropland expanded compared to permanent crops. Fertilizers and irrigation increased with agricultural emissions with pollution impact on both soil and water as the cultivated soil is almost steep slope easily facilitating erosion. For more environmental friendly practices in case like Rwanda, with a rapid growing population only relying on subsistence agriculture, it is suggested to:

(i) Reduce the incidence of fertilizers with more emphasis on organic farming systems.
(ii) Increase the area of permanent crops, agroforestry and bench terraces for soil erosion control and water quality management.
(iii) Transform the sector from household size into group cooperatives to improve its professionalism.
(iv) Promote institutional and technical assistance to improve local farmer's awareness on timely and appropriate fertilizers to apply and irrigation and their impact on natural resources.

Initiation and/or reinforcement of the polluter pay principle and Increase the awareness and share the

Table 3. Estimated heavy metals of the reviewed studies.

Places	Zn	Cr	Cu	Mn	Fe	Cd	Pb	Sources
Lake Muhazi	0.04	0.00	0	0.34	0.75	0.02	0	
Akagera Transboundary River	0.55	0.01	0.41	14.64	0.56	0.96	0.04	Usanzineza et al. (2011), Nshimiyimana et al.(2010) , Sekomo et al. (2011), RNRA (2012)
Nyabugogo River	0.10	n/s	0.02	n/s	n/s	0	n/s	
Congo Basin (Rwanda)	0.05	n/s	0.02	0.08	1.4	n/s	n/s	
Nile Basin (Rwanda)	0.21	n/s	0.02	0.23	1.32	n/s	n/s	RNRA (2012)
Kadahokwa	0.04	0.03	0.04	n/s	n/s	0.01	0.04	Uwimana et al. (2010)
EU and WHO Standards (mg/L)	5	0.05	2	0.05	0.3	0.03	0.01	WHO (2004) and Wyness et al. (2003)

n/s: not specified, Zn: Zinc, Cr: Chromium, Cu: Copper, Mg: Manganese, Fe: Iron, Cd: Cadmium, Pb: Lead, EU: European Union, WHO: World Health Organization and mg/l: milligram per liter. For both Tables 2 and 3, the highlighted Bold values indicate those exceeding the standards set out by the European Union (EU) and the World Health Organization (WHO).

responsibilities on environmental protection between policy makers, those in charge of implementation and the local community.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Chemical properties and fermentation behavior of the composts prepared by three composting methods in Malawi

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Soil fertility improvement is one of the urgent issues in Malawi. Since composting by locally available materials is needed for this purpose, the composts were prepared from maize stalk and cattle dung by using three common methods in Malawi. The composts had high alkalinity and relatively high NO_3^- and K contents. N% of composts was determined from the composition of maize stalk and cattle dung, and is expressed by the equation N% = 0.55 - 0.01 x % maize stalk + 0.03 x % cattle dung (r^2 = 0.37). Maize stalk is rich in acid digestion fiber (ADF) (39.3%), which accounts for its slow decomposition, whereas cattle dung is mainly composed of ash (73.5%), which is rapidly degradable inorganic material. The temperature change during fermentation was indicative of compost maturity. Mature composts were exposed to fermentation temperatures exceeding 50°C for a longer period, and had lower pH and higher EC and available N content. The germination rate of rape seeds measured with compost extract was compatible with the absorption value at 465 nm, indicating that these methods are simple and practical for testing compost maturity. The EC difference between wet and dry composts was also useful for identifying the end of fermentation. Low C/N (23) compost demonstrated rapid fermentation relative to high C/N (40, 60) composts, indicating the importance of adjusting C/N in compost fermentation.

Key words: Compost, fermentation, acid digestion fiber, maturity.

INTRODUCTION

The low soil fertility in Malawi is partly attributable to poor crop residue management. An improvement of soil fertility status through organic matter application therefore has been demonstrated (Snapp et al., 1998; Kanyama-Phiri, 2005; Vanlauwe et al., 2015). However organic matter based technologies including compost application despite the fact that compost application is promising for enhancing soil fertility, have remained few (Kumwenda et

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al., 1997).

In 2012, Japan International Cooperation Agency (JICA) conducted a baseline survey of crop residue utilization in the northern districts of Malawi and found that 52.1% of 424 farmers were not utilizing maize residues (70 farmers practiced burning and 151 farmers left the residues in their farms). To confront this situation, the Department of Land Resource Conservation in Malawi has been encouraging compost production and use. The interest in composting has greatly increased as a result of the need for environmentally acceptable animal waste treatment technologies and also the demand for organic fertilizers in organic agricultural production (Sabiiti, 2011),

Simple composting technologies, such as pit composting, are practiced by smallholder farmers in Malawi. However, composting is not commonly practiced due to several reasons, including the lack of knowledge (Mustafa-Msukwa et al., 2011) and difficulty of acquiring materials. Many farmers in Malawi find it difficult to secure compost materials even though maize residue is widely available as maize accounts for approximately 60% of the cropped area (Kumwenda et al., 1997). Therefore, compost making with maize residue will be most feasible in Malawi. However, composts vary considerably in terms of physical and chemical characteristics, which are influenced by material composition and maturity (Integrated Waste Management Board, 2002; Gigliotti et al., 2005). To increase compost use among smallholders, good-quality compost obtainable by advanced maturity techniques is sought.

Compost maturity is primarily governed by compost material and fermentation. A number of maturity and stability indicators have been proposed, including C/N ratio, microbial activity, germination index, cation exchange capacity (CEC), humic substance, dissolved organic matter, NH_4^+ and NO_3^-, and NH_4^+/NO_3^- ratio (Harada and Inoko, 1980; Zucconi et al., 1981; Iglesia and Perez, 1992; Hue and Liu, 1995; Bernal et al., 1998; Paredes et al., 2000; Eggen and Vethe, 2001; Benito et al., 2003; Smith and Hughes, 2004; Goyal et al., 2005; Tang et al., 2006; Pullicino et al., 2007).

Although, many maturity indicators have been proposed, no single maturity indicator can be applied universally as compost materials are so diverse. Therefore, it is necessary to determine the suitable compost maturity indicator for each type of compost. The Sustainable Land Management Promotion Project (SLMP), a collaborative effort between Malawi and Japan, was implemented in November 2011 with the aim of promoting sustainable land management technologies among smallholder farmers in the country. Its objective was to improve soil fertility through compost application. Different types of composts were produced primarily from maize residue. This study was carried out with the following objectives in mind: 1) to characterize compost materials and compost in terms of chemical fertility; 2) to examine fermentation rate by monitoring temperature change, and 3) to identify adequate maturity parameters for use in the field.

MATERIALS AND METHODS

Study site

Compost making was carried out at Mkondezi (MKD) Research Station of the Department of Agricultural Research Services (DARS). MKD is located 11°36'S, 34°18'E at the altitude of 471 m. Mean annual rainfall in MKD from 2002 to 2013 was 1,439 mm. The annual average temperature is 23.5°C.

Compost preparation

Composts were made with three methods (Changu, Windrow and Bokasi) (NRAES, 1992; Japan International Cooperation Agency, 2005; Nalivata, 2007) and environments (shade, plastic and open) (Figure 1). There is no uniform preparation procedure in these three method and varies a lot depending on a place where composting is carried out. Materials used for Changu and Windrow in this study were maize stalk, cattle manure, ash and virgin soil. In addition to these, grass was added for Bokasi. The effects of legume residue (soybean) were also examined by comparing composts with legume addition and no-addition composts. In Changu, the materials were piled up and shaped into cones and were turned regularly during preparation. In Windrow, the materials were layered to form long narrow piles. In Bokasi, the materials were used in small amounts for compost making in a short period (NRAES, 1992).

Compost material and compost analysis

The chemical analysis of compost and compost materials, namely, maize stalk, cattle dung, virgin soil, and soybean residue, was carried out at Lunyangwa Station, DARS. pH, Na and electrical conductivity (EC) were measured after shaking fresh compost samples for 1 h at the soil-water ratio of 1:5. K obtained by NH_4Ac extraction and NO_3^- and Na obtained by water extraction were measured with ion meters (HORIBA LAQUAtwin, models B-731 for K, B-741 for NO_3^-, and B-722 for Na). Available P and S were determined with a spectrophotometer (Bellstone WSP-UV800A) using extracts obtained with Mehlich III (Mehlich, 1984). Total C and N contents were determined by the Walkley-Black method (Walkley and Black, 1934) and by the micro Kjeldahl method (AOAC 1995), respectively. Available N was measured by the boiling decoction method (Yamaki, 2008).

Fermentation involves organic matter decomposition by microbial activity. Therefore, the quality of organic matter greatly affects the decomposition process. Decomposability varies depending on the kind of material; for example, glucose, sugar and protein are easy to decompose, whereas cellulose, lignin, etc. are refractive and need a long time for decomposition. In order to examine the decomposability of compost materials, insoluble lignin and cellulose contents in the compost materials were determined by the Van Soest method (Van Soest, 1996).

Temperature change during fermentation

Temperature changes during composting reflect the fermentation condition and are related to compost quality. Temperature changes were monitored for two consecutive years from 2013 through 2014, from the start of compost making until the time the temperature

Figure 1. Three composting methods (Changu, Windrow, Bokasi) and three environments (shade, open and plastic).

showed no more changes even after turning. Measurement was conducted two times a day at 8 a.m. and 2 p.m., but only data of 8 a.m. were used for analysis as the temperature changes at both times were identical.

Compost maturity

A number of parameters were used to assess compost maturity, including color, odor, shape, water content, pH, EC, K, PO_3^-, N, C/N, germination rate and NH_4^+. Composts were extracted with hot water and the extracts were used in the germination test of rape seeds. The extracts were also used for absorption measurements with a spectrophotometer. Compost maturity indices were determined at the end of fermentation. Ten grams of fresh compost samples was diluted with 100 mL of boiled water and the mixture was filtered after one hour. Absorption of the filtrate was measured at 465 nm with the spectrophotometer. A germination test was conducted using the same filtrate on a Petri dish containing 50 rape seeds on filter paper.

As the C/N ratio of compost material contributes to compost maturity (Guo et al., 2012), three C/N ratios (23, 40, 60) were prepared by changing the mixture ratio of maize residue, rice bran, cow dung to test their effects on fermentation. The difference in ECs between fresh and dry composts is correlated with NH_4^+ generated during fermentation (Yamada et al., 2012). As fermentation proceeds, the difference in ECs becomes smaller due to reduced NH_4^+ generation, and this could be used as a simple indicator of compost maturity. After EC of fresh compost sample was measured, the sample was dried at 105°C and dry EC was measured.

Statistical analysis

Statistical analysis was conducted using JMP 8.0.2 version for Windows (SAS Inc., 2009). Correlation analysis was conducted for the chemical characteristics of composts and the fermentation indices. The Tukey-Kramer HSD test was performed for the F-test at the significance level of either 0.1 or 0.5.

RESULTS AND DISCUSSION

Material composition of compost

Table 1 shows the amounts of compost materials used to make one heap each of the three types of composts. A large amount of compost materials were used to make one heap of Windrow, whereas small amounts were used for Changu and Bokasi. Maize stalk and cattle dung contents varied among the three types of composts. Bokasi had much lower cattle dung content than Windrow or Changu, whereas maize stalk content was almost the

Table 1. Amounts of compost materials (kg) to make one heap each of three types of composts (2014).

Material (kg)	Changu		Windrow		Bokasi	
	+ L	- L	+ L	- L	+ L	- L
Maize stalk	40	152	200	480	32	32
Cattle dung	114	114	228	115	10	10
Ash	2.5	11	7	3	20	20
Virgin soil	15	15	50	50	50	50
Soybean residue	18		175		28	
Grass					33	33
Total amount (kg)	189	292	660	648	173	145
Maize stalk (%)	21.1	52.1	30.3	74.1	18.5	22.1
Cattle dung (%)	60.2	39.0	34.5	17.7	5.8	6.9

+ L – with legume, - L – no legume

Table 2. Chemical composition of compost materials.

Material	pH	EC	K^+	NO_3^-	Na^+	C	N
		µS/cm	mg/l	mg/l	mg/l	%	%
Maize stalk	7.5	119	38	58	400	14.4	0.06
Cattle dung	8.7	247	68	160	210	0.15	0.23
Virgin soil	6.6	53	7	13	100	0.12	0.025
Soy bean residue	7.4	234	73	63	590	11.6	0.15

same regardless of compost type.

Chemical characteristics of compost materials

General chemical characteristics

Maize stalk pH is 7.5 and cattle dung pH, 8.7 (Table 2). The high pH of cattle dung originates from the high salt content, as shown by the EC value. Maize stalk has an average N content of 0.06%. As NO3- content in cattle dung is almost 20 times its content in virgin soil, compost made of materials containing cattle dung would improve soil chemical fertility. As a general characteristic of plant materials, K and S contents are also high. As K content in Malawi soil is extremely low (Mueller et al., 1993;

Gwosdz et al., 1996; Chilimba and Liwimbi, 2008), the addition of K by compost application would improve soil fertility.

P content in compost materials is low. More than 20 ppm would be necessary for maize growth (Staton, 2014). As all the compost materials have less than 10 ppm P content, P provision by another source would be necessary to achieve sustainable maize yield.

Characterization of compost materials by decomposability

As for organic constituents, not only element composition, but also decomposability should be examined because the fate of decomposition governs nutrient status and soil organic matter contents. Organic matter is composed of labile and refractive fractions according to decomposability (Figure 2). From the point of view of agriculture, labile organic matter stimulates microbial activity, which in turn activates the N cycle in soil. However, its rapid decomposition affects plant growth, leading to N deficiency, which is a common phenomenon when an excess amount of N-rich materials are incorporated into soils. Cattle dung is abundant in inorganic matter, its ash content being 73.5% (Table 3), and the inorganic matter is promptly absorbed by plants.

Refractive organic matter remains in soil for a long time and contributes to the formation of soil aggregates. Due to the presence of refractive organic matter, effective carbon sequestration in soil can be expected. In this regard, the evaluation of compost material decomposability is important. Acid detergent soluble organic matter is a promising indicator of labile organic matter. Portions that are insoluble in acid detergent are called acid digestion fiber (ADF). ADF contains lignin, which is reported to remain in soil for three years. Maize stalk is composed of 39.3% ADF and 54.3% acid-soluble organic matter (Table 3). On the other hand, maize bran contains a small amount of ADF (2.2%) and over 70% acid-soluble organic matter. The less degradable nature of maize stalk than maize bran will influence fermentation behavior if an excess amount of maize stalk is used for compost

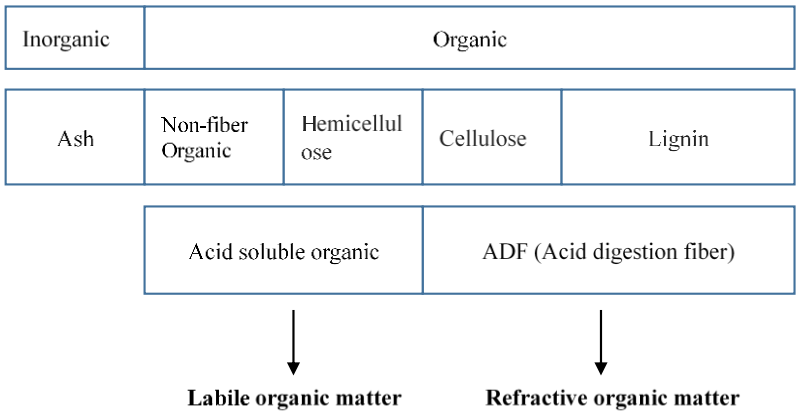

Figure 2. Inorganic and organic constituents in composts and lignocellulosic fibers in organic constituents.

Table 3. Composition of ash, acid-soluble organic matter and ADF in compost materials.

	Ash (%)	Acid-soluble organic (%)	ADF (%)
Maize stalk	6.4	54.3	39.3
Catttle dung	73.5	15.1	11.5
Maize bran	20.3	77.5	2.2

Table 4. Chemical properties of prepared composts (n = 2).

Method	Environment	pH	EC (μS/cm)	C (%)	N (%)	NO_3 (mg/l)	P (ppm)	K (mg/L)	Na (mg/l)	S (ppm)
Changu	Open	8.53	241.6	6.18	0.54	286	6.67	270	534	23.2
	Plastic	8.86	245.0	5.73	0.64	427	7.93	400	607	29.6
	Shade	9.45	168.5	5.97	0.80	625	4.26	325	580	17.9
Windrow	Open	9.11	157.0	3.16	0.53	440	3.38	415	430	20.3
	Plastic	9.37	184.0	6.81	0.47	585	4.04	285	345	22.3
	Shade	9.29	161.0	4.29	0.40	590	3.41	350	560	23.4
Bokasi	Open	9.40	133.5	6.69	0.43	213	5.87	440	375	13.1
	Plastic	9.32	151.5	1.14	0.46	490	8.18	380	495	19.6
	Shade	9.42	184.0	5.15	0.28	550	1.69	315	500	21.8

making.

Compost analysis

The low pH and the high EC in Changu Open/Plastic indicated advanced fermentation (Table 4). In the decomposition (oxidation) of compost materials having a large amount of salts, proton was consumed, resulting in a low pH.

N contents differed according to the compost making method. Changu and Windrow had almost the same N contents, whereas Bokasi showed lower mean N contents (Table 4). N% of composts is influenced by the contents of maize stalk and cattle dung. A larger amount of cattle dung and a smaller amount of maize stalk in Changu and Windrow resulted in more N-rich composts. From 70 compost samples, the following equation was formulated to estimate N% of compost.

N% = 0.55 - 0.01 x % maize stalk + 0.03 x % cattle dung (r^2 = 0.37)

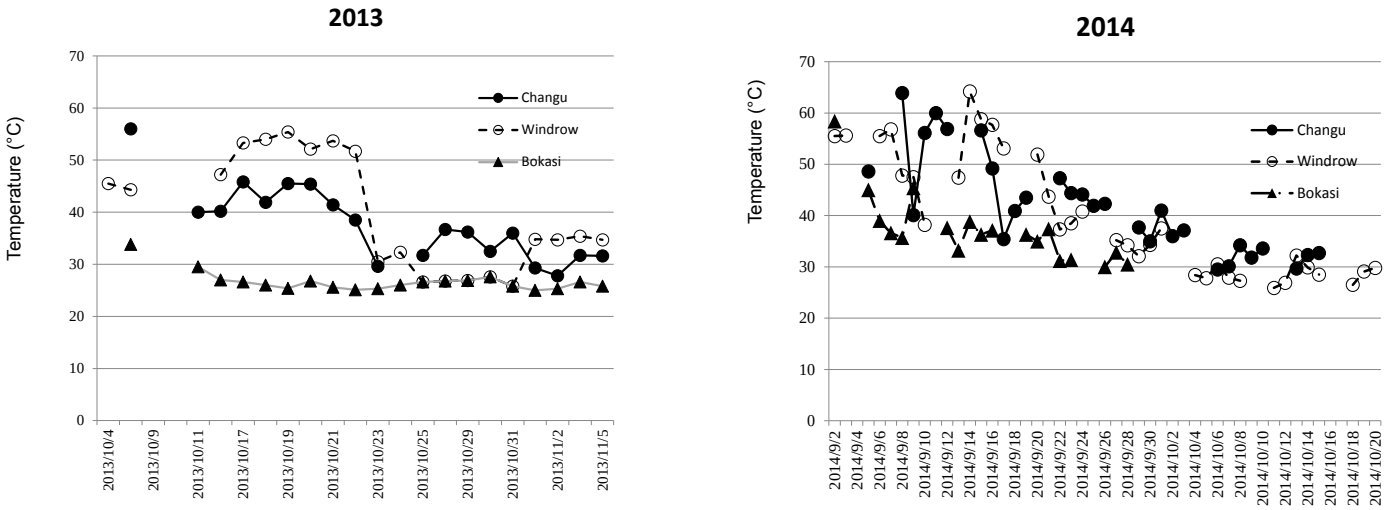

Figure 3. Changes in fermentation temperature of Changu, Windrow and Bokasi in Shade environment in 2013 (left) and 2014 (right).

K content in the Open environment was higher for Bokasi than Changu, whereas K content in plastic was low except for Changu. The results indicate that the increase in temperature brought about by plastic accelerated fermentation and more K was released by fermentation and lost by leaching.

NO3- content was highest in Shade and lowest in Open, indicating that NO3- is easily leached in the open environment. However, NO3- is relatively well preserved in plastic.

C% increased in the order of Windrow, Changu and Bokasi, which corresponded to % maize stalk in compost material (Table 1). The low C% for Bokasi + Plastic was a consequence of a high rate of decomposition. P content differed among the three methods: Changu showed the highest P values, with Bokasi being second and Windrow third.

Temperature changes during fermentation

Temperature monitoring was performed to observe fermentation behavior in the three types of composts. In 2013, the fermentation patterns of Changu and Windrow were almost the same, whereas no fermentation took place in Bokasi as indicated by the lack of temperature rise (Figure 3). The fermentation rate increased in the order of Bokasi, Changu and Windrow, and available N also increased in this order (Figure 5). Windrow was made with the largest amount of compost materials. Because of its large weight, more heat was stored inside the heap, which promoted fermentation.

Pathogenic bacteria, such as fecal coliform bacteria, died rapidly when cow dung temperature exceeded 50°C (Gong, 2007) and weed seeds lost their germination capacity at temperatures higher than 50°C (Feed Innovation Services BV, 2013). Therefore, the number of days with temperatures exceeding 50°C can be used as a key indicator of fermentation.

The number of days with temperatures higher than 50°C was significantly correlated with pH and EC (Table 5). As fermentation proceeds, pH decreases and EC increases. The pH decrease is attributable to the shift from NH_4^+ to NO_3^-, and the EC increase is due to increases in K, Na, Cl and NO_3^- contents during fermentation.

Fermentation temperatures of Bokasi were highest in plastic, followed by open and shade (Figure 4). Plastic promoted the fermentation of Bokasi by maintaining heat inside the compost heap. However, the temperature change in plastic was not markedly different from that in open for the case of Changu. The effects of the environment differed depending on the method, possibly because of the different total volumes and compositions of compost materials (Table 1).

Fermentation rate

The number of days where temperatures exceeded 50°C was related to the fermentation rate. Changu had the highest fermentation rate; it recorded more than 10 days where temperatures exceeded 50°C, whereas Bokasi recorded merely 2 days of over 50°C (Figure 6). Legume addition promoted fermentation and consequently, the number of days where temperatures exceeded 50°C was increased. pH was increased due to the accelerated fermentation by legume addition, but the contents of other nutrients did not change.

The legume effect on fermentation was significant in Changu and Windrow, but not in Bokasi (Figure 6), however there was no significant difference among the

Table 5. Correlation matrix of compost chemical characteristics and fermentation rate.

	Days >50°C	pH	EC	K	NO^{3+}	Na	TC	P	S	TN	C/N
pH	-0.57**										
EC	0.48**	-0.71**									
K	-0.41	0.22	0.09								
NO^{3+}	-0.06	0.19	0.11	0.14							
Na	-0.01	0.08	0.24	0.19	0.20						
TC	-0.18	0.15	0.15	0.09	-0.26	0.16					
P	0.38	-0.44**	0.41**	-0.01	-0.29	0.00	-0.07				
S	0.41	-0.04	0.30	0.05	0.04	0.20	0.07	0.13			
TN	0.01	-0.17	0.34	0.13	-0.04	0.39	0.35	0.16	0.15		
C/N	-0.18	0.28	-0.12	-0.09	0.04	-0.04	0.55**	-0.42**	-0.16	-0.46**	
Av. N	0.51**	-0.47**	0.38	-0.27	-0.31	-0.12	0.08	0.28	0.21	-0.03	0.06

* and ** indicate 1 and 5% level of significance, respectively.

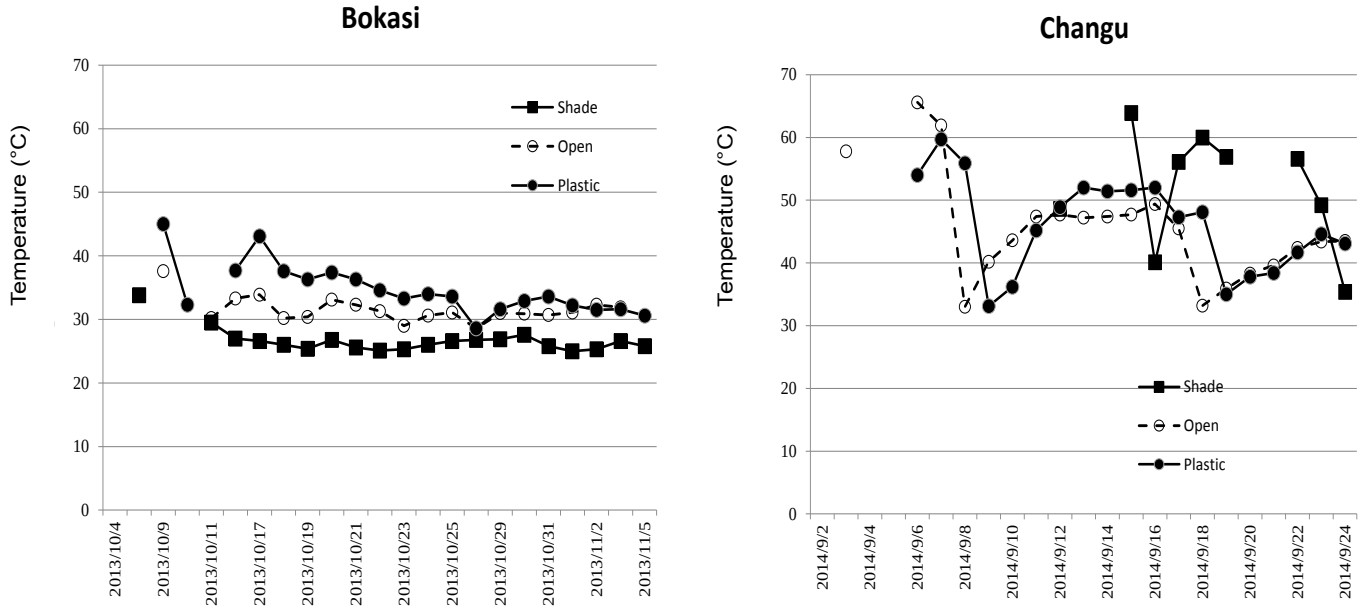

Figure 4. Changes in fermentation temperature in shade, open and plastic environments of Bokasi (left) and Changu (right) in 2014.

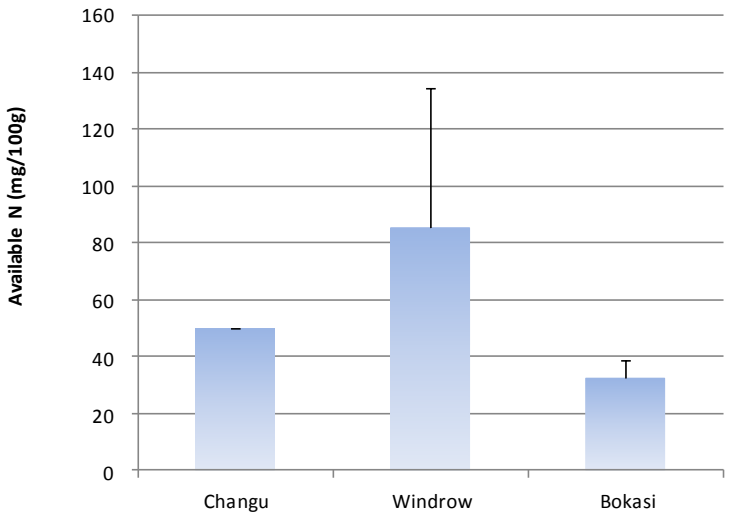

Figure 5. Available N in Changu, Windrow and Bokasi prepared in 2013 (n=3). Error bars indicate standard deviation.

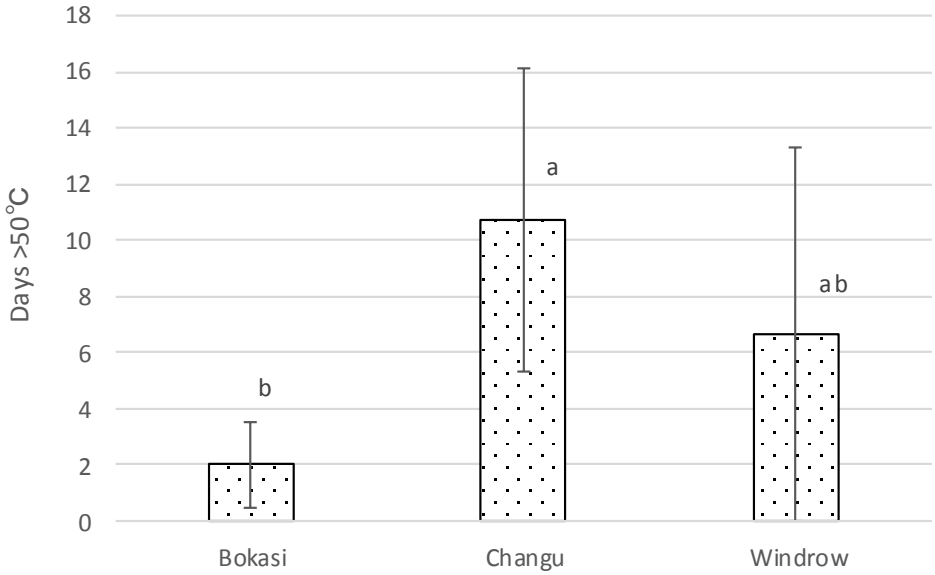

Figure 6. Fermentation temperature difference among the three systems (n=6 in Bokasi and Windrow, n=11 in Changu). Error bars indicate standard deviation and different letters show statistically significant difference between the methods.

environments (open, plastic and Changu), indicating that the environment had little influence on the fermentation.

Compost maturity

The germination test revealed different maturity levels among the three methods (Figure 7). The germination rate was 40% with distilled water, but was increased when the extracts of composts were used. Germination rates increased in the order of Bokasi, Changu and Windrow, and corresponded to the order of available N production (Figure 5). Absorption values of the extracts of composts measured at 465 nm were correlated with the germination rate, as expressed by the following equation.

Germination rate (%) = 9.36 x absorption value (465 nm) + 47.1 (r^2 = 0.38)

Both germination rate and absorption value would be a

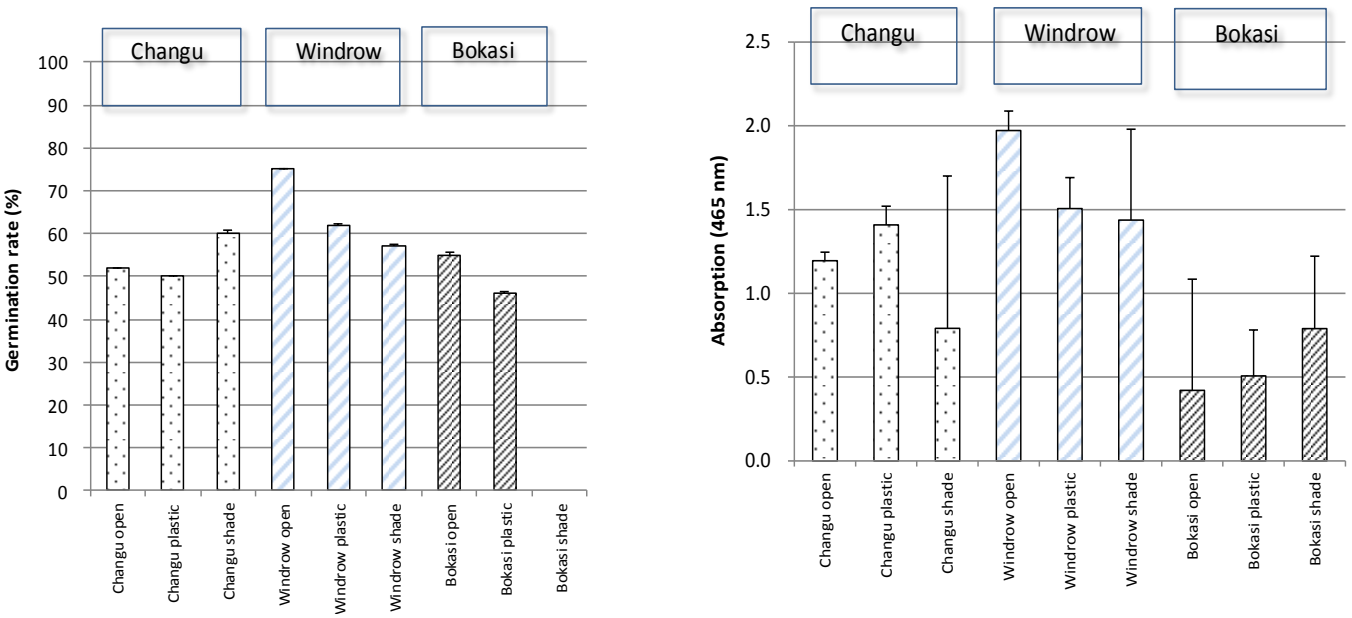

Figure 7. Germination rates and absorption values of composts. Error bars indicate standard deviation.

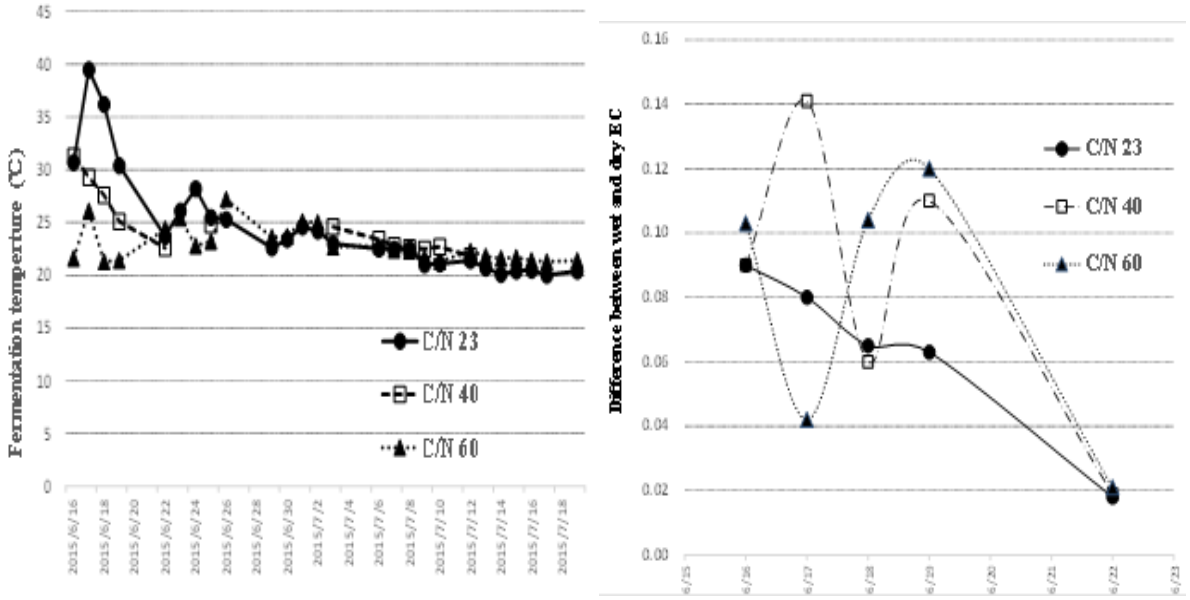

Figure 8. Changes in fermentation temperature (left) and difference between wet EC and dry EC (right) at different C/N ratios in composts.

practical indicator of compost maturity. For the determination of absorption value, a spectrophotometer is necessary, which is useful for handling a large number of compost samples.

Composts having various C/N ratios were prepared by changing the mixture ratio of maize stalk and cattle dung. C/N 23 compost showed a large temperature rise when compared with C/N 40 and C/N 60 composts (Figure 8). Fermentation temperature increased rapidly in the first week but became stable thereafter in C/N 23 compost. The temperature stability was brought about by the rapid decomposition of labile (easily degradable) substrates.

The difference between wet EC and dry EC was reduced as fermentation proceeded because NH_3 generated by compost fermentation was diminished. Fermentation was completed in one month because the difference between

the two EC values became nearly zero. EC differences in C/N 23 steadily decreased whereas the decrease was rather irregular in C/N 40 and C/N 60. The results indicate that C/N adjustment is important for compost maturity and fermentation at the initial stage.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Impact of natural toxin spinetoram 12 SC w/v (11.7 w/w) against *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben - Petersen) under laboratory conditions

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Insecticides are unavoidable in pest management programs especially when the pest crosses Economic Threshold Level (ETL). Nevertheless, often the plant protection products kill the natural enemy population making the pest to resurge and thus demanding more sprays. Therefore, insecticides used in integrated pest management (IPM) programs should be selective enough to spare the beneficial. Laboratory experiments were conducted with spinetoram 12 SC at 36, 45, 54 and 108 g a.i./ha; and various standard chemicals were used to assess the toxicity of spinetoram 12 SC to the egg parasitoid, *Trichogramma chilonis* and predatory green lacewing, *Chrysoperla zastrowi sillemi* during January 2013. In the present study, spinetoram 12 SC did not show harmful effects on *T. chilonis*. The results of the safety test experiment for *T. chilonis* on adult emergence and percent parasitization revealed that spinetoram 12 SC at 36, 45, 54 and 108 g a.i./ha had little adverse effect when treated at egg, larval and pupal stages. Treatment of parasitized eggs with spinetoram did not cause any ill effects to the developing parasitoids, adult emergence and emerged adults. The lowest egg mortality and highest egg hatchability of *C. zastrowi sillemi* was recorded by spinetoram at 36 g a.i./ha which was on par with spinetoram at 45 g a.i/ha. Effect of spinetoram on adult longevity and fecundity of *C. zastrowi sillemi* revealed that the adult longevity was the longest and number of eggs laid per five female was also more in untreated check (14.70 days 366.40 eggs), while it was 11.37, 9.40, 8.27 and 7.67 days and 145.97, 133.40, 105.67 and 93.70 eggs in spinetoram at 36, 45, 54 and 108 g a.i./ha, respectively.

Key words: Spinetoram 12 SC, *Trichogramma chilonis, Chrysoperla zastrowi sillemi,* safety.

INTRODUCTION

Insecticides because of their promising attributes, such as immediate kill, ease in availability and use, occupy a prominent place in integrated pest management. Insecticides often interfere with the activity of parasitoids

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and predators in the cropping ecosystem. Destruction of natural enemies of the insect pests due to excess use of pesticides is a major concern for entomologists as well as ecologists. Indiscriminate use of persistent insecticides disrupted the natural balance of pests and their natural enemies leading to pest outbreaks and resurgence (Geethalaxmi and Chandrasekaran, 2000). So understanding the toxicity of insecticides to natural enemies is important and relevant to develop a sound pest management programme.

Trichogramma chilonis Ishii is one such parasitoid highly useful in pest management especially of lepidopteran insects which causes a severe damage almost in all crops. *C. zastrowi sillemi* is a predator which highly useful in pest management especially of aphids. The larva of *C. zastrowi sillemi* (aphid lion) has relatively a broad range of prey acceptance which includes aphids, whiteflies, eggs of moths and other soft-bodied insects. Due to the polyphagous and voracious nature and vast geographical distribution, ease of mass multiplication and tolerance to some pesticides it has received much attention of farmers as well as researchers as a potential biological pest control agent.

Effectiveness of *C. zastrowi sillemi* as biological control agent has been demonstrated in field crops, orchards and in green houses and reported to give about 100% lepidopteran pest control when used along with *Trichogramma* spp. (Rincon, 1999). Inspite of all these benefits, *C. zastrowi sillemi* with many other beneficials has almost been eliminated from fields due to frequent use of some non-selective agrochemicals (Nasreen et al., 2005).

Spinetoram 12 SC is a new green insecticide fermented from microorganism, *Saccharapolyspora spinosa* and it belongs to the new chemical class of spinosyn compound. Spinetoram 12 SC has been reported as effective biological insecticide for the management of lepidopteran pests of chilli (Dharne and Bagde, 2011) and tomato (Visnupriya et al., 2013). However, hitherto research on natural enemies is limited. As a part of biointensive approach to the pest management, the safety of spinetoram in pest control needs to be evaluated. Considering the above aspects, present investigations were carried out to study the effect of spinetoram on parasitization and parasitoid emergence percentage of *T. chilonis* and effect of spinetoram on eggs, grubs and adults of *C. zastrowi sillemi* under laboratory conditions.

MATERIALS AND METHODS

Laboratory experiments were conducted with spinetoram 12 SC at 36, 45, 54 and 108 g a.i./ha; emamectin benzoate 5 SC 8.5 g a.i./ha; chlorpyriphos 20 EC 200 g a.i./ha; quinalphos 25 EC 200 g a.i./ha; indoxacarb 14.5 SC 75 g a.i./ha; novaluron 10 EC 75 g a.i./ha; and untreated control to assess the toxicity of spinetoram 12 SC to the egg parasitoid, *T. chilonis* and predatory green lacewing, *C. zastrowi sillemi*. For obtaining different doses, 0.6, 0.75, 0.9 and 1.8 ml of spinetoram 12 SC; 0.34 g of emamectin benzoate 5 SG; 2 ml of chlorpyripos 20 EC; 1.6 ml of quinalphos 25 EC; 1.04 ml of indoxacarb 14.5 SC; and 1.5 ml of novaluron 10 EC were dissolved in one litre of distilled water and these dilutions were used for toxicity experiments.

Impact of spinetoram 12 SC on immature stages of *T. chilonis*

The bioassay method described by Jalali and Singh (1997) was adopted with modifications. The eggs of *C. cephalonica* were pasted on paper cards of 21×30 cm size having thirty 7 × 2 cm rectangles. These egg cards were placed in plastic bags along with the nucleus card at 6:1 ratio for parasitization. The parasitized egg cards were cut into one centimeter square bits and for treating egg stage, two days old hundred percent parasitized eggs (eggs appearing black and plump) were sprayed with different concentrations of insecticides using an atomizer. For untreated check, distilled water was sprayed. The treated egg cards were shade dried for 10 min and then kept in a test tube of 10 × 1.5 cm size. In the same way larval (4 days old) and pupal (6 days old) stages of *T. chilonis* were treated. The number of parasitoids that emerged from each treatment was recorded after 24 and 48 h of treatment and percent emergence was worked out.

Fresh eggs were provided to these parasitoids at 6:1 ratio and the numbers of parasitized eggs were recorded after 24 and 48 h of treatment and the percent parasitization was worked out.

Impact of spinetoram 12 SC on *C. zastrowi sillemi*

Impact of spinetoram 12 SC on eggs of C. zastrowi sillemi

Laboratory studies were conducted to assess the effect of spinetoram on the eggs of *C. zastrowi sillemi*, as per the method described by Krishnamoorthy (1985). The eggs along with stalk collected on brown paper strips were sprayed with different insecticides viz., 0.6, 0.75, 0.9 and 1.8 ml of spinetoram 12 SC; 0.34 g of emamectin benzoate 5 SG; 2 ml of chlorpyripos 20 EC; 1.6 ml of quinalphos 25 EC; 1.04 ml of indoxacarb 14.5 SC; and 1.5 ml of novaluron 10 EC using an atomizer. Each treatment was replicated three times with 50 eggs per treatment. Untreated check was maintained by spraying distilled water. The number of grubs hatching from each treatment was recorded and percent hatchability was worked out.

Impact of spinetoram on grubs of *C. zastrowi sillemi*

Larval feeding method

Eggs of *C. cephalonica* were exposed to UV radiation of 15 W capacity for 15 min to kill the embryo and then sprayed with different concentrations of the insecticides with an atomizer. The treated eggs were shade dried for 15 min and then transferred to test tubes (one cc egg card/test tube of 2.0 × 15 cm size). In the control, the eggs were sprayed with distilled water. Second instar grubs of *C. zastrowi sillemi* were transferred to these test tubes at the rate of 10 per test tube. After complete feeding of the treated eggs, the grubs were provided with untreated *C. cephalonica* eggs until pupation. Observations were made on the grub mortality (12, 24 and 48 h after treatment), pupation and adult emergence (Suganyakanna, 2006).

Dry film method

The bioassay method described by McCutchen and Plapp (1988) was adopted with modifications. Glass vials of 20 ml capacity with 1 mm thickness were evenly coated with one milliliter of acetone

Table 1. Impact of spinetoram 12 SC on immature stages of egg parasitoid, *Trichogramma chilonis* Ishii on the adult emergence and parasitization.

Treatments	Doses (g a.i/ha)	Egg (2 days old egg)		Larval (4 days old egg)		Pupal (6 days old egg)	
		Adult emergence (%)	Parasitization (%)	Adult emergence (%)	Parasitization (%)	Adult emergence (%)	Parasitization (%)
Spinetoram 12 SC	36	86.9^{ab}	85.5^{ab}	90.1^{ab}	87.9^{ab}	88.9^{ab}	85.3^{ab}
Spinetoram 12 SC	45	84.9^{ab}	80.9^{b}	88.6^{b}	86.8^{ab}	86.2^{ab}	85.0^{ab}
Spinetoram 12 SC	54	77.6^{b}	73.4^{bc}	86.9^{b}	82.9^{b}	84.0^{b}	81.3^{b}
Spinetoram 12 SC	108	76.0^{bc}	71.4^{bc}	83.7^{bc}	81.3^{b}	80.1^{bc}	78.0^{bc}
Emamectin benzoate 5 SG	8.5	77.2^{b}	72.7^{bc}	86.9^{bc}	82.3^{b}	82.1^{b}	80.5^{b}
Chlorpyriphos 20 EC	200	66.6^{c}	63.3^{c}	73.4^{cd}	69.7^{c}	71.2^{c}	68.3^{c}
Quinalphos 25 EC	200	72.9^{bc}	66.5^{c}	79.5^{c}	75.0^{bc}	72.5^{c}	68.9^{c}
Indoxacarb 14.5 SC	75	62.6^{c}	60.1^{cd}	66.0^{d}	60.9^{cd}	63.3^{cd}	60.8^{cd}
Novaluron 10 EC	75	68.7^{c}	65.9^{c}	78.2^{c}	73.8^{bc}	73.1^{c}	70.3^{c}
Untreated control	-	91.4^{a}	91.7^{a}	96.0^{a}	91.6^{a}	91.8^{a}	90.3^{a}
CD (0.05%)	-	2.50	2.20	2.18	3.09	2.10	2.99
SEd	-	1.28	1.11	1.08	1.60	1.05	1.51

Data are mean values of three replications; In a column, means followed by a common letter are not significantly different by DMRT (P = 0.05); Values are arc sine $\sqrt{\text{percent}}$ transformed value.

solutions of insecticide formulations dried by rolling for few seconds. Second instar *C. zastrowi sillemi* grubs were released into the vials at 10 per vial, covered with muslin cloth and secured with a rubber band. For untreated check only acetone was used. Mortality observations were taken at 12, 24 and 48 h after treatment. After 24 h exposure of the grubs, 1 cc of *C. cephalonica* eggs were given as feed to the grubs. Percent mortality of the grubs was worked out and pupation (%) and adult emergence (%) were also worked out.

Impact of spinetoram 12 SC on adults of *C. zastrowi sillemi*

Five pairs of freshly emerged *C. zastrowi sillemi* adults were allowed in separate plastic containers. The adults were fed with 10 percent sucrose solution containing different concentrations of spinetoram formulation and other insecticides. In the untreated check, the adults were fed with 10 percent sucrose solution alone. The eggs laid in each treatment were collected daily by keeping a brown paper sheet of 21 × 6 cm size along the inner side of the plastic container. Observations were made on the adult longevity and fecundity at 12, 24 and 48 h after treatment (Suganyakanna, 2006).

RESULTS AND DISCUSSION

Impact of spinetoram 12 SC on immature stages of egg parasitoid, *T. chilonis* (Ishii) on adult emergence and parasitization

Treatment of spinetoram 12 SC at 36, 45, 54 and 108 g a.i./ha on 2 days old parasitized egg card (egg stage) of *T. chilonis* resulted in adult emergence of 86.9, 84.9, 77.6 and 76.0% (Table 1). The adult emergence however was 90.1, 88.6, 86.9 and 83.7% and 88.9, 86.2, 84.0 and 80.1% when 4 days old (larval stage) and 6 days old (pupal stage) parasitized egg card of *T. chilonis* was treated with spinetoram 12 SC at 36, 45, 54 and 108 g a.i./ha. Emamectin benzoate at 8.5 g a.i./ha recorded 77.2, 86.9 and 82.1% adult emergence, respectively when treated at egg, larval and pupal stages followed by quinalphos 200 g a.i/ha (72.9, 79.5 and 72.5% at egg, larval and pupal stage respectively) and novaluron (68.7, 78.2 and 73.1% at egg, larval and pupal stage respectively). Chlorpyriphos and indoxacarb treated eggs of *T. chilonis* however, achieved adult emergence of 66.6 and 73.4; 62.6 and 66.0; and 62.6 and 64.8% at egg, larval and pupal stages respectively. The untreated *T. chilonis* registered 91.4, 96.0 and 91.8% adult emergence from egg, larval and pupal stages, respectively.

The highest parasitization (from 90.3 to 91.7%) was recorded in the untreated check which was significantly superior to other treatments. This was

followed by spinetoram 12 SC at 36 g a.i./ha which recorded 85.3 to 88.0% parasitization. Spinetoram 12 SC at 45 g a.i./ha recorded 80.9, 86.8 and 85.0% parasitization when treated during egg, larval and pupal stages, respectively. Spinetoram 12 SC at 108 g a.i./ha recorded 71.4, 81.3 and 78.0% parasitization when treated during egg, larval and pupal stages, respectively and comparable with that of emamectin benzoate 5 SG at 8.5 g a.i/ha (72.7, 82.3 and 80.5% respectively).

The present investigation was in agreement with Hernandez et al. (2011) who reported that spinetoram 12 SC had no more toxic to the leaf miner parasitoid complex compared to other treatments and untreated control. In contrast, Hossain and Poehling (2006) found that spinetoram 12 SC negatively affects two endolarval leaf miner parasitoid immature stages. But Ruberson (2003) stated that spinosad had no negative effects on the development of *T. pretiosum* and appears to be entirely compatible with *T. pretiosum*. Elzen et al. (1998) who reported that spinosad at 75 g a.i./ha was safer to egg parasitoid *T. chilonis* than other insecticides (azinphos - methyl, imidacloprid, oxamyl, endosulfan and betacyfluthrin) and also Dhawan (2000) reported that spinosad was safe to predators (predatory bugs, spider and green lace wing) and parasitoids (*T. chilonis*) whereas conventional insecticides caused higher mortality. Spray droplets can cause harm to *Trichogramma* wasps and other parasitoids (Bret et al., 1997; Suh et al., 2000; Tillman and Mullrooney, 2000). However, once the deposits dry, they are generally safer for beneficial insects. Present study revealed that spinetoram 12 SC at lower doses (36 and 45 g a.i./ha) was less toxic to *T. chilonis.*

Impact of spinetoram 12 SC against *C. zastrowi sillemi*

Impact on egg hatchability and mortality

The results on the effect of spinetoram on *C. zastrowi sillemi* eggs are presented in Table 2. The lowest egg mortality was recorded by spinetoram at 36 g a.i./ha (4.2%) and 45 g a.i/ha (9.5%) which was followed by spinetoram at 54 g a.i/ha (13.2%), emamectin benzoate 5 SG at 8.5 g a.i/ha (13.8%) and spinetoram 108 g a.i/ha (18.4%). The next best treatments which registered moderate level of egg mortality were novaluron 10 EC at 75 g a.i/ha (24.0%), quinalphos 25 EC at 200 g a.i/ha (37.7%) and chlorpyriphos 20 EC at 200 g a.i/ha (38.4%). Indoxacarb 14.5 SC at 75 g a.i/ha (40.3%) was recorded highest egg mortality and the lowest egg mortality was in untreated check (2.9%).

The highest egg hatchability was recorded by spinetoram at 36 g a.i./ha (95.2%) and 45 g a.i/ha (90.1%) which was followed by spinetoram at 54 g a.i/ha (86.3%), emamectin benzoate 5 SG at 8.5 g a.i/ha (86.0%) and spinetoram 108 g a.i/ha (81.6%). The next best treatments which registered moderate level of egg hatchability were novaluron 10 EC at 75 g a.i/ha (76.0%), quinalphos 25 EC at 200 g a.i/ha (62.3%) and chlorpyriphos 20 EC at 200 g a.i/ha (60.4%). Indoxacarb 14.5 SC at 75 g a.i/ha (59.7%) was recorded lowest egg hatchability and the highest egg hatchability was in untreated check (97.1%).

Results are comparable with the findings of Elbarky et al. (2008) who found that spinetoram 12 SC (radiant) does not have harmful effect on population of lady beetles *Coccinella* spp., aphid lion, *Chrysoperla* spp. and rove beetle, *Paederus* spp.

Impact on adult longevity and fecundity of C. zastrowi sillemi

The studies conducted on effect of spinetoram 12 SC on adult longevity and fecundity of *C. zastrowi sillemi* revealed that the adult longevity was the longest in untreated check (14.7 days) and it was followed by 11.4, 9.4, 8.7, 8.3 and 7.7 days in spinetoram at 36, 45 g a.i/ha, emamectin benzoate 5 SG at 8.5 g a.i/ha, spinetoram 54 and 108 g a.i./ha respectively. Moderate level of adult longevity was recorded in novaluron 10 EC at 75 g a.i/ha (6.9 days), quinalphos 25 EC at 200 g a.i/ha (5.8 days) and chlorpyriphos 20 EC at 200 g a.i/ha (5.8 days). Adult longevity was shortest in indoxacarb 14.5 SC at 75 g a.i/ha (4.0 days) (Table 2).

The number of eggs laid per five female was also more in untreated check (366.4 eggs) and it was followed by spinetoram 36 g (146.0 eggs), 45 g (133.4 eggs), emamectin benzoate 5 SG at 8.5 g a.i/ha (106.1 eggs), spinetoram 54 g (105.7 eggs) and 108 g (93.7 eggs). Our results are in line with Medina et al. (2003) who investigated that spinosad at the highest concentration tested cause slight significant reduction in the adult life span and fecundity.

Impact on the grubs of C. zastrowi sillemi - Larval feeding method

C. zastrowi sillemi larval feeding method study revealed from 0.4 to 4.0 and from 3.2 to 9.5% mortality in spinetoram treatments at 12 and 24 HAT respectively (Table 3). At 48 HAT, the lowest mortality was recorded in spinetoram at 36 g a.i./ha (10.8%), while spinetoram at 45 g a.i./ha (16.2%) was followed by spinetoram at 54 g a.i./ha (19.2%). The next best treatments were emamectin benzoate 5 SG at 8.5 g a.i/ha (20.1%) and spinetoram at 108 g a.i/ha (22.3%). The standard check insecticides *viz.,* quinalphos 25 EC at 200 g a.i/ha, chlorpyriphos 20 EC at 200 g a.i/ha, indoxacarb 14.5 SC at 75 g a.i/ha and novaluron 10 EC at 75 g a.i/ha recorded > 30.00% mortality at 48 HAT.

Spinetoram 12 SC at 36 g (88.8%), 45 g (83.2%) and 54 g (79.8%) were recorded the highest pupation percentage followed by emamectin benzoate 5 SG at 8.5 g a.i/ha (79.5%), spinetoram 108 g (77.6%) and novaluron 10 EC at

Table 2. Impact of spinetoram 12 SC on the eggs and adults of *Chrysoperla zastrowi sillemi* (Esben - Petersen).

Treatments	Dose (g a.i/ha)	Eggs*		Adults**	
		Egg hatchability (%)	Egg mortality (%)	Adult longevity (days)	No. of eggs laid per 5 female
Spinetoram 12 SC	36	95.2^{ab}	4.2^{ab}	11.4^{b}	146.0^{b}
Spinetoram 12 SC	45	90.1^{b}	9.5^{b}	9.4^{c}	133.4^{c}
Spinetoram 12 SC	54	86.3^{c}	13.2^{c}	8.3^{d}	105.7^{d}
Spinetoram 12 SC	108	81.6^{d}	18.4^{d}	7.7^{e}	93.7^{e}
Emamectin benzoate 5 SG	8.5	86.0^{c}	13.8^{c}	8.7^{d}	106.1^{d}
Chlorpyriphos 20 EC	200	60.4^{f}	38.4^{f}	5.8^{g}	48.1^{g}
Quinalphos 25 EC	200	62.3^{f}	37.7^{f}	5.8^{g}	48.5^{g}
Indoxacarb 14.5 SC	75	59.7^{f}	40.3^{f}	4.0^{h}	45.6^{g}
Novaluron 10 EC	75	76.0^{e}	24.0^{e}	6.9^{f}	72.0^{f}
Untreated control	-	97.1^{a}	2.9^{a}	14.7^{a}	366.4^{a}
CD (0.05%)	-	1.18	2.20	2.52	3.00
SEd	-	0.71	1.10	1.30	1.53

In a column, means followed by a common letter are not significantly different by DMRT (P = 0.05); *Values are arc sine $\sqrt{\text{per cent}}$ transformed values; ** Values are $\sqrt{X+0.5}$ transformed values.

Table 3. Effect of Spinetoram 12 SC on the grubs of *C. zastrowi sillemi* by larval feeding method.

Treatments	Dose (g a.i/ha)	Larval feeding method*				
		Mortality (%)			Pupation (%)	Adult emergence (%)
		Hours after treatment (HAT)				
		12	24	48		
Spinetoram 12 SC	36	0.4^{a}	3.2^{b}	10.8^{b}	88.8^{b}	88.8^{b}
Spinetoram 12 SC	45	1.0^{ab}	4.3^{c}	16.2^{c}	83.2^{c}	83.2^{c}
Spinetoram 12 SC	54	2.1^{b}	7.3^{d}	19.2^{d}	79.8^{cd}	79.8^{cd}
Spinetoram 12 SC	108	4.0^{c}	9.5^{e}	22.3^{e}	77.6^{cd}	77.6^{cd}
Emamectin benzoate 5 SG	8.5	2.3^{b}	7.8^{d}	20.1^{d}	79.5^{cd}	79.0^{cd}
Chlorpyriphos 20 EC	200	7.1^{f}	13.6^{g}	38.8^{g}	61.2^{f}	59.0^{f}
Quinalphos 25 EC	200	6.2^{e}	11.2^{f}	30.7^{f}	69.3^{e}	69.3^{e}
Indoxacarb 14.5 SC	75	8.0^{g}	15.7^{h}	47.2^{h}	52.8^{g}	50.3^{g}
Novaluron 10 EC	75	5.2^{d}	10.0^{e}	24.1^{e}	75.0^{d}	73.0^{d}
Untreated control	-	0.4^{a}	0.4^{a}	4.7^{a}	94.0^{a}	94.0^{a}
CD (0.05%)	-	0.01	0.20	1.32	2.20	2.21
SEd	-	0.005	0.10	0.71	1.11	1.12

HAT - Hours after treatment; * Mean of three replications; in a column, means followed by a common letter are not significantly different by DMRT (P = 0.05); Values are arc sine $\sqrt{\text{per cent}}$ transformed values.

75 g a.i/ha (75.0%). The same trend was observed in adult emergence also. Sansone and Minzenmayer (2000) also reported that spinosad had the least impact on spiders as compared to indoxacarb and emamectin benzoate.

Dry film method

Experiment by dry film method revealed that, the lowest mortality of grubs (0.5, 4.7 and 9.1%) was recorded in lower dose of spinetoram at 36 g a.i./ha followed by spinetoram at 45 g a.i./ ha (2.2, 11.4 and 15.3%), spinetoram at 54 g a.i./ha (5.6, 16.5 and 22.7%) and emamectin benzoate 5 SG at 8.5 g a.i/ha (5.7, 16.9 and 22.7%) at 12, 24 and 48 HAT respectively (Table 4).

The pupation per cent was higher in untreated check (94.1%) while spinetoram at 36, 45, 54 and 108 g a.i./ ha recorded 90.9 to 72.0% pupation and other standard checks recorded 77.3 to 49.8% pupation. The same trend was also followed in adult emergence percentage. These findings are in conformity with the results of Gamal El-Kady et al.

Table 4. Effect of spinetoram 12 SC on the grubs of *C. zastrowi sillemi* by dry film method.

Treatments	Dose (g a.i/ha)	Dry film method*				
		Mortality (%)			Pupation (%)	Adult emergence (%)
		Hours after treatment (HAT)				
		12	24	48		
Spinetoram 12 SC	36	0.5[a]	4.7[b]	9.1[b]	90.9[b]	90.9[b]
Spinetoram 12 SC	45	2.2[b]	11.4[c]	15.3[c]	84.7[c]	84.7[c]
Spinetoram 12 SC	54	5.6[c]	16.5[d]	22.7[d]	77.3[d]	77.3[d]
Spinetoram 12 SC	108	11.3[d]	20.1[e]	28.0[e]	72.0[e]	72.0[e]
Emamectin benzoate 5 SG	8.5	5.7[c]	16.9[d]	22.7[d]	77.3[d]	77.3[d]
Chlorpyriphos 20 EC	200	15.8[f]	29.7[g]	42.4[g]	57.6[g]	56.0[g]
Quinalphos 25 EC	200	14.5[f]	25.9[f]	38.7[f]	61.0[f]	60.2[f]
Indoxacarb 14.5 SC	75	17.5[g]	34.3[h]	50.2[h]	49.8[h]	49.8[h]
Novaluron 10 EC	75	12.8[e]	22.3[e]	31.1[e]	68.9[e]	68.9[e]
Untreated control	-	0.5[a]	1.5[a]	5.9[a]	94.1[a]	94.1[a]
CD (0.05%)	-	0.03	0.22	1.61	1.90	2.01
SEd	-	0.015	0.12	0.83	0.92	1.00

HAT - Hours after treatment; * Mean of three replications; in a column, means followed by a common letter are not significantly different by DMRT (P = 0.05); Values are arc sine $\sqrt{\text{percent}}$ transformed values.

(2007) and Mahmoud and Osman (2007) who found that spinetoram 12 SC when applied at low rates (10 µg/ml) had low impact on most beneficial insects such as ladybirds, lacewings, big-eyed bugs or minute pirate bugs.

In conclusion, spinetoram 12 SC did not show any harmful effects on parasitization and parasitoid emergence of *T. chilonis* and spinetoram 12 SC was recorded lowest egg and grub mortality, highest egg hatchability and highest adult longevity of *C. zastrowi sillemi.*

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of pre-elite seed size and planting density on development and propagation efficiency of two virus-free potato cultivars in Sichuan Province, China

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The aim of the experiment is to develop a high-output and low-cost method for propagating elite potato seed in the field from pre-elite seeds. Field experiments were conducted in spring and autumn season of southwestern China (Yucheng in 2010 and Hanyuan in 2011). Randomized-block design was used with 3 seed-size rates, 4 planting densities and 2 cultivars of pre-elite seed from virus-free potato. During potato-growing periods, dry-matter accumulation, number, volume, and dry weight of tubers, and propagation efficiency of pre-elite seed from virus-free potato were measured. The results indicated that medium planting density with large pre-elite potato seed increased tuber volume and dry matter accumulation (per-plant), and prolonged rapid-growth period, resulting in a large amount of final growth. In addition, seed volume, number of plants harvested, rate of seed set per plant, and mass of individual tubers increased, reproductive yield and the coefficient of tuber number and weight increased but the weight coefficient decreased. Per-plant rate of seed set and propagation coefficients of weight, tuber number, and tuber weight decreased with increasing plant density, while number of plants harvested and reproductive output increased. The yields of elite seed of virus-free potato reached a peak in autumn season with large pre-elite seed and high planting density, and in spring season with large seed and medium planting density. Propagation coefficient of tuber numbers and weight reached a peak with large seeds and low planting density, but the highest propagation coefficient of weight was obtained with small pre-elite seeds and low planting density.

Key words: Virus-free potato, pre-elite seed, tuber size, planting density, propagation efficiency.

INTRODUCTION

Potato (*Solanum* spp.) is an important crop in Sichuan Province, China. Local governments and agricultural departments have placed high importance on potato production due to its advantages, which include

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adaptability to various agro-ecological regions, high yields, and diversity of uses, both fresh and processed. It is reported that the coverage of potato in Sichuan has expanded by 172.2% and potato yields have increased by 189.9% from 2005 to 2014 (Ministry of Agriculture, 2006, 2015). Although industrialized potato production has been developed in Sichuan to some extent, potato output in this province is approximately 20 t/ha^{-2} times lower than that in developed countries (Wu et al., 2012). The main reason for the lower output is that identical potato cultivars have been planted for many years, without any alteration. According to research by Xie et al. (2010), approximately 70% of farmers plant their own potato cultivars in Sichuan, but virus-free potato seeds are utilized only in a small area, which greatly limits the rapid industrialization of this crop.

Virus-free potato seeds are generally classified into 5 levels (The national standard of the People's Republic of China, 2006) or 3 levels (Xie et al., 2011), and different production technologies should be utilized for different seed levels or generations (Li, 2007; Yan et al., 2008). Some research has indicated that lowering planting density can result in an increased number of tubers per plantlet in all grades, improved plantlet survival, and increased numbers of stems per plant (Van der Veeken and Lommen, 2009). Muro et al. (1997) found that the number of tubers increased significantly with seed density, without a decrease in number of large-diameter tubers, and the number of the minitubers formed per unite area was in line with the increase in plant density (Jin et al., 2013). Wu et al. (2011) found that the size of minituber planted at the same density had significant influence on the occurrence of late blight and high yield of elite seed was achieved when the minitubers planted were 21 g or more. In another study, yield and number of tubers increased, but average weight of individual tubers decreased, as planting density increased (Yang et al., 2009). When virus-free seedlings of miniature potatoes were planted at a density of 400/m^2 (2 seedlings per hole), good growth, survival rate, and yield were obtained (Yan et al., 2006); at a constant planting density, high seed-tuber weight significantly affected tuber yield (He et al., 2007). Most research on the use of virus-free seedling products, pre-elite seed, or cultivated potato products as commodities has studied the effects of density or seed size on tuber number and yield; however, there have been few reports on the dynamics of tuber growth or the propagation efficiency of elite seed tuber in potato. Elite seeds are usually produced using the same methods as for commodity-potato production, but this approach is inefficient, and has a high cost and low output. The popularization of virus-free potato seeds is therefore limited to some extent. In this paper, we consider volume and planting density of pre-elite potato seed in an attempt to determine an efficient method for reproducing elite seed in the field. Our results may facilitate an increase in use and coverage of virus-free potato seed, which may further contribute to the industrialization of potato production in Sichuan, China.

MATERIALS AND METHODS

Materials and experimental sites

Zhongshu *2*, provided by the Potato Research and Development Center of Sichuan Agriculture University. Eshu 5, provided by the South China Potato Research Center, Hubei Province. Both Zhongshu 2 and Eshu 5 were pre-elite potato seeds obtained from virus-free potato plantlets. The soils in the experimental site were of medium fertility (it contained 30.54 g.kg^{-1} organic matter, 74.50 mg.kg^{-1} available N, 61.98 m g.kg^{-1}available P, 50.11 m g.kg^{-1} available potassium), and rice had been harvested before the potatoes were planted.

Experimental design

This experiment was conducted in Randomized-Block Design with 2-factors (A and B), and 3 replications, and each block comprised an area of 12 m^2. Factor A represented the volume of pre-elite potato seed, and included 3 levels: A1 (1-3 g), A2 (5-6 g), and A3 (9-10 g). Factor B represented planting density, included 4 levels: B1 (9 plants/m^2), B2 (12 plants/m^2), B3 (15 plants/m^2), and B4 (18 plants/m^2).

Cultivation and management

Zhongshu 2 seed was planted in autumn (28 September 2010) in Caoba Town, Yucheng District, altitude 600 m and harvested on 9 December 2010. Eshu 5 seed was planted in spring (10 January 2011) in Shuangxi Town, Hanyuan County, altitude 1600 m and harvested on 19 May, 2011. Planting density was established as described above, and rows were spaced 40 cm apart. The same management practices as used in large-scale production were used in the experimental blocks. Pure nitrogen fertilizer about 150 kg/ha, 70% fertilizer as a base fertilizer and a 30% as an additional fertilizer. P, K fertilizers about K_2O 135 kg/ha, and P_2O_5 45 kg/ha as a base fertilizer, after seeding, flowering and tuber bulking, when drought, irrigation 50, 80 and 80 mm, respectively. To protect and control potato disease, Choose a 58% frost spirit manganese zinc, 64% antivirus alum, and 72% du bangke dew, which were used alternately every 10 days, spray 2-3 times.

Observation and investigation

The seedling emerging rate was observed and uniform plants (with same emergence time, same plant size, and same plant growth) were tabbed as samples. Five sample plants were taken from each block every 10 to 12 days. The volume of tubers and roots were measured using the drainage method for each replicate, after which roots, stems, leaves, and tubers were dried for weighing. Plants harvested in all of the blocks were numbered and weighted, and their tubers were counted.

Data analysis

Data were analyzed using Microsoft Excel 2003, DPS 7.05, and SigmaPlot 12.0. Growth dynamics were fitted to the Logistic Equation $y = k/(1+ae^{-bt})$, where “t” represents the days after

seeding from which the date of maximum growth rate (t_0) and two inflection points (t_1 and t_2) were calculated.

$t_0=\ln(a)/b$ (t_0: the accumulation of dry matter or nutrient rate of the biggest moments).

t_1==-ln((2+$\sqrt{3}$)/a)/b(t_1: d^2y/d^2 in t1 time derivative to the maximum)

t_2=-ln((2-$\sqrt{3}$)/a)/b(t_2: d^2y/d^2 in t2 time derivative to the minimum).

RESULTS

Effect of volume of pre-elite seed and planting density on per-plant accumulation of dry matter

The dynamics of per-plant dry matter accumulation was met to the logistic equation $y = k/(1 + ae^{-bt})$, where "t" represents the number of days after seeding at which elite seeds were reproduced by pre-elite seeds. Accumulation of dry matter exhibited a slow-fast-slow curve (Figure 1), and was affected to some extent by volume of pre-elite seed and planting density.

The volume of pre-elite seed planted was larger, the fast-growth period ($t_1 – t_2$) lasted was longer, and the initial growth period ($t_0 – t_1$) became shorter, stimulating an increase in plant dry matter. Compared to small pre-elite seeds, large pre-elite seeds showed an initial growth period 5.8 days shorter, and a fast-growth period 17 d longer. In addition, the rate of dry matter was increased by 125% during the initial growth period, and the accumulation of dry matter increased by 90% during both the initial and fast-growth periods. The total weight of per-plant dry matter was increased by 90% on average.

The initial growth period tended to shorten with increasing planting density, and the fast-growth period tended to lengthen initially, and then ultimately to shorten. The accumulation of dry matter and growth rate of both initial and fast-growth periods revealed a tendency to increase at first, and then to decline. Low planting density (9 plants/m^2) resulted in the longest initial growth period, but middle-to-high planting densities (15 plants/m^2) resulted in the longest fast-growth period. Growth rate and rate of accumulation of dry matter reached their highest values with middle-to-low planting density (12 plants/m^2). At a planting density of 18 plants/m^2, the duration of the initial and fast-growth periods were the shortest, and growth rate and accumulation of dry matter during the initial and fast-growth periods were the slowest.

Effect of volume of pre-elite seed and planting density on tuber development

The process of potato tuber development met the logistic equation of $y = k/(1+ae^{-bt})$, in which "t" represents the number of days after formation of tubers, and this process was affected by both the volume and planting density of pre-elite seeds (Table 1). After initial formation, tubers increased in size and accumulated dry matter; the number of tubers per plant increased rapidly and reached a peak (t_0) within 10.1 to 14 days, with a very short fast-growth period. Enlargement of tubers (tuber volume) and accumulation of dry matter (tuber weight) increased slowly, with long fast-growth periods of 12.7 to 26.4 days and 9.2 to 25.5 days respectively, and reached a peak within 29 to 35.8 days and 28.1 to 37.9 days respectively. In order to obtain a high yield, it is thus advantageous to stimulate formation and enlargement of tubers, and to adequately prolong the fast-growth period of tuber enlargement and dry matter accumulation.

The initial, fast, and smallest growth period were reduced with increasing volume of seed planted. Compared to small seeds, large pre-elite seeds showed a fast rate of seeding within a short time; according to the average value of the 4 planting densities, large pre-elite seeds reproduced 38% more tubers than small ones.

Although tuber volume, dry matter, and tuber number were increased identically, but occurred earlier and shorter duration for large pre-elite seeds than small ones. In addition, large pre-elite seeds had longer and later fast and fastest growth periods, exhibited higher dry weight at harvest, and produced a greater volume of tubers. The fast-growth periods corresponding to tuber volume and weight were 13.1 and 14.3 days longer respectively in large pre-elite seeds than in small seeds. The time required for the maximum growth rate was increased by 5.9 d and 5.6 days respectively, and the harvested tuber volume and weight were increased by 103 and 110% respectively, when small pre-elite seeds were replaced by large seeds.

Volume of pre-elite seed exerted a stronger effect on development especially number of tubers than did planting density. The highest growth period, fast growth duration, and growth rate of tuber volume and weight increased initially and then declined. The fast-growth period was shortened and the growth rate was reduced by excessive planting density, which ultimately resulted in small tuber volumes and low per-plant dry weight.

Effect of pre-elite seed volume and planting density on biomass and distribution of dry matter in tubers

Biomass and distribution of tuber dry matter differed because of differences in the experimental seasons and sites (Table 2). Temperature in Hanyuan changed dramatically from day to night, and there was an abundance of sunlight in spring. The growing period was longer, and more dry matter accumulated and was distributed to tubers in Hanyuan than in Yucheng (in autumn season).

Biomass and distribution of dry matter into tubers were affected somewhat by volume and planting density of pre-elite seeds in both seasons. Biomass increased with

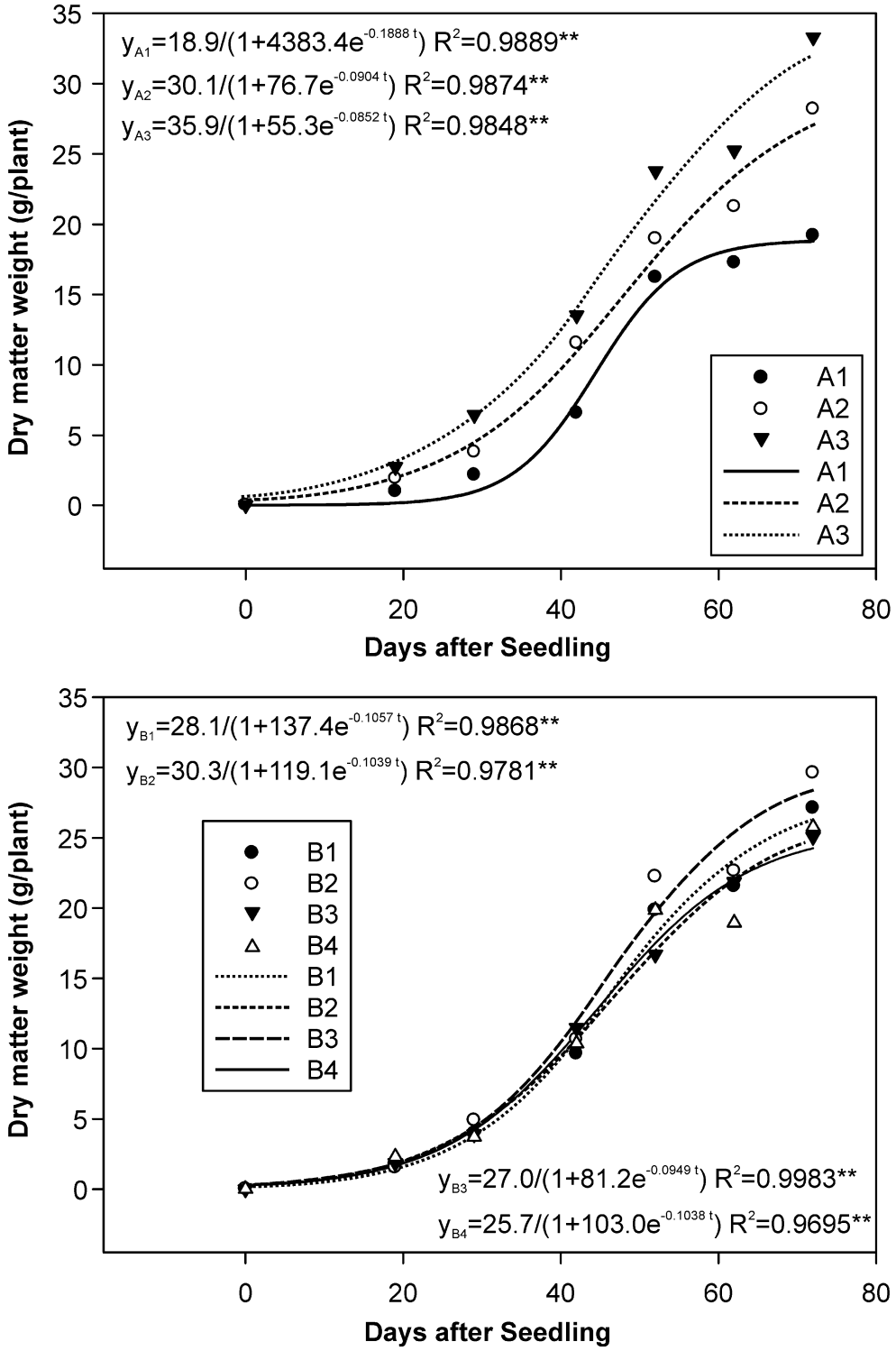

Figure 1. Effect of seed pre-elite seed volume and planting density on dry matter accumulation of individual plant of Zhongshu *2* (planted in autumn). **P < 0.01.

increasing volume of pre-elite seed. Biomass showed a tendency to increase with increased planting density, while the distribution ratio of dry matter to tubers showed the opposite pattern a decreasing trend although dry weight per plant also tended to decline. The biomass of large pre-elite seeds was 68% greater than that of small

Table 1. The growth coefficient of tubers of Zhongshu *2* meets logistic equation $y = k/(1 + ae^{-bt})$ in autumn.

Plant characteristics		Levels	k	a	b	R^2	t_0	t_1-t_2
Tuber number	Pre-elite seed volume (g)	1-3	2.98	117.437	0.34059	0.9948**	14.0	7.7
		5-6	3.77	7.2E+12	2.8085	0.9793**	10.5	0.9
		9-10	4.11	2.3E+23	5.2253	0.9268*	10.3	0.5
	Planting density(plants/m^2)	9	3.78	9.7E+08	1.8909	0.9247*	10.9	1.4
		12	3.81	1.5E+75	17.1712	0.9634**	10.1	0.2
		15	3.65	1.6E+09	1.9675	0.9871**	10.8	1.3
		18	3.43	1.5E+49	11.1948	0.9909**	10.1	0.2
Tuber volume	Pre-elite seed volume (g)	1-3	72.3	318.43	0.1986	0.9922**	29.0	13.3
		5-6	130.0	41.613	0.1088	0.9898**	34.3	24.2
		9-10	146.5	32.359	0.0995	0.9640**	34.9	26.4
	Planting density (plants/m^2)	9	93.6	263.117	0.2074	0.9464*	26.9	12.7
		12	134.9	43.576	0.1053	0.9788**	35.8	25.0
		15	114.0	32.743	0.1004	0.982**	34.7	26.2
		18	111.6	77.867	0.1345	0.9990**	32.4	19.6
Tuber weight	Pre-elite seed volume (g)	1-3	13	3475.5	0.2860	0.9762**	28.5	9.2
		5-6	24.1	46.004	0.1032	0.9793**	37.1	25.5
		9-10	27.3	45.309	0.1120	0.9747**	34.1	23.5
	Planting density (plants/m^2)	9	22.4	54.692	0.1164	0.9603**	34.4	22.6
		12	18.7	1012.8	0.2459	0.9575**	28.1	10.7
		15	22.9	52.886	0.1047	0.9943**	37.9	25.1
		18	19.6	65.425	0.1321	0.9784**	31.7	19.9

*P < 0.05, ** P < 0.01.

Table 2. Effect of seed pre-elite seed size and planting density on biomass and distribution of dry matter in tubers.

Seasons	Plant characteristics	Pre-elite seed volume (g)			Planting density (plants/m^2)			
		1-3	5-6	9-10	9	12	15	18
Spring	Biomass (dry weight, t/ha)	2.59^c	3.79^b	4.37^a	2.44^c	3.50^b	3.76^b	4.64^a
	Distribution of dry matter into tuber	75.3^b	78.6^a	76.4^b	77.6	77.1	76.7	75.7
Autumn	Biomass (dry weight, t/ha)	4.81^c	5.41^b	8.09^a	4.76^c	6.13^b	6.64^b	6.88^a
	Distribution of dry matter into tuber	83.5^b	80.5^c	85.7^a	86.1^a	85.8^a	82.2^b	78.9^c

The data were recorded from the final harvest of per plant for each treatment and the means of the three replications were shown. The different letter in the column of each growing season represents significant difference at P=0.05 by the LSD test (the value of LSD0.05 for each comparison was also presented).

seeds. Average biomass increased by 60%, and distribution of dry matter into tubers decreased by 4.6%, in treatment blocks with high planting densities.

Effect of pre-elite seed volume and planting density on propagation output

Propagation yields were over twice as high in spring as in autumn season. Yields were affected by seed volume and planting density in both spring and autumn, and increased with increasing pre-elite seed volume (Table 3). Reproductive yields of large pre-elite seeds were, on average, 76 and 51% greater than those of medium-sized and small seeds, respectively. It was clear especially in spring season that propagation yields increased with increasing plant density. In spring season the highest yields were at middle-to-high planting density with large pre-elite seeds, while in autumn season, the highest yields were at high planting density with large pre-elite

Table 3. Effect of pre-elite seed size and planting density on propagation yield.

Seasons	Pre-elite seed volume (g)	Planting density (plants/m^2)				
		9	12	15	18	Average
Autumn	1-3	6.86^{f}	7.53^{f}	7.84^{ef}	7.86^{ef}	7.52^{c}
	5-6	10.99^{de}	11.79^{cd}	12.36^{bcd}	13.12^{bcd}	12.07^{b}
	9-10	14.53^{abc}	15.49^{ab}	15.38^{ab}	17.85^{a}	15.81^{a}
	Average	10.80^{b}	11.60^{ab}	11.86^{ab}	12.94^{a}	
Spring	1-3	14.83^{e}	22.41^{cd}	22.49^{cd}	21.71^{cd}	20.36^{b}
	5-6	15.33^{e}	17.79^{de}	23.93^{bcd}	25.02^{bc}	20.52^{b}
	9-10	29.81^{ab}	34.96^{a}	34.54^{a}	34.02^{a}	33.33^{a}
	Average	19.99^{b}	25.05^{a}	26.99^{a}	26.92^{a}	

The data were recorded from the final harvest of per plant for each treatment and the means of the three replications were shown. The different letter in the column of each growing season represents significant difference at P=0.05 by the LSD test (the value of LSD0.05 for each comparison was also presented).

Table 4. Effect of seed pre-elite seed volume and planting density on propagation coefficients.

Treatments		Autumn			Spring		
		NNC	NWC	WWC	NNC	NWC	WWC
Pre-elite seed volume (g)	1-3	2.11^{c}	58.7^{c}	29.37^{a}	3.11^{b}	155.5^{b}	77.76^{a}
	5-6	2.76^{b}	93.9^{b}	17.08^{b}	3.25^{b}	154.3^{b}	28.04^{b}
	9-10	3.47^{a}	123.1^{a}	12.95^{c}	4.53^{a}	260.1^{a}	27.42^{b}
Planting density (plants/m^2)	9	3.37^{a}	119.9^{a}	25.77^{a}	4.32^{a}	222.1^{a}	49.41^{a}
	12	2.81^{b}	96.7^{b}	20.95^{b}	3.98^{b}	208.8^{a}	50.33^{a}
	15	2.71^{b}	79.1^{c}	17.30^{c}	3.33^{c}	179.9^{b}	42.74^{ab}
	18	2.23^{c}	71.9^{c}	15.17^{c}	2.89^{d}	149.5^{c}	35.16^{b}

NNC indicates the number-to-number propagation coefficient; NWI indicates the number-to-weight propagation coefficient; WWI indicates the weight-to-weight propagation coefficient. Different superscript letters indicate significant difference at P < 0.05.

seeds.

Effect of pre-elite seed volume and planting density on propagation coefficient

Seed volume and planting density did not only affect propagation yields, but also affected the number of tubers. Number of tubers per hectare was correlated positively with seed volume and planting density. Large pre-elite seeds produced 56 and 34% more tubers than did medium and small seeds, respectively. The number of tubers produced in the high-density planting treatment (18 plants/m^2) was 31 and 12% greater than that in 9 and 12 plants/m^2 respectively.

The number-to-number propagation coefficient (number of elite seeds reproduced per individual pre-elite seed) increased significantly with increasing volume of pre-elite seed, but decreased significantly with increased planting density (Table 4). The propagation coefficient was 53% higher in large pre-elite seeds than in small ones, and 50 and 27% higher at low planting density than at high and medium planting density, respectively.

The number-to-weight propagation coefficient (weight of elite seeds propagated per individual pre-elite seed) was positively correlated with volume of pre-elite seed, and negatively correlated with planting density. The number-to-weight coefficient was 79 and 54% higher in large pre-elite seeds than in small and medium-sized seeds, respectively, and 55 and 32% higher at low planting density than at high and medium-high planting densities, respectively.

The weight-to-weight propagation coefficient (the weight of elite seeds propagated by 1 kg of pre-elite seeds) was reduced with an increase in planting density and volume of pre-elite seeds. This coefficient was 165 and 137% higher in small pre-elite seeds than in large and medium-sized ones, and 49 and 25% higher at low planting density than at high and medium- high planting densities, respectively.

Table 5. Effect of seed pre-elite seed volume and planting density on components of tuber yield.

Treatments		Autumn			Spring		
		Plants harvested / m^2	Tubers / plant	Weight of one tuber (g)	Plants harvested / m^2	Tubers / plant	Weight of one tuber (g)
Pre-elite seed volume (g)	1-3	8.44^{b}	2.04^{c}	46.85^{b}	11.88^{b}	3.64^{b}	51.31^{ab}
	5-6	8.34^{b}	2.64^{b}	56.05^{a}	12.31^{b}	3.56^{b}	47.74^{b}
	9-10	9.79^{a}	2.98^{a}	59.15^{a}	14.03^{a}	4.38^{a}	57.94^{a}
Planting density (plants/m^2)	9	6.09^{c}	2.85^{a}	62.34^{a}	9.22^{d}	4.23^{a}	50.63^{a}
	12	8.24^{b}	2.55^{ab}	55.25^{ab}	11.11^{c}	4.29^{a}	52.70^{a}
	15	9.32^{b}	2.53^{ab}	50.16^{b}	12.57^{b}	4.04^{a}	53.97^{a}
	18	11.79^{a}	2.27^{b}	48.32^{b}	18.00^{a}	2.88^{b}	52.0^{a}

The data were recorded from the final harvest of per plant for each treatment and the means of the three replications were shown. The different letter in the column of each growing season represents significant difference at P=0.05 by the LSD test (the value of LSD0.05 for each comparison was also presented).

Table 6. The regression, correlation, and path coefficients of tuber yield and its components.

Coefficient	Propagation in autumn			Propagation in spring		
	Plants harvested	Tubers/ plant	Tuberweight	Plants harvested	Tubers/ plant	Tuber weight
Regression coefficient	1.2587	3.8186	0.2169	1.7077	5.9778	0.4397
Correlation coefficient	0.3635*	0.5815**	0.4915**	0.4145*	0.2177	0.6912**
Direct path coefficient	0.9047**	0.5928**	0.5685**	0.8102**	0.7257**	0.6519**
Contribution (%)	34.5	36.2	29.3	35.6	16.7	47.7

The intercept of regression equation in Yucheng and Hanyuan was -20.80 and -43.07 respectively. *P < 0.05, **P < 0.01.

Effect of pre-elite seed volume and planting density on composition of yield

The yield of elite seeds consisted of plants harvested, tubers per plant, and average weight per tuber. Propagation yield and its components were significantly affected by the size of pre-elite seed, planting density, and propagation region and season (Table 5). Propagation yields were higher in spring than in autumn, which we attribute to 2 factors. First, the average seedling rate in spring was 29% higher than that in autumn, leading to a 44% greater harvest. Second, the number of tubers formed by one plant was 51% greater in spring than autumn.

The larger the pre-elite seeds were, the higher the seedling rate, the more plants were harvested, the more tubers were formed by individual plants, and the greater the weight per tuber observed. Compared to small seeds, the number of plants harvested, tubers per plant, and weight per tuber produced by large pre-elite seeds were 17, 30, and 19% greater, respectively. When the same size of pre-elite seeds was used, the number of plants harvested increased with increasing planting density; the number of tubers per plant and the weight per tuber decreased in autumn season (in Yucheng), but increased initially and then declined in spring season (in Hanyuan). High planting density resulted in 95% more plants being harvested and 27% fewer tubers per plant, compared to low planting density.

Correlation and regression analysis

As revealed in Table 6, the reproductive yield of elite seeds was positively correlated with plants harvested, tubers formed per plant, and weight per tuber. Yield increased correspondingly by 1.26 to 1.71, 3.82 to 5.98, and 0.22 to 0.69 tons/ha, respectively, if 1 additional plant was harvested per m^2, 1 more tuber per plant was formed, and if weight per tuber increased by 1 g. The increased yield in Hanyuan in spring was higher than that in Yuchen in Autumn.

Propagation yield was extremely significant to the direct-path coefficient of plants harvested, tubers formed per plant, and weight per tuber. In addition, propagation yield was extremely significant to correlation coefficient of weight per tuber, and significant to the correlation coefficient of number of plants harvested. Propagation yield was extremely significant to the correlation coefficient of number of tubers formed per plant in

Yucheng but not in Hanyuan. Propagation yield in Yucheng was greatly impacted by tubers number per plant and least impacted by weight per tuber, but this scenario was reversed in Hanyuan. These results revealed that it is advisable to use different methods to propagate high yields of elite seeds in different regions. Harvested plants should consider in both Yucheng and Hanyuan; it would be preferable to increase the number of tubers formed per plant in Yucheng, and to increase he weight per tuber in Hanyuan.

DISCUSSION

It has been reported that seedling rate per tuber and number of stems per plant increased with increasing seed volume (Allen and Wurr, 1978), leading to high outputs (He et al., 2007; Qi et al., 2011; Yang and Tu, 2003). However, larger potato seeds are associated with lower propagation coefficients (Yang and Tu, 2003). Therefore, a reasonable volume of potato seeds can produce high yields. Many researchers believe that it is better to produce commercial potatoes using seeds that weigh from 30 to 50 g (He et al., 2007; Yang and Tu, 2003), or from 20 to 25 g (Qi et al., 2011). More larger sized minitubers (Pre-elite Seed) should be selected for elite seed I production, and when the minitubers planted were 21 g or more, the high yield of elite seed was achieved (Wu et al., 2011). But pre-elite potato seed is small generally no more than 20 g and usually between 3 to 5 g, so some way should be found to increase the yield of the elite seed.

With a 10 g increase in seed volume, it was found that yield of elite seed and the propagation coefficient of number-to-number and number-to-weight increased, but that the weight-to-weight propagation coefficient was reduced. Number of tubers, rather than weight, is widely used as a standard index for measurement and valuation in the production of pre-elite to elite seed. It is important to use the largest pre-elite seeds possible in propagating elite seeds.

Compared to small seeds, large pre-elite seeds have a greater number of bud eyes, store more water and nutrients, and are more adaptable to adverse conditions such as cold weather, drought, or flood. As a result, large pre-elite seeds have higher seedling rates, produced more plants for harvest, and exhibit a longer fast-growth period of per-plant dry weight, tuber enlargement, and dry-matter accumulation. These advantages result in an increased number of tubers per plant and weight per tuber, which further results in a high propagation yield of elite seeds.

A proper planting density can balance the relationship between individual and grouped plants, which is a key to producing high yields. Therefore, planting density is one of the most vital research subjects in potato cultivation (Bussan et al., 2007; Cheng and Su, 2009; Love and Thompson-Johns, 1999; Luo 2011). Planting density for high yield varies with differences among regions, seasons, and potato varieties. Planting areas where can plant potato one or two seasons in southern China generally support 6.0 to 7.5 and 7.0 to 9.0 plants/m^2 respectively. It is appropriate to increase planting density by 3.0 to 3.75 plants/m^2 (Allen and Wurr, 1978) during autumn.

It is well known that the reproductive ability of pre-elite seeds is low because of the small tubers associated with these seeds. The aim of propagating elite seeds is not only high output, but also high composition of medium-sized tubers to be used for sowing without cutting, rather than large tubers to be used for commercial production. So the ideal planting density for production of elite seed is not adequate for commercial potato production.

Within the range of 7 to 9 plants/m^2 examined in this study, reproductive yield of elite seeds increased with increasing plant density, but number-to-number, number-to-weight, and weight-to-weight propagation coefficients were reduced. A low planting density can bring about faster accumulation of dry matter and a greater number of tubers per plant than can high planting density; thus, low planting density can produce higher propagation coefficients due to its superiority at the level of the individual plant. On the other hand, low planting density produces a lower yield of elite seeds, due to fewer plants harvested.

Early planting of top-shoot cuttings with closer spacing (high plant density）was recommended for the multiplication of breeder seed potato (Al Mamun et al., 2016) and proper planting densities vary in achieving high yields and propagation efficiencies. The solution for high yield in Yucheng in autumn is to use large (9 to 10 g) pre-elite seeds and high planting density (18 plants/m^2), but the solution for high propagation efficiency is to use low planting density (9 plants/m^2) rather than changing the volume of seeds planted. In Hanyuan in spring, the method for obtaining high yield is to use large (9 to 10 g) pre-elite seeds and medium planting density (12 to 15 plants/m^2), and the recommended method for obtaining high propagation efficiency is the same as that for Yucheng. The proper planting density for producing elite seeds is commonly higher than that for commercial potato production, and is determined by factors that include production objectives, propagation region and season, and size of pre-elite seeds.

Approaches should be taken in different propagation regions and seasons to achieve high outputs of original potato seeds, due to differences in environmental conditions. In spring, the highest rate of propagation yield and coefficient was obtained in Hanyuan due to the long developmental period, so elite seeds in Hanyuan should be planted as possible in spring. Based upon the results of correlation and direct-path analysis, propagation of elite seeds should first consider enough harvest plants, followed by weight per tuber in Hanyuan and number of

tubers per plant in Yucheng.

Dry weight per plant, and volume and dry weight of tubers increased quickly when elite seeds were propagated by pre-elite seeds under the conditions of large pre-elite seed and medium-to-low planting density. Yield and number-to-number and number-to-weight coefficients of propagation increased with increasing size of pre-elite seeds, but weight-to-weight coefficient decreased, indicating that large pre-elite seeds should be the first choice for reproduction of original potato seeds. In addition, propagation yield increased but propagation coefficient decreased with increased planting density. Therefore, the ideal planting density should be comprehensively and systematically determined according to the objectives of reproduction, the size of pre-elite seeds, and cultivation season.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Cassava mosaic disease resistant clones' growth and yield are prone to early drought stress

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This study was conducted to evaluate the growth and yield stability of cassava mosaic disease (CMD) resistant breeding populations clones against early drought. Field trials were planted using 200 CMD resistant clones and 7 local landraces in a randomised complete block design with 3 replicates at the International Institute of Tropical Agriculture (IITA) station, Sendusu in Namulonge (Central Uganda) during the second rains of 2006 (2006B) and the first rains of 2007 (2007A). The 2007A crop suffered from drought stress in the first 4 months after planting (MAP). Data were taken on the leaf lobe length and width at 6 MAP and plant height at 12 MAP. Harvest was done at 12 MAP during which the number of storage roots per plant and storage root yield were recorded. Data were analysed using the Mann-Whitney U test to compare crop performance between the 2 seasons. The 2006B crop had significantly ($P<0.01$) longer leaf lobes, taller plant heights, higher number of storage roots per plant and higher storage root yield than the 2007A crop. There was no significant difference in the leaf lobe width. In this experiment, it was observed that the CMD resistant breeding clones were susceptible to early drought and thus it was recommended that selections should be done for higher water use efficiency.

Key words: Abscisic acid, *Manihot esculenta* Crantz, Stomatal conductance, water use efficiency, Uganda.

INTRODUCTION

The importance of cassava production as a source of income and household food security in Uganda (Fermont et al., 2009a) and the rest of sub Saharan Africa's (SSA) cannot be over emphasised. Cassava is widely believed to be a hardy crop against several environmental stresses, including drought (Purseglove, 1968; Onwueme, 1978). However, cassava has been reported to be susceptible to moisture stress especially at critical growth stages (Alves, 2002). This is likely to be a major production constraint in SSA smallholder cassava cropping systems in the near future given the predicted increase in extreme weather events with climate change,

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Table 1. Descriptive statistics for the experimental crop in 2006B and 2007A seasons.

Growth/Yield parameter	Minimum		Maximum		First quartile		Median		Third quartile	
	2006B	2007A	2006B	2007A	2006B	2007A	2006B	2007A	2006B	2007A
Leaf lobe length (cm)	10.3	5.4	25.7	26.0	15.0	14.8	16.3	16.2	18.3	17.7
Leaf lobe width (cm)	2.3	1.5	7.9	8.2	4.4	4.4	4.9	5.0	5.5	5.5
Plant height at 12 MAP (cm)	125.0	65.0	316.7	417.0	193.3	123.0	223.3	148.0	250.0	172.0
No. of roots per plant	0.0	0.0	18.0	13.0	4.0	2.0	6.0	4.0	8.0	6.0
Fresh tuber yield (t ha^{-1})	0.0	0.0	60.0	60.0	8.2	2.5	15.0	7.5	25.0	14.5

prolonged drought inclusive.

Breeding has been identified as a key strategy for controlling crop production stresses, especially in the low input cropping systems of the SSA region. A case in point is the cassava mosaic disease (CMD) pandemic that devastated Uganda's cassava production sector in the early 1990's (Otim-Nape et al., 1997), which was controlled courtesy of CMD-resistant materials developed through national and international collaborative cassava improvement programmes. Over forty thousand accessions have since been introduced and evaluated for resistance to CMD. Tolerance to multiple stresses is highly desirable among elite germplasm. It is thus imperative that future cassava improvement programmes have to draw from the CMD resistant breeding materials. This study compared the growth and yield of CMD resistant breeding clones in Uganda with and without early season drought stress as a way of evaluating their general stability against early moisture stress.

MATERIALS AND METHODS

Study was carried out at the International Institute of Tropical Agriculture (IITA) station in Namulonge (Wakiso district), central Uganda (0.53°N, 32.58°E; 1150 m above sea level). The annual rainfall received in the area averages 1200 mm in bimodal distribution. The first rainy season occurs between March and June, while the second one is between September and December. The soils are characterized as Rhodic Acrisols (FAO system of classification) on slopes averaging 4% in steepness.

320 important CMD-resistant clones were selected. These were subjected to cluster analysis based on disease reaction and yield. From each of the resulting 4 clusters, clones were randomly selected to constitute a total sample of 200 clones in direct proportion to each cluster's numerical size. In addition, 7 local landraces (*Alado-alado*, *Bao*, *Bukalasa*, *Njule*, *Nyaraboke*, *Tereka* and *Tongolo*) were included in the study. The selected clones were planted in the second rainy season of 2006. The spacing used was 1 × 1 m in a Randomized Complete Block Design (RCBD) replicated 3 times. For each genotype, a plot consisted of 10 plants in a row. The total trial area was 6300 m^2 with 2 border rows of genotype TMS I92/0067 (*Akena*). The set up was weeded using hand-hoeing whenever necessary. Neither pest nor disease attack on the crop was controlled. The trial was conducted in the second rainy season of 2006 (hereafter referred to as 2006B) and repeated in the first rainy season of 2007 (hereafter referred to as 2007A).

From the 8 central plants per plot, data were taken on leaf lobe length and width at 6 months after planting (MAP), and plant height at harvest (12 MAP), as described by Ferguson and Kawuki (2006). The number of storage roots per plant was obtained as an average of the total number of storage roots from each of 3 randomly sampled plants per plot. Fresh storage root yield data were taken at harvest, from the 8 middle plants per plot and extrapolated from the plot area to per hectare basis.

Daily rainfall data were obtained from meteorological station about 1 km away from the experimental field. The total rainfall received per month after planting was computed by summation from the daily records. The 2006B crop received a total of 1220 mm compared to 1400 mm for the 2007A crop. Although the 2006B crop experienced drought stress around 5 MAP, it received 164 mm more rainfall than the 2007A crop in the critical first 4 MAP (Figure 1) thus rendering the 2 crops' dataset ideal for the objective of the current study. The 2 seasons leaf lobe length and width, plant height and storage root yield were compared using Mann-Whitney U (Rank Sum) Test in SPSS 11.0.

RESULTS AND DISCUSSION

Based on the median values, the 2007A plants, which suffered early season drought stress, had leaves that were similar in size to those for the 2006B plants. However, the latter were taller, had more storage roots per plant that translated into higher fresh storage root yield than the former (Table 1).

The Mann-Whitney U test confirmed that the 2006B plants had significantly longer leaf lobes and were significantly taller than the 2007A plants. Furthermore, the test also confirmed that the 2006B plants significantly out-yielded the 2007B plants (Table 2).

Over the 2 seasons during which this study was conducted, 75% of the clones gave storage root yields less than 25 t ha^{-1}, with lower yields being recorded in 2007A than in 2006B (Table 1). The yields are within the range reported by Fermont et al. (2009b) in farmers' fields in Uganda, but are below on-station yields reported by Ntawuruhunga et al. (2006). The 2007A crop suffered drought stress early in the growing season (Figure 1), which depressed its aboveground growth, as is reflected in the significantly($P<0.01$) lower plant heights than in 2006B (Table 2) and lower number of storage roots (Table 2). In 2006B, the plant heights were in the range

Table 2. Comparison of the growth and yield parameters of 2006B and 2007A crops.

Growth/yield parameter	Mean rank		z-value	Significance level
	2006B	2007A		
Leaf lobe length	630.7	576.9	-2.679	0.01
Leaf lobe width	601.2	608.0	-0.339	0.73
Plant height at 12 MAP	835.5	347.2	-24.410	0.00
No. of roots per plant	651.6	445.1	-10.704	0.00
Fresh tuber yield	657.5	428.4	-11.835	0.00

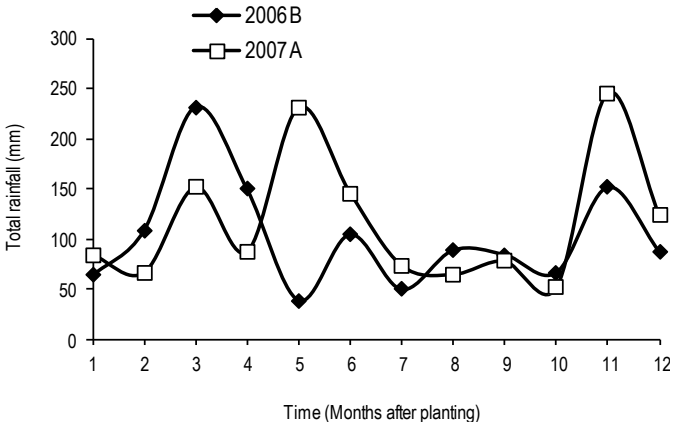

Figure 1. Total rainfall during the seasons 2006B and 2007A in Namulonge, Uganda.

130 to 320 cm (Figure 2). This is comparable to the cassava height range of 120 to 370 cm reported from elsewhere (Ramanujam, 1985; Pinho et al., 1995). In 2007A, 75% of the clones had a height of 130 cm or less.

Drought stress may have reduced both the source supply (that is, the amount of carbohydrates that were available for storage root formation) and the sink demand (that is, the number of storage roots), which resulted into the lower yields observed in the 2007A season. Alves (2002) observed that though cassava is relatively drought tolerant, storage root yield is adversely affected by drought stress within the first 5 months of growth. Connor and Palta (1981) reported that limited soil moisture supply led to lower stomatal conductance, suggesting restricted opening of the stomatal pore. Although this effectively controls further loss of water from the plant and hence maintains the leaf water potential, it comes at the expense of lower assimilate production due to restricted carbon dioxide entry into the leaves. This response has been attributed to the plant stress hormone abscisic acid, with the young leaves accumulating higher concentrations of the hormone than older leaves when water stressed (Alves and Setter, 2000). Cellier et al. (1998) reported genotypic differences in plant sensitivity to the hormone. This suggests that breeding approaches can be employed to improve water use efficiency in cassava so as to attain higher levels of drought tolerance.

CONCLUSION AND RECOMMENDATION

The clones exhibited sensitivity to early drought stress with serious yield decline. Since the clones are used regionally in cassava breeding programme, it is important for future work to focus on selecting for higher water use efficiency and hence drought tolerance, besides resistance to the biotic stresses for which they were developed.

Conflict of Interests

The authors have not declared any conflict of interests.

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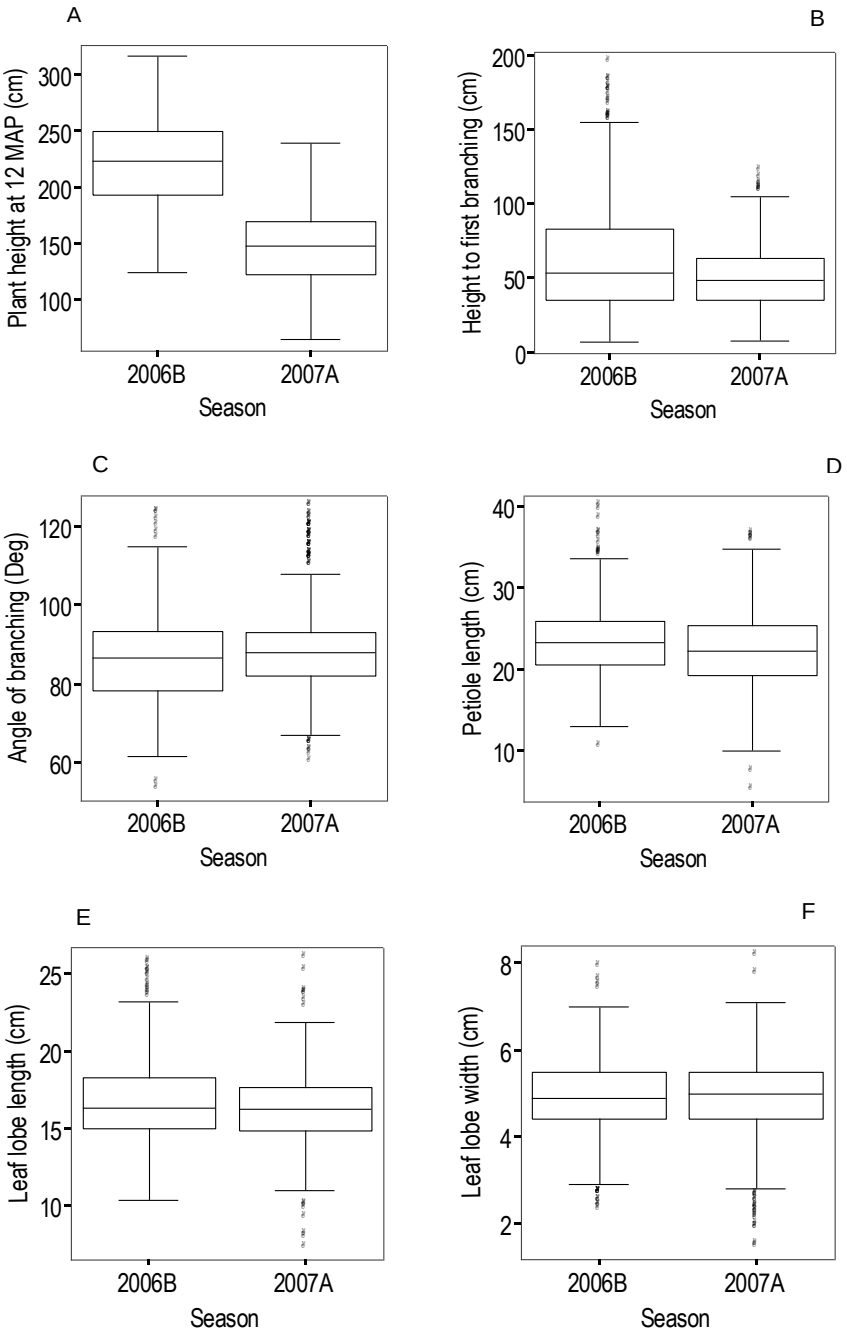

Figure 2. Comparison of cassava plant height (A), height to first branching (B) angle of branching (C), petiole length (D), leaf lobe length (E) and width (F) between seasons in Namulonge, central Uganda.

Tropical Agriculture.

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Full Length Research Paper

The role of *Crambe abyssinica* in the control of *Heterodera glycines* (Thylenchida: Heteroidae)

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The soybean cyst nematode (SCN), *Heterodera glycines*, is present in more than 150 municipalities in Brazil, and the long persistence of the cysts in the soil combined with the severe degree of parasitism induces high soybean production losses. The objective of this study was to evaluate the biofumigant effect of *Crambe abyssinica* on the SCN population in the second soybean crop season. The experiment was conducted on a rural property whose soil was naturally infested with SCN. *Crambe abyssinica* was planted in the second crop season following harvest, and in the subsequent crop season, 4 soybean varieties, 2 resistant and 2 susceptible to SCN, were planted. The nematode population was evaluated every month for 90 days after planting. In the second crop season, when *C. abyssinica* was in the field, there was a significant decrease in the number of adult SCN females and cysts. During the 90-day period after *C. abyssinica* cultivation, when plant residues were incorporated into the soil and the area was planted with soybeans, both the number of adult SCN females / 10 g roots and the number of cysts decreased. This result indicates that *C. abyssinica* reduced the nematode population during its time in the field.

Key words: Crambe, *Glycine max*, soybean nematode cyst, culture control.

INTRODUCTION

The acreage devoted to soybean [*Glycine max* (L.) Merr.] has grown more than for any other crop in Brazil over recent harvest seasons. However, some factors limit high grain yields in this crop, including the more than 100 nematode species, of approximately 50 genera, that are considered soybean pests worldwide. In Brazil, the most harmful nematodes to this crop are those that form galls (*Meloidogyne* spp.), cysts (*Heterodera glycines*), and root Lesions (*Pratylenchus brachyurus*) as well as reniform nematodes (*Rotylenculus reniformis*) (Dias et al., 2009).

The soybean cyst nematode (SCN), *H. glycines*, is a major soybean pest because of the damage it can cause and the ease of dissemination. It penetrates plant roots and hinders the absorption of water and nutrients, thereby reducing the size and number of pods and causing chlorosis and low productivity (Liu et al., 2012).

The control of phytonematodes over large areas, including those planted with annual crops, is most often

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performed with chemical nematicides, but no chemicals are registered for the control of *H. glycines* in soybean. Despite studies indicating the efficacy of biological agents in the control of other species, no biological nematicides are currently available for the control of SCN in soybeans (MAPA, 2015).

Another management strategy involves the use of resistant plant varieties, but due to the large genetic variability in SCN populations, such plants are not always available for the control of the particular nematode races present in a cultivated area. Therefore, crop succession and rotation are especially important strategies for the control of SCN populations (Franzener et al., 2005).

Crambe abyssinica belongs to the family Brassicacea, and its seed oil concentration is approximately 40%, which has favored its use as a raw material for the production of biodiesel (Lima et al., 2015). After harvest, its crop residues are incorporated into the soil, and because its tissues contain glucosinolates, this incorporation promotes biofumigation of the soil and the consequent reduction in nematode populations (Anita, 2012).

Glucosinolate metabolism results in the production of toxic substances, which act as natural barriers against pathogens and pests (Berbegal et al., 2008; Pitol et al., 2010), and Pal et al. (2009) argue that these compounds, which are primarily found in the Brassicaceae family, have several biological properties, including protection against pathogens and weeds. In the presence of the enzyme myrosinase, such as when plants are cut or chewed, glucosinolates are hydrolyzed to various products, including isothiocyanates, thiocyanates, and indoles.

Isothiocyanates (ITCs) are volatile compounds and toxic by-products of glucosinolate hydrolysis (Wu et al., 2011), and of the various ITCs that have been identified, allyl isocyanate (ITCA) has been found to be the most effective in the control of nematodes such as *Meloidogyne javanica*, *Tylenchulus semipenetrans*, *H. glycines, M. incognita, M. hapla, H. schachtii*, *Pratylenchus penetrans*, and *P. neglectus* (Zasada and Ferris, 2003; Yu et al., 2007).

The objective of this study was to evaluate the effect of *C. abyssinica,* succession culture with soybean in the reduction of SCN under field conditions.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Ipameri in the state of Goias, Brazil (latitude: 17°43'19" S, longitude: 48° 09'35" W, altitude: 764 m). In the previous crop season, soybean that was susceptible to *H. glycines* was grown in an area naturally infested with the species (890 cysts / 100 cm^3). The area was sampled to quantify the initial nematode population, after which *C. abyssinica* cv. FMS Brilhante was sown and variety of soybean NA7337RR (susceptible to SCN). During the crop cycle, soil and root samples were collected at 30, 60, and 90 days after planting (DAP), placed In labeled plastic bags and transferred to the Nematology Laboratory of the Federal Institute of Goias, Urutai Campus.

Female nematodes were collected by filtering the roots through 60 mesh sieves and washing them with a strong jet of water. They were subsequently transferred to beakers and counted using a stereoscopic microscope. To collect cysts from the soil, a 100-cm^3 aliquot was obtained and transferred to a 1-L becker, and the volume was completed with water. After 30 s, the supernatant was filtered using 20 mesh sieves about 60 mesh. This procedure was repeated 3 times, and after collection, the cysts were quantified using a stereoscopic microscope. The parameters evaluated were the number of adult females in the roots and the number of cysts in the soil.

Once sampling was complete, the reproduction factor (RF) of the SCN in *C. abyssinica* plants and for soybean variety NA7337 was calculated as RF = fp / ip, where RF is the reproduction factor, fp is the final population of the nematode, and ip is the initial population of the nematode (previous sampling). After harvest, the *C. abyssinica* plant residue was incorporated into the soil, and the second part of the experiment was performed. Four treatments were adopted: Soybean AS3730 and NS5959 (both without information on the SCN behavior), NA7337RR (susceptible to SCN), and P98Y51 (resistant to SCN races 1 and 3) in 6 replicates, totaling 24 plots, in a randomized block design. These treatments were adopted for to check if crambe had nematicide effect on the population of *H. glycines* or just nematostatic effect. Using variety with different behaviors in relation to *H. glycines*, the influence of root exudates in the population of this nematode was observed. This exudation favors the outbreak of juvenile when using susceptible variety.

Each plot consisted of 6.6 m long rows. The 2 end rows served as borders; the 2 central rows were used to evaluate productivity, and the 2 remaining rows were used to evaluate the SCN population. Two subsamples were collected from each row, totaling 4 subsamples per plot, and each sample contained both soil and roots.

The evaluations were performed 30, 60, and 90 days after sowing, and the samples were placed in labeled plastic bags and transferred to the Nematology Laboratory of the Federal Institute of Goias, Urutai Campus. The evaluated parameters were the number of female nematodes in the roots and the number of viable cysts in the soil, as previously detailed.

The data were subjected to analysis of deviance (ANODEV), and regression models were fit with predictors containing a simple linear effect. The Poisson distribution (Poisson regression) was assumed for the variables of number of female nematodes, eggs per female, and number of cysts, and the nominal level of significance was set at 5%. All analyses were performed using R software version 3.0.3 (Team RC, 2014).

RESULTS AND DISCUSSION

There was a linear effect ($p < 0.001$) of the duration of the plant in the field on the number of female SCNs in the roots (Figure 1A). After 30 days, the mean number of female nematodes was approximately 9-fold greater than at 90 days, indicating that the SCN population decreased over time.

In contrast to crambe, the population of *H. glycines* females in soybeans remained high in the three evaluation periods. It is higher at 60 days after planting. The number of cysts significantly increased over time. Going from 300 cysts/100 cm^3 soil at 30 days after planting to 907 cysts/100 cm^3 soil at 90 days after planting (Table 1).

Table 1. Population of females de *H. glycines* and cysts in crambe crop and soybeans at 30, 60 and 90 days after planting.

Assessment	Crambe			Soybean		
	Days after planting			Days after planting		
	30	60	90	30	60	90
Females (10 g/root)	68	13	7	590	775	580
Cysts (100 cm^3)	121	91	19	300	501	907

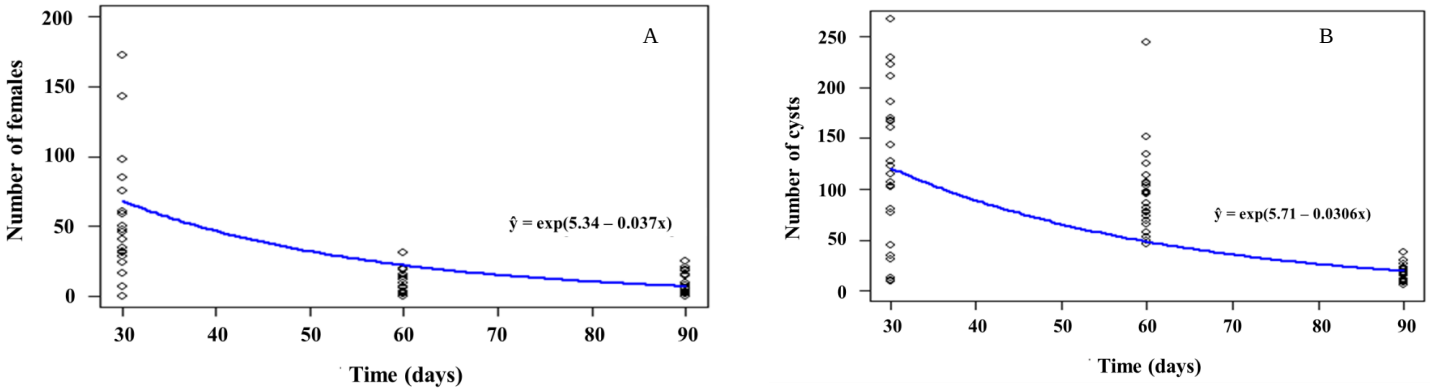

Figure 1. Analysis of deviance. (A) Number of female *H. glycines* as a function of the duration of *C. abyssinica* in the field. (B) Number of cysts as a function of the duration of *C. abyssinica* in the field.

A linear effect ($p < 0.001$) of the duration of the crops in the field on the number of cysts was also observed, but this relationship was inversely proportional (Figure 1B). After 30 days, the mean number of cysts was approximately 6-fold greater than at 90 days, following the same trend as the number of female nematodes.

This result demonstrates the activity of *C. abyssinica* against SCN because the number of viable cysts decreased from 890 cysts / 100 cm^3 to approximately 25 cysts / 100 cm^3 during the 90 days of cultivation. Similar results were found by Zasada and Ferris (2003), who observed a lethal effect of this crop on *H. schachtii* and *Globodera rostochiensis*, which was associated with compounds originating from the isothiocyanates.

After the crop residues were incorporated into the soil, the effect of the interaction ($p < 0.001$) between crop duration and variety on the number of adult females was evaluated in 10 g of roots (Figure 2). At 30 days, the mean number of female nematodes in variety AS3730 IPRO was lower ($p < 0.05$) than that in the other varieties, but no significant differences ($p > 0.05$) were observed between varieties NS5909 (without information on the SCN behavior), NA7337RR (susceptible on the SCN behavior), and P98Y51 (resistant on the SCN behavior). At 60 days, there was a significant difference ($p < 0.05$) among the varieties, and the number of female SCNs was higher in variety NA7337RR (susceptible). At 90 days, there were no significant differences ($p > 0.05$) among varieties NS 5959, AS3730, and NA7337RR, which indicates their relative susceptibility to SCN. However, the number of adult females remained low in all cultivars and times evaluated, indicating the harmful effect of *C. abyssinica* on female SCNs.

Wu et al. (2011) emphasize that the disadvantage of performing crop rotation with *Brassica* to control nematodes is that the levels of isothiocyanates released into the soil are unknown. In contrast, Zasada and Ferris (2003) evaluated the effects of various isothiocyanates on *Meloidogyne javanica* and *Tylenchulus semipenetrans* and found that low concentrations of these compounds (0.025 µmol / mL to 0.045 µmol / ml) decreased nematode populations. Zasada and Ferris (2009) worked with *M. incognit*a and managed to reduce the number of second stage juveniles, the egg mass, and the gall index using a concentration of 0.03 mmol / mL of isothiocyanate.

Our results indicate that *C. abyssinica* decreased the nematode population for up to 30 days after its cultivation and that the use of resistant plant varieties maintained the number of adult females at low levels. This result confirms the resistance of variety D (P98Y51), which linearly decreased the female population for 90 days. Poromarto and Nelson (2010) evaluated the invasion potential of *H. glycines* in several plant crops and found that SCN populations did not grow in *C. maritima*. This result corroborates the findings of the present study by inferring that *Crambe* sp. are poor hosts of and active against *H. glycines*. A similar result was observed by Wu

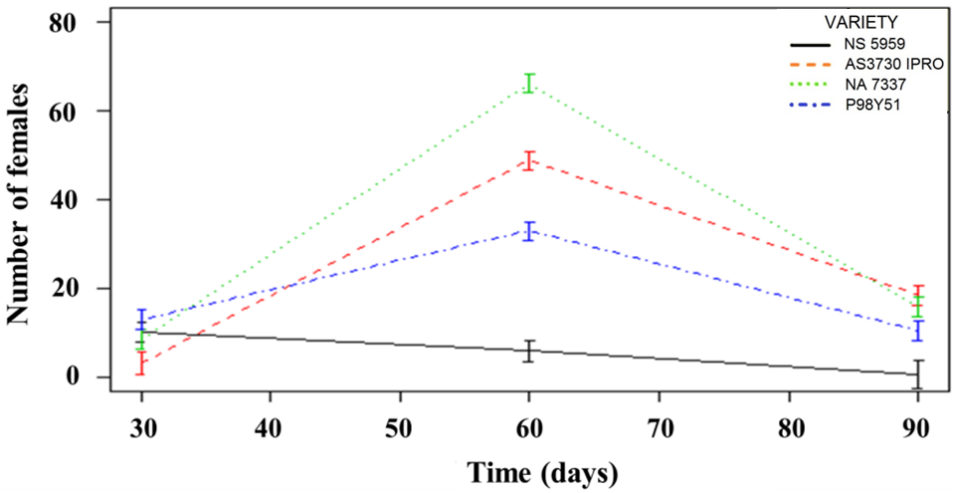

Figure 2. The number of female SCNs and the 95% confidence intervals for each combination of plant variety and duration in the field. Soybean varieties: NS5959; AS3730; NA7337RR; and P98Y51.

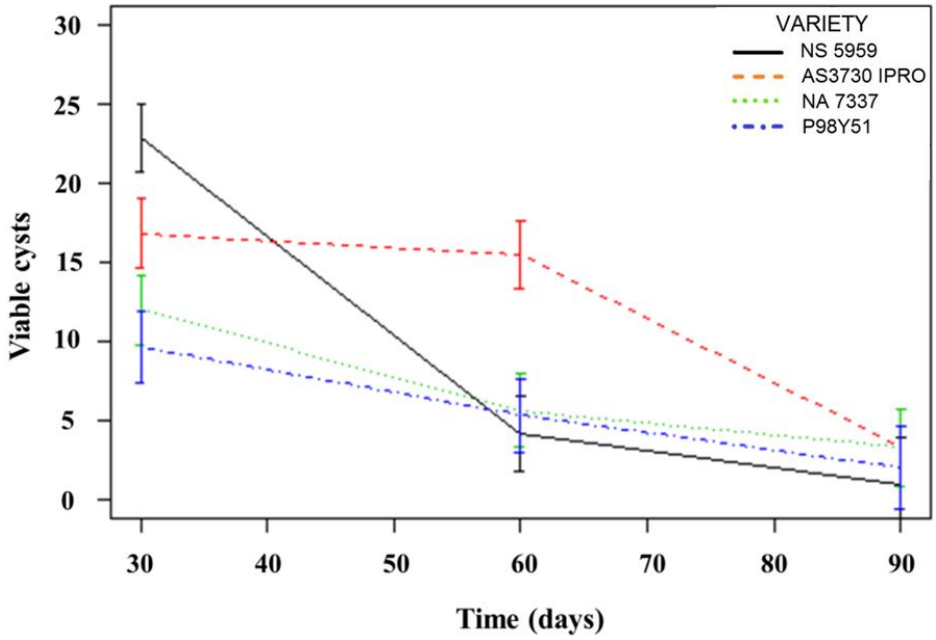

Figure 3. 95% confidence interval for each combination of plant variety and duration in the field. (A) Number of viable cysts. NS5959; AS3730; NA7337RR; and, P98Y51.

et al. (2011), who evaluated the mortality rate of second-stage juvenile *M. javanica* and found that it increased with the duration of exposure to *C. abyssinica* metabolites. The authors attributed this mortality to the presence of aliphatic isothiocyanates formed by the hydrolysis of glucosinolates.

Our results indicated a decrease in the number of viable cysts in all plant varieties from 30 to 90 days (Figure 3A). At 30 days, resistant variety D (P98Y51) and susceptible variety C (NA7337RR) had the lowest number of viable cysts ($p > 0.05$), while the varieties A (NS5959) and B (AS3730 IPRO) had the highest.

After 60 days, variety B (AS3730) differed significantly from the remaining varieties and had the greatest number of viable cysts, and at 90 days, the total population of viable SCN cysts was low, with no significant difference among the crop varieties. This result emphasizes the value of *C. abyssinica* in the control of *H. glycines*

because prior sampling indicated a population of 890 viable cysts / 100 cm^3. The number of female nematodes and cysts decreased in the presence of *C. abyssinica* in the field and in the subsequent soybean.

The harmful effect of *C. abyssinica* against many pathogens has been reported in the literature, and this effect has always been associated with the presence of isothiocyanates. Schroeder and MacGuidwin (2010) evaluated the LD_{50} and LD_{95} of isothiocyanates for juvenile *H. glycines* and found that even low concentrations are toxic to SCN populations.

Conclusion

The cultivation of *C. abyssinica* and the incorporation of its crop residues into the soil promotes biofumigation, which decreases the population of *H. glycines*.

Conflict of interests

The authors have not declared any conflict of interests.

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Evaluation of a lateral flow device for in-field detection of Banana Xanthomonas Wilt and its application in tracking the systemicity of *Xanthomonas campestris* pv. *musacearum*

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Early detection of Banana Xanthomonas Wilt (BXW) in the field and immediate destruction of infected plants or plant tissue are key control methods to prevent the introduction and spread of BXW. This requires rapid, cost-effective and an on- site diagnostic tool to detect the bacterium, *Xanthomonas campestris* pv *musacearum* (*Xcm*). Polymerase chain reaction (PCR) detection technique for BXW is efficient but requires expensive equipment and knowledgeable expertise; this limits PCR application to the laboratory. This study therefore was carried out to evaluate the enzyme-linked immunosorbent assay (ELISA) tool configured as a lateral flow device (LFD) for detection of *Xcm*. Studies on the systemicity of *Xcm* in banana were carried out using the BXW-LFD in a field trial of 300 banana plants of Pisang Awak inoculated with the *Xcm* at Kiifu Forest, Mukono District, Uganda. Pseudo-stem samples from symptomatic and asymptomatic suckers were collected and tested with the LFD and the results compared with conventional PCR using the GspDm BXW primers. The LFD was able to detect *Xcm* 3 days post inoculation (dpi), 2 cm above and below inoculation site, 15 to 35 days in the pseudo-stem, 35 to 42 days to reach the corm and 81 days in the lateral roots. The rate of *Xcm* movement in banana was found to be sigmoid in nature, leveling off as the bacteria moved down the pseudo-stem towards the corm. Conventional PCR was only 24% more sensitive than the LFD. The use of the BXW LFD can therefore boost BXW control measures through improved surveillance and quarantine services to arrest the introduction and spread of the disease within and between national borders.

Key words: Banana Xanthomonas Wilt lateral flow device (BXW LFD), Banana Xanthomonas wilt, *Xanthomonas campestris* pv musacearum, complete systemicity, incomplete systemicity, lateral flow device.

INTRODUCTION

Banana *Xanthomonas* wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm*) is a

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serious threat to the livelihood of millions in East and Central Africa, who depend on banana (*Musa* spp.) for food and income (Tushemereirwe et al., 2003). The disease causes up to 100% yield loss, attributed mainly to rapid death of the mother plant and premature fruit ripening and subsequent rotting of the bunch that renders the fruit completely inedible (Tripathi et al., 2009a). Additional symptoms of BXW include the progressive yellowing and wilting of leaves, shriveling and blackening of the male buds. None of the grown cultivars are resistant to BXW, although East African highland cultivars (AAA) are significantly less affected compared to Sukali Ndizi (AAB) and Pisang Awak (ABB) (Ssekiwoko et al., 2006a; Karamura et al., 2010). The causal bacterium (*Xcm*) was first detected on ensete (*Ensete ventricosum*), a close relative of banana that is native to the highlands of Ethiopia (Yirgou and Bradbury, 1968). Later it was reported in Central Uganda in 2001, and subsequently in other banana-growing areas of the Great Lakes region (Tushemereirwe et al., 2003; Ndungo et al., 2006; Reeder et al., 2007; Carter et al., 2010). Disease mainly spreads within fields primarily by insects such as bees, wasps, and cockroaches that frequently visit the male inflorescence while foraging for nectar. In the process, their limbs and appendages come in contact with bacterial ooze that is exuding from fresh scars left by the dehisced floral bracts (Tinzaara et al., 2006).

Field studies reveal that once inside the inflorescence, *Xcm* spreads systemically along the true stem downwards to the corm and subsequently into the entire root system (Ssekiwoko et al., 2006b; 2010). The infection process via the inflorescence to corm takes about 28 days, although the bacteria can survive in lower parts of the mat for over two years without causing visible symptoms (Ocimati et al., 2013a). Such prolonged durations of latency present a major concern, especially in the light of recent disease resurgence in growing areas wherein it had been contained (Tinzaara et al., 2013). Research shows that latent infections in host tissues can result in long distance spread through movement of infected planting materials (Mwangi, 2007; Lewis Ivey et al., 2010). Notably, inoculum concentrations below 10^4 colony-forming units per milliliter (cfu mL^{-1}) have great potential of causing latent infections (Ochola et al., 2014). Accurate detection of BXW is critical, since prompt deployment of cultural control options is strongly associated with faster subsidence of symptoms and reduced spread across fields in smallholder banana systems.

Majority of smallholder farmers in East and Central Africa rely on visual symptom expression for disease diagnosis. Unfortunately, studies reveal that by the time symptoms are expressed, *Xcm* is already fully established itself in the plant. Besides, symptoms are non-specific and quite easy to confuse with infections by Fusarium wilt (*Fusarium oxysporum* fsp. *cubense*), Moko disease (*Ralstonia solanacearum*) and Blood disease (*Pseudomonas solanacearum*). This consequently results in incorrect recommendations and deployment of inappropriate disease management options. Culture-based methods are not appropriate for a large number of samples in a short period of time, because it is time consuming. In addition, culture-based techniques are prone to give false negative results when used to assess latently infected plants due to the low bacterial load (Mwangi, 2007; Tripathi et al., 2007). Recent developed molecular approaches, that is, (Polymerase chain reaction) PCR need only slight amount of DNA for detection, however they are often costly, time consuming, and require tissue or DNA samples to be transported to the laboratory (Aritua et al., 2008; Lewis Ivey et al., 2010; Adikini et al., 2011; Adriko et al., 2011).

Multi-stakeholder efforts to address these limitations occasioned in the development of a novel, easy-to-use lateral-flow-device (LFD) for quick and accurate onsite detection of *Xcm* (Hodgetts et al., 2014). The immuno-chromatographic *Xcm*-LFD incorporates a polyclonal antibody (PAb) that detects a specific epitope on a glycoprotein antigen secreted during the active growth of *Xcm* inside the banana plant. In a positive reaction, *Xcm* binds with the PAb and conjugate-coloured particles to give a coloured complex seen as a line. The conjugate-coloured particles also migrate and bind with a second fixed control, hence the two observed coloured bands. Screenhouse experiments have proven that the *Xcm*-LFD takes guesswork out of disease identification and reduces the need for unnecessary laboratory testing of banana specimens (Hodgetts et al., 2014). As a basis of this study, the *Xcm*-LFD was evaluated to assess its application in field tracking *Xcm* spread among suckers emerging from infected mother plants and to generate knowledge on its capacity to detect latent infections in banana.

MATERIALS AND METHODS

Study site

The study was conducted at Kifu Forest Reserve (00°28'N and 32°44'E, 1250 m.a.s.l.) the only location in Uganda designated for controlled BXW epidemiological studies. Generally, the thick forest provides perfect seclusion from neighbouring farmers' fields, which minimizes any long-distance vector transmission (Ochola et al., 2014). The climate is warm-humid with an average temperature of 25°C and precipitation of 1560 mm per annum distributed in two seasons (March-June and August-November). The site is located in a crystalline basement characterized by metamorphosed granites and quaternary alluvial and lacustrine deposits. Kifu soils are mainly ferralsols with gleysols in the swamps (Okorio, 2000).

Experimental design and treatments

Tissue culture-derived plants of Pisang Awak (ABB-genome) were established at a spacing of 3 × 3 m in a newly opened field where banana has never been planted. This ensured that the subsequent infection symptoms are not the outcome of *F. oxysporum* fsp.

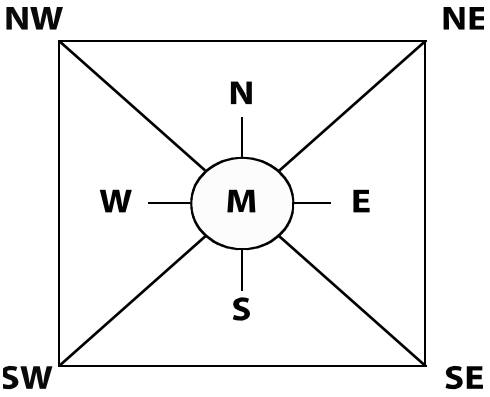

Figure 1. Orientation of treatments (NE, SE, SW and NW) with respect to the inoculated mother plant (M).

cubense. The field experiment consisted of four treatments and uninoculated control laid out in a randomized block design (RBD) with three replications. The four treatments were defined by the north-east (NE), south-east (SE), south-west (SW) and north-west (NW) orientation of the inoculated leaf petiole on the mother plant (Figure 1). The treatments and control (20 plants each) were randomly assigned per replication (that is, each replication = 100 plants). The chronological pattern of sucker emergence was closely monitored and each sucker geographically mapped in respect to its position on the mother plant.

Bacterial cultures and inoculum preparation

Bacteria were obtained from fresh bacterial ooze cultured and grown at 24°C for 72 h, on a semi- selective growth media, cellobiose cephalexin agar (CCA) (Mwebaze et al., 2006) containing yeast extract (1 gL^{-1}), glucose (1 gL^{-1}), peptone (1 gL^{-1}), NH_4Cl (1 gL^{-1}), $MgSO_4.7H_2O$ (1 gL^{-1}), K_2HPO_4 (3 gL^{-1}), agar (14 gL^{-1}), beef extract, (1 gL^{-1}), cellobiose, (10 gL^{-1}), cephalexin (40 mgL^{-1}), 5-fluorouracil (10 mgL^{-1}) and cycloheximide (120 mgL^{-1}). Pure *Xcm* colonies were harvested into sterile water and with optical density at 600 nm adjusted with sterile water to 0.5 *c.* 1×10^8 colony-Forming-units (cfu) mL^{-1} using a spectrophotometer (Biomate-3, Thermo Electron Corporation, USA).

Inoculation of banana plants

The point of inoculation on the petiole of the youngest leaf was cleaned with 95% ethanol and 1 mL of the bacterial cell suspension injected into the plant with an insulin syringe (Micro-Fine Plus, 0.33 x 12.7 mm, Beckton Dickinson, USA) (Ochola et al., 2014). Information on *Xcm* colonization of distal parts of banana including the suckers was collected for a period of two months.

Tracking the movement of *Xcm* in banana

Tracking the movement of *Xcm* began 2 days post inoculation (dpi). This was done by first measuring 2 cm from the point of inoculation downwards direction and cutting deep into the leaf stalk/pseudostem using a sterile knife. The plant tissue is then placed into the extraction buffer bottle that comes with the LFD kit and shaken for a minute. A drop of the tissue-extraction buffer mixture is placed onto the LFD and results read 5 min after. Blue twin bands indicated that the sample was positive for *Xcm* and one single band means the sample was negative for *Xcm*. Tracking *Xcm* was done every two days.

Detection of *Xcm* in suckers

Corm and pseudostem tissues were collected aseptically and tested from each sucker using a knife sterilized in sodium hypochlorite (NaOCl) to avoid cross contamination. Symptomatic and asymptomatic suckers whose samples tested positive were labeled, put into sampling bags, and taken to the laboratory for PCR confirmation using GspDmF/R primers (Adriko et al., 2011). These same suckers were also visually diagnosed (Visual Diagnosis, plants were determined to be unhealthy or healthy based on the symptoms seen) and any symptoms were noted down for purposes of comparison with LFD and PCR test results.

Relative sensitivity and specificity of LFD and PCR

Sensitivity, in this experiment was the probability that a test result will be positive when the disease is present (true positive) while specificity was the probability that a test result will be negative when the disease is not present (true negative). Sensitivity was calculated as true positive/ (true positive + false negative) while specificity was calculated as true negative/ (true negative + false positive).

Genomic DNA extraction

Plant genomic DNA was extracted using the CTAB method (Murray and Thompson, 1990). 300 mg of banana pseudo-stem tissue was frozen with liquid nitrogen and ground to a fine paste using mortar and pestle. The fine powder was then placed in 2 ml eppendorfs and 500 µl of CTAB buffer (2.0 g CTAB (Hexadecyl trimethyl-ammonium bromide), 1 M Tris pH 8.0, 0.5 M EDTA pH 8.0 (Ethylenediaminetetra Acetic acid Di-sodium salt) and 5 M NaCl, at pH 5) added to the mixture. The CTAB/plant extract mixture was then incubated in a water bath at 65°C for 30 min. 1.5 µl RNase at 10 mg/ml was added to each tube and incubated at room temperature for 10 min. The eppendorfs were then placed in the centrifuge to spin at 12000 rpm for 5 min to bring down cell debris. The supernatant transferred to clean eppendorfs and to each tube 250 µl of Chloroform: Iso Amyl Alcohol (24:1) added and the solution mixed by inverting. After mixing, the tubes were spun at 13000 rpm for 1 min. The upper aqueous phase that contains the DNA was then transferred to a clean tube and to each tube 5 µl of 7.5 M Ammonium Acetate was added followed by 500 µl of ice-cold absolute ethanol. The tubes were inverted several times slowly to precipitate the DNA for 5 min and then centrifuged at 13000 rpm for 1 min to pellet the DNA. The extraction yielded was 50 ng/µl of DNA. The DNA was then resuspended in sterile DNase free water of 100 µl and stored at 4°C.

PCR and cycling conditions

To test for the presence of *Xcm* in the plant samples, conventional PCR was run against the plant DNA samples using primers GspDm-F/R (Adriko et al., 2011). PCR reactions were composed as follows: each 25 µl PCR reaction contained 1 µl of DNA template, 12.5 µl of 2 × PCR Master Mix (GoTaq), 1 µl of each forward and reverse primer from a stock of 10 µM and 9.5 µl of nuclease-free water. Water was used as a non-template control (in the place of DNA) for each run. The PCR reactions were performed in a

GeneAmp PCR system 9700, Applied Biosystems. PCR amplicons of 5 µl were then separated by agarose gel electrophoresis in 1% agarose gels in 1x TBE (Tris/borate/EDTA) at 130 V for 60 min. O'gene ruler 1kb plus DNA ladder was used to evaluate the sizes of the PCR amplicons under ethidium bromide. 250 bp PCR amplicons were expected for any positive result.

RESULTS

Symptom expression after artificial inoculation

Wounding is essential for entry of *Xcm* into banana whether by vector-mediation via the inflorescence or through contaminated tools. The inoculated sites at the leaf stalk showed signs of cell death, typical of hypersensitive reactions to the bacteria. About 28% inoculated mother plants displayed typical BXW symptoms on their leaves. The incubation period ranged between 14 and 21 days post inoculation (dpi). Although *Xcm* is also introduced in intensively managed systems by contaminated tools, data reveals that artificial wounding followed by pathogen introduction may not necessarily result in disease.

Tracking the movement of *Xcm* in banana

The LFD allowed for screening of a reasonable number of plant samples in the field without need for movement of infected banana tissues for laboratory testing. Results of testing were achieved in 5 minutes. LFD was deployed for the determination of the speed of movement of *Xcm* from the inoculated leaf petiole downwards to the corm. The device was sensitive to detect *Xcm* within 2 cm proximity of the site of infection after 3 days post inoculation (dpi). The bacteria was noticed to move at 0.4 cm daily for the initial 12.5 days post inoculation (dpi), thereafter the rate of movement tended to rapidly increase at 20.5 dpi from 3.2 to 7.8 cm at 32 dpi before starting to level off. Since the distance between inoculated petiole and corm among plants was fairly uniform (280-320 cm), *Xcm* was predicted to rich the corm from between 35 and 42 dpi. The pattern of *Xcm* movement downwards the infected mother plant was sigmoid in nature.

Xcm colonization of lateral shoots

BXW symptom expression on lateral shoots was observed from 81 days onwards. Moreover, in some instances all suckers on the mat remained asymptomatic even after the demise of the mother. In general, the pattern of spread from the infected mother to lateral suckers was however random irrespective of the orientation of wounding site for *Xcm* to quickly access nutrients.

Comparison of the visual, LFD and PCR diagnosis of *Xcm*

On-site testing of 16 randomly selected mats confirmed a random pattern of BXW transmission from infected mother to suckers. Testing of the asymptomatic and symptomatic suckers proved that LFD and PCR were more sensitive than using visual diagnosis (VD).

LFD and PCR testing relative to VD

Out of 83 pseudo-stem samples from the suckers, 30 were positive and 53 negative by LFD while 21 were positive and 62 negative by PCR. Of the 30 that were positive by LFD, only 28 were diagnosed as positive by VD. Of the 53 that were negative by LFD, 51 were diagnosed as negative VD. Of the 21 that were positive by PCR only 16 had diagnosed as positive by VD. Of the 62 that were diagnosed as negative by PCR, only 48 were negative by VD (Table 1).

Relative sensitivity and specificity of LFD and PCR

Of the 30 that were positive by LFD, 17 were positive by PCR while of the 53 negative by LFD, 49 were found to be negative by PCR. Of the 21 positive by PCR, 16 were positive by LFD and of the 62 negative by PCR, 48 were negative by LFD. PCR detection of *Xcm* by the primers GspDmF/R was 24% more sensitive than the LFD. However the LFD was 13% more specific than the PCR (Table 2).

DISCUSSION

Xcm infection and tracking the movement of Xcm

A scenario in which artificial wounding and introduction of *Xcm* into the leaf petiole does not result in symptom expression and disease, suggests that infection via wounding may actually be passive or accidental. In this study, localized cell death in response to wounding and pathogen invasion of the leaf petiole was typical of hypersensitive (Hpr) defense mechanism of the plant. Specifically, restricted movement or entrapment of bacteria within 2-cm diameter of the site of inoculation is consistent with secretion of reactive oxygen species (ROS) that play a major role in signal transduction pathway that elicits programmed cell death. Studies show that when induced responses occur very early, Hpr is of great benefit to the plant, and reduces the subsequent pathogen attack (Heath, 2000; Gechev et al., 2006). Moreover, the transient nature of Hpr-mediated cell death portrayed by a later rapidity in *Xcm* movement suggests that the bacterium was able to totally decompose the

Table 1. Sensitivity and specificity of LFD and PCR relative to VD.

LFD vs VD and PCR vs VD	LFD	VD	95%CI	PCR	VD	95% CI
Total = 83						
+	30	28		21	16	
-	53	51		62	48	
Sensitivity	TP/(TP+FN)*100 28/(28+2))*100 = 93.3%		77.89 - 98.99%	TP/(TP+FN)*100 16/(16+5)*100 = 76.19%		52.83 - 91.69%
Specificity	TN/(TN+FP)*100 51/(51+2)*100 = 96.23%		87 - 99.43%	TN/(TN+FP)*100 48/(48+14)*100 = 77.42%		65.02 - 87.06%

LFD, Lateral flow device; VD, visual diagnosis; PCR- polymerase chain reaction; CI, confidence interval; TP, true positive; FP, false positive; TN, true negative; FN, false negative. + means pseudo stem samples that tested positive while – means pseudo stem samples that tested negative. Note that sensitivity = true positive/ (true positive + false positive), specificity = true negative/ (true negative + false negative).

Table 2. Comparison of sensitivity and specificity relative to PCR and LFD.

LFD vs PCR and PCR vs LFD	LFD	PCR	95%CI	PCR	LFD	95% CI
Total = 83						
+	30	17		21	17	
-	53	49		62	49	
Sensitivity	TP/(TP+FN)*100 17/(17+13)*100 = 56.67%		37.44 - 74.52%	TP/(TP+FN)*100 17/(17+4)*100 = 80.95%		58.08 - 94.44%
Specificity	TN/(TN+FP)*100 49/(49+4)*100 = 92.45%		81.77% - 97.86%	TN/(TN+FP)*100 49/(49+13)*100 = 79.03%		66.81 - 88.33%

LFD, Lateral flow device; VD, visual diagnosis; PCR- polymerase chain reaction; CI, confidence interval; TP, true positive; FP, false positive; TN, true negative; FN, false negative. + means pseudo stem samples that tested positive while – means pseudo stem samples that tested negative. Note that sensitivity = true positive/ (true positive + false positive), specificity = true negative/ (true negative + false negative).

oxidative activity of associated ROS.

The infection pathway in inflorescence-infected plants involves the systemic movement of *Xcm* along the true stem, into the leaf sheaths then downwards to the corm and subsequently into entire root system (Ssekiwoko et al., 2006; Ocimati et al., 2013a). However, despite being equally systemic, leaf petiole-internal spread was actually bi-directional within proximity of the area of infection. Internally, banana petioles are pierced by large air canals that are separated by narrow longitudinal partitions and lateral plates of stellate parenchyma that contain very few vascular bundles (Ennos et al., 2000). This most likely allows for the pathogen to rapidly spread and multiply in adjacent parenchyma tissues and intercellular spaces. Our findings show that the *Xcm* bacterium takes about 39 days to travel from the point of inoculation to reach the corm, and an extra 42 days for expression of typical symptoms in lateral shoots. These results contravene the claim that symptom development in attached lateral shoots may take up to 24 months (Ocimati et al., 2013a). The predicted increased systemicity under tool-mediated infections is likely to have severe implications on successful disease control particularly in intensively managed smallholder systems with relatively high probability for deployment of contaminated cutting tools.

Random nature of infection of lateral roots

Astoundingly some lateral shoots emerging from infected mother plants remained disease free - a phenomenon now referred to as incomplete systemicity (Ocimati et al., 2015; Sivirihauma, 2013). Insights into the causes of incomplete systemicity apparently revealed that the bacterium was capable of surviving for long periods in lower parts of the mat without causing visible symptoms (Ocimati et al., 2013a). Latent infection of plants by pathogens has been long recognized (Gäumann, 1951). Most latent pathogens tend to persist and later produce symptoms of disease when prompted by environmental or nutritional conditions or changes in phenology of the host (Agrios, 2005). Although this study has not corroborated the occurrence of latent infections, it has reliably confirmed the random nature of *Xcm* spread to lateral shoots emerging from previously infected mother. A number of reasons could account for this observation:

(1) Presence of other microbes and endophytes in the corm hence competition for nutrients, and (2) existing differences in the type of plant cells found in corm making it difficult for *Xcm* to move. However, according to Ssekiwoko et al. (2006), *Xcm* does not actually spread easily once in the inner cylinder and cortex of the corm. Ocimati et al. (2013b), also went on to prove that there was actually low transmission efficiency of *Xcm* from the corm or corm roots.

LFD application in the use of single diseased stem removal (SDSR)

Consequently, proposed a less labor intensive novel control is in practice known as the single diseased stem removal (SDSR), involving the removal of only the visibly diseased plants on the mat in order to reduce the inoculum level and lower disease incidence to an acceptable level. In context of latency and random infection pattern of lateral shoots, reliance on disease symptom expression to manage the XW is not sufficient (Ocimati et al., 2013a). Field application of SDSR requires early detection of BXW symptoms and tracking the systemic movement of the bacteria towards the corm. This study has demonstrated the LFD to be an essential field diagnostic kit that can be used to guide the implementation of SDSR. Since it takes about 14 to 21 days for symptom expression, and 35 to 42 days for *Xcm* to get to the corm, it is recommended that farmers deploy the LFD the moment foliar symptoms are expressed to first ascertain how far the bacterium has moved down the pseudostem towards the corm. Thereafter, SDSR must be implemented provided the bacteria have not reached within 45 cm from the corm.

However, this study has also revealed that the efficacy of SDSR in preventing further colonization of the mat is likely to be undermined by the random pattern of *Xcm* expression in lateral shoots. Since latently infected plants appear asymptomatic before diagnosed by traditional symptoms, culture-based techniques and the lateral flow device (LFD).

LFD sensitivity and specificity

Xcm was detected 3 dpi and in respect to the point of inoculation (2 cm from the point of inoculation) by the LFD, it is safe to assume that the LFD is sensitive enough to detect even smaller concentrations of *Xcm* bacterial inoculum in any suspected plant tissue. This is especially in regards to the fact that transmission of the *Xcm* within fields is commonly by contaminated tools and insect vectors that are suspected of carrying bacteria inoculum of more than a concentration of 10^8 cfu/ml or as low as 10^4 cfu/ml respectively. This level of LFD sensitivity was proven useful where *Xcm* was detected in asymptomatic plants. This is important for early detection of BXW in the field and immediate destruction of the affected plant, as a key control method of BXW.

Conventional PCR detection by GspDmF/R primers (Adriko et al., 2011) was found to be more sensitive than the LFD detection. This should be expected as studies have shown PCR to be at least 10 times more sensitive than antibody based methods of detection (Omrani et al., 2009) and is usually the confirmatory test. However due to its high sensitivity PCR is also able to pick up more false positives and negatives than antibody based methods (Alvarez and Kaneshiro, 1999; Kaneshiro, 2003). In some instances antibody based methods or immunodiagnostic assays can be used a confirmatory tests next to PCR (Alvarez, 2004). The LFD was only 24% more sensitive than the PCR and is therefore still reliable and precise enough to be used in the field. PCR detection of *Xcm* can then be used as a confirmatory test of the LFD.

Management of BXW using the cultural methods such as destruction and infected plant materials and use of clean plant materials has only slowed down the spread of the disease (Karamura et al., 2010; Tinzaara et al., 2009). The LFD will be useful in the integrated approach that not only involves cultural control methods but also surveillance of disease outbreak and routine screening at borders. The LFD provides rapid detection of the *Xcm* pathogen without the need for the laboratory environment. Quick results of the detection are achieved in less than 10 min.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of compost-biochar mixes and irrigation on the growth and yield of Amaranthus (*Amaranthus hybridus*) under two growing temperatures

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An experiment was carried out to study the sensitivity of *amaranthus* to different sources of soil nutrients and different amounts of irrigation water at different temperatures. Nitrogen (N) rich materials (compost/poultry manure) and carbon (C) rich material (biochar) used included poultry manure + rice husk biochar (PM+RB), poultry manure + sawdust biochar (PM+SB), rice husk compost + rice husk biochar (RC+RB), sawdust compost + sawdust biochar (SC+SB) mixed at 10 ton ha^{-1} N rich material to 5 ton ha^{-1} C rich material. Rice husk compost only, Sawdust compost only (at 10 ton ha^{-1} for each of RC and SC), NPK (400 kg ha^{-1}) and no amendments as Control were also used. Two irrigation amounts (0.1124 mm and 0.225 mm per pot), were imposed resulting in 12 treatment combinations, in a completely randomized design with 4 replicates. The experiment was repeated under two different temperatures of 37 and 30°C in the glass house and pot house, respectively. Data on growth, yield, water use and nutrient leaching were collected. PM+RB produced the tallest plants (31.67 cm) with 0.1124 mm irrigation at 30°C. PM+SB treated plants had more leaves (17) with 0.1124 mm amount of irrigation water at 37°C. NPK treated plants gave the highest stem girth (5.87 cm) and highest SPAD value (42.5%) with 0.1124 mm amount of irrigation water at 37°C. Leaf area index was highest (43) at 30°C for plants receiving NPK and 0.225 mm amount of irrigation water. NPK treated plants gave the highest fresh biomass of 36.93 g at 30°C but lowest biomass (13.01 g) at 37°C. PM+SB gave the highest fresh biomass weight of 16.7 g at 37°C and highest volume of leachate (123 ml) with 0.225 mm irrigation water at 30°C. At 37°C, SC gave the highest leachate volume (166 ml). The study indicates a good potential for sustaining crop yield with organic materials under increasing temperature and declining water resources that may be associated with changing climate.

Key words: *Amaranthus*, compost, biochar, climate change, irrigation frequency.

INTRODUCTION

Many countries in Africa have experienced rapid growth and diversification of agricultural production due to demands from both domestic and global markets. As a consequence, interventions in Africa have focused on

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exotic fruits and vegetables, for export markets. A serious downside to replacement of native crops by globally marketable crops is a reduction in diversity of seed stocks and vulnerability of cropping systems to climate variability. African Indigenous Vegetables (AIVs) or nutriceutical plants including *amaranthus* play a significant role in the health and food security of the underprivileged in both urban and rural settings. Surveys in East Africa show that AIVs display a higher profitability than exotic vegetables, and production of AIVs is highly relevant for small-scale farmers especially women as they require little financial input compared to exotic vegetables (Shackleton et al., 2009). The market potential for AIVs is very good. Several studies have highlighted great potential of AIVs especially due to their natural adaptation to the local conditions (Smith and Eyzaguirre, 2007; Nyarko and Quainoo, 2012).

Although environmental conditions are known to influence crop growth and global climatic changes are assumed to worsen cropping conditions in sub-Saharan African with dry spells becoming more frequent, only one paper published recently deals with the impact of soil moisture stress on AIVs (Olufolaji and Ojo, 2010). A few others focused on nutrient management (Ojo et al., 2007). It can be concluded that most aspects of the cultivation or cropping systems of AIVs are heavily under emphasized. The rain-fed production systems found throughout Sub-Saharan Africa are considered the most vulnerable to climate changes; choice of cropping system will become a key sustainability parameter and AIVs are obvious choices. The success of AIVs is strongly dependent on its sensitivity to key environmental variables especially water and nutrients (Olufolaji and Ojo, 2010).

However, the response of AIVs to climate sensitive variables are mostly not comprehensively determined or documented. Under depleting water and nutrient resources, recovery of nutrients and water from organic residues could enhance agro-sustainability. Organic soil amendments are critical in the development of sustainable urban production systems because they are important sources of carbon and nitrogen (De Lucia et al., 2013; Yadav et al., 2011). They are known to be important in regulating pH and contaminants transport in the soil. Sustained application of organic soil amendments are reported to boost yields, enhance N use efficiency, build up organic matter and reduce the accumulations of NO_3-N, salts and environmental contaminants such as heavy metals (Liang et al., 2012). Sawdust and rice husk are organic materials that when composted with poultry manure becomes enriched with nitrogen needed by plants. When these materials are charred to form biochar, they become important sources of carbon that could be mixed with composted N rich materials for improved productivity and carbon sequestration. Furthermore, addition of biochar to N rich materials has been shown to reduce leaching of N in soils (Agegnehu et al., 2015; Dempster et al., 2012; Yuan and Xu, 2012). It would therefore be important to also study growth and yield of *amaranthus* under different temperatures, water, nitrogen and carbon sources. The main objective of this study was to determine the response of *amaranthus* to different compost and biochar mixes at different growing temperatures. A second objective was to determine the influence of the amount of irrigation water and its interaction with compost-biochar mixes on the leaching potential and water use efficiency during the growing period of *amaranthus*

MATERIALS AND METHODS

Description of study sites

The study was set up as a pot experiment at the University for Development Studies (UDS), Nyankpala campus, Tamale, Ghana, glass house (at an average day time temperature of 37°C) and pot house (at an average day time temperature of 30°C). The experiment was carried out from October, 2014 to December, 2014.

Soil was collected from Kamina urban garden site in Tamale. The soil physical and chemical properties are presented in Table 1. As shown in the table, the soil is sandy loam and slightly acidic. The level of N is very low (0.06%). The data suggest that organic matter content is low (as indicated by organic carbon level of 0.58%) and P is very limited.

Poultry manure (PM) produced from a layer farm at University for Development Studies (UDS), Tamale, Ghana, Nyankpala campus, and commercial sawdust compost (SC) and rice husk compost (RC) compost produced at UDS, Nyankpala campus were used as nitrogen based soil mixes. Rice husk biochar and sawdust biochar (used as carbon based mixes) were produced following the method of the Japan International Research Center for Agricultural Sciences (JIRCAS, 2010).

Experimental design and treatments

The treatment combinations was 8 × 2 (mixes × irrigation), resulting in a 16 treatment combinations with 4 replications in a completely randomized design (Table 2). The experiment was then set up in the glass house (at 37ºC), and the same set up was repeated in the pot house (at 30ºC). The 0.225 mm irrigation represents full irrigation and 0.1125 represents halve of the crop water requirement. The two temperature values are fairly representative of the average temperatures for the dry and wet seasons (respectively) in northern Ghana. Plastic pots of a top diameter of 25 cm, a bottom diameter of 15 m and a height of 20 cm were filled with good top soil after mixing with respective treatments.

Field management

The soil and compost were sieved through a 4 mm sieve prior to mixing. At the plant house, pots were given either 0.1125 or 0.225 mm depending on treatment at two days interval. At the glass house, pots were given either 0.1125 or 0.225 mm daily depending on the treatment). As a result of the higher temperature in the glass house, the media dried out faster and therefore the irrigation frequency was higher for the glasshouse environment. As shown in Table 1, soil texture and characteristics were also obtained using the hydrometer method (Milford, 1997). Each pot had three holes at the bottom to allow free drainage, however the pots were placed in

Table 1. Properties of the soil used for the experiment.

pH (CaCl2)	C (%)	N (%)	P (mg/kg)	C/N	Sand (63-2000 µm)	Silt (2-63 µm)	Clay (<2 µm)
5.22±0.15	0.58±0.08	0.06±0.01	3.79±0.04	10.15±0.71	34.17±4.40	58.88±4.55	6.58±0.19

Table 2. Experimental layout at the glass house* (37°C).

Nutrient mix	Replication 1		Replication 2		Replication 3		Replication 4	
	Irrigation mm		Irrigation mm		Irrigation mm		Irrigation mm	
	0.1125	0.225	0.1125	0.225	0.1125	0.225	0.1125	0.225
Poultry manure (10 ton ha^{-1}) + Rice husk biochar (5 ton ha^{-1})	T1R1**	T9R1	T1R2	T9R2	T1R3	T9R3	T1R4	T9R4
Poultry manure (10 ton ha^{-1}) + sawdust biochar (5 ton ha^{-1})	T2R1	T10R1	T2R2	T10R2	T2R3	T10R3	T2R4	T10R4
Sawdust compost (10 ton ha^{-1}) + sawdust biochar (5 ton ha^{-1})	T3R1	T11R1	T3R2	T11R2	T3R3	T11R3	T3R4	T11R4
Rice husk compost (10 ton ha^{-1}) + rice husk biochar (5 ton ha^{-1})	T4R1	T12R1	T4R2	T12R2	T4R3	T12R3	T4R4	T12R4
Sawdust compost only (at 10 ton ha^{-1})	T5R1	T13R1	T5R2	T13R2	T5R3	T13R3	T5R4	T13R4
Rice husk compost only (at 10 ton ha^{-1})	T6R1	T14R1	T6R2	T14R2	T6R3	T14R3	T6R4	T14R4
NPK (400 kg ha^{-1})	T7R1	T15R1	T7R2	T15R2	T7R3	T15R3	T7R4	T15R4
Control (no nutrient added)	T8R1	T16R1	T8R2	T16R2	T8R3	T16R3	T8R4	T16R4

*Same set up was repeated at the pot house (30°C). **Treatments were completely randomised. The treatment code T1R1 means Treatment 1 in replication 1. Same description is applicable to the other codes.

plastic basins to aid leachate collection. Three weeks old *amaranthus* seedlings were transplanted into each pot containing the respective organic mixes and irrigation treatments.

Data collection

Plant heights and number of leaves were measured following the methods of Abubakari et al., 2012. Stem girth was also measured using electronic calipers. Relative chlorophyll content {Soil Plant Analysis Development (SPAD)} was measured every weeks using a Minolta chlorophyll meter (model SPAD 502). Volume of leachate was measured using a measuring cylinder. All the parameters were measured at four weeks after transplanting (WAT). Fresh leaf and fresh root weight were measured as an above ground and below ground biomass respectively using an electronic balance at the end of the growing season (6 weeks after transplanting. Dry leaf weight and dry root weight were determined after oven drying leaves and roots biomass for 24 h at 60ºC.

Water use efficiency was calculated using the following formula:

Water Use Efficiency = Mb/Cw (kg/m^3)

Where: WUE=water use efficiency, Mb=Sum of weight (dry weight) leaves, stems, roots in kg and Cw=cumulative amount of water applied (m^3).

Laboratory and data analysis

Total N was determined using the Kjeldahl digestion method (Okelabo et al., 1993). Organic C was determined by the modified Walkley-Black Wet oxidation method as outlined by Nelson and Sommers (1982). Phosphate was determined by the colorimetery method (Watanabe and Olsen (1965). EC was determined by inserting the Electrode of the EC meter into the compost sample suspension (Rowell, 1994). Crison Basic EC meter CM39P was used for the determination of EC. Crison Basic pH meter, PH29P was used for the determination of pH. The concentrations of nutrients in compost and in soil samples (nitrate nitrogen, ammonia nitrogen) were done using UV/VIS Spectrophotometer. Nitrate as nitrogen was determined by the Hydrazine Reduction Method (Cataldo et al., 1975). Ammonia as ammonia nitrogen was determined by the indophenol blue method (Koroleff, 1976). Chemical analysis of the compost and biochar was carried out before transplanting (Table 3). Genstat version 9.2 was used to carry out the ANOVA for the data generated.

RESULTS

Although there were no significant differences

Table 3. Quality of the compost, biochar and manure used for the experiment.

Quality	Compost		Biochar		Manure
	Sawdust	Rice husk	Sawdust	Rice husk	Poultry
Total Nitrogen (%)	2.46±0.09	1.68±0.27	0.17±0.02	0.38±0.02	4.37±0.28
Organic Carbon (%)	38.40±1.40	35.66±4.04	35.54±0.05	40.8±1.15	25.40±1.00
Phosphorus mg/L	64±0.08	45±0.01	42.77±0.01	43±1.0	105±0.03
NO_3-N (mg/kg)	1117.81±26.87	827.89±5.90	-	-	-
NH_4-N (mg/kg)	38.28±2.73	76.45±2.77	-	-	-
Carbon nitrogen ratio	15.76±4.33	19.31±2.11	209.05±15.55	107.36±1.2	5.58±1
pH	7.28±0.09	6.85±0.06	-	-	-
Electrical conductivity	3.80±0.27	3.19±0.11	-	-	-

Table 4. Effect of mixes and irrigation water on plant height (cm) at 4 weeks after transplanting.

Treatment	37°C		30°C	
	(0.1125 mm)	(0.225 mm)	(0.1125 mm)	(0.225 mm)
CONTROL	9.83	10.43	23.9	13.55
NPK	17.28	3.58	27.02	25.4
PM+RB	13.88	20.43	31.67	27.23
PM+SB	20.08	19.58	26.65	26.08
RC	20.05	19.55	26.97	21.3
RC+RB	21.55	18.7	31.10	25.65
SC	20.83	21.23	27.50	23.82
SC+SB	17.53	18.98	26.40	23.20
FPr	0.04		0.064	
LSD (5%)	7.6		4.157	

among treatments (p=0.064) lower plant height (13.55 cm) was obtained in the Control treatment supplied with 0.225 mm irrigation at 30°C (Table 4). When the same treatment was supplied with 0.1125 mm of water, growth was 23.9 cm at same temperature of 30°C. At 37°C, the Control treatment gave significantly (p=0.04) the lowest plant height of 9.83 cm (with 0.1125 mm) and 10.43 cm (with 0.225 mm). At 30°C, Poultry manure amended with Rice husk biochar gave the highest plant height of 31.67 cm (with 0.1125 mm irrigation) and 27 cm (with 0.225 mm irrigation). Plant height under PM+RH was 13.88 cm and 20.43 cm (with 0.1125 mm and 0.225 mm, respectively) at 37°C. Rice compost mixed with Rice husk biochar gave similar plant height as the PM+RB at 30°C. There were no significant differences (p=0.438) in number of leaves among all treatments under 30°C. However, under 37°C, NPK treatment supplied with 0.225 mm irrigation was significantly lower (p=0.02) in plant height (3.25) than all other treatments (Table 5).

There were no significant differences (p=0.058) in stem girth for all treatments under 30°C. However, stem girth (0.82 cm) was significantly lowest (p<0.001) for NPK supplied with 0.225 mm irrigation under 37°C. Furthermore there were reduction in stem girth under 37°C for the Control and PM+RB supplied with 0.1125 mm irrigation (Table 6).

There were no significant differences (p=0.726) in SPAD meter values of treatments at 30°C. The application of NPK together with 0.1125 mm amount of irrigation water gave Significantly (p=0.003) the highest SPAD meter value (42.5%) and the same treatment with 0.225 mm amount of irrigation water gave significantly (p=0.003) the lowest SPAD value of 9.7% (Table 7).

The Control treatment supplied with 0.225 mm amount of irrigation water at 30°C gave significantly the lowest (p=0.003) Leaf area index (LAI) of 6.76 and NPK supplied with 0.225 mm irrigation gave significantly the highest LAI of 43 (p=0.003). The Control treatment supplied with 0.1125 mm and 0.225 mm amount of irrigation water and the NPK treatment supplied with 0.225 mm amount of irrigation water gave significantly the lowest (p<0.001) LAI of 2.84, 4.88 and 1.07 respectively under the 37°C. The NPK treatment (with 0.1125 mm) and the PM+RB (with 0.225 mm) gave significantly highest LAI at 37°C (p=0.003). All treatments except NPK gave a lower LAI with 0.1125 mm amount of irrigation at 37°C (Table 8).

Table 5. Effect of mixes and amount of irrigation on leaf number at 4 weeks after transplanting.

Treatment	37°C		30°C	
	(0.1125 mm)	(0.225 mm)	(0.1125 mm)	(0.225 mm)
Control	12.75	13.25	15.50	13.25
NPK	12.75	3.25	16.75	15.75
PM+RB	11.00	13.25	13.50	15.75
PM+SB	17.00	11.50	15.50	15.25
RC	12.50	13.50	15.75	14.75
RC+RB	14.00	14.00	14.75	14.75
SC	14.50	13.75	12.75	14.25
SC+SB	12.25	13.75	14.25	14.25
FPr	0.02		0.438	
LSD (5%)	4.984		2.874	

Table 6. Effect of mixes and amount of irrigation water on stem girth (cm) at 4 weeks after transplanting.

Treatment	37°C		30°C	
	(0.1125 mm)	(0.225 mm)	(0.1125 mm)	(0.225 mm)
Control	2.26	3.74	3.97	3.14
NPK	5.87	0.82	4.54	5.63
PM+RB	3.75	4.23	5.37	4.66
PM+SB	4.66	3.89	4.52	4.28
RC	4.71	5.2	4.44	4.72
RC+RB	4.36	4.52	4.49	3.86
SC	4.18	4.44	4.31	4.36
SC+SB	4.35	4.89	4.21	4.05
FPr	<.001		0.058	
LSD (5%)	1.697		0.8666	

Table 7. Effect of amendment and irrigation on leaf SPAD at 4 weeks after transplanting.

Treatment	37°C		30°C	
	(0.1125 mm)	(0.225 mm)	(0.1125 mm)	(0.225 mm)
Control	20.2	25.8	30.57	27.32
NPK	42.5	9.7	35.02	34.8
PM+RB	27.1	34.1	36.17	31.35
PM+SB	36.0	27.4	30.45	32.76
RC	31.9	28.3	27.22	26.35
RC+RB	28.5	30.5	30.02	29.25
SC	31.5	31.3	31.45	30.0
SC+SB	30.4	29.9	28.65	26.7
FPr	0.003		0.726	
LSD (5%)	13.24		5.346	

The NPK treatment gave significantly the highest fresh and dry leaf weight of 36 g (p=0.013 and 4.17 g (p=0.007) respectively at 30°C, but this value was lowered by almost two-thirds at 37°C. Poultry manure

Table 8. Effect of amendment and irrigation on LAI at 4 weeks after transplanting.

Amendment	37°C		30°C	
	(0.1125 mm)	(0.225 mm)	(0.1125 mm)	(0.225 mm)
Control	2.84	4.88	17.78	6.76
NPK	22.25	1.07	24.58	43.00
PM + RB	8.90	22.57	21.52	23.23
PM+SB	13.92	15.47	14.09	18.48
RC	13.64	17.98	25.34	17.94
RC+RB	12.52	14.26	17.07	13.56
SC	10.23	9.94	15.67	15.91
SC+SB	10.35	14.55	21.31	12.47
FPr	<.001		0.003	
LSD (5%)	8.073		9.875	

Table 9. Effect of amendment on fresh and dry leaf weight per plant (g) at harvest.

Amendment	37°C		30°C	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	3.94	0.91	6.78	1.25
NPK	13.01	1.63	36.93	4.17
PM+RB	15.73	2.85	24.22	3.74
PM+SB	16.71	2.96	22.77	3.17
RC	13.65	2.7	18.26	2.50
RC+RB	10.76	2.2	11.85	1.86
SC	10.79	2.22	12.01	2.22
SC+SB	11.51	2.13	15.6	2.15
FPr	0.013	0.007	<.001	<.001
LSD (5%)	9.278	1.073	5.659	0.8001

mixed with sawdust biochar gave significantly the highest fresh leaf weight of 16.71 g (p=0.013) and dry leaf weight of 2.96 g (p=0.007) under 37°C. The fresh leaf, dry leaf, fresh root and dry root weight were not significant under the two irrigation regimes and hence the data is not presented (Table 9). Root weight was significantly highest (1.97 g), p<0.001 under PM+RB treatment and lowest (0.5 g) in the Control treatment at 30°C. At 37°C, fresh root weight was significantly highest (2.92 g) in the RC treatment and lowest (0.88 g) in the Control treatment (p<0.001). However, SC+SB treatment gave significantly (p<0.001) highest dry root weight (0.76 g) with NPK treatment recording the lowest dry root weight of 0.18 g (Table 10).

At 30°C Poultry manure mixed with sawdust biochar with 0.225 irrigation, gave a volume of leachate (123.2 ml) significantly higher (p<0.001) than the Control (65.9 ml), NPK (48.2 ml) and Rice husk compost (54.7 ml. At 37°C, Sawdust compost with 0.225 mm of irrigation, gave a volume of leachate (166.1 ml) significantly higher than NPK, PM+RB, PM+SB, RC and SC+SB treatments. At 37°C, NPK treatment with 0.1125 mm amount of irrigation water, significantly (p<0.001) gave the lowest volume of leachate of 5.9 ml (Table 11). At 30°C, NPK and PM+RB treatments gave significantly (p<0.001) the highest water use efficiency of 600 kg m^{-3} each. At 37°C, the Control treatment and NPK treatment gave significantly the lowest water use efficiency of 90 kg m^{-3} and 100 kg m^{-3} respectively (Table 12).

DISCUSSION

The compost used for the study had good qualities compared to compost produced elsewhere (Leconte et al., 2009). The soil used for the study is generally low in N, organic carbon and is slightly acidic and hence needed aditional inputs of N and C (Abubakari et al., 2011; Abubakari et al., 2012). Other studies reported that compost and biochar have ameliorating effect on poor soils and that plant growth and nutrient uptake were enhanced by addition of organic materials to soils (Brito et al., 2014; Agegnehu et al., 2015). According to Schulz et al. (2014) addition of compost to soils increases the pH

Table 10. Effect of amendment on fresh and dry root weight per plant (g) at harvest.

Amendment	37°C		30°C	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	0.88	0.26	0.50	0.26
NPK	1.48	0.18	1.91	0.49
PM+RB	2.64	0.59	1.97	0.54
PM+SB	2.36	0.54	1.56	0.51
RC	2.92	0.74	1.27	0.61
RC+RB	2.31	0.58	1.22	0.41
SC	1.84	0.45	1.10	0.47
SC+SB	2.37	0.76	1.29	0.47
FPr	<.001	<.001	<.001	0.143
LSD (5%)	0.849	0.24	0.6171	0.2311

Table 11. Effect of amendment on volume of leachate per pot (ml) 2-4 weeks after transplanting.

Amendment	37°C		30°C	
	(0.1125 mm)	(0.225 mm)	(0.1125 mm)	(0.225 mm)
Control	16.3	135.0	10.9	65.9
NPK	5.9	115.8	11.8	48.2
PM+RB	45.1	57.0	13.2	95.3
PM+SB	42.3	102.5	14.1	123.2
RC	13.2	64.1	19.7	54.7
RC+RB	32.8	135.3	15.7	119.9
SC	19.4	166.1	25.1	99.4
SC+SB	17.7	113.6	11.9	109.9
FPr	<.001		<.001	
LSD (5%)	34.10		20.41	

Table 12. Effect of amendment on water use efficiency of *amaranthus*.

Amendment	37°C	30°C
	Water use efficiency (kg m^{-3})	Water use efficiency (kg m^{-3})
Control	90	200
NPK	100	600
PM+RB	250	600
PM+SB	220	450
RC	170	340
RC+RB	200	310
SC	240	440
SC+SB	210	360
FPr	0.006	<.001
LSD (5%)	0.0923	0.1398

and makes nutrient.

Although the Control treatment gave the lowest plant height, leaf number, stem girth and SPAD meter value, treatment effects was not significant for these parameters at 30°C. At 30°C and with 0.1125 mm of irrrigation, poultry manure + rice husk biochar, rice husk compost + rice husk biochar and sawdust compost increased plant height by 32.4, 30, and 15%, respectively compared to

13% for NPK. However, at 37°C plant height decreased by compared to the control, the increament in plant height were 41.1%, 119.1%, 111.8% and 75.7 for poultry manure + plus rice husk biochar, rice husk compost plus rice husk biochar, sawdust compost and NPK respectively. NPK treamtments receiving 0.225 mm amount of irrigation water had significantly lowest plant height and number of leaves at 37°C. As NPK treatment receiving the 0.225 mm irrigation also had significantly higher volume of leachate as shown in Table 11, leaching of nutrients at higher temperature could have contributed to the poor performance of the NPK treatment. Combine use of compost and biochar is been reported to be more beneficial (Fischer and Glaser, 2012; Schulz and Glaser, 2012). Optimum use of compost and biochar improves nutrient and water retention (Liu et al., 2012). Use of organic amendents has also been shown to decreased volume of leachate and cumulative leaching volume was found to be inversely related to the above and below ground biomasss. Treatment effects on LAI index was significant at both 30 and 37°C. NPK gave significantly, the lowest LAI at 0.225 mm irrigation at 37°C, although it gave significantly highest LAI at 30°C. PM+RB which gave the lowest volume of leachate at 0.225 mm irrigation at 37°C, gave significantly the highest LAI under the same conditions.

The effect of the treatments on yield follows the same pattern as observed for growth parameters, with NPK showing significantly highest fresh and dry weight of leaves at 30°C. However, at 37°C, plant height increased 298.2%, 323%, 172.5% and 173.3 for PM+SB, PM+RB, RC+RB and SC respectively. This suggests addition of biochar to manure plays a significant role in reducing leaching and promoting yield of *amaranthus* (Agegnehu et al., 2015). It also suggests that addition of biochar will help when there is a rise in temperature due to climate change. Similar results were obtained for fresh and dry root weight at 30 and 37°C, but with PM+RB recording the highest fresh root weight. Root development appear to be generally higher at higher temperature (37°C) than at lower temperature (30°C), and addition of biochar appears to promote growth and yield high temperature. Water use is more efficient at 30°C, and NPK treatment is best in water utilization for higher productivity than organic treatments at this temperature. However, at 37°C, NPK treatment had poor water use efficiency and organic amendments especially poultry manure and rice husk biochar and sawdust compost are better in WUE.

Conclusion

The results suggest combination of nitrogen and carbon rich organic materials at appropriate irrigation levels have profound effect on plant growth and development especially under different temperature conditions. At lower temperatures inorganic fertilizers are very important in promoting growth yield. However, at higher temperatures organic materials rich in N (especially poultry manure) and C (sawdust biochar) are critical in retaining water and nutrients, promoting root develpment and enhancing fresh leaf weight of *amaranthus*. Although the organic materials rich in N and C gave higher volume of leachate under higher temperatures, the slow nutrient release potential could have reduced nutrient leaching. Therefore,at higher temperature as is the case in most Savanna areas of Northern Ghana where this experiment was conducted or as may occur in other areas due to climate change and rising temperatures, N rich materials such as poultry manure, rice husk compost, sawdust compost and the addition of C rich matrials (such as sawdust biochar) could be used to sustain growth and yield of *amaranthus*. The results also suggest that at higher temperatures and with full irrigation as typified by the application of 0.225 mm irrigation root development could be sustained by the use of rice husk compost amended with rice husk biochar.

Conflict of Interests

The authors have not declared any conflict of interests.

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