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Review

Review on prevalence of bovine trypanosomosis in Ethiopia

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Ethiopia is one of African country with nine regions and two city administration of which five regions were infected with more than one species of tsetse flies. Known species in Ethiopia are five in number namely *Glossina pallidipes*, *G .morsitans, G. fuscipes, G. tachinoides* and *G. longipennis.* Most tsetse transmission is cyclic and begins when blood from a trypanosome-infected animal is ingested by the fly. The clinical feature of the disease follows the level or burdens of tsetse challenge species. The main feature is anemia results in a progressive drop in packed cell volume, a non-specific but useful indicator in endemic areas. The most sensitive rapid method is examining a wet mount of the buffy coat area of a PCV tube after centrifugation, looking for motile parasites. The prevalence of trypanosomosis in enzootic area can be reduced by parasite control, vector control, host resistance protection prophylactic treatment and good husbandry management system. The methods of tsetse fly control involved bush clearing, elimination of game animals on which tsetse feed, and the sterile male technique (sterile insect techniques). Since female tsetse only mates once in a lifetime, this technique is theoretically able to eradicate a targeted tsetse species. Trypanotolerant animals are very important in tsetse fly challenging areas, but most countries did not accept them due to their low production of milk than indigenous breed. In conclusion, prevalence of trypanosomosis is devastating diseases of cattle in Ethiopia with both direct and indirect economic losses.

Key words: Bovine, trypanosomosis, nagana, tsetse fly, protozoa.

INTRODUCTION

Trypanosomosis has long been recognized as a massive constraint on animal husbandry, livestock production and mixed farming in vast areas of rural sub-Saharan Africa (Oluwafemi, 2014). Ethiopia is known for its large and diverse livestock resource endowments. Livestock is primarily kept on small holdings where it provide drought power for crop production, manure for soil fertility and fuels, serves as a sources family diet and sources of cash income (from livestock and livestock products). Despite large livestock population, Ethiopia fails to optimally utilize this resource due to different constrains facing the livestock subsector (Bezabih et al., 2015).

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Since more than 90% of crop production in Ethiopia are dependent on animal draught power mainly on ploughing oxen, many large fields lie fallow due to lack of these animals in trypanosomiasis infested area (Kenaw et al*.,* 2015), which worsen the food supply and living conditions in affected areas.

Trypanosomes are flagellated protozoan parasites that live in the blood and other body fluids of vertebrate hosts (OIE, 2013). Bovine trypanosome is one of the diseases that are caused by this flagellated protozoal parasite belonging to the genus trypanosome (Jember et al*.,* 2013). This group of diseases caused by protozoa of the genus Trypanosoma affects all domestic animals. The major veterinary species are *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma bruceibrucei, and Trypanosoma simiae. Trypanosoma bruceirhodesiense and Trypanosoma bruceigambiense* are zoonotic, with people as the predominant host. Animals are mainly affected by tsetse-transmitted trypanosomes and in geographic areas where tsetse-transmitted trypanosomiasis occurs (Merck, 2005).

In Ethiopia, trypanosomosis is widespread in domestic livestock in the Western, South and South-western lowland regions and the associated river systems (that I, Abay, Ghibe Omo and Baro/Akobo) (Tekle, 2012; Langridge, 1976). The tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33° and 38°E and latitude 5° and 12° N (Langridge, 1976). The infested area extends from the southern part of the Rift Valley, around the south-western corner of the country and along the western lowlands and escarpments to the Blue Nile (Bezabih et al*.,* 2015; Getachew, 2005).

Out of the nine region of Ethiopia, five (Amhara area, Benshangul-Gumuzs, Gambella, Oromia and Southern Nations Nationalities and Peoples' Regional State) are infected with more than one species of tsetse flies (Bitew et al*.,* 2011). Currently about 220,000 km^2 areas of the above mentioned regions are infested with five species of tsetse flies namely *Glossina pallidipes*, *Glossina morsitans, Glossina fuscipes, Glossina tachinoides* and *Glossina longipennis* (NTTICC, 2004; Abebe, 2005). Locally in Amharic language trypanosomosis in cattle referred, as "Gendi" is a serious constraint to livestock production in areas of the north and southwest Ethiopia at an altitude of below 2000 m.a.s.l (Tadesse, 2010). The most prevalent trypanosome species in tsetse infested areas of Southern region of Ethiopia (Tadesse, 2010) and south west Oromia are *T. congolense, T. vivax* by Denu et al*,* 2012. The objective of this paper is to summarize the available research evidence on trypanosomosis in cattle and reveals the gap that needs future research attention

Trypanosomosis in Ethiopia

In Ethiopia, trypanosomosis is widespread in domestic livestock in the Western, South and South-western lowland regions and the associated river systems (Tekle, 2012). Tsetse infested areas lie in the lowlands and in river valley of Abbay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo. Out of the nine regions of Ethiopia five (Oromia, SNNPR, Amhara, Beninshangul Gumuz, and Gambella) are infested with more than one species of tsetse flies (NTTICC, 2004; Keno, 2005; Abebe, 2005). The most important trypanosome species affecting cattle in Ethiopia are *T. congolense, T. vivax*and *T. brucei (*Alemayehu et al*.,* 2012). Cattle bitten by tsetse flies develop fever, anaemia, lose weight, and progressively become weak and unproductive. Breeding animals frequently abort or may become infertile. Several affected cattle die of anaemia, congestive heart failure or inter-current bacterial infections that frequently take advantage of the weakened immune system (Chaka and Abebe, 2005).

Bovine trypanosomiasis

Bovine trypanosomosis is a disease that affects cattle resulting from infection with protozoa of the genus Trypanosoma transmitted primarily by tsetse fly and also by other haematophagous fly (Urquart et al*.,* 1995). *T. vivax, T. congoiense, T. bruceibrucei* and *T. simiae* are the four main species responsible for African trypanosomoses affecting virtually all domestic mammals. *T. vivax and T. congoiense* are the main pathogens of cattle (Radostitis et al., 2007).

Epidemiology of trypanosomosis

Modes of transmission, vectors and ecological preference

The epidemiology of African trypanosomosis is determined mainly by the ecology of the tsetse fly which is found only in tropical Africa (Radostitis et al*.*, 2007). Most tsetse-fly transmission is cyclic and begins when blood from a trypanosome-infected animal is ingested by the fly. The trypanosome alters its surface coat, multiplies in the fly, then alters its surface coat again, and becomes infective (Merck, 2005). Tsetse flies (genus *Glossina*) are restricted to Africa from about latitude 15° N to 29° S. The three main species that inhabit relatively distinct environments are: *G. morsitans* usually found in savanna country, *G. palpalis* prefers areas around rivers and lakes, and *G. fusca* lives in high forest areas. All three species transmit trypanosomes, and all feed on various mammals (Merck, 2005). The riverine species (*G. palpalis, G. tachinoides*, and *G. fuscipes*) (Honigberg, 1986) are important as vectors of bovine (Radostitis et al*.*, 2007). Trypanosomosis is an important disease of livestock in Ethiopia (Alemayehu et al*.,* 2012). There are six pathogenic *T. equiperdum* and *T. rhodesiense* but the most species of trypanosomes are discovered in

Ethiopia, which are namely *T.vivax, T. congolense, T. brucei, T. evansi*, and important trypanosomes which are found in country are *T. vivax and T. congelense* (Abebe, 2005; Eticha and Aki, 2016).

Vector and parasitic survey

Monoconical standard traps were to be deployed in the study area for tsetse fly trapping. All the traps were baited uniformly with octenol (1-oct-3-nel), acetone and three weeks old cow urine. All odors were placed on the ground about 30 cm upwind of the trap. The apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day (Desta, 2014). Blood sample was collected by puncturing of the marginal ear vein of each animal with a lancet and drawn directly into heparinized capillary tube and centrifuged with capillary hematocrit centrifuge. Positive samples were further processed for thin blood smear for confirmation of trypanosome species using their morphological characteristics with Giemsa staining techniques (Tekle, 2012; Fayisa et al*.,* 2015).

Biology of tsetse fly

Tsetse-fly, in general adult tsetse, are narrow, yellow to dark brown flies (Veterinary Entomology, 2015) 6 to 15 mm in length and have along, rigid, forward projection proboscis. The thorax is a dull greenish brown color and is marked with inconspicuous stripes and spots (FEAV, 2015). The 23 known species of tsetse flies can be divided into three groups, each with different habits and requirements. *G. palpalis* group are riverine species which feed primarily reptiles and ungulates. Flies of the *G. morsitans* group are savannah and dry thorn-bush species which is mainly on large animals. Members of *G. fusca* group occur in rainforest, preferring dense shade and riverine thickets (FEAV, 2015). Life cycle of both male and female flies suck blood and although the various species of tsetse may have some host preferences, generally they will freedom a wide variety of animals (Urqhart et al., 1995). The puparial period can range from 20 days (at 30°C) to 47 days (at 20°C) (on average 30 days at 24°C). Development in the puparium is generally unsuccessful below about 17°C and above about 32°C. The entire life cycle from egg to adult usually takes about 30 days (Leak, 1999).

Pathogenesis

Infected tsetse inoculate metacyclic trypanosomes into the skin of animals, where the trypanosomes reside for a few days and cause localized inflammation (chancres).They enter the lymph and lymph nodes, then the bloodstream, where they divide rapidly by binary fission. In *T congolense* infection, the organisms attach to endothelial cells and localize in capillaries and small blood vessels. *T brucei* species and *T vivax* invade tissues and cause tissue damage in several organs (Merck, 2005; Radostitis et al., 2007). African animal trypanosomosis or nagana ("nagana") which is a Zulu word that means "powerless (useless"‘, “to be in low or depressed spirits”) is caused by *T. congolense*, *T. vivax* and *T. brucei* spp. (Steverding, 2008). Antibody developed to the glycoprotein coat of the trypanosome kills the trypanosome and results in the development of immune complexes. Antibody, however, does not clear the infection, for the trypanosome has genes that can code for many different surface-coat glycoproteins and change its surface glycoprotein to evade the antibody (Eshetu and Begejo, 2015).

Diagnosis of trypanosomosis

Clinical findings and lesions

The general clinical picture is as follows but there are many variations determined by the level of tsetse-fly challenge, the species and strain of the trypanosome, and the breed and management of the host (Radostitis et al*,* 2007). Severity of disease varies with species, age of the animal infected and the species of trypanosome involved. The incubation period is usually 1 to 4 week. The primary clinical signs are intermittent fever, anemia, and weight loss. Cattle usually have a chronic course with high mortality, especially if there is poor nutrition or other stress factors (Merck, 2005). The anemia results in a progressive drop in packed cell volume, a non-specific but useful indicator in endemic areas (Radostitis et al., 2007). Necropsy findings vary and are nonspecific. In acute and fatal cases, extensive petechiation of the serosal membranes, especially in the peritoneal cavity, may occur. Also, the lymph nodes and spleen are usually swollen (Merck, 2005). Definitive diagnosis of the disease is ultimately dependent on the detection of the trypanosome in blood samples from infected animals (Diseases of Cattle, 1981; Abebe, 2005).

Parasitological diagnosis

A presumptive diagnosis is based on finding an anemic animal in poor condition in an endemic area. The most sensitive rapid method is to examine a wet mount of the buffy coat area of a Packed Cell Volume (PCV) tube after centrifugation, looking for motile parasites. Other infections that cause anemia and weight loss, such as babesiosis, anaplasmosis, theileriosis, and haemonchosis, should be excluded by examining a stained blood smear (Merck, 2005).

Serological diagnosis

Various serologic tests measure antibody to

trypanosomes, but their use is more suitable for herd and area screening than for individual diagnosis. Rapid agglutination tests to detect circulating trypanosome species-specific antigens in peripheral blood are available for both individual and herd diagnosis, although their reliability remains varied (Merck, 2005). Another alternative is a series of standard serological tests to detect anti-trypanosome antibodies in sera or other body fluids. The three tests used most often are the indirect immuno-fluorescent antibody test (IFAT), the capillary agglutination test (CAT), and the ELISA (Radostitis et al., 2007).

Molecular technique

Molecular techniques for trypanosome detection and differentiation have been developed, but they are not generally available for routine field use (Merck, 2005). Dried blood spots on filter papers are also a useful source of DNA for the detection of *T. congolense* and *T. brucei* by Trypanosome and Trypanosomiasis (2014) PCR But the test is expensive and can only be done in specialized laboratories (Radostitis et al., 2007).

Control of trypanosomosis

Parasite control

The control of trypanosomosis in enzootic countries involves control of tsetse fly population, prophylactic treatment, good husbandry of animals at risk, and use of trypano-tolerant animals (Radostitis et al*.,* 2007). Most have a narrow therapeutic index, which makes administration of the correct dose essential (Merck, 2005). Therapeutic drugs for the treatment of trypanosomosis includes diminazenum aceturate, homidium bromide and homidium chloride. Prophylactic drugs for cattle include homidiumbromide, homidium chloride and isometamidium (Ayisheshim et al., 2015).

Vector control

Till date five species of *Glossina namely G. morsitans submorsitans, G. pallidipes, G. fuscipes fuscipes, G. tachinoides,* and *G. longipennis* are known to exist in Ethiopia. These vectors cyclically transmit four species of trypanosomes (*T. congolense, T. vivax, and T. brucei* of livestock and *T. rhodesiense* of human). The occurrence of trypanosomosis in the region was attributed to the existence of cyclical vectors, *G. pallidipes* and *G. f. fuscipes*. However*, G. pallidipes* was the predominant and most widely distributed vector (Abebe et al*.,* 2017). Control of tsetse has been successfully attempted in some African countries, but reinvasion is frequent if the land is not properly utilized. The earliest methods involved bush clearing and elimination of game animals on which tsetse feed (Radostitis et al., 2007). Another method is the sterile male technique. Since the female tsetse only mates once in a lifetime, this technique is theoretically able to eradicate a targeted tsetse species in areas where other methods have been used to reduce its density, but it is expensive (Urqhart et al*.,* 1995).

Host resistance protection

Trypano-tolerant animals are being used to establish ranches in areas where tsetse challenge is not too heavy, but they have not been readily accepted in some countries, supposedly because they are smaller in size and they produce less milk than other indigenous breeds and crosses with exotic breeds (Radostitis et al., 2007). They are infected by tsetse flies but do not show clinical disease. However, these breeds have not been readily accepted because they are small in size and low in milk producing. Cross breeding is however a common practice (OIE, 2013). The four Ethiopian cattle breeds Abigar, Gurage, Horro and Sheko in aspects are related to trypano-tolerance (Eshetu and Begejo, 2015).

Over view of economic Impacts

Tsetse flies infest 10 million square kilometers of Africa involving 37 countries. Hence, nagana is today the most important disease of livestock in the continent (Disease of Cattle, 1981). Since nagana is a wasting disease, affected animals are chronically unproductive in terms of milk, meat, manure, and traction and the mortality rate can be high (Jano, 2016; Radostitis, et al., 2007). The disease in Africa costs livestock producers and consumers an estimated US$1340 million each year (Ayisheshim et al., 2015).

DISCUSSION

In African Animal Trypanosomosis (AAT), some cattle breeds like the West African Taurine such as N'Dama, have the ability to control the development of the disease better than Zebu and exotic taurine breeds (Dagnachew et al., 2015). This capacity to better control the infection and disease in cattle which is demonstrated to have major genetic components, was called trypano-tolerant and defined as the traits that confers the capacity to survive and remain productive despite a still active trypanosome transmission in endemic areas.

Trypano-tolerance occurs in some African bovine breeds (Bos Taurus) such as longhorn (N'Dama) and shorthorn (Baoule) cattle, which entered into African continent before Zebu cattle (Courtin et al., 2008). According to literatures, the most prevalent trypanosome species of Bovine in tsetse-fly infested areas in different parts of Ethiopia were *T. congolense* and *T. vivax*

(Abebe, 2005). The risk of infection with trypanosomes during the dry season was lower than the late rainy season, with a statistically significant difference for *T. congolense* infection in both seasons as reported by Ayele et al. 2012. The prevalence of trypanosomosis was affected by agro-climatic zone.

Higher prevalence in lowland areas is related to the fact that animals in lowland areas are more challenged by vectors than higher altitudes. This is related to the temperature difference between these areas as temperature is one of the most important biotic factors that limit the distribution of vectors. A number of studies have shown the effect of age on the prevalence of trypanosome infections in cattle. The highest prevalence was observed in middle age group (2<x<4 years). The lower prevalence in younger group may be related to the husbandry system in which young animals were usually kept around their house with lower fly challenge (Melaku and Abebe, 2012).

The high ratio of *T. congolense* in tsetse-infested area may be ascribed to the more efficient transmission of *T. congolense (*Aki and Godeso, 2016*)* by major cyclical vectors than *T. vivax* in East Africa. An increased prevalence of *T. vivax* infections in cattle has been noted during rainy season which is attributed to higher density of tsetse flies and/or the abundant presence of mechanical vectors, such as tabanids and stomoxys spp (Mulaw et al., 2011). The high proportion infection rate of *T. congolense* in cattle might be attributable to the high number of serodems of *T. congolense* relative to *T. vivax* which could also be due to the possible development of better immune response to *T. vivax* by the infected animals.

Further, it might be attributed to the efficient transmission of *T. Congolense* by cyclical vectors than *T. vivax* in tsetse-infested areas*.* Previous reports indicated that *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia, respectively (Aki and Godeso, 2016). Ecological conditions for tsetse on the edge of a fly belt are usually less favorable resulting in a high mortality rate of tsetse and favoring the transmission of trypanosome species with a short developmental cycle such as *T. vivax* (Cherenet et al*.,* 2010).

CONCLUSSION AND RECOMMENDATIONS

Bovine trypanosomosis caused by *T. congolense* and *T. vivax* was found to be an important disease of cattle in rift valley basin in South, South West, West and North West of Ethiopia. Prevalence of trypanosomosis progress is high in bovine and impact of the disease on productivity of infected animals. Since trypanosomosis is worldwide problem and causes great economic lose due to infectious and death of animals as well as medication costs, agricultural and loss of production. To reduce its effects, the following measures are recommended:

1. Improve management practices such as rearing, feeding, housing, medication and restriction movement density population of tsetse
2. Increase awareness creation those animal rearing society especially pastoral community
3. Importing modern and latest drugs for trypanosomosis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sowing seasons × maturity groups on quantitative traits in soybean

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The world economic importance of soybean (*Glycine max* (L).) crop is consolidated, and tests to verify the best sowing season for yield gain of cultivars are demanded. The aim of this study is to analyze the effect of sowing season on soybean cultivars of different maturity groups, since the determination of the optimum time for planting soybeans and the cultivar most suited to the region under study can increase yield components and consequently productivity. The experiment was conducted in Currais, State of Piauí, Brazil, and involved evaluation of 12 treatments resulting from the interaction between: 1) sowing seasons: 11/22/2014; 11/29/2014; 12/6/2014; 12/13/2014; 12/20/2014 and 12/27/2014 and 2) two cultivars of maturity groups 8.2 and 8.6. The experiment was a randomized complete block design with four replications, in subdivided plots, and the nested effect in the plot was sowing dates. Data were analyzed using ANOVA and Tukey's test ($p \leq 0.05$). Interaction was significant for number of pods, pod length, dry mass of stem, dry mass of pods and number of grains per plant, but not for productivity and one thousand seed mass. In the agricultural year 2015/2016, the climatic factors worked directly on the components of soybean production, and it is possible to adopt any period of November and December for its planting.

Key words: *Glycine max*, climatic elements, yield components, photoperiod.

INTRODUCTION

Soybean (*Glycine max* (L.)) is one of the most cultivated crops in the world. In Brazil, the cultivated area is 32,092.9 ha, with a productivity of 2,998 kg ha^{-1} and 96,228 tons produced in 2014-2015 (CONAB, 2015).

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Among the Brazilian states, Piauí stands out for the growing expansion of soybean in the Cerrado area (Alcântara et al., 2012). The planted area, productivity and production are 673,700 ha, 2,722 kg ha^{-1} and 1,833.8 tons, respectively (CONAB, 2015). The Cerrado in the State of Piauí presents Central Brazil Tropical climate which is hot with average above 18°C every month, semi humid with 4 to 5 dry months (EMBRAPA, 2015). It is located in the MATOPIBA region (encompassing the States of Maranhão, Tocantins, Piauí and Bahia) and it stands out in the Brazilian scenario due to its flat topography, deep soils and favorable weather for the cultivation of major crops of grain and fiber (Borghi et al., 2014), which allowed agricultural expansion in this region.

Soybean yield depends on the sowing season, since plant development and production are related to the climatic elements and the different soybean maturity groups (Chen and Wiatrak, 2010; Kapoor et al., 2010). In this sense, it is necessary to determine the best time for planting, so that climatic conditions are favorable for the development of soybean and for a higher production of grains (Alcântara et al., 2012). In the Piauí Cerrado, sowing is traditionally between November 15 and December 15 (Cruz et al., 2010a). Therefore, the objective of this study was to evaluate the effect of sowing time on the yield components of soybean cultivars of different maturity groups produced in Serra do Pirajá, Cerrado microclimate. Since soybean is a crop that depends intrinsically on climatic conditions, sowing time plays a key role in its development and final productivity.

MATERIALS AND METHODS

Location of the experiment and soil analysis

The experiment was conducted in the crop year 2014-2015 at São João Farm, in Currais, State of Piauí (9° 1' 59'' S, 44° 41' 18'' W, and 590 m). Climatic data regarding air temperature, relative humidity (%) and rainfall (mm) were collected daily at the farm's Portable Automatic Weather Station during the study period. The estimated average values that each cultivar received of these elements in different sowing dates, both in the vegetative and reproductive stage, were calculated with Excel® 2010. The chemical properties of the soil were analyzed and fertilization was done according to soil analysis. The concentrations obtained were OM = 12.6 g dm^{-3}; pH $CaCl_2$ = 4.3, P = 8.4 ppm, S = 9.4 ppm, K = 1.2 mmolc dm^{-3}, Ca = 9.7 mmolc dm^{-3}, Mg = 2.3 mmolc dm^{-3}, Al = 3.0 mmolc dm^{-3}, H + Al = 35.4 mmolc dm^{-3}, SB = 13.2, CEC = 48.6 mmolc dm^{-3}, V = 27.1%, m = 6.2%, Cu = 1.6 ppm, Fe = 210.1 ppm, Mn = 2.5 ppm, Zn = 0.4 ppm, total sand = 630 g kg^{-1}, silt = 60 g kg^{-1} and clay= 310 g kg^{-1}.\

Adopted statistic

The experiment was a split plot randomized complete block with four replications. Each plot consisted of 25 m rows 0.5 m apart, and the subplots were 10 rows per cultivar. Seeds were inoculated and treated as follows: 4 doses of inoculant 5 × 10^9 CFU mL ha^{-1} + 140 mL ha^{-1} Standak Top®.

Sowing season and soybean cultivars

Sowing was done weekly from the onset of rainfall and there were six seasons (S): 11/22, 11/29, 12/06, 12/13, 12/20 and 12/27/2014. The soybean cultivars studied were C1, with a cycle of 110 to 115 days and maturity group 8.6; and C2 with a 100 days cycle and maturity group 8.2. The sowing was manual, 25 seeds m^{-1} and excess seedlings which were later thinned to 14 plants m^{-1} for C1 and 16 plants m^{-1} for C2, with a resultant final population of 300,000 and 330,000 plants ha^{-1}, respectively. The harvest was done manually when the plants reached the phenological stage R9. Pods were collected, stored in plastic bags and taken to the Laboratory of Plant Science of the Piauí Federal University (UFPI), where threshing was done.

Rated characters

Yield components were the following variables: pod length (PL); number of grains per pod (NGPO); number of grains per plant (NGPL); number of pods per plant (NPP); stem dry mass (SDM) and pod dry mass (PDM); mass of one thousand grains (MTG) and productivity (PROD) (Brasil, 2009; Alcântara et al., 2012; Souza et al., 2013). After obtaining data, the Shapiro Wilk test was performed, and next, the analysis of variance was run using the R statistical software. Data corresponding to pod numbers were transformed using the 1/x formula. Next, the significant interaction between season and cultivars was checked at $p \leq 0.05$, afterward, a statistical breakdown of treatments was performed and whenever significant, the comparison between mean values was made by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

The results of the analysis of variance evidenced significant interaction cultivar (C) × sowing season (S) for number of pods per plant, pod length, stem dry mass, pod dry mass and number of grains per plant (Table 1), indicating that climatic elements and maturity group interfered with the development of plants (Chen and Wiatrak, 2010).

The combined effect of C × S was compared to the recommendations of the cultivar with higher average performance of NPP, PL, SDM, PDM and NGPL and the best sowing season, represented by E3 (Table 2).

Unfolding of the interaction C × S

The highest mean number of pods per plant was found in sowing time 1 with C1, while for C2 the highest values were observed in sowing times 3, 4 and 6 (Table 2). This was probably because rainfall was better distributed during the vegetative stage in sowing times 3, 4 and 6

Table 1. Analysis of variance for the number of pods per plant (NPP), pod length (PL), stem dry mass (SDM), pod dry mass (PDM) number of grains per plant (NGPL) number of grains per pod (NGPO), mass of one thousand grains (MTG) and productivity (PROD).

VF	DF	Mean square							
		NPP	PL	SDM	PDM	NGPL	NGP	MTG	PROD
Cult (C)	1	218^{NS}	3.4^{NS}	150**	211*	5874*	0.5^{NS}	1110*	114608^{NS}
Sowing seasons (S)	5	342*	4.1^{NS}	19*	107*	4723*	0.2^{NS}	4478**	36398^{NS}
C × S	5	256*	6.8*	12*	124*	3887*	0.2^{NS}	89^{NS}	138512^{NS}
Error	33	67	2.5	3	41	1184	0.3	79	124526
VC (%)		30.8	3.9	36.39	36	32	21.3	6.59	13.94

^{NS}Non-significant; *p ≤ 0.05, **p ≤ 0.01 (Snedecor's F test). Cultivars (Cult), Sowing seasons (S), Interaction between cultivar and sowing seasons (C x S); Variation coefficient (VC); Degree Freedom (DF). Pod length (PL); number of grains per pod (NGPO); number of grains per plant (NGPL); number of pods per plant (NPP); stem dry mass (SDM) and pod dry mass (PDM); mass of one thousand grains (MTG) and productivity (PROD).

Table 2. Mean values of the interactions for the variables: number of pods per plant (NPP), pod length (PL), stem dry mass (SDM), pod dry mass (PDM), number of grains per plant (NGPL) of the cultivars C1 and C2 in 6 distinct sowing dates.

S	NPP (u)		PL (mm)		SDM (g)	
	C1	C2	C1	C2	C1	C2
1	33.42^{Aa}	$25A^{Ba}$	40.57^{Aa}	41.29^{Aba}	3.56^{Aa}	4.63^{Ba}
2	24.12^{ABa}	26.25^{ABa}	40.682^{Aa}	40.87^{Aba}	2.98^{Aa}	4.39^{Ba}
3	30.49^{ABa}	31.94^{Aa}	39.84^{Ab}	42.83^{Aa}	2.48^{Ab}	7.14^{Ba}
4	28.50^{ABa}	36.00^{Aa}	40.87^{Aa}	42.62^{Aa}	2.20^{Ab}	6.72^{Ba}
5	15.22^{Ba}	13.42^{Ba}	40.65^{Aa}	38.70^{Ba}	4.35^{Aa}	6.57^{Ba}
6	15.02^{Bb}	39.75^{Aa}	40.89^{Aa}	38.38^{Bb}	4.29^{Ab}	11.65^{Aa}
VC (%)	30.84		3.86		36.39	

S	PDM (g)		NGP (u)	
	C1	C2	C1	C2
1	17.97^{Aa}	16.24^{Ba}	85.08^{Aa}	85.08^{Aa}
2	14.08^{Aa}	14.36^{Ba}	69.42^{Aa}	69.42^{Aa}
3	15.25^{Ab}	34.49^{Aa}	89.16^{Ab}	89.16^{Ab}
4	12.88^{Aa}	19.30^{Ba}	87.83^{Aa}	87.83^{Aa}
5	16.97^{Aa}	17.76^{Ba}	124.66^{Aa}	124.66^{Aa}
6	17.19^{Aa}	17.38^{Ba}	125.37^{Aa}	125.37^{Aa}
VC (%)	35.87		31.87	

Uppercase letters: comparisons between sowing seasons; lowercase letters: comparisons between cultivars (Tukey's test; p ≤ 0.05). Sowing (S); Variation coefficient (VC).

(Figure 1). Regarding S1 with C1, these discrepant results could be explained with respect to the cultivar's maturity group because even though it was subjected to water stress, it may have been favored by the rainy season at the defining moments of this variable. Thus, rainfall may have promoted the high number of pods for plants at this date, since the high availability of water increase yield components. Likewise, stress conditions will cause a negative influence on the biological yield of soybeans, by damaging the final production of the plants (Siahbidi et al., 2013). The other climatic elements did not affect the studied variables, since temperatures in the range 20 to 30°C and adequate humidity are critical to the growth and development of soybean (Alcântara et al., 2012; Taiz and Zeiger, 2013; Battisti and Sentelhas, 2014) (Figures 1 and 2).

Sowing season 6 was a conflicting point, which generated good results in C2 (Table 2). It occurred due to

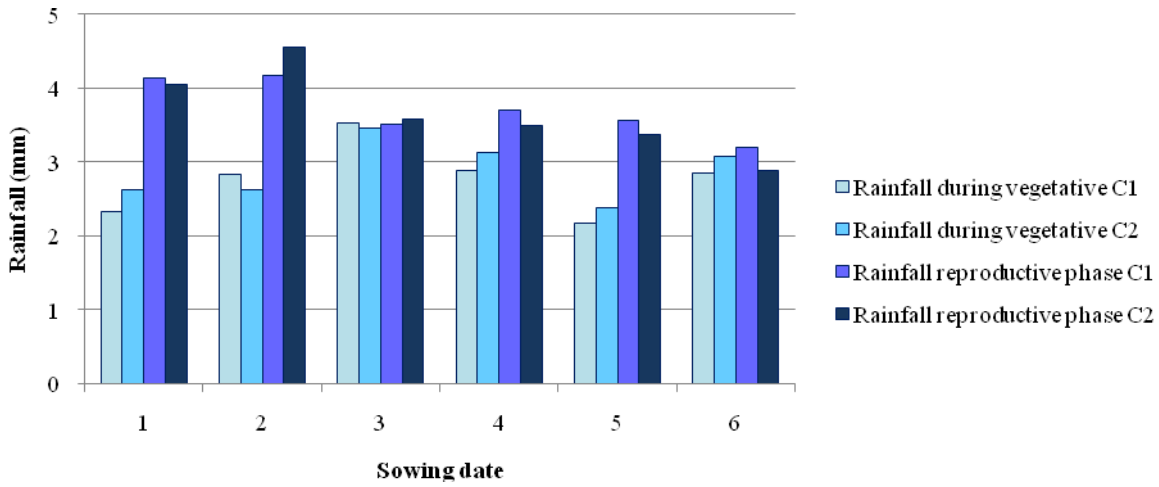

Figure 1. Temporal evolution of average rainfall during the vegetative and reproductive stages of the cultivars C1 and C2 during each sowing season.

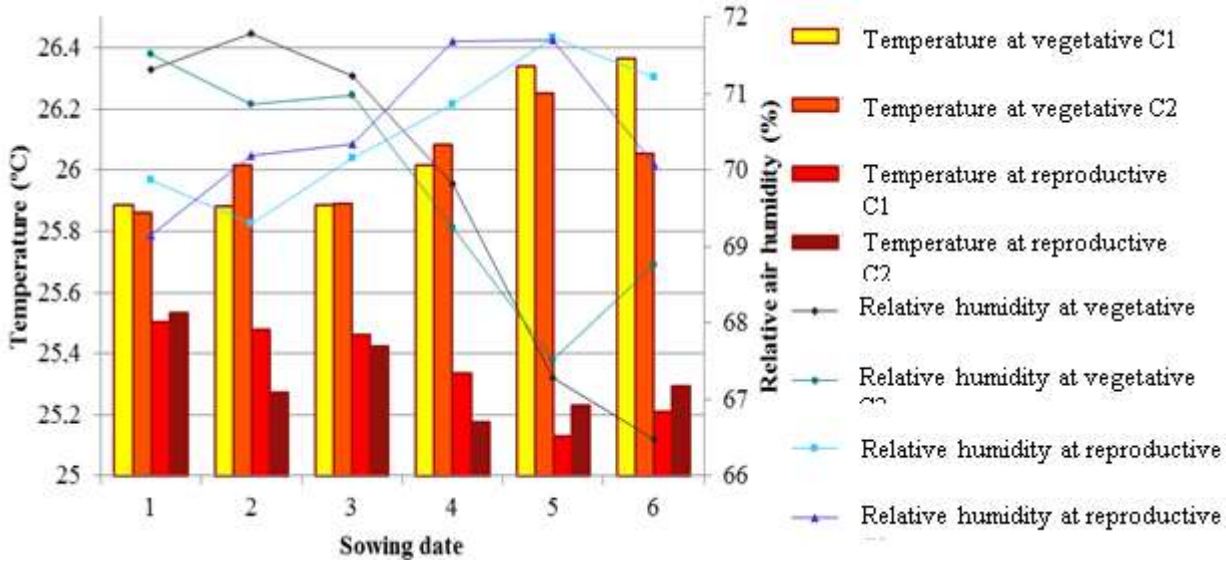

Figure 2. Temporal evolution of average temperature and relative humidity at vegetative and reproductive stages of the cultivars C1 and C2 during each sowing season.

the adequate rainfall during the vegetative stage, causing higher NPP. Although rainfall was less significant than in other dates, there is a possibility that C2 took greater advantages because its maturity group is greater than C1, enabling it to make better use of the water. The difference in duration of the phenological stages between cultivars (maturity group) is a major factor in determining yield components, suffering direct influence on genetic and environmental factors (Chen and Wiatrak, 2010).

In pod length, sowing dates 3 and 4 brought higher averages for C2, matching with NPP, while sowing seasons 5 and 6 had lower PL values. However, sowing season did not have effect on C1 (Table 2). These differences probably occurred due to sowing date to have greater influence on the results than the maturity group (Cruz et al., 2011a). Stem dry matter in S6 had a higher average for C2 (Table 2) than other sowing dates. The plants of that date were larger than the result of well-

distributed rainfall during the growth period (Figure 1). This fact is justified because water participates in the physiological processes of the plant, such as cell expansion, favoring stem growth and increasing the dry mass of the plant. Thus, sowing time and maturation cycle affect crop development (Cruz et al., 2010b; Taiz and Zeiger, 2013).

For pod dry mass, S3 had higher values for C2 while other seasons had lower values, the same was observed for C1 (Table 2). This is explained by the balance in water availability, both at vegetative and reproductive stages (Figure 1) since the accumulation of PDM occurs until the beginning of the R6 stage, and thereafter remains unchanged. If the availability of water for the plant is adequate throughout the cycle, the plant will have higher dry mass. Thus, small temporal differences of sowing time and maturity group contributed to this variation on average (Kurihara et al., 2013). For the number of grains per plant, the highest averages were observed in sowing seasons 3, 4 and 6 in C2 (Table 2), similar results for number of pods, because if the plant has a high NPP, it will probably also have a greater NGPL. This can be explained because the yield components (NPP, NGPL) are positive and related with the overall productivity, and reducing these components will cause reduction in grain yield (Kobraei et al., 2011).

Concerning the cultivars, there were significant differences in NPP for S6, where C2 presented higher mean value than C1 (Table 2). Conflicting results were observed in PL, which produced the highest mean for C2 and the lowest for C1 at the same sowing date. However, the results in S3 were the opposite. For SDM, C2 also achieved higher yield than C1, in sowing seasons 3, 4, and 6. C2 also had higher values than C1 in PDM and NGPL at S3. Although both cultivars are early-maturing varieties, the difference of a few days in the sowing date caused different results in yield components. This may have occurred because of different effects of climatic elements on each maturity group (Figures 1 and 2). This difference of days between the two cultivars favored more the maturity group 8.6 than the 8.2, because of rainfall and temperature, since soybean production is largely dependent on these elements (Kirnak et al., 2008; Bellaloui et al., 2011; Khan et al., 2011).

In general, S3 had higher mean values for most variables evaluated for both cultivars studied. As for the cultivars, C2 was statistically superior to C1 in every variable analyzed. However, differences between both seasons and cultivars were found, which supports the hypothesis of the influence of sowing date, due to changes in climatic elements and the length of the crop cycle (Chen and Wiatrak, 2010; Hu and Wiatrak, 2012). Furthermore, climatic elements and the cycle of each cultivar are related to physiological processes of the plants. In this experiment, water was a limiting factor in the production of photosynthates and their translocation in the phloem, restricting better results in yield components (Taiz and Zeiger, 2013).

Source of variation of the mass of one thousand grains (MTG) and productivity (PROD) for the factors cultivars (C) and sowing seasons (S)

The mass of one thousand grains showed no significant interaction between sowing date and cultivars; however, there was statistical difference for the factors separately (Table 1). Hence, ages 1 and 2 had the highest mean value for the mass of a thousand grains, while the lowest MTG values were obtained in sowing seasons 5 and 6 (Figure 3). Regarding cultivars, C2 had a higher mean value in agreement with the other results (NPP, PL, SDM, PDM and NGPL) (Table 2).

The amount of rainfall decreased in the later sowing dates (Figure 1), meaning that the amount of water available in dates 3, 4, 5 and 6 was much lower than that in the first two sowing seasons. The resulting decrease in MTG probably occurred because the plants need greater water accumulation in stages R1 to R7, and yield components are highly affected by periods of water stress, as they are the key elements to raise productivity in the field (Alcântara et al., 2012; Siahbidi et al., 2013).

For productivity, there was no difference between sowing dates and cultivars (Figure 4), meaning that all sowing dates and cultivars produce good results in productivity, that is, sowing may be done from late November to late December, based on the data of the 2014-2015 crop year for these cultivars.

Although sowing seasons 1 and 2 exhibited significantly different mean values for the mass of one thousand grains, the productivity was not different, that is, the results were similar between sowing dates 3, 4, 5 and 6, even with lower MTG values. The greater productivity of sowing dates 3, 4, 5 and 6 is the result of a greater number of grains per plant, despite the lower MTG value.

Interaction effects were verified for the variables NPP, PL, SDM, PDM and NGPL, but not for MTG and PROD (Table 1). This was probably due to the uneven rainfall during the crop cycle in sowing dates, as seen in Figure 1, with a greater accumulation of rainfall in the first three weeks from R1. However, the later sowing seasons benefited from rainfall in the vegetative stage, favoring an increase in dry matter production and contributing with productivity. If the rainfall events had continued well distributed until the end of March, an interaction between MTG and PROD in seasons 3, 4, 5 and 6 could have occurred, because this is the period where weather conditions are best for soybean plants and their development favors a high grain yield (Meotti et al., 2012). Nonetheless, to verify this, further experiments are

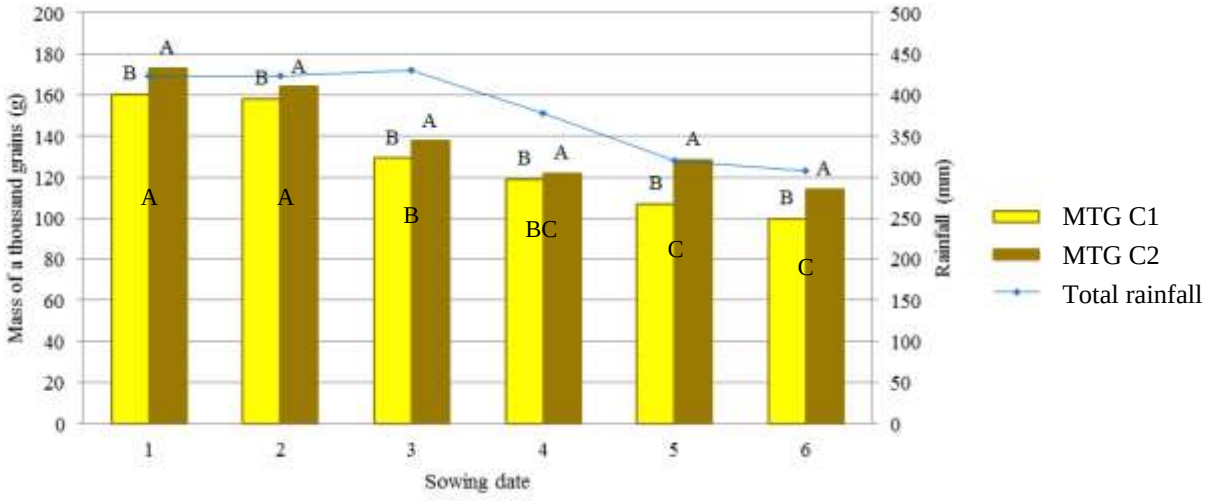

Figure 3. Mass of one thousand grains as a function of sowing season and total rainfall from sowing to harvest. Letters above the columns refer to cultivars, letters in the middle of the column refer to seasons.

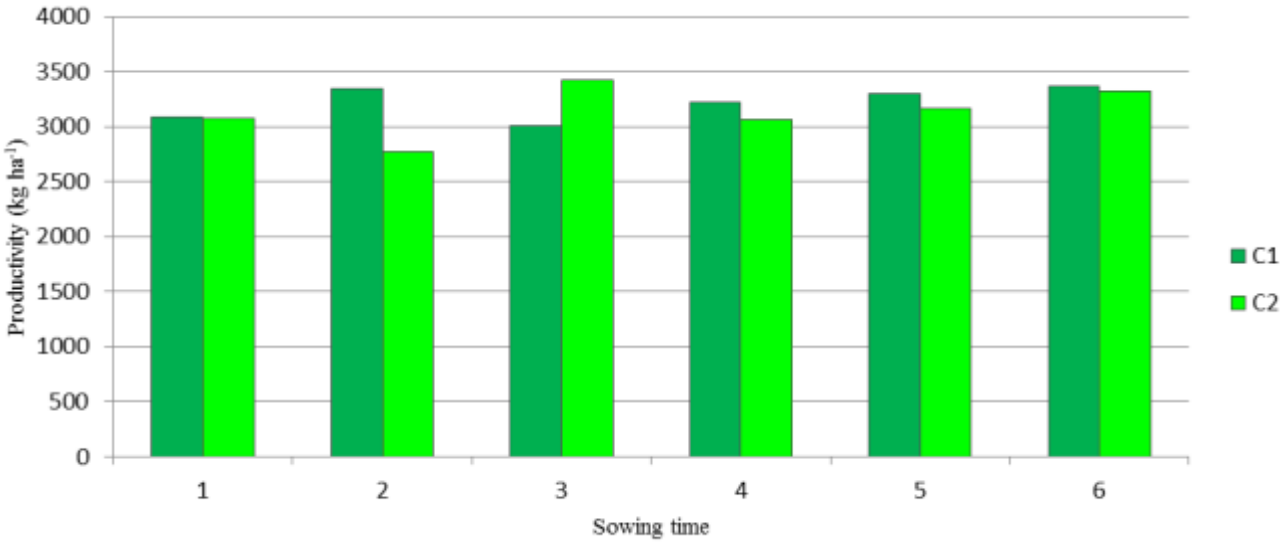

Figure 4. Average productivity for each sowing season for cultivars C1 and C2.

required to evaluate the results with irrigation if the rains ceased before grain filling.

Conclusion

Soybean sowing in the vicinity of Currais, State of Piauí, Cerrado microclimate, can be performed between November 20 and December 27 without changing productivity. Further, climatic elements work directly on yield components and on grain production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Response of bread wheat to integrated application of vermicompost and NPK fertilizers

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A greenhouse pot experiment was conducted to determine effects of vermicompost, inorganic fertilizers and their combinations on nutrient uptake, yield and yield components of wheat. A factorial combination four levels (0, 2, 4 and 6 tha-1) of vermicompost and four levels (0, 33.33, 66.67 and 100% ha-1) of the recommended NPK fertilizers was laid out in RCB design with three replications. Bread wheat variety, Kekaba was used as a test crop. Main effect results indicated that both vermicompost and NPK fertilizers significantly increased yield components, yield and nutrient uptake of wheat. Vermicompost applied at 2, 4 and 6 tha-1 increased grains yield of wheat by 11, 17 and 26% over control respectively whereas 33.33, 66.67 and 100% NPK fertilizers increased the grain yield by 10, 24 and 30%, respectively over the control. Vermicompost applied at 6 tha-1 resulted in the highest nutrient uptake and it increased grain uptake of N, P and K by 51, 110 and 89% over control respectively whereas among fertilizer rates, the highest uptake was produced by 100% NPK treatment and it increased the N, P and K uptake in the grain by were 79, 100 and 96% over control respectively. Combined application of vermicompost and NPK fertilizers has also significantly increased nutrient uptake, yield and yield components of wheat. It is concluded that wheat responds significantly to application of vermicompost and NPK fertilizers suggesting that nutrient contents of experimental soil is low for optimum production of wheat. Field verification and demonstration of results are recommended.

Key words: Soil fertility, Nutrient uptake, Grain and biomass yield, Yield components.

INTRODUCTION

Bread wheat is one of the major cereal crop produced in Ethiopia. According to central statistics authority (CSA) of Ethiopia, it is ranked fourth in terms of area cultivated and total production in 2014/2015 main cropping season (CSA, 2015). Wheat grains are used to prepare traditional food and beverages such as Dabbo (homemade bread), Enjera and Nifro, Tela, etc. It is also being used by food processing industries to prepare local bread, biscuits, pasta and macaroni. Despite, large area of land cultivated and suitable climate for wheat production in Ethiopia, the country is unable to produce sufficient amount of wheat grain to meet its annual

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domestic need. Thus, it is forced to import 30 to 50% of its annual demand for wheat grain (White et al., 2001). The low productivity of wheat (<2 tha^{-1}) is the main reason for the current wide gap between demand and supply for wheat grain in Ethiopia.

Decline in soil fertility among others is the main cause of very low productivity wheat in the country. Application of inorganic fertilizer especially those containing N and P have long been practiced to improve soil fertility for enhanced wheat and other crop production as these nutrients are the most limiting nutrients in almost all Ethiopia soils (ATA, 2014). However, fertilizers were applied irrespective of soil and crop types as well as agroecology. Such kind of blanket application of fertilizers are unrealistic due to the fact that the amount and type of fertilizer that should be applied can widely vary based on soil and crop type, and agroecology. Thus, developing site specific fertilizer recommendations are important for economic and environmental sound use of these inputs.

However, inorganic fertilizers were found to be more effective in increasing crop productivity when they are applied along with organic fertilizers. This is especially important for Ethiopia as nearly all soils in the arable lands of the country are highly depleted of organic matter. According to Gete et al. (2010), despite five times increase in fertilizer application in the Ethiopia, national cereal yields increased only by 10% since the 1980s. This was attributed to declining soil organic matter (IFPRI, 2010). This is because soil organic matter (SOM), in addition to improving the physicochemical properties of the soil and serving as nutrient sources, they hold nutrients from fertilizers applied in such a way that they are protected from loss through leaching and other pathways, and taken up by plants.

Organic fertilizers such as farm yard manure (FYM) and vermicompost can serve as a source of SOM and source of nutrients needed for the growth and production of crops. However, it is difficult to have sufficient amount of FYM that can supply adequate amount of nutrients needed by crops in smallholder famers' fields. Thus, integrate applications of inorganic and organic fertilizers are import to ensure adequate and balanced supply of nutrient to crops. With integrated nutrient management approach, the inorganic fertilizer can supplement with readily available nutrients to plants at early stages whereas organic fertilizers at later growth stages of plant that can boost yield and reduce the associated risks of chemical fertilizers (Mitiku et al., 2014). Integrated application of inorganic and organic fertilizers increases fertilizer use efficiencies, ensure balanced nutrient supply to crops, improve soil sustainability, etc. There are several literatures indicating the multiple advantages with integrated application of organic and inorganic nutrient sources over that obtained with sole application of either source (Kumar et al., 2015; Singh et al., 2011; Sangiga and Woomer, 2009). Therefore, the objectives of this experiment were to determine the effects of integrated applications of vermicompost and NPK fertilizers on the yield components and yields of wheat and to determine the effect of integrated application of vermicompost and NPK fertilizers on the uptake of N, P and K by wheat.

MATERIALS AND METHODS

Brief descriptions of the study site

The experiment was conducted in the greenhouse at tissue culture micro-propagation laboratory, Mekelle, Northern Ethiopia. Composite soil samples for pot experiment were collected randomly from farmlands of Mekan village, Enda-Mehoni district, Southern Tigray, Ethiopia. The sampling sites were located between 12°43'28" to 12°46'12" N and 39°29'18" to 39°33'35" E.

Physicochemical properties of soil and vermicompost used in this experiment

Prior to starting the experiment, the soil and vermicompost samples were analysed for their selected physicochemical properties following standard laboratory procedures (Jones, 2002) and the results are summarized in Table 1. The soil belongs to sandy clay loam textural class. The soil reaction (pH) was moderately alkaline and that of vermicompost was neutral (Tekalign, 1991).

Organic carbon (OC) content of the soil was found to be low whereas it was very high in vermicompost. Cation exchange capacity (CEC) of the soil was found to be high as outlined by (Hazelton and Murphy (2007). The soil TN content was in medium range but it was very high in the vermicompots (Berhanu, 1980). The available and total P contents of the soil and vermicompost were rated as medium (Cottenie, 1980) and very high (Murphy, 1968), respectively. Moreover, the total and exchangeable K contents of vermicompost and the soil were in medium ranges (FAO, 2006), respectively.

Treatments and experimental design

The treatments consisted of factorial combinations of four levels (0, 2, 4, and 6 tha^{-1}) of vermicompost (VC) and four levels (0, 33.3, 66.6, and 100%) of recommended NPK fertilizers. For vermicompost levels were coded as VC0, VC1, VC2, and VC3, whereas the fertilizer levels were coded as NPK0, NPK1, NPK2, and NPK3. The 100% recommended fertilizer rates (NPK3) for wheat production were equivalent to 64:20:50 kg NPK ha^{-1}. The experiment was laid out in RCB design with three replications.

Vermicompost was processed by earthworm *(Eisinea fetida)* using cow manure, *Lantana camara* leaves and wheat straw as main feedstock. Urea, TSP, and KCl were used as nitrogen (N), phosphorus (P), and potassium (K) sources in this experiment. Plastic pots with size 30 × 20 × 28 cm which were perforated at the bottom were filled up with 4 kg of soils. Then eight seeds of wheat variety, Kekaba were planted on pot as a test crop and later thinned to five seedlings after germination. The moisture contents of pots were regularly monitored and watered with distilled water as required.

Plant sampling and nutrient analysis

Plant samples were collected at harvest to determine the uptake of nitrogen, phosphorus, and potassium in the plant tissue. The above ground biomass of all the five plants from each pot were collected and partitioned into grain and straw. The grain and straw samples

Table 1. Some initial physicochemical properties of the soil and vermicompost used in the pot experiment.

Parameter	Sample source	
	Soil	Vermicompost (VC)
Textural class	Sandy clay loam	-
Moisture content (%)	-	38
pH	7.48	6.78
EC (ds m^{-1})	0.05	2.77
CEC (cmol(+) kg^{-1})	30.6	-
OC (%)	0.98	11.37
Total N (%)	0.06	1.41
Total P (%)	-	0.78
Av P (ppm)	9.26	-
Total K (%)	-	1.02
Exc.K (cmol(+) kg^{-1})	0.34	-

were washed with distilled water to clean contaminants, separately air-dried and oven dried to remove the moisture until constant weight was attained. The plant sample was ground and passed through 0.5 mm sieve for laboratory analysis. Plant phosphorus and potassium concentrations were analyzed through wet digestion method as described in Jones (2002). The P in the digest was determined by spectrophotometer, K by flame photometer and total nitrogen was analysed by Kjeldahl method (Bremner and Mulvaney, 1982).

Data collection

Data on total number of tillers per plant (TNTPP), effective number of tillers per plant (ENTPP), plant height (PHT), spike length (SPL), number of seeds per spike (NSPSP), above ground biomass (AGBYLD), and grain (GYLD) data were collected. The grain yield was divided to the biological yield and multiplied by 100% to calculate harvest index (HI) of wheat. Furthermore, nutrient (NPK) uptake data were obtained by multiplying the concentration of each nutrient in the straw and grain of wheat in each pot with the corresponding straw and grain yields.

Statistical data analyses

Data on yield component, AGBYLD, GYLD, HI, and nutrient uptake data were subjected to analysis of variance (ANOVA) using SAS software Version 9.0 (SAS, 2002). Mean were separated using least significance difference (LSD) method at 0.05 probability level using the same software.

RESULTS AND DISCUSSION

Effects on yield components of wheat

The results of main effects data showed both vermicompost and NPK fertilizers significantly affected the yield component data of wheat grown in the greenhouse experiment (Table 2). VC3 produced the highest PHT, SPL, and NSPSP of wheat which was followed by VC2 and VC1 in that order and the least values of these parameters were produced in the control treatment. VC3 increased these parameters by 6, 16 and 36% over the control, respectively. However, vermicompost did not significantly affect TNTPP and ETNPP (Table 2).

On the contrary, NPK fertilizers significantly increased TNPP and ETNPP of wheat relative to the control treatment. The highest number of TNTPP (2.1) and ETNPP (1.9) were produced by NPK3 treatment. The results are in agreement with the findings of Niamatullah et al. (2011) who observed a significant difference in number of tillering and productive tillers of wheat due to NPK levels. This could be due to the priming effect of chemical fertilizers on availability of nutrients especially mineral N that could have contributed to the vegetative growth and tiller initiation of wheat unlike vermicompost. Similarly, PLH, SPL and NSPP of wheat have been significantly increased by all NPK fertilizer doses. The highest values of these parameters were produced by NPK and it increase PLH, PLH and NSPP by 14, 43 and 43% over the control, respectively. The magnitudes of increases in these parameters are far higher than that produced by the highest dose of vermicompost which is VC3. This happened due to immediate availability of nutrients contained in chemical fertilizers than those in the organic fertilizers such as vermicompost in this case.

PLH, SPL and NSPP of wheat have been significantly affected by interaction effects of vermicompost and NPK fertilizers (Table 3). All treatment combinations of vermicompost and NPK fertilizers significantly increased PLH, SPL, and NSPP of wheat compared to the control. However, the highest increases of the parameters were obtained with treatments involving VC3 + NPK3, VC2 + NPK2, and VC2 + NPK3 in that order. But these treatments were statistically at par among each other with respect to their effects on these parameters. The results are in agreement with several reports indicating that combined application of organic and inorganic fertilizers

Table 2. Main effects of vermicompost and NPK fertilizer levels on TNPP, ENTPP, PLH, SPL and NSPSP.

Treatment	TNTPP	ENTPP	PHT (cm)	SPL (cm)	NSPSP
Vermicompost level					
VC0	1.62[a]*	1.05[a]	65.45[b]	6.36[c]	23.33[c]
VC1	1.63[a]	1.07[a]	65.92[b]	6.96[b]	29.27[b]
VC2	1.70[a]	1.08[a]	69.38[a]	7.22[ab]	29.38[b]
VC3	1.73[a]	1.09[a]	69.35[a]	7.42[a]	31.85[a]
LSD (0.05)	ns	ns	2.92	0.35	2.26
NPK fertilizer level					
NPK0	1.00[c]	1.00[b]	61.43[c]	5.58d	17.27[c]
NPK1	1.54[b]	1.00[b]	67.51[b]	6.85[c]	28.05[b]
NPK2	1.96[a]	1.02[b]	71.12[a]	7.53[b]	34.14[a]
NPK3	2.17[a]	1.29[a]	70.05[ab]	7.99[a]	34.36[a]
LSD (0.05)	0.26	0.13	2.92	0.35	2.26
VC*NPK	ns	ns	*	**	*
CV (%)	18.99	15.16	5.19	6.08	9.55

*Means followed the same letter (s) are not significantly different each other at 0.05 probability level.

Table 3. Interaction effects of vermicompost and NPK fertilizers on PHT, SPL and NSPP.

Treatment combination	PHT (cm)	SPL (cm)	NSPSP
VC0 × NPK0	57.9[h]*	5.7[e]	15.1[f]
VC0 × NPK1	61.4[fgh]	5.5[de]	19.7[f]
VC0 × NPK2	69.9[abcd]	7.0[c]	27.9[e]
VC0 × NPK3	72.6[abc]	7.9[ab]	31.3[cde]
VC1 × NPK0	61.1[gh]	5.5[de]	16.8[f]
VC1 × NPK1	68.3[abcde]	6.9[c]	31.8[cde]
VC1 × NPK2	67.4[cde]	7.4b[c]	33.2[bcd]
VC1 × NPK3	66.9[cdefg]	7.9[ab]	35.2[abc]
VC2 × NPK0	62.6[efgh]	5.6[de]	17.9[f]
VC2 × NPK1	67.6[bcde]	7.8[abc]	29.1[de]
VC2 × NPK2	73.8[a]	7.5[bc]	37.4[ab]
VC2 × NPK3	73.5[a]	8.2[a]	33.1[bcd]
VC3 × NPK0	64.1[defg]	6.2[d]	19.3[f]
VC3 × NPK1	72.7[abc]	7.3[bc]	32.3[cde]
VC3 × NPK2	73.4[a]	8.3[a]	38.0[a]
VC3 × NPK3	67.2[cdef]	7.9[ab]	37.8[a]
LSD (0.05)	5.83	0.71	4.52
CV (%)	5.19	6.09	9.55

*Means followed the same letter (s) are not significantly different each other at 0.05 probability level.

produce significantly higher values of yield components of crops including wheat that obtained from sole application organic or inorganic fertilizers (Dastmozd et al., 2015; Yavarzadeh and Shamsadini, 2012).

Effects on biomass, grain yield, and HI

Main effects of vermicompost and NPK fertilizers on the biomass and grain yield of wheat are presented in Table 4. All vermicompost rates produced significantly higher AGBYLD and GYLD of wheat than the control. But the highest values of these parameters were obtained with VC3 followed by VC2 and VC1 in that order. This is in agreement with findings of Joshi et al., (2013) and Yousefi and Sadeghi (2014) who reported that application of vermicopost to soil significantly increases the yield of wheat. Besides, different studies have also

Table 4. Main effects of vermicompost and NPK on biomass and grain yield and harvest index (HI).

Treatment	AGBYLD (g pot^{-1})	GYLD (g pot^{-1})	HI (%)
Vermicompost level			
VC0	39.3^{d}*	16.9^{d}	42.7^{b}
VC1	42.1^{c}	18.6^{c}	44.2^{ab}
VC2	44.2^{b}	19.6^{b}	44.3^{ab}
VC3	45.6^{a}	21.1^{a}	46.2^{a}
LSD (0.05)	1.34	0.85	2.07
NPK fertilizer level			
NPK0	38.1^{d}	16.4^{d}	43.1^{b}
NPK1	41.4^{c}	18.0^{c}	43.6^{b}
NPK2	45.0^{b}	20.3^{b}	45.0^{ab}
NPK3	46.8^{a}	21.4^{a}	45.6^{a}
LSD (0.05)	1.34	0.85	ns
VCXNPK	**	ns	ns
CV (%)	3.75	5.36	5.62

*Means followed the same letters are not significantly different each other at 0.05 probability level.

demonstrated the beneficial effect of application of vermicompost at different rates on the yields of other crops such as tomato (Arancon and Edwards, 2005; Kashem et al., 2015), maize (Reshid, 2016), and barley (Mitiku et al., 2014). As vermicopost is a source of different essential plant nutrients, its application in soil with low nutrient content especially in NPK will definitely increase the growth, yield and yield components of crops including wheat.

However, in addition to being sources of different nutrients, vermicompost is also supposed to contain growth promoting hormones (Edwards et al., 2004) which might facilitate higher nutrient uptake by plants and this could be an addition factor for the positive effect of vermicompost on crops. Both vermicompost and NPK fertilizers have significantly increased HI of wheat (Table 4). VC3 produced the highest HI than that produced by all other treatments including control. Similarly, the highest HI was produced by NPK3 than that produced by all other treatment.

Similarly, all NPK fertilizers rates produced significantly higher ABGYLD and GYLD of wheat than the control (Table 4). NPK3 produced the highest yield than that produced by all other fertilizer treatments and it increased the AGBYLD and GYLD by 22.8 and 30.5% over the control, respectively. It also resulted in significantly higher HI value of wheat.

The positive effects of vermicompost and NPK fertilizers application on wheat seen in this experiment suggest that the study soils are low in its nutrient contents particularly of NPK. The result of initial soils analyses data (Table 1) also proves this claim.

Vermicompost by NPK fertilizer interaction effect was highly significant (P<0.001) for biomass yield of wheat (Table 5). Accordingly, the highest AGBYLD was produced by treatment involving VC2 + NPK3 which was statistical at par with biomass yield produced by VC3 + NPK2, VC1 + NPK3, VC2 + NPK2, and VC3 + NPK3 and all these treatments were statistically at par among each other with respect to ABYLD of wheat produced by them. However, they produced significantly higher ABYLD of wheat than that produced by sole application vermicompost and NPK fertilizers. The result suggests that there was a synergistic interaction between the two nutrient sources in availing nutrients to the growing wheat and the finding is in agreement with report of Davari et al (2012) and Davis et al. (2011). In line with the current finding, Seal et al. (2014) reported that straw yield, which is the major constituent of biological yield, was also significantly increased by the combined application of vermicompost and NPK fertilizers.

Effects on nutrient uptakes

The uptakes of N, P and K by the straw and grain yield of wheat were significantly affected (P ≤ 0.01) by the main effects of vermicompost and chemical fertilizers (Table 6). VC1, VC2, and VC3 increased grain N uptake by 22, 35, and 51%, respectively over the control. Similarly, these treatments increased the grain P uptake by 22, 45, and 71% over the control, respectively and the grain K uptake by 33, 48, and 53% over the control, respectively. There were also significant increases in the straw uptake of N, P and K due to vermicompost application. The apparent increased uptake of nutrients due to application VC indicates that there was net mineralization of nutrients from vermicompost.

Table 5. Interaction effects of vermicompost and NPK fertilizers on AGBYLD (g pot^{-1}) of wheat.

NPK fertilizer level	Vermicompost Levels				
	VC0	VC1	VC2	VC3	Mean
NPK0	34.57^{h}*	35.47^{h}	38.77^{fg}	43.60^{dc}	38.10
NPK1	36.90^{gh}	41.47^{de}	43.07^{cde}	44.03^{dc}	41.37
NPK2	40.50^{ef}	44.57^{bc}	47.03^{ab}	47.90^{a}	45.00
NPK3	45.13^{bc}	46.93^{ab}	48.10^{a}	46.93^{ab}	46.77
Mean	39.28	42.11	44.24	45.62	42.81

*Means followed the same letter (s) are not significantly different each other at 0.05 probability level.

Table 6. Effects of vermicompost and NPK fertilizers on uptake of N, P and K by grains and straw of wheat.

Treatment	Nutrient uptake					
	Nitrogen (g pot^{-1})		Phosphorus (g pot^{-1})		Potassium (g pot^{-1})	
	Straw	Grain	Straw	Grain	Straw	Grain
Vermicompost level						
VC0	0.055^{c}*	0.306^{d}	0.007^{c}	0.009^{d}	0.432^{c}	0.08^{d}
VC1	0.078^{b}	0.373^{c}	0.011^{a}	0.011^{c}	0.476^{b}	0.107^{c}
VC2	0.091^{a}	0.412^{b}	0.009^{b}	0.014^{b}	0.514^{a}	0.132^{b}
VC3	0.098^{a}	0.462^{a}	0.011^{a}	0.019^{a}	0.543^{a}	0.151^{a}
LSD (0.05)	0.008	0.0375	0.001	0.0012	0.0368	0.0107
NPK fertilizer level						
NPK0	0.044^{d}	0.28^{d}	0.007^{b}	0.009^{d}	0.400^{d}	0.082^{d}
NPK1	0.068^{c}	0.35^{c}	0.008^{b}	0.011^{c}	0.472^{c}	0.098^{c}
NPK2	0.093^{b}	0.422^{b}	0.012^{a}	0.015^{b}	0.525^{b}	0.131^{b}
NPK3	0.116^{a}	0.502^{a}	0.012^{a}	0.018^{a}	0.568^{a}	0.161^{a}
LSD (0.05)	0.008	0.0375	0.001	0.0012	0.0368	0.0107
VC × Fertilizers	*	ns	**	ns	ns	ns
CV (%)	11.96	11.60	12.84	11.02	9.01	10.95

*Means followed the same letter (s) are not significantly different each other at 0.05 probability level.

Similarly, all NPK treatments have significantly increased the uptake of N, P and K by the straw and grain of wheat compared with the control or NPK0 (Table 6). However, the highest uptake of N, P and K by straw and grain of wheat was produced by NPK3 followed by NPK2 and NPK1 in that order. These treatments increased the grain N uptake by 79, 50 and 25% over the control (NPK0), respectively. These treatments increased the grain P uptake by 100, 67, and 22% over the control, respectively and the grain K uptake by 96, 60, and 20% over the control, respectively. The finding is in line with Sheoran et al. (2015) and Laghari et al. (2010) who reported that applications of NPK have significantly increased grain nutrient uptake of wheat.

Conclusion

Application of vermicompost significantly increased the yield components, yield and nutrient uptake of wheat grown in the greenhouse suggesting that there was net mineralization of nutrients contained in the vermicompost and availed to the growing wheat. The results also suggest that the soil used in the experiment was low in essential plant nutrients. Similarly, application of NPK fertilizers significantly increases the yield components, yield and nutrient uptake of wheat indicating insufficient amount of N, P and K in soil used in the study. This was confirmed by results of initial soil analyses data of experimental soil which showed that low levels of N, P and K as well as low level of soil organic matter content. There was a significant interaction between vermicompost and NPK fertilizers for above ground biomass yield of wheat and optimum yield was produced by treatment combination of VC2 + NPK2. The result suggests that there was synergistic interaction between vermicompost and NPK fertilizer in increasing nutrient availability to the growing wheat. The finding further

indicates that the full recommended dose can be decreased to 67% and the vermicompost dose can be decreased by 50% to achieve the same yield produced by 100% vermicompost and NPK fertilizer doses applied alone. Further verification and demonstration of the current results in the field are recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Application of ethanolic extract of propolis typified in nutrition and vegetative growth of beans

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There is little information about the effects of the ethanolic extract of propolis (EEP) in common bean. The aim of this study was to investigate the effects of foliar spray of ethanol extract of propolis (EEP) on vegetative growth, water loss and yield of black type common bean. The experiment was conducted at Farm Dona Isabina from February to May 2012. The experimental design was a randomized block with five replicates, applying five concentrations (0, 3, 6, 9 and 12%) of EEP, and the extract was made with 10% of crude propolis (which originated from the coast of Paraná State -Brazil) and 90% alcohol, 96°GL. Only chlorophyll content and stem diameter were split plot in time. The EEP also significantly increased the levels of Mg in the leaves, increasing the concentrations of chlorophyll in leaves and vegetative growth, which served as an energy threshold, increasing the productivity of the mighty bean for 426 kg/ha.

Key words: Water content, beeswax, drought stress.

INTRODUCTION

The cultivation of beans has increased each year especially in Mato Grosso State in Brazil. The expansion in these agricultural areas and the fragility of its harvest have been facing pest and disease attacks. Application of agricultural defensives and fertilizers has generated negative effects in the water quality, throughout the accumulation of pesticides molecules, plant equilibration and the natural biological control (Manzoni et al., 2006; Gama et al., 2013).

Due to the detriment of the use of pesticides and nutrients, many researchers have evaluated various alternatives to replace the use of chemicals, like grout, raw cow's milk, milk whey, cow urine, biological insecticide based on *Bacillus thuringiensis*, Bordeaux mixture, melted coconut soap and vegetable extracts (Zatarim et al., 2005; Sousa et al., 2012). However, the current alternatives are few; hence there is a need to provide new options and have more studies about the benefits of the use of its alternatives (Pereira et al., 2008).

With the intuition to offer new alternatives in the pest and disease control as well as plants nutrition, Pereira et

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Table 1. Levels of pH, macronutrients (mg/dm^3), aluminum (cmol/dm^3), potential acidity organic material (g/dm^3) and base saturation (%) of soil analysis of the sample withdrawal of 0 to 10 cm soil of the experiment in Fazenda Dona Isabina, Santa Carmen-MT, Brazil.

pH	P	K	Ca	Mg	Al	H	H+Al	OM	BS %
5.41	3.69	37.00	1.76	0.71	0.05	4.30	4.35	26.70	37.05

Table 2. Sand, silt, clay and micronutrients content (mg/dm^3) of soil analysis, sampled between 0 to 10 cm from soil of the experiment conducted at Dona Isabina Farm, Santa Carmen - Mato Grosso, Brazil.

Sand	Silt	Clay	Zn	Cu	Fe	Mn	B	S
436	147	417	4.13	0.54	103.39	8.37	0.43	10.18

al. (2008) proposed the application of foliar ethanolic extract of propolis (EEP) in coffee leaves to reduce the incidence and severity of brown eyes spot in coffee plant seedlings. Propolis is a resinous substance used by bees to protect themselves against predators, parasites and especially in aseptic beehive (Galvão et al., 2007). The chemical composition of propolis is complex as it has been found to contain more than 200 compounds altogether, in relation to plant diversity found around the hive (Menezes et al., 2009). This feature enables numerous properties that are favorable for human health, such as: antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, hypotensive, anticancer, anti-HIV, anticariogenic, among others (Castro et al., 2007; Endler et al., 2009).

The first results of the application of propolis extract to coffee are promising. For example, Pereira (2004) verified in glass slide chambers that propolis extract can be responsible for 100% reduction in germination of uredinosporos *Hemileia vastatrix* Berk & Br, coffee rust in coffee plants. Another study by Pereira et al. (2008) verified a reduction of 66 and 46% in the incidence of rust and gray leaf spot, respectively, with the application of EEP with 16% of the crude propolis extract in coffee. They also found an increase in the vegetative growth of seedlings which was attributed to the presence of propolis.

In this light, this study aims to investigate the effect of ethanolic extract of propolis (EEP) on vegetative growth, nutrition and yield in a variety of common black bean type.

MATERIALS AND METHODS

Study area

The experiment was conducted between February and May of 2012, on Farm Dona Isabina, located at Santa Carmen - MT, (11º51'S, 55°30', height 372 m). The climate, according to Koopen classification is AW (Tropical wet and dry), characterized by the presence of two seasons: the "rainy" and "dry". All the evaluations were accomplished in the Laboratory of Animal Nutrition and Forage at University of Mato Grosso (UFMT), Sinop – Mato Grosso, Brazil.

The experimental design used was randomized blocks with five replications while the treatments included five EEP concentrations (0, 3, 6, 9 and 12%) with extract made with 10% crude propolis (originating from the coast of Paraná) and 90% alcohol 96°GL. The chlorophyll content and stem diameter were split plot scheme in time with the experimental plot consisting of eight rows of five meters long, totaling 20 m². Only the three central rows of the plot were considered for evaluation, eliminating two feet on each row end and two lateral lines (borders). Thus, the plot area was 12 m².

Before the beginning of planting, soil analyses was carried out considering 20 cm depth, collecting five simple soil samples and a composed sample. Results of the foliar analysis are as shown in Tables 1 and 2.

At the moment of sowing, ten bean seeds/m^3 were deposited and row spacing was 0.50 m as suggested by Embrapa (2005). The variety of bean used in the experiment was BRS Valente, black type while weed control occurred within that recommended for this crop.

At the same time of sowing, fertilizer application was done using 200 kg/ha of the formulated 16-16-16. Also, 30 days after germination, 50 kg/ha of nitrogen was applied. Thereafter, insecticides were used to control the leafroller (*Bonagota cranaodes*) and the cucurbit beetle (*Diabrotica* speciosa).

Evaluation of the vegetative growth was done every fortnight from V4 to R8 with a consideration of the following traits: plant height, stem diameter, leaf area, number of leaves, fresh and dry mass of shoots of bean. To obtain these measurements, five plants/plot of the first row and the right side per plot were chosen.

Plant height was collected using a measuring tape, collecting the cervical length to the apical meristem of plants. Also, stem diameter was measured with the use of a digital caliper, collecting the data at 5 cm above the ground. After plant field evaluation, plants were cut close to the soil, packed in paper bags and taken into the laboratory.

The samples collected on the field were immediately weighed to estimate the fresh mass level of the shoot after the leaves were detached and counted for the number of leaves/plant. It was possible to obtain leaf area in cm², using leaf area meter LI-COR Model LI -3010. Finally, the samples were reconditioned on paper bags and placed in a forced oven at 60°C until constant mass, after which dry mass of shoots was obtained.

The chlorophyll contents of the leaves was assessed fortnightly, from V4 to R8 using a chlorophyll measurer (Mark Falker CFL1030 model). To obtain these variables, ten sheets of the third node below the apical meristem of 10 plants/plot were measured.

At the beginning of flowering time, when plants were at R6 growth stage, the relative water content of leaves was assessed following the methodology proposed by Tuner (1986). For this variable, three fully expanded leaves were collected on the middle

Table 3. Concentrations of macro (g/kg) and micro nutrients (mg/kg) in the propolis originating from the coast of Paraná.

Element	Concentration
Nitrogen	11.9
Phospor	8.9
Potassium	2.5
Calcium	1.99
Magnesium	0.535
Sulfur	40
Copper	5.6
Zinc	24
Manganese	166
Iron	195

third of the plants. Leaves were placed in plastic containers and packed in a cooler with ice to prevent it from losing water because of the time spent on transportation to the laboratory. Thereafter, three disks of leaf tissue was withdrawn using a metal ring with 1.3 cm diameter, avoiding the presence of ribs or any damage to them.

After obtaining these disks they were weighed on a precision balance (0.0005 g) to obtain the fresh mass (m_f). Shortly after, the same disks were placed in a beaker with distilled water for 12 h to reach swelling. Thereafter, they were weighed to obtain the turgid mass t (m_t). Finally, the disks were placed in paper bags and maintained in a forced-circulation oven at 60°C until they reached constant mass, for obtaining dry mass content (m_s). With the estimation of m_f, m_t e m_s, relative water content (RWT) was also estimated considering the following formula:

$$RWT\ (\%) = \frac{mf - ms}{mt - ms} \times 100$$

Where: *mf* is the fresh mass; *mt* is turgid mass and *ms* is dry mass.

Evaluation of the nutritional status of the leaves was accomplished according to Malavolta et al. (1997) by collecting the first mature leaf from the branch tip at the beginning of flowering time, at R1 or 42 days after plant emergence. For this, up to 30 sheets/plot were sampled. The collected leaves were placed in a paper bag, taken to the greenhouse and subjected to a temperature of 55°C for drying until constant mass was achieved.

The samples of leaves were sent to Procafé Foundation in Varginha - Minas Gerais State, Brazil, for analytical determinations of the levels of macro and micro in bean leaves, according to Malavolta et al. (1989). Nitrogen was determined by semimicro-Kjeldahl method, phosphorus by colorimetric method, sulfur by turbidimetric, and potassium by photometry and flame emission. Nutrients like Ca, Mg, Cu, Mn and Zn were determined by the method of atomic absorption spectrometry. The determination of nutrient contents of propolis followed the same methodology used in the assessment of foliar nutrients.

Harvesting, threshing and uprooting, was manually performed, when the grains were approximately 18% moisture. After harvesting, the beans had the moisture adjusted to 13% in forced circulation oven at 60ºC in the laboratory. Thus, it is possible to obtain productivity in grams per plot and was converted into kg/ha.

Sample preparation

Propolis was used originally from the coast of Paraná, and the ethanolic extract was prepared using 90% alcohol, 96°GL and 10% of crude propolis. After this preparation, the extract was stored for about a month due to extraction of the chemical properties of propolis (Marcucci et al., 2008) yet to occur.

Concentration analysis

The spray with EEP diluted in water was applied fortnightly on the leaf plants. The first application was done when plants had three trifoliate leaves; the second, when plants reached V4 growth stage; and the third and last was done near the harvest of the beans, when plants were at R6 growth stage.

Statistical analysis

Data were subjected to analysis of variance at 5% probability using SISVAR ® software (Ferreira, 2000). For quantitative variables, the models were chosen based on the significance of the regression coefficients using the "t" test in adopting the level of 7% probability and determining the value of r^2 (SQRegression / SQtreatment).

RESULTS

The foliar application of EEP had not significantly altered the foliar concentrations of nitrogen, phosphorus, potassium, sulfur, zinc, iron, manganese, copper and boron. This result can be attributed to low concentrations of these elements in propolis (Table 3).

The EEP changed quadratically, increasing the Mg content in bean leaves. The maximum efficiency of the application of EEP occurred at a concentration of 6.2%, with an accumulation of 0.697 dag/kg Mg (Figure 1). The relative water content (R1) was under the levels of water stress plants (Figure 1B) (Maia et al., 2007). The EEP linearly increased water content in the leaves of bean, along with addition of 1.35% in water content in leaves with every 1% increase in EEP added to spray liquid (Figure 1B).

The application of propolis promoted an increase of two units in chlorophyll content and stem diameter of bean plants when the concentration of 6.0% of EEP (Figure 2A and B) was considered.

Foliar application of EEP increased quadratically for fresh and dry mass of shoots. The maximum accumulation of fresh and dry matter by plants occurred at 7.68 and 7.49% EEP concentrations, showing values of 6.59 ton/ha (Figure 3).

The EEP increased the number of pods/plant while the maximum efficiency of EEP occurred at a 6.82 and 7.25% concentration level with observed values of 14.59 pods/plant. With the increase in number of pods/plant, productivity also increased for 426 kg/ha, which represents 22.6% above control, considering a concentration of 8.22% of EEP (Figure 4).

DISCUSSION

Regarding the nutrient content in the bean leaves, the use of the EEP concentrations changed only the Mg

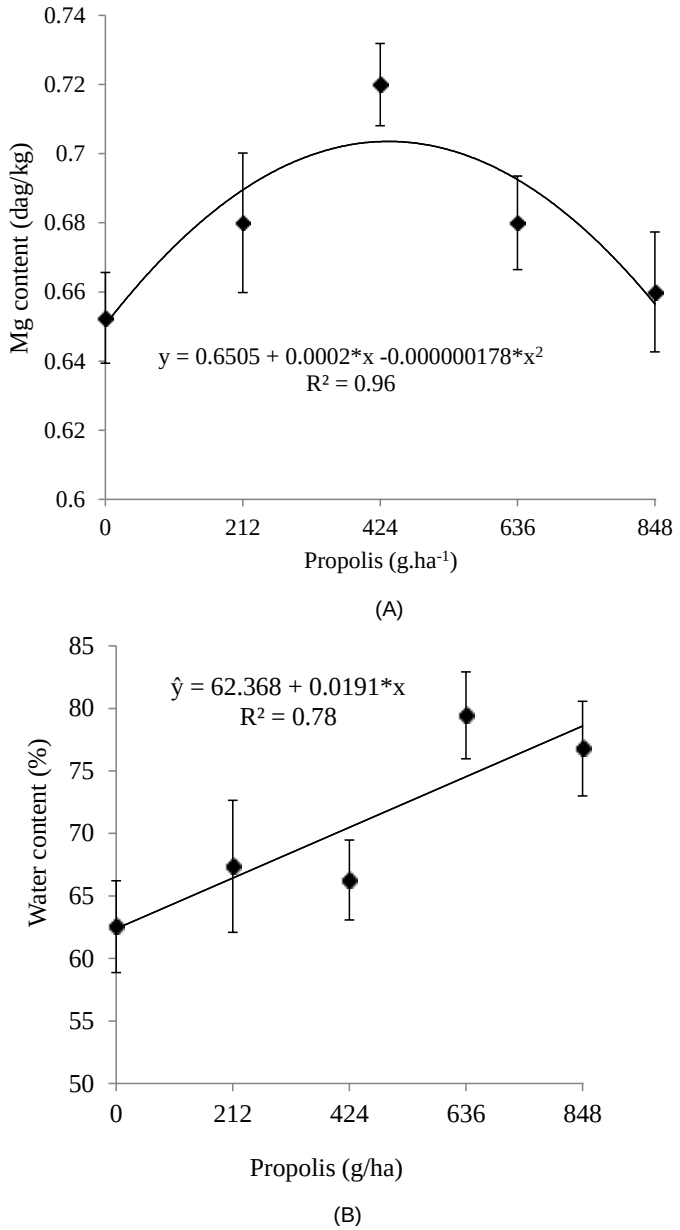

Figure 1. Leaves Mg content (dag.kg^{-1}) (A) and water content (B) in the black bean leaves of the BRS Valente variety, under the application of five concentrations of EEP (water 0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

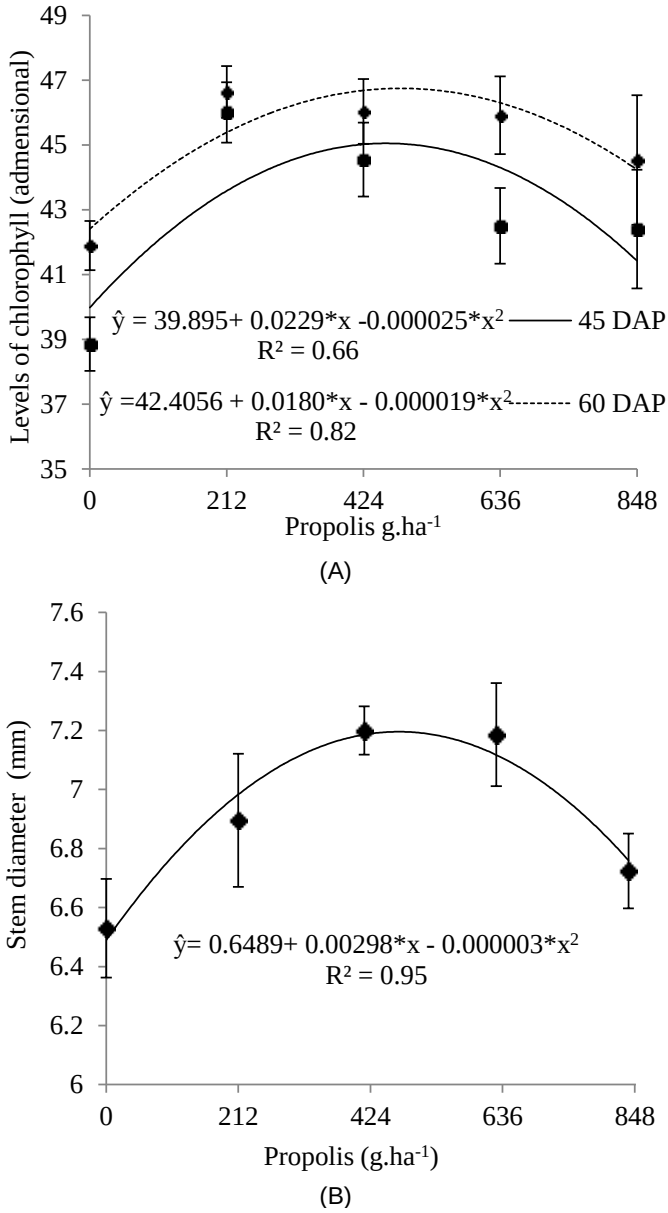

Figure 2. Levels of chlorophyll in leaves (admensional) (A) and stem diameter of beans, variety "BRS Valente", black type (B) under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen – MT.

content. Different results were found by Pereira and Farias (2013) on application of an EEP 10% crude propolis from Rondônia diluted in water to 2.5%. They also found a quadratic pattern in the concentrations of nitrogen and zinc in leaves of coffee plants due to the presence of significant levels of this nutrient in the composition of propolis.

The accumulation of Mg (Figure 1A) was due to the presence of this element in the constitution of propolis applied (Table 1), which was observed in the formation of propolis in other studies (Marcucci, 2008; Pataca, 2006; Gong et al., 2012; Korn et al., 2012).

The EEP reduced water loss from the leaves which occurred by forming a film of wax that accumulates on the skin of leaves (Pereira and Farias, 2012; 2013; Pereira et al., 2008).

The increase in chlorophyll content and vegetative growth (Figure 2A and B), observed by the increase of stem diameter and dry mass of shoots was due to the increase in foliar Mg (Table 1). This occurs because the Mg is present as central atom of the complex porphyrin derivatives which are chlorophyll, and thus a key element

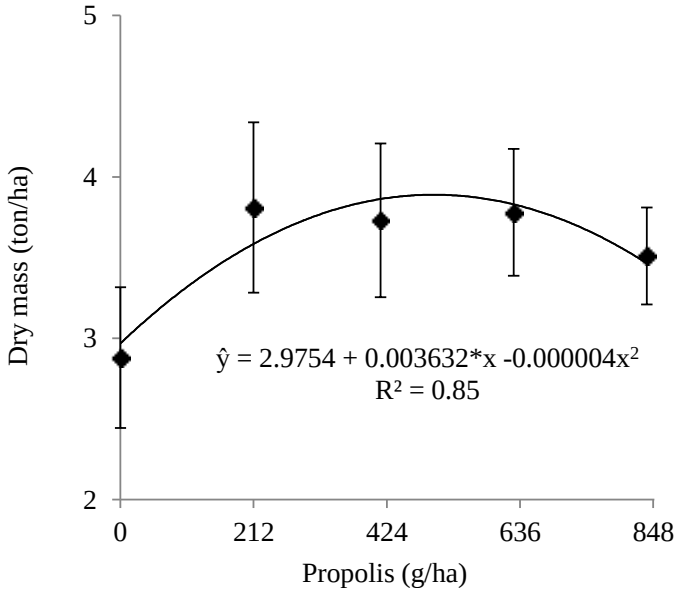

Figure 3. Dry mass of shoots of bean plants, variety BRS Valente, black type, under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

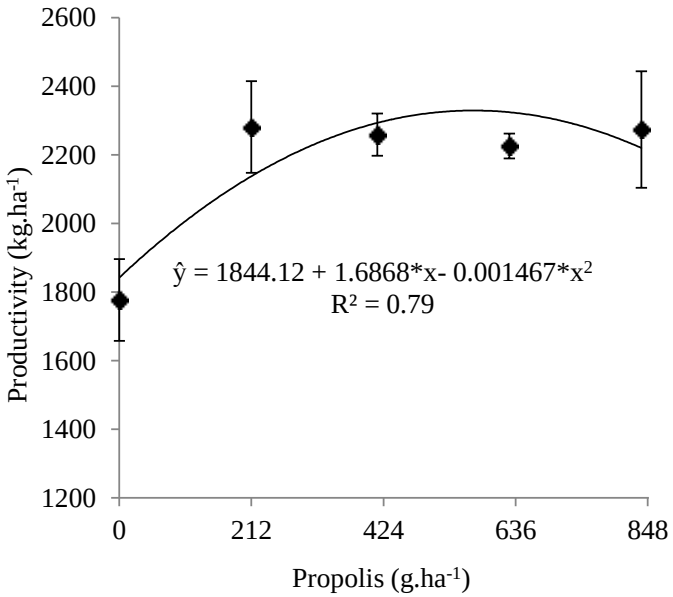

Figure 5. Productivity (kg/ha) of dry bean variety BRS Valente, black type, under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

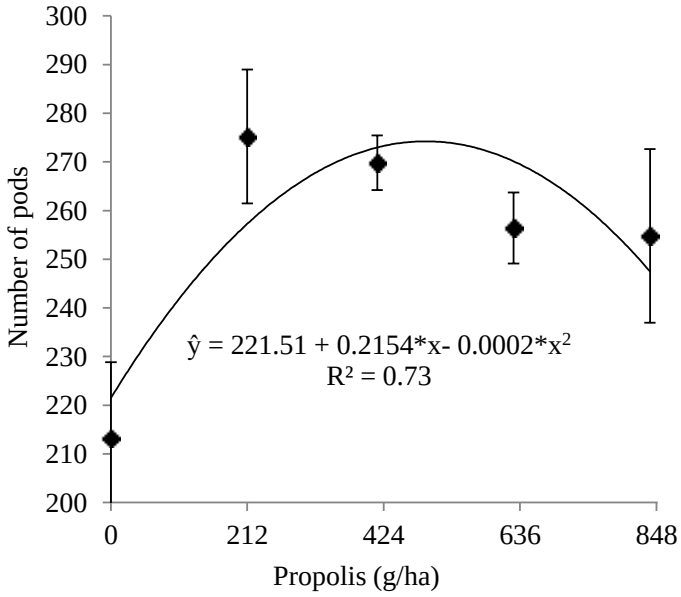

Figure 4. Number of pods of bean plants, variety BRS Valente, black type, under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

in the composition of chlorophyll (Taiz and Zeiger, 2004; Streit et al., 2005). In addition to the structural function, the Mg is present in almost all the enzymes responsible for metabolism and growth of bean plants (Boaro et al., 1996).

Thus, increases in the levels of Mg enhanced the levels of assimilates, which served as the threshold energy for the production of green matter in plants (Silveira et al., 2003; Soratto et al., 2004; Carvalho et al., 2008; Santana and Silveira, 2008).

With higher amounts of chlorophyll content, relative water content and shoot dry mass, promoted by the application of EEP, consequently productivity reached high levels (Figures 5). Results observed by other studies (Pereira et al., 2008, Pereira and Farias, 2013; Marini et al., 2012) concluded that applying EEP on coffee and bean seedlings can be responsible for higher vegetative growth, attributing them to the presence of nutrients and the formation of a film wax on the leaves.

However, it is important to note that different results can be found by other authors in the application of propolis in plants. These differences in chemical composition of propolis (Pereira, 2004; Pereira et al., 2008) are likely due to propolis plant origin, which has changed its composition according to the vegetation of the region where it was collected (Park et al., 1995), genetics of queen bees (Koo and Park, 1997) and methods used for extracting the propolis extract (Park et al., 1997).

Conclusion

This study thus revealed that the original propolis from Paraná Coast of Brazil was responsible for higher foliar concentrations of Mg and water content in bean leaves. The EEP is related to the number of pods at harvest, thus it can enhance the productivity of the BRS Valente bean for 426 kg/ha. The high levels of productivity can be

attributed to the maintenance of water and Mg content in the “BRS Valente“ bean leaves. The application of ethanol extract of propolis, can become a viable alternative for applications in organic and conventional agriculture to reduce crops environmental impacts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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