academicJournals

Vol. 13(1), pp. 21-26, 4 January, 2018
DOI: 10.5897/AJAR2017.12613
Article Number: CFE8ACE55269
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

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

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Received 25 July, 2017; Accepted 10 October, 2017

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

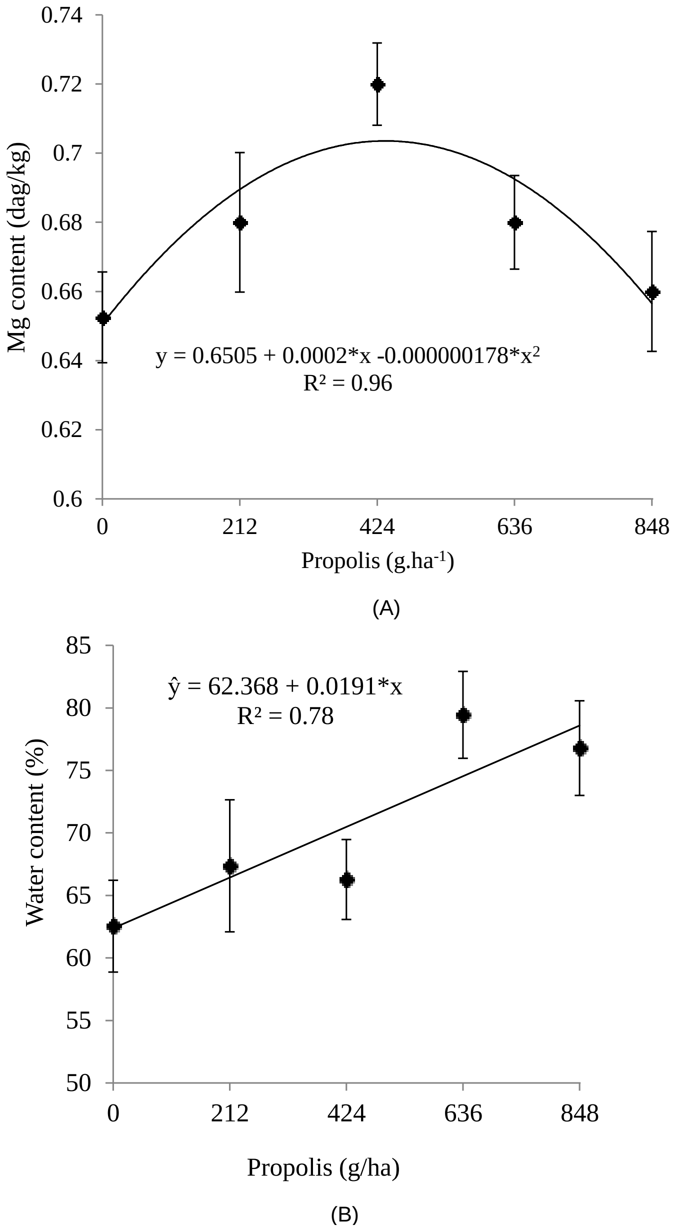

Figure 1. Leaves Mg content (dag.kg^{-1}) (A) and water content (B) in the black bean leaves of the BRS Valente variety, under the application of five concentrations of EEP (water 0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

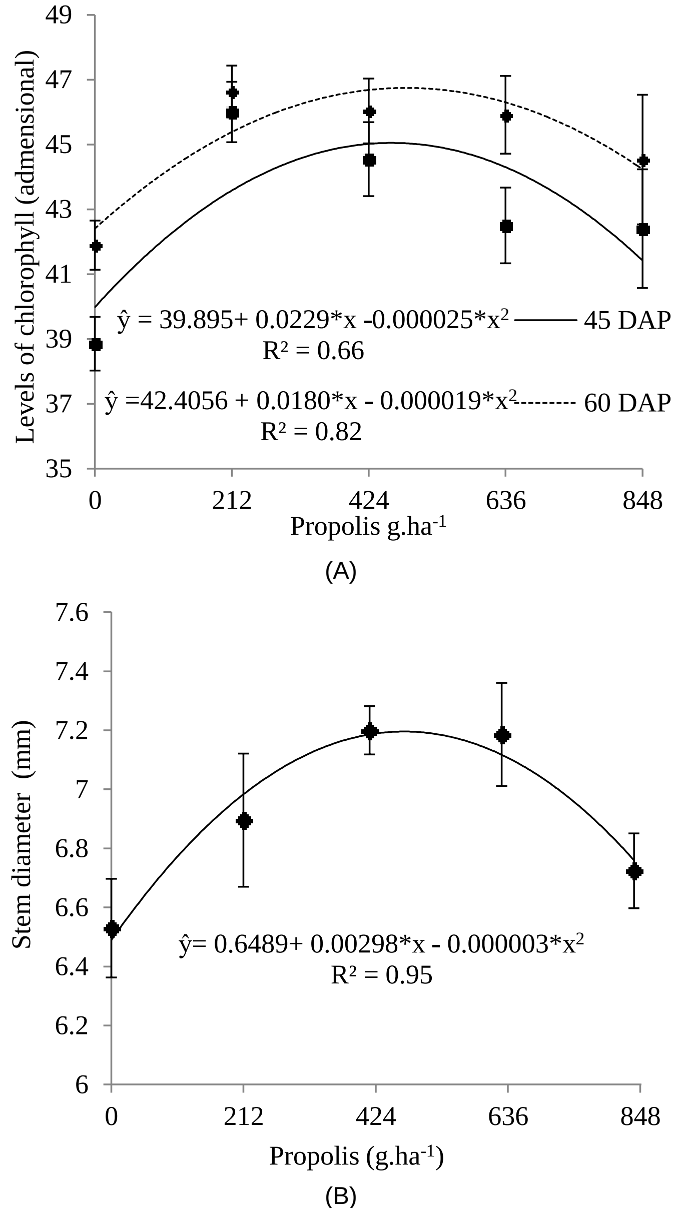

Figure 2. Levels of chlorophyll in leaves (admensional) (A) and stem diameter of beans, variety "BRS Valente", black type (B) under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen – MT.

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

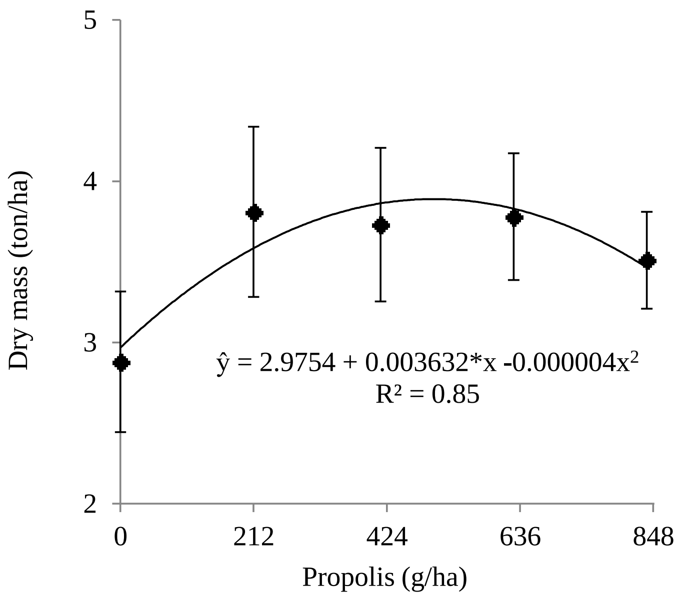

Figure 3. Dry mass of shoots of bean plants, variety BRS Valente, black type, under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

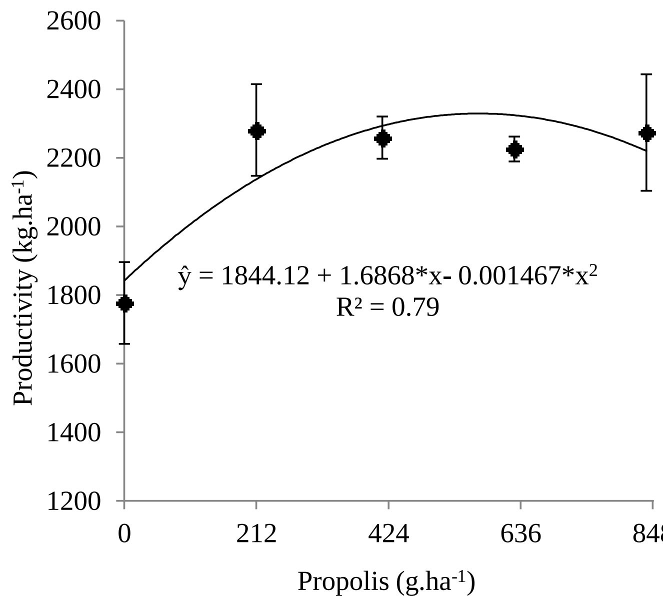

Figure 5. Productivity (kg/ha) of dry bean variety BRS Valente, black type, under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

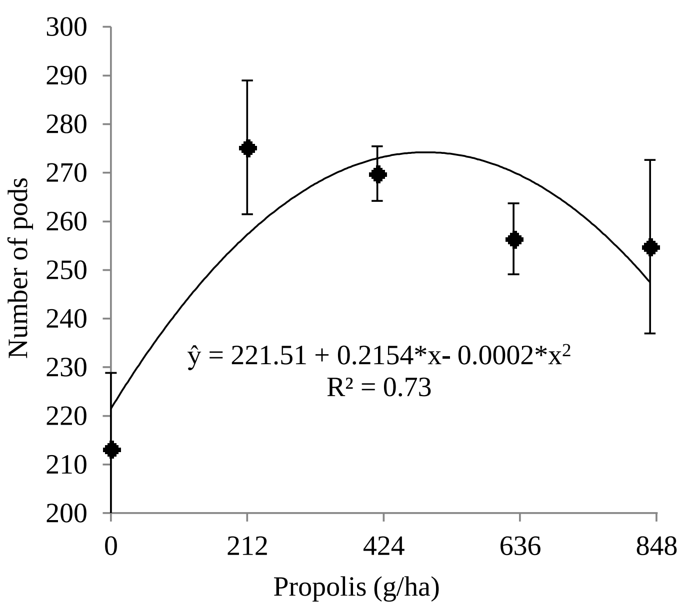

Figure 4. Number of pods of bean plants, variety BRS Valente, black type, under the application of five concentrations of EEP (0, 3.0, 6.0, 9.0 and 12.0%), Santa Carmen-MT.

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.

ACKNOWLEDGEMENTS

To Agropel Grain ® Company, Agronorte ® by ceding site and materials to conduct the experiment; Embrapa for their assistance in conducting the experiment; Course of Animal Science UFMT / Campus Sinop-MT, Brazil.

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