academicJournals

Vol. 16(13), pp. 615-622, 29 March, 2017
DOI: 10.5897/AJB2017.15972
Article Number: E8AB24663411
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Bioactivity of ethanolic extracts of *Euphorbia pulcherrima* on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

Viviane Tavares Almeida[1,2*], Vânia Maria Ramos,[1,2], Matheus Barbosa Saqueti[1,2], Pedro Henrique Gorni[1], Ana Cláudia Pacheco[1] and Renato Marcos de Leão[1,2]

[1]Agronomy Department, College of Agricultural Sciences, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, São Paulo, Brazil.
[2]Laboratory of Agricultural Entomology, College of Agricultural Sciences, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, São Paulo, Brazil.

Received 12 December, 2016; Accepted 24 January, 2017

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a polyphagous species which attacks many economically important crops in several countries. This insect is an important pest of corn, and currently the most widely used control method is chemical. In order to minimize environmental impacts, other forms of control have been tried, and accordingly, the investigation of plants with insecticidal effects becomes relevant. Thus the present study was conducted in order to evaluate the effect of the ethanolic extract of *Euphorbia pulcherrima* (poinsettia) leaves in fall armyworm biology. Extracts were prepared from leaves of the plant *E. pulcherrima* collected at different phenological stages (vegetative and reproductive), oven dried, crushed and then solubilized in ethanol, yielding the ethanol extract. The extracts were set aside in 0.5 and 1% concentrations for each phenological stage of the plant, incorporated into an artificial diet and offered to the larvae of *S. frugiperda*. The extract of vegetative and reproductive phase of *E. pulcherrima* leaves in concentrations of 0.5 and 1%, has showed that it affected mortality in the larvae, increasing the larval period and reducing the weight of larvae and pupae and viability of the eggs of the caterpillars. Ethanolic extract of *E. pulcherrima* leaves in the reproductive phase of the plant is effective to reduce the *S. frugiperda* population.

Key words: Botanical insecticide, plant extracts, mortality, poinsettia, pest biology.

INTRODUCTION

Among all species of insect pests, some stand out because of the negative impact they cause to agribusiness (attack crops with higher planted area) and the differentiated amount of crops that attack (Zarbin and Rodrigues, 2009). According to these criteria, seven of the top ten pest species are of the Lepidoptera order

*Corresponding author. Email: vivianetavaresdealmeida@gmail.com Tel: (055 18) 99719-0723.

(moths): *Spodoptera frugiperda* (J.E. Smith), *Spodoptera eridania* (Cramer), *Mocis latipes* (Guenée), *Agrotis ipsilon* (Hufnagel), *Corcyra cephalonica* (Stainton), *Plodia interpunctella* (Hubner), *Elasmopalmus lignosellus* (Zeller), *Procornitermes triacifer* (Silvestri), *Diabrotica speciosa* (Germar) and *Acromyrmex landolti* Forel (Zarbin and Rodrigues, 2009). Among these pests, the main pest of Brazilian agriculture can be considered the moth *S. frugiperda*, also known as fall armyworm, due to attack different crops, especially grasses (Vendramim et al., 2000), which together represent 97% of all planted area in the country. Their outbreaks have caused significant losses also in crops such as cotton, soybean and cultivated Solanaceae members (Pogue, 2002; Barros et al., 2010), besides using alternative hosts to remain in agricultural ecosystems.

The control methods of this insect focus primarily on the use of synthetic insecticides of high cost and with high risk of toxicity and environmental contamination (Viana and Prates, 2003). Therefore, it has been carried out researches on control measures with less environmental impact, and in this sense, the plants emerge as an important alternative for the management of this pest. According to Torres et al. (2001), natural products extracted from plants are sources of substances which may be used in pest control, being compatible with integrated pest management programs (IPM) as an option to minimize the negative effects of indiscriminate use of insecticides.

Currently, there are several researches involving insecticide plants in the control of *S. frugiperda*, which show promising results (Viana and Prates, 2003; Santiago et al., 2008). By testing various aqueous extracts of Meliaceae on *S. frugiperda*, Góes et al. (2003), revealed the existence of some plants with toxic activity, highlighting, among them *Trichilia pallida*, in addition to *Azadirachta indica* extract, which prevents the insect molting, leading them to death. Another action mechanism of the active ingredients from botanical insecticides is to affect certain organs or insect molecules, and in this case, it is act hindering the growth and development by interfering with cellular metabolism (Aguiar-Menezes, 2005). Depending on the concentration used, some extracts can reduce the viability of eggs, nymphs, larvae and pupae. The reduction of the eggs and the oviposition inhibition are important effects from plant extracts on the reproduction of insects (Costa et al., 2004).

The aim of this study was to evaluate the effect of ethanolic extract of *E. pulcherrima* leaves collected in different phenological stages on *S. frugiperda*.

MATERIALS AND METHODS

Trial place

The experiment was conducted at Universidade do Oeste Paulista (UNOESTE) in Presidente Prudente (22º7'39" S, 51º23'8" W, 471 ma.s.l.) São Paulo, Brazil, in the laboratory of Agricultural Entomology (LEA), using a room with controlled temperature of 26.0°C ± 1.0°C, humidity 60% ± 10% and 12 h photoperiod. The caterpillars used in the experiment were reared in the laboratory from the company BUG - Agentes Biológicos®.

Obtaining extract

To obtain the extract, fully expanded leaves of *E. pulcherrima* were collected in the plants hatchery of the Universidade do Oeste Paulista (UNOESTE) in vegetative and reproductive phases. The leaves were stored in kraft paper bags and dried in a kiln at 60°C for 48 h and therefore crushed (grinded) in a knife mill (Willye®) to a particle size of 0.45 mm, to obtain a fine powder that was then stored in sealed glass containers and kept at 24°C in a dark room until the manipulation of the extracts.

The obtained plant powder was macerated in ethanol solution and filtrated once a week. The filtering was performed on a conventional glass funnel, using as filter germination paper. After filtering, the ethanol was replaced in the bottle until it covers 4 cm of the volume filled by the powder. This procedure was performed to exhaustion to obtain the ethanolic extract (Santana et al., 2013).

The obtained solvent was evaporated under reduced pressure on a rotary evaporator (Quimis – Q344B), a procedure for obtaining the pure ethanolic extract. The extracted content was stored according to each phenological stage of the plant and spared to be added to the artificial diet (Parra, 1999).

Application of extract

The extract was weighed in the amounts of 5 and 10 g, respectively, corresponding to concentrations of 0.5 and 1% (w/v), which were added in 1 L of the diet, forming 5 treatments according to Table 1. The mixture was poured into gerbox containers, which stood for 40 min in the laminar flow hood with UV light for germicidal function, and soon after it was stored in the refrigerator until the inoculation of the larvae.

After preparation and cooling, the diet was cut into cubes containing on average 4 g of diet. The cubes were added individually in plastic pots of 75 ml, after that, larvae in second instar were placed under artificial diet *ad libitum*. Each treatment consisted of 50 repetitions, each repetition using one of the larvae.

Evaluated parameters

Observations were made every day to record the larval and pupal mortality. The caterpillars were weighed on the 3rd, 6th, 9th and 12th day after the start of the experiment (Precision Scale - Shimadzu AUY 220). Twenty four hours after the formation of pupae, they were observed under a binocular microscope for determining the sex (Butt and Cantu, 1962), and then they were weighed and placed in Petri dishes. Immediately after the formation of pupae the remaining diet and feces were weighed to determine the food intake and weight of stools.

For each treatment, seven male and female the pupae were used of same age to form seven moths couples of same age and placed inside of PVC cages (10 cm diameter x 15 cm high) coated with filter paper for oviposition purpose. Each pair was fed with an aqueous solution containing 10% honey. The cages were covered at its upper end with "voil" fabric and its base closed with plastic wrap and adhesive tape.

Every day, the egg masses were collected, transferred to plastic pots of 75 ml and stored in a room at 26°C, 60% of relative humidity and 12 h of photoperiod. The eggs of the second mass were

Table 1. Treatments containing extract of the leaf of *Euphorbia pulcherrima*, offered in an artificial diet for *Spodoptera frugiperda* larvae.

Treatment	Extract (%)	Phenological stage
TT	0	-
VE0.5	0.5	Vegetative
VE1	1.0	Vegetative
RE0.5	0.5	Reproductive
RE1	1.0	Reproductive

TT (control), VE0.5% and VE1% (extract of the vegetative phase), RE0.5% and RE1% (extract of the reproductive phase).

Table 2. Total mortality (%) and larval period (days) of *S. frugiperda* larvae fed with an artificial diet containing ethanolic extract of *E. pulcherrima* leaves.

Extract	Mortality (%)	Larval period (days)
TT	0[a]	13.816±0.119[b]
VE0.5%	22±0.059[b]	15.820±0.328[b]
VE1%	12±0.046[b]	19.045±0.425[a]
RE0.5%	24±0.061[b]	19.657±0.712[a]
RE1%	26±0.062[b]	18.432±0.588[a]
	P-value = 0.001864692	P-value = 1.1791E-21

Means followed by the same letter in the column do not differ significantly by the Kruskal-Wallis test at 1%.

counted, placed in plastic bowls (75 mL) until the eggs hatch, in order to measure their viability.

Experimental design and statistical analysis

The experimental design was completely randomized with 5 treatments. In the test with the caterpillars they were 50 repetitions and in the test with moths couples the number of repetition was 7.

After that, all the parameters were submitted to Shapiro-Wilk test; then it was performed nonparametric means comparison by Kruskal-Wallis test, using Action 2.9 program (Estatcamp, 2015).

RESULTS AND DISCUSSION

The ethanolic extract of *E. pulcherrima* leaves collected in the two phenological phases of the plant (vegetative and reproductive) with 0.5 and 1% concentration have caused higher larvae mortality compared to the control (Table 1). D'Incao et al. (2012) have reported that the cold aqueous extract of *E. pulcherrima* applied to leaf discs of *Neonotonia wightii* (perennial soybean) caused 58.5% mortality of *S. frugiperda*. Soares et al. (2011) have observed the influence of *Rosmarinus officinalis* 10% essential oil on *S. frugiperda* found mortality of 30%. The mortality value displayed can be correlated with the concentrations used in this study; perhaps higher concentrations produce greater larvae mortality rate. Prates et al. (2003) have studied the correlation among different concentrations of neem leaf aqueous extract (*A. indica*) on *S. frugiperda* mortality and concluded that the increasing concentration in this product leads to increased mortality rate.

Regarding to the larval period, the treatments VE1%, RE0.5% and RE1% caused a prolongation on the larval stage of 5.2, 5.8 and 4.7 days, respectively, compared to the other treatments (Table 2). According to Torres et al. (2001) this extension may be related to the presence of growth inhibitors, low conversion of ingested food or by contain toxic substances that interfere with the lower food intake. This result is corroborated by Piubelli (2004) and Hoffmann-Campo et al. (2006) who have studied biological aspects of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), fed with diets with the addition of the rutin flavonoid, observed an extension in feeding time.

The extension of the larval period in the field can leave the insects vulnerable for longer periods to parasitoids, predators and entomopathogenic organisms attack. The emerging adults may be in asynchrony compared to the normal population, and consequently copulation could be more difficult or lead to inbreeding by mating individuals of the same generation (Rodríguez and Vendramim, 1996). The number of insect generations in the

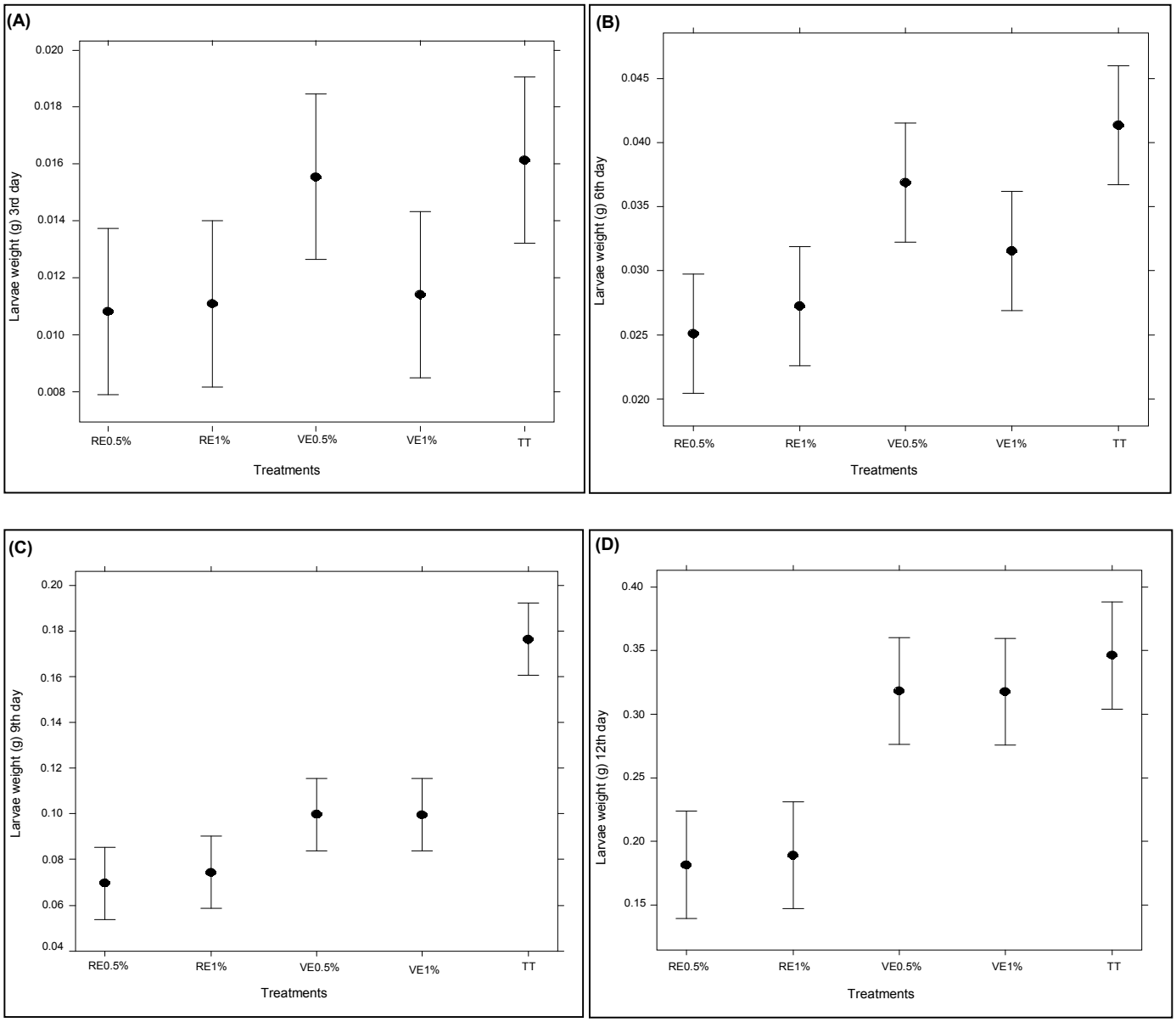

Figure 1. Weight (g) of S. *frugiperda* larvae on the 3rd, 6th, 9th and 12th day, feeding with artificial diet containing *E*. *pulcherrima* extracts from leaves. Legend: TT (control), VE0.5% and VE1% (extract of the vegetative phase), RE0.5% and RE1% (extract of the reproductive phase). P-value <0.01 by Kruskal-Wallis test.

agricultural cycle can be reduced, as was stated by Tanzubil and McCaffery (1990).

In the 3rd, 6th, 9th and 12th day the caterpillars were weighted to check the gain and/or weight loss ethanolic extracts. On the 3rd day there was no difference among treatments, probably because of the molecules present in treatments have not yet been metabolized by the caterpillars (Figure 1a). From the 6th day increased weight was observed for the control treatment (0.041g), showing the influence of the extracts on the larvae weight fed with VE0.5% (0.036g), VE1% (0.031g) , RE0.5% (0.025g) and RE1% (0.027g) (Figure 1b). It can be affirm that the RE0.5% treatment was statistically superior to treatment VE0.5%, since there was 30% reduction in weight.

The body weight of the 9th day, it was again confirmed the influence of the extracts, verifying that the control treatment showed the highest body weight (0.176 g) and treatments VE-0.5%, VE-1%, RE-0.5%, and RE-1% led to reduced weights for 0.099, 0.099, 0.074 and 0.069 g, respectively (Figure 1c). Similarly, Bogorni and Vendramim (2003) using extracts from six species of *Trichilia* spp. comparing with *A. indica* (neem), have also found reduced weight for *S. frugiperda* in treatments

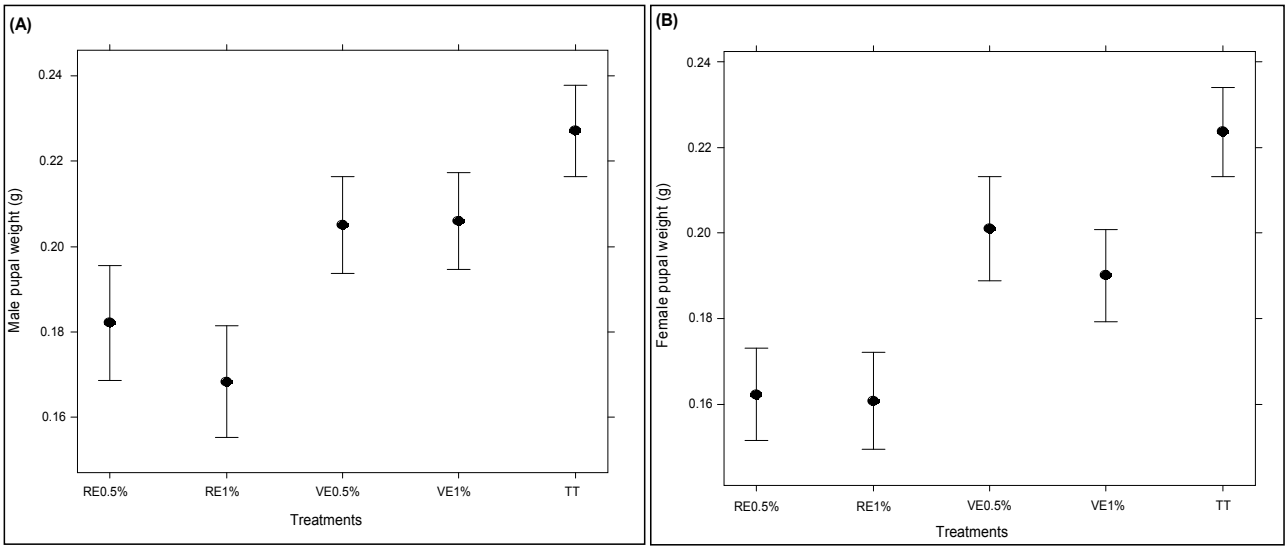

Figure 2. Weight (g) of male and female pupae of S. *frugiperda*, feeding at the larval stage with artificial diet containing E. *pulcherrima* extracts from leaves. Legend: TT (control), VE0.5% and VE1% (extract of the vegetative phase), RE0.5% and RE1% (extract of the reproductive phase). P-value <0.01 by Kruskal-Wallis test.

where neem seed extracts and *T. pallens* leaves were used.

Growth inhibition and poor weight gain can be attributed to reduced feeding and impaired ability to convert nutrients into biomolecules to form the tissues of insects, preventing growth and weight gain (Martinez and Emden, 2001). For Tanzubil and McCaffery (1990), significant weight reduction caused by the extracts indicates that the insects, in need of degrading possible secondary metabolites present in the extracts, can be diverted for this purpose, resources that would be used to gain biomass.

On 12th day, the last weighting was made, and the treatments RE0,5% and RE1% resulted in larvae with lower weights in relation to other treatments, being 0.181 and 0.189 g the results, respectively. Control, VE-0.5% and VE-1% the respective weight was 0.346, 0.318 and 0.317 g (Figure 1d). As in the 6th day, it was observed that the treatment of reproductive stage containing *E. pulcherrima* extract with the concentration of 0.5% was superior to the treatment with the highest concentration (1%) of the leaf extract collected in the vegetative phase. This can be attributed to the amount of metabolites present in the leaves, since different phenological stages of plants influence the amount and the dynamic of secondary metabolites that are present in them (Gobbo-Neto and Lopes, 2007). Tavares et al. (2005) analyzed the essential oil of three chemo types of *Lippia alba* and found an increase in the percentage of limonene during the flowering season, however the percentage of citral, carvone and linalool has slightly decreased during the reproductive phase.

Németh et al. (1993) have studied wild specimens of *Achillea crithmifolia* under different environmental conditions and developmental stages; they found that the rate of camphor in the essential oil decreased as the plants have advanced in their phenological stages. For the essential oil 1.8-cineol, the observed behavior was the opposite.

Based on the current study we can conclude, that, it is more efficient to use the extract made from the leaves of plants in the reproductive stage, since the caterpillars had reduced body weight.

Regarding the weight of pupae, there was a reduction in male pupae weight for treatments RE-0.5% (0.182 g) and RE-1% (0.168 g) compared to treatments with VE-0.5% and VE-1% which showed weight of 0.205 g, and the control treatment that obtained the highest weight (0.227 g) (Figure 2a). In the same direction, the weight of females pupae were significantly higher in the control treatment (0.223 g), followed by treatments VE-0.5% (0.201 g) and VE-1% (0.190 g). The treatments RE-0.5% (0.182 g) and RE-1% (0.168 g) have caused the greatest reduction of the weight of female pupae (Figure 2b). Ramos-López et al. (2010) observed a gradual decrease in weight of *S. frugiperda* pupae exposed to different extracts of *Ricinus communis* (Euphorbiaceae). Several studies using plant extracts on *S. frugiperda* reported reduction in pupae weight (Rodriguez and Vendramim, 1996; Vendramim and Scampini, 1997; Roel et al., 2000). However, D'Incao et al. (2012), evaluating the average weight of *S. frugiperda* pupae observed that the cold aqueous extract of *E. pulcherrima* applied to leaf discs of *Neonotonia wightii* (perennial soybean) was not different compared to the control.

The weight reduction in the pupal period is probably

Table 3. Food consumption (g) and weight of stool (g) of *S. frugiperda* larvae, fed with artificial diet containing ethanolic extract of *E. pulcherrima* leaves.

Extract	Food consumption (g)	Weight of feces (g)
TT	3.125±0.152[a]	1.023±0.068[a]
VE0.5%	3.073±0.084[a]	0.856±0.051[a]
VE1%	3.113±0.114[a]	1.018±0.053[a]
RE0.5%	3.034±0.087[a]	0.623±0.081[b]
RE1%	3.213±0.079[a]	0.630±0.049[b]
	P-value = 0.314352123	P-value = 4.51015E-07

Means followed by the same letter in the column do not differ significantly by the Kruskal-Wallis test at 1%.

related to the effects of substances in plant extracts ingested by the caterpillars during the larval stage. The toxic effect of insecticidal plants affects usually more larval stages than pupal stages, due to the fact that caterpillars are going to ingest the nutrients present in the food (Rodriguez and Vendramim, 1996; Céspedes et al., 2000; Martinez, 2001). This effect reflects in the insect morphology, reducing the weight of male and female pupae, as found in this study. The feeding reduction or low food conversion caused by plant extracts may interfere in the pupae weight. If the weight is lower than the control, it is suggested that the chemical compounds present in the plant might have caused a decrease in food consumption by the larvae. Consequently, pupae with low weight would become small adults, and possibly there will be problems in the mating behavior of these individuals compared to those with normal, weight pupae, leading to less females fertilized (Rodriguez and Vendramim, 1996).

After weighting the pupae, the food and feces that were left over in the pots were also weighted to measure food consumption and stool weight. Food intake was not affected by the treatments, but the caterpillars that received treatments containing extract of *E. pulcherrima* leaves in reproductive stage were reduced by 40% (RE-0.5%) and 39% (RE-1%) of excreted feces comparing to the control (Table 3). Probably, this result has occurred because the extracts have affected the digestibility of food by *S. frugiperda*. So, despite the fact that the larvae have fed normally, the food was kept for longer time in the gut for degradation of secondary metabolites present in the extracts. Similar type of results were presented by Sâmia (2013) using *Copaifera langsdorffii* aqueous extracts in 2nd instar *S. frugiperda* larvae, leading to reduced weight of feces excreted; the author states that this reduction may be related to food deterrence caused by some substance present in aqueous extracts, possibly enzyme inhibitors. Tirelli et al. (2010) using tannic fractions of *Schinus terebinthifolius* have found a reduction in excreted feces in the control treatment, but these treatments did not decrease the food consumption.

It is interesting to note that, despite the regular amount of food intake in larval stages, because the applied extracts of *E. pulcherrima* did not have proper utilization of the food, since its weight has been reduced.

Observing the fecundity of *S. frugiperda,* even not perceived any statistical significance, the number of eggs and egg masses decreased in the treatments containing extract, especially of reproductive stage, and the viability of the eggs was significantly influenced by the treatment with extract 1% of *E. pulcherrima* on reproductive stage, preventing 100% of the eggs from hatch (Table 4). Similar type of results were found by Alves et al. (2012), who has observed the low viability of *S. frugiperda* eggs from larvae fed diets containing methanolic extracts of *C. langsdorffii* leaves and bark of fruits. Silva et al. (2010) have found a reduction in the eggs viability from *S. frugiperda* larvae fed on artificial diet containing methanolic extract of *Piper hispidum* at concentrations 0.001, 0.006, 0.03, 0.2 and 1%. Santiago et al. (2008), using aqueous extract 10% of *Chenopodium ambrosioides* and *Licania rigida* on *S. frugiperda,* have found lower viability compared to the control treatment, indicating a possible negative effect on insect fertility. According to Costa et al. (2004), the viability of eggs and other parameters of fertility and fecundity are important effects of plant extracts on the reproduction of insects, which can be associated with eating disorders due to nutritional deficiency.

The study of the effects of plants with insecticidal properties should not aim only the mortality of insects, because for this purpose the amount required of the product is more than that used in this study making it practically an unviable technique. The main objective is that the plants have effects in reducing feed, fecundity and fertility, causing damage to future generations (Vendramim et al., 2000).

In this experiment, the extract to 1% of *E. pulcherrima* leaves collected in the reproductive phase, decreased the number of *S. frugiperda* eggs, and prevented them completely, proving its potential to efficiently reduce the populations of this particular insect in agricultural areas.

Table 4. Number of egg masses per couple (average), number of eggs per couple (average), and eggs viability (%) of *S. frugiperda* larvae fed on an artificial diet containing ethanolic extract of *E. pulcherrima* leaves.

Extract	Number of egg masses per couple (average)	Number of eggs per couple (average)	Viability (%)
TT	3.57±0.947[a]	547±114.8[a]	99.3±0.002[a]
VE0.5%	2.28±0.837[a]	303±159.6[a]	98.5±0.005[a]
VE1%	1.42±0.428[a]	264±173.4[a]	97.9±0.005[a]
RE0.5%	1.28±0.521[a]	106±54.40[a]	97.7±0.007[a]
RE1%	0.85±0.459[a]	136±68.20[a]	0[b]
	P-value = 0.122258	P-value = 0.069288	P-value = 0.000

Means followed by the same letter in the column do not differ significantly by the Kruskal-Wallis test at 1%.

Conclusions

1) The extract of vegetative and reproductive stages of *E. pulcherrima* leaves 0.5 and 1% concentrations has caused larval mortality of *S. frugiperda* up to 26%.
2) The larval period was prolonged by treatment with a vegetative extract 1% (19 days) and reproductive extract 0.5 and 1% (19 and 18 days respectively).
3) The larval weight was reduced by 40% with the reproductive extract at concentrations 0.5 and 1%.
4) Treatments with leaf extract of the reproductive phase (0.5 and 1%) reduced the weight of pupae.
5) The same extract above resulted in reduced rates of feces excreted by the larvae.
6) The leaf extract of the reproductive phase (1%) had as effects on *S. frugiperda* fertility, reduction of the number and decrease of the viability of the eggs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the grant of scholarship to the first author.

REFERENCES

Aguiar-Menezes EDL (2005). Inseticidas botânicos: seus princípios ativos, modo de ação e uso agrícola. Embrapa Agrobiologia, P 58.
Alves DS, Carvalho GA, Oliveira DF, Sâmia RR, Villas-Boas MA, Côrrea AD (2012). Toxicity of copaiba extracts to armyworm (*Spodoptera frugiperda*). Afr. J. Biotechnol. 11(24):6578-6591.
Barros EM, Torres JB, Bueno AF (2010). Oviposição, desenvolvimento e reprodução de *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) em diferentes hospedeiros de importância econômica. Neotrop. Entomol. 39(6):996-1001.
Bogorni PC, Vendramim JD (2003). Bioatividade de extratos aquosos de *Trichilia spp*. sobre *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) em milho. Neotrop. Entomol. 32(4):665-669.
Butt BA, Cantu E (1962). Sex determination of lepidopterous pupae. Washington, DC: USDA pp12.
Céspedes CL, Calderón JS, Lina L, Arand E (2000). Growth inhibitory effects on fall armyworm *Spodoptera frugiperda* of some limonoids isolated from *Cedrela spp*. (Meliaceae). J. Agr. Food Chem. 48(5):1903-1908.
Costa ELN, Silva RD, Fiuza LM (2004). Efeitos, aplicações e limitações de extratos de plantas inseticidas. Acta Biol. Leopold. 26(2):173-185.
D'incao MP, Quadros B, Fiuza L (2012). Efeito agudo e crônico de três diferentes extratos de *Euphorbia pulcherrima* sobre *Spodoptera frugiperda* (J.E.SMITH, 1797) (Lepidoptera, Noctuidae). In I Simpósio de Integração das Pós-Graduações do CCB/UFSC**.** Florianópolis P.28.
Estatcamp (2015). Action 2.9 Software. Available in: < http://www.portalaction.com.br>. Accessed: March 10, 2016.
Gobbo-Neto L, Lopes NP (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim. Nova. 30(2):374.
Góes GB, Neri DKP, Chaves JWN, Maracajá PB (2003). Efeito de extratos vegetais no controle de *S. frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Rev. Caatinga 16(1/2):47-49.
Hoffmann-Campo CB, Ramos Neto JA, Oliveira MCND, Oliveira L J (2006). Detrimental effect of rutin on *Anticarsia gemmatalis*. Pesqui. Agropecu. Bras. 41(10):1453-1459.
Martinez SS, Emden HV (2001). Redução do crescimento, deformidades e mortalidade *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) causadas por Azadiractina. Neotro. Entomol. *30*(1).
Martinez SS (2001). The use of plants with insecticidal and repellent properties in pest control. Inst. Agronômico do Paraná, pp4.
Németh É, Bernáth J, Héthelyi É (1993). Diversity in chemotype reaction affected by ontogenetical and ecological factors. Acta Hortic. 344:178-187.
Parra JRP (1999). Técnicas de criação de insetos para programas de controle biológico. ESALQ/FEALQ. pp137.
Piubelli GC (2004). Bioatividade de genótipos de soja resistentes a *A. gemmatalis* hübner (lepidoptera: noctuidae) e interações de suas substâncias químicas com inimigos naturais. 2004. pp126. (Doctoral dissertation, Tese (Doutorado) – Universidade Federal do Paraná, Curitiba).
Pogue M (2002). A world revision of the genus *Spodoptera Guenée* (Lepidoptera: Noctuidae). Mem. Am. Entomol. Soc. 43:1-202.
Prates HT, Viana PA, Waquil JM (2003). Activity of neem tree (*Azadirachta indica*) leaves aqueous extract on *Spodoptera frugiperda*. Pesqui. Agropecu. Bras. 38(3):437-439.
Ramos-López MA, Pérez S, Rodríguez-Hernández GC, Guevara-Fefer P, Zavala-Sanchez MA (2010). Activity of *Ricinus communis* (Euphorbiaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Afr. J. Biotechnol. 9(9):1359-1365.
Rodriguez HC, Vendramim DJ (1996). Toxicidad de extractos acuosos de Meliaceae en *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Manejo Integr. Plagas 42:14-22.
Roel AR, Vendramim JD, Frighetto RTS, Frighetto N (2000). Efeito do extrato acetato de etila de *Trichilia pallida* Swartz (Meliaceae) no desenvolvimento e sobrevivência da lagarta-do-cartucho.

Bragantia 59(1):53-58.
Sâmia RR (2013). Bioatividade de extratos aquosos de copaíba (Leguminosae) na biologia de *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae). pp51. (Master dissertation, Dissertação (Mestrado) – PPGEA- Universidade Federal de Lavras, Minas Gerais).
Santana LCLR, Silva OA, Brito MRM, David JPL, David JM, Galvão KCS, Freitas RM (2013). Avaliação do potencial antioxidante, atividade antimicrobiana e antihelmíntica do extrato etanólico padronizado das folhas de *Mikania glomerata* Sprengel. Rev. Bras. Farm. 94(2):120-129.
Santiago GP, Padua LDM, Silva PRR, Carvalho EMS, Maia CB (2008). Efeitos de extratos de plantas na biologia de *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae) mantida em dieta artificial. Cienc. Agrotec. 32(3):792-796.
Silva LV, Bednaski A, Oliveira JBB, Santos BS, Roel AR, Dourado DM, Matias R (2010). Efeito de *Piper hispidum* (Piperaceae) sobre lagartas de *Spodoptera frugiperda* (J.E.Smith, 1797) (Noctuidae) em dieta artificial. In XXIII Congresso Brasileiro de Entomologia. Natal.
Soares CSA, Silva M, Costa MB, Bezerra CES (2011). Ação inseticida de óleos essenciais sobre a lagarta desfolhadora *Thyrinteina arnobia* (Stoll)(Lepidoptera: Geometridae). Rev. Verde 6(2):154-157.
Tanzubil PB, McCaffery AR (1990). Effects of azadirachtin and aqueous neem seed extracts on survival, growth and development of the African armyworm, *Spodoptera exempta*. Crop Prot. 9(5):383-386.
Tavares ES, Julião LS, Lopes D, Bizzo HR, Lage CLS, Leitão SG (2005). Análise do óleo essencial de folhas de três quimiotipos de *Lippia alba* (Mill.) NE Br.(Verbenaceae) cultivados em condições semelhantes. Rev. Bras. Farmacogn. 15(1):1-5.
Tirelli AA, Alves DS, Carvalho GA, Sâmia RR, Brum SS, Guerreiro MC (2010). Efeito de frações tânicas sobre parâmetros biológicos e nutricionais de *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Ciênc. Agrotec. 34(6):1417-1424.
Torres AL, Barros R, Oliveira JV (2001). Efeito de extratos aquosos de plantas no desenvolvimento de *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Neotrop. Entomol. 30(1):151-156.
Vendramim JD, Scampini PJ (1997). Efeito do extrato aquoso de *Melia azedarach* sobre o desenvolvimento de *Spodoptera frugiperda* (JE Smith) em dois genótipos de milho. Rev. de Agric. 72(2):159-170.
Vendramim JD, Castiglioni E, Guedes JC (2000). Aleloquímicos, resistência de plantas e plantas inseticidas. Bases e técnicas do manejo de insetos. Santa Maria: Pallotti pp. 113-128.
Viana PA, Prates HT (2003). Desenvolvimento e mortalidade larval de *Spodoptera frugiperda* em folhas de milho tratadas com extrato aquoso de folhas de *Azadirachta indica*. Bragantia 62(1):69-74.
Zarbin PHG, Rodrigues MACM (2009). Feromônios de insetos: tecnologia e desafios para uma agricultura competitiva no Brasil. Quim. Nova 32(3):722-731.