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Bioactivity of a water extract of boldus (*Peumus boldus* Molina) against *Spodoptera frugiperda* (J.E. Smith) and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae)

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The insecticidal properties of water-extract of *Peumus boldus* Molina and its effect on the development cycle and feeding habits of *Spodoptera frugiperda* J.E. Smith and *Helicoverpa zea* Boddie were evaluated under laboratory conditions in concentrations of 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0% (w/w). *Spodoptera frugiperda* was the most susceptible with 75% mortality at 7 d at 8% *P. boldus* concentration, while *H. zea* had only 30% mortality. LC_{50} was 2.31 mL kg^{-1} for *S. frugiperda* and 16.05 mL kg^{-1} for *H. zea*. When the extract concentration increased in the diet, larval size and weight, percentage of pupation and number of adults decreased, and the time required to reach those states was greater. Neonate larvae fed primarily on the diet with the lower extract concentration and the control was preferred by more than 50% of larvae. Inhibition of feeding, growth, weight gain of 3rd instar larvae as well as new biomass production decreased with concentration of the extract.

Key words: Fall armyworm, corn earworm, botanical insecticides, nutritional indexes.

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda* J.E. Smith) and the corn earworm (*Helicoverpa zea* Boddie) are two important pests that affect a large quantity of crops, damage both plant foliage and fruits of many crops (Abd-Elghafar et al., 1993; Prates et al., 2003). To control these pests, farmers mainly use synthetic insecticides of chemical groups such as organophosphates, carbamates, and pyrethroids (Rodríguez and Vendramim, 1996). However, irrational use has resulted in problems like chemical residues in food, biological disequilibrium, intoxication and development of insect resistance (Roel and Vendramim, 2006).

The search for alternative methods includes the use of natural products that are both effective and less environmentally aggressive, such as plant extracts (Roel and Vendramim, 2006). For Lepidoptera, research has focused on the Meliaceae family with species such as *Azadirachta indica* A. Juss. (Viana et al., 2007), *Guarea trichilioides* L., *Guarea guidonia* (L.) Sleumer, *Melia azedarach* L. (Rodríguez and Vendramim, 1997; De Brito et al., 2004), *Trichilia havannensis* (Jacq.) (Caballero et al., 2008), and *Trichilia pallida* Swartz (Roel and Vendramim, 1999; Roel et al., 2000; Roel and Vendramim, 2006). However, in the last few years, there have been studies with plants of other plant families, such as *Quassia amara* L. (Simaroubaceae) (Mancebo et al., 2000; Souza et al., 2007), *Annona cherimola* Mill. (Annonaceae) (Álvarez-Colom et al., 2007), and *Anacardium occidentale* L. (Anacardiaceae) (De Brito et al., 2004). Many of these species have demonstrated great crop protection potential in laboratory, field, and greenhouse, although few are found in countries with temperate or cold climates. Additionally, many of these extracts are obtained with solvents such as hexane, acetone or methanol, instead of water (Dos Santos et al., 2008; Pedreira et al., 2008). As a result, they cannot be elaborated by farmers because their development requires specialized equipment and rigorous security conditions. The main advantage of water extracts is that even small farmers can prepare them, reducing production costs, health risks and dependence on manufactured insecticides (Viana et al., 2007).

Boldo (*Peumus boldus* Molina, Monimiaceae) is a Chilean native with insecticidal effect on *Sitophilus zeamais* Motschulsky (Páez et al., 1990; Silva et al., 2003; 2005; 2006; Pérez et al., 2007), *Xanthogaleruca luteola* Müll. (Chiffelle et al., 2011), and for third instar larva of *Spodoptera littoralis* Boisduval (Zapata et al., 2006) and fungicidal properties to *Penicillum* spp., *Fusarium* spp., *Aspergillus niger* and *A. flavus* (Leite de Souza et al., 2005). However, the effect of this plant's extract on *S. frugiperda* and *H. zea* has never been reported, and thus the objective of the present report was to evaluate

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the biological activity of water extracts of boldo leaves on larva from these species under laboratory conditions.

MATERIALS AND METHODS

Plant material

Dehydrated boldo leaves were obtained in the fruit and vegetable market in the city of Texcoco, State of Mexico, Mexico. The taxonomic confirmation was performed according to Vogel et al. (2005). Pérez et al. (2007) demonstrated that this leaves do not lose their insecticidal properties if they are maintained dehydrated and without grinding. The dehydrated foliage was triturated the same day it was used: it was ground with an electric coffee grinder (KSM2-BLK, Braun, Naucalpan, Mexico) and homogenized with a 250 µ sieve (DUAL Manufacturing, Chicago, Illinois, USA).

The extraction was performed following Prates et al. (2003). A total of 10 g boldo powder was placed 30 min in 100 mL distilled water at boiling point and was left to steep for 24 h. Then the solution was filtered with a Whatman nr 10 paper filter and used as the stock solution (100%).

Insects and toxicity assays

Spodoptera frugiperda and *H. zea* larva were obtained from a colony in the Laboratory of Toxicology of Insecticides of Entomology and Acarology Program, Colegio de Postgraduados en Ciencias Agrícolas, Montecillo Campus, Mexico, maintained in a bioclimatic chamber at 27 ± 1 ºC, 70 ± 5% RH, and 14:10 h photoperiod.

For the bioassays, 20-mL plastic cups (Envases Cuevas, Ecatepec, Mexico) were used. Ten milliliters of artificial diet (Tobacco Bollworm, Southland Products, Lake Village, Alaska, USA) were mixed with the boldo extract at 0, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0% (v/v). The mixture was performed at 40 °C to avoid degradation of active compounds (Martinez and van Emden, 2001). Once the diet was cooled and solidified, a neonate larva (< 24 h-old) was placed in each cup, covered with a perforated cover (0.5 cm diameter), with organza fabric set between the cup and cover to provide ventilation. Mortality was evaluated 7 d after inoculation; larvae were considered dead when they failed to move after being prodded gently with a dissection needle for 15 s. Six boldo concentrations and untreated control were evaluated with 20 replicates and the methodology was repeated five times on different days (100 cups per treatment). To estimate LC_{50} and LC_{90}, data were subjected to Probit analysis (Finney, 1971) using the SAS PROC PROBIT procedure (SAS Institute, 1998).

Effect on the life cycle

The effect of the boldo extract on the life cycle of each species was also evaluated in 100 plastic cups (20 mL) per treatment as described above. Larvae were allowed to feed for 72 h, and every 48 h five cups were randomly sampled to measure larval weight and length. Once the control larva reached 75% pupation, the remaining experimental units were divided in 10 replicates of five cups each. In each repetition, percentage of larva that reached pupal stage, their weight, the number that became adults and the time between larva-pupa and pupa-adult stages were determined.

Choice test for first instar larvae

To evaluate the feeding preference of neonate larvae, a choice test was performed using 5 cm diameter and 1.5 cm in height plastic Petri dishes (Industrias Technicare, Atizapan de Zaragoza, State of Mexico, Mexico) (Gore et al., 2005). Plugs of 1.5 cm diameter and 0.25 cm height of each of concentration were randomly and equidistantly arranged inside the dishes, which were covered with perforated lids and internally covered with organza fabric for ventilation and a < 24 h larva was placed in their center. Larvae feeding preferences were recorded for five consecutive days, after which plugs were dried in oven at 40 °C for 48 h to obtain their dry weight, to compare with dry weight of a set of dehydrated plugs not exposed to insects. Each treatment had 10 replicates per insect species and the methodology was repeated five times over time. In each repetition, treatments were randomly arranged inside the dish to avoid interference of external factors like light or temperature.

Choice test for third instar larvae

For this independent experiment, 2 mL of diets with each of the seven concentrations were set in each cavity of a 2 × 2 cm plastic ice cube tray. Plastic 9 cm diameter × 3 cm height Petri dishes with the bottom lined with a piece of moistened Whatman nr 10 filter paper to maintain relative humidity were used with perforated lids on top with organza fabric for gas exchange. Two diet cubes were placed on each Petri dish: one with the corresponding concentration (0 control, 0.25, 0.5, 1.0, 2.0, 4.0, or 8.0% of the extract in the diet) and the other without treatment. Each Petri dish was infested with one third instar larva, which was allowed to feed for 72 h. The remaining diet was dried at 40 °C for 72 h to obtain its dry weight, which was compared with the respective dry weight of 20 cubes of diet not exposed to larvae and dehydrated at the beginning of the bio-assay. Each treatment had 20 replicates and the experiment was repeated five times on different days. The weight results were used to calculate the feeding dissuasion index (FDI; Sadek, 2003):

$$FDI = ((Ic - It)/(Ic + It)) \times 100$$

and the feeding inhibition index (FII; Raffa and Frazier, 1988):

$$FII = ((Ic - It)/Ic)) \times 100,$$

where Ic = Ingestion of untreated diet, and It = ingestion of treated diet.

No choice tests

This experiment was performed using third instar larva of both insect species, and was performed as previously indicated, but with the difference that a larva in each Petri dish had access to two diet cubes of same treatment. The dry weight results were used to calculate the FII (Raffa and Frazier, 1988), growth inhibition index (GII):

$$GII = [(Wc - Wt)/Wc] \times 100$$

where Wc = weight (g) of control larva and Wt = weight (g) of treated larva; relative consumption rate (RCR):

$$RCR = FIL/(ILW \times T)$$

where FIL = feed intake (g) of larva, during the experimental period, ILW = initial larva weight (g) and T = experimental period (d); larval weight increase rate (WIR):

$$WIR = \Delta W/(ILW \times T)$$

where ΔW is increase in larval weight during the experimental period, ILW is initial larval weight (g), and T is experimental period (d) (Farrar et al., 1989), and the consumed feed conversion efficiency (CFE; Waldbauer, 1968).

$$CFE = (WIR/RCR) \times 100$$

Experimental design and statistical analysis

To evaluate the effect of boldo extract on *S. frugiperda* and *H. zea*, a randomized complete blocks experimental design was used. Data were transformed to $\sqrt{x} + 0.5$ to achieve variance homogeneity, and then an ANOVA ($\alpha = 0.05$) and a Tukey test ($p \leq 0.05$) were performed with the software Statistical Analysis System (SAS Institute, 1998).

RESULTS

Toxicity

The highest larval mortality of *S. frugiperda* (75%) was obtained with the 8% concentration, with no significant differences with 2% and 4% concentrations that achieved 57.5% and 67.5%, respectively (Table 1). The LC_{50} and LC_{90} were 2.31 and 12.72 mL kg^{-1}, respectively (Table 1). *Helicoverpa zea* presented low susceptibility to the water extract of *P. boldus* since the highest concentration resulted in only 30% mortality (Table 1). Lower concentrations produced < 15% mortality and did not differ significantly from control. Its low toxicity was even clearer with LC_{50} and LC_{90}, 16.05 and 82.3 mL kg^{-1}, respectively (Table 1).

Effect on the life cycle

In *S. frugiperda* the mixture of diet with different concentrations of the water extract of *P. boldus* did not affect significantly larval size or weight compared with control (Table 2). However, 8% concentration resulted in 60% less pupation, which was significantly lower (F = 2.9; df = 10;24; P = 0.0025) than the control and the other extract concentrations. However, pupae formed in the 8% treatment had a similar weight to others treatments. During the pupa-adult stage, it was observed that as the concentration increased, adult emergence diminished: control, 0.25% and 0.5% concentrations presented 100% of adult emergence, while 2%, 4%, and 8% presented lower emergence values (F = 7.39; df = 10;24; P < 0.001) with 60%, 55%, and 40% emergence, respectively (Table 2). Finally, the time between one stage and another was also affected since the time to transform larva into pupa increased with higher concentrations (F = 4.99; df = 10;24; P = 0.0001): the control lasted 8.6 d and 8% concentration took 13 d. During development from pupa into adult, the longest time was 21.4 d for 8% concentration, which was higher (F = 2.98; df = 10;24; P = 0.0138) than the other treatments, which did not present significant differences among themselves.

Helicoverpa zea showed lower larval sizes (F = 9.54; df = 8;12; P = 0.0004) when extract concentration in the diet increased. The largest size occurred in the control with 47.6 mm and the smallest at 8% concentration with 29.7 mm. Larval weight presented the same tendency, although only 8% concentration (0.31 g) showed significant differences with the rest of the treatments (Table 2). Additionally, for all the concentrations greater or equal to 0.5%, the number of larvae that reached pupal stage were significantly less (F = 2.19; df = 8;12; P = 0.001) than the control and 0.25%. The pupae that developed into adults were not different between control, 0.25% and 0.5% concentrations with values of 100%, 77.7%, and 66.6% development respectively; and they had significantly higher development to adults when compared with the remaining treatments (F = 5.59; df = 8;12; P = 0.0042) distinguishing 8% with only 11% pupation. The time for larva-pupa development increased when the *P. boldus* concentration increased and was lower with the control

Table 1. Dose mortality values produced by incorporating *Peumus boldus* water extract with insect artificial diet used against *Spodoptera frugiperda* and *Helicoverpa zea* neonates at 7 d after inoculation.

Concentration (%)	*Spodoptera frugiperda*	*Helicoverpa zea*
Control	0.0 ± 0.0c	0.0 ± 0.0b
0.25	0.0 ± 0.0c	0.0 ± 0.0b
0.50	15.2 ± 5.5bc	0.0 ± 0.0b
1.00	25.3 ± 8.3b	2.5 ± 2.5b
2.00	57.5 ± 4.7a	5.0 ± 3.3b
4.00	67.0 ± 6.6a	12.5 ± 5.5b
8.00	75.0 ± 6.5a	30.0 ± 7.2a
n[†]	200	200
b ± SD[¶]	0.83 ± 0.2	0.57 ± 0.29
$LC^{\S}_{50}$	2.31	16.05
(95% LC)[&]	(1.81-3.029)	(9.55-55.6)
$LC^{\S}_{90}$	12.72	82.3
(95% LC)[&]	(8.27-24.6)	(30.7-1.073)
$Pr > \chi^{2\Phi}$	0.0001	0.0001

Within the same column, values with the same lower-case letter are not significantly different according to Tukey test ($p \leq 0.05$).
[†]Total number of insects treated.
[¶]Probit adjustment slope (b) and standard error of slope (ES).
[§]Lethal concentration (g boldo kg^{-1} diet).
[&]Confidence limits at 95%.
[Φ]Probability that a log dose-probit line adjusts to a straight line.
SE: Standard error.

Table 2. Larval size and weight, percent pupation, pupal weight, time between larval and pupal stages and between pupal and adult stages, and percentage of adult emergence of and *Spodoptera frugiperda* and *Helicoverpa zea* fed artificial diet mixed with *Peumus boldus* water extract.

	Concentration	Larval size	Larval weight	Pupation	Pupal weight	Larva-Pupa (LP)[1] DDI[3]	Pupa-adult (PA)[2] DDI[3]	Adult emergence[4]
	%	mm	g	%	g			%
S. frugiperda	Control	38.2 ± 3.60a	0.57 ± 0.03a	100 ± 0.0a	0.27 ± 0.006a	8.6 ± 0.40c	18.6 ± 0.40b	100 ± 0.0a
	0.25	34.7 ± 0.75a	0.54 ± 0.02a	100 ± 0.0a	0.29 ± 0.007a	9.0 ± 0.40c	19.0 ± 0.0b	100 ± 0.0a
	0.50	34.2 ± 2.30a	0.53 ± 0.10a	100 ± 0.0a	0.29 ± 0.015a	9.4 ± 0.0c	19.0 ± 0.0b	100 ± 0.0a
	1.00	33.7 ± 4.20a	0.50 ± 0.11a	95 ± 5.0a	0.28 ± 0.004a	10.2 ± 0.48bc	19.0 ± 0.0b	80 ± 9.3ab
	2.00	32.1 ± 3.70a	0.45 ± 0.18a	90 ± 6.1a	0.27 ± 0.012a	11.4 ± 0.40ab	19.4 ± 0.40b	60 ± 6.1bc
	4.00	31.2 ± 6.20a	0.39 ± 0.10a	90 ± 6.1a	0.26 ± 0.016a	11.4 ± 0.40ab	19.8 ± 0.48b	55 ± 9.3c
	8.00	27.2 ± 2.60a	0.34 ± 0.03a	60 ± 12.7b	0.28 ± 0.006a	13.0 ± 1.26a	21.4 ± 0.74a	40 ± 10.0c
H. zea	Control	47.6 ± 1.34a	0.724 ± 0.04a	100 ± 0.0a	0.425 ± 0.02a	19.0 ± 1.00d	21.7 ± 0.33e	100 ± 0.0a
	0.25	38.3 ± 1.64b	0.719 ± 0.02a	66.7 ± 13.3a	0.395 ± 0.03ab	21.3 ± 0.66c	23.3 ± 0.66d	77.7 ± 11.1ab
	0.50	36.6 ± 2.90bc	0.712 ± 0.02a	44.0 ± 11.0b	0.353 ± 0.01ab	22.6 ± 0.66bc	24.6 ± 7.68d	66.6 ± 19.2ab
	1.00	33.9 ± 3.07cd	0.734 ± 0.08a	44.0 ± 22.0b	0.342 ± 0.03ab	24.0 ± 0.0ab	26.6 ± 0.0cd	44.4 ± 11.1bc
	2.00	32.4 ± 1.21cd	0.687 ± 0.05a	33.0 ± 19.1b	0.334 ± 0.06ab	24.6 ± 0.66a	26.0 ± 0.66bc	44.4 ± 11.1bc
	4.00	30.8 ± 0.43cd	0.663 ± 0.09a	33.0 ± 19.1b	0.352 ± 0.02ab	25.3 ± 0.66a	27.3 ± 0.66ab	22.1 ± 11.1c
	8.00	29.7 ± 0.86d	0.314 ± 0.07b	33.0 ± 19.1b	0.287 ± 0.07b	26.0 ± 0.0a	28.6 ± 0.66a	11.1 ± 11.1c

Values with the same letter within a column are not significantly different according to Tukey test ($p \leq 0.05$).
[1]LP: Time lapse (d) in which 50% of the larvae reached pupal stage.
[2]PA: Time lapse (d) in which 40% of the pupae reached adult stage.
[3]DDI: Days after infestation.
[4]Was considered as 100% the number of pupas obtained in each concentration.
± Standard error.

(19 d) than with other treatments (F = 13.67; df = 8;12; P < 0.0001). The longest period (26 d) occurred at 8% concentration. This same relation occurred with the time between pupa-adult with the control: 21.7 d vs. 27.3 and 28.6 d for 4% and 8%, respectively (Table 2).

Choice tests first instar larva

Spodoptera frugiperda neonates presented a clear preference toward the control and the treatments with lower extract concentrations. At 24 and 48 h, control had 40% and 45% preference, while concentrations between 0.25% and 4% diminished as the concentration increased (Table 3). Between 72 and 120 h, the larvae were only found in the control, 0.25% and 0.5%, and control diet was always the most preferred with the maximum of 60% at 120 h. After 72 h, 1%, 2%, and 4% concentrations were selected by the larvae, while the treatment with 8% concentration never presented larvae at any of the evaluations. Diet consumption diminished when concentration increased. The lowest consumption (0.002 g) was at 8%, significantly smaller (F = 2.14; df = 10;24; P = 0.0478) than control that presented the highest value with 0.030 g.

The preference of *H. zea* was also greater when the extract concentration in the diet diminished. The highest preference was observed for the control with 60% (F = 44.82; df = 6;2; P < 0.001). Between 48 and 120 h, no larva was detected in concentrations > 1% (Table 3). After 72 h, there no differences were recorded in the preference

Table 3. Presence of *Spodoptera frugiperda* and *Helicoverpa zea* neonates and consumption of insect artificial diet mixed with different concentrations of *Peumus boldus* water extract.

	Concentration	24 h	48 h	72 h	96 h	120 h	Diet intake
				%			g
S. frugiperda	Control	40.0 ± 14.14a	45.0 ± 5.0a	50.0 ± 12.9a	55.0 ± 18.9a	60.0 ± 20.0a	0.03 ± 0.014a
	0.25	20.0 ± 14.14a	25.0 ± 9.5ab	30.0 ± 5.7ab	30.0 ± 10.0ab	30.0 ± 20.0ab	0.03 ± 0.004ab
	0.50	15.0 ± 10.00a	10.0 ± 10.0b	20.0 ± 14.1ab	15.0 ± 9.5ab	10.0 ± 0.0b	0.02 ± 0.006abc
	1.00	10.0 ± 5.00a	10.0 ± 5.7b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.01 ± 0.003abc
	2.00	10.0 ± 10.00a	5.0 ± 5.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.01 ± 0.004bc
	4.00	5.0 ± 5.00a	5.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.009 ± 0.004bc
	8.00	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.002 ± 0.0008c
H. zea	Control	60.0 ± 11.5a	66.7 ± 6.7a	46.67 ± 13.3a	46.67 ± 13.3a	46.67 ± 13.3a	0.042 ± 0.013a
	0.25	26.6 ± 6.6b	26.7 ± 6.7b	46.67 ± 17.6a	46.67 ± 17.6a	46.67 ± 17.6a	0.036 ± 0.005ab
	0.50	6.7 ± 6.6c	6.7 ± 6.7c	6.67 ± 6.7b	6.67 ± 6.7ab	6.67 ± 6.7b	0.036 ± 0.003ab
	1.00	6.7 ± 6.6c	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.009 ± 0.006bc
	2.00	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.003 ± 0.003c
	4.00	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.002 ± 0.002c
	8.00	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.001 ± 0.001c

Within a column, values with the same letter are not significantly different according to Tukey test ($p \leq 0.05$).
Diet intake was measured by difference between dry weight before and after consumption by larvae.
± SE: Standard error.

between control, 0.25% and 0.5%. There were no differences in consumption between control, 0.25% and 0.5% concentrations. There were significant differences with the other treatments that fluctuated between 0.009 g for 1% and 0.001 g for 8%.

Choice tests third instar larva

The FDI and FII in *S. frugiperda* increased with the extract concentration in the diet (Table 4). For FDI, the minimum inhibition was 18.5% at 0.25% concentration and the maximum was 50.2% with the 8% concentration. For FII, the minimum value was 28.9% and the maximum 63.1% for 0.25% and 8% concentrations, respectively. In *H.* zea the lowest FDI was 16.3% with the 0.25% treatment, not different from the concentrations < 8%, which had the maximum FDI value of 40.3%. FII had a maximum value of 57.3% at 8% concentration, significantly greater for all the remaining concentrations (Table 4).

Table 4. Feeding dissuasion index (FDI) and feeding inhibition index (FII) of diet with different concentrations of *Peumus boldus* water extract incorporated into insect artificial diet against third-instar larvae of *Spodoptera frugiperda* and *Helicoverpa zea*.

	Concentration	FDI mean ± SE	FII mean ± SE
		%	
S. frugiperda	Control	--	--
	0.25	18.5 ± 8.6a	28.9 ± 10.8a
	0.50	34.1 ± 14.3a	46.1 ± 15.4a
	1.00	35.1 ± 10.3a	48.9 ± 13.3a
	2.00	38.4 ± 1.3a	55.5 ± 1.4a
	4.00	48.0 ± 10.4a	62.9 ± 9.5a
	8.00	50.2 ± 14.3a	63.1 ± 13.5a
H. zea	Control	--	--
	0.25	16.3 ± 4.1b	27.4 ± 5.7b
	0.50	24.9 ± 8.0ab	37.7 ± 11.6ab
	1.00	25.4 ± 10.5ab	37.7 ± 13.9ab
	2.00	25.9 ± 2.1ab	40.5 ± 2.7ab
	4.00	27.3 ± 7.8ab	41.3 ± 8.8ab
	8.00	40.3 ± 3.0a	57.3 ± 3.1a

Within a column, values with the same letter are not significantly different according to Tukey test ($p \leq 0.05$).
SE: Standard error.
Feeding inhibition index (FDI) = [(intake of non treated feed - intake of treated feed)/(intake of non treated feed + intake of treated feed)] × 100.
Feeding inhibition index (FII) = [(intake of non treated feed - intake of treated feed)/intake of non treated feed)] × 100.

No choice test

The highest RCR in *S. frugiperda* was produced in the control with 0.17 g g^{-1} d^{-1} (g diet g^{-1} larva daily) being significantly ($F = 8.26$; df = 9;27; $P < 0.0001$) greater than the rest of the extract concentrations (Table 5). The FII presented direct proportionality with *P. boldus* concentration, reaching 60.9% with 8% extract and the minimum of 14.1% occurred with the 0.25% treatment. The 8% concentration was not significantly different from the concentrations of 2 and 4%, although it was with the lower concentrations ($F = 11.1$; df = 8;23; $P = 0.0001$). The lowest GII occurred at 0.25% of extract with inhibition of 14.3%, a value that is statistically similar to the value at 0.5%; the highest inhibition was 46.9% at 4 and 8% of *P. boldus* although this value was not significantly different from 1% ($F = 3.12$; df = 8;23; $P = 0.0276$). The increase in larva weight (WIR) was smaller with higher extract concentrations in the diet: the control presented the highest weight increase of 0.21 g g^{-1} d^{-1} being significantly different ($F = 10.98$; df = 9;27; $P = 0.0001$) to all extract concentrations. Finally, CFE was not significantly higher

Table 5. Relative consumption rate (RCR), feeding inhibition index (FIA) and growth (GII), larval weight increase rate (WIR) and consumed feed conversion efficiency (CFE) by third-instar larvae of *Spodoptera frugiperda* and *Helicoverpa zea* exposed to artificial diet with different concentrations of *Peumus boldus* water extract.

	Concentration	RCR mean ± SE	FII mean ± SE	GII mean ± SE	WIR mean ± SE	CFE mean ± SE
	%	g g^{-1} d^{-1}		%	g g^{-1} d^{-1}	%
S. frugiperda	Control	0.17 ± 0.008a	--	--	0.21 ± 0.020a	90.5 ± 5.8a
	0.25	0.08 ± 0.006b	14.1 ± 0.29d	14.3 ± 5.7c	0.09 ± 0.009b	78.7 ± 6.8ab
	0.50	0.07 ± 0.003b	36.1 ± 3.15c	24.4 ± 3.7bc	0.07 ± 0.010bc	74.7 ± 12.3ab
	1.00	0.07 ± 0.009b	44.1 ± 7.60bc	32.9 ± 4.7abc	0.06 ± 0.020bc	69.4 ± 20.2abc
	2.00	0.06 ± 0.006b	47.2 ± 1.10abc	41.5 ± 2.0ab	0.04 ± 0.020c	36.3 ± 20.2bc
	4.00	0.06 ± 0.010b	55.0 ± 5.50ab	46.9 ± 6.9a	0.03 ± 0.010c	34.6 ± 10.3bc
	8.00	0.05 ± 0.010b	60.9 ± 6.50a	46.9 ± 8.8a	0.02 ± 0.009c	28.6 ± 6.4c
H. zea	Control	0.128 ± 0.020a	--	--	0.11 ± 0.020a	96.0 ± 20.4a
	0.25	0.089 ± 0.010ab	75.3 ± 5.5a	7.4 ± 3.6b	0.09 ± 0.020ab	90.3 ± 15.1a
	0.50	0.081 ± 0.020ab	67.4 ± 12.7ab	9.5 ± 2.7b	0.05 ± 0.006ab	74.4 ± 15.3a
	1.00	0.076 ± 0.030ab	52.0 ± 20.7ab	15.1 ± 6.7b	0.04 ± 0.004b	72.0 ± 28.4a
	2.00	0.056 ± 0.007ab	39.2 ± 9.2b	15.5 ± 3.5b	0.03 ± 0.010b	71.4 ± 28.8a
	4.00	0.053 ± 0.005ab	34.2 ± 4.2b	18.7 ± 3.3b	0.03 ± 0.008b	56.9 ± 20.3a
	8.00	0.039 ± 0.009b	33.6 ± 1.4b	51.2 ± 7.6a	0.02 ± 0.009b	49.4 ± 15.7a

Within a column, values with the same letter are not significantly different according to Tukey test ($p \leq 0.05$).
SE: Standard error
g g^{-1} d^{-1} = diet consumed (g)/(initial larva weight (g) × feeding period (d)).
Relative consumption rate (RCR) RCR = IaL/(PiL × T), where IaL = feed intake during the experimental period (g), PiL = initial larva weight (g) and T = experimental period (d).
Feeding inhibition index (FII) FII = [(intake of non treated feed - intake of treated feed)/intake of non treated feed)] × 100.
Growth inhibition index (GII) GII = ((Pc-Pt)/Pc) × 100, where Pc = control larva weight (g) and Pt = treated larva weight (g).
Larval weight increase rate (WIR) WIR = ΔP/(PiL × T), where ΔP = increase in larval weight during the experimental period, PiL = initial larval weight (g) and T = experimental period (d).
Consumed feed conversion efficiency (CFE) = (TIP/TCR) × 100.

in the control (90.5%) than with the extract concentrations 0.25%, 0.5%, and 1% but it did with the remaining concentrations (F = 2.49; df = 9;27; P = 0.0047).

The diet consumption (RCR) by *H. zea* diminished as the extract concentration increased. The control presented the highest value of 0.128 g g^{-1} d^{-1}, different only from the 8% of extract (0.039 g g^{-1} d^{-1}) (F = 2.0; df = 9;18; P = 0.044) (Table 5). Feeding inhibition (FII) increased with *P. boldus* concentration, reaching its maximum inhibitory value of 75.3% with the 8% extract. The remaining of the treatments did not surpass 40%. The GII values for extract concentrations between 0.25% and 4%, there were no significant differences surpassing 20% (F = 6.1; df = 8;15; P = 0.0003), although all were significantly lower than 8% with 51.2% inhibition. The same trend was observed in WIR, where the control presented the highest increase in larval weight with 0.11 g g^{-1} d^{-1}, although it did not differ significantly with treatments of 0.25% and 0.5%. The lowest increase was recorded for 8% with 0.02 g g^{-1} d^{-1}, which was statistically different only with the control (F = 3.65; df = 1;18; P = 0.003). Even when the CFE did not present significantly differences, it was inversely proportion to the extract concentration: the control had 96% and the 8% treatment had 49.4%.

DISCUSSION

The mortality of *S. frugiperda* is lower than the one obtained by Zapata et al. (2006), who mixed artificial diet with *P. boldus* powder at 4% concentration obtaining mortality > 80% in *S. littoralis* larva. In our case the LC_{90} implied that a 13% concentration is necessary to obtain 90% mortality. Additionally, toxicity of water *P. boldus* extract is similar to that obtained in *S. frugiperda* with other species such as *Azadirachta indica* (Prates et al., 2003) and higher than found for the extract of *Talisia esculenta* (A. St.-Hil.) Radlk. (Dos Santos et al., 2008), *Sapindus saponaria* L. (Dos Santos et al., 2008) and *Croton ciliatoglandulifer* Ortega (Euphorbiaceae) in *S. littoralis* (Huerta et al., 2002).

Even though there are no previous reports about the toxicity of some derivative of *P. boldus* on *H. zea*, our results indicate that the mortality is lower than the one achieved with other species such as *A. indica* and *Anonna* spp., which killed 100% of larva at 1% concentration (Grzywacz et al., 2005).

The diminishment in larval and pupal weights, together with the increase in the duration of *S. frugiperda* development cycle at higher *P. boldus* concentrations coincides with the results found with other plant species such as *Croton ciliatoglandulifer* (Huerta et al., 2002), *Ricinus communis* L. (Pedreira et al., 2008) and meliaceas of the genus *Trichilia* spp. (Rodríguez and Vendramim, 1996) and *Guarea* spp. (Rodríguez and Vendramim, 1997), which indicates that these extracts have growth regulating properties.

In choice test in neonate larvae according to Gore et al. (2005), the spectrum of choice is wider at the beginning due to the period of larval adaption to the environment but finally if the compound has deterrent properties the larvae will be concentrated in lesser concentrations and control.

The FDI values of third instar larvae of *S. frugiperda* are lower than found by Zapata et al. (2006), who mixed *P. boldus* powder with the diet of *S. littoralis* in concentrations of 1%, 2%, and 4% obtained values of 60.2%, 73.8%, and 96.2% respectively. In contrast, FII values presented opposite behavior because same authors found that same concentrations did not surpass 22% with inhibition values below the results found in this study.

In no choice test our FII values are lower than found by Zapata et al. (2006) for *S. littoralis* larva: 68.9% and 78.1% for 2% and 4%, respectively, and our GII values were also lower than those obtained by these authors: 81.1% and 86.7% at concentrations 2% and 4%.

CONCLUSIONS

The results obtained for the feeding preference bioassays for both species indicate that a higher extract concentration produces greater feeding inhibition, resulting in lower food consumption and assimilation. The lower production of new biomass results in lower growth and a drop in larval weight. Therefore, *Peumus boldus* extract presents greater toxicity to *Spodoptera frugiperda* in comparison with *Helicoverpa zea*. Even when our results for their effect on the cycle and anti-feeding activity make them valid alternatives for plant protection since their effect on F_1 will produce an impact of the pest, these results should be validated in the field in order to demonstrate to farmers that they are valid alternatives.

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