Bioactivity of Boldo (*Peumus boldus* Molina) (Laurales: Monimiaceae) on *Spodoptera frugiperda* (J.E. Smith) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)

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Source: Southwestern Entomologist, 35(3):215-231. 2010.
Published By: Society of Southwestern Entomologists
DOI: http://dx.doi.org/10.3958/059.035.0301
URL: http://www.bioone.org/doi/full/10.3958/059.035.0301

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Abstract. Insecticidal properties of powdered boldo, *Peumus boldus* Molina, were evaluated against larvae of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* (Boddie). Bioassays assessed development, feeding preferences, and mortality of neonate and third instars. For both species, most mortality (65 and 67.5%, respectively) was obtained with 8% boldo concentration incorporated into an artificial insect diet, and the LC_{50} and LC_{90} for fall armyworm were 6.8 and 25.9 g boldo kg^{-1}diet and 3.8 and 35.6 g boldo kg^{-1}diet for corn earworm. With increased concentration of boldo, larvae were shorter, weighed less, and had anatomical abnormalities, and fewer pupated. Concentrations of 4 and 8% boldo resulted in fewer adults of both species. In feeding preference tests, neonates selected the diet with the least concentration of boldo powder, and larvae fed less with higher concentrations. In tests for insect preference, the greatest concentrations of boldo resulted in the greatest indexes of feeding inhibition and growth, while the least indexes resulted in increases in larval weight and greater efficiency of conversion of ingested food in the diet percentage used to produce new larval biomass.

Resumen. Se evaluaron las propiedades insecticidas del polvo de boldo, *Peumus boldus* Molina, contra larvas de *Spodoptera frugiperda* (J. E. Smith) y *Helicoverpa zea* (Boddie). Se realizaron bioensayos para evaluar mortalidad, efecto en el desarrollo, y las preferencias alimentarias en larvas neonatas y de tercer instar. En ambas especies, la mayor mortalidad (65 y 67.5% respectivamente) se obtuvieron con la concentración de 8% de boldo (p/p) cuando esta se incorporó a la dieta. La CL_{50} y CL_{90} para *S. frugiperda* fueron 6.8 y 25.9 g boldo kg^{-1}dieta y 3.8 y 35.6 g boldo kg^{-1}dieta para *H. zea*. Cuando se incrementó la concentración de boldo el tamaño y peso de la larva se redujo, el porcentaje de pupación disminuyó y las larvas mostraron anormalidades anatómicas. Las concentraciones de 4 y 8%

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obtuvieron, en ambas especies, un menor número de adultos. En las pruebas de preferencia alimentaria, las larvas neonatas seleccionaron la dieta con la menor concentración de polvo de boldo y el menor consumo de dieta se observó en las mayores concentraciones. En los experimentos de selección las mayores concentraciones tuvieron los mayores índices de inhibición de la alimentación y del crecimiento mientras que los menores índices produjeron un incremento del peso larval y una mayor eficiencia de conversión del alimento ingerido para producir nueva biomasa larval.

Introduction

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), are two polyphagous insect species that affect major crops, including maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), soybeans (*Glycine max* L.), tomatoes (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), and sweet potatoes (*Ipomoea batata* L.) (Bergvinson 2005). The larvae feed on the foliage and, in extreme cases, can cause total crop loss. These species are controlled mainly with pyrethroids and to a lesser degree with organophosphorates (Cook et al. 2004) and other synthetic insecticides. However, development of resistance has limited the use of these insecticides (Yu 1992, Abd-elghafar et al. 1993, Al-Sarar et al. 2006, Pietrantonio et al. 2007). Although genetically modified cultivars of cotton and maize capable of expressing *Bacillus thuringiensis* Berliner δ-endotoxins that protect them from these two pests have been developed (Buntin et al. 2001, Gore et al. 2001, Adamcyk and Gore 2004), the search for low-risk conventional insecticides continues to be necessary, because both species have demonstrated tolerance to different strains of *B. thuringiensis* (Matten et al. 2008, Tabashnik et al. 2008).

The effectiveness of botanical insecticides against larvae in the genus *Spodoptera* has been evaluated. The most promising extracts are those of *Azadirachta indica* J. (Martínez and van Emden 2001), *Melia azedarach* L. (Schmidt et al. 1997, De Brito et al. 2004, Souza et al. 2007), *Brassica alba* (L.) Boiss. (Shadia and Sharaby 1997), *Carica papaya* L. (Franco-Archundia et al. 2006), *Geranium pelargonium graveolens* (Linn) (Shadia and El-Aziz 1998), *Reynoutria* sp. (Pavela et al. 2008), and teocintle (*Zea diploperennis* L.) (Farías-Rivera et al. 2002). Some botanical insecticides tested against *Helicoverpa* spp. were derived from *A. indica* (Barnby and Klocke 1987), *M. azedarach* (McMillian et al. 1969), and *Trichilia havanensis* Jacq (López-Olguín et al. 1997). These studies demonstrated the potential of plants as sources of insecticidal compounds that may be useful in organic and integrated pest management programs.

Boldo, *Peumus boldus* Molina, of the Monimiaceae family, is a perennial tree native to Chile. It contains antioxidant (Young et al. 2000, Quezada et al. 2004, Russo et al. 2004, Vogel et al. 2005b), anticarcinogenic (Russo et al. 2004), anti-inflammatory (Young et al. 2000, Vogel et al. 2005b), and anti-microbial (Vogel et al. 2005b, Mazutti et al. 2008) properties. According to Vogel et al. (1997, 1999, 2005a), the main active components of boldo are essential oils and alkaloids present in different concentrations in the foliage and bark depending on the season of the year. Boldo also has insecticidal activity against maize weevil, *Sitophilus zeamais* Motschulsky (Páez et al. 1990; Pérez et al. 2007; Silva et al. 2003, 2005, 2006), and against larvae of *Spodoptera littoralis* Boisd. (Zapata et al. 2006), as well as fungicidal activity against *Penicillum* spp., *Fusarium* spp., *Aspergillus niger*

P.E.L. Tieghem, and *A. flavus* Link (Leite de Souza et al. 2005). However, its potential against many other crop pests has not been studied. Therefore, the objective of this study was to evaluate biological activity in a laboratory to gain insight into whether powdered boldo leaves could serve as a source of insecticidal products against larvae of fall armyworm and corn earworm, two important polyphagous pests.

Materials and Methods

Plant Material. Dehydrated boldo leaves were acquired in the fruit and vegetable market of the city of Texcoco, state of Mexico, Mexico. The taxonomic identification of the samples was verified according to Vogel et al. (2005b). Mature whole leaves were selected and dehydrated to maintain their insecticidal properties, following the method of Pérez et al. (2007). Boldo leaves were ground in an electric coffee mill (Braun KSM2BLK, Braun de México y Cía de C.V., Naucalpan, Estado de Mexico, Mexico) and filtered through a 250-micron mesh (DUAL Manufacturing Co., Chicago, IL).

Insects. Larvae of fall armyworm and corn earworm were acquired from a colony in the Laboratory of Insecticide Toxicology of the Entomology and Acarology Program of the Colegio de Postgraduados, Campus Montecillo, where they were kept in a bioclimatic chamber under controlled conditions of 27 ± 1 ºC, 70 ± 5% relative humidity, and a photoperiod of 14:10 light:dark hours.

Toxicity of Boldo Powder. Boldo powder was incorporated into insect artificial diet (Tobacco Budworm, Southland Products, Lake Village, AR) when the diet had cooled to 40ºC to prevent degradation of the active compounds (Martínez and van Emden 2001). A Multipette (Eppendorf AG, Hamburg, Germany) was used to pour 10 ml of the mixture into each 20-ml cup (Envases Cuevas S.A. de C.V., Ecatepec, Estado de Mexico, Mexico) to obtain concentrations of 0, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0% by weight. After the diet cooled and solidified, a 24-72 hour-old larva was placed into each cup, making a set of cups of fall armyworm and another of corn earworm. Each cup was closed with a perforated cover (0.5 cm diameter), and organza fabric was put between the cup and cover to provide ventilation. Mortality was assessed every 48 hours until 75% of the larvae in the nontreated check (0%) reached the pupal stage. Larvae were considered dead when they failed to move after being prodded with a dissection needle for 15 seconds. Cups were placed in a randomized complete block design inside a bioclimatic chamber. For each insect species, there was a total of six boldo concentrations and a nontreated check, each with 20 replications. The experiment was replicated five times on different days (100 cups per treatment). To estimate the LC_{50} and LC_{90} values, the data were subjected to Probit analysis (Finney 1971) using the PROC PROBIT procedure of the Statistical Analysis System software (SAS Institute 1998).

Effect of Boldo Powder on Insect Development. In a separate study, using the concentrations of boldo and methodology described, 100 cups of diet were prepared for fall armyworm and corn earworm. A <24-hour-old larva of either species was put into each cup. Larvae were allowed to feed for 72 hours, and every 48 hours thereafter five cups per treatment were selected at random to record individual larval weight and length. Once 75% of the larvae on the check diet reached pupation, the remaining treatments with boldo powder were divided into 10 groups of five cups each. For each group, every 24 hours we recorded the percentage of larvae that reached the pupal stage, individual pupal weight, number of pupae that reached the adult stage, and time from larva to pupa and from pupa to adult.

Feeding Preference Tests. *Choice Tests.* These studies used the previously described concentrations against neonate and third instars of both species. To evaluate the feeding preference of neonates, a bioassay was used with a larval selection arena consisting of a plastic Petri dish (Industrias Technicare, Atizapan de Zaragoza, Estado de Mexico, Mexico) 5 cm in diameter and 1.5 cm deep, similar to that described in Gore et al. (2005). A 24-hour-old neonate was placed in the center of the dish while plugs of diet (1.5 cm diameter x 0.25 cm thick), of each boldo concentration, were randomly arranged around the inner wall of the dish. The cover of the Petri dish was perforated (2.5 cm diameter) and the holes were covered with organza fabric for ventilation. For five consecutive days, the larval feeding preference for each of the boldo concentrations was recorded for 5 minutes per day. Feeding preference was measured by assessing the amount of diet consumed. The seven diet plugs were dried for 48 hours at 40 ºC in an oven and weighed. Initial and final weights of 20 samples per treatment were compared. Each treatment was replicated 10 times and the test was repeated five times on different days.

The experiment was repeated with third instars of both species, using diet of each of the boldo concentrations poured into 2-ml ice-cube molds (2 x 2 cm) (Imperial Plastic® Inc, Lakeville, MN). Once the diet solidified, two diet cubes, one with one of the boldo concentrations and another untreated, were placed in a Petri dish (9 cm in diameter, 3 cm deep) with a perforated cover of organza fabric. The third instar was allowed to feed freely for 72 hours on its preferred diet. The remainder of the diet, after the larvae fed on it, was dried for 72 hours at 40 ºC in an oven. Each treatment was replicated 20 times, and the test was repeated five times on different days (100 cups per treatment). With the obtained values, the antifeedant index (AFI) $AFI = ((C-T)/(C+T))*100$ (Sadek 2003) and the deterrence index (DI) $DI = ((C-T)/C))*100$ (Raffa and Frazier 1988) were calculated, where C = intake of nontreated feed and T = intake of treated feed. To calculate the indexes, diet consumption was obtained as the difference between the initial and final amounts of diet of each of the 20 individual samples.

No-Choice Tests. This evaluation was a separate study with third instars of both insect species. This test was as for the ‘Choice test’, but the larva in each Petri dish had access to two cubes of diet of the same treatment concentration to ensure they had sufficient food during development. Initial and final larval and diet weights were obtained and the following were calculated: antifeeding index (AFI); growth inhibition index (GII), $GII = ((Wc-Wt)/Wc)*100$, where Wc = weight of check larva (g) and Wt = weight of treated larva (g); Relative consumption rate (RCR) $RCR = I/WiL*T)$, where I = feeding intake during the experimental period (g), WiL = initial weight of larva (g), and T = feeding period (days); relative growth rate (RGR) $RGR = \Delta W/WiL*T$, where ΔW = increase in larval weight during the experimental period and efficiency of conversion of ingested food (ECI) $ECI = (RGR/RCR)*100$ (Waldbauer 1968, Raffa and Frazier 1988, Farrar et al. 1989).

Experimental Design and Statistical Analysis. All of the tests were arranged as randomized complete blocks. To achieve homogeneity of variances, the data were transformed to $\sqrt{x} + 0.5$ and subjected to analysis of variance ($\alpha = 0.05$) and a Tukey test of comparison of means with a significance of 95% ($P \leq 0.05$) using Statistical Analysis System software (SAS Institute 1998).

Results

Toxicity of Boldo Powder. Powder of *P. boldus* incorporated into artificial diet at 8% (w:w) produced 65% mortality of fall armyworm and 67.5% of corn earworm. For corn earworm, there were no significant differences between mortality produced at the 4 and 8% concentrations of boldo, but significantly more larvae fed 8% boldo died than did larvae fed lower concentrations ($F = 11.56$; df = 6, 22; $P < 0.001$). For both species, the other concentrations (≤2%) of boldo resulted in death of <40% of the larvae (Table 1). For fall armyworm, the LC$_{50}$ and LC$_{90}$ values, in grams of boldo per kilogram of diet, were 6.89, and 25.9, respectively. For corn earworm, the LC$_{50}$ and LC$_{90}$ values were 3.8 and 35.6 g boldo kg^{-1} diet, respectively.

Effect of Boldo Powder on Insect Development. *Fall Armyworm*. Larvae were longest (38.3 mm) in the nontreated check but not significantly longer than larvae fed boldo concentrations <4% (Table 2). The shortest larvae (5.6 mm) were those fed diet with 8% boldo powder ($F = 28.08$; df = 6,20; $P < 0.0001$), significantly different from the other treatments. Larvae weighed significantly less when fed boldo in concentrations ≥2% (0.0012-0.22 g). Percentage of pupation (85-100%) did not differ significantly among treatments with concentrations of boldo <4%. Insects fed on boldo at concentrations ≥4% did not pupate ($F = 57.38$; df = 6,27; $P < 0.0001$), and 8% concentration induced anatomical abnormalities. This treatment

Table 1. Dose Mortality Values Produced by Incorporating *Peumus boldus* Molina Powder with Insect Artificial Diet Used against *Spodoptera frugiperda* (J.E. Smith) and *Helicoverpa zea* (Boddie) Neonates

	P. boldus concentration (w:w)	Mortality (%) ± SE	
		Spodoptera frugiperda	*Helicoverpa zea*
	Check	0.0 ± 0 A b	0.0 ± 0 A d
	0.25	0.0 ± 0 B b	10.0 ± 4.1 A d
	0.5	2.5 ± 2.5 A b	10.0 ± 5.5 A d
	1	2.5 ± 2.5 A b	15.0 ± 5.5 A cd
	2	12.5 ± 5.5 A b	37.5 ± 7.7 A bc
	4	17.5 ± 6.5 A b	52.5 ± 7.9 B ab
	8	65.0 ± 9.3 A a	67.5 ± 7.5 A a
n[†]		200	200
b ± SE[¶]		1.5 ± 0.2	1.3 ± 0.14
LC[§]$_{50}$ (95% CL)[&]		6.89 (5.27-10.3)	3.8 (2.74-6.03)
LC[§]$_{90}$ (95% CL)[&]		25.9 (15.5-65.1)	35.6 (17.5-122.1)
$P > \chi^{2}$[Φ]		0.0001	0.0001

Within the same row, values with the same upper-case letter are not significantly different (Tukey, $p \leq 0.05$).

Within the same column, values with the same lower-case letter are not significantly different (Tukey, $p \leq 0.05$).

[†] = 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.

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 *Spodoptera frugiperda* (J. E. Smith) Fed Insect Artificial Diet Mixed with Different Concentrations of *Peumus boldus* Molina Powder

Concentration (%)	Larval size ± SE (mm)	Larval weight ± SE (g)	Pupation ± SE (%)	Pupal weight ± SE (g)	Larva-Pupa* ± SE (LP)[1] DDI[3]	Pupa-adult ± SE (PA)[2] DDI[3]	Adult emergence ±SE (%)
0 (check)	38.26 ± 8.5a	0.59 ± 0.03a	100 ± 0.0a	0.27 ± 0.007a	9.5 ± 0.5a	20.0 ± 0.57a	90 ± 5.3a
0.25	37.17 ±7.5 a	0.57 ± 0.03a	95 ± 6.6a	0.28 ± 0.003ab	10.5 ± 0.5a	20.0 ± 0.57a	85 ± 5.0a
0.5	36.23 ± 7.3a	0.57 ± 0.07a	90 ± 6.6a	0.28 ± 0.004ab	10.5 ± 0.5a	20.0 ± 0.57a	85 ± 5.0a
1.0	35.87 ± 8.8a	0.55 ± 0.11a	85 ± 11.5a	0.29 ± 0.005ab	11.0 ± 0.0a	20.5 ± 0.50a	75 ± 9.6ab
2.0	29.30 ± 7.0a	0.22 ± 0.02b	85 ± 11.5a	0.3 ± 0.003ab	11.5 ± 0.5a	23.5 ± 0.50b	45 ± 12.6b
4.0	17.70 ± 3.7b	0.077 ± 0.02b	0.0 ± 0.0b				
8.0	5.60 ± 0.6c	0.0012 ± 0.001b	0.0 ± 0.0b				

Within a column, values with the same letter are not significantly different (Tukey, $p \leq 0.05$).

[1] LP = Time lapse (days) in which 50% of the larvae reached pupa stage.

[2] PA2 = Time lapse (days) in which 40% of the pupae reached adult stage.

[3] DDI = Days after infestation.

resulted in intermediate stages (Figs. 1a,b,c) with the body covered by the larval exuvium, thus preventing molting (Fig. 1d), and the cuticle becoming black (Fig. 1e). In the treatments in which larvae reached the pupal stage (i. e., ≤2%), larval and pupal weights did not differ significantly by treatment. Time from pupa to adult was significantly different (*F* = 7.97; df = 4,19; *P* = 0.0022) between the 2% boldo (23.5 days) and lesser concentrations (Table 2). Emergence of adults was observed with ≤2% boldo concentrations and ranged from 45 (2%) to 90% with the check diet. Emergence of adults was significantly less with the 2% than the 0.5% or lower concentrations of boldo in the diets (*F* = 20.42; df = 6,27; *P* = <0.0001).

Corn Earworm. The largest larvae (47.6 mm) resulted when fed nontreated check diet, but not significantly different from larvae fed 0.25, 0.5, or 1% boldo powder. The shortest length was 12.5 mm, observed 8% boldo. Weight of larvae fed the nontreated check diet was not significantly different from those fed concentrations ≤4%, with a minimum of 0.25 g and a maximum of 0.73 g (Table 3). The 8% concentration of boldo resulted in the least larval weight (0.08 g). With the nontreated check diet, 100% of the larvae pupated, while none reached the pupal stage when fed 4 or 8% diet concentration. Pupae weighed significantly less (*F* = 6.5; df = 4,14; *P* = 0.0037) with the 2% (0.28 g) than with lesser concentrations of boldo. There were no significant differences among the treatments in time between the larval and pupal stage, fluctuating between 13.6 and 19.0 days. The shortest time between pupal and adult stages (21 days) was observed for the nontreated check. More adults (66.6%) emerged from the nontreated check, but this was not statistically different from that observed for the 0.25% concentration, where 52.7% reached the adult stage.

Feeding Preference Tests. *Fall Armyworm. Choice Tests.* During the entire experimental period, 24 to 120 hours, the boldo concentrations most preferred

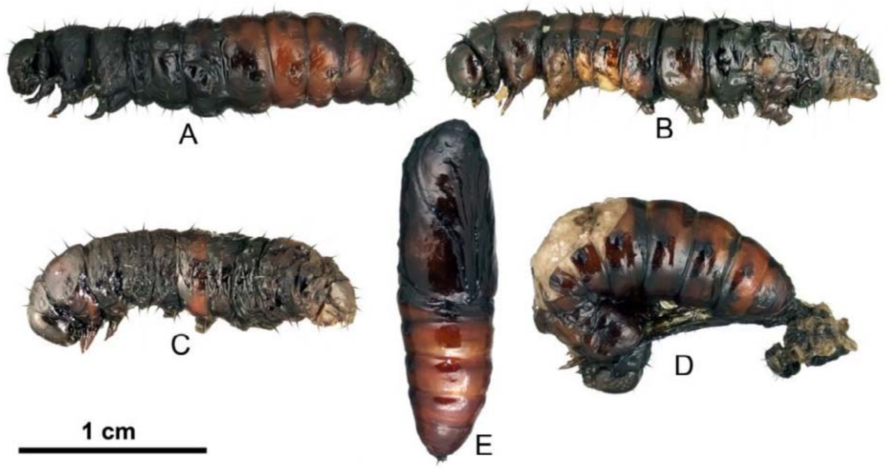

Fig. 1. Larva-pupa intermediates of *Spodoptera frugiperda* fed diet treated with *Peumus boldus* powder at 8%. A, B, and C intermediate stages, D larval exuvium covers the body, and E cuticle turned black.

Table 3. 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 *Helicoverpa zea* (Boddie) Fed Artificial Diet Mixed with *Peumus boldus* Molina Powder

Concentration (%)	Larval size ± SE (mm)	Larval weight ± SE (g)	Pupation ± SE (%)	Pupal weight ± SE (g)	Larva-Pupa ± SE (LP)[1] DDI[3]	Pupa-adult ± SE (PA)[2] DDI[3]	Adult emergence ± SE (%)
0 (check)	47.6 ± 1.3a	0.73 ± 0.007a	100 ± 0.0a	0.42 ± 0.027 a	13.6 ± 0.7a	21 ± 0.0a	66.6 ± 8.3a
0.25	41.9 ± 1.2a	0.63 ± 0.078a	66.6 ± 0.0ab	0.38 ± 0.004 a	13.6 ± 0.7a	22 ± 0.66b	52.7 ± 13.8ab
0.5	38.3 ± 2.7a	0.6 ± 0.205ab	61.1 ± 20.0abc	0.37 ± 0.013 a	14.0 ± 0.7a	25 ± 0.0b	25.0 ± 0.0bc
1.0	37.9 ± 3.9ab	0.5 ± 0.021ab	58.3 ± 8.3 abc	0.37 ± 0.010 a	16.0 ± 1.0a		0.0 ± 0.0c
2.0	26.8 ± 1.1bc	0.26 ± 0.127ab	44.4 ± 22.2abc	0.28 ± 0.096 b	19.0 ± 2.0a		0.0 ± 0.0c
4.0	18.5 ± 2.0cd	0.25 ± 0.115ab	0.0 ± 0.0c				
8.0	12.5 ± 0.4 d	0.08 ± 0.046b	0.0 ± 0.0c				

Within a column, values with the same letter are not significantly different (Tukey, $p \leq 0.05$).

[1] LP = Time lapse (days) in which 50% of the larvae reached pupal stage.

[2] PA2 = Time lapse (days) in which 40% of the pupae reached adult stage.

[3] DDI = Days after infestation.

by neonates (L1) were ≤0.5% (Table 4). Consumption of the 8% boldo concentration diet, 0.03 g, was significantly less (F = 9.53, df = 6,20; P = 0.0004) than the other treatments including the nontreated check, which was 0.05 to 0.18 g.

Table 4. Presence of *Spodoptera frugiperda* and *Helicoverpa zea* Neonates and Consumption of Insect Artificial Diet Mixed with Different Concentrations of *Peumus boldus* Molina Powder

Insect	Concentration (%)	% consumption by larvae ± SE		Diet intake* (g)
		24 hours	48-120 hours	
Spodoptera frugiperda	0 (check)	49.9 ± 25.0 a	41.6 ±16.6a	0.18 ± 0.004a
	0.25	16.6 ± 16.6ab	41.6 ± 22.0a	0.07 ± 0.01a
	0.5	16.6 ± 8.3ab	16.6 ± 8.3ab	0.06 ± 0.01a
	1.0	16.6 ± 8.3ab	0.0 ± 0.0b	0.05 ± 0.01a
	2.0	0.0 ± 0.0b	0.0 ± 0.0b	0.05 ± 0.01a
	4.0	0.0 ± 0.0b	0.0 ± 0.0b	0.05 ± 0.02a
	8.0	0.0 ± 0.0 b	0.0 ± 0.0b	0.03 ± 0.006b
Helicoverpa zea	Check	44.4 ± 22.2a	55.5 ± 22.2a	0.22 ± 0.07a
	0.25	55.5 ± 22.2a	44.4 ± 22.2a	0.09 ± 0.015b
	0.5	0.0 ± 0.0b	0.0 ± 0.0b	0.07 ± 0.012bc
	1.0	0.0 ± 0.0b	0.0 ± 0.0b	0.06 ± 0.028bc
	2.0	0.0 ± 0.0b	0.0 ± 0.0b	0.017 ± 0.009bc
	4.0	0.0 ± 0.0b	0.0 ± 0.0b	0.015 ± 0.005bc
	8.0	0.0 ± 0.0b	0.0 ± 0.0b	0.007 ± 0.007c

Within a column, values with the same letter are not significantly different (Tukey, $p \leq 0.05$).

*Diet intake was measured by difference between dry weight before and after consumption by larvae.

For third instars (L3), the antifeedant index (AFI) of 8% boldo concentration was significantly greater (47.35%, F = 3.69, df = 5,23; P = 0.0141) than the other concentrations whose AFI fluctuated between 15 and 40% (Table 5). The lowest deterrence index (DI) was observed with the check diet (F = 3.95, df = 5,23; P = 0.0106), which was significantly less than the other concentrations containing boldo that ranged from 51.3 to 65.5%.

No-choice Tests. There were significant statistical differences in the relative consumption rate (RCR) between the nontreated check and concentrations of boldo ≥2.0% (F = 7.67; df = 6, 27; P = 0.0001), which ranged from 2.9 to 1.7 g g^{-1} d^{-1} (diet consumed (g)/(initial larva weight (g) * feeding period (d)) (Table 6). The greatest AFI (65.2%) was observed with the 8% concentration of boldo, but this was not significantly different from that with the 1 or 4% concentration of boldo. Growth inhibition index (GII) was significantly affected by the concentration of boldo. This effect was dose dependent because this parameter increased progressively with greater concentrations of boldo. The greatest GII (67.8%) was reached at 8% boldo which was not significantly different from 4% but was greater than 0.25 to 2% of boldo (F = 6.76; df = 5,23; P = 0.0008). Relative growth rate (RGR) was also dose dependent ($F = 9.73$; df = 6,27; $P < 0.0001$) with the check having a RGR of 1.7 and the 8% diet 0.37. The efficiency of conversion of ingested food (ECI) fluctuated between 22 and 37%, with no statistical differences among treatments.

Corn Earworm. Choice Tests. For neonates (L1), at 24 and 48 hours, there were no statistical differences in preferences between the check and 0.25% concentration, but all other diets were significantly less preferred (Table 4). Diet intake was significantly greater for the nontreated check than any other treatment, with a value of 0.22 g ($F = 17.99$; df = 6,20; $P < 0.0001$).

For third instars (L3), the greatest antifeeding index (AFI) and deterrence index (DI) were observed with 2.0 to 8.0% concentrations of boldo, ranging from 46.0 to 81.9% for AFI and from 62.5 to 87.9% for DI (Table 5). Between 4 and 8% concentrations of boldo, there were no statistical differences; however, from 0.25 to 1%, the values were significantly different from the 8% diet for AFI ($F = 4.28$; df = 8,23; $P = 0.0128$) and DI ($F = 5.12$; df = 8,23; $P = 0.0062$) .

No-Choice Tests. There were no significant differences in relative consumption rate (RCR) between treatments, and the range was between 1.37 and 3.57 g g^{-1} d^{-1} (Table 6). The greatest AFI (60.6%) was observed with the 8% concentration of boldo, but it was not significantly different from the 2 or 4% concentration. There were no statistical differences in growth inhibition index (GII) among treatments even when the values fluctuated between 22.1 and 56.4%. Relative growth rate (RGR) values were not statistically different among the boldo treatments, but treatments containing boldo were significantly different from the

Table 5. Antifeedant Index (AFI) and Deterrence Index (DI) of Diet with Different Concentrations of *Peumus boldus* Molina Powder Incorporated into Insect Artificial Diet against Third-instar Larvae of *Spodoptera frugiperda* (J. E. Smith) and *Helicoverpa zea* (Boddie)

Insect	Concentration (%)	AFI mean ± SE (%)	DI mean ± SE (%)
S. frugiperda	Check	--	--
	0.25	15.0 ± 4.1a	25.8 ± 6.5b
	0.5	35.3 ± 6.1a	51.3 ± 6.7a
	1.0	36.7 ± 3.5a	53.4 ± 3.7a
	2.0	39.6 ± 8.8a	55.0 ± 4.3a
	4.0	40.3 ± 3.7a	57.1 ± 3.7a
	8.0	47.3 ± 6.1b	65.5 ± 5.9a
H. zea	Check	--	--
	0.25	28.9 ± 0.8b	44.9 ± 0.9b
	0.5	29.7 ± 0.7b	45.8 ± 0.8b
	1.0	33.3 ± 4.3b	49.6 ± 4.5b
	2.0	46.0 ± 4.8ab	62.5 ± 4.6ab
	4.0	75.5 ±28.6ab	78.4 ± 15.7ab
	8.0	81.9 ± 14.2a	87.9 ± 9.2a

Within a column, values with the same letter are not significantly different (Tukey, $p \leq 0.05$).

Antifeedant index (AFI) AFI = [(intake of nontreated feed - intake of treated feed)/ (intake of nontreated feed + intake of treated feed)] *100.

Deterrence index (DI) DI = [(intake of nontreated feed - intake of treated feed)/ intake of nontreated feed)] *100.

Table 6. Relative Consumption Rate (RCR), Antifeedant Index (AFI), Growth Inhibition Index (GII), Relative Growth Rate (RGR), and Efficiency of Conversion of Ingested Good (ECI) by Third-instar Larvae of *Spodoptera frugiperda* (J. E. Smith) and *Helicoverpa zea* (Boddie) Exposed to Artificial Diet with Different Concentrations of *Peumus boldus* Molina Powder In which Larvae Fed In Only One Type of Boldo Concentration Diet

Insect	Concentration (%)	RCR mean ± SE $g\ g^{-1}\ d^{-1}$	AFI mean ± SE (%)	GII mean ± SE (%)	RGR mean ± SE $g\ g^{-1}\ d^{-1}$	ECI mean ± SE (%)
S. frugiperda	Check	4.7 ± 0.43a	-	-	1.7 ± 0.13a	37 ± 0.04a
	0.25	3.7 ± 0.29ab	21.8 ± 6.3 b	8.2 ± 8.8c	1.3 ± 0.14ab	35 ± 0.01a
	0.5	3.3 ± 0.69ab	22.8 ± 14.6b	19.9 ± 6.6bc	1.1 ± 0.10abc	33 ± 0.05a
	1.0	3.3 ± 0.68ab	29.8 ±14.2ab	24.9 ± 9.7bc	1.0 ± 0.15bc	32 ± 0.05a
	2.0	2.9 ± 0.18bc	42.5 ± 11.2ab	30.8 ± 7.8bc	0.9 ± 0.12bc	33 ± 0.04a
	4.0	2.7 ± 0.39bc	49.5 ± 1.8ab	48.5 ± 11.4ab	0.6 ± 0.18cd	27 ± 0.08a
	8.0	1.6 ± 0.14c	65.1 ± 2.9a	67.8 ± 4.47 a	0.3 ± 0.07d	22 ± 0.04a
H. zea	Check	3.5 ± 0.61a	-	-	1.9 ± 0.17a	88 ±0.60a
	0.25	3.0 ± 0.11a	13.1 ± 3.3c	22.1 ± 7.7a	1.0 ± 0.12b	83 ± 0.28a
	0.5	2.6 ± 0.97a	17.6 ± 8.6bc	38.2 ± 0.97a	0.8 ± 0.015b	49 ± 0.23a
	1.0	2.2 ± 0.44a	44.8 ± 4.2ab	41.3 ± 11.4a	0.7 ± 0.18b	42 ± 0.08a
	2.0	1.5 ± 0.08a	46.7 ± 2.5a	43.2 ± 8.9a	0.7 ± 0.14b	41 ± 0.09a
	4.0	1.6 ± 0.28a	51.8 ± 8.1a	50.8 ± 11.3a	0.6 ± 0.17b	37 ± 0.07a
	8.0	1.3 ± 0.34a	60.6 ± 9.7a	56.4 ± 9.9a	0.5 ± 0.15b	36 ± 0.05a

Within a column, values with the same letter are not significantly different (Tukey, $p \leq 0.05$).

$g\ g^{-1}\ d^{-1}$ = diet consumed (g)/(initial larva weight (g) * feeding period (days)).

nontreated check ($F = 2.57$; df = 9,18; $P < 0.05$), which had a value of 1.96 g g^{-1} d^{-1}. The efficiency of conversion of ingested food (ECI) fluctuated between 36 and 88%, but there were no significant differences among any treatments.

Discussion

Boldo Powder Toxicity. Results indicated that toxicity against fall armyworm and corn earworm is dependent on concentration of boldo powder (Table 1). These trends agree with Zapata et al. (2006) who found boldo powder at a concentration of 4% caused 80% mortality of *S. littoralis* Boisduval larvae. Mortality was affected by plant concentration and insect species. Based on LC_{50} values, fall armyworm was less sensitive to boldo than was corn earworm, but at LC_{90} both were equally sensitive, given that the fiducial limits at 95% overlapped.

Effect of Boldo Powder on Insect Development. *Fall Armyworm.* A similar trend of larval weight reduction was found using similar concentrations for *S. littoralis* (Zapata et al. 2006). The number of larvae that pupated showed an inverse relationship to the concentration of powder in the diet, while pupal weight and time to reach pupation showed no significant differences (Table 2). This trend is similar to that found with extracts of several plants of the genus *Trichilia* used against fall armyworm (Roel et al. 2000, Bogorni and Vendramim 2005, Roel and Vendramim 2006).

Corn Earworm. Results (Table 3) are similar to those of Barnby and Klocke (1987) and Grzywacz et al. (2005) who evaluated different concentrations of azadirachtin against tobacco budworm, *Heliothis virescens* (Fabricius). They found that as the concentration in the diet increased, the number of pupae and adults decreased, as did their weights, while the time to adulthood increased.

Tests of Feed Election. *Fall Armyworm. Choice Tests.* A neonate (L1) during the first 24 hours is adapting to the new environment and searches more widely (Gore et al. 2005). After 48 hours in our tests, the neonates adapted to the environment and ceased movement to remain feeding on the nontreated check and the 0.25 and 0.5% concentrations, possibly indicating a repellant effect of boldo because the larvae preferred to feed on diet with lower concentrations of boldo. But the difference between initial and final weight indicated only a small difference in diet consumption between evaluations.

For third instars (L3), both antifeedant index (AFI) and deterrence index (DI) showed decreasing trends in larval feeding with increasing concentrations of boldo (Table 5). AFI was less than that documented by Zapata (2005), who recorded values of 96.2% for *S. littoralis* larvae, while the DI of our study (57%) was greater than that obtained by Zapata et al. (2005) with the 4% concentration of boldo (14.7%). The AFI of our study was also less than that obtained with other plant species, such as those of the Aristolochiaceae family (Raffa and Frazier 1988), *A. indica* (Bomford and Isman 1996), and *Adhatoda vasica* Nees against *S. littoralis* (Sadek 2003). The DI value was slightly greater than that found by Raffa and Frazier (1988) who, with a 1% concentration, obtained 51.8% feeding deterrence of fall armyworm.

No-choice Tests. The RCR values (Table 6) showed that the larvae fed mainly on the nontreated diet, indicating that the greater the concentration of boldo powder, the less the consumption by larvae. Similar results were found with boldo in *S. littoralis* larvae (Zapata 2005). This trend was also found with other species such as *Reynoutria* sp. (Pavela et al. 2008), *A. vasica* (Sadek 2003), and ursinic acid isolated from lichens (Emmerich et al. 1993). Therefore, a lower intake of diet is caused by feeding inhibition (AFI), which results in less growth (GII), and less weight of the larvae (RGR). Zapata (2005) obtained the same results with *S. littoralis* larvae fed powder of other native plants from Chile, such as *Cestrum parqui* L'Héritier and *Drimys wintery* J.R. Forster & G. Forster. The inverse relationship between ECI and powder concentration with no significant differences agreed with Shea and Romeo (1991) and Emmerich et al. (1993) for non-protein amino acids from *Calliandra* spp. against fall armyworm and ursinic acid against *S. littoralis*, respectively. However, Sadek (2003) and Pavela et al. (2008) obtained the same decreased trend, but significantly different from the nontreated check.

Corn Earworm. Choice Tests. The preference test (Table 4) showed that the diet mixed with different concentrations of boldo powder repelled neonate (L1) corn earworm larvae. Unlike fall armyworm larvae, corn earworm did not exhibit a period of initial adaptation because, from the start until 120 hours, they remained on the nontreated check and the 0.25% boldo powder diet. This behavior explains the greater amount of feeding by the larvae on the check diet and on that which contained the least concentration of boldo powder.

For third instars (L3), the deterrence (DI) and inhibition (AFI) indexes were >80%, indicating that the corn earworm larvae fed mainly on the nontreated diet. As the concentration of boldo increased, it was less attractive as food for the larvae.

No-choice Tests. Diet intake (RCR) decreased with higher concentrations of boldo powder, producing less growth (GII) and weight (RGR); thus, feeding inhibition (AFI) and concentration of boldo in the diet were directly correlated. The efficiency of conversion of ingested food index (ECI), even though there were no significant differences, decreased as the amount of powder increased in the diet. This implied that the increase in boldo powder in the diet did not significantly influence the percentage in which it was used by the larvae to produce new biomass. This differs from Shea and Romeo (1991) who indicated that the greater the concentration of botanical insecticide, the less the amount of ingested food was transformed into biomass. This can explain the decrease in weight and size of corn earworm larvae.

Our results suggested that *P. boldus* might be useful for control of fall armyworm and corn earworm larvae because of its toxic action, effect as a growth regulator or feeding inhibitor, as well as its repellent properties at greater concentrations. By deterring newly emerged larvae from feeding, development was affected and should decrease the impact of the pest on the crop. However, these results should be validated in field tests to determine the potential of boldo as a crop protectant.

Acknowledgment

The authors thank Lauro Hernández Pérez of the Laboratory of Insecticide Toxicology and M. S. Jorge Valdez Carrasco of the Laboratory of Insect Morphology of the Colegio de Postgraduados for his technical support.

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