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Insecticide effect of wild plant powders on bean weevil (*Zabrotes subfasciatus* Boheman; Coleoptera: Bruchidae) *in vitro*

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The loss of stored grain is one of the most common problems faced by small farmers. The major pest of stored bean is the bean weevil (*Zabrotes subfasciatus*) as it can lead to total loss if left uncontrolled. The insecticide effect of 13 plant powders obtained from 11 different plant species was evaluated under laboratory conditions. The effect was assessed in terms of mortality, emergence of first-generation insects, percentage of damaged grain, ovicide effect, repellent, residual effect, and seed germination. A fully randomized experimental design with 52 treatments derived from a 13 × 4 factorial design (13 plant powders by four doses: 0.0, 0.1, 0.5 and 1.0%) was used. Results from residual effect and germination tests were analyzed with repeated-measures analyses. The highest percent of mortality was obtained with 0.1, 0.5 and 1.0% of *Chrysactinia mexicana,* and 0.5 and 1.0% of *Gliricidia sepium* powders. The lowest adult emergence was obtained with leaf powder of *C. mexicana, G. sepium, Guazuma ulmifolia, Dyssodia pentachaeta, Dyssodia acerosa, Heterotheca inuloides* and *Zinnia acerosa,* as well as with fruit powder of *Crescentia alata* and *Melia azedarach.* Setting 20% as the threshold for maximum grain damage, the *C. mexicana* leaf powder reduced damage when administered at 0.5 and 1.0%. *C. mexicana, D. acerosa, D. pentachaeta, G. ulmifolia* (fruit), *H. inuloides, L. tridentata, D. stramonium* and *Z. acerosa* powders had a significant repellent effect on *Z. subfasciatus*. The toxic effect of *C. mexicana* and *G. sepium* lasted 4 weeks. Grain germination was only reduced by *G. sepium*. Finally, 1.0% of *C. mexicana* led to the highest mortality of *Z. subfasciatus* eggs and the lowest percentage of damaged grain.

Key words: Plant insecticides, weevil, bean, *Zabrotes subfasciatus*, stored grain.

INTRODUCTION

The common bean (*Phaseolus vulgaris*) is grown and consumed in most developing countries, where it constitutes a major protein source (Schmale et al., 2006; Cardona, 2007). However, a sizeable amount of stored grain is often lost by pest infestation. In Mexico, the bruchid *Zabrotes subfasciatus* Boheman (bean weevil) is one of the most common bean pests (Schoonhoven et al., 1983; López-Pérez et al., 2007). *Z. subfasciatus*

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grows exponentially on untreated grain and can account for up to 100% damage (Hall et al., 1997). Bean weevil populations can be significantly reduced by chemical pesticides (Daoust et al., 1985); however, continuous indiscriminate use of these chemicals might lead to harmful side effects on human health as some have potential carcinogenic or teratogenic effects, cause sterility, affect the nervous system leading to memory loss (Castillo et al., 2002), and may cause death by acute or chronic poisoning. They also negatively affect the environment, as they build up along the food chain and in soil and water bodies (Waterhouse et al., 1996). Pesticide components are also responsible for the development of pesticide resistance in insects (Bourguet et al., 2000), and also destroy beneficial parasites, predators and pollinators that are natural components of ecosystems (Freemark and Boutin, 1995). The use of natural insecticides could be an alternative for preventing the excessive accumulation of toxic residues in the environment (Macedo et al., 2002; Singh et al., 1985). Plant insecticides provide potential benefits such as being harmless to both farmers and consumers, and are also environmentally innocuous (Lagunes, 1994). Therefore, over the past years, it has been a growing interest in using plants as a source of pesticides that are safer for human health and the environment (Ottaway, 2001). Such products are easy to obtain and are prepared at low cost. In this work, we aimed to evaluate the insecticide effect of leaf powders of *Chrysactinia mexicana* A. Gray*, Zinnia acerosa* (DC.) A. Gray*, Datura stramonium* L.*, Dyssodia pentachaeta* (DC.) B.L. Robins, *D. acerosa* DC., *Heterotheca inuloides* ssp. *rosei* B. Wagenkn*, Gliricidia sepium* (Jacq.) Walp. and *Larrea tridentata* (DC.) Cov., as well as leave and fruit powders of *Guazuma ulmifolia* Lam. and *Melia azedarach* L., and fruit powder of *Crescentia alata* Kunth*.* Their effect was assessed in terms of mortality, emergence of first-generation insects, percent of damaged grain, grain germination, ovicide effect and repellence index on the bean weevil *Z. subfasciatus*, under controlled conditions.

MATERIALS AND METHODS

Stored grain

We used beans of the "Flor de mayo" commercial variety, produced at the Agronomy Faculty, Universidad Autónoma de San Luis Potosí. The grain that had 16% moisture content, was washed and cleaned off impurities, and kept at -4°C for 48 h to eliminate other insects that could interfere with the experiments' results.

Insects

Experimental insects were bred from a sample obtained from infested stored grain supplied by a local producer. Weevils were put into glass jars containing 1 kg of bean grain, covered with cloth mesh and kept in a room at 22.5 ± 2.5°C, 62.5 ± 2.5% r.h. and under 12/12 h photoperiod. Emerged insects were then used to infest additional grain until a sufficiently large population was obtained. For bioassays, the sex of adults was determined following the criteria by Decelle (1951): males are greyish in colour and smaller (2 mm length) than females, which are black with cream-coloured spots on the elytra, and measure 3 mm in length.

Plants

Flowering or fruiting specimens of 11 different plant species growing in natural habitats were collected at various locations and environments in the San Luis Potosí and Querétaro States, Mexico (Table 1). The taxonomic identity of each species was confirmed by Mr. José García, a taxonomist from the "Isidro Palacios" Herbarium (SLPM) at the Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí. One specimen of each species was deposited in the SLPM herbarium. Plant materials (foliage and fruits) were air-dried, protected from direct sunlight, and then stored at 4°C until processed. Dried material was ground in a standard blender (Osterizer ®, model L-83 Pulse-matic, Niles ILL, USA) to obtain a fine powder that could pass through a #40 mesh sieve.

Bioassays

Percent of mortality, F1 emergence and damaged grain

One hundred grams (100 g) of Flor de Mayo beans were placed into a 250-ml glass jar and the corresponding plant powder treatment was added. The mixture was thoroughly mixed and 10 adult couples of *Z. subfasciatus* younger than 24 h old were added; jars were capped with fabric mesh (Lagunes and Rodríguez, 1989). To estimate the treatments' toxicity, mortality was evaluated after 6 days of infestation by counting the number of living and dead insects in each treatment replicate. Insect mortality due to causes other than the plant powder was corrected for using Abbott's equation (Abbott, 1925):

$$CPM = ((X - Y)/(100 - Y))*100$$

Where: CPM = Corrected percent of mortality, X = percent of mortality in the treatment, and Y = percent mortality in the control treatment. According to Lagunes (1994), plant powders can be considered as promising insecticides when they cause a corrected percent of mortality ≥ 40. Emergence of first-generation (F1) insects and the proportion of damaged grain were determined after 50 days in the same samples used to assess percent of mortality. Percentage of emergence was estimated as:

$$PE = (X/Y)*100$$

Where X = Number of insects that emerged in the treatment, and Y = number of insects that emerged in the control treatment. Treatments where first-generation emergence was ≤ 50% were considered as promising (Lagunes, 1994).

To estimate the percentage of damaged grain, the numbers of healthy and damaged grains were counted and Adams and Schulten's formula was applied (Silva et al., 2003):

$$PDG = 100(NDG / TNG)$$

Where PDG = Percent of damaged grain, NDG = number of damaged grains, and TNG = total number of grains.

Since no standard guidelines to assess the significance of economic loss caused by grain damage was available, 20% threshold was set as the minimum grain damage to be prevented by the effect of plant powder. Statistical data from a previous study

Table 1. Plant species evaluated.

Family	Scientific name	Common name	Used part
Asteraceae	*C. mexicana* A. Gray	Damianita	L
Asteraceae	*D. acerosa* DC.	Pricklyleaf dogweed	L
Asteraceae	*D. pentachaeta* DC.	Hartweg's pricklyleaf	L
Asteraceae	*H. inuloides* ssp. *rosei* B. Wagenkn	Árnica	L
Asteraceae	*Z. acerosa* (DC.) A. Gray	Desert zinnia	L
Bignoniaceae	*C. alata* Kunth	Cuatecomate, jícaro	F
Leguminosae	*G. sepium (Jacq.) Kunth*	Quickstick	L
Meliaceae	*M. azedarach* L.	Chinaberrytree	L, F
Solanaceae	*D. stramonium* L.	Jimsonweed	L
Sterculiaceae	*G. ulmifolia* Lam.	Bastardcedar	L, F
Zygophylaceae	*L. tridentata* (DC.) Cov.	Creosote bush	L

Used part: L = leaves, F = fruits.

carried out in Veracruz State revealed that infestation by *Z. subfasciatus* caused grain damage ranging from 20% up to total harvest loss (Bonet et al., 2005).

Ovicide effect

Ovicide effect was evaluated by placing 100 g of bean grain together with 10 insect couples within 250 ml glass jars. Insects were left to mate for 10 days and then all insects were removed. Afterwards, the corresponding plant powder treatment was added to the jar and thoroughly mixed. Larvae emergence after 50 days was evaluated (Silva-Aguayo et al., 2005).

Repellent index

The repellent potential was assessed by means of the Mazzonetto and Tavares' method (Silva-Aguayo et al., 2005). Five circular plastic boxes measuring 5 cm in diameter and 1.5 cm in height were used; the central box was connected to the other four by means of 10 cm long plastic tubing arranged diagonally. Doses of the plant powder to be tested were placed in the outer boxes, together with 30 g of bean grain. Fifty 24 h old insects were released in the central box, and after 24 h, the number of insects within each lateral box was recorded. To further increase the reliability of results, the Mazzonetto's formula (Silva-Aguayo et al., 2005) was applied:

$$RI = 2G / (2G + P)$$

Where, RI = Repellent index, G = percentage of insects in the plant powder treatment and P = percentage of insects in the control treatment. A plant powder is considered as neutral when RI = 1, attractant when RI > 1 and repellent when RI < 1.

Residual effect of plant powder

These three effects were assessed only for treatments that caused at least 40% mortality (0.1, 0.5 and 1.0% of *C. mexicana,* and 0.5 and 1.0% of *G. sepium*). To evaluate the residual effect, the same model used for assessing mortality was used; bean grains were mixed with plant powder within 250 ml jars and 10 insect couples were added. After 24 h, all insects were removed from the jar and the number of living and dead insects was counted; then, the original plant powder-grain mixture was thoroughly mixed and 10 new weevil couples were added. This procedure was repeated on days 7, 13, 19, 25, 31 and 37 (Silva-Aguayo et al., 2004).

Grain germination

Grain germination was evaluated to determine whether plant powders affected seed viability. Thirty (30) seeds previously subjected to each treatment were placed in a Petri dish fitted with wet filter paper and then kept under controlled conditions (12/12 photoperiod, 26 ± 2°C). The number of germinated seeds was counted daily for 7 days (Silva et al., 2003; Araya and Emana, 2009).

Statistical analyses

Mortality, emergence and damaged grain figures were analyzed according to a fully random experimental design with 52 treatments resulting from a factorial arrangement of 13 different plant powders (that is, leaves of *C. mexicana, Dyssodia acerosa, D. pentachaeta, H. inuloides* ssp. *rosei, Z. acerosa, D. stramonium, L. tridentata* and *G. sepium,* fruits of *C. alata,* and leaves and fruits of *M. azedarach* and *G. ulmifolia*, and four different doses (0.0, 0.1, 0.5 and 1.0%), using the "MIXED" SAS (1999) procedure. Germination and residual-effect data were analyzed as a repeated-measures (days 1 to 6 and every 6 days, respectively) in a fully randomized experimental design and six treatments (0.0, 0.1, 0.5 and 1.0% of *C. mexicana*, and 0.5 and 1.0% of *G. sepium*), using the "MIXED" SAS (1999) procedure, and five jars per treatment, with each jar considered as an experimental unit. Averages of the main factors and their interactions were analyzed with the LSMEANS SAS (1999) routine. Each experimental procedure was repeated three times (4 weeks period between runs).

RESULTS

Mortality, adult emergence of *Z. subfasciatus,* and percent of damaged grain

All main effects and associated interactions for mortality, emergence of *Z. subfasciatus* adults and damage grains

Table 2. Percentage of mortality and emergence of *Z. subfasciatus,* and damaged grain.

Species	Mortality (%)			Emergence (%)			Damaged grain (%)		
	Doses (%)			Doses (%)			Doses (%)		
	0.1	0.5	1.0	0.1	0.5	1.0	0.1	0.5	1.0
C. alata (F.)	14.0	17.5	20.5	39.0	40.0	32.0	56.5.	50.0	50.0
C. mexicana (L.)	49.5[c]	66.0[b]	84.0[a]	20.0	12.0	6.0	30.0	14.0	8.0
D. stramonium (L.)	17.0	25.0	30.0	95.5[a]	98.0[a]	99.0[a]	100[a]	100[a]	100[a]
D. acerosa (L.)	13.0	13.0	15.0	39.0	23.0	21.0	55.5	38.0	26.0
D. pentachaeta (L.)	20.0	20.0	22.0	42.0	38.0	35.0	59.0	50.5	49.0
G. sepium (L.)	35.0	48.0[c]	56.0[b]	16.0	15.0	15.0	25.0	25.0	22.0
G. ulmifolia (L.)	18.0	20.0	20.0	27.0	40.0	45.0	41.0	53.0	62.0
G. ulmifolia (F.)	25.0	38.0	38.0	22.0	21.0	16.5	42.5	30.0	26.0
H. inuloides (L.)	16.0	16.0	20.0	31.0	38.0	38.0	66.0	55.0	55.0
L. tridentata (L.)	20.0	26.0	31.0	28.0	23.0	18.0	41.0	40.0	28.0
M. azedarach (L.)	20.0	27.0	29.0	70.5[b]	50.0	31.5	79.0[b]	70.0[b]	47.0
M. azedarach (F.)	19.0	27.0	27.0	39.0	38.0	30.0	62.0	60.0	55.0
Z. acerosa (L.)	8.0	11.0	11.0	46.0	48.0	50.0	72.0[b]	66.0	58.0
Control	2.2	2.1	2.4	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]
SE	10.5	10.8	10.4	9.3	10.2	10.7	10.1	10.9	10.5
P-value	P***, D***, P×D **			P***, D**, P×D **			P***, D***, P×D **		

F = fruits, L = leaves. Treatments with different letter by column and row are statistically different ($P < 0.05$); P = Plant, D = Doses, P×D = interaction, ***$P < 0.0001$, **$P < 0.001$; *SE* = Standard error of the mean.

Table 3. Ovicide effect and damaged grain decreased due promising treatments.

Species	Emergence F1 (%)				Damage grain (%)			
	Doses (%)				Doses (%)			
	0.0	0.1	0.5	1.0	0.0	0.1	0.5	1.0
C. mexicana	100[a]	22.3[b]	13.5[c]	6.9	100[a]	27.8[b]	16.3[c]	9.4
G. sepium	100[a]	*	15.0[c]	14.3[c]	100[a]	*	24.8[b]	26.2[b]
SE	2.05	2.05	2.05	2.05	3.71	3.71	3.71	3.71
P-value	P=0.05, D***, P×D[NS]				P**, D*, P×D[NS]			

*Treatments excluded because they were not promising. Treatments with different letter by column are statistically different, $P < 0.05$, P = Plant, D = Doses, P×D = interaction, ***$P < 0.0001$, **$P < 0.001$, *$P < 0.01$, [NS] Non-significant. *SE* = Standard error of the mean.

were significant at $P < 0.05$. Mortality, F1 emergence and damaged-grain data are shown in Table 2. Powders were considered prominent when they caused a mortality superior to 40%. The highest percentages of dead insects were recorded with 0.1, 0.5 and 1.0% of *C. Mexicana* (49.5, 66.0 and 84%, respectively), and 0.5 and 1.0% of *G. sepium* treatments (48.0 and 56.0%, respectively). The highest F1 emergence percentages were observed with 0.1% of *M. azedarach* (leaves), and 0.1, 0.5 and 1.0% of *D. stramonium* treatments (95.5, 98.0 and 99.0%, respectively); emergence was lower than 50% in the remaining treatments. The lowest percentages of damaged grain were recorded with 0.1, 0.5 and 1.0% of *C. mexicana* (30.0, 14.0 and 8.0%, respectively) and 0.5 and 1.0% of *G. sepium* treatments (25.0, and 22.0%, respectively).

Ovicide effect

All main effects for ovicide effect were significant at $P < 0.05$. Results for the ovicide and grain-protection effects are shown in Table 3. *C. mexicana* and *G. sepium* powders led to an emergence lower than 25%, although the lowest F1 emergence and damaged-grain values were recorded with the 1.0% of *C. mexicana* treatment.

Repellent index

Results from the repellent experiment are shown in Table 4. Treatments with a stronger repellent effect against *Z. subfasciatus* were those with leaf powder of *C. mexicana, D. acerosa, L. tridentata, M. azedarach, Z. acerosa* and

Table 4. Repellent index (RI) at 1.0% dose.

Repellent	RI	Neutral	RI	Attractive	RI
C. mexicana†	0.02	*H. inuloides*†	0.97	*G. ulmifolia*♣	1.10
D. acerosa†	0.16	*D. pentachaeta* †	1.03	*M. azedarach*♣	1.18
L. tridentata†	0.52			*D. stramonium*†	1.30
M. azedarach†	0.54			*G. sepium* †	1.34
Z. acerosa†	0.61			*C. alata*♣	1.43
G. ulmifolia†	0.95				

†Leaves; ♣fruits.

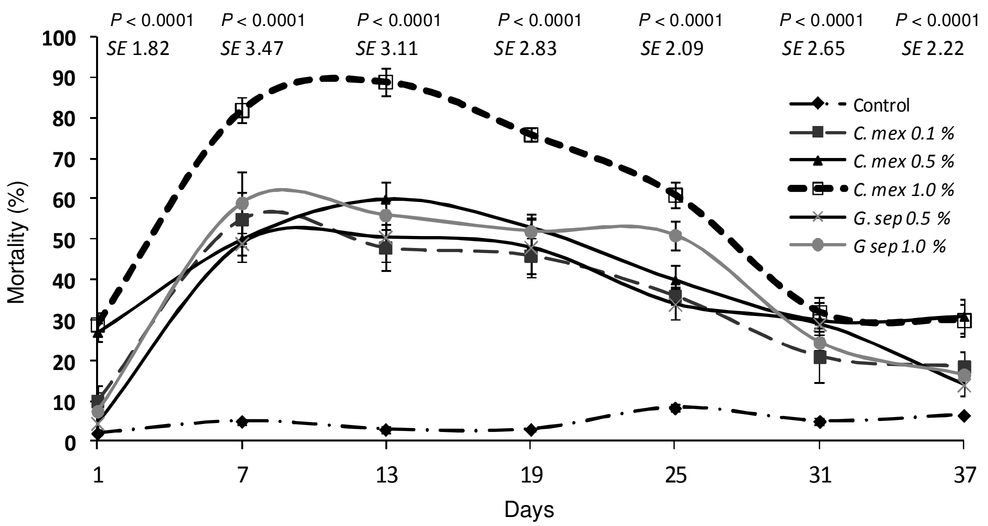

Figure 1. Residual effect of *C. mexicana* and *G. sepium* powders over mortality of *Z. subfasciatus*. *SE* = Standard error of the mean.

G. ulmifolia at 0.1, 0.5 and 1.0%. On the other hand, leaf powders of *D. stramonium* and *G. sepium* as well as fruit powders of *C. alata*, *M. azedarach* and *G. ulmifolia* were attractant to *Z. subfasciatus*. In all cases, the 0.1 and 0.5% doses were statistically identical to the 1.0% dose.

Residual effect

All main effects and associated interactions for residual effect over the mortality of *Z. subfasciatus* adults were significant at $P < 0.05$. The residual effect was evaluated only for *C. mexicana* and *G. sepium* powders, as these were the only treatments that led to a mortality of 40% in *Z. subfasciatus* (Figure 1). The highest effect was observed with 1.0% of *C. mexicana*. The insecticide effect of these treatments lasted for 4 weeks (25 d) and then it dropped under 40% of mortality.

Grain germination

All main effects and associated interactions for grain germination levels were significant at $P < 0.05$, except in days 1 and 2. Bean germination was negatively affected only by the 1% of *G. sepium* treatment (Table 5).

DISCUSSION

Using plants with insecticide properties is a well-established practice for controlling crop pests without causing environmental impacts such as contamination of water bodies, development of insecticide resistance and environmental release of toxic chemicals (Nascimento et al., 2008).

The obtained results in this work revealed that *C. mexicana* and *G. sepium* powders have an insecticide effect on *Z. subfasciatus*. It has been documented that *C. mexicana* is also effective against other weevil species, such as the maize weevil *Sitophilus zeamais,* where *C. mexicana* in the 1.0% concentration cause 98% of mortality (Juárez-Flores et al., 2010). Villaviscencio et al., (1995) analyzed the essential oil from *C. mexicana* and identified four different monoterpenes (quercetaget-3-glycoside, quercetaget-7-glycoside, 6-hydroxykaempferol

Table 5. Effect of *C. mexicana* and *G. sepium* powders over grain germination.

Time (days)	Germination (%)							*P*-value
	Control	*G. sepium*		*C. mexicana*			*SE*	
	0.0%	0.5%	1.0 %	0.1%	0.5%	1.0%		
1	0	0	0	0	0	0	0	-
2	2	0	0	0	0	0	0	P^{NS}, D^{NS}, $P \times D^{NS}$
3	100ª	60^c	0	50^d	100^a	75^b	6.99	P***, D^*, $P \times D^{NS}$
4	100ª	67^c	50^d	84^b	100ª	100ª	9.35	P***, D***, P×D**
5	100ª	75^b	72^b	100ª	100ª	100ª	11.20	P^*, D***, $P \times D^{NS}$
6	100ª	100ª	80^b	100ª	100ª	100ª	14.28	P***, D*, $P \times D^{NS}$

Treatments with different letter by row are statistically different, $P < 0.05$. P = Plant, D = Doses, P×D = interaction, ***$P < 0.0001$, **$P < 0.001$, *$P < 0.01$, NS Non-significant; *SE* = Standard error o-f the mean.

7-glycoside, 6-hydroxykaempferol 7-acetylglycoside, 6-hydroxykaempferol 7-glycoside) which caused an anti-food reaction on *S. zeamais*, likely related to the high mortality and the reduced F1 emergence observed in our experiments. To note, *C. mexicana* has also displayed fungicide effects on *Aspergullis flavus* due to the presence of piperitone (Cárdenas-Ortega et al., 2005). Lagunes (1994) found that *G. sepium* exerted an insecticide effect on *Prostephanus truncatus*, *Spodoptera frugiperda* and *Acanthoscelides obtectus,* probably due to the presence of estigmastanol glycoside and 3′,4′-dihydroxy-*trans*-cinnamic acid octacosylester (Herath and de Silva, 2000). Sabillón and Bustamante (1996) observed that *G. sepium* powder can control other pests: aphids (bark), *Culex* sp. (fruit) and rodents (whole plant). Sharma et al. (1998) found that dried leaves of *G. sepium* have a toxic effect against larvae of *Anopheles stephansi, Aedes aegypti* and *Culex quinquefasciatus*. The insecticide effect (low mortality, low emergence) observed in some treatments [*C. alata* (fruits), *D. acerosa, D. pentachaeta, G. ulmifolia* (leaves and fruits), *H. inuloides, L. tridentata, M. azedarach* (fruits) and *Z. acerosa*], might result from alterations in biological activity that inhibit or restrain breeding, or that partially or totally reduce female fertility (Silva et al., 2003). Lagunes (1994) tested leaf powder of *C. alata, D. stramonium*, *M. azedarach* and *H. inuloides* at 1.0%, but these treatments failed to produce observable toxic effects on *Z. subfasciatus,* which agrees with findings in the present investigation.

The repellent effect of *C. mexicana* on *Z. subfasciatus* is consistent with the results obtained in the mortality, emergence and damaged-grain experiments, in which powder of these species could have caused an anti-food effect by preventing the insect's contact with grain and hence preventing the grain from being infested by females. By contrast, the attractant effect of *D. stramonium* could be related to the higher infestation and lower mortality of adult insects observed with this species' powder Silva-Aguayo et al. (2005) investigated the effect of leaves and seeds of *D. stramonium* on *S. zeamais* and their findings are comparable with those obtained in our experiments, as they observed a low mortality (below 2%) and high emergence (77.7%).

The residual-effect bioassays, conducted only for treatments which caused > 40% mortality, revealed that the insecticide effect remained only until the 4th week. This is in agreement with the results obtained by Silva-Aguayo et al. (2005), which show that powder of three *Chenopodium* species had effect on the maize weevil *S. zeamais* for 30 days; the decrease in toxicity might be due to the degradation or volatilization of plant chemicals.

Only the *G. sepium* powder delayed germination of bean seeds. It is worth mentioning that, according to international standards, seeds treated with plant powder resulting in a 10% reduction of germination shall not be marketed as seed for planting (González, 1995).

Finally, regarding the ovicide effect, the scarce emergence observed might be mainly due to a high mortality of eggs and, probably, also of larvae of *Z. subfasciatus.*

Studies conducted with *C. mexicana* on the mortality of adults, larvae and eggs of other insect species are scarce. The insecticide properties of *C. mexicana* on different stages of other pest species deserve further investigation.

Conclusions

Our results show that plant powders of *C. mexicana* and *G. sepium* can reduce *Z. subfasciatus* populations in stored grain, as well as the emergence of the subsequent generation. Both materials have a protective effect on stored grain for up to 25 days. In addition, *C. mexicana* showed a repellent and ovicide effect without affecting seed germination.

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