MANAGEMENT OF THE MEXICAN BEAN WEEVIL BY ADDING AROMATIC PLANT DERIVATIVES IN TWO DRY FORMULATIONS †

[MANEJO DEL GORGOJO MEXICANO DEL FRIJOL MEDIANTE LA ADICIÓN DE DERIVADOS DE PLANTAS AROMÁTICAS EN DOS FORMULACIONES SECAS]

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SUMMARY

Background. *Zabrotes subfasciatus* (Boh., 1833) (Coleoptera: Chrysomelidae: Bruchinae), is considered one of the most important pest of stored beans. **Objective.** This study reports the possible toxicity and repellence of powders prepared from eight plant species against the Mexican bean weevil in two formulations (dry powder and sachets). **Methodology.** A 10 × 2 factorial design (10 species × 2 formulations) with 8 repetitions in a completely randomized design was employed. Pots with no powder were used as a negative control, and a pyrethroid insecticide [K-Obiol® 2 DP (deltamethrin, 0.5 g a.i. ton^{-1})] was applied as a positive control. **Results.** A mixture of powdered *Chenopodium ambrosioides* L., *Ruta graveolens* L. and *Mentha pulegium* L. added to bean grains was confirmed to be toxic to *Z. subfasciatus* adults with promising grain protector properties. *C. ambrosioides* powder had the same effect when in a sachet. A mixture of powdered *R. graveolens*, *M. pulegium* and *C. ambrosioides* with the beans inhibited weevil oviposition. The same effect was achieved for *M. pulegium* and *C. ambrosioides* in sachets. A mixture of powdered *C. ambrosioides*, *M. pulegium*, *R. officinalis* and *R. graveolens* repelled *Z. subfasciatus* adults from bean grains. **Implications.** This is the first report of using botanical derivatives by means of sachets or dry formulations, a pre-commercial purpose for aromatic plants with insecticidal/repellent activities. **Conclusions.** Sachets containing powdered *C. ambrosioides* and *M. pulegium* efficiently controlled the Mexican bean weevil in stored beans and constitute an useful tools for domestic grain stock or post-harvest management of organic grains.
Keywords: botanical insecticides; *Zabrotes subfasciatus*; *Phaseolus vulgaris*; *Chenopodium ambrosioides*; stored-grain integrated pest management.

RESUMEN

Antecedentes. *Zabrotes subfasciatus* (Boh., 1833) (Coleoptera: Chrysomelidae: Bruchinae), es una de las plagas más importantes de los frijoles almacenados. **Objetivo.** Este estudio informa la posible toxicidad y repelencia de polvos preparados a partir de ocho especies de plantas contra el gorgojo mexicano del frijol en dos diferentes formulaciones (polvo seco y bolsa pequeña). **Metodología.** Se empleó un diseño factorial de 10 × 2 (10 especies × 2 formulaciones) con 8 repeticiones en un diseño completamente al azar. Se utilizaron macetas sin polvo como control negativo, y se aplicó un insecticida piretroide [K-Obiol® 2 DP (deltametrina, 0.5 g i.a. ton^{-1})] como control positivo. **Resultados.** Se confirmó que una mezcla de polvo de *Chenopodium ambrosioides* L., de *Ruta graveolens* L. y de *Mentha pulegium* L. añadida a los granos de frijol fue tóxica para adultos *Z. subfasciatus* con promisorias propiedades protectoras de granos. El polvo de *C. ambrosioides* tuvo el mismo efecto cuando estaba en bolsa pequeña. Una mezcla de polvo de *R. graveolens*, de *M. pulegium* y de *C. ambrosioides* con los frijoles inhibió la oviposición del gorgojo. El mismo efecto se logró para *M. pulegium* y *C. ambrosioides* en bolsa pequeñas. Una mezcla de polvos de *C. ambrosioides*, de *M. pulegium*, de *R. officinalis* y de *R. graveolens*

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repelieron adultos de *Z. subfasciatus* de granos de frijol. **Implicaciones.** Este es el primer informe sobre el uso de derivados botánicos mediante bolsas o formulaciones secas, un propósito pre comercial para plantas aromáticas con actividades insecticidas/repelentes. **Conclusiones.** Las bolsas pequeñas que contenían polvo de *C. ambrosioides* y de *M. pulegium* controlaron eficientemente el gorgojo mexicano del frijol en frijoles almacenados y constituyen una herramienta útil para el almacenamiento doméstico de granos o el manejo poscosecha de los granos orgánicos.
Palabras clave: insecticidas botánicos; *Zabrotes subfasciatus*; *Phaseolus vulgaris*; *Chenopodium ambrosioides*; manejo integrado de plagas de granos almacenados.

INTRODUCTION

The Mexican bean weevil, *Zabrotes subfasciatus* (Boh., 1833) (Coleoptera: Chrysomelidae: Bruchinae), is a primary pest species of stored beans in South America (Lopes *et al*., 2016; Gonçalves *et al*., 2017; Luz *et al*., 2017). This insect is responsible for great losses in the grain/seed quality and quantity due to larvae feeding inside of the bean, reducing the grains nutritional value and seeds viability and vigor (Barbosa *et al*., 2000; Wong-Corral *et al*., 2013). The direct losses caused by this pest species were estimated to be 35% in Mexico, Central America, and Panama and between 7 and 15% in Brazil (Van Schoonhoven and Cardona, 1982).

Chemical control is the most commonly used management strategy in the warehouse in Brazil and elsewhere (Braga *et al*., 2011; Ribeiro *et al*., 2016; Luz *et al*., 2017); however, the increase in insecticide use leads to greater selection pressure and an increase in the number of populations that are resistant to the insecticidal active ingredients, such as pyrethroids and organophosphates (Perry *et al*., 2011), a scenario that has stimulated the study and development of alternative strategies (Ribeiro *et al*., 2013). Additionally, the practice of applying many chemicals near consumption often poses a risk to workers and consumers due to potentially toxic residues on the grains (Stejskal *et al*., 2015).

Pest management with botanical insecticides has attracted great social interest because they are generally safer and have greater selectivity than synthetic products (Isman, 2008; Isman, 2015; Ribeiro *et al*., 2016). Such products generally do not require highly qualified personnel, are inexpensive and have few environmental effects, and they can be produced at the same location as where the grains are stored (homemade preparations), making them easy to use (Ribeiro *et al*., 2014). Additionally, the difficulty of obtaining new synthetic molecules and the consequent high production costs have also stimulated studies with botanically derived insecticides (Ribeiro *et al*., 2013; Ribeiro *et al*., 2016; Singh, 2017).

Considering the economic importance of *Z. subfasciatus* as a key pest of common beans (*Phaseolus vulgaris* L., Fabaceae), the development of research on botanical insecticides that are compatible with integrated pest management (IPM) is timely and can provide tools to mitigate the undesirable effects of synthetic insecticides, mainly benefitting small farmers in under development countries. Some papers have been reported the bioactivity of powders of botanical origin for *Z. subfasciatus* management; however, to date, there are no studies describing the possible effects of these botanical derivatives using sachets or dry formulations – a pre-commercial purpose for aromatic plants with insecticidal/repellent activities.

Although the literature has increasingly reported research on botanical pesticides and other areas of biologically active natural product in the last years, few formulations have been elaborated and driven progress toward the development of commercial products (Isman and Greinesen, 2014). In light of this perspective, this study evaluated the repellent and insecticidal properties of powders prepared from parts of eight aromatic plants applied as two dry formulations (powder mixed with the bean grains or in a sachet) on the Mexican bean weevil management. Dry formulations prepared from insecticidal plants should be constituted a useful tool for insect pest management in domestic grain stock or post-harvest of organic grains.

MATERIALS AND METHODS

Test insects and plant material

The *Z. subfasciatus* used in the bioassays were obtained from colonies kept in a climate-controlled room [temperature 27 ± 2 °C, relative humidity (RH) 70 ± 10% and 12 L: 12 D h photoperiod]. Common bean cv. 'Bolinha' was used as feeding and oviposition substrates for *Z. subfasciatus* rearing. However, the cultivar IAC Pérola was used for the tests. Previously to their use in the bioassays, the beans were disinfested and hygroscopically equilibrated (~ 13% humidity) according to procedure described by Ribeiro *et al*. (2013)

The plant material were collected from Patrocínio, Minas Gerais State, Brazil (18º56'17.4''S 46º59'34.5''W), as described in Table 1. To prepare the dried formulations, the plant materials were dried (separately by plant species) in an oven MA035/1 model (Marconi Equipamentos para Laboratório Co., Piracicaba, SP, Brazil) at 40 °C for 48 to 72 hours and subsequently ground in a knife mill model 4 Wiley® (Thomas Scientific Inc., Philadelphia, PA, USA). The plant powder obtained was separately stored in sealed glass and kept refrigerated until use (approximately 5 days after collection). In addition, in the Table 1 is highlighted the yield for each plant species after processing.

Bioassays

Effects on biological parameters

For the evaluation of the effect of the plant powders on *Z. subfasciatus* biology, there were two types of pots: plastic containers (4 × 5 cm) with 10 g of beans mixed with 0.3 g of each plant powders (0.3%, w w^{-1}) or containers with 10 g of beans and 0.3 g of plant powder packed in a white, porous, polyester sachet (1.0 × 2.5 cm). The discriminatory concentration used was stablished based on previous studies (Mazzonetto and Vendramim, 2003; Procópio *et al*., 2003; Baldin *et al*., 2009).

A 10 × 2 factorial design (10 species × 2 formulations) with 8 repetitions in a completely randomized design was employed. Pots with no powder were used as a negative control, and a pyrethroid insecticide [K-Obiol® 2 DP (deltamethrin, 0.5 g a.i. ton^{-1})] was applied as a positive control. Due to its particular mode of action (contact), the pyrethroid was not used in the sachet trials.

The containers were infested with five newly emerged *Z. subfasciatus* couples (<24 h of age) and placed in another chamber under the previously described controlled conditions. Mortality was evaluated at five days after infestation. Twenty days after infestation, the total number of hatched and unhatched eggs per container was observed (Lara, 1997).

The oviposition preference index (OPI) was also calculated using the formula OPI = [(T-P/T+P)] x 100, where T is the total number of eggs in a treatment group and P is the number of eggs in the control group (Baldin *et al*., 2008; Baldin *et al*., 2009). This index varies from +100 (very stimulating) to zero (neutral) to –100 (totally deterrent). A treatment was classified by comparing the average of number of eggs in the treatment with the average in the negative control and with consideration of the standard error of the mean.

Beginning at thirty days after infestation, the pots were inspected daily to determine the number of emerged adults, the insect dry weight, the dry weight of the grain consumed and the cycle egg-adult of *Z. subfasciatus*. The newly emerged individuals were packed in glass vials and immediately stored at -4 ºC to rapidly interrupt their life cycle. At the end of emergence, the flasks were opened and placed in an oven at 50 ºC for 48 h to determine the dry weight of the emerged insects.

After the end of emergence, the grains were packed in paper bags and placed in oven at 50 ºC for 48 h. Bean consumption by the larvae was determined by comparing the grain weight of the infested pots with the grain weight of the not infested pots, discarding the average losses due to humidity.

Repellent effect

In the repellence assay, powder of each plant species was separately tested using an arena adapted from Procópio *et al*. (2003) constituted by five plastic containers. Four containers were each packed with 10 g of beans. Powder of each tested plant (0.3 g) was applied to two side containers, and symmetrically opposite containers without powder were used as controls. Ten recently emerged *Z. subfasciatus* couples (<24 h of age) were added into a central container, and the number of insects per container was counted 24 hours after infestation. The assays were repeated ten times for each plant species, and the results were analysed as a completely randomized design. Sachets were not used in this assay because of the arena. Moreover, the commercial product K-Obiol® 2 DP was not evaluated in this assay because it is not recommended for this purpose.

Data analysis

For the data analysis, a pre-adjustment of the model was first performed using normal distribution of the data. Furthermore, both the normality of the residues with the Shapiro-Wilk test and the homogeneity of the variances with the Levene test were observed. When the data did not show normality and/or homoscedasticity, a transformation was sought based on the method of maximum power of Box-Cox (Box and Cox, 1964). After the assumptions, the data were subjected to an analysis of variance (ANOVA), and the means were compared using the Tukey test (P< 0.05).

The repellence assay was analysed by Student "t" test. The repellence index (RI) (adapted from Lin *et al*., 1990) was calculated by RI = 2G / (G + P), where G = % of insects in the test plants and P = % of insects in the control. The classification range (IClass) was based on the RI and the standard deviation and determined for the treatment means by the formula: $IClass = 1 \pm t_{(n-1;\alpha=0.05)} \times DP/\sqrt{n}$, where t is the tabulated value of "t"; SD is standard deviation; and n is the number of repetitions. The powder was considered neutral when the RI value was within the IClass range; repellent when the RI value was less than the minimum IClass; and attractive when the RI was higher than the maximum IClass. All these analyses were carried out using the SAS statistical software (SAS Institute, 2001).

Table 1. List of botanical species and plant parts collected from Patrocínio, Minas Gerais State, Brazil (18º56’17.4’’S 46º59’34.5’’W) and tested against *Zabrotes subfasciatus* (Boh., 1833) as well as yield of dried powder after processing.

Botanical species	Family	Plant part	Common name	Collector	Herbarium number*	Yield (%, w/w)
Campomanesia adamantium O. Berg	Myrtaceae	Leaves	‘Gabiroba’	Bernardes; Araujo	HUFU 61.674	34.0
Chenopodium ambrosioides L.	Chenopodiaceae	Aerial part	‘Erva-de-Santa Maria’	Bernardes; Araujo	HUFU 61.679	18.9
Keithia denudata L.	Lamiaceae	Aerial part	‘Poejo do campo’	Bernardes; Araujo	HUFU 61.672	27.6
Lippia alba (Mill) N.E. Brown	Verbenaceae	Leaves	‘Cidreira de folha’	Bernardes; Araujo	HUFU 61.678	30.5
Mentha pulegium L.	Lamiaceae	Leaves and branches	‘Poejo de quintal’	Bernardes; Araujo	HUFU 61.681	17.7
Rosmarinus officinalis L.	Lamiaceae	Aerial part	‘Alecrim’	Groppo Júnior	SPFR 11912	20.3
Ruta graveolens L.	Rutaceae	Aerial part	‘Arruda’	Bernardes; Araujo	HUFU 66.679	21.6
Schinus terebinthifolius Raddi	Anacardiaceae	Leaves and branches	‘Aroeira-mansa’	Bernardes; Araujo	HUFU 61.673	34.6

***** Voucher specimens were deposited in the Herbarium Uberlandense (HUFU), in Uberlândia, Minas Gerais State, Brazil, or in the Herbarium de Franca, in Franca, São Paulo State, Brazil.

Table 2. Mortality of *Zabrotes subfasciatus* (Boh., 1833) adults after five days exposed to bean grains impregnated with powders and in contact with sachets containing different aromatic plant species.

Botanical species	Means (±SE) of dead individuals (n= 10)[1]	
	Powder	Sachet
Campomanesia adamantium	0.0 ± 0.00 a A	0.6 ± 0.26 a B
Schinus terebinthifolius	0.3 ± 0.16 a A	0.1 ± 0.13 a A
Keithia denudata	0.4 ± 0.26 a A	0.1 ± 0.13 a A
Lippia alba	0.4 ± 0.18 a A	0.3 ± 0.16 a A
Rosmarinus officinalis	1.9 ± 0.35 b A	0.4 ± 0.26 a B
Mentha pulegium	5.6 ± 0.42 c A	1.0 ± 0.27 a B
Ruta graveolens	8.5 ± 0.38 d A	0.4 ± 0.26 a B
Chenopodium ambrosioides	10.0 ± 0.00 d A	10.0 ± 0.00 b A
Positive control (K-Obiol® 2 DP)[2]	10.0 ± 0.00 d	-
Negative control (without powder)	0.1 ± 0.13 a A	0.6 ± 0.18 a A

Botanical species = F: 7.45; *P*: 0.0027

Application form = F: 2.36; *P*: 0.0418

Interaction (botanical species × application form) = F: 13.25; *P*: 0.0145

[1] Original data. Means followed by the same lowercase letter in a column or uppercase letter in a row are not significantly different by Tukey's test ($P \geq 0.05$);

[2] Sachets were not evaluated.

RESULTS AND DISCUSSION

Treatments with *Rosmarinus officinalis* L., *M. pulegium*, *R. graveolens* and *C. ambrosioides* powders and the K-Obiol® 2 DP insecticide was toxic to the adult *Z. subfasciatus* and differed from the other treatments in the Mexican bean weevil adult survival assay (Table 2). It is noteworthy that powders from *R. graveolens* and *C. ambrosioides* caused 85% and 100% mortality, respectively, by the end of the evaluation period, similar to the K-Obiol® 2 DP - our positive control. The insecticidal potential of *R. graveolens* powder on *Z. subfasciatus* was recently reported by Silva *et al*. (2016), who obtained 100% mortality using the same test method as in this study. However, only *C. ambrosioides* powder in a sachet was toxic to adult *Z. subfasciatus*, causing 100% mortality in the bioassay endpoint (Table 2). The plant × formulation interaction was significant for *C. adamantium*, *R. officinalis*, *M. pulegium* and *R. graveolens*, indicating that placing the powder in a sachet altered its efficacy against the Mexican bean weevil.

For *C. ambrosioides*, the maintenance efficacy (100%) for both formulations indicates that the main mortality factors are volatile because they were effective even when in a sachet. It is likely that for *R. graveolens*, the adult toxicity is due to the contact action between the powder and the tegument of the insects, and the volatiles are less active, which explains the mortality reduction (8.5 to 0.4) when the powder is formulated in a sachet. Procópio *et al*. (2003) evaluated the control of *Z. subfasciatus* by powder from leaves, flowers and fruits of *C. ambrosioides* in concentrations of 3%, 1.5% and 0.37% (w w^{-1}) and found 100% insect mortality on the fifth day of exposure, and the F_1 generation was completely inhibited. The results obtained by these authors for lower concentrations were similar to those found in the present study for a concentration of 0.3% (w w^{-1}). Selase and Getu (2009) also obtained 97% mortality of *Z. subfasciatus* with powder from leaves of *C. ambrosioides* at a concentration of 10% (w w^{-1}). Lima-Mendonça *et al*. (2013) evaluated the insecticidal activity of powders of 10 plant species and found that powdered flowers and leaves of *C. ambrosioides* at a concentration of 10% (w w^{-1}) was the only treatment that caused 100% mortality of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) adults on the first day after exposure. These studies showed results similar to previous studies and reinforced the potential of *C. ambrosioides* in the management of the Mexican bean weevil.

Placing powders in sachets to control *Z. subfasciatus* was efficacious only for *C. ambrosioides*, indicating that the bioactivity of powdered *R. officinalis*, *M. pulegium* and *R. graveolens* is probably related not only to volatiles but also to contact between the weevil and the powder. Baldin *et al*. (2009) studied the effect of 11 plant powders on the control of *Acanthoscelides* *obtectus* (Say) (Coleoptera: Bruchidae) and reported that the plant powders mixed with the beans probably affected the viability of the eggs as well as the larvae of *A. obtectus*.

A significant reduction in the total number of eggs was also found with *R. officinalis*, *R. graveolens*, *M. pulegium,* and *C. ambrosioides* powders (Table 3), in a similar level with our positive control (K-Obiol® 2 DP). On the other hand, only *C. ambrosioides* and *M. pulegium* powders in sachets differed from the control and the other treatments, reducing the number of eggs deposited by the insects (Table 3). The interaction between the plants and the formulation was not significant for *R. officinalis* and *M. pulegium* (Table 3), indicating that the formulation did not change the effects of the botanical derivatives on insect oviposition. Probably, volatile compounds from *M. pulegium* and *C. ambrosioides* have a direct action in inhibiting oviposition of *Z. subfasciatus*. Baldin *et al*. (2008) evaluated the effects of powdered leaves and branches of 17 botanical species on various biological characteristics of *Z. subfasciatus* in common beans and emphasized that specific negative effects from each plant can occur during a specific phase of the life cycle or behaviour of an insect.

The oviposition preference index indicates that the treatments (i.e., powder formulations) prepared from *C. ambrosioides*, *M. pulegium, R. graveolens* and the commercial insecticide showed greater deterrence of oviposition in the weevils, while *S. terebinthifolius* and *Keithia denudata* powders stimulated this behaviour compared to the negative control (Table 3). Treatments with *R. officinalis* and *C. adamantium* powder was also a deterrent, but at a lower level. Treatment with *C. ambrosioides* and *M. pulegium* powder in a sachet was a greater deterrent to insect oviposition, and these results suggest that activity is related to the existence of secondary compounds present in the powder, and contact with the weevil is not required (Table 3). According to Lara (1991), volatile compounds from plants or vegetable powders can have deterrent effects on insect feeding and oviposition, changing their behaviour while searching for a host.

Lower percentages of larval viability were observed in the powder formulation of *R. officinalis* (61.03%) and the sachet formulation of *R. graveolens* (62.64%) (Table 4). Treatments with *C. ambrosioides*, *M. pulegium* and the insecticide K-Obiol® 2 DP completely inhibited weevil oviposition of and therefore did not have viability data (Table 4). Treatment with *R. officinalis* powder significantly reduced larval viability compared to the other treatments, as did *R. graveolens* powder in a sachet. The effect of formulation on larval viability was not verified. Overall, the plant powders did not affect the average egg to adult development period, regardless of formulation (Table 4). The same was observed for the weight of the emerged adults (Table 4), indicating that a feeding deterrent was not evident.

Table 3. Oviposition (±SE) and classification of botanical species by oviposition preference of *Zabrotes subfasciatus* (Boh., 1833) in bean grains impregnated with powders and in contact with sachets containing different aromatic plant species.

Botanical species	Means (±SE) of eggs/container[1]			
	Powder	OPI[2]	Sachet	OPI[2]
Schinus terebinthifolius	127.1 ± 10.93 a A	Neutral	100.3 ± 8.26 ab B	Stimulant
Keithia denudata	123.5 ± 8.76 a A	Neutral	97.8 ± 4.52 ab B	Neutral
Lippia alba	110.8 ± 6.35 ab A	Neutral	70.9 ± 2.91 c B	Neutral
Campomanesia adamantium	92.6 ± 3.48 bc A	Deterrent	78.0 ± 4.10 bc B	Neutral
Rosmarinus officinalis	80.5 ± 3.28 cd A	Deterrent	83.4 ± 6.91 abc A	Neutral
Ruta graveolens	0.0 ± 0.0 e A	Deterrent	86.9 ± 4.62 abc B	Neutral
Mentha pulegium	0.0 ± 0.0 e A	Deterrent	0.8 ± 0.75 d A	Deterrent
Chenopodium ambrosioides	0.0 ± 0.0 e A	Deterrent	0.3 ± 0.25 d A	Deterrent
Positive control (K-Obiol® 2 DP) [2]	0.0 ± 0.0 e	Deterrent	-[3]	-
Negative control (without powder)	119.6 ± 6.74 ab A	-	82.6 ± 6.68 abc B	-

Botanical species = F: 8.66; *P*: 0.0008
Application form = F: 22.54; *P*: 0.0046
Interaction (botanical species × application form) = F: 17.01; *P*: 0.0227

[1] Means followed by same lowercase letter in a column or uppercase letter in a row were not significantly different by Tukey's test ($P \geq 0.05$);
[2] Oviposition preference index;
[3] The insecticide K-Obiol® 2 DP was used only in mixture of grains (contact).

Treatment with *C. ambrosioides*, *M. pulegium*, *R. officinalis* and *R. graveolens* powder attracted a smaller percentage of insects in relation to the control (Table 5), allowing their classification as repellents. *C. adamantium* was found to be an attractant, whereas *Lippia alba, K. denudata* and *Schinus terebinthifolius* were neutral. Mazzonetto and Vendramim (2003) evaluated the bioactivity of powders made from 18 botanical species on the weevil *A. obtectus* and reported that the powders made from the aerial parts of *C. ambrosioides*, the leaves of *R. graveolens*, and the fruits of *C. reticulata* and *C. sinensis* repelled adult *A. obtectus*. Lima-Mendonça *et al*. (2013) analysed the repellent and insecticidal effects of 10 botanical species and reported that *C. ambrosioides* powder repelled adult *S. zeamais*.

This study verified a high potential for the use of *C. ambrosioides*, *R. graveolens* and *M. pulegium* powders in the alternative control of *Z. subfasciatus*. Terpenes such as ascaridol (Souza *et al*., 2015), 2-undecanone and 2-nonanone (Silva *et al*., 2014) and oxygenated terpenes (Teixeira *et al*., 2012) are present in these species, and their bioactivity has been previously reported in the literature. In addition, this study demonstrated that changing the formulation from a powder to a sachet did not affect the efficacy of *C. ambrosioides* powder, making the possibility of applying it in weevil management more likely. This is the first report of using botanical derivatives by means of sachets or dry formulations, a pre-commercial purpose for aromatic plants with insecticidal/repellent activities.

The isolation and characterization of biologically-active compounds from some plant extracts including *C. ambrosioides* was made and active compounds against stored grain pests were found. The fractions isolated from *C. ambrosioides* contained mainly long-chain hydrocarbons and fatty acid derivatives (i.e., heptadecane, ethyl hexadecanoate and arachidonic acid) that were as toxic as nicotine sulphate, malathion and pirimiphos-methyl against *Tricolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) and *Sitophilus granarius* (Linnaeus, 1875) (Coleoptera: Curculionidae) (Peterson *et al*., 1989). The compositions of essential oil from *C. ambrosioides* and their potential effects was determined, and the main components found were α-terpinene, *p*-cymene and *trans*-ascaridol. The predominance of monoterpene hydrocarbons and oxygenated monoterpenes was observed (Santiago *et al*. 2014). According to Viegas Júnior (2003), monoterpenes have been isolated and proved its toxicity against the different insects may cause mortality between 40 to 100%.

CONCLUSION

Mixing *C. ambrosioides*, *R. graveolens* and *M. pulegium* powders with bean grains is toxic to adult *Z. subfasciatus* and protective against weevil attack. *M. pulegium* powder mixed with bean grains inhibit weevil oviposition, and the same effect is shown by using in sachets with this species. Mixtures of *C. ambrosioides, M. pulegium, R. officinalis* and *R. graveolens* powders make bean grains repellent to adult *Z. subfasciatus. C. ambrosioides* powder exhibits similar efficiency when the powder is placed in a sachet. Sachets of *C. ambrosioides* and *M. pulegium* powders are also efficacious for weevil control in stored beans. The use of plant species to control stored grain pests appears to be quite promising. Considering the ease of cultivation and preparation of the powders of the species evaluated in this study, this practice is indicated as an efficacious tool for the alternative control of weevils in stored beans, mainly in domestic grain stock or post-harvest of organic grains. Despite the promising results, further studies are needed to evaluate both the persistence of these botanical derivatives in the different forms of use (dry powder or sachets) and the possibility of associating these natural products with other strategies, including diatomaceous earth-based insecticides (inert dusts).

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Table 4. Means (±SE) of larval viability, adult emergence, egg-adult development period and dry weight of *Zabrotes subfasciatus* (Boh., 1833) adults obtained in bean grains impregnated with powders and contact with sachet containing powders of different aromatic plant species.

Botanical species	Larval viability (%)[1,2]		Period of egg-adult development (days)[2]		Weight of the adults (mg)[2]	
	Powder	Sachet	Powder	Sachet	Powder	Sachet
Schinus terebinthifolius	93.48 ± 7.54 a A	83.65 ± 6.72 a A	48.1 ± 0.59 a A	46.9 ± 0.64 a A	1.2 ± 0.03 a A	1.1 ± 0.08 a A
Keithia denudata	93.09 ± 6.33 a A	86.99 ± 5.57 a A	46.7 ± 0.49 a A	47.5 ± 0.96 a A	1.1 ± 0.01 a A	1.1 ± 0.02 a A
Lippia alba	90.73 ± 8.22 a A	83.90 ± 6.98 a A	47.7 ± 0.83 a A	47.8 ± 1.15 a A	1.2 ± 0.01 a A	1.0 ± 0.03 a A
Campomanesia adamantium	85.21 ± 7.48 a A	84.90 ± 6.15 a A	48.3 ± 0.70 a A	46.6 ± 1.00 a A	1.3 ± 0.02 a A	1.1 ± 0.04 a A
Rosmarinus officinalis	61.03 ± 5.21 b A	73.66 ± 2.49 a A	48.1 ± 0.59 a A	46.0 ± 0.68 a A	1.5 ± 0.29 a A	1.1 ± 0.02 a A
Ruta graveolens[3]	-	62.64 ± 4.27 b	-	47.0 ± 0.80 a	-	1.0 ± 0.04 a A
Chenopodium ambrosioides [4]	-	-	-	-	-	-
Mentha pulegium [4]	-	-	-	-	-	-
Positive control (K-Obiol® 2 DP) [4]	-	-	-	-	-	-
Negative control (without powder)	91.89 ± 5.24 a A	87.80 ± 7.04 a A	48.6 ± 0.57 a A	45.9 ± 0.58 a A	1.2 ± 0.02 a A	1.1 ± 0.02 a A
Botanical species =	F: 34.15; *P*: 0.0021		F: 14.47; *P*: 0.0784		F: 23.12; *P*: 0.1241	
Application form	F: 6.88; *P*: 0.0312		F: 26.41; *P*: 0.4421		F: 50.37; *P*: 0.0662	
Interaction (botanical species × application form)	F: 5.65; *P*: 0.1074		F: 36.97; *P*: 0.0845		F: 19.32; *P*: 0.1546	

[1] The data were transformed into arcsine $(x + 0.5)^{1/2}$ for analysis. [2] Means followed by the same letters are not significantly different by Tukey's test ($P \geq 0.05$);
[3] Treatment with powder caused mortality of all individuals;
[4] Treatment with both formulations caused mortality of all individuals.

Table 5. Attractiveness (%) of bean grains impregnated with powders of different botanical species to *Zabrotes subfasciatus* (Boh., 1833) adults.

Botanical species	Attracted insects[1]		R.I. [2] (M ± SE)	I.Class. [3]	C.I. [4]
	Treated [2]	Control [3]			
Campomanesia adamantium	77	23	1.54 ± 0.13	0.72 – 1.28	A
Chenopodium ambrosioides	12	88	0.25 ± 0.04	0.92 – 1.08	R
Keithia denudata	44	56	0.87 ± 0.16	0.66 – 1.34	N
Lippia alba	60	40	1.19 ± 0.12	0.75 – 1.25	N
Mentha pulegium	17	83	0.34 ± 0.12	0.74 – 1.26	R
Rosmarinus officinalis	19	81	0.39 ± 0.18	0.61 – 1.39	R
Ruta graveolens	26	74	0.51 ± 0.18	0.61 – 1.39	R
Schinus terebinthifolius	60	40	1.20 ± 0.13	0.73 – 1.27	N

[1] Percentage of attracted insects;
[2] Repellence Index (mean ± standard error);
[3] Classification index;
[4] Classification: R = repellent, N = neutral, A = attractive.

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