

# Toxicity and repellency of essential oils to *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) in *Phaseolus vulgaris* L.

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## ABSTRACT

The effects of tangerine (*Citrus reticulata* Blanco), lemon (*Citrus medica limonum* Lush), pear orange (*Citrus sinensis* L. Osbeck), red copaiba (*Copaifera langsdorffii* Desf.), rosemary (*Baccharis dracunculifolia* De Candolle), eucalyptus (*Eucalyptus globulus* Labillardière and *E. citriodora* Hook), lemongrass (*Cymbopogon citratus* Stapf.) and citronella (*Cymbopogon nardus* Linnaeus) oils at several concentrations on *Zabrotes subfasciatus* (Boheman) were studied. In toxicity tests, grains of *Phaseolus vulgaris* L. cv. Rajadinho were impregnated with oils and infested with adults of *Z. subfasciatus* up to 24 hours old. All tested oils were effective in reducing the viable egg-laying and adult emergence of this pest, in function of the concentrations used, highlighting *E. citriodora* and *E. globulus* oils which caused 100% effectiveness from 0.5 mL Kg<sup>-1</sup> concentration. In repellency tests, two arenas consisting of plastic containers, connected symmetrically to a central box by two plastic tubes were used. In one of the boxes, untreated beans were placed and on the other ones beans treated with each oil concentration were used. In the central box, five couples of *Z. subfasciatus* were released. Grains of *P. vulgaris* treated with oils of *E. citriodora*, *C. citratus* and *C. oleifera* reduced the attraction percentage of *Z. subfasciatus* adults, while the *E. globulus* increased this percentage. The percentages of reduced viable eggs ranged from 17.9% (*C. medica limonum*) to 93.3% (*C. nardus*), while the reduction on the number of emerged insects was 23.9% and 95.9%, respectively for these same oils.

**KEYWORDS:** Botanical insecticides, bean weevil, bioactivity, behavioral effect.

# Toxicidade e repelência de óleos essenciais a *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) em grãos de *Phaseolus vulgaris* L.

## RESUMO

Estudaram-se os efeitos dos óleos de tangerina 'Cravo' (*Citrus reticulata* Blanco), limão-siciliano (*Citrus medica limonum* Lush), laranja 'Pêra' (*Citrus sinensis* L. Osbeck), copaiba-vermelha (*Copaifera langsdorffii* Desf.), alecrim-do-campo (*Baccharis dracunculifolia* De Candolle), eucalipto (*Eucalyptus globulus* Labillardière e *Eucalyptus citriodora* Hook), capim-santo (*Cymbopogon citratus* Stapf.) e citronela (*Cymbopogon nardus* L.), em diversas concentrações, sobre *Zabrotes subfasciatus* (Boheman). Nos testes de toxicidade, grãos de *Phaseolus vulgaris* L. cv. Rajadinho foram impregnados com os óleos e infestados com adultos de *Z. subfasciatus* com até 24 horas de idade. Todos os óleos foram eficientes na redução da postura de ovos viáveis e na emergência de adultos desta praga, em função das concentrações utilizadas, destacando-se *E. citriodora* e *E. globulus*, que a partir da concentração de 0,5 mL Kg<sup>-1</sup> já causaram 100% de eficácia. Nos testes de repelência utilizaram-se arenas compostas por dois recipientes plásticos, interligados simetricamente a uma caixa central por dois tubos plásticos. Numa das caixas colocaram-se grãos de feijão não tratados e, na outra, feijões tratados com cada uma das concentrações de óleo testadas. Na caixa central foram liberados cinco casais de *Z. subfasciatus*. Os grãos de *P. vulgaris* tratados com os óleos de *E. citriodora*, *C. citratus* e *C. oleifera* reduziram a porcentagem de adultos de *Z. subfasciatus* atraídos, enquanto o óleo de *E. globulus* aumentou essa porcentagem. As porcentagens de redução de ovos viáveis variaram de 17,9% (*C. medica limonum*) a 93,3% (*C. nardus*), enquanto que a redução do número de insetos emergidos foi de 23,9% e 95,9%, respectivamente, para esses mesmos óleos.

**PALAVRAS-CHAVE:** Inseticidas botânicos, caruncho-do-feijão, bioatividade, efeito comportamental.

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## INTRODUCTION

The Bruchinae are distributed in almost all continents except in Antarctica and New Zealand, being the largest number of species found in the Neotropical Region (Kingsolver 2002). The species of stored products may have originated in various regions of the world, but due to transport from one country or continent to another together with the goods they have become cosmopolitan. It is believed that many of these species occur in populations, geographic subspecies or breeds that differ in food preference, climatic conditions and susceptibility to insecticides (Hill 1990).

*Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) is a major primary pest of the stored bean *Phaseolus vulgaris* L. in tropical regions. It infests grains and seeds in storage units causing quantitative losses (weight loss), which can be quite variable depending on the degree of infestation and susceptibility of seeds or grains from such species. Qualitative losses are more difficult to be assessed, and they consist of vigor and germination reduction, changes in physical appearance, nutritional losses, presence of insects, mites and their fragments, fungal infection and mycotoxin contamination, impurities and other foreign materials (Lazzari 1997).

Botanical insecticides containing different compounds derived from plants secondary metabolism have been tested in order to control this pest in stored beans with promising results (Weaver *et al.* 1994; Oliveira and Vendramim 1999; Barbosa *et al.* 2002; Paranhos *et al.* 2005, Santos *et al.* 2007; Silva *et al.* 2008; Paul *et al.* 2009), as an alternative to protective chemical insecticides, which are generally more toxic and likely to cause harmful effects to applicators, consumers and the environment. Among the most promising compounds, the monoterpenes and its analogues are remarkable. They are quite volatile, lipophilic and act by contact, ingestion and fumigation, causing mortality, repellence and effects on the insect biology, physiology and behavior. They can be used as dry powders, extracts, essential oils and oil emulsion (Brito *et al.* 2006; Isman 2006; Rajendran and Sriranjini 2008; Selase and Getu 2009).

This study aimed to evaluate the effects of some essential oils on mortality, number of viable eggs and emergence of *Z. subfasciatus*, as well as its repellency to adults on grains of *P. vulgaris*.

## MATERIAL AND METHODS

***Zabrotes subfasciatus* rearing.** Insects were reared for several generations on grains of *P. vulgaris* cv. Rajadinho in the Agricultural Entomology Laboratory of the Federal Rural University of Pernambuco (UFRPE), Brazil, at  $27.1 \pm 1$  °C,  $74.2 \pm 5.3\%$  of RH (Humidity Relative) and 12 h of

photophase, stored in glass containers, sealed with perforated plastic lids and lined with fine cloth to allow gas exchange. Adults were confined for six days, subsequently they were removed, and the grains stored until adult emergence.

**Infestation removal and hygroscopic equilibrium.** Beans properly selected (broken grains with impurities were eliminated) used for rearing and experiments were placed in plastic bags and kept in a freezer at  $-5$  °C for seven days to eliminate possible insect infestation from field. After removal from the freezer, the grains were transferred to glass flasks and kept in laboratory temperature, which varied between 26 and 28 °C for seven days in order to reach the hygroscopic equilibrium. The equilibrium with air humidity is normally reached after seven days.

**Origin of plant essential oils.** Oils from tangerine (*Citrus reticulata* Blanco) (Rutaceae), lemon (*Citrus medica limonum* Lush) (Rutaceae), pear orange (*Citrus sinensis* L. Obesck) (Rutaceae), red copaiba (*Copaifera langsdorffii* Desf.) (Fabaceae), rosemary (*Baccharis dracunculifolia* De Candolle) (Asteraceae), eucalyptus (*Eucalyptus globulus* Labillardière and *Eucalyptus citriodora* Hook.) (Myrtaceae), lemongrass (*Cymbopogon citratus* Stapf.) (Poaceae), and citronella (*Cymbopogon nardus* (L.) (Poaceae) were purchased from Aromalandia Ltd. (Belo Horizonte, MG, Brazil).

**Contact toxicity tests.** Experiments were conducted at  $30.1 \pm 1.8$  °C,  $67.2 \pm 4.7\%$  Relative Humidity and 12 h photophase. Oils were tested at the concentrations 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mL kg<sup>-1</sup> grains of *P. vulgaris* cv. Rajadinho, selected in preliminary experiments. The treatment of grains with the essential oils was performed using plastic containers. Oils were manipulated using an automatic pipette and then the containers were manually agitated during two minutes. Each 20 g portion of *P. vulgaris* grains was infested with five couples of *Z. subfasciatus* up to 24 h old. Six days after the confinement, live and dead insects were counted and then discarded. The quantification of viable and unviable eggs was performed after 16 days, examining all grains of each plot under stereomicroscope. The viable eggs are opaque whereas the non-viable are translucent. Adult emergences were measured at 40 days after infestation. The oils were separately tested in a fully randomized design, consisting of six treatments and six replications. Results were submitted to regression analysis, being selected those equations that best represented the biological response, based on its significance (F and P) and higher coefficient of determination (R<sup>2</sup>). All tests were conducted using the statistical program SAS version 8.02 (SAS Institute 2001).

**Repellency tests.** The same oils used above were studied. The experiments were conducted at the same range of temperature, relative humidity and photoperiod. Arenas consisting of two plastic containers, connected symmetrically

to a central box by two plastic tubes were used. In one of the boxes, 20 g of beans without oil (control) were placed and on the other one, the same amount of treated grains was used. In the central box five couples of *Z. subfasciatus* up to 24 h old were released. The oils were tested separately in a complete randomized design, consisting of two treatments: the best oil concentration in the no-choice test and the control, using 10 replicates. After six days, insects maintained in each container were counted for repellency evaluation, and then discarded; the beans were stored in plastic containers. The viable and unviable eggs were evaluated in all grains of the plot after 16 days, and emerged adults quantified at 40 days after infestation.

Percentages of adults attracted to the control and to each concentration of oil were submitted to the analysis of choice frequency and evaluated by the chi-square test using the SAS. The number of viable eggs and the number of emerged insects were subjected to analysis of variance; data from *B. dracunculifolia*, *C. citratus*, *C. langsdorffii*, *C. nardus* e *C. reticulata* were transformed into square root of  $x + 0.5$ , in order to achieve homogeneity in variances, and analyzed by the *t* test. The average percentage reduction of oviposition and adult emergence was calculated using the formula adapted from Obeng-Ofori (1995):  $PR = [(NC - NT) / (NC + TN) \times 100]$ , where PR = average percentage of reduction in oviposition and/or emergence, NC = average number of eggs and/or emerged insects in the control, and NT = average number of eggs and/or emerged insects in the treatment.

## RESULTS AND DISCUSSION

**Contact toxicity tests.** Mortality rates of *Z. subfasciatus* on grains of *P. vulgaris* treated with plant essential oils varied with both oil type and concentration used. Regression equations (significant,  $P \leq 0.05$ ) that explain oils performance on adult mortality, number of viable eggs, and emerged adults were obtained (Table 1). No significant regression equations were established for the essential oils of *E. globulus*, *E. citriodora*,

*C. nardus*, *C. citratus*, *B. dracunculifolia* and *C. sinensis*, as all of them caused 100% mortality rates and total reduction on the number of viable eggs and emerged insects from the lowest concentration (0.5 mL kg<sup>-1</sup> beans) not fitting the statistical model. Although presenting 100% efficiency, the results demonstrated the need to test these oils at lower concentrations, for the establishment of regression equations.

In Brazil there are only four products registered for control of *Z. subfasciatus* in stored beans, being three inorganic precursors of phosphine fumigants (extremely toxic), used to curative control, and a pyrethroid (deltamethrin), as a protective insecticide (AGROFIT 2011). Hence, the results of this work, with essential oils that may features less impact on environment and health of applicators and consumers, are crucial as alternative products.

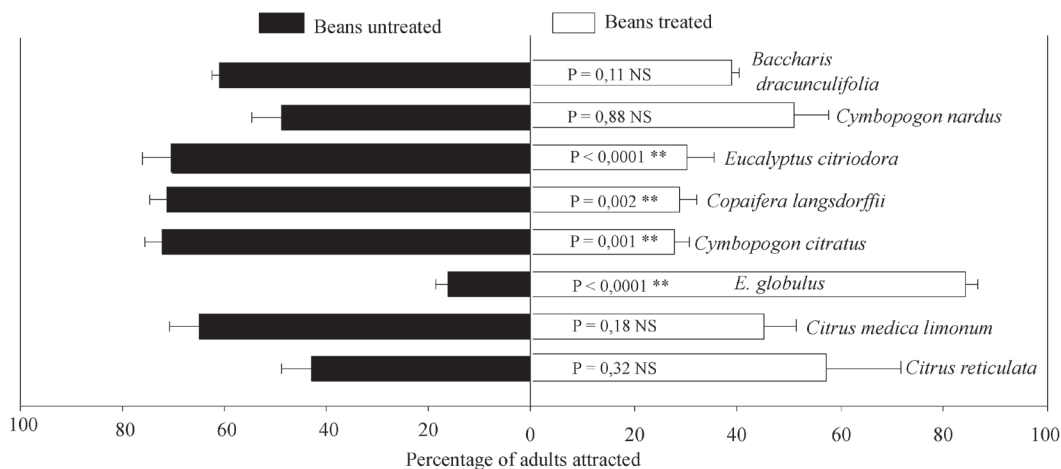
Some studies have shown the bioactivity of plants on *Z. subfasciatus*. Black pepper powders [*Piper nigrum* (L.)] (Piperaceae), cinnamon leaves (*Cyannomum zeylanicum* Blume) (Lauraceae) (Oliveira *et al.* 1999) and neem oil (*Azadirachta indica* A. Juss) (Meliaceae) (Barbosa *et al.* 2002) were effective on *Z. subfasciatus*. According to Mazzonetto (2002), the powders of *Chenopodium ambrosioides* L. (Chenopodiaceae), *Mentha pulegium* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), and rue (*Ruta graveolens* L.) (Rutaceae) caused 100% mortality rates and prevented oviposition of *Z. subfasciatus* on *P. vulgaris* grains. Crushed leaves of *Tetradenia riparia* (Hochst.) (Lamiaceae) species at 4% concentration reduced fertility and fecundity of *Z. subfasciatus* on *P. vulgaris* grains (Weaver *et al.* 1991). The root powder of *Senecio salignus* DC. (Asteraceae) showed contact toxicity on *Z. subfasciatus*, with LC<sub>50</sub> (Lethal Concentration 50) ranging between 0.11% and 0.08%, but it had neither fumigant nor repellent effect (López-Pérez *et al.* 2007). The linalool compound, one of the major components of *Ocimum canum* L. (Lamiaceae) caused 98% and 100% mortality rates to adults of *Z. subfasciatus* at concentrations of 500 and 700 µg cm<sup>-2</sup>, respectively (Weaver *et al.* 1991).

**Table 1** - Equations for mortality rates (%), number of viable eggs and adult emergence, and their respective adjusting parameters (F, Fisher test; P value and significance; R<sup>2</sup>, coefficient of determination) of *Z. subfasciatus* submitted to *P. vulgaris* beans treated with several plant essential oils. Temp: 30.1 ± 1.82 °C, RH (Relative Humidity): 67.2 ± 4.68%, and 12 h photophase.

| Treatments                    | Parameters            | Equations                               | F <sup>P</sup>          | R <sup>2</sup> |
|-------------------------------|-----------------------|---|-------------------------|----------------|
| <i>Copaifera langsdorffii</i> | Mortality             | $\hat{y} = 2.30 + 42.38x$               | 157.96<sup>0.0001</sup> | 0.82           |
|                               | Number of viable eggs | $\hat{y} = 126.24 + 165.57x - 47.94x^2$ | 107.24<sup>0.0001</sup> | 0.87           |
|                               | Emergence             | $\hat{y} = 114.76 - 151.85x + 44.13x^2$ | 112.44<sup>0.0001</sup> | 0.87           |
| <i>Citrus reticulata</i>      | Mortality             | $\hat{y} = 4.70 - 21.63x + 17.26x^2$    | 22.11<sup>0.0001</sup>  | 0.57           |
|                               | Number of viable eggs | $\hat{y} = 147.60 - 66.70x$             | 187.92<sup>0.0001</sup> | 0.85           |
|                               | Emergence             | $\hat{y} = 114.59 - 52.07x$             | 151.23<sup>0.0001</sup> | 0.82           |
| <i>Citrus medica limonum</i>  | Mortality             | $\hat{y} = 2.85 - 27.19x + 26.19x^2$    | 139.79<sup>0.0001</sup> | 0.89           |
|                               | Number of viable eggs | $\hat{y} = 150.04 - 67.20x$             | 259.22<sup>0.0001</sup> | 0.88           |
|                               | Emergence             | $\hat{y} = 119.35 - 53.39x$             | 219.63<sup>0.0001</sup> | 0.86           |

The fumigant effect of essential oils has been also investigated, opening new perspectives in the control of stored grain pests with plants of the families Apiaceae, Lamiaceae, Lauraceae and Myrtaceae, aiming mainly to control immature and adults of *Tribolium castaneum* Herbst, *Rhizopertha dominica* Fabricius, *Sitophilus oryzae* Linnaeus and *Sitophilus zeamais* Motschulsky (Rajendran and Sriranjini 2008), and that can be tested for the management of *Z. subfasciatus*. Essential oils from plants of the genus *Eucalyptus* reduced the proportion of viable eggs and emerged adults of *Z. subfasciatus* due to its fumigant effect (Brito *et al.* 2006). The essential oil from leaves and stems of *Croton grewoides* Baill. (Euphorbiaceae) also presented fumigant effect to adults of *Z. subfasciatus*, with LC<sub>50</sub> values of 4 and 13.77µl/L air, respectively (Silva *et al.* 2008).

**Repellency tests.** The oils of *C. citratus* ( $\chi^2 = 10.17$ ;  $P < 0.01$ ), *C. angsdorffii* ( $\chi^2 = 9.22$ ;  $P = 0.002$ ) and *E. citriodora* ( $\chi^2 = 8.33$ ;  $P < 0.0001$ ) reduced the number of adults of *Z. subfasciatus* attracted to *P. vulgaris* beans compared to the control; however, *E. globulus* ( $\chi^2 = 26.14$ ;  $P < 0.001$ ) increased the attraction of adults (Figure 1). Treatments of *C. reticulata* ( $\chi^2 = 0.98$ ;  $P = 0.32$ ), *C. medica limonun* ( $\chi^2 = 1.74$ ;  $P = 0.18$ ), *C. nardus* ( $\chi^2 = 0.02$ ;  $P = 0.88$ ) and *B. dracunculifolia* ( $\chi^2=2.44$ ;  $P=0.11$ ) were considered neutral, since *Z. subfasciatus* showed indifferent choice. The oils of *B. dracunculifolia* (DF= 18,  $t = -3.06$ ,  $P < 0.006$ ), *C. citratus* (DF = 11.5,  $t = -10.59$   $P < 0.0001$ ), *C. langsdorffii* (GL= 11.5,  $t = 10.14$   $P < 0.0001$ ), *C. nardus* (DF = 9.28,  $t = 8.97$   $P < 0.0001$ ), *E. citriodora* (DF = 18,  $t = 11.03$   $P < 0.0001$ ), and *C. reticulata* (DF= 10.8,  $t = -2.64$ ,  $P < 0.02$ ) significantly reduced the number of viable eggs when compared with the control (Table 2). *C. nardus* did not reduce the number of



**Figure 1** - Percentage of adults of *Zabrotes subfasciatus* attracted to *Phaseolus vulgaris* beans treated and untreated with essential oils. \*\* Statistically significant by  $\chi^2 =$  chi-square test. NS = Not significant.

**Table 2** - Effect of plant essential oils on egg-laying of viable eggs of *Z. subfasciatus* in grains of *P.vulgaris*. Free-choice Test.

| Treatment                        | Conc.(mL Kg <sup>-1</sup> ) | Oviposition ( $\pm$ SE <sup>1</sup> ) |                  | Reduction (%) |
|----------------------------------|-----------------------------|---------------------------------------|------------------|---------------|
|                                  |                             | Control                               | Oil              |               |
| <i>Baccharis dracunculifolia</i> | 0.5                         | 61.54 $\pm$ 12.25*                    | 15.53 $\pm$ 7.88 | 59.7          |
| <i>Cymbopogon citratus</i>       | 0.5                         | 79.26 $\pm$ 6.49 *                    | 3.92 $\pm$ 2.51  | 90.6          |
| <i>C. nardus</i>                 | 0.5                         | 70.36 $\pm$ 7.70 *                    | 2.43 $\pm$ 0.98  | 93.3          |
| <i>Copaifera langsdorffii</i>    | 0.5                         | 86.90 $\pm$ 7.24 *                    | 8.25 $\pm$ 2.70  | 82.7          |
| <i>Eucalyptus citriodora</i>     | 0.5                         | 92.94 $\pm$ 5.79 *                    | 5.83 $\pm$ 5.02  | 88.2          |
| <i>E. globulus</i>               | 0.5                         | 51.74 $\pm$ 8.75                      | 26.51 $\pm$ 8.84 | 32.2          |
| <i>Citrus medicum limonun</i>    | 1.5                         | 44.60 $\pm$ 7.59                      | 30.98 $\pm$ 6.83 | 17.9          |
| <i>C. reticulata</i>             | 1.5                         | 66.15 $\pm$ 14.63*                    | 29.89 $\pm$ 4.59 | 37.75         |

\* Significant means in t test ( $P \geq 0.05$ ) compared to control.

<sup>1</sup> Standard Error.



adults attracted to *P. vulgaris* beans treated with this essential oil, but it was the most effective in reducing the egg-laying of viable eggs (93.3%), followed by *C. citratus* (90.6%), *E. citriodora* (88.2%) and *C. angsdorffii* (82.7%); for the other oils, percentages of reduction ranged between 17.9% and 59.7% (Table 2). This behavior was also observed in the adult emergence of *Z. subfasciatus* for *C. nardus* (DF = 9.16,  $t = 8.51$ ,  $P < 0.0001$ ), *C. citratus* (DF = 11.3,  $t = -9.61$ ,  $P < 0.0001$ ), *E. citriodora* (DF = 18,  $t = 9.68$ ,  $P < 0.0001$ ), *C. oleifera* (DF = 11,  $t = 8.21$ ,  $P < 0.0001$ ) and *B. dracunculifolia* (DF = 12.7,  $t = -3.37$ ,  $P < 0.005$ ) which reduced emergence in 95.9%, 92.4%, 88.4%, 85% and 72.4%, respectively. For the other oils the reduction ranged from about 23.97% to 37.5% (Table 3). The oils of *C. medica limonun* (DF = 18,  $t = -1.38$ ,  $P < 0.18$ ; DF = 18,  $t = -1.19$ ,  $P < 0.25$ ) e *E. globulus* (DF = 18,  $t = 1.62$ ,  $P < 0.12$ ; DF = 18,  $t = 1.18$ ,  $P < 0.09$ ) caused no significant reduction on the number of viable eggs, neither on the number of *Z. subfasciatus* adults emerged.

The repellency is an important feature of both plant essential oils and powders for the management of *Z. subfasciatus*, since they play a role directly in the reduction of egg-laying and hence the emergence of adults, making difficult the establishment of pest populations in grain storage units. The oils of cinnamon leaves and bay leaves (*Laurus nobilis* L.) at 2.5 mL kg<sup>-1</sup> grains of *P. vulgaris* exerted repellent effect over 70% on adults of *Z. subfasciatus* (Oliveira and Vendramim 1999). Essential oils from *Lippia origanoides* Kunth, *E. citriodora*, and *Tagetes lucida* Cav. collected in Colombia caused repellency to *S. zeamais* (Nerio et al. 2009). Oils extracted from *Laurus nobilis* L., *Citrus bergamia* (Risso) Wight & Arn., *Foeniculum vulgare* Mill., and *Lavandula hybrida* L. were repellent to adults of *S. zeamais*, *Cryptolestes*

*ferrugineus* Stephens, and larvae of *Tenebrio molitor* L. (Cosimi et al. 2009). Nerio et al. (2010) published a recent review about repellency activity of essential oils. This author noted that the compounds of *Cymbopogon* spp., *Ocimum* spp. and *Eucalyptus* spp. were the most repellent to several stored product pests. Due to the own insecticidal properties, plant essential oils can be very useful in integrated management of *Z. subfasciatus* in stored beans, mainly in small farms, as a promising alternative to synthetic chemical insecticides. However, further studies are a priority regarding to the collecting processes, drying, storage and preparation of plant material, quantification of bioactive compounds, toxicology studies, formulations, and safety. The main goal is to provide consumers with high-quality beans without the presence of toxic waste.

## CONCLUSIONS

1) Oils of *C. reticulata*, *C. medica limonum*, *C. sinensis*, *C. langsdorffii*, *B. dracunculifolia*, *E. globulus* and *E. citriodora*, *C. citratus*, *C. nardus* significantly reduced viable egg-laying and the adult emergence of *Z. subfasciatus*, depending on the concentrations.

2) *C. citratus*, *E. citriodora* and *C. oleifera* showed the highest repellent effect on adults of this pest.

3) *E. globulus* showed an attractive effect on adults of *Z. subfasciatus*.

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**Table 3** - Effect of plant essential oils on the emergence of adults of *Z. subfasciatus* in grains of *P. vulgaris*. Free-choice Test.

| Treatment                        | Conc.(mL Kg <sup>-1</sup> ) | Emergence ( $\pm$ SE <sup>1</sup> ) |                  | Reduction (%) |
|----------------------------------|-----------------------------|-------------------------------------|------------------|---------------|
|                                  |                             | Control                             | Oil              |               |
| <i>Baccharis dracunculifolia</i> | 0.5                         | 50.87 $\pm$ 12.22*                  | 8.14 $\pm$ 5.69  | 72.4          |
| <i>Cymbopogon citratus</i>       | 0.5                         | 70.82 $\pm$ 6.67*                   | 2.79 $\pm$ 2.4   | 92.4          |
| <i>C. nardus</i>                 | 0.5                         | 63.80 $\pm$ 7.64*                   | 1.32 $\pm$ 0.73  | 95.9          |
| <i>Copaifera langsdorffii</i>    | 0.5                         | 74.22 $\pm$ 7.85*                   | 6.02 $\pm$ 2.66  | 85.0          |
| <i>Eucalyptus citriodora</i>     | 0.5                         | 79.03 $\pm$ 6.14*                   | 4.86 $\pm$ 4.22  | 88.4          |
| <i>E. globulus</i>               | 0.5                         | 43.76 $\pm$ 8.80                    | 25.61 $\pm$ 7.39 | 26.2          |
| <i>Citrus medica limonun</i>     | 1.5                         | 36.14 $\pm$ 7.84                    | 23.93 $\pm$ 7.18 | 23.97         |
| <i>C. reticulata</i>             | 1.5                         | 9.32 $\pm$ 13.17*                   | 26.99 $\pm$ 4.16 | 37.46         |

\* Significant means in t test ( $P \geq 0.05$ ) compared to control.

<sup>1</sup> Standard Error.

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