Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
http://periodicos.ufersa.edu.br/index.php/sistema

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)

FUMIGATION TOXICITY OF ESSENTIAL OILS AGAINST *Rhyzopertha dominica* (F.) IN STORED MAIZE GRAIN[1]

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ABSTRACT - The *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) is a primary pest of stored grains in many regions of the world. In this work we evaluated the fumigant activity of essential oils of *Ocimum basilicum* L., *Citrus aurantium* L., *Mentha spicata* L. and *Croton pulegiodorus* Baill on adult *R. dominica* in stored maize. Tests were conducted to determine lethals concentrations (CL_{50} and CL_{100}) and mortality (fumigation).The fumigation test was done in containers made of glass containing 10 individuals of *R. dominica*, where essential oils were applied at different concentrations: *O. basilicum* and *M. spicata* (5, 10, 15, 20, 30 and 40µL/L of air), *C. aurantium* (10, 20, 30, 40, 50 and 60µL/L of air) and *C. pulegiodorus* (0, 20, 30, 50, 70 and 90µL/L of air). After 48 hours of exposure to the oils the percentage of insect mortality was evaluated. According to LC_{50} and LC_{100} the toxicity of essential oils decreased in the following order: *O. basilicum*> *M. spicata*> *C. pulegiodorus*> *C. aurantium.* The essential oil of *O. basilicum* exhibited strong fumigant toxicity against *R. dominica* adults, with a LC_{50} value of 17.67 µL/L air and LC_{100} value of 27.15 µL/L air. The *C. aurantium* essential oil required higher concentrations than *O. basilicum*, *M. spicata* and *C. pulegiodorus* to kill insects. However, all oils evaluated presented fumigating property to promote the control of *R. dominica* and demonstrated potential use in the management of this coleoptera.

Keywords: Botanical insecticides. Fumigant effect. Bostrichidae. Stored grains.

TOXICIDADE POR FUMIGAÇÃO DE ÓLEOS ESSENCIAIS SOBRE *Rhyzopertha dominica* (F.) EM GRÃOS DE MILHO ARMAZENADOS

RESUMO – *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) é uma praga primária de grãos armazenados em muitas regiões do mundo. Este trabalho teve como objetivo avaliar a atividade fumigante dos óleos essenciais de *Ocimum basilicum* L., *Citrus aurantium* L., *Mentha spicata* L. e *Croton pulegiodorus* Baill. sobre adultos de *R. dominica* em grãos de milho armazenados. Para isso, testes para determinação das concentrações letais (CL_{50} e CL_{100}) e mortalidade (fumigação) foram realizados em câmaras constituídas por recipientes de vidro, contendo 10 indivíduos de *R. dominica*, com óleos essenciais em diferentes concentrações: *O. basilicum* e *M. spicata* (5, 10, 15, 20, 30 e 40 µL/L de ar), *C. aurantium* (10, 20, 30, 40, 50 e 60 µL/L de ar) e *C. pulegiodorus* (0, 20, 30, 50, 70 e 90 µL/L de ar). Após 48 h de exposição aos óleos, avaliou-se a percentagem de mortalidade dos insetos. Com base nas CL_{50} e CL_{100}, a toxicidade dos óleos essenciais decresceu na seguinte ordem: *O. basilicum* > *M. spicata* > *C. pulegiodorus* > *C. aurantium*. O óleo essencial de *O. basilicum* exibiu forte toxicidade por fumigação sobre adultos de *R. dominica*, com valores de CL_{50} de 17,67 µL/L de ar e de CL_{100} de 27,15 µL/L de ar. O óleo essencial de *C. aurantium* requereu concentrações mais altas que os óleos de *O. basilicum*, *M. spicata* e *C. pulegiodorus* para matar os insetos. Contudo, todos os óleos avaliados apresentaram propriedades fumigantes para controlar a *R. dominica* e demonstraram potencial de utilização no manejo deste coleóptero.

Palavras-chave: Inseticidas botânicos. Efeito fumigante. Bostrichidae. Grãos armazenados.

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[1]Received for publication in 05/09/2015; accepted in 02/06/2016.
Paper extracted from the masters thesis of the first author.
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INTRODUCTION

Maize is very important as it constitutes a staple food for humans and animals, and is the most consumed cereal worldwide. Maize production is among the main agricultural activities practiced in Brazil (CONAB, 2012). However, many maize farmers are small-scale farmers who grow low-input crops by planting seeds saved in one year during the next, usually intercropped with other species. In this sense, it favors the incidence of pests that cause large losses by attacking at all stages of their cycle (NÉRI et al., 2005; OLIVEIRA et al., 2007).

Damage incurred by stored grain is definitive and unrecoverable, since pest feeding causes qualitative and quantitative losses, such as grain weight loss, decrease in nutritional value and germination capacity of seeds, and product devaluation (GALLO et al., 2002; LORINI, 2008; SCHEEPENS et al., 2011). Of the insect pests that affect maize during storage, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are responsible for physical, physiological, and sanitary deterioration (LORINI, 2008).

The *R. dominica* beetle is notable because it attacks various cereals and is considered one of the most destructive corn pests owing to its high incidence and the great difficulty in avoiding the losses that it causes to grains (LORINI, 2008). This insect is mainly controlled using synthetic insecticides (organophosphates or pyrethroids) and fumigation, phosphine being remarkable as the main fumigant used (LORINI; GALLEY, 1999; GONÇALVES et al., 2007). However, studies revealed phosphine resistance in Brazilian *R. dominica* populations (ATHIÉ et al., 2001; PIMENTEL et al., 2008), which could render the use of these products unviable if alternative methods are not implemented.

Recently, studies with powders, essential oils, and plant-based extracts have been developed in integrated pest management programs, mainly for the control of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), *R. dominica*, and *S. zeamais* (ALMEIDA et al., 2005; BRITO et al., 2006; NAIMA et al., 2013). In Brazil, there are various plant families with insecticidal action that can be used for their essential oils, such as, Piperaceae, Myrtaceae, Lauraceae, Euphorbiaceae, Verbenaceae, Lamiaceae, Rutaceae, and Poaceae (COITINHO et al., 2011).

The genera *Ocimum* and *Mentha,* belonging to the family Lamiaceae, have recently been investigated with regard to their insecticidal properties against diverse insect pests (KÉITA et al., 2001; KHALFI et al., 2006; LOPEZ et al., 2008; OGENDO et al., 2008; BENAYAD et al., 2012; ESMAILI et al., 2013). Similarly, essential oils of the genus *Citrus*, family Rutaceae, have been described to have insecticidal properties against insect pests of stored grains (PRATES et al., 1998; FOROUZAN et al., 2013; RINGUELET et al., 2014). Several studies involving the family Euphorbiaceae have been also conducted to evaluate its potential in essential oil production, the genus *Croton* being the most tested in controlling insect pests (BRITO, 2014; MAGALHÃES, 2014). However, there is still no information about the use of these essential oils against *R. dominica* in Brazil.

In this context, the present study aims to evaluate the insecticidal effect of essential oils of basil (*Ocimum basilicum*), bitter orange (*Citrus aurantium*), spearmint (*Mentha spicata*), and "velaminho" (*Croton pulegiodorus*) on *R. dominica* adults by fumigation of stored maize grains.

MATERIAL AND METHODS

The study was performed at the Entomology Laboratory of the Serra Talhada Academic Unit (UAST)/Federal Rural University of Pernambuco (UFRPE), Brazil, and the following steps were conducted:

a) Elimination of infestation and balance of grain moisture content - maize grains, 2013/14 crop season, from farmers of Serra Talhada, PE (07° 59′ 31″ S, 38° 17′ 54″ W, and 429 m), were used for rearing and bioassays with *R. dominica*. For this, the grains were cleaned (sieved), dried, and placed in plastic bags and kept in a freezer (-10 °C) for 7 days, to eliminate subsequent infestations of insects from the field. After this period, the grains were placed in glass flasks and kept at room temperature (28 ± 2 °C and 65 ± 5% moisture content).

b) *R.* dominica rearing - the insects were reared in maize grains at 30 ± 2 °C, 60% relative humidity, and 12-hour photoperiod, in biochemical oxygen demand (BOD) climatic chambers, placed in closed containers with perforated plastic lids and the inside lined with thin material to allow gas exchange. They remained confined for 7 days to induce oviposition and were then removed and the containers stored until emergence of the next generation. This procedure was performed for five generations to ensure the number of adults necessary to execute the experiments.

c) Essential oil obtainment - essential oils of basil (*O.* basilicum), bitter orange (*C. aurantium*), and spearmint (*M. spicata*) were acquired from the company Terra-Flor Indústria e Comércio de Aromaterápicos Ltda. Basil essential oil was obtained by the vapor distillation extraction method using the leaves; spearmint essential oil was obtained by vapor distillation using the stems and leaves; bitter orange essential oil was obtained by cold pressing the peels. The plant material used to produce essential oil of "velaminho" (*C. pulegiodorus*) was harvested at Triunfo, PE (07° 50′ 17″ S, 38° 06′ 06″ W, and 1004 m), Brazil. The species was identified by a specialist of the Brazilian Semiarid Herbarium (HESBRA), which was deposited under Voucher - S.S. Matos 104. "Velaminho" essential oil was extracted from fresh leaves by hydrodistillation using a modified Clevenger equipment, with 200 g in 3 L of distilled water for 2 hours. The obtained fractions were separated from water by dichloromethane and dried with anhydrous sodium sulfate (Na_2SO_4), and then placed in a rota-evaporator, where dichloromethane was removed, obtaining the essential oil.

Chromatography provided by the Terra-Flor company indicates that the basil essential oil exhibited estragole (72.67%) and linalool (22.41%) as major compounds. The spearmint essential oil mainly comprised menthol (35.20%), isomenthone (18.71%), and menthyl acetate (6.22%). Bitter orange essential oil exhibited limonene (90.78%) and myrcene (3.87%) as main compounds. According to Dória et al. (2010), "velaminho" essential oil had β-caryophyllene (20.96%), bicyclogermacrene (16.89%), and germacrene D (10.55%) as major components.

d) Fumigation - to evaluate the fumigant effect, glass containers (bomboniere) were used as fumigation chambers (1 L), where 10 *R. dominica* adults were confined. Preliminary tests were conducted with all essential oils, in which several concentrations were evaluated, including intermediaries, with less replicates, and the most promising were used in the definitive tests. Based on the preliminary tests, essential oils were tested at the following concentrations: basil (5, 10, 15, 20, 30, and 40 µL/L of air); bitter orange (10, 20, 30, 40, 50, and 60 µL/L of air); spearmint (5, 10, 15, 20, 30, and 40 µL/L of air), and "velaminho" (10, 20, 30, 50, 70, and 90 µL/ L of air). A porous fabric (tulle) was placed between the lids and respective containers themselves to avoid direct contact of the insects with the oils. The containers were sealed with plastic film (PVC) and adhesive tape in order to prevent vapors from escaping. The experiment was conducted in completely randomized design, with six treatments (concentrations in µL/L of air) and 10 replicates, in which insect mortality was evaluated after 48 hours, following the method proposed by Coitinho et al. (2011). The toxicity ratio (TR) was obtained using the quotient between the LC_{50} of the essential oil that exhibited lowest toxicity and the LC_{50s} of the remaining essential oils. The results were subjected to analysis of variance using the ASSISTAT program and probit analysis using the STATPLUS program to obtain the lethal concentrations (LC_{50} and LC_{100}).

RESULTS AND DISCUSSION

Basil essential oil led to an increase in mortality with increasing concentration, with 100% mortality observed for 30 and 40 µL/L of air (Figure 1A). Bitter orange essential oil caused up to 45% mortality at the highest concentration (Figure 1B). Spearmint led to a linear increase in mortality until the 20 µL/L of air concentration, with 70% insect mortality (Figure 1C). "Velaminho" essential oil also showed an increase in mortality with increasing concentrations, with 74 and 80% mortality at the higher concentrations (Figure 1D).

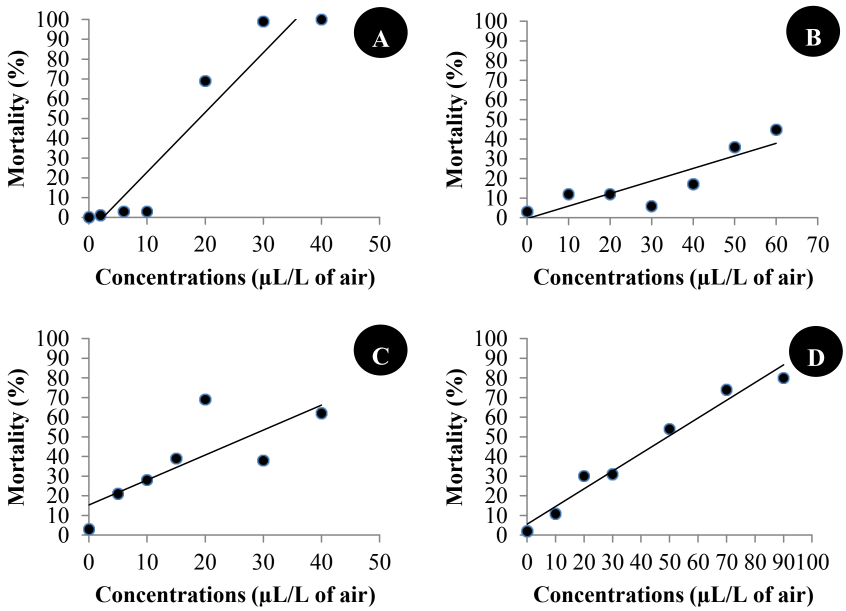

Figure 1. Mortality of *Rhyzopertha dominica* following fumigation with different concentrations of the essential oil of *Ocimum basilicum*: y = 3.0262x – 7.4043 and R^2 = 0.9131 (A); *Citrus aurantium*: y = 0.6393x – 0.4643 and R^2 = 0.7673 (B); *Mentha spicata*: y = 1.2683x – 15.401 and R^2 = 0.6097 (C); and *Croton pulegiodorus*: y = 0.9009x + 5.5374 and R^2 = 0.9716 (D).

According to the determined lethal concentrations (LC_{50} and LC_{100}), toxicity of the essential oils decreased in the following order: *O. basilicum* > *M. spicata* > *C. pulegiodorus* > *C. aurantium* (Table 1). The essential oils of *O. basilicum* and *M. spicata* exhibited the lowest LC_{50} values, which resulted in higher toxicities achieved at lower concentrations, leading to 50% mortality. LC_{100} followed the same principle, with the highest toxicities achieved for the essential oils of *O. basilicum* and *M. spicata*, exhibiting the lowest concentrations, leading to 100% mortality. The toxicity ratio (TR) values were between 3.93 and 1.42 (Table 1). All tested essential oils exhibited potential to cause mortality in *R. dominica*; however, the essential oils of *C. pulegiodorus* and *C. aurantium* required higher concentrations to reach this goal.

Table 1. Toxicity (fumigation) of essential oils of *Citrus aurantium*, *Croton pulegiodorus*, *Mentha spicata*, and *Ocimum basilicum* against *Rhyzopertha dominica* in stored maize grains.

Essential oils	N	Angular coefficient ± SEM	LC_{50} (95%CI) µL/L of air	LC_{100} (95%CI) µL/L of air	TR (LC_{50})	X^2	P
C. aurantium	700	0.0245±0.0125	69.36	130.68	-	23.12	0.0001
C. pulegiodorus	700	0.0242±0.0075	48.66	110.57	1.42	3.30	0.0001
M. spicata	700	0.0255±0.0163	27.51	86.31	2.52	16.92	0.0001
O. basilicum	700	0.1583±0.0294	17.67	27.15	3.93	73.47	0.0001

N = number of individuals; SEM = standard error of the mean; CI = confidence interval; TR = toxicity ratio; χ^2 = chi-square; P = probability.

Several studies with essential oils from species of the genus *Ocimum* exhibited satisfactory results regarding their insecticidal effect against insect pests. Kéita et al. (2001) evaluated the fumigant effect of *O. basilicum* and *O. gratissimum* in controlling *C. maculatus* and obtained 80 and 70% mortality with 25 µL, respectively. Rozman et al. (2007) reported toxicity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *R. dominica,* and *S. oryzae* in fumigation with linalool. For *R. dominica*, linalool was highly effective and caused 100% mortality at the lowest used concentration (0.1 mL/720 mL of volume). Ogendo et al. (2008) obtained 98, 99, and 100% mortality, respectively, against *R. dominica*, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), and *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae), in using 1 µL/L of air of *O. gratissimum* essential oil.

Relevant results have been described for *Mentha* essential oils in controlling diverse pests of stored products. Khalfi et al. (2006) observed an insecticidal effect of the composition of *M. spicata* essential oil in different proportions of 1.8 cineol and carvone against *R. dominica.* The results indicated that insecticidal activity of this essential oil led to a synergistic effect of both compounds, since composition of the essential oil differed depending on the extraction time. Benayad et al. (2012) evaluated the chemical composition and insecticidal effect of essential oils of *M. suaveolens* and *M. pulegium* against *S. oryzae* and *R. dominica*. The essential oils were very toxic for the two Coleoptera species within the first 24 hours, with 100% mortalities when 50 µL and 12 µL were used, respectively.

The essential oils of bitter orange and "velaminho" exhibited higher concentrations than did the other oils used to cause mortality in *R. dominica*. Essential oils from fruit peels of some citrus species were reported to have insecticidal properties against insect pests of stored grains. Prates et al. (1998) evaluated cineol (from *Eucalyptus* spp.) and limonene (from *Citrus* spp.) to determine possible fumigant activity, contact, and ingestion against *R. dominica* and *T. castaneum*. These authors observed that limonene was more effective against *T. castaneum* than *R. dominica*. The toxic effects of these substances are mediated by penetrating the insect body through the respiratory system (via a fumigant), the cuticle (by contact), or the digestive system (via ingestion). Forouzan et al. (2013) evaluated the potential of essential oil from *Citrus reticulata* peels against *R. dominica* during different exposure periods and concluded that this essential oil was indicated as a good fumigant against adults of this insect.

Brito (2014) evaluated the effect of essential oils of *C. pulegiodorus* and *O. basilicum* on *C. maculatus* and *Zabrotes subfasciatus* Boh. (Coleoptera: Chrysomelidae) at different concentrations (0.0, 5, 10, 15, and 20 µL/L of air). According to this author, the fumigant effect of *O. basilicum* caused 100% mortality in *C. maculatus*, but there was no significant difference for the concentrations of *C. pulegiodorus* essential oil. According to the same author, 100% mortality in *Z.*

subfasciatus was observed for all used concentrations of both essential oils. Using essential oils of *C. pulegiodorus* and *O. basilicum* against *S. zeamais* and *T. castaneum*, Magalhães (2014) observed 90% mortality in *S. zeamais* at 20 µL concentration of *C. pulegiodorus* essential oil. On the other hand, there was no significant difference between the tested concentrations against *T. castaneum*. Campos et al. (2014) observed 100% mortality in *C. maculatus* adults using essential oil of *Baccharis articulata.*

Insect mortality is only one of the effects to be achieved in pest control with insecticidal plants (HUANG et al., 2002; KETOH et al., 2005). According to these authors, these plants also affect feeding, oviposition, and growth, and lead to decrease in the emergence of adults, among other effects not occurring with conventional insecticides, which are usually more toxic and cause insect mortality only.

CONCLUSIONS

Essential oils of *O. basilicum*, *C. pulegiodorus*, and *M. spicata*, at the highest concentrations, cause mortality higher than 70% in *R. dominica*, whereas the essential oil of *C. aurantium* causes mortality lower than 45%.

Oils of *O. basilicum* and *M. spicata* exhibit the lowest LC_{50} and LC_{100} values for mortality of *R. dominica*.

All studied oils in the fumigation bioassays demonstrate potential to control *R. dominica.*

ACKNOWLEDGEMENTS

The authors would like to thank UFRPE, FACEPE and CNPq for financial support.

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