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Bioactivity of Five Essential Oils Against *Bruchidius incarnatus* (Bohemann, 1833)

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Abstract

In the world, the faba bean beetle *Bruchidius incarnatus* (Coleoptera: Bruchidae) is an important insect-pest, especially on faba bean *Vicia faba* (*Leguminosae*) and it can infest field crops and cause severe damage in storage. Essential oils can be an alternative method to synthetic insecticides for pest management, due to their efficiency and environmental safety. The aim of the current study was to evaluate the toxicity and repellent activity of essential oils of camphor (*Eucalyptus globules*), castor (*Ricinus communis*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and mustard (*Brassica rapa*) against *B. incarnatus* adults. The treatments which contained essential oils at 0.5, 1, 2 and 4% and acetone (control) were applied. All essential oils with 4% concentration repelled the *B. incarnatus* adult except castor oil. The percentage of repellence was higher when used essential oil of cinnamon with 2 and 4% concentration compared with other essential oils and concentrations. In residual film experiment, the cinnamon oil had the highest toxicity rate on *B. incarnatus* adult fallowed by clove, camphor, mustard and the lowest effect was by castor oil. Based on our results, I can conclude that essential oils of camphor, cinnamon, clove and mustard have potential for use in the integrated management of *B. incarnatus* adult.

Keywords: botanical insecticides, essential oil, faba bean beetle, repellency, storage grains, toxicity

Introduction

The faba bean beetle, *Bruchidius incarnatus* (Coleoptera: Bruchidae) is associated with faba bean storage, where it can attack the whole faba bean grains in field and storeroom. Traditional organophosphates, such as malathion and pirimiphos-methyl are the most commonly used residual insecticides in stored grains (Arthur, 1996; Santos *et al.,* 2009). Chemical insecticides can cause pest resistance, environmental and food contamination and toxicity to non-target organisms (Pimentel *et al.*, 2009; Tavares *et al.*, 2010). Plants produce secondary metabolites many of which can have insecticidal properties, as an alternative to synthetic insecticides (Potenza *et al.*, 2004). Plant extracts and essential oils have traditionally been used to kill or repel stored product insects (Arabi *et al.*, 2008; Fouad *et al.*, 2012; Tapondjou *et al.*, 2005; Tinkeu *et al.*, 2004). The insecticidal constituents of many essential oils against stored product insects are mainly monoterpenoids such as limonene, linalool, terpineol, carvacrol and myrcene (Ahn *et al.*, 1998; Regnault-Roger and Hamraoui, 1995). Essential oils of several medical plant displayed considerable toxic, fumigant and repellent effects on adults of *Bruchidae* family (Mahfuz and Khalequzzaman, 2007; Mahmoudvand *et al*., 2011; Sabbour and Abd-El-Aziz, 2010).

The objective of this study was investigation the adulticidal of essential oil of camphor (*Eucalyptus globulus*), castor (*Ricinus communis*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and mustard (*Brassica rapa*) plants on *B. incarnatus* adult.

Materials and methods

The insect

Parent adults of faba bean beetle, *Bruchidius incarnatus* were obtained from laboratory stock cultures maintained at plant protection department, Faculty of Agriculture, Sohag University, Sohag, Egypt. They were reared in an environmentally controlled room at 25±2°C, 70±10% relative humidity (RH) and darkness. The food media used was whole faba bean grains.

Essential oils

The essential oils of camphor (*Cinnamomum camphora*), castor (*Ricinus communis*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and mustard (*Brassica rapa*) were purchased from an commercial company (Obour City, Egypt).

The repellency test

The test was done in Petri dishes (9 cm diameter), containing filter papers inside (Whatman Nº 1, 9 cm diameter) in the dimension of the dishes. Solutions were prepared at concentrations of 0.5, 1, 2 and 4%. On one half of filter paper, uniformly, 0.5 mL of each concentration of the essential oils was applied, and on the other half only acetone

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was applied. The treated and control half-discs were left at 10 minutes for the solvent to evaporate. On the center of each dish, 10 newly unsexed adults of *B. incarnatus* were placed. The treatments were repeated ten times. The repellency assay was placed in an environmentally controlled room at 25±2ºC, 70±10% RH and darkness. The number of beetles present in the control half (NC) and the treated half (NT) were recorded after 2 and 4 hours (h) (Olivero-Verbel *et al.*, 2010).

Contact with a treated surface

Using a precision microsyringe, one mL of either each oil solution at 0.5, 1, 2 and 4% or acetone (control) was applied to the surface of a Petri dish (9 cm diameter, surface 63.6 cm^2) corresponding to dosages of 0.08, 0.16, 0.32 and 0.64 μL of oil/cm^2. Each dish was left without direct sunlight for 10 min, after which 10 newly unsexed adults *B. incarnates* were placed in each one. The dishes were closed with a glass cover and kept in an environmentally controlled room (25±1°C, 70±10% RH and darkness). The mortality (%) was evaluated 24, 48, 72 and 96 h after starting the test (Tapondjou *et al.*, 2005).

The design was entirely randomized with five oils with five concentrations and ten replications for each, with 10 adults of *B. incarnates* for each replicate. Data obtained were corrected using Abbott's formula (1925).

Statistical analysis

The data from the toxicity of the essential oils on contaminated surfaces were calculated using PROBIT analysis (Finney, 1971). The median lethal dose (LD_{50}) was obtained by PROC PROBIT model using SAS software (SAS Institute 2002). The data of repellent test were compared by the paired t-test at 5% probability using SAS software (SAS Institute, 2002). The percentage of repellency (PR) values were classified into classes of repellency 0, I, II, III, IV or V, where, class 0 (PR ≤ 0.1%), class I (PR = 0.1-20%), class II (PR = 20.1-40%), class III (PR = 40.1-60%), class IV (PR = 60.1-80%) and class V (PR = 80.1-100%), and negative PR values were treated as zero (Juliana and Su, 1983; Obeng-Ofori and Reichmuth, 1997; Benzi *et al.*, 2009).

Results and discussion

Percentages of repellence (PR) values are shown in Fig. 1, Fig. 2 and Tab. 1. Four essential oils exhibited repellent activity against *B. incarnates* adult after 2 and 4 h. Data

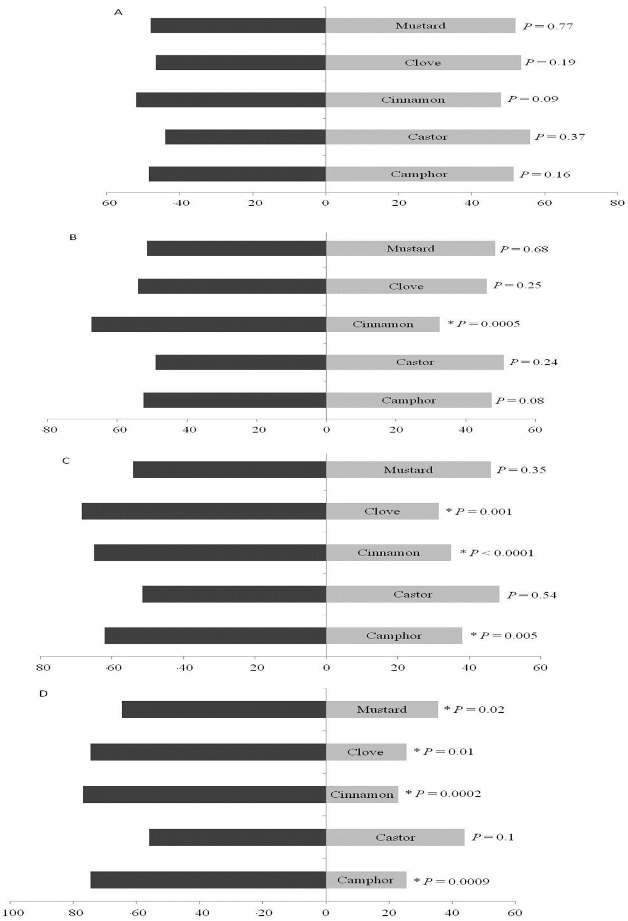

Fig. 1. Preference (%) of *Bruchidius incarnatus* for a half filter paper treated or not with five essential oils with concentrations (A) 0.5 % (B) 1 %, 2 % and (c) 4 % after 2 h, in free choice test. * Significant values at 5 % probability by t-paired test ($p < 0.05$)

Tab. 1. Mean percent repellency (PR) values for five essential oils tested on adults of *Bruchidius incarnatus* (*Coleoptera: Bruchidae*) in free-choice test

Concentrations / Essential oils	After 2 hours				After 4 hours			
	0.5 %	1 %	2 %	4 %	0.5 %	1 %	2 %	4 %
Camphor	13 I	21 II	30 II	44 III	0	5 I	24 II	49 III
Castor	4 I	8 I	3 I	18 I	0	0	3 I	12 I
Cinnamon	18 I	55 III	65 IV	63 IV	4 I	35 II	30 II	54 III
Clove	0	12 I	43 III	36 II	0	8 I	37 II	49 III
Mustard	4 I	11 I	27 II	37 II	0	3 I	8 I	29 II

Note: Classes of Repellency: class I (PR = 0.1-20 %), class II (PR = 20.1-40 %), class III (40.1-60 %);
Negative PR values were treated as zero

in Tab. 1 showed that cinnamon oil had generally a more effective repellent (63%) after 2 h against adult *B. incarnatus*. However, castor oil had less PR values in all concentrations been used. The rest of essential oils had a moderate repellent action. A non significant difference showed between the essential oils with 0.5% concentration against *B. incarnates* after 2 and 4 h of treatment. In generally, the efficacy in respect of the repellency followed in the order: cinnamon > clove > camphor > mustard > castor.

The classes of repellency were higher with the cinnamon oil at 4% (classes IV) after 2 h of treatment compared with those from essential oils and other essential oils at 0.5, 1 and 2% (classes III, II and I) (Tab. 1).

The LD_{50} for *B. incarnatus* beetles was recorded after 24, 48, 72 and 96 h from the beginning of treatment (Tab. 2). The essential oils from all five medical plants increased the mortality of the *B. incarnatus* adults. The LD_{50} was decreased gradually in all the essential oils with increasing the days of exposure. Cinnamon oil revealed the highest residual toxicity effect followed by clove oil, camphor, mustard and the lowest effect was recorded in case of castor oil.

Our results showed that four of the tested essential oils (cinnamon, clove, camphor and Mustard) had a significant

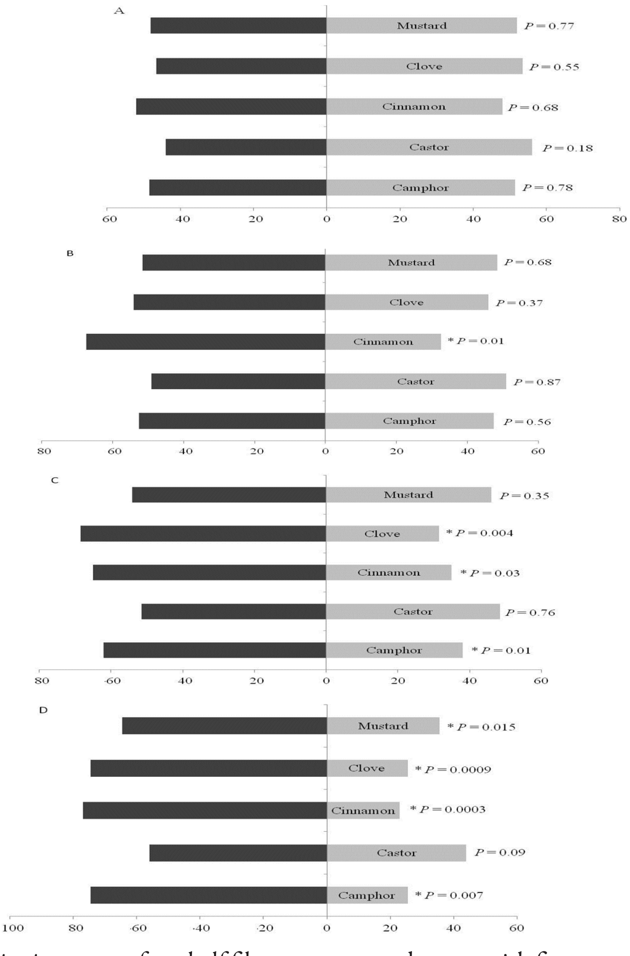

Fig. 2. Preference (%) of *Bruchidius incarnatus* for a half filter paper treated or not with five essential oils with concentrations (A) 0.5 % (B) 1 %, 2 % and (c) 4 % after 4 h, in free choice test. * Significant values at 5 % probability by t-paired test ($p < 0.05$)

Tab. 2. Essential oils, LD_{50} (μL/Cm2) of Camphor, Castor, Cinnamon, Clove and Mustard on a treated surface on the faba bean beetle, *Bruchidius incarnatus* (*Coleoptera: Bruchidae*) adults

Duration (h)	Oils	LD_{50} (μL/Cm^2)	Slope ± SE	X^2	P value
24 h	Camphor	0.64 (0.52-0.88)	2.92±0.45	66.94	0.003
	Castor	2.08 (1.20-7.60)	1.82±0.40	41.95	0.30
	Cinnamon	0.52 (0.43-0.65)	2.22±0.25	44.57	0.21
	Clove	0.86 (0.62-1.56)	2.15±0.39	66.08	0.003
	Mustard	0.67 (0.53-0.98)	2.59±0.41	69.82	0.001
48 h	Camphor	0.62 (0.51-0.81)	2.31±0.26	30.38	0.81
	Castor	1.44 (0.96-3.22)	1.91±0.35	28.01	0.88
	Cinnamon	0.37 (0.32-0.43)	2.49±0.24	44.74	0.21
	Clove	0.50 (0.42-0.61)	2.43±0.26	47.78	0.13
	Mustard	0.59 (0.48-0.80)	2.68±0.38	62.86	0.007
72 h	Camphor	0.51 (0.43-0.62)	2.45±0.26	37.59	0.49
	Castor	1.25 (0.88-2.40)	2.05±0.35	26.79	0.19
	Cinnamon	0.30 (0.27-0.34)	2.99±0.25	46.65	0.16
	Clove	0.41 (0.35-0.51)	2.80±0.33	57.95	0.02
	Mustard	0.60 (0.48-0.83)	2.35±0.33	57.72	0.02
96 h	Camphor	0.46 (0.39-0.55)	2.47±0.26	35.32	0.59
	Castor	0.94 (0.73-1.42)	2.42±0.36	34.39	0.64
	Cinnamon	0.30 (0.26-0.33)	3.02±0.26	48.87	0.11
	Clove	0.38 (0.33-0.46)	3.04±0.34	58.21	0.02
	Mustard	0.55 (0.46-0.73)	2.71±0.37	60.72	0.01

repellent and toxic effect on *B. incarnates* adults, suggests a wide spectrum of action from these essentials oils. Cinnamon oil had a highest possess repellency as well as toxicity effects against *B. incarnates* adult followed by clove > camphor > mustard and a lowest possess repellency as well as toxicity effects was castor oil. Cinnamon powder also was showed generally a more repellent effective on adults of *Sitophilus granarius*, *Rhyzopertha dominica* and *T. castaneum* (Shayesteh and Ashouri, 2010). The powders of *Piper nigrum*, *Capsicum annuum* and *C. zeylanicum* (Cinnamon plant) showed a repellent effect on *S. zeamais* (Salvadores *et al.*, 2007). Cinnamaldehyde isolated from cinnamon oil was considered contact toxicity to both *T. castaneum* and *S. zeamais* (Huang and Ho, 1998).

The clove oil had also repellent activity on three important stored grain insect pests, *R. dominica*, *S. oryzae* and *T. castaneum* (Zeng *et al.*, 2010). As well as, oil of clove is toxic to *S. oryzae* and *R. dominica* (Sighamony *et al.*, 1986). However, extracts from clove plant had insecticidal effect to *T. castaneum* and *S. zeamais* (Ho *et al.*, 1994), with the main chemical components of clove essential oil are phenylpropanoids such as carvacrol, thymol, eugenol, eugenol acetate, iso-eugenol and caryophyllene (Chaieb *et al.*, 2007; Olivier *et al.*, 1999).

The monoterpene camphor might have broad insecticidal activity against stored-product insects and act as a fumigant in *Asplenium haussknechtii* oil. Camphor from several Artemisia species reported that is toxic against stored-product beetles (Dunkel and Sears, 1998; Kordali *et al.*, 2006; Negahban *et al.*, 2007). Effect of mustard oil also has been reported on *B. incarnatus* (Sabbour and Abd-El-Aziz, 2010), *Callosobruchus chinensis* (Ali *et al.*, 1983) and *S. zeamais* (Costa *et al.*, 2006). The presence of Allyl isothiocyanate (AITC), the main toxic compound formed from allyl glucosinolate hydrolysis (Mayton *et al.*, 1996), was considered insecticidal substance biofumigation (Noble *et al.*, 2002). The highest concentrations of AITC are found in some mustard, horseradish and wasabi species (Olivier *et al.*, 1999; Yu *et al.*, 2003).

In our results, the LD_{50} value was decreased gradually in all the essential oils with increasing the days of exposure. This results agree with the results obtained by Arannilewa *et al.* (2006) whose reported that an increase of mortality of *S. zeamais* adult associated with increasing the days of exposure in all concentrations of tested essential oils. Also, Arabi *et al.* (2008) and Ahmed (2006) reported that mortality of *S. oryzae* and *O. surinamensis* adults, respectively, was increased with the increase of the concentrations of camphor oil and increased the time of exposure.

Conclusions

Based on the present study, it could be concluded that essential oils of cinnamon, clove, camphor and mustard pose potential repellent and toxic activity against adults of *B. incarnatus* with higher effective was found by using cinnamon oil. The study demonstrates that these essential oils can play an important role in protection of faba bean grains from adults of *B. incarnatus*.

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