



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(5): 738-742

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Received: 28-07-2018

Accepted: 29-08-2018

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Larvicidal effects of eight essential oils against *Plodia interpunctella* and *Tribolium castaneum*, serious pests of stored products worldwide

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Abstract

Fumigant toxicity of essential oils obtained by hydrodistillation from eight plant species viz. *Artemisia maritima* L., *Cinnamomum zeylanicum* Blume, *Citrus hystrix* DC., *Colebrookea oppositifolia* Sm., *Pelargonium hortorum* Bailey, *Rabdosia rugosa* Wall. Ex Benth, *Thuja occidentalis* L. and *Zanthoxylum armatum* DC. Was tested against different immature stages of *Tribolium castaneum* (Herbst) and *Plodia interpunctella* (Hubner). 8-10 day and 18-20 day old larvae of *P. interpunctella* and *T. castaneum* were used for the bioassay. *R. rugosa* oil gave 80.03±5.6% mortality at 100 µl/ml followed by 65.47±2.9% mortality against 18-20 day old larvae of *T. castaneum* at similar concentration after 120 hrs. At the lowest concentration of 10 µl/ml *R. rugosa* oil produced 53.8.33±2.2% mortality for 8-10 day old larvae and a mortality of 40.55±3.9% respectively was obtained for 18-20 day old larvae of *P. interpunctella* after similar time period. Among eight essential oils tested *R. rugosa* had the highest toxicity followed by *A. maritima*, *C. zeylanicum*, *Z. armatum*, *T. occidentalis*, *P. hortorum*, *C. oppositifolia* and *C. hystrix* against both the insect pests. The results revealed increase in tolerance as the immature stages grow older. 18-20 day old larvae were least susceptible to all the treatments as compared to 8-10 days old larvae of both insects. Larvae of *T. castaneum* were found to be more susceptible for all treatments than *P. interpunctella*.

Keywords: Fumigant toxicity, larvicidal activity, essential oils, immature stages, pests

1. Introduction

Stored cereals, oilseeds stored for future uses and pulses, spices, dried fruits, tree nuts and their processed products are important as food and trade purposes but suffer qualitative and quantitative losses due to insect pests [4, 15, 23, 30]. There are over 600 species of beetles and 70 species of moths capable of causing quantitative and qualitative losses [25]. Indian meal moth, *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae), is distributed world-wide and is a serious stored-product pest of grain and seeds as well as flour and other milled products [20]. Larvae of *P. interpunctella* are able to penetrate and infest a wide range of packaged foods [6] and have a great economic impact due to direct product loss and indirect factors such as cost of pest control and loss of sales due to consumer complaints [27]. *Tribolium* species are major pests of stored grains and grain products in the tropics [10]. The red flour beetles cannot feed on whole undamaged grain; however, often found among dust, fines, and dockage and larvae feed on fine materials and broken grain kernels. During the past few decades, control of these insect pests relies heavily on the use of synthetic pesticides and fumigants. However, with growing evidence many conventional pesticides have adversely affected the environment. Therefore requirements for safer means of pest management have become crucial. Thus the use of safe, low toxicity botanical pesticides is now emerging as one of the prime means to protect crops, their products and the environment from pesticide pollution, a global problem [24]. Botanical insecticides are naturally occurring insecticides that are derived from plants [12]. The insecticidal activity of essential oils and plant extracts against different stored-product pests has been evaluated [28, 26, 31]. Thus, repellents, fumigants, feeding deterrents and insecticides of natural origin are rational alternatives to synthetic insecticides

Many essential oils isolated from various plant species belonging to different genera contain relatively high amount of monoterpenes. Essential oils have potential fumigant activity against a large number of stored product insects [11, 18] and their monoterpenoid components are highly volatile, strongly toxic to insect pests but exhibits very low toxicity to warm-blooded animals [16,17]

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Therefore, the present study was carried out to investigate the fumigation toxicity of eight essential oils extracted from *Artemisia maritima*, *Cinnamomum zeylanicum*, *Citrus hystrix*, *Colebrookea oppositifolia*, *Pelargonium hortorum*, *Rabdosia rugosa*, *Thuja occidentalis* and *Zanthoxylum armatum* against different immature stages of *Tribolium castaneum* and *Plodia interpunctella*.

2. Materials and methods

2.1 Extraction of essential oils

Essential oils were extracted from leaves of *A. maritima*, *C. oppositifolia*, *R. rugosa*, *P. hortorum*, *R. rugosa*, *T. occidentalis* and *Z. armatum*, bark of *C. zeylanicum* and fruit peel of *C. hystrix* from the local areas of Shimla district of Himachal Pradesh, India. The plant material were dried in shade at room temperature (30±5 °C) and grounded by domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample in 1:10 plant material/water volume ratio for 4 hrs distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (2.9% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4°C for further analysis.

2.2 Test insects

Laboratory cultures of *P. interpunctella* and *T. castaneum* were maintained at 28±2 °C and 68±2% relative humidity. Test insects of *T. castaneum* were reared on whole meal wheat flour plus brewer's yeast (19:1) and *P. interpunctella* was reared on a diet of 80% ground rice, 10% glycerin, 5% yeast in plastic containers (30 cm length × 20 cm width × 8 cm height). Mouth of the containers was covered with fine mesh cloth for ventilation as well as to prevent escape of the insects. 8-10 and 18-20 days old larvae of *P. interpunctella* and *T. castaneum* respectively were used in the experiment.

2.3 Fumigant toxicity of essential oils

A stock solution of all the essential oils was prepared by dissolving 100 µl of essential oils in 1 ml of methanol. Plastic jars of 250 ml capacity with screw lids were used as exposure chambers. Different doses of each oil 10, 30, 50 and 100 µl prepared in solvent were applied to a circular filter paper (What man No. 1) and after evaporating the solvent for 5-10 minutes the treated filter paper discs were then introduced into the plastic jars and attached to the inner surface of the

screw lid of the jar by using adhesive tape. 1 ml solvent alone was used as a control. In each jar a small glass Petri dish containing about 10 larvae (8-10 and 18-20 days old) of *T. castaneum* and *P. interpunctella* were introduced. After 48 hrs of exposure to essential oils vapours, the larvae of *T. castaneum* and *P. interpunctella* were transferred to clean vials and larval mortality was recorded after 120 hrs.

2.4 Statistical analysis

All the data concerning mortality were corrected by using Abbott's formula [1]. Tests for fumigant toxicity were performed in triplicate and data presented are mean ± SE. The mean values were compared by one-way ANOVA and Tukey's multiple comparison tests using software SPSS, version 11.5.

3. Results

3.1 Larvicidal activity of essential oils against *T. castaneum*

R. rugosa and *A. maritima* oil were found to be highly effective against larvae of *T. castaneum*. *R. rugosa* oil gave 80.03±5.6 at 100 µl/ml after 120 hrs followed by 72.28±4.9 (50 µl/ml), 70.17±2.3 (30 µl/ml) and 68.33±3.8 (10 µl/ml)% mortality against 8-10 days old larvae whereas 65.47±2.9 (100 µl/ml), 62.09±1.8 (50 µl/ml), 55.17±4.2 (30 µl/ml) and 50.30±2.6 (10 µl/ml)% mortality was obtained for 18-20 days old larvae of *T. castaneum*. 50 µl/ml *A. maritima* and *C. zeylanicum* oil resulted in 72.09±4.4 and 69.16±3.4% mortality among 8-10 days old larvae and mortality decreased to 60.43±4.5 and 57.48±1.9 respectively for 18-20 days old larvae. *Z. armatum* oil at 30 µl/ml produced 61.34±1.8% mortality after 120 hrs for 8-10 days old larvae and 48.32±5.4% mortality for 18-20 days old larvae followed by *T. occidentalis* oil resulted in 58.09±4.8 and 45.10±2.2% mortality for 8-10 and 18-20 days old larvae respectively at similar concentration. 52.26±2.6 and 38.18±5.2% mortality was obtained by *P. hortorum* oil at 10 µl/ml for 8-10 and 18-20 days old larvae after 120 hrs while 50.10±2.3 and 36.21±4.4% mortality was obtained by *C. oppositifolia* oil for the same age of larvae. *C. hystrix* oil at 10 µl/ml gave 45.21±2.4% mortality after 120 hrs for 8-10 days old larvae that reached to 55.03±3.8% at an increased concentration of 100 µl/ml whereas 32.43±1.8 and 45.39±1.4% mortality was obtained for 18-20 day old larvae at similar increased concentrations and time interval (Table 1).

Table 1: Larvicidal activity of eight essential oils against *T. castaneum* (Values are mean± SE).

	% Mortality ± SE		
	Doses µl/ml	Exposure time (120 hrs)	
		8-10 day old larvae	18-20 day old larvae
<i>C. hystrix</i>	10	45.21±2.4 ^a	32.43±1.8 ^b
	30	48.54±3.5 ^a	36.21±3.4 ^b
	50	52.09±1.2 ^b	40.52±4.8 ^a
	100	55.03±3.8 ^b	45.39±1.4 ^a
<i>C. oppositifolia</i>	10	50.10±2.3 ^b	36.21±4.4 ^b
	30	54.17±1.7 ^b	40.22±5.6 ^a
	50	58.32±1.6 ^b	45.32±1.7 ^a
	100	62.07±4.4 ^c	50.54±2.5 ^c
<i>P. hortorum</i>	10	52.26±2.3 ^b	38.18±5.2 ^b
	30	56.23±1.9 ^b	42.21±2.6 ^a
	50	60.09±4.1 ^c	48.03±3.5 ^c
	100	64.34±1.6 ^c	53.29±2.3 ^c
<i>T. occidentalis</i>	10	55.32±3.7 ^b	41.33±4.6 ^a
	30	58.09±4.8 ^b	45.10±2.2 ^c
	50	63.13±1.3 ^c	51.43±1.2 ^c

	100	68.02±5.3 ^d	55.09±4.8 ^c
<i>Z. armatum</i>	10	59.08±5.5 ^b	43.17±2.2 ^a
	30	61.34±1.8 ^b	48.32±5.4 ^c
	50	65.23±3.5 ^d	54.09±1.7 ^c
	100	71.23±2.6 ^d	58.44±4.9 ^c
<i>C. zeylanicum</i>	10	63.23±2.3 ^c	45.08±1.2 ^a
	30	65.48±1.5 ^d	51.33±5.4 ^c
	50	69.16±3.4 ^d	57.48±1.9 ^c
	100	74.45±2.4 ^{bc}	60.00±4.5 ^{bc}
<i>A. maritima</i>	10	65.24±1.9 ^d	48.11±2.8 ^c
	30	68.35±2.1 ^d	54.22±5.2 ^c
	50	72.09±4.4 ^d	60.43±4.5 ^{bc}
	100	76.01±3.8 ^{bc}	62.48±1.6 ^{bc}
<i>R. rugosa</i>	10	68.33±3.8 ^d	50.33±2.6 ^c
	30	70.17±2.3 ^d	55.17±4.2 ^c
	50	75.28±4.9 ^{bc}	62.09±1.8 ^{bc}
	100	80.03±5.6 ^{cd}	65.47±2.9 ^{cd}
Control		0.00±0.00 ^{ab}	0.00±0.00 ^{ab}

% values are mean ($n = 3$) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

3.2 Larvicidal activity of essential oils against *P. interpunctella*

Essential oil of *R. rugosa* and *A. maritima* at a lowest concentration of 10 µl/ml produced 53.83±2.2 and 55.50±4.1% mortality for 8-10 days old larvae of *P. interpunctella* and a mortality of 40.55±3.9 and 38.09±4.8% respectively was obtained for 18-20 days old larvae after 120 hrs. *C. zeylanicum* oil produced 64.33±2.1% mortality for 8-10 day old larvae at 100 µl/ml after 120 hrs followed by 58.16±4.4 at 50 µl/ml, 55.35±2.5 at 30 µl/ml and 52.09±1.1 at 10 µl/ml. Whereas for *Z. armatum* oil 60.32±3.9% mortality was obtained for 8-10 days old larvae at 100 µl/ml, 55.52±4.6 at 50 µl/ml, 52.43±1.3 at 30 µl/ml and 50.06±2.2% at 10 µl/ml. *T. occidentalis* oil at 30 µl/ml gave 48.29±4.7% mortality against 8-10 day old larvae after 120 hrs and also decreased to 32.15±3.4 with the increasing age of 18-20 days

old larvae followed by *P. hortorum* oil giving 45.32±1.4 and 30.43±2.2% mortality for the same age of larvae after 120 hrs. At a highest concentration of 100 µl/ml *C. oppositifolia* oil gave 50.32±4.2% mortality among 8-10 day old larvae and 34.14±3.6 in 18-20 day old larvae followed by *C. hystrix* oil producing 45.43±1.7 and 30.17±1.3% larval mortality respectively after 120 hrs of treatment (Table 2). Among eight essential oils tested, *R. rugosa* had the highest toxicity followed by *A. maritima*, *C. zeylanicum*, *Z. armatum*, *T. occidentalis*, *P. hortorum*, *C. oppositifolia* and *C. hystrix* against insect pests. The results obtained also showed that tolerance increases as the immature stages grow older and 18-20 day old larvae were least susceptible to all the treatments as compared to 8-10 day old larvae. Larvae of *T. castaneum* were found to be most susceptible towards all the treatments than *P. interpunctella*.

Table 2: Larvicidal activity of eight essential oils against *P. interpunctella* (Values are mean± SE).

	% Mortality ± SE		
	Exposure time (120 hrs)		
	Doses µl/ml	8-10 day old larvae	18-20 day old larvae
<i>C. hystrix</i>	10	38.18±4.5 ^b	20.28±4.8 ^a
	30	40.33±2.6 ^b	23.06±2.4 ^a
	50	42.18±5.3 ^a	26.13±3.7 ^a
	100	45.43±1.7 ^a	30.17±1.3 ^b
<i>C. oppositifolia</i>	10	40.55±5.7 ^b	24.43±5.1 ^a
	30	42.32±3.3 ^a	28.06±2.4 ^a
	50	45.48±1.5 ^a	30.17±1.8 ^b
	100	50.32±4.2 ^c	34.14±3.6 ^c
<i>P. hortorum</i>	10	43.10±2.2 ^a	28.12±2.8 ^a
	30	45.32±1.4 ^a	30.43±2.2 ^b
	50	48.22±4.4 ^c	32.66±1.7 ^b
	100	54.19±1.2 ^c	37.22±4.3 ^d
<i>T. occidentalis</i>	10	46.13±2.8 ^a	30.22±1.2 ^b
	30	48.29±4.7 ^a	32.15±3.4 ^b
	50	52.18±3.4 ^c	35.37±1.5 ^d
	100	57.07±5.2 ^d	40.03±5.3 ^d
<i>Z. armatum</i>	10	50.06±2.2 ^c	32.26±4.8 ^b
	30	52.43±1.3 ^c	35.08±3.5 ^d
	50	55.52±4.6 ^c	38.21±1.6 ^d
	100	60.32±3.9 ^d	43.51±5.7 ^{bc}
<i>C. zeylanicum</i>	10	52.09±1.1 ^c	34.52±1.9 ^d
	30	55.35±2.5 ^c	38.09±2.4 ^d
	50	58.16±4.4 ^d	40.47±5.6 ^d
	100	64.33±2.1 ^{bc}	45.03±1.6 ^{bc}
<i>A. maritima</i>	10	55.50±4.1 ^c	38.09±4.8 ^d

	30	58.12±1.3 ^d	40.08±3.2 ^d
	50	60.08±5.4 ^d	43.11±2.6 ^{bc}
	100	68.17±3.2 ^{bc}	48.09±1.9 ^{cd}
<i>R. rugosa</i>	10	58.33±2.2 ^d	40.55±3.9 ^d
	30	62.29±4.8 ^d	42.31±5.5 ^d
	50	65.40±3.1 ^{bc}	48.08±2.4 ^{cd}
	100	71.04±1.8 ^{cd}	54.22±4.2 ^{de}
Control		0.00±0.00 ^{ab}	0.00±0.00 ^{ab}

% values are mean ($n = 3$) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

4. Discussion

Different doses of 10, 30, 50 and 100 µl/ml of essential oils were tested against 8-10 and 18-20 days old larvae of two insect species and larval mortality was recorded after 120 hrs. Many researchers pointed that some of plant essential oils had shown strong larvicidal effects against storage pests [29, 5, 2, 3]. *R. rugosa* and *A. maritima* oil at 100 µl/ml were found to be highly effective with highest mortality of 80.03±5.6 and 76.01±3.8% respectively against 8-10 day old larvae of *T. castaneum* followed by 71.04±1.8 and 68.17±3.2, for *P. interpunctella*. Whereas against 18-20 day old larvae of *T. castaneum* 65.47±2.9 and 62.48±1.6% mortality was obtained followed by 54.22±4.2 and 48.09±1.9% mortality for *P. interpunctella* for same age of larvae and concentrations of essential oils after 120 hrs. Hexane extracts of star anise produced very low mortality in 10, 12 and 14-days old *T. castaneum* larvae and there was a significant tendency of the larvae to become less susceptible as they grew older [9]. The results obtained during the present investigation also showed that tolerance increases as the immature stages grow older and 18-20 day old larvae were less susceptible to all the treatments as compared to 8-10 day old larvae. Similarly adults of *T. castaneum* were more susceptible to garlic oil than the larvae, which became progressively more tolerant with age [8]. Petroleum ether extracts from fruits of *Piper nigrum* and seeds of *Jatropha curcas* had LC₅₀ values of 12.5 and 13.2 µl/ml against larvae of *Corcyra cephalonica* [13]. *T. occidentalis*, *Z. armatum* and *C. zeylanicum* oil at similar concentration showed moderate activity and resulted in 56-60% mortality against 18-20 day old larvae of *T. castaneum* followed by 40-45% in *P. interpunctella*. *P. hortorum* and *C. oppositifolia* obtained highest mortality of 64.34±1.6 and 62.07±4.4% against *T. castaneum* among 8-10 day old larvae and 53.29±2.3 and 50.54±2.5% in 18-20 day old larvae. In related studies fumigant toxicity of the essential oils from *Lavandula hybrida*, *Rosmarinus officinalis* and *Eucalyptus globulus* against the larvae and pupae of *Acanthoscelides obtectus* and a significant decrease in susceptibility of insects to essential oil vapours with increasing age was observed [13]. In the present study *C. hystrix* oil revealed a weaker toxicity towards the larvae of all insect species. Larvicidal activity of five plant essential oils against *T. castaneum* and *T. confusum*, and found that *E. viminalis* showed highest and *E. sargentii* lowest toxicity to the larval stages and also found that different concentration at different exposure time resulted in variable mortality in pest species [7]. Similar findings were reported during the present investigations. Toxicity of essential oils against insect pests investigated in our study may be attributed to their major monoterpenes components. Some major compounds of the test oils, such as carvacrol, camphor, 1, 8-cineole, α-pinene, p-cymene, piperitenone oxide, and terpineol possessed insecticidal effects against the test insects [21, 19, 14].

5. Conclusion

The present study concluded that the eight essential oils used resulted in high mortality of both the larval stages of *P. interpunctella* and *T. castaneum*. Of the eight essential oils tested *R. rugosa* was found to be highest toxic followed by *A. maritima*, *C. zeylanicum*, *Z. armatum*, *T. occidentalis*, *P. hortorum*, *C. oppositifolia* and *C. hystrix* against both the insect pests. As the immature stages grow older they tend to become more resistant. 8-10 day old larvae were more susceptible to all the treatments as compared to 18-20 days old larvae. Larvae of *T. castaneum* showed more susceptibility towards all treatments than *P. interpunctella*.

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