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## Ecofriendly approach for rice weevil (*Sitophilus oryzae*) (Coleoptera: Curculionidae) management using fumigant oils

NAVEENKUMAR B PATIL\*, TOTAN ADAK, G GURU PIRASANNA PANDI,  
G BASANA GOWDA, MAYABINI JENA

*Crop Protection Division, ICAR–National Rice Research Institute, Cuttack, Odisha 753 006, India*

### ABSTRACT

Laboratory studies were conducted to evaluate the contact and fumigant activities of some essential oils, viz. orange oil (*Citrus sinensis* L.), eucalyptus oil (*Eucalyptus oblique* L'Her) and cinnamon oil (*Cinnamomum verum* Presl) against rice weevil, *Sitophilus oryzae* (L.). In contact bioassay at 24 h after treatment, the highest mortality (95.8%) was recorded at 0.75  $\mu\text{l}/\text{cm}^2$  with eucalyptus oil, followed by orange oil (93.40%) which was at par with eucalyptus oil (93.2%) at 0.50  $\mu\text{l}/\text{cm}^2$ . On the other hand, cinnamon oil at all the tested concentrations (0.75, 0.50 and 0.25  $\mu\text{l}/\text{cm}^2$ ) showed significantly less effect on *S. oryzae* by registering 16.63, 13.33 and 6.67% mortality, respectively at 24 h after treatment. In fumigation assay also, eucalyptus oil recorded the highest mortality of 26.1% followed by orange oil (13.3%) and cinnamon oil (10.43%) after 24 h exposure at the highest concentration used in the study (2  $\mu\text{l}/\text{cm}^2$ ). Fumigation toxicity trend remained same at different time intervals (48, 72, 96 and 120 h after treatment) for all the treatments, wherein increasing trend of mortality was observed as the time lapsed. Even 120 h after treatment, only eucalyptus oil was able to kill more than 80% of adult beetles in comparison to 65 and 50% mortality, respectively, for orange and cinnamon oil at the highest concentration tested (2  $\mu\text{l}/\text{cm}^2$ ). Thus, eucalyptus oil has higher contact and fumigant toxicity potential, and it can be included in the IPM programmes for effective stored grain pest management.

**Key words:** Essential oils, Fumigant action, Management, Rice weevil, Rice storage, Storage pest, Toxicity

Storage insects alone cause post-harvest losses of 10% annually in developing countries (Nanda et al., 2012). As a control measure, many synthetic chemical insecticides and fumigants are being widely used against various insect pests. However, due to their slow biodegradable nature which results in environmental pollution and also with concern to human health hazards, many insecticides have been replaced by modern ones (Daglish et al., 2013). Further, due to the problem of resistance to insecticides, there is a growing demand to develop newer eco-friendly and safer control approach for stored pest management. Natural products are an important source of novel active compounds that can be used to control stored grain insect pests (Rajendran and Sriranjini, 2008; Rajashekar et al., 2013). Hence there is a need to develop selective products of natural origin for the

management of storage pests in rice.

The most widespread and destructive insect pest of stored grains is rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Control of this pest is primarily dependent on repeated application of synthetic insecticides (Hasan and Reichmuth, 2004). Methyl bromide and aluminium phosphide fumigants have been used for decades to control stored pests (Islam et al., 2009) and belong to the most effective treatments to protect stored food, feedstuffs and other agricultural commodities. Growers are moving away from using methyl bromide as post-harvest fumigant because of its ozone-depleting nature. Repeated use of phosphine leads to development of pest resistance (Ignatowicz, 1999).

Plant essential oils, traditionally been used to kill or repel insects (Isman, 2006) are being considered as an alternative to stored-grain conventional pesticides because of their low toxicity to warm-blooded mammals and their high volatility (Shaaya et al., 1997;

\*Corresponding author e-mail: [patil2850@gmail.com](mailto:patil2850@gmail.com)

Li and Zou, 2001). The toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used (Don-Pedro, 1996; Lee et al., 2004). Among the essential oil components, the monoterpenoids have drawn the greatest attention for fumigant activity against stored-product insects (Ahn et al., 1998; Rajendran and Sriranjini, 2008). Several reports indicate that monoterpenoids cause insect mortality by inhibiting acetylcholine esterase enzyme (AChE) activity (Houghton et al., 2006). The monocyclic monoterpene 1, 8-Cineole (eucalyptol) is the major component of different species of *Eucalyptus* having fumigant action against *Tribolium castaneum* (Herbst) (Rajendran and Sriranjini, 2008). In the present study, insecticidal activity of three essential oils viz. eucalyptus, orange and cinnamon oil were assayed against the major stored-product insect *S. oryzae* under laboratory conditions.

## MATERIALS AND METHODS

### *Insects*

The insect species were collected from the NRRI paddy godown and the insect culture was maintained in the Grain Entomology Laboratory of the institute. Throughout the study period, culture was maintained at  $28 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  RH. Initially, 50 pairs of freshly emerged adults were placed in a jar containing rice (*Oryza sativa* L.) grains (0.5 kg). The open end of jars were covered with muslin cloth and remained sealed for a maximum period of 7 days to allow mating and oviposition. Then parental stocks were removed and the remaining content of each jar (rice grains and freshly laid eggs) was kept for further multiplication and insects were collected and reused for sub-culturing. The subsequent progenies of beetles were used for conducting the experiment.

### *Test materials*

Industrially extracted oils of eucalyptus (*Eucalyptus oblique* L'Her), orange (*Citrus sinensis* L.) and cinnamon oil (*Cinnamomum verum* Presl) were obtained from market (NICE chemicals Private Limited). Only 95% pure oils were used throughout the experiment. The pure essential oils were diluted with acetone to obtain the required doses for contact and fumigant activity assay.

### *Bioassays*

*Contact toxicity of plant oils:* The insecticidal

activity of eucalyptus, orange and cinnamon oils against adults of *S. oryzae* L. was evaluated by direct contact application assay. The experiment was carried out in a completely randomized design with three replications. All tests were conducted in petridishes with a surface area of  $63\text{ cm}^2$ . One day before the tests, bottom of the dishes were covered with the CEM I 52.5 N material to create the concrete surface (Kavallieratos et al., 2016). Each essential oil was prepared in acetone (100  $\mu\text{l}$ ) at different concentrations (0.25, 0.50 and  $0.75\ \mu\text{g}/\text{cm}^2$ ). The concrete surface of individual petridishes was sprayed with different concentration of oil solution as a fine mist using Atlas hand atomizer, and 10 adults each of *S. oryzae* (7–10 days old) were placed in each petridish and covered with a lid. Control was sprayed with 100  $\mu\text{l}$  acetone only. Adult mortality was recorded 24, 48, 72, 96 and 120 h after treatments.

### *Fumigant activity of the tested plant oils*

The vapour toxicity of the eucalyptus, orange and cinnamon oils were evaluated as per Kim et al. (2003) and Usha Rani and Rajasekharreddy (2010). In brief, small diet cups (3.6 cm diameter  $\times$  4 cm) were used as fumigation chambers and groups of 10 adults were placed in diet cups and covered with a nylon 60 mesh cloth. Each filter paper (Whatman No. 1, cut into 10  $\text{cm}^2$  pieces) treated with each test oils (2, 1, 0.50 and  $0.25\ \mu\text{l}/\text{cm}^2$ ) previously dissolved in acetone (100  $\mu\text{l}$ ), was placed at the bottom of a polyethylene cup (5.0 cm diameter  $\times$  9 cm), and a diet cup containing adult insects was put into the polyethylene cup. This prevented direct contact of the test adults with the test compound. Each polyethylene cup was then sealed with a lid. Controls received only 100  $\mu\text{l}$  acetone. All the tests were carried out at  $28 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  RH. Mortality was ensured by probing insect body with a slender paintbrush. Dead insects were counted every 24 h for a total period of 120 h post-treatment. There were three replicates per treatment.

Statistical analysis of the toxicity data was performed in MSTAT-C. Mortality data were converted into arcsine values to perform one-way ANOVA, to find the significant differences (Gomez and Gomez, 1984).

## RESULTS

### *Fumigant toxicity of the tested plant oils*

Mortality of *S. oryzae* adults due to fumigation at different dosages of eucalyptus, orange and cinnamon oil ranged from 3.27 to 82.97% (Table 1). At 24 h after treatment, eucalyptus oil recorded significantly highest mortality (26.10%), followed by orange oil

CONTROLLED ATMOSPHERE AND FUMIGATION IN STORED PRODUCTS

Table 1 Fumigant toxicity of essential oils against *Sitophilus oryzae* adults at 28°C

Treatments	Per cent mortality (hours after treatment)				
	24	48	72	96	120
Eucalyptus oil @ 2 µl/cm <sup>2</sup>	26.10 (30.72 <sup>a</sup> )	33.23 (35.20 <sup>a</sup> )	43.77 (41.42 <sup>a</sup> )	56.37 (48.66 <sup>a</sup> )	82.97 (65.62 <sup>a</sup> )
Eucalyptus oil @ 1 µl/cm <sup>2</sup>	10.30 (18.72 <sup>c</sup> )	13.10 (21.22 <sup>c</sup> )	16.50 (23.97 <sup>d</sup> )	20.03 (26.59 <sup>c</sup> )	53.50 (47.01 <sup>d</sup> )
Eucalyptus oil @ 0.5 µl/cm <sup>2</sup>	6.57 (14.85 <sup>d</sup> )	12.93 (21.08 <sup>c</sup> )	13.13 (21.25 <sup>e</sup> )	13.43 (21.50 <sup>e</sup> )	26.57 (31.03 <sup>e</sup> )
Eucalyptus oil @ 0.25 µl/cm <sup>2</sup>	3.50 (10.78 <sup>e</sup> )	3.37 (10.57 <sup>e</sup> )	3.20 (10.30 <sup>i</sup> )	6.53 (14.81 <sup>e</sup> )	26.37 (30.90 <sup>e</sup> )
Orange oil @ 2 µl/cm <sup>2</sup>	13.27 (21.36 <sup>b</sup> )	16.57 (24.02 <sup>b</sup> )	26.43 (30.94 <sup>b</sup> )	30.23 (33.36 <sup>b</sup> )	65.53 (54.05 <sup>b</sup> )
Orange oil @ 1 µl/cm <sup>2</sup>	6.53 (14.81 <sup>d</sup> )	13.60 (21.64 <sup>c</sup> )	20.33 (26.80 <sup>c</sup> )	19.87 (26.47 <sup>c</sup> )	53.07 (46.76 <sup>d</sup> )
Orange oil @ 0.5 µl/cm <sup>2</sup>	3.50 (10.78 <sup>e</sup> )	3.50 (10.78 <sup>e</sup> )	10.57 (18.97 <sup>f</sup> )	10.20 (18.63 <sup>f</sup> )	26.47 (30.96 <sup>e</sup> )
Orange oil @ 0.25 µl/cm <sup>2</sup>	3.37 (10.57 <sup>e</sup> )	3.40 (10.63 <sup>e</sup> )	5.57 (13.65 <sup>h</sup> )	6.50 (14.77 <sup>e</sup> )	20.20 (26.71 <sup>i</sup> )
Cinnamon oil @ 2 µl/cm <sup>2</sup>	10.43 (18.84 <sup>c</sup> )	16.40 (23.89 <sup>b</sup> )	16.60 (24.04 <sup>d</sup> )	16.47 (23.94 <sup>d</sup> )	56.47 (48.72 <sup>c</sup> )
Cinnamon oil @ 1 µl/cm <sup>2</sup>	6.67 (14.96 <sup>d</sup> )	13.53 (21.58 <sup>c</sup> )	13.43 (21.50 <sup>e</sup> )	13.33 (21.42 <sup>e</sup> )	40.33 (39.43 <sup>e</sup> )
Cinnamon oil @ 0.5 µl/cm <sup>2</sup>	3.27 (10.41 <sup>e</sup> )	6.40 (14.65 <sup>d</sup> )	10.10 (18.53 <sup>f</sup> )	10.10 (18.53 <sup>f</sup> )	33.43 (35.33 <sup>f</sup> )
Cinnamon oil @ 0.25 µl/cm <sup>2</sup>	0.00 (0.00 <sup>f</sup> )	3.23 (10.36 <sup>e</sup> )	6.73 (15.04 <sup>e</sup> )	6.57 (14.85 <sup>e</sup> )	23.53 (29.02 <sup>h</sup> )
Control	0.00 (0.00 <sup>f</sup> )	0.00 (0.00 <sup>f</sup> )	0.00 (0.00 <sup>j</sup> )	0.00 (0.00 <sup>h</sup> )	0.00 (0.00 <sup>j</sup> )
SEm±	0.61	0.58	0.59	0.49	0.49
CD (P=00.1)	2.39	2.29	2.34	1.96	1.94

Figures in parentheses are Arcsine transformed values

Table 2 Contact toxicity of essential oils against *Sitophilus oryzae* adults at 28°C

Treatments	Per cent mortality (hours after treatment)				
	24	48	72	96	120
Eucalyptus oil @ 0.75 µl/cm <sup>2</sup>	95.77 (78.13 <sup>a</sup> )	96.07 (78.56 <sup>a</sup> )	100.00 (90.00 <sup>a</sup> )	100.00 (90.00 <sup>a</sup> )	100.00 (90.00 <sup>a</sup> )
Eucalyptus oil @ 0.5 µl/cm <sup>2</sup>	93.17 (74.85 <sup>b</sup> )	95.13 (77.26 <sup>b</sup> )	95.43 (77.66 <sup>b</sup> )	96.13 (78.66 <sup>b</sup> )	96.87 (79.80 <sup>b</sup> )
Eucalyptus oil @ 0.25 µl/cm <sup>2</sup>	90.27 (71.82 <sup>c</sup> )	93.50 (75.23 <sup>c</sup> )	92.83 (74.47 <sup>c</sup> )	94.07 (75.90 <sup>c</sup> )	96.43 (79.11 <sup>c</sup> )
Orange oil @ 0.75 µl/cm <sup>2</sup>	93.40 (75.11 <sup>b</sup> )	95.97 (78.41 <sup>ab</sup> )	100.00 (90.00 <sup>a</sup> )	100.00 (90.00 <sup>a</sup> )	100.00 (90.00 <sup>a</sup> )
Orange oil @ 0.5 µl/cm <sup>2</sup>	86.97 (68.84 <sup>d</sup> )	90.30 (71.85 <sup>d</sup> )	92.23 (73.82 <sup>c</sup> )	94.30 (76.19 <sup>c</sup> )	95.97 (78.41 <sup>c</sup> )
Orange oil @ 0.25 µl/cm <sup>2</sup>	56.97 (49.00 <sup>e</sup> )	75.27 (60.18 <sup>e</sup> )	83.37 (65.93 <sup>d</sup> )	85.67 (67.75 <sup>d</sup> )	87.33 (69.15 <sup>d</sup> )
Cinnamon oil @ 0.75 µl/cm <sup>2</sup>	16.63 (24.07 <sup>f</sup> )	43.43 (41.23 <sup>f</sup> )	53.27 (46.87 <sup>e</sup> )	56.57 (48.77 <sup>e</sup> )	58.83 (50.09 <sup>e</sup> )
Cinnamon oil @ 0.5 µl/cm <sup>2</sup>	13.33 (21.42 <sup>g</sup> )	36.60 (37.23 <sup>g</sup> )	46.33 (42.90 <sup>f</sup> )	49.67 (44.81 <sup>f</sup> )	53.67 (47.10 <sup>f</sup> )
Cinnamon oil @ 0.25 µl/cm <sup>2</sup>	6.67 (14.96 <sup>h</sup> )	23.53 (29.02 <sup>h</sup> )	33.27 (35.22 <sup>g</sup> )	35.57 (36.61 <sup>g</sup> )	37.00 (37.46 <sup>g</sup> )
Control	0.00 (0.00 <sup>i</sup> )	0.00 (0.00 <sup>i</sup> )	0.00 (0.00 <sup>h</sup> )	0.00 (0.00 <sup>h</sup> )	0.00 (0.00 <sup>h</sup> )
SEm±	0.61	0.51	0.57	0.53	0.62
CD (P=00.1)	2.46	2.06	2.30	2.16	2.49

Figures in parentheses are Arcsine transformed values

(13.27%) and cinnamon oil (10.43%) when fumigated at 2 µl/cm<sup>2</sup>. Trend of fumigation toxicity remained the same at different time intervals of 48, 72, 96 and 120 h after treatment. Control treatment showed the least mortality. Increasing trend in mortality was observed as the time lapsed. Significantly highest mortality was registered under eucalyptus oil treatment at 2 µl/cm<sup>2</sup> 120 h after treatment which showed the potentiality as fumigant. Decreasing trend in mortality was observed with the decrease in concentration of essential oils. As a fumigant, eucalyptus oil showed good results when

compared to orange and cinnamon oil against *S. oryzae*.

*Contact toxicity of the tested plant oils*

At 24 h after treatment mortality of adult *S. oryzae* ranged from zero to 95.77% (Table 2) and significantly highest mortality was recorded with Eucalyptus oil (95.77%), followed by orange oil (93.40%) when sprayed at 0.75 µl/cm<sup>2</sup> which was on par with eucalyptus oil (93.17 %) treated at 0.50 µl/cm<sup>2</sup>. In contrast, cinnamon oil at 0.75, 0.50 and 0.25 µl/cm<sup>2</sup> showed very less effect on *S. oryzae*, causing

16.63, 13.33 and 6.67% mortality, respectively, at 24 h after treatment. Control treatment showed no mortality. Eucalyptus oil and orange oils at 0.75  $\mu\text{l}/\text{cm}^2$  exhibited 100% mortality at 72, 96 and 120 h after treatment, showing higher efficacy as contact toxicants among the oils tested. Increasing trend in mortality was observed at different temporal intervals of 48, 72, 96 and 120 h after treatment.

## DISCUSSION

Essential oils are far safer for mammals compared to toxic fumigants like methyl bromide and phosphine currently used across the globe, which pose problems like adverse environmental disturbances, the possibility of carcinogenicity and increasing development of resistance in target pests. Therefore, there is an urgent need for new strategies to focus on a search for alternative fumigants for the control of stored-product insects.

In the current study, the three essential oils, namely, eucalyptus, orange and cinnamon demonstrated fumigant and contact toxicity to *S. oryzae*. In contact bioassay at 24 h after treatment, the highest mortality (95.8%) was recorded at 0.75  $\mu\text{l}/\text{cm}^2$  with eucalyptus oil, followed by orange oil which was a par with eucalyptus oil at 0.50  $\mu\text{l}/\text{cm}^2$ . On the other hand, cinnamon oil at all the concentrations showed significantly less effect on *S. oryzae*. Fumigation toxicity trend remained the same at different time intervals (48, 72, 96 and 120 h after treatment) for all the treatments, wherein increasing trend of mortality was observed as the time lapsed. Even 120 h after treatment, only eucalyptus oil was able to kill more than 80% of adult beetles in comparison to 65 and 50% mortality, respectively, for orange and cinnamon oil at the highest concentration (2  $\mu\text{l}/\text{cm}^2$ ). The present findings are in line with Ali et al. (2016), who reported that the percent mortality of *S. granarius* L. reached 91.2, 95.0 and 91.2% when 1 week old adults were exposed to higher concentration of *Thuja*, *Eucalyptus* and peppermint oils, respectively, comparing to zero per cent in the control after 24 h. After 72 h, the mortality was 100% at the highest concentration of 100, 30 and 15  $\mu\text{l}$  of respective volatile oils. Perera and Karunaratne (2016) evaluated essential oil from leaves of *Ruta graveolens* L. to evaluate its toxic activities against the rice weevil, *Sitophilus oryzae*. In contact and fumigation toxicity bioassays, highest doses of the oil tested (150 and 200  $\mu\text{l}$ ) were extremely effective in inducing 100% mortality of weevils within an hour and over 90% within half an hour compared

to that of the control which showed no mortality. The efficacy in respect to contact and fumigation toxicity at the lowest dose (50  $\mu\text{l}$ ) after one hour was 93% and 91%, respectively. Similarly, Andrea et al. (2011) reported in topical application assays, the essential oil of *Cymbopogon citrates* (leaf) had greater toxicity ( $\text{LC}_{50}$  -0.027  $\mu\text{l}/\text{ml}$ ) and shorter exposure time than the oils of the *Zingiber officinale* (root) and *Mentha* sp. (leaf). After 24 h and 48 h, 70% and 100% mortality of *S. oryzae* was recorded respectively. In fumigation assays, essential oil of *Z. officinale* had a lower  $\text{LC}_{50}$  (1.18  $\mu\text{l}/\text{cm}^2$ ) and 70% mortality after 24 h exposure.

The toxicity of essential oils to stored product insects is influenced by the chemical composition of the oil and plant part to be used (Don-Pedro, 1996; Lee et al., 2004). From the previous reports on the insecticidal and repellent properties of monoterpenoids and phenolic acids, it can be stated that common chemicals found in oil of coriander as well as eucalyptus such as monoterpenoids, 1,8-cineole, alpha pinene, carvone, linalool, etc., and phenolic acids, quercetin, caffeic acid, protocatechuic acid are responsible for the insecticidal activity of the essential oils. Tapondjou et al. (2005) demonstrated that essential oils consisting of 1, 8-cineole, terpineol and a-pinene as major constituents show toxic and repellent properties. Lee et al. (2004) reported 1, 8-cineole for its fumigant toxicity against major stored grain insects. Obeng-Ofori et al. (1997) found 8-cineole to be highly repellent and toxic to *Sitophilus granarius* (L.) and *S. zeamais* Motschulsky. The results obtained indicate good potential for the use of essential oils as both fumigant and contact toxic agents against *S. oryzae* adults.

Chaubey (2011) reported fumigation of *S. oryzae* adults with sublethal concentration of *Cuminum cyminum* (Apiaceae) and *Piper nigrum* (Piperaceae) essential oils significantly inhibited AChE activity. The insecticidal activity of many plant essential oils might be attributed to monoterpenoids (Subramanyam et al., 1994; Tripathi et al., 2003; Lee et al., 2004; Waliwitiya et al., 2005; Tong and Coats, 2010). Due to the high volatility, they have fumigant activity that might be of importance for controlling stored product insects (Koul, 2004). Monoterpenoids were reported earlier as fumigants and contact toxicants on various insect pests (Rice and Coats, 1994).

Several researchers reported the toxicity and protectant potential of essential oils extracted from different plants against major stored product insects (Talukder et al., 2004; Islam and Talukder, 2005; Isman, 2006; Rajendran and Sriranjini, 2008; Usha Rani et al., 2011). Further work would focus on its penetration

into insect cuticle and grain, metabolic target in the insect body as well as its effects on mammals fed on treated materials and the usefulness for commercial application.

### CONCLUSION

Though the examined essential oils had contact as well as fumigant activity, the fumigant toxicity of the oils were much more potential in shorter period (24 h). From this, we conclude that the plant essential oils can be potential alternative to synthetic fumigants in future.

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