

TOXICITY, OVIPOSITIONAL BEHAVIOUR AND ELECTROPHYSIOLOGICAL RESPONSE OF RICE MOTH CORCYRA CEPHALONICA (STAINTON) ADULTS TO ESSENTIAL OILS

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ABSTRACT

Rice moth *Corcyra cephalonica* (Stainton) is a serious pest on storage commodities. The management of *C. cephalonica* relies on the use of chemical pesticides. Insecticide resistance and its effect on nontarget demand for compounds that are clean, and green. Essential oils (EOs) viz., *Trachyspermum ammi* (Ajowan), *Piper betle* (betel), *Eucalyptus citriodora* (citridora), *Cymbopogan nardus* (citronella), *Pelargonium graveolens* (geranium), *Rosmarinus officinalis* (rosemary) and *Ocimum basilicum* (sweet basil) were investigated for their cidal activities against the egg and adult stages of *C. cephalonica*. On ovicidal action, among the EOs tested *P. betle* was found to have higher cidal action to eggs (EC₅₀ 3.79 mg/ dm³), followed by *T. ammi* EO (EC₅₀ 4.17 mg/ dm³). *P. graveolens* and *P. betle* EOs caused the highest fumigant toxicity in adults with the lowest LC₅₀ values (12.58 and 18.01 mg/ dm³). All the EOs tested caused an antennal response in *C.cephalonica* female adults. Among the Eos, *R. officinalis* and *P. graveolens* elicited the highest antennal response in female moths (1.3 mV and 0.84 mV). In the behavioural assay, the female moths showed reduced preference for the oviposition substrate treated with EOs. The desirable cidal, physiological and behavioural response of EOs on *C. cephalonica* adults makes them a viable alternative to synthetic pesticides.

Key words: *Corcyra cephalonica*, electroantennography, essential oils, ajowan, betel, citridora, citronella, geranium, rosemary, sweet basil, fumigant toxicity, ovicidal activity.

Rice moth Corcyra cephalonica (Stainton). (Lepidoptera: Pyralidae) is a serious pest of rice, wheat, maize, sorghum, groundnuts, cotton seeds, coffee, spices, and cocoa beans (Cox et al., 1981; Allotey and Kumar, 1985; Srivastava and Subramanian, 2016). The larvae form silken cocoons and feed by residing within, thereby converting whole grains into webbed mass and making them unfit for human consumption (Tripathi, 2018). Insecticides are used to scale down the damage caused by pests to stored products (Rajendran and Sriranjini, 2008), but their indiscriminate use results in the development of insecticide resistance, buildup of pesticide residues and detrimental effects on non-targets and the environment (Park et al., 2003). This problem can be addressed by searching for alternative approaches that are clean and green. The risks associated with synthetic insecticides have led to a growing environmental movement towards sustainable pest control alternatives. Essential oils (EOs) derived by hydro distillation of plant parts have

mono and sesquiterpenes (Bakkali et al., 2008; Reis et al., 2016) that cause insecticidal, repellent, ovicidal, feeding deterrent and ovipositional deterrence (Tak et al., 2015; Feng et al., 2019). They have multiple physiological targets in insects that diminish the buildup of insecticide resistance (Kiran and Prakash, 2015). The insecticidal effects of the plants belonging to Apiaceae, Geraniaceae, Lamiaceae, Myrtaceae, Piperaceae, and Poaceae have been investigated (Basaid et al., 2021). Studies on the biological effect of essential oils on C. cephalonica adults were limited to screening for its insecticidal activity (Allotey and Azalekor, 2000; Attia et al., 2020; Perera et al., 2021), but there are other attributes in EOs that could be exploited for their use in pest management. The adult females of C. cephalonica rely on olfactory cues to search its habitat and suitable substrate for egg laying. Screening of EOs that cause a neurophysiological response that aids in ovipositional repellence and ovicidal activity will facilitate deciphering the biological effect of EOs

that could be used for pest management. In the current study, the electrophysiological and ovipositional responses of *C. cephalonica* adult females to essential oils (EOs) viz., ajowan *Trachyspermum ammi*, betel *Piper betle*, citriodora *Eucalyptus citriodora*, citronella *Cymbopogon nardus*, geranium *Pelargonium* graveolens, rosemary *Rosmarinus officinalis*, and sweet basil *Ocimum basilicum* are reported. In addition, ovicidal and adulticidal activities of essential oils against *C. cephalonica* have been evaluated.

MATERIALS AND METHODS

The rice moth C. cephalonica were reared as suggested by Lalitha and Ballal (2015). About, 3 kilograms of milled jowar seeds (Sourced from the local market in Bengaluru) were mixed with 100 grams of roasted ground nut powder, 5 grams of yeast, 5 grams of wettable sulphur and 0.05 grams of streptomycin sulphate. The diet mixture was loaded into a sterilized wooden rearing box. Each box was mixed with 1 CC of C. cephalonica eggs. The rearing temperature was maintained at $28 \pm 2^{\circ}$ C and 75 % \pm 5 RH. On emergence, the moths were fed with 20 % honey solution containing vitamin E and transferred to an egg-laying chamber. Essential oils (EOs) viz., Ajowan, Trachyspermum ammi, betel, Piper betle, citriodora, Eucalyptus *citriodora*, citronella, *Cymbopogon nardus*, geranium, Pelargonium graveolens, rosemary, Rosmarinus officinalis and sweet basil, Ocimum basilicum were sourced from Southern Spice Products, Madurai, Tamil Nadu, India. Dichloromethane (HPLC-grade), acetone and Dimethyl 2,2-dichlorovinyl phosphate (DDVP) (PESTANAL®) analytical standards were sourced from Sigma Aldrich.

The ovicidal activity was assessed as suggested by Perera et al. (2021) with minor modifications. Briefly, freshly laid eggs of C. cephalonica (20 nos.) were collected using a fine brush and transferred to the filter paper (30 mm dia., Whatman no.1) placed in the bottom of 50 ml plastic sample container (Tarsons) having screw cap lid. EOs ranging from (1,2,4,8 and 16 mg/ dm³) were loaded on a filter paper disc (2x 2) cm) attached to the underside of the lid. Acetone-treated eggs were maintained as the negative control and DDVP (2,2-dichlorovinyl dimethyl phosphate) was treated as the positive control. The setup was tightly sealed using parafilm and placed in an incubator at $28\pm 1^{\circ}$ C and RH $65\pm 5\%$. Five replications were maintained per treatment. The number of hatched and unhatched eggs was counted after 72 hr of treatment. A test for adult fumigant toxicity was conducted as suggested by Liu and Ho (1999). EOs were diluted in acetone to achieve concentrations of 4,8,16,32,64,128 mg/dm³. The test concentrations were fixed based on the preliminary range-finding test. Adults of C. cephalonica (24 hr after eclosion) were collected and starved for 2 hr. Twenty uniform-sized moths were separated and placed in a 1 dm³ jar. One hundred microliter aliquots with the concentrations mentioned above were loaded on Whatman No. 1 filter paper (2 cm diameter) fixed on the underside of a one-liter jar lid. DDVP (2,2-dichlorovinyl dimethyl phosphate) was a positive control and acetone alone was a negative control. To prevent direct contact of the test insect with the treated filter paper, it was covered with a wire mesh. The lids were tightly sealed with a parafilm and kept under laboratory conditions (28± $2 \circ C$, RH 6± 5%). Five replications were maintained/ treatment, and the insect mortality was recorded after 12 hr of exposure. The mortality data were subjected to probit analysis to determine the LC_{50} (Finney, 1971).

An electroantennographic system (Syntech) with a dual-electrode probe was used to record the antennal response of adult females of C. cephalonica (2 days old) to EOs. The whole antenna was excised from the head and mounted on a ground electrode, the tip of the flagellum was attached to the recording electrode using conductive gel (Spectra 360 Parker) and the base was attached to the ground electrode. The clean air (activated charcoal filtered) was continuously flushed over the antennae. The EOs were diluted in HPLC-grade dichloromethane to achieve a concentration of 100 ng. Dichloromethane alone was used as a control. Aliquots were placed on Whatman filter paper strips (Advantec 5C, 110 mm) Japan of 2 cm length and 4 mm dia dried for 5 min in a fume hood, and then it was inserted into the Pasteur pipettes. This setup was connected to a stimulus controller (CS 05 Syntech) by a Tygon silicone tube. The first puff was blown off after 30 sec of loading filter paper. After sixty sec, antennae were exposed to vapour phase of the stimulus through a pipette placed 15 mm upstream from the antennae that had a continuous air stream (pulse time 0.5 sec, continuous flow 25 ml/ s, pulse flow 21 ml/s) as suggested by Venugopal and Subaharan (2019). Between the stimulus puffs, a time delay of 20 sec was maintained. The antennal responses were recorded through a high-impedance probe that was in turn connected to an amplifier (IDAC-4, Syntech), and the signals were recorded with EAG software (Syntech). Responses were expressed as a summated response of neurons, sorted according to shape and amplitude, emitted during 1 sec after the onset of the stimulation. The control stimulus was provided at the beginning, middle and end of each session. EOs were exposed with four replications/ stimuli/ antennae in a randomized manner.

The ovipositional response C. cephalonica moths to EOs was evaluated by a no-choice test with minor modifications from the method suggested by Stamopoulos (1991). The essential oils were diluted in acetone to achieve 0.002% concentration. Test solutions were loaded on filter paper stripes and placed in plastic sample containers (Tarsons) (121 ml capacity). Two g of broken rice kernels were used to induce oviposition. Three pairs of newly emerged moths (female: male 1:1) were released into each container, filter paper treated with acetone was used as negative control and Azadirachta indica (neem) essential oil as a positive check. The containers were stored at 28° C and $65 \pm 5\%$ RH. The moths were removed from the experimental containers 72 hr after treatment and dissected to count the number of eggs retained in the lateral oviducts. The number of eggs laid, eggs retained in lateral oviducts and eggs produced (number of eggs laid plus the number of eggs retained at lateral oviducts) were recorded. The egg and adult toxicity data were subjected to probit analysis (Finney, 1971) to determine dose-response and chi-square values. The EAG response of antennae to EOs was compared using a one-way ANOVA followed by Tukey's Post hoc test (p<0.05) using SPSS software version 16.0.

RESULTS AND DISCUSSION

The non-motile egg stage of the pest is an easy target to employ pest management measures. Identifying the EOs having ovicidal activity will be a robust strategy to contain the pest. Among the EOs tested, *P. betle* (EC₅₀ 3.79 mg/ dm³) caused the highest ovicidal action followed by *T. ammi* (EC₅₀ 4.17 mg/dm³), *E. citriodora* (EC₅₀ 4.72 mg/ dm³) *R. officinalis* and *O. basilicum* (EC₅₀ 4.92 mg/ dm³). EOs of *C. nardus* and *P.* graveolens had an EC_{50} of above 5 ppm. The chemical insecticide DDVP caused the highest ovicidal effect with the lowest EC_{50} of 0.15 mg/dm³ (Table 1). The ovicidal and ovipositional repellence of EOs to insect pests was reported by Pavela and Benelli (2016) and Senthil-Nathan (2020). EOs of P. betle caused cidal action on Musca domestica (Subaharan et al., 2021) and Callosobruchus maculatus eggs (Gragasin et al., 2006). The eggs of Tribolium confusum and Ephestia kuehniella exposed to R. officinalis EO had a negative impact on hatch rates (Tunc, 2000). The ovicidal action of EOs occurs through the entry into eggs through the posterior pole and disrupts embryonic development, which ultimately results in the death of the embryo (Credland, 1992). Furthermore, EOs are neurotoxins and ovicidal activity occurs during the development of the embryonic nervous system (Papachristos and Stamopoulos, 2004).

In the adult fumigant toxicity assay, all the EOs tested were toxic to C. cephalonica adults (Table 2). On the order of toxicity, P. graveolens caused the highest adult toxicity with the lowest LC₅₀ 12.58 mg/ dm³ followed by P. betle (LC₅₀ 18.01 mg/ dm³), O. basilicum (LC₅₀ 20.02 mg/ dm³). EOs of *E. citriodora*, *C. nardus* and R. officinalis recorded an LC₅₀ of over 20 ppm/ dm³. The chemical insecticide DDVP caused the highest ovicidal effect with the lowest LC₅₀ (0.69 mg/ dm³) (Table 2). EOs have been reported to exhibit fumigant toxicity against Sitophilus oryzae and the Drosophila suzukii (Lee et al., 2003; Kim et al., 2016). The terpenes present in EOs are the major contributing factors for fumigant toxicity as they have a high penetration ability into the biological targets (Rajendra and Sriranjini, 2008; Chaudhari et al., 2021; Singh et al., 2021). In our investigation, P. graveolens EO was effective against adult moths of C. cephalonica (LC₅₀ 12.58 mg/ dm³). P. graveolens EO was reported to be toxic to adults of Tribolium castaneum (Abouelatta et al., 2020), S.

Test sample	EC ₅₀	95% CI	Chi-	Р	LC ₅₀	95% CI	Chi-	Р
	(mg/dm^3)		square	value	(mg/dm^3)		square	value
T. ammi EO	4.17	3.06 - 5.69	2.643	0.488	20.02	16.28 - 24.61	1.49	0.827
P. betle EO	3.79	2.47-5.23	2.002	0.572	18.01	11.66 - 26.41	0.48	0.993
E. citriodora EO	4.72	3.49 - 6.18	0.659	0.883	21.89	17.11 - 27.67	5.70	0.336
C. nardus EO	7.30	5.30 - 10.48	0.340	0.952	31.24	25.06 - 40.27	1.65	0.799
P. graveolens EO	5.56	3.45 - 8.68	0.092	0.993	12.58	9.67 - 15.95	3.75	0.588
R. Officinalis EO	4.92	3.77 - 6.26	3.113	0.374	31.22	24.33 - 39.49	3.50	0.622
O. basilicum EO	4.92	3.45 - 6.73	0.798	0.850	20.02	16.28 - 24.61	1.53	0.909
DDVP	0.15	0.13 - 0.18	1.43	0.092	0.69	0.51 - 0.93	2.5	0.639

Table 1. Fumigant toxicity of essential oils on C. cephalonica eggs

CI - Confidence Interval; df – Degree of freedom (z = 3); p = 0.05; df = 3

oryzae and *Rhyzopertha dominica* (Michaelraj et al., 2007). The fumigant toxicity of *T. ammi* EO against adult stages of *T. castaneum* was reported by Chaubey et al. (2007). Fumigant toxicity in essential oils occurs by the entry of the vapours into the insect body through the respiratory route (Chaudhari et al., 2021). The fumigant effect of EOs may be due to the neurotoxic effect that occurs by acting on GABA, octopamine synapses and inhibition of acetylcholinesterase activity. The fumigant toxicity is desirable action considering the distribution and nature of pest feeding in the stored commodities.

The electroantennogram (EAG) is used to measure the electrical response of the insect antennae to volatile organic compounds (VOCs). The antennal response of the adult female moths of C. cephalonica to EOs was assessed by an electroantennogram. Female adults of C. cephalonica exposed to EOs at 100ng caused an antennal response (Fig. 1). R. officinalis elicited significantly highest antennal response compared to control, followed by P. betle, P. graveolens and C. nardus EOs (2.45, 1.70, 1.54 and 1.51 mV) (F = 22.45; df = 7; P < .005). Antennal response of female C. cephalonica to EOs revealed a dose-dependent response (Fig. 2). The EAG responses of female moths to the tested EOs increased with increasing concentration. The antennal response of adult females Calliphora vomitoria to EO of R. officinalis to (Bedini et al., 2020) and Philaenus spumarius to P. graveolens (Ganassi et al., 2020) were reported. Previous reports suggest that the electroantennographic response of the C. cephalonica to plant volatiles increases with increasing concentrations of the chemical stimulus until a saturation level was reached (Roelofs, 1984; Nebapure, 2018; Chen et al., 2021). Previous results of the EAG experiments



Fig. 1. Mean antennal response of female *C. cephalonica* exposed to essential oils. Means followed by the same alphabet in a bar do not differ significantly by Tukeys HSD (P < 0.05)





indicated that the antennae of *Tuta absoluta* respond to VOCs from the host plant and these volatiles at minute quantity act as an attractant or repellent to *T. absoluta*. Hence, the compounds detected by the EAG experiment may contribute to the altering of the preference for the selection of habitat or oviposition sites. Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity in insects is attributed to the presence of monoterpenes and sesquiterpenes (Nerio et al., 2010).

Essential oils have a significant effect on fecundity and egg production. Female moths of C. cephalonica laid less number of eggs when exposed to EOs of *P. betle*, followed by C. nardus, P. graveolens, E. citriodora and *O. basilicum* (F = 36.60; df = 8; P < .005). The EOs of R. officinalis and T. ammi were less effective among the oils tested. Similarly, EOs of P. betle, C. nardus, P. graveolens and E. citriodora had the highest per cent of egg retention in lateral oviducts (47.54, 43.41 41.22 and 39.45 % respectively) when compared to adults in control (F=28.23; df = 8; P < .005). The EOs tested had an impact on egg production as compared to the control (232). Egg development was low in moths exposed to EOs of O. basilicum (140), E. citriodora (143.33), P. graveolens (143.66) and C. nardus (144.66) (df = 8; F= 20.46; P < .005) (Table 3). Aedes aegypti exposed EOs of *P. betle* and *Etlingera elatior* at 100ppm caused a reduction in oviposition (Bezerra-Silva et al., 2016; Martianasari and Hamid, 2019). EOs of Cinnamon zeylanicum, Zingiber officinale and Rosmarinus officinalis are reported to have oviposition-deterrent activities to Anopheles stephensi, A. aegypti, and Culex quinquefasciatus (Prajapati et al., 2005). Gragasin et al. (2006) reported reduced fecundity in S. oryzae and R. dominica females exposed to P. betle EO. The extracts of P. betle caused a reduction in A. aegypti fecundity and oviposition deterrence (Vasantha-Srinivasan et al., 2018; Martianasari and Hamid, 2019), which may be attributed to physiological impairment (respiratory activities, hormonal disruption and behavioural changes) (Viteri Jumbo et al., 2018). The reduced Toxicity, ovipositional behaviour and electrophysiological response of rice moth *Corcyra cephalonica* (Stainton) 5 Sowmya M et al.

Treatment	Mean eggs	Total egg	% of eggs
	laid female	production female	retained± SE
Control	212.33± 7.21ª	232.00± 3.21ª	8.51±2.10°
T. ammi EO	101.33 ± 7.21^{cd}	150.00 ± 7.75^{bc}	32.77 ± 3.04^{b}
<i>P. betle</i> EO	77.66 ± 8.17^{d}	147.33 ± 9.35^{bc}	47.54 ± 2.12^{a}
E. citriodora EO	87.00 ± 7.02^{cd}	143.33 ± 8.35^{bc}	39.45 ± 1.30^{ab}
C. nardus EO	81.66 ± 6.17^{cd}	144.66 ± 8.35^{bc}	43.41 ± 2.93^{ab}
P. graveolens EO	85.00 ± 10.58^{cd}	143.66 ± 6.06^{bc}	41.22 ± 4.87^{ab}
<i>R. officinalis</i> EO	117.33±9.70°	143.00 ± 8.00^{bc}	$18.16 \pm 2.86^{\circ}$
<i>O. basilicum</i> EO	94.66± 3.17 ^{cd}	140.00± 3.51°	32.29± 2.91 ^b
Azadirachta indica EO	156.33 ± 3.84^{b}	174.66 ± 5.60^{b}	$10.45 \pm 0.68^{\circ}$

Table 3. Effect of essential oils on fecundity and egg retention in C. cephalonica

Means within a column followed by the same letter not significantly different (Tukeys HSD p < 0.05); values $\overline{x} \pm SE$

fecundity of *C. cephalonica* exposed to EOs in our study is due to the retention of eggs in lateral oviducts. In our study, *R. officinalis* EO retained less number of eggs (25.66) in lateral oviducts of *C. cephalonica*. In contrast, *R. officinalis* EO showed the highest number of egg retention in *Acanthoscelides obtectus* (Papachristos and Stamopoulos, 2002). The negative physiological and behavioural impacts, egg resorption and structural alterations in the follicle were reported to cause egg retention (Dieng et al., 2022). *E. citriodora* and *O. basilicum* EOs recorded reduced egg production, which might be attributed to the altered respiratory patterns, repellence and behavioural changes brought about by exposure to essential oils (Correa et al., 2015; Silva et al., 2017).

The fumigant toxicity of EOs against the egg and adult stages of *C. cephalonica* has been established. Based on the findings, it can be concluded that EOs like *P. betle* and *P. graveolens* can effectively be used to manage *C. cephalonica* considering its ovicidal and fumigant toxicity. Additionally, the EOs tested caused a physiological response in antennae and they caused ovipositional deterrence an attribute that could be used as a prophylactic treatment in storage structures to scale down the dependence on synthetic pesticides, which may have harmful effects on human health and the environment. However, further research is needed to develop stable formulations of EOs that can have better spatiotemporal efficacy when used in warehouses to manage *C. cephalonica*.

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AUTHOR CONTRIBUTION STATEMENT

Sowmya M: Investigation and data generation. Bindu O S: Conceived the draft. Subaharan K: Conceived and designed the research. Vinay Kumar T M, Senthoorraja R and Varshney R: Resource supply and manuscript preparation and correction. Chalapathi Rao N B V: Conducting experiments. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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