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IMPACT OF ESSENTIAL OILS ON BIOLOGICAL TRAITS OF *TRICHOGRAMMA CHILONIS* ISHII

Sowmya M[1,2], Bindhu O S[2], Subaharan K[1]*, Vinay Kumar T M[1,2], Soundarya Y M[1], Varshney R[1], Navik O[1], Amala U[1] and Sushil S N[1]

[1]ICAR- National Bureau of Agricultural Insect Resources,
H A Farm Post, Bellary Road, Hebbal, Bengaluru 560024, Karnataka, India
[2]Department of Chemistry and Biochemistry, Jain (Deemed-to-be University),
Bengaluru 560027, Karnataka, India
*Email: Kesavan.Subaharan@icar.gov.in (corresponding author): ORCID ID 0000-0003-1828-6644

ABSTRACT

***Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) reared on factious host rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is an egg parasitoid on lepidopteran pests. The essential oils, viz., ajowan *Trachyspermum ammi*, betel *Piper betle,* and sweet basil *Ocimum basilicum* and neem oil were assessed for their toxicity and biological traits like parasitism and emergence on *T. chilonis*. Among the EOs tested, *T. ammi* caused highest toxicity to *T. chilonis* (LC_{50} 0.316 ppm) followed by *P. betle* (LC_{50} 0.567 ppm). At sublethal dose, they had an impact on parasitism and adult emergence of *T. chilonis*. Whilst *O. basilicum* and neem oil were relatively safe at sublethal doses to *T. chilonis*. This study highlights the need to maintain a waiting period before the release of *T. chilonis* in EOs treated substrates.**

Key words: *Corcyra cephalonica*, essential oils, IPM, *Ocimum basilicum*, parasitoids, *Piper betle*, selectivity, *Trachyspermum ammi*, *Trichogramma chilonis*, Trichogrammatidae

Rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is a secondary storage pest that prefers to feed on damaged or broken grains (Rajasekhar et al., 2016). Defilement by larval defecation, webbings, exuviae, and cadavers decreases human acceptance. Chemical insecticides are generally used for the management of *C. cephalonica* in storage (Meena et al., 2016). Indiscriminate use of synthetic insecticides has led to the development of insecticide resistance, pest resurgence, (Gonzalez et al., 2014) negative impact on beneficial insects and human health (Rajak et al., 2023). Alternatively, the bioactive compounds derived from plants are employed as effective pest control as they have little negative impacts on the environment and non-targets (Giunti et al., 2022). In Europe and Africa infestation by *C. cephalonica* of rice stored in paper bags and jute bags in supermarkets is managed by the release of *T. evanescens* (Adarkwah et al., 2015). However, the use of *Trichogramma* as parasitoids, against *C. cephalonica* in storage structures remains relatively limited under natural conditions in India. EOs were reported to be used to manage *C. cephalonica* as they possess toxicity and ovipositional repellence (Sowmya et al., 2023). Generally, EOs are considered low-risk products as they present low toxicity to non-target vertebrates and show little persistence in the environment (Isman, 2020). But their impact on non-targets needs further investigation (Siviter and Muth, 2020) as integrating the natural enemies (parasitoids), along with plant-based insecticides is an effective method to manage stored product pests (Parreira et al., 2018b). Previous studies reveal that direct exposure of *Trichogramma* to EOs exhibit contact (Cruz et al., 2021) and fumigant toxicity (Poorjavad et al., 2014), but prolonged exposure to sublethal doses of EOs to *Trichogramma* had an impact on biological parameters like parasitism, development, longevity, fecundity, behaviour, and mating (Parreira et al., 2019). The present investigation aims to evaluate the impact of EOs viz., ajowan, *T. ammi*, betel, *P. betle*, and sweet basil, *O. basilicum* essential oils on the biological traits of *T. chilonis*.

MATERIALS AND METHODS

Essential oils (EOs) viz., ajowan *Trachyspermum ammi*, betel *Piper betle,* and sweet basil *Ocimum basilicum* were sourced from Southern Spice Products, Madurai, Tamil Nadu, India. Acetone was procured from Sigma Aldrich. Imidacloprid (Pestanal®) from Sigma Aldrich. *Corcyra cephalonica* larvae were reared on milled jowar grins as described by Lalitha and Ballal (2015) and the eggs of *C. cephalonica* were used to rear *T. chilonis* as described by Dupatne et al. (2023). The mortality of *T. chilonis* exposed to essential oils was evaluated as suggested by Poorjavad et al. (2014) with minor modifications. Briefly, twenty adults of *T. chilonis* (24-48 hr after emergence) of uniform size were collected and released into a glass tube (55 ml). EOs and neem oil diluted in acetone were loaded onto filter paper strips (2 x 2 cm) and placed in a glass tube (150 x 25 mm) to reach a concentration of 0.04-1.28 ppm for EOs and 500-16,000 ppm for neem oil. Synthetic insecticide imidacloprid was maintained as a standard check (Ray et al., 2023). Acetone-treated filter papers were maintained as a control. The open end of the tubes was covered with cotton plugs lined with muslin cloth and placed upright so that treated filter papers remain at the bottom. Each treatment was replicated three times. The mortality of parasitoids was recorded after 24 hr of exposure. Median lethal concentration (LC_{50}) was determined by probit analysis (Finney, 1971).

The effect of EOs and neem oil on *T. chilonis* parasitism was investigated as described by Parreira et al. (2018b) with minor modifications. UV sterilized *C. cephalonica* eggs pasted on the paper cards were sprayed sublethal dose (LC_{20}) of EOs and neem oil (*T. ammi* 0.094, *P. betle* 0.15, *O. basilicum* 0.16 and neem oil 2837.60 ppm, respectively) with a hand-held atomizer. The cards treated with acetone alone were maintained as control. The cards were dried for 10 minutes under a fume hood and then placed in the glass tubes containing 3 pairs of 24 hr old *T. chilonis* adults. The experiment was replicated five times. After 24 hr of exposure, the adult parasitoids were removed from the tube. The parasitized eggs were distinguished from unparasitized eggs by the change in colour (turned black) and the exit holes on the *C. cephalonica* eggs. The reduction in parasitism was calculated as suggested by Parreira et al. (2019). The effect of essential oils on the emergence of *T. chilonis* was assessed the same as the method suggested in the fumigant toxicity bioassay but with slight modification where the tubes were placed horizontally. The EOs and neem oil diluted in acetone to achieve the concentration corresponding to their sublethal dose at LC_{20} (*T. ammi* 0.094, *P. betle* 0.15, *O. basilicum* 0.16 and neem oil 2837.60 ppm, respectively) were loaded on a filter paper strip (2×2 cm) were placed in the glass tube having 20 *C. cephalonica* eggs parasitized by *T. chilonis*. After 96 hr of exposure, the number of parasitoids that emerged was recorded and the % emergence were calculated as suggested by Parreira et al. (2019). The adult toxicity data were subjected to probit analysis (Finney, 1971). The emergence and parasitism of *T. chilonis* from treated eggs of *C. cephalonica* were 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

In the fumigant toxicity assay, all the tested EOs showed varying levels of toxicity to adult *T. chilonis.* Among the EOs tested, *T. ammi* EO was highly toxic with the lowest LC_{50} 0.316 ppm followed by *P. betle* EO (LC_{50} 0.567 ppm). Neem oil was found to be the least toxic (LC_{50} 8049 ppm). Imidacloprid caused high toxicity with LC_{50} 0.010 ppm**.** Increased toxicity of EOs *T. ammi* and *P. betle* may be attributed to volatile terpenes that have an impact on *T. chilonis*. Fumigant toxicity of EOs caused by *O. basillicum* and *Thymus vulgaris* on *T. evanescens* was reported by Oudenhove et al. (2023). The fumigant toxicity by EO is due to their ability to penetrate the biological targets (Chaudhari et al., 2021; Sowmya et al., 2023). The toxicity of EOs on *T. chilonis* may be attributed to their neurotoxic effect, as the terpenes present in EOs inhibit the acetylcholine esterase enzyme, resulting in the continuous firing of the neurons that leads to mortality (Chintalchere et al., 2021). Further, the lipophilic nature of EOs facilitates easy penetration into the insect cuticle and interacts with enzymes or receptors disrupting the physiological process (Castilhos et al., 2018). The present study supports earlier findings of the toxic effect of neem seed oil on *T. chilonis* adults, neem seed oil at 5% caused 40-52% mortality in *T. chilonis* adults (Raghuram and Singh, 1999).

The impact of sublethal dose of EOs exposure on parasitism by the females of *T. chilonis* was assessed. Among the EOs tested *T. ammi* treated eggs resulted in 55% parasitism by *T. chilonis* as compared to 86% parasitism in control. This was followed by EOs *P. betle*, *O. basilicum*, and neem oil causing 63, 65, and 68% parasitism respectively and they were at par ($F = 19.39$; $df = 4$; $p<.005$) (Table 1). The EOs have been reported to have an impact on the parasitism of *Trichogramma*. Exposure to sublethal doses of EOs viz., *Capara guianensis, Citrus sinensis*, *Origanum vulgare*, and *Zingiber officinale* reduced parasitism in *T. galloi* (Parreira et al., 2018a). EOs of *C guianensis* and *O. vulgare* reduced parasitism in *T. pretiosum* (Parreira et al., 2018b). Maize fall army worm, *Spodoptera frugiperda* eggs treated with *Cymbopogan winterianus* and *C. citratus*, were less preferred for parasitism by *T. pretiosum*; the reduction of parasitism was due to the

Table 1. Effect of essential oils on biological traits and toxicity to *T. cholonis*

Test sample	LC_{50} (ppm)	95% CI	% Parasitism± SE *	% Reduction in parasitism	% Emergence± SE*	% Reduction in emergence
Control	-	-	86± 0.37[a]	-	99± 0.20[a]	-
T. ammi EO	0.316	0.256 – 0.395	55± 0.63[c]	36.04	37± 0.50[c]	62.22
P. betle EO	0.567	0.446- 0.763	63± 0.50[bc]	26.74	44± 0.37[c]	55.55
O. *basilicum* EO	0.790	0.574 – 1.240	65± 0.54[bc]	24.41	69± 0.66[b]	30.30
Neem oil	8049	6532.62-10386.01	68± 0.50[b]	20.93	61± 0.66[b]	38.38
Imidacloprid	0.010	0.008-0.012	-	-	-	-

**T. chilonis* exposed to sublethal dose of LC_{20}; CI - Confidence Interval; Means within a column followed by the same letter not significantly different (Tukeys HSD, $p< 0.05$), EO-essential oil

presence of active compounds like citral, citronellal, and geraniol (Sombra et al., 2022). *Trichogramma pretiosum* females detected the volatile compounds from EOs by olfactory receptors in antennae or tarsi and the perception of these compounds by the receptors led to a reduced preference for parasitism or ovipositing on host eggs exposed to EOs (Sombra et al., 2022). Reduction of parasitism rate on *C. cephalonica* treated eggs observed now may be due to the volatile compounds on the treated surface of the eggs perceived by the olfactory receptors leading to non-preference for treated host eggs.

The EOs recorded inhibition in the emergence of *T. chilonis* adults from parasitized *C. cephalonica* eggs as compared to untreated eggs. Among the EOs tested, *T. ammi* and *P. betle* EOs caused 62.62 and 55.5% reduction in emergence followed by neem oil (38.3%) and *O. basilicum* EO (30.3%), respectively (Table 1). The reduced emergence might be due to the diffusion of EO through the chorion (outer layer) of the eggs, thereby disrupting the development of the embryo and the immature stages of natural enemies leading to paralysis and death (Perreira et al., 2019). Further, essential oils have been reported to act as insect growth regulators (IGRs) disrupting the normal growth and development of insects, often by interfering with their biosynthetic processes at various stages of their life cycle which impacts the emergence of adult insects. Among the EOs tested, *O. basilicum* was less harmful to the emergence of *T. chilonis* as compared to *T. ammi* and *P. betle*. These present findings corroborate with those of Bibiano et al. (2022) where *O. basilicum* EO was selective to *T. pretiosum* emergence. The selectivity of EOs may be attributed to the physiological differences between the insect pest and the parasitoid. The physiological differences can result in variations in how chemical compounds are absorbed, penetrate, and interact within their respective bodies (Carvalho et al., 2019). *Ocimum basilicum* EO had less impact on parasitism and the emergence of *T. chilonis* and this could be considered for its use with parasitoids. The impact of EOs on the adult stages of *T. chilonis* adults has been established; EO of *T. ammi* and *P. betle* were toxic to *T. chilonis*. When expose to sublethal dose *T. ammi* caused < 60% parasitism and < 40% of adult emergence, whilst *O. basilicum, P. betle* and neem oil caused > 60% parasitism. This indicates that there should be a waiting period to be followed before the release of parasitoids after the application of EO as both bioagents and EOs cannot be applied in tandem to manage *C. cephalonica*. Further studies are needed to fix the safe interval period for the release of parasitoids after the application of EOs in storage structures.

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AUTHOR CONTRIBUTION STATEMENT

SM investigation and data generation. BS and SK conceived and designed the research. RV and ON provided resources and manuscript preparation. VKTM, SYM, AU and SSN manuscript preparation and correction. The final manuscript was read and approved by all the authors.

CONFLICT OF INTEREST

No conflict of interest.

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