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Research Article

Safety evaluation of horticultural mineral oil against *Trichogramma chilonis* Ishii. and *Bracon brevicornis* Wesmael

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ABSTRACT: Horticultural Mineral Oils (HMOs) are highly refined paraffinic oils that aids in management of pests and diseases and are used as a tool in integrated pest management. The safety of the HMOs to the natural enemies is important. So, the present study was taken up to evaluate the safety of HMO to the parasitoids, *Trichogramma chilonis* Ishii. and *Bracon brevicornis* Wesmael. The safety evaluation methods, such as egg card bioassay, dipped surface residue bioassay, and dry film residue method, were performed in the laboratory at Department of Entomology, College of Agriculture, Padannakkad, and treatments were HMO 2%, HMO 2.5%, HMO 3%, HMO 3.5%, HMO 4%, HMO 4.5% and control. The lowest mortality percentage and highest adult emergence percentage of *Trichogramma chilonis* were accomplished with HMO 2%, which was on par with the control, followed by HMO 2.5% and HMO 3%. Same results were obtained in the laboratory bioassay of *Bracon brevicornis,* which also suggested an increased mortality percentage at higher concentrations.

KEYWORDS: Horticultural mineral oil, laboratory bioassay, *Trichogramma chilonis, Bracon brevicornis,* pest management

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INTRODUCTION

Horticultural mineral oils are highly refined paraffinic oils that effectively manage pests and diseases. They have also been referred to as petroleum spray oils. These oils have been used in agriculture for about 100 years. These act as contact insecticides that interfere physically rather than chemically with the respiration of insects (Johnson, 1980).

Horticultural Mineral Oils (HMOs) work by suffocation when the spray forms a coating of oil on the insect's body, blocking the spiracles. HMOs also may interact with insect fatty acids, disrupting cell membranes and metabolism. Mineral oils create a thin layer on the insects' eggs' surface where they stop the exchange of gases and also act as oviposition deterrents (Helmy *et al*., 2012). They are safe to mammals (including humans), some birds, and reptiles and are less phytotoxic (Sridharan *et al*., 2015). It is reported that mineral oils are comparatively safer to groundwater or soil, and no pest resistance has been recorded so far (Helmy *et al*., 2012). HMO significantly controls San Jose Scale and European Red Mite in apple (Khurshid *et al*., 2023). It is also reported to manage Asian citrus psyllid, *Diaphorina citri* very effectively due to its repellent and antifeedent properties (Hu *et al*., 2023). Open field study in tomato showed the ability of HMO to control leaf miner *Tuta absoluta*, resulting in the lowest fruit damage (Ngugi *et al*., 2023). Mineral oil was less toxic to coccinellid beetles, *Pharoscymnus anchorago* (Ben Chaaban *et al*., 2025). Predator of tomato pests, *Macrolophus pygmaeus* nymphs resulted in less mortality after contact exposure with paraffinic oil (Betsi & Perdikis, 2023). Though HMOs furnishes a prominent role in integrated pest management, only a few studies have been done to evaluate its toxicity against parasitoids.

Hymenopteran parasitoids comprise the important part of natural enemies and they play a crucial role in biological pest control strategies. Among hymenopterans, the genus *Trichogramma* and *Bracon* are effective against several crop pests and help to reduce their population on a wide range of agricultural crops. In India, about 45 species of *Trichogramma* and 1083 species of *Bracon* are described (Ahmed *et al.,* 2023; Gupta, 2023).

Since the selective nature of insecticides varies between target and non-target pests, especially natural enemies, it is essential to ensure safety against the latter. Since research studies are scarce in this area, our study mainly aims to get a clear understanding of the safety of HMOs against

parasitoids. In this backdrop, the present research has been undertaken to evaluate the toxicity of HMO to two parasitoids of lepidopteran pests, namely, *Trichogramma chilonis* Ishii. and *Bracon brevicornis* Wesmael.

MATERIALS AND METHODS

Laboratory bioassay for safety evaluation of HMO was carried out on the parasitoids *viz., Trichogramma chilonis* Ishii. and *Bracon brevicornis* Wesmael. in the laboratory, Department of Entomology, College of Agriculture, Padannakkad, Kasargod, Kerala. Cristol TSO, commercial horticultural mineral oil obtained from Krishna Antioxidants Pvt. Ltd., Mumbai, which meets NPOP and USDA standards, was used. The design was CRD with 7 treatments and 3 replications.

Toxicity to *Trichogramma chilonis*

Egg card bioassay

The bioassay methodology used was according to Basappa (2007) and Asrar *et al.* (2022) with modifications. Egg cards of *Corcyra cephalonica* parasitised by *Trichogramma chilonis* were collected from the State Biocontrol Laboratory, Mannuthy, Thrissur, Kerala used for bioassay. Different treatment solutions were prepared and egg cards having fifty eggs were dipped in these solutions for five seconds. The treated egg cards were shade dried for ten minutes and kept inside respective test tubes (15 × 2.5 cm) at 25 ± 2ºC temperature and 60 ± 5% relative humidity for adult emergence. Number of adults which emerged from these egg cards was recorded. The percentage of emergence of *Trichogramma chilonis* adults was computed for each treatment.

Dipped surface residue bioassay

The bioassay methodology used was according to Thakur and Pawar (2000) and Asrar *et al.* (2022) with modifications. Whatman No. 1 filter papers were dipped in different treatment solutions and kept in test tubes (15 × 2.5 cm) after drying. Fifty newly emerged adult *Trichogramma* were released into test tubes. The mouth of the test tubes was fastened with fine muslin cloth and tied with rubber bands. Cotton swabs dipped in honey solution were kept in the test tube as food supplement. Laboratory conditions were maintained at 30 ± 2ºC temperature and 70 ± 5% relative humidity. Observations were recorded at 2, 8, and 24 h after the treatment. The mortality of adults at these intervals was determined, and percentage mortality was computed.

Table 1. Treatments imposed at the laboratory bioassay

S. No.	Treatments
1	HMO at 2 per cent
2	HMO at 2.5 per cent
3	HMO at 3 per cent
4	HMO at 3.5 per cent
5	HMO at 4 per cent
6	HMO at 4.5 per cent
7	Control

Toxicity to *Bracon brevicornis*

Dry film residue method

The bioassay methodology adopted followed the method of Shankarganesh *et al.* (2017) and Dabhi *et al.* (2013) with modifications. The culture of *Bracon brevicornis* was collected from the Biological Control Laboratory, Divison of Entomology, Central Plantation Crops Research Institute, Kasargod, Kerala. All treatment solutions were individually sprayed inner side of test tubes (15 × 2.5 cm) and were left to dry for one hour to ensure complete evaporation of water and formation of a thin film of HMO before introducing parasitoids. Ten freshly emerged parasitoids were released into respective test tubes. Honey-soaked cotton swabs were placed inside the test tubes as food supplement. The test tubes were covered with muslin cloth and secured with rubber bands. Laboratory conditions were maintained at 32 ± 2ºC temperature and 68 ± 5% relative humidity. Mortality of adult parasitoids was recorded at 2, 8, and 24 h and percentage mortality was calculated.

Statistical analysis

Abbots formula was used to correct data on mortality (Abbott, 1925). The data were analysed using Analysis Of Variance (ANOVA) after arcsine transformation, and significant means were separated by Tukey's HSD test at 5 per cent level of significance. Grapes version 1.1.0 software (Gopinath *et al*., 2021) was used to compare the significance of each treatment.

RESULTS

Toxicity to *Trichogramma chilonis*

Egg card bioassay

Data on the percentage emergence of *Trichogramma chilonis* on different concentrations of horticultural mineral is presented in Figure 1. From the figure, it can be concluded that HMO treatment at 2 per cent had higher percentage of adult emergence (94.67), which was on par with control (97.99) followed by HMO at 2.5 per cent (84.00) and HMO at 3 per cent (70.00). HMO at 3.5 per cent with an adult emergence of 18.67 was on par with HMO at 4 per cent (12.00). The lowest percentage of adult emergence (8.00) was recorded in treatment having HMO at 4.5 per cent concentration.

Dipped surface residue bioassay

The mortality percentage of *Trichogramma chilonis* adults at 2, 8, and 24 h intervals is computed and presented in Table 2.

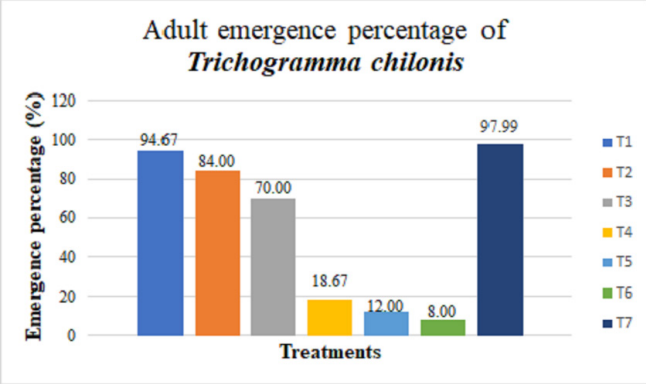


Figure 1. Adult emergence percentage of *Trichogramma chilonis* under different concentrations of HMO.

HMO treatment at 4.5 per cent with a mortality of 88.67 per cent was highest, which was followed by HMO treatment at 4 per cent (70.67 per cent) two hours after the treatment. Treatment having HMO at 3.5 per cent with a mortality of 38.67 per cent was statistically on par with HMO treatment at 3 per cent (27.33 per cent). The lowest percentage of mortality was found in HMO treatment at 2 per cent (2.00 per cent), which was statistically on par with HMO treatment at 2.5 per cent (6.00 per cent). Control showed zero percentage of mortality.

Eight hours after the treatment, HMO at 4.5 per cent showed 90.00 per cent mortality, which was followed by HMO treatment at 4 per cent (74.00 per cent). Treatment having HMO treatment at 3.5 per cent with a mortality of 41.33 per cent was statistically on par with HMO treatment at 3 per cent (29.33 per cent). The lowest percentage of mortality was found in HMO treatment at 2 per cent (2.67 per cent), which was statistically on par with HMO treatment at 2.5 per cent (7.33 per cent). Zero percentage of mortality was observed in control.

Twenty four hours after the treatment, HMO at 4.5 per cent showed 94.67 per cent mortality, which was followed by HMO at 4 per cent (77.33 per cent). Treatment having HMO treatment at 3.5 per cent (47.33 per cent) was statistically on par with HMO treatment at 3 per cent (32.67 per cent). The lowest percentage of mortality among treatments was found in HMO treatment at 2 per cent (6.67 per cent), which was statistically on par with HMO treatment at 2.5 per cent (10.00 per cent) and control (3.33 per cent).

The table data also shows a trend of increasing cumulative mortality in treatments. After two hours, HMO at 4.5 per cent resulted in highest mortality (88.67 per cent) and this increased to 90 per cent and 94.67 per cent cumulative mortality at eight and twenty four hours respectively. Similarly, 70.67 per cent mortality caused by HMO at 4 per cent after two hours also increased to 74 per cent and 77.33 per cent at eight and twenty four hours respectively. HMO at 3.5 per cent, HMO at 3 per cent, HMO at 2.5 per cent and HMO at 2 per cent also showed the same trend.

Table 2. Percentage mortality of *Trichogramma chilonis* adults under different concentrations of HMO

Treatments	Percentage mortality of *Trichogramma chilonis* adults		
	2 HAT	8 HAT	24 HAT
HMO 2%	2.00 (8.13) ^{de}	2.67 (9.27) ^{de}	6.67 (10.57) ^{d}
HMO 2.5%	6.00 (14.05) ^{d}	7.33 (15.68) ^{d}	10.00 (14.96) ^{d}
HMO 3%	27.33 (31.50) ^{c}	29.33 (32.77) ^{c}	32.67 (33.37) ^{c}
HMO 3.5%	38.67 (38.41) ^{c}	41.33 (39.99) ^{c}	47.33 (42.42) ^{c}
HMO 4%	70.67 (57.48) ^{b}	74.00 (59.56) ^{b}	77.33 (61.16) ^{b}
HMO 4.5%	88.67 (70.97) ^{a}	90.00 (72.07) ^{a}	94.67 (77.59) ^{a}
Control	0.00 (0.41) ^{e}	0.00 (0.41) ^{e}	3.33 (10.40) ^{d}
Tukey's HSD (0.05%)	**11.40**	**9.85**	**10.59**

HAT – Hours after treatment. Means followed by similar letters are not significantly different. Figures in parenthesis denote arcsine transformed values.

Toxicity to *Bracon brevicornis*

Dry film residue method

The mortality percentage of *Bracon brevicornis* adults at 2, 8 and 24 h intervals is computed and presented in Table 3.

HMO treatment at 4.5 per cent showed 80.00 per cent mortality which was statistically on par with HMO treatment at 4 per cent (63.33 per cent), two hours after the treatment. Treatment having HMO treatment at 3.5 per cent (13.33 per cent) was statistically on par withHMO treatment at 3 per cent (10.00 per cent) and HMO treatment at 2.5 per cent (6.67 per cent). The lowest percentage of mortality was found in HMO treatment at 2 per cent (0.00 per cent) which was statistically on par with HMO treatment at 2.5 per cent and HMO treatment at 3 per cent. Zero percentage of mortality recorded in control.

Eight hours after the treatment, HMO treatment at 4.5 per cent showed 86.67 per cent mortality which was followed by HMO treatment at 4 per cent (63.33 per cent). Treatment having HMO treatment at 3.5 per cent (16.67 per cent) was statistically on par with HMO treatment at 3 per cent (16.67 per cent) and HMO treatment at 2.5 per cent (10.00 per cent). The lowest percentage of mortality was found in HMO treatment at 2 per cent (0.00 per cent) when compared to control having zero percentage of mortality.

Table 3. Percentage mortality of *Bracon brevicornis* adults under different concentrations of HMO

Treatments	Percentage mortality of *Bracon brevicornis* adults		
	2 HAT	**8 HAT**	**24 HAT**
HMO 2%	0.00 (0.91) [c]	0.00 (0.91) [d]	0.00 (0.91) [d]
HMO 2.5%	6.67 (12.59) [bc]	10.00 (18.43) [c]	13.33 (21.15) [c]
HMO 3%	10.00 (15.30) [bc]	16.67 (23.86) [c]	20.00 (26.57) [c]
HMO 3.5%	13.33 (21.15) [b]	16.67 (23.86) [c]	23.33 (28.78) [c]
HMO 4%	63.33 (52.78) [a]	63.33 (52.78) [b]	70.00 (57.00) [b]
HMO 4.5%	80.00 (63.44) [a]	86.67 (68.86) [a]	86.67 (68.86) [a]
Control	0.00 (0.91) [c]	0.00 (0.91) [d]	0.00 (0.91) [d]
Tukey's HSD (0.05%)	**18.51**	**9.31**	**10.48**

HAT – Hours after treatment. Means followed by similar letters are not significantly different. Figures in parenthesis denote arcsine transformed values

Twenty four hours after the treatment, HMO treatment at 4.5 per cent showed 86.67 per cent mortality which was followed by HMO treatment at 4 per cent (70.00 per cent). Treatment having HMO treatment at 3.5 per cent (23.33 per cent) was statistically on par with HMO treatment at 3 per cent (20.00 per cent) and HMO treatment at 2.5 per cent (13.33 per cent). The lowest percentage of mortality was found in HMO treatment at 2 per cent (0.00 per cent) when control showed zero percentage of mortality.

While considering the time period of observation from two hours to twenty four hours, the table data also shows a trend of increasing cumulative mortality in treatments. After two hours, HMO at 4.5 per cent resulted in highest mortality (80.00 per cent) and this increased to 86.67 per cent cumulative mortality after eight hours and it remained same after twenty four hours. Similarly, 63.33 per cent mortality caused by HMO at 4 per cent after two hours remained same after eight hours and it increased to 70 per cent after twenty four hours. HMO at 3.5 per cent, HMO at 3 per cent, HMO at 2.5 per cent and HMO at 2 per cent also showed an increasing trend.

DISCUSSION

Laboratory bioassay experimental studies regarding the safety of HMO against parasitoids are scarce. The results obtained from current study suggest that lower concentrations of HMO at 2 per cent, 2.5 per cent and 3 per cent cause lower mortality of parasitoids compared to higher concentrations.

Egg card bioassay and dipped surface bioassay of *Trichogramma chilonis* suggest an increase in the adult emergence and a decrease in the mortality of adults respectively, with decreasing concentrations. These results are in tune with the observations made by Al Dabel *et al.* (2008), who observed the decrease in the mortality of adult parasitoid *Trichogramma brassicae* at 2 per cent concentration. In their study, nC24 and nC27 named oils were used and resulted in 8.3 per cent and 12.7 per cent mortality percentage.

The dry film residue method of *Bracon brevicornis* also gave similar results, indicating that the lower concentration of HMO is safer than higher concentrations. Similar findings were recorded by Muthukumar *et al.* (2020) where they stated that HMO at 0.5 per cent concentration resulted in zero mortality percentage of bitter gourd gall midge parasitoids *Bracon* sp. and *Aprostocetus diplosidis* under laboratory conditions. Rugno and Qureshi (2024) also reported that HMO is safer compared to other globally used insecticides based on the topical and field-tested residual studies on the parasitoid *Tamarixia radiata* released against the Asian citrus psyllid *Diaphorina citri*. The nC24 horticultural mineral did not significantly affect the abundance and diversity of parasitoid species (Liang *et al*., 2010). Thomson *et al.* (2002) also conducted a field trial with nC21 Sunspray Ultra–Fine HMO on greedy scale *Hemiberlesia rapax* and its parasitoid *Encarsia citrina*. They concluded that the HMO had no toxicity against *Encarsia citrina.* This is in concordant with the results of the field experiment in apple done by Sofi and Khan (2021), who found that arbofine extra HMO at 0.5 per cent is safer to *Encarsia pernicious* and *Aphytis proclia,* parasitoids of san jose scale *Quadraspidiotus perniciosus.* It resulted in a least cumulative mean mortality of 26.46 per cent.

Even though HMO causes significant mortality of parasitoids in higher doses in the present study, it can be considered negligible compared to the effect of chemical pesticides on parasitoids. Abamectin and fenpyroximate at 0.06 per cent and 0.01 per cent resulted in significant mortality of parasitoid *Anagyrus vladimiri* compared to mineral oil formulations which are safer at even 1 per cent concentration (Singh *et al*., 2023).

CONCLUSION

Horticultural Mineral Oil **(**HMO) at lower concentrations is safer to parasitoids, *Trichogramma chilonis* Ishii. and *Bracon brevicornis* Wesmael compared to higher concentrations. This study provided experimental evidence based on the results of different bioassay methods. The absence of explicit research in this area can be considered both as a limitation and as an opportunity for various future investigations. The outcome of this study encourages judicious use of HMO in integrated pest management.

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