Evaluation of the safety of horticultural mineral oil to the predatory mite, *Neoseiulus longispinosus* and acaropathogen, *Acremonium zeylanicum*

ABSTRACT

Laboratory bioassay to evaluate the safety of Horticultural mineral oil (HMO) to the predatory mite, *N. longispinosus* revealed that HMO is relatively safer to the predator. At the highest concentration evaluated (3.0%) HMO resulted in 38.89 per cent and 22.22 per cent mortality of eggs and adults respectively. Horticultural mineral oil alone and in combination with neem oil was found to be inhibitory to the acaropathogen, *A. zeylanicum*. The combination treatment, HMO (3.0%) + neem oil (2.0%) recorded significantly higher inhibition of 86.44 per cent as against a significantly lower 46.67 per cent inhibition by HMO at 2.5 per cent. No visible phytotoxic effects were observed on okra plant at higher concentration of HMO (6%). The study revealed that HMO is relatively safer to the predatory mite, acaropathogen and to the okra plant. The HMO can be incorporated into IPM programmes for pest management.

Keywords: Bioassay, Inhibitory, Mortality, Phytotoxic

1. INTRODUCTION

The phytoseiid predator, *Neoseiulus longispinosus* (Evans) (Mesostigmata: Phytoseiidae) is one of the most potent predators of tetranychid mite in tropics and subtropics [1]. A recent study at Kerala Agricultural University (KAU) revealed the potential of *N. longispinosus* as a biocontrol agent against *Tetranychus urticae* on cucumber in polyhouse [2]. Moreover, an acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans was isolated from *Tetranychus urticae* on brinjal in polyhouse in Thrsissur district [3]. Evaluation of the same in the laboratory and polyhouse against *T. truncatus* revealed that *A. zeylanicum* can be a potential candidate in biological control spider mites on vegetable crops [4, 5]. Bioassay studies revealed that HMO at 2.0 and 2.5 per cent possess appreciable efficacy against both egg and adult stages of *T. truncatus* and is relatively safer to the predatory mite, *Neoseiulus longispinosus* [6]. Spider mites belonging to the genus *Tetranychus* have emerged as a major pest of vegetable crops causing considerable yield loss [7]. Farmers depend solely on acaricide molecules for mite management in polyhouses, which can lead to high residue levels and cause resurgence and resistance [29]. Hence, there is an increasing interest in natural pesticides which are derived from plants and microorganisms, since they are perceived to be safer than the synthetic pesticides. Mineral oils are been used for centuries to control several insect and mite pests of different ecosystem [8]. Horticultural mineral oils (HMO) have been used for pest control initially as dormant oil sprays for deciduous tree crops [9]. Currently, horticultural mineral oil reported resurgence in interest in insect pest control as they are compatible with modern sustainable management practices [10]. HMO can be an effective tool for mite management in vegetable

crops [11] without any risk of phytotoxicity and limited effects on beneficial organism because of recent advances in refined technology.

2. MATERIAL AND METHODS

To evaluate the safety of HMO to *N. longispinosus* and to the acaropathogen, *Acremonium zeylanicum*, laboratory bioassays were carried out separately on egg and active stages of the predator and *in vitro* evaluation, respectively (Fig. 1). For this, two best concentrations of HMO *viz.*, 2.5 and 3.0 per cent and its combinations with neem oil *viz.,* HMO (2.5 %) + neem oil (2.0%) and HMO (3.0%) + neem oil (2.0%) selected from the laboratory bioassay [12] against *T. truncatus* were evaluated along with neem oil 2.0 per cent alone and untreated control. The commercial formulation of horticultural mineral oil, Cristol TSO manufactured by Krishna Antioxidants Pvt. Ltd. Mumbai was used. The experiment was laid out in a Completely Randomized Design (CRD) with six treatments and three replications.

To check the ovicidal effect of HMO on the predatory mite, *N. longispinosus* the eggs of uniform age of *N. longispinosus* were obtained by transferring five gravid females of the predatory mite on to mulberry leaves with *T. truncatus* in petri plate arenas and they were removed after 24 h. In each Petri plate, six eggs of predator were retained and the excess eggs were removed. The leaves with *N. longispinosus* eggs were then sprayed with different treatments using a hand atomizer [13]. The number of predator eggs hatched in each treatment was recorded at 24 and 48 h after treatment under a stereo binocular microscope (LEICA EZ4 HD) and per cent mortality were calculated. The data on per cent mortality was subjected to analysis of variance.

The adulticidal effect of HMO on the adults of *N. longispinosus* was studied following leaf dip bioassay method [13]. Six adults of *N. longispinosus* were released on each treated leaves with the aid of fine brush. They were provided with adequate number of *T. truncatus* as food. All treatments were replicated three times. Mortality of predatory mites was recorded at 24, 48 and 72 h under a stereo binocular microscope (LEICA EZ4 HD). The data on per cent mortality was subjected to analysis of variance

An *in vitro* evaluation was carried out to test the inhibitory effects of HMO on the growth of the acaropathogen, *A. zeylanicum*. The neem oil was disinfected under UV light for one hour before mixing with SDAY medium to avoid contamination. Requisite quantity of each formulation was mixed with the 100 ml medium and 20ml was plated on each Petri plate. For perfect mixing of neem oil with the medium, Tween 20, a non-ionic surfactant @ 0.2 per cent was added [14]. Then, mycelial disc of 5mm diameter of *A. zeylanicum* was cut with sterile cork borer and placed aseptically at the centre of the plate containing poisoned medium and the plates were sealed with parafilm. The experiment was laid out in Completely Randomized Design (CRD) and each treatment was replicated thrice. Petri plates without poisoned medium served as untreated check. Inoculated plates were incubated at room temperature. Observations on mycelial growth of fungal pathogen were recorded next day onwards till the control plates showed 90mm growth and the per cent inhibition on growth of pathogen was calculated using the formula given by Vincent [15].

Per cent inhibition = $\frac{C-T}{C} x100$

where,

C = Diameter of fungal growth in control
T = Diameter of fungal growth in treatment

Phytotoxic effect of HMO at different concentrations of 2, 3, 4, 5 and 6 per cent was tested on potted okra plant along with an untreated control. The crop was raised in the grow bag as

per the Packages of Practices Recommendations by Kerala Agricultural University. The experiment was laid out in a Completely Randomized Design (CRD) with six treatments and three replications (five plants per replication). Emulsions of HMO were prepared by mixing appropriate quantities of HMO with water to obtain different treatments. Treatments were applied on 45 days old okra plants using a hand sprayer to run off. Observations on appearance of scorching on leaf tips and surfaces, yellowing, wilting, vein clearing and necrosis were recorded on 1, 3, 7, 10 and 15 days after application.

3. RESULTS AND DISCUSSION

Safety of HMO to the predatory mite was evaluated in the laboratory separately on eggs and adults. The effect of the best five treatments identified on the eggs of *N. longispinosus* was evaluated under laboratory conditions. At 24 h, hatchability of eggs did not vary significantly among treatments. By 48 hours all the treatments recorded significantly lower hatchability compared to untreated control. The treatment, HMO 3.0 per cent + neem oil 2.0 per cent recorded the highest egg hatchability (77.78%). This was followed by HMO 2.5 per cent, neem oil 2.0 per cent and HMO 2.5 + neem oil 2.0 per cent all of which recorded 66.67 per cent hatchability and HMO 3.0 per cent (61.11 %). However, the treatments did not differ significantly. Based on the egg hatchability at 48 h, the per cent mortality was derived. All the treatments resulted in significant mortality of predator eggs compared to control. Though highest mortality was recorded at HMO 3.0 per cent (38.89%), mortality did not vary significantly among treatments. HMO 2.5 per cent, neem oil 2.0 per cent and HMO 2.5 + neem oil 2.0 per cent recorded 33.33 per cent mortality each, while HMO 3.0 per cent + neem oil 2.0 per cent recorded 22.22 per cent mortality each (Table 1).

The safety of HMO to the gravid females of *N. longispinosus* was assessed under laboratory conditions. Twenty-four hours of treatment, mortality was recorded only in the treatments HMO 3.0 per cent + neem oil 2.0 per cent as well as neem oil 2.0 per cent, both causing 5.55 per cent mortality. After 48 h of treatment, highest mortality of 22.22 per cent was observed in HMO 3.0 per cent + neem oil 2.0 per cent followed by HMO 3.0 percent, neem oil 2.0 per cent and HMO 2.5 per cent + neem oil 2.0 per cent all recording 16.67 per cent mortality and HMO 2.5 per cent (11.11 %). However, there was no significant difference among the treatments. After 72 h, all the treatments caused significantly higher mortality of *N. longispinosus* compared to control. Highest mortality was recorded in the treatment HMO 3.0 per cent + neem oil 2.0 per cent (27.78%), followed by HMO 2.5 + neem oil 2.0 per cent, HMO 3.0 per cent and neem oil 2.0 per cent all of which recorded mortality of 22.22 per cent each. However, HMO 2.5 per cent recorded a mortality of 16.67 per cent. There was no significant difference among the treatments (Table 2).

The effect of HMO and its combinations with neem oil on the growth of fungus, *A. zeylanicum* was evaluated using poisoned food technique. The per cent inhibition of fungus ranged from 46.67 to 86.44 per cent in the treatments (Table 3). The inhibition of the acaropathogen was significantly more in the treatments with combinations of HMO and neem oil. The treatment, HMO 3.0 per cent + neem oil 2.0 per cent recorded the highest degree of inhibition of 86.44 per cent, followed by HMO 2.5 per cent + neem oil 2.0 per cent which recorded 78.81 per cent inhibition. This was followed by HMO 3.0 per cent (66.48 %) and neem oil 2.0 per cent (56.26 %). HMO 2.5 per cent recorded significantly the lowest growth inhibition of 46.67 per cent (Fig. 2).

Foliar application of HMO up to 6 per cent did not show any phytotoxic symptoms like scorching, yellowing, wilting, vein clearing or necrosis on 45 day old plants during the present investigation. Phytotoxic effect of mineral oil was tested on okra up to 20 per cent and reported that mineral oil up to 5 per cent was safe and showed no phytotoxic symptoms

[16]. However, mineral oil at 7, 10, 15, and 20 per cent, produced leaf injury to tip and leaf surface on 30 and 40-day old okra plants. In tea, HMO upto 2.0 per cent showed no phytotoxic symptoms up to 63 days of spraying under field conditions [17]. Dormant oils which were heavier and less refined were customarily used on woody plants during the dormant season. However, these older oils have been replaced with more refined, light-weight oils that have potential application to plant foliage. The impurities in the oil that is associated with plant injury, such as aromatic compounds and compounds containing sulfur, nitrogen or oxygen, are removed during refinement techniques for their safety to plants [18]. The HMO used in the present study is a refined product which could explain its lack of phytotoxicity on okra.

The study revealed that HMO is relatively safe to both eggs and adults of *N. longispinosus*. HMO at the highest concentration evaluated (3.0 %) recorded significantly higher egg mortality of 38.89 per cent. But it resulted in a mortality of only 22.20 per cent in the case of adult mite. In case of adults, highest mortality was recorded in treatment, HMO 3.0 per cent + neem oil 2.0 per cent (27.78 %). However, in the study HMO resulted in very high mortality of both eggs (100 %) and adults (HMO 2.5 and 3.0%) of *T. truncatus*. The results clearly indicate that HMO is relatively safer to the predatory mite, *N. longispinosus.* Safety of mineral oil to phytoseiid predatory mites was reported earlier by several workers. The combination of *Neoseiulus californicus* with petroleum spray oils produced significant control of *Tetranychus marianae* and did not affect the survival of *N. californicus* [19]. Nicetic *et al*. [20] reported that 0.5 per cent petroleum spray oil applied fortnightly to roses gave excellent protection from *T. urticae* Koch (Acarina: Tetranychidae) but did not affect predatory mites, *Phytoseiulus persimilis* (Acarina: Phytoseiidae). Moreover, Morse *et al.* [21] found that the predatory mite *Euseius stipulates* Athias-Henriot (Acarina: Phytoseiidae) was not significantly affected by oil sprays. In brinjal field treated with HMO 2.0 per cent, though the population of the phytoseiid predator, *N. longispinosus* was found to be significantly lower compared to the control plot, their population was significantly higher than in plots treated with the acaricides such as abamectin, fenazaquin, difenthiuron, fenpyroximate, proparite and hexythiazox [22]. Lower toxicity of HMO to the phytoseiid predator, *P. persimilis* was also reported by Cote *et al*. [23]. In addition to its high efficacy against red spider mite, *Tetranychus truncatus* HMO was also found to be safe to phytoseiid predator, *N. longispinosus* [12].

The study also revealed antagonistic effect of HMO on the acaropathogen, *A. zeylanicum*. HMO in combination with neem oil recorded significantly higher inhibition of the acaropathogen. However, HMO alone recorded significantly lower levels of inhibition with HMO at 2.5 per cent proving to be the safest treatment for the fungus. In a related study conducted by Kumar *et al.* [24], petroleum oils showed no negative impact on the growth parameters (CFUs and radial diameter) of an entomopathogenic fungus, *Isaria fumosorosea*. However, Andrew *et al*. [25] observed that direct exposure of entomopathogenic fungus, *Lecanicillium muscarium* for 24 h to spray oil (petroleum oil) had resulted in very low spore germination, suggesting variable response of different species of fungus to HMO. Horticultural mineral oil is reported to be having fungicidal and fungistatic activity. In combination with systemic fungicides, mineral oil facilitated foliar penetration [26]. Mineral oils have been reported to control both powdery mildew infestation and offer protection against two spotted mite [27].

Table 1. Effect of horticultural mineral oil on eggs of *Neoseiulus longispinosus*

Treatments	Mean hatchability (%)		Mean mortality (%)
	24 h	48 h	48 h
T_1 - HMO 2.5%	27.78	66.67 [b]	33.33 [a]

	(31.54)	(55.21)	(34.78)
T_2 - HMO 3.0%	11.11 (16.45)	61.11 [b] (51.49)	38.89 [a] (38.51)
T_3 - HMO 2.5% + neem oil 2.0%	50.00 (45.00)	66.67 [b] (55.21)	33.33 [a] (34.78)
T_4 - HMO 3.0% + neem oil 2.0%	27.78 (27.14)	77.78 [b] (62.18)	22.22 [a] (27.81)
T_5 - Neem oil 2.0%	27.78 (27.14)	66.67 [b] (55.21)	33.33 [a] (34.78)
T_6 – Control	38.89 (38.51)	100 [a] (88.83)	0.00 [b] (0.76)
CD value (p=0.05)	NS	14.55	14.55

Means followed by same letter in the column do not differ significantly.
Figures in the parentheses are arcsine transformed values.

Table 2. Effect of horticultural mineral oil on adults of *Neoseiulus longispinosus*

Treatments	Mean mortality (%)		
	24 h	**48 h**	**72 h**
T_1 - HMO 2.5%	0 (0.71)	11.11 (2.99)	16.67 [a] (3.56)
T_2 - HMO 3.0%	0 (0.71)	16.67 (3.56)	22.22 [a] (4.70)
T_3 - HMO 2.5% + neem oil 2.0%	0 (0.71)	16.67 (3.56)	22.22 [a] (4.70)
T_4 - HMO 3.0% + neem oil 2.0%	5.56 (1.85)	22.22 (4.70)	27.78 [a] (5.26)
T_5 - Neem oil 2.0%	5.56 (1.85)	16.67 (3.56)	22.22 [a] (4.70)
T_6 - Control	0 (0.71)	0 (0.71)	0.00 [b] (0.71)
CD value (p=0.05)	NS	NS	15.72

Means followed by same letter in the column do not differ significantly.
Figures in the parentheses are square root transformed values.

Table 3. *In vitro* evaluation of horticultural mineral oil against *Acremonium zeylanicum*

Treatments	Radial growth (mm)	Per cent inhibition over control (%)
T_1 - HMO 2.5%	48.00 [b]	46.67 [e]
T_2 - HMO 3.0%	30.16 [d]	66.48 [c]
T_3 - HMO 2.5% + neem oil 2.0%	19.06 [e]	78.81 [b]
T_4 - HMO 3.0% + neem oil 2.0%	12.20 [f]	86.44 [a]
T_5 - Neem oil 2.0%	39.36 [c]	56.26 [d]
T_6 - Untreated control	90.00 [a]	-

Means followed by same letter in the column do not differ significantly.

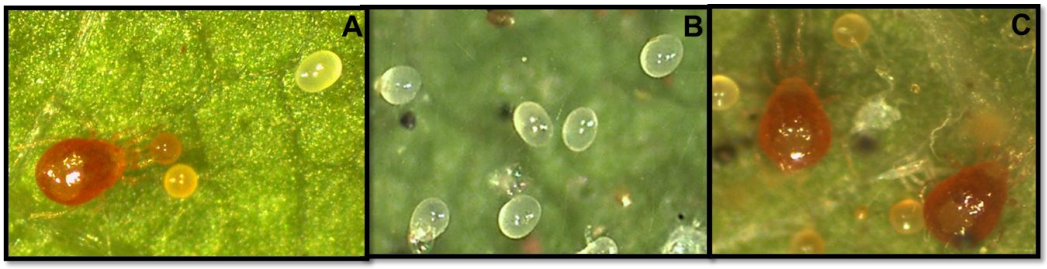

Figure 1. A) *N. longispinosus* attacking *Tetranychus truncatus* egg B) Eggs and C) Gravid females of *N. longispinosus* (35X)

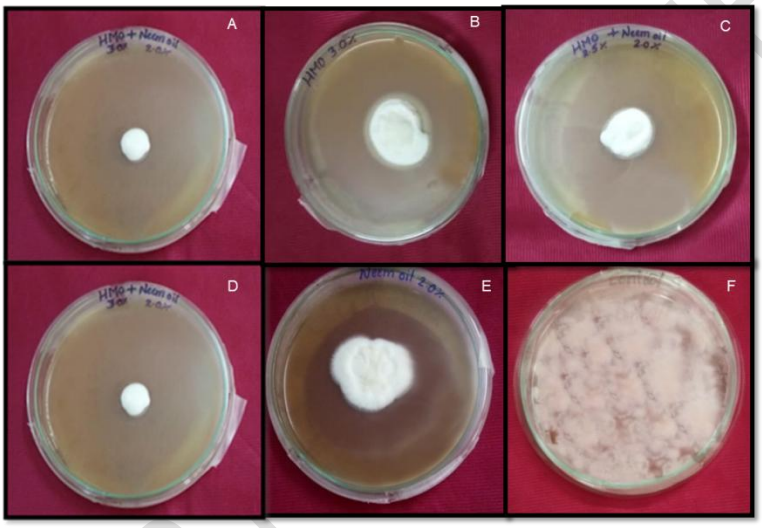

Figure 2. Mycelial growth of *Acremonium zeylanicum* in poisoned SDAY media A) HMO 2.5%; B) HMO 3.0% C) HMO 2.5%+neem oil 2.0%; D) HMO 3.0%+neem oil 2.0%; E) Neem oil 2.0%; F) Control

4. CONCLUSION

The high efficacy of HMO against the spider mites and its relative safety to the predatory mite, *N. longispinosus* and to the acaropathogen, *A. zeylanicum* brought out in the study suggests that HMO can be an effective tool for mite management in vegetable crops.

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