

**BIOEFFICACY OF HORTICULTURAL MINERAL OIL  
AGAINST THE SPIDER MITE, *Tetranychus truncatus*  
(PROSTIGMATA: TETRANYCHIDAE) ON OKRA**

By

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(2016-11-120)**

**THESIS**

**Submitted in partial fulfilment of the requirement  
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2018**

## DECLARATION

I, Kavya Yadav G. A. hereby declare that the thesis entitled “**Bioefficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae) on okra**” is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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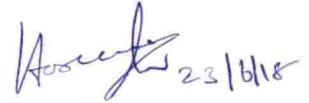
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## CERTIFICATE

Certified that this thesis entitled “**Bioefficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae) on okra**” is a bonafide record of research work done independently by Ms. Kavya Yadav G. A. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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## CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. Kavya Yadav G. A, a candidate for the degree of **Master of Science in Agriculture** with major field in Agricultural Entomology, agree that the thesis entitled "**Bioefficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae) on okra**" may be submitted by Ms. Kavya Yadav G. A. in partial fulfilment of the requirement for the degree.



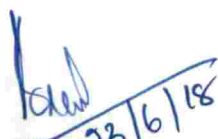
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*An inch forward but miles to go...*

*This thesis is only a stepping stone of my journey...*

*Dedicated to*

***“My inspiring  
Angel mother”***

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# Introduction

# 1. INTRODUCTION

Okra is cultivated in an area of 5,11,000 hectares in India with a production of 58,49,000 metric tonnes (GOI, 2017). Among several factors responsible for the low production of okra, the damage inflicted by insect and mite pests has been considered important (Varadaraju, 2010). More than hundred species of insects have been reported as pests of okra (Santhoshkumar *et al.*, 2013). However, among them, only to a few insects such as leaf hopper, aphid, whitefly and shoot and fruit borer are considered as economically important.

Among the mite pests, spider mites belonging to the genus *Tetranychus* have emerged as a major pest of okra causing considerable yield loss (Ghosh *et al.*, 1996; Srinivasa and Sugeetha, 1999; Kumaran *et al.*, 2007). Recent studies at Kerala Agricultural University indicate that *Tetranychus truncatus* Ehara has emerged as the predominant species of mite infesting vegetable crops in Kerala, including okra (Bennur *et al.*, 2015).

The spider mites colonise undersurface of the leaves and cause significant damage by feeding on sap. This results in yellowing and speckling of leaves, webbing, premature leaf fall, stunted growth, reduction in photosynthetic activity and ultimately death of the whole plant (Damirel and Cabuk, 2008). Apart from its polyphagous nature, high reproductive potential and short life cycle, factors such as change in climatic conditions and over-use of plant protection chemicals also help to compound the mite problem.

Though conventional pesticides offer good control, they have high residue levels and cause resurgence and resistance (Khajehali *et al.*, 2011; Sharma and Bhullar, 2018). Moreover they cannot be recommended during the later stages of the crop, when mite damage typically intensifies. Consequently, biocontrol agents, botanicals and mineral oils are increasingly being evaluated against the mites.

The phytoseiid predator *Neoseiulus longispinosus* (Evans) (Mesostigmata: Phytoseiidae) is one of the most potent predators of tetranychid mite in tropics and subtropics (Mallik *et al.*, 1998). 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 (Lenin and Bhaskar, 2017). Recently, an acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans was isolated from *Tetranychus urticae* on brinjal in polyhouse in Thrissur district (Krishna *et al.*, 2014). 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 (Sherief *et al.*, 2017; Sherief and Bhaskar, 2018).

Mineral oils have been used for centuries to control insect and mite pests on several crops (Egho and Emosairue, 2010). Use of highly refined horticultural mineral oils and agricultural mineral oils is an important tool in managing certain pest problems (eg., scales, aphids, mites) on fruit trees, shade trees and woody ornamental plants (Agnello *et al.*, 1994).

With recent advances in technology, refinement of petroleum oil to summer spray oils commonly called as horticultural mineral oils (HMOs) or agricultural mineral oils (AMOs) has made it possible to use them all the year round, without any risk of phytotoxicity (Davidson *et al.*, 1991; Agnello, 2002). Oils have several advantages over conventional pesticides, such as low mammalian toxicity, low residual toxicity, minimal risk of resistance development and limited effects on beneficial organism (Beattie *et al.*, 2002).

The present study was undertaken in order to explore the possibility of using horticultural mineral oil (HMO) against spider mites affecting okra with the following objectives

1. Evaluate the efficacy of horticultural mineral oil (HMO) against the spider mite, *Tetranychus truncatus* under laboratory and field conditions
2. Evaluate the phytotoxicity of HMO on okra
3. Evaluate the safety of HMO to the predatory mite, *Neoseiulus longispinosus*
4. Evaluate the safety of HMO to the acaropathogen, *Acremonium zeylanicum*

# Review of Literature

## 2. REVIEW OF LITERATURE

### 2.1 Horticultural mineral oil (HMO) in pest management

Petroleum based mineral oils have a long history of effective use for variety of insect pest management (Agnello, 2002). Horticultural mineral oils (HMO) have been used for pest control initially as dormant oil sprays for deciduous tree crops (Davidson *et al.*, 1991). Currently, horticultural mineral oil reported a resurgence in interest in insect pest control as they are compatible with modern sustainable management practices (Beattie *et al.*, 2002). However, HMO appears to be an underexploited tool in vegetable pest management.

#### 2.1.1 Horticultural mineral oil in insect pest management

In a field trial conducted to evaluate the efficacy of mineral oil against insects and mites in a season long programme on kiwi fruit at Hamilton, New Zealand, mineral oil at 2.0 per cent was found to significantly reduce the infestation of armoured scale, caterpillars, thrips and mites and was found to be on par with the standard chemical control (Tomkins *et al.*, 1996).

Seasonal mineral oil application in apple orchard was found to suppress the population of white leaf hopper, codling moth and woolly aphid (Fernandez *et al.*, 2005).

Horticultural mineral oil alone and in combination with fungicides and insecticide was evaluated at 0.5 and 1.0 per cent concentration on tomato to evaluate the response of insect pests. The population of silver leaf whitefly, green peach aphid, southern army worm and pepper weevil were significantly reduced (Stansly and Conner, 2005).

Horticultural mineral oil, Purespray Green @ 2 per cent resulted in complete mortality of the eggs of obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae) under laboratory conditions (Wins-Purdy *et al.*, 2009).

The effectiveness of three mineral oils namely, premium motor spirit, dual purpose kerosene and automotive gas oil were evaluated against four major insect pests of cowpea in Delta State University, Abraka, Southern Nigeria. The results showed that mineral oil at 0.4 per cent effectively controlled the cowpea aphid, *Aphis craccivora*, the legume pod borer, *Maruca vitrata*, legume bud thrips, *Megaleurothrips sjostedti* and pod sucking bugs (*Clavigralla tomentosicollis*, *Anoplocnemis curvipes* Fab, *Aspavia armigera* Fab and *Nezara viridula* L) (Egho and Emosairue, 2010).

Horticultural mineral oil (Mak all season) @ 1.5, 2.0 and 2.5 per cent concentrations significantly reduced thrips on citrus (Rao *et al.*, 2013).

The repellency characteristics of five mineral oils was studied against *Diaphorina citri* (Hemiptera: Liviidae) in laboratory bioassay. The results showed that various HMO's had significant behavioral repellent effects against *D. citri*. Repellency was significantly correlated with the carbon number distribution but not with their emulsifying agent (Ouyang *et al.*, 2013).

James *et al.* (2015) tested the costs and benefits of frequent low-volume applications of HMO for management of Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in comparison with grower standard (GS) (mixture of insecticide and HMO) for management of *Diaphorina citri* in Florida. It was found that HMO and GS treatments significantly reduced the Asian citrus psyllid and yields were greater for HMO treated than untreated control.

Horticultural mineral oil alone and in combination with neem oil and pongamia oil at 2 per cent concentration were found to cause significant mortality of the whitefly, *Bemisia tabaci* on okra both in the laboratory and pot culture (Sridharan *et al.*, 2015).

### 2.1.2 Horticultural mineral oil in mite management

Agnello *et al.* (1994) evaluated highly refined horticultural petroleum oil, sunspray ultra fine against apple red mite, *Panonychus ulmi*. Effective control was achieved with three applications of oil at 3 and 2 per cent, starting at the petal fall stage and continuing on 2-3 week schedule. One per cent oil provided control under conditions of moderate population pressure but required an additional spray in late July under severe population pressure. However lower concentrations of 0.05 and 0.25 per cent resulted in unacceptable mite numbers by midsummer.

Seasonal application of mineral oil in apple orchard was found to reduce the incidence of phytophagous tetranychid and eriophyid mites (Fernandez *et al.*, 2005).

Application of HMO in tomato and pepper significantly reduced the broad mite, *Polyphagotarsonemus latus* (Stansly and Conner, 2005).

A study was undertaken with acaricides and oils like mineral oil and Fish Oil Rosin Soap (FORS) against yellow mite *Polyphagotarsonemus latus* infesting chilli crop. After seven days of treatment chlorfenapyr, diafenthiuron, milbemectin, dicofol, fenazaquin and mineral oil (1 and 1.5%) recorded less number of mites (1 to 4.4 per 6 leaves) including eggs and active stages compared to untreated check with 17.7 per 6 leaves (AINPAA, 2009).

Studies on the effect of different HMO on winter egg hatchability of European red mite indicated that in arbofine treated twigs, 92.55 per cent eggs remained unhatched followed by HP HMO and servo with 87.7 and 83.37 per cent unhatched eggs as compared to untreated plants where maximum 78.1 per cent winter egg hatchability was recorded (AINPAA, 2013).

In apple, application of mineral oils was found effective against European red mite, *Panonychus ulmi*. Rilso-999 @ 1.5 per cent resulted in 85 per cent mortality or unhatching of diapausing eggs of *Panonychus ulmi* in Mashroba region of Himachal



Pradesh followed by mak all season and orchol-13 with 77 and 70 per cent ovicidal effect respectively (AINPAA, 2013).

Bioefficacy of HMOs and acaricides was evaluated against *T. urticae* on nethouse brinjal (BH-50) at Punjab Agricultural University (PAU), Ludhiana. Acaricides viz., fenazaquin @150 and 200 ml/acre resulted in maximum reduction in mite population followed by spiromesifen @ 200ml/acre. Among mineral oils, arbofine at different concentrations was found to be better in reducing mite population at all the doses, sparrow and BP mak were found more effective at the highest dose of 200ml/acre (AINPAA, 2013).

Field evaluation of HMO (Mak all season) against citrus rust mite, *Phyllocoptruta oleivora* in mandarin fruits revealed that per cent infestation was significantly lower in HMO 2.5 per cent (8.1% -14.8 %) compared to other treatments (20.0% - 55.8 %) (Rao *et al.*, 2013).

Petroleum based horticulture oil, servo agro spray oil was evaluated against tea red spider mite, *Oligonychus coffeae*. Servo agro spray oil @ 0.5, 1.0 and 1.5 per cent resulted in 92.04, 95.46 and 98.86 per cent mortality of eggs respectively. Significant mortality of adults was observed after 6 h (43.33% -83.33 %) and 48 h (63.33%-100 %) of treatment (Roy *et al.*, 2015).

### **2.1.3 Comparative efficacy of horticultural mineral oil with novel acaricides**

Citrus rust mite, *P. oleivora* (Damavandian, 2005) and citrus red mite, *P. citri* (Damavandian, 2007; Damavandian and Jafarabadi, 2007), could be more effectively controlled by mineral oil spray compared to synthetic pesticides.

A field trial was conducted to evaluate newer molecules, horticultural mineral oil (HMO) and NSKE at vegetable research farm Ludhiana. Fenazaquin 10 EC at 400g a.i. ha<sup>-1</sup> recorded 77.2 per cent reduction in egg population, which was on par with dicofol.

NSKE 5 per cent and HMO 1 per cent were on par with each other and HMO recorded 53.84 per cent reduction in the egg counts (AINPAA, 2011).

A field trial was conducted in Bangalore to evaluate the efficacy of novel acaricides and HMO against spider mite infesting okra. Propargite (425g ai/ha), HMO (1%) and ecomite (0.2%) were found to reduce the mite population significantly (AINPAA, 2011).

The potential of different mineral oils alone and in combinations with reduced rates of insecticides were evaluated in order to find alternatives to chlorpyrifos for the control of light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). Mineral oils demonstrated good ovicidal properties comparable with chlorpyrifos l-cyhalothrin and g-cyhalothrin. The mixtures of mineral oils with reduced rates of the above insecticides maintained residual efficacy (Taverner *et al.*, 2012).

A study was conducted to evaluate the efficacy of different acaricides and HMO against *T. urticae* on okra. Spiromesifen @200 ml/acre resulted in maximum per cent mortality of *T. urticae* two days after spraying followed by HMO @1000 and 1500ml/acre and fenpyroximate @ 200ml/acre (AINPAA, 2013).

Polyhouse trial at Himachal Pradesh, to assess the efficacy of three acaricides *viz.*, hexythiazox (0.005 %), fenazaquin (0.0025 %) and propargite (0.057 %) and one HMO, servo 1 per cent were evaluated against spider mites in beans. The maximum kill of mites was recorded in fenazaquin and propargite with a mite count of 0.8 mite/ leaf on third day of treatment as against precount of 39.2 and 41.0 mites/ leaf, respectively. However, HMO (servo 1 %) showed 3.6 mites/ leaf on 3 days after spray (AINPAA, 2013).

Studies were conducted on the effect of horticultural mineral oils on winter egg hatchability of European red mite. Three HMO's *viz.*, servo, HP summer oil and arbofine (2 % each) alone and in combination, and one insecticide carbosulfan 0.02 % (Marshal 20EC) were tested against winter eggs of mite. Results indicated that HMO played major role in controlling egg hatching in European red mite during late winter. In arbofine

treated twigs, 92.55 per cent eggs could not hatch followed by servo 1 % + carbosulfan 0.057 % (88.9 % unhatched eggs), HP HMO (unhatched 87.7 %) and servo (unhatched 83.37 %) as compared to untreated plants where maximum winter egg hatching was recorded (78.1 % egg hatch) (AINPAA, 2013).

A field trial was conducted in Coonor to evaluate the bio effectiveness of acaricides and different mineral oils against carnation mite (*T. urticae*) on the variety Darjeeling. Among the mineral oils tested, HMO 3 per cent (Bharat Petroleum oil) recorded 20.67 per cent reduction in mite population over control, 10 days after spray and found to be very effective in reducing the spider mite population in carnation (AINPAA, 2013).

A study was conducted in Ooty to evaluate entomopathogenic fungi (EPF), predatory mites and mineral oil under protected cultivation against carnation mite on carnation variety Master. Mineral oil (2.0%) treated plot resulted in mean mite population of 3nos./2 cm<sup>2</sup> leaf area as compared to untreated check with 16.29nos./2 cm<sup>2</sup> after two rounds of spray and revealed promising features of mineral oil in reducing mite population followed by EPF and predatory mites (AINPAA, 2013).

A field trial was conducted on the bioefficacy of horticultural mineral oils viz. Mak all season and H.P summer oil 1 per cent each and acaricides viz., propargite 0.057%, fenazaquin 0.0025% and hexythiazox 0.005% against two spotted spider mite (*Tetranychus urticae*) on strawberry. Results clearly indicated that two applications of HMOs at 1.0 per cent suppressed two spotted mite on strawberry (AINPAA, 2013).

Different acaricides and mineral oil were tested against *T. urticae* infesting brinjal plants in Bangalore during 2013. Acaricides viz., propargite and spiromesifen reduced the overall mite population by 0.78 and 1.05 mites/leaf respectively. HMO at 1 and 2 per cent resulted in 2.61 and 2.44 mean mite population respectively, on *T. urticae* within three days of treatment (Kavya *et al.*, 2015).

Comparison studies were conducted on the use of conventional pesticides with mineral oil sprayed to control important insect pests of citrus. Population diversity of

citrus pests was significantly higher in conventional orchards compared to mineral oil sprayed orchards (Damavandian, 2016).

Singh *et al.* (2017) evaluated the bio-efficacy of six pesticides along with HMO against two-spotted spider mite on rose under poly house conditions. Fenazaquin at 0.0025 per cent concentration was found to be the most effective recording 60.94 mean per cent reduction of active stages of mite after 10 days of spray, while HMO at 2.5 percent concentration recorded 55.60 per cent reduction.

## 2.2 Phytotoxicity of horticultural mineral oils

A field experiment was carried out to ascertain the phytotoxic effect of different concentrations of mineral oil *viz.*, 0.25, 0.5, 1.0, 1.25 and 1.5 per cent on 60 days old healthy plants of okra and chilli. Mineral oil @ 1.25 and 1.5 per cent showed mild phytotoxicity on chilli and okra plants two days after treatment. However, the plants recovered from scorching effect after 5 days (Sudha, 2008).

Five different HMO's *i.e.* BP mak, sparrow, servo IOC and arbofine all @ 1, 2, 3, 4 and 5 ml/L were sprayed on 30 days old brinjal crop (Hybrid BH-50) at Entomological Research Farm, PAU, Ludhiana, under field conditions. All the oils at higher concentrations of 4 and 5ml/L caused low to moderate phytotoxicity symptoms in the form of chlorotic patches, bronzing and necrosis (AINPAA, 2013).

Horticultural mineral oil (servo agro spray oil) up to 2 per cent when used for the management of tea mite, *Oligonychus coffeae* showed no phytotoxic symptoms up to 63 days of spraying under field conditions (Roy *et al.*, 2015).

Different concentrations of HMO *viz.*, 3, 5, 7, 10, 15 and 20 per cent were tested for phytotoxic symptoms on potted okra plants. Foliar application of HMO 3 and 5 per cent produced no phytotoxic symptoms on 30 and 45 day old plants. However, HMO at 7, 10, 15 and 20 per cent produced injury to tips and surfaces of leaves with a rating of 2.0, 2.0, 3.33, and 6.33 on 30 day old plants and 2.0, 2.0, 3.0, 5.0 on 45 day old plants, respectively (Sridharan *et al.*, 2015).

### 2.3 Safety of HMO to natural enemies

Different concentrations of neem up to 0.1 g a.i/l showed no negative effects on the survival of predatory mite, *Iphiseiodes zuluagai* (Venzon *et al.*, 2005).

According to Stansly *et al.* (2002), use of HMO was the best way of protection of beneficial arthropods with minimal effects instead of broad-spectrum insecticides and acaricides. HMO was reported to be one of the few groups to which insect resistance has never been reported (Willett and Westigard, 1988; Helmy *et al.*, 2012).

The use of petroleum spray oils in navel orange orchard in South China was found to increase the species richness of natural enemies and reduce the use of chemical pesticide (Chen *et al.*, 2009).

Leong *et al.* (2012) reported that mineral oils controlled *Diaphorina citri* without any negative effect on citrus trees. The study also showed that mineral oils have low toxicity to primary parasitoids *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae).

It was reported that the population growth of phytoseiid predatory mite was higher and faster in the mineral oil sprayed orchards compared to conventional orchards (Damavandian, 2010).

In a study conducted to evaluate the safety of acaricides and horticultural mineral oils against predator, *Neoseiulus longispinosus*, HMO (servo 1%) proved to be relatively safe to the predator. It recorded only 28.2 per cent mortality compared to 40-60 per cent mortality of predators with synthetic acaricides *viz.*, hexythiazox (39.2%), endosulfan (43.8%), fenazaquin (54.7%) and carbosulfan (59.5%) (AINPAA, 2013).

Horticultural mineral oil at 2.0 per cent significantly reduced spider mite population on brinjal 10 days after application, and proved to be safe to the predatory mite, *Neoseiulus longispinosus* (Kavya *et al.* 2015).

Biosafety studies on mineral oil alone and in combination with botanicals to the predator, *Chrysoperla carnea* showed that mineral oil (2.0 %) resulted in 89.09 per cent egg hatchability of *C. carnea*. Mineral oil + neem oil and mineral oil + karanj oil produced 89.44 and 93.52 egg hatchability respectively. Among different treatments, mineral oil + karanj oil (2.0%) recorded the highest mortality of grubs (16.67 %) at 24 h after treatment compared to an untreated check with lower mortality of grubs (Sridharan *et al.*, 2015).

Rao *et al.* (2014) reported that MAS-HMO (upto 1.0 per cent) was safe to the grubs of *Cheilomenes sexmaculata* as mortality of grubs was nil. HMO at 2.0 per cent (18.86% grub mortality) recorded significantly low mean mortality of grubs of *C. sexmaculata* than imidacloprid @ 0.009% (41.1%).

## **2.4 Plant based oils for mite management**

NSKE and mahua oil at 4 per cent were effective against adults of *T. urticae* resulting in mortality ranging from 35 to 65 per cent (Chandrashekarappa, 1995).

Chandrashekar (1997) reported that neem, pongamia and mahua oils at 3, 4 and 5 per cent were effective against adults of *T. urticae* on French bean. After 72 h of treatment, mahua oil and pongamia oil at 5 per cent caused complete mortality followed by 4 per cent mahua oil (97% mortality), neem oil (90% mortality) and pongamia oil (87% mortality).

Citronella oil 0.4 and 0.6 per cent was more consistent in inducing the walk-off response in the adult females of *T. macfarlanei* upto 24 h, as more than 50 per cent of the individuals attempted to move out of treated okra leaves (Sugeetha, 1998).

Srinivasa and Sugeetha (1999) reported that neem oil, pongamia oil and mahua oil at 3 per cent were effective against *T. macfarlanei* under laboratory conditions causing a mortality of 68-76 per cent, 72 hours after treatment.

Yathiraj and Jagadish (1999) reported the effect of various plant extracts on *T. urticae* under laboratory conditions and indicated neem seed kernel extract (NSKE) at 5 and 1 per cent resulted in mortality of 60.25 and 51.20 per cent mite respectively.

Among the neem products evaluated for efficacy in controlling red spider mites (*Tetranychus* spp.) on okra, neem oil (1%) showed significantly higher mortality (79.60%) of mites 24 h after treatment compared to achook and nimbecidine which were on par with each other (Umamaheshwari *et al.*, 1999). Kumaran *et al.* (2007) reported that among the neem products tested, neem oil and NSKE were least effective against red spider mite on okra, however they were superior to pongamia oil.

Field experiment on cowpea for managing *T. ludeni* using botanicals proved that neem oil 5 per cent could be successfully used for suppressing the mite population. It was followed by neem garlic 2 per cent. But after five days, the mite population increased which showed the relevance of requirement of repeated application of botanicals (Abhilash, 2001).

Among the various botanicals tested for ovicidal action against *T. macfarlanei*, neem oil proved superiority by recording maximum egg hatch inhibition of 55.18 per cent. Neem oil also caused significantly higher mortality of adults (21.67 %) at 24 h which was on par with NSKE (18.33 %) (Patil, 2005).

Among the different biorationals tested against tetranychid mites on brinjal, neem oil 2 per cent and NSKE 5 per cent were superior over nimbecidine, azadirachtin, pongamia oil, *Lecanicillium lecanii* (Zimmerman) Gams and Zare and *Metarhizium anisopliae* (Metschnikoff) Sorokin in reducing the mite population. But, they were found inferior to standard check, dicofol. The analysis of yield and net return of various biorationals have indicated that neem oil and NSKE recorded higher yield and maximum net returns (Prasanna, 2007).

Onkarappa (1999) reported that 24 h after application, mahua oil (1 to 2%) and neem oil (2%) caused 80-88 and 74 per cent reduction in the number of adults of *T. urticae*, respectively, on rose grown in open fields.

Among the various botanicals studied for efficacy against red spider mite on brinjal in Karnataka, neem oil 2 per cent exhibited maximum acaricidal action (Patil and Nandihalli, 2009). At Varanasi, among the various botanicals tested against spider mites, NSKE 5 per cent proved to be the best, followed by azadirachtin and mahua oil respectively (AINPAA, 2009).



# Materials & Methods

### 3. MATERIALS AND METHODS

The present study entitled “Bioefficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) on okra” was carried out in the Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara during 2017-2018. The objectives of the investigation were to evaluate the bioefficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus*, to test the phytotoxicity on okra as well as to evaluate its safety to the predatory mite, *Neoseiulus longispinosus* and the acaropathogen, *Acremonium zeylanicum*. The details of the materials used and methods adopted for conducting various experiments based on the objectives set forth in this study are presented here under.

#### 3.1 Bioefficacy of horticultural mineral oil (HMO) against *Tetranychus truncatus* on okra

The commercial formulation of horticultural mineral oil, Cristol TSO manufactured by Krishna Antioxidants Pvt. Ltd. Mumbai was used for laboratory and field evaluation in the present study. Cristol TSO is approved for use in organic agriculture according to NPOP and USDA standards. Cristol is high grade paraffin oil recommended against red spider mites and other insect pests (Plate 1). Bioefficacy of HMO against *T. truncatus* was evaluated in the laboratory as well as in the field.

##### 3.1.1 Mass culture of *Tetranychus truncatus*

*Tetranychus truncatus* was mass cultured in the laboratory on mulberry leaves placed in plastic trays (40×25cm<sup>2</sup>) lined with moistened synthetic absorbent sponge (Plate 2). Mulberry leaves were placed with their abaxial surface on wet sponge. Gravid females collected from the nucleus culture maintained in the Acarology laboratory of AINPAA (All India Network Project on Agricultural Acarology) were released on the leaves. Leaves were replaced with fresh ones once every four to five days by placing old leaf with mites above the new leaf where upon the mites moved to fresh leaves on their



Plate 1. Horticultural mineral oil



Plate 2. Laboratory culture of *Tetranychus truncatus* on mulberry leaves



Plate 3. Mass culturing of *Tetranychus truncatus* on okra plants in polyhouse

own, so that *T. truncatus* got transferred to the new leaf naturally. In the polyhouse, *T. truncatus* population was maintained on okra plants (variety Arka Anamika) (Plate 3).

### **3.1.2 Evaluation of horticultural mineral oil in laboratory**

Laboratory bioassays were conducted to evaluate the bioefficacy of HMO alone and in combinations with neem oil on egg and gravid females of *T. truncatus* (Table 1) (Plate 4). The experiment was laid out in a Completely Randomized Design (CRD) with fourteen treatments and three replications (Plate 5 and 6).

#### **3.1.2.1 Ovicidal effect of horticultural mineral oil**

The effect of HMO on the eggs of *T. truncatus* was studied in the laboratory following topical application method.

Eggs of uniform age were obtained by transferring ten gravid females of *T. truncatus* each from laboratory culture using moistened camel hair brush (zero size) on to three mulberry leaf bits ( $5 \times 5 \text{cm}^2$ ) placed in Petri plates lined with moistened cotton pad. A thin layer of wet cotton was provided all around the leaf bits to prevent the escape of mites. The female mites were removed after 24 h. Twenty five eggs were retained per leaf bit after removing the excess eggs. Leaf bits containing *T. truncatus* eggs were sprayed with appropriate treatments using a hand atomizer (2ml/bit) (Plate 7). All the treatments were replicated three times. Observations on hatchability of eggs were recorded at 24, 48, 72 and 96 h of treatment under a stereo binocular microscope (LEICA EZ4 HD). Per cent mortality of eggs was calculated as follows

$$\text{Per cent mortality} = \frac{25 - \text{no. of eggs hatched}}{25} \times 100$$

##### **3.1.2.1.1 Data analysis**

The data on per cent mortality of eggs was then subjected to analysis of variance.

**Table 1. Different concentrations of horticultural mineral oil evaluated against red spider mite, *Tetranychus truncatus* under *in vitro* condition**

Sl. No.	Treatments
1.	HMO 0.5 %
2.	HMO 1.0 %
3.	HMO 1.5 %
4.	HMO 2.0 %
5.	HMO 2.5 %
6.	HMO 3.0 %
7.	HMO 0.5 % + neem oil 0.5 %
8.	HMO 1.0 % + neem oil 1.0 %
9.	HMO 1.5 % + neem oil 1.5 %
10.	HMO 2.0 % + neem oil 2.0%
11.	HMO 2.5 % + neem oil 2.0%
12.	HMO 3.0 % + neem oil 2.0%
13.	Neem oil 2.0%
14.	Control



Plate 4a. *T. truncatus*, adult with eggs (35X)



Plate 4b. Eggs of *T. truncatus* (25X)



Plate 4c. Adults of *T. truncatus* (25X)

Plate 4. *Tetranychus truncatus*



**Plate 5. Horticultural mineral oil treatments**



**Plate 6. Experimental layout of laboratory bioassay**





**Plate 7. Topical application method**



**Plate 8. Leaf dip method**



### 3.1.2.2 Adulticidal effect of horticultural mineral oil

The effect of HMO on gravid females of *T. truncatus* was studied in the laboratory following leaf dip bioassay.

Mulberry leaf bits of  $7 \times 7 \text{cm}^2$  were dipped for 30 seconds in each of the treatment solution, air dried for 30 minutes and then placed in Petri plates lined with moistened cotton pad (Plate 8). Twenty five gravid females of *T. truncatus* of uniform age taken from laboratory culture were released on each treated leaf bit by using moistened camel hair brush. To prevent the escape of mites from treated leaves, a thin layer of wet cotton was provided all around the leaf margin. All treatments were replicated three times. Observations on mortality of mites were recorded at 24 h interval for seven days under a stereo binocular microscope (LEICA EZ4 HD).

#### 3.1.2.2.1 Data analysis

The data on per cent mortality was subjected to analysis of variance.

### 3.1.3 Evaluation of horticultural mineral oil under field condition

A field experiment was carried out to test the efficacy of HMO against *T. truncatus* on okra (variety Arka Anamika) at College of Horticulture, KAU, Vellanikkara during March, 2018. Two best concentrations of HMO namely 2.5 and 3.0 per cent and its combinations with neem oil *viz.*, HMO 2.5 per cent + neem oil 2 per cent and HMO 3.0 per cent + neem oil 2.0 per cent identified in the laboratory bioassay against *T. truncatus* were evaluated along with neem oil 2.0 per cent alone, an acaricide spiromesifen @ 0.02 per cent and a control treatment with water spray (Table 2). The crop was raised as per the Package of Practices Recommendations (KAU, 2016) at a spacing of  $60 \times 30 \text{cm}$  in plots of  $2 \times 2 \text{m}$  size. The experiment was laid out in Randomized Block Design with seven treatments and four replications (Plate 9). Mites were released on 45 days old okra plant at the rate of 25 active mites/leaf by stapling mite infested mulberry leaf bit of  $3 \text{cm}^2$  size each on top, middle and bottom leaf of okra plant. Treatments were imposed two weeks after the release of mites using a hand sprayer.

The number of mites from three windows of 1cm<sup>2</sup> each from top, middle and bottom leaves of randomly selected five plants per replication. The mite count was recorded *in situ* by using a hand lens of 10X magnification one day before spraying and 1, 3, 7, 10 and 14 days after spraying.

### 3.1.3.1 Statistical analysis and interpretation of data

Data on mean population of mites were transformed using square root transformation. Population difference on one, three, seven, ten and fourteen days after treatment were tested by one way ANOVA. The mean per cent reduction in population of mites over pre count was also worked on seven and fourteen days after treatment application.

$$\text{Per cent reduction} = \frac{\text{Pre count} - \text{Post count}}{\text{Pre count}} \times 100$$

**Table 2. Different concentrations of horticultural mineral oil evaluated under field condition**

Sl. No.	Treatments
1.	HMO 2.5 %
2.	HMO 3.0 %
3.	HMO 2.5 % + neem oil 2.0%
4.	HMO 3.0 % + neem oil 2.0%
5.	Neem oil 2.0%
6.	Spiromesifen 240 SC - 0.02%
7.	Control



Plate 9. Layout of field experiment

### **3.2 Evaluation of phytotoxicity of horticultural mineral oil under pot culture experiment**

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 during August to October, 2017. The crop was raised in the grow bag as per the Packages of Practices Recommendations (KAU, 2016). The experiment was laid out in a Completely Randomized Design (CRD) with six treatments and three replications (five plants per replication) (Plate 10). 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.

### **3.3 Evaluation on safety of horticultural mineral oil to the predatory mite, *Neoseiulus longispinosus***

#### **3.3.1 Maintenance of *Neoseiulus longispinosus* culture**

*Neoseiulus longispinosus* was multiplied on *T. truncatus* culture maintained on mulberry leaves. The mulberry leaves were placed on plastic trays lined with moistened synthetic absorbent sponge and gravid females of *T. truncatus* were released (Plate 11). Four days after release of prey mite, six gravid females of *N. longispinosus* were released on prey culture. Predatory mite culture was monitored daily and prey mites were replenished periodically. Leaves were replaced with fresh ones in every five days. For replacing mulberry leaf, old leaf was placed above the new leaf so that the prey mite, *T. truncatus* and the predatory mite, *N. longispinosus* got transferred to the new leaf naturally.





Plate 10. Layout of pot culture experiment



Plate 11. Culturing of *Neosieulus longispinosus* on mulberry leaves in laboratory

### 3.3.2 Laboratory bioassay

Laboratory evaluation of safety of HMO to *N. longispinosus* was carried out separately on egg and active stages of the predator (Plate 12). The two best treatments of HMO alone and its combinations with neem oil identified in the laboratory bioassay against *T. truncatus* were evaluated along with neem oil 2.0 per cent (Table 3). The experiment was laid out in a Completely Randomized Design (CRD) with six treatments and three replications.

#### 3.3.2.1 Ovicidal effect of horticultural mineral oil on *Neoseiulus longispinosus*

Ovicidal effect of HMO on the predatory mite, *N. longispinosus* was studied following topical application method.

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 plates arenas as already described under 3.1.2.1. 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. 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 mortality was calculated.

##### 3.3.2.1.1 Data analysis

The data on per cent mortality was subjected to analysis of variance

#### 3.3.2.2 Adulticidal effect of horticultural mineral oil on *Neoseiulus longispinosus*

The effect of HMO on the adults of *N. longispinosus* was studied following leaf dip bioassay method as already described. 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).



Plate 12a. *N. longispinosus* attacking *Tetranychus truncatus* egg (35X)

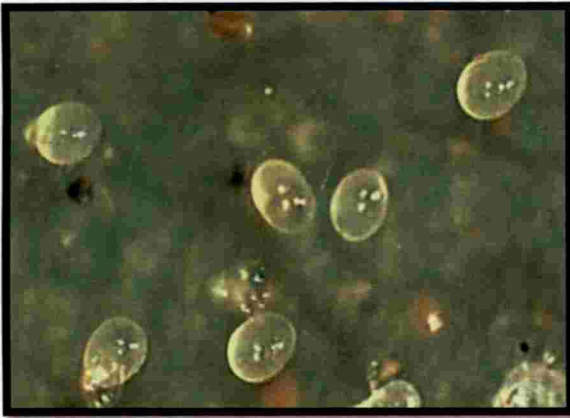


Plate 12b. Eggs of *N. longispinosus*  
(35X)



Plate 12c. Gravid females of *N. longispinosus*  
(35X)

Plate 12. *Neoseiulus longispinosus*



### 3.3.2.2.1 Data analysis

The data on per cent mortality was subjected to analysis of variance.

**Table 3. Different concentrations of horticultural mineral oil evaluated for safety to predatory mite, *Neoseiulus longispinosus* and to the acaropathogen, *Acremonium zeylanicum***

Sl. No.	Treatments
1.	HMO 2.5 %
2.	HMO 3.0 %
3.	HMO 2.5 % + neem oil 2.0%
4.	HMO 3.0 % + neem oil 2.0%
5.	Neem oil 2.0%
6.	Control

## 3.4 Safety of horticultural mineral oil to the acaropathogen, *Acremonium zeylanicum*

### 3.4.1 Maintenance of *Acremonium zeylanicum* culture

The pure culture of the acaropathogen, *A. zeylanicum* procured from the Acarology laboratory, AINPAA was used for the study (Plate 13). The acaropathogen was sub cultured on specific medium, Sabouraud Dextrose Agar with the addition of 2 per cent Yeast extract (SDAY) (Appendix 1) in slants and on Sabouraud Dextrose in conical flasks (SDY). To maintain the virulence, spore suspension was prepared by grinding the broth culture of *A. zeylanicum* with thick fungal mat in an ordinary sterilized mixer. The suspension was shaken thoroughly with a drop of 0.05 per cent Tween 80 for uniform dispersion of spores. The suspension of spores was filtered through a double layered muslin cloth and sprayed on *T. truncatus* culture maintained on mulberry leaves. The fungus was reisolated from the infected (moribund) mites on SDAY media. The medium was prepared and autoclaved at 121° C temperature and 15 PSI pressure for 20 minutes. After sterilization, the medium was cooled and approximately 20 ml was transferred to sterilized Petri dishes and the dishes were inoculated with pure culture of fungus under aseptic condition. All the plates were incubated at room temperature



(26±1°C) and observed for fungal growth from next day onwards. The purity of the culture was confirmed by preparing slides and observing under phase contrast microscope (LEICA ICC50). The pure culture of *A. zeylanicum* was maintained in SDAY slants by subsequent subculturing and was used for further work.

### 3.4.2 *In vitro* evaluation on sensitivity of *Acremonium zeylanicum* to horticultural mineral oil

An *in vitro* evaluation was carried out to test the inhibitory effects of HMO on the growth of the acaropathogen, *A. zeylanicum*. 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 against *T. truncatus* were evaluated along with neem oil 2.0 per cent alone and an untreated control (Table 3).

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 (Neves *et al.*, 2001). 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 (1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

where,

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment



Plate 13. *Acremonium zeylanicum* culture

# Results

## 4. RESULTS

The results of the study on bioefficacy of horticultural mineral oil (HMO) against the spider mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae) on okra are presented in this chapter.

### 4.1 Evaluation of horticultural mineral oil against red spider mite, *Tetranychus truncatus*

Horticultural mineral oil was evaluated for its efficacy against *T. truncatus* both in laboratory and field.

#### 4.1.1 Laboratory bioassay

Bioassay studies were carried out in the laboratory to study the effect of HMO on eggs and gravid females of *T. truncatus* separately.

##### 4.1.1.1 Ovicidal effect of horticultural mineral oil on *Tetranychus truncatus* eggs

The results of the ovicidal bioassay showed that the eggs did not hatch upto 48 h in any of the treatments. Significantly higher egg hatchability was recorded in control treatment (94.66%) at 72 h after treatment application followed by HMO 1.0 per cent (2.66%), HMO 0.5 per cent (1.33%) and the combination of HMO 0.5 per cent + neem oil 0.5 per cent (1.33%) which were on par with each other. However no hatching was observed in other treatments at 72 h (Table 4).

Control treatment recorded significantly higher hatchability of 98.67 per cent after 96 h as well. This was followed by; neem oil 2.0 per cent (6.67%), HMO 1.5 per cent + neem oil 1.5 per cent (4.0%), HMO 0.5 per cent + neem oil 0.5 per cent (4.0%), HMO 1.0 per cent (2.66%) and HMO 0.5 per cent (1.33%) which were on par with each other. Eggs did not hatch in other treatments at 96 h.

Based on the egg hatchability at 96 h, per cent mortality of eggs was worked out. Significant mortality of eggs was observed in all the treatments except control, which ranged from 93.33 per cent to a maximum of 100 per cent. Highest mortality of 100 per

cent was recorded in treatments HMO 1.5 per cent, HMO 2.0 per cent, HMO 2.5 per cent, HMO 3.0 per cent and the combinations HMO 1.0 per cent + neem oil 1.0 per cent, HMO 2.5 per cent + neem oil 2.0 per cent, HMO 3.0 per cent + neem oil 2.0 per cent which were on par with each other. The treatments HMO 0.5 per cent (98.67%), HMO 1.0 per cent (97.33%) and the combinations HMO 2.0 per cent + neem oil 2.0 per cent (97.33%), HMO 1.5 per cent + neem oil 1.5 per cent (96%) and HMO 0.5 per cent + neem oil 0.5 per cent (96%) recorded mortality on par with the treatments that recorded 100 per cent mortality. These treatments were also on par with neem oil 2.0 per cent which recorded a mortality of 93.33 per cent. The lowest egg mortality of 1.33 per cent was recorded in control treatment.

**Table 4. Effect of horticultural mineral oil on eggs of *Tetranychus truncatus***

Treatments	Mean hatchability (%)				Mean mortality (%)
	24 h	48 h	72 h	96 h	96 h
T <sub>1</sub> - HMO 0.5%	0 (0.76)	0 (0.76)	1.33 <sup>b</sup> (4.35)	1.33 <sup>bc</sup> (4.35)	98.67 <sup>ab</sup> (85.64)
T <sub>2</sub> - HMO 1.0%	0 (0.76)	0 (0.76)	2.66 <sup>b</sup> (5.98)	2.67 <sup>bc</sup> (5.98)	97.33 <sup>ab</sup> (84.01)
T <sub>3</sub> - HMO 1.5%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>4</sub> - HMO 2.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>5</sub> - HMO 2.5%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>6</sub> - HMO 3.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>7</sub> - HMO 0.5% + neem oil 0.5%	0 (0.76)	0 (0.76)	1.33 <sup>b</sup> (4.35)	4.0 <sup>bc</sup> (9.57)	96.00 <sup>ab</sup> (80.42)
T <sub>8</sub> - HMO 1.0% + neem oil 1.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>9</sub> - HMO 1.5%+ neem oil 1.5%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	4.0 <sup>bc</sup> (9.57)	96.00 <sup>ab</sup> (80.42)
T <sub>10</sub> - HMO 2.0%+ neem oil 2.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	2.66 <sup>bc</sup> (5.98)	97.33 <sup>ab</sup> (84.01)
T <sub>11</sub> - HMO 2.5% + neem oil 2.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>12</sub> - HMO 3.0% + neem oil 2.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	0.00 <sup>c</sup> (0.76)	100 <sup>a</sup> (89.23)
T <sub>13</sub> - Neem oil - 2.0%	0 (0.76)	0 (0.76)	0.00 <sup>b</sup> (0.76)	6.67 <sup>b</sup> (11.96)	93.33 <sup>b</sup> (78.04)
T <sub>14</sub> - Control	0 (0.76)	0 (0.76)	94.67 <sup>a</sup> (79.14)	98.67 <sup>a</sup> (85.64)	1.33 <sup>c</sup> (4.35)
CD value (p=0.05)	NS	NS	7.13	9.98	9.98

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

#### 4.1.1.2 Adulticidal effect of horticultural mineral oil on gravid females

The effect of HMO on the gravid female is furnished in the Table 5.

One day after treatment, HMO 3.0 per cent recorded significantly higher mortality of 65.33 per cent, followed by HMO 2.5 per cent (54.67%). This was followed by neem oil 2.0 per cent (42.67%), HMO 3.0 per cent + neem oil 2.0 per cent (38.67%) and HMO 2.0 per cent (34.67%) which were on par with each other (Table 5). The treatment HMO 2.5 per cent + neem oil 2 per cent recorded mortality (30.67%) on par with HMO 2.0 per cent and the combination HMO 3.0 per cent + neem oil 2.0 per cent. The next best treatments in the order of mortality were HMO 2.0 per cent + neem oil 2.0 per cent (26.67%), HMO 1.5 per cent (24.00%), HMO 1.5 + neem oil 1.5 per cent (20.00%), HMO 1.0 per cent + neem oil 1.0 per cent (18.67%), HMO 0.5 per cent + neem oil 0.5 per cent (16.00%) and HMO 0.5 per cent (14.67%).

On second day of treatment also, highest mortality was observed in the treatment HMO 3.0 per cent (73.33%), followed by HMO 2.5 per cent and neem oil 2.0 per cent (58.67 %) which were on par with each other. The treatments, HMO 3.0 per cent + neem oil 2.0 per cent (48.00%), HMO 2.0 per cent (46.67%), HMO 2.5 per cent + neem oil 2.0 per cent (40.00%) were on par with each other. Mortality recorded by treatments HMO 2.0 per cent + neem oil 2.0 per cent (33.33%), HMO 1.5 per cent (32.00%), and HMO 1.5 per cent + neem oil 1.5 per cent (29.33%), HMO 1.0 per cent (28.00%) were on par with each other. Both the treatments, HMO 0.5 per cent + neem oil 0.5 per cent and HMO 1.0 per cent + neem oil 1.0 per cent recorded a mortality of 24.00 per cent. The least mortality 17.33 per cent was observed in the treatment HMO 0.5 per cent.

After three days of treatment application, HMO 3.0 per cent recorded a significantly higher mortality of 77.33 per cent mortality. Mortality recorded in treatments *viz.*, neem oil 2.0 per cent (69.33%), HMO 3.0 per cent + neem oil 2.0 per cent (62.60%) and HMO 2.5 per cent (62.67%) were on par with each other. This was followed by HMO 2.0 per cent (53.33%), HMO 2.5 per cent + neem oil 2.0 per cent (45.30%), HMO 2.0 per cent + neem oil 2.0 per cent (37.33%), HMO 1.5 per cent (37.33%), HMO 1.5 per cent + neem oil 1.5 per cent (34.67%), HMO 1.0 per cent

(33.33%), HMO 1.0 per cent + neem oil 1.0 per cent and HMO 0.5 per cent + neem oil 0.5 per cent (30.60%). However, HMO 0.5 per cent alone resulted in least mortality of 18.67 per cent.

On fourth day of treatment, highest mortality was observed in HMO 3.0 per cent (81.33%). The treatments HMO 2.5 per cent (78.67%), neem oil 2.0 per cent (72.00%) and HMO 3.0 per cent + neem oil 2.0 per cent (69.33%) were on par with each other and HMO 3.0 per cent. The next best treatments were HMO 2.0 per cent (57.33%), HMO 1.5 per cent + neem oil 1.5 per cent (42.67%), HMO 2.0 per cent + neem oil 2.0 per cent (41.33%) and HMO 1.5 per cent (40.00%) and were on par with each other. HMO 1.0 per cent (34.67%), HMO 0.5 per cent + neem oil 0.5 per cent and HMO 1.0 per cent + neem oil 1.0 per cent (33.33%) recorded mortality on par with each other. HMO 0.5 per cent (20.00%) recorded the least mortality.

Five days after treatment HMO 3.0 per cent proved the best and resulted in 88.00 per cent mortality. This was followed by neem oil 2.0 per cent (74.67%), HMO 3.0 per cent + neem oil 2.0 per cent (73.33%) and HMO 2.5 per cent (72.00%) which were on par with each other. However, HMO 2.0 per cent and HMO 2.5 per cent + neem oil 2.0 per cent resulted in mortality of 60.00 and 57.33 per cent, respectively and were on par with each other. This was followed by HMO 2.0 per cent + neem oil 2.0 per cent (46.67%) and HMO 1.5 per cent (45.33%), HMO 1.5 per cent + neem oil 1.5 per cent (44.00%), HMO 0.5 per cent + neem oil 0.5 per cent (40.00%), HMO 1.0 per cent + neem oil 1.0 per cent (37.33%) and were on par with each other. HMO 1.0 per cent recorded 36.00 per cent mortality, while the lowest mortality was recorded in HMO 0.5 per cent (20.00%).

On sixth day of treatment, HMO 3.0 per cent recorded the highest mortality of 90.67 per cent. Mortality recorded in treatments neem oil 2.0 per cent (78.67%), HMO 2.5 per cent (77.33%) and HMO 3.0 per cent + neem oil 2.0 per cent (76.00%) were on par with each other. This was followed by HMO 2.5 per cent + neem oil 2.0 per cent (62.67%) and HMO 2.0 per cent (61.33%) which were on par with each other. The next best treatments in the order of mortality were HMO 2.0 per cent + neem oil 2.0 per cent (52.00%), HMO 1.5 per cent + neem oil 1.5 per cent (50.67%), HMO 1.5 per cent



(48.00%), HMO 1.0 per cent + neem oil 1.0 per cent (42.67%), and were on par with each other. HMO 0.5 per cent + neem oil 0.5 per cent (41.33%) and HMO 1.0 per cent (37.33%) were on par with each other. HMO 0.5 per cent recorded 25.33 per cent mortality.

After seven days of treatment, HMO 3.0 per cent recorded significantly higher mortality of 92.00 per cent. This was followed by, HMO 2.5 per cent (84.00%), neem oil 2.0 per cent (81.33%), and HMO 3.0 per cent + neem oil 2.0 per cent (77.33%) which were on par with each other. The next best treatments in the order of mortality were HMO 2.0 per cent (65.33%), HMO 2.5 per cent + neem oil 2.0 per cent (64.00%), HMO 2.0 per cent + neem oil 2.0 per cent (58.67%), HMO 1.5 per cent + neem oil 1.5 per cent (54.67%), HMO 1.5 per cent (50.67%), HMO 0.5 per cent + neem oil 0.5 per cent (45.33%) recorded mortality on par with each other. HMO 1.0 per cent + neem oil 1.0 per cent (44.00%), and HMO 1.0 per cent (40.00%) were on par with each other. The lowest mortality was recorded by treatment HMO 0.5 per cent (26.67%).

**Table 5. Effect of horticultural mineral oil on gravid females of *Tetranychus truncatus***

Treatments	Mean mortality (%)													
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT							
T <sub>1</sub> - HMO 0.5%	14.67 <sup>i</sup> (22.47)	17.33 <sup>h</sup> (24.46)	18.67 <sup>i</sup> (25.57)	20.00 <sup>i</sup> (26.49)	21.33 <sup>i</sup> (27.48)	25.33 <sup>h</sup> (30.11)	26.67 <sup>i</sup> (31.04)							
T <sub>2</sub> - HMO 1.0%	18.67 <sup>hi</sup> (25.57)	28.00 <sup>ef</sup> (31.91)	33.33 <sup>e</sup> (35.21)	34.67 <sup>e</sup> (36.01)	36.00 <sup>e</sup> (36.85)	37.33 <sup>g</sup> (37.62)	40.00 <sup>h</sup> (39.21)							
T <sub>3</sub> - HMO 1.5%	24.00 <sup>gh</sup> (28.89)	32.00 <sup>def</sup> (34.42)	37.33 <sup>de</sup> (37.65)	40.00 <sup>de</sup> (39.21)	45.33 <sup>de</sup> (42.32)	48.00 <sup>ef</sup> (43.85)	50.67 <sup>efg</sup> (45.38)							
T <sub>4</sub> - HMO 2.0%	34.67 <sup>cde</sup> (36.04)	46.67 <sup>c</sup> (43.08)	53.33 <sup>c</sup> (46.92)	57.33 <sup>bc</sup> (49.24)	60.00 <sup>c</sup> (50.81)	61.33 <sup>cd</sup> (51.58)	65.33 <sup>c</sup> (53.98)							
T <sub>5</sub> - HMO 2.5%	54.67 <sup>b</sup> (47.69)	58.67 <sup>b</sup> (50.04)	62.67 <sup>b</sup> (52.36)	78.67 <sup>a</sup> (64.08)	72.00 <sup>b</sup> (58.20)	77.33 <sup>b</sup> (61.71)	84.00 <sup>b</sup> (66.81)							
T <sub>6</sub> - HMO 3.0%	65.33 <sup>a</sup> (53.94)	73.33 <sup>a</sup> (58.92)	77.33 <sup>a</sup> (61.59)	81.33 <sup>a</sup> (64.43)	88.00 <sup>a</sup> (69.73)	90.67 <sup>a</sup> (72.64)	92.00 <sup>a</sup> (73.92)							
T <sub>7</sub> - HMO 0.5% + neem oil 0.5%	16.00 <sup>i</sup> (23.47)	24.00 <sup>ig</sup> (29.28)	30.67 <sup>e</sup> (33.59)	33.33 <sup>ef</sup> (35.21)	40.00 <sup>de</sup> (39.21)	41.33 <sup>fg</sup> (40.00)	45.33 <sup>fgh</sup> (42.32)							
T <sub>8</sub> - HMO 1.0% + neem oil 1.0%	18.67 <sup>hi</sup> (25.57)	24.00 <sup>ig</sup> (29.28)	30.67 <sup>e</sup> (33.61)	33.33 <sup>ef</sup> (35.25)	37.33 <sup>de</sup> (37.62)	42.67 <sup>efg</sup> (40.77)	44.00 <sup>gh</sup> (41.54)							
T <sub>9</sub> - HMO 1.5% + neem oil 1.5%	20.00 <sup>ghi</sup> (26.49)	29.33 <sup>ef</sup> (32.78)	34.67 <sup>e</sup> (36.04)	42.67 <sup>cde</sup> (40.76)	44.00 <sup>de</sup> (41.54)	50.67 <sup>ef</sup> (45.38)	54.67 <sup>def</sup> (47.68)							
T <sub>10</sub> - HMO 2.0%+ neem oil 2.0%	26.67 <sup>efg</sup> (31.07)	33.33 <sup>de</sup> (35.26)	37.33 <sup>de</sup> (37.65)	41.33 <sup>de</sup> (39.98)	46.67 <sup>d</sup> (43.07)	52.00 <sup>de</sup> (46.15)	58.67 <sup>cde</sup> (50.01)							
T <sub>11</sub> - HMO 2.5% + neem oil 2.0%	30.67 <sup>def</sup> (33.62)	40.00 <sup>cd</sup> (39.18)	45.33 <sup>cd</sup> (42.31)	52.00 <sup>cd</sup> (46.15)	57.33 <sup>c</sup> (49.24)	62.67 <sup>c</sup> (52.34)	64.00 <sup>cd</sup> (53.15)							
T <sub>12</sub> - HMO 3.0% + neem oil 2.0%	38.67 <sup>cd</sup> (38.44)	48.00 <sup>c</sup> (43.84)	62.67 <sup>b</sup> (52.37)	69.33 <sup>ab</sup> (56.41)	73.33 <sup>b</sup> (58.92)	76.00 <sup>b</sup> (60.71)	77.33 <sup>b</sup> (61.64)							
T <sub>13</sub> - Neem oil - 2.0%	42.67 <sup>c</sup> (40.74)	58.67 <sup>b</sup> (50.01)	69.33 <sup>b</sup> (56.49)	72.00 <sup>a</sup> (58.20)	74.67 <sup>b</sup> (59.88)	78.67 <sup>b</sup> (62.63)	81.33 <sup>b</sup> (64.50)							
T <sub>14</sub> - Control	0.00 <sup>j</sup> (0.76)	0.00 <sup>h</sup> (0.76)	0.00 <sup>g</sup> (0.76)	4.00 <sup>g</sup> (9.57)	6.67 <sup>g</sup> (14.44)	10.67 <sup>i</sup> (18.46)	12.00 <sup>j</sup> (20.09)							
CD value (p=0.05)	5.11	5.27	4.71	8.79	5.57	6.10	5.76							

DAT= Days after treatment. Means followed by same letter in the column do not differ significantly. Figures in parenthesis are arcsine transformed values.

#### 4.1.2 Evaluation of horticultural mineral oil on okra under field conditions

A study was conducted to evaluate the field efficacy of the best treatments identified from the laboratory study along with a synthetic acaricide, spiromesifen against the red spider mite, *T. truncatus* on okra during March, 2018. The results of the experiment are presented in Table 6.

The mean population of *T. truncatus* before the application of treatments ranged from 17.73 to 18.59 per cm<sup>2</sup> leaf area.

One day after spraying, all the treatments significantly reduced the population of mite as compared to untreated control. The mean mite population ranged from 4.85 to 18.51 per cm<sup>2</sup> leaf area. The lowest mean mite count of 4.85/cm<sup>2</sup> leaf area was recorded by HMO 3.0 per cent + neem oil 2.0 per cent followed by HMO 2.5 per cent + neem oil 2.0 per cent (5.15/cm<sup>2</sup> leaf area) which were on par with each other. The HMO 2.5 and 3.0 per cent recorded 5.46 and 5.33 mites/cm<sup>2</sup> leaf area respectively and were on par with each other and also with HMO 2.5 + neem oil 2.0 per cent, HMO 3.0 + neem oil 2.0 per cent and neem oil 2 per cent (5.43 mites/cm<sup>2</sup> leaf area). The acaricide spiromesifen 240 SC recorded mite count of 5.88 mites/cm<sup>2</sup> leaf area which was on par with HMO 2.5 per cent, HMO 3.0 per cent and neem oil 2.0 per cent.

Three days after spraying, the plants treated with combination of HMO + neem oil harboured significantly lower mite population compared to other treatments. HMO 3.0 per cent + neem oil 2.0 per cent and HMO 2.5 per cent + neem oil 2.0 per cent recorded 1.59 and 1.43 mean mite per cm<sup>2</sup> leaf area respectively. This was followed by HMO 2.5 per cent (2.49/cm<sup>2</sup> leaf area) and HMO 3.0 per cent (2.31 mites/cm<sup>2</sup> leaf area) which were on par with each other and the above treatments. The acaricide, spiromesifen 240 SC recorded 2.87 mites/cm<sup>2</sup> leaf area and was on par with neem oil 2 per cent (3.10 mites/cm<sup>2</sup> leaf area) and with HMO 2.5 and 3.0 per cent.

At seven days after treatment, all the treatments significantly reduced the mite population as compared to untreated control (15.53 mites/cm<sup>2</sup> leaf area). The lowest mite population of 1.04 per cm<sup>2</sup> leaf area was recorded by HMO 2.5 per cent + neem oil 2.0 per cent followed by HMO 3.0 per cent (1.08/cm<sup>2</sup> leaf area), HMO 3.0 per cent + neem oil 2.0 per cent (1.11/cm<sup>2</sup> leaf area) and HMO 2.5 per cent (1.33/cm<sup>2</sup> leaf area). However, these treatments did not differ significantly. Spiromesifen 240 SC (1.82/cm<sup>2</sup> leaf area) and neem oil 2.0 per cent (2.32 mites

/cm<sup>2</sup> leaf area) were on par with each other and differed significantly from treatments of HMO and combination of HMO + neem oil.

Per cent reduction in mite count after seven days of treatment application was worked out. By seventh day of treatment, HMO 2.5 per cent + neem oil 2.0 per cent recorded 96.21 per cent reduction in the mite count closely followed by HMO 3.0 per cent + neem oil 2.0 per cent (93.95%), HMO 3.0 per cent (93.90%) and HMO 2.5 per cent (92.60%). This was followed by treatments *viz.*, spiromesifen 240 SC (89.99 %) and neem oil 2.0 per cent (87.26 %).

Similar trend was observed on ten days after spraying where treatments of HMO and combination of HMO + neem oil continued to record lower mean mite counts. The treatments, HMO 2.5 per cent (0.70/cm<sup>2</sup> leaf area), HMO 3.0 per cent (0.73/cm<sup>2</sup> leaf area), HMO 2.5 per cent + neem oil 2.0 per cent (0.79/cm<sup>2</sup> leaf area) and HMO 3.0 per cent + neem oil 2.0% (0.87/cm<sup>2</sup> leaf area) recorded mean mite count on par with each other which was significantly lower. Spiromesifen 240 SC and neem oil 2.0 per cent recorded mean mite counts of 1.57 and 2.04 per cm<sup>2</sup> leaf area respectively which were on par with each other, but significantly differed from treatments of HMO and combination of HMO + neem oil.

At fourteen days of spraying, all the treatments recorded significant reduction in mite population as compared to untreated control (12.86 / cm<sup>2</sup> leaf area). HMO 2.5 per cent recorded lowest mean mite count of 0.54 per cm<sup>2</sup> leaf area, followed by HMO 3.0 per cent (0.58/cm<sup>2</sup> leaf area), HMO 3.0 per cent + neem oil 2.0 per cent (0.59 /cm<sup>2</sup> leaf area) and HMO 2.5 per cent + neem oil 2.0 per cent (0.67/cm<sup>2</sup> leaf area) which were all on par with each other and significantly superior over spiromesifen and neem oil. Mite population count in spiromesifen 240 SC (1.62/cm<sup>2</sup> leaf area) and neem oil 2.0 per cent (1.74/ cm<sup>2</sup> leaf area) were on par with each other.

Per cent reduction in mite count after fourteen days of treatment application was worked out. By fourteenth day, the highest reduction in mite population over untreated control was observed in HMO 2.5 per cent (96.99%) followed by, HMO 3.0 per cent + neem oil 2.0 per cent (96.79%), HMO 3.0 per cent (96.72%) and HMO 2.5 per cent + neem oil 2.0 per cent (96.21%), spiromesifen 240 SC (91.08 %) and neem oil 2 per cent (90.42 %).

Table 6. Effect of various treatments against *Tetranychus truncatus* on okra

Treatments	Mean no. of mite/cm <sup>2</sup> leaf area					Per cent reduction on 7 DAT	Mean no. of mite/cm <sup>2</sup> leaf area		Per cent reduction on 14 DAT
	Pre count	1DAT	3 DAT	7DAT	10 DAT		14 DAT		
		(4.24)	(2.33)	(1.56)	(1.15)		(0.83)	(1.01)	
T <sub>1</sub> - HMO 2.5%	17.98 (4.24)	5.46 <sup>bc</sup> (2.33)	2.49 <sup>bc</sup> (1.56)	1.33 <sup>c</sup> (1.15)	0.70 <sup>c</sup> (0.83)	92.60	0.54 <sup>c</sup> (1.01)	96.99	
T <sub>2</sub> - HMO 3.0%	17.73 (4.21)	5.33 <sup>bc</sup> (2.31)	2.31 <sup>bc</sup> (1.49)	1.08 <sup>c</sup> (1.04)	0.73 <sup>c</sup> (0.84)	93.90	0.58 <sup>c</sup> (1.03)	96.72	
T <sub>3</sub> - HMO 2.5% + neem oil 2.0%	17.76 (4.21)	5.15 <sup>c</sup> (2.27)	1.59 <sup>c</sup> (1.22)	1.04 <sup>c</sup> (1.02)	0.79 <sup>c</sup> (0.88)	94.14	0.67 <sup>c</sup> (1.08)	96.21	
T <sub>4</sub> - HMO 3.0% + neem oil 2.0%	18.36 (4.28)	4.85 <sup>c</sup> (2.20)	1.43 <sup>c</sup> (1.16)	1.11 <sup>c</sup> (1.05)	0.87 <sup>c</sup> (0.93)	93.95	0.59 <sup>c</sup> (1.04)	96.79	
T <sub>5</sub> - Neem oil 2.0%	18.21 (4.26)	5.43 <sup>bc</sup> (2.33)	3.10 <sup>b</sup> (1.75)	2.32 <sup>b</sup> (1.51)	2.04 <sup>b</sup> (1.43)	87.26	1.74 <sup>b</sup> (1.49)	90.42	
T <sub>6</sub> - Spiromesifen 240SC - 0.02%	18.19 (4.26)	5.88 <sup>b</sup> (2.42)	2.87 <sup>b</sup> (1.66)	1.82 <sup>b</sup> (1.34)	1.57 <sup>b</sup> (1.24)	89.99	1.62 <sup>b</sup> (1.45)	91.08	
T <sub>7</sub> - Control	18.59 (4.31)	18.51 <sup>a</sup> (4.29)	17.91 <sup>a</sup> (4.23)	15.53 <sup>a</sup> (3.94)	12.35 <sup>a</sup> (3.51)	16.46	12.86 <sup>a</sup> (3.65)	30.83	
CD value (p=0.05)	NS	0.15	0.43	0.18	0.21	0.34	0.26	0.65	

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

## 4.2 Evaluation of the phytotoxic effect of HMO under pot culture experiment

Phytotoxic effect of HMO was tested on potted okra plants (variety Arka Anamika) during August to October, 2017. Application of HMO at 2, 3, 4, 5 and 6 per cent did not show any phytotoxic symptoms like scorching, yellowing, wilting, vein clearing or necrosis on 45 day old plants.

## 4.3 Evaluation of safety of horticultural mineral oil to the predatory mite, *Neoseiulus longispinosus*

Safety of HMO to the predatory mite was evaluated in the laboratory separately on eggs and adults

### 4.3.1 Ovicidal effect

The effect of the best five treatments identified in 3.3.1 on the eggs of *N. longispinosus* was evaluated under laboratory conditions, the results of which are presented in Table 7.

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 7. Effect of horticultural mineral oil on eggs of *Neoseiulus longispinosus***

Treatments	Mean hatchability (%)		Mean mortality (%)
	24 h	48 h	48 h
T <sub>1</sub> - HMO 2.5%	27.78 (31.54)	66.67 <sup>b</sup> (55.21)	33.33 <sup>a</sup> (34.78)
T <sub>2</sub> - HMO 3.0%	11.11 (16.45)	61.11 <sup>b</sup> (51.49)	38.89 <sup>a</sup> (38.51)
T <sub>3</sub> - HMO 2.5% + neem oil 2.0%	50.00 (45.00)	66.67 <sup>b</sup> (55.21)	33.33 <sup>a</sup> (34.78)
T <sub>4</sub> - HMO 3.0% + neem oil 2.0%	27.78 (27.14)	77.78 <sup>b</sup> (62.18)	22.22 <sup>a</sup> (27.81)
T <sub>5</sub> - Neem oil 2.0%	27.78 (27.14)	66.67 <sup>b</sup> (55.21)	33.33 <sup>a</sup> (34.78)
T <sub>6</sub> - Control	38.89 (38.51)	100 <sup>a</sup> (88.83)	0.00 <sup>b</sup> (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.*

#### 4.3.2 Effect of horticultural mineral oil on gravid females of *Neoseiulus longispinosus*

The safety of HMO to the gravid females of *N. longispinosus* was assessed under laboratory conditions and the results of the study are presented in the Table 8.

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 per cent, 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 8. Effect of horticultural mineral oil on adults of *Neoseiulus longispinus***

Treatments	Mean mortality (%)		
	24 h	48 h	72 h
T <sub>1</sub> - HMO 2.5%	0 (0.71)	11.11 (2.99)	16.67 <sup>a</sup> (3.56)
T <sub>2</sub> - HMO 3.0%	0 (0.71)	16.67 (3.56)	22.22 <sup>a</sup> (4.70)
T <sub>3</sub> - HMO 2.5% + neem oil 2.0%	0 (0.71)	16.67 (3.56)	22.22 <sup>a</sup> (4.70)
T <sub>4</sub> - HMO 3.0% + neem oil 2.0%	5.56 (1.85)	22.22 (4.70)	27.78 <sup>a</sup> (5.26)
T <sub>5</sub> - Neem oil 2.0%	5.56 (1.85)	16.67 (3.56)	22.22 <sup>a</sup> (4.70)
T <sub>6</sub> - Control	0 (0.71)	0 (0.71)	0.00 <sup>b</sup> (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.*

#### 4.4 Assessment of sensitivity of *Acremonium zeylanicum* to horticultural mineral oil

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 9). 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 (Plate 14).

**Table 9. *In vitro* evaluation of horticultural mineral oil against *Acremonium zeylanicum***

<b>Treatments</b>	<b>Radial growth (mm)</b>	<b>Per cent inhibition over control (%)</b>
T <sub>1</sub> - HMO 2.5%	48.00 <sup>b</sup>	46.67 <sup>c</sup>
T <sub>2</sub> - HMO 3.0%	30.16 <sup>d</sup>	66.48 <sup>c</sup>
T <sub>3</sub> - HMO 2.5% + neem oil 2.0%	19.06 <sup>e</sup>	78.81 <sup>b</sup>
T <sub>4</sub> - HMO 3.0% + neem oil 2.0%	12.20 <sup>f</sup>	86.44 <sup>a</sup>
T <sub>5</sub> - Neem oil 2.0%	39.36 <sup>c</sup>	56.26 <sup>d</sup>
T <sub>6</sub> - Untreated control	90.00 <sup>a</sup>	-

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



HMO 2.5%



HMO 3.0%



HMO 2.5%+neem oil 2.0%



HMO 3.0%+neem oil 2.0%



Neem oil 2.0%



Control

**Plate 14. Mycelial growth of *Acremonium zeylanicum* in poisoned SDAY media with different concentrations of horticultural mineral oil and its combinations with neem oil**

# Discussion

## 5. DISCUSSION

The results of present study on the efficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae) on okra are discussed in the light of available literature.

### 5.1 Evaluation of horticultural mineral oil against the spider mite, *Tetranychus truncatus*

In the laboratory bioassay, HMO alone at different concentrations and in combination with neem oil, recorded significant mortality of eggs and gravid females of *T. truncatus*. The acaricidal activity of HMO was concentration and time dependent.

HMO exhibited very high ovicidal action against *T. truncatus* at all the concentrations evaluated. While it recorded 100 per cent kill of eggs at concentration of 1.5 per cent and above, of greater significance could be the fact that HMO induced near total mortality of eggs even at a relatively low concentration of 0.5 per cent (Fig.1).

Ovicidal effect of HMO against *T. urticae* was reported by Roy *et al.* (2015). They evaluated the efficacy of HMO (servo agro spray oil) at concentrations 0.5, 1.0 and 15 per cent in the laboratory against different stages of *T. urticae*, and reported 98.86 per cent mortality of eggs at the highest concentration of 1.5 per cent. The findings of the present study agree with the above observations.

The efficacy of HMO against adult mites showed a marked variance as compared to that against eggs (Fig. 2). The mortality of 92.00 per cent seven days after treatment, also differed with previous report by Roy *et al.* (2015) who reported 100 per cent mortality at 1.0 per cent concentration 24 h after exposure. However, this difference could be due to the differences in bioassay methods followed. While the above study followed topical application against both eggs and adults, the present study used leaf dip bioassay for adults which limit the exposure of adult mites to HMO considerably, there by resulting in lower mortality rates.

The modes of action of petroleum oil against arthropods have been investigated by several workers. Interference with hormonal activity, water balance and coagulation of protoplasm has been proposed as reason for mortality of eggs (O'kane and Baker, 1934). A more recent study by Al Dabel *et al.* (2008) has suggested that ovicidal action could be due to the adverse effect of oil, on respiration by eggs. Asphyxiation following blocking of spiracles has been suggested the most plausible cause for adult mortality (Roy *et al.*, 1943; Stadler and Buteler, 2009; Taverner *et al.*, 2001) though cell level disruption (van Overbeek and Blondeau, 1954; Najar-Rodríguez *et al.*, 2007) also are not ruled out.

Smith and Pearce (1948) observed that the quantity of oil as well as duration of exposure were critical for egg mortality. The differential mortality of eggs and adults observed in the present study corroborate the above observation. The active adults absorb only a fraction of HMO applied on the leaf surface which would explain their lower mortality.

The above fact has been underlined by the linear relationship between time as well as concentration and mortality of gravid females. Mortality of *T. truncatus* has been consistently higher at higher concentration. Similarly, at the same concentration, the mortality increased from day one to day seven across all treatments involving HMO.

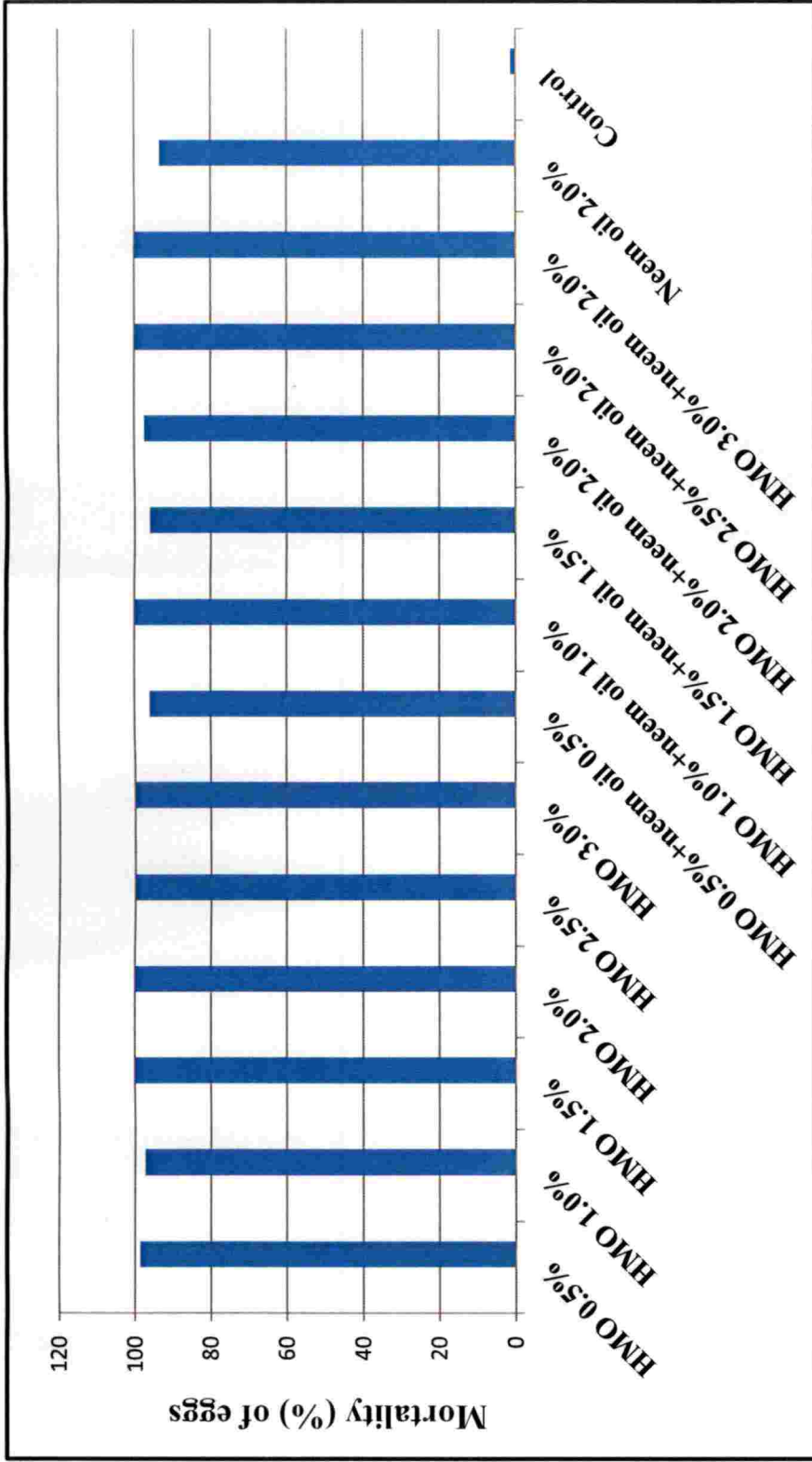
The significantly higher mortality of combinations of HMO and neem oil than that recorded by neem oil alone suggests synergistic effect against eggs of *T. truncatus*. However, the values being compared with that of HMO alone negates such a claim. This holds true against adult mites as well. Combination of HMO with neem oil did not cause any significantly higher mortality of adult and for most part, were less effective than HMO or neem oil (2%) alone.

In the field experiment, HMO alone (2.5 and 3.0%) and in combination with neem oil (2.0%) were significantly superior to both the acaricide spiromesifen and neem oil (Fig. 3). Kavya *et al.* (2015) evaluated the efficacy of acaricides and HMO at 1.0 and 2.0 per cent concentration against *T. urticae* on brinjal and found that HMO significantly reduced mite infestation and was on par with spiromesifen. The higher concentrations of HMO used in the present study could be the reason for higher efficacy over the acaricide.

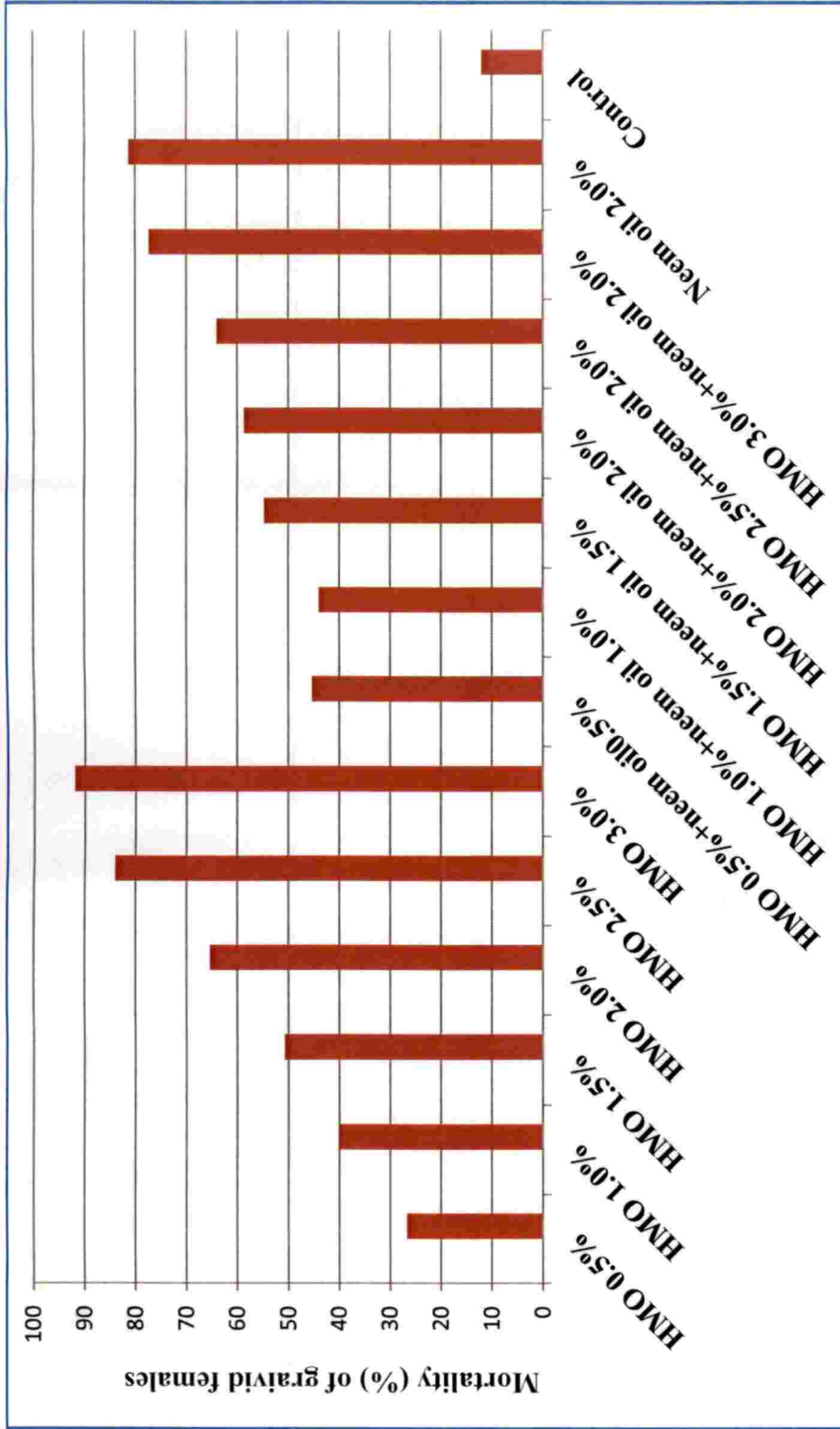
Spiromesifen, an inhibitor of lipid biosynthesis, is highly toxic to eggs and immature stages of spider mites, while it acts more slowly against adult females, causing reduction in fertility and fecundity (Marcic *et al.*, 2011). Krishna and Bhaskar (2013) reported a higher reduction in egg count (15.40%) of *T. urticae* due to spiromesifen, while it recorded very low adult mortality (3.40%). Similarly, Sato *et al.* (2011) studied the toxicity of spiromesifen to different developmental stages, and found egg stage of *T. urticae* was most sensitive.

The field study has indicated that HMO possesses greater persistence in field though a confirmatory call for further studies. While there was progressive reduction in mite count in all treatments up to 10 DAT, mite population showed an increase in both spiromesifen treated plots as well as control plots. This attribute of HMO, if confirmed, augers well for HMO in mite pest management.





**Fig. 1.** Ovicidal effect of horticultural mineral oil and its combination with neem oil on *Tetranychus truncatus* in the laboratory



**Fig. 2.** Adulticidal effect of horticultural mineral oil and its combination with neem oil on *Tetranychus truncatus* in the laboratory

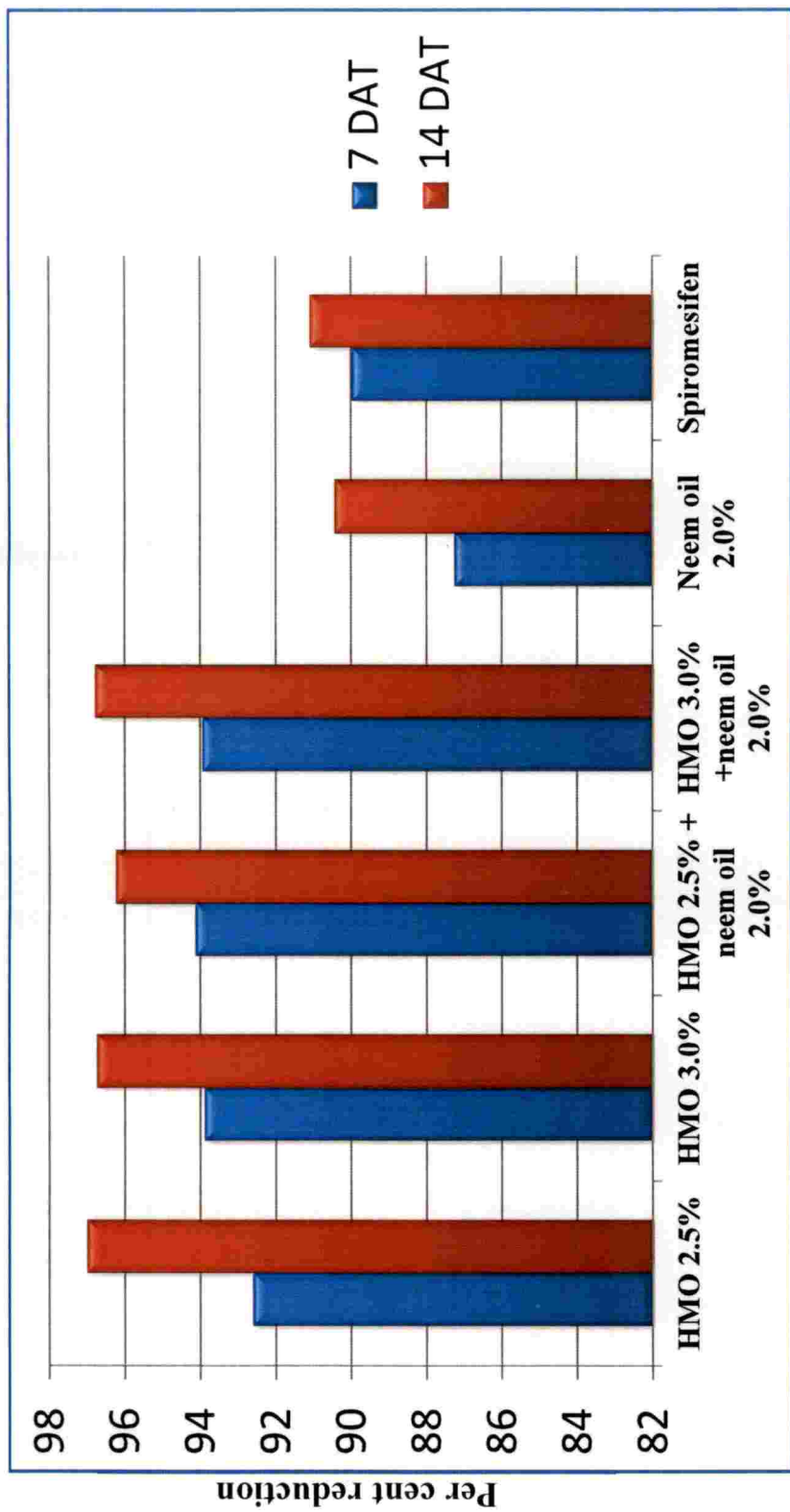


Fig. 3. Effect of various treatments against *Tetranychus truncatus* on okra

## 5.2 Phytotoxicity of horticultural mineral oil on okra

In the present study, foliar application of HMO up to 6 per cent did not show any phytotoxic symptoms when sprayed on 45 day old okra plants. Sridharan *et al.* (2015) tested the phytotoxic effect of mineral oil on okra up to 20 per cent and reported that mineral oil up to 5 per cent was safe and showed no phytotoxic symptoms. 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 (Roy *et al.*, 2015).

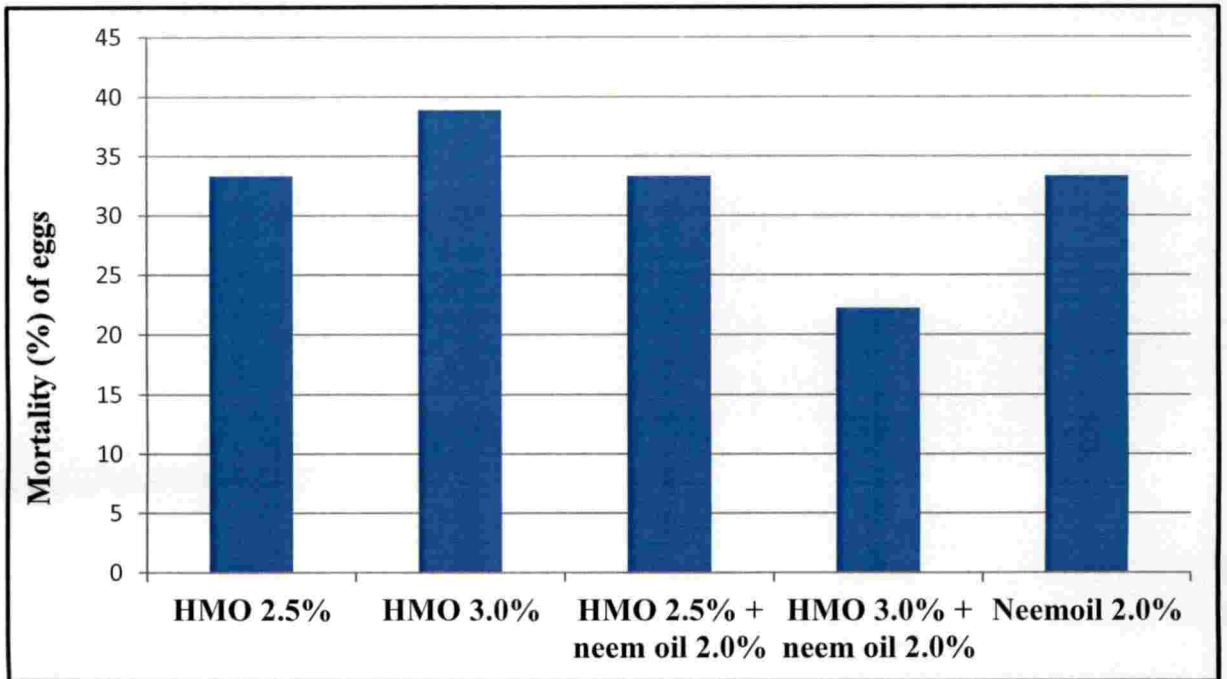
Dormant oils which were heavier and less refined were customarily used on woody plants during the dormant season. However, these older oils have been replaced with more refined, light-weight oils that have potential application to plant foliage. The impurities in the oil that is associated with plant injury, such as aromatic compounds and compounds containing sulfur, nitrogen or oxygen, are removed during refinement techniques for their safety to plants (Sarwar and Salman, 2015). The HMO used in the present study is a refined product which could explain its lack of phytotoxicity on okra.

### 5.3 Evaluation of safety of horticultural mineral oil to the predatory mite, *Neoseiulus longispinosus*

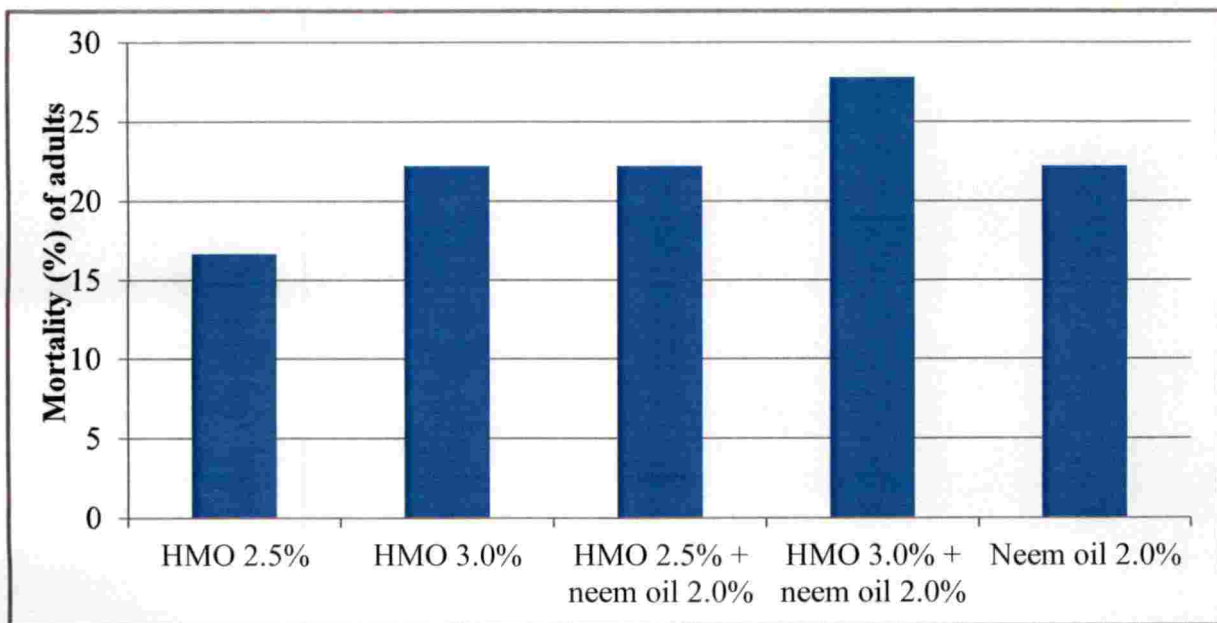
The present study revealed that HMO is relatively safe to both eggs and adults of *N. longispinosus*. HMO at the highest concentration evaluated (3.0 %) recorded significantly higher egg mortality of 38.89 per cent (Fig. 4). But it resulted in a mortality of only 22.20 per cent in the case of adult mite. In case of adults, highest mortality was recorded in treatment, HMO 3.0 per cent + neem oil 2.0 per cent (27.78%) (Fig. 5). 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 were 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* (Reddy and Bautista, 2012). Nicetic *et al.* (2001) 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.* (1987) 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 (Kavya *et al.*, 2015). Lower toxicity of HMO to the phytoseiid predator, *P. persimilis* was also reported by Cote *et al.* (2002).



**Fig. 4. Ovicidal effect of horticultural mineral oil on *Neoseiulus longispinosus***



**Fig. 5. Adulticidal effect of horticultural mineral oil on *Neoseiulus longispinosus***

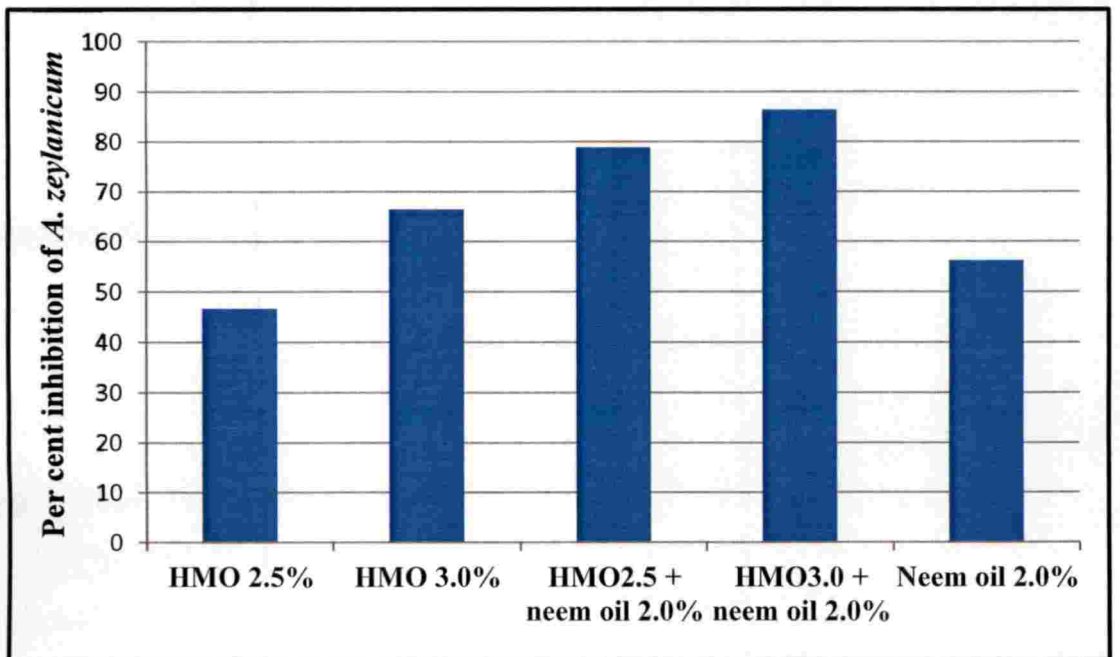
#### 5.4 Safety of horticultural mineral oil to the acaropathogen, *Acremonium zeylanicum*

The present study revealed antagonistic effect of HMO on the acaropathogen, *A. zeylanicum*. HMO in combination with neem oil recorded significantly higher inhibition of the acaropathogen (Fig. 6). 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.* (2017), petroleum oils showed no negative impact on the growth parameters (CFUs and radial diameter) of an entomopathogenic fungus, *Isaria fumosorosea*. However, Andrew *et al.* (2008) 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 (Vawdrey *et al.*, 2004). Mineral oils have been reported to control both powdery mildew infestation and offer protection against two spotted mite (Nicetic *et al.*, 2001).

The overall results suggest that HMO at 2.5 per cent could be used for the management of red spider mite in okra. However, more studies are to be conducted further on the residual effect of HMO to arrive at a recommendation.





**Fig. 6. Inhibitory effect of horticultural mineral oil on *Acremonium zeylanicum***

# Summary

## 6. SUMMARY

The present study entitled “Bioefficacy of horticultural mineral oil against the spider mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae) on okra” was carried out in the Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara during February 2017 - June 2018 to evaluate the bioefficacy of horticultural mineral oil against the spider mite, *T. truncatus* Ehara and to test the phytotoxicity on okra as well as safety to the predatory mite, *Neoseiulus longispinosus* Evans and the acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans

The salient findings of the study are summarized hereunder:

- The laboratory bioassay conducted to evaluate the efficacy of HMO at different concentrations namely HMO alone (0.5,1.0, 1.5, 2.0, 2.5 and 3.0 %) and combination treatments viz., HMO (0.5%) + neem oil (0.5%), HMO (1.0%) + neem oil (1.0%), HMO (1.5%) + neem oil (1.5 %), HMO (2.0%) + neem oil (2.0%), HMO (2.5%) + neem oil (2.0%) and HMO (3.0%) + neem oil (2.0%) as well as neem oil alone at 2.0 per cent against *T. truncatus* indicated HMO had appreciable ovicidal and adulticidal action against *T. truncatus*
- Horticultural mineral oil alone, at concentrations of 1.5, 2.0, 2.5 and 3.0 per cent and combinations of HMO with neem oil viz., HMO (2.5%) + neem oil (2.0%), HMO (3.0 %) + neem oil (2.0%) and HMO (1.0%) + neem oil (1.0%) recorded 100 per cent mortality of eggs.
- Adulticidal effect of HMO was concentration and time dependent. HMO at 3.0 per cent recorded the highest mortality of 92.00 per cent and was significantly superior to HMO at 2.5 per cent (84.00%) and neem oil 2.0 per cent (81.33%), which were on par with each other.

- In the field experiment, plots treated with HMO at 2.5 (92.60%) and 3.0 per cent (93.90%) as well as combination treatments HMO 2.5 per cent + neem oil 2.0 per cent (94.14%) and HMO 3.0 per cent + neem oil 2.0 per cent (96.79%) recorded significant reduction in mite population and were superior to plots treated with either spiromesifen (91.08%) or neem oil alone at 2.0 per cent (90.42%).
- Foliar application of HMO up to 6 per cent did not show any phytotoxic effects on okra plants.
- Laboratory bioassay to evaluate the safety of 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.
- The high efficacy of HMO against the spider mite *T. truncatus* as well as its relative safety to the predominant natural enemy brought out in the study suggests that HMO can be an effective tool for mite management in vegetable crops.

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# Appendix



## **APPENDIX 1**

The composition of Sabouraud Dextrose Agar (SDAY) medium is given below

1. Peptone : 10 g
2. Dextrose : 40 g
3. Yeast extract : 20 g
4. Agar : 20 g
5. Distilled water : 1000 ml

**BIOEFFICACY OF HORTICULTURAL MINERAL OIL  
AGAINST THE SPIDER MITE, *Tetranychus truncatus*  
(PROSTIGMATA: TETRANYCHIDAE) ON OKRA**

by

**KAVYA YADAV G. A.  
(2016-11-120)**

**ABSTRACT OF THE THESIS**

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## ABSTRACT

Spider mites of the family Tetranychidae are devastating pests of horticultural crops demanding suitable control measures. Synthetic acaricides, while effective, often cause resistance and resurgence, besides leaving residues on fruits and vegetables. Consequently, several natural products are being evaluated as safer alternatives for the management of mite pests. Petroleum derived mineral oils, for instance, have been used for centuries against insect and mite pests in several crops. However, phytotoxicity concerns have limited their use to a few selected crops. Recent advances in processing of mineral oils have mitigated these apprehensions, enabling their wider application in crop pest management, including vegetables.

A study was undertaken at Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2017-18 in order to evaluate the bioefficacy of horticultural mineral oil (HMO) against the spider mite, *Tetranychus truncatus*. The study also aimed to test the phytotoxic effect of HMO on okra and its safety to important natural enemies such as the predatory mite, *Neoseiulus longispinosus* and the acaropathogenic fungus, *Acremonium zeylanicum*.

Laboratory bioassays were conducted to evaluate the efficacy of HMO alone and in combination with neem oil against eggs and adults of *T. truncatus*. The results showed that HMO had appreciable ovicidal and adulticidal action against *T. truncatus*. Mineral oil alone, at concentrations of 1.5, 2.0, 2.5 and 3.0 per cent and combinations of HMO with neem oil viz., HMO 2.5 per cent + neem oil 2.0 per cent; HMO 3.0 per cent + neem oil 2.0 per cent and HMO 1.0 per cent + neem oil 1.0 per cent recorded 100 per cent mortality of eggs. In the case of adults, HMO at 3.0 per cent recorded the highest mortality of 92.00 per cent and was significantly superior to HMO at 2.5 per cent (84.00%) and neem oil 2.0 per cent (81.33%), which were on par with each other.

In the field experiment, plots treated with HMO at 2.5 (92.60%) and 3.0 per cent (93.90%) as well as HMO 2.5 per cent + neem oil 2.0 per cent (94.14%) and HMO 3.0 per cent + neem oil 2.0 per cent (96.79%) recorded significant reduction in mite

population and were superior to plots treated with either spiromesifen (91.08%) or neem oil alone at 2.0 per cent (90.42%).

The phytotoxic effect of HMO was tested on 45 day old potted okra plants by foliar application at concentrations of 2, 3, 4, 5 and 6 per cent. None of the concentrations showed phytotoxic symptoms on okra.

Laboratory bioassay to evaluate the safety of HMO to the predatory mite, *Neoseiulus longispinosus* revealed that HMO is relatively safer to the predator than to *T. truncatus*. HMO at 3.0 per cent killed 38.89 per cent of eggs while HMO 3.0 per cent + neem oil 2.0 per cent caused the highest mortality (27.78%) in adults.

Combinations of HMO with neem oil were found to be more inhibitory to the acaropathogen, *Acremonium zeilanicum* than HMO alone. HMO 3.0 per cent + neem oil 2.0 per cent 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.

The high efficacy of HMO against the spider mite *T. truncatus* as well as its relative safety to the predominant natural enemy brought out in the study suggests that HMO can be an effective tool for mite management in vegetable crops.

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