Acta Phytopathologica et Entomologica Hungarica 50 (1), pp. 127–137 (2015)
DOI: 10.1556/038.50.2015.1.12

Evaluation of Petroleum Based Horticulture Oil for the Management of the Tea Red Spider Mite, *Oligonychus coffeae* Neitner (Acarina: Tetranychidae)

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(Received: 7 September 2014; accepted: 10 November 2014)

The Servo agro spray oil (petroleum based horticulture oil) was evaluated in the laboratory against tea red spider mite, *Oligonychus coffeae* to determine its effect on adult mortality, viability of eggs, oviposition deterrence and repellent properties. The same was also evaluated in the field to determine its effect on mite population. Direct spray method was used in the laboratory at concentrations of 0.5, 1.0, and 1.5%. Mortality of *O. coffeae* was both concentration and time dependent. Deposition of eggs by adult mites on treated leaf surfaces decreased significantly and the viability of eggs was also significantly reduced. In addition, different concentrations of the Servo agro spray also exhibited repellent properties against adult mites. In the field Servo agro spray oil significantly reduced the mite population and its bio-efficacy was comparable to that of the synthetic pesticide (propargite 57 EC). No phytotoxic effect was observed when tea bushes were sprayed with different doses of Servo agro spray. Tea samples were taint free. Quality (appearance of liquor, flavour, taint and taste) of made tea was not adversely affected by treatments with Servo agro spray oil. Therefore Servo agro spray oil may be a potential agent to be used in the sustainable management of tea red spider mite.

Keywords: *Oligonychus coffeae*, Servo agro spray oil, tea, anti-mite properties, phytotoxic effect.

Oligonychus coffeae Nietner (Acari: Tetranychidae), tea red spider mite, is one of the important pests distributed all over the world. *O. coffeae* has been known as an important pest of approximately 133 crops planted in tropical and subtropical regions (Roy et al., 2014), including tea, cashew nut, camphor laurel, citrus, coffee, jute, African oil palm, Tasmanian blue gum, cotton, rubber, indigo, mango, cassava, castor bean, Singapore almond, grapevine etc. Tea (*Camellia sinensis* (L) O. Kuntze) is the most preferred and principal host plant of *O. coffeae*. Nymphs and adults of *O. coffeae* lacerate cells, producing minute characteristic reddish brown marks on the upper surface of mature leaves, which turn red in severe cases of infestation, resulting in crop loss (Roy et al., 2014). This pest occurs on tea round the year, although population density may vary depending on season. Their number increases as the weather warms up and decreases remarkably once rain set in. Chemical control is the primary mode of management of *O. coffeae* and a wide

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range of acaricides belonging to different chemical groups are currently in use to control this pest. However, the indiscriminate use of these chemicals has given rise to a number of problems including resurgence of primary pests or mite syndrome, secondary pest outbreak, resistance development, undesirable residues on made tea and increased costs of application which causes a serious drain on the fragile economy of developing countries.

Beside these Central Insecticide Board (CIB), Environmental Protection Agency (EPA), Food and Agricultural Organization (FAO), World Health Organization (WHO), European Economic Commission (EEC/EC), etc. have fixed the MRL values for different pesticides in tea and also restricted the uses for many products. Hence to minimize the chemical pesticide load, efforts are directed towards finding commercially acceptable alternatives to these chemical pesticides.

Emphasis is given on utilizing petroleum based horticulture oils which can offer a safe and effective alternative to conventional pesticides for controlling the major insect or mite pests within an integrated pest management programme. Oil sprays have been used for over a century to control insect pests. The use of petroleum products in agriculture commenced with the use of kerosene in the late 1880s (Buteler and Stadler, 2011) but most horticulture oils used today are petroleum based. Most oil based products sold as pesticides are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide and Rodenticide Act. Exemptions are granted to edible oils and other specific exempt ingredients that are considered to pose minimum risk to humans (http://www.epa.gov/). During this time there have been significant changes in the way oils are formulated, their quality and in the emulsifiers used (Beattie, 2005). Petroleum-based spray oils pose a number of advantages over conventional pesticides as they have very low mammalian toxicity and the oil deposited are broken down within a week to a simple form with low residual activity on made tea. Moreover there has been no report on development of pest resistance and are having less adverse effect on natural enemies than broad spectrum insecticides (Beattie and Smith, 1993; Beattie and Hardy, 2004; Buteler and Stadler, 2011).

Sufficient information regarding the potential of such petroleum based horticulture oil like Servo agro spray oil as acaricide is still lacking. So the present study aimed to explore the possibility of using Servo agro spray for tea red spider mite management.

Materials and Methods

Maintenance of O. coffeae

The mites were collected from the tea fields of Tocklai Tea Research Institute Jorhat, Assam, India. A culture of tea red spider mite was maintained in the laboratory following the detached leaf culture method of Roy et al. (2010a). From the stock, adult mites were transferred onto fresh tea leaves (6 cm^2) placed on moistened cotton pads (ca. 1.5 cm thick) in plastic trays ($42 \times 30 \times 6.5$ cm). Rearing trays were kept under controlled conditions, where temperature and humidity were maintained at 25 ± 2 °C, 75 ± 5% RH, and 16L: 8D photoperiod. Withered leaves were replaced regularly with new ones at 4 days interval.

Studies on anti-mite properties of Servo agro spray oil

Servo agro spray oil was procured from recognized distributor of the Indian Oil Corporation Ltd, New Delhi, India.

Effect of Servo agro spray oil against adult tea red spider mite

Thirty to forty healthy adult tea red spider mites (24-h old) were released onto healthy detached mature tea leaves of the cultivar TV1 from the culture maintained in the laboratory. The mature leaves were padded with water-soaked cotton. A final count of the mite population was taken after proper settlement of mites for 4 h. Distilled water solutions of Servo agro spray oil was used in three selected concentrations, expressed in percentage (0.5, 1.0, 1.5%). Each concentration and control (distilled water) was sprayed on both surfaces of the leaf using a glass atomizer (constant pressure 2.5 kg/cm^2).

The numbers of live mites were counted after 6 h, 24 h, and 48 h of treatment. The *O. coffeae* adults were considered dead if no movement was apparent by probing with the tip of a fine brush which was assessed visually with the help of a stereomicroscope. Each treatment and control was replicated five times. The data were expressed as per cent mortality of mite at each treatment, in relation to untreated control using Abbott's formula (Abbott, 1925).

Effect of Servo agrospray oil on the eggs of tea red spider mite

To assess the ovicidal properties of the Servo agro spray oil, 15 gravid female tea red spider mites were released on a TV1 mature leaf (fourth leaf from the top of tea plant) and allowed it for 12 h for oviposition. On those leaves 30 eggs were selected for treatment and were sprayed with different concentrations of Servo agro spray oil using a glass atomizer (constant pressure 2.5 kg/cm^2). Hatchability was observed for both treated and untreated control batches of eggs for a period of 12 days after treatment. Those eggs that did not hatch after this period were considered as non-viable (Das, 1959).

Each treatment was replicated five times and in all cases, distilled water was sprayed as control. From the record of egg hatchability, corrected percent mortality was calculated out using Abbott's formula (Abbott, 1925).

Bioassay on repellency and ovipositional deterrency

The ovipositional deterrence and repellency test of Servo agro spray oil was made according to the choice tests method described by Roh et al. (2012, 2013) with slight modification. Tea leaf discs (mature TV1) were placed with surface upwards in a Petri-dish lined with moist cotton wool. One half of each leaf (lamina/blade one side of mid rib) was treated separately with different concentration of Servo agro spray oil (0.5, 1.0, and 1.5%), while the other half was treated only with water which served as control. Twenty gravid females of *O. coffeae* were placed in the center i.e. on the midrib of each leaf. Each treatment was comprised of five replicates, and 10 female mites were released on each replication.

The observations on repellency (mites which had left the treated portion were considered as repelled) were taken after 24 h of treatment and the numbers of eggs laid on each half were recorded after 3 days.

The discrimination quotient (DQ) was calculated using the following formula (after Roobakkumar et al., 2010; Roy and Mukhopadhyay, 2012): $DQ = [(C–T)/ (C + T)]$ where C = number of eggs on control surface of the leaves; T = number of eggs on treated surface of the leaves.

Field evaluation of Servo agro spray oil

The field trial was conducted in the Borbhetta Experimental Estate (26.72° N latitude, 94.195° E longitude) in the months of October to November, 2013 to evaluate the efficacy of Servo agro spray oil (0.5, 1.0, 1.5 and 2.0%) along with a standard treatment of propargite 57 EC (organosulfurs: ®Allmite, EID Parry India Ltd., at the Tea Research Association recommended dose of 1:400), and an untreated control (water spray with teepol) against tea red spider mite. Mixed Assam tea clones TV1, –9, –1, –23 and –30 (100 × 65 cm space) were chosen for the field trials following a randomized block design (RBD) with three replication plots per treatment. Each plot in the experiment was separated by two buffer rows of non-experimental tea. There were 60 bushes per plot for the chosen test substances including the standard and untreated control. Infestation level of tea red spider mite was varied from plot to plot. Hence, after selection of the plots a pretreatment count was taken in the respective plots and two rounds of foliar spray were applied at 15-day intervals with a hand-operated calibrated knapsack sprayer (hollow cone NMD 60450 nozzle, droplet diameter 1.6 mm, droplet size 140 ml, discharge 450 ml/min at 40 psi pressure and distance between nozzle and target 30–45 cm) at 400 l/ha. Post-treatment observations were taken for 2 weeks after each treatment. Observations on mite population were made on both adaxial and abaxial surfaces of 30 randomly collected mature leaves per 50 bushes for each test substance in each plot (Das 1960; Roy et al., 2010b). Mean population reduction of mites per treatment was calculated using the following formula: mite population reduction = [(pretreatment population count – post-treatment population count) / pretreatment population count] × 100. The data thus obtained were subjected to Analysis of Variance (ANOVA) following RBD and critical difference (CD; P = 0.05) was calculated (Snedecor and Cochran, 1989).

Phytotoxic effect

A score method was followed to assess the phytotoxic effect of the test substances and was observed in the field trials of tea red spider mite at weekly intervals after spraying and continued for 63 days. The following scale was used to assess the phytotoxicity symptoms (Roy et al., 2010b).

Phytotoxic symptom	**Score (%)**
Injury to leaf tips	20
Injury to leaf surfaces	20
Leaf wilting	20
Necrosis	10
Vein clearing	10
Epinasty	10
Hyponasty	10

Weekly observations of the phytotoxic symptoms in each plot and their corresponding scores were pooled and compared with the untreated control. Finally, the phytoxic effect of Servo agro spray oil was graded as per the following:

Percentage	Grade
0–10	1
11–20	2
21–30	3
31–40	4
41–50	5
51–60	6
61–70	7
71–80	8
81–90	9
91–100	10

Tainting and organoleptic test

A field experiment was conducted to determine whether Servo agro spray oil imparted any taint to black tea. There were five treatments namely, four concentrations of Servo agro spray oil (0.5, 1.0, 1.5 and 2%) along with untreated control (water spray). Spraying was carried out with hand operated knapsak sprayer using a spray volume of 400 litre/ha. Tea shoots were harvested on 7th and 14th day after spraying and processed separately in a mini CTC machine. The samples were forwarded to a tea taster for assessment of taint as positive or negative and for organoleptic test. Leaf-infusions and liquor strength were considered for organoleptic test and score was given as 1–2 being poor, 3–5 being moderate, 6–8 being good and 10 being very good.

Results

The adults were exposed to different concentrations of Servo agro spray oil (0.5%, 1.0% and 1.5%) to determine the percent mortality and observations were taken at different intervals (24, 48, 72 h, and 1 week) (Table 1). The acaricidal activity of Servo agro spray oil was concentration and time dependent. Adult mortality (43.33–83.33%) was observed at 0.5%, 1.0% and 1.5% concentration of the oil solutions after 6 h of exposure and this increased significantly to 63.33–100% after 48 h of exposure. After 48 h, all mites in the control group were still alive. Therefore mortality percentages were directly proportional to the concentrations and also with time after treatment (Table 1).

Oils show affinity to the insect/mite body surface and penetrate their cuticle (Stadler and Buteler, 2009), dissolve internal lipids (Taverner et al., 1999) and eventually penetrate internal cell structures (Taverner et al., 2001; Taverner, 2002). The mortality of adult mites may be due to the following reasons: 1) Suffocation by spiracle blockage which is usually held as the most accepted theory on the mode of action of mineral oils (Roy et al., 1943; Stadler et al., 1996; Taverner et al., 2001); 2) Effect on the integument: symptoms observed on the integument after topical treatment with sub-lethal doses of oils include cell membrane disruption and darkening (Van Overbeek and Blondeau, 1954;

Table 1
Adulticidal activity of Servo agro spray oil against tea red spider mites

Concentration (%)	Adult mortality (%)* ± SD		
	6 h	24 h	48 h
0.5	43.33 ± 11.55^{c}	63.33 ± 15.28^{c}	76.67 ± 11.55^{c}
1	80.0 ± 10.00^{b}	100.00 ± 0.00^{b}	100.00 ± 00^{b}
1.5	83.33 ± 5.77^{b}	100.00 ± 0.0^{b}	100.00 ± 00^{b}
Control	0.0 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}

* Mean of five observations. The difference among doses in same time is grouped at same column with small letters. Means followed by the same letter do not differ significantly at $P = 0.05$ according to Tukey's multiple comparison test (HSDa)

Table 2
Ovicidal activity of Servo agro spray oil against tea red spider mites

Concentration (%)	Adult mortality (%)[a]		
	Percent hatchability (mean* ± SD)	Percent egg mortality	Corrected percentage egg mortality
0.5	7.78 ± 2.31^{d}	92.22	92.04
1	4.44 ± 1.32^{c}	95.56	95.46
1.5	1.11 ± 1.01^{b}	98.89	98.86
Control	98.78 ± 3.85^{a}	2.22	–

* Mean of five replications; Means followed by the same letter in a column do not differ significantly at $P = 0.05$ according to Tukey's multiple comparison test (HSDa)

Stadler et al., 1996; Najar-Rodríguez et al., 2007). Given their lipophilic nature, petroleum based horticulture accumulate in cell membranes and thus affect their structural and functional properties (Mazella et al., 2005). As shown by Najar-Rodríguez et al. (2007) *in vitro,* oils are able to penetrate the cell membranes, accumulate inside the cytoplasm and cause cell dehydration and DNA condensation inside the nucleus. 3) Disruption of the synaptic transmission of nerve ganglia (Najar-Rodríguez et al., 2008).

The eggs of tea red spider mite were sprayed with the Servo agro spray oil at different concentrations and their influence on percent hatchability is shown in (Table 2).

The egg hatchability was reduced to 92.04%, 95.46% and 98.86% at 0.5%, 1% and 1.5% concentrations (Table 2). Ovicidal action of Servo agro spray oil may be due to respiratory effects of oils on eggs which seemed to stop the embryonic development and killed the embryo in the eggs (Smith and Pearce, 1948; Al Dabel et al., 2008). Petroleum based horticulture oils found to be responsible for decreased respiration rate, presumably through mechanical interference with normal gaseous exchange. Topical application of 2% Purespray Green Horticultural mineral oil (Petro-Canada) also led to almost complete egg mortality in the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae) (Harris) through both contact toxicity and suffocation (Wins-Purdy et al., 2009).

The repellency test in the choice bioassay showed that Servo agro spray oil had significant effects on the activity of the mites at 0.5, 1 and 1.5% concentrations (Table 3). Similarly, the total number of eggs laid was significantly lower on the side of the leaf surface where 0.5, 1 and 1.5% of the oil was applied than on the control side (Table 3).

In the present study, the DQ value was high at higher concentration i.e. 1.5% (0.86) followed by 1%, and 0.5% Servo agro spray oil (0.72, and 0.47, respectively). The present study suggested that Servo agro spray oil has a repellent effect that discourages egg deposition and feeding. The residual film may inhibit insects from attaching to plant surfaces (Trammel, 1965). Also, it should be noted that "arrested activity" in insects is one recurrent symptom caused by Petroleum based horticulture oils that has been reported directly or indirectly by many authors in laboratory toxicity tests (Xie and Isman, 1995; Stadler et al., 1996; Taverner et al., 2001; Najar-Rodríguez et al., 2007; Najar-Rodríguez et al., 2008).

The data on the bioefficacy of Servo agro spray oil under field conditions against *O. coffeae* are summarized in (Table 4). All concentration of Servo agro spray oil (0.5, 1.0, 1.5, 2.0%) significantly reduced mite population in compared to untreated control and

Table 3

Repellency (%) and ovipositional deterrence of Servo agro spray oil against tea red spider mite

Concentration (%)	% of adults moved on to leaves after 24 h			Avg. no. of eggs/female after 72 h			
	Control	Treated	*P* value	Treated	Control	*P* value	DQ value
0.5	70.00 ± 7.07	30.00 ± 7.07	< 0.0001*	1.73 ± 0.28	4.74 ± 0.54	< 0.0001*	0.47
1	72.5 ± 7.5	27.5 ± 7.50	< 0.0001*	0.833 ± 0.12	5.21 ± 0.79	< 0.0001*	0.72
1.5	77.5 ± 6.29	22.5 ± 6.29	< 0.0001*	0.33 ± 0.15	4.34 ± 0.97	< 0.0001*	0.86

Means with '*' are significantly different between treated and untreated by *t*-test (mean ± S.E., $P \leq 0.05$)

Table 4
Bio-efficacy of Servo agro spray oil against tea red spider mite *(Oligonychus coffeae)* in field condition

Treatment	Concentration (%)	Pretreatment number of the living mites*	Post-treatment mite and mite reduction (MR)*							
			I week		II week		III week		IV week	
			number of the living mites	MR (%)	number of the living mites	MR (%)	number of the living mites	MR (%)	number of the living mites	MR (%)
Servo agro spray oil	2	133	40	69.9	61	54.1	22	83.4	2	99.0
	1.5	118	43	63.5	72	38.9	28	76.2	15	87.2
	1	121	72	40.5	92	23.9	45	62.8	32	73.5
	0.5	113	84	25.6	97	14.1	46	59.2	63	44.2
Propargit 57% EC	0.25	124	46	62.9	72	42.0	32	74.5	3	97.6
Untreated control	Water spray	125	135	–6.4	138	–10.9	145	–16.0	163	–30.6
Critical difference ($p = 0.05$)				3.50		6.25		3.58		5.05
CV %				8.88		12.29		12.28		21.73

* Mean value of three observations (30 leaves per observations)

at 1.5, 2.0% concentrations of Servo agro spray oil the percent reduction was at par to that of propargite used as standard (Table 4). The four concentrations of Servo agro spray oil caused various degrees of reduction in number of *O. coffeae*, and offered various levels of protection to tea in sprayed plots throughout the period, compared to the unsprayed control plots and check plots conventionally sprayed with synthetic acaricide. Reduction in the populations of the pest in the field may be due to collective effect of acaricidal, ovicidal, and ovipositional deterrence of the Servo agro spray oil.

The control plots, where only water was sprayed, also had fluctuations in the population of tea red spider mite (after 14 days of 1st spray), which may be due to physical action of washing or drowning of the pest in the water spray. No phytotoxic symptoms were observed up to 63 days after spraying of Servo agro spray oil (0.5, 1, 1.5 and 2% – score – 0–5%; grade-1) under field conditions.

Further, made tea samples prepared after treatment had no taint and achieved an organoleptic score of 6.5–7.0, indicating good liquor, strength, color and quality. Although petroleum based horticultural oils have been found to be effective against numerous orchard pests including scales and mites (Beattie et al., 1995; Beattie, 1990; Beattie and Smith, 1993), whiteflies (Larew and Locke, 1990; Liang and Liu, 2002), aphids (Najar-Rodríguez et al., 2007), psylla (Zwick and Westigard, 1978; Weissling et al., 1997), and fruit-feeding Lepidoptera (Davidson et al., 1991; Al Dabel et al., 2008), but to best of our knowledge, this is the first report of petroleum based horticultural oil in details showing acaricidal activity against the tea red spider mite, *O. coffeae* form tea plantation of North East India. As the chosen petroleum based horticultural oil (Servo agro spray oil) is produced by Indian Oil Corporation (IOC), is also shipped to different parts of tea growing region of Assam and approved for organic farming by USOCA, it could effectively be utilized in the IPM programme of tea.

Acknowledgements

The authors are grateful to Dr. N. Muraleedharan, Director, Tocklai Tea Research Institute, for providing the facilities and kindly reviewing the ms and offering his valuable suggestion for its improvement.

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