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Oviposition deterrents for the management of citrus leaf miner *Phyllocnistis citrella S*tainton (Lepidoptera: Gracillariidae)

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ABSTRACT: Experiments were carried out to evaluate neem oil (3%), azadirachtin(0.03%), Neem Seed Kernel Extract(7%), Neem cake extract (10%), Horticultural mineral oil (HO)(1%), *Bacillus thuringiensis* (*Bt*) (0.3%), *Bt* (0.3%) + HO (1%), Spinosad 45SC (0.009%), Spinosad 45SC (0.009%) + HO (1%) and imidacloprid 17.8SL (0.009%) for deterrence to citrus leaf miner oviposition on rough lemon seedlings under caged and nursery conditions. Under laboratory conditions(caged), maximum oviposition deterrence of 84.52% was observed with neem oil (3%) followed by imidacloprid 17.8 SL (0.009%) with 83.33 % deterrence. Under nursery conditions, neem oil (3%), spinosad 45 SC (0.009%) + HO (1%) and imidacloprid 17.8 SL (0.009%) were found effective in controlling leaf miner infestation up to 15 days after spraying.

KEYWORDS: Neem products, microbials, oviposition deterrence, *Phyllocnistis citrella*

INTRODUCTION

Citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the serious pests among 27 species of insect and mite species, particularly on nursery and young plantations of citrus (Sharma et al.,2006). The pest attacks mainly plants of the family Rutaceae and within that family, it is mostly attracted to the genus *Citrus* and its commercially grown cultivars. In India, more than 80 % of the *Citrus reticulata* Blanco (Nagpur mandarin) nurseries are found severely affected by leaf miner infestation, especially in Central India (Shivankar *et al.,*2002). The larvae bore through the leaf epidermis, ingesting the sap and produces silvery mined areas. Citrus leaf miner (CLM) may prevent young leaves from expanding; causing them to remain curled and twisted. Leaf mines are characterized by twisted galleries and the epidermis appears as a silvery film. Leaf mining causes retardation of plant growth especially nursery stock ready for field planting and renders the leaves unsightly.

A host of control measures have been deployed for the management of CLM. Among them, biopesticides including botanicals, can offer a safe and effective alternative to conventional insecticides controlling major insect pests. In past literatures, various products like fish oil, rosin soap (3.3%v/v),

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pongamia, mahua (Katole *et al*., 1993), neem seed cake extract (2%) and pongamia seed extracts (2%) were found effective against CLM on lime (Singh and Azam, 1986; Dhara *et al.,* 1990; Jothi *et al.,* 1993). Chakravarthi *et al. (*1998) have reported that use of azadirachtin 0.03% and neem oil gave high level of control of *Diaphorina citri* Kuwayama upto 90%. Spraying *Melia azadirachta* seed oil 0.5% emulsion was effective (93.6% and 96%) against *Planococcus citri* without any sign of phytotoxicity (Well *et al.,* 1989). The efficacy of petroleum spray oils was tested against citrus psylla on calamondin trees (*Citrus madurensis*) and found that 1st and 2nd instars were highly susceptible (Priore and Pandolfo, 1972-73). But still, a limited number of studies have dealt with the use of botanicals against the citrus leaf miner. Hence, the present study was carried out to find out the effect of different botanicals, oilsand microbial formulations in deterring the CLM adults from ovipositing on the young flush leaves and further evaluation of these botanicals/oils along with insecticides on rough lemon (*Citrus jambheri* Lush) seedlings under protected nursery conditions.

MATERIALS AND METHODS

Oviposition deterrence studies were conducted initially to arrive at the optimum dosage of neem based products viz., neem oil (1%,2%,3%), Azadirachtin 1EC (0.01%, 0.02%, 0.03%), NSKE (3%,5%,7%)andHO (0.3%, 0.5%,1.0%) under caged conditions and further evaluation was carried out under nursery (semi-field) conditions during rainy season(season-I), winter(season-II) and spring (season-III), 2014-15 at ICAR-CCRI, Nagpur.

Oviposition deterrence of neem based products and oils against CLM under caged/ semi-field conditions

Rearing techniques for CLM was followed as described by Urbaneja*et al*. (1999). Initially for arriving at the appropriate dosage, five pairs of newly emerged CLM moths were releasedonto one year treatedold rough lemon seedlings inside cages of 3 x1.5 x 1.5 ft size. The different doses tested were Neem oil 1,2& 3%, Azadirachtin 1EC (0.01, 0.02 &0.03%), Neem Seed Kernal Extract (NSKE) (3, 5 & 7%), Horticultural oil (Mak All Season, a product of Bharat Petroleum) (0.3, 0.5 & 1.0%) each along with water spray as control. The concentrations of the respective molecules were prepared freshly, sprayed using a hand held sprayer (one litre capacity) on the seedlings till runoff. Each treatment was replicated four times with10 seedlings in each replication. A multi-vitamin drop mixed with 10 % (w/v) of sucrose solution was also provided as a food for adult feeding. Five pairs of newly emerged CLM moths were released in each cage for egg laying. Observations were taken 48 hours after treatment (HAT) on number of eggs laid on new leaves from shoot tip of 10 cm length from both treated and control seedlings.

Effective doses of each treatment from above cage studies viz.,neem oil 3%, azadirachtin0.03%, NSKE 7%, Neem cake extract 10%, Horticulture mineral oil 1% and imidacloprid 0.009% along with water spray as control were selected and sprayed on 1.5 years old rough lemon seedlings raised under protected nursery sheds(100m X 10 m) at CCRI farm with 15 seedlings per replication inside muslin cloth cages (3 x 1.5 x 1.5 ft size) with four replications. Egg count was taken 48 HAT per seedling by counting number of eggs laid on new leaves from shoot tip of 10 cm length and oviposition deterrence (%) was calculated using the formula of Williams et al.(1986):

$$\frac{\text{No. of eggs in control - No. of eggs in treatment}}{\text{No. of eggs in control}} \times 100$$

Efficacy of botanicals/oils/insecticides against CLM in protected nursery

The best dose of each treatment viz., Neem oil (3%), Azadirachtin 1 EC(0.03%), NSKE(7%) and Horticultural oil 1% along with *Bacillus thuringiensis* (*Bt*) formulation (0.3%), *Bt* + HO (1%), spinosad 45SC (0.009%), spinosad 45SC (0.009%)+ HO (1.0%), imidacloprid 17.8SL (0.009%) on one year old rough lemon seedlings.. The use of spinosad 45SC (Spintor, Bayer Company), a bio-rational insecticide containing natural material active against pest populations has

increased significantly because of eco-friendly mode of action. But CLM is protected inside the leaf, hence the use of mineral oil (HO) was included in our study as a surfactant to increase the penetration of insecticides through the epidermis of the citrus leaf. Imidacloprid 17.8SL (Confidor, Bayer Crop Science Limited) having translaminar mode of action served as a check.

Observations on % infestation by citrus leaf miner were recorded at 7, 10 and 15 days after spraying (DAS)**.** The data generated were statistically analyzed (http://stat.iasri.res.in/sscnarsportal) using completely randomized design (CRD) for cage studies and randomized block design (RBD) for nursery experiments. The data on egg count and % infestation were transformed to square root and arc sine, respectively as per the method followed (Gomez and Gomez, 1984) and means were separated by Duncans Multiple Range Test (DMRT) for inference.

RESULTS

Oviposition deterrence of neem based products and oil against citrus leaf miner under caged/semi-field conditions

Under caged conditions, neem oil 3%, azadirachtin 0.03%, NSKE 7% and HO 1.0% was found significantly the best doses for deterring the CLM adults from oviposition with an egg count/10 cm shoot of 0.79, 0.85,1.50 and 0.80, respectively (Table.1). The best insecticide/botanical dose was furtherevaluated for oviposition deterrence under caged conditions in protected nursery(semi-field condition). Out of the seven treatments, egg count/ 10 cm shoot was significantly low in neem oil 3% (0.60) but was at par with imidacloprid 17.8 SL (0.009%) (0.70) and HMO1% (0.80) treated seedlings (P=0.05). Maximum oviposition deterrence (%) of 84.52 was observed in the case of rough lemon seedlings sprayed with neem oil (3%) followed by imidacloprid 17.8 SL (83.33%) (Table.2). Spray with horticultural mineral oil 1% was found significantly next best in repelling the leaf miner adults from ovipositing the leaves with 80.91% deterrence. Neem cake extract (10%) and NSKE (7%) were found to have the lowestdeterrence of 66.67 and 64.28 %, respectively.

Table1. Oviposition deterrence of different doses of botanicals/oils against citrus leaf miner, *Phyllocnistis citrella*

Treatment	Egg count/ 10cm shoot
T1 Neem oil 1%	1.80(1.32)^{c}
T2 Neem oil 2%	1.20(1.08)^{b}
T3 Neem oil 3%	0.79(0.65)^{a}
T4 Water spray	4.20(2.04)^{d}
CD=0.222(0.01%); CV=13.95	
T1 Azadirachtin 1EC (0.01%	1.90(1.37)^{b}
T2 Azadirachtin 1EC (0.02%)	1.60(1.25)^{b}
T3 Azadirachtin 1EC (0.03%)	0.85(0.91)^{a}
T4 Water spray	4.20(2.04)^{c}
CD=0.199(0.01%); CV-11.75	
T1 NSKE 3%	3.00(1.71)^{b}
T2 NSKE 5%	2.80(1.65)^{b}
T3 NSKE 7%	1.50(1.21)^{a}
T4 Water spray	4.20(2.04)^{c}
CD=0.282(0.01%); CV=15.12	
T1 HO 0.3%	2.10(1.43)^{c}
T2 HO 0.5%	1.30(1.12)^{b}
T3 HO 1.0%	0.80(0.88)^{a}
T4 Water spray	4.20(2.04)^{d}
CD=0.260(0.01%); CV=15.64	

NSKE- Neem Seed Kernel Extract,
HO- Horticulture mineral oil
*Values in parentheses are square root transformed

Efficacy of insecticides/botanicals against citrus leaf miner in the protected nursery

During monsoon 2014, neem oil 3%, spinosad 45 SC + HO (1%) and imidacloprid 17.8 SL @ 0.009% recorded 24.06, 25.36 and 24.39% leaf miner infestation 7 days after spraying (DAS) but was at par with Horticultural oil 1%(28.63). At 10 DAS, neem oil 3% and spinosad 45 SC + HO 1% recorded an infestation of 16.11 and 17.15%, respectively. Similarly, at 15 DAS neem oil 3%,

Table 2. Oviposition deterrence (%) of insecticides/botanicals against citrus leaf miner, *Phyllocnistis citrella* on rough lemon seedlings

Sl.No.	Treatments	Egg count/10 cm shoot (after 48 hours)	Oviposition deterrence (%)
1.	Neem oil (3%)	0.60(0.76)[a]	84.52
2.	Azadirachtin (0.03%)	0.95(0.97)[b]	79.76
3.	NSKE (7%)	1.50(1.20)[c]	64.28
4.	Neemcake extract (10%)	1.40(1.14)[c]	66.67
5.	Horticulture Mineral Oil (1%)	0.80(0.88)[ab]	80.91
6.	Imidacloprid (0.009%)	0.70(0.82)[ab]	83.33
7.	Water spray	4.20(2.04)[d]	-
	CD (P=0.05)	0.16	
	CV	15.42	

*Values in parentheses are square root transformed

spinosad 45 SC + HO 1% and imidacloprid 17.8 SL followed by HO 1% were found significantly effective in reducing leaf miner infestation (Table.3).

During winter 2014, neem oil 3% (24.68), horticultural oil 1% (26.27), spinosad 45 SC + HO (1%) (25.31) and imidacloprid 17.8 SL (24.39) recorded significantly less infestation at 7 DAS (Table 3). At 10 DAS, imidacloprid 17.8 SL, spinosad 45 SC + HO 1% and neem oil 3% were the effective module with significantly lowest infestation of 17.55, 17.16 and 16.12%, respectively. Only imidacloprid 17.8SL (25.16%) was found effective in keeping the leaf miner infestation levels below 30% ETLat 15DAS. But during spring 2015, imidacloprid 17.8 SL recorded lowest infestation at 7 DAS (31.72%) and 15 DAS (29.96). At 10 DAS, an infestation of 29.99% was observed when sprayed with neem oil 3% and spinosad 45 SC + HO 1% (Table 3).

Under nursery conditions over the three seasons (pooled mean data for monsoon and winter, 2014; spring, 2015), at 7 DAS, infestation was significantly low in seedlings treated with imidacloprid 17.8 SL (0.009%) (26.83%) but was at par with neem oil (3%) with 27.03%, spinosad 45 SC + HO (1%) with 28.44%; HO 1% with 29.85% and NSKE (7%) with 33.48%. At 10 DAS, neem oil still recorded an infestation of 20.74 % only and spinosad45 SC(0.009%) + HO (1%) with 21.43 % while imidacloprid 17.8 SL (0.009%) followed by neem oil (3%) and spinosad45 SC (0.009%) + HO (1%) were the effective at 15 DAS. Azadirachtin (1%EC) @ 0.03 % was found less effective as indicated by infestation levels of 40.45 % at 7 DAS to 38.46 %at 15 DAS while NSKE (7%) with only 33.48 to 39.01 % up to 15 DAS. Sprays with *Bt* + HO recorded an infestation which ranged between 28.88 to 35.49% while HO 1 % (alone) with 29.49 to 35.11% indicated that *Bt* as sole (37.63 to 43.58%) insecticide may not be effective in controlling the pest. But in combination with HO, better results were observed in our studies.

DISCUSSION

In the context of several pressures like pesticide resistances accelerated the search for environmentally safe, toxicologically selective and efficacious pesticides (Dias *et al.,* 2005). Neem products have several advantages compared to conventional synthetic pesticides such as their extremely low mammalian toxicity (Thoeming and Poehling, 2006) and a very low probability of pest resistance (Feng and Isman, 1995). It is evident from the above studies that neem oil (3%) has

Table 3. Efficacy of botanicals/insecticides against citrus leaf miner, *Phyllocnistis citrella* during monsoon, 2014, winter, 2014 and spring, 2015 in protected nursery at ICAR- CCRI, Nagpur

Treatment	Per cent infestation															
	Monsoon 2014				Winter 2014				Spring 2015				Pooled mean			
	Pre-treatment	7 DAS	10 DAS	15 DAS	Pre-treatment	7 DAS	10 DAS	15 DAS	Pre-treatment	7 DAS	10 DAS	15 DAS	Pre-treatment	7 DAS	10 DAS	15 DAS
Neem oil (3%)	46.33 (42.89)	24.06 (29.33)^{a}	16.11 (23.65)^{a}	24.29 (29.53)^{a}	45.52 (42.42)	24.68 (29.76)^{a}	16.12 (23.65)^{a}	29.63 (32.97)^{b}	46.00 (42.69)	32.34 (28.67)^{ab}	29.99 (25.00)^{a}	34.22 (31.67)^{c}	45.95 (42.67)	27.03 (31.27)^{ab}	27.03 (26.84)^{a}	29.38 (32.77)^{abc}
Azadirachtin (0.03%)	44.62 (42.02)	39.29 (38.87)^{d}	36.69 (37.27)^{e}	42.66 (40.78)^{d}	44.68 (41.94)	41.28 (39.97)^{cd}	36.69 (36.79)^{c}	35.67 (36.66)^{c}	45.00 (42.12)	40.77 (42.67)^{d}	37.85 (37.66)^{e}	37.06 (36.33)^{d}	51.33 (45.67)	40.45 (39.49)^{c}	37.08 (37.51)^{d}	38.46 (38.31)^{def}
Horticultural oil (HO 1%)	46.66 (43.08)	28.63 (32.27)^{ab}	28.66 (32.33)^{cd}	27.00 (31.25)^{ab}	47.07 (43.31)	26.27 (30.79)^{a}	25.37 (30.21)^{b}	41.67 (40.20)^{de}	47.66 (43.65)	34.65 (32.33)^{bc}	34.44 (32.00)^{c}	36.66 (35.66)^{d}	47.13 (43.35)	29.85 (33.08)^{ab}	29.49 (32.84)^{bc}	35.11 (36.25)^{cde}
NSKE (7%)	38.78 (38.51)	32.39 (34.67)^{bc}	30.15 (33.29)^{cd}	41.00 (39.81)^{d}	37.57 (37.80)	32.39 (35.12)^{b}	27.30 (31.49)^{b}	37.99 (38.04)^{cd}	39.33 (38.84)	35.65 (34.00)^{c}	34.85 (32.66)^{c}	38.05 (38.00)^{d}	38.56 (38.38)	33.48 (35.34)^{abc}	30.77 (33.66)^{cd}	39.01 (38.65)^{ef}
Bacillus thuringiensis (Bt)(0.3%)	40.62 (39.59)	41.00 (39.81)^{d}	37.22 (37.59)^{e}	41.66 (40.18)^{d}	42.38 (40.62)	43.05 (40.99)^{d}	37.22 (37.59)^{c}	46.38 (42.92)^{ef}	43.33 (41.16)	40.19 (41.67)^{d}	38.44 (38.66)^{e}	42.70 (46.00)^{e}	46.56 (43.01)	41.41 (40.05)^{c}	37.63 (37.83)^{d}	43.58 (41.30)^{gf}
Bt (0.3%) + HO (1%) (35.92)^{bcde}	44.70 (41.95)	36.37 (37.06)^{cd}	25.74 (30.47)^{c}	31.48 (34.12)^{c}	44.27 (41.71)	35.67 (36.65)^{bc}	26.47 (30.96)^{b}	35.16 (36.36)^{c}	44.66 (41.93)	34.44 (32.00)^{bc}	34.44 (32.00)^{c}	36.66 (35.66)^{d}	51.21 (45.72)	35.49 (36.55)^{bc}	28.88 (32.46)^{bc}	34.43
Spinosad 45SC (0.009%)	44.03 (41.57)	41.33 (40.00)^{d}	32.39 (34.68)^{de}	30.22 (33.33)^{bc}	44.52 (41.85)	36.52 (34.57)^{b}	34.21 (35.78)^{c}	34.22 (35.77)^{bc}	45.00 (42.12)	34.43 (32.00)^{bc}	36.86 (36.00)^{d}	33.41 (30.33)^{bc}	52.65 (46.60)	40.76 (39.64)^{c}	34.49 (35.95)^{cd}	32.62 (34.82)^{bcd}
Spinosad 45SC (0.009%) + HO (1%)	43.33 (41.16)	25.36 (30.20)^{a}	17.15 (24.45)^{a}	25.71 (30.46)^{a}	46.18 (42.81)	25.31 (30.17)^{a}	17.16 (24.45)^{a}	30.00 (33.18)^{b}	46.00 (42.70)	34.64 (32.33)^{bc}	29.99 (25.00)^{a}	31.30 (27.00)^{ab}	47.95 (43.82)	28.44 (32.16)^{ab}	21.43 (27.38)^{a}	29.00 (32.56)^{ab}
Imidacloprid 17.8 SL (0.009%)	45.49 (42.41	24.39 (29.56)^{a}	21.33 (27.44)^{b}	24.47 (29.61)^{a}	45.22 (42.25)	24.39 (29.56)^{a}	17.55 (24.75)^{a}	25.16 (30.07)^{a}	45.33 (42.31)	31.72 (27.67)^{a}	30.86 (26.33)^{b}	29.96 (25.00)^{a}	45.67 (42.51)	26.83 (31.15)^{a}	23.25 (28.67)^{ab}	26.53 (30.98)^{a}
Control	45.57 (42.45)	63.11 (52.61)^{e}	49.00 (44.42)^{f}	47.66 (43.66)^{e}	45.58 (42.46)	63.12 (52.61)^{e}	47.76 (43.71)^{d}	48.89 (44.37)^{f}	45.66 (42.50)	44.61 (49.33)^{e}	41.94 (44.67)^{f}	45.76 (51.33)^{f}	48.82 (44.32)	56.95 (49.03)^{d}	46.23 (42.83)^{e}	47.44 (43.53)^{g}
CD(P=0.05)	NS	4.09	2.91	2.79	NS	3.34	2.34	2.82	NS	2.65	0.66	2.33	NS	5.34	4.22	3.58
CV		6.54	5.22	4.62		5.37	4.27	4.44		4.25	1.10	3.72		8.46	7.31	5.71

DAS-Days after spraying; Figures in the parentheses are arc sine transformed values; In a column, means followed by same letter (s) are not significantly different at p=0.05 by DMRT

oviposition deterrent effect on CLM adults. The efficiency of neem based products, including emulsified neem oil, NSKE etc for citrus leaf miner management has been confirmed in earlier studies (Jayanthi and Verghese, 2004; Nath and Sinha, 2011). Katole *et al.* (1993) tested the efficacy of some botanicals and synthetic insecticides against infestation of CLM on Nagpur mandarin and found that neem oil was effective in the control of CLM which is in line with our findingthat neem oil (3%) waseffective in deterring the leaf miner adults from ovipositing within 48 hours after spray.Efficacy of Neem oil (1%) has been reported against citrus leaf miner to cause a percent reduction of 33.1, 28.7 and 26.4 at 3, 7 and 14 DAS, respectively(Patil, 2013). Among synthetic insecticides, imidacloprid 17.8 SL (0.009%) was significantly effective in deterring the oviposition by adults as well as checking the pest population up to 15 DAS. Feeding deterrence of sub-lethal concentrations of imidacloprid in plant tissue has been reported for adult Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama on citrus(Boina *et al.*, 2009). According to them,sublethal concentrations of imidacloprid in plant tissue negatively affectsthe development, reproduction, survival and longevity of ACP, which contributes to population reductions over time . Reduction in citrus leaf miner infestation may be due to either feeding deterrence or oviposition deterrence effect of imidacloprid.

Horticultural oil (HO) has shown to work as a temporary oviposition deterrent in nursery seedlings upto 10 DAS (32.84%) in our studies as sole sprays or in combination. Oviposition deterrence as a result of oil deposits as physical barrier has also been demonstrated with Asian citrus psyllid, *D. citri* and citrus leaf miner earlier (Rae et al., 1996). Horticultural mineral oil (Mak all Season) @1.5% reduced the infestation of citrus up to 11 days, @ 2.0 % against psylla up to 7 days and @ 2.0 % against citrus thrips and mites in citrus nurseries. Only limitation with horticultural oils is that they should be used with care to avoid phytotoxicity on the seedlings when temperature is high (>40^{o}C) (Rao et al., 2014).

The botanicals/oils with oviposition deterrent and ovicidal activity are really a valuable weapon to protect the seedlings under nursery conditions before the plants are damaged by the pests. The present findings of this study indicated that timely intervention with selective botanicals /oils/ insecticides during the active adult emergence targeting oviposition may help to combat the rapid multiplication of citrus leaf miner and thereby protect the young flush leaves in citrus during the peak flushing seasons.

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