SYNERGISTIC EFFECT OF INSECTICIDE-BOTANICAL MIXTURES ON CITRUS LEAF MINER, *Phyllocnistis citrella* Stainton

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ABSTRACT: Efficacy of neem seed kernel extract individually and in combination with synthetic pyrethroid, cypermethrin in different ratios (half the recommended dose and standard full dose) were evaluated experimentally on citrus leaf miner, *Phyllocnistis citrella* Stainton on acid lime (*Citrus aurantifolia* (Christon) Swing) during 2002 - 2003 at Indian Institute of Horticultural Research, Bangalore, India. Observations were made on the number of live mines, dead mines, adult emergence and leaf damage. The results clearly showed that the combination treatment viz., neem seed kernel extract 4% + cypermethrin 0.5 ml/l (full dose) was found to be the best combination to manage *P. citrella* infestation with minimum leaf damage. Further, this treatment also recorded minimal fresh infestation, high larval mortality and low adult emergence. The present work shows the strong synergistic effect of the neem seed kernel extract with cypermethrin to minimize the leaf miner damage.

Key Words: Botanicals, citrus, *Phyllocnistis citrella*, synthetic pyrethroid

INTRODUCTION

In India, the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae), is a regular pest in citrus nurseries and >80% of nurseries in central India are affected by leafminer. During serious infestation leaf damage exceeds 87% and moderate infestation lowered yield by 30-40% in the following year (Shivankar et al., 2002).

Various control methods have been proposed for the management of CLM in citrus; these include: cultural practices, chemical control, use of petroleum spray oils and biological control (Beattie et al., 1995; Rae et al., 1996; Ateyyat and Mustafa, 2001; Shivankar et al., 2002). Considerable work has been done on chemical control. Many synthetic insecticides have been tested against CLM (Borle and Khosdasker, 1977; Bhumannavar, 1987; Muthaiah et al., 1998). Many workers have recommended two or more insecticidal sprays for the control of CLM. Although most of the recommended chemicals were reported to control the CLM, some eggs and early instars are bound to escape and these surviving stages lead to reinestation within a week of insecticidal application (Boulahia et al., 1996). At present there is a growing concern to reduce environmental hazards due to pesticide usage. So need based limited number of sprays is the need of the hour to reduce insecticidal pressure in citrus ecosystem. Use of botanicals like neem, which conserve natural enemy complex is now being emphasized to reduce or phase out
the use of broad-spectrum insecticides in citrus IPM programs. The extracts of neem seed contain azadirachtin, a triterpenoid, which was found effective especially against lepidopterans (Schmutterer, 1990) and thus has potential as an important source of botanical insecticide in the management of CLM. However, the minimal persistence of neem is one of the important limiting factors in pest management programmes (Rembold, 1989). Therefore, a study was undertaken with the objective of finding out how best neem could be integrated in the management schedule along with currently prevailing effective synthetic pyrethroid viz., cypermethrin to reduce insecticidal pressure and to evolve an effective, and persistent CLM management.

MATERIALS AND METHODS

A study to evaluate the bioefficacy of neem seed kernel extract in combination with a synthetic pyrethroid viz., cypermethrin, an insecticide commonly being used by farmers for the management of CLM was carried out at the citrus orchard of Indian Institute of Horticultural Research, Bangalore (12°58′N; 77°35′E), India during 2002 - 2003. The experiments were conducted on 12-year-old acid lime (Citrus aurantiifolia (Christon) Swing). A control without any spray was maintained as check. Two trials were conducted. The treatments along with doses are given in Table 1. Neem seed kernel extract was prepared by grinding neem seed kernels and mixing @40 g per liter of water, and leaving over night. The mixture was filtered through muslin cloth the next morning to obtain 4% NSKE. Neem seed kernel extract was chosen for testing over its commercial formulations because of the superiority of NSKE in earlier trials (Jayanathi and Verghese, 2004).

The experiment was laid out in a randomized block design, and the treatments replicated thrice. Each tree was considered as a unit of replication. Single spray was given during emergence of new leaves. Observations were taken at weekly interval on the total number of live mines, dead mines and mines with emerged holes from all the leaves on ten randomly selected terminal shoots per plant. Each such shoot had a range of 8 – 10 leaves. Larvae when alive are creamy yellow in color, while dead one gets discolored with a brownish to blackish tinge. These are discernible with a hand lens, even when larvae are within a mine. From these, the mean number of live mines, dead mines and mines with emerged holes were calculated and was transformed using log (x+1), to normalize the distribution. The data are presented and discussed with respect to days after spray (DAS) i.e., 7 DAS, 14 DAS and 21 DAS. Further, the plants were visually graded for percent leaf damage (i.e., 28 days after spray, 28 DAS) by scoring the newly sprouted leaves from ten randomly selected shoots per plant. On each shoot, first eight leaves were taken from the tip and each leaf was graded for CLM incidence depending upon percentage severity of the damage, ranging from zero (no CLM incidence) to 100 (total damage with leaf curled and twisted). The data were transformed to arc sine values. The data of two trials were subjected to ANOVA with least significant difference at P = 0.05 as test criterion.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatments</th>
<th>Dosage (ml/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cypermethrin</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>2</td>
<td>Neem seed kernel extract</td>
<td>40 g/l</td>
</tr>
<tr>
<td>3</td>
<td>Cypermethrin + Neem seed kernel extract</td>
<td>0.5 ml + 40 g</td>
</tr>
<tr>
<td>4</td>
<td>Cypermethrin + Neem seed kernel extract</td>
<td>0.25 ml + 40 g</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Data of the two field trials on number of live mines, dead mines, adults emerged and per cent leaf damage due to leaf miner are combined and presented in Figs 1-3 and Table 2.
Table 2: Efficacy of different treatments on *P. citrella*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Per cent leaf damage at 28 days after spray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypermethrin (0.5 ml/l)</td>
<td>21.33 (27.47)</td>
</tr>
<tr>
<td>NSKE (4%)</td>
<td>30.00 (33.14)</td>
</tr>
<tr>
<td>Cypermethrin (0.5 ml/l) + NSKE (4%)</td>
<td>10.00 (18.14)</td>
</tr>
<tr>
<td>Cypermethrin (0.25 ml/l) + NSKE (4%)</td>
<td>19.67 (26.26)</td>
</tr>
<tr>
<td>Control</td>
<td>99.67 (88.19)</td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td>6.31</td>
</tr>
</tbody>
</table>

Live mines:

At 7 days after the spray, all the treatments were significantly superior (p<0.05) in preventing fresh infestation except cypermethrin (0.25 ml/l) in combination with NSKE which recorded 1.10 live mines/shoot. However, it was superior to untreated control which recorded 1.80 live mines/shoot. The treatments viz., cypermethrin (0.5 ml/l) + NSKE 4%, cypermethrin (0.5 ml/l) recorded lowest number of mines (0.17 and 0.20 respectively) and significantly superior to other treatments (p<0.05) in preventing fresh infestation in second trial.

Fig. 1 Effect of different treatments on fresh infestation of citrus leaf miner, *P. citrella*

Fig. 2. Effect of different treatments on larval mortality of citrus leaf miner, *P. citrella*
At 14 days after spray, cypermethrin (0.5 ml/l + NSKE 4%), cypermethrin (0.5 ml/l) were still significantly superior (p<0.05) and found to be effective in preventing fresh infestation. Interestingly NSKE alone also found statistically on par with the above treatments in preventing fresh mining by *P. citrella*.

At 21 days after spray also, the plants treated with cypermethrin (0.5 ml/l) + NSKE 4%, cypermethrin (0.5 ml/l) showed lowest number of live mines followed by cypermethrin (0.25 ml/l) + NSKE 4%. In case of NSKE 4% alone a slight increase in number of fresh mines was observed.

**ii. Dead mines:**

At seven days after the spray, in case of dead mines also similar trend was observed like live mines. The treatments viz., cypermethrin (0.5 ml/l) + NSKE 4%, cypermethrin (0.5 ml/l) recorded highest number of dead mines compared to other treatments, and found to be on par with each other.

At 14 days after the spray, the maximum number of dead mines was recorded on cypermethrin (0.63) treated plants followed by other treatments which recorded 0.47 dead mines/shoot. The untreated control recorded only 0.10 dead mines/shoot.

At 21 days after the spray, the treatment cypermethrin (0.25 ml/l + NSKE 4%) recorded highest number of dead mines (0.83/shoot) followed by cypermethrin (0.5 ml/l + NSKE 4%) (0.57/shoot) and NSKE 4% (0.43). The treatment cypermethrin (0.5 ml/l) alone recorded the lowest number of dead mines (0.17/shoot) at 21 days after spray. Interestingly, the treatment where NSKE 4% was combined with cypermethrin (half of the recommended dose) recorded as second best treatment though at 7 DAS it could not cause any larval mortality.

**iii. Adult emergence**

At seven days after the spray, the effect of treatments was observed on emergence on adult *P. citrella*. Here, cypermethrin (0.5 ml/l) and cypermethrin (0.5 ml/l) + NSKE 4% recorded zero adult emergences and were superior to all the other treatments. Among the treatments, highest emergence was recorded in NSKE 4% treated plants (0.30/shoot) followed by cypermethrin 0.25 ml/l + NSKE 4% (0.20/shoot).

At 14 days after the spray, cypermethrin 0.5 ml/l + NSKE 4% was found superior with zero adult emergence followed by cypermethrin (0.5 ml/l) alone which recorded 0.07/shoot.

At 21 days after the spray, all treatments were found effective in bringing down the adult emergence by 21 days after spray. However, cypermethrin (0.5 ml/l) + NSKE 4% was found to be superior with 0.07/ shoot and was on par with NSKE 4% (0.17/ shoot) and cypermethrin (0.5 ml/l) alone (0.13/ shoot). Highest emergence was recorded in control (0.53/ shoot).
iv. Leaf damage:

All treatments were superior over control regarding reduction in per cent leaf damage. Cypermethrin 0.5 ml + NSKE 4% recorded the lowest leaf damage (10.00%) followed by cypermethrin 0.25 ml/l + NSKE 4% (19.67%) and cypermethrin alone (21.33%).

The combination of NSKE and cypermethrin was significantly superior to that of individual treatments showing a phenomenon of synergism. At seven days after spray, cypermethrin 0.5 ml/l + NSKE 4% was found to be effective treatment with minimum number of live mines/shoot (0.17 mines/shoot), and maximum number of dead mines (0.90 mines/shoot) and with zero emergence followed by cypermethrin alone. However, the treatments viz., NSKE alone and in combination with cypermethrin at sub-optimal dose (0.25 ml/l) were not as effective as above treatments. At 14 days after spray also these two treatments were found to be on par with each other in preventing the fresh infestation, with high mortality of existing mines and with low emergence. At 21 days after spray also, cypermethrin 0.5 ml/l + NSKE 4% was found to be superior over other treatments with low live mines, high dead mines and low emergence. Even this treatment was found to be effective over cypermethrin alone by causing highest mortality even at 21 days after spray as cypermethrin alone recorded lowest number of dead mines/shoot. Interestingly, though the adult emergence was lowest in the cypermethrin treatment a slow increase in adult emergence was observed by 21 DAS unlike NSKE treated plants. In NSKE treated plants though the initial reduction in adult emergence as not as high as cypermethrin but a negative trend was observed over a period of time i.e., by 21 DAS. In the treatment where NSKE was added to cypermethrin, similar trend was observed i.e., slow down of the adult emergence over a period. The overall grading on leaf damage caused by P. citrella, 28 days after the spray revealed that cypermethrin 0.5 ml/l in combination with NSKE 4% was significantly superior in reducing the leaf damage when compared to other treatments. This study clearly suggests a probable synergistic interaction between neem seed kernel extract and cypermethrin against P. citrella. Synergistic interactions have been reported for different neem extracts with a variety of materials, including piperonyl butoxide, common vegetable oils and several different insecticides, including pyrethrins and synthetic pyrethroids. However, combinations of pyrethroids with neem extracts are not always synergistic. Synergism of neem was shown by Qadri and Rao (1980), who report a combination of pyrethrins and neem seed extract is more effective against certain household and stored product pests than would be expected from the individual contributions of the components.

Thus, by considering the overall efficacy of all treatments, the treatment combination, which involved cypermethrin and NSKE at recommended full doses (0.5 ml/l and 4% respectively) not only prevented the fresh infestation, but also caused high larval mortality and thereby reducing the adult emergence of P. citrella. This may be because of the reason that extracts of neem seed contain Azadirachtin, a triterpenoid that controls insects in larval and pupal stages by interfering with the metabolism of ecdysone, therefore preventing normal development. Further, it can also work as repellent or antifeedant on the adult stage of many insect pests (http://www.msue.msu.edu/ipm/CAT02_frt/F04-09-02.html). However, the necessity of reaplication of neem formulations due to its short residual life may be overcome by mixing it with chemical insecticides. Jayanthi and Verghese (2004) also recommended neem formulations as follow-up sprays during new flush emergence for the efficient management of P. citrella.

In conclusion, our results demonstrated that the use of NSKE in mixture with chemical insecticides can be used for effective control of the P. citrella damage for longer duration and it could be a useful alternative to repeated sprayings of broad-spectrum insecticides. It also circumvents the fear of commercial azadirachtin fortified with sublethal dose of insecticides.

As CLM is potentially serious pest of citrus, and the continuous use of pesticides induces
resistance, management strategies for the CLM should incorporate an integrated approach with spray combinations which are synergistic to enhance the insecticidal efficacy and to slow down resistance development if any. The importance of utilizing botanicals like neem in improving insecticidal efficacy is indisputable. The present study clearly showed the efficacy of integrating the neem with synthetic insecticides to achieve sustained control of CLM and thereby reducing insecticidal pressure in citrus ecosystem.

REFERENCES


http://www.msue.msu.edu/ipm/CAT02_frt/F04-09-02.html