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Evaluating the Efficacy of Insecticides Against the Apple Leaf Miner, *Lyonetia clerkella* Linn. (Lepidoptera: Lyonetiidae) in Kashmir, India

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ABSTRACT

Lyonetia clerkella Linn. (Lepidoptera: Lyonetiidae) is one of the most destructive insect pests in many parts of the Kashmir valley. In order to find an effective control agent against this lepidopteran pest, we determined the effectiveness of four synthetic insecticides, viz. Dichlorvas 76EC, Chlorpyriphos 20EC, Dimethoate 30EC, and Cartap hydrochloride 4% GR under both field as well as in laboratory conditions. The distilled water was also used as a control in the experiments. The mortality caused by these insecticides was recorded in both larval as well as in adult bioassay experiments. In the larval bioassay experiment, the highest mortality was caused by Cartap hydrochloride 4% GR (93.13%) followed by Dimethoate 30EC (88.97%), whereas in adult bioassay experiment, the highest mortality was caused by Dimethoate 30EC (94.03%). Results obtained in the present study are promising and may be used as a control strategy for the management of this severe leaf miner pest; however, no recommendations concerning the potential use of insecticides for apple orchards can be given, and further studies are needed in this respect, especially under field conditions.

INTRODUCTION

Kashmir is the northernmost geographical region of South Asia that is located between the Great Himalayas and the Pir Panjal mountain range (Khanday & Buhroo 2014). Kashmir is famous for the deliciousness of its temperate fruits in every part of India. Thousands of fruit orchards consisting mainly of apple (*Malus domestica*) can be seen at many parts of the valley. Besides apple, other prominent fruit trees that are cultivated in most regions of this Himalayan valley are- *Cydonia oblonga*, *Prunus persica*, *P. domestica*, *P. avium*, *P. armeniaca*, Pyrus *communis*. A wide variety of sub-tropical fruits can also be seen in this part of the world.

A wide variety of insect pests can also be seen on apple trees from the onset of foliage till the end of the autumn period. The effect of their damage to the apple trees varies from mild to severe infestation. These pests not only are the cause of damage to the host plants but simultaneously reduce the annual yield of the latter in the valley of Kashmir. Among these insect pests, *L. clerkella* is one of the most destructive and widespread in many parts of the valley (Ahmed & Bhat 1987). This pest is also distributed in many parts of the world such as Japan, South Korea, Taiwan, China, north-western Siberia, India, Europe, Turkey and Madagascar where it damages different species of fruit trees including apple, pear, cherry, plum, peach, and quince (Inoue *et al* 1982; Agata *et al* 2007). Apple leaf miner is considered a major insect pest in the commercial peach orchards in Japan where it causes heavy defoliation prior to harvest (Naruse 1978; Naruse & Hirano 1990). In East Asia, this miner pest is reported to cause leaf abscission just after the emergence of few larvae per leaf (Akira & Takehiko 1989). Furthermore, the mines formed by the larvae of *L. clerkella* were found to be one of the major causes that reduce the photosynthetic capacity of leaves and cause their premature abscission (Parrella 1987; Spencer 1973; Parrella & Jones 1987).

The female moth of *L. clerkella* sits on the underside of the leaf and laid eggs singly into the parenchyma. The hatched caterpillars penetrate into the mesophyll and form serpentine mines in the upper half of the leaves. The three pairs of thoracic blackish legs differentiate the third instar larvae from the first and second instars and these prologs are easily observed through the foliar epidermis. Mature 3rd instar larvae abandon their mines and form silken cocoons under the leaves or on the bark.

In Kashmir, insect pests of apple trees are controlled by a wide range of conventional pesticides. Due to the lack of recommendations on insecticides against the apple leaf miner pests, farmers continue to use whatever is available without considering the effectiveness. A wide range of insecticides is being applied to apple trees when growers see the slightest symptoms of infestation caused by different species of insect pests. However, broad-spectrum insecticide applications have declined the rate of parasitism and developed resistance within a fly population followed by an increase in the density of leaf miner pests (Oatman & Kennedy 1976; Murphy & LaSalle 1999). The objective of the present study was to determine the efficacy of four pesticides against the various life stages of *L. clerkella* in Kashmir.

MATERIALS AND METHODS

Chemical Treatments:

In the present study, experiments were conducted in the field as well as in the experimental rooms. During the experimental period (first week of March, 2016 to the second week of April, 2016) red delicious apple plants with an average height of 2.5 feet were planted in plastic pots. The methodology was followed as per the method adopted by Adachi 2002. The experimental pots were kept in the open place and were watered once every three days till the maturation of their leaves. Seven-liter transparent plastic bottles were used to cover the foliage of these experimental plants. Ventilations with the dimensions of 10 x 10 cm were kept at opposite directions of these bottles and covered by one layer of nylon mesh. The aperture diameter of the mesh was 0.5 mm. The bottles were cleaned and then mounted upon the experimental host plants.

Based on the current and potential use of insecticides for the management of leaf miners, four insecticides were selected. Commercial formulations of these insecticides were diluted with distilled water and applied at field rates based on the recommended label dilutions (Table 1).

Table 1: Different type of insecticides with their label dilutions

Insecticide	Trade name	Concentration
Dichlorvas 76EC	NUVAN	0.3ml +100ml H_2O
Chlorpyriphos 20EC	COROBAN	0.1ml +100ml H_2O
Dimethoate 30EC	ROGOR	0.1ml+100ml H_2O
Cartap hydrochloride 4% GR	DARTRIZ 4G	0.1g +100ml H_2O

Larval Leaf Miner Bioassay:

Following the methods described by Cox *et al.* (1995) with some modifications, the larval leaf miner bioassay was carried out. Fifteen Red delicious apple plants were selected for this experiment and were distributed in five groups; each group consisted of three plants. These groups were then placed in five different experimental rooms and the latter was labeled as experimental room one (R_1), experimental room two (R_2), experimental room three (R_3), experimental room four (R_4) and experimental room five (R_5). The rooms were cross ventilated and had free circulation of air and light. Plants in the rooms were kept 10 feet apart from each other. The potted plants covered by transparent plastic bottles were exposed to 20 mixed sex *L. clerkella* adults each for oviposition. After 12 hrs, moths were removed from their respective bottles and leaves were regularly monitored with a high-power magnifying glass for the appearance of mines.

The plants were kept in natural conditions at an average room temperature of 25 ± 3 ^{0}C and 16L: 8D photoperiod. After eight days, the well visible mines on each plant were counted and the diluted insecticides were applied on the leaves by using a small-sized brush. Plants placed in the experimental room 1, 2, 3 and 4 (R_1 to R_4) were treated with Dichlorvas 76EC, Chlorpyriphos 20EC, Dimethoate 30EC, and Cartap hydrochloride 4% GR respectively. As a control, distilled water was applied to host infested plants in experimental room 5 (R_5). Twelve days after spraying, the number of cocoons formed inside the bottles was counted and the percentage of larval mortality for each insecticide used was determined by the following formula:

$$\frac{\textit{Total No. of live mines before chemical treatment} - \textit{Total No. of cocoons formed after chemical treatment}}{\textit{Total No. of live mines before chemical treatment}} \times 100$$

In the field, 15 apple trees with numerous visible mines were selected and divided into five groups; each group consisted of three trees. These groups were selected at random and then labeled as G_1, G_2, G_3, G_4, and G_5. The trees of each group were labeled as T_1, T_2, and T_3. The distance between these groups was about 40-50 feet and, in each group, trees were located 10-15 feet apart from one another. The height and age of these trees were 6-8 feet and eight years respectively. From each of these trees, only 30 infested leaves were selected and tagged for chemical sprays. Trees of the 1st, 2nd, 3rd and 4th groups (G_1 to G_4) were treated with Dichlorvas 76EC**,** Chlorpyriphos 20EC, Dimethoate 30EC, and Cartap hydrochloride 4% GR respectively. The 5th group of trees was kept as control and treated with distilled water.

Ten days after spraying, infested leaves of the trees in each group were examined carefully and the number of empty mines was counted. Furthermore, solutions of chemicals were prepared in the same way as mentioned above and sprayed with the help of a sprayer. Percentage mortality of the larvae for each insecticide used was determined by the following formula:

$$\frac{\text{Total number of live mines before chemical treatment} - \text{Total number of empty mines after chemical treatment}}{\text{Total number of live mines before chemical treatment}} \times 100$$

Adult Leaf Miner Bioassay:

For adult leaf miner bioassay, the method described by Tran and Takagi (2005) was followed. Fifteen potted plants covered by transparent plastic bottles were distributed in 5 groups (3 plants in each group) and placed in the above mentioned 5 experimental rooms. These plants were applied with diluted insecticides and then two hours after spraying, each plant was exposed to 20 mixed-sex *L. clerkella* adults (12-24 hrs old). The plants were kept in natural conditions at an average room temperature of 25 ± 3 ^{0}C and 16L: 8D photoperiod. Mortality was recorded 48 hrs from the onset of exposure. No insecticide was used in one group treated as control.

Statistical Analysis:

Means of different parameters were compared for significant differences using student's t-test for two variables and for more than two variable comparisons one way ANOVA followed by Tukey's test. The significance level was set at $P<0.05$. All the statistical analyses were carried out in SPSS (Version 16).

RESULTS

Larval Leaf Miner Bioassay:

Larval mortalities at the recommended dilutions of the four tested insecticides used in the rearing rooms are shown in Table 2. Out of these four insecticides, Cartap hydrochloride 4% GR and Dimethoate 30EC caused a relatively high larval mortality, 93.13% (±3.30 SD) and 88.97% (±3.30 SD) respectively. The other two insecticides i.e., Chlorpyriphos 20EC and Dimethoate 30EC caused comparatively low larval mortality. Chlorpyriphos 20EC produced mortality of 13.60% (±2.62 SD), followed by Dichlorvas 76EC which only killed 10.70% (±1.11 SD) caterpillars. Thus, the two insecticides, Chlorpyriphos 20EC and Dichlorvas 76EC had no significant effect on the larvae of *L. clerkella* while Cartap hydrochloride 4% GR and Dimethoate 30EC were found to have a significant effect on the larval mortality under laboratory conditions. Furthermore, no statistically significant difference was found between the insecticides, Cartap hydrochloride 4% GR and Dimethoate 30EC (p>0.05) (Table 2).

Table 2: Relative efficacy of 4 insecticides at their recommended dilution on the larvae of *L. clerkella* under laboratory conditions

Insecticide	Trade name	Concentration	%age mortality (Mean ± SD)
Cartap hydrochloride 4% GR	DARTRIZ 4G	0.1g +100ml H_2O	93.13± 3.30^a
Chlorpyriphos 20EC	COROBAN	0.1ml +100ml H_2O	13.60 ± 2.62^b
Dichlorvas 76EC	NUVAN	0.3ml +100ml H_2O	10.70 ± 1.11^b
Dimethoate 30EC	ROGOR	0.1ml+100ml H_2O	88.97 ±3.30^a

Means that do not share a same letter are significantly different

Adult Leaf Miner Bioassay:

In the case of adult moths of *L. clerkella* (Table 3), Dimethoate 30EC was found more effective than the other three insecticides used in the experiment and produced 94.03% (±4.04 SD) mortality. Cartap hydrochloride 4% GR caused 86.07% (±5.29 SD) mortality. The other two insecticides, chlorpyriphos 20EC and Dichlorvas 76EC were also found to have caused mortality of 75.31% (±5.03 SD) and 69.33% (±3.06 SD) respectively. No statistically significant difference (p>0.05) was found between these three insecticides, Dimethoate 30EC, Cartap hydrochloride 4% GR and chlorpyriphos 20EC (Table 3).

Table 3: Relative efficacy of 4 insecticides at their recommended dilution on the adult moths of *L. clerkella* under laboratory conditions

Insecticide	Trade name	Concentration	%age mortality (Mean ± SD)
Cartap hydrochloride 4% GR	DARTRIZ 4G	0.1g +100ml H_2O	86.07 ± 5.29^{ab}
Chlorpyriphos 20EC	COROBAN	0.1ml +100ml H_2O	75.31 ± 5.03^{bc}
Dichlorvas 76EC	NUVAN	0.3ml +100ml H_2O	69.33 ± 3.06^{c}
Dimethoate 30EC	ROGOR	0.1ml+100ml H_2O	94.03 ± 4.04^{a}

Means that do not share a same letter are significantly different

The same insecticides with their recommended dilutions were used in the field (Table 4). Here, Cartap hydrochloride 4% GR was found to have a profound effect and produced high larval mortality of 89.07% (±4.30 SD). Dimethoate 30EC caused 83.53% (±4.08 SD) mortality. The other two pesticides, chlorpyriphos 20EC and Dichlorvas 76EC were found insignificant which caused only 13.30% (±3.30 SD) and 9.97% (±2.35 SD) larval mortalities respectively (Table 4). Furthermore, no statistically significant difference was found between Cartap hydrochloride 4% GR and Dimethoate and similarly, no such difference was found between chlorpyriphos 20EC and Dichlorvas 76EC ($p>0.05$).

Table 4: Relative efficacy of 4 insecticides at their recommended dilution on the larvae of *L. clerkella* in the field

Insecticide	Trade name	Concentration	%age mortality (Mean ± SD)
Cartap hydrochloride 4% GR	DARTRIZ 4G	0.1g +100ml H_2O	89.07 ± 4.30^{a}
Chlorpyriphos 20EC	COROBAN	0.1ml +100ml H_2O	13.30 ± 3.30^{b}
Dichlorvas 76EC	NUVAN	0.3ml +100ml H_2O	9.97 ± 2.35^{b}
Dimethoate 30EC	ROGOR	0.1ml+100ml H_2O	83.53 ± 4.08^{a}

Means that do not share a same letter are significantly different

DISCUSSION

In the present study, four insecticides at their recommended dilutions were used against the larvae of *L. clerkella* in the experimental rooms. The used insecticides are; Cartap hydrochloride 4% GR, Chlorpyriphos 20EC, Dichlorvas 76EC, and Dimethoate 30EC. Only two of these insecticides, Cartap hydrochloride 4% GR and Dimethoate 30EC were found to have a significant effect under laboratory conditions and caused a relatively high larval mortality, 93.13 ± 3.30% and 88.97 ± 3.30% respectively. Similar results (Cartap hydrochloride 4% GR and Dimethoate 30EC) were also obtained against the leaf miner, *Chromatomyia horticola* in Japan where larval mortalities of the two pest populations located at the two places, Shizuoka and Kagoshima were treated with different insecticides at their recommended dilutions (Saito 2004). In the Shizuoka population, Cartap hydrochloride 4% GR was found to have produced high larval mortality of about 99.11% while as Dimethoate 30EC killed 100% larvae for the Kagoshima population. In the present study, the other two insecticides namely, Chlorpyriphos 20EC and Dichlorvas 76EC had no significant effect on the larvae of *L. clerkella*. Furthermore, Dimethoate 30EC and Cartap hydrochloride 4% GR were also found

significant against the adult moths of *L. clerkella* which produced 94.03 ± 4.04% and 86.07 ± 5.29% mortality respectively. Furthermore, Chlorpyriphos 20EC and Dichlorvas 76EC were also found significant on adult moths and produced mortality of 75.31 ± 5.03% and 69.33 ± 3.06% respectively. In the field, Cartap hydrochloride 4% GR and Dimethoate 30EC were found to have a profound effect on the caterpillars and produced high larval mortality of 89.07 ± 4.30% and 83.53 ± 4.08% respectively. However, the other two pesticides, Chlorpyriphos 20EC and Dichlorvas 76EC were found insignificant and killed only about 10 –14% larvae.
Seventeen insecticides at their recommended dilutions were used on the larvae of stone leek leaf miner, *Liriomyza chinensis*. Among these pesticides, Dimethoate produced more than 93% mortality. Dimethoate and Cartap were also found to have a profound effect on the adults of this leaf miner. While Dimethoate caused 100% mortality, Cartap was found to have produced 90% female and 100% male mortality (Tran & Takagi 2005). Chlorpyriphos 20EC was found highly effective on the larvae of serpentine leaf miner, *L. trifolii* and caused the mortality of 74.90%.

In the present study, very low larval mortality was observed in the plants treated with Dichlorvas and Chlorpyriphos, although they resulted in significant adult moth mortality. This could be attributed to the fact that these two chemicals are contact pesticides and their very small percentages reach the mesophyll where the larvae are present. High larval mortalities are observed in the plants treated with systemic insecticides Viz, Dimethoate (83.53±4.08) and Cartap hydrochloride (89.07±4.30). Similar results against the miner larvae have also been reported by Ghewande *et al* (1987), Shrivastava *et al* (1988).

Based on these preliminary results, the use of Dimethoate and Cartap hydrochloride could be recommended for the control of *Lyonetia clerkella*. However, further experiments need to be carried out in order to achieve the best results in the management of the said leaf miner.

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