Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 1532-1537

Received: 10-03-2019
Accepted: 12-04-2019

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# Studies on efficacy of green insecticides on mulberry silkworm, *Bombyx mori* L. through rearing performance

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**Abstract**
The bioassay study was conducted in the Department of Sericulture, UAS, GKVK, Bengaluru during 2018, to test the efficacy of different insecticides on mulberry leaf roller *Diaphania pulverulentalis* (Hampson). The effective insecticides with optimum dose were sprayed on mulberry plants and fed to silkworm *Bombyx mori* L at 10, 15, 20, 25 and 30 days after spraying (DAS) to find the waiting period of such chemicals on silkworms. The results revealed that among the eight insecticides tested, lambda-cyhalothrin and emamectin benzoate were highly toxic to silkworms with 100 per cent mortality even after 30 DAS. However, the insecticide Chlorofenapyr (1.5 ml/liter) recorded less mortality (12.09%) of silkworms at 10 days after spray. Similarly, Azadirachtin and Novaluron (10.00% & 9.67%, respectively) at 25 DAS. Whereas, the insecticides Profenofos and fipronil at 30 DAS recorded less mortality rate of 11.00 per cent and 10.67 per cent respectively, which were on par with that of standard check and untreated control. Similar trends were observed with respect to moulting duration (h), instar duration (h) and cocoon yield (g/100cocoon) of silkworms. The findings of present study clearly indicated that the insecticide Chlorofenapyr was proved to be less toxic to silkworms even at 10 DAS followed by Azadirachtin and Novaluron at 25 days after spraying and Profenofos and fipronil at 30 days after spraying. However, lambda- cyhalothrin and emamectin benzoate were highly toxic to silkworms even after 30 DAS. Chlorofenapyr, Azadirachtin and Novaluron could be the alternate insecticides to be thought to manage the mulberry leaf roller in view of the ban on Dichlorvos the safest insecticide recommended in mulberry.

**Keywords:** Insecticide, silkworm mortality, rearing performance and safety period

## Introduction
Sericulture is an integral part of agrarian country like India. It helps to improve the livelihood and socio-economic development of the farming community in general and Karnataka in particular. At present throughout country 86.04 Lakh person get employment by sericulture [15], in an acre of irrigated mulberry cultivation gives employment and earns net returns of around Rs 60,000 per year, which is all most all more compared to farm sectore crops. As per the recent report of CSB, India ranks second place in silk producing countries after to the china, which shares about 12.00 per cent of the raw silk production in worldwide, during 2018, significantly marked growth has noticed in Indian sericulture industry, it follows around, 31906 MT of total raw silk production, Foreign Exchange Earnings (Rs. 1649. 48 cores per annum). The productivity and profitability of silkworm rearing depends on quality and yield of mulberry. Mulberry is the sole food of silkworm *Bombyx mori* L. Insect pests are common in sericulture ecosystems and they are detrimental to the health and productivity of mulberry inturn silkworm also. Among the various pests the defoliators are considered to be major as they cause extensive damage to the mulberry. These defoliating pests cause around 10-30 per cent leaf yield loss either by depletion in nutritive value or defoliation, Leaf yield loss due to *Diaphania pulverulentalis* (Hampson) is upto 30 per cent [14], The insecticides applied for the control of mulberry pests have greater impact on silkworm. The silkworm, *B. mori* L. had least resistance to insecticides and its production was reduced by more than 30 per cent annually because of insecticide poisoning [2]. The susceptibility of *B. mori* L. to novel group of insecticides particularly Pyrrole, Avermectin, Phenylpyrazole and insect growth regulators is not ascertained much till today by looking at this time, age old insecticide Dichlorvos 76 EC is extensively recommended in chemical management of defoliators on mulberry since a very

long time. As per the G.O.I notification on 28th December 2016, Dichlorvos 76 EC usage is to be completely banned 31st December 2020 onwards. This study is planned to find out the best suitable alternate insecticide group against defoliators of mulberry, and identify molecules which have less residual action and relatively safer to silkworm, and natural enemies associated with mulberry ecosystem.

## Materials and Methods

The laboratory experiment was conducted in the Department of Sericulture, UAS, GKVK Bengaluru during 2018. The silkworms (PM × CSR2) were reared using V-1 mulberry leaves. The selected insecticides through bioassay studies (Table.1) were sprayed to mulberry plants with recommended dose. Silkworm rearing was conducted by feeding the mulberry leaves sprayed with selected insecticides from treated plots at 10, 15, 20, 25 and 30 days after spraying (DAS) from third instar onwards. The rearing experiment was laid in a Completely Randomized Block Design (CRD) with nine treatments and three replications. The standard disinfection and rearing methods were followed as per the procedure [3].

**Table 1:** Insecticidal treatment evaluated for determining their relative safety to silkworm.

| Treatment | Insecticide group | Name of the insecticide | Dosage |
|---|---|---|---|
| $T_1$ | Pyrethroids | Lambda-cyhalothrin. 2.5% EC | 1.0 ml/liter |
| $T_2$ | Pyrrole | Chlorfenapyr. 10% EC | 1.5 ml/liter |
| $T_3$ | Avermectin | Emamectin benzoate 5% SG | 0.4 gm/liter |
| $T_4$ | Organophosphate | Profenofos 50% EC | 1.00 ml/liter |
| $T_5$ | Neem ( Plant origin ) | Azadirachtin 0.03% EC | 2. 0 ml/liter |
| $T_6$ | Phenylpyrazole | Fipronil 5% SC | 0.75 ml/liter |
| $T_7$ | Insect growth regulators. | Novaluron 10% EC | 0.5 ml/liter |
| $T_8$ | Organophosphate | Dichlorvos 76% EC (Standard check) | 2.63 ml/liter |
| $T_9$ | - | Untreated control (Check) | - |

## Statistical analysis

The data obtained were analysed by following standard statistical tools. Completely Randomized Block Design (CRD) and the percentage values were subjected to arcsine transformation. The mean values of the experiments were separated by using Duncan's Multiple Range Test (DMRT)[4].

**Plate 1:** Imposition of treatments on mulberry at Department of Sericulture, UAS, GKVK, Bengaluru.

**Plate 2:** General view of silkworm rearing laboratory

## Results and Discussions

### Larval mortality (%)

The larval mortality recorded was minimum in batches of silkworms fed on mulberry sprayed with chlorfenapyr at 10 DAS (12.09%) which was on par with that of untreated control (8.99%) and Dichlorvos (11.27%). Similarly, the mortality recorded was 10.00 per cent with Azadirachtin at 25 DAS, 9.67 per cent with novaluron at 25 DAS, 11.00 per cent with profenofos at 30 DAS and 10.67 percent with fipronil at 30 DAS which were on par untreated control and standard check dichlorvos at respective DAS. The larval mortality recorded was significantly more (100%) in silkworm batches fed on leaves sprayed with Lambda–cyhalothrin and emamectin benzoate even at 30 DAS followed by profenofos, azadirachtin and fipronil at 10 DAS (100%). (Table. 2 and Fig. 1). This might be due to the toxic molecules present in the mulberry leaf for longer period in such chemicals. Similar result with regard to the mortality rate (100%) due to insecticides poisoning was noticed in early instar larvae fed with insecticides sprayed leaves [13]. The Pyrethroid based pesticide Lambda–cyhalothrin is highly detrimental to chawki worms of *B. mori* L. because of its long residual action and caused complete mortality and most of the larvae died without moulting[5], the toxic residue of insecticides may result in rupture of the integument, complete cessation of feeding and incomplete ecdysis. Novaluron exposure impairs the midgut and may affect the physiological functions of this organ [12].

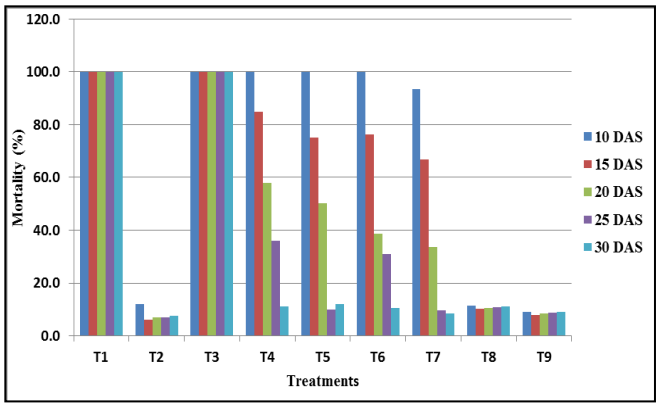


**Fig 1:** Effect of insecticidal treatments on mortality (%) of silkworms

**Table 2:** Mortality (%) of silkworms as influenced by feeding of mulberry sprayed with selected insecticides.

| Treatments | Mortality (%) | | | | |
|---|---|---|---|---|---|
| | **10 DAS** | **15 DAS** | **20 DAS** | **25 DAS** | **30 DAS** |
| T1-Lambda-cyhalothrin. 2.5% EC (1.00 ml/liter) | 100.00$^{a}$ (89.44) | 100.00$^{a}$ (89.44) | 100.00$^{a}$ (89.44 ) | 100.00$^{a}$ (89.44 ) | 100.00$^{a}$ (89.44 ) |
| T2-Chlorfenapyr. 10% EC (1.5 ml/liter) | 12.09$^{c}$ (20.29 ) | 6.00$^{c}$ (13.96 ) | 7.10$^{f}$ (15.21) | 7.00$^{e}$ (16.17) | 7.67$^{d}$ (10.71) |
| T3-Emamectin benzoate 5% SG (0.4 gm/liter) | 100.00$^{a}$ (89.44 ) | 100.00$^{a}$ (89.44 ) | 100.00$^{a}$ (89.44 ) | 100.00$^{a}$ (89.44) | 100.00$^{a}$ (89.44) |
| T4- Profenofos 50% EC (1.00 ml/liter ) | 100.00$^{a}$ (89.44 ) | 84.89$^{b}$ (71.69) | 58.00$^{b}$ (64.23) | 36.00$^{b}$ (45.64) | 11.00$^{bc}$ (28.00) |
| T5-Azadirachtin 0.03% EC (2.0 ml/liter) | 100.00$^{a}$ (89.44 ) | 75.00$^{c}$ (74.79) | 50.33$^{c}$ (51.43) | 10.00$^{d}$ (34.52) | 12.00$^{b}$ (24.01) |
| T6-Fipronil 5% SC (0.75 ml/liter) | 90.00$^{b}$ (71.44) | 76.30$^{c}$ (75.18) | 38.55$^{d}$ (41.75) | 31.00$^{c}$ (39.20) | 10.67$^{bc}$ (21.30) |
| T7-Novaluron 10% EC (0.5 ml/liter) | 93.33$^{b}$ (77.66 ) | 66.78$^{d}$ (57.56) | 33.67$^{e}$ (43.05) | 9.67$^{de}$ (25.61) | 8.33$^{cd}$ (21.30) |
| T8-Dichlorvos 76% EC (2.63 ml/liter) | 11.27$^{c}$ (19.50) | 10.20$^{e}$ (18.59) | 10.53$^{f}$ (18.79) | 10.84$^{d}$ (19.12) | 11.00$^{bc}$ (19.84) |
| T9- Untreated control | 8.99$^{c}$ (17.50) | 8.00$^{c}$ (17.34) | 8.33$^{f}$ (17.34) | 8.67$^{de}$ (17.34) | 9.00$^{cd}$ (17.34) |
| F Test | * | * | * | * | * |
| Sem ± | 1.50 | 1.67 | 1.41 | 1.13 | 0.79 |
| CD at 0.05 | 4.48 | 4.98 | 4.21 | 3.36 | 2.35 |
| CV (%) | 3.34 | 4.40 | 4.83 | 5.00 | 4.07 |

*- 5 per cent level of significance; DAS- Days after spray;
Figures in the parentheses are arcsine transferred values;
Numbers with same alphabets are statistically on par

1. Swinging of entire silkworm body

2. Chain type excreta

3. Vomiting gut juice

4. Incomplete ecdysis

5. Dead silkworm

**Plate 3:** General Symptoms exhibited by silkworms fed on mulberry leaves sprayed with insecticide


**Table 3:** Effect of insecticidal treatments in mulberry on Instar duration of silkworm hybrid (PM × CSR2)

| Treatments | 3rd Instar duration | | | | | 4th Instar duration | | | | | 5th Instar duration | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 Das | 15 Das | 20 Das | 25 Das | 30 Das | 10 Das | 15 Das | 20 Das | 25 Das | 30 Das | 10 Das | 15 Das | 20 Das | 25 Das | 30 Das |
| $T_1$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $T_2$ | 71.00 | 70.66 | 72.33 | 70.00 | 72.00 | 96.00 | 95.00 | 91.66 | 94.10 | 92.00 | 193.20 | 196.66 | 190.76 | 189.50 | 186.00 |
| $T_3$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $T_4$ | - | 82.00 | 81.00 | 74.33 | 72.33 | - | 123.00 | 116.66 | 99.00 | 96.66 | - | 229.50 | 226.00 | 213.00 | 184.65 |
| $T_5$ | - | 78.10 | 76.40 | 71.10 | 69.20 | - | 107.10 | 104.00 | 97.33 | 94.00 | - | 215.33 | 205.56 | 190.00 | 184.00 |
| $T_6$ | 85.10 | 80.76 | 78.33 | 74.43 | 71.00 | 122.66 | 121.33 | 110.66 | 104.66 | 97.66 | 228.66 | 221.00 | 211.66 | 200.66 | 185.00 |
| $T_7$ | 79.10 | 76.43 | 76.10 | 72.66 | 71.33 | 112.33 | 106.33 | 100.43 | 96.20 | 93.43 | 221.00 | 205.66 | 197.00 | 186.66 | 176.33 |
| $T_8$ | 72.33 | 71.66 | 71.66 | 70.66 | 73.30 | 93.66 | 97.30 | 94.66 | 93.30 | 93.33 | 196.66 | 188.00 | 188.00 | 187.33 | 186.30 |
| $T_9$ | 70.33 | 70.76 | 70.66 | 71.00 | 70.33 | 93.33 | 96.00 | 93.00 | 94.00 | 95.66 | 190.00 | 181.66 | 183.00 | 186.33 | 180.33 |
| F Test | * | * | * | * | NS | * | * | * | * | NS | * | * | * | * | NS |
| Sem ± | 1.54 | 1.13 | 1.31 | 0.82 | - | 1.49 | 1.67 | 1.67 | 1.18 | - | 1.81 | 2.32 | 2.49 | 2.45 | - |
| CD at 0.05 | 5.04 | 3.48 | 4.05 | 2.54 | - | 4.86 | 5.15 | 5.16 | 3.64 | - | 5.90 | 7.17 | 7.67 | 7.55 | - |
| CV (%) | 3.57 | 2.60 | 3.01 | 1.99 | - | 2.49 | 2.73 | 2.86 | 2.11 | - | 1.52 | 1.96 | 2.16 | 2.19 | - |

*- Significant at 5 per cent; DAS- Days after spray;

**Table 4:** Effect of insecticidal treatments in mulberry on moulting duration (h) of silkworm hybrid (PM × CSR2)

| Treatments | 3rd Moult duration (h) | | | | | 4th Moulting duration (h) | | | | | Single cocoon weight (g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 Das | 15 Das | 20 Das | 25 Das | 30 Das | 10 Das | 15 Das | 20 Das | 25 Das | 30 Das | 10 Das | 15 Das | 20 Das | 25 Das | 30 Das |
| $T_1$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $T_2$ | 23.25 | 23.10 | 23.1 | 21.23 | 22.3 | 23.76 | 23.53 | 22.85 | 22.93 | 22.30 | 1.57 | 1.40 | 1.60 | 1.58 | 1.62 |
| $T_3$ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $T_4$ | - | 29.00 | 27.76 | 25.30 | 23.32 | - | 29.66 | 28.16 | 26.43 | 24.33 | - | 1.22 | 1.33 | 1.27 | 1.65 |
| $T_5$ | - | 29.10 | 26.66 | 22.45 | 22.13 | - | 28.66 | 27.50 | 26.10 | 23.93 | - | 1.28 | 1.57 | 1.59 | 1.65 |
| $T_6$ | 31.50 | 30.33 | 27.16 | 25.70 | 23.66 | 32.18 | 29.26 | 26.11 | 23.26 | 22.43 | 1.24 | 1.41 | 1.31 | 1.44 | 1.72 |
| $T_7$ | 32.76 | 30.00 | 27.45 | 23.66 | 21.71 | 29.73 | 28.36 | 25.33 | 22.56 | 23.44 | 1.27 | 1.40 | 1.44 | 1.62 | 1.71 |
| $T_8$ | 22.30 | 20.33 | 22.80 | 23.88 | 22.76 | 21.33 | 22.50 | 22.85 | 23.23 | 21.45 | 1.56 | 1.38 | 1.59 | 1.59 | 1.59 |
| $T_9$ | 21.5 | 22.33 | 23.00 | 22.73 | 22.86 | 22.33 | 21.54 | 21.78 | 22.20 | 22.15 | 1.63 | 1.50 | 1.62 | 1.62 | 1.61 |
| F Test | * | * | * | * | NS | * | * | * | * | NS | * | * | * | * | * |
| Sem ± | 0.68 | 0.73 | 0.68 | 0.64 | - | 0.66 | 0.64 | 0.64 | 0.68 | - | 0.04 | 0.03 | 0.04 | 0.04 | 0.03 |
| CD at 0.05 | 2.22 | 2.26 | 2.10 | 1.99 | - | 2.18 | 2.00 | 1.98 | 2.12 | - | 0.13 | 0.10 | 0.13 | 0.12 | 0.11 |
| CV (%) | 4.53 | 4.87 | 4.86 | 4.84 | - | 4.54 | 4.32 | 4.47 | 5.04 | - | 4.85 | 4.40 | 4.99 | 4.71 | 3.72 |

**Larval parameters**

**Larval duration (h)**

The third instar larval duration recorded in the batches of silkworms fed on mulberry sprayed with Chlorofenapyr 10% EC at 10 DAS (71 h) which was on par with that of Dichlorvos (72. 33 h) standard check. The treatments with novaluron (72.69 h) and Azadirachtin (71.10 h) at 25 DAS recorded on par result with that control, which might be due to reduction of residual toxicity over a period noticed in mulberry leaf. Similarly at 30 DAS the treatments with profenofos and fipronil recorded (72.33 h and 71.00 h respectively) which were on par to that of standard check and untreated control. (Table. 3). However, the treatments with Lambda–cyhalothrin and emamectin benzoate proven completely toxic even after 30 DAS. Similar trend was observed with regard to total larval duration.

The fourth instar larval duration recorded in the batches of silkworms fed on mulberry sprayed with Chlorofenapyr 10% EC at 10 DAS (95 h) which was on par with that of Dichlorvos (93. 66 h) standard check. The treatments with novaluron (96. 20 h) and Azadirachtin (97.33 h) at 25 DAS recorded on par result with that control, which might be due to reduction of residual toxicity over a period noticed in mulberry leaf. Similarly at 30 DAS the treatments with profenofos and fipronil recorded (96. 66 h and 97. 66 h respectively) which were on par to that of standard check and untreated control. (Table. 3). However, the treatments with Lambda-cyhalothrin and emamectin benzoate proven completely toxic even after 30 DAS. Similar trend was observed with regard to total larval duration.

The Fifth instar larval duration recorded in the batches of silkworms fed on mulberry sprayed with Chlorofenapyr 10% EC at 10 DAS (193. 20 h) which was on par with that of Dichlorvos (196. 66 h) standard check. The treatments with novaluron (186. 66 h) and Azadirachtin (190. 00 h) at 25 DAS recorded on par result with that control, which might be due to reduction of residual toxicity over a period noticed in mulberry leaf. Similarly at 30 DAS the treatments with profenofos and fipronil recorded (184. 65 h and 185.00 h respectively) which were on par to that of standard check and untreated control. (Table. 3). However, the treatments with Lambda–cyhalothrin and emamectin benzoate proven completely toxic even after 30 DAS. The extended larval instar duration might be due to presence of higher residue of insecticide in the initial days of spray and its effect decreases as the number of day's increases [8]. Similarly, the larval period was extended slightly when the silkworm, *Bombyx mori* L. was fed with pesticides treated mulberry leaf. The pesticide interferes with the release of the hormones essential for metamorphosis resulting in delaying of instar duration [12].

**Moulting Duration (H)**

The third moulting duration differed significantly among different treatments. A normal moulting duration was recorded in silkworm batches reared on mulberry leaves fed with untreated control (21. 5 h) followed by Dichlorvos at 10 DAS (22. 30 h), chlorofenapyr at 10 DAS (23.25 h). The remaining treatments showed detrimental result with regard to larval weight due to highest insecticide toxicity at 10 DAS. Similarly in treatments after 25 DAS with Azadirachtin (22. 45 h) and novaluron (23. 66 h) recorded on par result with that of Dichlorvos (23. 88 h) and untreated control (22. 73 h) at 25 DAS. Feeding silkworms with the mulberry leaves treated with insecticides at 30 days after spraying showed marked improvement in larval weight in case of profenofos (23.32 h) and fipronil (23.66 h) which were on par to standard check Dichlorvos (22.76 h) at 30 DAS. The results indicated that residual effect of insecticides resulted in delay in moulting. The production of deformed insects, resulting in inhibition of growth at high dose of insecticide toxicity had been reported in various insect species [9].

Similarly, in fourth moulting duration also in chlorofenapyr at 10 DAS (23. 76 h), Azadirachtin (26. 10 h), novaluron (72.66 h), profenofos (24. 33 h) and fipronil (22. 43 h) showed on par result to that of standard check and untreated control (Table. 3). This might be due to gradual reduction of pesticide toxicity over a period of time and increased activity of SOD, GPX and GST could be involved in free radicals scavenging in larvae leading to oxidative stress by dichlorvos insecticide [10].

**Single cocoon weight (g)**

The single cocoon weight was significantly high in untreated control (1.63 g) followed by standard check dichlorvos at 10 DAS (1.56 g) which were on par with the treatments chlorofenapyr at 10 DAS (1.57 g), azadirachtin at 25 DAS (1.59 g) and novaluron at 25 DAS (1.62 g) with respective days after spraying. Similarly progressive increase in the single cocoon weight over a period of time due to reduction in insecticidal residue was noticed in profenofos at 30 DAS (1.65 g) and fipronil at 30 DAS (1. 71 g) which was on par to the untreated control (1. 61 g) and standard check at 30 DAS (1. 59 g) (Table.4). This might be due to feeding of silkworms with mulberry leaves harvested from insecticide sprayed plots after safe waiting period showed a significant improvement with respect to larval and cocoon parameters [6-10].

**Conclusion**

The present study clearly indicated that the insecticides lamda-cyhalothrin and emamectin benzoate were highly toxic to silkworms even after 30 days after spray. However, the insecticide chlorofenapyr proved to be relatively safer to silkworm rearing at 10 days after spraying followed by Azadirachtin and Novaluron at 25 days after spray. However, 30 days waiting period is suggested for profenofos and fipronil based on the present findings. Chlorfenapyr, Azadirachtin and Novaluron could be the alternate insecticides to be thought to manage the mulberry leaf roller in view of the ban on Dichlorvos the safest insecticide recommended in mulberry.

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