PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–08
https://doi.org/10.14719/pst.5754

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RESEARCH ARTICLE

Bioefficacy and persistent toxicity of newer insecticide against thrips, *Pseudodendrothrips mori* (Niwa) and leaf webber, *Diaphania pulverulentalis* (Hampson) in mulberry cultivation

Prety Rekha Narzary[1], S Manimegalai[1], B Vinothkumar[2]*, A Suganthi[3], P Radha[4], R Shanmugam[1], A Thangamalar[1] & J Kousika[3]

[1]Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam 641 301, Tamil Nadu, India

[2]ICAR - Krishi Vigyan Kendra, Ooty, The Nilgiris 641 001, Tamil Nadu, India

[3]Pesticide Toxicology Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[4]Department of Forest Biology and Tree Improvement, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam 641 301, Tamil Nadu, India

*Email: vinothkumar@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY
Received: 13 October 2024
Accepted: 19 October 2024
Available online
Version 1.0 : 31 December 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE
Narzary PR, Manimegalai S, Vinothkumar B, Suganthi A, Radha P, Shanmugam R, Thangamalar A, Kousika J. Bioefficacy and persistent toxicity of newer insecticide against thrips, *Pseudodendrothrips mori* (Niwa) and leaf webber, *Diaphania pulverulentalis* (Hampson) in mulberry cultivation. Plant Science Today.2024;11 (sp4):01-08. https:/doi.org/10.14719/pst.5754

Abstract

Two field trials were conducted in two locations to assess new insecticide molecules' efficacy against thrips and leaf webber in the mulberry ecosystem. Results indicated that fipronil 5SC @ 50 g a.i. /ha showed the highest percentage reduction in the population of thrips (87.13 and 88.41 % in the first and second trials, respectively) over untreated control. In contrast, for leaf webber, flubendiamide 39.35 SC @ 48 g a.i./ha showed the highest effectiveness with 85.56 and 89.90 per cent reduction over control in the first and second trials, respectively. Persistence was observed through laboratory bioassay. Results revealed that the order of persistent toxicity of insecticides against thrips based on persistent toxicity index was fipronil 5 SC > spinetoram 11.7 SC > thiacloprid 21.7 SC > dimethoate 30 EC. Emamectin benzoate 5SC and dimethoate 30 EC recorded the shortest persistency of 10 days after treatment against mulberry leaf Webber, while chlorantraniliprole 18.5 SC and flubendiamide 39.35 SC registered the longest persistency of 25 and 20 days, respectively at the recommended dose. Higher concentrations of pest–resistant proteins and enzymes were recorded in the insecticide-treated mulberry plants than in the untreated plants. To conclude, fipronil 5 SC and thiacloprid 21.7 SC were the most effective in checking the population of thrips. At the same time, emamectin benzoate 5SC and flubendiamide 39.35 SC were most efficient against leaf webber, besides having less persistent toxicity than other treatments. Hence, these insecticides may be recommended to manage thrips and leaf webber in the mulberry ecosystem.

Keywords

bioefficacy; insecticides; mulberry; persistent toxicity; pests; PR proteins

Introduction

For ages, mulberry plants (*Morus alba* L.,) have been known as an essential food source for the mulberry silkworm (*Bombyx mori* L.), to produce more silk (1). Pest infestation in the mulberry drastically reduces the quality of the leaves, thereby reducing the silk quality (2). Mulberry is a plant known to be infested by more than 28 pests, of which thrips *Pseudodendrothrips mori* Niwa (Thysanoptera: Thripidae) and leaf webber *Diaphania pulverulentalis* Hampson (Lepidoptera: Pyralidae) are considered as significant pests causing economic yield loss to the plant (3). The presence of *P. mori* causes a decrease in weight and leaf area, causing a 20 to 50 per cent decrease in leaf yield in mulberry (4, 5). Mulberry leaf webber is a significant

defoliator pest of mulberry (6, 7). Since 1995, this pest has been reported to cause infestation in mulberry, mainly in the southern silk-producing states of India like Tamil Nadu, Karnataka and Andhra Pradesh (8). Karnataka has recorded a leaf webber incidence of 27.85%, followed by Andhra Pradesh of 20.98% and Tamil Nadu of 16.48%. It causes considerable yield loss (24.18 per cent in field condition and 34.83% in glass house) and when the incidence is at its highest (September-November). The pest completes multiple overlapping generations from June to December with 1 to 2 larvae per leaf (9).

Organophosphate pesticides comprise 34 per cent of global insecticide use, with around 100 types deployed for pest control in agriculture and horticulture (10-12). (Dichlorvos (2, 2-dichloroethenyl dimethyl phosphate, $C_4H_7Cl_2O_4P$), an organophosphate (OP) insecticide, has been widely utilized by farmers who practice sericulture and is frequently advised to battle mulberry pests because of its limited persistence, fumigant activity and knockdown effect, lasting less than 10 days (13-16). However, recent studies have shown that thrips have developed resistance to Dichlorvos (17-19). Furthermore, the chemical is highly poisonous to insect pests' natural enemies, which causes their number to disappear in mulberry habitats (20). Furthermore, Dichlorvos is currently prohibited in India as per gazette notification no. Vide S.O. 3951 (E), dated 08.08.2018 of the Ministry of Agriculture and Farmers Welfare, Government of India regulation (21).

The newer insecticide molecules lack label claims for mulberry based on information from the Indian government's Central Insecticide Board and Registration Committee (http://cibrc.nic.in/) (22). It is crucial to evaluate their bioefficacy and optimize their doses as alternatives to organophosphate insecticides for managing pests before incorporating them into the management spray schedule of mulberry crop pests, which often require multiple pesticide applications (23). Therefore, discovering new molecules with high bioefficacy and high persistent toxicity against target pests that reduce the crop susceptibility to pests and protect it from them becomes essential for producing quality mulberry leaves and, hence, quality silkworm cocoons.

Materials and Methods

Study on bioefficacy of insecticides

There were two field trials at two locations, one each at Kariyampalayam (11 ^{0}12'10.8 ⊠ N, 77 ^{0} 5' 2.8° E) (Trial I) and the other at Kurukkiliyampalayam (11 ^{0}14'20.76 ° N, 77 ^{0} 4' 40.8° E) (Trial II), Tiruppur District, Tamil Nadu, India to test the bioefficacy of listed newer insecticide molecules against mulberry thrips (Table 1). Similarly, two field trials were conducted, each at Department of Sericulture, Forest College and Research Institute, Mettupalayam (11 ^{0}11'24 ° N, 77 ^{0} 33'36° E) (Trial I) and Alangombu (11 ^{0}18'37.08 ° N, 76 ^{0} 59' 40.2° E) (Trial II), Tamil Nadu, India to test the bioefficacy of newer insecticide molecules against mulberry leaf webber. The trials for mulberry thrips were conducted from March to July 2023 and against leaf webber from September to January 2024 using the V1 mulberry variety. Three replications of the study were conducted utilizing Randomized Block Design (RBD). Insecticides used in the current study were selected based on ad hoc recommendations of the Central Insecticide Board Registration Committee, CIBRC in similar crops emamectin benzoate 5SG @ 10 g a.i. ha^{-1}, flubendiamide 39.35 SC @ 48 g a.i. ha^{-1}, chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}, fipronil 5 SC @ 50 g a.i. ha^{-1}, thiacloprid 21.7 SC @ 72 g a.i. ha^{-1}, spinetoram 11.7 SC @ 50 g a.i. ha^{-1}, broflanilide 20 SC @ 25 g a.i. ha^{-1}, dimethoate 30 EC @ 300 g a.i. ha^{-1} and untreated control. The formulations were obtained from local pesticide dealers in Coimbatore. The insecticide dilution required for various bioassays was made afresh by adding water to the appropriate pesticide formulations to dissolve them.

Two rounds of spraying were done at 15-day intervals after the 30th day of pruning, using a pneumatic knapsack sprayer @ 500 litres of spray fluid per hectare. Ten plants per treatment were randomly selected from every trial plot and labelled for observation. Pest population data and percentage reduction compared to the control group were recorded. The number of insects per three leaves (top, middle and bottom) was counted during pre-treatment count (PTC) a day before the commencement of first spraying and again at 1, 3, 5, 7, 10 and 14 days after spraying (DAS).

Data on the population were converted into (Öx + 0.5) transformed values. The data was analyzed in a randomized block design (RBD) using the IBM SPSS 21 program for the least notable variation (Critical difference). Two locations' worth of data was merged replication-wise. Duncan's Multiple Range Test (DMRT) was employed to discern the average values (24). The means in the tables separated by the same alphabet between the treatments do not differ substantially at the five-percentile level.

2.2. Persistent toxicity study

The effective chemicals were taken for the persistent toxicity study based on the field experiment. Clip–on cage method was used to assess insecticides' persistent toxicity, viz., fipronil 5 SC, thiacloprid 21.7 SC, spinetoram 11.7 SC and dimethoate 30 EC against *P. mori*. Clip-on cages made from clear blister pack lids from pharmaceutical tablets were used to fix the thrips on treated mulberry leaves. These aeration packs had openings at the top for air and were sized 1.5 cm in length, 1.0 cm in width and 0.5 cm in height. The mulberry leaf was inserted between the cage and a transparent plastic sheet was fastened with paper clips to hold the clip-on cages in place. The death rate was recorded after ten apterous thrips of the third instar were inserted into every cage and every 48 hours. A new batch of thrips was introduced every 48 hours until no death was observed. The corrected per cent mortality was determined using Abbott's formula in Equation 1(25).

$$\text{Per cent corrected mortality} = \frac{\%\ \text{Test mortality} - \%\ \text{Control mortality}}{(100 - \text{Per cent control mortality})} \times 100$$

(Eqn. 1.)

To evaluate the persistent toxicity of flubendiamide 39.35 SC, chlorantraniliprole 18.5 SC, emamectin benzoate 5 SG and dimethoate 30 EC against *D. pulverulentalis*, a pot culture experiment was carried out. Thirty-day-old saplings were planted in the 30 cm wide earthen pots @ 3 saplings per pot. Fifteen days after transplanting, insecticides were sprayed in the recommended dose on the fifteen potted plants each. Leaf samples were gathered on 1, 2, 3, 4, 5, 7, 10, 15, 20, 25 and 30

days after spray and bioassay was conducted. Six well bioassay tray was used for the bioassay study. Two three-cm leaf bits from each treatment were placed gently inside the well over the agar medium and one larva was released per well. After 24 hours of exposure, live larvae were fed untreated leaves. For each treatment thirty larvae were used and percent larva mortality was observed.

2.3. Pest resistant proteins in mulberry

Based on the results of the above two studies, insecticides with high efficacy and comparatively less persistent insecticides alone were taken to induce pest resistance protein study. The upregulation of defensive enzymes and proteins involved in pest resistance after an infestation of *P. mori* and *D. pulverulentalis* on mulberry (V_1 variety) treated with effective insecticides at recommended doses along with salicylic acid and jasmonic acid @ 150 ppm was sprayed to the plants in pot culture and the insects were released on them. The treatment details were T_1- fipronil 5SC @ 50 g a.i. ha^{-1} + *P. mori*, T_2- thiacloprid 21.7 SC@ 72 g a.i. ha^{-1} + *P.mori*, T_3- spinetoram 11.7 SC @ 50 g a.i. ha^{-1} + *P. mori*, T_4 - salicylic acid @ 150 ppm + *P.mori*, T_5 - jasmonic acid @ 150 ppm + *P.mori*, T_6 - *P. mori* infested plant and T_7-Untreated control for induction of protein due to infestation of thrips, T_1-emamectin Benzoate 5SG @ 10 g a.i. ha^{-1} + *D. pulverulentalis,* T_2-flubendiamide 39.35 SC @ 48 g a.i. ha^{-1} + *D. pulverulentalis,* T_3- chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1} + *D. pulverulentalis,* T_4-salicylic acid @ 150 ppm + *D. pulverulentalis,* T_5- jasmonic acid @ 150 ppm + *D. pulverulentalis,* T_6 -*D. pulverulentalis* infested plant and T_7-untreated control for induction of protein due to leaf webber infestation studies. Three replications in a completely randomized block design (CRD) were used in the experiment. Thirty days after transplanting, the treatments were sprayed on potted plants at the previously indicated dosages using an atomizer at 15 millilitres per plant. After 24 hours, insects were released at the rate of 3 larvae per plant near the leaf petiole of the young shoot.

The amount of protein in mulberry leaves treated with various treatments was estimated using the Bradford method (1976) (26). The Folin-ciocalteau reagent method was followed for total phenol estimation. Peroxidase and polyphenol oxidase were estimated using the following methods (27, 28).

Results

3.1. Study on bioefficacy of insecticides

The thrips population varied from 33.67 to 38.67 nos. per plant during Trial 1 with non-significant differences among various treatments at one day before imposing the treatments (Table 1). Fipronil 5 SC @ 50 g a.i. ha^{-1}reduced the thrips population significantly and the mean population after the first spray was 4.78 nos. per plant, followed by spinetoram 11.7 SC @ 50 g a.i. ha^{-1} (6.61 nos. per plant) and thiacloprid 21.7 SC @ 72 g a.i. ha^{-1} (8.34 per plant), whereas the population in untreated control was 38.28 nos. per plant. The order of relative efficacy was based on a percent reduction over untreated control after the second spray, which was Fipronil 5 SC @ 50 g a.i. ha^{-1}(87.13%) > spinetoram11.7 SC @ 50 g a.i. ha^{-1} (82.25%) > thiacloprid21.7 SC @ 72 g a.i. ha^{-1} (68.74%) > dimethoate 30 EC @ 300 g a.i. ha^{-1} (62.83%) > chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1} (55.34%) > broflanilide 20 SC @ 25 g a.i. ha^{-1} (49.98%) > flubendimaide 39.35 SC @ 48 g a.i. ha^{-1} (47.44%) > emamectin benzoate 5 SG @ 10 g a.i. ha^{-1} (41.97%).

The population of thrips before the application of insecticides ranged from 24.00 to 30.33 per plant (Table 1) in Trial 2. Mean population during first spray infers that fipronil 5 SC @ 50 g a.i. ha^{-1}(7.39 thrips per plant), spinetoram11.7 SC @ 50 g a.i. ha^{-1} (7.33 thrips per plant) and thiacloprid 21.7 SC @ 72 g a.i. ha^{-1} (8.06 thrips per plant) were on par in controlling *P. mori* population than the other insecticides. After two rounds of spraying, fipronil

Table 1. Bioefficacy of newer insecticide molecules against mulberry thrips, *P. mori*

Treatments	Trial 1						Trial 2					
	First Spray			Second Spray			First Spray			Second Spray		
	PTC	Mean	PRC	PTC	Mean	PRC	PTC	Mean	PRC	PTC	Mean	PRC
Emamectin benzoate 5SG @ 10g a.i.ha^{-1}	34.00^{a} (5.83)	17.83^{h} (4.22)	42.18	35.33^{a} (5.94)	17.61^{f} (4.20)	41.97	28.00^{a} (5.29)	13.72^{f} (3.70)	55.93	26.00^{a} (5.10)	13.22^{f} (3.64)	54.63
Flubendiamide 39.35 SC @ 48g a.i.ha^{-1}	33.67^{a} (5.80)	15.72^{g} (3.96)	46.85	35.67^{a} (5.97)	15.67^{e} (3.96)	47.44	27.00^{a} (5.20)	10.89^{de} (3.30)	63.45	26.33^{a} (5.13)	11.283^{e} (3.36)	64.10
Chlorantraniliprole 18.5 SC @ 30g a.i.ha^{-1}	34.33^{a} (5.86)	14.00^{f} (3.74)	53.10	37.00^{a} (6.08)	13.67^{d} (3.70)	55.34	26.67^{a} (5.16)	11.61^{e} (3.41)	59.41	26.00^{a} (5.10)	12.34^{ef} (3.51)	58.33
Fipronil 5 SC @ 50g a.i.ha^{-1}	38.67^{a} (6.22)	4.78^{a} (2.19)	88.53	40.67^{a} (6.38)	4.06^{a} (2.01)	87.13	30.33^{a} (5.51)	7.39^{a} (2.72)	90.71	27.00^{a} (5.20)	6.00^{a} (2.45)	88.41
Thiacloprid 21.7 SC @ 72g a.i.ha^{-1}	36.33^{a} (6.03)	8.34^{c} (2.89)	73.11	34.33^{a} (5.86)	7.89^{b} (2.81)	62.83	28.33^{a} (5.32)	8.06^{ab} (2.84)	80.19	27.00^{a} (5.20)	8.28^{bc} (2.88)	78.83
Spinetoram 11.7 SC @ 50g a.i.ha^{-1}	37.33^{a} (6.11)	6.61^{b} (2.57)	80.24	40.67^{a} (6.38)	6.11^{b} (2.47)	82.25	29.33^{a} (5.42)	7.33^{a} (2.71)	86.87	26.67^{a} (5.16)	6.95^{ab} (2.64)	83.00
Broflanilide 20 SC @ 25g a.i.ha^{-1}	35.33^{a} (5.94)	11.89^{e} (3.45)	61.22	32.67^{a} (5.72)	11.61^{c} (3.41)	49.98	27.67^{a} (5.26)	10.11^{cd} (3.18)	69.39	26.33^{a} (5.13)	9.78^{d} (3.13)	69.22
Dimethoate 30 EC @ 300g a.i.ha^{-1}	36.33^{a} (6.03)	10.06^{d} (3.17)	68.60	39.00^{a} (6.24)	10.06^{c} (3.17)	68.74	28.00^{a} (5.29)	8.89^{bc} (2.98)	75.70	26.67^{a} (5.16)	9.17^{cd} (3.03)	73.75
Untreated control	37.67^{a} (6.14)	38.28^{i} (6.19)	-	33.33^{a} (5.77)	42.66^{g} (6.53)	-	24.00^{a} (4.90)	25.22^{g} (5.02)	-	25.00^{a} (5.00)	24.11^{g} (4.91)	–
SEm±	-	0.56	-	-	0.62	-	-	0.45	-	-	0.45	–
SEd	-	0.79	-	-	0.88	-	-	0.64	-	-	0.64	–
CD (LSD) 5%	-	1.69	-	-	1.87	-	-	1.36	-	-	1.37	–
C.V.		6.90	-	-	7.52	-	-	6.87	-	-	7.06	–

PTC-Pre Treatment Count, PRC-Percentage Reduction to Control; The population values enclosed in parenthesis are √X + 0.5 transformed values; the Number followed by the same alphabet in the column denotes statistically insignificant.

5 SC @ 50 g a.i. ha^{-1} recorded an 88.41 per cent reduction of thrips nymphs over untreated control, followed by spinetoram11.7 SC @ 50 g a.i. ha^{-1} (83.00%) and thiacloprid 21.7 SC @ 72 g a.i. ha^{-1} (78.83%). Dimethoate 30 EC @ 300 g a.i. ha^{-1}, broflanilide 20 SC @ 25 g a.i. ha^{-1}, flubendimaide 39.35 SC @ 48 g a.i. ha^{-1}, chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1} and emamectin benzoate 5 SG @ 10 g a.i. ha^{-1}recorded 73.75, 69.22, 64.10, 58.33 and 54.63 per cent reduction over untreated control.

The population of leaf webber before imposing treatment ranges from 6.20 to 7.80 in the different treatment plots (Table 2). Flubendiamide 39.35SC @ 48 g a.i. ha^{-1} recorded the least mean population of webbers (1.11 larvae per plant) during first spray at Trial 1 and it was on par with emamectin benzoate5 SG @ 10 g a.i. ha^{-1} (1.17 larvae per plant) and chlorantraniliprole 18.5SC @ 30 g a.i. ha^{-1} (1.22 per plant) followed by standard check dimethoate 30 EC @ 300 g a.i. ha^{-1} (1.67 per plant). After the second spray, flubendiamide 39.35 SC @ 48 g a.i. ha^{-1}, chlorantraniliprole 18.5SC @ 30 g a.i. ha^{-1} and emamectin benzoate 5 SG @ 10 g a.i. ha^{-1} recorded more than 80 percent reduction of webbers over untreated control, followed by dimethoate 30 EC @ 300 g a.i. ha^{-1} (78.70%), spinetoram11.7 SC @ 50 g a.i. ha^{-1} (74.56%), fipronil 5 SC @ 50 g a.i. ha^{-1}(73.90%), thiacloprid21.7 SC @ 72 g a.i. ha^{-1} (73.68%) and broflanilide 20 SC @ 25 g a.i. ha^{-1}(70.53 %).

Leaf webber population during pre-treatment count ranged from 6.20 to 7.60 larvae per plant at Trial 2 (Table 2). After the first round of insecticide spray, at 14 DAT, flubendiamide 39.35 SC @ 48 g a.i. ha^{-1}treated plots recorded the least mean population of 0.94 larvae per plant, whereas untreated control plots registered 7.07 larvae per plant. It was followed by chlorantraniliprole 18.5SC @ 30 g a.i. ha^{-1} (1.44 larvae per plant) was on par with emamectin benzoate 5 SG @ 10 g a.i. ha^{-1}(1.28 larvae per plant). The same trend was observed during the second spray. The order of relative efficacy of test insecticides based on the mean population during the second spray was flubendiamide 39.35 SC @ 48 g a.i. ha^{-1} (89.90%) > chlorantraniliprole 18.5SC @ 30 g a.i. ha^{-1} (85.74 %) > emamectin benzoate5 SG @ 10 g a.i. ha^{-1}(78.64%) > dimethoate 30 EC @ 300 g a.i. ha^{-1} (75.56%) > thiacloprid 21.7 SC @ 72 g a.i. ha^{-1} (74.25%) > spinetoram 11.7 SC @ 50 g a.i. ha^{-1} (73.68%) > fipronil 5 SC @ 50 g a.i. ha^{-1}(72.60%) > broflanilide 20 SC @ 25 g a.i. ha^{-1} (72.21 %).

3.2. Persistent toxicity study

The persistent toxicity study revealed that toxicity of fipronil 5 SC persisted for up to 25 days in the mulberry leaves and produced mortality of mulberry thrips (Table 3). More than 80 per cent mortality was observed during the first seven days after treatment in fipronil 5SC, whereas in thiacloprid 21.7 SC, mortality was observed till 20 DAT. On 15 DAT, 12.45 and 2.35 per cent mortality was observed in spinetoram 11.7 SC and dimethoate 30 EC, respectively. The order of relative efficacy (ORE) based on persistent toxicity index (PTI) in thrips was: fipronil 5 SC (PTI: 514.30) > thiacloprid 21.7 SC (PTI: 410.89) > spinetoram 11.7 SC (PTI: 350.83) > dimethoate 30 EC (PTI: 323.90).

The toxicity of flubendiamide 39.35 SC against the larvae of *D. pulverulentalis* remained up to 20 days after treatment (DAT). In contrast, mortality was observed in leaf webber larvae till 25 DAT in chlorantraniliprole 18.5 SC (Table 3). Up to 3 DAT, more than 90 per cent mortality of the test population was observed in flubendiamide 39.35 SC and chlorantraniliprole 18.5 SC, which declined later. The toxicity of emamectin benzoate 5 SG and dimethoate 30 EC against *D. pulverulentalis* persisted up to 10 DAT with 3.33 and 6.67 per cent mortality, respectively. The ORE based on PTI against leaf webber was chlorantraniliprole 18.5 SC (PTI: 483.25) > flubendiamide 39.35 SC (PTI: 446.60) >emamectin benzoate 5 SG (PTI: 263.30) > dimethoate 30 EC (PTI: 256.60).

Table 2. Bioefficacy of newer insecticide molecules against leaf webber, *D. pulverulentalis*

Treatments	Trial 1						Trial 2					
	First Spray			Second Spray			First Spray			Second Spray		
	PTC	Mean	PRC	PTC	Mean	PRC	PTC	Mean	PRC	PTC	Mean	PRC
Emamectin benzoate 5SG @ 10g a.i.ha^{-1}	7.00^{a} (2.65)	1.17^{a} (1.08)	83.33	7.50^{a} (2.74)	1.39^{ab} (1.18)	81.49	6.70^{a} (2.59)	1.44^{b} (1.20)	78.46	6.50^{a} (2.55)	1.39^{c} (1.18)	78.64
Flubendiamide 39.35 SC @ 48g a.i.ha^{-1}	8.30^{a} (2.88)	1.11^{a} (1.05)	86.61	7.70^{a} (2.77)	1.11^{a} (1.05)	85.56	6.20^{a} (2.49)	0.94^{a} (0.97)	84.78	6.60^{a} (2.57)	0.67^{a} (0.82)	89.90
Chlorantraniliprole 18.5 SC @ 30g a.i.ha^{-1}	7.50^{a} (2.74)	1.22^{a} (1.11)	83.71	7.90^{a} (2.81)	1.33^{ab} (1.15)	83.12	7.30^{a} (2.70)	1.28^{b} (1.13)	82.49	7.40^{a} (2.72)	1.06^{b} (1.03)	85.74
Fipronil 5 SC @ 50g a.i.ha^{-1}	8.10^{a} (2.85)	2.28^{d} (1.51)	71.87	8.30^{a} (2.88)	2.17^{de} (1.47)	73.90	6.80^{a} (2.61)	1.89^{d} (1.37)	72.23	7.30^{a} (2.70)	2.00^{f} (1.41)	72.60
Thiacloprid 21.7 SC @ 72g a.i.ha^{-1}	7.30^{a} (2.70)	1.83^{bc} (1.35)	74.89	7.60^{a} (2.76)	2.00^{de} (1.41)	73.68	7.50^{a} (2.74)	1.95^{d} (1.39)	75.56	6.90^{a} (2.63)	1.78^{d} (1.33)	74.25
Spinetoram 11.7 SC @ 50g a.i.ha^{-1}	7.80^{a} (2.79)	2.06^{cd} (1.43)	73.63	7.20^{a} (2.68)	1.83^{cd} (1.35)	74.56	7.40^{a} (2.72)	1.95^{d} (1.39)	73.72	7.60^{a} (2.76)	2.00^{f} (1.41)	73.68
Broflanilide 20 SC @ 25g a.i.ha^{-1}	7.60^{a} (2.76)	2.28^{d} (1.51)	70.04	8.10^{a} (2.85)	2.39^{e} (1.54)	70.53	6.80^{a} (2.61)	2.06^{d} (1.43)	69.75	6.80^{a} (2.61)	1.89^{ef} (1.37)	72.21
Dimethoate 30 EC @ 300g a.i.ha^{-1}	7.40^{a} (2.72)	1.67^{b} (1.29)	77.48	7.30^{a} (2.70)	1.56^{bc} (1.25)	78.70	7.30^{a} (2.70)	1.67^{c} (1.29)	77.17	7.50^{a} (2.74)	1.83^{de} (1.35)	75.56
Untreated control	7.70^{a} (2.77)	7.87^{e} (2.80)	-	7.60^{a} (2.76)	7.78^{f} (2.79)	-	6.90^{a} (2.63)	7.07^{e} (2.66)	-	7.80^{a} (2.79)	7.32^{g} (2.70)	–
SEm±	-	0.08	-	-	0.13	-	-	0.06	-	-	0.03	–
SEd	-	0.12	-	-	0.18	-	-	0.09	-	-	0.04	–
CD (LSD) 5%	-	0.26	-	-	0.39	-	-	0.19	-	-	0.10	–
C.V.	-	6.33	-	-	9.62	-	-	4.91	-	-	2.69	–

PTC-Pre Treatment Count, PRC-Percentage Reduction to Control; The population values enclosed in parenthesis are √X + 0.5 transformed values; Number followed by the same alphabet in the column denotes statistically insignificant.

Table 3. Persistent toxicity of effective insecticides

Treatments	Percent Mortality (%)									P Days	T (%)	PTI	RE	ORE
	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	15 DAT	20 DAT	25 DAT	30 DAT					
***P. mori* on mulberry**														
Fipronil 5 SC	100.00	93.19	89.80	86.40	65.99	45.58	25.17	8.16	0.00	25	20.57	514.30	1.58	1
Spinetoram 11.7 SC	100.00	89.90	73.06	52.86	22.56	12.45	0.00	0.00	0.00	15	23.38	350.83	1.08	3
Thiacloprid 21.7 SC @ 72 g a.i./ha	96.60	82.99	76.19	69.39	52.38	28.57	4.77	0.00	0.00	20	20.54	410.89	1.26	2
Dimethoate 30 EC @ 300 g a.i./ha	100.00	86.54	62.96	49.49	22.56	2.35	0.00	0.00	0.00	15	21.59	323.9	1.00	4
***D. pulverulentalis* in mulberry**														
Flubendiamide 39.35 SC @48 g a.i./ha	100.00	90.00	83.33	66.67	53.33	43.33	10.00	0.00	0.00	20	22.33	446.60	1.74	2
Chlorantraniliprole 18.5 SC @ 30 g a.i./ha	100.00	93.33	86.67	73.33	60.00	46.67	20.00	3.33	0.00	25	19.33	483.25	1.88	1
Emamectin benzoate 5 SG @ 10 g a.i./ha	96.67	73.33	50.00	33.33	3.33	0.00	0.00	0.00	0.00	10	25.66	256.60	1.00	4
Dimethoate 30 EC @ 300 g a.i./ha	93.33	80.00	56.67	26.67	6.67	0.00	0.00	0.00	0.00	10	26.33	263.30	1.02	3

DAT-Days after treatment, P-Period of persistence toxicity, T-Mean Per cent Toxicity, PTI-Persistent Toxicity Index, RE-Relative Efficacy, ORE-Order of relative efficacy.

3.3. Pest-resistant proteins in mulberry

The protein content in mulberry leaves tested against *P. mori* ranged between 136.20 mg g^{-1} FW (Salicylic acid @ 150 ppm + *P. mori*) (standard check) to 83.00 mg g^{-1} FW (*P. mori* infested plant) (Table 4). The protein content was comparatively high (122.90 mg g^{-1} FW) in infested mulberry plants treated with fipronil 5 SC, followed by thiacloprid 21.7 SC (116.80 mg g^{-1} FW) and spinetoram 11.7 SC (102.80 mg g^{-1} FW) over untreated control (93.90 mg g^{-1} FW).

Phenol content was highest in the thrips-infested plants (4.23 mg CE g^{-1} FW) and lowest in those treated with fipronil 5 SC (2.97 mg CE g^{-1} FW) (Table 4). The content of peroxidase and polyphenol oxidase also differed significantly between the treatments. The highest amounts of peroxidase and polyphenol oxidase activity were observed in plants treated with fipronil 5 SC (1.39 µM min^{-1} protein and 3.77 µM min^{-1} protein), followed by thiacloprid 21.7 S (1.17 and 2.80 µM min^{-1} protein) and spinetoram 11.7 SC (0.88 and 1.66 µM min^{-1} protein). Pest-infested plants recorded the lowest activity (0.19µM min^{-1} protein and 0.72 µM min^{-1} protein).

Similar trends could be observed for the PR protein contents in mulberry against *D. pulverulenlatis* (Table 5). The highest protein content was recorded in infested mulberry treated with emamectin benzoate 5 SG (71.80 mg g^{-1} FW), followed by flubendiamide 39.35 SC (65.30 mg g^{-1} FW) and chlorantraniliprole 18.5 SC (50.7 mg g^{-1} FW). The least protein content was observed in *D. pulverulenlatis*-infested plants (15.30 mg g^{-1} FW). Treatment phenol content ranged from 4.07 mg CE g^{-1} FW (emamectin benzoate 5 SG) to 1.85 mg CE g^{-1} FW (*D. pulverulenlatis* infested plants). Polyphenol oxidase and peroxidase activity in the treatments was higher in the insecticide-treated plants than in the infested ones. The contents were highest in emamectin benzoate 5 SG (1.45 and 3.66 µM min^{-1} protein), which was followed by flubendimaide 39.35 SC (1.22 and 2.87 µM min^{-1} protein) and chlorantraniliprole 18.5 SC (0.90 and 1.72 µM min^{-1} protein). The lowest polyphenol oxidase and peroxidase activity was recorded in the *D. pulverulenlatis*-infested plants (0.33 and 0.76 µM min^{-1} protein).

Table 4. Estimation of pathogenesis–related (PR) proteins against *P. mori*

Treatments	Protein (mg g^{-1} FW)	Phenol (mg CE g^{-1} FW)	Polyphenoloxidase (µM $min^{-1}mg^{-1}$ protein)	Peroxidase (µM $min^{-1}mg^{-1}$ protein)
Fipronil 5SC @ 50 g a.i. ha^{-1} + *P.mori*	122.90	2.97	1.39	3.77
Thiacloprid 21.7 SC @ 72 g a.i. ha^{-1} + *P.mori*	116.80	3.12	1.17	2.80
Spinetoram 11.7 SC @ 50 g a.i. ha^{-1} + *P.mori*	102.80	3.56	0.88	1.66
Salicylic acid @ 150 ppm + *P.mori*	129.30	3.82	1.71	5.39
Jasmonic acid @ 150 ppm + *P.mori*	136.20	3.76	2.31	6.59
P.mori infested plant	83.00	4.23	0.19	0.72
Untreated control	93.90	3.91	0.69	0.99
SE(m) ±	0.226	0.168	0.263	0.847
CD (p = 0.05)	0.552	0.412	0.644	2.073

FW-Fresh weight

Table 5. Estimation of pathogenesis–related (PR) proteins against *D. pulverulentalis*

Treatments	Protein (mg g^{-1} FW)	Phenol (mg CE g^{-1} FW)	Polyphenoloxidase (µM $min^{-1}mg^{-1}$ protein)	Peroxidase (µM $min^{-1}mg^{-1}$ protein)
Emamectin benzoate 5SG @ 10 g a.i. ha^{-1} + *D.pulverulentalis*	71.8	4.07	1.45	3.66
Flubendiamide 39.35 SC @ 48 g a.i. ha^{-1} + *D.pulverulentalis*	65.3	3.61	1.22	2.87
Chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1} + *D.pulverulentalis*	50.7	3.27	0.90	1.72
Salicylic acid @ 150 ppm + *D.pulverulentalis*	86.9	4.93	1.78	5.42
Jasmonic acid @ 150 ppm + *D.pulverulentalis*	94.8	4.47	2.03	6.79
D.pulverulentalis infested plant	15.3	1.85	0.33	0.76
Untreated control	31.1	2.91	0.75	0.94
SE(m) ±	10.93	0.39	0.23	0.86
CD (p = 0.05)	26.75	0.95	0.55	2.12

FW-Fresh weight

Discussion

Fipronil 5 SC @ 50 g a.i. ha^{-1}has shown significantly the highest efficacy against *P. mori* among all the insecticides. The present findings showed that fipronil 5 SC @ one mL/L effectively reduced thrips populations from 29.60 to 3.01 per top three leaves, achieving a 90.01% reduction over untreated controls after two applications at 30 and 45 days in mulberry, cotton, chillies and protected cultivation (17, 29-31). In comparative studies, fipronil 5 SC at one mL/L resulted in 100 per cent mortality of thrips within five days, outperforming dichlorvos 76 EC (32).These findings underscore the strong bioefficacy of fipronil 5 SC as a promising solution for thrips management in mulberry and diverse agricultural contexts.

The highest efficacy against *D. pulverulentalis* was observed in flubendiamide, followed by chlorantraniliprole and emamectin benzoate. The findings conform with those who reported that in soybean fields, an application of 60 g a.i. ha^{-1} of flubendiamide 20 WG effectively protected crops from defoliators for up to 15 days (33-35). Chlorantraniliprole 18.5 SC significantly reduced capsule damage in sesame to 3.25 per cent and larval populations in cauliflower to 0.20 larvae per plant seven days after the second spray during the first season (36). Flubendiamide 39.35 SC showed the highest efficacy against *D. pulverulentalis* in the study, which is in confirmation with the findings which showed that emamectin benzoate and spinetoram highly toxic to fall armyworm but with lower persistence in maize, recommending flubendiamide and chlorantraniliprole for early crop stages and emamectin benzoate and spinetoram for later stages to avoid residue build-up (37-39). Emamectin benzoate has been identified as the most potent among the tested insecticides, showing 100 per cent mortality in initial and residual activity against the black cutworm, with a longer half-life compared to indoxacarb and chlorantraniliprole (40). Additionally, emamectin benzoate effectively inhibits the population growth of the fall armyworm at low concentrations, significantly reducing fecundity and prolonging developmental stages (41).

Pest-resistant proteins were higher in insecticide-treated plants than in pest-infested ones. Applying specific insecticide formulations in mulberry planting effectively reduces pest infestations, promoting healthier plant growth and potentially increasing leaf protein content. Pest damage significantly affects the biochemical composition of mulberry, including protein levels, with reported protein contents ranging from 63.80 to 107.30 mg g^{-1} in fresh leaves (42). Healthy mulberry genotypes like S13 (49.05 mg g^{-1}) and Chinese White (48.55 mg/g) exhibit high protein levels, but infestations can drastically lower these values (43). An increase in the protein content may result from a possible change in its synthesis pattern to overcome the injury and develop resistance (44). Insect damage reduces protein content in leaves by disrupting metabolic functions, which either decreases protein synthesis or causes the plant to mobilize proteins for tissue repair and resistance (45). The decline in protein levels might also result from insects rapidly utilizing these proteins for reproduction or the protein breakdown by proteolytic enzymes secreted by the insect (46). Research indicates that healthy mulberry plants generally exhibit higher phenol content compared to those affected by pests. For instance, the study highlights that leaf roller pest infestation in mulberry plants reduces total phenols, among other metabolic elements, detracting from the leaves' quality (44). Wild mulberry resources, typically less affected by pests due to their natural resilience, have higher total polyphenol contents than cultivated varieties (47). Furthermore, the study reveals that mulberry plants grown in open fields, which are more exposed to natural environmental stress, including pests, have higher polyphenol and anthocyanin contents compared to those cultivated in greenhouses, suggesting that some level of environmental stress can enhance phenolic compound production (48). When mulberry shoots are wounded, there is an immediate increase in peroxidase activity at the cut surface due to the release of latex from laticifers, followed by de novo synthesis of the enzyme in neighbouring tissues (49). Additionally, whitefly infestation and the associated Tomato yellow leaf curl virus (TYLCV) disease in tomato plants, closely related to mulberry in defence responses, show increased peroxidase activity, particularly in mature leaves (50).

Conclusion

The research study highlights the effectiveness of newer insecticide molecules in managing mulberry pests, specifically the mulberry thrips (*P. mori*) and leaf webber (*D. pulverulentalis*). Among the tested insecticides, Fipronil 5 SC demonstrated the highest efficacy against *P. mori*, while Flubendiamide 39.35 SC was most effective against *D. pulverulentalis*. These insecticides not only provided significant pest control but also exhibited prolonged persistence activity. Furthermore, they caused less physiological stress to the mulberry plants, evidenced by higher peroxidase and polyphenol oxidase activity, alongside increased protein and phenol content compared to untreated ones. Consequently, fipronil and flubendiamide can be recommended for foliar application within the mulberry crop management schedule, offering a sustainable approach to pest control as a last resort of an integrated pest management (IPM) system.

Acknowledgements

The author thanks the Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, for supporting this work and utilizing the laboratory facilities. The author also acknowledges the Ministry of Tribal Affairs, Govt. of India, for the funding assistance as a fellowship.

Authors' contributions

PRN contributed to the investigation, validation, formal analysis and research writing. SM and BV were responsible for conceptualizing, overseeing and leading the research activity planning. AS played a key role in methodology development, investigation and mentoring. RS and AT provided field evaluation facilities and offered mentorship. PR supported the research through methodology development and investigation, while JK contributed to formal analysis and mentoring.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this work, the authors used Quillbot to paraphrase the content. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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