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MP YeshikaDepartment of Sericulture, UAS,
GKVK, Bangalore, Karnataka,
India**KG Banuprakash**Department of Sericulture, UAS,
GKVK, Bangalore, Karnataka,
India**K Murali Mohan**Department of Agricultural
Entomology, UAS, GKVK,
Bangalore, Karnataka, India

Field evaluation of novel insecticides against *Maconellicoccus hirsutus* green in mulberry ecosystem and their safety to silkworm *Bombyx mori* L.

MP Yeshika, KG Banuprakash and K Murali Mohan

Abstract

Five novel insecticides along with dichlorvos as standard check were evaluated against pink mealybug, *Maconellicoccus hirsutus* (Green) under field conditions. The treatment dinotefuron 20 SG @ 0.25 g/l recorded the highest mealybug mortality at both 7 DAS (78.78 %) and 15 DAS (99.44 %) owing to its unique mode of action. Phytotoxicity symptoms assessed on mulberry revealed that all the insecticides except flonicamid 50 WG (0.15 and 0.3 g/l) had no phytotoxic effect on mulberry plants at both 7 and 15 DAS. When the insecticide treated leaves were fed to silkworms reared on 10, 20, 30 and 40 Days after Spraying (DAS) of insecticides to mulberry plants, Flonicamid 50 WG @ 0.3 g/l recorded 100 percent silkworm mortality and zero percent mortality was recorded in all the other treatments. Effective Rate of Rearing of 100 percent was recorded in silkworms fed on mulberry leaves from treatments pymetrozine 50 WG @ 0.3 g/l at 10 DAS, azadirachtin 1% @ 2 ml/l and dichlorvos 76 EC @ 2.63 ml/l at 30 DAS.

Keywords: novel insecticides, *Maconellicoccus hirsutus*, mulberry phytotoxicity, dinotefuron

Introduction

Mulberry (*Morus alba* L.) foliage is the only food for the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Many sucking pests cause damage to mulberry among which, pink mealybug causes severe damage leading to a significant loss in mulberry leaf yield (12-25 %) by sucking the sap from vascular tissues cause depletion in nutritive value of mulberry [14]. Mealybugs are often referred to as “hard to kill” insects and are soft, small-bodied sap feeders that constitute the second largest family of scale insects (Hemiptera: Coccoidea), with more than 2,000 described species [4]. Mealybug incidence caused an estimated loss in leaf yield of 4500 kg / ha / yr amounting to 34.24 percent, thus depriving the farmer from rearing about 450 dfls /ha /yr (10-15 %) [13]. Dichlorvos 76 EC (DDVP) is one of the oldest and safest chemical molecule which is being predominantly used in sericulture for the management of pests including pink mealy bug. Being a contact and fumigant poison, it is most effective on insects with biting and chewing mouthparts, but mealybug being a sucking pest, its mortality is due to the fumigant action only [11]. Dichlorvos is an excessive insecticide which has been notified for the ban from import and manufacture with effective from January 2019 and a complete ban from usage on all crops with effective from December 2020 [1]. Therefore, it is important to find molecules as alternative in place of prohibited dichlorvos. Keeping these views, the present investigation was carried out, where five novel insecticides viz., buprofezin 25 SC, pymetrozine 50 WG, flonicamid 50 WG, dinotefuron 20 SG and azadirachtin 1% along with dichlorvos 76 EC as standard check in different dilution concentrations were evaluated against pink mealybug under field conditions along with their waiting periods and residual toxicity to silkworm *B. mori* L. were also evaluated.

Material and methods

a) Experimental layout

The experiment was conducted at the Department of Sericulture, UAS, GKVK, Bengaluru, India with well established mulberry garden of V₁ variety. The field experiment was laid out in Randomized Block Design with 13 treatments, each replicated three times and fed to Kolar Gold the commercial cross breed (PM × CSR₂) silkworm *B. mori* L.

Corresponding Author:**MP Yeshika**Department of Sericulture, UAS,
GKVK, Bangalore, Karnataka,
India

The rearing of silkworms was laid out in Completely Randomized Design with 13 treatments and each replicated three times.

b) Treatment details

Insecticides like buprofezin 25 SC @ (1 ml/l and 2 ml/l), pymetrozine 50 WG @ (0.3 g/l and 0.6 g/l), flonicamid 50 WG @ (0.15 g/l and 0.3 g/l), dinotefuron 20 SG @ (0.12 g/l and 0.25 g/l), azadirachtin 1% @ (1 ml/l and 2 ml/l), dichlorvos 76 EC @ (1.32 ml/l and 2.63 ml/l) and untreated control were sprayed on the mulberry plants and care was taken by holding a polythene cover along the treated plants

while spraying to ensure that there was no drifting of chemicals. Mealybugs were artificially inoculated to the mulberry plants 15 days after pruning by camel hair brush.

c) Observations in the field

Pre treatment count on 35th days after pruning and Post treatment count of mealybugs in the treated mulberry garden was taken to find out the mortality of mealybugs on 7th and 15th day after the spray on five plants per replication. Mealybug mortality and percent reduction over control was calculated by following Henderson and Tilton formulae [7].

$$\text{Mortality of bugs (\%)} = \frac{\text{Population at pre count} - \text{Population after spray}}{\text{Population at pre count}} \times 100$$

$$\text{Protection over control (\%)} = \frac{\text{Population in control} - \text{Population after spray}}{\text{Population in the control}} \times 100$$

Phytotoxic symptoms like leaf injury on tips or surface of leaves, wilting of the plants, vein clearing or discolouration on the leaves, necrosis, epinasty and hyponasty were taken into consideration and observations were made on these symptoms on 7th and 15th DAS of insecticides. These symptoms were recorded based on the standard scoring method with 0-10 scale.

d) Rearing of silkworm

Before the commencement of silkworm rearing the appliances were sun dried and rearing room, appliances were thoroughly cleaned and the floor was washed using 2 percent bleaching powder solution. Then the entire rearing room was later disinfected by following standard procedure [3]. The rearing room was kept air tight for 24 hours and then the room was kept open and used for rearing. The third instar larvae were provided with chopped mulberry leaves of required quantity and quality. After 30 minutes of initial feeding, 90 larvae were transferred to each experimental tray in three replications along with the mulberry leaves. Later, in order to assess extent of toxicity in insecticides to silkworm and to determine the safety period for effective molecules, the silkworm rearing was carried out. Observations such as larval instar duration (h), larval mortality (%), matured larval weight (g) and ERR (%) were recorded during rearing.

Results and discussion

Mealybug mortality (%)

The post-treatment count on 7 DAS recorded significantly highest mealybug mortality of 78.78 percent in the treatment dinotefuron 20 SG @ 0.25 g/l. This was followed by the insecticide azadirachtin 1% @ 2ml/l (72.34%), dichlorvos 76 EC @ 2.63 ml/l (72.04%), pymetrozine 50 WG @ 0.6 g/l (71.39%), dinotefuron 20 SG @ 0.12 g/l (70.99%), buprofezin 25 SC @ 1 ml/l (70.78%), flonicamid 50 WG @

0.3 g/l (70.59%) and dichlorvos 76 EC @ 1.32 ml/l (69.41%) which were statistically on par with each other. Buprofezin 25 SC @ 2 ml/l (66.44%) and pymetrozine 50 WG @ 0.3 g/l (66.05%) recorded on par values for mealybug mortality. Significantly lowest mealybug mortality among the insecticides treated on the 7 DAS was recorded in the treatment flonicamid 50 WG @ 0.15 g/l of about 56.60 percent. At 15 DAS, there was significant difference between the treatment where dinotefuron 20 SG @ 0.25 g/l exhibited highest mealybug mortality (99.44%) which was statistically on par with dichlorvos 76 EC @ 2.63 ml/l (98.94%) and azadirachtin 1% @ 2 ml/l (97.71%). Whereas flonicamid 50 WG @ 0.15 g/l exhibited the lowest mealybug mortality (82.75%) (Table 1 and Fig.1).

Most of the insecticides assessed were found effective on mealybugs. Among the treatments dinotefuron 20 SG @ 0.25 g/l recorded the highest mealybug mortality at both 7 DAS (78.78%) and 15 DAS (99.44%) This was followed by the same insecticide dinotefuron 20 SG applied at lower concentration of @ 0.12 g/l recording 70.99 percent mortality at 7 DAS and 96.25 percent at 15 DAS (Table 1 and Fig.1). Dinotefuron 20 SG is a third generation neonicotinoid with strong systemic and translaminar action. Upon ingestion, it acts on the nervous system of the insect and disrupts the normal functioning of nervous system by mimicking the functions of acetylcholine. This molecule is known to be effective on multiple sucking insects in addition to mealybugs feeding on crops. These results are in conformity with the findings [6], where field experiment conducted for evaluation of dinotefuran at 15, 20, 25 and 30 g a.i. per ha, along with imidacloprid at 25 g a.i. per ha, acephate at 400 g a.i. / ha against brown plant hopper of rice suggested that, dinotefuran 25 g a.i. per ha was found effective against brown plant hopper compared to other insecticides.

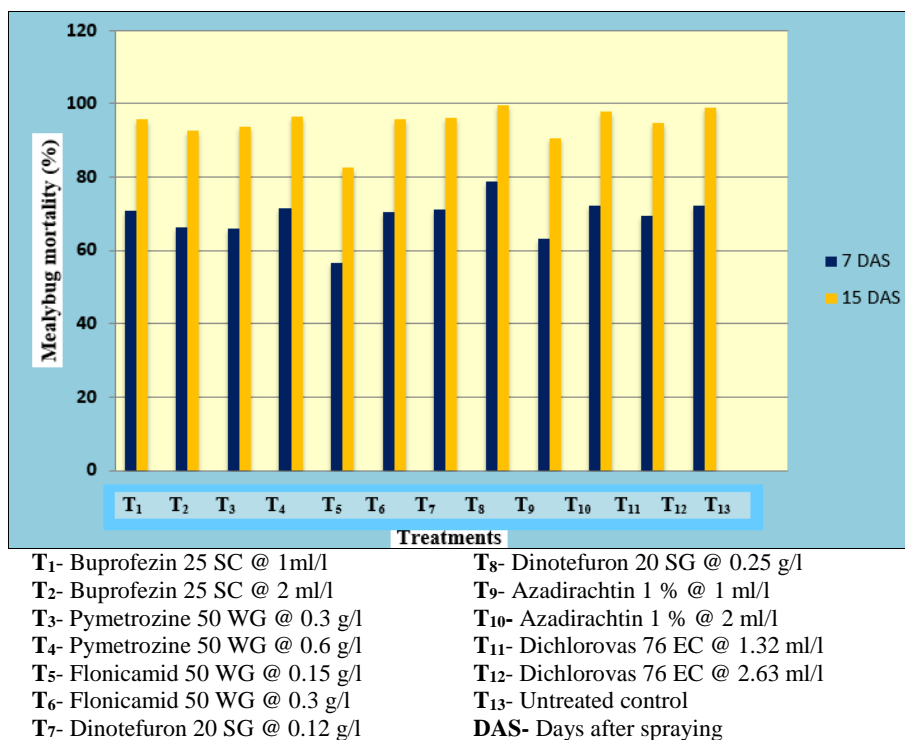


Fig 1: Effect of insecticides on mealybug mortality (%) in field on 7 DAS and 15 DAS

Table 1: Efficacy of novel insecticides on mealybug mortality (%) in mulberry field ecosystem

Treatments		Mealybug mortality (%)	
		7 DAS	15 DAS
T ₁	Buprofezin 25 SC @ 1ml/l	70.78 ^b	95.84 ^{bc}
T ₂	Buprofezin 25 SC @ 2 ml/l	66.44 ^{cd}	92.58 ^{de}
T ₃	Pymetrozine 50 WG @ 0.3 g/l	66.05 ^{cd}	93.57 ^d
T ₄	Pymetrozine 50 WG @ 0.6 g/l	71.39 ^b	96.36 ^{bd}
T ₅	Fonicamid 50 WG @ 0.15 g/l	56.60 ^e	82.75 ^f
T ₆	Fonicamid 50 WG @ 0.3 g/l	70.59 ^b	95.85 ^{bc}
T ₇	Dinotefuron 20 SG @ 0.12 g/l	70.99 ^b	96.25 ^{bc}
T ₈	Dinotefuron 20 SG @ 0.25 g/l	78.78 ^a	99.44 ^a
T ₉	Azadirachtin 1 % @ 1 ml/l	63.34 ^d	90.71 ^e
T ₁₀	Azadirachtin 1 % @ 2 ml/l	72.34 ^b	97.71 ^{ab}
T ₁₁	Dichlorvos 76 EC @ 1.32 ml/l	69.41 ^{bc}	94.83 ^{cd}
T ₁₂	Dichlorvos 76 EC @ 2.63 ml/l (std. check)	72.04 ^b	98.94 ^a
T ₁₃	Untreated control	0.00 ^f	0.00 ^g
F-test		*	*
SE.m ±		1.365	0.808
CD at 5 %		3.984	2.183

*Significant at 5 %, DAS- Days after spraying.

Phytotoxicity

The death of leaf tissue *i.e.*, necrosis was observed on the mulberry leaves treated with flonicamid 50 WG @ 0.3 g/l about 0.67 which was followed by flonicamid 50 WG @ 0.15 g/l about 0.13 on 7 DAS (Table 2). Remaining insecticides did not exhibit any necrosis symptoms. On 15 DAS flonicamid 50 WG @ 0.3 g/l recorded highest necrotic symptoms on mulberry leaf of about 7.60 and which was followed by flonicamid 50 WG @ 0.15 g/l which exhibited necrosis of about 4.87. The rest of the chemicals did not exhibit any necrosis on the mulberry leaves. Epinasty symptoms on mulberry leaf were observed on the plants treated with flonicamid 50 WG @ 0.3 g/l (0.93) and it was followed by flonicamid 50 WG @ 0.15 g/l (0.13) after 7 days of spraying. On the 15th day after spraying of the insecticide, highest epinasty symptoms was observed in the leaves treated with flonicamid 50 WG @ 0.3 g/l (2.07) and it was followed by

flonicamid 50 WG @ 0.15 g/l (0.73). Rest of the insecticides showed zero percent epinasty on both 7th and 15th day after spray of insecticides proving them to be safe to mulberry (Plate 1).

The results demonstrated that all the insecticides except flonicamid 50 WG had no phytotoxic effect on mulberry plants at both 7 DAS and 15 DAS. Fonicamid 50 WG exhibited only necrosis and epinastic symptoms and did not show other symptoms such as leaf injury on tip or surfaces, wilting, vein clearing and hyponasty. These observed results suggest that though flonicamid is effective on mealybugs, it may not be suitable for application on mulberry plant owing to its phytotoxicity. However, it was observed that when Dinotefuran 20% SG @ 300 ml and 350 ml / ha was applied on tea bushes against tea mosquito bug, *Helopeltis theivora* (Heteroptera: Miridae), it did not produce any phytotoxic symptom [10].

Table 2: Efficacy of insecticides on phytotoxicity on mulberry under field condition

Treatments		Phytotoxicity (Scoring method) [#]			
		Necrosis		Epinasty	
		7 DAS	15 DAS	7 DAS	15 DAS
T ₁	Buprofezin 25 SC @ 1ml/l	0.00	0.00	0.00	0.00
T ₂	Buprofezin 25 SC @ 2 ml/l	0.00	0.00	0.00	0.00
T ₃	Pymetrozine 50 WG @ 0.3 g/l	0.00	0.00	0.00	0.00
T ₄	Pymetrozine 50 WG @ 0.6 g/l	0.00	0.00	0.00	0.00
T ₅	Fonicamid 50 WG @ 0.15 g/l	0.13	4.87	0.13	0.73
T ₆	Fonicamid 50 WG @ 0.3 g/l	0.67	7.60	0.93	2.07
T ₇	Dinotefuron 20 SG @ 0.12 g/l	0.00	0.00	0.00	0.00
T ₈	Dinotefuron 20 SG @ 0.25 g/l	0.00	0.00	0.00	0.00
T ₉	Azadirachtin 1 % @ 1 ml/l	0.00	0.00	0.00	0.00
T ₁₀	Azadirachtin 1 % @ 2 ml/l	0.00	0.00	0.00	0.00
T ₁₁	Dichlorovas 76 EC @ 1.32 ml/l	0.00	0.00	0.00	0.00
T ₁₂	Dichlorovas 76 EC @ 2.63 ml/l (std. check)	0.00	0.00	0.00	0.00
T ₁₃	Untreated control	0.00	0.00	0.00	0.00
F-test		*	*	*	*
SE.m ±		0.019	0.272	0.029	0.045
CD at 5 %		0.055	0.780	0.082	0.128

*Significant at 5 %, DAS- Days after spraying.

[#] Scoring: 0= none, 1=0-10%, 2= 11-20%; 3=21-30%; 4=31-40%; 5=41-50%; 6=51-60%; 7= 61-70%; 8= 71-80%; 9= 81-90%; 10=91-100%.



Plate 1: Phytotoxicity symptoms exhibited by fonicamid 50 WG @ 0.3 g/l on 7 DAS on mulberry

Larval mortality (%)

The silkworm larval mortality was recorded from batch of worms fed with treated leaves harvested 10 days after spraying showed variation in the mortality rates among the treatments. Fonicamid 50 WG @ 0.3 g/l recorded 100 percent silkworm larval mortality. However, in all other treatments including fonicamid 50 WG @ 0.15 g/l, no larval mortality was recorded. No mortality was recorded in all the treatments including Fonicamid 50 WG @ 0.3 g/l when silkworms were reared with the leaves harvested 20, 30 and 40 days after spraying (Table 3; Plate 2; Plate 3).

Fonicamid insecticide was toxic to silkworms only when

used at 0.3 g /l. However, at lower concentration of 0.15 g /l it was found safer. Though fonicamid is a selective feeding blocker specific to sucking insects with repellent action, it appears that silkworms have lower tolerance limits for this molecule. Other studies have revealed that insecticides belonging organophosphates produce toxic symptoms on silkworms when sprayed on mulberry plant. It was reported that toxicity of diazinon, dichlorvos, phoxim and triazophos chemicals on silkworms when plants were treated with these insecticides [9]. Similarly, residual toxicity of dimethoate and dichlorvos insecticides on silkworms was also reported by [8]. In the present investigation except fonicamid, all the new insecticide molecules were found safer on silkworms. The toxicity was not noticed even at 10 DAS. This clearly suggests that silkworms were not affected by the application of these insecticide molecules. The new molecules that were tried in this study possess unique mode of action against insects, as they are highly selective on homopteron insects including mealybugs and have limited action on biting & chewing type of insects [6]. When efficacy of insecticides and botanicals were used for regulating whitefly incidence on mulberry, treatments with neem oil @1500 ppm and azadirachtin 1 % recorded the lowest mortality of silkworms with 10.8 % and 11.6 % at 7 DAS, 6.3 % & 8.3% 14 DAS, respectively [2].

Table 3: Effect of insecticides treated mulberry on silkworm mortality (%) reared on leaves harvested at different days after spraying (DAS)

Treatments		Silkworm larval mortality (%)			
		10 DAS	20 DAS	30 DAS	40 DAS
T ₁	Buprofezin 25 SC @ 1ml/l	0	0	0	0
T ₂	Buprofezin 25 SC @ 2 ml/l	0	0	0	0
T ₃	Pymetrozine 50 WG @ 0.3 g/l	0	0	0	0
T ₄	Pymetrozine 50 WG @ 0.6 g/l	0	0	0	0
T ₅	Fonicamid 50 WG @ 0.15 g/l	0	0	0	0
T ₆	Fonicamid 50 WG @ 0.3 g/l	100	0	0	0
T ₇	Dinotefuron 20 SG @ 0.12 g/l	0	0	0	0
T ₈	Dinotefuron 20 SG @ 0.25 g/l	0	0	0	0
T ₉	Azadirachtin 1 % @ 1 ml/l.	0	0	0	0
T ₁₀	Azadirachtin 1 % @ 2 ml/l	0	0	0	0
T ₁₁	Dichlorvos 76 EC @ 1.32 ml/l	0	0	0	0
T ₁₂	Dichlorvos 76 EC @ 2.63 ml/l (std. check)	0	0	0	0
T ₁₃	Untreated control	0	0	0	0

DAS- Days after spraying.

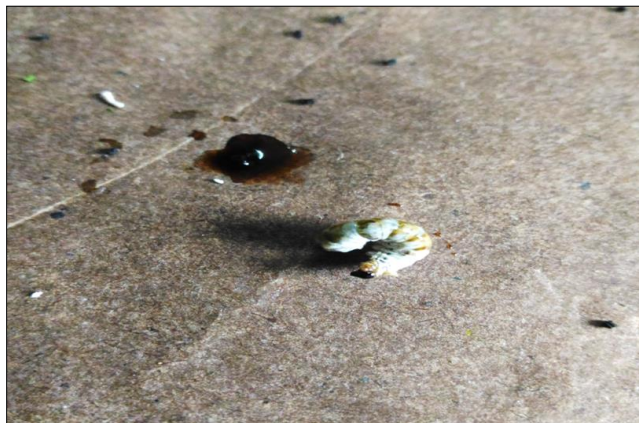


Plate 2: Third instar silkworm larval mortality in the treatment flonicamid 50 WG @ 0.3 g/l



Plate 3: Third instar silkworm larvae in untreated control

Effective rate of rearing (ERR) (%)

The silkworm batch reared by feeding mulberry leaves 10 DAS of insecticides exhibited significant difference in ERR percentage. The maximum ERR was observed in the treatment of pymetrozine 50 WG @ 0.3 g/l (100 %). Every other treatment was found statistically on par with each other except flonicamid 50 WG @ 0.3 g/l (0) and 0.15 g/l (87.00 %, which was lowest). On 20th DAS the silkworm batches reared

exhibited non-significant results among the treatments. The maximum ERR was observed in the treatment dinotefuron 50 SG @ 0.25g/l (97.77 %) and Dinotefuron 20 SG @ 0.12 g/l recorded the lowest ERR of 93.30 percent. ERR for 30 DAS was found non-significant between the treatments. The treatment azadirachtin 1 % @ 2ml/l and dichlorvos 76 EC @ 2.63 ml/l recorded highest ERR (100.00 %), the least was found with Buprofezin 25 SC @ 2ml/l (93.30 %) and untreated control recorded the ERR of 95.50 percent. ERR for 40 DAS was also found non-significant between treatments. Flonicamid 50 WG @ 0.3 g/l and Dichlorvos 76 EC at both concentrations exhibited highest ERR (98.87 %) and the least was recorded in the treatment Dinotefuron 50 SG @ 0.12 g/l (94.43 %) ERR of 100 percent was recorded in silkworms fed on mulberry leaves from treatments pymetrozine 50 WG @ 0.3 g/l at 10 DAS, azadirachtin 1% @ 2 ml/l and dichlorvos 76 EC @ 2.63 ml/l at 30 DAS. However, there was no significant difference with regard to ERR percent among the treatments at 20, 30 and 40 DAS. At 10 DAS, lowest ERR of 87 percent was recorded in flonicamid 50 WG @ 0.15g/l (Table 4).

Studies on potential efficacy of new pesticides for the control of mulberry whitefly and its impact on silkworm rearing found that 0.015 percent thiamethoxam, a neonicotinoid treated silkworms recorded a higher ERR of 94.35 percent followed by dichlorvos @ 0.1 % (92.96 % ERR), which is similar in the present study also [12]. Neem formulations recorded ERR similar with untreated control during the present investigation. Similar observations were made with bioassay of 1% neem oil treated mulberry leaves resulted in highest ERR percent (93% ERR) among other insecticides viz., monocrotophos, acephate and dichlorvos [2].

Contrarily, adverse effects of pesticide application on ERR percent of silkworm *B. mori* was reported by [5]. She reported that, when worms fed from fourth and fifth instar onwards, at different days after spraying recorded minimum ERR of 39.07 percent when treated with methyl demeton (0.05 %) and maximum ERR of 47.59 percent was recorded with higher concentration of nimbicidin (6 ppm).

Table 4: Effect of insecticides on Effective Rate of Rearing (ERR) (%)

Treatments		Effective Rate of Rearing (%)			
		10 DAS	20 DAS	30 DAS	40 DAS
T ₁	Buprofezin 25 SC @ 1ml/l	96.63 ^a	96.67	93.30	96.60
T ₂	Buprofezin 25 SC @ 2 ml/l	96.60 ^a	95.53	96.60	94.40
T ₃	Pymetrozine 50 WG @ 0.3 g/l	100.00 ^a	95.50	98.87	97.77
T ₄	Pymetrozine 50 WG @ 0.6 g/l	94.40 ^a	97.77	96.63	97.73
T ₅	Flonicamid 50 WG @ 0.15 g/l	87.00 ^b	93.87	97.53	96.67
T ₆	Flonicamid 50 WG @ 0.3 g/l	0.00 ^c	95.53	95.53	98.87
T ₇	Dinotefuron 20 SG @ 0.12 g/l	95.53 ^a	93.30	98.87	94.43
T ₈	Dinotefuron 20 SG @ 0.25 g/l	97.77 ^a	97.77	98.87	97.73
T ₉	Azadirachtin 1 % @ 1 ml/l.	96.63 ^a	96.63	98.87	94.40
T ₁₀	Azadirachtin 1 % @ 2 ml/l	97.77 ^a	96.60	100.00	95.50
T ₁₁	Dichlorvos 76 EC @ 1.32 ml/l	98.87 ^a	96.63	96.60	98.87
T ₁₂	Dichlorvos 76 EC @ 2.63 ml/l. (std. check)	96.63 ^a	95.50	100.00	98.87
T ₁₃	Untreated control	96.60 ^a	95.50	95.50	94.40
F-test		*	NS	NS	NS
SE.m ±		1.927	2.094	1.559	2.117
CD at 5 %		5.607	6.088	4.532	6.153

*Significant at 5 %, NS – Non significant, DAS - Days after spraying.

Conclusion

The selected insecticides were sprayed at two different concentrations on mulberry plants infested with mealybug among which the treatment dinotefuron 20 SG @ 0.25 g/l recorded the highest mealybug mortality at both 7 DAS (78.78 %) and 15 DAS (99.44 %) owing to its unique mode of action. Phytotoxicity symptoms assessed on mulberry revealed that all the insecticides except flonicamid 50 WG (0.15 and 0.3 g/l) had no phytotoxic effect on mulberry plants at both 7 DAS and 15 DAS. Though flonicamid is effective on mealybugs, it may not be suitable for application on mulberry plant owing to its phytotoxicity.

Among the various treatments, larval mortality was noticed only when worms were fed with leaves harvested at 10 DAS in the treatment flonicamid 50 WG @ 0.3 g/l(100 %) and no larval mortality was noticed in any of the insecticide treatments when fed with leaves harvested at 20, 30 and 40 DAS. Though flonicamid is a selective feeding blocker specific to sucking insects with repellent action, it appears that silkworms have lower tolerance limits for this molecule. Among the insecticide treatments, consistently the maximum ERR was observed in the treatment of Dinotefuron 50 SG @ 0.25g/l (97.77 %) and Dinotefuran belongs to neonicotinoid group of pesticides. By considering these facts Dinotefuron 20SG can be used as an effective substitute for DDVP in Sericulture.

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