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Effect of Toxicity of Various Insecticides on the Rearing Performance of *Bombyx mori* L.

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ABSTRACT

The bioassay study was conducted to test the efficacy of different insecticides on mulberry leaf roller *Diaphania pulverulentalis* (Hampson). The effective insecticides with selected 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 impact 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 which is on par with that of untreated control (8.99 %) and standard check Dichlorvos (11.27 %). Similarly, the larval mortality recorded in batches of silkworms fed on mulberry leaves sprayed with Azadirachtin and Novaluron (10.00 % & 9.67 %, respectively) at 25 DAS was on par with that of standard check and untreated control. Whereas, the insecticides Profenofos and fipronil at 30 DAS recorded less mortality rate of 11.00 per cent and 10.67 per cent, respectively. Similar trends were observed with respect to rearing performance 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

SERICULTURE is an integral part of the rural economy in an agrarian country like India. India is the second largest silk producing country next only to China. 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. 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 (Bing *et al.,* 2010). The residue of pesticides in the mulberry leaves could affect growth and quality of economic characteristics of cocoons. The susceptibility of *B. mori* L. to novel group of insecticides particularly Pyrrole, Avermectin, Phenylpyrazole and insect growth regulators is not ascertained. The age old insecticide Dichlorvos 76 EC is extensively recommended for management of defoliators pests of mulberry. As per the Government of India notification dated 28th December, 2016 Dichlorvos 76 EC usage is to be completely banned from 31st December, 2020 onwards. In order to find suitable alternate insecticides for mulberry leaf roller management and identify molecules which have less residual action and relatively safer to silkworm, the present study was conducted.

Material 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 out lined by Dandin *et al.* (2003).

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	1.00 ml/ liter
T_2	Pyrrole	Chlorfenapyr. 10 % EC	1.50 ml/ liter
T_3	Avermectin	Emamectin benzoate 5 % SG	0.40 gm/ liter
T_4	Organo - phosphate	Profenofos 50 % EC	1.00 ml/liter
T_5	Neem (Plant origin)	Azadirachtin 0.03 % EC	2 .00 ml/ liter
T_6	Phenylp - yrazole	Fipronil 5% SC	0.75 ml/ liter
T_7	Insect growth regulators.	Novaluron 10 % EC	0.5 ml/liter
T_8	Organo phosphate	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) (Duncan, 1955).

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

Results and Discussion

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 per cent with fipronil at 30 DAS which were on par with 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. The findings are in close conformity with the reports of Sathish *et al.* (2014) who reported 100 per cent mortality in chawki stage in the experiment with insecticides. 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 (Jothi *et al.,* 2013). 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 (Santorum *et al.,* 2019).

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 (1.0 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

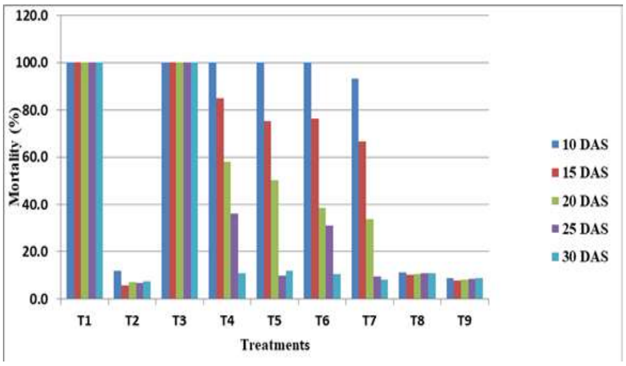

Fig. 1: Effect of insecticidal treatments on mortality (%) of silkworms

Swinging of entire silkworm body

Vomiting gut juice

Chain type excreta

Plate 2 : General Symptoms exhibited by silkworms fed on mulberry leaves sprayed with insecticide

Larval Parameters

3rd Instar Larval Weight (g/10 larva) : The third instar larval weight was significantly high in the batches of silkworms fed on mulberry sprayed with Chlorofenapyr 10 per cent EC at 10 DAS (1.38 g) which was on par with that of Dichlorvos (1.39 g) standard check. The treatments with novaluron (0.68 g) and fipronil (1.01 g) recorded least larval weight on 10 DAS, which might be due to high residual toxicity noticed in mulberry leaf. Similarly at 25 DAS the treatments with Azadirachtin (1.29 g), novaluron (1.28 g) and fipronil (1.27 g) recorded the larval weight which were on par with Dichlorvos (1.32 g) and untreated control (1.30 g). At 30 DAS all the insecticides proved safer result as the larval weight (1.28 to 1.31 g) was on par with that of Dichlorvos (1.31g) and untreated control (1.38 g) (Table 3). However, the treatments with Lambda - cyhalothrin and emamectin benzoate recorded complete mortality even after 30 DAS. This might be due to decreased in the levels of inorganic elements in the haemolymph (Kordy *et al.,* 2014). It was clear from the study that larval weight was reduced progressively as the concentration of insecticides was increased and feeding larvae with leaves treated with insecticides had a negative response on silkworms with respect to growth and development (Kumutha *et al*., 2013).

4th Instar Larval Weight (g/10 larva) : The 4th instar larval weight differed significantly among different treatments. Maximum 4th instar larval weight was recorded in silkworm batches reared on mulberry leaves fed with untreated control (21.16 g) followed by Dichlorvos at 10 DAS (20.83 g), chlorofenapyr at 10 DAS (17.66 g). 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 (20.36 g) and novaluron (21.19 g) recorded on par result with that of Dichlorvos (22.18 g) and untreated control (21.50 g) 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 (20.95 g) and fipronil (21.38 g) which were on par to standard check Dichlorvos (23.38 g) at 30 DAS (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 (Muthusami *et al*., 2010).

5th Instar Larval Weight (g/10 larva) : The batch of silkworms fed on mulberry leaves sprayed with chlorofenapyr at 10 DAS recorded significantly higher larval weight (21.22 g) which was on par with that of untreated control (24.96 g) and Dichlorvos at 10 DAS (24.65 g). Whereas azadirachtin (23.11 g) and novaluron (25.73 g) treatment proved safer to growth and development of silkworm growth at 25 DAS as these observations were on par with Dichlorvos (24.95 g) and untreated control (25.90 g) at 25 DAS. (Table 3). These results are in accordance with the findings of Maria *et al*. (2000). The mulberry leaves treated with chemicals and fed to silkworms after 30 days of spraying resulted in marked improvement in larval weight, cocoon and economic parameters of silkworms rather than feeding the larvae with leaves after 10 days of spraying (Anitha, 2015).

Cocoon Parameters

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).

Single Shell Weight (g) : The shell weight recorded was significantly more in the cocoons harvested from the batches of silkworms fed on mulberry leaves sprayed with profenfose at 30 DAS (0.32 g), fipronil at 30 DAS (0.30 g), azadirachtin at 25 DAS (0.31 g), novaluron at 25 DAS (0.28 g) and chlorofenapyr at

TABLE 3

Effect of insecticidal treatments in mulberry on larval weight of silkworm hybrid (PM × CSR2)

Treatments	3rd Instar larval weight (g/ 10 larvae)					4th Instar larval weight (g/ 10 larvae)					Maximum larval weight on 5th day of 5th instar (g/ 10 larvae)				
	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	1.38^{b}	1.33^{a}	1.36^{a}	1.30^{a}	1.31^{a}	17.66^{b}	18.94^{a}	21.38^{a}	19.00^{dc}	20.39^{c}	21.22^{b}	23.38^{b}	21.33^{b}	24.54^{b}	25.10^{a}
T_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_4	-	1.11^{b}	0.91^{d}	1.20^{b}	1.29^{a}	-	5.83^{d}	10.84^{c}	17.58^{e}	20.95^{c}	-	12.26^{f}	16.25^{d}	23.37^{c}	25.28^{a}
T_5	-	0.86^{c}	1.12^{c}	1.29^{a}	1.30^{a}	-	5.73^{d}	16.86^{b}	20.36^{bcd}	21.30^{c}	-	13.10^{fg}	19.25^{c}	23.11^{d}	25.11^{a}
T_6	1.01^{c}	1.10^{b}	1.15^{c}	1.27^{ab}	1.28^{a}	10.43^{c}	7.66^{c}	16.46^{b}	19.54^{cd}	21.38^{c}	12.66^{cd}	15.91^{e}	20.09^{bc}	24.32^{bc}	24.66^{a}
T_7	0.68^{d}	1.06^{b}	1.22^{bc}	1.28^{ab}	1.28^{a}	12.16^{c}	17.38^{b}	17.13^{b}	21.19^{abc}	21.78^{bc}	11.85^{d}	18.96^{d}	19.68^{bc}	25.73^{a}	24.07^{a}
T_8	1.39^{b}	1.35^{a}	1.29^{ab}	1.32^{a}	1.31^{a}	20.83^{a}	20.41^{a}	21.43^{a}	22.18^{a}	23.38^{ab}	24.65^{a}	24.93^{b}	26.70^{a}	24.95^{ab}	26.16^{a}
T_9	1.48^{a}	1.38^{a}	1.37^{a}	1.30^{a}	1.38^{a}	21.16^{a}	21.48^{a}	21.83^{a}	21.5^{ab}	24.72^{a}	24.96^{a}	26.90^{a}	27.66^{a}	25.90^{a}	27.78^{a}
F Test	*	*	*	*	NS	*	*	*	*	*	*	*	*	*	NS
Sem ±	0.02	0.04	0.03	0.02	-	0.64	0.39	0.51	0.57	0.54	0.60	0.52	0.62	0.37	-
CD at 0.05	0.08	0.13	0.11	0.09	-	2.09	1.20	1.57	1.75	1.67	1.96	1.60	1.91	1.15	-
CV(%)	3.92	6.33	5.16	4.00	4.73	6.75	4.91	4.92	4.83	4.27	5.48	4.70	4.97	2.65	5.28

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

TABLE 4

Effect of insecticidal treatments in mulberry on cocoon parameters of silkworm hybrid (PM × CSR2)

Treatments	Single cocoon weight (g)					Single shell weight (g)					Single cocoon silk filament length (m)				
	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	1.57 ^{a}	1.40 ^{ab}	1.60 ^{a}	1.58 ^{a}	1.62 ^{ab}	0.29 ^{a}	0.23 ^{c}	0.25 ^{b}	0.31 ^{ab}	0.30 ^{a}	669.18 ^{a}	693.90 ^{a}	695.98 ^{a}	701.64 ^{a}	700.27 ^{ab}
T_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_4	-	1.22 ^{d}	1.33 ^{c}	1.27 ^{c}	1.65 ^{ab}	-	0.27 ^{b}	0.26 ^{b}	0.29 ^{bc}	0.32 ^{a}	-	560.55 ^{c}	629.06 ^{c}	667.51 ^{b}	688.22 ^{cd}
T_5	-	1.28 ^{cd}	1.57 ^{ab}	1.59 ^{a}	1.65 ^{ab}	-	0.23 ^{c}	0.24 ^{b}	0.31 ^{ab}	0.31 ^{a}	-	588.53 ^{bc}	664.29 ^{b}	696.10 ^{a}	680.83 ^{d}
T_6	1.24 ^{b}	1.41 ^{ab}	1.31 ^{c}	1.44 ^{b}	1.72 ^{a}	0.12 ^{b}	0.21 ^{c}	0.26 ^{b}	0.25 ^{d}	0.30 ^{a}	438.77 ^{c}	576.33 ^{cd}	630.38 ^{c}	661.68 ^{b}	705.44 ^{a}
T_7	1.27 ^{b}	1.40 ^{ab}	1.44 ^{bc}	1.62 ^{a}	1.71 ^{a}	0.13 ^{b}	0.22 ^{c}	0.24 ^{b}	0.28 ^{c}	0.32 ^{a}	575.45 ^{b}	608.41 ^{b}	660.06 ^{b}	705.40 ^{a}	698.45 ^{abc}
T_8	1.56 ^{a}	1.38 ^{bc}	1.59 ^{a}	1.59 ^{a}	1.59 ^{b}	0.28 ^{a}	0.29 ^{ab}	0.31 ^{a}	0.30 ^{ab}	0.31 ^{a}	668.28 ^{ab}	695.92 ^{a}	694.26 ^{a}	692.62 ^{a}	694.39 ^{bc}
T_9	1.63 ^{a}	1.50 ^{a}	1.62 ^{a}	1.62 ^{a}	1.61 ^{ab}	0.30 ^{a}	0.31 ^{a}	0.33 ^{a}	0.32 ^{a}	0.32 ^{a}	686.52 ^{a}	698.73 ^{a}	702.86 ^{a}	693.26 ^{a}	701.68 ^{ab}
F Test	*	*	*	*	*	*	*	*	*	NS	*	*	*	*	*
Sem ±	0.04	0.03	0.04	0.04	0.03	0.006	0.008	0.007	0.008	-	7.00	7.71	16.13	7.11	3.39
CD at 0.05	0.13	0.10	0.13	0.12	0.11	0.02	0.02	0.02	0.02	-	22.82	23.77	49.70	21.92	10.46
CV (%)	4.85	4.40	4.99	4.71	3.72	5.259	5.68	4.73	4.73	4.13	1.99	2.11	4.18	1.78	0.84

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

10 DAS (0.29 g) (Table 4) which were on par with that of untreated control and standard check dichlorvos in respective days after spraying. The variation in shell weight might be due to the variation in cocoon weight and varied toxicity level over the period of days, in respective treatments. These findings are in accordance with the observations reported by Muthuswami *et al*. (2010).

Single Cocoon Silk Filament Length (m) : The single cocoon silk filament length was significantly high (669.18 m) in the treatment chlorofenapyr at 10 DAS which was on par with untreated control followed by standard check dichlorvos in the respective DAS interval. The observations recorded in respect of single cocoon filament length in treatments Azadirachtin (664.29 m) and Novaluron (660.06 m) at 25 DAS, profenofos (688.22 m) and fipronil (705.44 m) at 30 DAS were on par with each other. However the treatments, Lamda-cyhalothrin and emamectin benzoate proved to be highly toxic to silkworm even after 30 DAS (Table 4). This might be due to the residual effect of insecticide, poor quality leaves which in turn resulted in poor coccon quality.. Similar observations were made by Roxelle *et al.* (2013) and Anitha (2015).

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. Chlorofenapyr, Azadirachtin and Novaluron could be the alternate insecticides to be ideal to manage the mulberry leaf roller in view of the ban on Dichlorvos the safest insecticide recommended in mulberry.

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