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Effect of pesticide's residue on commercial parameters of the silkworm, *Bombyx mori* L

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Abstract
Bombyx mori L. is highly susceptible to the effect of pesticides even when applied at minimal quantity. However, the complete elimination of pesticide drift is impossible and insecticides that are employed in other crops, especially those that are applied through aerial spraying, can damage sericulture activity. The present study focused on effect of pesticides on rearing of silkworm and its biochemical and physiological changes due to residual nature of pesticides. The pesticide chlorfenapyr 10EC caused maximum larval mortality (13.93%), followed by novluron 10EC (12.35%), fenazaquin 10EC (11.17%), dinotefuron 20SG (9.39%). The results indicated that, novluron 10EC and chlorfenapyr 10EC were highly toxic even after waiting period. However, the rearing parameters recorded with dimethoate 30EC, carbofuron 3G, azadirechtin 0.03EC which were on par with control with water spray and absolute control. The biochemical and physiological parameters of the silkworm haemolymph recorded minimum with novluron 10EC except peroxidase enzyme which was recorded highest than other pesticides, that was reflected through the performance of silkworm reared on the leaves of treated plants. The larval, cocoon and biochemical parameters recorded significantly better values in those silkworms fed with leaves harvested from carbofuron 3G treated plants. The findings of present study clearly indicated that the insecticide carbofuron 3G was safe to silkworms followed by dimethoate 30EC and azadirachtin 0.03EC.

Keywords: Silkworm, novluron, dinotefuron, azadirechtin

Introduction
Mulberry is infested by several pests and these pests affect the growth of mulberry and cause considerable damage to the plant and loss in leaf yield. The insecticides applied for the control of mulberry pests in turn have a greater impact on silkworm growth and development. Apart from this, mulberry grown adjacent to the commercial crops, when plant protection measures taken on the commercial crops may cause drift to the mulberry garden. Further, which affect the growth, development, fecundity and survival of *B. mori*. The studies have focused on the effect of insecticides on *B. mori* dealing with toxicity, retardation of growth and development, fecundity, mortality, food utilization and economic parameters (Dutta *et al*., 2003) [5]. To overcome this problem, safe waiting period should be followed for leaf harvest (Narasimhanna, 1988) [15]. Sik *et al*. (1976) [19] reported that, more than 1.40% of yield reduction in sericulture is due to side effect of pesticide application, 49.40% was due to the application of different pesticides in rice fields, 21.20% in fruit gardens and 12.30% in olericulture.

Materials and Methods
Experimental layout
The experiment was conducted at College of Sericulture, Chintamani with well-established mulberry garden of V1 variety fed to Kolar Gold the commercial cross breed (PM × CSR2) and biochemical parameters were analysed at Advanced Centre for Plant Biotechnology, UAS, GKVK, Bengaluru. The experiment was laid out in randomized complete block design with 9 treatments, each replicated three times.

Treatment details
Pesticides like carbofuron 3G @ (12 g/plant), dimethoate 30 EC @ (2 ml/L), novluron 10 EC @ (0.5 ml/L), azadirachtin 0.03 EC @ (2 ml/L), fenazaquin 10 EC @ (1.5 ml/L), dinotefuron 20 SG @ (0.25 g/L), chlorfenapyr 10 EC @ (1.5 ml/L), control with water spray and absolute 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 due to wind and the treated leaves were fed to the silkworms.

Silkworms were reared up to fourth instar by following standard rearing practices (Dandin and Giridhar, 2014) [4]. After fourth moult the larvae were separated batch-wise, 210 larvae per treatment were transferred to each experimental tray in three replications along with the mulberry leaves. Later, in order to assess extent of residual toxicity of pesticides to silkworm, the silkworm rearing was carried out. The mulberry leaves treated with pesticides were fed to silkworms separately, as per schedule. Observations such as fifth instar duration (h), larval weight (g), fifth instar larval mortality (%), survival percentage, cocoon weight (g), pupal weight (g), shell weight (g), cocoon shell ratio (%), deformed moths (No.), fecundity (No.) were recorded during rearing.

Results and discussion

Fifth instar larval duration (h)

Significant difference was observed with respect to fifth instar larval duration among the treatments. The larval duration was minimum in the treatments of silkworms fed on mulberry leaves sprayed with pesticide novluron 10EC (T_3) (154.86 h) followed by fenazaquin 10EC (T_5) (159.78 h) and chlorfenapyr 10EC (T_7) (173.22 h). The larval duration was maximum in the treatments of silkworms fed on untreated leaves *i.e.* absolute control (T_9) (204.28 h) followed by control with water spray (T_8) (202.03 h) and carbofuron 3G (T_1) (200.01 h). However, the fifth instar larval duration recorded in the treatments of silkworms fed on mulberry leaves sprayed with dimethoate 30EC (T_2), azadirechtin 0.03EC (T_4) and dinotefuron 20SG (T_6) (199.64 h, 198.08 h and 184.30 h, respectively) (Table 4). These results are supported with the findings of Rahmathulla and Suresh (2012) [17] and Maqbool *et al*. (2015) [12] who reported reduction in feeding time in the most active feeding stage (fifth instar) in the bivoltine races resulting in a shorter larval duration. The variations in larval duration could be due to difference in the quality of leaf and seasonal variations.

Fifth instar larval weight (g)

Significantly less larval weight was recorded in the treatments of silkworm fed on mulberry leaves sprayed with chlorfenapyr 10EC (T_7) (2.13 g) followed by fenazaquin 10EC (T_5) (2.22 g) and azadirechtin 0.03EC (T_4) (2.33 g). Among all the treatments significantly highest larval weight was recorded in the treatment control with water spray (T_8) (3.25 g) followed by absolute control (T_9) (3.17 g) and dimethoate 30EC (T_2) (3.07 g). However, the treatment with dimethoate 30EC (T_2) (3.07 g) and carbofuron 3G (T_1) (2.93 g) were found to be safe to silkworms with respect to larval weight. It was clear from the present study that larval weight was reduced when larvae fed with pesticides treated leaves and exhibits a negative response on silkworm growth. The results are also in accordance with Kumutha *et al*. (2013) [10] who reported highest larval weight was recorded in control (32.32 g) as compared to the batches of silkworms fed on mulberry leaves treated with Vijaya Neem (0.005%) (28.02

Larval mortality (%)

The per cent larval mortality recorded was maximum in treatments of silkworms fed on mulberry sprayed with chlorfenapyr 10EC (T_7) (13.93%) followed by novluron 10EC (T_3) (12.35%) and Fenazaquin 10EC (T_5) (11.17%). Further, the mortality recorded was 9.39 per cent with Dinotefuron 20SG (T_6), 9.09 per cent with Dimethoate 30EC (T_2) and 8.46 per cent with Azadirechtin 0.03EC (T_4). The larval mortality recorded was significantly lower in treatments of silkworms fed on leaves sprayed with absolute control (T_9) (5.10%) followed by control with water spray (T_9) (5.24%) and carbofuron 3G (T_1) (5.42%) (Table 4) (Plate 1). The varied mortality among silkworm batches in different treatments might be due to the toxic molecules persists in the mulberry leaf for longer period as residue. The findings are in close conformity with the reports of Jyothi *et al*. (2013) [8] who reported that, 58.00% larval mortality was observed when silkworms fed with Spinosad 9 DAS (0.1ml). Gayathri and Rajendra (2011) [6] who reported that highest larval mortality was recorded in silkworms fed with 10 DAS Mancozeb sprayed leaves in fifth instar (23.20%).

Table 1: Larval parameters of fifth instar silkworm (*B. mori* L.) as influenced by feeding pesticide treated mulberry leaves

Treatments	Fifth instar larval duration (h)	Fifth instar larval weight (g)	Larval Mortality (%)	Survival at spinning (%)
T_1 - Carbofuron 3G	200.01	2.93	5.42	94.58
T_2 - Dimethoate 30EC	199.64	3.07	9.09	90.91
T_3 - Novluron 10EC	154.86	2.54	12.35	87.66
T_4 - Azadirechtin 0.03EC	198.08	2.33	8.46	91.54
T_5 - Fenazaquin 10EC	159.78	2.22	11.17	88.84
T_6 - Dinotefuron 20SG	184.30	2.82	9.39	90.61
T_7 - Chlorfenapyr 10EC	173.22	2.13	13.93	86.08
T_8 - Water spray	202.03	3.25	5.24	94.76
T_9 - Absolute control	204.28	3.17	5.10	94.90
F - test	*	*	*	*
SEm ±	6.50	0.12	0.42	0.42
CD @ 5%	19.48	0.36	1.24	1.24

Survival at spinning (%)

The per cent survival of silkworms at spinning recorded was minimum in treatments of silkworms fed on mulberry sprayed with chlorfenapyr 10EC (T_7) (86.08%) followed by novluron 10EC (T_3) (87.66%) and Fenazaquin 10EC (T_5) (88.84%). Further, the survival per cent recorded was 90.61 per cent with Dinotefuron 20SG (T_6), 90.91 per cent with Dimethoate 30EC (T_2) and 91.54 per cent with Azadirechtin 0.03EC (T_4). The survived larvae recorded was significantly higher in treatments of silkworms fed on leaves sprayed with absolute control (T_9) (94.90%) followed by control with water spray (T_9) (94.76%) and carbofuron 3G (T_1) (94.58%) (Table 1). The findings are in close conformity with the reports of Narayanaswamy *et al*. (2017) [16] recorded increased levels of silkworm survival per cent (PM × CSR2) in fifth instar when NSKE at 4 per cent was applied on mulberry.

Cocoon weight (g)

The single cocoon weight was significantly high in control with water spray (T_9) (1.94 g) followed by absolute control (T_8) (1.85 g). Similarly, increase in the single cocoon weight over a period of time due to reduction in insecticidal residue in dimethoate 30EC (T_2) (1.81 g) and carbofuron 3G (T_1) (1.78 g) (Table 2). This is in conformity with Kariappa and Narasimhanna, (1978) [9] who reported that feeding silkworms with mulberry leaves harvested from dimethoate sprayed plots after safe waiting period showed a significant improvement in respect of larval weight, cocoon weight and shell weight as compared to the infested control. The results are also in accordance with Bandyopadhyay *et al*., (2005) [3] reported significant decline in the commercial characters like cocoon weight when silkworm larvae were fed with monocrotophos, acephate and dichlorvos and azadirachtin 1500 ppm & 300 ppm.

Shell weight (g)

Shell weight also differed significantly between the treatments where in maximum shell weight was recorded in control with water spray (T_8) (0.38 g), followed by absolute control (T_9) (0.34 g) and dimethoate 30EC (T_2) (0.31 g). However, lowest shell weight was recorded in novluron 10EC (T_3) (0.23 g), followed by chlorfenapyr 10EC (T_7) (0.25 g) and fenazaquin 10EC (T_5) (0.26 g) (Table 2). The variation in shell weight might be due to the cocoon weight recorded in respective treatments. These findings are in accordance with Muthuswami *et al*. (2010) [14]. In the current investigation azadirechtin 0.03EC (T_4) (0.30 g) recorded significantly lower shell weight compared to other treatments. These findings are in line with the findings of Yeshika *et al*. (2020) [20] who reported that azadirachtin 1% recorded significantly lower shell weight at 10, 20, 30 and 40 DAS as compared to untreated control. Similarly, Narayanaswamy *et al*. (2017) [16] reported significantly lower shell weight when worms were fed with NSKE 4% treated leaves harvested after 16, 17, 18 and 19 DAS. In another study, similar to the current findings, buprofezin 25SC at 1 ml/l showed decline in the shell weight when larvae of silkworm *B. mori* were fed with treated leaves harvested at 20, 30 and 40 DAS (Maria *et al*., 2000) [13].

Table 2: Cocoon parameters as influenced by feeding silkworm with pesticide treated mulberry leaves

Treatments	Cocoon weight (g)	Shell weight (g)	Cocoon shell ratio (%)	Pupal weight (g)
T_1 - Carbofuron 3G	1.78	0.30	17.32	1.61
T_2 - Dimethoate 30EC	1.81	0.31	17.60	1.64
T_3 - Novluron 10EC	1.56	0.23	14.92	1.44
T_4 - Azadirechtin 0.03EC	1.51	0.30	19.54	1.35
T_5 - Fenazaquin 10EC	1.50	0.26	17.53	1.32
T_6 - Dinotefuron 20SG	1.72	0.29	16.88	1.57
T_7 - Chlorfenapyr 10EC	1.46	0.25	16.89	1.30
T_8 - Water spray	1.94	0.38	19.94	1.73
T_9 - Absolute control	1.85	0.34	18.28	1.68
F - test	*	*	NS	*
SEm ±	0.06	0.01	0.59	0.06
CD @ 5%	0.19	0.04	1.77	0.18

Cocoon shell ratio (%)

Shell ratio differed significantly between treatments and maximum shell ratio was recorded in cocoons obtained from control with water spray (T_8) (19.94%) followed by Azadirechtin 0.03EC (T_4) (19.54%) and absolute control (T_9) (18.28%). The present results are in close conformity with the findings of Bandyopadhyay *et al*. (2013) [2] who observed 19% shell ratio when silkworms were fed with 1% neem oil treated mulberry leaves. Minimum shell ratio was recorded in novluron 10EC (T_3) (14.92%), followed by dinotefuron 20SG (T_6) (16.88) and chlorfenapyr 10EC (T_7) (16.89) (Table 2). This might be due to residual effect of pesticide to the silkworms resulting in construction of poor quality cocoons and reduced shell ratio. This is in conformity with Roxelle *et al*. (2013) [18] and Anitha (2015) [1]. The results are also in accordance with Gayathri (2007) [6] reported that cocoon shell ratio was significantly lower in treatments with organophosphate insecticide and methyl demeton compared to untreated control.

Pupal weight (g)

Pupal weight recorded was highest in control with water spray (T_8) (1.73 g), followed by absolute control (T_9) (1.68 g) along with other insecticides *viz*., dimethoate 30EC (T_2) (1.64 g) and carbofuron 3G (T_1) (1.61 g). These findings are in conformity with the findings of Gayathri (2007) [6] reported that application of methy demeton, dimethoate and DDVP did not differ significantly among them in respect of pupal weight. Contrarily, when the larvae were fed with 4th instar onwards, there was a significant increase in pupal weight with increase in days after the spray. The lowest pupal weight was observed in chlorfenapyr 10EC (T_7) (1.30 g), followed by fenazaquin 10EC (T_5) (1.32 g) and azadirechtin 0.03EC (T_4) (1.35 g) (Table 2). Similar to the present findings, Maria *et al*. (2000) studied the effects of buprofezin on the growth of silkworm and concluded that among the different insecticides treated buprofezin gave higher pupal weight of 1.5 g / cocoon. Similarly, Yeshika *et al*. (2020) [20] who reported the lowest pupal weight was observed in flonicamid 50WG at 20, 30 and 40 days after spraying.

Fecundity (No.)

The present study revealed that the adults emerged from silkworm larvae fed with pesticides treated leaves laid less number of eggs. The highest fecundity was recorded in control with water spray (T_8) (510.35), followed by absolute control (T_9) (503.15) and dimethoate 30EC (T_2) (497.85). However, lowest fecundity was recorded in treatment with novluron 10EC (T_3) (326.65), followed by chlorfenapyr 10EC (T_7) (382.70) and fenazaquin 10EC (T_5) (415.65) (Table 2). These findings are in agreement with the findings of Zheng *et al*. (2022) [21] who reported that, when a given concentration of dimethoate was treated, the number of total eggs, fertilized eggs and hatching per cent were significantly lower than those in the control group. Similarly, Kumutha *et al*. (2013) [10] reported number of eggs laid by control moth was 547±4.12.

Whereas the eggs laid by moths emerged from larvae fed with Dichlorovos treated leaves were 504.4±2.84 and 485±4.71 for concentrations of 0.0004% and 0.0005%, respectively. Similarly, Kuribayashi (1981) [11] reported the ovicidal action of some pesticides treated during fifth instar larval instar.

Deformed moths (No.)

The number of deformed moths recorded was maximum in Novluron 10EC (T_3) (11.00), followed by Chlorfenapyr 10EC (T_7) (10.34). However, less number of deformed moths was recorded in control with water spray (T_8) (0.33) followed by absolute control (T_9) (0.34) and Carbofuron 3G (T_1) (2.00). The results are also in accordance with Kuribayashi (1981) [11] who reported that larvae treated with 8 ppm to 30 ppm solutions of ethylmercuric phosphate and methylmercuric chloride exhibits abnormalities in moth's behavior, wings, copulation, egg laying and subsequent embryonic development were affected (Plate 3).

Table 3: Grainage parameters of silk moth (*B.mori* L.) as influenced by feeding silkworm with pesticide treated mulberry leaves

Treatments	Fecundity (No.)	Deformed moths (No.)
T_1 - Carbofuron 3G	482.55	2.00
T_2 - Dimethoate 30EC	497.85	2.67
T_3 - Novluron 10EC	326.65	11.00
T_4 - Azadirechtin 0.03EC	467.80	4.34
T_5 - Fenazaquin 10EC	415.65	6.34
T_6 - Dinotefuron 20SG	457.00	4.34
T_7 - Chlorfenapyr 10EC	382.70	10.34
T_8 - Water spray	510.35	0.33
T_9 - Absolute control	503.15	0.34
F - test	*	*
SEm ±	20.01	0.15
CD @ 5%	59.98	0.44

Conclusion

The present study clearly indicated that the pesticides chlorfenapyr 10 EC and novluron 10 EC had negative effects on rearing performance even at safe waiting period. This clearly indicated that whenever the pesticides are used in crops near to mulberry garden there is needs to suggest taking extra care during spraying. Since dinotefuron 20SG @ 0.25 g/L and azadirachtin 0.03 EC @ 2 ml/L are performing well with respect to rearing and cocoon parameters they can be used as an effective novel molecules to replace DDVP and dimethoate 30 EC in sericulture.

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