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Effect of sub lethal exposure to dimethoate along with botanical extract on larval growth and development of *Bombyx mori* L

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Abstract
Dimethoate, an extensively used systemic and contact organophosphorus insecticide, is exploited in agriculture in order to control in sectpests and acarids due to its rapid action and degradation. It is well-known from the literature study that Organophosphate toxicity of Dimethoate exert its influence by interfering the acetyl cholinesterase activity which splits the neurotransmitter acetylcholine. The silkworm *Bombyx mori* L. is a domesticated insect which was identified for the commercial production of mulberry silk. Silkworms are influenced by acute toxicity or sublethal doses of insecticides through leaf contamination often showed retardation of growth and development. An experimental study was performed in order to assess the effect of insecticides on the silkworm *Bombyx mori* L fed with the mulberry leaves treated with organophosphorus insecticide like Dimethoate as well as botanical pesticide such as Neem oil. Identical sublethal concentrations of Dimethoate 30% EC (0.001%, 0.002%, 0.003%) and neem oil (0.01%,0.02&0.03 %) were prepared in deionized water and silkworm larva for IIIrd, IVth& Vth instars were subjected to get exposed for feeding to those pesticide sprayed leaves. The larval weight and duration were influenced by insecticides treated leaves depending on the varied concentrations of the pesticides. It has been observed that Neem oil had been found to exhibit more effect on the silkworm larval weight and duration in comparison with Dimethoate.

Keywords: dimethoate, Organophosphates, *Bombyx mori* L, sub lethal, mulberry, neem oil

Introduction
The silkworm *Bombyx mori* L. is an important economic sericigenous insect which is monophagous and depends solely on mulberry leaf. Highly nutritive mulberry leaves make the silkworms more resistant to any type of disease as well as enable them to improve silk productivity (Kaur*et al*.,2013) [5] Yield of Mulberry Leaves may be excessively reduced by the infestation of several pests which affect the growth of mulberry plantation as a whole also. The pesticides applied in order to regulate mulberry pests in turn have a substantial effect on the health of silk worms. Pesticides are generally applicable at a nominal level to avoid mortality in silkworms. It has been estimated thatpesticides sprayed over 98% and herbicides exceeding95% generally affect few non-targeted insect species also (Miller, 2004) [10] The silkworm *Bombyx mori* respond to toxicity of the pesticides and affected by organophosphates and other insecticides (Peng, *et al*.,2011) [14]. Zhang, *et al*., 2008) [21] studied about the acute toxicity of various organophosphate and pyrethroid pesticides to B. mori and suggested that limited use of these pesticides in close proximity to agricultural land enabling sustainable expansion of the sericulture industry. Recommended insecticides like dichlorvos and alternately dimethoate and botanicals like neem are generally used to control the pests in mulberry (Dandin *et al*., 2003 Li, *et al*. 2010; Ghelani, *et al*.,2006) [3, 7, 4].A study of Mukhopadhyay, S.K;&Santha Kumar, M.V (2010)[12]revealed that coccinellid beetles(coleopteran) when exposed to Dimethoate (0.1%) had been emerged as highly toxiccausing 100% mortality of that beetles(coleopteran) whereas dichlorvos (0.1%) was comparatively less toxic with regards to dimethoate. Although Dimethoate is used to suppress sucking insects, their toxicity on larvae of lepidoptera is comparatively less and hence it is known to be used for protection of Mulberry species. Despite the fact that the insecticide is moderately toxic to Lepidopteran, the sensitivity of the silkworm to the insecticide cannot be overlooked (Shanmuga Sundaram, *et al*., 2015; Mandal and Bhatacharya, 2003; Tiwari, *et al*., 2006) [16, 8, 17] had used neem derived from botanicals to control several pests efficiently.

Neem oil is identified as a effective insecticide due to its repellent, insecticidal, nematocidal, bactericidal, and fungicidal activities (Pascoli, *et al*.,2019) [13]. It has been reported that azadirachtin (an extracts derived from neem seeds) is highly sensitive for Lepidopteran insects (Adhikari Kripa 2020) [1]. Pesticides are safe to silk worms if they are applied on mulberry plants and need an investigation. However, proper information is not available to determine the sublethal effect on direct mortality or diminished reproduction, growth and survival. Hence in the present study an investigation was carried out to find out the effectiveness and safety of this bio-pesticide. Considering the above fact, an experimental study was initiated using sublethal concentration of organophosphate insecticides, along with a botanical insecticide and its impact on the larval parameters of silkworm *B mori* L. related to toxicity.

Materials and methods
Twenty DFLs (disease free layings) egg of silk worm (*Bombyx mori* L) breed $M_{12}W$ was collected from Phulpahari Sericulture complex of Paschim Midnapore, Govt of W.B and were reared up to 3rd instar in the DDE Zoology Laboratory, Vidyasagar University. The eggs were

incubated carefully by maintaining 80-85% relative humidity and 25^0C temperature, and also disinfected with 2% formaldehyde solution for five minutes. Uniformly developed eggs were selected, packed and covered with a black cloth to ensure uniform hatching. The larvae were provided with chopped tender leaves with highest moisture % during young stage (I^{st} to III^{rd} instars). Rearing of indigenous white nistari ($M_{12}W$) was carried out following standard procedure developed by CSR &TI, Mysore. Silkworms were supplied mulberry leaves for feeding three times a day at 6 a.m., 12 noon and 8 pm as proposed by Rajan et al., (2001) [15]. The experiment was conducted to investigate the effect of two pesticides namely Dimethoate and Neem oil extract.III^{rd} instars larvae (soon after the second ecdysis) of uniform size from a pooled batch were subdivided into seven batches of 100 larvae each. Silkworm larvae of III^{rd} instars stage were exposed to sublethal concentrations of Dimethoate (0.001%, 0.002%, 0.003 %.) and neem oil (0.01%, 0.02 % and 0.03 %) through feeding with pesticide-soaked leaves ones per 1^{st} day day and they were then relocated to rearing tray containing fresh mulberry leaves. At the same time control batch was also maintained through feeding with untreated larvae dipped in deionizer water. Observations like weight of matured larval(g) and larval period were recorded during the experimental rearing for III^{rd}, IV th and V^{th} instars of silkworm, *Bombyx mori* L. The experiment related to the rearing of silkworm larva for each concentration was replicated three times and thus obtained data were subjected to ANOVA in order to determine the significant difference between the various parameters of the treated and control batch of silkworm larva.

Results and Discussion

In the present investigation impact of pesticides on the growth of silk worm was traced and it was observed that in all the concentrations tested, (Dimethoate and neem oil) there was change in larval weight due to the ingesting of pesticide-soaked leaves (Table 1).

All the treatments with induced sub lethal dose showed lower larval weight and which decreased gradually from third to fifth instars. The mean weight of third instars larvae in the control batches was 0.92 g where as it was reduced to 0.82 g at higher concentration of dimethoate (0.003% concentration) which was 10.86 % over control and at lower concentration (1%) of neem oil. i.e 0.80 g which was 13.04% over control. The mean body weight of the larva of fourth instars in the control group was 1.07 g where as it was reduced to 1.05 g at higher concentration of dimethoate (0.003% concentration) which was 1.86 % over control and at lower concentration (1%) of neem oil. i.e 0.99g which was 7.47 % over control. The average weight of fifth instars in the larvae of control batches was 3.03 g where as it was reduced to 2.99g at higher concentration of dimethoate (0.003% concentration) which was 1.32 % over control and at lower concentration (0.01%) of neem oil. i.e 2.97 g which was 1.98 % over control. Larval weight recorded for different instars indicated significant variations in larval weight of *Bombyx mori* L in response to concentration of pesticides. The results revealed that the larval weights had been decreased progressively at gradual increment of concentration of pesticide from lower to higher. Significant decrease in larval weight with the effect of pesticides are in conformity with the study with Maria, *et al*., (2000) [9] where it had been recorded that the insecticide buprofezin treated leaves reduced the weight of fifth instars larva.

Table 1: Impact of sublethal concentration of pesticides on the growth of silkworm *Bombyx mori* L (Var: $M_{12}W$)

Pesticides	Concentration%	Larval weight					
		Average wt. of III^{rd} instar larvae (3rd days)	% Change over control	Average wt. of IV^{th} instar larvae (4th day)	% Change over control	Average wt. of V^{th} instar larvae (5th day)	% Change over control
Control		0.92		1.07		3.03	
Dimethoate (30 % EC)	0.001%	0.85	7.60%	1.05	1.86%	2.99	1.32%
	0.002%	0.83	9.78%	1.04	2.80%	2.97	1.98%
	0.003%	0.82	10.86%	1.02	4.67%	2.95	2.64%
Neem oil (crude)	0.01%	0.8	13.04%	0.99	7.47%	2.93	3.30%
	0.02%	0.79	14.13%	0.98	8.41%	2.92	3.63%
	0.03%	0.77	16.30%	0.96	10.28%	2.9	4.29%
F test		**		**		**	
CD 5 %		0.021		0.015		0.026	
CD 1 %		0.029		0.022		0.037	
SE (Mean)		0.012		0.009		0.015	
CV%		0.427		0.856		0. 502	

Table 2: Impact of sublethal concentration of pesticides on the larval duration of silkworm *Bombyx mori* L (Var: $M_{12}W$)

Pesticides	Concentration%	Larval period					
		Average of III^{rd} instar larval duration	% Change over control	Average of IV^{th} instar larval duration	% Change over control	Average of V^{th} instar larval duration	% Change over control
Control	DW	3.15	-	5.16	-	6.21	-
Dimethoate (30 % EC)	0.001%	3.23	2.53%	5.23	1.35	6.23	0.33
	0.002%	3.25	3.17%	5.24	1.55	6.27	0.96
	0.003%	3.27	3.8	5.26	1.93	6.29	1.28

Neem oil (crude)	0.01%	3.28	4.12	5.28	2.32	6.3	1.45
	0.02%	3.3	4.76	5.29	2.52	6.31	1.61
	0.03%	3.32	5.39	5.31	2.91	6.33	1.93
F- Test		*		**		*	
CD 5 %		0.088		0.037		0.066	
CD 1 %		NS		0.052		NS	
SE (Mean)		0.049		0.021		0.037	
CV%		1.516		0.394		0.592	

The consumption of pesticide sprayed leaves had extended the normal developmental duration of silkworm. A significant lengthy larval period was observed after consumption at different concentrations of pesticides (dimethoate and neem) soaked leaves as compared to control diet.

Larval period for IIIrd, IVth and Vth instars were found to be expanded with higher concentration of pesticides in the feed. Third instar Larval period from 3.15 days in control treatment was extended to3.27 days due to the treatment with dimethoate at higher concentration0.003% and 3.32 days due to neem at higher concentration 0.03%. (Table2). Larval instar duration recorded at fourth instar indicated significant variations among the treatments. Longest fourth instar larval duration of silkworms in the Dimethoate (30 % EC) treated leaves @ 0.003% (5.26 hrs.) which was 1.93 % over control along with neem oil @ 0.03 % (5.31 days) i.e.,2.91% over control Persistence of lengthy larval period for V^{t} instar silkworm in higher concentration of neem oil (0.03%) showed 6.33 which was 1.93% over control. Prolonged larval period in higher concentration of the application like neem derivatives in silkworm rearing bed corroborates with the similar findings of Kumutha, *et al* 2013 [6] who recorded that Larval period was extended due to due to neem-based insecticide like Vijay neem at higher concentration. Despite the fact that neem based insecticide containing azadirachtin have a pivotal role in crop protection (Mosesso,*et al*,.2012) [11]but an inhibitory effects of azadirachtin against the development of 5th-instar silkworm larvae were observed by Zhang,*et al.*, (2017) [20]

Extended larval duration were observed when the silkworms were fed with insecticide treated leaves at IIIrd, IV th and Vth instars (Yeshika, *et al*.,2019) [19] Several studies revealed that neem oil although a natural derivatives obtained from neem tree, but simultaneously showed the lethal toxicity to the pupal stage of insects which results several morphological deformities such as distorted adults, partial ecdysis, blocking of moulting of larvae, that defers and hinders adult formation (Boulahbel, *et al*., 2015) [2]

Complementary to the results in this study, another study (Unal, 2009) [18] suggested that azadirachtin is was extremely effective to inhibit lepidopteran larval growth and reduces the feeding activity of T. pityocampa showing that azadirachtin has antifeedant and growth inhibition properties against T. pityocampa. The longest larval period was recorded when third instar larvae were exposed to the treatment with azadirachtin 1% (Yeshika, *et al*., 2019) [19].

Conclusion

The pesticide toxicity through contamination of mulberry leaves causes various types of damage to silkworms, depending on effectiveness and quantity of pesticides. Overall, it is clear from the present investigation due to sublethal effects of insecticides diminished larval weight, prolonged larval duration occurred in every stage of late age larval instar but sublethal doses of both insecticides and botanicals don't haveany contribution tomortality of the insects. From the present experimental study it is also clear that the botanicals like neem oil has been identified to give an adverse effect on the growth and rearing performance of silkworm. The effectiveness of toxicity of the pesticides remain on mulberry leaves diminishes with time and finally fade away, although the efficacy of toxicity varies greater immediately after treatment. Therefore, in order to enhance the efficacy of those pesticides without reaching harm to silkworms, a suitable pesticide for the purpose of pesticidal control should be used by a proper technique at the time when risk of causing residual toxicity to silkworms would be diminished.

Abbreviations: **Var**- Variety; **EC**- Emulsifiable concentrate; DFLs-disease free layings;
$M_{12}W$ -Indigenous silkworm races known as white nistari

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Conflict of Interest

The authors declare that there are no conflicts of interest that is relevant to the content of this article.

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