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Effect of Temperature on Toxicity of Dimethoate 30% EC to Mulberry Silkworm, *Bombyx mori* (Linn.)

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Abstract:
In order to assess the influence of temperature on the acute toxicity of Dimethoate 30% EC on mulberry silkworm, *Bombyx mori* (Linnaeus, 1758), a laboratory study was conducted under three temperature conditions *viz*., 20±1°C, 25±1°C, 30±1°C with a constant range of relative humidity from 60 to 80%. Identical concentrations of Dimethoate 30% EC *viz*, 125, 250, 500, 1000, 2000 and 4000 ppm were prepared in deionised water and evaluated on the early 3rd instar larvae of silkworm by following leaf dip method. The larvae were exposed to treated mulberry leaves for 24 hours and incubated separately under different temperature conditions. Observation on larval mortality was made on 24 and 48 hours after treatment. The results of the experiment showed that there was a temperature response toxic effect noticed on the silkworm larvae. The 48 hour LC_{50} values of Dimethoate 30% EC were 1322, 1242, 997 ppm for 20±1°C, 25±1°C, 30±1°C respectively. Similarly the LC_{95} values were 4319, 3521, 2217 ppm respectively. The 48 hour LC_{50} and LC_{95} values of Dimethoate 30% EC were significantly minimum at higher temperature range tested (30±1°C). It is concluded that the temperature has a pronounced effect on toxicity of Dimethoate 30% EC on silkworm.

Keywords: *Bombyx mori,* Dimethoate 30% EC, Leaf dip bioassay, Silkworm, Temperature, Toxicity.

1.0 Introduction:
The mulberry silkworm, *Bombyx mori* is a monophagous insect feeding only on mulberry leaves due to the presence of the protein, morin (Tribhuwan & Mathur, 1989). The silkworm, *Bombyx Mori* L. is valued high in the economic view, as it converts leaf protein into valuable silk. The basic research works conducted using silkworm as a laboratory model due to its historical and economic importance and the world wide usage (Ramarethinam, 2002). *Bombyx mori* L feeds on mulberry plants which is grown widely as an important commercial crop.(Earanna & Govindan, 2002). Various pesticides are applied on mulberry plants to control various pests which may in turn affect silkworm, *Bombyx mori* when it is used for silkworm rearing (Jagadish Naik *et al.*, 2013). Genetic stability of the silkworm strains plays a major role in it's adaptability to the environment fluctuations (Li *et al*., 2001; Gangwar *et al*., 2009).

The predominant pests on Mulberry being, mealy bug is "hard to kill" and affect the quality of mulberry leaves and leads to unpalatable to the silkworm larvae. The tukra infected mulberry leaves with symptoms of minute mealy bugs, infected mulberry garden, curling of apical leaves. The mulberry infested with *M. hirsutus* (green) is a major pest of mulberry in southern parts of India and has become regular pest of mulberry in Andhra Pradesh and Tamil Nadu and other southern states especially during warmer. It has been reported that most of the mulberry varieties were susceptible for the mealy bug, *M. hirsutus* (green) attack (Mukhopadhyay *et al*, 2008). Usage of high potent insecticides on a crop like mulberry is practically impossible as it may cause death of silkworms (Dhahirabeevi, 1991). To combat the insect pests which are affecting mulberry crop especially sucking insect pests, Dimethoate, systemic insecticide with well known mechanism of action is used. Though, Dimethoate is used for controlling sucking insects, their toxicity on lepidopteron larvae is comparatively less and Hence it is used to protect Mulberry species. Despite the fact that the insecticide is moderately toxic to Lepidopteron, the sensitivity of the silkworm to the insecticide cannot be overlooked.

Intensive and careful domestication over centuries has apparently deprived this commercial insect of the opportunity to acquire thermo tolerance (Kumari *et al*. 2001). Humidity and temperature largely determine the growth of the silkworm and success of silkworm rearing (Kenten 1955). Higher or lower temperature than 25°C acts as a stress factor and increases the susceptibility of silkworm to viral infection and poor cocoon production (Steinhaus 1958). However the suitable, normal temperature for silkworm rearing in tropical countries varied from 20 to 30°C. Many studies were conducted against the silkworm with various agrochemicals. However, Toxicity of pesticides (which is used for controlling mulberry pests) influenced by temperature on silkworm larvae is not in glut. Therefore, a study was conducted to assess the toxicity of Dimethoate 30% EC in three different levels of temperature (within the range of silkworm rearing) on mulberry silkworm larvae. The main objective of the study is to compare the influence of temperature on the toxicity of Dimethoate 30% EC to mulberry silkworm larvae .

2.0 Materials and Methods:

The study was conducted at Ecotoxicology laboratory, International Institute of Biotechnology And Toxicology (IIBAT). The 3rd instar larvae of silkworm *Bombyx mori* were procured from State government sericulture unit, Vaniyambadi, Tamilnadu, India through a commercial agent. Required number of healthy larvae was acclimated one day before in respective test temperatures with mulberry leaves as food. A non replicated range finding test was performed prior to the definitive test, with different concentrations of Dimethoate 30% EC ranging from 10 to 5000 ppm to assess the LC_{50} range of the same (Data not shown). Based on the results of range finding test, identical concentrations of Dimethoate 30 EC *viz*., 125, 250, 500, 1000, 2000 and 4000 ppm (geometric factor of 2.0) were selected for definitive test for each temperature conditions. The test item was prepared in deionised water. Three replicates was maintained for each concentration and control with 10 larvae per replicate.

Fresh undamaged mulberry leaves free from insecticide application were collected from IIBAT farm, wiped with clean wet cotton to remove dust particles and air dried for few seconds. Fresh air dried leaves were dipped in aqueous solution of the test concentrations for about 30 seconds. Mulberry leaves dipped in deionised water were maintained as control. The treated leaves were air dried, chopped into pieces and placed in a plastic container of dimension 12.5 × 10 cm covered with perforated plastic lids. Ten 3rd instar silkworm larvae (which were starved for three hours before the test start) per test container were released on the leaves in order to get exposed (contact poisoning) and to feed (Oral poisoning) on the treated leaves for 24 hours. After the exposure period (24 hours), the uneaten leaves were removed and fresh mulberry leaves (untreated) were given to individual treatments as larval feed. The test containers were kept in air conditioned room where three different temperatures *viz*., 20±1°C, 25±1°C, 30±1°C with constant Relative Humidity, 60-80% maintained up to 48 hours of treatment. Observation on larval mortality was done on 24 and 48 hours after treatment. The survived larvae were transferred to the fresh leaves with the help of soft brush after 24 hours assessment.

2.1 Statistical Analysis:

Probit analysis was done using the software ECOSTATS in SAS environment to estimate LC_{50} and LC_{95} values. Mean mortality from the three temperatures and data on LC_{50} and LC_{95} with 95% confidence limits were analysed using one way ANOVA $P<0.05$. Means were compared by applying Duncan's Multiple Range Test (DMRT) to know the significant difference between the temperatures (Steel & Torrie, 1981).

3.0 Results and Discussion:

The results showed that the 'concentration response' mortality of silkworm larvae observed in all the temperature levels (**Table 1**) which was on par with observation made by Vishwanath *et al*., 1987. Mortality of silkworm larvae increased (both in 24h and 48h) with increase in temperature (**Figure 1**). Among the concentrations tested, significant larval mortality was observed from the concentration 250 ppm onwards compared to control in all the three conditions tested. The LC_{50} for 24 hours after exposure for 20, 25 and 30° C were 1560, 1535, 1350 ppm, respectively. Similarly, the LC_{95} value was 4977, 4945, 4141 ppm, respectively. There was no significant difference on 24 hour LC_{50} and LC_{95} values between the temperatures tested. However, significant difference was observed in 48 hour LC_{50} and LC_{95} values between the temperatures. The higher temperature (30°C) recorded significantly minimum LC_{50} and LC_{95} of 997 and 2217 ppm, respectively compared to 20 and 25°C (**Table 2**).

Table 1: Mortality of Dimethoate 30% EC on the larvae of *Bombyx mori*

Time points (Hours)	Temperature (°C)	Mortality (%) with different Concentrations (ppm)						
		Control (Deionised water)	125	250	500	1000	2000	4000
24	20±1	0	0	3.33a (2.89)	6.67a (2.89)	15.00b (5.00)	61.67 (7.64)	96.67 (5.77)
	25±1	0	0	0b	1.67b (2.89)	43.33a (5.77)	51.67 (10.41)	93.33 (5.77)
	30±1	0	0	0b	0b	53.33a (7.64)	63.33 (7.64)	93.33 (5.77)
	LSD	**N.S.**	**N.S.**	**3.35**	**4.70**	**12.45**	**N.S.**	**N.S.**
48	20±1	0	0	6.67a (2.89)	8.33a (2.89)	16.67c (5.77)	73.33a (5.77)	100
	25±1	0	0	0b	3.33b (2.89)	55.00b (5.00)	61.67b (7.64)	100
	30±1	0	0	0b	0b	71.67a (7.64)	83.33a (7.64)	100
	LSD	**N.S.**	**N.S.**	**3.35**	**4.70**	**12.45**	**14.15**	**N.S.**

Mean of three replications; Figures in parentheses are Standard Deviation
Means followed by similar letter are not statistically different by ANOVA,
LSD (Least Significant Difference) N.S- Not significantly different

The silkworm, *Bombyx mori* is a poikilothermic insect and environmental factors such as temperature, humidity, light, air, feed quality and quantity have intimate influence on its growth and development (Rahmathulla. *et al*, 2004). Our study clearly indicates that apart from growth and development of silkworm, toxicity also greatly influenced by environmental factors particularly temperature. Venugopala & Krishnaswamy (1987) who studied the effect of high temperature during later development stages of silkworm stating that there is direct correlation between metabolic responses of the poikilothermic insect to temperature. The present study also proved the same by exhibiting high mortality with increasing temperature indicating that the metabolic responses of the insects due to the chemical stress. The seasonal variations in temperature and relative humidity and feeding of different mulberry leaves of different varieties also influence the performance of silkworm larvae (Sarkar *et al*., 1995; Zaman *et al*., 1996). In our study, it is proved the same as the variation in the environmental temperature influence the toxicity of silkworm larvae against chemical stress.

Table 2: Data on LC_{50} and LC_{95}

Lethal Concentration	Hours after treatment with different temperature							
	24 hours			LSD	48 hours			LSD
	20±1°C	25±1°C	30±1°C		20±1°C	25±1°C	30±1°C	
LC_{50} (ppm) (Fiducial Limits)	1560a (1010 – 2667)	1535a (1024 – 2423)	1350a (832 – 2211)	**233.48**	1322a (679 – 3254)	1242a (742 – 2099)	997b (556 – 1608)	**103.46**
LC_{95} (ppm) (Fiducial Limits)	4977a (2843 - >5000)	4945a (2929 - >5000)	4141a (2440 – >5000)	**1078.1**	4319a (2163 - >5000)	3521a (2088 - >5000)	2217b (1438 - >5000)	**1068.7**

LC_{50} & LC_{95} – Concentration required to kill 50 and 95% of the test population, respectivelyFiducial Limits – with 95% confidence interval
Means followed by similar letter (column wise) are not statistically different ANOVA,
LSD (Least Significant Difference)

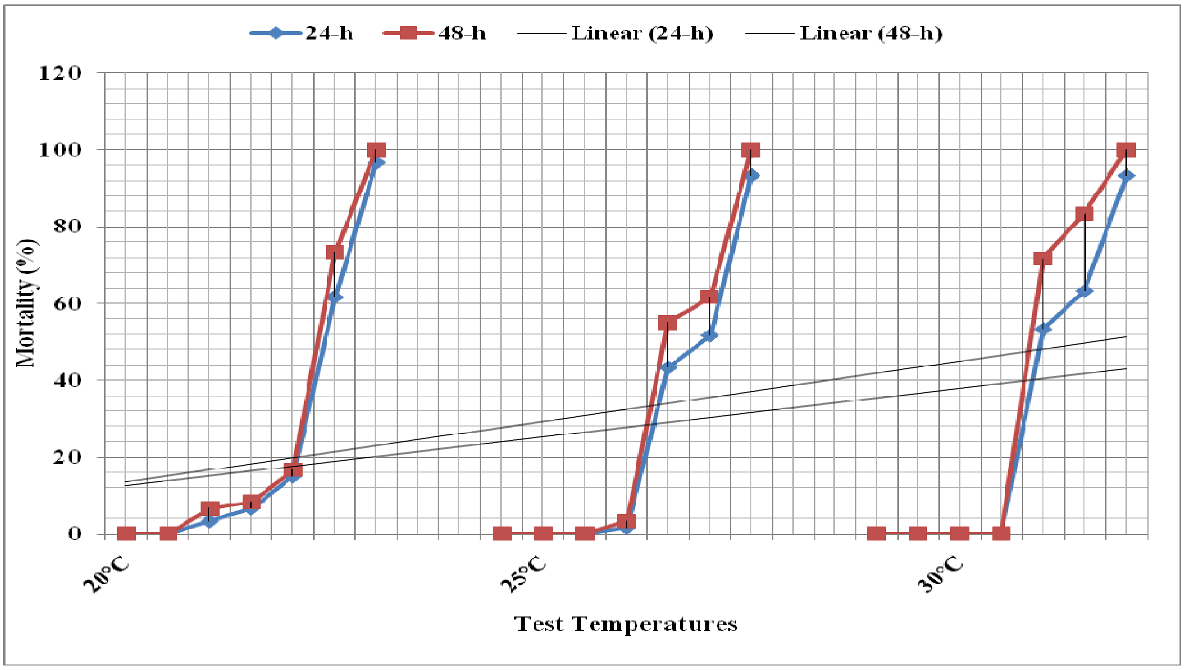

Figure 1: Silkworm mortality Trend with temperature on Dimethoate 30 EC

Hussain *et.al* (2011a) studied that higher temperature, especially more than 30°C in the breeding conditions did not favor the larval growth and finally lead to larval death. He (Hussain *et.al* (2011b) also indicated in his another study that the variations in rearing conditions of temperature and RH resulted in poor performance of non-feeding stages (pupa & adult) of moth. Chatterjee (2009) studied that increase of pathogenecity at high humidity level lead to increase in toxicity of biopesticides on silkworms. This may be due to high physiological activities of the insects at higher temperature. Similar way in our study, toxicity of chemical pesticides on silkworm increased with temperature.

Application of chemical insecticides against mulberry pests may pose residual problem on leaves which in turn may affect silkworm, *B. mori*. Sufficient waiting period must be given to avoid mortality of silkworm (Yokoyama, 1962). Waiting period for utilization of insecticide sprayed leaves for silkworm rearing was found to vary from 10 to 15 days (Munivenkatappa *et. al*., 1989). Several workers screened synthetic insecticides with less persistent toxic chemicals and among this Dichlorovos were toxic to mealy bugs and safer to silkworms (Dhahirabeevi, 1991; Datta 1993; Ramkishore *et. al*., 1995). Silkworm suffered due to insecticide poisoning when fed with leaves sprayed with Monocrotophos (0.05%) even 40 days after spraying (Ali, 1995). Feeding silkworms with Phosalone 0.07% treated leaves caused complete mortality of larvae even after 10 days after spraying (Asiamariam, 1999). In the present study, complete larval mortality was observed in the highest concentration of 4000 ppm (0.4%) Dimethoate 30EC at 48 hours after treatment indicated that toxicity of Dimethoate (which is applied for the control of sucking pests) on lepidopteron larvae is less when compared to other insecticides. Similar LC_{50} of Dimethoate was observed in the toxicity study on silkworms under normal temperature (25±2°C) conducted by YanyanChi *et al*, 2015. Vyjayinthi & Subramanyam 2002 and Datta *et.al*., 2003 studied the effect of insecticides on growth, fecundity, mortality, food utilization and economic parameters of silkworm. Bizhannia *et.al*., (2005) studied that larval feeding on mulberry leaves contaminated with Carbendazim residue showed a decrease on economic and biological characteristics of silkworm *Bombyx mori*. Sengupta *et. al*., (1990) concluded that Parathion (0.01%) spray dissipated within 13 days and found safer to silkworm depending upon prevailing weather conditions.

4.0 Conclusion:

It is concluded from the present investigation that the temperature in rearing conditions is greatly influencing the toxicity of chemical pesticide like Dimethoate on silkworm larvae. Among the temperature tested, the highest temperature, 30±1°C was found to influence more than the other temperatures tested in determining the toxicity of pesticide. By chance pesticide treated leaves are bound to offer to the silkworms (situations such as the residue of unknown chemical on the leaves or when the treated leaves are offered first time after the pesticide application or accidental feeding of treated mulberry leaves, unknowingly), we recommend that the rearing temperature can be minimized at lower end of the rearing condition for the particular period of feeding to avoid additional stress due to the pesticide. However, the other parameter such as growth, cocoon spinning ability and quality of cocoon etc, cannot be ruled out.

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