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Research article

PERMETHRIN INDUCED NEURONAL INEFFICIENCY IN THE SUPRAOESOPHAGEAL GANGLION OF MULBERRY SILKWORM, *BOMBYX MORI*. L

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Abstract

The primary target for pyrethroid insecticides is the nervous system. Available literature on the toxicity of pyrethroids to mulberry silkworm, are confined to specific tissues like haemolymph, fat body, eggs of silk moth and no study is indicated on supraoesophageal ganglion tissue, hence this study was conducted. In this study the changes occurring in the level of cholinergic and GABAergic transmitters (ACh and GABA); their connected enzymes viz., ChAT, AChE, GAD were studied in the supraoesophageal ganglionic tissue of silkworm *Bombyx mori* upon permithrin intoxication. Findings indicate that suppression of acetyl cholinesterase has led to the accumulation of ACh in greater quantities. Contrary to the cholinesterase suppression, a profound increase in GABAergic system was evident in the nervous tissue suggesting existence of an imbalance of excitatory-inhibitory interplay in the supraoesophageal ganglia due to the toxic insult caused by permethrin; this substantially affects the growth, development, fecundity and silk production. In conclusion, the permethrin induced neurotoxicity resulted a series of perturbations in silkworm neurotransmitter metabolism.

Keywords: Acetylcholine, *Bombyx mori*, GABA, GAD, neuronal transmission, permethrin.

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Introduction

One of the major constraints in silk production is the susceptibility of the silkworms to attack of different pests, parasitoids, predators and pathogens. Even mulberry silkworms which can be cultured in indoor condition are not free from such constraints. Growth and development of silkworm to a great extent depend on quality and quantity of food consumed and utilized. Mulberry fields often been contaminated with insecticides and problems have occurred in sericulture due to intoxication of silkworms fed on contaminated leaves [1]. Based on the available data on the toxicity of insecticides, silkworm *Bombyx mori* has shown to be the highly sensitive. According to Watnabe [1] the acute sensitivity of the silkworm to insecticides may be exploited in toxicity tests of insecticides.

Pyrethroid insecticides have been used in agricultural and home formulations for more than 30 years and account for approximately one-fourth of the worldwide insecticide market [2]. Permethrin being most photo- stable compound and possess high insecticidal activity. According to experimental evidences, nervous system is the prime target for pyrethroid action [3]. The toxicological studies made on economically important insects like *Bombyx mori* are mostly confined to the assessment of factors like lethality, fecundity and the retardation of growth and development [4]. Even data available on the biochemical studies impound to selected tissues like haemolymph, fat body and eggs of silk moth [5-7]. Very little is known about the physiology of nervous system due to pesticide(s) induced toxicity in the developing larvae of *Bombyx mori*. The genes coding for neurotransmitters, *e.g.*, acetylcholine, γ-aminobutyric acid and dopamine, and neuropeptides, *e.g.*, prothoracicotropic hormone (PTTH) and diapause hormone (DH), were found in the nervous system of the silkworm. These transmitters play a vital role in the regulation of sight, smell, feeding, cocooning, mating, excretion and other life activities of the silkworm by transferring signals. Interference of toxic substances might bring alterations in transmitter system. To the best of our knowledge no assessment is made on the effects of permithrin on neuronal activity of mulberry silk worm. Thereby this study aimed to address the alterations occurring in the excitatory (Cholinergic) and inhibitory transmitter system in the supraoesophageal ganglion of silk moth *Bombyx mori* due to the effect of permethrin a synthetic pyrethroid.

Materials and Methods

Laboratory reared forth instar larvae of *Bombyx mori* L (maintained at 26 $\pm$ 2°C and fed adlibitum (12 D: 12L photoperiodic condition) on fresh mulberry leaves (*Morus alba*) in the weight range of 1-1.3g were supplemented insecticide permethrin (3-phenoxy benzyl-cis, trans-3- (2,2-dichlorovinyl)-2-, 2 diethyl cyclopropane carboxylate, NRDC-143; EC 25%, Bharath pulvirising, Mills P.Ltd, Mumbai,India) sprayed leaves at sub lethal doses (1 ng/g wt and 5ng/g wt (LD50 = 15ng /g wt). They were grouped in two, after 24 hr of intoxication group-I larvae (4^{th} instar) were dissected and supraoesophageal ganglia 'the brain' was excised at 0° C and used immediately for the analysis of different biochemical parameters.

The activity levels of the enzyme AChE (Acetyl cholinesterase) and ChAT (Choline acetyl transferase) were determined by adopting the methods given by Ellman et al.,(1961) [8] and Hestrin's (1949) [9] respectively using acetyl-thiocholine as substrate. GABA (gamma-aminobutyric acid) and GAD (glutamic acid decarboxylase) levels were determined by paper chromatographic method of Roberts et al. (1950) [10] as given by Colowick and kapalan (1965) [11]. The total proteins were determined by the method of Lowry et al., (1951) [12].

The group –II intoxicated larvae were allowed to cocoon and economic traits such as cocoon weight, cocoon shell weight, pupa weight, and cocoon shell rate were assessed by using the formulae. The data obtained were analyzed statistically by two way ANOVA and Post-hoc test.

$$\text{Weight of single cocoon} = \frac{\text{Weight of 10 male cocoons + Weight of 10 female cocoons (gm)}}{\text{No. of cocoons taken (20)}}$$

$$\text{Single shell weight} = \frac{\text{Total shell weight of 10 male cocoon + 10 female cocoon shell (gm)}}{\text{Total no of cocoons taken (20)}}$$

$$\text{Shell ratio (\%)} = \frac{\text{Single shell weight (gm) X 100}}{\text{Single cocoon weight (gm)}}$$

Results and Discussion

The silkworm, *Bombyx mori*, has a ventral nerve cord type nervous system, which is simpler in structure than that of vertebrates; however, the simple system controls the complex behavior of the silkworm. Therefore, *B. mori* is an ideal model animal for neurobiology study. The silkworm nervous system, including the central nervous system, the peripheral nervous system and the sympathetic nervous system, plays a role in the regulation of sight, smell, feeding, cocooning, mating, excretion and other life activities of the silkworm by transferring signals. In this study the changes occurring in the different neurotransmitter levels and enzymes connected to their metabolism in the supraoesophageal ganglia (brain) of both control and permithrin administered silkworm larvae are measured (Table-1).

Table: 1 Permethrin induced changes in excitatory-inhibitory transmitter levels in the supraoesophageal ganglia of *Bombyx mori* .L

Parameters	Control	Permethrin exposed	
		(1ng/g wt)	(5ng/g wt)
AChT (U moles Ach synthesized/g wt of tissue /hr)	2.84 +0.021	3.57 +0.031 (+25.70) *	4.17 +0.042 (+46.83) *
AChE (U moles Ach hydro/min/mg protein)	3.54 +0.12	2.49 +0.19 (-29.66) *	1.59 +0.12 (- 55.08) *
ACh (U moles/g wt of tissue)	1.27 +0.13	2.59 +0.21 (+103.9) *	2.12 +0.0.19 (+66.93) *
GABA (U moles/g wt of tissue)	7.59 +0.56	12.59 +0.24 (+65.88) *	16.59 +0.56 (+118.58) *
GAD (U moles of GABA synthesized/g wt of tissue /hr)	11.59 +0.21	12.59 +0.24 (+8.63) *	14.19 +0.49 (+22.43) *
Total Proteins (mg/g wt of tissue)	12.56 +0.91	18.59 +0.99 (+48.01) *	20.59 +1.21 (+63.93) *

Values are mean + S.D of 12 observations. Values in parenthesis indicate % change;'+' sign indicate increase and '-'sign indicate decrease over controls.*P<0.05.

The results show that application of pyrethroid does not affect the survivability of silkworm, whereas economic traits found to be affected and showed decreased whole cocoon weight (25.45%), cocoon shell weight (33.33%), pupa weight, (66.07%), and shell ratio (24.39%).

Acetyl cholinesterase (AChE) is an important enzyme in the nervous system of insects, terminating nerve impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine (ACh). Organophosphate insecticides like malathion, DDVP, Diazinone, Anthio and parathion were shown to inhibit esterases in the haemolymph, while chlorinated hydrocarbons and pyrethroids exhibited no change [13]. Apart from the catalytic action in hydrolyzing ACh, AChEs role is also indicated in cell proliferation, differentiation, and responses to various stresses [14-15]. The dose dependent decrease in AChE activity was recorded in supraoesophageal ganglion of silkworm larvae was evident as a result of permethrin toxicity. Permithrin exposure in lower doses (1ng/g) produced 29.66 % decrease in AChE activity while 55.08% decrease noticed upon higher dose permethrin exposure (5ng/g). The inhibition of AChE resulted in excessive accumulation of ACh, leading to hyper activeness in silk worms. The increases found in the level of ACh in toxicated larvae paralleled with the inhibition of cholinesterase enzyme, indicating altered neuronal transmission as a result of toxic burden. It is evident from the results that the neuronal activity is rendered inefficient due to accumulation of Ach and the findings of this study corroborates with results of earlier studies where authors have shown a similar decreases in the activity of AChE and accumulation of Ach in the nervous tissue of insects on pyrethrum exposure [15]. *In-vivo* studies made to assess the effects of sumithion (an organophosphate) on esterases of haemolymph, integument, silk gland, ganglia and mid-gut of silkworm also reported similar inhibition of haemolymph esterases upon sumithion toxicity [16].

Gamma-amino-butyric acid (GABA) is a very important inhibitory neurotransmitter in both vertebrate and invertebrate nervous system. Bombyx mori appears to have the largest insect GABA-R gene family known to date, including three RDL, one LCCH3, and one GRD subunit. In this study, GABA level found to be substantially augmented by permithrin exposure (table2) in a dose dependent manner, while no change was established in the activity of GAD. Preponderance of studies have showed " type I" effect, upon permithrin toxicity which may be due to synaptic action between the lateral line sense organ, and the afferent fiber for which the transmitter is either glutamate or GABA is necessary[17]. In this study upon permethrin intoxication, the supraoesophageal ganglionic proteins found to be increased (Table 2), indicating the altered biochemistry of synapses which encompass deleterious effects on the physiology of the insect in general and neuronal functioning in particular.

Table: 2 Permethrin induced changes in the economic traits of silkworm *Bombyx mori* L

	Control	**Permethrin exposed (ng/g wt)**	
		(1ng/g wt)	**(5ng/g wt)**
Cocoon pupa	2.32+0.04	1.59+0.05 (31.46%)*	1.32+0.06 (43.10%)*
Pupa	1.12+0.09	0.77+. 05 (31.25%)*	0.38+0.01 (66.07%)*
Cocoon	1.10+0.06	0.94+0.04 (14.45%)	0.82+0.04 (25.45%)
Shell weight	0.30+0.01	0.22+0.01 (26.67%)	0.20+0.01 (33.33%)*
Shell ratio	27.27	23.40	24.39

Values are mean + S.D of 12 observations. Values in parenthesis indicate % change and '-' signs indicate decrease over controls.*P<0.05

It is also clear from the results that permethrin intoxication has affected both the excitatory transmitter system and also the inhibitory transmission. From the decreased AChE activity and increased ACh content found in the experimental insects, it can be envisaged that the electrical activity and the potentiality of neuronal response has been affected due to the toxic impact of the

insecticide. It appears that the cholinergic transmission has been profoundly inhibited due to the toxic insult. The results also depict the accumulation of GABA (table-2) in the neural tissue this eventually leads to labeling the neurons as inhibitory in nature [18]. Pyrethroid insecticides are also shown to disrupt the nerve function by modifying the gating kinetics of transitions between the conducting and non-conducting states of voltage-gated sodium channels [19]. From the results, it is envisaged that, being a neurotoxin, Permithrin intoxication appear to cause inhibition of sodium ion movement through the nerve membrane leading to hyper stimulation of nervous system and potentiated the inhibitory transmission in the supraoesophageal ganglia of the developing larva (4^{th} instar).

Thus, the present study results clearly indicate an existence of an imbalance of excitatory-inhibitory interplay in the supraoesophageal ganglia due to the toxic impact of permethrin. Its intoxication in developing larvae cause decreased neuronal efficiency, which in turn may affect the production of silk. The present study results also confer the decrease in the production of silk proteins as evidenced by the decreased weight of the cocoon weight, shell weight and ratio.

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Conflict Of Interest

Authors declare no conflict of interest neither for financial and personnel relations**.**

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