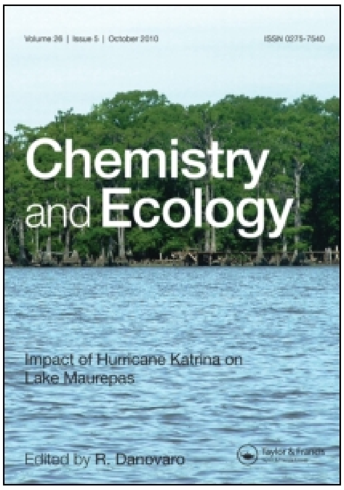

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STUDIES ON THE EFFECT OF HEXACHLORO-CYCLOHEXANE ON THE GROWTH AND SILK QUALITIES OF SILKWORM, *BOMBYX MORI* L.

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Toxicity of an organochloride insecticide, hexachlorocyclohexane (HCH) on the alterations in the growth and silk qualities of silkworm, *Bombyx mori* L were investigated. HCH yielded higher growth constants (K), indicating impairment of growth of silkworms and silk gland. The decrement in fibroin content is significant. HCH treatment also resulted in considerable reduction in the cocoon, pupal and shell weights, survival and emergence of pupae and number of eggs laid and deterioration in quality and quantity of silk thread.

KEY WORDS: Hexachlorocyclohexane (HCH), silkworm *(Bombyx mori)*, growth, cocoon production.

INTRODUCTION

In the recent years the use of pesticides in agriculture and public health has been studied with increasing interest; however, the hazards involved in the use of pesticides cannot be ignored. The pesticides employed are not always specific and the indiscriminate application of insecticides can often lead to residue problems both in the host plant as well as in non-target species. Although governmental agencies recommend that insecticides should not be sprayed on mulberry plantations, there is likely to be contamination resulting from air-borne drift when neighbouring fields are sprayed. In these circumstances, it is essential to have a knowledge of the effects of such insecticides on the growth of the silkworm (*Bombyx mori*).

In Andhra Pradesh, HCH under a trade name 'gammexane' is being used indiscriminately to control pests on tomato, brinjal (aubergine) and chilli crops. The mulberry crop is grown on small pieces of land often located in the middle of fields raising food crops and incidents when farmers unknowingly sprayed gammexane dust on mulberry have occurred. When gammexane sprayed leaves are fed to silkworms, they develop a series of toxic symptoms which eventually lead to death. Earlier investigations involving *Bombyx mori* revealed that the acetylcholineacetylcholinesterase system was impaired due to HCH stress and this might be a contributory factor to toxic effects in the silkworm (Reddy and Ramamurthi, 1984). Initial excitement, rapid swinging of the head and anterior part of the body, ejection of gastric juices through the mouth, paralysis and eventually death was observed in silkworms treated with HCH (2.8 μg/larva, 24 h LD_{50}) by topical application (Reddy and Ramamurthi, 1984).

This paper presents the results of topical application of a sublethal concentrations of HCH (0.7 μg/larva; 1/4th of 24 h LD_{50}) on the growth and cocoon production of silkworm.

MATERIALS AND METHODS

Bioassays

Disease-free layings of the silkworm moth [LR(PM) × $NB_4.D_2$] were purchased from Government grainage centre, Palamaner (A.P). The silkworms were reared on mulberry leaves in the laboratory following Krishnaswami (1978). A few larvae of V instar (newly moulted) were collected at random for cellular rearings. Separate groups of larvae (approx. 400 larvae) were maintained for control and experimental groups.

HCH (1,2,3,4,5,6 – hexachlorocyclohexane) (technical grade) obtained from M/S ICI (India) was used in the studies. The HCH was first dissolved in acetone and diluted with distilled water so that the final concentration of acetone was 0.05%. Acetone was found to be less toxic than other solvents for topical applications on silkworms (Buchfield *et al.*, 1952). The silkworm larvae were placed on filter paper in a Petri dish. A 20 μl solution containing 0.7 μg of HCH (1/4th of 24 h LD_{50}) was applied topically on the dorsal part of the anterior abdomen of each larva. Controls were treated similarly with 20 μl of the solvent (0.05% acetone). Topical applications were made during 0–24, 48–72 and 120–144 h of the V instar. The experimental, as well as the control, worms were fed *ad libitum* on uncontaminated mulberry leaves. The treated larvae were used for the assay after 24 h of the HCH treatment.

Growth rate studies

Larval growth rate of the V instar was recorded for control and experimental groups at 0–24, 48–72 and 120–144 h by determining weight with an electrical balance (Adiar Dut & Co. Pvt. Ltd., Calcutta). Posterior silk gland weight was also recorded during these periods for both control and experimental groups. A logistic growth curve expressing growth of silkworm or growth of posterior silk gland was drawn to fit the growth of body weight and posterior silk gland weight. Growth constant (K) values (see Table I) were determined according to Ueda and Suzuki (1967) after giving due consideration to biological and mathematical aspects.

Estimate of fibroin content

Total fibroin levels in posterior silk glands were estimated according to Shigematsu *et al.* (1965). Posterior silk gland of a single larva was isolated and soaked overnight in cold 5% trichloroacetic acid solution. The coagulated intraluminal fibroin was peeled out of the silk gland tissue and washed several times with 0.3% acetic acid. From the crude glandular fibroin, sericin was extracted twice with 0.2% sodium carbonate at 100 °C for 1 hour (Coleman and Howitt, 1947). The remaining fibroin was washed several times with hot and then with cold distilled water and finally with alcohol. The lipids were extracted twice with alcohol : ether (3:1) at 37 °C and the fibroin was washed

Table I K (constant) values [a] obtained for the control and HCH treated V instar larvae

Time of HCH application (age of V instar)	*Worm growth*	*Silk gland growth*
Control	11.73[b] ± 0.82	16.82 ± 0.96
0–24 h	12.04**[c] ± 0.91 (2.65)	18.14** ± 0.92 (7.85)
48–72 h	12.73*** ± 0.91 (8.53)	18.97*** ± 1.09 (12.78)
120–144 h	14.81*** ± 0.88 (26.26)	26.51*** ± 1.11 (57.61)

[a] K values were calculated based on the logistic curves (Figure 1 and Figure 2) using the formula:

$$\log \frac{X}{A\text{-}X} = K\,(t - t_0)$$

where X = growth quantity
A = maximum growth quantity
t_0 = time at A/2
K = constant

[b] Values are mean ± S.D. of 20 larvae.
[c] Values are significant at $P < 0.01$** and $P < 0.001$***.
[d] Values in parantheses are % change over control.

with ether and dried. The level of fibroin was determined gravimetrically after drying at 110 °C for 5 hours and expressed as milligrams per larva.

Evaluation of cocoons and their products

The other parameters such as larval duration, percent of cocooning, weight of cocoon, weight of pupa, weight of shell, emergence of the moth, fecundity, percentage of raw silk, reelability of silk thread, size of filament (denier), were evaluated according to standard methods followed by the State Department of Sericulture (Krishnaswami, 1978).

Statistical treatment

The data obtained was tested by Student's t-test and the significance was represented at $P < 0.01^{(**)}$ and $P < 0.001^{(***)}$ levels.

RESULTS AND DISCUSSION

The logistic curves obtained according to Ueda and Suzuki (1967) for V instar silkworm larvae and for the growth of the posterior silk gland are presented in Figure 1. The derived growth constant (K) values, denoting the accumulation of matter resulting from assimilation and metabolism, indicates some impairment between assimilation and metabolism in HCH treated worms. The V instar larval growth curve exhibited a sigmoid curve, while HCH applications shifted the curve towards the right indicating reduction in maximum growth, and a longer duration to attain maximum growth. The 'K' values were higher for the silkworm when HCH was

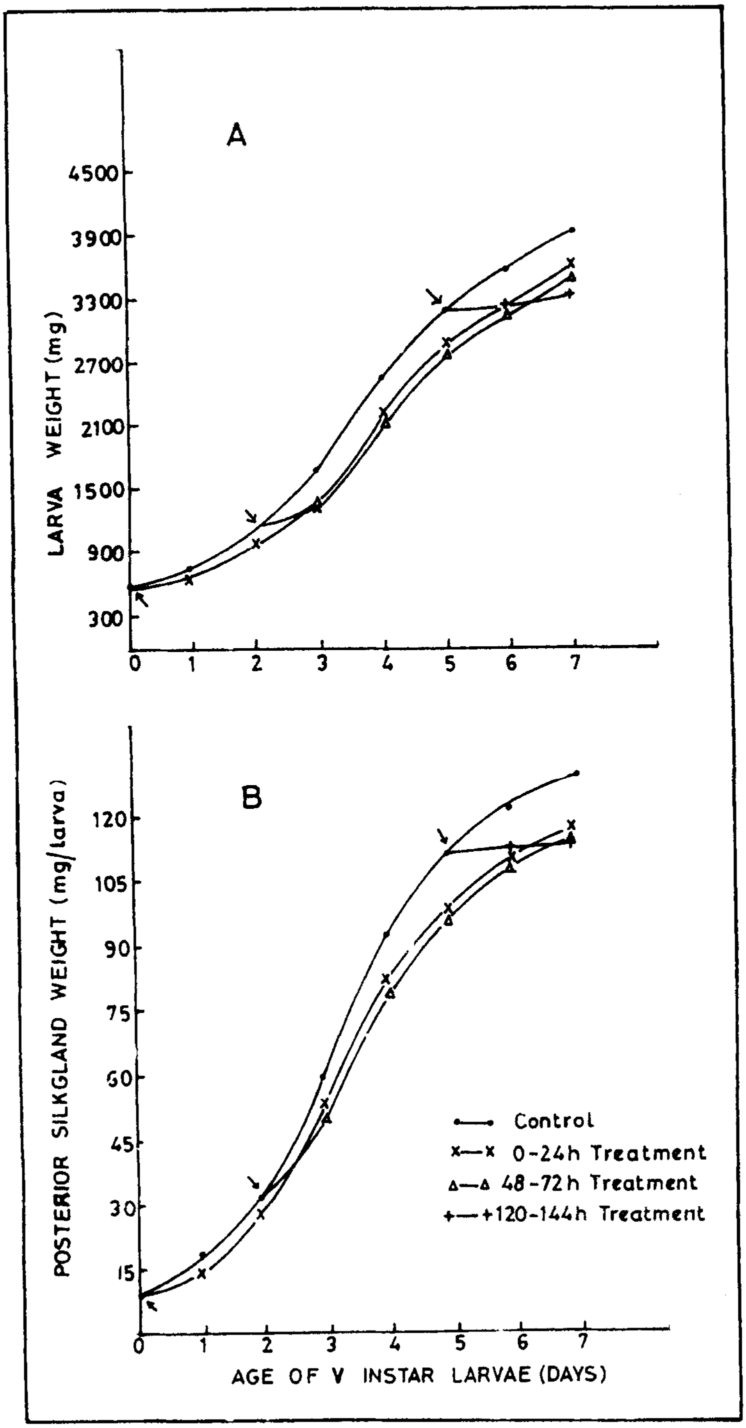

Figure 1 Logistic curves expressing the growth rate of V instar larvae (a) and posterior silk gland (b) after sublethal topical application of HCH. Each curve represents the average growth of 20 larvae. ↑ indicates day of application of HCH.

applied at a later stage of the V instar (Table I). Kashi (1972) also found that tolerance to malathion decreased as the silkworm larvae grow. Reddy *et al.* (1989) reported that the tolerance of the V instar of *Bombyx mori* to carbaryl is reduced as the larvae mature.

Fibroin content of the silk gland is an index of gland growth (Shimura, 1978). The fibroin content increased hyperbolically with age, reaching a maximum during day 5 of the V instar. HCH treatment reduced the fibroin content significantly (Fig. 2), especially when HCH was applied to later stages.

It is evident from the results (Table II) that the duration of larval life increased due to HCH treatment. The effect was greater if the insecticide was administered at a later time in the V instar stage. Considerable reduction in cocoon weight and cocoon shell ratio were also observed due to HCH treatment. The survival rate of the pupae was affected drastically by the treatment, and was lowered as the time of application was advanced. The emergence of moths and the number of eggs laid by the females are reduced in the treated silkworms (Table II). The number of normal eggs laid by the treated silkworms also decreased significantly.

HCH treatment reduces the reelability of the cocoon (Table III) and the reduction is more pronounced as the day of treatment within the V instar is advanced. The length of reelable silk thread and also the weight of the silk decreased as the time at which

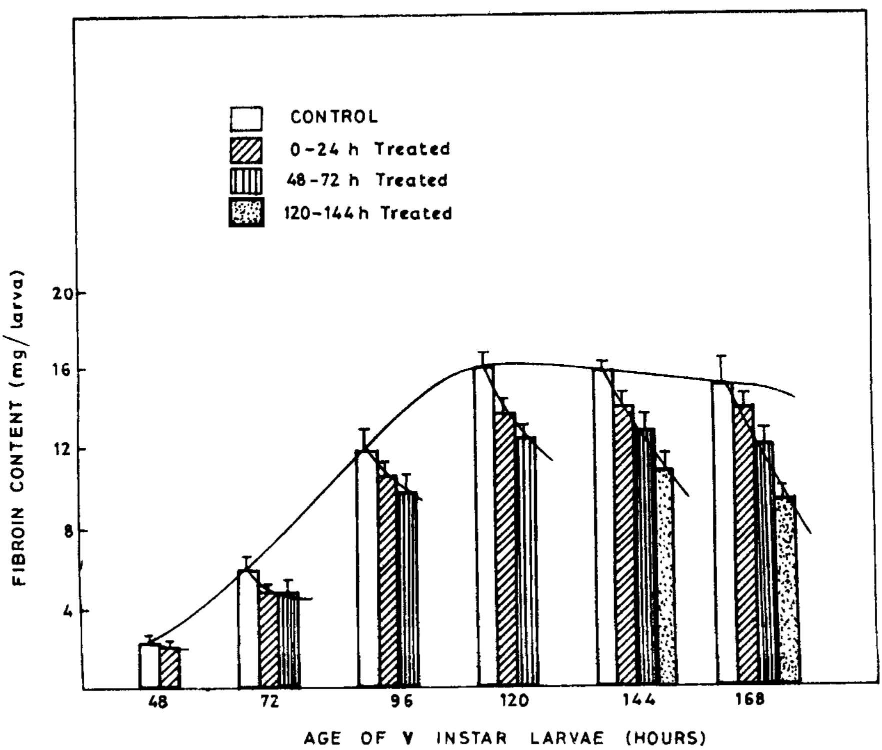

Figure 2 Effect of HCH on the dry weight of fibroin content in the posterior silk gland of V instar larvae *Bombyx mori*.
Each histogram represents the mean and S.D. of 10 individuals.

Table II Effect of sublethal topical application of HCH in different days of V instar larvae of *Bombyx mori* and on duration of V instar, cocooning, emergence and egg laying.

	Control (n=50)	*0–24 h (n=50)*	*48–72 h (n=50)*	*120–144 h (n=65)*
Duration of V instar (days)	7.5	7.5	8.0	9.0
% cocooning	96	92	88	70
Cocoon weight (g)	1.90 ±0.005	1.90 ±0.008	1.75** ±0.007	1.47*** ±0.006
Cocoon shell ratio (%)	20.6	20.4	19.7	17.4
Survival of pupae (%)	98	94	77	55
Emergence (%)	97	92	41	18
Number of eggs laid	420 ±30	412 ±24	290*** ±19	192*** ±20
Normal eggs (%)	85	79	70	52

Values are significant at $P<0.01$** and at $P<0.001$***

Table III Effect of sublethal topical application of HCH in different days of V instar larvae *Bombyx mori* on cocoon characteristics.

Time interval of HCH application in V instar	*Length of silk thread (metres/cocoon)*	*Reeled silk weight (mg)*	*Denier* [e]
Control	570.48 ± 62.72 [a]	1929 ± 140	2.34
0–24 h	565.57[b] ± 78.28 (−0.86) [d]	1876[b] ± 123 (−2.75)	2.21
48–72 h	492.45***[c] ± 66.34 (−13.68)	1609*** ± 127 (−16.59)	1.92
120–144 h	420.15*** ± 91.81 (−26.53)	1123*** ± 137 (−41.78)	1.87

[a] Values are mean ± S.D. of 12 individuals
[b] Values are not significant
[c] Values are statistically significant at $P<0.001$***
[d] Values in parantheses are % change over the controls
[e] The size of the denier of silk thread is measured gravimetrically by taking a constant length of 450 metres and weighing it in units of denier 0.05 g or for convenience 9000 metres in gram units. In practice it is calculated as:

$$\text{Denier} = \frac{\text{weight of reeled silk (g)} \times 9000}{\text{total length of silk} \times 1.13}$$

1.13 is a constant.

the insecticide was administered is delayed. Consequently, the denier of the silk is decreased following HCH application.

Besides abiotic and biotic factors, the spinning characters (cocooning, reelability, silk quality etc.) are dependent on intrinsic factors of the *milieu interior* (Horie and Watanabe, 1980). Reduction in reelability following HCH treatment could be explained by the loss of body water which is released to harden the liquid silk (Yokayama, 1963). Earlier, Reddy and Ramamurthi (1984) reported significant reduction in haemolymph volume and body water content of *Bombyx mori* during HCH treatment. When the amount of body water is reduced by HCH treatment, the spinning worms suffer water debt and consequent impairment of cocoon and silk qualities.

Kuriabayashi (1982) has demonstrated that organomercuric compounds, when administered during the larval stage, caused reproductive abnormalities in the silkworm. He also suggested that these compounds are transferred to the tissues, in particular to gonadial tissue, accumulate there and are not biodegraded. Their accumulation may bring about changes in the metabolism of reproductive cells and may sometimes produce dominant lethal mutations which carry over to succeeding generations. Abnormalities in hatchability, mortality, cocooning and egg laying habits of the next generation were also observed in silkworms during ethylmercuric chloride poisoning (Kuriabayashi, 1982). Similar results were reported in silkworms during application of carbaryl (a carbamate insecticide) (Reddy *et al.*, 1980) and phosphomidon (an organophosphorus compound) (Srinivas and Rao, 1992). In the light of these observations, any pesticidal molecule like HCH could have adverse effects on the metabolism of *Bombyx mori* leading to reduction in biomass and loss in bioproductivity. It is possible that the silkworm, which has undergone several genetic transformations and adaptations in the course of domestic rearing over millions of generations (Gamo, 1973; Tazima *et al.*, 1975; Yokayama, 1979), may have lost all degradative enzyme machinery for HCH, making it susceptible to HCH toxicity.

It is clear that HCH is a very effective insecticide and even at sub-lethal doses produces deleterious effects on the growth of silkworms. Although sublethal doses of HCH do not cause visible toxic symptoms, they lead to sub-standard and inferior cocoons, resulting in low quality silk. It is therefore recommended that farmers be advised not to use HCH in or near mulberry fields. It is also recommended that neighbouring farmers avoid the use of HCH in order to keep mulberry plantations free from HCH contamination arising from drift.

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