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STUDIES ON THE EFFECT OF NERIUM FLOWERS PRESENCE IN REARING BED ON HAEMOLYMPH TREHALOSE DURING FIFTH INSTAR OF MULTIVOLTINE SILKWORM, *BOMBYX MORI*

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The phytochemical effect of nerium flowers on haemolymph trehalose of fifth instar larvae of pure Mysore race was studied by continuously exposing silkworms from 1st day of instar I and upto harvest of cocoons to fully bloomed flowers of nerium introduced in the rearing bed. However, after III moult the worms were separated into batches and reared separately on leaf and shootlets in trays. In the shoot rearing minimum increase of the trehalose 19.5% was found on 5th day while maximum content of 34.8% was found on 1st day. However, with leaf rearing method, the lowest of 26.2% was found on the 5th day and highest increase of trehalose content (33.23%) was found on the 3rd day.

INTRODUCTION

India is rich with a variety of useful flora. There is a vast literature on the medicinal plants which contain biologically active principles. These botanical extracts are one of the alternatives to synthetic insecticides.

The phytochemicals of certain medicinal and aromatic plants are well known for their effect on insect population which make significant physiological, biochemical and even behavioural changes among them.

Silk is a commercial product of high demand, which has become a part of the research and biotechnology. The presence of nerium flowers (*Nerium indicum* Miller) (Apocynaceae) a non-host plant in the breeding environment of *Earias vittella* (F.) has affected the embryonic development of the noctuid species (Singh *et al.*, 1987; Ivan & Krishna, 1989; Singh & Krishna, 1989). Based on these reports, it is proposed to investigate to what extent these flowers would influence the silkworm, an economically important insect.

MATERIALS AND METHODS

Newly hatched larvae that were obtained from grainage (Elkathurty P_1 station) of pure Mysore. Silkworms were reared on mulberry leaves in wooden trays covered with paraffin paper in the laboratory at 24 - 28^{0}C and relative humidity 70 - 90%. In the beginning of the experiment, these larvae were divided into two batches of four lots, 100 each and feeding methods followed were leaf method upto III moult. In each tray one lot of the insects were permitted to complete its larval development in the presence of required quantity of fresh nerium flowers placed in the rearing bed daily during morning (5.30 a.m.) and evening feeding 4 p.m.). During the spinning, nerium flowers were placed on the mountage at close vicinity of the worms, while maintaining uniform distribution simultaneously larvae of all stages were allowed to develop free of nerium flowers, which is considered as control. The observations were recorded at 24 hrs interval during V instar haemolymph trehalose was estimated on 1st, 3rd, 5th day of V instar. The haemolymph was drawn by pricking the caudal horn and one of the prolegs of the larva and the sample was collected in test tubes, kept on ice. Minimum of five such samples were collected. Haemolymph trehalose was estimated by anthrone reagent as detailed by Wyatt & Kalf (1967) using trehalose (BDH,

England) as standard.

RESULTS AND DISCUSSION

Changes in the trehalose content of silkworm haemolymph during V instar silkworm after exposing to nerium flowers are present in Table I. The experiments were carried out on 1st, 3rd and 5th day of V instar larvae which consistently showed an increase of trehalose content. In the shoot rearing minimum content of 34.8% was found 1st day. However, with leaf rearing method, the lowest of 26.2 was found on the 5th day and highest increase of trehalose content (33.23%) was found on the 3rd day.

Table I : Changes in trehalose content in the haemolympnh of V instar exposed to nerium flowers (Expressed as mg/100 ml of haemolymph) (Mean values).

S.No	Age of V instar larvae	Nerium flowers during shoot rearing			Nerium flowers during leaf rearing		
		Control	Treatment	PC	Control	Treatment	PC
1.	1st	189.764 ± 7.423	255.872 ± 7.414	34.837	182.894 ± 3.750	232.654 ± 1.326	27.207
2.	2nd	230.762 ± 7.479	240.442 ± 3. 261	25.865	230.396 ± 1.373	307.226 ± 3.991	33.231
3.	3rd	205.840 ± 4.304	246.164 ± 11.198	19.589	207.642 ± 1.179	262.058 ± 8. 397	26.206

Each value is the mean of 05 observations, mean ± S.D.; PC denotes percentage increase over control.

Trehalose is the most characteristic and important reserve metabolite of insects (Wyatt & Kalf, 1957; Saito, 1963). The concentration of trehalose on the insect haemolymph depends on its rate of synthesis and utilization (Coutchie & Crow, 1979).

The trehalose content was high during the active feeding stage of the silkworm larva, suggesting its synthesis from its monosaccharide precursors derived from digested food. Further, decline of the trehalose concentration during prepupal stage (5th day) could be due to the higher energy requirement at larval-pupal moult, which must be derived from endogenous sources (Pant & Agarwal, 1965). The gradual decrease of haemolymph trehalose concentration from 3rd to 5th day in worms exposed to nerium flowers could be mainly due to gradual decline in feeding. Similar reduction and delayed build up of haemolymph trehalose concentration were reported after treatment of diflubenzron (Subrahmanyam & Rao, 1986a) in young ones of *Achaea janata* and *Spodoptera litura* respectively. It is also assumed that volatile compounds enanating from nerium flowers interact with the physiological process regulating the postembryonic development of the silkworm. Another possibility which cannot be ruled out, is that in shoot rearing, the biomass content is higher than leaf rearing which may be blocking the impact of nerium flowers. The effect of odour of nerium flowers on the haemolymph trehalose may also be due to the interaction of volatile compounds of neriodorin ($C_{22}H_{32}O_2$) and neriodorein ($C_{23}H_{34}O_{11}$) with physiological process of the silkworm (Ian & Krishna, 1989).

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