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INFLUENCE OF JUVENILE HORMONE ANALOGUE, METHOPRENE ON THE BIOCHEMICAL CHANGES AND ECONOMIC CHARACTERS OF SILKWORM *BOMBYX MORI* L.

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ABSTRACT: The influence of methoprene on the changes in trehalose,glycogen content and economic characters of silkworm were studied in CSR2 x CSR4 (Bivoltine x Bivoltine), and PM x CSR2 (Multivoltine x Bivoltine) hybrids. Application of methoprene caused significant ($P< 0.05$) increase in trehalose and glycogen contents, both in haemolymph and fat body tissues. The increase in trehalose was about 48% and 37% in haemolymph after 72 hrs of treatment in CSR2 x CSR4 and PM x CSR2 respectively. In the fat body 25 & 26 % increased trehalose content was observed after 96 hours of treatment in CSR2 x CSR4 and PM x CSR2 respectively. Significant ($P< 0.05$) increase in glycogen content also observed between treated and control hybrids in both tissues. Administration of methoprene (JH) also influenced the prolongation of 5th instar larval period by 24 hours in PM x CSR2 and 30 hours in CSR2 x CSR4 and resulted in significant increase in the cocoon weight (6.45 and 10.30%), shell weight (9.25 and 14.53%) and shell percentage (2.56 and 3.80%) in both the hybrids. Thus the methoprene administration prolonged the larval period and increased the cocoon characters in silkworm hybrids. The differential behaviour of trehalose and glycogen content suggested that these disaccharides might play distinct physiological roles during course of development.

Key words: Methoprene, Silkworm, Trehalose, Glycogen, Larval weight, Cocoon weight

INTRODUCTION

Ecdysone and juvenile hormone (JH) are the two major circulating hormones in insects, which control majority of the growth and developmental activities of the insects. JH has been considered to be an exclusive insect hormone and thus has attracted much attention also in plant and grain protection-oriented research. It is clearly a pleiotropic master hormone of insects, which governs most aspects of their integration with the ecosystem and affects decisive life history parameters during their entire life cycles (Hartfelder, 2000). It also regulates diverse traits in insects such as the synthesis of yolk protein, uptake of the molecule into the developing egg, diapause, flight, development, reproductive features and dispersal polymorphisms (Denlinger 1985; Nijhout, 1999; Wyatt and Davey 1996; Era and Cisper 2001; Wheeler and Nijhout 2003).

Methoprene is a long chain hydrocarbon ester (1, isopropyl 2E, 4E-11 methoxy-3,7,11-trimethyl-2, 4-dodecadienoates) which acts as insect growth regulator. It is especially effective against dipteran insects and has been widely used for the control and eradication of numerous pests and insects that affect humans and livestock and in the storage of various agricultural products (Garg and Donahue, 1989).

Hormones like thyroid (Thyagaraja *et al*., 1991) and methoprene JH analogue, (Akai *et al*., 1985; Miranda *et al*., 2002) have long been utilized for the improvement of silk production in the silkworm *Bombyx mori (L).* The juvenile hormones reportedly alter physiological processes essential for insect development and appears to act especially on insects (Siddall 1976). In view of its physiological alteration in insects and biological significance in silkworm, *Bombyx mori* the present study is being sought after to determine the effects of the topical application of methoprene on the changes of glycogen and trehalose content and economic characters of silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

In the present study two silkworm hybrids *viz.,* PM x NB4D2 (Multivoltine x Bivoltine) and CSR2 x CSR4 (Bivoltine x Bivoltine) were used.

Treatment of animals

Methoprene was administered to the larvae at the rate of 0.25 µl/ larva. The dose was applied after 24 hrs beginning of fifth instar larvae in PM x NB4D2 and after 48 hrs in CSR2 x CSR4 as per earlier report (Nair *et al.,*.1999) Prior to the treatment, the excreta and left over leaves were removed.

Collection of tissues

The haemolymph and fat body tissues were collected in the fifth instar larvae after 24, 48, 72 and 96 hours of treatment. Haemolymph was collected in a pre-chilled tube with thio- urea (anti- coagulant) by amputating one of the thoracic legs. Simultaneously, the larvae were dissected and the fat body was removed and kept at 0.9% saline at 6.5 pH. The collected samples were kept at -81ºC.

Estimation of trehalose and glycogen

The trehalose content in haemolymph and fat body was estimated by following the method of Roe (1955). The glycogen content in haemolymph and fat body was estimated by following the method of Montgomery (1957).

RESULTS

Trehalose content

Methoprene treated larvae showed significant ($p<0.05$) increase of trehalose content in both haemolymph and fat body tissues as compared to non treated control larvae. There was an improvement of 46 & 56, 29 & 60 and 48 & 48%, 25and 29%in the trehalose content both in haemolymph and fat body tissues of treated larvae as compared to the control larvae after 24, 48, 72 and 96 hours of treatment in CSR2 x CSR4. In PM x NB4D2, the percentage improvement was 55 & 56, 35 & 83, 37 & 47 and 26 & 43% respectively. Irrespective of voltinism, both hybrids showed similar trend. Moreover it was observed that the trehalose content showed increased drift during the developmental period in both haemolymph and fat body. The trehalose content showed increase in quantity from after 24 hrs of treatment to till 72 hrs of treatment in haemolymph, thereafter a sharp decline at 96 hrs (Fig.1a). However in fat body, no such decline was observed, but continued to increase up to 96 hours of after treatment. However, a similar trend in both the hybrids was observed (Fig .1b).

Glycogen content

Glycogen content also showed significant increase ($p<0.05$) over control in both the tissues and the hybrids. In CSR2 x CSR4, an improvement of 30 & 42, 31 & 20,34 & 18 and 27 & 43% in the glycogen content in the haemolymph and fat body tissues of treated larvae as compared to the control larvae after 24, 48, 72 and 96 hours of treatment was observed. Whereas in PM x NB4D2, the percentage improvement was 35 & 14, 36 & 11, 36 & 17 and 22 & 43%. However, a steady and stable increase in glycogen amount was observed between 24 to 96 hrs of after treatment in both the tissues (Fig.2a,b).

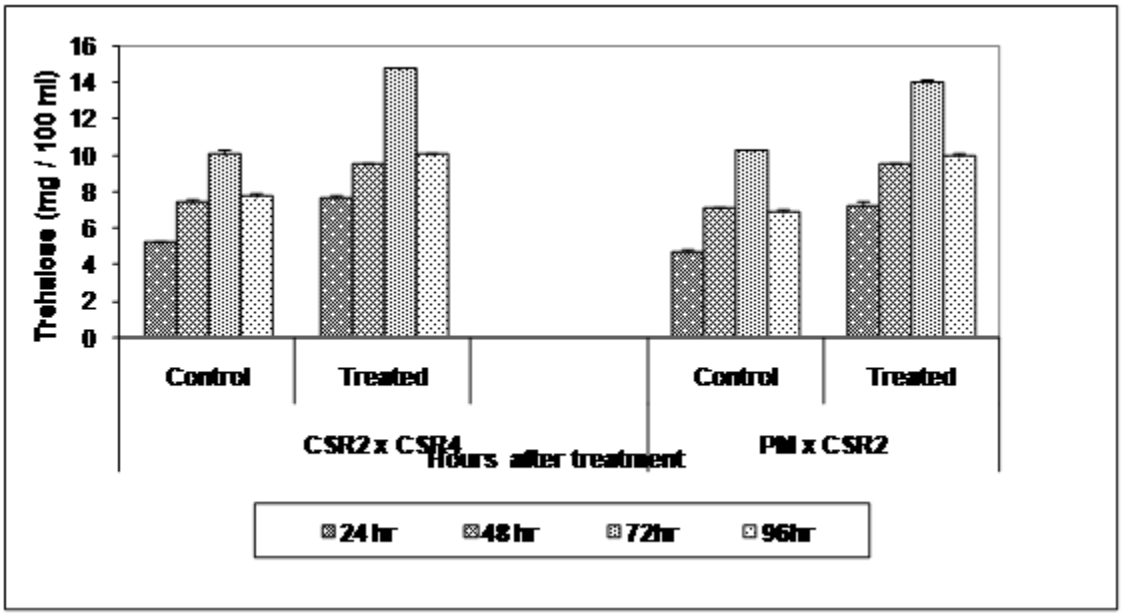

Fig 1a : Effect of methoprene on the changes of trehalose content in haemolymph of silkworm hybrids. Where CSR2 x CSR4 is bivoltine hybrid and PM x CSR2 is multivoltine x bivoltine hybrid. Values are mean ± SE based on three replicates (n = 3).

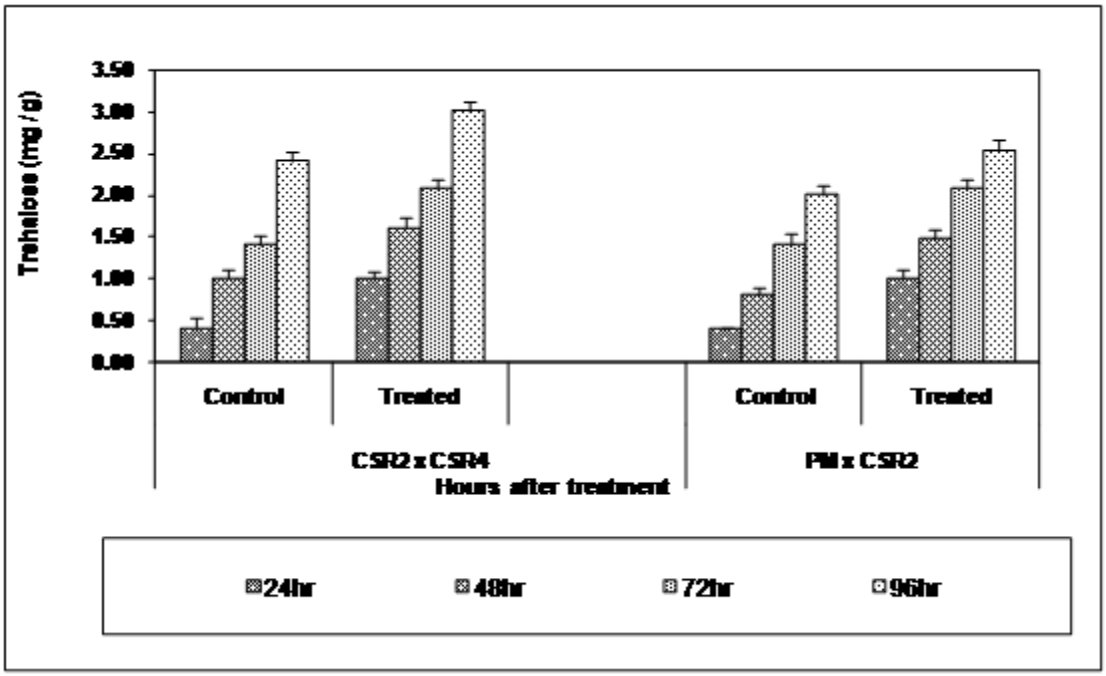

Fig1b : Effect of methoprene on the changes of trehalose content in fatbody of silkworm hybrids. Where CSR2 x CSR4 is bivoltine hybrid and PM x CSR2 is multivoltine x bivoltine hybrid. Values are mean ± SE based on three replicates (n = 3).

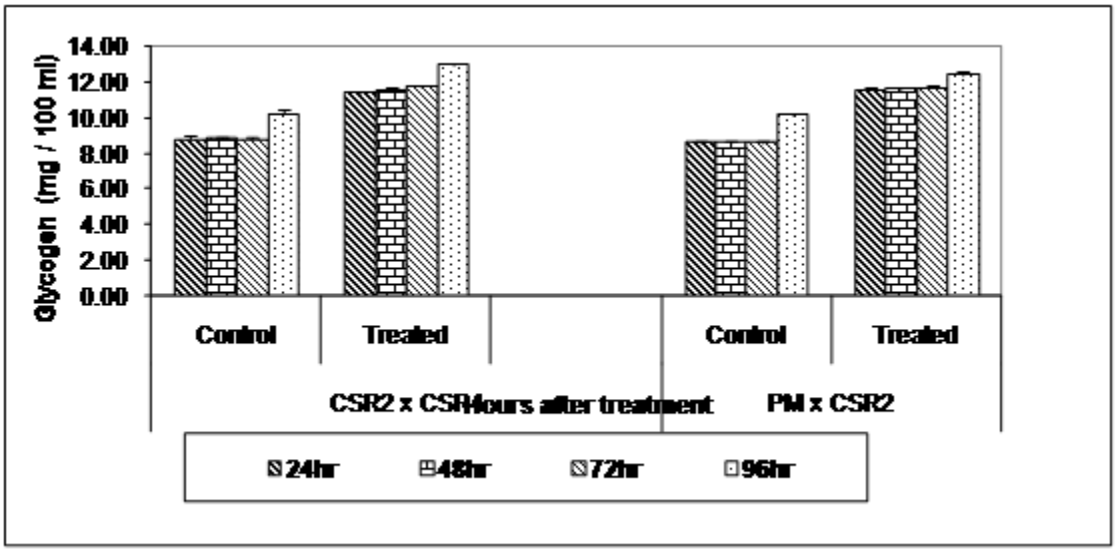

Fig.2a. Methoprene induced changes on the glycogen content in haemolymph of silkworm hybrids.Where CSR2 x CSR4 is bivoltine hybrid and PM x CSR2 is multivoltine x bivoltine hybrid. Values are mean ± SE based on three replicates (n = 3).

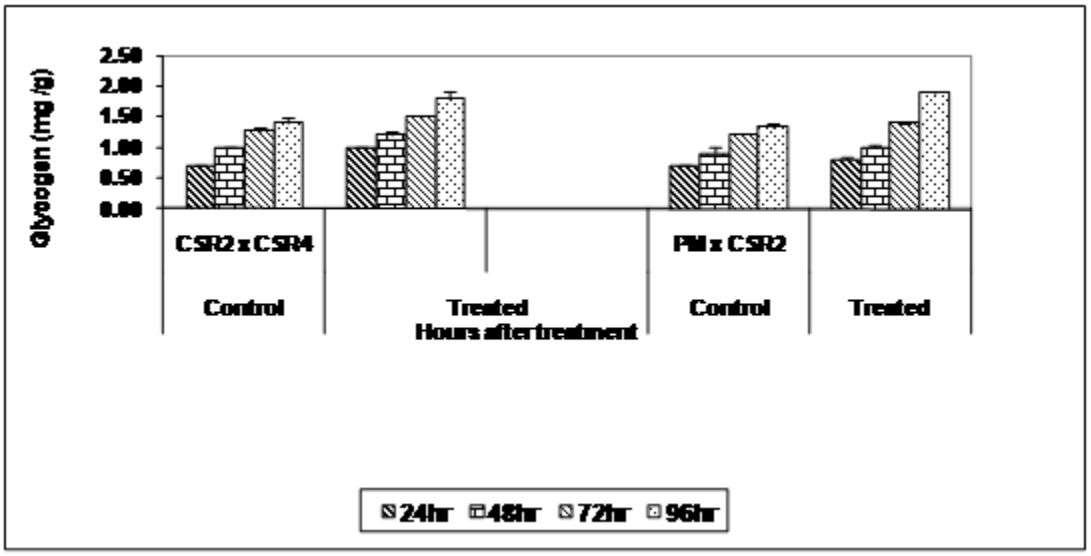

Fig2b: Methoprene induced changes on the glycogen content in fat body of silkworm hybrids. Where CSR2 x CSR4 is bivoltine hybrid and PM x CSR2 is multivoltine x bivoltine hybrid. Values are mean ± SE based on three replicates (n = 3).

Economic characters

The administration of methoprene (JH) brought out significant changes in larval period, cocoon weight and shell weight of silkworm larvae. It influenced the prolongation of 5th instar larval period by 24 hours in PM x CSR2 and 30 hours in CSR2 x CSR4. Besides, significant increase in the cocoon weight (6.45 and 10.30%), shell weight (9.25 and 14.53%) and subsequent increase in shell percentage (2.56 and 3.80%) as against the control 5th instar larvae of PM x CSR2 after 24 hours and in the 5th instar larvae of CSR2 x CSR4 after 48 hours respectively were noted (Fig.3).

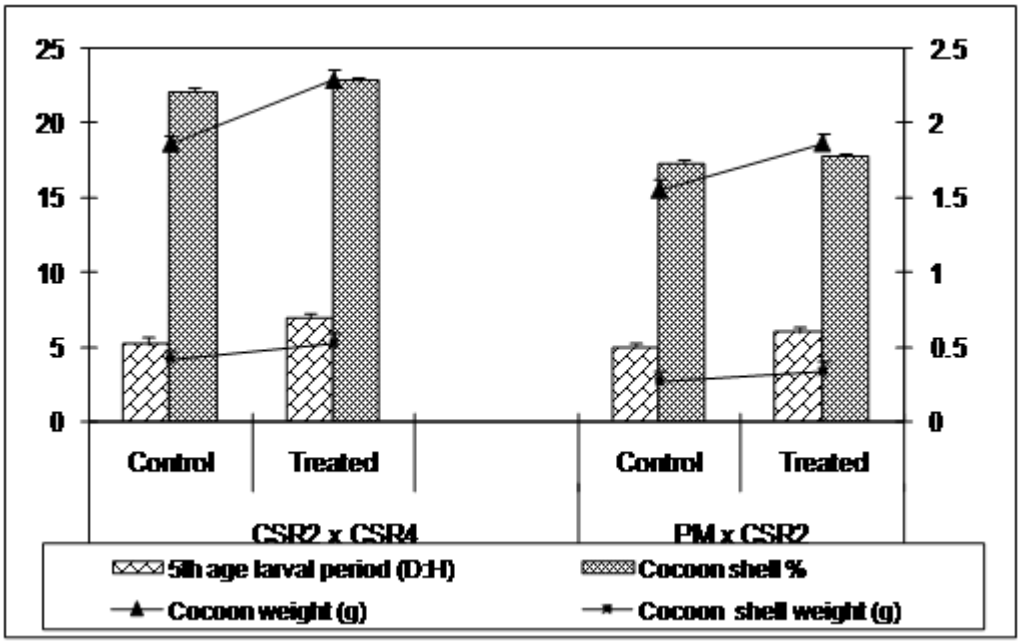

Fig 3.Methoprene induced changes of quantitative parameters of silkworm hybrids
Where CSR2 x CSR4 is bivoltine hybrid and PM x CSR2 is multivoltine x bivoltine hybrid. Values are mean ± SE based on three replicates (n = 3).

DISCUSSION

It is a well known fact that many factors such as weather conditions, feeding habit, diet, chemicals may affect the enzymatic and non-enzymatic compound of insect body (Zibaee *et al.,* 2008). Methoprene is a JH analogue, that has been used extensively in insect endocrinology to investigate JH regulation of morphology, reproduction, development, and metabolism (Smith and Nijhout 1981; Zera and Tiebel 1988; Wyatt and Davey 1996). Trehalose is a non-reducing disaccharide present in diverse organisms ranging from bacteria, fungi to invertebrates, in which it serves as an energy resource and membrane protectant. Glycogen is a polysaccharide and it is the principal storage form of glucose in animal cells.

This study demonstrates that juvenile hormone analogue, methoprene (JHA) is proved to be very active at low concentrations and induces physiological changes when applied to fifth instar larvae of silkworm, *Bombyx mori L.*. The physiological effects on insects caused by the application of juvenile hormone analogues vary according to the product rate (Cappellozza *et al.,* 1997), insect strain (Gaaboub *et al.,* 1985; Sarangi, 1988), application method and time (Kotikal and Devaiah, 1986). This analogue has a significant effect on glycogen content and trehalose in haemolymph and fat body tissues of *Bombyx mori* L. The trehalose and glycogen contents increase significantly in both the tissues of the silkworm larvae treated with JH methoprene. This observation corroborates the findings of Gordon and Burford (1984) where they reported that, application of juvenile hormone analogue (methoprene) significantly increased the concentration of carbohydrates in the haemolymph of the latter fourth-instar larvae and reduced the haemolymph carbohydrate concentration of 24-h-old pupae relative to controls in early fourth-instar larvae of *Aedes aegypti.* In *Gryllus firmus* decrease *in vivo* biosynthesis of total lipid and triglycerides, increase in absolute and relative biosynthesis of phospholipid, increase of oxidation of fatty acids, and decrease *in vitro* specific activities of six lipogenic enzymes and a transaminase after methoprene application was observed (Zero and Zhao 2004). This proves that JH alters the biochemical pathway in insects.

Further the increase of glycogen content in the fat body during feeding period *in Philosoma ricini* was observed, which may be due to the glycogenesis and metabolic shift from lipogenesis to glycogenesis in the fact body tissues of mid last instar larvae (Inagaki and Yamashita, 1986). Moreover, the amount of trehalose present in the haemolymph is directly related to the glycogen content of the fat body which is influenced by a number of endogeneous organic and inorganic factors (Kochi and Kaliwal 2006).

Hence, the increase in glycogen and trehalose content may be considered as a stimulatory effect of methoprene, as the trehalose and glycogen contents are influenced by various organic and inorganic factors (Downer 1979). Secondly it might be due to metabolic shift from lipogenesis to glycogenesis, where methoprene affects the lipid metabolism (Zero and Zhao 2004) resulting in reduction of total lipids due to the activity of lipogenic enzymes.

The present study also demonstrates that the application of methoprene causes increase in cocoon weight, shell weight, and extension of the fifth instar larval period. This has been reported by many investigators in silkworm using various JH analogues (Akai *et al*., 1985; Chowdhary *et al*., 1986; Nair *et al.,* 1998; 2008; Chengamma *et al.,* 2000). The extension is highly related to the quantity, i. e., increase in the quantity resulted in linear increase of the larval period of the treated larvae. Chatterjee and Datta (1992) have reported that the production of silk cocoon and other quantitative parameters of silkworm are very much dependent on the metabolism of the carbohydrates. This aspect is supported by our results which show that the methoprene treated larvae recorded higher quantum of glycogen and trehalose , and which inturn contribute to the higher cocoon weight and shell weight in silkworm. Moreover, the effect of the juvenile hormone analogue on insects is through the influence on their morphogenesis, i. e., causing an increment in weight gain, and a portion of this biomass weight gain is directed to the growth of silkgland and its metabolism (Kajiura and Yamashita 1989) resulting in increased cocoon weight and shell weight. Further according to Mamath *et al.* (2008), methoprene enhances protease, aspartate aminotransaminase (AAT) and alanine aminotransaminase (ALAT), adenosine triphosphate synthase (ATPase) and cytochrome-c-oxidase (CCO) activity levels in silkworm indicating overall surge of oxidative metabolism. Hence, in the present study stimulating capacity of the JHA hormones on various characteristic of the silkworm contributing to the quality silk yield .Our present study once again prove that the appropriate application of JH methoprene brings about significant improvement in silk production.

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