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EFFECT OF DIETARY AZASTEROIDS ON THE GROWTH AND DEVELOPMENT OF THE GRAM POD BORER *HELICOVERPA ARMIGERA* (HUBNER)

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ABSTRACT

Effect of two azasteroids, 25-azacholestane and 25-azacoprostane was studied on growth and development of an economically important phytophagous pest, *Helicoverpa armigera* (Hubner) causing extensive damage to crops like cotton, pigeon pea, chickpea and others. 25-Azacholestane caused an increase in larval mortality (36% in control to 52% at 25 ppm), decrease in pupation (64% in control to 48% at 25 ppm) and decrease in adult emergence (60% in control to 28% at 25 ppm). Larval and pupal duration was significantly more as the azacholestane concentration increased to 25 ppm ($p<0.001$). Similar results were observed in case of 25-azacoprostane treatment also. Both the azasteroids were found to have inhibitory effect on the growth and development of *H. armigera*. Formation of larval-pupal intermediates and adults with abnormal wings was also observed. Addition of 0.1% cholesterol along with 10 ppm of 25-azacoprostane in the diet reversed the inhibitory effect of the azasteroid.

Key words: *Helicoverpa armigera*, 25-azacholestane, 25-azacoprostane, sterol, cholesterol, larval duration, larval mortality, pupal duration, pupal mortality, adult emergence, metabolism, utilization, inhibition, IPM.

One of the basic tenets of insect biochemistry is the inability of insects to synthesize cholesterol de novo (Jing and Behmer, 2020; Li and Jing, 2020). Insects fulfil their cholesterol requirements from dietary sources which in case of phytophagous insects is the plant sterols (Rath and Agarwal, 1988; Jing and Behmer, 2020). Dominant plant sterols such as sitosterol, stigmasterol and campesterol are converted to cholesterol by the dealkylation pathway using several enzymes (Svoboda and Feldlaufer, 1991; Svoboda et al., 1995; Behmer et al., 1999). Cholesterol is essential for growth and development of insects being a constituent of cell membranes and as a precursor for ecdysteroid synthesis (Svoboda and Thompson, 1985; Tarlochan et al., 1998; Gilbert et al., 2002; Behmer and Nes, 2003; Toprak et al., 2020; Entringer et al., 2021; Goel et al., 2021). Azasteroids are known to inhibit the pathway of conversion of phytosterols to cholesterol, thereby affecting growth, moulting and development in some insects (Svoboda and Robbins, 1971; Svoboda et al., 1972; Thompson et al., 1975; Agarwal et al., 1990). These compounds also inhibit phytosterol biosynthesis (Darnet, 2020), have antifungal properties (Burbiel and Bracher, 2013). Therefore, the role of these azasteroids on sterol metabolism pathways, targeting insect growth, could be many folds. In insects, the hormonal regulation of vital processes such as metabolism, moulting, reproduction and diapause are unique which is not found in other higher groups of animals. Compounds which could target these processes, have specificity and high biological activity to higher animals could be a promising candidate for safe insect pest management practices. However, limited literature is available regarding effects of azasteroids on *Helicoverpa armigera* (Hubner). Therefore, two azasteroids namely 25-azacholestane and 25-azacoprostane were studied for their effect on the growth and development in *H. armigera* , a major and economically important pest of cotton, pulses and many other crops worldwide (Ravi et al., 2005; Haile et al., 2021). These compounds were given in the artificial diet to study their effect on the larval duration, larval mortality, percent pupation, pupal duration, adult emergence and any other abnormality. The azasteroids or similar compounds, targeting the requirement of sterols in insects, could be exploited as an alternative method for pest management (Kuthiala et al.,1987; Agarwal et al., 1990; Svoboda and Weirich, 1995) and also for better understanding of sterol biology in other animals including humans (Jing and Behmer, 2020).

MATERIALS AND METHODS

The larvae of *H. armigera* used in these studies (procured from culture maintained in Department

of Zoology, University of Delhi) were reared on an artificial diet (Rath, 1988). Due to the cannibalistic nature of the larvae, they were reared individually in glass vials (7.5 cm h x 2.5 cm dia.) plugged with cotton containing 7-9 ml diet, till pupation (Rath and Agarwal, 1988). The culture of *H. armigera* was maintained in the laboratory under the controlled conditions of temperature, 26.1± 2°C, 70± 5% RH and a photoperiod regime of 16L:8D (Rath, 1988). On pupation, the pupae were sexed and kept in glass jars (20 cm h x15 cm dia.) till adult emergence and egg laying (5 to 10 pairs/jar). Cotton soaked with 10% honey solution was placed in glass vials and hung on the sides of the jar. The freshly hatched first instar larvae from the stock culture were then transferred and reared till adult emergence on an artificial diet same as the stock diet which was fortified with varying concentrations of the azasteroids. The rearing conditions remained same as the stock culture.

The azasteroids tested individually were 25-azacholestane and 25-azacoprostane (gifted by Dr J A Svoboda, Insect and Nematode Hormone Laboratory, USDA, Beltsville, Maryland, USA) ranging from 5 to 50 ppm of the wet weight of the diet in case 25-azacholestane and 5 to 25 ppm in case of 25-azacoprostane. Insects reared on stock diet without the azasteroids served as control. Minimum of 25 larvae were tested for each concentration and 7 to 9 ml of the test diet was given to each larva. Daily observations were made on the larval mortality, larval duration, pupal duration, % pupation and adult emergence. Any other morphological abnormalities seen were also recorded. Another set of experiment was conducted in which the freshly hatched larvae of *H. armigera* were reared on an artificial diet same as the stock but fortified with 10 ppm of 25-azacoprostane and 0.1% cholesterol (CDH, purity>99%) of the fresh weight of the diet. Daily observations were made of the various parameters as above for the growth and development of these insects. Statistical analysis of the data was done using Mann-Whitney Rank Sum test.

RESULTS AND DISCUSSION

When 25-azacholestane was added to the artificial diet in concentrations ranging from 0 to 50 ppm, it was seen that the larval mortality increased from 36% in the control to 52% at 25 ppm of 25-azacholestane (Fig. 1). However, a further increase in 25-azacholestane to 50 ppm caused a decrease in larval mortality. Pupation decreased from 64% in control to 48% at 25 ppm. At 50 ppm azacholestane the pupation again increased to 64% (Fig. 1). The adult emergence also showed a similar pattern of decrease till 25 ppm but at 50 ppm increased to 52%, though still less than the control (Fig. 1). The larval duration significantly increased in the groups whose diet was treated with 5, 10 and 25 ppm of 25-azacholestane ($p < 0.001$) as compared to the control group (Fig. 2). However, a further increase of azacholestane to 50 ppm in the diet did not show any significant difference with that of control. In both male and female, the pupal period increased significantly at 5 and 10 ppm azacholestane ($p < 0.001$) as compared to the control. In case of male pupae, though the duration decreased at 25 and 50 ppm azacholestane as compared to 5 and 10 ppm, it was still significantly higher than control ($p = 0.045$) at 25 ppm. While no significant difference was observed at 50 ppm ($p = 0.094$). In female pupae, similar pattern like male pupae was observed, however, the duration was significantly higher at both 25ppm ($p = 0.009$) and 50 ppm ($p < 0.001$) of azacholestane as compared to the control.

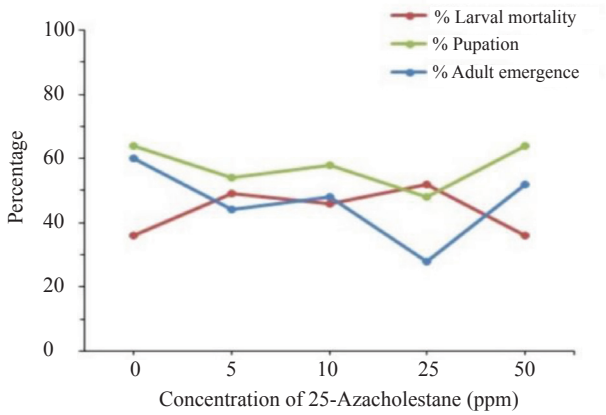

Fig. 1. Effect of 25-azacholestane on growth and development of *Helicoverpa armigera*

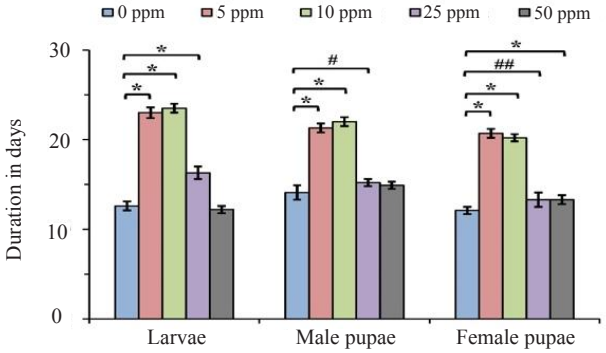

Fig. 2. Larval and pupal duration of *H. armigera* reared on an artificial diet containing 25-azacholestane. The data is presented in the bar graphs as mean± S.D. Statistical analysis was done by Mann-Whitney Rank Sum Test between the control and other groups for larvae, male pupae and female pupae individually *$p < 0.001$, ##$p < 0.01$, #$p < 0.05$.

Figures 3 and 4 show the effect of the second azasteroid, 25-azacoprostane on growth in *H. armigera*. When 25-azacoprostane was added to the diet at concentrations ranging from 0-25 ppm, the larval mortality increased from 10% to 27.3%. However, when 0.1% cholesterol was additionally provided in the diet containing 10 ppm of 25-azacoprostane, there was a reduction in the larval mortality to 16% (Fig. 3). The azacoprostane treatment caused a decrease in % pupation and adult emergence as compared to the control. In the presence of additional 0.1% cholesterol, the pupation and adult emergence increased to 84% and 72% respectively (Fig. 3). The larval duration significantly increased in the groups whose diet was treated with 5, 10 and 25 ppm of 25-azacoprostane ($p < 0.001$) as compared to the control group (Fig. 4).

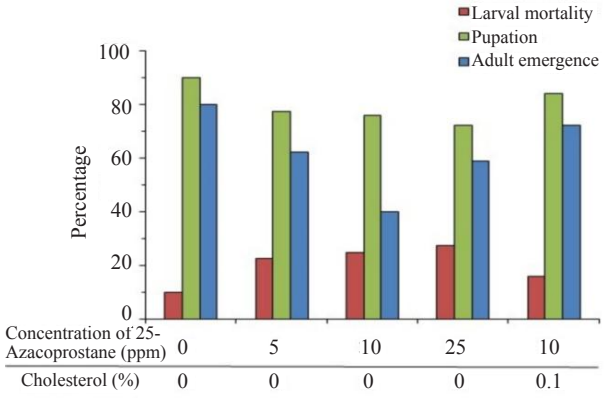

Fig. 3. Larval mortality, pupation and adult emergence of freshly hatched *H. armigera* larvae reared on an artificial diet containing 25-azacoprostane and cholesterol

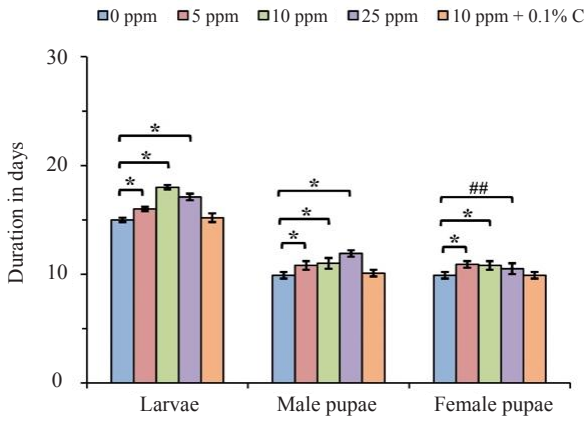

Fig. 4. Larval and pupal duration of *H. armigera* reared on artificial diet containing 25-azacoprostane and cholesterol**.** The data is presented in the bar graphs as mean± S.D. Statistical analysis was done by Mann-Whitney Rank Sum Test between the control and other groups for larvae, male pupae and female pupae individually.
*$p < 0.001$, ## $p < 0.01$.
C – Cholesterol

(a) dorsal view (b) lateral view

Fig. 5. Larval-pupal intermediate of *H. armigera* reared on an artificial diet containing 25-azacholestane or 25-azacoprostane

However, when 0.1% cholesterol was added along with 10 ppm of 25-azacoprostane in the diet, no significant difference in larval duration was observed as compared to control. The pupal period , in both male and female increased significantly with 5 ppm ($p < 0.001$), 10 ppm ($p < 0.001$) and 25 ppm (male pupae: $p < 0.001$; female pupae: $p = 0.009$) azacoprostane as compared to the control. In both male and female pupae, no significant difference in pupal duration was observed when 0.1% cholesterol was added with 10 ppm azacoprostane as compared to the respective control groups (Fig. 4). It was also observed that the presence of the azasteroids in the diet resulted in larval-pupal intermediates (Fig. 5) and abnormal wing formation in adults.

Compounds such as azasteroids are known to inhibit the metabolism of sterols in plants (Darnet et al., 2020), nematodes (Choi et al., 2003) and insects either by acting directly on the enzyme Δ^{24}-sterol reductase or at some other step in sterol metabolism, transport or utilization (Svoboda and Weirich, 1995). In insects, they block the formation of the moulting hormone or inhibit sterol transport and utilization which is essential for insect growth and development (Entringer et al., 2021; Toprak and Musselman, 2021). A retardation in growth and development due to azasteroids similar to that seen in our study on *H. armigera* has been reported in many insects (Al- Izzi and Hopkins, 1982; Kuthiala et al., 1987; Goel and Agarwal, 1987; Agarwal et al.,1990) and nematode, *Caenorhabditis elegans* (Choi et al., 2003). In some insects growth and development of the larvae was not inhibited even though the analyses of the sterols revealed that the Δ^{24}-sterol reductase enzyme was inhibited considerably. It appears then that a significant limitation in availability of cholesterol was not sufficient in itself to disrupt normal development but in addition ecdysteroid biosynthesis or metabolism might also be affected (Svoboda et al., 1972; Tarlochan et al., 1998; Gilbert et al., 2002; Entringer et al., 2021). Larval-pupal intermediates and abnormal wing formation in adults due to azasteroid treatment also indicate that the growth,

development and moulting of *H. armigera* is inhibited by these azasteroids. Similar results have been reported in other insects like *Diatraea grandiosella* (Chippendale and Reddy, 1973), *Anthonomous grandis* (Earl et al., 1967), *Epilachna varivestis*. (Walker and Svoboda, 1973), *Spodoptera litura* (Kuthiala *et al*, 1987). However at a higher concentration of 50 ppm of azacholestane, the larval mortality decreased in *H. armigera*. A lesser inhibitory effect of 25-azacholestane at higher doses is still obscure and may need further investigations. However, 25-azacoprostane had no effect on honey bee growth, development, sterol utilization or metabolism (Svoboda et al., 1987). When cholesterol was added to the diet in addition to the normal sterol content of the diet and 10 ppm of 25- azacoprostane, the growth of *H. armigera* was similar to that of control. This may indicate that the inhibitory effects of azasteroids on the growth of *H. armigera* could be a result of the reduction in the amount of cholesterol available to the insect due to azasteroids. This could be due to the inhibition of the enzyme Δ^{24}-sterol reductase and hence when additional cholesterol was added to the diet, the inhibitory effect of the azasteroid was nullified.

Similar studies of addition of cholesterol completely nullifing the inhibitive effects of 25-azacholesterol, has been reported in *Epilachna varivestis* (Walker and Svoboda, 1973). However, addition of cholesterol did not reverse the inhibitive effects of 25-azacholesterol on the boll weevil, *Anthonomus grandis* Boheman (Earl et al., 1967) and the housefly, *Musca domestica* (Svoboda et al., 1972). The ability of steroidal moulting hormone inhibitors or similar compounds to disrupt the pathway of sterol utilization and metabolism in agricultural and medicinal pests can be exploited for developing novel insect pest management practices (Yang et al., 2016; Entringer et al., 2021). Our studies also suggest azasteroids to have potential to inhibit sterol utilization and metabolism pathways as observed by the inhibitive effect on the growth and development of *Helicoverpa armigera*. The studies and investigations for alternative pest management strategies in general and for *H. armigera* in particular is an ongoing process. The use of biopesticides (Agale et al., 2021), botanical and semiochemicals (Edosa, 2019) and our present study using azasteroids could be explored as one of the futuristic approaches for pest management. Therefore, such studies with azasteroids and similar compounds along with field validations can open avenues to be exploited for safe pest management technologies. Research and studies on sterol metabolism and its inhibition in insects may also help to elucidate the knowledge of comparative biochemical and physiological processes of steroids in other organisms including humans.

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AUTHOR CONTRIBUTION

RR, RS, VG and RG helped to design and perform the experiments. RS and VG prepared the figures and analysed the results. RR wrote the manuscript. All authors have read the manuscript and agree to its publication.

CONFLICT OF INTEREST

RR, RS, VG, RG hereby declare that there is no conflict of interest.

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