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EFFECT OF JUVENILE HORMONE AGONIST ON THE ACCESSORY SEX GLANDS OF MALE *SPODOPTERA MAURITIA* BOISD

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ABSTRACT

Low concentration of pyriproxyfen, the juvenile hormone agonist dissolved in acetone was topically applied on the abdomen of the day 0 pupae (tanned) of male *Spodoptera mauritia* (Boisd) which is a sporadic pest of paddy to study its effects on the accessory sex glands (ASGs). The ultrastructure of the ASGs dissected out on day 6 appeared non- functional with degenerated epithelium showing large empty spaces, dense granules, condensed chromatin clumps, reduced number of RER and mitochondria in contrast to that of the control.

Key words: *Spodoptera mauritia*, sporadic pest, paddy, pyriproxyfen, acetone, insect growth regulators, IPM, tanned pupa, ultrastructure, lumen, reproduction, vesicular RER, secretory vesicles.

Insect growth regulators (IGRs) function in a variety of methods, including as chitin synthesis inhibitors, ecdysone agonists, and juvenile hormone mimics (Soin et al., 2010). Pyriproxyfen is an important IGR which mimics juvenile hormone of insects and disrupts metamorphosis and emergence of adults in the target insects. Hence, they have been used at large to check insect pests and as larvicide to control vectors (Devillers and Devillers, 2020). Though pyriproxyfen prevents maturation it is regarded to be less effective for mature insects (Hallman et al., 2015). Successful reproduction of many insects can be deranged by manipulating their reproductive development and physiology through judicious use of IGRs. Previous studies of Gillot (1988), Happ (1984) and Parthasarathy et al. (2009) have shown the importance of accessory sex glands (ASGs) in the reproductive success of insects. The present study was conducted to evaluate whether pyriproxyfen (PPN) which is a widely used juvenile hormone agonist, causes any specific disruptive effect on the development of accessory sex glands in the males of *Spodoptera mauritia* (Boisd).

MATERIALS AND METHODS

Stock colony of *S. mauritia* was reared in the laboratory on a diet of *Ischaemum aristatum* collected from nearby paddy fields. The insects were sexed and males were segregated in the 5th instar stage and were kept on the same diet and maintained at room temperature, RH 90± 3% and 12:12 light: dark photoperiod regime. Pyriproxyfen used was received from Dr V M Kannan, Biochemistry and Molecular Biology lab, Department of Zoology, University of Calicut, Kerala, India. Other chemicals were procured from the Chemind Laboratory Chemicals and Equipment, Thrissur, Kerala. The female moths were caught by light trap and used to raise the colony. The study was conducted on day 0 tanned pupae which were divided into experimental and control comprising of 10 pupae each. These pupae were topically treated with 2 µl of 0.04µg of pyriproxyfen (dissolved in acetone). The control pupae received equal quantity of acetone only. Both the control and experimental pupae were dissected open on day 6. The mid region of the ASGs were fixed in 3% gluteraldehyde in 0.1 M phosphate buffer (pH between 7.2 and 7.4). The fixation was pursued in the cold (4ºC) and processed for transmission electron microscope (TEM) studies. The ultrastructure studies were conducted in the TEM Lab of Sree Chitra Thirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.

RESULTS AND DISCUSSION

The ultrastructure of ASGs of the control pupae showed numerous mitochondria, a few tubular RER, abundant vesicular RER, Golgi apparatus, lipid droplets and many darkly stained secretory vesicles. The nuclei were larger with a few heterochromatin patches without prominent nucleolus (Fig. 1). The ASGs of the experimental pupae showed degenerated epithelium, condensed chromatin clumps in the nuclei, highly reduced number of RER and mitochondria. In some cells dense granules were found inside the nuclei. Large empty spaces were seen in between the cells. In addition

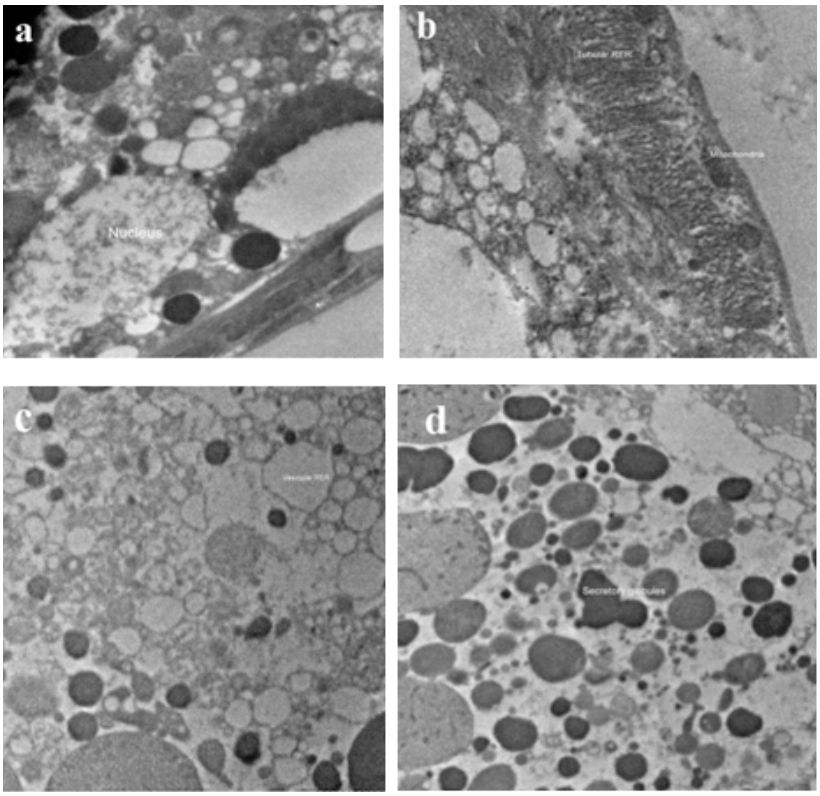

Fig. 1. Ultrastructure of ASG of control pupae showing
a) Nucleus, b) Tubular RER, Mitochondria, c) Vesicular RER, d) Secretory globules

to these, overall shrinkage of cells were observed (Fig. 2). In control pupae, ASGs are well equipped with protein synthetic machinery and are actively engaged in protein synthesis as seen in Day 0 adult as studied earlier by the author (Mathew, 2008). In the present study the ASGs of control pupae did not show a lumen or intracellular secretory globules. Though accumulation of secretion is not seen in the lumen at this pupal stage early studies of the author show that a fairly large sized lumen filled with many secretory globules is seen in the day 0 adult. Previous studies show that the relationship between the appearance of abundant RER and secretory globules can be attributed to the high synthetic activity of the cell (Federer and Chen, 1982). Vesicular RER may represent the intracellular storage of the secretory materials (Gillot, 1988). The presence of large number of RER was reported in the accessory sex glands of *Ceratitis capitata* and *Achoris grisella* (Marchini, 2003; Fernandez and Cruz –Landim, 2005). The occurrence of numerous mitochondria are also reported in ASGs (Lai Fook, 1982; Fernandez and Cruz –Landim, 2005).

JH plays a critical role in the reproduction of male insects through their accessory gland secretions. But it is also reported that juvenile hormone or its analogues/ agonists, when applied during the vulnerable period of physiological development, can disrupt the normal growth and development (Negeshi et al., 1976). Pyriproxyfen inhibits the emergence of adults in *Aedes aegypti* (Abdullah and Bary, 2022). In *Bombyx mori*, pyriproxyfen treatments have affected the normal development of ovary and caused reproductive disorders (Qian et al., 2020). Pyriproxyfen reduced pupation rate and inhibited adult emergence in *Anopheles gambiae* (Bahati et al., 2022). The early studies of the author in *S. mauritia* showed that the topical treatments of 0.1 µg PPN on the newly ecdysed pupae resulted in adultoids (adults failed to emerge from the pupal case) and 1 µg PPN on the newly ecdysed pupae resulted 100% mortality (Mathew, 2008). In the current study, it is well evident that the topical application of pyriproxyfen caused considerable disruption in the development of the male ASGs. The chromatin clumps inside the nucleus is an indication of necrosis and pyknosis. Empty spaces in the cells are clear signs of degeneration. Previous studies show that in *Tenebrio molitor* development and differentiation of ASGs occurring in pupal stage are characterized by two bouts of mitosis (Happ and Happ 1982; Happ et al., 1985). The second bout of mitosis coincides with the peak of ecdysteroid in the pupal stage (Delbecque et al., 1978). It is assumed that in the present study, the topical application of pyriproxyfen

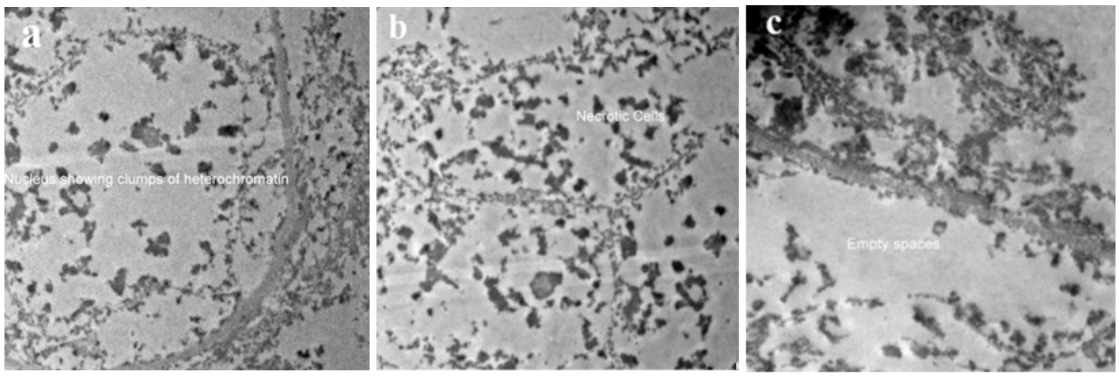

Fig. 2. Ultrastructure of ASG of treated pupae showing a) Nucleus showing clumps of heterochromatin, b) Necrotic cells, c) Empty Spaces

might have caused an imbalance in the endogenous critical titre of the juvenile and ecdysteroid hormones adversely affecting the natural bout of mitosis.

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