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ORIGINAL RESEARCH ARTICLE

Evaluation of the histopathological and biochemical effects of fenoxycarb in the ovaries of *Spodoptera mauritia* (Lepidoptera: Noctuidae)

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Abstract
Insect development is disrupted by juvenile hormone (JH) and their mimics in several ways. Many of them have been thoroughly investigated and are already being used to manage pest insects with commercially available agents. This research aims to explore the potentials of juvenile hormone analogue fenoxycarb on morphological, histopathological, and biochemical changes in the ovary of *Spodoptera mauritia*. Newly emerged female pupae were treated topically with sublethal doses (LD_{10}, LD_{25}) of fenoxycarb to determine their effects on reproduction. The results reveal that this juvenile hormone analogue affects the normal development of the ovary tissue by reducing the number of oocytes and oogonia in the ovaries of *S. mauritia*. Fenoxycarb treated pupae showed a substantial decrease in the reclaimed adultoids ovaries development and reduction in length of ovarioles, area of basal oocytes and the total number of eggs laid. A microscopic examination exhibited reduced pulsating movements, tumour-like bulbous masses and the germarium region exhibited hypertrophy. Histological investigation of ovaries indicated a degeneration of ovarian follicle cells, deformed oocytes with deteriorated trophocytes, malformed egg chamber, vacuolated ooplasm and defective vitellogenesis in malformed adult female's ovarioles. The effect of fenoxycarb could be correlated with quantitative depletion of proteins, lipids, and carbohydrates in gonads of the treated groups and there were no significant changes in sodium dodecyl sulphate(SDS)- protein pattern. This study forms baseline data suggesting that fenoxycarb respond considerably for the control of the lepidopteran pest of paddy (*S. mauritia*) effectively.

Keywords Juvenile hormone analogue · Fenoxycarb · *Spodoptera mauritia* · Ovarian biochemistry · Ovarian histology · SDS-protein pattern

Introduction

Insect juvenile hormones (JH) are essential for development and reproduction.The hormone retains larval characteristics of the insects that make it possible for the larval form's continued growth (Wigglesworth 1964). Further, it can also act like an adult gonadotropin (Dahm et al. 1976). JH plays a prominent role in coordinating the different tissues activities to produce the gametes and behaviours related to mating and oviposition occurring at the appropriate time. This involves JH responses in the fat body, nervous system, muscles, gonads and reproductive accessory glands. Juvenile hormone prevents significant morphological and physiological changes from the larva to the adult. It has entirely different adult female functions, regulating oocyte growth and maturation, including vitellogenin production and its uptake by developing oocytes (Tobe and Stay 1985).

Insects treated with exogenous JH during low endogenous JH titer disrupt metamorphosis and ovaries development. Additional doses of either juvenile hormones or juvenoids administered to larvae and pupae cause severe abnormalities in the ovaries (Metwally et al. 1972; Rohdendorf and Sehnal 1972). Synthetic formulations of Juvenile hormone, fenoxycarb is a potent juvenile hormone analogue (JHA) that prevents insects from attaining the reproductive stage. The destructive effect of juvenoids overpowers the homeostatic

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effects. A second mechanism may be the juvenile hormone's imperfect mimicking ability, leading to its antagonistic action that disrupts the moulting and slows down the developmental process (Bruce and Gary 1981) and also inhibits ovarian development (Govind 2014).

In the last two decades, studies on the insect growth regulators were mainly targeted on the biochemical and organism level mode of action. It is well known that many of the IGRs affect insects by controlling or inhibiting particular biochemical pathways or process essential for insect development and growth. The biochemical effects of IGRs are crucial for understanding the mode of action of a specific IGR. Even though the endocrine system is the first site of action for JHA insecticides, several biochemical and physiological changes in different metabolic pathways have been reported (Kim 2001; Leonardi et al. 2001). The biochemical effects of juvenoids are complex and differ from one analogue to another, as they act as juvenile hormone agonists or antagonists or both. The JHAs interfere with essential biochemical mechanisms such as the secretion and transportation of natural JHs from the secretion site to the target site, degradation, excretion and feedback control. The response to various analogues also differs between species (Mondal and Parween 2000).

In insects, the nurse cells transfer nutrients to the growing oocytes for female reproductive maturation. Yolk formation is a vast process that results in a 100-fold increase in each oocyte volume over many days. It exhausts the reserves of proteins, lipids, and carbohydrates accumulated by larvae and stored in pupae. A period of rapid yolk deposition, or vitellogenesis, is followed by the relatively slow oocyte growth period. The oocyte obtains nutrients and genetic determinants during insect oogenesis to promote embryonic growth and egg maturation. The oogenesis of insects is divided into distinct stages, such as oogonic previtellogenesis, vitellogenesis and choriogenesis. Different biochemical and morphological changes occur during these stages in the oocyte. These phases are controlled by juvenile hormones (JH), ecdysteroids, and neurosecretions to varying degrees (Nijhout 1994). In insects, the juvenile hormone is a suitable candidate for researching the flexibility of endocrine responses to intraspecific conflicts as it has significant effects on both reproductive and behavioural processes. JH plays an important and diverse role in the reproduction of adult insect species and their nutrition physiology (Wheeler 1996) by controlling vitellogenesis in the fat body and ovary vitellogenin uptake (Tobe and Stay 1985; Nijhout 1994).

Further, the juvenile hormone titers transport, and feedback regulation in many pest insects seemed altered by the JHAs. For example, the rice swarming caterpillar (*S. mauritia*) is a major pest and creates extreme paddy damage in the nursery stages. The loss of production due to this pest is very high in the southern part of India. The pest species *S. mauritia* has been used for primarily toxicological evaluation of JHA, fenoxycarb (Banu et al. 2019a, b). The present study is an extended work of the same and mainly focused on the effects of fenoxycarb on histopathological variation and certain biochemical aspects. Therefore, the primary aim of this current study, is to clarify the role of the sublethal doses (LD_{10}, LD_{25}) of fenoxycarb on the ovarian development of *S.mauritia*.

Materials and methods

Test organism

Collection, rearing, and maintenance: The fertilized adults of *S. mauritia* were collected from paddy fields by using light traps. The adult moths were fed with 10% honey solution. Mating pairs were kept in separate beakers covered with muslin cloths for colony maintenance**.** The females laid eggs on the cloth and sides of the beaker, and the eggs hatched into larvae in 2–3 days. After then the larvae were reared in plastic tubs and were fed with the alternate host plant, *Ischaemum aristatum* and reared in the laboratory, at room temperature and $90 \pm 3\%$ RHand 10:14 light:dark photo-periodusing a light source (500 lx X 2).In the later stage(from 3rd day) of the 6th instar male larvae, the yellow coloured testis is externally visible on the dorsal side through the slightly transparent skin. By checking the presence of testis, male and female larvae were separated. Females were maintained in separate labelled tubs and allowed to pupate.

In the present study, 0-1 h old newly molted untanned pupae of *S. mauritia* females were selected for the treatment to assess the effect of excess titer of the JHA, fenoxycarb.

Chemical

The experiment was performed with the juvenile hormone analogue, **Fenoxycarb** (Ethyl (2- (4 phenoxyphenoxy)-ethyl) carbamate) analytical standard, purchased from Sigma Aldrich.

Treatments of pupae with fenoxycarb

The newly ecdysed (0–1 h) untanned pupae of *S. mauritia* were obtained from laboratory stock culture. The fenoxycarb dissolved in acetone, was subjected to topical treatments with a series of doses(0.001 µM to 10 µM) and the LD values was calculated (Banu et al. 2019b), the obtained LD values were used for the present study. In this experiments two concentrations LD_{10} and LD_{25}(0.4 ng and 0.7 ng) of the compound were used. Using a Hamilton microsyringe,

5 µl of the compound was applied topically to the abdominal area of freshly ecdysed pupae. The control pupae were maintained with equal quantities of solvent. Four replicates (25 pupae in each replicate) were used for each concentration. The experimental and control pupae were kept in separate beakers covered with muslin cloths and checked daily for mortality (by checking its undulating movement of the posterior area when gently touched on the head head surface & further verified by noting their adult emergence after eight days) and morphological abnormalities until adult emergence. The emerged female adults of day two old were taken for further analysis.

Surgical techniques

The day two (48 h) old adult females emerged from treated pupae, and controls were anaesthetized by using ethyl acetate and pinned with the dorsal side in a wax-lined Petri dish for dissection. The dissections were performed with sterilized instruments in insect ringer's solution (NaCl—0.65 gm, KCl—0.025 gm, CaCl—0.03 gm, $NaHCO_3$—0.025 gm dissolved in 100 ml distilled water). A longitudinal cut was performed on the dorsal side of the abdomen, then vertically cut opened the abdomen and removed the ovaries using fine forceps under a stereoscopic microscope. The isolated ovaries were rinsed with insect ringer solution and subjected to further analysis.

Sublethal effects of fenoxycarb on the ovary of *S. mauritia*

Gonadosomatic index (GSI)

The isolated ovaries obtained from experimental as well as control groups (10 individuals each) were rinsed with insect ringer solution and fat bodies removed, blotted dry with filter paper, and the weight was measured using a digital weighing balance (Shimadzu). The gonadosomatic index (GSI) was calculated using the equation of (Strum 1978).

$$\text{GSI} = \frac{\text{Weight of gonad}}{\text{Weight of insect}} \text{x} 100$$

The experiments were repeated four times, and the mean value of GSI was calculated for each experimental group from the data obtained from 10 individuals.

Length of ovarioles

The newly molted pupae were treated with two doses of fenoxycarb ($LD_{10,}$ LD_{25}), and the linear growth of the ovary was studied after 48 h of adult emergence. The total length of ovarioles of 48 h old control and treated female adults was measured by stretching the ovary on a glass slide using graph paper under a dissection microscope. The length of ovaries from treated groups was compared with the control and vehicle control groups.

Area of basal oocyte

The experimental and control sets of the ovaries were subjected to the basal oocyte area measurements with the Image processing software, Fiji Image J (Schindelin et al. 2012).

Histopathology of the ovary

Ovaries of day two adults were dissected out and transferred to Bouin's fluid. They were then separated into individual ovarioles, dehydrated in graded alcohol series, infiltrated with Paraffin wax (melting point 58°C), and finally embedded in wax. The ovary samples were cut into serial sections of 5 µm thickness using a Reichert Rotary Microtome, and the sections were stained with hematoxylin and eosin. The sections of control and treated ovaries were photographed under a ZeissAxio-Scope A.1 microscope.

Egg lying capacity

Adults who emerged after pupal treatment with two doses ($LD_{10,}$ LD_{25}) of fenoxycarb were allowed to mate with normal males. Mating pairs were separated and kept in separate glass beakers covered with muslin cloths. The fertilized females lay eggs on the cloth and sides of the beaker. The effect of fenoxycarb treatment on egg-laying capacity was noted. The number of eggs laid was counted from control and treated sets and was recorded for statistical analysis.

Biochemical analysis

Protein quantification and SDS-PAGE profile

Ovarian tissues (3 ovaries were pooled and weighed) were homogenized in 2 vol of 10 Mm phosphate buffer, pH 6.8, and supplemented with protease inhibitor PMSF at a concentration of 1 Mm. The homogenates were centrifuged at 4 °c for 20 min at 20,000 rpm, and the supernatant was subjected to analysis.

Bradford (1976) method was used to quantify protein, with Bovine Serum Albumin (BSA) as the standard. For the micro method, 0.5 ml of protein solution was added to the 0.5 ml of dye and mixed. Optical density (OD) was taken at 595 nm with a microplate reader (Synergy™ HT, Bio-Tek Instruments and calculated the protein concentration in the extract by comparison with the standard curve for BSA. The estimated protein values were calculated in µg/µl.

Treated and control tissue samples were denatured in sample buffer (Lammeli 1970). The samples were analyzed on 10% SDS-PAGE in a mini vertical system (Genie). Electrophoresis was performed at a constant current of 15 mA in stacking gel and 25 mA in separating gel. The stained gels were photographed using a canon camera. The approximate molecular weight of bands and intensity were analyzed using Fiji Image J software.

Lipid determination

Lipid estimation of the treated samples was assayed using the protocol of (Van Handel 1985). Standards and samples were transferred into a 96 well plate and read at 490 nm in a SYNERGY HT microplate reader.

Carbohydrate determination

A phenol–sulfuric acid (Gerhardt et al. 1994) method was used to quantify carbohydrates, with glucose as the standard. Read the OD at 630 nm in Synergy HT microplate reader.

Statistical analysis

The data were analyzed using descriptive statistics, the SPSS version of the package (IBM SPSS 20). The statistical significance of differences between individuals was determined using a one-way ANOVA test.

Results

Sublethal effects of fenoxycarb on the ovary of *S. mauritia*

Gonadosomatic index (GSI)

The vital measurement to indicate gonadal development and maturity is an essential indicator of an organism's health. In the present analysis, the fenoxycarb treated pupae of *S.mauritia* showed a substantial decrease in the reclaimed adultoids ovaries development. In the control and vehicle or acetone treated control groups, the female's GSI values were 37.29 ± 1.44 and 35.59 ± 1.05.GSI compared to 28.61 ± 1.28 in a lower dose (LD_{10}) and 22.01 ± 1.68 in a higher dose (LD_{25}). Data show a significant dose-dependent decrease in fenoxycarb treated groups (Fig. 1).

Morphological features of ovary

The microscopic examination of the *S. mauritia* (control) adult ovaries showed a pair of polytrophic meroistic ovarioles. Each ovary comprises four elongated functional units

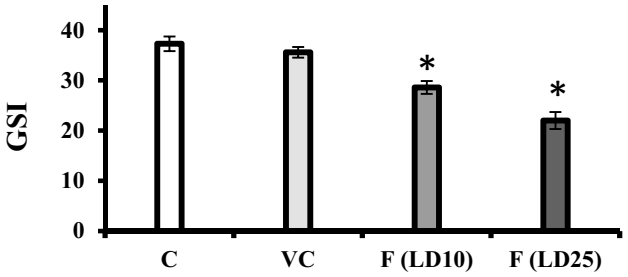

Fig. 1 Gonado somatic index (GSI) of control and fenoxycarb treated females pupae of *S. mauritia*

in which oogenesis occurs. As a rule, four easily identifiable regions are distinguished in the individual ovariole: a terminal filament, germarium, vitellarium, and an ovariole pedicel connecting the ovariole to the lateral oviduct. The two lateral oviducts link to the median oviduct. In addition, a chain of developing ova witha spherical shape was found in the normal ovariole. The critical structures associated with the female reproductive system are the bursa of copulatrix, spermatheca, paired colleterial glands, two genital openings with ovipore and vulva.

The sublethal doses of fenoxycarb ($LD_{10,}$ LD_{25}) applied to newly moulted pupae caused them to emerge as two-day-old abnormal adult females or adultoids (Morphological malformations in pupal instar includes abnormal pupae unable to moult into adults, Larval pupal mosaics, abnormal pupae with the deformed body); ovaries dissected showed marked abnormalities. In contrast with the controls, fenoxycarb's application resulted in incomplete ovarian development, and numerous anomalies, such as defective ovaries with fewer ova found (Fig. 2); the ovarioles exhibited reduced pulsating movements. A transparent thread like tube with tumour-like bulbous masses was found in the basal part of the ovarioles. Moreover, several constrictions were seen along the ovarioles.The germarium region exhibited hypertrophy in the higher dose-treated samples. The pedicel and the lateral oviducts were very much reduced in the treated sets.

The fenoxycarb effect on ovarian size was dose-dependent, and pronounced reduction in length in treated groups was evident compared to controls (Table 1).

Effects of fenoxycarb on the ovarian histopathology

The treatment of *S. mauritia* pupae with fenoxycarb showed, severe histopathological changes in the malformed adult female's ovarioles. Several abnormalities were observed in the early and late stages of oocyte development. Numerous small and large-sized vacuoles were noted in the trophocytes and the oocytes. The adult ovariole of control insects had developing oocytes, occupying one-third of the nurse-cell

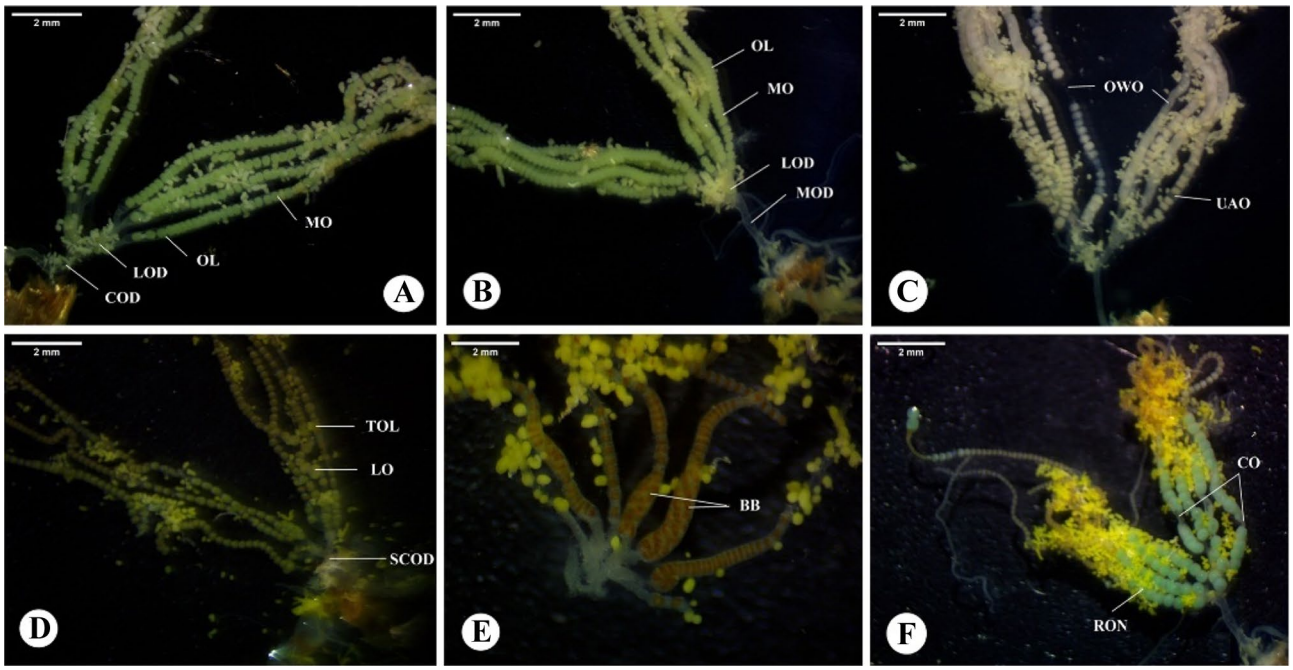

Fig. 2 Ovarian morphology of *Spodoptera mauritia* on post-treatment with fenoxycarb

oocyte complex containing trophocytes and follicular epithelial cells. The trophocytes of the control ovaries were composed of trophocytic nuclei, and each egg enclosed in the egg chamber. Section through the bulbous base of an ovariole removed from the treated sample showed the following abnormalities, the presence of undifferentiated follicles arranged in different rows, all follicles present in the compound egg chamber were highly disorganized, the follicular epithelial cells were enlarged and ruptured in appearance, the multilayered follicular epithelium was evident in the compound egg chamber's follicles and with ooplasm filled with numerous vacuoles of varying sizes, each follicular strand differs from the other in its shape; poorly deposited yolk in the oocytes (Fig. 3).

A normal ovariole contains ovarian follicles with vitellogenic oocytes surrounded by follicle cells and trophocytes. The treated set showed the follicle change to rectangular, and trophocytes compress to form a disc-like cap. The deteriorated oocytes are surrounded by follicular epithelial cells and undergo necrosis. The oocytes exhibited curved egg surface formed as a hollow cavity, with malformed follicle cells, and a lack of proper arrangement of the egg chamber. Malformed vitellogenic oocytes with vacuoles due to vitelline loss is also evident. The fenoxycarb treated ovarioles showed a deformed oocyte with folded follicular epithelium and vacuolization within the ooplasm (Fig. 4). Higher magnified section of follicle in the middle region of untreated ovariole with vitellogenic oocytes containing yolk granules. In fenoxycarb treated individuals, the ovaries displayed degenerated oocytes with a mesh of vacuoles in the ooplasm. The folded follicular epithelium and enlarged spaces between oocyte and follicular cells are evident. Most deformed oocytes are characterized by a wavy surface and follicular epithelium that are widely separated and abnormally arranged. Significant gaps between the oocyte and follicular cells and numerous small peripheral vacuoles within the ooplasm are evident (Fig. 5).

Histological studies show that the follicles are in the advanced stage of maturation. The follicular epithelium becomes flattened and shows signs of hypertrophy. The oocyte

Table 1 Effect of fenoxycarb on Length of ovarioles and basal Oocyte Area of 48 h old adult *Spodoptera mauritia*

	Control	Vehicle Control	Fenoxycarb (LD_{10})	Fenoxycarb (LD_{25})
Length of ovarioles(mm)	61.02 ± 1.05^{c}	60.41 ± 0.94^{c}	56.125 ± 0.91^{b}	49.77 ± 1.18^{a}
Basal Oocyte Area (mm^2)	80.26 ± 0.54^{c}	79.59 ± 0.46^{c}	70.03 ± 1.85^{b}	61.78 ± 1.67^{a}

Data expressed Mean ± Standard Error; The data were analyzed by one-way ANOVA using SPSS software (IBM SPSS 20). Values that are followed by different letters with the column are significantly different ($P < 0.05$, Post hoc test)

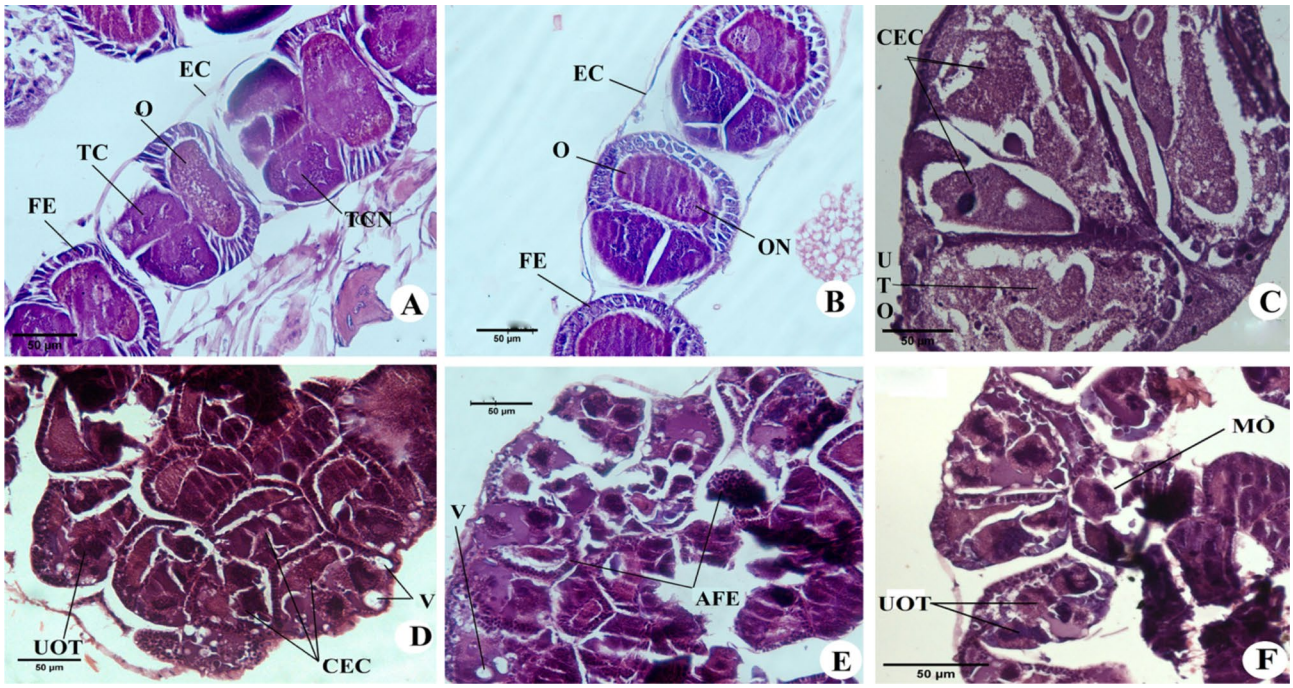

Fig. 3 Section through the basal part of 48 h old adult and adultoid ovariole: Developing oocyte (O) with oocyte nucleus(ON) surrounded by follicular epithelium (FE). The trophocytes with trophocytic nuclei (TCN). Each egg enclosed in an egg chamber (EC)

showed loss of vitelline,which resulted in numerous vacuole formations and appeared like a mesh inside the ooplasm. Deformed oocytes are observed with the abnormal chorion, interconnected oocytes (ICO), and fusion of ooplasm, malformed egg chamber, and irregular shape (Fig. 6).

Examination of the basal region revealed that mature oocytes showed a prominent vitelline membrane and ooplasm filled with highly basophilic yolk bodies. Fenoxycarb treated samples shows irregularly shaped oocyte with vacuolated ooplasm (Fig. 7). Mature basal oocytes

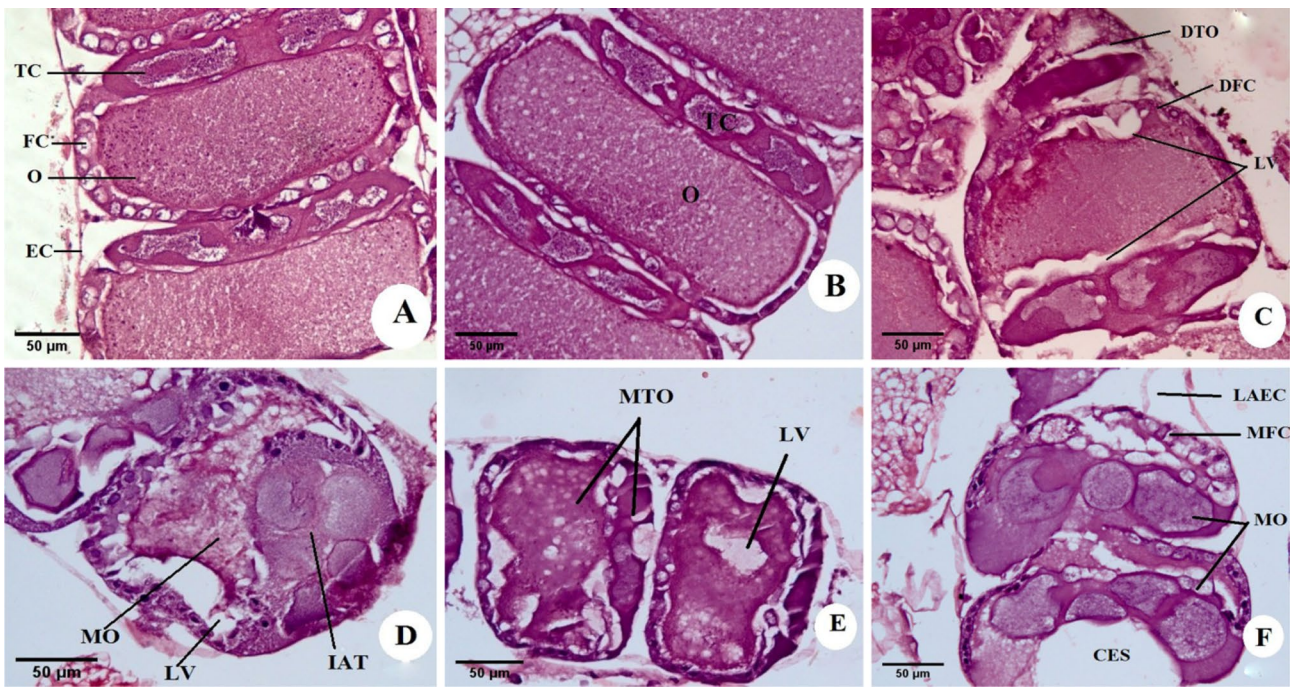

Fig. 4 Section through the middle part of the ovariole:Showing vitellogenic Oocyte (O) surrounded by follicle cells (FC) and Trophocytes (TC)

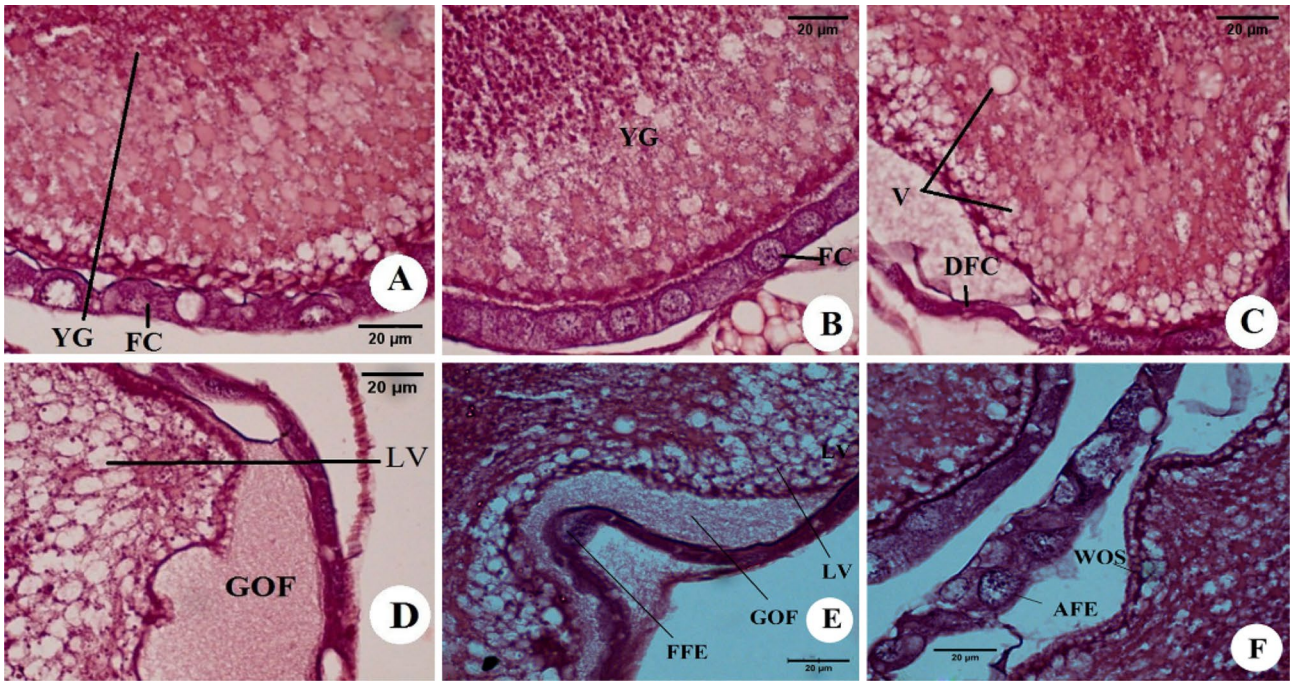

Fig. 5 Higher magnification of the section of follicle through middle region of ovariole: Vitellogenic oocytes containing yolk granules (YG) and follicular cells (FC)

are filled with yolk granules and surrounded by a thick vitelline membrane. A higher concentration of fenoxycarb treated samples showed oocyte cytoplasm with numerous small peripheral vacuoles and a large area at the centre. Also, oocytes revealed cavities, invaginated oocyte surface, and folded vitelline membrane (Fig. 8).

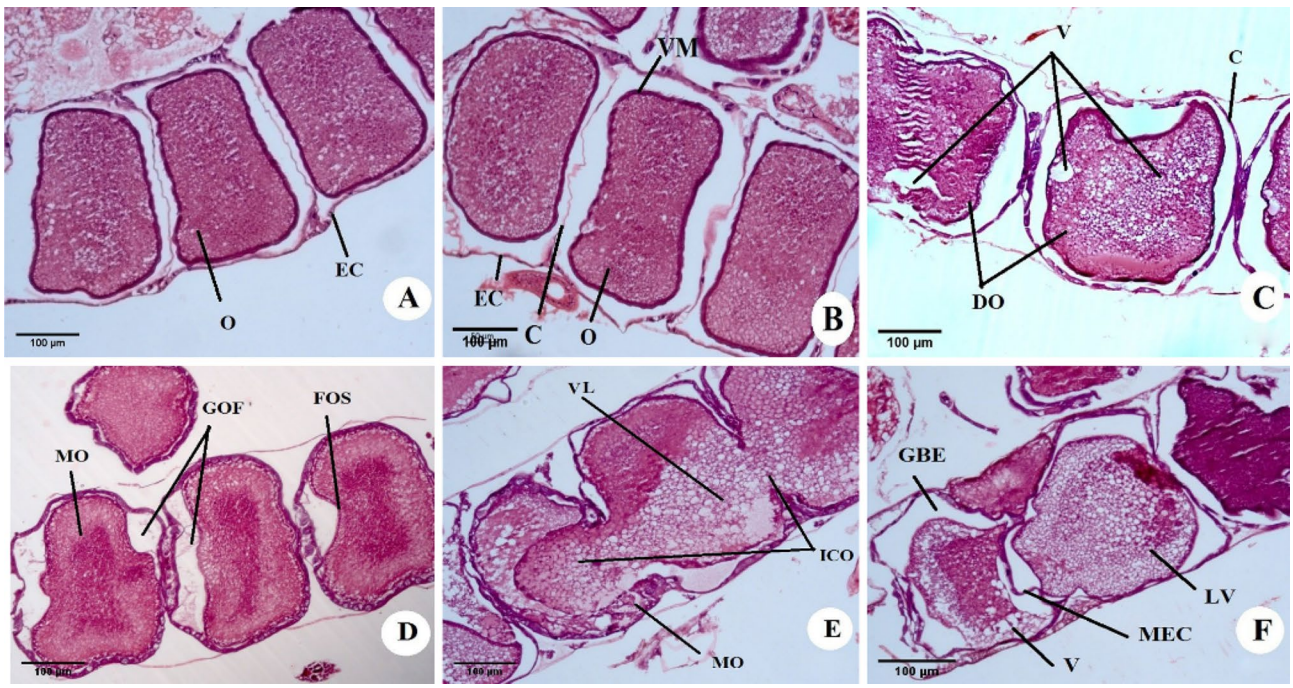

Fig. 6 Section of ovariole representing the general structure of mature oocyte enclosed within the vitelline membrane (VM), chorion (C) egg chamber (EC)

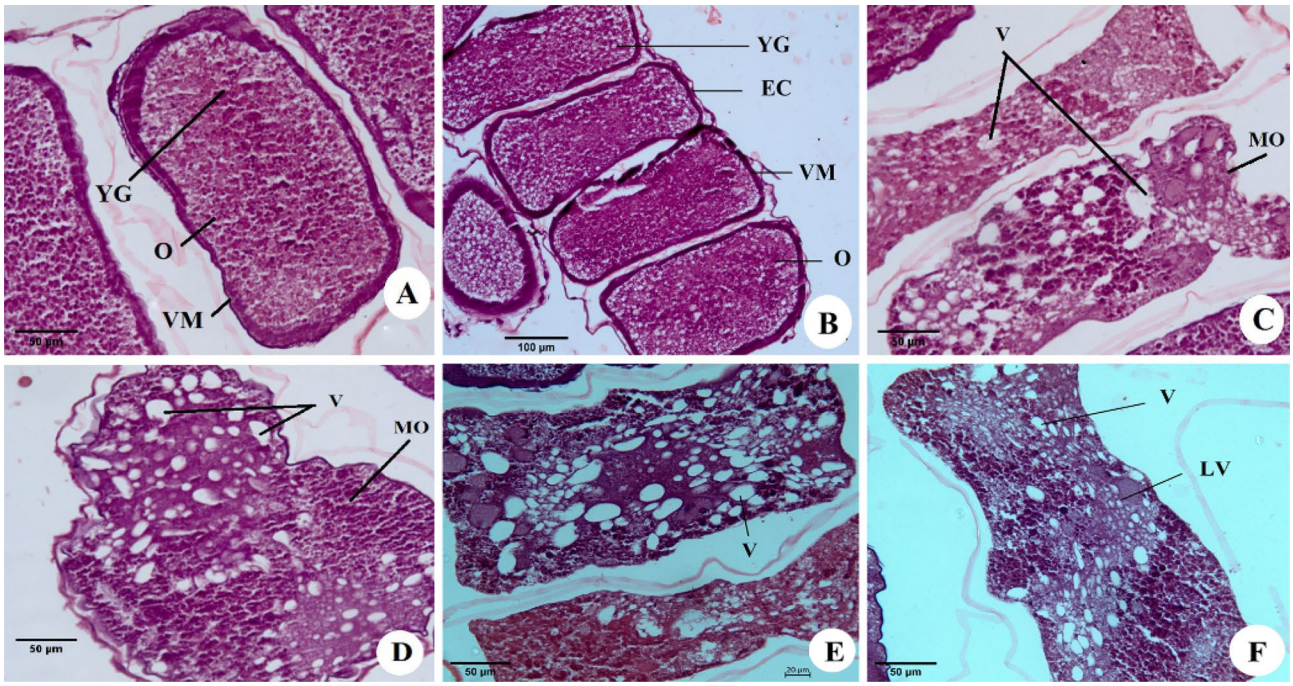

Fig. 7 Section of ovariole with basal oocytes with ooplasm containing full of yolk granules (YG) and bordered by the vitelline membrane (VM)

Area of basal oocyte

The area of basal oocytes was recorded from the histological sections of the 48 h old adult that emerged from treated, untreated(control) and acetone treated pupae; the result demonstrates a significant reduction in basal oocytes area in the LD_{10} and LD_{25} treated groups when compared to the control and acetone treated controls (Table 1).

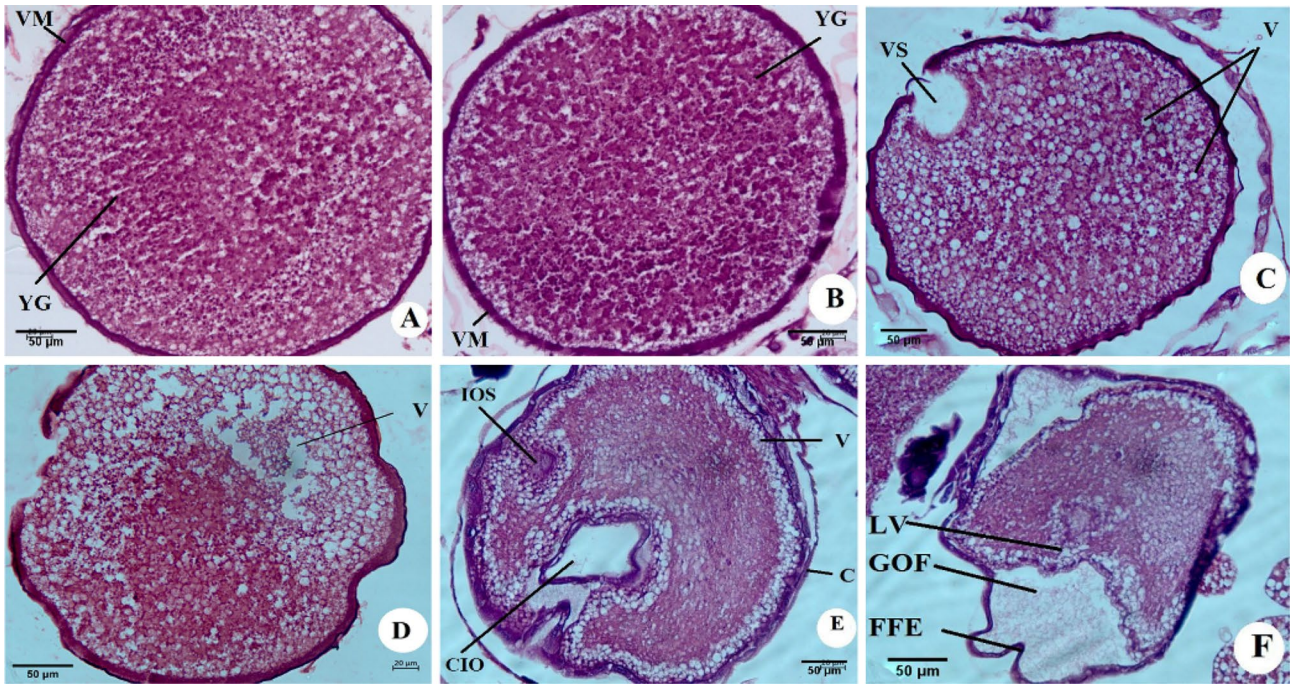

Fig. 8 Transverse section of mature basal oocytes filled with yolk granules (YG) surrounded by a thick vitelline membrane (VM)

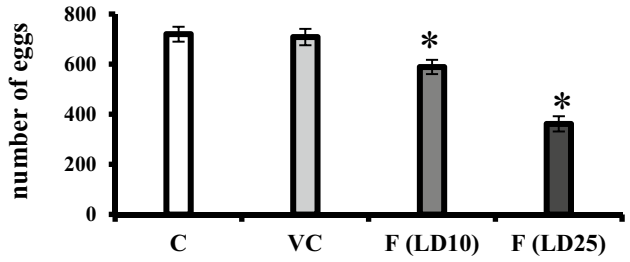

Fig. 9 Rate of the oviposition of control and fenoxycarb treated *S. mauritia*

Egg lying capacity

The total number of eggs per control and acetone treated female was 720 ± 29.77 and 708 ± 32.7, respectively. In fenoxycarb-treated individuals,the total number of oviposited eggs was significantly reduced to 589 ± 28.35 and 361 ± 30.32 for lower (LD_{10})and higher doses(LD_{25}), respectively (Fig. 9).

Fenoxycarb's application to *S.mauritia* induced histomorphological deformities in ovaries which caused reduction in reproductive capacity of adult females.

Biochemical analysis

The temporal variation in *S. mauritia* tissue nutrient levels between control and fenoxycarb treated sets showed high sensitivity of biochemical indices to the pesticide. The total protein, lipid, and carbohydrate contents of the ovary of 48 h adult insect emerged from treated pupae are presented in Table 2.

The amount of total soluble protein (µg/µl) in the ovary at 48-h old adult was 186.6 ± 7.2 and 191.11 ± 7.4 in control and acetone treated samples while in fenoxycarb treated ovarian samples it was 157.37 ± 5.9 and 127.87 ± 6.7 respectively in LD_{10} and LD_{25} doses (Table 2).

The mean total lipids were 246.72 ± 6.69 and 240.44 ± 7.11 (µg/µl) in control and vehicle control groups of the 48 h of the adult. In a dose–response relationship, the lipid content in LD_{10} and LD_{25} of fenoxycarb treatments caused a significant reduction to 149.16 ± 5.36 and 98.92 ± 8.10, respectively (Table 2).

In carbohydrate estimation, a remarkable reduction was noticed in the fenoxycarb treated sets. Control and vehicle control samples had a carbohydrate concentration of 5.22 ± 0.53 and 5.31 ± 0.3(µg/µl) while in the treated samples it reduced to 3.97 ± 0.39 and 2.78 ± 0.31(µg/µl) in LD_{10} and LD_{25} in a dose-dependent manner (Table 2).

Electrophoretic SDS protein pattern

The ovarian tissue SDS protein profile of the 48 h old females of *S.mauritia* emerged from different concentrations of fenoxycarb treated pupae is depicted in Fig. 10. A & B. The electrophoretogram displayed nine bands with approximate molecular weight (Mw) ranging between 108 and 13 kDa. The electrophoretogram shows that the percentage of protein concentration in the two bands of the middle range (76 kDa, 41 kDa) showed a slight increase. Likewise, the two proteins of the low content (23 kDa, 17 kDa) also showed a small increase. Two bands of the high range (108 kDa, 60 kDa) displayed a slight decrease so that there is no sign of up or down-regulation. There are no noticeable changes in the other three bands (52 kDa, 30 kDa, 13 kDa) compared to the control.

The above results show that fenoxycarb treatment inhibited protein, lipid, and carbohydrate content in the ovary of *S. mauritia*, which can predict impaired adult performance and imperfect reproductive capability.

Discussion

Insect reproduction and development are influenced by the titers of JH and ecdysteroid in the haemolymph, which is crucial for their proper growth and metamorphosis. Several studies have shown that the exogenous application of JHA/ agonists results in severe deformities in the reproductive organs and eggs formation. The present study showed that the sublethal treatments of fenoxycarb on newly molted pupae induced various abnormalities in adult development. The study demonstrated a reduction in gonadosomatic index and morphological aberrations in the ovaries of the adults of *S. mauritia*. The observed decrease in ovariole growth suggests inhibition of the mitotic activity of ovarian cells.

Table 2 Effect of fenoxycarb on concentrations of biochemical parameters of 48 h old adult ovary of *Spodoptera mauritia*

	Control	Vehicle Control	Fenoxycarb (LD_{10})	Fenoxycarb (LD_{25})
Total soluble proteins (µg/µl)	186.62 ± 7.2	191.11 ± 7.4	157.53 ± 5.9*	127.87 ± 6.7*
Total lipids (µg/µl)	246.72 ± 6.6	240.44 ± 7.1	149.13 ± 5.3*	98.92 ± 8.1*
Total carbohydrates (µg/µl)	5.22 ± 0.53	5.31 ± 0.31	3.97 ± 0.39*	2.78 ± 0.30*

Data expressed Mean ± Standard Error; The data were analyzed by one-way ANOVA using SPSS software (IBM SPSS 20). The asterisks show significantly different from control sample by one-way ANOVA

* $P < 0.05$

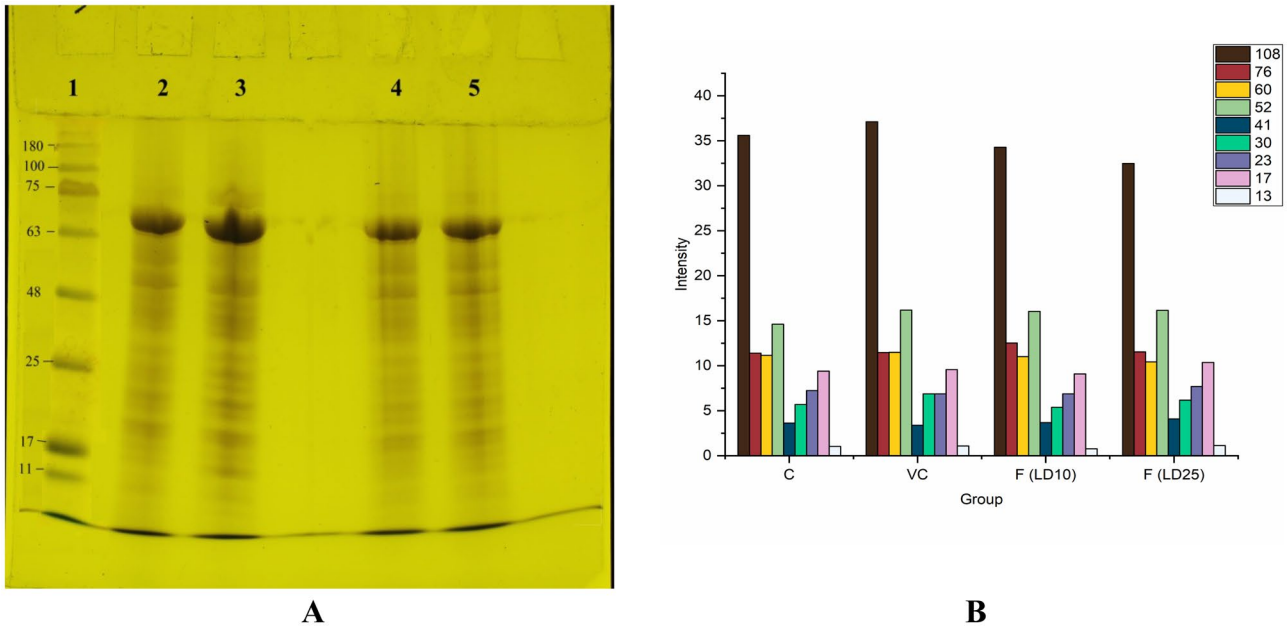

Fig. 10 **A** Electrophoretogram of SDS protein profile of *S. mauritia* control and fenoxycarb treated ovarian samples. **B** Percentage of protein intensity with a specific molecular weight in control and treated protein samples of *S. mauritia*

Enlargement of germaria indicated that the fenoxycarb treatment did not obstruct the proliferation of undifferentiated cells; instead, the production of their excessive cells resulted in tumour formation like protuberances. Similar anomalies were reported in JHA, hydroprene treated pupae of *S. mauritia* (Mathai and Nair 1990). Our study shows that adult ovaries growth was significantly suppressed due to fenoxycarb treatment in pupae of *S.mauritia*. Similarly, earlier reports on cockroaches evidenced that the impact of JHA, pyriproxifen on ovarioles, accessory glands, and oviducts were developed abnormally (Fathpour et al. 2007).

From the results, it is clear that JHA hinders the differentiation of oogonial cells similar to how JH inhibits morphogenesis during insects metamorphosis. It is possible that fenoxycarb affects the mitotic activity of ovarian cells as a result of excess JH titre, or JHA,which might interfere with ovarian cell division and differentiation. The arrested ovarian development leading to necrosis of oocytes and follicular cells by fenoxycarb is comparable to those reported by Verma (1990). Similarly, topical application of fenoxycarb to adult *Dysdercus similis* reduced yolk deposition with atrophied oocytes, resulting in decreased adult reproductive capacity and fecundity. The degeneration of follicular epithelium affects the normal uptake of nutrients from haemolymph (Govind 2014). Treatments with JHAs, juvenoid RO8-9801 and methoprene completely inhibited oocyte yolk absorption in *Blattella germanica* (Maiza et al. 2004). Qian et al. (2020) suggested that pyriproxyfen exposure could influence ovarian tissue development following a decline in the number of oocytes and oogonia in the ovaries of pyriproxyfen-fed silkworms.They inferred that the treatment impaired the transcription level of genes related to ovary development affecting the absorption of nutrients, energy metabolism, ovarian growth and egg formation, which ultimately led to reproductive disorders in silkworms. The present findings revealed a dose-dependent inhibitory effect of fenoxycarb in the oviposition rate of *S. mauritia*. These results are in agreement with the report of pyriproxyfen topical treatment in *S. litura* that inhibited embryogenesis, leading to a decrease in the egg-laying rate (Xu et al. 2015). The studies on the effects of pyriproxyfen on *Aphis glycines* (Richardson and Lagos 2007) and methoprene on *Rhyzopertha dominica* (Chanbang et al. 2008) showed a reduction in fecundity. The study suggests that decreased ovarian growth and oogenesis is responsible for the decline in fecundity.

The present study showed a sizeable decrease in the adult's ovaries of *S. mauritia* from fenoxycarb treatment. Biochemical profiling of total protein, lipids, and carbohydrates showed significant reductions in a dose-dependent manner relative to the controls. These findings are similar to juvenile hormone analogue pyriproxyfen treatment in Indian meal moth *Plodia interpunctella*, which significantly reduced total protein and lipids (Ghasemi et al. 2010). Pyriproxyfen decreased lipid content in *Corcyra cephalonica* (Mandal and Chaudhuri 1992), *Plodia interpunctella*

(Ghasemi et al. 2010), *Eurygaster integriceps* (Zibaee et al. 2011), and *Leptinotarsa decemlineata* (Fotouhi et al. 2015). Previous study suggested that pyriproxyfen prevented *Schistocerca gregaria* nymphs from achieving a standard carbohydrate content in the hemolymph. The lack of nutrients supplied by hemolymph to the growing ovaries is one of the key reasons for reducing ovarian size in insects treated with JH, JHA, or growth regulator of plant origin (Telfer et al. 1981) or due to a deficiency of materials produced by the ovaries (Indrasith et al. 1988). Protein, lipid, and carbohydrate deficiencies may contribute to abnormal oogenesis (Kunkel and Nordin 1985; Kanost et al. 1990). This hypothesis has been verified with the diminution of three main biochemical compounds: lipid, carbohydrate and protein in the ovaries of *S. mauritia* which caused abnormal ovarian development in treated groups. Evidently, from these reports, it is clear that fenoxycarb treatment caused several morphological, histopathological and biochemical ill effects in the ovarian development of *S. mauritia* that affect adult females to become sterile.

Conclusion

The application of fenoxycarb to *S. mauritia* pupae induces histomorphological deformities and causes adult females to become sterile. These responses are due to the high titre of the compound that inhibits JH production. Fenoxycarb treated sets showed a high sensitivity of biochemical indices. The effect of fenoxycarb could be correlated with quantitative depletion of proteins, lipids, and carbohydrates in gonads of the treated groups. We infer from the overall findings that fenoxycarb is a promising JHA for controlling the rice pest, *S. mauritia*. Based on the above results, more studies on this compound are recommended in future, including field trials to manage insect pests effectively.

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Declarations

Competing interests The authors of this study announce no conflict of interest about the publication and dissemination of information.

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