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Ultra Structural Studies of the Ovaries of Susceptible and Insecticide-Resistant *Plutella xylostella* (Linnaeus)

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ABSTRACT

The present study was aimed to compare the ultra-structural changes in ovaries of susceptible and insecticide-resistant *P. xylostella.* Ovaries of the adults of *P. xylostella* from the susceptible and insecticide-resistant populations were dissected, prepared chemically and were observed under scanning electron microscope. The length and breadth of the ovarian follicles were measured and their size was calculated. Considerable variations in shape, size and appearance of ovarioles in susceptible and resistant moths were recorded. Ultra structural studies revealed reduction in dimensions i.e. length, breadth and consequent size of the ovarian follicle in resistant moths as compared to the susceptible one. The variations in the dimensions of ovarian follicles in resistant and susceptible moths are indicators of influence of insecticides there upon.

Key words: Ovarioles, *Plutella xylostella,* Scanning electron microscope.

INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) is cosmopolitan, economically important (Liu *et al.,* 2002; Liang *et al.,* 2003) and most noxious pest of cole crops in Punjab, India and rest of the world where it has posed a very serious threat to the crops causing over US $ 1 billion worth damage globally per annum and has become very challenging to control (Khaliq *et al.,* 2007). *P. xylostella* is distributed worldwide in areas of different climatic zones including tropical, subtropical and temperate and has the ability to migrate among different climatic zones (Chu, 1986; Honda, 1990; Honda *et al.,* 1992, Chapman *et al.,* 2002, Coulson *et al.,* 2002). The crop damage of 90% has been reported in fields with heavy infestation of Diamondback moth (Verkerk and Wright, 1996; Gautam *et al.,* 2018). *P. xylostella* has become one of the most difficult insect pest in the world to control in the past 50 years. Management of diamondback moth is mainly based on insecticides use and the dependence on this single approach has led to increase in application rates resulting in decreased effectiveness and eventual breakdown of control efficiency (Fletcher, 1914; Saxena *et al.,* 1989; Gautam *et al.,* 2018). It became the first crop pest in the world to develop resistance to DDT and ranks among the top 20 most resistant insect species reported so far (Mota-Sanchez *et al.,* 2002). This exceptional pest status is due to the diversity and abundance of host plants, lack or disruption of its natural enemies, its high reproductive potential, up to 20 generations per year and its proven ability to rapidly evolve resistance to insecticides (Shelton, 2004). Presently, it is known to have developed resistance to more than 46 insecticides from all groups including new generation insecticides such as neonicotinoids, avermectins, macrocyclic lactones, IGRs and Bt formulations etc. (Jallow and Hoy, 2007; Oliveira *et al.,* 2011). There also exists a knowledge gap in regards to comparative study of ovaries of susceptible and insecticide-resistant populations of *P. xylostella*. This knowledge may help to know the sub-lethal influences of different insecticides on ovarioles, which ultimately affect insect reproduction.

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MATERIALS AND METHODS

Collection, rearing and maintenance of populations of *P. xylostella*

Susceptible and resistant populations of *P. xylostella* were reared and maintained in screen house at Entomological Research Farm. For maintenance of susceptible population, larvae of *P. xylostella* were collected from cauliflower/ cabbage fields of Amritsar region and reared for 22 generations in insecticide free environment. The resistant population was maintained after collecting *P. xylostella* from cauliflower/cabbage fields of Amritsar region subjected to intense insecticidal pressure (Chandi *et al.,* 2012). To check the relative toxicity for both the populations, resistant ratio was calculated for test insecticides and it was found that the maximum level of resistance in *P. xylostella* was noted for fenvalerate (515.86 fold) followed by quinalphos (90.26 fold), endosulfan (20.78 fold) and the minimum resistance

was shown to spinosad (14.25 fold) (Chandi and Singh, 2013).

Culture of *P. xylostella* was maintained on the cauliflower leaves kept in glass jars (10 ×15 cm) placed in a Plant Growth Chamber at 27°C and 65% RH. Each jar was covered with a piece of *dasuti* cloth fastened with rubber bands around its rim. Food was changed daily till the onset of pupation. The leaf portions bearing pupae were transferred into other glass jars and those remaining attached to the walls of the jars were allowed to emerge as adults as such. The emerging adults were sexed on the basis of the differences in the brightness of diamond patterns on their forewings. The males are dark brown in colour with a pattern of three consecutive white diamond shaped spots on its back. The female moth is tan coloured and its diamond patterns are less distinct as compared to the males. Moreover, tip of abdomen was pointed in females, whereas it was slanting in males (Lal, 1992). Also, size of the females was bigger than that of the males. The emerging adults were sexed and transferred into new jars for mating and oviposition on the same day. A piece of cauliflower leaf placed in each jar acted as stimulant for egg-laying. A cotton-swab dipped in 10% honey solution was hung from top of the *dasuti* cloth covering the mouth of the jar which provided food to the adults. The leaf with eggs laid upon was removed daily and replaced with a new one to facilitate further oviposition.

Study of ultra-structure of ovary/ovariole of *P. xylostella*

Ovaries of the adults of *P. xylostella* from the susceptible and insecticide-resistant populationswere dissected and were observed under scanning electron microscope (SEM-S3400N) in Electron microscopy and Nanoscience laboratory of the Punjab Agricultural University, Ludhiana. For scanning electron microscopy (SEM), the sample was prepared chemically by following the method mentioned by Santos and Gregorio (2002) with some modifications. Ovaries of the virgin females of *P. xylostella* from the susceptible and resistant populations were dissected and then fixed in a fixative solution (2% glutaraldehyde solution in 0.1 M Sodium cacodylate buffer (pH 7.3) at 4°C for 24 hours. After fixation, the ovaries were washed in buffer solution (0.1 M Sodium Cacodylate buffer) and it was repeated thrice giving 15 minutes for each wash. The post-fixation was done in 1% osmium tetroxide for 2 hours at 4°C. The dehydration process of the specimens was done in a graded series of ethyl alcohol solution by immersing in 30%, 50%, 70%, 80%, 90%, 95% and absolute solution for 20 minutes each at 4°C. After dehydration, the specimen was allowed to dry by keeping it in vacuum desiccators for 24 hours. Then, the specimen was mounted on metallic (aluminium) stub having a double sticky carbon tape on its surface. The specimen was coated with a thin layer of approximately 20 nm to 30 nm of gold in ion sputter coater (E-1010). The specimen was viewed and imaged using Scanning Electron Microscope operated at an accelerating voltage of 15 kV using secondary electron detector.

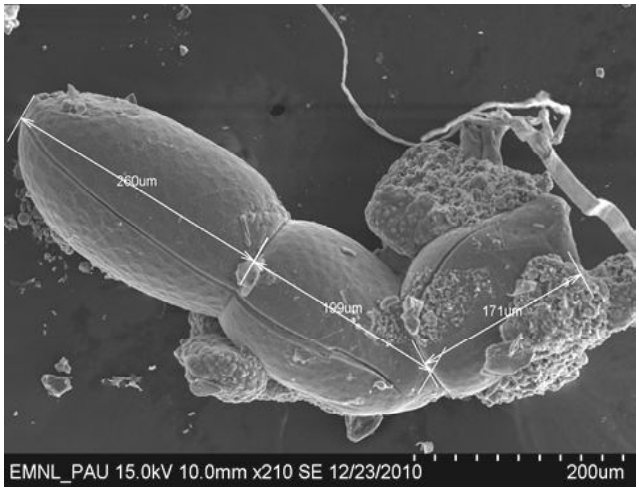
The length and breadth of the ovarian follicles were measured and their size was calculated by applying the formula used by Loeb *et al.* (1984)

$$\text{Size of ovarian follicle} = 4/3\ \pi\ (ab^2)$$

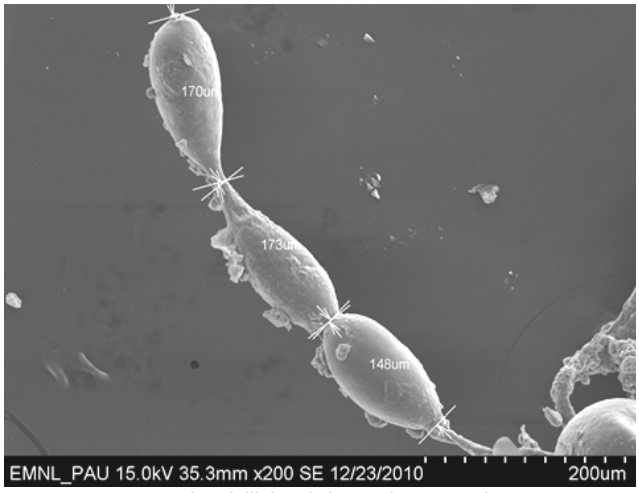
Where 'a' is the radius of the long and 'b' is the short dimension of the same follicle.

RESULTS AND DISCUSSION

Microscopic examination of an ovary of *P. xylostella* revealed three ovarioles therein. Each ovariole appeared to be a slender thread like structure containing a chain of follicles or eggs chambers. Spermatheca, a globular sac was attached through a duct to proximal end of the vagina of *P. xylostella.* Considerable variations in shape, size and appearance of ovarioles in susceptible and resistant moths were recorded (Table 1, Plate 1). Length, breadth and consequent size of the ovarian follicle was appreciably small in adult moths from the resistant population as compared



Ovarian follicle of the susceptible moth



Ovarian follicle of the resistant moth

Plate 1: Ovarian follicles of susceptible and resistant moths of *P. xylostella*.

to those from the susceptible one; the mean size of ovariole in the resistant moths was recorded as 0.0015 mm^3 ± 0.001 as compared to the corresponding value of 0.0097 mm^3 ± 0.005 in the susceptible moths. The shape of ovarian follicle in resistant female moths appeared to be constricted and shrinked whereas those in the susceptible moths looked globular and bigger in appearance.

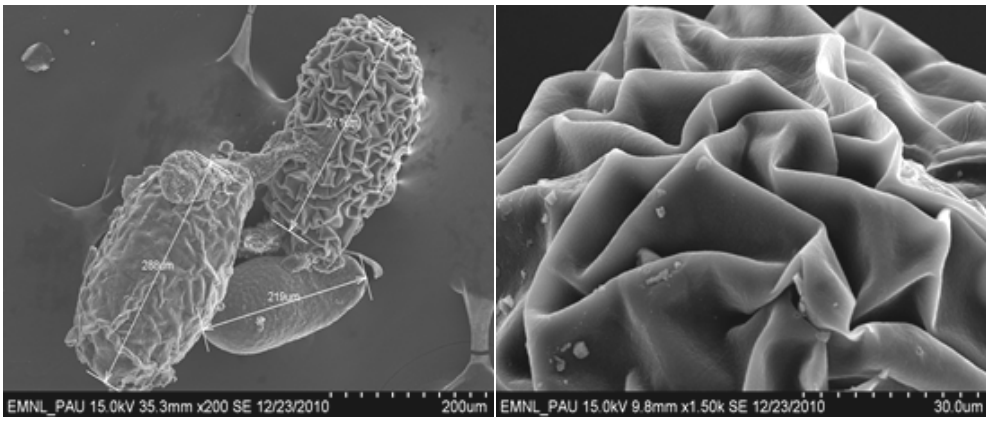
Variations in the dimensions of ovarian follicles in resistant and susceptible moths are indicators of influence of insecticides there upon. As observed under high resolution, surface of ovariole of resistant moths appeared to be wrinkled and creased in comparison to relatively smoother surface in case of susceptible females (Plate 2). The smaller size of eggs laid by the resistant moths could be related to the reduced size of the ovarian follicles as observed in ultra structural studies. Perveen and Miyata (2000) also observed that sublethal doses of chlorfluazuron affected ovarion development and oogenesis in the *Spodoptera litura* and found that in newly emerged LD_{10} or LD_{30} treated adults; the total length of ovariole was significantly reduced in comparison to that of the control. The basic factors responsible for the reduction in ovarion weight were reduction in the size of basal oocytes and thickness of their follicular epithelium and reduction in protein content of ovarion constituents as compared with the controls. Earlier, Perlak *et al.* (1993) found a reduction in the sizes of ovaries of Colorado potato beetles, *Leptinotarsa decemlineata* (Say) fed on transgenic potato carrying gene coding for cryIII endotoxin protein of bacteria, *Bacillus thuringiensis* var. *tenebrionis*. Singh *et al.* (2017) conducted ultra-structural studies of ovaries of *P. xylostella* in spinosad-treated females and untreated females and found that follicles in the treated *P. xylostella* were somewhat rounded as compared to those in the ovaries of control females, where they appeared to be elongated. Also, size of the developing follicles was measured under scanning electron microscope and observations revealed that there was appreciable decrease in length and increase in breadth of the follicle in ovarioles from the untreated lot of *P. xylostella.*

Table 1: Development of ovarian follicle in susceptible and insecticide-resistant populations of *P. xylostella*.

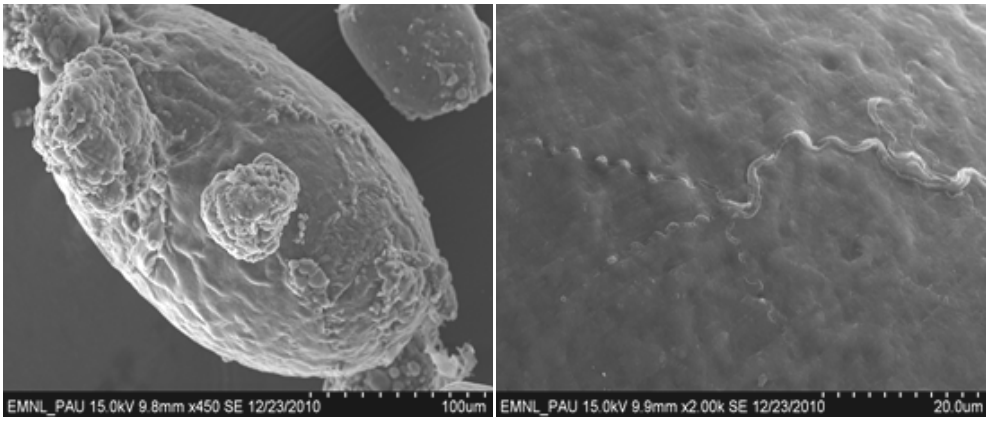
Dimensions of ovarian follicle	Population of *P. xylostella*		t-test
	Susceptible	Resistant	
Mean* length (µm)	221.6 ± 24.833	138.9 ± 25.198	7.39
Mean* breadth (µm)	143.6 ± 12.772	70.41 ± 5.661	16.56
Mean* size** (mm^3)	0.0097 ± 0.005	0.0015 ± 0.001	10.85

*Based on ten replications, each comprising single female.

**Size is calculated as per method described by Loeb *et al.* (1984).



The resistant moth



The susceptible moth

Plate 2: Surface of ovarian follicles of susceptible and resistant moths of *P. xylostella*.

In a study conducted on impact of pesticides on two populations of *Chrysoperla externa* (Neuroptera, Chrysopidae) by Moura *et al.* (2011) it was revealed that abamectin led to malformations in micropyle and chorion morphology of *C. externa* eggs, on other hand no deformities were observed in untreated ones. Ultrastructural examinations done by Meena and Singh (2014) showed different deteriorating effects on oocytes i.e. degeneration of follicular cells and vacuolization in developing ovarian follicles. Kaur (2015) reported considerable variations in shape, size and appearance of ovarioles in LC_{50} treated and control moths were recorded. Length, breadth and consequent size of the ovarian follicle was appreciably small in adult moth from the LC_{50} treated (0.025%) as compared to those from the control one; the mean size of ovariole in the treated moths was recorded as 0.0004 mm^3 as compared to the corresponding value of 0.0012 mm^3 in the control moths. The constricted and shrunken appearance of ovarian follicle was observed in treated female moths. Aziza and Abdel (2012) observed the histological abnormalities in ovaries of *S. littoralis,* due to pyriproxyfen treatment in the form of oocytes shrinkage. This factor was responsible for reduction in size of ovariole follicles in the treated moths. Ovarian development of *S. litura* was inhibited with pyriproxyfen treatment and about 40 per cent of female adults showed morphological ovarian abnormalities (Nomura and Miyata, 2000). Ghazawi *et al.* (2007) observed disintegration and destruction of ovarian follicle cells and mitochondria in female of *Heteracris littoralis* when treated topically with serial concentrations of azadirachtin. Xu *et al.* (2015) when examined oocytes of common cutworm, *S. litura* using transmission electron microscopy after treatment with 100 µg of pyriproxyfen; found increased amount of rough endoplasmic reticulum and mitochondria in the primary oocytes. Maiza *et al.* (2004) found that treatment with RH-0345 and benfuracarb can lead to significant reduction in the number, size of oocytes and the volume of the basal oocyte in *Blattella germanica*. Methoprene distorted the ovarian development since it caused a significant reduction in the number of oocytes. The complete inhibition of yolk absorption by oocytes and formation of empty cavities and autophagic vacuoles in oocytes was also observed. Hence ultra-structure studies revealed reduced dimensions of the ovarian follicles in resistant moths, which could lead to reduced egg size and fecundity.

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