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Efficacy of botanical and microbial fungal bio-pesticide against a key pest of *Helicoverpa armigera* (Hubner)

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Abstract
Objective: To study the histopathological approach of Botanical biopesticides Lantana camera and microbial fungus Beauveria bassiana to reveal the mode of action on insect tissues.
Methods: In our experiment, both biopesticides botanical Lantana camera and microbial entomopathogenic fungus Beauveria bassiana (Balsamo) were applied on Helicoverpa armigera (Hubner) larva. The whole-body section of untreated and treated larvae was taken to observe their effect on various tissues by histopathological techniques.
Results: Larvae were treated with the highest dose of 1. 0 ml leaf and flower extract and 0.250 x 108 conidia/ml. Hematoxylin, Eosin, and Mallory triple stain were used for the histopathological protocol. Slanting section of the body exposed noticeable change in a variety of tissues. Extract affected the complete loss of peritrophic membrane, disorganized columnar epithelium, structural and fat bodies, and damaged integument. The fungus also targeted to cuticular fraction, which was well obvious by injured exocuticle, endocuticle, and statement of conidia in big amounts on the outside surface as well as in the crypts of the cuticular wall. The overall effect brought about the liquefaction of the body tissues making the infected larvae turgid which upon slight disturbance, ruptured releasing the infected conidia into the environment.
Conclusion: The histopathological studies have revealed that integument is the main target tissue for the action of both biopesticides. However, it was also observed in the histomicrographs that extensive tissue destruction occurred in the body and epithelium of the insect.

Keywords: helicoverpa armigera, biopesticides, lantana camera, entomopathogenic fungi, beauveria bassiana, histopathology

Introduction

Organic pesticides are derived from plants, microorganisms or other natural substances that have been shown to be safe for humans and the environment. They contain end pathogenic viruses, bacteria, fungi and plant secondary metabolites, which are very important as an alternative to chemical pesticides and are an integral part of many pest control programs. Kaur and Padmaja, (2008) [18] study the indiscriminate utilize of chemical pesticide is assuming the serious cause of concern to health and environmental safety. A feasible substitute to chemical pesticide is integrated pest running. Kumar, *et.al*., (2019) [19] also observed that biopesticides are environmentally safer from synthetic pesticides. The pathogenic effect of these microorganisms on target insects depends on the species. The activity of microbial end pathogens occurs as a result of insect infestation or intestinal invasion, followed by the death of the host as a result of pathogen multiplication, e.g., insects. Botany is the use of herbal products or products that value pesticides, inal medicine or properties. Phyto-pesticides completely separate bioactive photoelectric power plants or new formats that work against pests and pathogens (Prakash and Rao 1996 and Salako *et al*., 2008) [33, 37]. In general, botany works in one of two ways, either as contact venom when sprayed on an insect, or as stomach venom when ingested by an insect. They infect insects very quickly to stop feeding. They do not cause death for hours or days, but they often cause immediate freezing or cessation of feeding. With this background in mind, in the present investigation, the leaf and flower extracts of one commonly grown plant *Lantana camara* (L.) was screened to assess its insecticidal properties against the key pest *Helicoverpa armigera* (Hubner). *Lantana Camara* (L.) is a significant weed of which there are 650 varieties in over 66 countries. It occurs in high summer rainfall and drained sloping sites (Dwivedi, 2008) [9]. This property provides this weed suitable Inhabitat of south Rajasthan where it grows profusely. The presence of tannin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides makes this plant a highly reactive biological agent (Kumar and Maneenmagalai, 2008) [19]. Its antifeedant property (Dong et. al., 2005) [8], repellant (Deka et. al., 1998) [6], insecticidal (Abdel et. al., 2005), and nematicidal (Oanmar, 2005) properties are well known. Ayalew,(2020) Their research shows that methanol juice is highly toxic to the benefits of the grain, followed by the juice of ethanol and ethyl acetate. L. Chamomile Leaf Methanol Extract Has High Extractive Secondary Metabolism. Same as Fungal microbial biopesticides also work like pathogenic. some studies exposed the insecticidal possible of *B. bassiana* as my pesticides and business endophytic fungi (Jaber and Ownley 2018) [17]. *Beauveria bassiana* generally infects through the integument. The fungus also invades the larval alimentary canal of H. zea to cause starvation and nutrient depletion that may lead to larval death (Cheung and Grula, 1982) [4]. *Beauveria bassiana* produces Mycotoxins *culture media is produced by Beauveria paciana. Toxic compounds rapidly weaken the insect after a hemolymph attack (Roberts, 1981)* [45]*. However, t. In the larvae of B. z. Sampling and Crowley (1979) stated that beaver production was insufficient to inhibit the pathogenic development of Pasiana. Dead larvae, pupae, and adults are formed from infected insects and are surrounded by outer mycelium and white conidia within a day or two of death.*

Conidia are caused by conidiophores growing from the surface of the insect. Puviria paciana Lepidopterus is highly contagious to insect larvae and is contagious to cochlear predators. (Pingel and Lewis, 1996) [32].

Infection by *Beauveria bassiana* infection has been shown to directly invade insect host interactions through the growth of hyphae, which appears to facilitate mechanical and enzymatic activity. (Lefeliva *et al*., 1934; Pekrul and Grula, 1979; Pendl and Boucias,1993, Vey and Fargues,1977, Wagner and Lewis *et al*., 2000) [23, 30, 31, 41, 42].

In the present investigation, we used leaf and flower extracts of the plant *Lantana Camara* (L.) and fungal biopesticide *Beauveria bassiana* (Balsamo) to see their insecticidal properties against a pest of national status, that is *Helicoverpa armigera* (Hubner). *Helicoverpa armigera* (Hubner) It is one of the most destructive and high polyphonic pests of field crops. It causes serious damage and loss of food, fiber, oil, food, vegetables, horticultural, ornamental, aromatic, and medicinal plants. Its main physiological, behavioral, and ecological features are polyphagia, high mobility, high depression, and facial diaphysis, which can help even in unstable habitats. (Neoliya *et al*., 2007) [27]. It is approximate that *Helicoverpa armigera* alone is answerable for losses over Rs. 3500 million annually in India, despite the heavy application of pesticides inputs (Kumar and Kapur, 2003) [21]. *Helicoverpa armigera* alone financial records for the expenditure of half of the total pesticide used in India for the guard of dissimilar crops (Suryavanshi *et al*., 2008 and Vikrant et.al., 2018) [39, 40].

Material and Methods

After screening of both biopesticides botanical *Lantana camera* parts i. e., leaf and flower and entomopathogenic fungal pesticides *Beauveria bassiana* against different developmental stages, the effects of both Biopesticides on different tissues were studied by histopathological techniques. Histopathological work was carried out by standard methods of microtomy. Whole-body sections of untreated and treated (leaf, flower extracts, and fungal treated) fourth instar larvae, were taken to observe their effect on various tissues of the insect. With the help of histopathological protocols firstly fixed tissues in Gilson Fixative (Gatenby and Beams 1950) and tissues were transferred separately in15 ml fixative was filled 25 ml capacity vial. After 24 hours tissues were kept in Lugol's Iodine solution for washing. Washed tissues were dehydrated for the removal of water, which is an essential step for subsequent treatment. Graded series of ethyl alcohol was used and the tissues were gradually transferred from 30 percent to absolute alcohol passing through 50, 70, and 90 percent alcoholic grades. Xylene, an organic solvent was used as a cleaning or dealcoholization of tissues since it is miscible with both ethanol and paraffin for impregnation. For paraffin infiltration of tissues, the oven was adjusted just above the melting point of paraffin used i. e., 620C. The paraffin wax was mixed with beeswax in the ratio 3:1 and was filtered twice to get rid of the impurities. The clearing agent i. e., xylene was replaced by paraffin during infiltration. The tissues were kept in 50:50 – xylene: wax for one hour and were transferred to pure wax for two hours which had two changes. Tissues were embedded in 'L'pieces and paper rafts/boxes for a hard matrix to avoid distortion during microtomy. The embedded tissue block was trimmed and cut the sections of 7 microns thickness with the sharp razor of the microtome. Sections ribbon were spread on a clean albuminized slide and kept overnight for drying and further used double staining (Haematoxylin – Eosin) method for Staining, Dehydration, and Mounting. And observe tissue under a microscope.

Result

Examination of Untreated and Treated Fourth Instar Larvae of *Helicoverpa armigera* (Hubner) Body Wall.

The histoarchitecture of controlled tissue revealed an uninterrupted that is made up of cuticular integument. The insect's body's outermost part is made up of epicuticle, exocuticle, and endocuticle. (Plate-1,Fig-1,2).The innermost part is the epidermis (Plate-1, Fig-3). Setae are hair-like structures that arise from pits on the epicuticular surface in a sort of ball and socket nature and the spine are also noncellular and small outgrowths of epicuticle (Plate -1, Fig-3, 4). All the three sub-layers of the cuticle are firmly attached giving a band-like appearance to the integument. (Snodgrass, 2003). The epidermis is single-layered under the endocuticle which is made up of a single epithelial layer with nuclei at the base of the cells. It becomes less distinct and found as a thin layer under the endocuticle in a later stage (Plate-1, Fig: 1 to 4)

Histopathological changes in the body wall of *Helicoverpa armigera* (Hubner) treated with leaf and flower extracts of *Lantana Camara* (L.)

As we observed in our investigation the botanical *Lantana Camara* (L.) acts as a stomach poison. So, the effect of the leaf and flower extracts of *Lantana Camara* (L.) started from midgut to integument. Leaf extract treatment to fourth-instar larvae although exhibited similar damage in different tissues as that of flower treatment but there were some differences observed. Maximum damage was recorded in the integumentary structure of the treated larvae although there were little differences in the leaf and flower treated larval skin.

Body wall treated with leaf extract (Plate-2, Fig.1 & 2)

There was a gradual degeneration of complete integument so that no demarcation between integument and its different layers in the body cavity could be observed. The fragile nature of the body wall from the outer surface was well evidenced by the thinning of exocuticle and endocuticular layers due to degeneration of the cuticular matrix. Endocuticle gets detached from the exocuticle forming a space between the two. The epidermis became very thin and almost degenerated. The loss of chitinous content in the endocuticle was well evidenced by disarticulated integument (Plate-2, Fig. 1). The endocuticle became very thin and delicate, there was no demarcation between exocuticle and endocuticle, and loosening was evidenced by forming space in between the two. The important difference that could be observed was that leaf-treated integument setae became reduced but swelled and other layers also revealed a swollen nature. The trichogen cells of the setae are showing diffused and degenerated appearance Lamellated arrangements and pore canals depicted loosening with diffused spaces. (Plate-2, Fig. 2)

Body wall treated with flower extract (Plate – 2, Fig.3 & 4)

Here, more loss of cuticular structures can be observed. The integument became thin, delicate, and less dense with loosening of exocuticle from endocuticle. There was a complete disintegrate between the different histoarchitectural structures of the integument. If the epicuticle is observed, the setae became short and setal cells completely lost their identity. The cells became diffused and almost disappeared later on. (Fig. 3) Spines were almost reduced and gave a noncorrugated appearance to the skin that was rough and corrugated in control. (Fig. 4) Loss of chitinous content was well evident in the disintegrating and loosening of Exo and endocuticle. The endocuticle became fragile, loose with lamellae arranged loosely by large spaces. It caused loss of tight binding and pore canals started diffusing with lamellae. (Fig. 4) Apodemes and lowermost layer of endocuticle started loosening from the inner epidermal layer creating space in between the two layers. (Fig. 4)

Histopathological changes in the body wall of *Helicoverpa armigera* (Hubner) treated with entomopathogenic *Beauveria bassiana* (Balsamo)

Larvae were sacrifice after treating with the utmost dose of 0.250 x 108 conidia/ml. Hematoxylin, Eosin, and Mallory triple stain were used for the histopathological protocol. Sloping sections of the body exposed noticeable change in a variety of tissues. The body wall of the treated larva exhibited significant changes where penetration germination and establishment of fungus were well depicted. There was a complete disintegrate between the different histoarchitectural structures of the integument. we observed the main objective of fungus infection was the cuticular part, which was well evident by injured exocuticle, endocuticle, and statement of conidia in large amounts on the external surface as well as in the crypts of the cuticular wall. The overall effect brought about the liquefaction of the body tissues making the infected larvae turgid which upon slight disturbance, ruptured releasing the infected conidia into the environment. The entire process took 3 to 10 days.

The fragile nature of the body wall from the outer surface was well evidenced by the thining of exocuticle and endocuticle layers due to degeneration of the cuticular matrix. The endocuticle became detached from the exocuticle forming a space within the layers (Plate-3, Fig.1). The epidermis became very thin and almost degenerated. If the epicuticle is observed the setae became short and setal cells completely lost their identity. The cells became diffused and almost reduced and gave a noncorrugated appearance to the cuticle that was rough and corrugated in control (Plate-3, fig-2). The endocuticle became fragile, loose with lamellae arranged loosely by large space. It caused loss of tight binding and pore canals started diffusing with lamellae (Plate-3, fig.3). The lowermost layer of the endocuticle started loosening from the inner epidermal layer creating space in between the two layers (Plate-3, Fig.4). The above changes in the cuticle were due to a fungal attack that revealed marked step-by-step action on the insect histoarchitectural design.

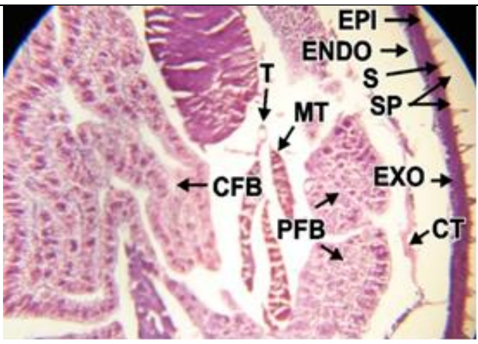

Fig. 1 Whole body section of fourth instar showing general histoarchitectural design of the body cavity along with various tissues. Epicuticle (EPI), Exocuticle (EXO), Endocuticle (ENDO), Muscular tissues (MT), Peripheral fat bodies cells (PFB) , Connective tissues (CT), Central fat bodies (CFB), Trachea (T), Spines (SP) and Seta (S) x5

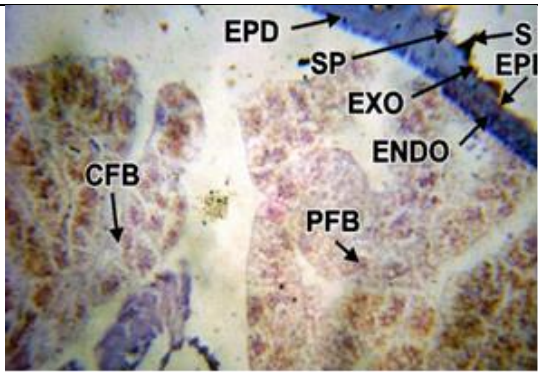

Fig. 2 Detailed structure of body wall showing stiff Setae, Spine and Fssatty layers. Epicuticle (EPI), Exocuticle (EXO), Endocuticle (ENDO), Epidermis (EPD) are very well distinguished. Spine (SP) and Setae (S) with Peripheral fat bodies (PFB), Central fat bodies (CFB) x 40

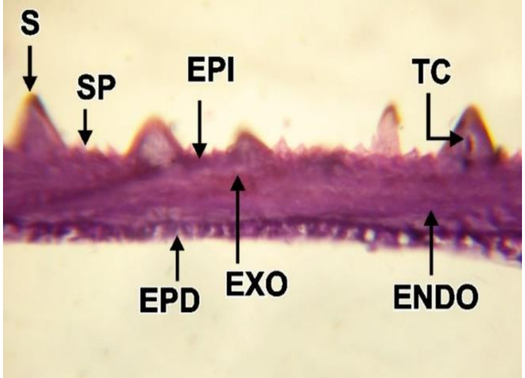

Fig. 3 Magnified view of body wall to show the details of setae and spines along with cuticular layers. Note the trichogen cell of the seta responsible for its secretion. Trichogen cell (TC), Epicuticle (EPI), Exocuticle (EXO), Endocuticle (ENDO), Epidermis (EPD), Seta (S), Spine (SP), × 10

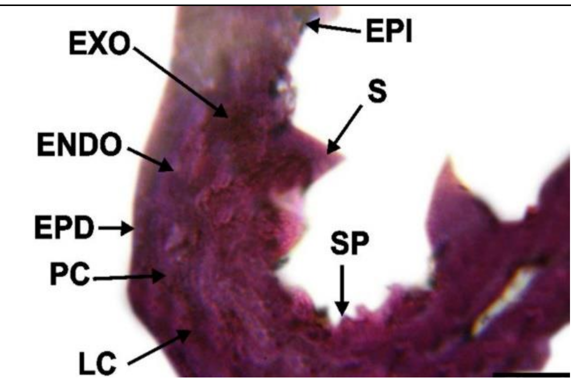

Fig. 4 Magnified view of a pocket of integument with different cuticular layers. Lamellate nature of cuticle and pore canals are also visible. Endocuticle (ENDO), Exocuticle (EXO), Epicuticle (EPI), Epidermis (EPD), Setae (S) and Spine (SP), Lamellar cuticle (LC), Pore canals (PC).

Plate 1: Histomicrograph of a portion of body wall of fourth instar larva (normal) of Helicoverpa armigera (Hubner) presentation cuticular layers and their preparations.

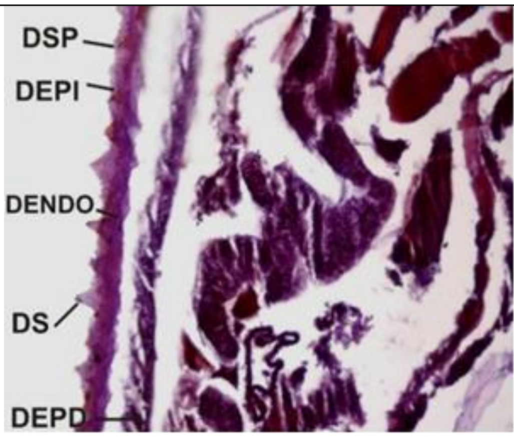

Fig (1) Magnified view of the body wall showing clear degeneration of the layers.Degenerating Epicuticle, (DEPI)., Degenerating Endocuticle (DENDO), Detached Epidermis(DEPD), Degenerating Spine(DSP) and Degenerating Setae(DS) x 20

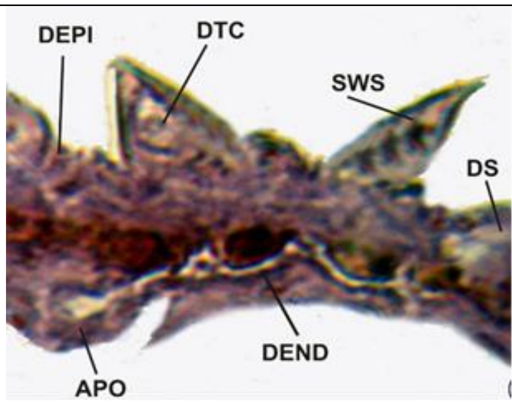

Fig (2) Magnified view of body wall showing detailed changes in its integument after treatment.Degenerating Epicuticle (DEPI), Degenerating Spine(DS),Swollen Seta(SWS), ,Degenerating and Swollen Spine (DSSP), Diffused Trichogen Cells (DTC), Degenerating Endocuticle (DEND), Apodeme(APO) x 40

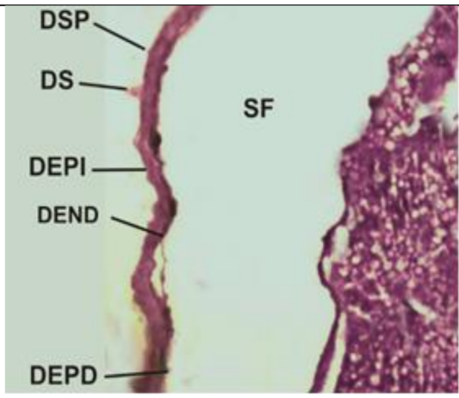

Fig (3)Cuticle is so thin that large space is formed between body wall and visceral tissues is seen DegeneratingEpicuticle, (DEPI), Endocuticle (DEND), Detached Epidermis(DEPD), Spine(DSP) and Setae(DS) Space Formation(SF) x 10

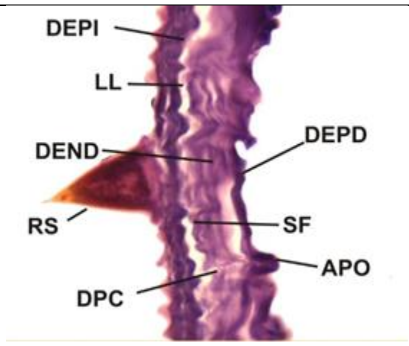

Fig (4) Magnified view of body wall shows degeneration.
Degenerating Epicuticle (DEPI),Loosening of layers(LL), Degenerating Endocuticle (DENDO),Degenerating Epidermis(DEPD), Space Formation(SF), Apodeme(APO),Diffused Pore canals(DPC),Reduced Seta(RS) x 40

Plate 2: Histomicrograph of fourth instar larva of *Helicoverpa armigera* (Hubner) treated with flower (1.0ml) extract of *Lantana camara* (L.) showing damaged body wall.

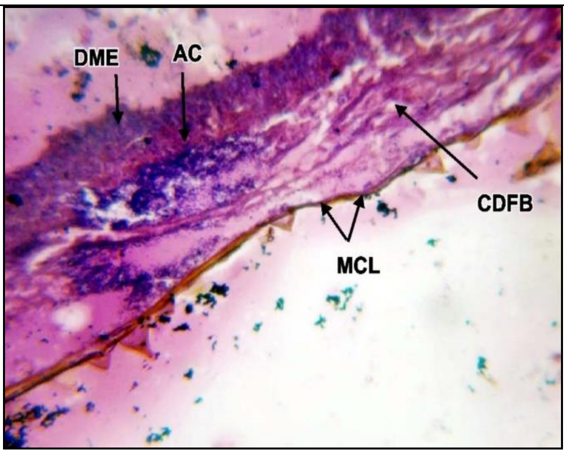

Fig. 1 T. S. of body section passing through body wall showing complete loss of cuticular architecture. Merged cuticular layers Loosing their identity (MCL), completely damaged Fat body cells (CDFB), Aggregation of conidia (AC) Degenerating midgut epithelium (DME)x40.

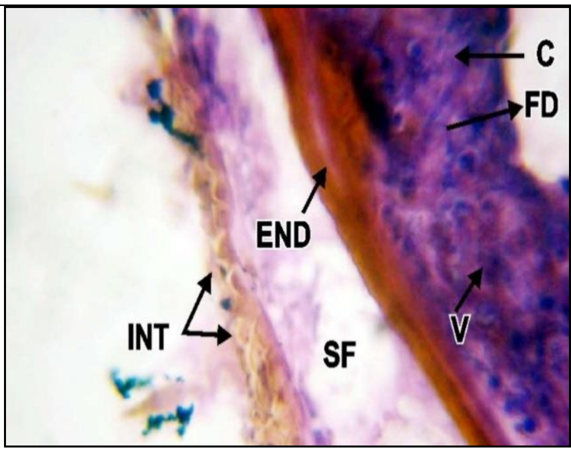

Fig.2 Integument (INT) loosing its entire architecture with Space formation (SF), Fungus developing (FD), below Endocuticle (END) in epidermal area. Some fungal germination is seen on outer most layer too. Increasing vacuolization (V) and number of Conidia (C) are seen x40.

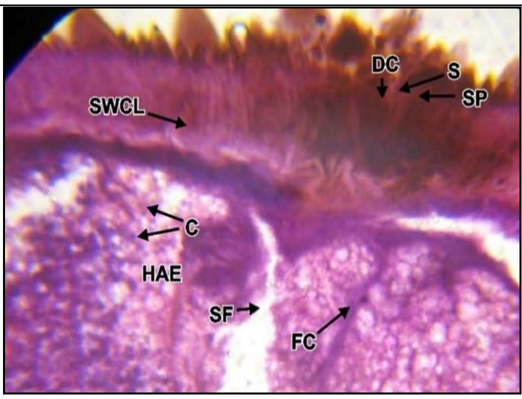

Fig. 3 Elaborated view of fungal attack on larval skin. Cuticular layers are merged and swollen (SWCL), Fungus has established itself completely in the fat cells (FC) Conidia (C) heavily spread in Haemocoel (HAE), deposition of Conidia (DC) within Seta (S) and Spine (SP), Space formation (SF). x 40.

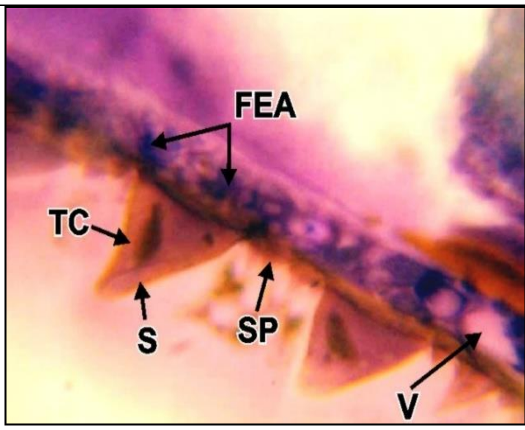

Fig. 4 Further magnification of same portion showing invasion of fungus in Epidermal area (FEA), Large vacuoles (V) can be seen in Epidermis, Trichogen cells (TC) almost degenerated in Setae (S) and Spine (SP) are reducedx100

Plate 3: Histomicrograph of fourth instar larva of *Helicoverpa armigera* (Hubner) treat with *Beauveria bassiana* (Balsamo) showing changes in the body wall.

Discussion

Histological studies were conducted on the fourth instar larvae of *Helicoverpa armigera* (Hubner) using the most effective dose that emerged out from the screening test with botanical biopesticides plant *Lantana Camara* (L.) (leaf and flower) extracts and microbial fungus *Beauveria bassiana*.

When 1. 0ml of leaf and flower extracts were given through food to the fourth instar larvae of *Helicoverpa armigera* (Hubner), and 7µ section were cut and discoloured with hematoxylin and eosin, various degrees of cellular disruptions were apparent. They were expressed in terms of damage in all cuticular structures like integument, trachea, peritrophic membrane, to noncuticular structures like epidermis, fat cells, and basement membrane, suggesting the action of plant extracts on the majority of insects tissue

Grainge *et. al.*, (1984) [14] report that around 1800 plants species have been reported to have insecticidal values and they act as stomach, contact poison, or antifeedants and repellants. Rajashekar *et.al.*, (2014) [36] Because they are rich sources of vital energy molecules, plants can provide an alternative to the pest control agents currently in use. In our investigation, we found that basically, the extracts are acting as a stomach poison in addition to some antifeedant activities. Although there was not a complete cessation of feeding. The insects started showing changes, one to two days after treatment. Hence, there is a strong possibility that insects are acting as a stomach poison.

The effect of plant extracts starts right from the peritrophic membrane although the body wall was the last target of the activity of the extracts. But the fact that all the major chitinous structures related to chitin synthesis and deposition are present here, this part revealed elaborated destruction in its histoarchitecture after treated. The body wall became fragile. The outer corrugated appearance was lost. Setae and spine lost their identity, The thick chitinous endocuticle became thin sheet-like with feeble architecture. The laminated structure that provided strength was disorganized that resulted in the diffused pore canal formation.

Integument of insect is often involved in defense towards predators and pathogenic agents and checks the entry of foreign attack. Generally, it forms the first contact point from the interaction between an insect and natural enemies. The low mechanical strength after treatment indicated that the extracts have attacked the vital structure of the insect that is chitin synthesis. Since bug cuticle fulfills a huge diversity of functions (Gorb, 2001 and Neville, 1975) [13, 28]. It does not only build the exo- skeleton but also forms an significant constituent of a assortment of mechano-receptors. Loss of hairs and spines in our study show that the mechano-receptor goods of the skin was loaded which strength lead to the poor articulation of body parts like the head, mouthparts, and legs. These structures suffered a lot during the treatment and it was well evident in our investigation where treated larvae developed weak mouthparts, stumpy legs with a swollen base, and flaccid and smooth skin that could be easily ruptured at any point. The dissolution of the spines and setae with complete loss of cuticular identity and swelling of epidermal layers and formation of reduced and swollen setae were very prominent with leaf treatment than that of the flower treatment.

The adjacent lamellae in the endocuticle were partially or completely digested in flower-treated tissues. The laminar layer of the endocuticle revealed a hazy appearance with diffused pore canals. This might have resulted in the inadequate transportation of important materials for the synthesis of upper layers. (Plate -2, Fig. 3&4)

Many processes take place on the surface of an insect bite. Separation and repair of wax layers, endocrine tanning, endocrine formation, scratch healing (Denell 1958 and Wigglesworth 1957) [43].

In order for cells to participate in these processes, they must have a definite path through freely woven endocrinologists or through the perforated canals of solid clots. These perforated channels may have anchors to attach the epithelium to the endothelial cell. (Locke, 1961) [24]. Hence, it can be very well speculated that after treatment with plant extracts the pore canals were disturbed and they were unable to attach endocuticle with epithelium, hence there was a large space formation in both layers. These weak pore canals and weak lamellar layer brought about the loosely and weakly developed integument. The epidermal cells that secrets the endocuticle are also responsible for spine formation through trichogen cells that were are almost damaged with undifferentiated cells with degenerating nuclei. Gulka *et. al.*, (1981) [15] reported that diflubenzuron which is a chitin synthesis inhibitor insecticide caused similar disruption. Ghiradelle (2005) also suggested that in all butterflies and moths, scales and bristles are made up of nonliving insect cuticles and disturbance in the epidermal layer brings disturbed chitin synthesis thereby weak cuticles with feeble spines and scales. Similar space formations and delamination of endocuticle and epidermis were also observed in our investigations.

Since the toxic nature of the extracts is related to growth regulators the formation of intermediates, death during molting with attached exuviae and the weak nature of the integument manifests the chition inhibition during developmental stages. Several reports are available to prove the effects of growth regulators on chitin inhibition. (Khaled, 2009) [19].

Further protein is an important component of chitin and hemolymph of insects is an important vehicle for protein transportation, the affected insect is not able to transport proper proteins at the required site thereby developing weak, fragile, and deformed larval changes in histological architecture.

Hence, the overall effects of plant extracts on the insect's tissues indicate the chitin inhibitory nature of extracts in addition to general toxicity like insecticides.

Next, the histopathological studies of *Beauveria bassiana* (Balsamo) revealed that it is consisted conidia, hyphal bodies, and hyphae. These hydrophobic conidia are attached to all body region, with a favourite for surface contain setae and spines. Conidia be able to penetrate directly through the integument.

Microscopic spores of the fungus attach themselves to the body of the insects and upon getting favorable conditions, hydrophobic conidia germinate and establish themselves in 24 to 48 hours in a white mycelial mass turning whole insects cadaver into a white cushiony structure, hence called white muscardine. In the present investigation, we are considering the exact mode of action of this fungus on insect tissues. We can attack the fungus conidia by sticking to a cut surface, swelling from the inside, destroying all the cells in the body and resuscitating or resuscitating an insect bite.

Dannon *et.al.* (2020) [5] Host infection with the fungus manifests itself in four stages: grafting, germination, differentiation, infiltration, and multiplication. Freimoser *et al.,(*2003) [10] The life cycle of forced biotropic insect pathogens can be divided into three distinct stages: host infection, plant growth within the host insect, and re-infection by insect bites.

The setae became delicate, soft with destroyed trichogen cells. The integument of insect is the main structure involved in defense and protection due to its mechanical strength of chitin. Snordgrass, (1953) also observed Setae and spines are also defensive structures made up of chitin and secreted by cells of the epidermis. The low mechanical strength after treatment indicated that the pathogen has attacked the vital structure of the insect that is chitin synthesis. Since pest cuticle fulfills a large range of function (Gorb 2001 and Neville, 1975) [13, 28].

The mechanical strength of the cut layers is low due to their weakness. The epithelium is transformed and the exocuticle and endocuticle are separated from the epithelium. Creating this space was more smooth and vulnerable. The tension in the bark is completely lost.

Ultimately, the structure of the cuticle became fragile with no identity in between different cuticular layers. Seta swelled with small trichogen cells and conidia made their presence on the cuticle and inside cuticle by rupturing the endocuticle. Attack of this pathogen on articulating structures has also been reported by many workers.

Madeleine (1963) Conidium hydrophobic conidia are establish in all parts of the body, charitable favourite to surface and spines. Conidia were able to penetrate directly through the integument. Germinated conidia were observed on the larval body surface at 24, 48, and 72 hr post-inoculation. In the colonization events in this study, the formation and multiplication of hyphal bodies inside the host body were illustrious. It was further observed in our studies that conidia also started germinating inside the spine and setae. The spine and setae were bloated with germinating conidia inside. A large space was formed between Exo and endocuticle. At some parts, we observed dense invasion of conidia and fungal mycelia inside the cuticle, and spines were blotted fully under the attack of entomopathogen.

Holder and Keyhani. (2005) [16] the basic initial stages of colonization involve merging and joining the host surface. Passive hydrophobic reactions are mediated by hydrophobins that initially cause insect bites in conidia. Early spores multiply; the hydrophobic layer decomposes and deposits new cell wall material (Wosten, 2001) [44]. Conidia (or in some cases Plastospora) are associated with insect bites by electromagnetic and chemical forces (Mascarin and Jaronski 2016) [26]. Later, with the production of mucus, they caused an epithelial change (Wraight and Roberts 1987) [45], followed by conidia edema. When the conditions are right, the appearance of conidia or plastospores can lead to the formation of genital tubes. In fact, under chemical stimuli the conidia emerge, regenerate, and the embryonic tube is formed (Mascarin and Jaronski 2016) [26].

To conclude, the histopathological studies have revealed that integument is the main target tissue for the action of both botanicals *Lantana camera (*L.) and microbial pesticides *Beauveria bassina (Balsamo*). However, it was also observed in the histomicrographs that extensive tissue destruction occurred in the body wall of the insect. These damages were from the center towards the periphery, which was also evident by the development of various morphological and behavioral abnormalities developed after the insect was severely infected.

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