Egypt. J. Plant Prot. Res. Inst. (2020), 3 (2): 731 - 742

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Contact and feeding effects of chlorantraniliprole, methoxyfenozid and spinosad on some histological changes in cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ARTICLE INFO

Article History

Received: 8/4/2020

Accepted: 11/6/2020

Keywords

Coragen, chlorantraniliprole, methoxyfenozid, runner, spinosad, *Spodoptera littoralis*, histopathological effects, midgut and cuticle.

Abstract:

The contact and ingestion effect of chlorantraniliprole, methoxyfenozid and spinosad on the 4th larval instar of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) were studied. Half and a quarter off the recommended rates of three insecticides (Coragen, runner and spinosad) were prepared, and the two methods of contact and food contamination were applied in laboratory to compare their effects on the histo-formation of both the midgut and the integument of the 4th instar larvae *S. littoralis*. Histomorphological changes have been observed within 48 hrs. in both applications (contact and/or ingestion) on the midgut and integument tissue formation of the treated larvae. The treatment with the tested insecticides caused in general, destruction of both goblet and columnar cells of midgut. Epithelial layer showed detached of the basement membrane, disorganized and accompanied by major vacuolization. Separation of both basement and peritrophic membrane. Lysis in the peritrophic membrane led to the mixing of the components of the lumen with the lysis cells of the midgut membrane. It progressively degeneration in the epithelial lining of the midgut. The tested insecticides affected the integument as disturbance with abnormal deposition showing different thickness, fissure in hypodermal cells and endocuticle distortion. These histopathological effects are presumed to be responsible for the food utilization and reduction in growth caused by Runner, coragen and spinosad. Therefore, be concluded that these insecticides have sublethal effects on *S. littoralis* that may affect on the dynamic population in the field by reductions in both survival and reproduction.

Introduction

Cotton, *Gossypium hirsutum* L. is one of the most important economic crops in Egypt and the world since cotton is cultivated in over 100 countries (Zidan *et al.*, 2012). The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most polyphagous destructive pests which attacked crops belonging to 40 families such as cotton, vegetables which considered the most valuable crops in the country (Azab *et al*., 2001). The intensive use of insecticides against *S. littoralis* has led to

the development of insect resistance to many registered pesticides (Sparks and Nauen, 2015). Insecticide resistance is a long-standing and expanding problem for pest arthropod control, that needed to the development of new insecticides such as chlorantraniliprole (CAP). It is primarily active on chewing pests by both ingestion and contact. The target insect rapidly stops feeding, disrupts calcium homeostasis in nerve and muscle cells becomes paralyzed, and ultimately die occurs within 24-72 hours (Bassi *et al.*, 2009). Spinosad is a secondary metabolite produced under natural fermentation conditions by the actinomycete *Saccharopolyspora spinosa*. Routes of insecticides entry include both topical and ingestion. Signs of spinosad poisoning include initial flaccid paralysis, followed by tremors and eventual death (Thompson *et al.*, 1995). Spinosad has been applied to over 200 different crops. It has been used to control caterpillars in various crops, in addition, it is quick degradation, low toxicity to humans, and low doses of use. IGRs considered as the third generation of insecticides because their mode of action differs from other insecticides with low toxicity to natural enemies and less polluted. methoxyfenozide which belongs to dibenzoyhydrazine, developed as a non-steroidal agonist of the insect moulting hormone of caterpillar, acts on binding to ecdysone receptor protein/ ultraspiracle protein and most effective after ingestion larvae cease feeding within 2-5 days. These insecticides which belonged to different groups with a different mode of action including coragen, runner and spinosad are selected for a comparative study to clarify their effects on the histopathological structures of cuticle and midgut of the 4th instar larvae of the cotton leafworm *S. littoralis* .

Materials and methods

1.Test insect:

Field strain of cotton leafworm, *S. littoralis*, were collected from Qalyubia Governorate and reared under laboratory condition for one generation at 25±2°C in the Department of Cotton Leafworm, Plant Protection Research Institute, Dokki, Egypt, Rearing procedure was carried out according to Ghoneim (1985).

2. Insecticides used:

Coragen 20 % SC. Common name: Chlorantraniliprole (rynaxypyr), Dupont. Recommended rate, 60ml /feddan Tracer 24% SC, Common name: Spinosad 24% Dow Agro Sciences LLC. Recommended rate, 37.5ml / 100 L. Runner 24% SC : Common name: Methoxyfenozid, Dow Agro Sciences LLC. Recommended rate, 50ml /feddan.

3. Laboratory bioassay:

The three tested compounds coragen, runner and spinosad which belonging to different groups were diluted with water to prepare (0.0075 and 0.00375%), (0.00625 and 0.00313%) and (0.0188 and 0.00938%) half and a quarter off the recommended rates of each, respectively. These insecticides were prepared freshly before treatments conducted as two methods, contact and ingestion. Contact bioassay modified to be like Rashwan *et al.* (2013), by using 4 glass jars for each concentration, adding suitable amount of insecticides on them to make insecticide residual film on the glass jar. At the same manner, water instead of insecticides as control. All treatments and the control were left to dry under the room temperature. The 4th instar larvae were placed into each jar for 48 hrs. Ingestion bioassay was carried out by dipping castor bean leaves in the tested concentration solutions for 20 sec, the treated leaves were left to dry under room temperature. The 4th instar larvae were replicated four times and fed for 48 hrs. on treated leaves. At the same time, four replicates of larvae were fed on untreated leaves and used as a control. The survived larvae in both the two treatments as well as untreated larvae were collected and preserved to perform histological preparations.

4. Histological examination of larval midgut using light microscope:

To examine histological changes of both the midgut and the integument 4th instar larvae, *S. littoralis* induced by the tested insecticides, a histological technique was done at the Animal Health Research Institute. Histological cuts of 5 µm were performed with a microtome and dyed with hematoxylin-eosin, according to previously described protocols (Rodríguez-Santiago, 2002). Slices were observed with an optical microscope with the lens of 40× and microphotographs were taken.

Results and discussion

1. Histological examination of the normal midgut 4th instar larvae of *Spodoptera littoralis*:

The midgut of 4th larval instar of *S. littoralis* was shown in Figure (1) the outer sheath of the intestine, includes two types of muscles, the outer one is a layer of longitudinal muscles, and the inner one is a layer of circular muscles. This followed internally by a thin basement membrane where the epithelium rested. The epithelium consists of a layer of epithelial cells, which are elongated and columnar in shape. Each cell consists of a dark round nucleus occupying the middle part of each cell. There are other types of epithelial cells, the regenerative or the imaginal cells, which are small, found between the bases of the columnar cells. The regenerative cells are present individually or in clusters of few cells, each cell contains a large nucleus surrounded with granular cytoplasm. The lumen of the ventriculus is surrounded by the peritrophic membrane, which envelops the food materials and protects the epithelial cells from contact with the food mass. In general, the epithelium layer in the untreated specimen was thicker than that in treated. Microscopic examination showed that the histological structure of the normal midgut of the 4th instar larvae of *S. littoralis* is represented in (Figure, 1). It consists of two layers of muscle fibers, the outer longitudinal fibers and the inner circular ones. The circular muscle fibers are very close to the basement membrane of the epithelial cells. There is a wide space between the longitudinal fibers. The peritrophic membrane is followed by an epithelial layer of cells which lines the cavity of the midgut. There is a peritrophic membrane surrounding the lumen which protects the epithelial cells from contact with the food mass.

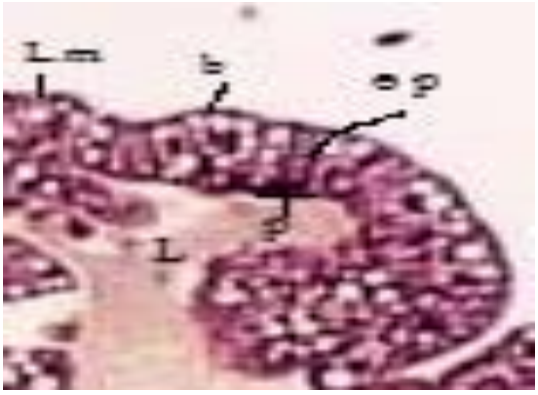

Figure (1): Histomorphological structure of the normal midgut of *Spodoptera littoralis* 4th instar larva. (L.m: Longitudinal muscle b: Basement membrane ep: Epithelial cell p: Peritrophic membrane L: lumen)

2. Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with "Coragen" insecticide:

The midgut of the 4th larval instar of *S. littoralis* treated via contact with coragen at concentrations 0.0075% and 0.00375%, respectively is shown in Figure (2 a,b). Figure (3 a,b) showed treatment with coragen by ingestion. It was noticed several histological changes compared to the control. In general, coragen was affected on midgut structure as, some epithelial cells disintegrated and necrosis, others cell boundaries were destructed and lysis, elongation of epithelial cells and appearance of vacuoles. Also, the peritrophic membrane was destructed and detached from the epithelial cell. Some complete separation and lysis of the peritrophic membrane led to release the lysis cells into lumen components.

These disturbances in the midgut structure and necrosis cells led to a disturbance in the shapes, enzyme secretion and its function.

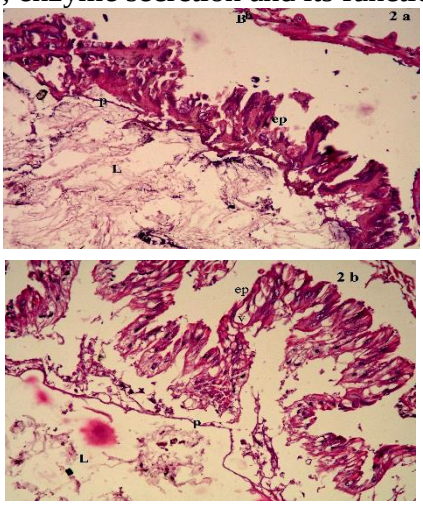

Figure (2a,b): Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with coragen insecticide by contact. a. Completely separation for the basement membrane and necrosis of some cells. Scattering of the nuclear content of the epithelial cell. Partially destruction in the peritrophic membrane. b. Disappearing of basement membrane, cell boundaries, proliferation of columnar cell lining midgut, increase of goblet cell, separation of peritrophic membrane and appearance of vacuoles.

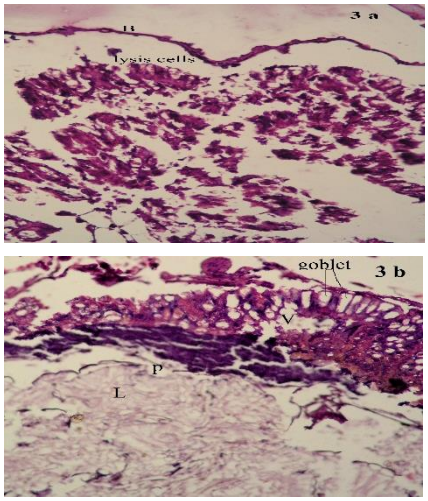

Figure (3a,b): Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with coragen insecticide by ingestion. a. Severe destruction of cells lining midgut with cell necrosis, complete destruction for epithelial layer and basement membrane completely separated. b. Some of the epithelial cells became detached from the wall of the midgut and full of the vacuoles, the muscle fibers lost their typical striation pattern, where they became irregular and broad. Sever increase of goblet cells.

Coragen showed effects on the midgut structure by both the contact and ingestion treatments with different degree leading to completely losing its function, increase of goblet cells and destruction for boundaries cell and vacuolization with low concentrations. on the other hand, almost of cell necrosis, lysis and undistinguished specially in ingestion. Increasing goblet cells with losing basement membrane led to losing its permeability, transportation and more effective with high concentration by ingestion than contact.

3. Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with "Runner" insecticide:

The two concentrations of the compound runner produced great damage in all layers of the midgut wall as shown in (Figure, 4 a,b and Figure, 5 a,b) by contact or ingestion treated. Separation of both basement and peritrophic membrane with the appearance of vacuoles. The most effective concentration was 0.00625 and 0.00313% by ingestion and contact, respectively.

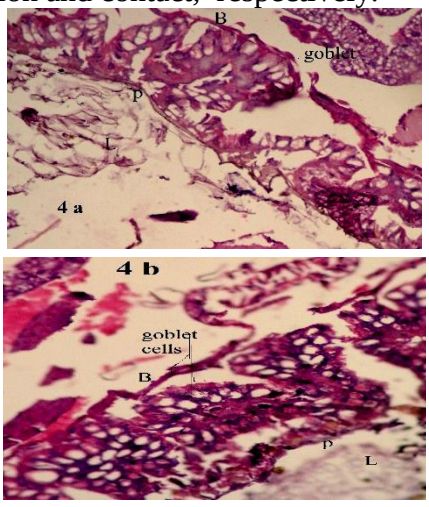

Figure (4a,b): Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with runner insecticide by contact. a. Sever increase of goblet cells. Partial disruption of both basement and peritrophic membrane in both concentrations. b. Sever proliferation of cell lining midgut with an increase of goblet cells.

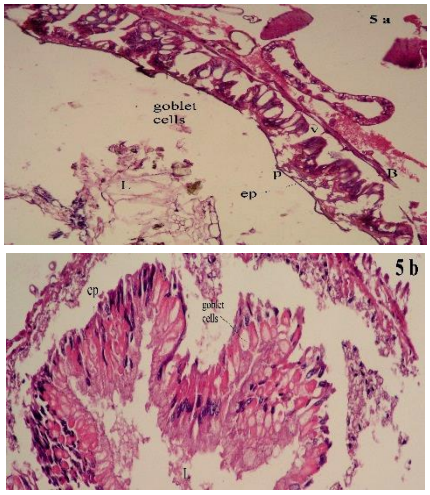

Figure (5a,b): Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with runner insecticide by ingestion. a. Necrosis of some cells lining midgut as well as an increase in goblet cell appearance of vacuoles, disruption of columnar cells. b. An increase of goblet cells, epithelial cells collected in clusters, separation in the basement membrane, lysis in the peritrophic membrane.

4. Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with "Spinosad" insecticide:

The midgut 4th instar larvae, *S. littoralis* treated with spinosad at concentrations 0.00188 and 0.00938%), respectively, are shown in (Figure, 6 a,b and Figure, 7 a,b) by contact or ingestion treated.

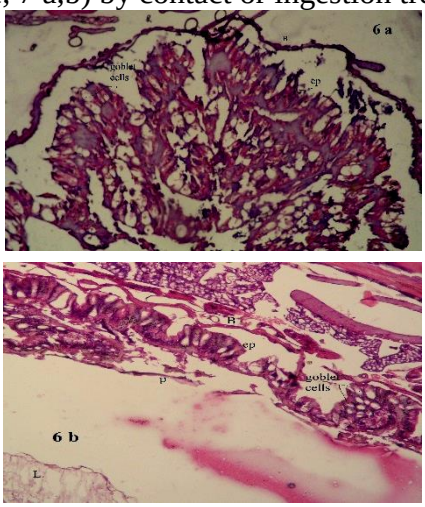

Figure (6 a,b): Histomorphological changes in the midgut 4th instar larvae of *Spodoptera littoralis* treated with spinosad by contact a. Sever proliferation of cells lining midgut, separation of the basement membrane. b. Necrosis of some cells lining midgut.

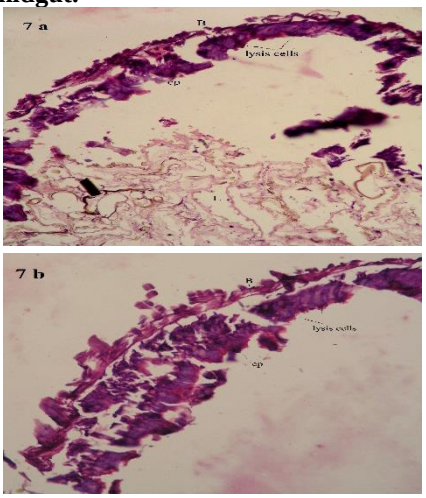

Figure (7a,b): Histomorphological changes in the midgut 4th instar larvae, *Spodoptera littoralis* treated with Spinosad insecticide by ingestion. a. Most of the cells lining midgut showing necrosis, epithelial cell undistinguished, partially disrupted basement membrane, complete separation of the peritrophic membrane. b. Sever damage in cells lining midgut.

So, spinosad more effective on mid gut by ingestion than contact in both concentrations.

5. Histomorphological changes in the integument 4th instar larvae of *Spodoptera littoralis* induced by the tested insecticides:

Normal integument structure of *S. littoralis* larva is represented in Figure (8).

It consists of an outermost distinct layer, the epicuticle and the inner layer called procuticle. Procuticle, composed of exocuticle which is hardened and endocuticle remains flexible and colorless. Lastly the hypodermal layer is a distinct layer (Epidermal and oenocytes) which composed of columnar or cuboidal cells (Chapman, 2004).

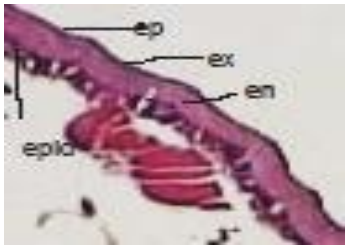

Figure (8): Histomorphological structure of the normal integument of *Spodoptera littoralis* 4th instar

larva. (ep: epicuticle, ex: exocuticle, en: endocuticle, epid: epidermal cell).

6. Histomorphological changes in the integument *Spodoptera littoralis* treated with "Coragen" insecticide:

Histomorphological changes in the integument 4th instar larvae *S. littoralis* are shown in (Figure, 9 a,b and Figure, 10 a,b). Disturbance, thin or thickness of cuticle occurred. On the other hand, by ingestion, the thickness of the cuticle decreased with abnormal deposition.

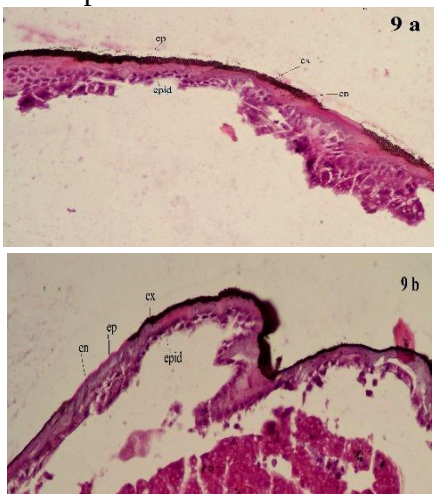

Figure (9 a,b): Histomorphological changes in the integument of the 4th larval instar of *Spodoptera littoralis* treated with coragen by contact. a. Shows disturbance, thin or thickness of cuticle occurred. b. The thickness of the cuticle decreased with abnormal deposition.

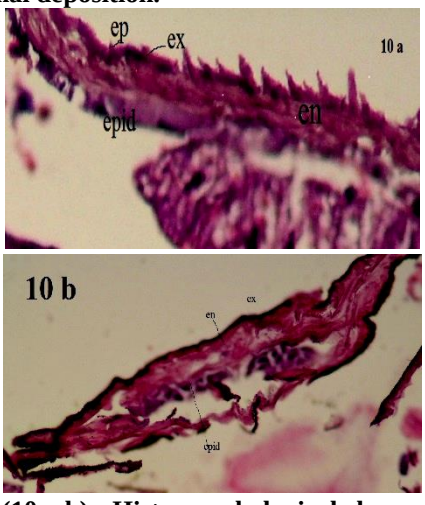

Figure (10 a,b): Histomorphological changes in the integument of the 4th larval instar of *Spodoptera littoralis* treated with coragen by ingestion. a. thickening of a fibrous layer with irregular cuticle deposition and present of some fat cells. b. Distortion, the thickness of the exocuticle decreased with abnormal deposition.

7. Histomorphological changes in the integument *Spodoptera littoralis* treated with "Runner" insecticide:

Histomorphological changes in the integument 4th instar larvae *S. littoralis* are shown in (Figure, 11 a,b and Figure, 12 a,b) with Runner insecticide by contact and/or ingestion treatments. Runner treatments shewed clear a thin layer of exocuticle with abnormal deposition in both contact and ingestion treatments. lack of fat cells in case of contact than in ingestion.

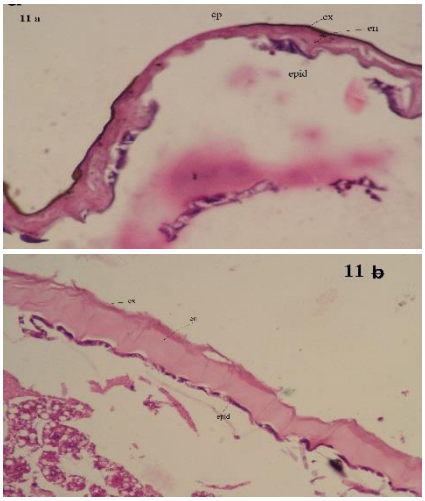

Figure (11a,b): Histomorphological changes in the integument 4th instar larvae *Spodoptera littoralis* treated with runner insecticide by contact. a. The cuticle thickness decreased with abnormal deposited, lack of fat cell, distortion of endocuticle separated from epidermal cells. b. Appearance of the fat layer, with a thin layer of exocuticle deposition and partial separation of endocuticle from the epidermal cell.

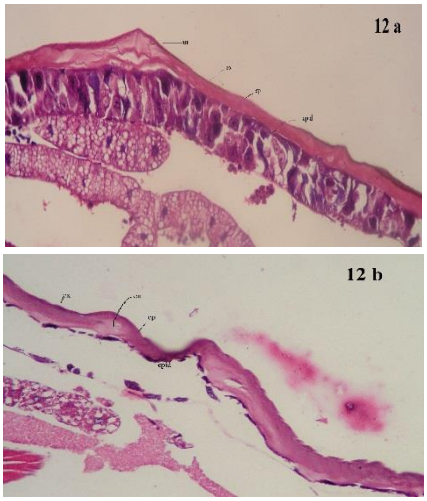

Figure (12 a,b): Histomorphological changes in the integument 4th instar larvae *Spodoptera littoralis* treated with runner insecticide by ingestion. a. Less effect on both hypodermal and fat cells. b. Decrease in the thickness and mild thickening of fibrous layers.

8. Histomorphological changes in the integument *Spodoptera littoralis* treated with "Spinosad" insecticide:

Histomorphological changes in the integument 4th instar larvae *S. littoralis* are shown in (Figure, 13 a,b and Figure, 14 a,b) with spinosad insecticide by contact and/or ingestion.

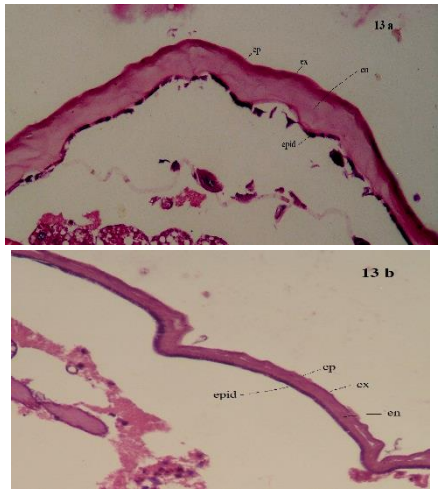

Figure (13 a,b): Histomorphological changes in the integument 4th instar larvae *Spodoptera littoralis* treated with spinosad insecticide by contact. a. Slight effect on the hypodermal cell, mild thickening layer of exocuticle. b. Thin layer of exocuticle with less irregular cuticle deposition and less distortion in endocuticle with the epidermal regular layer.

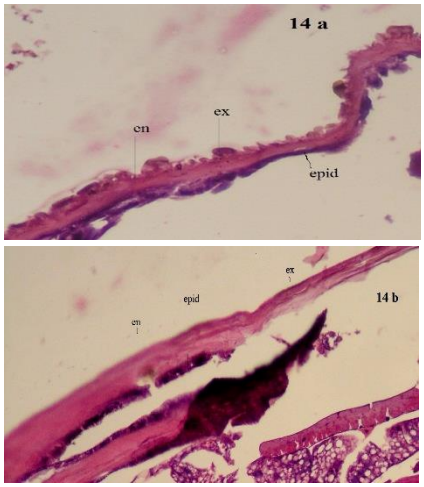

Figure (14a,b): Histomorphological changes in the integument 4th instar larvae *Spodoptera littoralis* treated with spinosad insecticide by ingestion. a. Swelling of some epidermal layers with decrease fat cells, an abnormal deposit of thin exocuticle layer with swelling. b. A thin layer of exocuticle, an increase of fat cell. Partial effective on endocuticle with separation from a hypodermal cell.

Our results showed that the highest concentration used for each tested insecticide was the most effective and produced very histomorphological disturbances in the mid-gut of 4th instar larvae *S. littoralis* treated with the tested compounds. The most recorded observations are destruction in all layers of the midgut, separation of both basement and peritrophic membranes which may lead to losing its permeability properties and losing its function by a disturbance in the potassium transport out of the haemolymph into the gut lumen by goblet cells which responsible for this function (Rosaiah and Mukkerjee,1985). The appearance of vacuoles and lysis in the epithelial cell was noticed. This disturbance, distortion and cell lysis in the midgut structure may be led to disturbance associated with its function and gradually death.

These results are like many previous studies for each of, Abou El-Ghar *et al*. (1994), Abou El-Ghar *et al*. (2013). Ghoneim *et al*. (2015) and Begum and Qamar (2016). They reported that such as these tested insecticides caused vacuolization of the midgut epithelium of *S. littoralis* larvae, in

addition to the sloughing off scattered groups of the midgut epithelium into the gut lumen and disappearance of the cell boundaries. Malinowski (2004) revealed that methoxyfenozide, teflubenzuron and diflubenzuron are reacting with receptors on the brush border membrane of insect midgut, reaches the target tissue mainly by alimentary canal. The ingestion of toxicant by the insects releases a toxic peptide, which binds to sites on the microvilli membranes of the midgut causing cytolysis. Epithelial cells progressively degenerated until it was totally disrupted, and larvae died. The destruction of both goblet and columnar cells of midgut together with pathological effects on microvilli (Federici, 1993). The disruption of the microvilli caused vacuolation appearance of the midgut epithelium of *S. littoralis* larvae as a result of reduced absorptive capacity of the midgut epithelium. , disappear of the cell boundaries and sloughing off scattered groups of the epithelial cells into the lumen. (Abou El-Ghar *et al*., 1994). Coragen insecticide acts via disrupting calcium homeostasis in central neurons rather than inhibiting AChE as anticholinergic compounds and causes decreased antioxidant and biotransformation enzyme activities (Doyotte *et al*., 1997; Li *et al*., 2011 and Andreia *et al.,* 2015). On the other hand, methoxyfenozide and rynaxypyr exhibited the least contact toxicity, whereas spinosad was the slowest. Rynaxypyr was more effective at 24 h as ingestion (Temple *et al.,* 2009 and Rashwan *et al.,* 2013). Rynaxypyr have contact effect (residue film on glass) and ingestion (feeding on insecticide-treated leaves). This leads to paralysis and subsequent death of the insect. IGR's may have calcium-binding properties and may disorganize the cementing substance between cells and tissues of the membrane, which may disturb permeability properties of the plasma membrane and lead to water loss causing dehydration and possibly vacuolization, that explained the coragen shrinkage due to dehydration may lead to the collapse of the lumen of midgut epithelium. Also, affect the lipid layer of the membrane, which may ultimately destroy specific permeability properties of the plasma membrane. disrupted midgut tissues would function abnormally, and the enzyme secretion and nutrient absorption would be disrupted (Mordue and Blackwell, 1993 and Abd-El-Aziz *et al.*, 2017). The differences between the inactive ingredients are due to their mode of action and method of penetration. So, histopathological changes are one of the most definitive indicators of fat changes, vacuolation and destruction of epithelial cells and their boundaries are highly recognized in both epidermal and midgut cells of tested insects and give a good explanation for the recorded disturbance in the lipid, chitin synthesis, protease lead to disturbances in the function of the internal organs as cell damage (Anitha *et al*., 1999; Abd-El-Aziz *et al*., 2013 and 2017). The degeneration of the epithelial cells and decay of its boundaries, caused slight and severe disintegration of the epithelium, fading of the boundaries of epithelial cells and detachment of epithelial cells. This might reflect the variable susceptibility to different insecticides. The present histopathological destruction on cuticle and midgut caused by the tested insecticides may suggest that any of these insecticides can cause the death of an insect when entering tissues either ingestion or contact in adequate amounts. Coragen, runner and spinosad have high toxicity and are considered promising for controlling *S. littoralis* and 48 hrs. after treatment is enough to promote morphological abnormalities and death. Appearance lack of fat cell may be explained the larvae could use it as a source of energy to get rid old cuticle in case of starvation.

Cuticle forms layers on the surface or very close to the surface of secretory cells, and that cuticular deposition may be completed at a distance from the secret or

sites. (Khedr, 2011). IGR's interferes with the exoskeleton chitin by contact, through the alimentary canal. The penetration of insecticides through midgut walls to haemolymph of the treated larvae is the first position to be affected by these compounds, as well as the midgut (peritrophic membrane). In fact, epidermal cells provide the chemical precursor for chitin synthesis process which is extracellular. Moulting (Ecdysis) is initiated with separating epidermal cells from the old cuticle during apolysis process (Chapman, 2004).

Insect growth regulators are known to affect through digestion and utilization of ingested food, IGR acts on the peritrophic membrane by affecting its chitin-protein structure, hindering its role in protecting secretory cells from damage. Indoxacarb caused abnormalities in the shape of the exocuticle and the hypodermal cells separated from the endocuticle , distortion in the endocuticle and some fissure were revealed blockage of its formation in case of methoxyfenozide (Hassan, 2009). While spinetoram has slight effect on the cuticle as compared with other compounds, decreased the thickness of the cuticle, separated some hypodermal cells from the endocuticle. Abd-El-Aziz *et al*. (2013 and 2017) and El-Shourbagy (2019) reported that the disturpance in LDH, indicate excessive disturbance of tissues also, total carbohydrate, chitinase and protease enzymes were significantly disrupted as *S. littoralis* treated with coragen ,runner , neem, thyme and bitter and rose bengal. These components are necessary for building cuticle and lake or disruption of them postulated the reason of deformations, increasing abnormal forms due to disturbance in cuticle deposition. Khedr and Mead (2015) mentioned that inability of discarding the old cuticle or precocious molt lead to abnormal chitin deposition. That clear as partially ecdysed larvae with big batches of new cuticle without normal coloration, the extrusion of body fluids that led to mortality. In the same trend the larval deaths by Novaluron may be due to the failure of larvae to moult owing to the inhibition of chitin formation .a prohibition of feeding and continuous starvation where it suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis leading to the production of abnormalities (Ghoneim *et al*., 2000 and 2017) or interfere with the synthesis or deposition of chitin on the exoskeleton or other chitinized internal structures or to the inability to shed their exocuticle or to swallow volumes if air for splitting the old cuticle and expand the new one during ecdysis (Adel, 2012 and Zorzetti *et al*., 2015)

Many studies on *S.littoralis* with coragen , IGR's and other insecticides according to the symptoms as a result of treatment were carried out . Ceases within hours of ingestion, paralysis and may not die for several days, die due to inability to feed, molting acceleration or incomplete molt, lethargic and develop discolored areas or bands between their larvae body by Dhadialla *et al*., 1998; Kumar and Santharam ,2008; Abd-El-Aziz *et al.*, 2013; Rashwan, 2013; Khaled and Farag, 2015 and Abd-El-Aziz *et al*., 2017 revealed their effect to disturbance in enzyme activities and carbohydrates hydrolyzing enzymes which inhibit the functions in all insect tissues during metamorphosis processes in the cuticle by inhibition of chitin synthesis due to reducing of chitinase, protease lipids. These components are necessary for building cuticle and digestion of old endocuticle in the molting process. So, any disturbance or lake in these enzymes may attribute to abnormal endocuticular deposition and abnormal molting by disrupted the binds to the ecdysone receptor which initiates the molting process. Another reason, the larvae die due to dehydration, starvation and prevented from shedding its old cuticle within 2-5 days. Also, great damage occurred in cuticular layer,

exocuticle and endocuticle was in distinguish, endocuticle separated from hypodermis , partial degeneration with fissure and distortion of epidermal cell as a result of treatment with rose bengal for 5 hrs. (El-Shourbagy, 2019) .

The effect of the tested insecticides on the midgut of *S. littoralis* larvae by contact proved their ability to interfere with the exoskeleton chitin then the alimentary canal can be affected. Via ingestion of insecticides the penetration from midgut walls to haemolymph of the treated larvae could be influenced on peritrophic membrane to lose its permeability and caused abnormal structure in midgut. These disturbance, distortion or cell lysis in the midgut structure led to disturbance associated to its function, enzyme secretion, cell degeneration ,cell necrosis and gradually die. These abnormal structural may reflect the functional differences between the midgut epithelium of both treated and untreated larvae. The larvae treated with coragen become shrinking, hard, dark in color that may due to dehydration specially in the starved larvae.

The present histomorphological destruction on cuticle and midgut caused by the investigated insecticides may suggest that any of these insecticides can cause death of an insect when entering tissues either ingestion or contact in adequate amounts. Coragen, runner and spinosad have high toxicity and are considered promising for controlling *S. littoralis* and 48 hrs. of treatment enough to promote morphological abnormalities and death. Appearance lack of fat cell may explain the larvae could use it as a source of energy to get rid old cuticle in case of starvation.

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