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Feeding deterrence and midgut histomorphological alterations in *Spodoptera littoralis* larvae treated with separate and combined imidacloprid and spinosad

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ABSTRACT

The widespread agricultural polyphagous insect pest *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) infests various crops especially the Egyptian cotton. Safer alternatives for insect pest control are needed due to the drawbacks of current insecticide used in agriculture settings. The neonicotinoid imidacloprid and the spinosyn biopesticide spinosad are widely used in crop systems to fight against a broad spectrum of phytophagous insect pests. Although spinosad and imidacloprid have been used in separate trials against *S. littoralis*, nothing is known about the impact of their binary mixing. Thus, the current research investigated the effects of imidacloprid at sublethal levels and spinosad alone and in combination on the antifeedant activity and the histomorphological changes probably caused in the midgut of *S. littoralis* 4th-instar larvae. Using the leaf-dip technique approach, on castor-bean

Ricinus communis leaves that had been treated, larvae were fed for three days in a row (treatment period). In case of the midgut histological study, for two days in a row, new, untreated leaves were substituted for the treated ones (recovery period). The most effective antifeedant was imidacloprid. Which was about 1.30, 1.41, and 1.29 times more than that of spinosad on the 1st , 2nd , and 3rd day post-treatment, respectively. Beside, imidacloprid was about 1.46, 1.11, and 1.18 more than that of the combined imidacloprid and spinosad on the 1st , 2nd , and 3rd day post-treatment, respectively. Compared to the midgut of untreated larvae (controls), the treated larvae's midgut displayed changes after 3 days of treatment, 1st and 2nd day of recovery. Muscle layer disintegration, epithelial cell disarray, peritrophic membrane separation, basement membrane detachment, and epithelium vacuolization were among the histological abnormalities. Combining spinosad and imidacloprid would reduce the amounts of each pesticide when used separately. This might result in less environmental degradation and prevent the development of resistance.

INTRODUCTION

In the world's tropical and subtropical regions, cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most devastating pests [1]. As a leaf feeder that will consume practically any herbaceous plant, the larval stage was well-known [2]. About 90 host plant species from 40 families are infested, including crops and vegetables with high economic value [3]. This pest is a year-round resident in Egypt, It is regarded as one of the most harmful pests to cotton, the country's main cash crop [3].

In Egypt, pesticides have been the principal tool for controlling *S. littoralis* [4,5]**.** Unfortunately, due to the improper application of these pesticides, *S. littoralis* has evolved a resistance to organophosphates, carbamates, and synthetic pyrethroids

[6,7,8,9,10]**.** In addition, due to the usage of these chemical pesticides, a number of issues have arisen, including environmental contamination, human health risks including cancer, and immune system abnormalities [11] and harmful effects on beneficial insects [12].

To address these issues, as replacements for usage in integrated management strategies, new pesticides have been developed and licensed that mimic natural products or come from biotic agents with new modes of action [13,14,15,16]**.** These pesticides include spinosad and imidacloprid.

One of the most used neonicotinoids in the globe is imidacloprid [17]. It is a neurotoxin that affects pest insects' central nervous systems. It is a chloronicotynil systemic insecticide agonist of the nicotinic acetylcholine receptors (nAChRs), increasing Na+ entrance and K+ exit, causing irreversible blockage of postsynaptic receptors, leading to convulsions and paralysis, and eventually, insect death [18,17]**.** In multiple modes of administration, such as foliar, seed/soil treatments, and other methods, imidacloprid is one of the most promising and efficient pesticides against lepidopterous insect pests [19,20,21].

A combination of macrocyclic lactones (spinosyn A and spinosyn D) that were naturally fermented from the soil actinomycete *Saccharopolyspora spinose* is known as spinosad [22]**.** It was chosen as a potential natural insecticide since it works both via contact and ingestion [23]**.** Its major target site seems to be a subtype of the nAChRs, and a second putative secondary target site at the gamma-aminobyturic acid (GABA)-gated chloride channel suggests that its mechanism of action is similar to that of the neonicotinoids, leading to continuous activation of motor neurons, which stops feeding, tremors in most body muscles, and eventually paralysis and death of insect [24]**.** Thus,

the mode of action of spinosad renders it a useful agent to manage resistance in pests. Spinosad has a broad spectrum nematocidal, acaricidal and insecticidal properties [25]**.** It is a stomach poison with some contact action; thus, It has been approved for use in more than 30 nations to combat pests that feed on leaves in the orders Lepidoptera, Coleoptera, Diptera, and Thysanoptera [26,27,28]**.**

The toxic and biological effects of separate imidacloprid and spinosad against *S. littoralis* have been investigated by several authors [29,21]**,** with only one study using combined imidacloprid and spinosad in this respect has been conducted by [30]**.** Applying a binary mixture of insecticides has been hypothesized to minimize the concentrations of each insecticide when applied alone, leading to decreasing environmental pollution and heading off the development of resistance [30]**.** Moreover, to the best of our knowledge, no study has been carried out regarding evaluating the antifeedant activity of both separate and combined imidacloprid and spinosad as well as evaluating the combined effect of imidacloprid and spinosad on the midgut histology of *S. littoralis* larvae. Based on these findings, the present study aimed at evaluating the antifeedant activity of imidacloprid and spinosad, either separately or in combination, against *S. littoralis* larvae. We also evaluated the effects of separate and combined treatments with these pesticides on the histological architecture of the midgut.

MATERIALS AND METHODS

2.1. Insect culture

Eggs from the Cotton Leafworm Research Division were used to generate a stock colony of *S. littoralis*, Plant Protection Research Institute, Assiut, Egypt. Before beginning the experiments, larvae were raised for 30 generations on the *Ricinus communis* L.

(Euphorbiaceae) leaves of the castor bean, in the insectaries of the Zoology Department, Faculty of Science, Assiut University. Adults were fed on a 10% sucrose solution. Insects were maintained at 27 ± 2°C, 65 ± 5% relative humidity, and 16-h light: 8-h dark photoperiod according to [3]**.** A branch of oleander *Nerium oleander* L. (Apocynaceae) was placed in the cage as an oviposition site. Every day, egg masses were gathered and stored in 90-ml plastic cup until hatching.

2.2. Insecticides

Two insecticides were tested, imidacloprid and spinosad. Imidacloprid (Imaxi 35% SC) was produced by Syngenta Agrochemical Co., Ltd., while spinosad (Tracer 24% SC) was created by Dow AgroSciences Co., UK. Two insecticides were purchased from Green Vally Company for agriculture & trade , El-Haram, (Giza), Egypt.

2.3. Antifeedant assay

Four groups of 300 newly molted 4th-instar larvae of *S. littoralis* each were set up and starved overnight. The three groups were given leaves treated with the LC_{50} of imidacloprid (352.18 ppm), LC_{50} of spinosad (175.34 ppm) and combined LC_{25} of imidacloprid (271.02 ppm) + LC_{25} of spinosad (99.06 ppm), respectively, using leaf-dip technique according to [30]**.** The 4th group served as the control, in which larvae were treated with distilled water using leaf-dip technique. One hundred larvae from each treatment were used in three separate replications. Control experiment was also replicated three times with 100 larvae each. Larvae were fed on treated castor-bean leaves for three successive days. Before and after the treatment period, the leaves were weighed daily and the larvae were weighed separately (i.e., 3 days). The amount of consumed food and

larval weight were calculated. The antifeedant index (AFI) was calculated according to [31,32] as follows:

$$\mathbf{AFI = [(C-T) / (C + T)] X 100 (in \%)}$$

where:

C: the food consumed in the control;

T: the food consumed in the treatment.

2.4. Starvation percentage assay

Five hundred newly molted 4th-instar larvae of *S. littoralis* were starved overnight, divided into 5 groups of 100 larvae each, three groups for the treatments with the LC_{50} of imidacloprid, LC_{50} of spinosad and LC_{25} of imidacloprid + LC_{25} of spinosad, using leaf-dip technique [30]**,** one group for the control and one group as starved larvae. Each group was replicated 10 times with 10 larvae each. Castor bean leaves were treated and fed to larvae for 24, 48, and 72 hours. Control larvae were fed on untreated leaves for 24, 48 and 72 h. While starved larvae were left without feeding for 72 h. Before conducting experiments, all larvae were previously weighed. After reweighing the larvae, the starving percentages of the examined larvae were estimated using the formula [33,34] as follows:

$$\mathbf{Starvation\ (\%) = C – E/C – S\ X\ 100}$$

where:

C = Mean weight gain of control larvae after 24h ,48h, and 72 h;

E = Mean weight gain of treated larvae at each insecticide treatment after 24, 48, and 72 h;

S = Mean weight gain of starved untreated larvae after 24, 48, and 72 h.

2.5. Histopathological studies

Four hundred newly molted 4th-instar larvae of *S. littoralis* were starved overnight, divided into 4 groups of 100 larvae each, three groups for the treatments (LC_{50} of imidacloprid, LC_{50} of spinosad and combined LC_{25} of imidacloprid + LC_{25} of spinosad, respectively) using leaf-dip technique according to [30]**,** and one group as the control. Larvae were fed for three successive days. They were then fed for two successive days on fresh untreated leaves (recovery period). After 3 days of treatments and recovery period, larvae were collected and used for histopathological examination of the middle portion of the midgut. Control groups were also examined. For each treatment, ten duplicates of ten larvae each were evaluated. A parallel control of untreated larvae (10 replicates of 10 larvae each) was also run. Both treated and control larvae were dissected with a stereomicroscope (Olympus, Japan) in Ringer's saline solution. The sections of midguts center were promptly fixed in aqueous Bouin's solution for 24 hours, followed by washing in distilled water. They underwent ethanol dehydration in grades of 30, 50, 70, 90%, and absolute before being embedded in paraffin wax. A rotary microtome (Model 2030; Leica, Germany) was used to cut transverse slices at a thickness of 5 m. Hematoxylin and eosin staining (Merck) was applied routinely. Photos of treatments compared to controls were acquired using an Olympus light microscope (Japan).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the data using IBM-SPSS Statistics, version 25 (IBM, Armonk, New York, NY, USA). When there were significant differences between the treatments, the least significant difference (LSD) test at a 5% level was used to significantly separate the mean values.

RESULTS

3.1. Antifeedant assay

Table 1 shows the antifeeding activity of the treatments with imidacloprid and spinosad separately and in combination against the 4th instar larvae of *S. littoralis* after 24, 48 and 72 hours of feeding on treated castor bean leaves. Data revealed that all treatments exhibited antifeeding properties.

The antifeeding effect of imidacloprid was the most pronounced one at all the interval times, where this activity was about 1.30, 1.41 and 1.29 times more than that of spinosad, and was about 1.46, 1.11, and 1.18 times more than that of their binary mixture on the 1st, 2nd and 3rd day post-treatment, respectively. The antifeeding effects of imidacloprid were significantly different at all the intervals ($P \leq 0.003$). On the 1st and 3rd day of treatment, the antifeeding effects of imidacloprid was significantly different from both that of spinosad ($F_{2,6} = 19.4$, $P = 0.0024$, LSD = 4.12) and the combined imidacloprid + spinosad ($F_{2,6} = 17.1$, $P = 0.0033$, LSD = 3.10. The antifeeding properties of spinosad and the combination of spinosad and imidacloprid, however, did not vary significantly. But on the 2nd day of treatment, the difference between the three antifeeding effects due to the three treatments with separate imidacloprid, spinosad and combined imidacloprid + spinosad was significant ($F_{2,6} = 37.1$, $P = 0.0004$, LSD= 2.31) .

Table 1: Antifeeding activity of imidacloprid and spinosad separately and in combination against 4th -instar larvae of *S. littoralis* at 3 days post- treatments

Treatments	Antifeedant index (%)			
	Days post-treatments			Mean*
	1st	2nd	3rd	
Imidacloprid	78.71 ± 1.24 a	66.86 ± 1.18 a	77.53 ± 2.22 a	74.37
Spinosad	60.75 ± 2.95 b	47.35 ± 2.02 c	59.87 ± 1.54 b	55.99
Imidacloprid+ Spinosad	53.84 ± 3.90 b	60.39 ± 1.58 b	65.72 ± 2.63 b	59.98
P	0.0024	0.0004	0.0033	
F	19.4	37.1	17.1	
LSD	4.12	2.31	3.1	
df	2,6	2, 6	2,6	

Data are expressed as mean ± SE (n= 3). * Total mean of each treatment at different time intervals. Values are statistically analyzed by one-way ANOVA, where means within each column followed by different letters are significantly different ($P< 0.05$) using LSD.

3.2. Starvation percentage assay

Data in Table 2 show the starvation percentage of the 4th -instar larvae of *S. littoralis* treated by the imidacloprid or spinosad separately and in combination after 24, 48 and 72 hours of feeding on treated castor bean leaves.

Data revealed that the average of starvation percentage of larvae treated by imidacloprid was about 1.16 and 1.10 times more than that of larvae treated with spinosad and their mixture, respectively (Table 2). On the 1st day of treatment, the starvation percentages of larvae due to the three treatments were approximately the same ~ 92%. But on the 2nd and 3rd day of treatments, the starvation percentages of larvae treated by imidacloprid were 84%, and 80.69%, those of larvae treated by spinosad were 64.73% ,and 64.66, and those of larvae treated by a mixture of imidacloprid and spinosad

were 81%.and 72.51% ,respectively. Therefore, imidacloprid proved to be more effective antifeedant than spinosad.

Table 2: Starvation percentage of the 4th *S. littoralis* larvae treated with imidacloprid and spinosad separately and in combination at different time intervals.

Treatments	Time	Average weight (mg)	Difference* (mg)	Starvation (%)	Average (%)
Imidacloprid	0 min	97.60	-----	-----	
	24h	93.82	–3.78	92.05	85.58
	48h	97.88	+0.28	84.00	
	72h	105.52	+7.93	80.69	
Spinosad	0 min	96.33	-----	-----	
	24h	92.47	–3.86	92.22	73.87
	48h	112.35	+16.02	64.73	
	72h	127.08	+30.75	64.66	
Imidacloprid+ Spinosad	0 min	96.03	----	----	
	24h	92.19	–3.83	92.16	81.89
	48h	98.76	+2.73	81.00	
	72h	115.59	+19.56	72.51	
Control larvae	0 min	97.83	-----	-----	
	24h	136.10	+38.27	-----	
	48h	166.73	+68.90	-----	------
	72h	220.68	+122.85	-----	
Starved larvae	0 min	97.56	-----	-----	
	24h	90.15	–7.41	-----	------
	48h	84.76	–12.79	-----	
	72h	77.98	–19.58	-----	

* Difference: Average weight after 24, 48, and 72 h of treatment – Average weight at zero time of treatment

3.3. Histopathological effects on the midgut

3.3.1. Light microscopy of normal midgut histology

Light microscopic analysis reveals that the midgut of *S. littoralis* fourth instars has the histological structure seen in (Fig. 1). It is composed of a unicellular layer (epithelium) resting upon a basement membrane. This membrane is surrounded by two layers of muscle fibers, the outer longitudinal fibers and inner circular ones (musculosa). Three different cell types make up the epithelium: columnar, goblet and regenerative cells. The columnar cells are cylindrical, each cell contains large coarsely nucleus occuping a central position within the cell. These cells have a striated border (microvilli) brushing the inner surfaces of the cells to enhance the columnar cells' absorption surface, and the gap between them serves as a kind of filter. The goblet cells are somewhat calyx-shaped and are seen in great numbers between the columnar cells, each has in its mesal part a large ampulla opening by a narrow neck through a small aperture on the inner surface. The nucleus of each cell lies at the basal end of the cell. The regenerative cells are clusters of small-sized cells resting on the basement membrane between the bases of the other cells. They are round or elongated, and each contains a large nucleus surrounded by a small amount of strongly basophilic cytoplasm. The peritrophic membrane, a thin layer of unattached material that tightly encircles the food mass inside the lumen of the midgut, is present.

3.3.2. Effect of imidacloprid, spinosad and their mixture on the midgut histology

After 72 hours of treatment, 4th-instar *S. littoralis* larvae exposed to imidacloprid showed a variety of histological markers in the midgut (Figs. 2A, B). These signs became more obvious on the 1st -day of recovery (Figs. 2C, D) and on the 2nd -day of recovery (Figs.

2E, F), compared to those of the control (Fig.1). The muscle layer, particularly the circular muscle layer, degenerated in some parts round the gut (Figs. 2A, B & D). The epithelium became loosed and detached (Figs. 2B & D). The columnar cells lost their organization, and their nuclei were degenerated (Figs. 2A, B, C & F). Moreover, their cytoplasm became vacuolated (Figs. 2B, C & E). These cells ultimatly became necrotic and faintly stained with haematoxylin and eosin (Figs. 2C, D & E). The regenerative cells lost their nidi appearance (Figs. 2A, B & D). Also, the goblet cells become disorganized. The microvilli lost their brush appearance and became fragmented (Figs. 2A, C, E & F). The peritrophic membrane was pushed inward to the lumen leaving a wide space away from the epithelium (Fig. 2A, B, C & D).

Similarly, treatment of *S. littoralis* 4th instars with the spinosad resulted in numerous histological alterations (Fig.3) compared to those of the control (Fig. 1). The histopathological signs in the larval midgut appeared similar at all intervals post-treatment, after 72 h of treatment (Figs.3A&B), on the 1st -day of recovery (Figs. 3C & D) and on the 2nd -day of recovery (Figs. 3E &F). The most prominent sympotoms were strong vacuolation of the columnar cells, particularly at the apical portions (Figs. 3.B, C, E & F). Some epithelial cells showed pyknotic nuclei, while others seemed to have histolysis and cytoplasmic vacuolation (Figs. 3 A, B, C, D & F). Also, it appeared that the apical brush border of the epithelial cells was destroyed (Figs. 3A, C, D & E). In some specimens, the epithelium became loosed and detached (Figs. 3A,&D). The muscle fibers were separated from each other, leaving a degenerated area in-between (Figs. 3A, C & E). The peritrophic membrane was pushed inward to the lumen, leaving a wide space

away from the epithelium (Figs. 3A, C & D). The regenerative cells were scattered within the epithelium (Figs. 3C & F).

In comparison, treatment of 4th larval instars of *S. littoralis* with imidacloprid and spinosad mixture induced less histopathological effects at all intervals post-treatment, after 72 h of treatment(Figs.4A & B), on the 1st -day of recovery (Fig. 4C & D) and on the 2nd -day of recovery (Figs. 4E & F).

The most prominent sympotoms included destruction of the muscle layers (Figs. 4B,C&F) , disorganization of the epithelial cells (Figs. 4D, E & F), detachment of the peritrophic membrane and the basement membrane (Figs. 4A, B & C) and appearance of vacuoles (Figs. 4A, B, D, E & F). According to Figures 4A–F, certain epithelial cells showed cytoplasmic vacuolation and apparent histolysis, while other epithelial cells displayed pyknotic nuclei. Additionally, the epithelial cells' apical brush boundary was damaged (Figs. 4 A, B, D, & F).

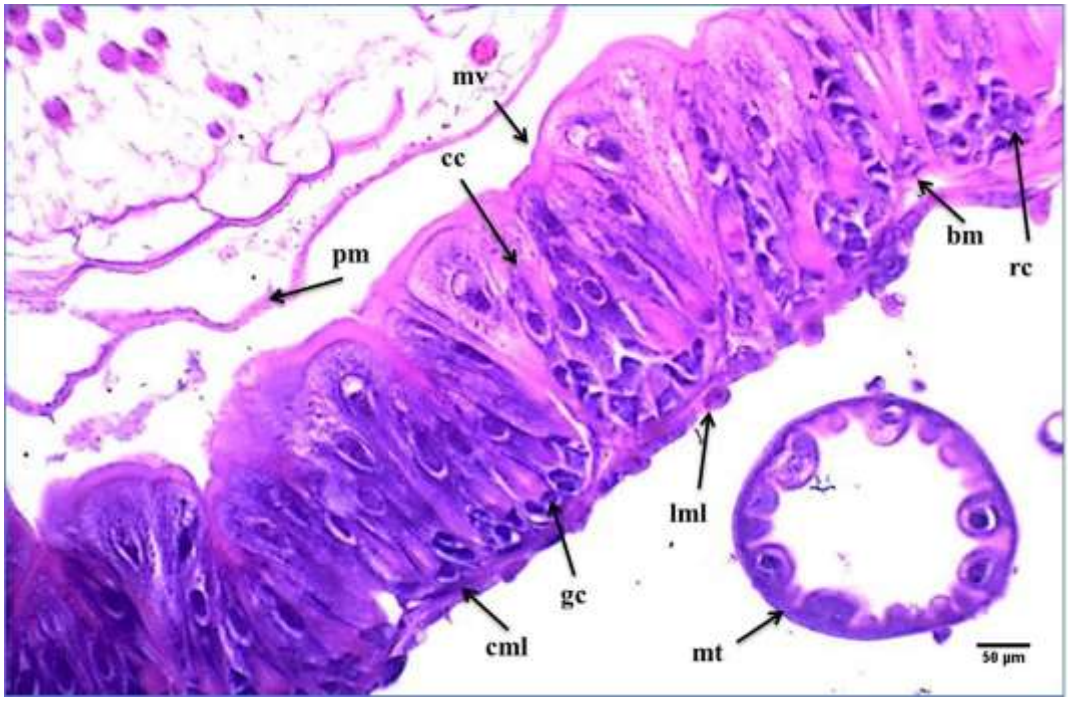

Fig. (1) : Transverse section through the midgut of healthy (the control) 4th -instar larvae of *S. littoralis* stained with Hematoxylin-Eosin (HE-X400).The epithelium layer resting upon a basement membrane (bm) and surrounded externally by outer longitudinal muscle layer (lml) and inner circular muscle layer (cml). The epithelium consists of columnar cells (cc) which have a striated border; microvilli (mv), regenerative cells (rc) and goblet cells (gc). The lumen of the gut is lined with peritrophic membrane (pm). Scale bar = 50 µm.

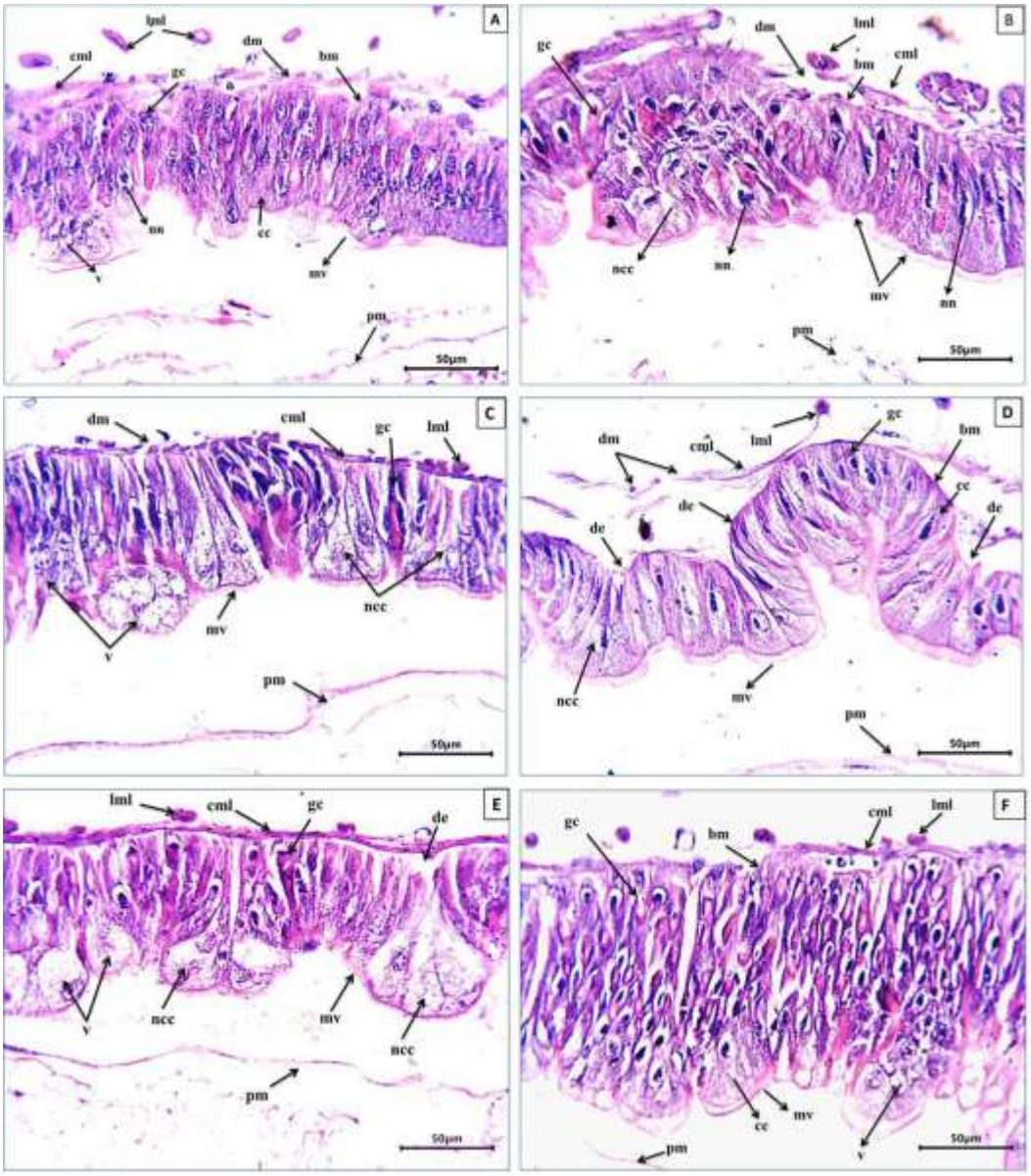

Fig. (2): Transverse section through the midgut of 4th -instar larvae of *S. littoralis* fed on castor-bean leaves treated with LC_{50} of imidacloprid at 72 h post-treatment (A & B), 1st day of recovery (C & D) and 2nd day of recovery (E & F) showing detachment of the epithelium (de), degeneration of circular muscle layer (dm), disorganization of columnar cells (cc) with vacuolated cytoplasm (v) and degenerating nucleus (nn), necrotic columnar cells (ncc), disorganization of regenerative cells (rc) leaving a cleft, widely-spaced peritrophic membrane (pm), microvilli (mv) were fragmented (H&E X400). Scale bar = 50 µm.

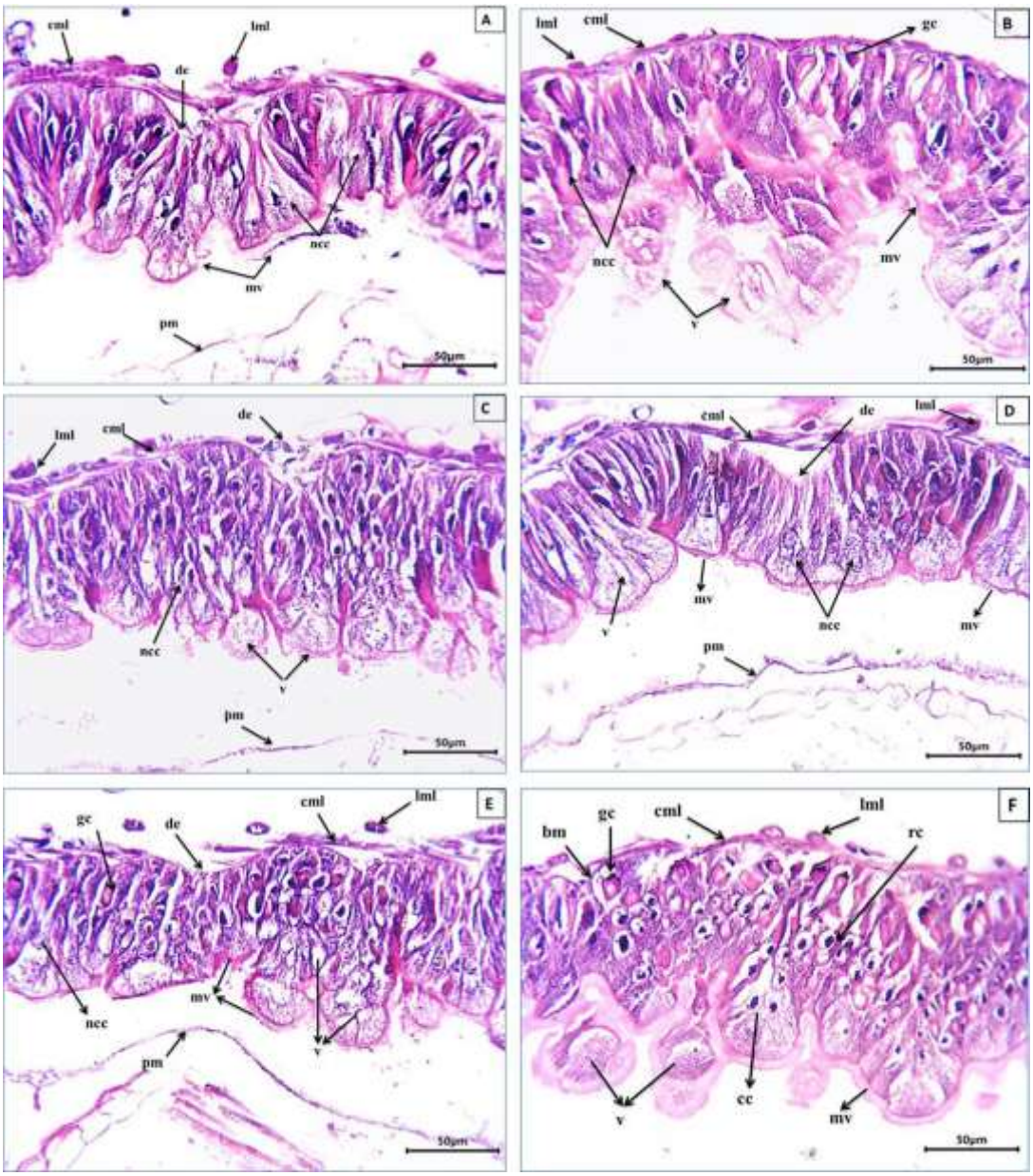

Fig. (3): Transverse section through the midgut of 4th -instar larvae of *S. littoralis* fed on castor-bean leaves treated with LC_{50} of spinosad at 72 h post-treatment (A & B), 1st day of recovery (C&D) and 2nd day of recovery (E & F) showing vacuolation of the apical portions of columnar cells (v), disorganization of columnar cell (cc) and loosing of the epithelium, necrotic columnar cells (ncc) with necrotic nucleus (nn), detachment of the circular muscle layer (dm), swollen goblet cell (gc), disorganization of regenerative cells (rc), widely-spaced peritrophic membrane (pm),and microvilli (mv) were fragmented (H & E X400). Scale bar = 50 µm.

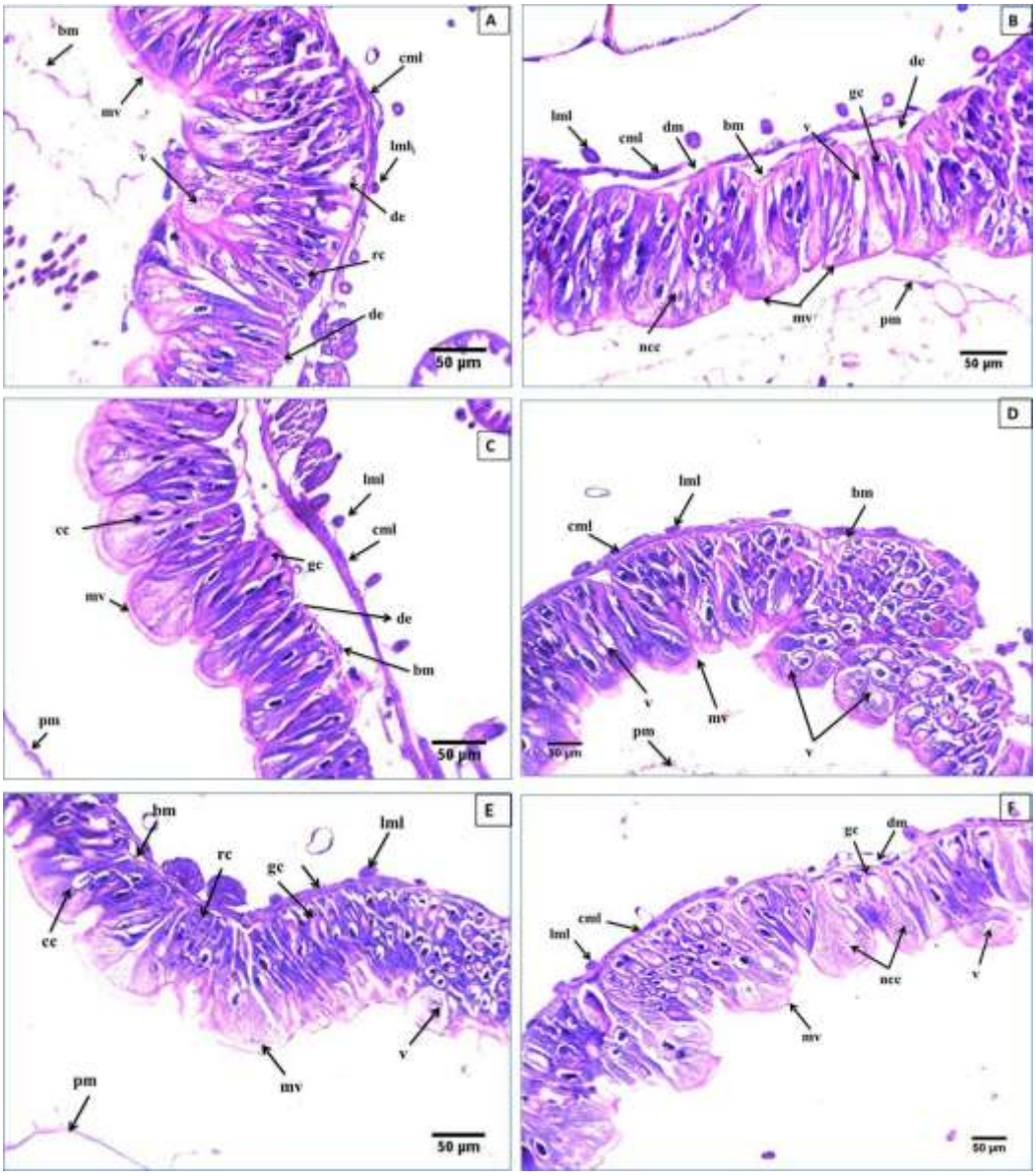

Fig. (4): Transverse section through the midgut of 4th -instar larvae of *S. littoralis* fed on castor-bean leaves treated with (LC_{25} imidacloprid + LC_{25} spinosad) at 72 h post-treatment (A & B), 1st day of recovery (C & D) and 2nd day of recovery (E & F) showing detachment of the epithelium (de), degeneration of circular muscle layer (dm), disorganization of columnar cells (cc) with vacuolated cytoplasm (v) and degenerating nucleus (nn), necrotic columnar cells (ncc), disorganization of regenerative cells (rc) leaving a cleft, goblet cell (gc) became swollen, widely-spaced peritrophic membrane (pm), microvilli (mv) were fragmented (H & E X400). Scale bar = 50 µm.

DISCUSSION

Insects that survive exposure to pesticides may endure behavioral and/or physiological changes in addition to the direct death caused by these substances [35]. This is especially true for newly developed insecticides, which have slower modes of action and may have more sublethal effects than acute ones [35,36,37]. Reduced eating or searching behavior may also be the consequence of behavioral changes brought on by exposure to or consumption of sublethal doses of a pesticide [38].

The first interaction between an insect and its host occurs during antifeedants. Instead of directly toxicating the insects, they starve them to death. Additionally, antifeedants shield crops until naturally occurring slow-acting pesticides start to have an antifeedant impact. For these reasons, research into antifeedants against polyphagous pests is becoming more and more popular [39]**.**

In the current study, imidacloprid, spinosad and their mixture showed antifeeding activities against *S. littoralis* larvae. We have not come across any direct reference on the effect of imidacloprid on feeding behavior of *S. littoralis* larvae. However, the antifeedant effects of imidacloprid have been reported for many insect species. For instance, imidacloprid were found to act as an antifeedant against variety of hemipteran insects, including aphids, whiteflies and green leafhoppers, at sublethal concentrations [40,41,42]**.** [43] described a similar antifeeding effect in the hemipteran brown plant hopper *Nilaparvata lugens*. The antifeeding effects of imidacloprid have also been observed for the coleopterans black maize beetles *Heteronychus arator* , and the false wireworms *Somaticus* sp., when feeding on stems of seed-treated maize plants [44,45]**.** Lepidopteran species showing this antifeeding response was *Heliothis virescens* [46]**.** On *Myzus*

persicae, low quantities of systemically administered imidacloprid had a reversible antifeeding effect. [40]**.** Similar antifeeding effects were described in *Myzus nicotianae* [36,47]. Female *Bemisia. tabaci* also avoided leaf discs treated with imidacloprid and stopped eating leaf discs treated with extremely low doses of imidacloprid administered systemically, but such antifeeding effects seem to be minimal when imidacloprid was delivered via the leaf-dip approach. [41]**.**

In the present study, spinosad exhibited antifeeding activities against 4^{th} -instar larvae of *S. littoralis* and these findings were consistent with those made by [29], who discovered that spinosad acts as an antifeedant to the fifth-instar *S. littoralis* larvae after 48 hours of feeding on cotton leaves taken from spinosad-sprayed field plots. [48] showed that high concentration of spinosad played a role as antifeedant to tested larvae of *Agrotis ipsilon.* Also, [49] analyzed the effect of flubendiamide, spinetoram, and spinosad on feeding inhibition in *Spodoptera litura* and *Spilarctia obliqua* larvae.

In the present study, the order of deterrence against 4^{th} -instar larvae of *S. littoralis* was imidacloprid > mixture (imidscloprid + spinosad) > spinosad. We have not come across any direct reference on the effect of imidacloprid + spinosad mixture on feeding behavior of 4^{th} -instar larvae of *S. littoralis*. However, the antifeeding effect of spinetoram was tested against fourth instar larvae of *S. littoralis* by [50]**,** who reported that spinetoram significantly reduced the amount of food ingested by the larvae; however antifeeding activity was less than that of indoxacarb and methoxyfenozide.

In the present study, *S. littoralis* larvae showed high starvation percentage in treatments by imidacloprid, spinosad and their mixture and this may be due to the antifeeding activities of these insecticides. [40,36] revealed that imidacloprid has a

significant impact on aphid feeding behavior at sublethal doses, which causes depression of honeydew excretion, wandering, and, eventually, starving death. The decrease in food consumed in *S. littoralis* larvae treated with imidaclopeid, spinosad and their mixture was concomitant with the decrease in larval weight [30] due to starvation and this reflects the antifeeding activity of these insecticides.

The midgut is the middle portion of the insect digestive tract where food is absorbed and digested. Some epithelium cells produce enzymes; others absorb the digested food [51]**.** Since the midgut has a more intensive digestion process and is where the majority of nutrient absorption takes place, the cellular alterations are more pronounced here. As a result, this area is the most susceptible to the effects of foreign substances. This observation suggests that the insecticides imidacloprid and spinosad induce morphological changes and subsequently impact the effectiveness of nutrient absorption. Likewise, we have not come across any direct reference on the histopathological effects of imidacloprid on midgut of 4^{th} -instar larvae of *S. littoralis*. However, in our current study, the histological changes induced in the midgut of the 4^{th} larval instars of *S. littoralis* treated with imidacloprid was similar to these obtained by [52] for the midgut of *Locusta migratoria* treated with the same insecticide. Also, the histopathological alterations of imidacloprid and tannic acid on the larvae of *Culex pipiens* showed that the treated larvae had cytopathological alterations of the midgut epithelium, muscular layers, and epithelial cells [53]**.**

[54] elucidated the effect of the LC_{50} of imidacloprid on the cytological and histological alterations of the mid-gut of *Podisus nigrispinus* (Heteroptera: Pentatomidae). This concentration induced histological changes in the mid-gut epithelium

as well as cytotoxic features, such as abnormal border epithelium, cytoplasmic vacuolation, and apocrine secretions in the first 6 h after exposure to the insecticide. Digestive cells in the mid-gut became apoptotic after 12 h of exposure.

The histological changes induced in the midgut of the 4th larval instars of *S. littoralis* treated with spinosad was similar to these obtained by [29] for 5th -instar larvae of *S. littoralis* treated with the same insecticide. The results agree with those reported by [55] **,** who recorded similar histological changes in the midgut of *S. littoralis* larvae treated with spinosad and tebufenozide, including disruption and stretching of the columnar epithelial cells, leading to peritrophic membrane tearing. Also, [56] showed many midgut histological aberrations in case of *S. littoralis* larvae treated with lufenuron and diflubenzuron, The muscle layers were destroyed, the epithelial cells were disorganized, the peritrophic membrane and the basement membrane were separated, and there was vacuolation among the histological changes.

The cytoplasmic extrusion at the apex of the columnar cells due to treatment with spinosad has also been described to other insect species as *Hyalophora cecropia* [57]**,** *Ephestia kuehniella* [58] and *Anticarcia gemnatalis* [59, 60]. According to these experts, cell degeneration during epithelial renewal is likely connected to the phenomena of cytoplasm loss. These patterns are brought on by the midgut's strong enzyme secretion activities, which try to repair the lining that is being assaulted [51,61,59] **.**

The nervous system or muscles are the primary target organ of most insecticides including neonicotinoids and spinosad [24,18] . The midgut is a composite organ, and the muscles that surround it are enervated by neurons. Alternately, the abnormalities seen in

the midgut may be explained by direct physiological effects of pesticide action. The overall decrease in growth, digesting, and gross food consumption efficiency brought on by spinosad and imidacloprid may be the result of these histological changes, which have been reported by [30].

CONCLUSION

Imidacloprid and spinosad alone or in combination are promising control agents to *S. littoralis* in terms of their acute toxic effects as well as their latent effects including antifeeding activities and impairment of the midgut histological architecture. These effects would ultimately lead to decreased growth of larvae and less damages to crops. Moreover, the binary combination of imidacloprid and spinosad would lead to potentially cheaper costs, less environmental contamination and deterrence of resistance. So, this mixture is advantageous in integrated management of *S. littoralis*. It is necessary to check in the field the laboratory results from this investigation.

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