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Research Article

Synthesis, formulation, evaluation of insecticidal activity of chromen derivatives against cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) and their mode of action under laboratory conditions

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Abstract
The toxicity of benzothiazole derivatives obtained from 2-cyanomethyl benzothiazole against cotton leafworm *Spodoptera littoralis* 2nd and 4th instar larvae has been documented. The main objective of this research was to formulate two previously reported chromen derivatives and test their biological activity against cotton leafworm *S. littoralis* 2nd and 4th instar larvae under laboratory conditions. According to the standard method, two chromen derivatives with two distinct substituents (salicylaldehyde and 2-hydroxy-1-naphthaldehyde) were synthesized. Their physical and chemical properties were evaluated, and both were formulated as 10 and 9.5 percent dustable powder formulations. Both formulae were then evaluated in the laboratory on cotton leafworm *S. littoralis* 2nd and 4th instar larvae for mortality and developmental effect percentage. Formulation (F_2) was more efficient than formulation (F_3) in both cases and for both stages. Furthermore, when comparing the developmental effects on the 2nd and 4th instar larvae, the 4th instar larvae demonstrated marked tolerance. Both stages were unable to complete their life cycle. Histopathological analysis of samples from the affected stages was performed to assess the mode of action of these formulations on 4th instar larvae at their LC_{50} values. Experimental data showed that Formulation (F_3) resulted in epidermal cells separated from the cuticular layer, necrosis, ruptured columnar cells with pyknotic nuclei, disrupted basement membrane, and weak epicuticle necrosis and separation. In comparison, formulation (F_2) revealed a midgut with vacuoles, damaged columnar, muscle cell necrosis, and a ruined peritrophic matrix. Thus, the cotton leafworm *S. littoralis* could be combated with the newly prepared formulations (F_2) and (F_3).

Keywords: Biological efficacy, Chemical substitution, Dustable powder, *Spodoptera littoralis*, Thiazole

INTRODUCTION

Egyptian cotton evolution began in the early 18th century, when Egypt encouraged crops to boost the country's economy. As a result, the quality became legendary and a trade-mark in the global market. Egyptian cotton is more than a crop; it is the history and future of modern Egypt's resurgence (Karvy, 2009). Egyptians refer to it as "white gold" (Ahmed and Delin, 2019). When compared to other cotton farmed throughout the world, Egyptian cotton has established a global reputation for being of the best lint quality over the previous century and a half (Abdel-Salam and El-Sayed Negm, 2009). Cotton still plays a significant role in Egyptian society, generating employment and tradable items to benefit the economy. For all its dealers, customers who buy cotton supplies, ransport the items, and raise and harvest the crop themselves are all involved (Baffes, 2005). It employs one million people directly and indirectly and spends 26 billion Egyptian pounds in agriculture and related businesses such as textiles, dyeing, and garment manufacturing. Cotton seeds are utilized

in the feed business (Czwartkowski *et al.,* 2022) and as oil seeds, making them an important crop for food security (El-Hamidi and Zaher, 2018). Egypt exports a major portion of its cotton (60–70%) (Raza *et al.,* 2020). Cotton crop size changed with market prices and the global economy (Hatab *et al.,* 2009)

Spodopteras littoralis (Boisduval), the cotton leafworm, is considered Egypt's most serious cotton pest (Hatem *et al.,* 2009). In Egypt and other Mediterranean and Middle Eastern areas, the cotton leafworm is a damaging pest to approximately 112 cultivated plants from various families (El-Zoghby *et al.,* 2011). Unfortunately, the infection rate could reach 119 048 egg-mass/ha, wreaking havoc on leaves, buds, flowers, and bolls (Temerak 2002; El-Sheikh 2012; El-Geddawy *et al.,* 2014; Ahmed *et al.,* 2015a, b).

It is wreaking havoc on North African vegetables, Egyptian cotton (Allam *et al.,* 2021), and Southern European glasshouse plant and flower output (Abdel-Mageed *et al.,* 2018). This insect feeds on approximately 80 plant species belonging to over 40 distinct families (CABI, 2019). In Egypt, cotton is being attacked as well as corn, peanuts, clover, vegetables, and a variety of fruits (El-Kholy *et al*., 2014).

To manage *S. littoralis* species, chemicals are utilized. The widespread use of broad-spectrum conventional insecticides against *S. littoralis* over the last 25 years has resulted in the development of resistance to a number of authorized chemicals for its control (Aydin and Gurkan. 2006), including organophosphate, carbamate, and pyrethroid (El-Zemaity *et al.,* 2003). These pesticides paralyse and kill targeted pests by blocking sodium channels (Wing *et al*., 2000; McKinley *et al*., 2002) or inhibiting the acetylcholinesterase enzyme (Nkya *et al.,* 2014). More focus should be given to the use of novel chemical pesticides with new and distinct modes of action against pests, as well as those that are less hazardous to the environment (Hassan, 2009).

Pesticides are a necessary part of contemporary agriculture. Plant pests and diseases can be efficiently reduced, resulting in higher agricultural yields. On the other hand, traditional pesticide formulations have many drawbacks, including high organic solvent content and low dispersibility. The majority of pesticides are lost to the environment, and less than 1% remain on the target. This ineffectiveness adds to substantial pollution of the environment (Reichenberger *et al.,* 2007, Sun *et al.,* 2016). As a result, efforts should be made to reduce pesticide waste, manufacturing costs, and pollution while simultaneously prolonging the period of pesticide activity on crops. Pesticide formulation is the process of converting a pesticide into a form that can be conveniently manufactured, stored, transferred, and applied in a practical manner to provide a safe, easy, and effective means of pest control at a low cost. To transform it into a product that can be kept, transported, and used in practical ways to achieve effective, safe, and cost-efficient results (Hazra and Purkait, 2019).

The current study aims to find new active ingredients, test them on *S. littoralis,* prepare them in the form of a local commercial formulation, and study their pesticidal effect and their mode of action in a trial to overcome resistance problems, with the goal of using them to control *S. littoralis* after laboratory and field studies are completed.

MATERIALS AND METHODS

Tested chemicals

a) Fine chemicals: *O*-aminothiophenol (2-aminobenzene-1-thiol, molar mass 125.19 $g.mol^{-1}$), malononitrile (propanedinitrile, molar mass 66.06 $g.mol^{-1}$), salicylic aldehyde (2-hydroxybenzaldehyde, molar mass 122.12 $g.mol^{-1}$), 2-hydroxy-1-naphthaldehyde (2-hydroxynaphthalene-1-carbaldehyde, molar mass 172.18 $g.mol^{-1}$), acetic acid (ethanoic acid, molar mass 60.05 $g.mol^{-1}$) and hexahydropyridine (piperidine, molar mass 85.15 $g.mol^{-1}$) were supplied by Sigma–Aldrich Co.

b) Diluent: The diluent was supplied by EL-Gomhoria Co., Cairo, Egypt.

c) Solvents: Absolute ethanol, acetone, xylene and DMF were supplied by EL-Gomhoria Co., Cairo, Egypt.

Chemistry part

Synthesis of 2-(benzo[d]thiazol-2-yl) acetonitrile 1

In 10 ml glacial acetic acid, a cold solution of 2-aminothiophenol (0.27 g, 1 mmol) and malononitrile (0.06 g, 1 mmol) in 100 ml ethanol were reacted. The reaction mixture was maintained at room temperature for 28 hours; the solid-formed product was filtered off, rinsed with ethanol, dried well, and purified from ethanol to yield acetonitrile 1.

Synthesis of 3-(benzo[d]thiazol-2-yl)-2H-chromen-2-imine and 2-(benzo[d]thiazol-2-yl)-3H-benzo[f] chromen-3-imine compounds

In 15 ml ethanol containing a few drops of piperidine, equimolar amounts of acetonitrile 1 (0.293 g, 1 mmol), salicylaldehyde (0.122 g, 1 mmol) and 2-hydroxynaphthaldehyde (0.172 g, 1 mmol) were heated for 3 hours. The obtained solid in each case was filtered out, dried thoroughly, and crystalized from ethanol to afford chromen derivatives 2 and 3.

Racané *et al.* (2012) synthesized 2-cyanomethyl benzothiazole 1 through the reaction of 2-mercaptoaniline (ortho aminophenol) and malononitrile in EtOH/AcOH at room temperature. Thiazole derivative 1 underwent condensation and cyclization after 3 hours of treatment with salicaldehyde and 2-hydroxy napthaldehyde in ethanol and piprideine under reflux for 1 hour, yielding chromen compounds 2 and 3 (Refat and Mohamed,

2015).

Physico-chemical properties of formulation components

Active ingredient

The physico-chemical properties of the synthesized chromen derivatives as active ingredients were determined as follows:

a) Solubility: It was calculated by measuring the volume of several solvents (xylene, distilled water, DMF, acetone and ethanol) for 100% solubility of a specific amount of active substance at 20 degrees Celsius (Nelson and Fiero, 1954). The following equation was used to compute the percent solubility:

% solubility = W/V x 100 …… (1)

where W= weight of active ingredient and V= volume of solvent required for complete solubility.

b) Free acidity or alkalinity: It was determined using the same methodology set in FAO and WHO guideline MT 191 (2010).

c) Melting point: The melting point was calculated using a Gallenkamp 9200 A electrical digital melting point instrument.

The physico-chemical properties of diluent

a) Surface activity: The surface activity of carriers or diluents was estimated using Hammett indicators (Malina *et al.,* 1956 and Anonymous, 1965). The PKa was determined using diphenyazodiphenylamine and dimethyl aminazobenzene at different levels; the former at the 1.5 level (yellow indicates safe, purple denotes hazardous or active), and the latter at the 3.3 level (yellow means safe, and red means unsafe or active).

b) pH: According to Dobrat and Martijn (1995), pH was determined using a Cole-Parmer pH/conductivity meter 1484-44.

c) Free acidity or alkalinity: It was determined as before.

d) Bulk density: Bulk density was obtained using FAO and WHO guideline MT 186 (2010).

e) Screen analysis: The particle size of the selected diluents was determined using the method described by Zaazoua *et al.* (1966).

Preparation of chromen compounds as dustable powder formulations (DP)

The new formulations were developed as dustable powder (DP) formulations using the dry mix method, which includes mixing the active ingredient with the appropriate diluent accessible (Furmidge, 1972). The active ingredients in the newly produced formulations were 10% and 9.5% (wt/wt).

The physicochemical characteristics of the new locally manufactured 10 and 9.5% dustable powder (DP) formulations were determined as follows:

a) Bulk density: Bulk density was determined as previously mentioned.

b) Free acidity or alkalinity: It was determined as stated before.

c) Screen analysis: This was determined by the same procedure reported before.

Bioassay

Rearing insects

The eggs acquired by a susceptible strain established in the cotton leafworm Department, Plant Protection Research Institute (PPI); Agriculture Research Centre (ARC), Giza, were used to create the *S. littorals* culture used in this work. This strain was cultured in the laboratory at a constant temperature of 25 ± 2 degrees Celsius and a relative humidity of 70±5 percent (El- Defrawi, 1964). Egg masses were maintained in petri dishes, two per plate, until hatching. The hatched larvae were moved to glass containers with a capacity of 2 liters, which were covered with muslin cloth and held together with elastic bands. Fresh castor leaves were supplied to the larvae on a daily basis (*Rieinus communis*). As the larvae grew older, they were permitted to pupate in glass containers containing two mish sections of dry saw dust, and the pupae were then put in another container. The eventually resultant moths were supplied with a 20 percent sugar solution to feed on and permitted to lay their eggs on fresh Nerium oleander leaves as a physical surface for moths mating, oviposition and resting egg-masses were gathered every two days and moved to petri dishes for another generation.

Five concentrations (500, 1000, 3000, 5000 and 6000 ppm) were taken from the two tested substances (F_2 and F_3) to evaluate the latent influence. Three replicates of each concentration were made, each with ten larvae, and another 3 replicates were used as controls without any substances added (untreated with freshwater). The 2^{nd}- and 4^{th}-instar larvae were tested. Fresh castor oil leaves were dusted with each concentration from the previously mentioned concentrations, and the larvae were permitted to feed on treated leaves for 2 days, while control larvae were left to feed on untreated leaves. To investigate the latent effect, a mortality count was taken every two days, and mortality % was determined. The total numbers of developed pupae and moths in each treatment were recorded to determine the developmental effect against both pupae and moths.

Histopathological studies

The 4^{th} instar larvae at their LC_{50} concentration were analysed for histopathological studies. Tissue preparation, sectioning, and staining were performed in accordance with the protocol of Lynch (1969 and Lilli, 1965). After 96 hours of treatment, the larvae were removed and placed in an alcoholic Bouins solution as

a fixative. The larvae were rinsed in ethanol solutions to dehydrate and remove the yellow colour of the Bouins solution. They were first immersed in 50% ethyl alcohol for two hours at 40 degrees Celsius (two changes) and then left for 24 hours. The larvae were then subjected to a series of alcoholic treatments, each lasting two hours at room temperature, beginning with 80 percent, then 90 percent, 96 percent, and finally 100 percent alcohol. The larvae were dehydrated before being immersed in a solution of amylacetate solution and soft paraffin wax and kept at 50°C for 24 hours. At 50°C, the larvae were deposited three times in soft paraffin wax at 24-hour intervals. The larvae were given a mixture of one part hard paraffin wax. The larvae were then embedded in the wax mixture that had been utilized in the previous phase. Serial 6-micron sections were cut on a microtome and placed on clean slides with Mayer's albumin. The sections were mounted on glass slides and stained with hematoxylin before being counterstained with an alcoholic solution.

Statistical analysis

The Abbott formula (1925) was used to obtain the corrected mortality percentage,

corrected mortality % = (1 – n in treatment after treatment/n in control after treatment) x 100 ……. (2)

where n= Insect population

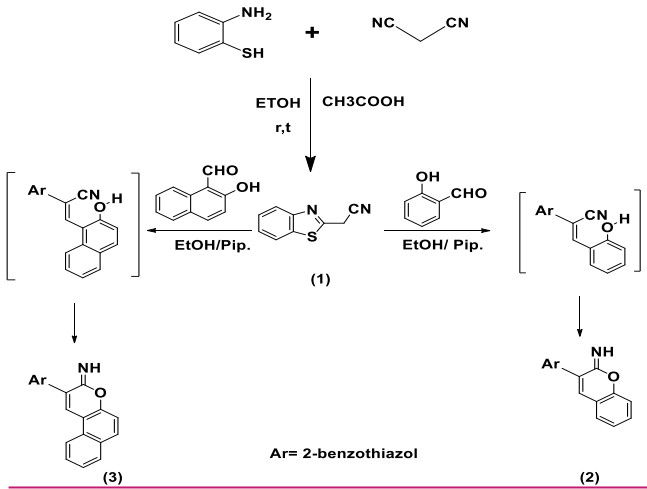
According to Finney (1971), the concentration inhibition regression lines were calculated, and the toxicity index was calculated according to (Sun, 1950) by the following equation:

Toxicity index = LC_{50} of the most powerful compound/ LC_{50} of the screened compound × 100 ……. (3)

RESULTS AND DISCUSSION

Chemistry Part

The reaction of ortho aminophenol with malononitrile gave 2-cyanomethyl benzothiazole 1 which on treatment with salicaldehyde and 2-hydroxy napthaldehyde afforded chromen compounds 2 and 3 as follows:



Formulation part

The physico-chemical characteristics of compounds (2) and (3) are shown in Table 1. Both were completely insoluble in various solvents with varying polarities. Furthermore, the two compounds' declared alkaline features appeared evident from the calculated value of their free alkalinity as sodium hydroxide. These findings indicated that solid formulations (dustable powder, wettable dispersible granules, and wettable powders) would be the best choice for formulating these active ingredients (Hamouda *et al.,* 2021).

The sorption of an active component onto a finely powdered, solid inert such as talc, clay, or chalk produces dustable powder (dust). As no mixing is required and the application equipment (e.g., hand bellows and bulb dusters) is light and simple, they are comparatively straightforward to use. As these formulations are not diluted with water before being applied in the field, their particle size is greater (approximately 25 to 35 microns) than wettable powder formulations (approximately 5 to 10 microns), and they can also give outstanding coverage (Hazra and Purkait, 2019).

Many diluents found the best one for making dustable powders for these compounds. The physico-chemical features of the selected diluent employed in the formulation processes are shown in Table 2. Its bulk density before and after compaction was 0.36 and 0.41, respectively, consistent with the WHO standard (1979), which indicated that the bulk density percent variation between before and after compaction should not exceed 60%. A weak alkaline characteristic was also detected in the diluent, as demonstrated by sodium hydroxide and an alkaline pH (8.33). The PKa test must be used in combination with the pH test for determining the physical parameters of diluents or carriers. The pH value determines the average acidic and alkaline sites in a solid form that do not neutralize each other, whereas the acidic and alkali sites of solid and inert material, as well as their interaction, are defined by PKa (Hamouda *et al.,* 2021). Additionally, the diluent's particle size screen revealed the entire capacity to pass through sieves ranging from 20 to 53 microns.

According to the measurements of the produced dustable powder formulations, both dustable powder formulations had alkaline properties (Table 3). The release rate of pesticides increased when the pH was below 7, and the release rate under alkaline conditions was slightly higher than that under neutral conditions. The pH response of PAA/chlorpyrifos/aminated mesoporous silicon during release was particularly strong: it was easily released under an acidic environment and showed the strongest slow-release effects under neutral conditions. As a result, pesticide release can be successfully controlled by adjusting the pH. In a study conducted by Zhao *et al.* (2006), Schiff base organic molecules were

Table 1. Physical properties of compounds (2) and (3) considered active ingredients

Compound	% Solubility (W/V)					Free alkalinity as % NaOH	Melting point °C
	Water	Acetone	DMF	Ethanol	Xylene		
2	-*	-	-	-	-	1.96	220
3		-	-	-	-	1.47	190

-*: means insoluble.

Table 2. Physical properties of the chosen diluent

PKa	pH	Free alkalinity as % NaOH	Bulk density		Screen analysis less than microns			
			Before compacting	After compacting	53	40	30	20
>1.5>3.3	8.33	0.0057	0.36	0.41	100	100	100	100

Table 3. Physical properties of the new dustable powder formulations

Compound	Bulk density		Free alkalinity as % NaOH	Screen analysis less than 74 microns
	Before compaction	After compaction		
F_3	0.09	0.17	2.5	73.4
F_2	0.29	0.47	13.23	73.6

used to control pesticide release. Since these substances are slightly more stable in alkaline than in acidic environments, the rate of pesticide release is higher in acidic environments (Huang *et al.,* 2018). The two formulations met the WHO specification (1979) regarding bulk density before and after compaction, as the percentage of bulk density before and after compaction should not exceed 60% of the density before compaction, and their particle size was less than 74 microns, as required by the same specification (Table 3).

Many studies have shown that the more efficient dry hydrophobic siliceous insecticides are finely divided, have a low bulk density, and have a low unit weight per unit volume (Hazra and Purkait, 2019). In addition, it was shown that fine particles stick to insects more readily than coarse powders. Furthermore, lower bulk density tricalcium phosphate (0.37 g/cm^3) was more successful than high bulk density tricalcium phosphate (0.52-0.9 g/cm^3) in treating *Tribolium castaneum* adults and *Tenebrio molitor L*. larvae (Li, 2018).

Biological activity

The mortality percentages of the two dustable powder formulations on the 2^{nd-} and 4^{th-} instar larvae of *S. littoralis* under laboratory conditions with serial concentrations calculated after 4 periods of treatment for 2, 4, 6 and 8 days are given in Table 4. The obtained results showed a direct relationship between the increase in concentration and the percentage of mortality for both formulations at both insect stages under study. Furthermore, by prolonging the period after treatment with all tested concentrations, the mortality percentages increased, with the maximum effect recorded 8 days after treatment. In addition, formulation (F_2) was more effective on both stages compared to formulation (F_3), as the percentage of mortality of the former was 96.42 and 57.14% on 2nd and 4th instar larvae , respectively, while it was 76.66 and 56% on the same stages for the latter formulation, respectively, with 6000 ppm after 8 days from treatment. On testing on the 2nd instar larvae of the cotton leafworm, *S. littoralis* Fadda *et al.* (2020) found that benzothiazole derivatives generated from 2-cyanomethyl benzothiazole showed good toxicity, with LC_{50} values of 54.5 and 57.2 ppm for mercapto derivatives.

The efficacy of dustable powder formulations (F_2) and (F_3) on *S. littoralis* 2nd and 4th instar larvae depending on their Ldp line is shown in Table 5 under laboratory conditions. Eight days after treatment, both formulations displayed the lowest LC_{50} values for both phases under investigation. On 2nd instar larvae, formulations (F_3) and (F_2) exhibited 2477 and 935 ppm, respectively, and 5626 and 4761 ppm on 4th instar larvae. These findings also revealed that formulation (F_2) was more effective than formulation (F_3) since its LC_{50} value was lower on both insect phases than the other formulation. When using the toxicity index, formula (F_3) exhibited 37.74% toxicity and 84.62% toxicity on 2nd and 4th instar larvae, compared to 100% for formula (F_2). For both phases, the slope values were likewise higher in the case of (F_2) than in the case of (F_3), indicating that the toxicity line is sharper in the first case than in the second one.

Table 4. Mortality percentages of the newly formulated dustable powder formulations (F_2) and (F_3) against 2nd and 4th instar larvae of *S. littoralis* under laboratory conditions

Tested formulation	Concentration ppm	Mortality %							
		2nd instar larvae				4th instar larvae			
		Days				Days			
		2	4	6	8	2	4	6	8
F_3	500	3.33	3.33	6.66	13.33	3.33	6.66	7.14	12.00
	1000	10.00	16.66	20.00	30.00	6.66	16.66	17.85	24.00
	3000	16.66	23.33	30.00	50.00	10.00	26.66	28.57	36.00
	5000	30.00	43.33	46.66	66.66	23.33	33.33	35.71	44.00
	6000	36.66	50.00	56.66	**76.66**	33.33	40.00	42.85	**56.00**
F_2	500	3.33	10.00	21.42	35.71	3.33	3.33	10.71	10.71
	1000	6.66	23.33	46.42	50.00	6.66	6.66	21.42	25.00
	3000	16.66	43.33	64.28	71.42	10.00	10.00	28.57	32.14
	5000	26.66	53.33	85.71	92.85	23.33	23.33	46.42	53.57
	6000	36.66	56.66	89.28	**96.42**	33.33	33.33	50.00	**57.14**
Control		0.00	0.00	3.33	**6.66**	0.00	0.00	3.33	**6.66**

Table 5. Comparison between the efficacy of the newly formulated compounds (F_2) and (F_3) against 2nd and 4th instar larvae of *S. littoralis* under laboratory conditions

Tested formulation	2nd instar larvae				4th instar larvae			
	LC_{50} ppm	LC_{90} ppm	Slope	Toxicity index	LC_{50} ppm	LC_{90} ppm	Slope	Toxicity index
F_3	2477	16509	1.5559±0.1534	37.74	5626	93998	1.048±0.152	84.62
F_2	935	4997	1.7617±0.1599	100	4761	109243	1.2089±0.1552	100

Table 6 shows the developmental effect of the new dustable powder formulations (F_2 and F_3) on *S. littoralis* 2nd and 4th instar larvae under laboratory conditions. The results revealed that the treatment of 2nd- and 4th- instar larvae with both formulations decreased pupal and adult formation. The pupa and adult formation reduction increases as the tested concentration increases, with the maximum effect recorded at 6000 ppm. On the other hand, treating 2nd instar larvae at 5000 and 6000 ppm revealed that the F_2 formulation was more effective against pupa formation than the F_3 formulation. At 5000 and 6000 ppm, the percentages dropped from 42.6 and 23.95 in the case of (F_3) to 5.65 and 3.21 in the case of (F_2). In the case of 4th instar larvae, however, no significant differences were detected between the effects of formulations F_3 and F_2 on pupa and adult formation. In addition, when 2nd and 4th instar larvae were treated with the investigated formulations, pupa formation was more influenced than adult formation. These findings also demonstrated that the 4th instar larvae were more resistant to both dustable powder formulations used than the 2nd instar larvae. In general, an inverse relationship was observed between the increases in concentrations of formulations (F_2) and (F_3) and the percentages of pupation and emergency of the adult. Furthermore, these findings revealed that formulation (F_2) was more effective on *S. littoralis* pupae and adults of 2nd and 4th instar larvae than formulation (F_3). Furthermore, it was established that for both formulations and both phases, the resultant pupae and adults were unable to complete their life cycle and died.

As shown in Fig. 1, the outer cuticle is above the epidermis, and the basement membrane is beneath the epidermis (Control) of *S. littoralis*. Fig. 2 (Control) displays the typical midgut of *S. littoralis* 4th instar larvae, which displays a normal columnar layer with brush border, goblet cells, circular and longitudinal muscles. The effect of the newly developed dustable powder formulation (F_3) with LC_{50} on *S. littoralis* 4th instar larvae is shown in Fig. 3-5. It revealed necrosis of epidermal cells separated from the cuticular layer (Fig. 3) and ruptured columnar cells with pyknotic nuclei and a damaged basement membrane (Fig. 4). In addition, there was weak epicuticle necrosis of epidermal cells and

Table 6. Effect of chemical residues of dustable powder formulations on the formation of pupae and adults of the 2nd and 4th instar larvae of *S. littoralis* under laboratory conditions

Tested formulation	Concentration ppm	Developmental effect %			
		2nd instar larvae		4th instar larvae	
		Pupa %	Adult %	Pupa %	Adult %
F_3	500	85.52	90.60	87.00	95.34
	1000	68.21	85.00	75.08	90.08
	3000	48.91	83.91	64.78	83.13
	5000	42.60	72.60	55.17	80.52
	6000	**23.95**	**61.52**	**43.86**	**72.21**
F_2	500	62.52	80.00	89.13	87.73
	1000	49.56	73.21	74.78	81.13
	3000	26.95	70.65	67.82	77.82
	5000	5.65	64.00	46.43	72.60
	6000	**3.21**	**56.00**	**42.86**	**70.26**
Control		92.21	99.00	93.00	100.00

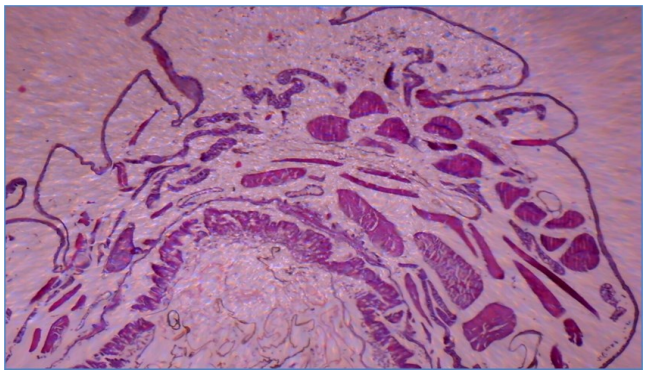


Fig. 1. *Cross section in the cuticle of untreated instar larvae of S. littoralis (HE 200X) showing a normal fiber layer and cellular layer (control)*

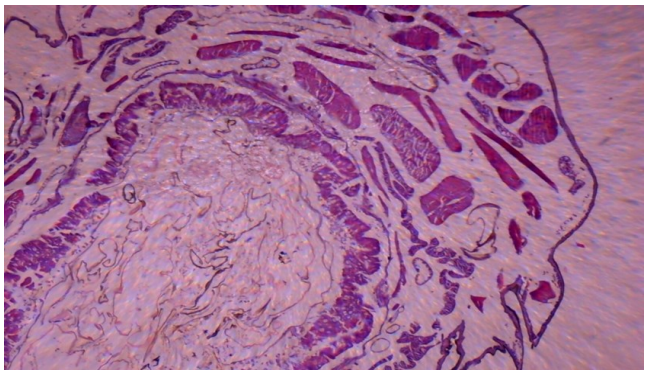


Fig. 2. *Cross section in the midgut of untreated instar larvae of S. littoralis (HE 200X) showing a normal columnar layer with brush border, goblet cells, circular and longitudinal muscles (control)*

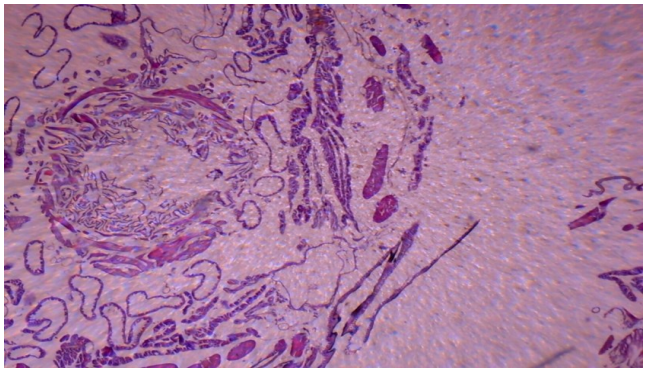


Fig. 3. *Cross section in the cuticle of the 4th instar larvae of S. littoralis treated with the LC_{50} of formulation (F_3) (HE 200X) showing necrosis of epidermal cells and separation from the cuticle*

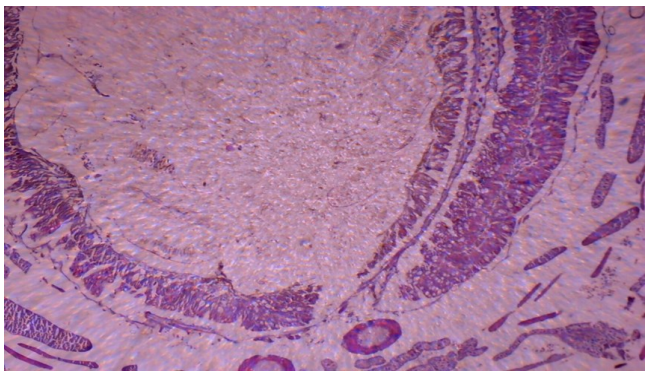


Fig. 4. *Cross section in the midgut of the 4th instar larvae of S. littoralis treated with the LC_{50} formulation (F_3) (HE 200X) showing ruptured columnar cells with pyknotic nuclei and a destroyed basement membrane*

separation from the cuticle (Fig. 5). The formulation (F_2) with the same concentration revealed several histological abnormalities, such as the midgut with vacuoles and damaged columnar cells (Figs. 6, 7) and the midgut with necrosis of muscle cells and destroyed peritrophic matrix (Fig. 8).

Conclusion

The present study concluded that among two newly formulated benzothiazole derivatives as dustable powders tested under laboratory conditions against 2nd- and 4th instar larvae of the cotton leafworm *S. littoralis*, the

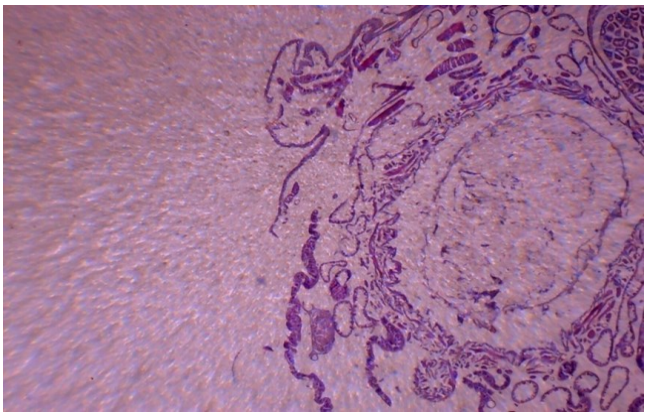


Fig. 5. *Cross section in the cuticle of the 4th instar larvae of S. littoralis treated with the LC_{50} formulation (F_3) (HE 400X) showing a weak epicuticle, necrosis of epidermal cells and separation of the cuticle*

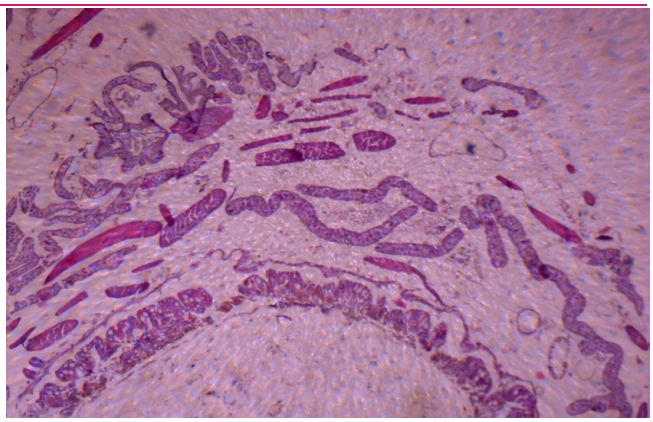


Fig. 6. *Cross section of the 4th instar larvae of S. littoralis treated with the LC_{50} formulation (F_2) (HE 200X), showing the midgut with vacuoles and destroyed columnar cells*

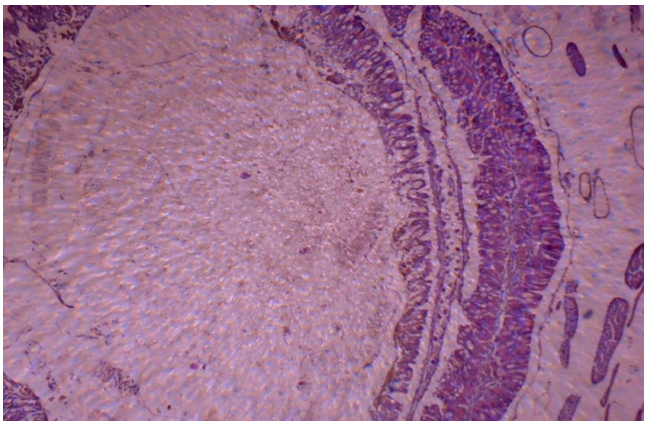


Fig. 7. *Cross section in the 4th instar larvae of S. littoralis treated with the LC_{50} formulation (F_2) (HE 200X), showing the midgut with vacuoles in the columnar cells, cells separated from the basement membrane, and necrosis of muscle cells*

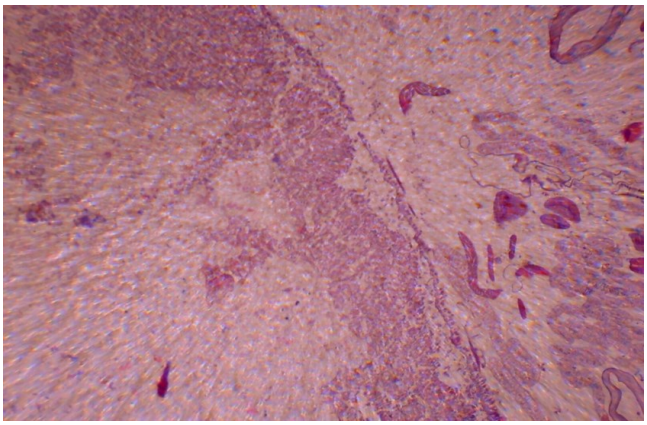


Fig. 8. *Cross section in the 4th instar larvae of S. littoralis treated with the LC_{50} of formulation (F_2) (HE 200X) showing the midgut with necrosis of muscle cells and destroyed peritrophic matrix*

formulation (F_2) was more efficient than formula (F_3) at both stages which may be attributed to the type of chemical substituent applied in each case. Comparing the developmental effect on 2nd- and 4th- instar larvae, the 4th- instar larvae showed substantial tolerance, as their growth is more complete than the 2nd- instar larvae. Neither stage was able to finish its life cycle. The histopathological analysis of the 4th instar larvae showed that the formulation (F_3) caused epidermal cells to separate from the cuticular layer, necrosis, ruptured columnar cells with pyknotic nuclei, damaged basement membrane, weak epicuticle necrosis, and separation. A midgut with vacuoles, damaged columnar, muscle cell necrosis, and a wrecked peritrophic matrix was revealed in formulation (F_2). Although both formulations were effective against 2nd- and 4th- instar larvae, F_2 was more successful than F_3; hence, compounds containing salicylaldehyde rather than 2-hydroxy-1-naphthaldehyde could be used to control the cotton leafworm *S. littoralis*.

Conflict of interest

The authors declare that they have no conflict of interest.

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