Egypt. J. Plant Prot. Res. Inst. (2019), 2 (4): 751 - 758

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Efficiency of certain insecticides and their histological effects against sugar beet beetle *Cassida vittata* (Coleoptera: Chrysomelidae) in sugar beet field

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ARTICLE INFO

Article History

Received: 15 / 10 / 2019

Accepted: 11 / 12 /2019

Keywords

Sugar beet , *Beta vulgaris* , *Cassida vittata*, insecticides, imatrade, flagtra, agriflex, dora and histology.

Abstract:

Sugar beet beetle *Cassida vittata* (Vill.) (Coleoptera: Chrysomelidae) is a serious pest on sugar beet causes losses in root yield and sugar content in Egypt. The present work dealt with the efficiency of four compounds representing different classes of insecticides against sugar beet beetle *C. vittata* larvae under field conditions at Dakahlia and Behira Governorates in 2015 season. The tested compounds were imatrade (35% SC) (Imidacloprid), flagtra (25% WP) (Thiamethoxam), agriflex (18.6% SC) (Thiamethoxam + Abamectin) and dora (48% EC) (Chloropyrefos). In each field, the results revealed that, all treatments were able to suppress the larval population comparison to the untreated control. The suppression varied according to the tested compound, dora caused 93.51% reduction after ten days post spray in the larval population followed by flagtra (87.15%). Generally, the highest reduction was recorded in dora treatment at two regions. The mean Reduction percentage were 89.64 and 91.09 % in Dakahlia and Behira Governorates, respectively. The histological examination of mid gut of 3rd instar larvae of *C. vittata* showed that all tested insecticide lead to several damages occurred in all the cells layers in the mid gut as separation of the basement membrane. In addition, imatrade (35% SC) possessed many mid gut cells with a weakly stained cytoplasm as well as a nucleus with decondensed chromatin and evident nucleoli. Agriflex (18.6% SC) due to increase proliferation of columnar cell. While, dora (48% EC) caused increase most of columnar cell lining mid gut were necrosis. Also, flagtra (25% WP) lead to increase in number of goblet cell, most of columnar suffer from necrosis.

Introduction

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops in the world. It considered as an important source of feed for livestock and pectin production from the pulp of sugar beet (Fouad *et al.*, 2011). The Egyptian Governments encourage sugar beet growers to increase the cultivated area with sugar beet for decreasing the gap between sugar production and consumption (Al-Habshy, 2013). Sugar beet quality is of great economic importance. Sugar beet is strategic sugar crops in Egypt because of its lower consumption of irrigation water and its shorter growing season. In Egypt, sugar beet is cultivated in 555.6 thousand faddan produce 909.9 Ton with an average production of 16.7 tons per faddan (Anonymous, 2016)

Sugar beet plants attack by numerous insect species during growing season such as beet fly *Pegomia mixta* (Vill.) (Diptera: Anthomyiidae) , the green beach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Cassida vettata* Vill. (Coleoptera: Chrysomelidoe) caused considerable damage in its yield (Sherief *et al.*, 2013). The sugar beet beetle *C. vettata* is one of the most destructive pest of sugar beet plant (El-Zoghbey *et al*., 2003). Both sugar beet beetle larvae and adults feed on the lower side of the sugar beet leaves, where, they eat the lower epidermis and inner tissue, but the upper epidermis remains intact looking like a glass. In addition, adults feed on leaves tissue, causing regular circular holes (Abo El-Ftooh *et al.*, 2013). Crop loss occurs due to leaf feeding and reduction in sugar content of infested plants (Ali *et al.* , 1993).

There are two important methods of insect control, biological and chemical methods. This pest is controlled in Egypt by conventional chemical insecticides. Use of insecticides has several disadvantages. It reduces population of predators and parasitoids of insect pests, leads to environmental pollution and development of pesticides resistant biotypes of insects (Abo El-Ftooh *et al*., 2013). Some conventional insecticides, i.e. Pirimiphos-methyl, monocrotophos, profenofos, methomyl were comparatively more effective against *C. vittata* under field conditions (Shaheen *et al*., 2011).

The main purpose of the current investigation is selecting the most suitable four compounds to contribute the integrated control operations to *C. vittata* larvae under the field condition and their effect on histology of this insect.

Materials and methods

The experiments were conducted at the farms of Dakahlia and Behira Governorates during 2014-2015 sugar beet growing season. Sowing dates were the 15th and 18th of October in Dakahlia and Behira, respectively. All normal agricultural practices were performed and no insecticide treatments were applied. These experiments were carried out to study the following to:

1. Efficiency of certain insecticides on the larvae of *Cassida vittata* :

The experiment was carried out to evaluate the efficacy of different compounds against the tortoise beetle *C. vittata* on sugar beet. An area of experimental was about 875 m^2 was cultivated with cable variety. The area was divided with randomized complete block design to 20 plots. The tested treatments are four compounds in addition to the control (untreated) as shown in Table (l). Each compound was applied with the recommended rate when the numbers of *C. vittata* were high. Spraying was directed to the plants using a knapsack sprayer was used for sprays at the morning hours during the two regions. Random samples were taken just before spraying the field and those for posttreatment counts were taken 1, 3, 7 and 10 days after application. Larvae were counted directly at random on 5 plant / plot.

2. Statistical analyses:

Reduction percent in *C. vittata* larval population after treated with four insecticides calculated according to **(Henderson and Tilton, 1955).**

Table (1): Tested insecticides, dosages, common name and trade name during 2014 - 2015 season.

Trade name	Common name	Rate of use/100L water
Imatrade (35% SC)	Imidacloprid	100 cm^2
Flagtra (25% WP)	Thiamethoxam	20 g
Agriflex (18.6% SC)	Thiamethoxam (3.3 2g/l) +Abamectin (15.24 g/l)	80cm^2
Dora (48% EC)	Chloropyrefos	330 cm^2
Untreated (control)	----	----

3. Histological technique for examination of mid gut, integument of larvae:

The 3rd instar larvae treated and untreated were obtained from the field after 24 hour of the treatment with recommended doses of all the tested compounds and transferred to the laboratory. Larvae were individually dissected in petri dish containing Ringer's solution to obtain mid gut by using fine entomological needles under a binocular dissecting microscope at 40X magnification and fixed in alcoholic Bouins solution for 12 hour and dehydrated through a graded series of ethanol and embedded in paraffin wax (42-48 Cº M.P) for half hr then changed 3 times in paraffin wax of melting point 58-60 ºC. 30 minutes each transverse sections of 6 microns thickness of the mid gut were stained with heidenhain's haematoxylin and eosin according to **Junqueira and Carneiro (1980).**

Results and discussion

1.Effect of certain insecticides on *Cassida vittata* larvae:

The efficiency of certain insecticides was examined against the *C. vittata* larvae in the sugar beet field in Dakahlia and Behira Governorates during 2015 season. The percent of reduction infestation is presented in Tables (2 and 3). The results showed that, all compounds were able to suppress the levels of infestation of *C. vittata* larvae to different degrees in comparison to that of untreated control. Generally, the highest reduction was recorded in dora treatment at two regions. The mean reduction % was 89.64 and 91.09 % in Dakahlia and Behira Governorates, respectively.

1.1. In Dakahlia Governorate:

The percentage reductions of *C. vittata* larvae population were showed in Table (2). All treatments declined alive larvae at 1, 3, 7 and 10 days post spraying comparing with control. The first day after treatment, dora was the most effective achieving 88.09% reduction in larvae. Three days after post spray, the highest reduction were with dora which recorded 89.52% followed by agriflex, imatrade and flagtra which recorded 83.92, 83.48 and 83.34%, respectively. Also the reductions after 7 and 10 days recorded the highest value with dora 90.95% followed by agriflex flagtra and imatrade which were 84.87, 84.29 and 83.93%, respectively.

Table (2): Reduction percentage in *Cassida vittata* larvae population after treated with certain compounds in the field sugar beet during 2015 season in Dakahlia Governorate.

Compounds	Rate of use/100L (recommended)	Reduction Post spraying (days)				Mean
		1	3	7	10	
Imatrade	100 cm^2	4.16	83.48	84.93	83.68	64.06
Agriflex	20 g	2.60	83.92	85.34	84.87	64.18
Flagtra	80cm^2	1.90	83.34	84.76	84.29	63.57
Dora	330 cm^2	88.09	89.52	90.00	90.95	89.64

1.2. In Behira Governorate:

The data in Table (3) did not differ from Behira Governorate. A significant decline in alive larvae was observed at 1, 3, 7 and 10 days post spraying with all tested insecticides. After one day post spray, dora showed the highest present of reduction (89.35%) while imatrade, agriflex and flagtra were recorded the lowest reduction. However, the reduction percentage reached 90.28, 86.70, 86.51 and 86.17% for dora, flagtra, imatrade and agriflex after three days post spray. Also the same trend, after ten days from application, dora caused highly decrease in the larval population where the percent reduction were 93.51% at recommended rate followed by flagtra was 87.15%, while agriflex and imatrade were the least effective (86.92 and 86.84%, respectively.) at recommended rate.

Table (3): Reduction percentage in *Cassida vittata* larvae population after treated with certain compounds on the field sugar beet during 2015 season at Behira Governorate.

Compounds	Rate of use/100L (recommended)	Reduction Post spraying (days)				Mean
		1	3	7	10	
Imatrade	100 cm^2	4.74	86.51	87.04	86.84	66.28
Ariflex	20 g	3.66	86.17	87.15	86.92	65.97
Flagtra	80cm^2	3.66	86.70	87.61	87.15	66.28
Dora	330 cm^2	89.35	90.28	91.20	93.51	91.09

The obtained results are in a harmony with that recorded by Asmahan and Qasem (2004) evaluated the efficiency of chlorfenapyr against eggs, larvae, pupae and adults of the tortoise beetle *C. vittata*. Results indicated that, chlorfenapyr was highly toxic effect. El-Kouly (1998) reported that the profenofos was the most efficient compound against all stages of *C. vittata* under field. Abdou (2009) mentioned that, Marshal the most effective to control the adult of *C. vittata,* marshal caused 82.3% decrease in the adult population followed by achook 68.5% while (Bancol, alkanz and chess) had moderate efficient against this pest. Abo El-Ftooh *et al.* (2013) who evaluated the effect of three pesticides (Radiant 12% SC, dursban 48% EC and mospilan 20% SP) against sugar beet beetle *C. vittata* at Behira Governorate. They found that, radiant SC 12% pesticide was more toxic against *C. vittata* (Larvae and adults). But the dursban EC 48% was superiority during some periods of experiment.

2.Effect of tested insecticides on histological structure of mid gut of *Cassida vittata* larvae:

The mid gut is the main origin for digestion and absorption of ingested food. Insects mid gut wall comprises of two muscular layers and an epithelial layer lining the lumen. The intestinal epithelium contains four types of cells; digestive, regenerative, endocrine and goblet cells.

2.1. In the control group:

The mid gut of 3rd instar larvae of *C. vittata* consists of two layers of muscle fibers, the outer longitudinal fibers and the inner circular one. The circular muscle fibers are very close to the basement membrane of the epithelial cells. There is wide space between the longitudinal fibers. The peritrophic membrane is followed by the epithelial layer which lining the cavity of the mid gut. The mid gut epithelium consisted of a single layer of digestive cells exhibiting a well developed brush border and cytoplasm with acidophilic regions. (Figure, 1)

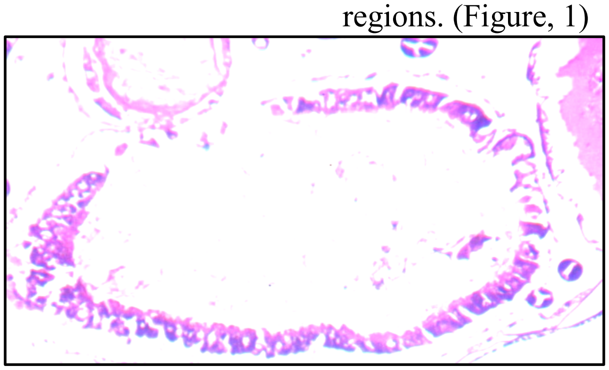

Figure (1): Transverse section in the mid gut of the control 3rd instar larvae of *Cassida vittata*.

2.2. Imatrade treatment:

Microscopic examination of the transverse section of the mid gut of 3rd instar larvae of *C.vittata* treated with imatrade (35% SC) (Figure,2) showing increase in number of goblet cells as well as necrosis of some columnar cells lining it. In addition, several damages occurred in all the cells layers in the mid gut as separation of the basement membrane. Imatrade treated larvae possessed many mid gut cells with a weakly stained cytoplasm as well as a nucleus with decondensed chromatin and evident nucleoli.

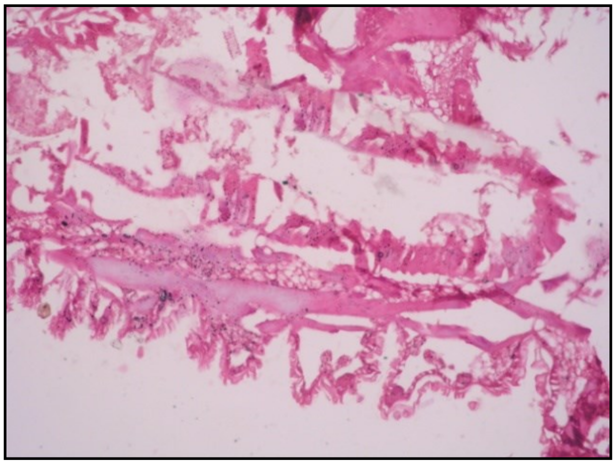

Figure (2): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with imatrade (35% SC).

2.3. Flagtra treatment:

The most affected tissue was the mid gut epithelium when compared with the untreated mid gut. Microscopic examination of transverse section in the mid gut on the 3rd instar larvae of *C.vittata* with flagtra (25% WP) (Figure, 3) illustrated the increase in no. of goblet cell, most of columnar suffer from necrosis.

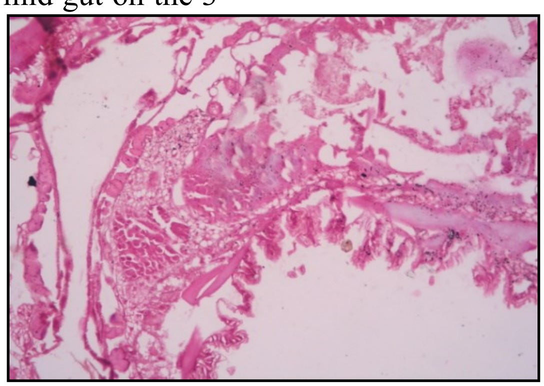

Figure (3): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with flagtra (25%WP).

2.3. Agriflex treatment:

Transverse section in the mid gut on the 3rd instar larvae of *C.vittata* which treated with agriflex (18.6SC%) (Figure, 4) showed that severe damage effects on the mid gut layers and increase proliferation of columnar cell. Epithelia cells separated and collected in groups.

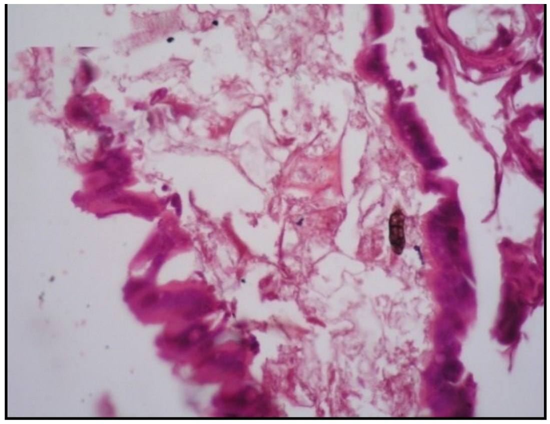

Figure (4): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with agriflex (18.6% SC).

2.4. Dora treatment:

Severe effects were found in the mid gut of the larval treated with In case treated the 3rd instar larvae of *C. vittata* by dora (48% EC) (Figure, 5). The mid gut showing increase most of columnar cell lining mid gut were necrosis. In addition destruction in the longitudinal, epithelial and columnar cells.

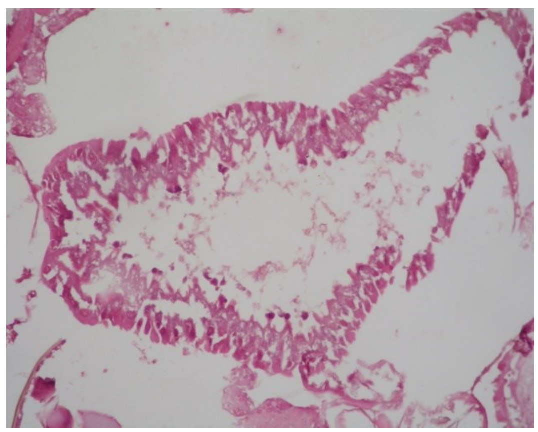

Figure (5): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with dora (48% EC)

These results are similar to that obtained by Sayed and Abd El- Aziz (2014) who reported that, microscopic examination of transverse sections in the mid gut of 3rd instar larvae of *C. vittata* treated with robust and marshal showed sever histological effects in all layers of the mid gut. And it showed separation of both basement and peritrophic membranes. Sharaby and El-Nujiban (2016) found that, the most affected tissue was the mid gut epithelium when compared with the untreated mid gut of *A. ipsilon* larvae. The epithelium possessed deeply stained nuclei, the regenerative cells were not pronounced and could not been identified in some areas at the base of the epithelial cells due to the sever destruction of the epithelium, goblet cells increased their secretion. And they added, Epithelial cells of the treated larvae were destroyed, large vacuoles were found between the epithelium and the muscular layer. Yasmeen and Amir (2018) who cleared that, the insecticide chlorpyrifos was provided in food to 3rd instar larvae *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) causing loosely attached epithelial layer in sections which became filled with vacuoles at higher concentrations in mid gut. In addition epithelial cells became more or less circular in shape and highly separated from each other but on increasing the concentration, significant elongation in epithelium cells and decrease in lumen of the mid gut were seen. The number of epithelial cells has been reduced.

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