THE COMPARATIVE EFFICACY OF SPINOSAD, LUFENURON AND HEXYTHIAZOX ON SOME STRAWBERRY PESTS UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT: The comparative efficacy of spinosad, lufenuron and hexythiazox on Spodoptera littoralis, thrips and aphids under laboratory and field conditions was carried out during this study. The toxicity and time-mortality relationship were investigated through testing a serious of prepared concentrations or recommended field rates. Data of mortality under laboratory conditions indicated that spinosad and lufenuron efficacy was highly similar in killing 90% of the tested instars while when compared at their corresponding LC50, spinosad showed 5.2 times lower in concentration than that of lufenuron after 72 h of treatment. Patterns of time-mortality showed generally that spinosad caused the highest mortality either with time or with concentrations followed by lufenuron taking time around 16 h to kill 50% of the tested 2nd instar. Results of field experiments showed that spinosad and lufenuron caused more than 50% reduction in thrips and aphid numbers after 10 days of treatment. This show that both spinosad and lufenuron have almost the same effect against cotton leafworm, thrips and aphids and can be used wisely in IPM programs for pest control of pests infesting strawberry.

Key words: Strawberry, S. littoralis, thrips, aphid, spinosad, lufenuron, hexythiazox, pest control.

INTRODUCTION

Strawberry is widely cultivated worldwide due to its attractive fragrance, sweet taste, and high economic benefit. Also, it is known to be rich in Vitamin C and has the medicinal properties in preventing cardiovascular, neurodegenerative and other human diseases such as aging, obesity and cancer (Zhang et al., 2008; Saber et al., 2016). Strawberries have a long cultivation cycle of 4–5 months that require many applications of different pesticides to prevent pests including the cotton leafworm and spider mites from becoming resistant (Wang et al., 2018). Thus, many chemical insecticides and acaricides (Xie et al., 2015; Saber et al., 2016) registered for use worldwide and have been introduced to control different strawberry pests cultivated in open field and greenhouse.

The cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae), is one of the most serious and destructive polyphagous agricultural pest of different field crops in Africa, Asia and Europe causing severe yield losses (Brown and Dewhurst, 1975; Carter, 1984). Numerous economically important crops and considerable feeding damage on different field, ornamental, and vegetable crops including strawberry throughout the year are reported from the insect attacking (Bayoumi et al., 1998; Pineda et al., 2007).

Many insecticide formulations belonging to different groups have been registered and used in Egypt for its control according to the approved agricultural pest control recommendations (El-Sheikh, 2015). Thrips and aphids are of the highest incidence of pests in strawberry and mainly damages the leaves and stems (Baskoy et al., 2019). They are small and feed on hundreds of plants including strawberry crops worldwide that need to be controlled due to the big losses they cause.
Due to their desired properties of having unique mode of action and less toxic effects to non-target organisms, insect growth regulators (IGRs) are considered successful substitutes to the conventional insecticides (Kai et al., 2009). Existing environmentally-friendly methods that have become fundamental for pest management helped in conventional pesticides to be withdrawn because of their undesirable effects on humans and non-target species, and overall environmental impact (Metspalu et al., 2013).

New products have advantages that include their greater selectivity to the target species and acting on specific insect biological processes such as moultng that makes them less harmful to natural enemies and humans comparing with conventional insecticides (Grafton-Cardwell et al., 2005). Lepidopteran larvae development is affected by exposure to lufenuron through the inhibition of the synthesis of new cuticle and production of infertile eggs (Tunaz and Uygun, 2004). Fermentation of the naturally occurring soil actinomycete, Saccharopolyspora spinosa Mertz and Yao, is producing a mixture of spinosyns A and D known as spinosad insecticide (Sparks et al., 1998; Thompson et al., 2000). This insecticide is currently registered in several countries and affect pests in two unique ways through nicotinic acetylcholine and GABA receptors (Salgado and Sparks, 2005; Osorio et al., 2008) causing significant increase in larval mortality and decrease in pupation and adult emergence of Spodoptera littoralis, as well as rapid death of leafminers, thrips, and foliage-feeding beetles (Copping and Menn, 2000; Dayan et al., 2009; El-Sheikh, 2015). For the efficacy study, the comparative effect of spinosad, lufenuron and hecithiazox were investigated on some pests’ attacking strawberry under field and laboratory conditions.

MATERIALS AND METHODS

Insecticides

Insecticide formulations were used in all assays in this study. The insecticides used were: Hecithiazox (Macomait 10 % WP, Nippon soda Co.), Lufenuron (Match 5 % EC, Syngenta Co), Spinosad (Tracer 24 %, SC, Dow Agrosciences Co.). Leaf dip technique was used in laboratory bioassays.

Insect Rearing

A laboratory colony of the Egyptian cotton leafworm, S. littoralis, was used in the current study. The colony was obtained from Syngenta company and reared for one generation in the lab on castor bean leaves at 27 ± 2°C and 65 ± 5% RH, with a photoperiod of 16:8 h (light: dark) before starting the experiments (El-Defrawi et al., 1964).

Laboratory Bioassay

Bioassays using a standard leaf disc adopted from the Insecticide Resistance Action Committee (IRAC) of the tested insecticides were performed on 2nd instar larvae of S. littoralis (IRAC, 2014). Eight to 10 concentrations from each insecticide were prepared starting with a stock solution of commercially available formulations by dissolving each insecticide in distilled water. The castor leaves, Ricinus communis, were cut into small pieces (~7 cm²) and dipped for 10 second in an insecticide concentration (Silva et al., 2011). After dipping, leaves were air dried at room temperature for 10 min. The leaves dipped in distilled water only were used in control experiment. Leaves treated with insecticides were then transferred to each Petri dish (9 cm diameter) where 10 newly moulted 2nd instar larvae of S. littoralis were transferred to each Petri dish. Three replicates were used per each concentration with a total of 30 larvae for each concentration including controls. Larvae were allowed to feed on treated and untreated disks for 24 h then clean leaves were introduced to each replicate for 48 h. Larval mortality was recorded after 24, 48, and 72 h post treatment.

Time-Mortality Assay

Time mortality relationships were determined using concentrations (1, 10 and 100 ppm) of the tested insecticides on 2nd instar larvae. For conducting this assay, concentrations from each insecticide were prepared in distilled water. Discs of castor leaves were dipped in each concentration for 10 second and introduced to total of 30 2nd instar larvae in triplicate (10 larvae/each replicate). Larvae were allowed to feed on treated (experimental) or untreated
Field Experiment

Field experiment was conducted to evaluate the effect of two insecticides in control of aphid and thrips on strawberry plants grown in a privet farm related to the International Company for Agricultural Production and Processing (ICAPP) located in El-Salheia El-Gededa district at Sharkia Governorate, Egypt. The two tested insecticides used were spinosad and lufenuron according to the recommended field rates. Experiments were done in a completely randomized design (CRD) and started by dividing the experimental areas into plots of 10 m²/plot. A knapsack sprayer with one nozzle beam was used in application of insecticide solutions at the rate of 50 ml/plot in three replicates (plots) per each insecticide. Numbers of insects were counted before treatment and the reduction in insect populations due to insecticide applications were compared after 1 day (initial effect) and after 3, 7 and 10 days post-treatment for evaluating the residual effects of these insecticides on aphid and thrips populations. Aphid and thrips populations were recorded in the early morning from terminal branches, leaves, stems of plants for each plot before and after treatment.

Statistical Analysis

Mortality data were obtained 24, 48, and 72 h after exposure to treated leaves and larvae were considered dead if they did not move when pushed with a fine brush. The mortality data were corrected for those in controls using the Abbott (1925) formula and were processed by probit analysis according to method described by Finney (1971) using computer software of LdP Line.

Lethal times of 50 and 90 values and their 95% confidence limits were estimated using Vistat 2.1 program (Hughes, 1990). Lethal concentrations of 50 or 90 values were considered significantly different when their confidence limits did not overlap. The percentages of aphid and thrips reduction were calculated in 1, 3, 7, and 10 days of exposure according the number of insects before treatment.

RESULTS AND DISCUSSION

Laboratory Experiments

The comparative efficacy of spinosad, lufenuron and hexathiazox on 2nd larval instars of Spodoptera littoralis under laboratory conditions is presented in Table 1. Data of mortality was compared at 24, 48 and 72 hours. After 24 and 48 h, hexathiazox did not give mortality data to be computed by LdP Line program for calculating lethal times. Accordingly, no data was obtained for this pesticide with low concentration tested (1 and 10 ppm), while for spinosad and lufenuron, their efficacy was not significantly different in killing 90% of the tested instars as their confidence limits were overlapped. When spinosad and lufenuron were compared at their corresponding LC50, spinosad showed significant effect compared with lufenuron as the concentration used for killing 50% was 5.2 times lower than that of lufenuron. For mortality data after 48 and 72 h, both spinosad and lufenuron showed the same effect for killing 50 and 90% of 2nd instars larva of S. littoralis with no statistical differences between them (after 48 h). While spinosad was more effective than lufenuron in killing 50% with no difference between them in killing 90% after 72 h depending on the overlap of their 95% CLs. Hexythiazox showed very low effect on the 2nd instar comparing with either spinosad or lufenuron. Toxicity regression lines of the tested pesticides (Fig. 1) against 2nd instar larvae showed patterns of the obtained values of 50, 90% mortality and slopes after 72 h of treatment.

Accumulative mortality of the 2nd instar of S. littoralis when exposed to concentrations of 1, 10, 100 ppm from spinosad, lufenuron or hexythiazox is presented in Fig. 2. Patterns of mortality showed to increase with increasing time and concentration. Generally, spinosad showed to cause the highest mortality either with time or with concentrations followed by lufenuron which showed very close mortality percentage specially with increasing time (60 and 72 h). In all cases, hexythiazox showed to cause low effect comparing with spinosad or lufenuron.
Table 1. Lethal concentrations (LC$_{50}$ and LC$_{90}$) of spinosad, lufenuron and hexythiazox after 24, 48, and 72 hours of treatment on the second larval instar of Spodoptera littoralis under laboratory conditions

| Insecticides | Lethal concentrations (µg ml$^{-1}$) (95 % CL)$^a$ | Slope ± SE | $\chi^2$ | p-value |
|--------------|-------------------------------------------------|------------|----------|---------|
|              | LC$_{50}$                                      | LC$_{90}$  |          |         |
| Spinosad     | 14.9 (8.8-28.1)                                | 8176.1 (1944.9-77450.5) | 0.7±0.08 | 6.1     | 0.200   |
| Lufenuron    | 78.1 (48.1-147.7)                              | 5230.6 (1748.0-27606.4) | 0.5±0.06 | 4.4     | 0.230   |
| Hexythiazox  | b                                               | -          | -        | -       |         |
| Spinosad     | 0.5 (0.1-0.4)                                  | 52.4 (24.5-436.5)    | 0.6±0.04 | 22.9    | 0.001   |
| Lufenuron    | 0.75 (0.09-3.6)                                | 323.4 (157.7-20252.0) | 0.5±0.04 | 17.9    | 0.001   |
| Hexythiazox  | b                                               | -          | -        | -       |         |
| Spinosad     | 0.02 (0.01-0.05)                               | 15.8 (6.1-56.8)      | 0.5±0.04 | 5.2     | 0.150   |
| Lufenuron    | 0.03 (0.003-0.122)                             | 29.7 (12.4-1180.5)   | 0.4±0.04 | 21.4    | 0.001   |
| Hexythiazox  | 1.27 (0.8-1.9)                                 | 103.4 (55.7-236.9)   | 0.7±0.06 | 3.1     | 0.546   |

$^a$ 95% confidence limits.

$^b$ data of bioassay of hexythiazox were not in a suitable range for probit analysis.

Fig. 1. Toxicity regression lines of spinosad (A), lufenuron (B), and hexythiazox (C) applied using dipping bioassay against 2$^{nd}$ instar larvae of Spodoptera littoralis after 72 h of treatment
Fig. 2. Accumulative mortality of second instar larvae of *Spodoptera littoralis* treated with 1 (A), 10 (B), and 100 (C) µg ml⁻¹ of spinosad, lufenuron, or hexythiazox under laboratory conditions.
Results of time-mortality relationship of spinosad, lufenuron and hexythiazox when tested at three concentrations (1, 10, and 100 ppm) on the 2nd instar larvae of *S. littoralis* are presented in Table 2. Hexythiazox showed to take very long time to kill 50 or 90% of the number tested exceeding 72 h with 1 and 10 ppm. Also, with the low concentration (1 ppm) of both spinosad and lufenuron took a long time (>72 h) to kill 90%. Spinosad showed to kill either 50 or 90% of the treated larvae faster than lufenuron or hexythiazox when tested at 10 ppm. When tested using high concentration (100 ppm), spinosad and lufenuron showed no differences in killing 50% taking 17 and 15 h, respectively.

New insecticide members showed to cause different levels of toxicity, e.g. a spinosyn group (spinosad) reported to acts on the gamma aminobutyric acid and nicotinic acetylcholine receptors. Also, it was reported to be effective against insect pests related to different orders such as Diptera, Lepidoptera, and some insect species of Coleoptera (Osorio et al., 2008).

Data presented in the current study showed that spinosad is the highly effective insecticide insignificantly followed by lufenuron. In the same context, it was reported that spinosad has strong insecticidal activity against lepidopteran larvae with a unique mechanism of action and relatively low toxic effects against non-target insects (Wang et al., 2009). Spinosad was found to be either more toxic or as effective as lufenuron, although it shows previously the slowest effectiveness in killing *S. littoralis* compared with lufenuron (El-Sheikh, 2015) which might due to the difference in strains susceptibility (field or laboratory), resistance to insecticide (Gunning and Balfe, 2002) or its unique mode of action at nicotinic acetylcholine and GABA receptors (Salgado and Sparks, 2005).

Data regarding lethal times of exposure to different concentrations of the tested pesticides (spinosad, lufenuron and hexythiazox) showed generally that spinosad was killing the tested insect faster than the other 2 pesticides. It was showed in the study of El-Sheikh (2015) that emamectin benzoate was high and faster in efficacy over spinosad or lufenuron when tested on 3rd or 5th instars of *S. littoralis* using contaminated artificial diet. Also, in a study on *S. exigua*, Saeed et al. (2012) found that emamectin benzoate was more toxic and killed 2nd instar larvae faster than lufenuron. On the other hand, lufenuron found to be more effective and faster in killing 2nd and 4th instar larvae of *S. littoralis* than other chitin synthesis inhibitors recommended in many countries (i.e., flufenoxuron and triflumuron) (Ahmad et al., 2008; El-Sheikh and Aamir, 2011).

**Field Experiment**

The efficacy of spinosad and lufenuron when tested under field conditions against thrips and aphid infesting strawberry was reported in Table 3. Before applying insecticides, the number of insects was recorded on plants and showed to be from 20-24 individuals/plant. After applying insecticides, the number of insects (thrips or aphids) was found to be decreased by the time. The reduction percentages in thrips and aphid were 50 and 55% after 10 days of treatment with spinosad, respectively. While the reduction in both thrips and aphid numbers was similar when exposed to lufenuron (58% reduction) after 10 days of treatment. The mean effect of both insecticides on thrips and aphid was ranged from 79 – 83%.

It was reported that spinosad has an ovicidal activity against pests related to lepidopteran order including *S. littoralis*, *Heliothis zea*, and *H. virescens* (Peterson et al., 1988; Bret et al., 1997; Pineda et al., 2004) as shown in the reduction of the fecundity and egg size of *Plutella xylostella* after treatment of 3rd instar larvae with spinosad at LC25 or LC50 values (Yin et al., 2008). Also, spinosad at concentration values ranged from LC10 to LC50 was found to be more effective on fecundity and hatchability compared with emamectin benzoate on 2nd instar larvae of *S. littoralis* (Korrat et al., 2012).

Five insect growth regulators (IGRs) including lufenuron were tested for their efficacy and persistence against 4th instar larva of *S. littoralis* in laboratory-field bioassays in comparison with pyridalyl (El-Zahy et al., 2021). Their experiments showed that pyridalyl caused the highest initial and mean residual effects, while lufenuron resulted in the lowest initial effect and mean residual activity.
Table 2. Lethal time (LT$_{50}$ and LT$_{90}$; hours) of spinosad, lufenuron and hexythiazox tested on second larval instar of *Spodoptera littoralis* under laboratory conditions

| Insecticides | LT$_{50}$ (h) (95% CL) | LT$_{90}$ (h) (95% CL) |
|--------------|------------------------|------------------------|
|              | Concentration (1 µg ml$^{-1}$) |                  |
| Spinosad     | 49 (46-51)             | >72                    |
| Lufenuron    | 54 (52-56)             | >72                    |
| Hexythiazox  | >72                    | >72                    |
|              | Concentration (10 µg ml$^{-1}$) |                |
| Spinosad     | 28 (26-30)             | 54 (52-56)            |
| Lufenuron    | 37 (33-41)             | 63 (59-67)            |
| Hexythiazox  | 55 (50-61)             | >72                    |
|              | Concentration (100 µg ml$^{-1}$) |               |
| Spinosad     | 17 (14-20)             | 37 (32-42)            |
| Lufenuron    | 15 (13-17)             | 49 (45-53)            |
| Hexythiazox  | 42 (38-46)             | 69 (60-77)            |

Table 3. Efficacy of spinosad and lufenuron on the reduction of thrips and aphids numbers on strawberry under field conditions

| Insecticides | Insect | Count before treatment (No/Plant) | Number after treatment/Plant (% reduction) | % residual effect* | % mean effect |
|--------------|--------|----------------------------------|--------------------------------------------|-------------------|---------------|
|              |        | 1 day | 3 days | 7 days | 10 days |                  |                |
| Spinosad     | Thrips | 20 ± 0 | 0 ± 0 | 0 ± 0 | 3 ± 0 | 10 ± 0 | 78 | 83 |
|              | Aphid  | 20 ± 0 | 0 ± 0 | 2 ± 0 | 5 ± 0 | 9 ± 0 | 73 | 80 |
| Lufenuron    | Thrips | 24 ± 1 | 1 ± 0 | 3 ± 1 | 5 ± 2 | 10 ± 2 | 75 | 80 |
|              | Aphid  | 24 ± 1 | 0 ± 0 | 3 ± 1 | 7 ± 2 | 10 ± 1 | 72 | 79 |

* Residual effect was calculated using the data of insect numbers recorded in 3, 7 and 10 days of the treatment.

Combination studies of spinosad with either bioinsecticides or adjuvants on onion field trials showed that spinosad was the most effective bioinsecticide with either neem oil or salts of fatty acids, providing the largest reductions (26–85%) in thrips densities and feeding damage (56–69%) as well as caused up to 26% increases in total onion yield (Iglesias *et al*., 2021).

Results of the current study showed that hexythiazox was the lowest in effect as presented in Tables 1 and 2 and Fig.1. Dunn *et al.* (2016) reported that hexythiazox has ovicidal, larvicidal and nymphicidal activities with high effects on different kinds of plant mites with low to moderate toxicity to mammals, birds, fish, and aquatic invertebrates. In agreement with the current findings, the study of Kumari *et al.* (2017) showed that hexythiazox was 711 times less effective than abamectin. Alzoubi and Cobanoglu (2008) reported that LC$_{50}$ values of hexythiazox against *T. urticae* 537.45 and 175.75 ppm after 24 and 72 h, respectively with high levels of resistance in *T. urticae* (>1000-fold) (Gough, 1990), *Panonychus ulmi* (>2500-fold) (Edge *et al.*, 1987) and in *Panonychus citri* (>24,000-fold) (Yamamoto *et al*., 1995).

In conclusion, previous reports stated that lufenuron is still at minimum levels of resistance and can be useful to manage *S. litura*, while spinosad showed increasing trends toward resistance, but still at moderate levels in most populations. As found in the current study, both spinosad and lufenuron were almost similar in their toxic effects on *S. littoralis*, thrips and...
aphid. Accordingly, these insecticides (spinosad and lufenuron) might be used wisely in IPM programs due to their desired characteristics regarding the environmental safety and rapid degradation processes (Schneider et al., 2004).

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