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

Ahmed M. Refaat*, E.A. El-Sheikh, D.A. Ragheb and A.A. Shalaby

Plant Prot. Dept., Fac. Agric., Zagazig Univ., Egypt

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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 LC_{50}, 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.

*** Corresponding author: Tel. :** +2 01018754514
E-mail address: Ahmed.elmesllamy@gmail.com

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 moulting 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 hexythiazox 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: Hexythiazox (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 been 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 2^{nd} 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 cm^2) 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 2^{nd} 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 2^{nd} 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 2^{nd} instar larvae in triplicate (10 larvae/each replicate). Larvae were allowed to feed on treated (experimental) or untreated (control) leaves for 24 h before transferring into clean leaves until 72 h post treatment. Larval mortality was recorded every 12 h of starting experiment. Mortality data were subjected for Vistat 2.1 program for calculating lethal times (**Hughes, 1990**).

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 m^2/plot. A knapsack sprayer with one nozzle beam was used in application of insecticide solutions at the rate of 50 ml/Faddan 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 2^{nd} 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 LC_{50}, 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 2^{nd} 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 2^{nd} instar comparing with either spinosad or lufenuron. Toxicity regression lines of the tested pesticides (Fig. 1) against 2^{nd} instar larvae showed patternes of the obtained values of 50, 90% mortality and slopes after 72 h of treatment.

Accumulative mortality of the 2^{nd} 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 spinsad 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	X^2	*p*-value
	LC_{50}	LC_{90}			
		After 24 hour			
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]	-	-	-	-
		After 48 hour			
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	-	-	-	-	-
		After 72 hour			
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.

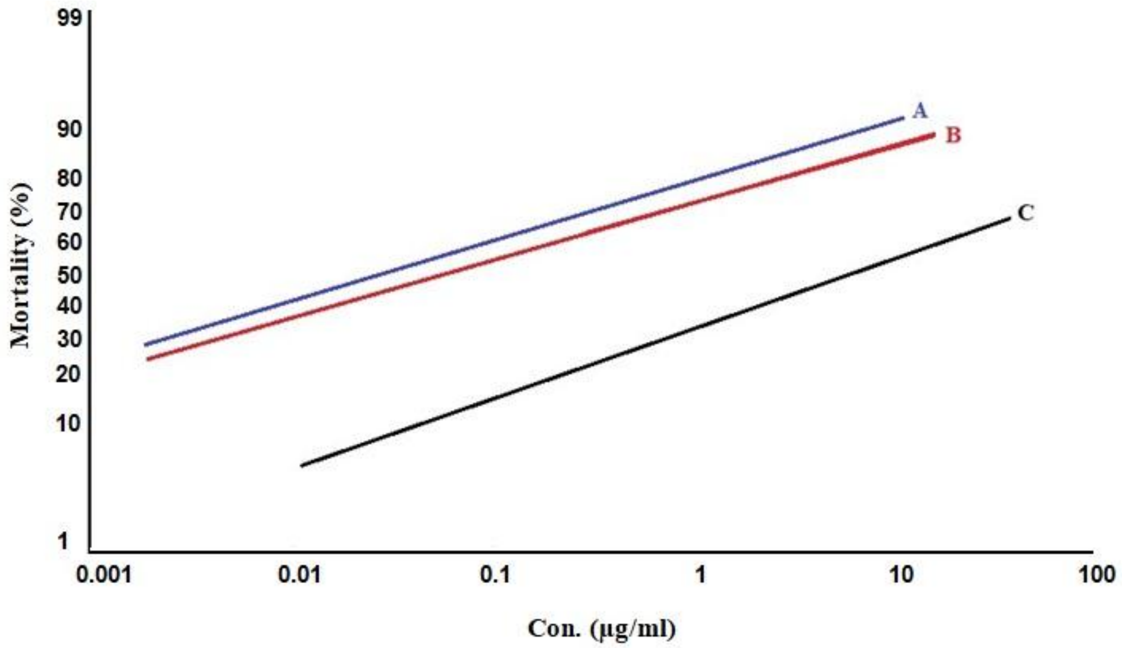

Fig. 1. Toxicity regression lines of spinosad (A), lufenuron (B), and hexythiazox (C) applied using dipping bioassay against 2nd instar larvae of *Spodoptera littoralis* after 72 h of treatment

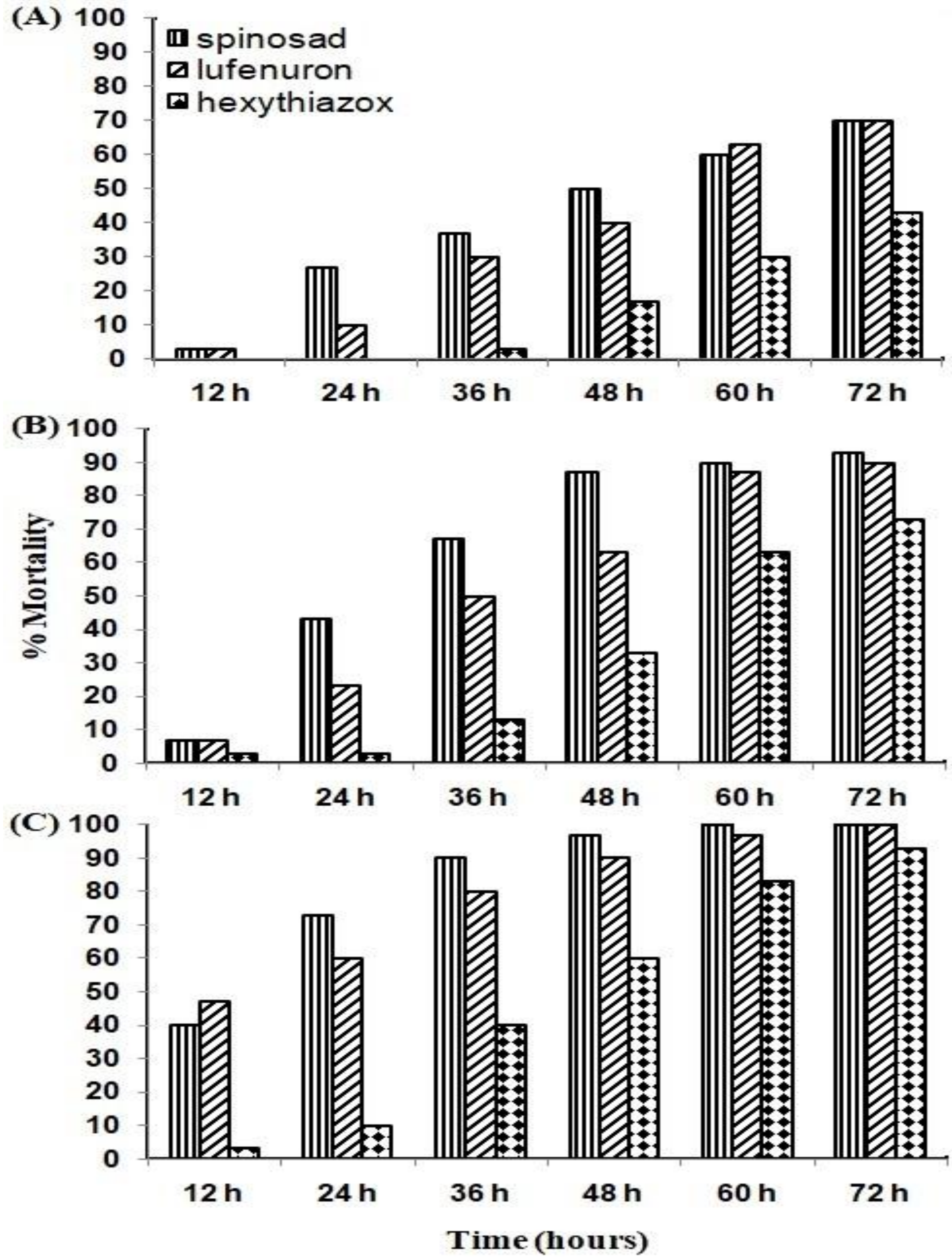

Fig. 2. Accumulative mortality of second instar larvae of *Spodoptera litoralis* treated with 1 (A), 10 (B), and 100 (C) µg ml^{-1} of spinosad, lufenuron, or hexythiazox under laboratory conditions

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 LC_{25} or LC_{50} values (**Yin *et al*., 2008**). Also, spinosad at concentration values ranged from LC_{10} to LC_{50} 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 larvae 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 (100)	0 ± 0 (100)	3 ± 0 (85)	10 ± 0 (50)	78	83
	Aphid	20± 0	0 ± 0 (100)	2 ± 0 (90)	5 ± 0 (75)	9 ± 0 (55)	73	80
Lufenuron	**Thrips**	24 ± 1	1 ± 0 (96)	3 ± 1 (88)	5 ± 2 (79)	10 ± 2 (58)	75	80
	Aphid	24 ± 1	0 ± 0 (100)	3 ± 1 (88)	7 ± 2 (71)	10 ± 1 (58)	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. **Dunnm *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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الفعالية النسبية للسبينوساد، الليفينيورون والهيكسيثيازوكس على بعض آفات الفراولة تحت الظروف المعملية والحقلية

احمد محمد رفعت- السيد عبدالمالك الشيخ- ديدير احمد راغب- على عطا شلبى

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

أجريت خلال هذه الدراسة مقارنة الفعالية النسبية للسبينوساد، الليفينيورون، وهيكسيثيازوكس على دودة ورق القطن، التربس والمن تحت الظروف المعملية والحقلية. تم دراسة العلاقة بين الوقت والموت من خلال اختبار عدد من التركيزات المحضرة أو المعدلات الحقلية الموصى بها. أشارت نتائج السمية تحت ظروف المعمل إلى أن فعالية مبيدات سبينوساد وليفينيرون متشابهة إلى حد كبير في قتل 90% من العمر اليرقى الثانى لدودة ورق القطن، بينما، عند مقارنتها على مستوى LC_{50}، أظهر مبيد اسبينوساد فعالية اعلى حيث كان التركيز المستخدم لقتل 50% أقل بمقدار 5.2 مرة من تركيز ليفينيرون وذلك بعد 72 ساعة من المعاملة. أظهرت أنماط العلاقة بين الوقت ونسبة الموت أن مبيد الإسبينوساد تسبب في أعلى معدل موت يرقى ثم مبيد ليفينيرون حيث استغرق وقتًا حوالي 16 ساعة لقتل 50% من اليرقات المختبرة. أظهرت نتائج التجارب الحقلية أن مبيد سبينوساد وليفينيورون تسبب في انخفاض بنسبة أعلي من 50% في اعداد التربس والمن بعد 10 أيام من المعاملة، ويدل ذلك على أن كلا من مبيد الإسبينوساد والليفينيورون لهما نفس التأثير ضد دودة ورق القطن، التربس، والمنّ ويمكن استخدام هذه المبيدات بحكمة في برامج المكافحة المتكاملة لبعض آفات الفراولة.

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