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Ovicidal and larvicidal activity of spinosad against egyptian cotton leafworm

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ABSTRAC

One of our objectives in this investigation is to determine the ovicidal action of spinosad (Tracer®) compared to chlorpyrifos (Dursban®) and triflumuron (Alsystin®) against 0-1 day old eggs of cotton leafworm, *Spodoptera littoralis*. The residual toxicity of these insecticides to the neonates is also evaluated. Toxicity of spinosad against 2nd, 3rd and 4th instar larvae of *S. littoralis* by dipping technique and against the 4th instar larvae by topical application was carried out. The joint toxic action of spinosad with cypermethrin, chlorpyrifos and methomyl against 4th instar larvae of *S. littoralis* was also studied. Spinosad has an ovicidal activity lower than chlorpyrifos and triflumuron. Although spinosad has low ovicidal activity, it has the highest residual activity against the neonates after 24h of hatching. Spinosad has the highest total mortality (% killed eggs + % neonate mortality after 24h of hatching) compared to chlorpyrifos and triflumuron. According to the LC_{50} values, susceptibility of *S. littoralis* larvae to spinosad is decreased considerably as the larvae advance in age and weight. Second instar larvae were more susceptible to spinosad than the 3rd instar larvae which were more susceptible than the 4th instar larvae. The LC_{50} values by dipping technique were 41.7, 138.1 and 196.7 ppm for 2nd, 3rd and 4th instar larvae, respectively. Toxicity of spinosad by topical application is enhanced by increasing the exposure time where LD_{50} values were 0.51, 0.12 and 0.09 µg/mg body weight after 24, 48 and 72h of treatment, respectively. Data revealed that dipping treatment exerts toxicity more than the topical application. In respect with the joint toxic action, the combination of spinosad and cypermethrin results in a synergistic effect or an additive effect as calculated by the co-toxicity factors. The combination of spinosad with chlorpyrifos or methomyl results in additive effect or antagonistic effect; this will shed some lights on the possible joint toxic action and the sequence of alternative spraying programs.

INTRODUCTION

Bioinsecticides represent the major segment of biopesticides and comprise the largest array of diverse microbial and natural products in the biopesticide armamentarium. Among the most promising alternative is the discovered bacterial insecticide spinosyns. Spinosad was discovered during the 1980s by Lilly Research Laboratories. Spinosyns are a fermentation broth of a soil sample that containing the microorganism *Saccaropolyspora spinosa*. The spinosyns are composed of a 12-membered macrocyclic ring as part of an unusual tetracyclic ring system, to which two different sugars are attached. This attributes set the spinosyns apart from other macrocyclic compounds. An extract of the fermentation broth that contains naturally occurring mixture of spinosyns A and D is called spinosad (Tracer), the first product of spinosyns in Dow Agrosciences. Spinosyn A has a broad insecticidal activity, but especially on lepidopterous pests such as tobacco budworm, the cotton bollworm, American bollworm, armyworms, loopers and rice stem borer (Sparks *et al.*, 1995; Sparks *et al.*, 1997a; Thompson *et al.*, 1995; DeAmicis *et al.*, 1997). Good activity is also observed against a variety of dipteran pests, thrips, fleas, and hymenopteran pests.

The development of multiple insecticide resistance in field strains of the *S. littoralis* to several insecticides constitutes a major challenge to the workers in the field of insect control. Most applications of insecticides for the control of *S. littoralis* are timed to control the larval instars, which are the most destructive stages of the insect on cotton and vegetable crops. However the larval stages have become extremely tolerant to the action of pesticides because of the intensive treatments with these chemicals. The search about other more effective insecticides to control this insect is very important. More consideration needs also to be given to the possibility of controlling the pest at other stages of its development, when it may be more susceptible to the chemicals used for control. This may also decrease the damage caused by the insect pest to plants to the level of no damage, with more emphasis on the critical thresholds of pest infestation. Several studies had been conducted to evaluate the ovicidal activity against many insect species (Wells and Guyer, 1962; Dittrich, 1967; Mitri and Kamel, 1970; El-Guindy *et al.*, 1983; Renkleff *et al.*, 1995; Canela *et al.*, 2000).

The main target of this study is to magnify the use of spinosyns in insect control through assessment of ovicidal and larvicidal activity of spinosad (both by dipping and topical application techniques) against *S. littoralis*.

MATERIALS and METHODS

Experimental insect: Cotton leaf worm, *Spodoptera littoralis*, larvae for test purposes was reared in the laboratory on castor bean leaves. The egg masses were collected daily and as they hatched on the oleander leaves the larvae were transferred to fresh castor oil leaves. The colony was kept at a temperature of 25±2 °C and 65±5 % RH (Eldefrawi *et al.*, 1964).

Tested insecticides: Formulated spinosad (Tracer® 24%SC) and technical grade (97.2%) were produced by Dow Agrosciences. Technical grades of chlorpyrifos (95.4%), methomyl (98.6%) and cypermethrin (87.9%) were produced by Dow Agrosciences, E.I.du Pont de Nemours&Co and Agrochem, respectively.

Ovicidal activity: Ovicidal activity of spinosad (Tracer®), chlorpyrifos (Dursban®) and triflumuron (Alsystin®) against the laboratory strain of *S. littoralis* egg masses was determined. The upper layers of each egg mass (0-24 hr old) were removed gently with a fine hair brush. The lower layer in each egg mass was counted by the binocular. The counted egg samples were dipped (5 seconds) in different concentrations of the tested compounds, while the control was dipped in water according to Dittrich (1967). Each treatment was replicated three times. Treatments and control were held in a plastic cups (9x4 cm) at 27±2°C, 65-75% RH and observed until hatching. The number of un-hatched eggs, dead neonates and live larvae were counted, and the mortality percentages were calculated.

Larvicidal activity: Toxicity of the formulated spinosad (Tracer 24% SC) against 2nd, 3rd and 4th instar larvae of *S. littoralis* was evaluated. Homogenous pieces of the castor oil leaves were dipped in a series of the spinosad concentrations for 10 sec., held vertically to allow excess solution to drip off and dried at room temperature. Treated castor oil leaf pieces were transferred to a plastic cups, and the appropriate number and weight of starved larvae were added. Each concentration was replicated three times. Mortality percentages were recorded after 24 hr of treatment. The toxicity of technical grades of spinosad, chlorpyrifos, methomyl and cypermethrin was also evaluated against the 4th instar larvae of *S. littoralis* by topical application. One microliter acetone solution of the tested insecticides was topically applied to the whole dorsum of each larvae using Arnold Hand Microapplicator. An acetone treated control was included in each series to

ascertain the effect of solvent and other laboratory experimental conditions on the larvae. The treated larvae were transferred to clean plastic cups with pieces of fresh castor oil leaves. Mortality counts were recorded (Eldefrawi *et al.*, 1964), corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971).

Joint toxic action of spinosad with chlorpyrifos, methomyl and cypermethrin against 4th instar larvae: Joint toxic action of the bioinsecticide (spinosad) with the synthetic insecticides (chlorpyrifos, methomyl and cypermethrin) against 4th instar larvae was investigated. Larvae were treated with spinosad at LC_{50} by dipping technique as described above, while synthetic insecticides at (LD_{10} or LD_{25}) were applied topically. Larvae were treated with synthetic insecticides in three different ways: spontaneously with spinosad; 24 hr before spinosad and 24 hr after spinosad treatments. Three control groups were subjected to calculate the expected mortalities. Co-toxicity factors (Mansour *et al.*, 1966) were calculated as follows:

$$Co\text{-}toxicity\ factor = \frac{observed\%mortality - exp\ ected\%mortality}{exp\ ected\%mortality} \times 100$$

This factor was used to categorize the results into three categories. A positive factor of 20 or more was considered potentiation, a negative factor of 20 or more implied antagonism and intermediate values (between -20 and +20) were considered as only additive.

RESULTS

Ovicidal activity: One of our objectives in this investigation was to determine the ovicidal action of spinosad compared to chlorpyrifos and triflumuron against 0-1 day old eggs. The residual activity of these insecticides to the neonates was also evaluated. According to the results in Tables (1, 2 and 3), spinosad has an ovicidal activity lower than chlorpyrifos and triflumuron. The highest used concentration (100 ppm as a.i) of spinosad caused 82 % eggs mortality. Chlorpyrifos at 50 ppm as a.i caused 100% mortality to treated eggs. Approximately 95% of treated eggs were not hatched treated with 50 ppm triflumuron. While spinosad has an ovicidal activity lower than chlorpyrifos and triflumuron, it has the highest residual activity against the neonates of *S. littoralis* after 24 h of hatching (Tables 1, 2 and 3). Spinosad and chlorpyrifos at 10 ppm caused 95.8 and 82.6 % mortality to new hatched eggs, respectively.

Table 1: Ovicidal and residual toxicity against neonates of spinosad against *Spodoptera* egg masses

Conc. ppm (a.i)	No. Treated eggs	No. hatched eggs	No. unhatched eggs	No. dead larvae	% unhatced eggs	% mortality 24 h from hatching	Total effect
Control	312	303	9	0	2.88±0.66	0	2.88±0.66
0.5	310	299	11	92	0.83±0.09	30.43±3.68	31.05±2.99
1.0	335	270	65	140	17.08±0.15	51.85±7.62	60.60±6.07
10	271	237	34	227	9.94±1.95	95.83±3.20	96.33±2.59
25	377	312	65	301	15.08±3.75	96.47±2.83	96.61±2.05
50	317	168	149	166	46.47±4.29	98.48±1.36	99.25±0.65
100	272	47	225	47	82.01±3.31	100 ± 0.0	100±0.0

All numbers has been corrected according to Abbott's equation 1925

Table 2: Ovicidal and residual toxicity against neonates of chlorpyrifos against *Spodoptera* egg massess

Conc. ppm (a.i)	No. Treated eggs	No. hatched eggs	No. unhatched eggs	No. dead larvae	% unhatced eggs	% mortality 24 h from hatching	Total effect
Control	272	265	7	0	2.57±1.47	0	2.57±1.47
0.5	323	292	31	58	6.70±1.45	19.86±1.14	25.18±3.60
1.0	259	199	60	57	20.73±1.25	28.60±1.78	43.40±2.13
10	270	195	75	161	25.41±4.99	82.56±3.20	87.41±2.54
25	231	6.0	225	4	97.55±4.12	66.67±3.34	99.59±0.68
50	262	0	262	-	100±0.0	-	100±0.0
100	258	0	258	-	100±0.0	-	100±0.0

All numbers has been corrected according to Abbott's equation 1925

Table 3: Ovicidal and residual toxicity against neonates of triflumuron against *Spodoptera* egg masses:

Conc. ppm (a.i)	No. Treated eggs	No. hatched eggs	No. unhatched eggs	No. dead larvae	% unhatced eggs	% mortality 24 h from hatching	Total effect
Control	216	208	8	0	3.67± 0.66	0	3.67±0.66
1.0	208	190	18	0	5.12± 1.17	0	5.12±1.17
10	220	156	64	4	26.37±0.81	2.36±1.25	28.25±1.22
25	182	102	80	2	41.82±0.91	1.93±1.67	42.96±0.09
50	192	8	184	0	95.68±0.80	0	95.68±0.80
100	172	7	165	1	95.80±0.82	0.11±0.19	96.38±0.13

All numbers has been corrected according to Abbott's equation 1925

Triflumuron has no residual activity against the new hatched larvae within 24 hr. Spinosad has the highest % total mortality after hatching at lower concentrations (0.5, 1.0, and 10 ppm) compared to chlorpyrifos and triflumuron, this is due to its residual activity against the newly hatched

larvae. Chlorpyrifos has the highest activity at the highest concentrations; this is due to the relative higher ovicidal activity than the other two compounds (Tables 1, 2 and 3).

Larvicidal activity: Toxicity of spinosad against 2nd, 3rd and 4th instar larvae of *S. littoralis* by dipping technique are shown in Tables (4 and 5). General pattern was observed for the three insect instars, where the toxicity is decreased by the increasing in the insect instars. For 2nd instar, spinosad gave 10, 30, 50, 83.3 and 96.7% mortality at concentrations 10, 25, 50, 100 and 200 ppm, respectively. For the 3rd instar, spinosad gave 16.7, 43.3, 60.0, 80.0 and 93.3% mortality at concentrations 50, 100, 200, 400 and 600 ppm, respectively. Spinosad at concentrations 50, 100, 200, 400 and 600 ppm gave 10, 25, 60, 65 and 85% mortality, respectively, against fourth instar larvae. Table (5) represents LC_{50} values with their lower and upper limits for *Spodoptera* larval instars. The second instar is more susceptible than the third and fourth instars with LC_{50} values of 41.7, 138.1 and 196.7, respectively. The values of the third and fourth instars are greater than the second instar in order of 3.5 and 5 fold, respectively.

Table 4: Insecticidal activity of spinosad (Tracer®) against different *Spopdoptera* larval instars* (dipping technique)

Conc. (ppm)	% Mortality ± SD		
	2nd instar	3rd instar	4th instar
10	10.0 ± 1.21	-	-
25	30.0 ± 1.34	-	-
50	50.0 ± 0.98	16.7 ± 1.56	10.0 ± 1.01
100	83.3 ± 0.85	43.3 ± 1.02	25.0 ± 0.78
200	96.7 ± 1.10	60.0 ± 0.95	60.0 ± 0.92
400	-	80.0 ± 0.91	65.0 ± 0.60
600	-	93.3 ± 0.98	85.0 ± 0.42
Slope	2.31	2.94	2.10
$(Chi)^2$	3.50	2.10	8.69

Larval weight average: 2nd instars 5 ±0.2mg, 3rd instars 20 ±0.4 mg, 4th 62 ±3.0 mg

Table 5: LC_{50} values of spinosad (Tracer®) against different *Spopdoptera* larval instars

Instars	LC_{50} (ppm)	Lower limit (ppm)	Upper limit (ppm)
2nd instar	41.7	36.6	47.3
3rd instar	138.1	118.8	158.7
4th instar	196.7	171.8	223.3

Insecticidal activity of spinosad against 4th instar *Spodoptera* larvae by topical application is shown in Tables (6 and 7). Toxicity of spinosad is enhanced by increasing the exposure time. Spinosad at 2.5, 5, 7.5, 15 and 20 µg/larva gave 10, 15, 20, 30 and 45% mortality after 24h of treatment, 25, 35, 45, 70 and 85% mortality after 48h of treatment, and 25, 45, 55, 85 and 95% mortality after 72h of treatment (Table 6). The LD_{50} values are 0.51, 0.12 and 0.09 µg/mg body weight after 24, 48 and 72h of treatment, respectively (Table 7). Data revealed that dipping treatment exerts toxicity faster than the topical application.

Table 6: Insecticidal activity of spinosad (technical) against 4th instar *Spodoptera* larvae* (topical application).

Dosage µg/larva	µg/mg body weight	% Mortality ± SD 24 hr	48 hr	72 hr
2.5	0.04	10 ± 1.26	25 ± 0.98	25 ± 0.45
5	0.08	15 ± 1.01	35 ± 0.96	45 ± 0.72
7.5	0.12	20 ± 0.98	45 ± 0.74	55 ± 0.63
15	0.24	30 ± 0.95	70 ± 0.63	85 ± 0.35
20	0.32	45 ± 0.91	85 ± 0.87	95 ± 0.63
Slope		1.25	1.84	2.39
(χ^2)		2.35	5.98	5.67

Larval weight average: 62 ± 3.0 mg.

Table 7: LD_{50} values of spinosad (technical) against 4th instar *Spodoptera* larvae (topical application)

Time after treatment (hr)	LD_{50} µg/mg body weight	Lower limit µg/mg body weight	Upper limit µg/mg body weight
24	0.51	0.35	0.99
48	0.12	0.10	0.14
72	0.09	0.08	0.10

Joint toxic action of spinosad with chlorpyrifos, methomyl and cypermethrin against 4th instar larvae: In order to reduce pesticide hazards and the development of resistant populations, insect control should be accomplished with fewer applications at lower doses. This aim might be realized, for example, by combining toxicants with other control agents with specific mode of action, such as certain bioinsecticides. In this investigation, the joint toxic action of spinosad with other synthetic insecticides from different insecticidal groups was determined. Results are shown in Table (8). It is clear that, when the 4th instar *Spodoptera* larvae treated with spinosad at LC_{50}, and cypermethrin at LD_{10} and LD_{25}, co-toxicity factors are + 38.3 and + 34.33, respectively.

Table 8: Joint toxic effects of cypermethrin, chlorpyrifos and methomyl with spinosad (LC_{50}) against 4th instar *Spodoptera* larvae.

Treatment		Insecticides + Spinosad			Larvae pre-treated with insecticides			Larvae post-treated with insecticides		
		Expected % M	Observed % M	CTF*	Expected % M	Observed % M	CTF	Expected % M	Observed % M	CTF
Cypermethrin	LD_{10}	47	65	+38.3	47	45	- 4.26	50	55	+ 9.09
	LD_{25}	67	90	+34.33	67	85	+ 26.87	70	85	+ 21.43
Chlorpyrifos	LD_{10}	62	70	+ 12.9	62	55	- 11.29	55	50	- 9.09
	LD_{25}	77	85	+ 10.39	77	90	+ 16.88	75	60	- 20.0
Methomyl	LD_{10}	62	70	+ 12.9	62	60	- 3.23	65	55	- 15.38
	LD_{25}	82	90	+ 9.76	82	90	+ 9.76	80	60	- 25.0

* CTF = Co-toxicity factor.

According to Mansour *et al.*, (1966), these values indicate that the combination between spinosad and cypermethrin at the same time resulting in a potentiation. When larvae were pre-and post- treated with LD_{25} of cypermethrin the co-toxicity factors are +26.87 and +21.43, respectively (potentiation). Co-toxicity factors are –4.26 and 9.09 when larvae pre-and post-treated with LD_{10} of cypermethrin (additive effect). Co-toxicity factors are +12.9, -11.3 and 9.1 (additive effect), when larvae spontaneously, pre- and post-treated with chlorpyrifos. When larvae were spontaneously, pre- and post –treated with LD_{25} of chlorpyrifos, co-toxicity factors are +10.4, +16.88 (additive) and –20.0 (antagonism). The Joint toxic action of methomyl with the median lethal spinosad dose against the 4th instar *Spodoptera* larvae was also investigated. The combination of methomyl at LD_{10} with spinosad gave additive effects with co-toxicity factors of +12.9, -3.23 and –15.38 when larvae were spontaneously, pre-and post-treated with methomyl, respectively. At LD_{25} of methomyl, the effect was additive when larvae were spontaneously and pre-treated by methomyl, and antagonism when the larvae were post-treated by methomyl. Co-toxicity factors are + 9.76, +9.76 and –25.0 when larvae were spontaneously, pre-and post-treated by methomyl.

Discussion

Several studies had been conducted to evaluate the ovicidal activity against many insect species (Wells and Guyer, 1962; Dittrich, 1967; Mitri and Kamel, 1970; El-Guindy *et al.*, 1983; Renkleff *et al.*, 1995; Canela *et al.*, 2000;..... etc..). In the present study we focused mainly on the possibility of controlling *S. littoralis* by spinosad at other stages (eggs), when it may be more susceptible. Results indicated that ovicidal activity of spinosad is lower than chlorpyrifos and triflumuron. Spinosad at 100 ppm caused 82.01% eggs mortality, while chlorpyrifos at 50 ppm revealed 100% eggs mortality, to *S. littoralis* 0-1 day old eggs. Approximately 95% of treated eggs by triflumuron were killed at 50 ppm. This is may refer to the low penetration ability of spinosad through the egg shell due to its high molecular weight (1478) compared to chlorpyrifos (350.6) and triflumuron (358.7). But, although spinosad has an ovicidal activity lower than chlorpyrifos and triflumuron, it has the highest residual activity against the neonates of *S. littoralis* after 24h of hatching. In previous studies, Pinela *et al.* (2000) stated that treatment of *S. littoralis* 0-24 h eggs at 10 mg a.i / litre or above caused 100% mortality of newly emerged larvae after the first day of hatching. When tobacco budworm eggs were treated with spinosad,

although only 49% egg mortality was observed, none of the larvae that emerged survived (Bret *et al.*, 1997).

Toxicity of spinosad against 2nd, 3rd and 4th instar larvae of *S. littoralis* by dipping technique was studied. Second instar larvae is more susceptible to spinosad than the 3rd and 4th instar larvae with LC_{50} values 41.7, 138.1 and 196.7 ppm for 2nd, 3rd and 4th instars, respectively. It is quite clear that the susceptibility of the larvae to spinosad decreased considerably as the larvae advance in age and weight. The LD_{50} values of spinosad against 4th instar larvae by topical application were 0.51, 0.12 and 0.09 µg / mg body weight, after 24, 48 and 72h of treatment, respectively. Spinosad was less active by topical application than dipping. Studies comparing the dippin versus topical toxicity of spinosyn A and cypermethrin (Sparks *et al.*, 1997b) in tobacco budworm larvae show that spinosyn A is as active as cypermethrin by injection, but about 5 fold less active than cypermethrin when applied topically. This apparent difference in the rate of penetration is confirmed by *in vivo* studies in last stadium *Trichoplusia ni* larvae examining the penetration of spinosyn A versus permethrin; at 4h post treatment > 30% of applied permethrin was internal while < 10% of applied spinosyn A was internal. Likewise, for topically applied spinosyns A, B and D, only 1.5-4% of the applied dose was present in the hemolymph 3h post treatment. As with *T. ni*, radiotracer studies with tobacco budworm larvae showed that spinosyn A penetrates at a slower rate (2% in 3h than cypermethrin 42% 3h).

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التأثير السام لمبيد الإسبينوساد على بيض ويرقات دودة ورق القطن

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أحد أهداف إجراء هذا البحث هو تقدير سمية مبيد الإسبينوساد (تريسر) على بيض دودة ورق القطن (عمر 0 – 1 يوم) بالمقارنة بمبيدات الكلوربيريفوس (دورسبان) والترايفلوميورون (ألسيستين). التأثير السام لمتبقيات هذه المبيدات على الفقس الحديث تم تقديرها أيضا. كذلك تم تقدير التأثير السام لمبيد الإسبينوساد على الأعمار الثانى والثالث والرابع للطور اليرقى بطريقة الغمر وعلى العمر الرابع للطور اليرقى بطريقة معاملة السطح (بالملامسة). وأخيرا تم تقدير التاثير السام المشترك لمبيد الإسبينوساد مع السيبرميثرين والكلوربيريفوس والميثوميل علي يرقات العمر الرابع لدودة ورق القطن. الإسبينوساد له نشاط إبادى على بيض دودة ورق القطن ولكن أقل من الكلوربيريفوس والترايفلوميورون0على الرغم من أن النشاط الإبادى للإسبينوساد على البيض أقل من الكلوربيريفوس والترايفلوميورون إلا أن النشاط الإبادى للمتبقى على اليرقات حديثة الفقس كان كبيرا. الإسبينوساد كان أعلى من الكلوربيريفوس والترايفلوميورون بالنسبة للموت الكلى (النسبة المئوية للبيض الميت + النسبة المئوية لموت اليرقات حديثة الفقس).كلما تقدمت اليرقات فى العمر والوزن كلما كانت أقل حساسية للإسبينوساد(المعاملة من خلال الغذاء). يرقات العمر الثاني كانت أكثر حساسية من العمر الثالث والتي كانت اكثر حساسية من العمر الرابع. التركيزات اللازمة لموت 50% من اليرقات (من خلال التغذية) كانت 7ر41 ، 1ر138 ، 7ر196 جزء فى المليون ليرقات العمر الثانى والثالث والرابع على الترتيب. الجرعات اللازمة لموت 50% من يرقات العمر الرابع المعاملة بالإسبينوساد (بالملامسة) كانت 0.51 و 0.12 و 0.09 ميكروجرام/ملليجرام من وزن اليرقة بعد 24 ، 48 ، 72 ساعة من المعاملة على الترتيب. الإسبينوساد كان فعلة بطى عند المعاملة بالملامسة عن المعاملة من خلال الغذاء. من خلال دراسة التأثير السام المشترك لمبيدات السيبرميثرين والكلوربيريفوس والميثوميل مع الإسبينوساد وجد أنه عموما عند خلط الإسبينوساد مع السيبرميثرين كان التأثير اما تنشيط أو أضافة. عند خلط الإسبينوساد مع الكلوربيريفوس والميثوميل كان التأثير اما اضافة أو تضاد. هذا سوف يوجة النظر الي عملية ترتيب رش المبيدات عند وضع برامج مكافحة الحشرات.