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EVALUATION OF ENVIRONMENTAL SAFE NATURAL COMPOUNDS IN CONTROLLING COTTON LEAFWORM *Spodoptera littoralis.*

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ABSTRACT

The toxicity of azadirachtin, pyridalyl and quercetin compounds were evaluated against the immature stages of the cotton leafworm, *Spodoptera littoralis,* field strain and laboratory susceptible strain. The results showed that azadirachtin was the most potent tested compound flowed by pyridalyl and the least one was quercetin. Azadirachtin at 15.0 ppm achieved 30.4% mortality of treated egg masses, while pyridalyl and quercetin at the same concentration caused 28.8% and 21.2% mortality of treated egg masses, for lab strain, respectively, while field strain at the same concentration caused 23.4%; 20.4% and 16.4% mortality of treated egg masses respectively. The toxicity of tested compounds were evaluated against 2^{nd} and 4^{th} larval instar of *S. littoralis,* cleared that the toxicity of the tested compounds increased with increasing the exposure time, decreased by increasing the stage of larval instars. The larvae stop eating after two days of treatment. Also, the tested compounds shows no pupal mortality after 24; 48, and 72 hrs of treatment, but significantly increased percentages of deformed of adults. Results proved that the three natural compounds (azadirachtin, pyridalyl and quercetin), are potentially potent for control of *S. littoralis*, as antifeeding. Generally, natural compounds, will produce a new trend to increase toxicity of the newly hatched larvae of *S. littoralis,* enhance the role of beneficial insects. These compounds could be used in the IPM programmes, in order to minimize the negative effects of conventional insecticides on environment components and to protect the natural enemies.

INTERODUCTION

The cotton leafworm *Spodoptera littoralis* is considered as one of the most serious and destructive phytophagous lepidopterous insect-pests in Egypt, not only for cotton plants but also for other field crops, vegetable and deciduous fruits, the larval stages is not only the major enemy of several economic crops but also attacks all parts of plants of more than 70 cultivated crops, the larval stages have become extremely tolerant to different classes of conventional insecticides have been used for many years to control this pest larvae which developed of exhibits multiple resistance to all insecticides used, (Abo Elghar *et al*., 2005). The search about other more effective insecticides to control this insect is very important, so, a number of new insecticides classes have been discovered and commercialized, noval insecticides including azadirachtin, pyridalyl and quercetin which characterized with their new and/or unique modes of action (Argentine *et al.*, 2002), therefore, attempts have been made to replace conventional pesticides by natural insecticides originating from plants, in this context, currently, attention is being focused on the use of neem-based botanical insecticide over 300 compounds have been isolated from various parts of the neem tree among them the terpenoids that compromise, (Wei-hong and Ban-

qian, 2006). The major active compounds commonly refused to as limonoids, Azadirachtin, the chief substance and the best known example of these limonoids is accumulated in the seed Kernels of *Azadirachta indica* (Sujanya *et al*., 2008). The alternative methods for controlling this pest have received a great deal of attention in the last couple of decades because of the deleterious side effect resulted from the intensive use of conventional insecticides and use of natural plant products as insect toxicants, antifeedants, oviposition deterrents, and insect growth inhibitors is considered one of the promising tactics in insect pest management. implemental of integrated pest control tactics will help to reduce pests and environmental hazard problems (Freeman and Andow, 1983; Klocke, 1987 and Srivastava *et al*., 1990).

The purpose of this investigation is to study the ovicidal activity of three natural compounds, azadirachtin, pyridalyl and quercetin against the egg stage and to evaluate their toxicity against 2^{nd} and 4^{th} larval instars as well as pupae for field strain of *S. littoralis,* and compared them with data obtained of lab strain.

MATERIALS AND METHODS

1. Test insect:

The susceptible laboratory strain of cotton leafworm, *Spodoptera littoralis* was provided from centeral lab of pesticides. Agricultural Research Center (ARC) Cairo, Egypt which was reared for several years on artificial diet under standard laboratory conditions of 27 ± 2 ^{o}C and 65-70% RH.

Field strain of cotton leafworm, *Spedoptera littoralis* egg masses were collected from cotton fields at Abeis area Alex. Proviance Egypt. Experiments were carried out using egg masses (24 hrs-old) and newly hatched larvae, chosen for bioassays, the Pupae (48 hrs-old) also investigated.

2. Chemicals and test insecticides:

Azadirachtin, Neemix 4.5, a 4.5% Azadirachtin EC formulation, was purchased from Thermotrilogy, USA; quercetin (50% EC) was purchased from Sigma Chemical Co. USA, and pyridalyl (Pleo, 50% EC) supplied from Sumitomo Chemical Co.

3. Bioassay tests:

3. 1- The determination of the ovicidal activity

Ovicidal activity of the azadirachtin, pyridalyl and quercetin, against the lab and field strains of *S. littoralis* egg mass (0-24 hrs old) was investigated. They were removed gently with a fine hair bruch. The lower layer in each egg mass was counted by the binocular. The counted egg samples were dipped (5 sec.) in different concentrations of the tested compounds, while control was dipped in water according to Dittrich (1967). Each treatment was replicated three times. Treatment and control were held in a clean plastic cup (9x4 cm) at 27 ± 2 ^{o}C, 65-75% RH and observed until hatching. The number of unhatched eggs, dead neonates and live larvae were counted, and the mortality percentages were calculated.

3.2-Toxicity of the tested compounds against larvae:

Toxicity of azadirachtin, pyridalyl and quercetin against 2nd and 4th instar larval of cotton leafworm, lab and field strains were starved for 6hrs before exposed test. The selected larvae were bioassayed using three replicates for each concentration with ten larvae in each replicate. Disc dipping technique was used (Tabashink and Chushing, 1987). Each castor leaves disc (2 cm^2) was dipped into the suspension of tested formulation for 10 sec. Tested concentrations were prepared in glass distilled water (GDW) (Toni and Fred, 1996). Disc were held vertically to allow excess solution to drip off and places on a rack to dry for at last 2 hrs. Treated discs were offered to starved larvae (on disc per cup) and left under constant conditions (27 ± 2 ^oC and 65-70% RH). Thereafter survivors were transferred with fresh castor oil plant leaves to clean cups and kept under the same conditions. Control larvae were allowed to feed on castor oil leave discs treated with distilled water. Mortality percentage was calculated for each concentration daily after 24; 48, and 72 hrs and corrected according to Abbott 's equation (Abbott, 1925) and subjected to probit-analysis using the computer program (Finney, 1971).

3.3-Toxicity of the tested compounds on pupae:

Using the residual film method to determine the LC_{50} values of 48 hrs-old *S. littoralis*, pupae with different concentrations of tested compounds. The experiment was left for 24; 48, and 72 hrs in pre-treated Petri-dishes. Concentrations-mortality percentages were calculated and corrected for natural mortality according to Abbott 's equation (Abbott, 1925). LC_{50} values were calculated by using probit-analysis method of Finney (1971).

RESULTS AND DISCUSSION

Ovicidal activity against egg masses:

Plant leaves containing egg masses were dipped in different concentrations of each tested compounds. Data in Tables (1 and 2) show the ovicidal activity of the tested compounds against 24 hrs-old eggs of *S. littoralis*. The results showed that, azadirachtin; pyridalyl and quercetin have low ovicidal activity. Percentages of unhatched eggs at 0.5 ppm were 12.4%; 9.8% and 7.6% for azadirachtin; pyridalyl and quercetin respectively against lab strain, for field strain at the same concentration caused 10.2%; 6.2% and 4.6% mortality of treated egg masses respectively, while percentages of unhatched eggs for lab strain at 1.0 ppm were 17.5%; 11.4% and 9.4% respectively at the same concentration caused 14.1%; 9.3% and 7.2% mortality of treated egg masses of field strain respectively, also, at 5.0 ppm were 21.4%; 14.5% and 11.0% respectively against eggs of *Spodoptera* lab strain, for field strain at the same concentration caused 17.4%; 11.1% and 9.4% mortality of treated egg masses respectively, at 10.0 ppm were 26.9%; 22.2% and 18.5% respectively against eggs of *Spodoptera* lab strain, for field strain at the same concentration caused 20.3%; 16.2% and 13.2% mortality of treated egg masses respectively, on the other hand, mortality percentages at 15.0 ppm were 30.4%; 28.8% and 21.2% respectively, against eggs of

Spodoptera lab strain, for field strain at the same concentration caused 23.4%; 20.4% and 16.4% mortality of treated egg masses respectively.

Significant differences in percentages of unhatched eggs were found between all tested concentrations of tested compounds and control eggs. *Spodoptera* eggs was most potent against azadirachtin in comparison to the pyridalyl and quercetin compounds. The lab strain of *Spodoptera* eggs are susceptible to three tested insecticides in comparison to the field strain, in general the susceptibility of *Spodoptera* eggs to tested compounds low ovicidal activity. These results are in agreement with which found by, Mitri, and Kamel 1970; El-Sayed 1985; Renkleff *et al*., 1995; Canela *et al*., 2000; Pinela *et al.,* 2000; Raslan, 2002; & Bueno, and Freitas, 2004.

Table (1): Ovicidal activity of some compounds against *Spodoptera* (lab strain) egg masses.

Conc. (ppm)	Azadirachtin		Pyridalyl		Quercetin	
	*H%	*UH%	H%	UH%	H%	UH%
Control	95.7	4.3	92.5	7.5	90.7	9.3
0.5	87.6	12.4	90.2	9.8	92.4	7.6
1	82.5	17.5	88.6	11.4	90.6	9.4
5	78.6	21.4	85.5	14.5	89.0	11.0
10	73.1	26.9	77.8	22.2	81.5	18.5
15	69.6	30.4	71.2	28.8	78.8	21.2

***H; Hatched eggs and * UH; Unhatched eggs**

Table (2): Ovicidal activity of some compounds against *Spodoptera* (field strain) egg masses..

Conc. (ppm)	Azadirachtin		pyridalyl		Quercetin	
	*H%	*UH%	H%	UH%	H%	UH%
Control	97.0	3.0	95.5	4.5	94.7	5.3
0.5	89.8	10.2	93.8	6.2	95.4	4.6
1	85.9	14.1	90.7	9.3	92.8	7.2
5	82.6	17.4	88.9	11.1	90.6	9.4
10	79.7	20.3	83.8	16.2	86.8	13.2
15	76.6	23.4	79.6	20.4	83.6	16.4

***H; Hatched eggs and * UH; Unhatched eggs**

Toxicity of tested compounds against 2nd and 4th larval instar of *S. littoralis*:

The results of the toxicity of the azadirachtin; pyridalyl and quercetin in terms of LC_{50} are given in table (3 and 4) for 2nd and 4th larvae of *S. littoralis*. LC_{50} values were 1.21; 4.34 and 7.44 ppm for azadirachtin; pyridalyl and quercetin respectively against 2nd instar larvae of cotton leafworm after 24hr for lab strain, but field strains LC_{50} values were 3.12; 6.23 and 8.34 ppm for three tested compounds respectively, while LC_{50} values were 0.41; 0.62 and 4.22 ppm after 48hr for lab strain respectively, for field strains LC_{50} values were 0.60; 0.83 and 6.23 ppm for three tested compounds respectively, also LC_{50} values were 0.0.61; 0.12 and 0.60 ppm after 72hr for lab strain respectively, for field strains LC_{50} values were 0.084; 0.32 and 0.81 ppm for three tested compounds respectively. LC_{50} values were 2.33; 5.55 and 8.67 ppm for azadirachtin; pyridalyl and quercetin respectively against 4th instar

larvae of *Spodoptera* after 24hr for lab strain, for field strains LC_{50} values were 5.43; 7.56 and 9.68 ppm for three tested compounds respectively, while LC_{50} values were 0.54; 0.77 and 5.23 ppm after 48hr for lab strain respectively, for field strains LC_{50} values were 0.72; 0.90 and 7.34 ppm for three tested compounds respectively, also LC_{50} values were 0.087; 0.33 and 0.72 ppm after 72hr for lab strain respectively, for field strains LC_{50} values were 0.095; 0.50 and 0.90 ppm for three tested compounds respectively,

According to the LC_{50} values, it is quite clear that the susceptibility of *Spodoptera* larvae to azadirachtin; pyridalyl and quercetin, in general, three tested compounds decreased LC_{50} values by increasing the posttreatment period of times, the larvae stopped feeding after two days, also, lab strain of *Spodoptera* larvae is more susceptible to three tested compounds in comparison to the field strain, general pattern was observed for the two instars, where the toxicity is decreased by the increasing in the insect instars, the second instar is more susceptible than the fourth instars. So may be these effect of compounds are a good control of Lepidopterous larvae, the lab and field strains of *Spodoptera* larvae is more susceptible to azadirachtin and pyridalyl in comparison to the quercetin. Generally, efficacy of tested compounds have a good toxicity for lab and field strains *Spodoptera littorallis*. Generally, it could be concluded that the use of tested compounds with biological insecticides (azadirachtin; pyridalyl and quercetin) instead of conventional hazardous insecticides; and these my reduce the environmental pollution and hazard management programs, using tested compounds looking forward in an intergrated pest. The present results are confirmed with the results of Sivori *et al*., 1999; Morduo and Nisbet 2000; Martinez *et al*., 2001; Saito *et al*., 2004; Isayama, *et al*., 2005; Sakamoto *et al*., 2005; Ahmed *et al*., 2006; Abdel-Rahim *et al.,* 2009; Abdu-Allah, 2010, and El-Naggar, 2013.

Table (3): LC_{50} values of tested compounds against *S. littoralis* 2nd larvae instar.

Compounds	LC_{50} (ppm)					
	24hr		48hr		72hr	
	Lab strain	Field strain	Lab strain	Field strain	Lab strain	Field strain
Azadirachtin	1.21	3.12	0.41	0.60	0.061	0.084
Pyridalyl	4.34	6.23	0.62	0.83	0.12	0.32
Quercetin	7.44	8.34	4.22	6.23	0.60	0.81

Table (4): Toxicity of tested compounds against *S. littoralis* 4th larvae instar.

Compounds	LC_{50} (ppm)					
	24hr		48hr		72hr	
	Lab strain	Field strain	Lab strain	Field strain	Lab strain	Field strain
Azadirachtin	2.33	5.43	0.54	0.72	0.087	0.095
Pyridalyl	5.55	7.56	0.77	0.90	0.33	0.50
Quercetin	8.67	9.68	5.23	7.34	0.72	0.90

Toxicity on pupae:

The toxic action of tested compounds against pupae of *S. littoralis*, was also investigated. Data in tables (5; 6 and 7) shows that treatment of 48hrs-old pupae of *S. littoralis*, with different concentrations of tested compounds. The data shows a significant differences in the percentages of pupal mortality or deformed adults about (35-45%) compared with untreated pupae for azadirachtin; pyridalyl and quercetin. It was observed from the present results that significicant differences in the percentages of pupal mortality or deformed adults slightly higher for pupae treated with azadirachtin than those treated with pyridalyl and quercetin. However, according to El-Zahi (2013) found that indoxacarb significantly produced the least percentage of normal pupae and deformed pupae (26.4 and 20.3%) at zero time from spray, also, Amer *et al.,* (2012) found that pyridalyl and emamectin benzoate had the highest persistent negative effect on pupation causing (20.4 and 42%) normal pupae, (12.9 and 10.3%) deformed pupae and (26.7 and 55.2%) normal adult, respectively.

Table (5): Pupicidal activity of the azadirachtin to 48 hrs-old of the cotton leafworm, *S. littoralis*.

Time posttreatment (hrs)	% Accumulative mortality of pupae											
	Control		Concentrations (ppm)									
			0.5		0.1		5.0		10.0		15.0	
	lab	field	lab	field	lab	field	lab	Field	lab	field	lab	field
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	1.3	3.3	2.1
48	0.0	0.0	0.0	0.0	1.1	0.0	1.9	1.0	2.9	2.3	6.8	4.3
72	0.0	0.0	8.0	3.5	12.7	9.2	19.8	16.0	32.1	25.4	34.9	28.7
% died pupae	0.0	0.0	8.0	3.5	13.8	9.2	21.7	17.0	37.5	29.0	45.0	35.1
% emerged adult	100	100	92.0	96.5	86.2	90.8	78.3	83.0	62.5	71.0	55.0	64.9
% nonemerged adult	0.0	0.0	8.0	3.5	13.8	9.2	21.7	17.0	37.5	29.0	45.0	35.1

Table (6): Pupicidal activity of the pyridalyl to 48 hrs-old of the cotton leafworm, *S. littoralis*.

Time posttreatment (hrs)	% Accumulative mortality of pupae											
	Control		Concentrations (ppm)									
			0.5		0.1		5.0		10.0		15.0	
	lab	field	lab	field	lab	field	lab	Field	lab	field	lab	field
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	2.8	1.5
48	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.2	2.5	1.7	3.6	2.7
72	0.0	0.0	5.5	1.1	9.5	4.4	17.2	10.3	24.8	18.5	28.7	23.2
% died pupae	0.0	0.0	5.5	1.1	9.5	4.4	18.7	11.5	28.3	20.2	35.1	27.4
% emerged adult	100	100	94.5	98.9	90.5	95.6	81.3	88.5	71.7	79.8	64.9	72.6
% nonemerged adult	0.0	0.0	5.5	1.1	9.5	4.4	18.7	11.5	28.3	20.2	35.1	27.4

Table (7): Pupicidal activity of the quercetin to 48 hrs-old of the cotton leafworm, *S. littoralis*.

Time posttreatment (hrs)	% Accumulative mortality of pupae											
	Control		Concentrations (ppm)									
			0.5		0.1		5.0		10.0		15.0	
	lab	field	lab	field	lab	field	lab	field	lab	field	lab	field
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	1.5	2.7	1.8
72	0.0	0.0	0.0	0.0	3.8	2.2	9.6	5.7	17.8	13.3	23.7	19.7
% died pupae	0.0	0.0	0.0	0.0	3.8	2.2	9.6	5.7	20.0	14.8	27.9	21.5
% emerged adult	100	100	100	100	96.2	97.8	90.4	94.3	80.0	85.2	72.1	78.5
% nonemerged adult	0.0	0.0	0.0	0.0	3.8	2.2	9.6	5.7	20.0	14.8	27.9	21.5

In conclusion, this investigation proved that tested compounds are successful botanical insecticides. The symptoms of toxicity included stop of feeding, delay or prevention of pupation, blackening the body, failure of molting to the next larval instar; formation of larval-prepupal intermediates and malformed pupae, so tested compounds are larvicidal activities, therefore, it could be used in the integrated management programs to control *S. littoralis*.to prevent or delay appearance of resistance to conventional pesticides. Some unpublished results showed that resistance to novel compounds are not possible after several applications because it have multi effects on insect, and there is more than one target for the compounds in insects to affect. However, it is better to use these compounds in sequences with other insecticides.

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تقيـــيم بعـــض المركبـــات الأمنـــة للبيئـــة لمكافحـــة دودة ورق القطـــن (ســـبدوبترا ليتولارس).

سهام منصور إسماعيل
المعمل المركزى للمبيدات – الصبحية – الإسكندرية

الهدف من البحث هو تقيم التأثير الأبادى لثلاثة من المركبات هى الأزديراختين والكواريستين والبيريداليل ضد الأطوار غير الكاملة للسلالتين المعملية والحقلية لحشرة دودة ورق القطن (سبدوبترا ليتولارس) بهدف تلاشى التأثيرات السلبية العالية للمبيدات التقليدية على مكونات البيئة والأعداء الطبيعية. أوضحت النتائج أن المركبات المختبرة لها سمية ضعيفة على البيض مقارنة بالكنترول فلقد أوضحت النتائج أن فى حالة الأزديراختين كانت النسبة المئوية للبيض غير الفاقس هى 30.4% عند تركيز 15.0 ppm بينما الكواريستين والبيريداليل عند نفس التركيز كانت النسبة المئوية للبيض غير الفاقس هى 28.8و 21.2 % على الترتيب بالنسبة للسلالة المعملية أما بالنسبة للسلالة الحقلية كانت النسبة 23.4و 20.4 و 16.4% على الترتيب, وعلى الرغم من أن المركبات تحت الدراسة كان لها سمية منخفضة على البيض إلا أن النشاط الإبادى المتبقى على اليرقات كان كبيراً. وقد تم تسجيل قيم التركيزات النصف مميتة (LC_{50}) لكلا" من يرقات العمر الثانى و يرقات العمر الرابع لدودة ورق القطن وذلك بالنسبة للسلالتين المعملية والحقلية، وقد أظهرت قيم الـ LC_{50} أن الأزديراختين كان أكثر سمية على اليرقات بالمقارنة بالكواريستين والبيريداليل فلقد أوضحت النتائج أن قيم الـ LC_{50} أنخفضت بدرجة ملحوظة فمن النتائج نجد أن الفاعلية تزداد بزيادة فترة التعرض لهذه المركبات وتقل بزيادة الأعمار اليرقية, أيضاً تم تقييم الفعل السام للمركبات المختبرة على طور العذراء أظهرت النتائج أنه بعد 24، 48 و 72 ساعة من المعاملة لم يلاحظ وجود فروق معنوية فى النسب المئوية للموت فى العذارى ولكن زادت معنويا' النسب المئوية للموت فى العذارى قبل أنبثاق الفراشات وقد كان الأزديراختين أكثر تأثير من الكواريستين والبيريداليل. وتشير النتائج إلى أن هناك تأثير سمى للمركبات النباتية المختبرة وخاصة الأزديراختين على الأطوار غير الكاملة لحشرة دودة ورق القطن, فمن هذه النتائج أتضح أن يمكن أستخدام هذه المركبات كمانع للتغذية وبذلك يمكن أستخدامها فى برامج المكافحة المتكاملة لدودة ورق القطن.

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