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Laboratory Evaluation of Some Insecticides Against Cotton Leafworm, *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae).

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ABSTRACT

The toxicological effects of some insecticide mixtures (chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide), against the 3rd instar of *Spodoptera* larvae were evaluated.

The results indicated that, LC₅₀ values for each insecticide were 12.44, 0.88, 0.08, 0.16 and 3.35 mg L⁻¹ respectively, and 0.458, 0.009, 0.0014 and 0.357 mg L⁻¹ respectively. Also, the LC₅₀ values of chlorpyrifos are increased by larvae weight increased. LC₅₀ values of chlorpyrifos as a function of post-feeding initiation of *Spodoptera* larvae to pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide after two days (single exposure) were 1.33, 1.99, 0.74, and 0.37 mg L⁻¹ respectively.

Therefore, post-feeding initiation of larvae to previous insecticides tremendously decreased LC₅₀ values of chlorpyrifos. Also, the percent inhibition of AChE from *Spodoptera* head capsules by chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide (LC₁₀ equivalent concentrations) were 44.5, 23.4, 19.4, 33.5, and 15.4%, respectively.

The percent inhibition of AChE from *Spodoptera* head capsules by chlorpyrifos either pre-treatment or post-treatment with pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide were increased. This indicates that the toxic potential of these pesticides for the tested organism is greater when present in a mixture than in an individual.

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisduval), (family: Noctuidae) is one of the most serious and destructive polyphagous insect pest that lives on a wide range of crops (Su and Sun; 2014). The insect causing considerable damage by feeding on leaves, fruiting points, flower buds, and, occasionally, also on bolls. So, it requires several insecticides and applications to control (Abou-Taleb; 2016). Insecticides have been heavily applied to control this pest; however, the development of insecticide resistance has resulted in control failures and field resistance cases to conventional insecticides (Ahmad and Arif, 2010).

Insecticide resistance has been a major factor influencing insect control and pest management for more than half a century. The first paper documenting insecticide

resistance was published 100 years ago, (Sparks and Nauen; 2015). Additionally, resistance to newer chemistry insecticides (spinosad, abamectin, indoxacarb, and tebufenozide) has been documented (Che *et al.*, 2013 and Su and Sun; 2014).

Also, the demise of conventional insecticides in horticultural crops has accelerated the development of novel insecticides with 'reduced risk' to human health. The 'reduced risk' alternatives are not always of lower risk to arthropods, particularly natural enemies, and may have variable effects (Bostanian *et al.*, 2009 and Lefebvre *et al.*, 2011).

Organophosphorus pesticides (OPs) are widely used in agricultural and non-agricultural applications (John and Shaik, 2015 and Garate *et al.*, 2020), for crop protection, and have contributed to dramatic increases in crop yields in modern agriculture (Deb and Das, 2013; Xuereb *et al.*, 2009). The toxicity of OPs is reflected by covalent binding to the active site of cholinesterase to inhibit the activity of the enzyme (Huynh and Nugegoda, 2012 and Richendrerfer and Creton, 2015), causing tremors, lacrimation and bradycardia, even death (Mogha *et al.*, 2016 and Yang *et al.*, 2018). Pesticide poisoning is a serious concern in modern agriculture. Although these hazardous compounds seem to be indispensable in crop production, the negative impact of pesticides in the human body is now a proven fact (Patra, *et al.*, 2020). Although new molecules have been developed which are less persistent and bio-degradable in nature, their efficiency needs to be studied before coming to any conclusion.

Therefore, the main objective of this study is to investigate, the efficiency of some insecticide mixtures (chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide), against the 3rd instar of *S. littoralis* larvae to reduce the number of insecticides used to reduce the hazard.

MATERIALS AND METHODS

Experimental Insects:

A susceptible strain of the *S. littoralis* has been reared for many years in the Plant Protection Research Institute. Larvae were fed castor bean leaves under controlled laboratory conditions (25 ± 2 °C, RH 65%) for several years avoiding exposure to any pesticides according to the method of Eldefrawi *et al.*, (1964).

Tested Insecticides:

Chlorpyrifos (Dursban 48% EC); and Methoxyfenozide (Runner 24% SC) were produced by Dow Agro Science Co. Pyridalyl (Pleo 50% EC) was produced by Sumitomo Chemical Co. Ltd. Chlorantraniliprole (Coragen 20% SC), was produced by DU PONT DU NEMOURS Co. Emamectin benzoate (Albin- X 5% WG), was produced by Shandong Sino-Agri United Biotechnology LTD.

Bioassay and Determination of Sublethal Doses of Tested Insecticides against *S. littoralis*:

A leaf dip bioassay method (Eldefrawi *et al.*, 1964) was used. Homogenous castor bean leaf pieces were dipped in six concentrations of each tested insecticide (prepared in water) for 10 sec. and dried at room temperature. Treated castor bean leaf pieces were introduced to ten 3rd instar larvae (30 ± 0.5 mg/larva), which had been starved for two hrs. The cups were covered with lids and maintained at 25 ± 2 °C. Each concentration was replicated four times. Mortalities of chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide were recorded after 24 hrs, corrected according to Abbott equation (Abbott, 1925), and subjected to probit analysis (Finney, 1971). The median lethal concentrations, confidence limits, and the slope were calculated.

Effect of Pretreatment with Sublethal Concentration Pyridalyl, Chlorantraniliprole, and Novaluron on Sensitivity *S. littoralis* to chlorpyrifos:

Castor bean leaves were dipped in the determined LC₁₀ equivalent concentrations of pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide. The 3rd instar larvae were fed on treated leaves for each treatment. After 24 hrs, surviving larvae were transferred to jars containing fresh leaves treated with a series of concentrations chlorpyrifos for the next three days, as well as, the control.

Inhibition of AChE from *Spodoptera* Larvae Laboratory Strain:

One hundred *Spodoptera* larvae from each concentration in replicates along with control were randomly chosen. LC₁₀ equivalent concentrations of each insecticide were used to *Spodoptera* larvae fed on for 6 hrs. Head capsules of *Spodoptera* larvae were cut, collected, and homogenized in glass homogenizer (1: 10 w/v) in 100 mM sodium phosphate buffer pH 7.4. The homogenate was centrifuged at 15000 rpm for 10 min at 4°C using Cryofuge 20-3, Heraeus Christ centrifuge. The supernatant was served as the enzymes source.

The method of Ellman *et al.* (1961) as modified by Brownson and Watts (1973) was used for assaying AChE activity. The rate of changes in absorption (at 412 nm was monitored) as a function of enzyme activity. Reaction mixture with a total volume of 3ml contained: 0.1ml enzymes source, and 1.5ml of each reagent (Acetylthiocholine iodide (ASChI) and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) prepared in the same buffer, quickly shaken, placed in position (Sequoia-Turner Model 340 spectrophotometer) for up to 5 minutes. An assay mixture without enzyme was used as a blank. Enzyme activity was calculated as Δ OD min-1mg protein-1. The percent inhibition of AChE by chlorpyrifos either pre-treated or post-treated with pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide were recorded, using the following formula.

$$I\% = \frac{(s-s_i)}{s} \times 100$$

Where: S is a specific activity with no inhibitor, Si is a specific activity with an inhibitor.

RESULTS AND DISCUSSION

Toxicity of chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide against 3rd instar of *S. littoralis* larvae were presented in table (1). LC₅₀ values; for chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate and methoxyfenozide were 12.44, 0.88, 0.08, 0.16 and 3.35 mg L⁻¹ respectively, and 0.458, 0.009, 0.0014 and 0.357 mg L⁻¹ respectively.

Table 1: LC₅₀ values of some insecticides against 3rd instar of *S. littoralis* larvae after 24 hrs.

Insecticide	LC ₅₀ (mg L ⁻¹) (95% F.L)	LC ₁₀ (mg L ⁻¹) (95% F.L)	LC ₉₀ (mg L ⁻¹) (95% F.L)	Slope ± SE
Chlorpyrifos	12.44(9.88-16.71)	1.28(0.55-2.07)	120.88(64.79-366.32)	1.30±0.19
Pyridalyl	0.88(0.66-1.20)	0.05(0.01-0.11)	14.67(6.84-65.17)	1.051±0.18
Chlorantraniliprole	0.08(0.06-0.09)	0.01(0.01-0.02)	0.51(0.35-0.93)	1.54±0.18
Emamectin benzoate	0.16(0.13-0.12)	0.03(0.01-.03)	1.05(0.74-1.70)	1.57±0.14
Methoxyfenozide	3.35(2.61-4.18)	0.49(0.27-0.74)	23.11(16.84-35.44)	1.53±0.14

Data in table (2) summarized LC₅₀ values of chlorpyrifos as a function of the weight of *S. littoralis* larvae after different exposure times. The results indicated that, when *Spodoptera* larvae weight increased, the LC₅₀ values of chlorpyrifos are increased.

Table 2: LC₅₀ values of chlorpyrifos as a function of the weight of *S. littoralis* larvae after different exposure times.

Larval weight (mg)	LC ₅₀ (mg L ⁻¹) (95% F.L)	LC ₁₀ (mg L ⁻¹) (95% F.L)	LC ₉₀ (mg L ⁻¹) (95% F.L)	Slope ± SE
20.0±0.5	12.44(9.88-16.71)	1.28(0.55-2.07)	120.89(64.79-366.32)	1.30±0.19
31.1±1.8	25.02(19.63-31.39)	2.75(1.62-4.11)	227.56(163.07-349.40)	1.34±0.11
45.7±2.4	33.24(26.16-41.09)	5.23(3.01-7.79)	211.18(156.88-314.31)	1.60±0.15
71.4±3.4	74.65(40.30-121.42)	15.21(3.62-20.23)	366.31(269.99-1211.29)	1.85±0.16

Data in table (3) are helpful in interpreting the interaction of pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide with chlorpyrifos. LC₅₀ values of chlorpyrifos against pretreated 3rd instar *S. littoralis* larvae with pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide at LC₁₀. Data indicated that LC₅₀ values of chlorpyrifos as a function of post-feeding initiation of *S. littoralis* larvae to pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide after two days (single exposure) were 1.33, 1.99, 0.74, and 0.37 mg L⁻¹ respectively. Therefore, post-feeding initiation of *S. littoralis* larvae to pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide tremendously decreased LC₅₀ values of chlorpyrifos. The results indicated that a combination of chlorpyrifos with pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide against *S. littoralis* larvae are useful in reducing the amount of insecticide used in *Spodoptera* larvae control programmers. Mixtures of insecticide usually used in the field to enhance the control when multiple pests are attacking simultaneously. Also, that, to increase the efficacy of control against a single pest, or to delay the insecticide resistance but without a good experimental evidence (Ishaaya *et al.*, 1985; Ahmad, 2004). Insecticides that have different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pest. When two compounds are mixed, they can either be potentiating or additive or antagonistic in an insect species. These effects can be different on different insect species or strains depending upon their physiology and the mechanism(s) of resistance they have developed. If a mixture is potentiating, it is a useful tool in enhancing control efficacy and combating insecticide resistance (Ahmad, 2004).

Table 3: LC₅₀ values of chlorpyrifos as a function of post-feeding initiation of *S. littoralis* larvae to (LC₁₀) of pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide.

Insecticide		Post initiation (days)		
		One	Two	Three
Pyridalyl	LC ₅₀ (mg L ⁻¹) (95% F.L)	4.48(3.98-5.02)	1.33(1.09-1.68)	-
	Slope ± SE	2.70±0.31	1.89±0.24	-
Chlorantraniliprole	LC ₅₀ (mg L ⁻¹) (95% F.L)	6.90(5.62-8.28)	1.99(1.63-2.34)	0.33(0.26-0.42)
	Slope ± SE	1.66±0.19	1.75±0.23	1.53±0.14
Emamectin benzoate	LC ₅₀ (mg L ⁻¹) (95% F.L)	4.09(3.25-5.01)	0.74(0.61-0.90)	-
	Slope ± SE	1.54±0.15	1.68±0.17	-
Methoxyfenozide	LC ₅₀ (mg L ⁻¹) (95% F.L)	3.20(2.57-3.86)	0.37(0.30-0.44)	0.06(0.05-0.07)
	Slope ± SE	1.80±0.16	2.05±0.30	3.396±0.60

Also, the percent inhibition of AChE from *Spodoptera* head capsules by chlorpyrifos, pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide (LC₁₀ equivalent concentrations) were showed in table (4). Inhibition percent were 44.5, 23.4, 19.4, 33.5 and 15.4%, respectively. The percent inhibition of AChE from *Spodoptera* head capsules by chlorpyrifos either pre-treatment or post-treatment with pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide were increased.

AChE is mostly a neuronal enzyme involved in the precise control of the neurotransmission in cholinergic synapses by hydrolyzing the neurotransmitter acetylcholine could be used as a marker for cholinergic function (Glavan, *et al.* 2020). In the natural environment, various pesticides are usually present in a mixed form and therefore their impact on the organisms may be synergistic, synergistic cumulative, or antagonistic depending on their chemical nature, structure, and mechanism of action. In the present study, these pesticides have altered the AChE activity. Previous studies found that chlorpyrifos caused an 80% decrease in AChE activity (Yen *et al.*, 2011; and Richendrfer and Creton 2015). These results were in agreement with those obtained by Barrania (2002). This indicates that the toxic potential of these pesticides for the tested organism is greater when present in a mixture than in an individual (Tiwari, *et al.* 2019). Several reports on the interaction of drugs and insecticides with AChE appeared. From these reports, significant evidence has accumulated indicating that AChE possesses, in addition to an anionic site in the catalytic center, peripheral anionic sites where ligands bind and exert a regulatory role on the enzyme activity (Eldefrawi, 1985).

Table 4: % inhibition of AChE caused by chlorpyrifos either pre-treated or post-treated with pyridalyl, chlorantraniliprole, emamectin benzoate, and methoxyfenozide.

Insecticide	%Inhibition± SE		
	alone	pre-treated	post-treated
Chlorpyrifos	44.5±0.02	-	-
Pyridalyl	23.4±0.18	78.2±0.40	82.6±0.42
Chlorantraniliprole	19.4±0.40	66.4±0.24	70.4±0.71
Emamectin benzoate	33.5±0.18	88.4±0.30	90.1±0.45
Methoxyfenozide	15.4±0.17	78.2±0.48	82.8±0.63

Means within the same column followed by the same letters are not significantly different according to the LSD_{0.05}.

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ARABIC SUMMARY

التقييم المعملی لبعض المبيدات الحشرية ضد يرقات دودة ورق القطن

أحمد عبد الحكيم برانيه

معهد بحوث وقاية النباتات- محطة البحوث الزراعية بايتاي البارود- مركز البحوث الزراعية

تم تقييم التأثيرات السامة لبعض مخاليط المبيدات الحشرية (الكلوربيريفوس، البيريديليل، الكلورانترانيلبيرول، الإيمامكتين بنزوات والميثوكسيفينوزيد) ، ضد يرقات العمر الثالث لدودة ورق القطن. أوضحت النتائج أن قيم التركيزات اللازم لموت 50 ٪ من اليرقات المعاملة هي 12.44 ، 0.88 ، 0.08 ، 0.16 و 3.35 مجم/لتر على التوالي. كما أوضحت النتائج أنه، كلما زاد وزن اليرقات تزداد قيم التركيز اللازم لموت 50 ٪ المقابلة من الكلوربيريفوس. كما أن المعاملة المسبقة لليرقات بالبيريديليل، الكلورانترانيلبيرول، الإيمامكتين بنزوات والميثوكسيفينوزيد كما كانت قلل قيم التركيز اللازم لموت 50 ٪ لليرقات المعاملة بالكلوربيريفوس كالاتي 1.33 ، 1.99 ، 0.74 و 0.37 مجم/لتر على التوالي.

أيضا ، كانت النسبة المئوية لتنشيط نشاط إنزيم أستيل كولين إستيريز المستخلص من رؤس يرقات دودة ورق القطن بواسطة الكلوربيريفوس، البيريديليل، الكلورانترانيلبيرول، الإيمامكتين بنزوات والميثوكسيفينوزيد (بالتركيز اللازم لموت 50 ٪) هي 44.5 و 23.4 و 19.4 و 33.5 و 15.4 ٪ على التوالي. المعاملة المسبقة بالكلوربيريفوس أو البيريديليل والكلورانترانيلبيرول وإيمامكتين بنزوات وميثوكسيفينوزيد يؤدي إلى زيادة قيم التركيز اللازم لتنشيط 50 ٪ من نشاط إنزيم أستيل كولين إستيريز المستخلص من رؤس يرقات دودة ورق القطن بواسطة الكلوربيريفوس. و هذا يشير إلى أن الإمكانيات السامة لهذه المبيدات الحشرية للكائن المختبر تكون أكبر عند وجودها في صورة خلأط عنها في الصورة الفردية.