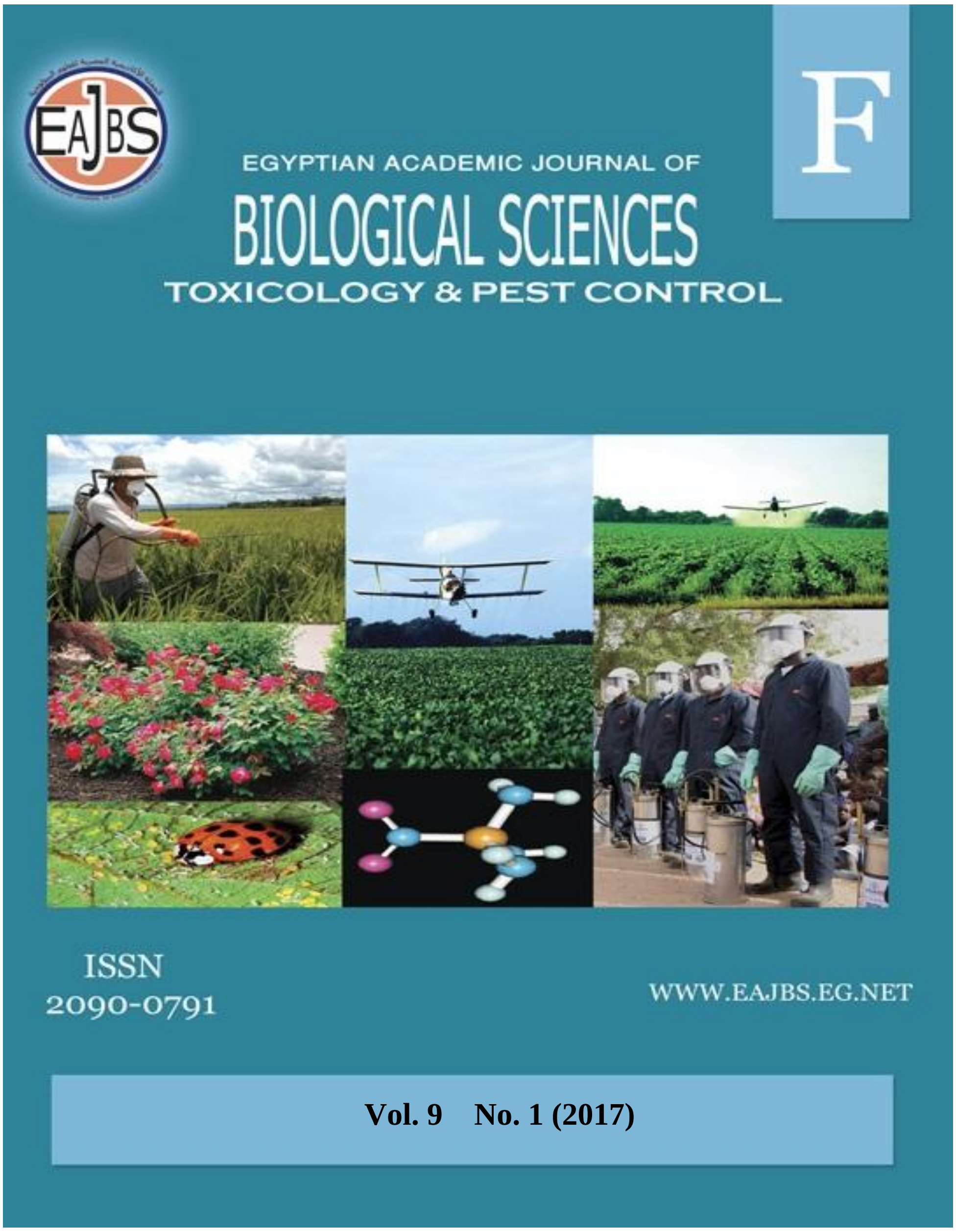

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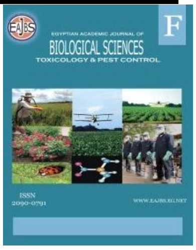

Assessment of Resistance Risk to Emamectin Benzoate,Indoxacarb and Spinetoram in Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.)

El-Sayed Mohammad Soliman Mokbel[1]; Amal Ibrahim Huesien[1]; Hassan Fouad Mohammed Abdel-Hamid[2] and Hanan. H. Osman[3]

1- Department of Standard Rearing, Central Agricultural Pesticides Laboratory, Agriculture Research Center, 12618 Giza, Egypt.
2- Cotton Pesticides Bioassay Dept., Plant Protection Res. Institute, ARC.
3- Cotton leaf worm Dept., Plant Protection Res. Institute, ARC.

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ABSTRACT

Cotton leaf worm, *Spodoptera littoralis* is a major polyphagous pest in Egypt. Resistance development to conventional insecticides led to introduce new pesticides with novel modes of action such as emamectin benzoate, indoxacarb and spinetoram. Assessment risk of resistance evolution to these insecticides has a great important for evaluating their future use on a pest population. To determine suitable larval stage for selection experiment. Bioassays were carried out against 1-st, 3-ed and 5-th larval instars. Resistance risk assessment to these insecticides was conducted by selecting a field collected population of *S. littoralis* (1-st instar) with the tested insecticides in the laboratory for six generations to estimate their realized heritability (h^2). Realized heritability (h^2) of resistance was 0.21, 0.37 and 0.33 for emamectin benzoate, indoxacarb and spinetoram, respectively. The rates of resistance development were compared using the response quotient (*Q*), which was estimated as 0.170 for both emamectin benzoate and spinetoram; while indoxacarb recorded *Q* value of 0.21. The projected rate of resistance development had been estimated with different values of slopes and realized heritability. Results suggest that a risk for resistance development to emamectin benzoate, indoxacarb and spinetoram may occur in *S. littoralis* under continuous selection pressure but that resistance development would be slower against emamectin benzoate and spinetoram than indoxacarb.

INTRODUCTION

Cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is a serious polyphagous agricultural pest (Carter, 1984). In Egypt, more than 40 insecticide formulations belonging to different groups have been registered and recommended to control the pest (Anonymous, 2012). Resistance evolution to conventional insecticides such as carbamates, organophosphates, and pyrethroids beside environmental hazards and public health restrictions led to a great necessitate introducing novel chemistries with reduced risk (Issa *et al*., 1984; Abo-El Ghar *et al*., 1986; Korrat *et al*., 2012).

Emamectin benzoate is a second-generation avermectin analog act as a chloride channel activator; leads to decrease neurons excit ability. So, the insect larvae stop feeding, irreversibly paralyzed, and lately died (Teran-Vargas *et al*., 1997; Grafton-Cardwell *et al*., 2005).

Indoxacarb, acts as sodium channel blocker, inhibiting sodium ion entry into nerve cells, resulting in paralysis and death of targeted pests. It has a good field activity against a number of Lepidoptera and exhibits reduced pesticide risk with low mammalian toxicity (Wing *et al*., 2000; McKinley *et al*., 2002). Spinetoram is a member of spinosyns which activate a unique site of the nicotinic acetylcholine receptors (Salgado *et al*., 1998).

Integration of these novel insecticides to avoid resistance development is critical for pest management strategies. Therefore, assessment of resistance risk before resistance occurs in the field, to recently introduced insecticides is of great important because it can provide valuable information aid to maintain susceptibility in field populations and consequently delay the development of resistance(Lai and Su, 2011;Sial andBrunner,2010). Resistance risk for an insecticide can be conducted throughout selection for resistance in laboratory throughout quantitative genetic techniques (Falconer and Mackay, 1996; Jutsum *et al*., 1998). Quantitative genetic can use selection experiments data to analyze the genetic variable and estimate realized heritability of resistance (Firkoi and Hayes, 1990).Realized heritability can be used to predict the rate of genetic change in population (Laiand Su, 2011).

Realized heritability (h^2), defined as the proportion of phenotypic variance accounted for by additive genetic variation (Firkoi and Hayes, 1990). Estimation of realized heritability provides a standardized way of analyzing and summarizing results from selection experiments (Tabashnik, 1992). The heritability parameters are important when estimating the resistance risk before predicting the continued effective use of a chemical on a particular pest. The susceptibility of pests to insecticides may change depending on selection pressure of these compounds on a population, and the heritability of resistance can be measured through generations with laboratory selection experiments. The rate of resistance evolution to an insecticide is proportional to the population's realized heritability (h^2) of resistance to that insecticide (Tabashnik and McGaughey 1994), so we can evaluate the resistance risks of insecticides by comparing their realized heritability of resistance to a particular pest strain.

In this study, we assessed the risk of resistance development to emamectin benzoate, indoxacarb and spinetoram in *Spodoptera littoralis* throughout selection tosix successive generations of a field population of the pest.

MATERIALS AND METHODS

Insecticides

The insecticides used in this study are given in Table (1).

Table1: Details of the used insecticides:

Active ingredient (common name)	Trade name	Manuf acturer	Chemical group	IRAC MOA
Emamectin benzoate	Biolarve 5% EC	CHEMVET	Avermectins	Group 6
Indoxacarb	Avant 15% SE	Dupont	Oxadiazines	Group22A
Spinetoram	Radiant 12% SC	Dow Agro Sciences	Spinosyns	Group **5**

IRAC MoA Classification Version 8.1, April 2016

Insects

In this experiment, *Spodoptera littoralis* population was collected at the larval stage from commercial cotton fields (*Gossypium hirsutum* L.) located in Sharqia governorate, East Delta area throughout season, (2015).Larvae were brought into the Central Agricultural

Pesticides Laboratory (CAPL), Dokki, Egypt, and reared on castor bean leaves at 25 ◦C, 65-70 % RH and a 14 : 10 h light : dark photoperiod. The emerged adults were kept in glass jars that were provided with tissue papers hung vertically for oviposition. They were fed on a solution containing 10% sugar solution in a soaked cotton wool ball.

Leaf dip bioassay

Leaf dip technique was used for larval bioassays to determine responses to the tested insecticides. Stock solution of each insecticide formulation was prepared using the tap water, and then serial of concentrations were prepared. The castor bean leaves were dipped into insecticide solution for 30 seconds, and allowed to dry. Leaves dipped into tap water served as control. At least six concentrations and five replicates were used to estimate each concentration-mortality line. Ten larval instars were transferred to petri dish; whereas treated leaf was placed. Petri dishes containing larvae were kept in the rearing chamber at 25±2 °C, 65-70% RH, and a photoperiod of 14:10 (L:D) h. until mortality and scored after24 hrs. Larvae failing to exhibit coordinate movement when probed with a soft camel hair brush was considered dead. Data were corrected by Abbott's (1925) formula. The data were analyzed by probit analysis (Finney, 1971).

Selection

The field population of *Spodoptera littoralis* was divided into three groups. One was selected with emamectin benzoate, while the second category was selected with indoxacarb and the third was selected with spinetoram. Selection was carried out up to 6 successive generations, by applying the median lethal concentration (LC_{50}) for the tested insecticide against 1^{-st}instar larvae for the first generation, and a new $LC_{50}$for eachinsecticide was used based on the resistance level from bioassay results every generation.

Estimation of realized heritability

Realized heritability (h^2) was estimated by using the method described by Tabashnik (1992) as follows: h2 = Response to selection(R) / Selection differential (S). Response to selection (R) was estimated as follows: R= (Log final LC_{50} - Log initial LC_{50}) /n. Where the final LC_{50} is the LC_{50} of population after n generations of selection and initial LC_{50} is for the parental population before selection. The selection differential (S) was estimated as follow: S = $i^{\delta}p$, Where i is the intensity of selection and is calculated according to Falconer (1989) and $^{\delta}p$ is the phenotypic standard deviation, calculated as: $^{\delta}p$ = $[1/2(\text{initial slope + final slope})]^{-1}$.The response to selection (R) can be estimated as follows = h^2S

Based on the response of *Spodoptera littoralis* to insecticidal selection in laboratory, predictions about the risk of resistance development were made under varying conditions of heritability and slope at different selection intensities in terms of number of generations required for a 10-fold increase in LC_{50} (*G*), which is the reciprocal of R (Tabashnik 1992):$G = R^{-1} = (h^2S)^{-1}$

For any particular value of *S*, the rate of resistance development will be directly proportional to $h^2$and inversely proportional to. *S* can be constant across insecticides for a particular intensity of selection only if the slope of the probit regression lines (and thus _*p*) is constant across insecticides, but slope is not necessarily constant across insecticides. Thus, response quotient (*Q*) was used to compare the rates of resistance development against emamectin benzoate, indoxacarb and spinetoram, which can be defined as Rdivided by I (Tabashnik and Mc Gaughey 1994): Q = R/i.

The value of *Q* enables comparing the rates of resistance evolution among different insecticides without reference to slope, and thus allows us to evaluate the durability of an insecticide against a particular pest population.

Effect of heritability on projected rate of resistance increase at constant slope value was assessed by drawing a graph between percent mortality and generations. Three values of h^2were used (one value was calculated from F1 to F6 and other two values were assumed theoretically and same procedure was adopted for effect of slope on projected rate of resistance evolution at calculated constant value of h^2.

Statistical Analysis

Mortality was corrected for control using Abbott's formula (Abbott1925). Data were analyzed by probit analysis (Finney, 1971) using probit analysis models using the software package EPA probit analysis version 1.5.Resistance factors were calculated as the resistant LC_{50}/ susceptible LC_{50}.

RESULTS

Toxicity of the tested insecticides against certain larval instars

Susceptibility test in the 1^{-st}, $3^{-ed}$and 5^{-th} larval instars of the cotton leaf worm, *Spodoptera littoralis* was carried out. Dataillustratedin Table (2) indicate that emamectin benzoate was more superior insecticidal than the other insecticides used against the tested larval instars. Spinetoram was more efficient than Indoxacarb on the 1^{-st} larval instar .on contrast indoxacarb was more efficient than spinetoramon the 3^{-ed} larval instar and the 3^{-ed} larval instar was more susceptible than the 1^{-st} larval instar. On the other hand spinetoram didn't give proper toxicity line in the range of the recommended dose against 5^{-th} instars.

Table 2: Susceptibility status in the 1^{-st}, $3^{-ed}$and 5^{-th} larval instars of the cotton leaf worm, *S. littoralis* to the tested insecticides

Insecticides	larva instar	Slope± SE	LC_{50} ($mgml^{-1}$)	Fiducial limit	Chi - Square	Regression Equation Y= a+b*x*
Emamectin benzoate						
	1^{-st} instar	1.16±0.26	0.001	0.000 - 0.001	4.43	8.53+1.16*x*
	3^{-ed} instar	1.43±0.33	0.04	0.02- 0.06	0.29	7.00+1.43x
	5^{-th} instar	1.21±0.21	0.06	0.03 - 0.09	1.68	6.48+1.21x
Indoxacarb						
	1^{-st} instar	1.03± 0.23	0.70	0.27 - 1.21	1.53	5.15+1.03x
	3^{-ed} instar	2.67± 0.66	0.29	0.18 - 0.38	1.01	6.44+2.68x
	5^{-th} instar	1.14±0.20	1.64	0.98 - 2.45	0.57	4.75+1.14x
Spinetoram						
	1^{-st} instar	2.19±0.41	0.12	0.08 - 0.16	3.16	7.02+2.19x
	3^{-ed} instar	1.22 ± 0. 20	8.03	5.23 - 14.19	4.62	3.89+1.22x
	5^{-th} instar	-	-	-	-	

5^{-th} instar larva showed mortality less than 10 % with spinetoram recommended concentration

Resistance selection to the tested insecticides in *S. littoralis*

Selection pressure was started by exposing the 1^{-st} larval instar to the median lethal concentration at (parent) and selection pressure was maintained for 6 consecutive generations. Resistance level was monitored every generation in respect to the parent generation. Sequential selection for 6 generations resulted in LC_{50} values increasing from 0.001 to 0.007, 0.70 to 7.43 and 0.12 to 0.87 (mg $Litre^{-1}$) for emamectin benzoate, indoxacarb and spinetoram, respectively. The resistance ratio increased to7, 10.6 and 7.25fold compared with parental field strain (Table 3).

Table 3: Toxicological profiles of the tested insecticides against first and six generations of *S. littoralis*, after consecutive selection experiment

Insecticides	F1		F6		RR (folds)
	Slope ± SE	LC50 (mg ml-1)	Slope ± SE	LC50(mg ml-1)	
Emamectin benzoate	1.15±0.26	0.001(0.000- 0.001)	1.25±0.25	0.007(0.003- 0.011)	7
Indoxacarb	1.03± 0.23	0.70(0.27 - 1.21)	2.48± 0.80	7.43(5.22 - 15.93)	10.6
Spinetoram	2.19±0.41	0.12(0.040 - 0.16)	1.54±0.25	0.87(0.62 - 1.26)	7.25

Realized heritability (h2)

Realized heritability of resistance (h^2) estimated over six generations of the three insecticidal selection showed the highest value in the indoxacarb selected strain with h^2 value of (0.37) decreasing to(0.28)in the case of spinetoram selected strain. While, the lowest value was (0.21) for emamectin benzoate selected strain(Table 4).

The response to selection (R) was highest in indoxacarb selected strain (0.170) and lowest emamectin benzoate selected strain (0.14).While the selection differential (S) was lower in indoxacarb selected strain (0.48) than spinetoram selected strain (0. 52) and emamectin benzoate selected strain (0.66).

Table 4: Estimation realized heritability (h2) and response quotient (Q) of resistance to the tested insecticides in *S. littoralis*

Insecticide	Estimate of mean response per generation		R	Estimate of mean selection differential per generation				S	h^2	*Q*
	Log initial LC_{50}	Log final LC_{50}		P	I	Mean slope	$^{\delta}p$			
Emamectin benzoate	-3	-2.15	0.14	50.0	0.80	1.20	0.83	0.66	0.21	0.17
Indoxacarb	- 0.154	0.87	0.17	50.0	0.80	1.75	0.57	0.46	0.37	0.21
Spinetoram	- 0.92	-0.06	0.14	50.0	0.80	1.86	0.53	0. 42	0.33	0.17

The mean values of *Q* for resistance against emamectin benzoate, indoxacarb and spinetoram were 0.17, 0.21and 0.17, respectively. These results indicate that resistance evolution would be slower against emamectin benzoate and spinetoram than indoxacarb; thus, emamectin benzoate and spinetoram would be more durable than in doxacarb against this particular population of *S. littoralis*.

Projected rates of resistance evolution

The projected rate of resistance development is directly proportional to h^2 and selection intensity. The projected rates of resistance development to emamectin benzoate illustrated in (Fig. 1A). When, assuming that emamectin benzoate mean slope = 1.2 (the mean slope of emamectin benzoate observed in this study) and h^2 (0.21).

When selection mortality = 95%, a tenfold increase in LC_{50} value would occur after only about3generations. Whereas, it would take about 7 generations for the same to happen at selection mortality = 50 %.

However, at similar slope and h^2 of (0.35) and selection mortality = 95%, a tenfold increase in LC_{50}value would occur after only about 2 generations. Whereas, it would take about 5 generations for the same to happen at selection mortality = 50 %.Likewise, similar findings would occur in only about (7 and 3) generations at (50 and 95%) selection intensity if (h^2 = 0.21). The projected rate of resistance evolution is inversely proportional to the slope of the probit line (Fig. 1B). In the case of emamectin benzoate, assuming that h^2 = 0.21 (the observedh^2 in this study) and selection mortality = 95%, a tenfold increase in LC_{50} value would occur after only 2 generations at a slope of 1.2, whereas, it would take 5 generations for the same to happen at a slope of (2.2).

Emamectin benzoate

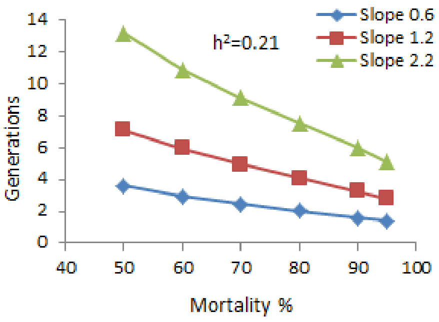

Fig. 1(A): Effect of slope on the number of generations of *S. littoralis* required for a tenfold increase in LC_{50} of emamectin benzoate (h^2 = 0.21) at different selection intensities

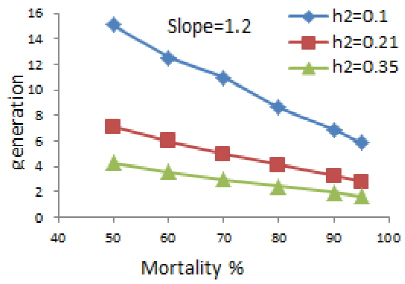

Fig. 1 (B): Effect of realized heritability (h^2) on the number of generations of *S. littoralis* required for a tenfold increase in LC_{50} of emamectin benzoate (slope = 1.2) at different selection intensities

While, at a slope of 0.6 it would take only 1 generation to get the tenfold increase in LC_{50}value. Likewise, Resistance predictions of indoxacrb illustrated in (Fig. 2) and spinetoram in (Fig. 3)

Indoxacarb

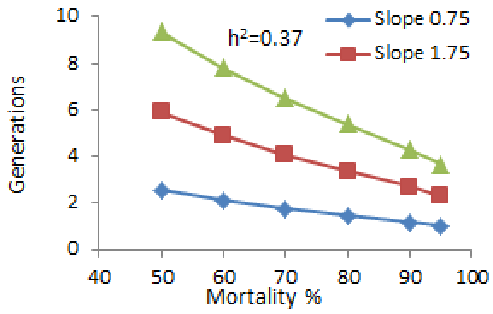

Fig. 2(A): Effect of slope on the number of generations of *S. littoralis* required for a tenfold increase in LC_{50} of indoxacarb ate (h^2 = 0.37) at different selection intensities

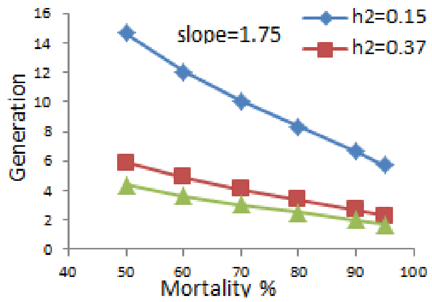

Fig. 2 (B): Effect of realized heritability (h^2) on the number of generations of *S. littoralis* required for a tenfold increase in LC_{50} of indoxacarb (slope = 1.75) at different selection intensities

spinetoram

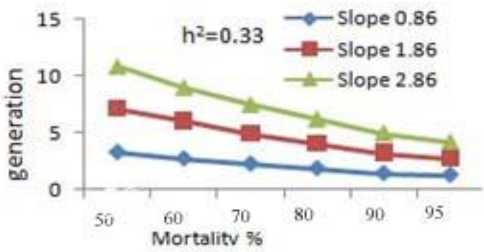

Fig. 3 (A): Effect of slope on the number of generations of *S. littoralis* required for a tenfold increase in LC_{50} of spinetoram (h^2 = 0.33) at different selection intensities

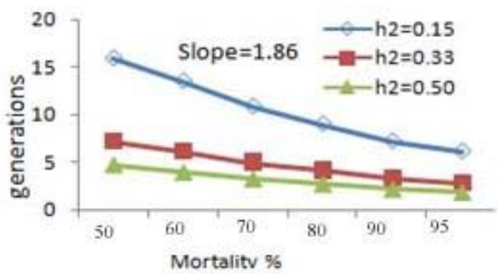

Fig. 3 (B): Effect of realized heritability (h^2) on the number of generations of *S. littoralis* required for a tenfold increase in LC_{50} of spinetoram (slope = 1.86) at different selection intensities

DISCUSSION

Emamectin benzoate, indoxacarb and spinetoram are novel insecticides used against lepidopteran insect pests. The results of present study revealed that, Emamectin benzoate was the most effective against 1^{-st} instar larvae followed by spinetoram and indoxacarb, respectively. In general, 1^{-st} instar larvae of *S. littoralis* were found to be more susceptible than 3^{-ed} and 5^{-th} instar larvae, with the exception of, indoxacarb which was more effective against 3^{-ed} instar than1^{-st} instar larvae. This may as result to indoxacarb parent molecule is a pro-insecticide with only weak activity on voltage gates sodium channels, which is rapidly bioactivated by target insects. Metabolic activation through esterase is resulting in an NH-derivative with potent insecticidal activity (Wing *et al*., 1998).so susceptibility of 3^{-ed}than 1^{-st} instar may due to esterase activity in1^{-st} instar larvae less than 3^{-ed} instar larvae.

Our findings revealed that selection of *S. littoralis* with the aforementioned insecticides for six consecutive generations, resulted in the development of 7, 10.6 and 7.25-fold resistance to emamectin benzoate, indoxacarb and spinetoram, respectively. Laboratory selection experiments data can be used to assess the resistance risk in insect species to a particular insecticide. Moreover, these data is analyzed by quantitative genetic techniques to obtain additive genetic variance (VA) and realized heritability (h^2) of resistance ((Jutsum *et al.*, 1998; Firkoi and Hayes, 1990).

Population genetic studies such as heritability of resistant genes used to predict the risk of resistance development and planning more effective resistance management programs (Askari-Saryazdi *et al*., 2015).Heritability provides a good indication for pest ability to develop resistance to insecticides (Johnson and Tabashnik, 1999). Realized heritability (h^2) provides the mean to compute selection experiments results throughout corporating selection strength and resistance development rate (Tabashnik 1992). The lower h^2 indicates high erphenotypic variance (VP) and loweradditive genetic variance (VA) and alleles which are responsible for resistance were rare in the field collected strain of *S. littoralis*. The lower h^2 (0.21), after 6 generations of selection with emamectin benzoate, indicated that *S. littoralis* strain have lower ability of resistance development to emamectin benzoate when compared with the other insecticides, spinetoram (h^2 = 0.33) and indoxacarb (h2 = 0.37). These results indicate that about 0.21, 0.37 and 0.33% of the total variation in emamectin benzoate, indoxacarb and spinetoram susceptibility was caused by additive genetic variation.in the present study higher h^2in indoxacarb resistance selection compared with emamectin benzoate and indoxacarb was as a result to the high value of R in indoxacarb.

Estimates of realized heritability (h^2) and slope of probit lines in conjunction with varying selection intensities can be used to project the rates of resistance development. Prediction based on h^2 must be interpreted cautiously because h^2 of resistance to a particular insecticide can vary between conspecific populations as well as within populations as a result to allele frequencies and environmental variation over time. So, the predictions made from quantitative genetic theory on the basis of $G= R^{-1}$ gives valuable information to develop strategies for managing pesticide resistance (Tabashnik, 1992). Estimating h^2 from laboratory selection experiments is necessary to assess the risk of insecticide resistance in pests (Lai and Su, 2011).The outcomes of the current experiment showed that *S. littoralis* populations have the ability to develop resistance to the aforementioned insecticides in the field. The previous results indicated that, resistance alleles to the tested pesticides were not rare.

Relatively quick response of selection with the tested insecticides suggests that the higher potential for resistance development to these insecticides. The higher values of response quotient (*Q*) for indoxacarb (0.21) compared with that both emamectin benzoate and spinetoram (0.17) suggests that resistance to indoxacarb could evolve faster than both emamectin benzoate and spinetoram in *S. littoralis*. The present study represents an early warning to serve the efficacy of these pesticides throughout designing effective resistance management programs.

In conclusion, the findings of the present work report the potential of the field population of S. *littoralis* to develop resistance against emamectin benzoate, indoxacarb and spinetoram. The field population can develop resistance more rapidly by increasing (h^2), intensity of selection and strain heterogeneity (decreasing slope value).So, tested insecticides must be used wisely and incorporate with no cross resistance pesticides in resistance management programs to control the target lepidopteran pests.

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ARABIC SUMMERY

تقييم مخاطر تطور المقاومة لمبيدات الإيمامكتين بنزوات والإندوكسيكارب والسبينوترام علي دودة ورق القطن

السيد محمد سليمان مقبل[1]،أمل إبراهيم حسين[١]،حسن فؤاد محمدعبدالحميد[٢]،حنان حسين عثمان [٣]

١- قسم بحوث التربية القياسية- المعمل المركزي للمبيدات- مركزالبحوث الزراعية- الجيزة- مصر
٢- قسم إختبار مبيدات آفات القطن - معهد بحوث وقاية النباتات –مركز البحوث الزراعية- الجيزة- مصر
٣- قسم دودة ورق القطن - معهد بحوث وقاية النباتات –مركز البحوث الزراعية- الجيزة- مصر

دودة ورق القطن من الآفات متعددة العوائل الهامة في مصر.وقد أدي تطور المقاومة للمبيدات التقليدية المستخدمة في مكافحة هذة الآفة إلي إدخال مبيدات جديدة ذات طريقة تأثير مختلفة مثل الإيمامكتين بنزوات ، الإندوكسيكارب والسبينوترام.ودراسة مخاطر تطور المقاومة لهذة المبيدات ذات أهمية كبيرة في كيفية الإستخدام الأمثل لهذة المبيدات بحيث يتم منع أو تاخير ظهور صفة المقاومة.وتم تقييم المبيدات محل الدراسة ضد أطوار يرقية مختلفة (عمر أول، ثالث و خامس)لتحديد أنسب الأطوار للإنتخاب.وتم الإنتخاب بتعريض العمر اليرقي الأول لمدة ستة أجيال متتابعة للإنتخاب بالمبيد وذلك لحساب درجة وروثية المقاومة (h^2) والتي سجلت قيما تبلغ ٠,٢١ ,٠,٣٧ و ٠,٣٣ للإيمامكتين بنزوات،الإندوكسيكارب والسبينوترامعلي الترتيب.ونتيجة لعدم ثبات قيمة ميل خط السمية للمبيدات المستخدمة تم إستخدام مقياس يستبعد تأثير الميل علي معدل تطور المقاومة وهو(Response quotient (Q والذي أعطي قيمة ٠,١٧ لكلا من الإيمامكتين بنزوات والإندوكسيكارب بينما كانت ٠,٢١ للإندوكسيكارب. كما تم التنبأ بإمكانية تطور المقاومة لهذة المبيدات عند قيم (h^2) وميل slopes مختلفة . وتظهر نتائج الدراسة قدرة الحشرة علي تطور المقاومة لهذة المبيدات كما تظهر أن تطور المقاومة لمبيد الإندوكسيكارب أسرع من كلا من الإيمامكتين بنزوات والسبينوترام بما يظهر أهمية الإستخدام الرشيد لهذة المبيدات ضمن برنامج للسيطرة علي المقاومة للعمل علي تأخير ظهور صفة المقاومة وتعظيم الإستفادة من هذة المركبات.