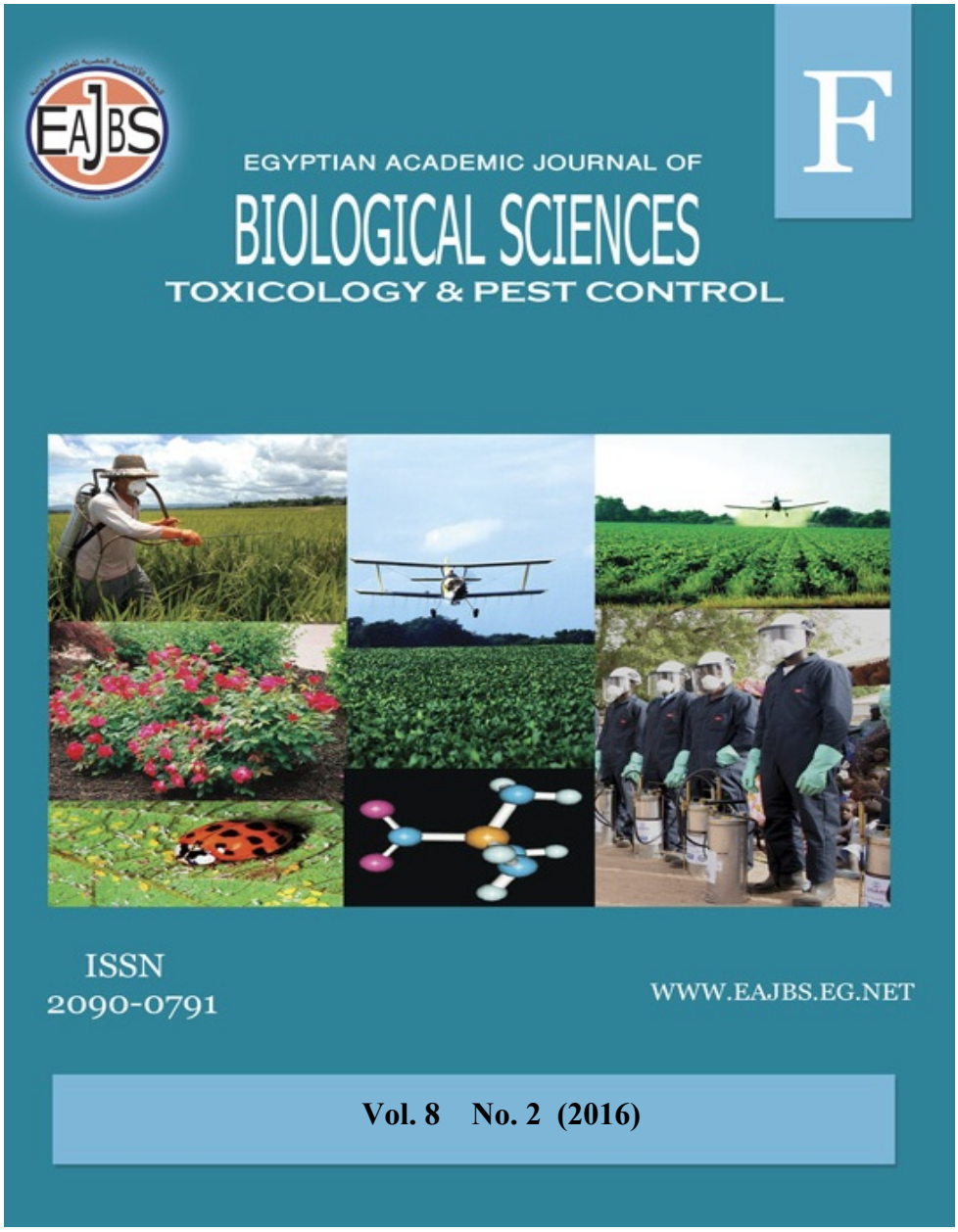

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Effect of Methoxyfenzoid, Indoxacarb and Emmamectin Benzoate on Carbohydrate and Phosphatase Enzymes of *Tuta Absoluta* (Lepidoptera: Gelechiidae).

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ABSTRACT

The effect of Methoxyfenzoid, Indoxacarb and Emmamectin Benzoate on Carbohydrate and Phosphatase Enzymes of field collected *Tuta Absoluta* was investigated. Toxicity results against third instar larvae showed LC_{50} values was (50.77 ppm, 2.33 ppm and 0.22 ppm) for Methoxyfenzoid, Indoxacarb and Emmamectin Benzoate respectively. Additional results, indoxacarb and emmamectin benzoate are faster to kill than methoxyfenzoid where LT_{50} was (1420.8 min 1469.9 min and 2629.3 min) respectively. The biochemical results showed high increase in trehalase and acid phosphatase (17.93, 7.83%) respectively and slight increase in invertase and amylase activities (2.51 and 0.039%) respectively when treated with indoxacarb comparing the other two insecticide.

INTRODUCTION

The tomato pinworm, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is one of the most destructive pest of solanaceae and it prefers tomato (*Solanum lycopersicum* L., becoming a major concern for tomato cultivation in Europe, Africa and the Middle East, recording control failure, development of resistance to a wide range of compounds, Siquera *et.al.* & 2000b and Silva *et.al.* 2015. Damages are caused through affecting the plant's photosynthetic capacity and development by all larval stage feeding on leaf mesophyll, stems and the growing tips forming mines and galleries. The fruit rot occurrence by pathogens attack and lowering tomato yield was the source of ' Marketing and processing misplace up to 80–100%, Desneux *et.al.* 2011. Resistance can be defined as the susceptibility change of the pest population reflects a repeated control failure of proper insecticide used with its label recommendation of application Contijo, *et.al.* 2013. The effective management needs a durable assessment to any new insecticide by many category of detection to follow and predict its more efficacy information on the proper pest, discovering new additionally resistance mechanisms and reduce the number of insecticides not desirable for control, this was infinitely by using biochemical approaches as well as toxicological measures.

Metabolism of carbohydrate hydrolyzing enzymes plays a principal role in digestion and utilization of carbohydrate in the insect midguts and is controlled by amylase, invertase and trehalase enzymes that basically essential for insect growth and development and its inhibition can stress on the insect's life cycle, Guven 2003.

The reduction in enzyme synthesis is due to the direct effect of toxic agents on it. The low enzyme activity may be used as a marker for resistant individual in populations Van Dyk and Pletschke 2011.

To measure the effect of insecticide it must be measure the inhibition on the carbohydrate hydrolyzing enzyme because it is from 5–20 times more sensitive to inhibitors than general protein synthesis. Silva *et al.* 2015, Campos *et al.* 2014.

The present study was designed to examine the changes in the activities of carbohydrate hydrolyzing enzymes as amylase, trehalase, invertase, acid and alkaline phosphatase after treatment with the median lethal concentration of the tested insecticides.

MATERIALS AND METHODS

Insects:

Collection of tomato plant leaves contain different developmental stages of *T. absoluta* larvae were obtained from heavy infested greenhouse tomato crop at Dokki Giza production station sustained by Egyptian agriculture research center, using intense chemical treatments to maintain plants free of pests and transferred to the laboratory for conducting a laboratory toxicity and biochemical bioassay. On the other side, a small batch of 3rd instar larvae were collected from Egyptian tomato fields didn't use insecticides considered as reference strain.

Toxicity Measurements:

Time mortality bioassay under insecticide field rate:

Insecticide time bioassay were carried out using filter paper impregnated with one concentration (according to recommended label rate cited in Table1) 1ml of insecticide dissolved in acetone and placed in petri dish and three replicate each one contain 30 3rd instar larvae of *T.absoluta* were used for each treatement. Mortality was checked every six hour, for two days. Every day larvae were provided with fresh leaves. Statistical Probit Analysis of Correlated Data: Multiple Observations over Time at One Concentration was carried out to estimate the Lethal times causing 50 and 90% mortality (LT_{50} and LT_{90}) values, using LDP-line software (Ehabsoft) including correction for control mortality.

Table 1: Chemical, Brand names, Mode of action and field dose of insecticides tested against *T.absoluta*.

Insecticide	Class	Formulations	Commercial names	Field Dose (g a.i./ha)	Primary mechanism
Methoxyfenzoid	Diacylhydrazine	24% SC	Runner	37.5 ml /100L	chitin synthesis inhibition
Indoxicarb	Oxadiazine	15% EC	Avaunt	30 ml /100L	Na channel blocking
Emmamectin	Avermectin	1.9% EC	Pasha	25 ml / 100L	GABA receptor blocking

Concentration-mortality bioassays:

Bioassays were performed using a leaf dip method according to (Reyes *et al.,* 2012, Silva *et.al.* 2011 and 2015 and Campos *et al.* 2014). Uninfected tomato Leaves were individually dipped in fresh solutions of the insecticide for 5s smoothly, ensuring the entire surface was equally covered and left to dry. Controls were dipped in water only. Subsequently, the treated leaves were placed in individually in 9 diameter petri dish.

Then, 10 3rd instars larvae were placed on each dish in three replicate and seven concentrations and kept on controlled conditions at 25 ± 2ºC and 16:8 h light: dark. Mortality was recorded 24 h after treatment with the faster-acting insecticides Indoxacarb and emmamectin benzoate (neurotoxic insecticide) and 48h for the slower-acting insecticides for methoxyfenozide (insect growth regulators) Silva *et al.,* 2011; under a binoclar. A larva was considered dead when it was not able to move normally. Statistical analysis of bioassay data was carried out to estimate the median lethal concentration (LC_{50} and LC_{90} values) using LDP-line (Ehabsoft), correction for control mortality was performed throughout probit transformations.

A large number of larvae were treated with LC_{50} that previously determined for each insecticide and about 0.5 g of healthy *T. absoluta* larvae survived after two days of treatment for methoxyfenzoid and after one day for indoxacarb and emmamectin benzoate and the control were collected in plastic dishes and kept in a freezer at (-20°C) until required.

Preparation of samples for biochemical studies:

The biochemical assay was done on the larvae homogenates of *T. absoluta* is that collected from different treatments. After centrifugation the supernatant was directly used for enzyme assay.

Enzymes measurements:

Carbohydratase assayed was based on the digestion of trehalose, starch, and sucrose by trehalase, amylase and invertase, respectively, according to the method described by Ishaaya and Swirski (1976).

Acid and alkaline phosphatase activities were estimated according to the method described by Powell and Smith (1954) Using disodium phenyl phosphate as substrate. The activity is expressed as ug phenol released/ mg body weight.

RESULTS AND DISCUSSION

Time mortality bioassay:

The LT_{50} and LT_{90} values (Tables 2) revealed that emmamectin benzoate was the fastest for kill larvae where LT_{50} (1420.8 min). For indoxacarb the LT_{50} occurred within 1469.9 min. Toxicity of methoxyfenzoid was delayed, with significantly higher LT_{50} 2629.3 min, compared with emmamectin and indoxacarb. Briefly at the recommended application rates the three insecticides tested proved effective against *T.absoluta* larvae.

Table 2: Lethal times causing 50 and 90% mortality ($LTime_{50}$ and $LTime_{90}$) with confidence limits of three insecticides against 3rd instar larvae of tomato pinworm *Tuta absoluta* (Meyrick).

	Min speed of action			
insecticides	LT_{50} (95% FL)	LT_{90} (95% FL)	Slope ± SE	$\chi 2$(df)
Methoxyfenzoid	2629.3(2088.9-3917.3)	12074.7(6727.0-34141.8)	1.93±0.37	5.36(3)
Indoxicarb	1469.6(1271.9-1686.2)	3912.9(3114.6-5631.8)	3.01±0.40	10.6(3)
Emmamectin	1420.83(1155.5-1740.7)	6253.9(4201.6-13162.9)	1.99±0.33	4.13(3)

Concentration-mortality bioassays:

The Toxic effect of the three insecticides against 3rd instars *T. absoluta* larvae were in Table 3. LC_{50} of methoxyfenzoid, indoxacarb and emmamectin benzoate LC_{50} values were 50.77, 2.33 and 0.022 ppm, respectively. The slope values were 1.85, 1.87 and 1.4. Resistance ratios (RR) were determined on the basis of the LC_{50} using the most susceptible population as reference. RR values were 1.88, 4.12, and 0.18 respectively.

The population consider susceptible to those insecticides because RR was low (under 5fold) according to Roditakis *et al.* (2013 a).

From the previous results it seems that indoxacarb were less efficient than the others, almost insecticide resistances were incorporated to this problem where some literature explains the same results. Contijo *et al.,* 2013 and Roditakis *et al.* 2013 found that LC_{50} of some tested insecticides against *T. absoluta* populations was less than 5 fold except for indoxacarb up to 10 fold of resistance. Gacemi and Guenaoui 2012, reported that three foliar applications of Emamectin benzoate were made at 7 days interval in a tomato greenhouse, showed a good activity on *Tuta absoluta* larvae with a mortality reached 87%. Fanigliulo and Sacchetti 2008**,** said, that Emamectin benzoate has a high control of *H. armigera* more than indoxacarb and spinosad. Although some sesarches noted that indoxacarb were potent with other insect pests.

Table 3: Susceptibility of 3rd instar larvae of tomato pinworm *Tuta absoluta* (Meyrick) to three tested insecticide.

Tested insecticides	Greenhouse population			Reference population			RR
	LC_{50} (95% FL)	Slope ± SE	$\chi 2$(df)	LC_{50} (95% FL)	Slope± SE	$\chi 2$(df)	
Methoxyfenzoid	50.77 (37.46 - 67.39)	1.857±0.296	2.805(3)	26.9(20.8- 34.5)	2.2±0.32	2.7(3)	1.88
Indoxicarb	2.33 (1.6 - 3.08)	1.873±0.304	1.057(3)	0.565(0.392-0.759)	1.7±0.29	0.56(3)	4.12
Emmamectin	0.022 (0.014 - 0.03)	1.400±0.271	1.712(3)	0.012(.009-0 .016)	1.7±0.28	1.67(3)	0.18

Enzyme assay:

Effect on carbohydrate enzymes activity:

As seen in (Table 4), larvae treatment with LC_{50} of methoxyfenzoid caused a significant decrease in trehalase and amylase activity near -20.18 % and -12.85 than that in untreated larva. However, activities of invertase were slightly decreased by -0.354% than the control, respectively. Meanwhile; treatment of Indoxacarb caused a much increase of trehalase activity about 17.93% and slight increase in invertase and amylase activities, 2.51%, and 0.039% respectively. And the treatment of emmamectin benzoate cause a little decrease of trehalase -2.96% and a little increase in invertase activity 0.978% and much decrease in amylase activity -25.42 % than that in untreated larvae.

Amylase enzyme is required to digest carbohydrates into glucose. Invertase enzyme hydrolyzes sucrose, forming fructose and glucose. Trehalase also plays a role in carbohydrate absorption, Guven 2003. This note is identical to Ishaaya and Ascher (1977), determine the effect of diflubenzuron on trehalase, amylase and invertase inhibition of *Tribolium castaneum*, and found them essential during molting to generate production of glucose for chitin build-up that might affect the molting process. These results are in agreement with previous research verified decrease in amylase, trehalase and invertase was observed in *T. absoluta* exoposed to the seed proteinaceous extracts from non-host plant, found to be 50% inhibition of α-amylase than host plant, Esmaeily, and Bandani, 2015.

Table 4: Carbohydrate hydrolyzing enzymes activity in homogenate of the 3rd instar larvae of larvae *Tuta absoluta* (Meyrick) after 48h of treatment with LC_{50} of each tested insecticide.

Carbohydratase activities (µg glucose / min /g body weight)						
insecticides	**Trehalase(Mean± SE)**	**%**	**Invertase(Mean ± SE)**	**%**	**Amylas(Mean ± SE)**	**%**
Control	688.444 ±19.307	---	1271.831±35.029	---	1857.823 ±43.113	---
Methoxyfenzoid	549.472 ±14.150a	-20.18	1267.325±31.885	-0.354	1615.995 ±35.377	-12.85
Indoxicarb	811.889 ±25.871	17.93	1303.764±37.680	2.51	1855.548 ±35.034	0.039
Emmamectin	668.034 ±20.407	-2.96	1284.269 ±38.911	0.978	1382.543 ±30.018	-25.42

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test

Effect on acid and alkaline phosphatase:

The activity of acid and alkaline phosphatase was being 54.136 and 39.047 µg phenol/ml/min, respectively in untreated *T. absoluta* 3rd instar larvae. Data in (Table 5), showed the activity of acid phosphatase that was much higher in larvae treated with indoxacarb than treatment with methoxyfenzoid, and emmamectin benzoate (58.375 µg phenol/ml/min) when compared with the untreated control. Alkaline phosphatase activity was reduced from 39.07 to 33.52 µg phenol/ml/min in larvae treated with LC_{50} Indoxacarb, making a-14.2% decrease. This enzyme's activity recorded a significant distinguishable increase from 39.07 to 53.13 µg phenol/ml/min (35.9% increases) by the treatment of methoxyfenzoid.

Phosphatases catalyse the hydrolytic cleavage of phosphoric acid esters called 'acid' or 'alkaline' phosphatases according to their activity related to pH optima and associate with many important physiological processes such as metabolism and cell signaling. It found in the membrane of insect gut plays an important role in insecticide toxicity, Koodalingam *et al*. 2012.

Gamil *et al.* 2011, found a decrease in the acid phosphatase, and content of carbohydrates and lipids increase in the alpha, beta esterase and the total protein was increased in *Spodoptera littoralis* (Boisd.) larvae treated with indoxacarb. Awad *et al.,* 2013, measures the *Schistocerca gregaria* digestive enzymes and chitinase that affected by treatment with farnesol. All insects can be develop resistance to the continuous insecticide exposure in the root of detoxifying variety of poisons with much its body enzymes each of them proper to a specific poison, and this call resistance mechanisms. The fall armyworm had developed multiple/cross resistance to both Bt toxins and conventional insecticides and the reduced alkaline phosphatase prove that the alkaline phosphatase could be a Bt resistance mechanism, Zhu *et al.* 2015. Furthermore the effect of Vectobar (*Bacillus thuringiensis* (Bt)-based formulations) against *Aedes aegypti* larvae, treated by LC_{50} and the level of alkaline phosphatase decreased (49%), Koodalingam *et al.* 2012.

Table 5: Phosphatase activity in homogenate of the 3rd instar larvae *Tuta absoluta* (Meyrick) after 48h of treatment with LC_{50} of each tested insecticide.

Phosphatase activities (µg phenol/min/g body weight)				
Tested insecticides	**Acid (Mean ± SE)**	**%**	**Alkaline (Mean ± SE)**	**%**
Control	54.136 ±1.117	--	39.072 ±0.823	---
Methoxyfenzoid	36.004 ±0.660	-33.5	53.130 ±1.212	35.9
Indoxicarb	58.375 ±1.135	7.83	33.520 ±0.792	-14.2
Emmamectin	47.731 ±1.045	-11.8	40.144 ±0.798	2.73

% of Increase or decrease than control = (Test – Control)/Control × 100;
Letters mean the significant differences between treatments according to Duncan's test

The different mode of action can play the same role on this enzymes inhibition that affect insect's balances. There are two possible explanations to these inhibitions: direct inhibition of enzyme synthesis and inhibition of the mechanism that controls the digestive enzyme secretion into the midguts, Liu *et.al.* 2008. After treatment with insecticides, the secretion and function of insect digestive enzymes becomes imbalanced and normal consumption and digestive processes are affected. When the activity of amylase affected, the degradation of carbohydrates also decreases. This leads to disturbance in

chitin building and failure of molting process to insect pests. For example: The activity of trehalase was reduced in *Bradysia odoriphaga* (Diptera treated with benzothiazole, which may explain why the trehalose fraction increased while the carbohydrate content decreased Zhao *et.al.* 2016. Other organism also can interrupt by the insecticide exposure, for instance endosulfan, Maneb and Mancozeb on *Bacillus subtilis* α-amylase was also seen at very low concentrations (0.048 ppm), G 2003.

From the result it showed that most treatment was lower than the control, Bemani *et al.* 2013, found that the trehalose content in pupae of fruit hull borer *Arimania camaroffi* treated with pyriproxyfen 0.52 mg/g fresh body weight was lower than control with 2.07mg/g.

From this study we can say that the inhibited digestive enzyme could be used as biochemical markers and indicators for monitoring pesticide residual present in *T. Absoluta*. Also insecticides can be used is that didn't display resistance or control failure and must be incorporated in rotational program or such management objects.

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ARABIC SUMMERY

تاثير مبيد ميثوكسي فنزويد واندوكساكارب وايمامكتين بنزوات علي نشاط انزيمات الكربوهيدرات والفوسفات في حشره ناخرات اوراق الطماطم توتا ابسولوتا.

حنان صلاح الدين طه و مني قطب الحادق

المعمل المركزي للمبيدات- مركز البحوث الزراعيه- الدقي- جيزه

تمت دراسه سميه مبيد الميثوكسي فنزويد واندوكساكارب وايمامكتين بنزوات علي حشره ناخرات اوراق الطماطم توتا ابسولوتا تم جمعها من الصوب الزراعيه . اظهرت النتائج المتحصل عليها قيم الجرعات النصفيه للموت للمبيد ميثوكسي فنزويد والاندوكساكارب والايمامكتين بنزوات (٥٠.٧٧ جزء في المليون و ٢.٣٣ جزء في المليون و ٠.٠٢٢ جزء في المليون علي التوالي.

كما اثبتت الدراسه ان ايمامكتين بنزوات واندوكساكارب كانا اسرع من الميثوكسي فنزويد من حيث زمن قتل اليرقات وكانت كالتالي (١٤٢٠.٨٣ دقيقه و ١٤٦٩.٦ دقيقه و ٢٦٢٩.٣ دقيقه) علي التوالي.

ومن حيث التاثيرات البيوكيماويه اظهرت الدراسه زياده كبيره في انزيم التريهالوز وانزيم الفوسفاتيز الحامضي (١٧.٩٣% و ٧.٨٣ %) علي التوالي وزياده طفيفه في نشاط انزيم الانفرتيز والاميليز (٢.٥١% و ٠.٠٣٩%) علي التوالي في حاله المعامله بمبيد الاندوكساكارب.