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# Toxicity of Certain Insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) with Relation to Carbohydrasis and Phosphatasis Enzyme

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## ABSTRACT

The present work is the studying the toxicities of three insecticides from different groups indoxacarb, emamectin benzoate and methoxyfenozide on *Tuta absoluta*. collected from field. The obtained result showed the toxicity of three insecticides against the third larvae instar of *T.absoluta*, the values of LC<sub>50</sub> for methoxyfenzoid, indoxacarb and emmamectin benzoate (50.77 ppm., 2.33 ppm. and 0.022 ppm.), respectively. Emmamectin benzoate and indoxacarb are more effective methoxyfenzoid. The biochemical result showed high increase in activity enzyme 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 by Indoxicarb comparing with the other two insecticide.

*Keywords: Tuta absoluta*, Indoxicarb, Methoxyfenzoid, Emmamectin benzoat, Insect growth regulators (IGR's), Acid phosphatase (AcP), Alkaline phosphatase (AlP) and Carbohydrates enzyme.

## **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 (Gebremariam, 2015). Tuta absoluta (Mevrick) is considered as one of the most important and devastating tomato insect pest (Cuthbertson et al., (2013) and Öztemiz.,(2013). Recording control failure, development of resistance to a wide range of compounds, (Silva et.al, 2015). Damages are caused through affecting the plant's photosynthetic capacity and development by all larval instars 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 (Gontijo 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.

Carbohydrates, protein and lipids are very efficiently utilized by insects and most species derive the main part of their nourishment from these nutrients depends. Amylase, invertase and trehalase were found to be the most important enzymes that play the major role in the digestion and metabolism of carbohydrates in insects (Wigglesworth, 1972; and Wyatt, 1967). These enzymes have received a great deal of attention in concern with digestion and utilization of carbohydrates in insect.

The low enzyme activity may be used as a marker for resistant individual in populations (Van Dyk, 2011). To measure the effect of insecticide it must be measure the inhibition on the carbohydrate hydrolyzing enzyme because it more sensitive to inhibitors than general protein synthesis from 5–20 times (Silva *et.al.*, 2015; and Campos *et.al.*, 2014). There is a relationship between the increase of insecticide resistance and the activity of detoxification enzyme, acid phosphatase (ACP) in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li, and Liu, 2007).

There for, that present study aims to infestigate the changes in the carbohydrate hydrolyzing enzymes amylase, invertase, trehalase, Acid phosphatase & alkaline phosphatase activity after treatment the field strain larvae of *T. absoluta* larvae by the insecticide median lethal concentration (LC<sub>50</sub>).

## MATERIALS AND METHODS

#### Insects

Collection tomato plant leaves contain different developmental stages of *T. absoluta* were obtained from heavy infested tomato crop (field strain) and transferred to laboratory to study the toxicity and biochemical assay.

## Insecticides used

## 1-The toxicological studies

Insecticides were carried out using filter paper impregnated with one concentration (according to recommended Dose cited in Table1) 1ml of insecticide dissolved in acetone and placed in petri dish, three replicate each one contain 10 larvae of the third larvae instar *T.absoluta* were used for each insecticide. Mortality was

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checked every six hour, for two days. Every day larvae were provided with fresh food. Statistical Probit Analysis of Correlated Data, The average mortality was corrected using Abbott's formula (1925). The corrected mortality average of each compound was statistically computed

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according to Finney (1971). Resistance ratio (RR) and lethal dose causing 50 and 90% mortality (LC<sub>50</sub> and Lc<sub>90</sub>) values using LDP-line software, Ehabsoft, (2005) including correction for control mortality.

GABA receptor blocking

Table 1. Chemical	, Brand names, Mode	of action and fie	eld dose of insection	cides tested	
Insecticide	Commercial names	Formulations	Field Dose	Class	Primary mechanism
Methoxyfenzoid	Rano	24% SC	37.5 ml/100L	Diacylhydrazine	chitin synthesis inhibition
Indoxicarb	Camvaal	15% EC	25  m/100L	Oxadiazine	Na channel blocking

75 ml / 100L

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1.9% EC

#### 2-The biochemical assay

Emmamectin benzoat

Bioassays were performed using a method according to (Reyes et al., 2012; Silva et.al. 2011 & 2015 and Campos et.al. 2014) leaves were dipped for in fresh solutions of the insecticide for 5 seconds in each aqueous concentration of the tested compound then left to dry on room temperature, then two leaves were placed in individually in 9 diameter Petri dish. Elementary controls were dipped in water only, 10 3rd instar larvae were carefully placed on every dish of the three replicate and seven concentrations and kept on controlled conditions at  $25 \pm 2^{\circ}$ C and 16:8 h light to dark. Mortality was recorded after 24 h from treatment for 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 binocular. The larvae were 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<sub>50</sub> and LC<sub>90</sub> values) using LDP-line (Ehabsoft, 2005), correction for control mortality were performed throughout probit transformations. A number of biochemical indices was measured in the present study to evaluate the physiological and biochemical effects of the tested insecticides against larvae of T. absoluta. For that a huge number of larvae were treated with LC<sub>50</sub> that previously determined for each insecticide and distilled water only for control and about 0.5 g of healthy T. absoluta larvae from each insecticide treatment that survived after two days from treatment with the LC50 of methoxyfenzoid and after one day treatment with the LC<sub>50</sub> of 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 collected larvae were homogenized in distilled water at 500 rpm using a Teflon homogenizer -(MECHIANIKA PRECYZYJNA Warszawa type MPN-

309-Poland) - surrounded with a jacket of crushed ice for 3 minutes. The larvae homogenates of T. absoluta were collected in cold tubes (on ice) previously coated with crystals of phenylthiourea to prevent melanization, then centrifuged at 6000 rpm for 10 min at 5°C using (BECKMAN GS-6R Centrifuge). After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at -20 °C until analysis of enzyme activities and determination of main component. Three replicates were carried out for each biochemical determination.

Avermectin

#### A-Determination of carbohydrate hydrolyzing enzymes activity :

The method 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).

#### B- Determination of acid and alkaline phosphatase activities

Acid phosphatase (AcP) and alkaline phosphatase (AlkP) activities were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown colour is produced.

## **Results and Discussion**

#### **Toxicological study:**

Table (2) show the toxicity effect of three insecticides against the third larvae instar of T.absoluta larvae, the values of LC<sub>50</sub> for methoxyfenzoid, indoxacarb and emmamectin benzoate (50.77 ppm., 2.33 ppm. and 0.022 ppm.), respectively. The slope values were (1.85, 1.87 and 1.4), respectively. Resistance ratios (RR) for methoxyfenzoid, indoxacarb and emmamectin benzoate were determined using the most susceptible population as reference. RR values were (18.8, 4.12, and 1.83), respectively.

Table 2. Resistance ratio (RR) of the third larvae instar larvae of tomato pinworm Tuta absoluta (Meyrick) tested by three different.

Tested	field	strain		Suscept	ible strain		- RR		
insecticides	LC <sub>50</sub> (95% FL)	Slope ± SE	χ2(df)	LC <sub>50</sub> (95% FL)	Slope± SE	χ2(df)	ЛЛ		
Methoxyfenzoid	50.77 (37.46 - 67.39)	1.857±0.29	2.805(3)	2.699(20.8-34.5)	2.2±0.32	2.7(3)	18.88		
Indoxicarb	2.33 (1.6 - 3.08)	1.873±0.30	1.057(3)	0.565(0.392-0.759)	1.7±0.29	0.56(3)	4.12		
Emmamectin benzoat	0.022 (0.014 - 0.03)	$1.400\pm0.27$	1.712(3)	0.012(.009-0.016)	1.7±0.28	1.67(3)	1.83		

The population consider susceptible to insecticides because RR was low (under 5 fold) according to Roditakis et.al., (2013). From the previous results it seems that emmamectin benzoate most efficient than the others, Methoxyfenzoid were less efficient. Contijo et.al., (2013) and Roditakis et.al., (2013) found that LC50 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 searches noted that indoxacarb were potent with other insect pests.

## **Biochemical study:**

## Effect on carbohydrate enzymes activity:

As show in table (3), larvae treatment with  $LC_{50}$  of methoxyfenzoid caused a significant decrease in trehalase and amylase activity (-20.18 %, 12.85 %), respectively comparative with than control. However, activities of invertase were slightly decreased by -0.35% than the control, respectively. on the other hand treatment by indoxacarb caused a much increase of trehalase activity about 17.93% and slight increase in invertase and amylase activities, (2.51%, 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.

 Table 3. Enzyme activity for Amylase, Trehalase and Invertase at homogenate of the third larvae instar of *Tuta absoluta* (Meyrick) treatment with LC<sub>50</sub> of each tested insecticide.

Carbohydratase activities (µg glucose / min /g body weight)											
Trehalase	(Mea	an± SE)	%	Invertase	(Me	an ± SE)	%	Amylase (	Mear	n ± SE)	%
445.36	±	0.10	-2.96	611.56	±	0.28	0.98	691.27	±	0.20	-25.42
366.31	±	0.41	-20.19	603.49	±	0.21	-0.35	808.00	±	0.25	-12.85
541.26	±	0.16	17.93	620.84	±	0.27	2.51	927.77	±	0.25	0.039
458.96	±	0.93		605.63	±	0.25		928.91	±	0.33	
	<b>Trehalase</b> 445.36 366.31 541.26	Trehalase         (Mea           445.36         ±           366.31         ±           541.26         ±	$\begin{array}{c cccc} \hline {\bf Trehalase} & ({\bf Mean \pm SE}) \\ \hline 445.36 & \pm & 0.10 \\ 366.31 & \pm & 0.41 \\ 541.26 & \pm & 0.16 \\ \end{array}$	$\begin{array}{c cccc} \hline {\textbf{Trehalase}} & (\textbf{Mean} \pm \textbf{SE}) & \textbf{\%} \\ \hline 445.36 & \pm & 0.10 & -2.96 \\ 366.31 & \pm & 0.41 & -20.19 \\ 541.26 & \pm & 0.16 & 17.93 \\ \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$						

\* % (decrease or increase)

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  $\alpha$ -amylase than host plant, Esmaeily, and Bandani, (2015).

#### Effect on acid and alkaline phosphatase:

The activity of acid and alkaline phosphatase was being (36.09 and 26.05) µg phenol/ml/min, respectively in untreated T. absoluta the third larvae instar larvae. Data in table (4), showed the activity of acid phosphatase that was much higher in larvae treated with indoxacarb than treatment with emmamectin benzoate, methoxyfenzoid, 38.92, 31.82 and 24.00 (µg phenol/ml/min), respectively. Alkaline phosphatase activity was reduced to 22.35 (µg phenol/ml/min) in larvae treated with Indoxacarb, making a (-14.2)% decrease. Alkaline phosphatase activity recorded a significant distinguishable increase to 35.42 µg (35.9% phenol/ml/min increases) treatment bv methoxyfenzoid when compared with the control.

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 LC50 and the level of alkaline phosphatase decreased (49%), Koodalingam et.al. 2012.

Table 4. Phosphatase activities at homogenate of the the third larvae instar of *Tuta absoluta* (Meyrick) treatment with  $LC_{50}$  of each tested insecticide.

Phosphatase activities (µg phenol/min/g body weight)									
insecticides	AcP act	ivity (Mo	ean ± SE)	%	AlP acti	vity (M	ean ± SE)	%	
Emmamectin benzoat	31.82	±	0.85	-11.8	26.76	±	0.80	2.73	
Methoxyfenzoid	24.00	±	0.46	-33.5	35.42	±	0.91	35.9	
Indoxicarb	38.92	±	0.96	7.83	22.35	±	0.49	-14.2	
Control	36.09	±	0.92		26.05	±	0.52		

\*% (decrease or increase)

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.*, (2015). Other organism also can interrupt by the insecticide exposure, for instance endosulfan, Maneb and Mancozeb on *Bacillus subtilis*  $\alpha$ -amylase was also seen at very low concentrations (0.048 ppm), Guven 2003.

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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 failing to control pest and must be incorporated in integrated pest management (IPM).

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سمية بعض المبيدات الحشرية على حفار أوراق الطماطم (Lepidoptera: Gelechiidae ) وعلاقتها بالكربو هيدرات وإنزيمات الفوسفاتيز هبه عبد الجليل الغنام و سامح مصطفى عبد النبى معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى – الجيزة

فى هذه التجارب تم دراسة سمية ثلاثة مبيدات حشرية من مجموعات مختلفة اندوكساكارب، ايمامكتين بنزوات وميثوكسى فينوزيد، على حفار أوراق الطماطم التى تم الحصول عليها من الحقل. وأظهرت النتائج التي تم الحصول عليها ان للمبيدات حشرية ضد العمر البرقى الثالث *T.absoluta وك*انت قيم (LC<sub>5</sub>) الجرعة النصفية المميته لميثوكسى فينوزيد، اندوكساكارب و ايمامكتين بنزوات هى (٥٩,٧٥ جزء في المليون، ٢,٣٣ جزء في المليون. و ٢،٢٠ جزء من المليون ) على التوالي. ايمامكتين بنزوات و اندوكساكارب أكثر كفاءة من ميثوكسى فينوزيد. وأظهرت نائج التحليل الكيمياتي زيادة كبيرة في نشاط انزيم التريهالوز وانزيم لفوسفاتيز الحامضي ( ١٧,٩٣ جزء في المليون ) على التوالي. ايمامكتين بنزوات و اندوكساكارب أكثر كفاءة من ميثوكسى فينوزيد. وأظهرت نتائج التحليل الكيمياتي زيادة كبيرة في نشاط انزيم التريهالوز وانزيم لفوسفاتيز الحامضي ( ١٧,٩٣ ٪ )، على التوالي و زيادة طفيفة في نشاط انزيم الانفرتيز و الأميليز ( ٢,٥٩ ٪ ، و ٩٠,٠٠ ٪ ) على التوالي . و ١٩٠٢ الماليون ب