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The Activity of Some Detoxification Enzymes in *Spodoptera Littoralis* (Boisd.) Larvae (Lepidoptera – Noctuidae) Treated With Two Different Insect Growth Regulators

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ABSTRACT

The susceptibility to IGRs, moulting hormone agonist, mimic (tebufenozide) and chitin synthesis inhibitors match (lufenuron) was studied in the 2nd and 4th larval instars of the cotton leafworm *Spodoptera littoralis* (Bosid). Obtained results revealed that match was the more toxic against 2nd larval instars at sub-lethal concentrations LC₂₅ and LC₅₀ (0.3 and 0.6 ppm, respectively) than mimic (1.1 and 1.5ppm, respectively). But LC₉₀ level was the same in the two IGRs. With respect to 4th larval instar, match induced the higher toxic effect at all sub lethal doses than mimic. Biochemical analysis showed that match and mimic exhibited a severe reduction in the activities of the detoxification enzymes, acid phosphatase and esterases (α and β), as compared to the control. The enzymatic activities were inhibited with the increase in the time post-treatment and also with the increase in dose. Therefore, the tested IGRs, tebufenozide and lufenuron, may be not detoxify by these enzymes.

Keywords: IGRs, Toxicity, Detoxification Enzymes, *Spodoptera littoralis*

INTRODUCTION

Among all cotton pests in Egypt, the cotton leaf worm, *Spodoptera littoralis*, is the most important. It is extremely polyphagous, and always appropriate to inflict excessive damage when it occurs in masses during certain years, commonly referred-to as “cotton worm monsoons (Amin and Salam, 2003). But the intensive use of broad-spectrum insecticides against *S. littoralis* has led the development of resistance to many registered pesticides for its control (Aydin and Gu˘ rkan, 2006) including IGRs (Temerak, 2002). IGRs differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system (Oberlander, *et al.*, 1997). Their

comprehensive effects and high selectivity as well as lower toxicity to non-target animals and the environment provide new tools for integrated pest management (Huang, *et al.*, 2008).

IGRs can be grouped according to their mode of action as: chitin synthesis inhibitors and substances that interfere with the action of insect hormone (i.e. JHa, and ecdysteroids) (Tunaz, H. and N. Uygun, 2004). Tebufenozide (mimic) belongs to a class of insect growth regulators (IGRs), bisacylhydrazine ecdysteroid agonists, mimicking the natural insect moulting hormone 20-hydroxyecdysone (20-E) (Dhadialla, *et al.*, 1998). Several studies have reported on the sublethal effects of ecdysteroid agonists on different important pest species from various insect orders (Knight, 2000; Sun, *et al.*, 2003). Due to a high specificity of ecdysteroid action

against Lepidoptera, the use in integrated pest management (IPM) is possible since the compound is selective for several beneficial insects (Smagghe, *et al.*, 2004). The lufenuron (match) is insect growth regulator that interferes with chitin synthesis, disrupt hormonal balance with exchanging in molting process, and inhibit the insect's growth (Oberlander and Silhacek, 1998).

The ineffectiveness of insect growth regulators in controlling insect pests, is attributed to the increased levels of enzymatic detoxification, (Biddinger, *et al.*, 1996). There is a relationship between the increase of insecticide resistance and the activity of detoxification enzymes (Xin-Ju and Hui-Min, 2011). General esterases are a large and diverse group of hydrolases that hydrolyze numerous substrates, including esters, and have no specific substrate (Walker & Mackness, 1983). A member of the esterase cluster probably plays a role in the detoxification of xenobiotic esters (Gacar and Taskan, 2009). Increased esterase activity is a major mechanism of insecticide insensitivity or even resistance in many insect species (Zhou, *et al.*, 2002).

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). Phosphatases are capable of transphosphorylation in addition to hydrolysis. Phosphatases thus play an important role in the metabolism of carbohydrates, phospholipids and nucleotides. Acid phosphatase is important in biological processes that need high level of energy, such as development, growth, gamete's maturation and histolysis (Ray, *et al.*, 1984).

The objective of this research was to assess the effect of tested IGRs: moulting hormone agonist, mimic (tebufenozide) and chitin synthesis inhibitors, benzoylphenylurea derivative, match

(lufenuron) on the activity of esterases (α - and β) and acid phosphatases of both 2nd and 4th instar larvae of the cotton leafworm *S. littoralis* (Boisd.) These enzymes were selected as an indicator of insecticide detoxification is based on their importance in the insecticide resistance. Further, the study of enzymes involved in the detoxification mechanism will help us to introduce appropriate control measures such as combinations of insecticides or synergists for a better and effective control programme during outbreaks of the pest.

MATERIAL AND METHODS

Test insect:

The culture of the cotton leafworm, *S. littoralis* (Bosid) was initiated from freshly collected egg masses supplied from the division of cotton leafworm, of Plant Protection Research Institute, Dokki, Egypt, formed the basis of a culture designed to provide insects used in the present investigation. Larvae reared on castor leaves and maintained under laboratory conditions of 27 ± 2 C° and R.H. 70 ± 5 % according to Bakr, *et al.*, (2010).

Tested Compounds:

One moulting hormone agonist, ecdyson agonist, mimic10% (tebufenozide) and the other chitin synthesis inhibitors, match5% (lufenuron) were used in this study

Susceptibility of cotton leafworm to IGRs:

Newly moulted 2nd and 4th larval instars were segregated from the stock colony in clean glass Petri dishes and starved for 24 hrs. Pieces of castor been leaves, were treated by the leaf-dipping technique in the different concentrations of tested compounds according to Bakr, *et al.*, (2010). Five concentrations of each IGRs (match and mimic) were used. Eighty of starved larvae, distributed in four replicates (20 larvae/replicate). Final mortality percentages of the treated and control larvae were recorded 24 hrs post-treatment. The data of susceptibility test

were subjected to probit analysis (Finney, 1971) to evaluate the values of LC_{25} , LC_{50} and LC_{90} .

Estimation of enzymatic activities:

Experiments were carried out to evaluate the effect of the tested IGRs residue on some detoxification enzymes of cotton leafworm. The castor bean leaves smeared with LC_{25} , LC_{50} and LC_{90} ppm concentrations were fed to larvae as 2nd and 4th instars. Acid phosphatase and esterases (α and β) were measured at different time intervals (6-12- 24-48 hrs) post treatment. The tested and control larvae were homogenized in distilled water (5 larvae/5 ml) using a chilled glass Teflon tissue grinder for 3 minutes. Homogenates were centrifuged at 3500 r.p.m for 10 minutes at 2C° in a refrigerated centrifuged. Phosphatases

(Acid and alkaline) and esterases (α and β) were determined according to the methods described by Powell and Smith (1954) and Van Asperen (1962), respectively.

RESULTS

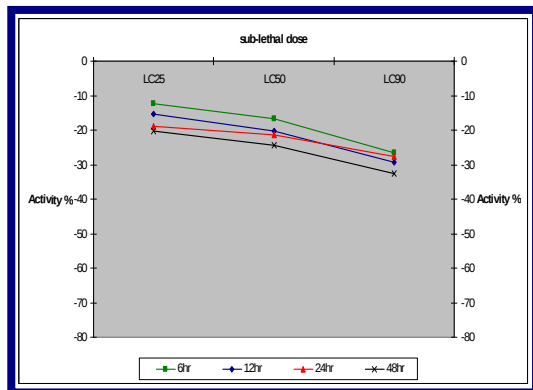
Toxicological activities of the tested IGRs against 2nd and 4th instar larvae are summarized in Table (1). Toxicity bioassays showed that match was the more toxic against 2nd larval instars at sub-lethal concentrations LC_{20} and LC_{50} (0.3 and 0.6 ppm, respectively) followed by Mimic (1.1 and 1.5ppm, respectively). Obtained results also, showed that the LC_{90} level was the same in match and mimic (2.5ppm). With respect to 4th larval instar, match induced the higher toxic effect at all lethal doses than mimic.

Table 1: Toxicity data of the tested IGRs against 2nd and 4th larval instar of *S. littoralis*.

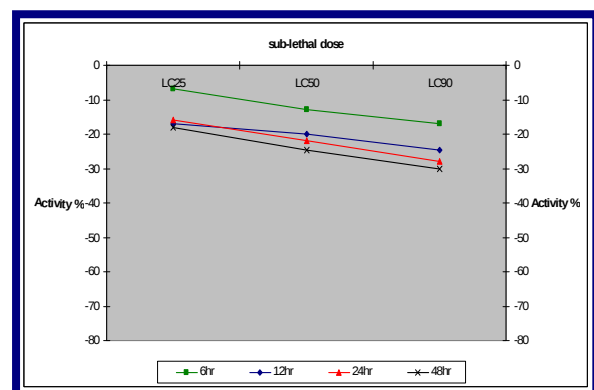
IGRs	Larval instar					
	2 nd instar			4 th instar		
Conc. (ppm)	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}
Match	0.3	0.6	2.5	1.1	2	6.3
Mimic	1.1	1.5	2.5	6.1	8.4	15

The influence of the two IGRs, mimic (tebufenozide) and match (lufenuron) on the activity of esterases (α -an β -esterases) and acid phosphatases of both of 2nd and 4th instar larvae of the cotton leafworm *S. littoralis* was

investigated. Results in Fig. (1A and B) showed inhibition in the activity of ACP in larvae (2nd and 4th) treated with mimic with the increase in the time post-treatment and also with the increase in dose.



(A)

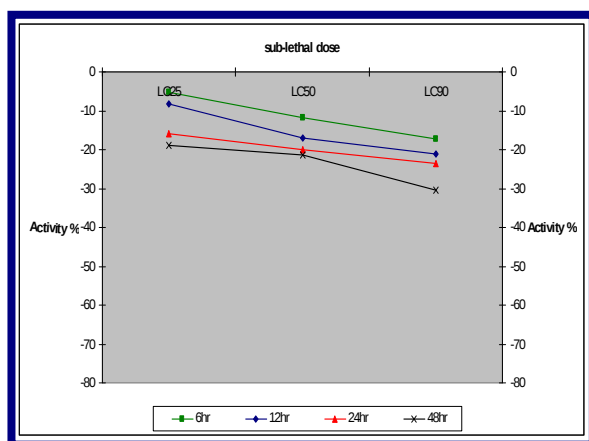


(B)

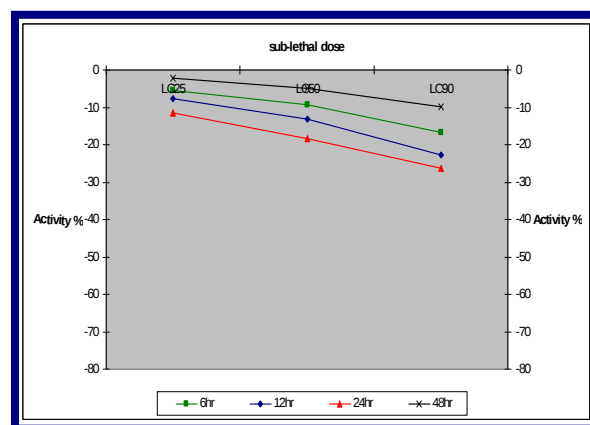
Fig. 1: Acid phosphatase activity of 2nd (A) and 4th (B) *S. littoralis* larval instars treated with Sub-lethal doses of Mimic at different time intervals.

The same effect of mimic on the activities of alpha esterase (Fig. 2A and B for 2nd and 4th larval instars, respectively) and beta esterase (Fig. 3A and B) for 2nd and 4th larval instars,

respectively) was observed as the activities of all enzymes decreased with the increase of doses and post time treatments.

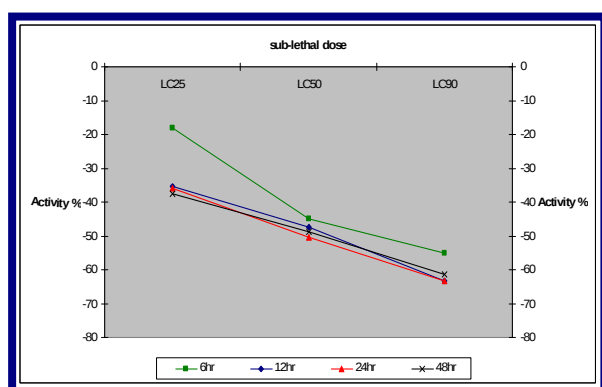


(A)

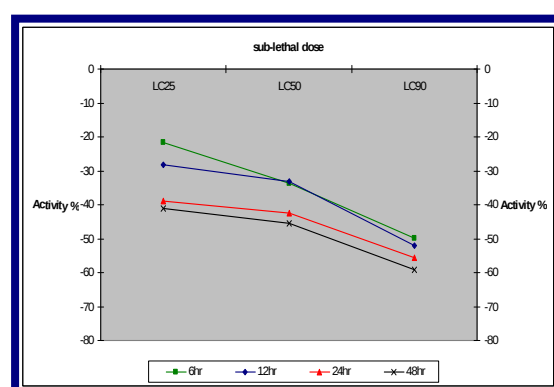


(B)

Fig. 2: α -esterase activity of 2nd (A) and 4th (B) *S. littoralis* larval instars treated with Sub-lethal doses of Mimic at different time intervals.



(A)



(B)

Fig. 3: β -esterase activity of 2nd (A) and 4th (B) *S. littoralis* larval instars treated with Sub-lethal doses of Mimic at different time intervals.

Results obtained in Fig. (4A) declared that treated 2nd larval instar with match induced a decreasing in acid phosphatase activity as the dose increase and the time post-treatment increase. Treated 4th larval instar with sub-lethal doses showed a reduction in the activity of the same enzyme (Fig. 4B). The activities of alpha esterase (Fig. 5 A and

B) in 2nd and 4th larval instars, respectively decreased also as the time post-treatment increase and with the increasing of the doses of match. The same result of severe reduction in the activity of beta esterase was observed in case of treatment of 2nd and 4th larval instars (Fig. 6 A and B, respectively).

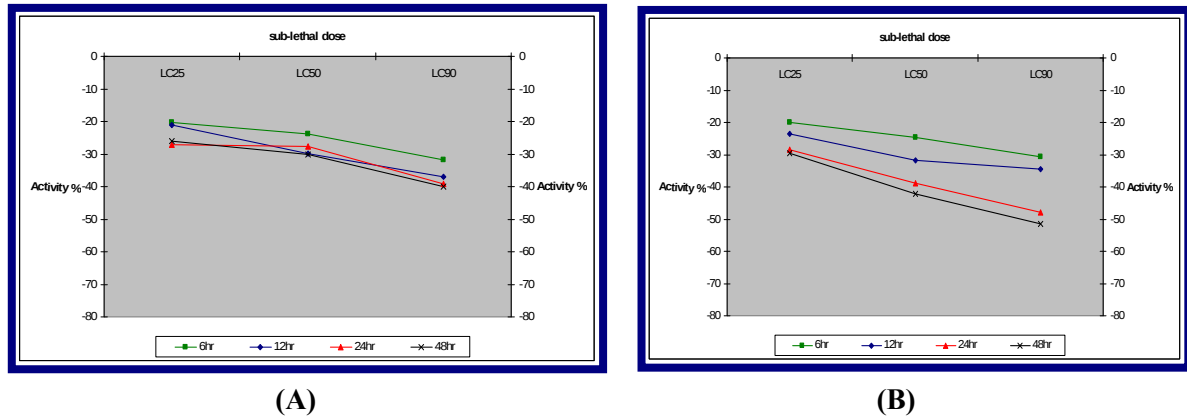


Fig. 4: Acid phosphatase activity of 2nd (A) and 4th (B) *S. littoralis* larval instars treated with Sub-lethal doses of Match at different time intervals.

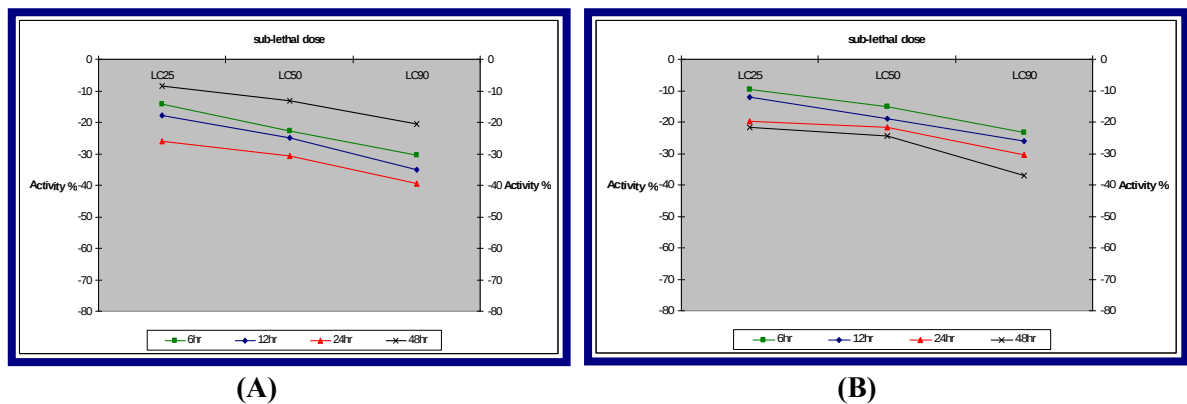


Fig. 5: α-esterase activity of 2nd (A) and 4th *S. littoralis* larval instars treated with Sub-lethal doses of Match at different time intervals.

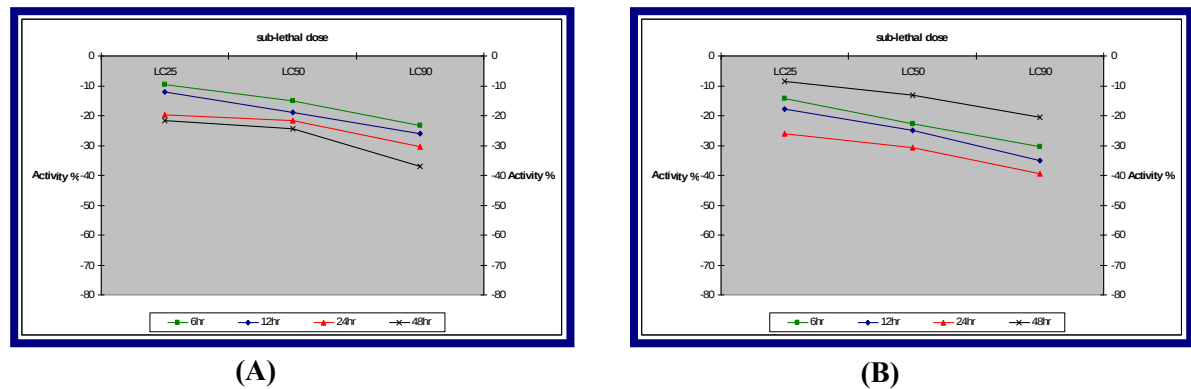


Fig. 6: β-esterase activity of 2nd (A) and 4th (B) *S. littoralis* larval instars treated with Sub-lethal doses of Match at different time intervals.

DISCUSSION

These results revealed that match was more toxic against the 2nd and 4th larval instars of *S. littoralis* at LC₂₀ and LC₅₀ than mimic and their toxicity increase depending on the concentration and the time after treatment. Lethal

concentration with 20, 50 and 90% kill values were used in the evaluation of their biochemical activity against two different detoxifying enzyme systems. Generally, effect of IGRs on detoxification enzymes in large group of pests was tested by many authors. El-Bermawy, (1994) had

perceived suppression in acid phosphatase activity in larval, pupal and adult stage of *Musca domestica* treated with BAY SIR 8514 and pyriproxyfen. The sublethal effects of methoxyfenozide on detoxification enzymes in *Spodoptera litura* were also studied by Jian-jun, *et al.*, (2009). After exposure to methoxyfenozide at LC₁₀ for 24 hrs and LC₃₀ for 48 and 96 hrs, the activities of esterase were significantly increased compared with control. The activities of microsomal multifunctional oxidase and glutathione S-transferases were also significantly enhanced when 3rd instar larvae were treated with methoxyfenozide at LC₁₀ for 24 h and LC₃₀ for 96 hrs, respectively. Assar, *et al.*, (2010) reported that match induced increase in the activity of acid phosphatase while mimic induced a significant reduction in the same enzyme in the house fly, *Musca domestica*. Our finding agree with the results were found by Bakr, *et al.*, (2010). They studied the effect of the sublethal doses LC₂₅, LC₅₀ and LC₉₀ of flufenoxuron (Cascade) on the activity of detoxification enzymes, acid phosphatase and the non-specific esterases (α , β esterases), of 2nd and 4th larval instars of *S. littoralis*. Their results showed that the activity of all enzymes decreased significantly in treated larvae at different times intervals post treatments.

Detoxification enzymes in insects are generally demonstrated as the enzymatic defense against foreign compounds and play a significant roles in maintaining their normal physiological functions (Li and Liu, 2007). Our results suggest that the two tested IGRs; moulting hormone agonist, mimic (tebufenozide) and chitin synthesis inhibitors, match (lufenuron) worked more or less in a similar manner on the activity of the tested detoxification enzymes acid phosphatase and esterases (α - and β -esterases). Their activities were dependent on the time and the pesticide dose. Generally, match and mimic exhibited a severe (high significant)

reduction in the activities of all enzyme as compared to the control.

The primary routes of insecticide resistance in all insects are alterations in the insecticide target site or changes in the rate at which the insecticide is detoxified. So far esterases, are known to be involved in the detoxification of the major groups of insecticides (Zhou *et al.*, 2002; Herron, *et al.*, 2004 and Pethuan, *et al.*, 2007). Also acid phosphatase (ACP) plays an important role in the detoxification process of toxic compounds entering the body (Zheng, *et al.*, 2007). The insect esterases can either cause broad-spectrum resistance to various insecticides through rapid binding and slow turnover of insecticide molecules (i.e., sequestration) or cause narrow-spectrum resistance to a very restricted range of insecticides containing a common ester linkage, such as malathion, through rapid metabolism of the insecticides (Karunaratne, *et al.*, 1995).

The suppression of detoxification enzymes indicated that these enzymes play no role in the detoxification of tested compounds and may be increase the susceptibility of insect pest to these insecticides (Abd-Elaziz and El-Siad, 2009). In conclusion, our results indicated that the tested IGRs tebufenozide and lufenuron induced a reduction in the activities of acid phosphatase and alpha and beta esterases. Therefore, the tested IGRs, tebufenozide and lufenuron, may be not detoxify by these enzymes.

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ARABIC SUMMERY

نشاط بعض الإنزيمات في إزالة السموم من يرقات دودة ورق القطن المعاملة يانثين من منظمات نمو الحشرات

رضا فضيل على بكر^{1,2}، منى قوزى عبدالعزيز²، نهاد محمد البرقي²، محمد عواد³، هشام محمد عبدالحليم²

1- قسم الحشرات – كلية العلوم – جامعة عين شمس

2- قسم الحشرات – كلية العلوم – جامعة بنها

3- قسم الحيوان – كلية العلوم – جامعة بنها

4- قسم الاحياء – كلية العلوم – جامعة الملك خالد – ابها – المملكة العربية السعودية

تناولت هذه الدراسة حساسية العمر اليرقي الثاني و الرابع من دودة ورق القطن الكبرى *سيودوتيرا ليتوراليس* لاثنتين من منظمات النمو، التيفنزويد اوالممك (مشابه لهرمون الانسلاخ) و اللوفيرنون اوالماتش (مثبط تكوين الكيتين). واثّر ذلك على بعض الانزيمات المضادة للسمية. وظهرت النتائج ان الماتش كان اكثر سمية ضد العمر اليرقي الثاني عند الجرعات LC_{25} و LC_{50} بينما تساوت نسبة الاماتة عند جرعة LC_{90} لكلا المبيدين. اما بالنسبة للعمر اليرقي الرابع فقد اظهرت النتائج ان هذا العمر أكثر حساسية للماتش عن الممك عند جميع الجرعات. كما اظهر التحليل البيوكيميائي انخفاض معنوي في نشاط بعض الانزيمات المضادة للاكسدة (الفوسفاتيز الحمضي والفا وبينا استيرز) في اليرقات المعاملة بكلا النوعين من منظمات النمو. وذلك بزيادة الجرعة وزيادة وقت مابعد المعاملة. ونستخلص من هذه النتائج ان هذه الانزيمات قد لاتلعب دورا في ازالة التسمم بهذه الانواع من منظمات النمو.