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Carbohydrate Hydrolyzing Enzymes As A Target for Pink Bollworm *Pectinophora gossypiella* (Saund.) Control Agents

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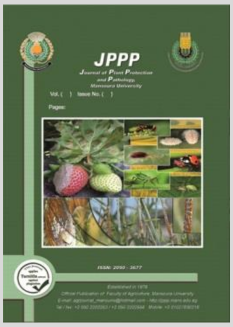

ABSTRACT

Most insect species depend on the digestive enzymes (invertase, trehalase and amylase) to utilize their uptake of carbohydrates. In the present investigation the activities of these enzymes are used as parameters to study the effect of the selected insecticides [*i.e.* Teflubenzuron (Nomolt 15%), emamectin benzoate (Proclaim 5% SG); azadirachtin (Saif oil 0.03% EC) and spinosad (Tracer 24% SC)] on pink bollworm, *Pectinophora gossypiella*. Results showed that invertase had the highest activity (62.03 mg glucose released/gm body weight/min), while amylase had the lowest one (37.91 mg glucose released/gm body weight/min). Treatment caused a reduction effect on the carbohydrate hydrolyzing enzymes, except in the case of trehalase whereas its activity increased significantly only by Saif oil. Tested insecticides could be arranged according to their percentages of changes in the enzymes activities as follows: Nomolt & Tracer > Proclaim > Saif oil. The present study clearly showed that the amylase enzyme appeared to be the most affected enzyme with high level of significant reduction followed by invertase than trehalase, these percentages were -83.42, -48.56 & -17.56; -73.45, +0.70 & -15.55; -19.91, -24.40 & +73.85 and -75.52, -69.01 & 53.55% for Nomolt, Proclaim, Saif oil and Tracer compounds, respectively.

Keywords: *Pectinophora gossypiella*, PBW, mid gut, biochemical and histological aspects.

INTRODUCTION

Chemical control is the traditional method for controlling pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), all over the world. This traditional method has a lot of disadvantages. In this situation, using new categories of insecticides which disrupt the target pest physiological processes, could be valuable as an alternative in the integrated pest management programs (Smagghe *et al.*, 2003). One of such physiological processes that gained attention in the recent years is the inhibition of carbohydrates hydrolyzing enzymes (amylase, trehalase and invertase), and subsequently their latent effects on the insect growth and biology (El-Barky *et al.*, 2008; Gaaboub *et al.*, 2012; Ibrahim *et al.*, 2014 and Assar *et al.* 2016 and Mohammadzadeh & Izadi, 2018). Having this kind of insecticides is desirable because it might be having a selectivity for target, not to non-target organisms. Also, studying insect's digestive enzymes could help in developing biotechnological processes to find new enzyme inhibitors or provide resistant varieties of host plants.

Inhibiting of these enzymes in some insect is reached to practical step in the insect management (Jouanian *et al.*, 1998).

Comprehensive knowledge about the carbohydrates hydrolyzing enzymes of *P. gossypiella* could offer new opportunities for a workable pest management for controlling this pest. Thus, this study is an attempt to understand the behavior of the carbohydrates hydrolyzing enzymes in full-grown larvae of *P. gossypiella* after exposure to some selected insecticides [*i.e.* Nomolt, Proclaim; Saif oil and Tracer] to reach a better understanding of the physiology of digestive and its relation to some biological aspects. This understanding may be hopefully lead to new strategies for control management of this pest.

MATERIALS AND METHODS

1. Insect: The laboratory strain of newly hatched larvae of the pink bollworm (PBW), *P. gossypiella*, used in this study was obtained from a laboratory colony of Bollworms Research Department, Plant Protection Research Institute; Agricultural Research Center (ARC), reared for several generations away from any contamination with insecticides and maintained at 26 ± 1ºC temperature, 70 ± 5% RH. The pink bollworm larvae were reared on a modified artificial diet as described by Rashad & Ammar (1985).

2. Insecticides: Four selected insecticides [*i.e.* Teflubenzuron (Nomolt 15%), emamectin benzoate (Proclaim 5% SG); Azadirachtin (Saif oil 0.03% EC) and Spinosad (Tracer 24% SC)] were obtained from their corresponding manufacturers and to use in the present studies.

3. Biochemical studies:

1. Sample for biochemical assay: to access the latent biochemical effects of the tested insecticides, newly hatched larvae (≈100 larvae) of PBW were allowed to feed on an artificial diet containing LC_{50} of the tested insecticides which estimated previously (El- Shennawy & Kandil, 2017), and then transferred to feed on control diet. Ten days after treatment, batches of treated larvae

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were homogenized in distilled water using a Teflon homogenizer. The homogenates were centrifuged at 500 rpm for 10 minutes at 5º C. The supernatants were immediately assayed to determine the biochemical analysis under the study. Untreated larvae were used as control.

2. **Technique of biochemical analysis:** The method used to determine the digestion of trehalose and sucrose by trehalase and invertase enzymes, respectively were described by Ishaaya & Swiriski (1976).

4. **Statistical analysis**: Analysis of variance (ANOVA) was conducted using Costat program software computer. Significant between differences were determined according to LSD values of Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUTION

1. Biochemical studies:

In insect, carbohydrates are of vital importance. It can be utilized by the insect body for energy production or conversion to proteins or lipids. Carbohydrates metabolism is mainly controlled by amylase, invertase and trehalase enzymes (Wigglesworth, 1972). In the current study, the activities of the carbohydrate hydrolyzing enzymes were used as a parameter to study the effect of the selected insecticides. Enzymes activities were investigated in the 10-days old larvae of PBW after treatment as a newly hatched larvae with the LC_{50} of the aforementioned insecticides. Results showed in Table (1) and illustrated in Figures (1, 2 & 3).

Table 1. Effect of LC_{50} of the tested compounds on amylase, invertase and trehalase activities (mg glucose released/gm body weight/min) of *P. gossypiella* larvae

Treatment	Amylase		Invertase		Trehalase		Mean	LSD (5%)
	Mean ± SD	%Change	Mean ± SD	%Change	Mean ± SD	%Change		
Control	$76.50^{Ba} \pm 4.40$	—	$138.67^{Aa} \pm 3.51$	—	$63.66^{Cb} \pm 5.81$	—	$92.96^{a} \pm 34.97$	9.37
Nomolt	$12.68^{Cd} \pm 0.40$	-83.42	$71.33^{Ac} \pm 7.04$	-48.56	$52.48^{Bb} \pm 6.76$	-17.56	$45.50^{c} \pm 26.39$	11.27
Proclaim	$20.31^{Cc} \pm 2.73$	-73.45	$139.64^{Aa} \pm 3.05$	+0.70	$53.76^{Bb} \pm 3.08$	-15.55	$71.24^{b} \pm 53.37$	51.92
Saif oil	$61.27^{Bb} \pm 2.31$	-19.91	$104.84^{Ab} \pm 7.75$	-24.40	$110.67^{Aa} \pm 21.83$	+73.85	$92.26^{a} \pm 26.11$	26.85
Tracer	$18.73^{Cc} \pm 1.71$	-75.52	$42.97^{Ad} \pm 3.94$	-69.01	$29.57^{Bc} \pm 2.10$	-53.55	$30.42^{d} \pm 10.78$	5.52
Mean	$37.91^{C} \pm 26.89$		$99.49^{A} \pm 39.45$		$62.03^{B} \pm 29.14$			
LSD (5%)	4.85		9.86		19.42			

Means within columns and rows with same letter (s) are not significantly different from each other at 5% level of probability.
Capital letter row
Small letter column

% Change $= \frac{Treatment - control}{Control}$ **X 100**

According to the results presented in Table (1) and illustrated in Figure (1) revealed that the invertase had the highest activity (total mean = 99.49 mg glucose released/gm body weight/min) followed by trehalase (total mean = 62.03 mg glucose released/gm body weight/min) then amylase (total mean = 37.91 mg glucose released/gm body weight/min). These results agree with that of Gaaboub *et al.* (2012) whereas the mean of the enzymes' activities in *Spodoptera littoralis* (Boisd.), were 1.636, 0.697 and 0.261 µg Glu/g body weight/min for invertase, trehalase and amylase, respectively. Also, the results agree with Ibrahim *et al.* (2014) and Assar *et al.* (2016) on *S. littoralis* and Shaurub & Abd El-Aziz (2015) on *Culex pipiens* (Linnaeus), According to Ishaaya & Swirski (1976), at optimal conditions, both invertase and trehalase activities in young females of the black scale, *Saissetia oleae* (Olivier), were about 8-fold much higher than that of amylase. They mentioned that this is indication to the significance of these enzymes in digestion of food and energy supply. Also, they stated that the host plants greatly affect the enzymes activities.

On the other hand, the present results don't agree with that of Taha & Al-Hadek (2016) whereas they found that *Tuta Absoluta* (Meyrick), had a higher amylase activity, followed by invertase then trehalase. These fluctuated results might be due to the variance in treatments, instar, tested times and used concentrations (Mohamady, 2000). Abdel-Fattah *et al.* (1986) stated that the higher activities for the three enzymes were at the initial time interval than the last one. While Azab *et al.* (2011) found that the activities of amylase and trehalase of whitefly *Bemisia tabaci* (Genn.) and aphids *Aphis gossypii* (Glover) were higher in the initial time, but the opposite is true in the case of invertase. These results are in accordance with recent results whereas the estimation of enzymes activities done on the full-grown larvae (Figure 1). Also, Żółtowska *et al.* (2012) found that in *Apis mellifera carnica* Pollman, amylase and trehalase had their highest activities in unsealed larvae and the lowest activities in prepupae then followed by an increase in enzymatic activities. Intense increases were occurred especially in the last stage of pupae and newly emerged imago. Rajitha & Savithri (2014) recorded increasing trend in the amylase activity up to 5th day then declined on the 6th day in the healthy larvae of *Bombyx mori*. L., Whereas the increased in trehalase activity was achieved from the 1st day to 6th day.

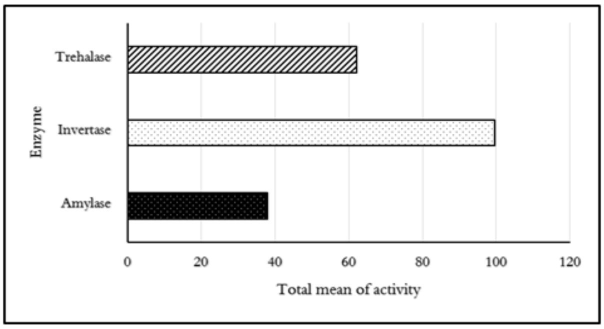

Figure 1. Total mean of the Amylase, Invertase and Trehalase activities of 10-days old *P. gossypiella* larvae treated as newly hatched with LC_{50} of tested compounds.

Since most insect species depend on the carbohydrate hydrolyzing enzymes (invertase, trehalase and amylase) to utilize the carbohydrates, the present study evaluated the effect of the tested insecticides on the enzymes' activities (Table, 1 and Figures 2 & 3).

Amylase activity: In the control check of PBW amylase activity was 76.50 mg glucose released/gm body weight/min. Tested compounds decrease significantly the amylase activity by 83.42, 73.45, 19.91 and 75.52 %, for Nomolt, Proclaim, Saif oil and Tracer, respectively. Means were 12.68, 20.31, 61.27 and 18.73 mg glucose released/gm body weight/min, respectively. Nomolt was the most effective compound in this respect, followed by Tracer and Proclaim; while Saif oil considered the least effective compound (Figure 3).

Invertase activity: Tested compounds tacked the same pattern as in amylase whereas they caused significant decrease in the invertase activity, except in the case of Proclaim where it caused increase in the enzyme activity but this increasing was insignificant. Means were 71.33, 139.64, 104.84 and 42.97 mg glucose released/gm body weight/min, for Nomolt, Proclaim, Saif oil and Tracer, respectively. Percentages of change were -48.56, +0.70, -24.40 and -69.01%, respectively. Also, it could be noticed that Tracer was the most effective compound in this respect, followed by Nomolt and saif oil (Figure3).

Trehalase activity: Insignificant decreases achieved in the case of Nomolt (52.48 mg glucose released/gm body weight/min) and Proclaim (53.76 mg glucose released/gm body weight/min). Meanwhile, Tracer significantly decreased the enzyme activity to 29.57 mg glucose released/gm body weight/min and the percentage of reduction was 53.55%. On the other hand, Saif oil significantly increased trehalse activity to 110.67 mg glucose released/gm body weight/min by 73.85% induction to the enzyme activity.

So, generally it could be concluded that tested compounds [*i.e.* Teflubenzuron (Nomolt 15%), emamectin benzoate (Proclaim 5% SG); azadirachtin (Saif oil 0.03% EC) and spinosad (Tracer 24% SC)] have a reduction effect on the carbohydrate hydrolyzing enzymes, except in case of trehalase whereas its activity increased significantly only by Saif oil. In addition, the tested insecticides could be arranged according to their percentages of changes in the enzymes activities as follows: Nomolt & Tracer > Proclaim > Saif oil (Table 1 & Figure 3).

Recent results are in agreement with that of Omer *et al.* (2006) who found that spintor decrease invertase activity in *P. gossypiella* and *Earias insulana* (Boisd.), Also, it caused abnormalities in the mid-gut tissue of larvae of the two insect species. Similar findings for *S. littoralis* were also reported by Abo El-Ghar *et al.* (1995) using abamectin, El-Barky *et al.* (2008) and Rashwan (2013) using spinetoram, and by Mead *et al.* (2008) using Tracer and triflumuron. Moreover, results of El-Didamony (2012) on newly hatched larvae of *E. insulana* showed that Proclaim decreased the activity of trehalase and invertase after ten days of treatment.

On the other hand, opposite results achieved by Dahi *et al.* (2009) who showed that avermectin derivative Methylamine avermectin (Radical 0.5% EC) increased trehalase and invertase activities estimated in 4th instar larvae of *S. littoralis*. Similar results were observed with variable insecticides on different insects by Abdel-Hafez *et al.* (1993), Mohamady (2000), Fahmy (2005), Tolba (2006) and El-Sheikh *et al.* (2013).

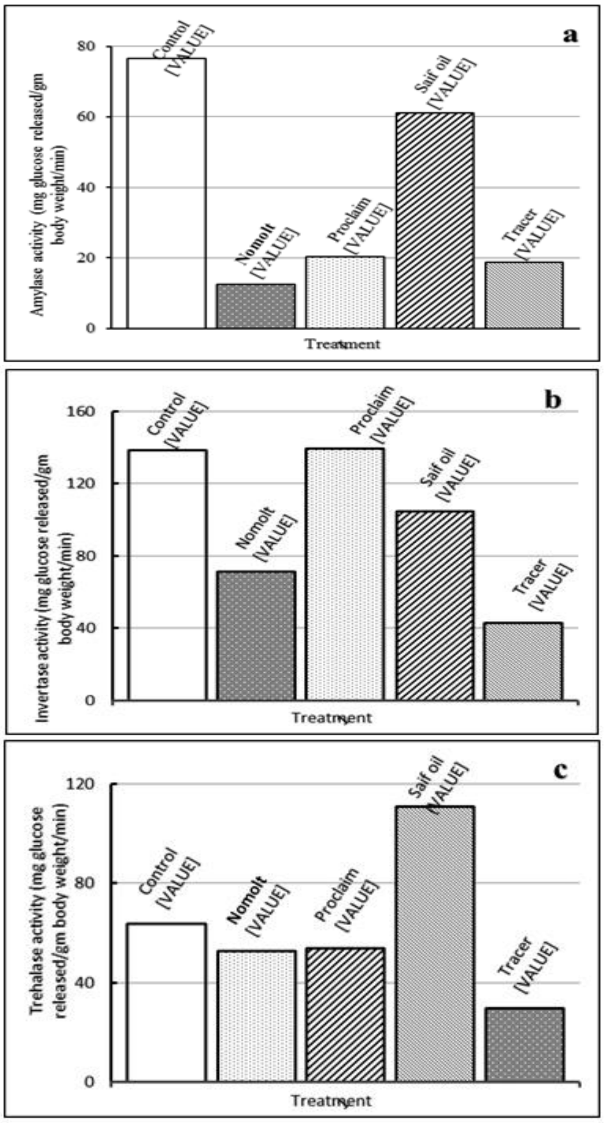

Figure 2. Amylase (a), Invertase (b) and Trehalase (c) activities of 10-days old *P. gossypiella* larvae treated as newly hatched with LC$_{50}$ of tested compounds.

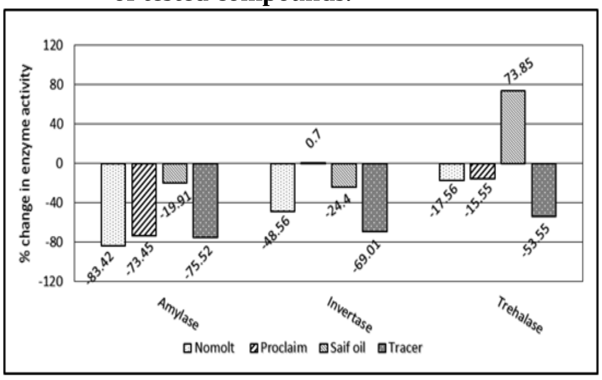

Figure 3. Percent of change (%) in the Amylase, Invertase and Trehalase activities of 10-days old *P. gossypiella* larvae treated as newly hatched with LC$_{50}$ of tested compounds.

Fluctuated results were achieved by Kandil *et al.* (2005 & 2012) who found that insect growth regulators (IGR) groups decreased the amylase activity, while increased the invertase and trehalase activities in newly hatched larvae of *P. gossypiella*. So, the activation effect of Saif oil on the trehalase activity in the present study could agree with these studies. Also, Assar *et al.* (2016) stated that teflubenzuron (as IGR), spinetoram and emamectin (as bioinsecticide) significantly increased the carbohydrates hydrolysing enzymes in the 4th instar larvae of *S. littoralis* except that for invertase by emamectin, and trehalase & amylase for spinetoram. These asymmetrical effects might be due to the alteration in treatments, instar, experiment times and the concentrations used (Abdel-Fattah *et al*., 1986; Mohamady, 2000, Heba, 2005 and Azab *et al.*, 2011).

Additionally, the present study clearly showed that the amylase appeared as the most affected enzyme with high level of significant reduction followed by invertase than trehalase. These percentages were -83.42, -48.56 & -17.56; -73.45, +0.70 & -15.55; -19.91, -24.40 & +73.85 and -75.52, -69.01 & 53.55% for Nomolt, Proclaim, Saif oil and Tracer, respectively (Table 1 & Figure 3). These results are in congruence with those of Abo El-Ghar *et al.* (1995) who observed marked decrease in the hydrolyzing enzymes of carbohydrate specifically amylase and invertase in the 5th instar larvae of *S. littoralis* after treated with sub-lethal concentrations of thuringeinsin and abamectin. Also, results of Azab *et al*. (2011) indicated that amylase in *Bemisia tabaci* (Genn.) and *Aphis gossypii* (Glover) appeared as the most affected enzyme in comparing with trehalase and invertase enzymes.

Additionally, Taha & Al-Hadek (2016) stated that emmamectin benzoate cause a slight decrease in trehalase activity by -2.96% and a slight increase in invertase activity by 0.978% and abundant decrease in amylase activity by -5.42% than that in untreated larvae of field *Tuta Absoluta*.

Generally, the predominant trend of the tested insecticides, which belong to different groups of insecticides, is the significant inhibition of carbohydrates hydrolyzing enzymes activities. This result puts the spotlight on the importance of studying the effect of control agents on carbohydrates hydrolyzing enzymes, in particular, due to the observed common effect on them despite the tested compounds belong to different groups of influence. And recently, trehalase competitive inhibitors were discovered and used in insects' control (Wegener *et al.*, 2010).

According to Isman, (2006) and Hosseini-Naveh *et al*. (2007) inactivation of digestive enzymes results in blocking of digestive enzymes, leading to poor nutrient utilization, development retardation, and death.

Carbohydrates are of vital importance to insects, where they require for energy growth, production, movement, longevity, and reproduction (Nation, 2008 and Chapman, 2012). It must be broken down to monomers like glucose to be absorbable via epithelial cells (Nation, 2008 and Sharifloo *et al.*, 2016). Some epithelium cells produce enzymes and others absorb the digested food (Terra & Ferreira 1994). Nasiruddin & Mordue (1993) reported the adverse effects of azadirachtin on midgut epithelial cells, which might disrupt enzyme secretion and nutrient absorption. Abouelghar *et al.* (2013) mentioned that the histological alterations in the larval midgut of *S. littoralis* 4th instar larvae induced by Tracer are supposed to be responsible for the decreasing in food utilization and subsequently growth. In addition, Ahmed (2020) found that Proclaim caused destruction in the epithelial columnar cells of PBW. So, destruction of the epithelial cells could disrupt enzyme secretion, meanwhile affect the metabolism of main metabolites accordingly affect the growth, longevity, development and reproduction.

Carbohydrates metabolism is controlled primarily by amylase, invertase and trehalase enzymes (Wigglesworth, 1972). Alpha-amylase (EC 3.2.1.1) is an enzyme that break down starch to oligosaccharides then hydrolyzed to glucose by glucosidases (Terra & Ferriera 2005 and Kaur *et al.*, 2014). Alpha-amylase, in physiological status, improve the insect's digestive performance, resulting in survival in different conditions and raise their biological fitness (Kaur *et al.*, 2014). As well, other physiological functions rather than digestion could be considered for α-amylase because it is active during non-feeding stages like pupa (Zhu *et al*., 2005).

Invertase enzyme (EC 3.2.1.26) hydrolyzes sucrose into monosaccharides; glucose and fructose so it could be utilized by insects (Heil *et al*., 2005). Trehalase (EC 3.2.1.28) has the vital function whereas it catalyzes the breakdown of trehalose into 2 glucose molecules. So, they activated during molting to generate glucose molecules which needs up for chitin build up. So, the defect in the enzyme's activities could impede the supply of glucose, which is needed for chitin build up (Wyatt, 1967 and Candy & Kilby, 1962). General carbohydrate metabolism disturbance expressed by reduction of carbohydrate hydrolyzing enzyme activities could be result of a series effect initiating mainly from chitin synthesis inhibition (Meisner *et al*., 1978). Hence, the inhibition of carbohydrate hydrolyzing enzymes documented in the present study might affect the molting process and then could explain the prolongation in immature stages occurred in PBW as illustrated in earlier biological studies (Kandil *et al*., 2005 & 2013; El-Barkey, 2009; El-Shennawy, 2009; Said *et al*. (2017) and El-Shennawy & Kandil (2017). For example, Teflubenzuron (Nomolt 15%) belongs to the benzoyl-urea groups of chitin synthesis inhibitors (CSIs). CSIs overlap with chitin synthesis (Gijswijt *et al*., 1979) and accordingly impede moulting, or produce a defective cuticle (Hammock & Quistad, 1981). According to Kandil *et al*. (2013) and Said *et al*. (2017) CSIs disturb deposition of chitin through ecdysis and caused malformation and prolonged larval and pupal duration. Gadallah *et al.* (2014) Teflubenzuron (Nomolt) has an important effect on development and moulting process in different stages insects. Also, it might induce irregular biological effects like hypertrehalosemia, hyperglycemia, loss of the ability to move and fly in the locust *L. migratoria* and house fly *M. domestica* (Kono *et al.*, 1994 and Wegener *et al.*, 2003), and death in the migratory locust *L. migratoria*, leaf worm moth *S. litura* and *M. brassicae* (Kono *et al.*, 1993 and Wegener *et al.*, 2003).

In conclusion, this study indicates to the central role carbohydrates hydrolyzing enzymes and its defects in the insect's physiology and subsequently the effects in growth and molting following treatment. Understanding of this is important for helping to approach a new management strategy like carbohydrates hydrolyzing enzymes inhibitors which could be planned to form the future groups of insecticides or serve in improved of genetically breeding of cotton varieties.

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الانزيمات الهاضمة للكربوهيدرات كهدف لعوامل مكافحة دودة اللوز القرنفلية *Pectinophora gossypiella* (Saund.)

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تعتمد معظم أنواع الحشرات على الإنزيمات الهاضمة (إنفرتيز، تريهاليز وأميليز) للاستفادة من الكربوهيدرات، وقد استخدم البحث الحالي نشاط هذه الإنزيمات كمعامل لدراسة تأثير المبيدات الحشرية المختارة [تيفلوبنزورون (نومولت 15٪)؛ بنزوات إيمامكتين (بروكليم 5%)؛ أذادراختين (سيف أويل 0.03%) وسبينوساد (تراسر 24٪ SC) على دودة اللوز القرنفلية، وقد أظهرت النتائج أن الانفرتيز كان أعلى الانزيمات نشاطا ، بينما كان الأميليز أقلهم نشاطا. هذا وقت تسببت المعاملة بالمبيدات المختبرة في تقليل نشاط إنزيمات التحلل المائي للكربوهيدرات بصفة عامة باستثناء التريهاليز في حالة المعاملة بالسيف أويل حيث زاد نشاطه بشكل ملحوظ، وقد أمكن ترتيب المبيدات المختبرة حسب نسب التغير الحادثة في نشاط الإنزيمات على النحو التالي: نومولت وتراسر > بروكلايم >سيف أويل. كما أظهرت الدراسة الحالية بوضوح أن الأميليز كان أكثر الانزيمات تأثرا نتيجة المعاملة حيث سجل أعلى نسبة انخفاض في نشاطه يليه الانفرتيز ثم الترهاليز، وكانت النسب كالتالي: -83.42، -48.56 و -17.56؛ -73.45، +0.70 و-15.55؛ -19.91، -24.40 و+73.85 و-75.52، -69.01 و53.55٪ لـ نومولت، بروكلايم، سيف أويل والتراسر، على التوالي.