Host-Plant Effect on Na^+, K^+-ATPase and AChE Sensitivity to Some Insecticides in *Spodoptera littoralis* Larvae.

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Received on: 8/4/2014 *Accepted: 17/6/2014*

ABSTRACT

The toxicity of cyhalothrin; chlorpyrifos, and thiodicarb insecticides were evaluated against 4th instar larvae of the cotton leafworm, *Spodoptera littoralis*. The larvae reared on different host-plants (cotton and tomato). The results showed that cyhalothrin was the most toxic compound followed by chlorpyrifos, and thiodicarb. The specific activity for Na^+,K^+-ATPase and AChE were determined. The sensitivity of Na^+,K^+-ATPase and AChE to tested insecticides were measured by the K_m; V_{max}; I_{50} and K_i values. The I_{50} values of cyhalothrin on Na^+,K^+-ATPase activity were 0.21; 0.36, and 0.55 µM for lab; El-Bustan (tomato fields), and Etai-Elbaroud (cotton fields) strains respectively. The I_{50} values of chlorpyrifos and thiodicarb on AChE activity were 0.32; 0.48; 0.67 µM, and 0.47; 0.56, and 0.70 µM for the tested three strains respectively. The K_i values of cyhalothrin on Na^+,K^+-ATPase activity were 14; 24, and 43 µM for lab; El-Bustan, and Etai-Elbaroud strains, respectively. The K_i values of chlorpyrifos and thiodicarb on AChE activity were 22; 35; 54 µM, and 36; 48; 60 µM for the tested three strains, respectively. Results proved that El-Bustan strain (tomato fields), was more sensitive against the tested insecticides than Etai-Elbaroud strain (cotton fields), this results of the present study may add some more steps to put the host-plants effect on insecticides activity, especially against this insect. So, the host-plants can be involved in important steps necessary for successful IPM programs applied against *S. littoralis*.

Key words: Na^+,K^+-ATPase- Cholinesterase activity- Cotton leafworm- host plant effect .

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* is one of the most destructive pests of numerous economic crops in the world (Azab *et al.*, 2001). Larvae of this pest can feed on -90 economically important plant species belonging to 40 families and the rate of development has a strong nutritional component (Brown and Dewhurst, 1975). Commonly, the control of such pest measures have largely been depending on the use of chemical pesticides has an important role in management insect pests attacking crops, which can easily be applied, give rapid control and have been successful against insects. Furthemore, the insecticides are the only tool for pest management that is reliable for emergency action when insect pest populations approach or exceed the action thereshold (Younis, *et al*., 2007; El-Naggar and El-Dewy, 2012, and El-Naggar, 2013). Insecticides conventional (pyrethroid; carbamate, and organophosphate insecticides) are widely used for control of the *Spodoptera littoralis* because different host plants or larval diets are known to affect susceptibility to conventional insecticides (El-Aw and Hashem, 2001; Ahmed, *et al*., 2006, and Zidan, *et al.,* 2012).

Therefore, the present study investigated the susceptibility of cotton leafworm, *Spodoptera littoralis* field strains (collected from cotton fields at Etai-Elbaroud area, and tomato fields at Abdel-Monem Ryad Village, El-Bustan area) compared with the laboratory strain to host-effect to the tested insecticides. The effect of two different host-plants on the activity and sensitivity of Na^+,K^+-ATPase and AChE to tested insecticides in both strains was investigated.

MATERIALS AND METHODS

1. Test pest:

The susceptible laboratory strain of cotton leafworm, *Spodoptera littoralis* was provided from centeral lab of pesticides, Agricultural Research Center (ARC) Cairo, Egypt which was reared for several years on artificial diet under standard laboratory conditions of 27 ± 2 °C and 65-70 % RH.

Field strains of cotton leafworm, *Spodoptera littoralis* egg masses were collected from cotton fields at Etai-Elbaroud (El-Boheira Governorate) and tomato fields at Abdel-Monem Ryad Village, El-Bustan (El-Boheira Governorate). The eggs were allowed to hatch larvae, the 4th instar larvae chosen for bioassays and biochemical assessments.

2. Chemicals and tested insecticides:

One carbamate-insecticide, thiodicarb provided as technical grade insecticide from JinHung Fin Chem. Co. LTd. Koria, one organophosphorus-insecticide, durban (chlorpyrifos 48 % EC) was obtained from Dow Chemical Co, and one pyrethroid (cyhalothrin) provided as technical grade insecticide from U.S.A. Environmental Protection Agency (EPA), were used in this study. Ouabain is a cardiac glycoside which specifically inhibits the

Na^+,K^+-ATPase (Mcllwain, 1963). A pure sample was obtained from Sigma Chem., Co. ST. loius.

3. Toxicity bioassay:

3.1. Toxicity of the tested insecticides against *S. littoralis*:

Cyhalothrin; chlorpyrifos and thiodicarb were bioassayed against the 4th instar larvae of *S. littoralis*. The castor leaves were dipped in different concentrations of the tested insecticides. Cyhalothrin and thiodicarb concentrations were prepared in pure acetone, while chlorpyrifos concentrations were prepared in distilled water. Treated and control were air-dried for 3 hrs, the treated leaves were placed in clean glass container at the laboratory conditions of 27±2 °C and 65-70 % RH. Ten larvae (lab and field strains) were used for each test with three replicate at least. Number of alive and dead larvae per each replicate was counted after 24, and 48 hr of treatment. Concentrations–mortality percentages were calculated and corrected for natural mortality according to Abbott equation (Abbott, 1925). LC_{50} values were calculated by using the of probit-analysis method of Finney (1971).

4. AChE preparation and activity assay:

Head capsoul from *Spodoptera littoralis* (fourth-instar larvae) was dissected and homogenized in Tris-HCl buffer (pH 7.4) at 30 larvae / 30 ml buffer, with polytron mixer (at 50 % power for 50 sec.), then subjected to low speed centrifuged at 5,000 rpm for 15 min at 4 °C. The resulting supernatant was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant centrifuged at 25,000 rpm for 1 hr at 4 °C. Pellets were resuspended in 1 ml of Tris-HCl buffer (pH 7.4) and stored at (-20 °C) for used as enzyme source.

The AChE activity measurement were done according to method reported by Ellman *et al*., (1961). This method is based on the hydrolysis of acetylthiocholine iodide (ATChI) as substrate by enzyme to produce thiocholine and acetic acid. Thiocholine reacts with 5,5-dithio bis-(2-nitrobenzoic acid), "DTNB" to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of color production as a function of enzyme activity is measured spectrophotometrically at λ 412 nm. Enzyme specific activity was computed as (O.D. λ_{412} / mg protein / hr).

5. Na^+,K^+-ATPase preparation and activity assay:

Head capsouls from *Spodoptera littoralis* fourth-instar larvae were dissected and homogenized in a solution of 0.32 M sucrose, 1 mM EDTA and 40 mM tris-HCl buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. Mitochondrial ATPase was prepared according to the method reported by Koch *et al*., (1969), by differential centrifugation of the homogenate at 8000 Xg for 10 min. The supernatant was then centrifuged at 20000 Xg for 30 min. The formed pellets were then suspended in the buffer and stored at (-20 °C) for use.

The ATPase activity was measured according to the method reported by Koch *et al*., (1969) with slight modification by Morshedy (1980) using tris-HCl buffer instead of imidazole buffer. Absorbancy of inorganic Phosphate (Pi) was measured at λ 750 nm (Taussky and Shorr, 1953). This method was based on the spectrophotometric determination of the inorganic Phosphate (Pi) liberated from the hydrolysis reaction of the ATP, mediated by the enzyme.

The ATPase activity was measured in total volume of 1 ml. The mitochondrial preparation was mixed with a reaction mixture (700 µl) containing 100 mM Na^+; 20 mM K^+; 5 mM Mg^{2+} chlorides; 40 mM tris-HCl buffer (pH 7.4) and 5 mM ATP. The volume was completed to 850 µl with buffer. The mixture was incubated for 15 min, in a shaking water bath at 37 °C. The reaction was stopped by adding 150 µl trichloroacetic acid (TCA, 30 %). Hydrolyzed Pi was determined according to the method, described by Taussky and Shorr, (1953). The activity of Mg^{2+}-ATPase was measured after the addition of 1 mM ouabain, whereas the activity of Na^+,K^+-ATPase was calculated as the difference between the total ATPase and Mg^{2+}-ATPase activities.

The protein content in prepared homogenates of *S. littoralis* was assayed spectrophotometrically by the method of Lowery *et al*., (1951) at λ 750 nm using Bovine Serum Albumin (BSA) as a standard protein.

6. *In vivo* inhibition of AChE and Na^+,K^+-ATPase activity:

The inhibition of AChE and Na^+,K^+-ATPase activity were determined in the 4th instar larvae using the values of each of the tested compounds. In the inhibition studies, 10 µl of the enzyme preparation was incubated with of the inhibitor for 30 min. The enzyme-inhibitor mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:-

$$\%\text{Inhibition} = \frac{V - Vi \times 100}{V}$$

Where:

(V) is the specific activity without inhibitor.

(Vi) is the specific activity in presence inhibitor

7. *In vitro* inhibition and kinetics of Na^+,K^+-ATPase and AChE activity:

The inhibition of AChE and Na^+,K^+-ATPase activity were determined in the 4th instar larvae using the values of each of the tested compounds. The method of Dixon and Weeb (1964) was adopted to draw the Dixon-plots by ploting 1/V versus concentrations of the inhibitor at two concentrations of the substrate. Acetylcholine iodide (the substrate

of AChE) was used at concentrations of 5 and 10 mM, while ATP (the substrate of ATPase) concentrations were 3.0 and 5.0 mM.

Estimation of I_{50} value (the concentration of the inhibitor which inhibits 50 % of the enzyme activity) was carried out by per incubating the enzyme with the inhibitor for 30 min. Using the following concentrations 0.1; 1; 5; 10, and 50 µM. K_i (the inhibition constant) values for each inhibitor were estimated from Dixon-plot.

Michaelis-Menten Kinetics (K_m and V_{max}) values were calculated by a linear regression of 6 points on each Lineweaver and Burk plot (1934).

RESULTS AND DISCUSSION

Toxicity of tested insecticides against *S. littoralis* larvae:

Toxicity results of the tested insecticides expressed in terms of LC_{50} are given in table (1). Cyhalothrin LC_{50} values after 24 hr are 0.33; 1.31, and 1.97 ppm for lab; Etai-Elbaroud, and El-Bustan strains respectively, while LC_{50} values after 48 hr are 0.061; 0.52, and 0.87 ppm respectively. Also, chlorpyrifos LC_{50} values after 24 hr are 0.75; 1.58, and 2.22 ppm for the tested three strains respectively, while LC_{50} values after 48 hr are 0.096; 0.78, and 1.14 ppm respectively. On the other hand, LC_{50} values of thiodicarb after 24 hr are 0.97; 2.11, and 2.97 ppm for the tested three strains respectively, while LC_{50} values after 48 hr are 0.24; 1.10, and 1.55 ppm respectively.

Present results demonstrated that the LC_{50} values of both tested areas were decreased, in general, it is clear that the cyhalothrin was the most potent tested insecticide followed by chlorpyrifos, and thiodicarb. The present results emphasize that during many years of selection pressure in the field, the resistance and/or tolerance levels to the conventional insecticides due to the intensive application of such for controlling *S. littoralis*. These results fully agreeded with Kaygisiz (1980), and McDonald (1981) who reported that synthetic pyrethroids were the most effective against 4th instar larvae of *S. littoralis*. Korkor *et al*., (1995), reported that synthetic pyrethroids were more toxic than other tested insecticides in controlling bollworms. Mascarenhas *et al*., (1998) found that several field strains of *Spodoptera exigue* (Hubner) exhibitied reduce susceptibility to chlorpyrifos and thiodicarb. Also, our results revealed that toxicity of the tested insecticides was higher for El-Bustan strain (tomato fields), than that of Etai-Elbaroud strain (cotton fields). These findings are in agreement with many investigators, El-Aw and Hashem, 2001; Sharma and Yadav, 2001 and Zang *et al*., 2005, whom reported that different host plants are known to affect susceptibility of insect pests to insecticides.

Specific activities of Na^+,K^+-ATPase and AChE:

Table (2) summarized the specific activity of Na^+,K^+-ATPase; Mg^{2+}-ATPase and AChE. The specific activity of the ATPase, isolated from lab strain, El-Bustan strain (tomato fields), and Etai-Elbaroud strain (cotton fields) of *S. littoralis*. The maximum values of specific activity of Na^+,K^+-ATPase were found in lab strain followed by El-Bustan strain (tomato fields), whereas that the values of Na^+ ,K^+- and Mg^{2+}-ATPase activities in brain preparations of the *S. littoralis* were recorded. Also the specific total activities of total ATPases (Na^+, K^+, Mg^{2+}-ATPases) were greatest in lab strain 45.86 ± 0.13 followed by El-Bustan strain (tomato fields) 38.85 ± 0.06 and least in the Etai-Elbaroud strain (cotton fields), 28.51 ± 0.43. In contrasy Mg^{2+}-ATPase specific activity was more higher in Etai-Elbaroud strain followed by El-Bustan strain and lab strain.

Data presented in table (2) show the specific activity of the AChE in the brain of the 4th larval instar of lab; El-Bustan, and Etai-Elbaroud strains of *S. littoralis*. The results show that there were significant differences in AChE specific activity between the strains. AChE activities were higher in the lab, and El-Bustan strains (tomato fields), the values are 31.86 ± 0.05 and 26.56 ± 0.37 respectively, than Etai-Elbaroud strain (cotton fields), the value is 14.28 ± 0.12.

Table 1: Toxicity of tested insecticides against the *S. littoralis* 4th instar larvae.

***S. littoralis* strains locations**	**LC_{50} (ppm)**					
	cyhalothrin		**Chlorpyrifos**		**thiodicarb**	
	24hr	**48hr**	**24hr**	**48hr**	**24hr**	**48hr**
Laboratory	0.33	0.061	0.75	0.096	0.97	0.24
El-Bustan	1.13	0.52	1.58	0.78	2.11	1.10
Etai-Elbaroud	1.97	0.87	2.24	1.14	2.67	1.55

Table 2: Na^+,K^+-ATPase and AChE specific activities *Spodoptera* brain of 4th larval instar.

***S. littoralis* strains locations**	**Specific activities ± S.D**			
	Total ATPase	**Na^+,K^+-ATPase**	**Mg^{2+}-ATPase**	**AChE**
Laboratory	45.86 ± 0.13	36.82 ± 0.04	09.04 ± 0.01	31.86 ± 0.05
El-Bustan	38.85 ± 0.06	28.30 ± 0.14	06.60 ± 0.06	26.56 ± 0.37
Etai-Elbaroud	28.51 ± 0.43	24.21 ± 0.52	4.17 ± 0.08	14.28 ± 0.12

Na^+,K^+-ATPase specific activity (Pi µmole mg^{-1} protein hr^{-1}).
AChE specific activity (λ_{max} 412 mg^{-1} protein hr^{-1}).

The *in vivo* inhibition of brain *S. Littoralis* Na^+,K^+-ATPase and AChE activity:

The *in vivo* inhibitory effect of the LC_{50} values of tested insecticides against the *Spodoptera littoralis* 4th instar lab; El-Bustan, and Etai-Elbaroud strains to the larval Na^+,K^+-ATPase and AChE are shown in table (3). The data declared that cyhalothrin exhibited significant reduction in Na^+,K^+-ATPase activity, with inhibition percentages of 93.6%; 81.4% and 70.2% respectively for lab; El-Bustan, and Etai-Elbaroud strains, respectively. On the other hand, in the case of AChE, the significant reduction in its activity, was recorded for chlorpyrifos and thiodicarb with inhibition percentages of 85.7%; 72.2% and 63.1% for the tested three strains respectively, while the percentages of AChE inhibition were 80.5%; 67.4% and 58.2% respectively in the case of thiodicarb. It is clear that, the inhibition against El-Bustan strain (tomato fields), strongest than Etai-Elbaroud strain (cotton fields) these results are in agreement with many investigators, Graflon-Cardwell, *et al*., 2004, El-Dewy 2006, and Saleem, *et al*., 2008.

Kinetic parameters of Na^+,K^+-ATPase and AChE inhibition:

The kinetic studies were conducted to evaluate the effects of cyhalothrin on Na^+,K^+-ATPase activity and chlorpyrifos and thiodicarb on AChE activity in the brain of both tested strains of *S. littoralis* 4th larvae. Table (4) shows the obtained Lineweaver-Burk (L-B) plots for Na^+,K^+-ATPase and AChE and statistical analysis of the obtained values of K_m (Michaelis-Menten, constant) and V_{max} (maximum velocity) of the Na^+,K^+-ATPase and AChE. The K_m values for Na^+,K^+-ATPase and AChE were generally higher in two tested field strains than lab strain. The change in K_m values of Na^+,K^+-ATPase and AChE between the tested strains indicate change in the affinities. Our results are strongly emphasized by the kinetic studies of Gonzalez *et al*., (1990) found that the calculated K_m of 0.22mM for AChE of gastropod *Concholepas concholepas*.

The present results show that the V_{max} values of Na^+,K^+-ATPase and AChE are obviously higher. This points to the high substrate turnover which may reflect the physiological importance of the Na^+,K^+-ATPase in the function of the nervous tissue of the *S. littoralis* larval brain (El-Aw and Hashem, 2001, and El-Dewy 2006). The V_{max} values were generally higher in two tested strains than lab strain. This fact indicated that the number of active sites on the Na^+,K^+-ATPase and AChE of the 4th larvae brain was increased in the fields strains. Such change may be followed by decrease in the insect susceptibility which could be altered by field application of the carbamate; organophosphorus and pyrethroide insecticides. In addition, *S. littoralis* Na^+,K^+-ATPase and AChE were found to have higher and lower values in El-Bustan strain (tomato fields), than Etai-Elbaroud strain (cotton fields), host-plants influence in this concern (El-Aw and Hashem, 2001, and Zang, *et al*., 2005).

The *in vitro* inhibition of brain *S. littoralis* Na^+,K^+-ATPase and AChE activities:

To characterize more details about the *in vitro* inhibition of Na^+,K^+-ATPase and AChE by the tested insecticides, the I_{50} and K_i values of each inhibitor was estimated from the graphical method of Dixon and Weeb, (1964) (table 5). The K_i values were 14; 24, and 43 µM for lab strain; El-Bustan strain (tomato fields), and Etai-Elbaroud strain (cotton fields) respectively in the case of cyhalothrin, while the K_i values were 22; 35, and 54 µM for the tested three strains respectively in the case of chlorpyrifos. Also the K_i values were 36; 48, and 60 µM for the tested three strains respectively in the case of thiodicarb. The obtained data proved that each of cyhalothrin; chlorpyrifos, and thiodicarb competitive by inhibited of Na^+,K^+-ATPase and AChE activity. The present results are in accordance with those reported by Zhu and Brindley (1992) who reported competitive inhibition of AChE purified from *Lygus hesperus* by six Op_s compounds.

Table 3: *In vivo* inhibition of brain *Spodoptera* larvae 4th instar Na^+,K^+-ATPase and AChE activity.

S. littoralis strains locations	%Inhibition (LC_{50})		
	Na^+,K^+-ATPase	AChE	
	cyhalothrin	chlorpyrifos	thiodicarb
Laboratory	93.6%	85.7%	80..5%
El-Bustan	81.4%	72.2%	67.4%
Etai-Elbaroud	70.2%	63.1%	58.2%

Table 4: Michaelis-Menten, kinetics of the Na^+,K^+-ATPase and AChE of larval brain of *S. littoralis*.

S. littoralis strains locations	Na^+,K^+-ATPase		AChE	
	K_m (mM)	V_{max} (mM)	K_m (mM)	V_{max} (mM)
Laboratory	0.28	5.5	0.44	4.6
El-Bustan	0.37	4.2	0.60	3.3
Etai-Elbaroud	0.51	3.1	0.82	2.0

Table 5: *In vitro* inhibition of brain *Spodoptera* larvae Na^+,K^+-ATPase and AChE activities by tested insecticides.

***S. littoralis* strains locations**	**Na^+,K^+-ATPase**		**AChE**			
	cyhalothrin		**chlorpyrifos**		**thiodicarb**	
	I_{50} (µM)	**K_i (µM)**	**I_{50} (µM)**	**K_i (µM)**	**I_{50} (µM)**	**K_i (µM)**
Laboratory	0.21	14	0.32	22	0.47	36
El-Bustan	0.36	24	0.48	35	0.56	48
Etai-Elbaroud	0.55	43	0.67	54	0.70	60

Table (5) show the *in vitro* interaction of cyhalothrin; chlorpyrifos and thiodicarb on Na^+,K^+-ATPase and AChE activity of *S. littoralis* 4th instar brain respectively. The I_{50} values for cyhalothrin against Na^+,K^+-ATPase were 0.21; 0.36, and 0.55 µM for lab strain; El-Bustan strain (tomato fields), and Etai-Elbaroud strain (cotton fields) respectively. The corresponding I_{50} values for chlorpyrifos and thiodicarb against three strains larval AChE were 0.32; 0.48, and 0.67 µM respectively, and were 0.47; 0.56, and 0.70 µM for lab; El-Bustan, and Etai-Elbaroud strains respectively.

In comparing the inhibitory potency of cyhalothrin; chlorpyrifos, and thiodicarb against Na^+,K^+-ATPase and AChE activity respectively, it is clear that cyhalothrin showed to be the strong inhibitor for *S. littoralis* Na^+,K^+-ATPase activity, thus causing a decrease in the unidirectional transport of Na^+ and K^+ through cell membranes. On the other hand, the I_{50} values of each cyhalothrin; chlorpyrifos, and thiodicarb were more lower in lab strain than in the field strains. Also El-Bustan strain (tomato fields), is more succeptible than that of Etai-Elbaroud strain (cotton fields).

In general, these results suggest that host-plant has major role in *S. littoralis* susceptibility to the tested insecticides. These findings have been confirmed by the results of many authors such as Liburd, *et al*., 2000; El-Aw, and Hashem 2001, Graflon-Cardwell, *et al*., 2004, and Zang, *et al*., 2005.

In general, it may be suggest that host-plant could be used in the integrated pest management (IPM) programs, in order to maximize the effect of conventional insecticides when applied against *S. littoralis*.

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الملخص العربى

تأثير النبات العائل على مدى حساسية أنزيم الصوديوم- بوتاسيوم أدينوسين ترای الفوسفاتيز وأنزيم الأسيتايل كولين أستريز لبعض المبيدات الحشرية فى يرقات دودة ورق القطن

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الهدف من البحث هو دراسة تأثير نوعان مختلفان من النبات العائل(القطن والطماطم) على نشاط إنزيمين من أهم الأهداف البيولوجية فى الحشرة وهما إنزيم الصوديوم-بوتاسيوم ادينوسين ترای الفوسفاتيز وإنزيم الأستايل كولين أستريز وأيضاً على مستوى حساسية يرقات العمر الرابع لدودة ورق القطن لثلاثة مبيدات حشرية من مجاميع مختلفة وهى سيهالوثرين، كلوروبيروفوس والثيودى كارب حيث تم أستخلاص كلا الإنزيمين من رأس يرقات العمر الرابع لدودة ورق القطن وذلك مابين سلالتين أحدهما جمعت من حقول الطماطم بمنطقة البساتين والآخرى من حقول القطن بمنطقة إيتاى البارود وتمت مقارنة النتائج المتحصل عليها بسلالة معملية حساسة. ولقد أوضحت النتائج أن قيم التركيزات النصف مميتة (LC_{50}) أظهرت أختلافاً محسوساً حيث كانت سلالة البساتين أكثر حساسية من سلالة إيتاى البارود وذلك فى حالة الإنزيمين، وقد أوضحت النتائج المتحصل عليها أن أعلى معدل نشاط نوعى للإنزيمين كان فى سلالة البساتين بينما كان هذا المعدل أقل فى سلالة إيتاى البارود وذلك بالمقارنة بالسلالة المعملية الحساسة، وكذلك فقد تم تقدير قيم الـ I_{50} فوجد بالنسبة لتأثير السيهالوثرين على نشاط الإنزيم الصوديوم-بوتاسيوم ادينوسين ترای الفوسفاتيز كانت ٠.٠٣٦، ٠.٥٥ و٠.٢١ ميكروموار بالنسبة للبساتين و إيتاى البازود والسلالة الحساسة على التوالى بينما فى حالة الكلوربيرفوس على نشاط أنزيم الأسيتايل كولين أستريز كانت ٠.٤٨، ٠.٦٧ و ٠.٣٢ ميكروموار بالنسبة للسلالتين الحقلية والسلالة المعملية على التوالى بينما لمبيد الثيودى كارب على نشاط أنزيم الأسيتايل كولين أستريز كانت ٠.٥٦، ٠.٧٠ و ٠.٤٧ ميكروموار بالنسبة للسلالتين الحقلية والسلالة المعملية على التوالى. كذلك تم دراسة بعض الثوابت مثل ثابت ميخائيل K_m وأقصى نشاط نوعى V_{max} وكذلك تم تقدير ثابت التثبيط K_i فكانت فى حالة السيهالوثرين ٢٤، ٤٣ و ١٤ ميكروموار بالنسبة للسلالتين الحقلية والسلالة المعملية على التوالى بينما فى حالة الكلوربيرفوس كانت ٣٥، ٥٤ و ٢٢ ميكروموار بالنسبة للسلالتين الحقلية والسلالة المعملية على التوالى أما بالنسبة لمبيد الثيودى كارب كانت ٤٨، ٦٠ و ٣٦ ميكروموار بالنسبة للسلالتين الحقلية والسلالة المعملية على التوالى. ومن هذه النتائج نجد أن هناك أختلافات مرتبطة بالنبات العائل هذه الأختلافات ظهرت فى القيم الحركية للإنزيمين تحت الدراسة وكذلك فى مدى تأثير المبيدات تحت الدراسة على يرقات دودة ورق القطن، ولذلك يمكن القول أن النبات العائل يجب وضعه فى الأعتبار كأحد العناصر الهامة عند وضع برامج المكافحة المتكاملة لدودة ورق القطن حتى نحصل على أعلى تأثير للمبيدات بأقل التركيزات وبهذا نقلل من تلوث البيئة.