HOST-PLANT EFFECTS ON THE KINETICS AND INHIBITION OF ACETYLCHOLINESTERASE IN THE COTTON WHITEFLY *BEMISIA TABACI* GENNADIUS.

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ABSTRACT

The influence of two host-plants (tobacco and tomato) on the enzymatic kinetics and the inhibition of acetylcholinesterase (AChE) was investigated in the whole body homogenates of Bemisia tabaci male and female adults. The obtained results revealed that K_m values of AChE from both B. tabaci sexes reared on tobacco plants were significantly higher than those of tomato plants. It is noticed that, K_m values of tobacco culture increased to be 3.18 and 3.23 folds for females and males respectively when compared with the same sex from tomato culture. This may reflect a relative lower affinity of AChE from both sexes of tobacco culture to the substrate and in return lower activity than those of AChE from both sexes reared on tomato culture. On the contrary, V_{max} of females from tomato culture increased significantly to be about 1.3 folds of that of females from tobacco culture. The same trend was also recorded in the case of males but with no significant difference between V_{max} values of the two tested host-plants. In the same host-plant, males AChE were relatively lower in their affinity to the substrate than those of females. These latter findings may draw the attention to the criticism of not only segregated effect(s) of sex or host-plant alone but also to the combined sex: host-plant influence in this concern.

The in vitro inhibition of AChE by an organophosphorus insecticide, chlorpyrifos, and a carbamate insecticide, carbaryl, was investigated. The obtained results showed that, irrespect of carbaryl effect on males from tobacco culture, this insecticide showed to be the strongest inhibitor against AChE of both sexes of B. tabaci adults from tomato culture and only of females from tobacco culture. The lowest recorded I_{50} value for carbaryl was that against AChE from females reared on tobacco plants (0.13 µM) followed by females from tomato culture (0.30 µM) and then by males from tomato culture (0.71 µM). The highest I_{50} value was that of chlorpyrifos against AChE from male adults of tomato culture (251 µM) followed by that of females from tomato culture (56.2 µM) by the same insecticide. Therefore, it could be concluded that, chlorpyrifos exerted a very weak inhibitory effect against AChE of both B. tabaci male and female adults reared on tomato plants. The obtained I_{50} values of chlorpyrifos, against B. tabaci adults from tobacco culture were very low (0.668 and 5.96 µM) when compared to those of tomato culture. These findings revealed that chlorbyrifos caused its strong toxic effect on B. tabaci adults AChE reared on tobacco in comparison to AChE from B. tabaci tomato culture. This may reflect a host varying effect on AChE sensitivity to inhibition by chlorbyrifos. Moreover, within the same host-plant, AChE of B. tabaci female was more sensitive than that of males towards the two tested insecticides.

Generally, it could be concluded that the present study showed the existence of host-correlated variation phenomenon in *B. tabaci* AChE kinetics and inhibition by the two tested insecticides.

Key words: *Bemisia tabaci, host-plant, Acetylcholinesterase, Kinetics, inhibition, insecticides.*

INTRODUCTION

The economic importance of whiteflies on the Egyptian agriculture has been recognized since 1930's (Priesner and Hosny, 1932), but viruliferous whiteflies have become very important pest during the last twenty five years. At least, twenty-one aleyrodid pests have been documented in Egyptian cropping system (Idriss *et al.* 1997). Three of them (*B. tabaci, B. argentifolii, Trialeurodis vaporariorum*) are able to transmit plant viruses (Bock *et al.*, 1974; Brown *et al.*, 1995a; McGrath and Harrison, 1995). Besides, *B. tabaci, B. argentifolii, Trialeurodis ricini* are vectors of plant geminiviruses (Idriss *et al.*, 1997). These geminiviruses are the largest and most economically significant group of plant viruses that cause devasting plant diseases to many crops in Egypt. In general, geminiviruses have become a significant group of plant diseases due to the modification of the ecology and other behavioural aspects of their natural vectors (Gerling, 1990).

For all the previously mentioned information about the increasing economic importance of the subject whitefly species in the Egyptian agriculture, and in the light of the previously recorded phenomena of host-correlated variations in the subject whitefly (i.e. morphology, susceptibility, ...etc.), the present study was planned and achieved in order to gain some forward steps towards a successful integrated pest management (IPM) programme(s) of this pest. These phenomena clearly referred to the abruptive physiology of the whiteflies, and in return their promising, highly important role in enriching our knowledge of insect genetics and physiology.

The present study was conducted to evaluate the possible effects of host-plant on acetylcholinesterase (AChE) kinetics for both sexes of *B. tabaci* adults cultured on two different host-plants. Besides, experiments were carried out to investigate the host-correlated variations in relation with *B. tabaci* AChE inhibition by checking the potency of chlorpyrifos and carbaryl, as examples of OP- and carbamate- insecticides, respectively. Results of these investigations will surely add to the knowledge about insecticide susceptibility of the subject aleyrodid in concideration with host-correlated variations phenomenon. As AChE plays a key role in terminating excitatory neurotransmitter action in insect synapses (Gerschenfeld, 1973). This enzyme forms the primary site of action for the most widely used chemical

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control agents in whitefly control, the systemic insecticides. Insects that used esterase to resist insecticides may alter and/or elevate AChE (Iwata and Hama, 1972; Devonshire and Moores, 1982; Moores *et al.*, 1988, Ffrench-Constant and Roush, 1990; Yu and Nguyen, 1992; Zhu *et al.*, 2000; Vontas *et al.*, 2001).

The present study along with all previous ones aimed to gain further knowledge and better understanding of the whitefly host-correlated variation phenomena in order to control this deleterious pest through suitable successful pest integrated management programmes (IPM) safe enough to man and environment.

MATERIALS AND METHODS

I- The whitefly *B. tabaci* Genn. Laboratory culture:

The subject insect has been taken from a laboratory culture of the whitefly *Bemisia tabaci* Gennadius, which has been first established by El-Helaly (1966) and which is still bred apart of any chemical treatments on tobacco plants *Nicotiana tabacum* in greenhouses at 25±7°C, 65±5% RH and under natural light conditions. The identification of the mother culture was achieved by El-Helaly *et al.* (1971).

- The tomato culture of *B. tabaci* Genn.:

Adults of B. tabaci from the laboratory tobacco culture have been reared on tomato plants since 1999 in order to establish a new tomato culture to fulfil the requirements of present investigation about physiological host-correlated variations in AChE of the subject insect.

II- Insect homogenate preparation:

Newly emerged *B. tabaci* adults [20 adults/0.3 ml buffer] of either sex (~14 and 20 ng body weight of males and females, respectively) were homogenized in ice cold 0.1M phosphate buffer, pH 7.0. Homogenization was achieved at low temperature by means of manually driven, glass minute homogenizer (1ml) especially developed for the whitefly adults.

III- Determination of Acetylcholinesterase (AChE) kinetics:

Enzyme activity was measured using the spectrophotometric method of Ellman *et al.* (1961). The method is based on the hydrolysis of acetylthiocholine iodide (ATChI) as a substrate of AChE producing thiocholine iodide that reacts with 0.1M DTNB [5,5 dithiobis- (2-nitrobenzoic acid)] producing yellow color as function of the enzyme activity. The color intensity was measured spectrophotometrically at 412 nm as a rate of enzyme activity. The changes in absorbance were recorded at the beginning of the reaction and after 30 min. interval. The reaction mixture was kept at 37°C. The affinity of AChE in whole body homogenates to the substrate were estimated using 0.1 to 1X 10^{-3} M range of ATChI concentrations. The lineweaver-Burk plot (L-B) was drawn by plotting 1/v versus 1/[S] where [S] is the molar concentration of (ATChI). Values of K_m (Micaeli's constant) and V_{max} (maximum velocity) were estimated from L-B regression lines.

Protein content in the whole body homogenates of the subject whitefly adults was assayed spectrophotometrically by the method of Lowry *et al.* (1951), at 750 nm wavelength, using bovine serum albumin (BSA) as a standard protein.

Statistical analysis:

Values of K_m (the affinity) and V_{max} (the hydrolylising efficiency) were obtained by a least squares linear regression of double receprocal plots of the points (Lineweaver and Burk, 1934). A *t*-test was adopted to compare the responses of AChE in both tested host-plants.

IV- *In vitro* inhibition of AchE activity:

The inhibition of AChE activity was determined in both *B. tabaci* sexes from tobacco and tomato cultures using the organophosphorus insecticide, chlorpyrifos and the carbamate insecticide, carbaryl as inhibitors.

Estimation of I_{50} value (the concentration of the inhibitor which inhibit 50% of the enzyme activity) was carried out by preincubating the inhibitor with the enzyme (insect homogenate) at 37°C prior to the addition of Ellman's reagent (1961) (DTNB: at concentration of 0.1M & ATChI: at concentration of 10^{-3} M).

Insect homogenate was preincubated with chloropyrifos for 30 min and with carbaryl for 15 min. at 37°C before the substrate was added. The residual activity was then measured as described before in the determination of AChE kinetics. The inhibitors were used at 10^{-7} to 10^{-3} M concentrations. The percentage of the *in vitro* inhibition was calculated with respect to the activity in the absence of the inhibitor, using the equition of Ellman *et al.* 1961.

- Tested inhibitors:-

The organophosphorus insecticide, chlorpyrifos, (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosophothioate) and the carbamate insecticide, cabaryl, (1-naphthalenyl methylcarbamate) were provided as technical grade insecticides from Frunol-chemie (W. Germany) and JinHung Fine Chem., Co. LTd Korea., respectively.

RESULTS AND DISCUSSION

1- Host-plant effect on the AChE kinetics of *B. tabaci* adults.

The Kinetic studies were conducted to evaluate the possible effects of host-plant on AChE activity in both sexes of *B. tabaci* adults. This was conducted taking into consideration the previously observed host-correlated variation in the susceptibility of the subject insect to insecticides (El-Helaly, 1973; El-Meniawi, 1992; Anthony *et al.*, 1998).

The catalytic properties of AChE were estimated in whole body homogenates of male and female adults of two populations reared on tobacco and tomato plants. This was achieved by measuring reaction rates (V) over a range of ATChI concentrations, as a substrate (S), under optimum enzymatic activity conditions.

Fig.(1) shows the obtained Lineweaver-Burk (L-B) plots for both sexes of the subject insect adults from the two tested host plants. Table (1) summarizes the statistical analysis of the obtained values of K_m and V_{max} of AChE derived from all aforementioned L-B regression lines.

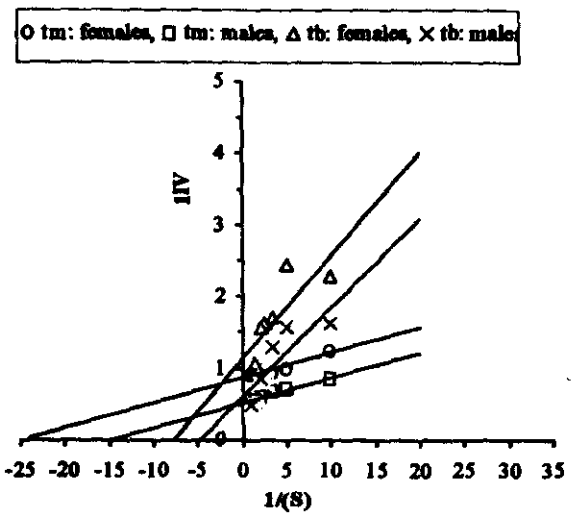

Fig.(1): Lineweaver-burk plots of AChE activities of *B. tabaci* female and male adults from the two tested cultures.

Tb-female: females from tobacco culture, Tb-male: males from tobacco culture, Tm-male: males from tomato culture, Tm-female: females from tomato culture, 1/(S) = the inverse of ATChI concentrations in 0.1 to 1X 10^{-3} M, 1/v = the inverse of AChE activities in µM of ATChI.

Table (1): Host-plant effect on AChE kinetics of *Bemisia tabaci* adults from both tobacco and tomato cultures.

Host-plant	K_m		V_{max}	
	female	male	female	male
Tobacco	0.127	0.21	0.88	1.69
Tomato	0.04	0.065	1.15	1.89
t-calculated	16.82**	16.37**	6.44**	1.45$^{n.s.}$

$t_{0.05}$= 2.776, $t_{0.01}$= 4..604

K_m: Michaeli's constant= ATChI concentration X10^{-3} M

V_{max}: Maximum velocity of the enzyme activity expressed as O.D. λ 412 µM/mg protein/min.

Statistical analysis of the obtained results revealed that K_m values of AChE from both *B. tabaci* sexes reared on tobacco plants were significantly higher than those from tomato plants. It is noticed that K_m values in tobacco culture increased to 3.18 and 3.23 folds for females and males respectively when compared with the same sex from tomato culture. This may reflect a relative lower affinity of AChE from both sexes of tobacco culture to the substrate and in return lower activity than those of AChE from both sexes reared on tomato culture.

On the contrary, V_{max} of females from tomato plants increased significantly to about 1.3 folds of that of females from tobacco culture (Table 1). The same trend was also recorded in the case of males but with no significant difference between V_{max} values of the two tested host-plants.

The shift of the Michaeli's Mentin constants was sufficient to explain the changes which occurred due to host-plant. The observed reduction in K_m values of either sex from tomato culture indicated that the enzyme aquired more affinity to ATChI. The V_{max} values of AChE in both sexes from tomato culture were raised referring to a probable increase in the number of active sites due to the effect of host-plant, but this needs further investigations. In other words, these findings are almostly host than sex correlated.

Present results suggest that the two tested host-plants induced some alteration in the AChE nature in both sexes of the subject whitefly adults probably by increasing the active centers of the enzyme molecules. This alteration might lead to meet its defined physiological functions. Taking into consideration the probability of the presence of certain allelochemicals in each tested hot-plant.

Moreover, the present results show that in the same host-plant, males AChE are relatively lower in their affinity to the substrate than those of females as K_m values in males exceeded those of females within the same culture. Therefore, it could be concluded that the kinetics of *B. tabaci* AChE were found to be different in either males and females of both tobacco or tomato cultures. This may refer to some probable

difference in the reproductive biotic potentials of either sex. In addition, *B. tabaci* AChE were found to have higher V_{max} and lower K_m values in tomato than tobacco host-plants. These latter findings may draw the attention to the criticism of not only segregated effect(s) of sex or host-plant alone but also to the combined sex: host-plant influence in this concern. In other words, the already noticed increasing effect of tobacco host-plant on female AChE K_m value compared with that of tomato females has been also noticed here for male AChE. Therefore, it could be concluded that irrespective of sex, host-correlated variation in AChE is pronounced in the subject aleyrodid *B. tabaci* adults, and in return worth further investigation.

At this point of discussion, it could be clearly concluded that the obtained results proved the existence of both studied phenomena of sex- and host-correlated variations in the AChE kinetics of the subject whitefly *B. tabaci*. In addition, the recorded similar kinetical attitude in both sexes which may reflect probable effects for such kinetical attitude in relation to the subject whitefly toxicity should not be overlooked.

As far as the writer is aware, no information is available in the literature about the influence of host-plants on the AChE kinetics in Homopteran insects. However, kinetics of general esterase by using α-naphthyl acetate as a substrate were found to be affected by host-plants in *Bemisia tabaci* (El-Meniawi, 1992); *Spodoptera littoralis* (El-Aw and Hashem, 2001); *Aonidiella aurantii* (Grafton-Cardwell *et al.*, 2004). In addition, changes in the electrophoretic profiles of general esterase due to host-plant have been reported (Costa and Brown, 1991; Perring *et al.*, 1992; El-Meniawi, 1992; Wool *et al.*, 1993; Brown *et al.*, 1995b).

2-AChE inhibition by organophosphate (OP) and carbamate (CR)- insecticides of *B. tabaci* tobacco and tomato cultures.

From the obtained I_{50} values (Table 2), remarkable inhibition of the enzyme activity by the two tested insecticides was observed with relatively varying degrees between the tested host-plants.

Table (2): The I_{50} (μM) values of the tested pesticides (chlorpyrifos and carbaryl) against AChE activity of *Bemisia tabaci* adults from both tobacco and tomato cultures.

Host-plant	Sex	Tested pesticides	
		Chlorpyrifos	Carbaryl
Tobacco	Female	0.668	0.13
	Male	5.96	31.6
Tomato	Female	56.2	0.30
	Male	251	0.71

In comparing the inhibition potency of the two tested insecticides, it is clear that, irrespect of carbaryl effect on males from tobacco culture, this insecticide showed to be the strongest inhibitor against AChE of both sexes of *B. tabaci* adults from tomato culture and only of females from tobacco culture. The lowest recorded I_{50} value (the highest inhibition potency) for carbaryl was that against AChE from *B. tabaci* tobacco females (0.13 μM) followed in ascending order by females from tomato culture (0.30 μM) and then by males from tomato culture (0.71 μM). These results reflect that carbaryl had the highest inhibition potency against AChE from females of tobacco culture.

On the other hand, the highest I_{50} value was that of chlorpyrifos against AChE from male adults reared on tomato plants (251 μM) followed by that of females from tomato culture (56.2 μM) by the same insecticide. Therefore, it could be concluded that, chlorpyrifos exerted a very weak inhibitory effect against AChE of both *B. tabaci* male and female adults from tomato culture. Concerning the inhibitory potency of the OPs insecticide, chlorpyrifos, against *B. tabaci* adults from tobacco culture, it is clear that the obtained I_{50} values were very low (0.668 and 5.96 μM) when compared to those of *B. tabaci* tomato culture (56.2 and 251 μM). This may reflect a host varying effect on AChE sensitivity to inhibition by chlorbyrifos. These findings revealed that chlorbyrifos causing its strong toxic effect on AChE *B. tabaci* adults reared on tobacco in comparison to AChE from *B. tabaci* tomato culture. Such varying effect of the tested insecticides on *B. tabaci* AChE in respect to host-plant could be understood in the light of the results of El-Helaly, (1971 and 1973); El-Meniawi (1992); Anthony *et al.* (1998); Byrne and Devonshire (1997) about the host-correlated variations in the insecticide susceptibility.

Moreover, it was also noticed that, within the same host-plant, AChE of *B. tabaci* female was more sensitive than males towards the two tested insecticides (Table 2). As I_{50} values in the case of chlorpyrifos were 0.668, 56.2 μM for females and 5.96, 251 μM for males from tobacco and tomato cultures, respectively. Also, in the case of carbaryl, I_{50} values were 0.31, 0.30 μM for females and 31.6, 0.71 μM for males from tobacco and tomato cultures, respectively. These findings could be understood in the light of the highest total protein content in *B. tabaci* females when compared to those of males (Table 3).

Table (3): Means of protein content mg/ml as affected by the interaction between sex, and host-plant.

Host-plant	Female	Male
Tobacco	2.51 b ± 0.110	1.43 c ± 0.160
Tomato	3.57 a ± 0.104	1.49 c ± 0.191

$L.S.D_{0.05} = 0.19$

Means followed by the same letter (s) are not significantly different according to L.S.D at 0.05 level of probability.

In discussion of the present findings on AChE inhibition with respect to changes in the kinetic parameters in the former item, it could be concluded that, the changes in K_m values between the two tested host-plants indicate change in the affinity of such esterase to ATChI. However, the tobacco culture had generally higher K_m values, or, in other words, had lower affinities to ATChI within the same sex. This indicates that the AChE affinity to ATChI may vary in response to changing the sort of host-plant under which *B. tabaci* adults were reared.

The greater affinity of tomato culture (or males than females within the same culture) enzyme for the substrate, as indicated by its lowest K_m values would have a greater protective influence on its interaction with tested insecticides than that expected for tobacco culture enzyme. If this reflects the situation *in vivo*, this greater affinity for substrate means that AChE should be partially protected from inhibitors by any substrate present in the synapse. This suggestion may be explained with the highest I_{50} values in the case of *B. tabaci* tomato culture when compared with tobacco culture. Acceptable or rejection of such latter comment surely needs further investigation. However, such latter comment looks acceptable in the light of the studies of Devonshire and Moores,1984 and Byrne and Devonshire, 1997, who concluded that the low K_m value could be afforded some degree of protection from the weaker inhibitors such as monocrotophos. Taking into consideration the conclusion in the study of Byrne and Devonshire (1997), that increased release of Acetylcholine would also be expected in a hyperactive nervous system following OP- or carbamate- poisoning, and this might partially alleviate the inhibition even more. The study of Grafton-Cordwell *et al.* (2004) about the effect of host-plant tissue on the activity of inhibition of AChE in *Aonidiella aurantii* suggested that this insect is using increased amounts of esterase enzymes, including AChE, to sequester OP and carbamate insecticides, rather than modified AChE.

In general, the present study proved the existence of host-correlated variation phenomenon in AChE kinetic parameters and inhibition by the tested insecticides. In addition, the present investigation revealed the interaction between the effects of the differences in both sexes and host-plants.

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الملخص العربي

تأثير النبات العائل على تنشيط وتثبيط انزيم الأستيل كولين استيريز فى حشرة ذبابة القطن البيضاء *Bemisia tabaci* Genn.

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تم دراسة تأثير نوعان مختلفان من النبات العائل (دخان وطماطم) على كل من تنشيط وتثبيط إنزيم الأستايل كولين استيريز، فى متجانس أجسام الحشرات الكاملة لحشرة ذبابة القطن البيضاء وذلك لكل من الذكور والإناث.وقد أوضحت النتائج المتحصل عليها أن قيم الـ K_m لهذا الإنزيم المستخلص من كل من ذكور وإناث الحشرة موضع الدراسة والمرباه على نبات الدخان كانت تفوق معنوياً مثيلتها فى الحشرات المرباه على نبات الطماطم. فقد لوحظ أن قيم الـ K_m لكل من الإناث والذكور المرباه على الدخان كانت تعادل ٣,١٨، ٣,٢٣ مرة قدر مثيلتها من نفس الجنس فى حالة مزرعة الطماطم. وهذا ربما يعكس إنخفاض ميل الإنزيم للإرتباط بمادة التفاعل فى كلا جنسي الحشرة المرباه على الدخان بالمقارنة بتلك المرباه على الطماطم. وعلى العكس من ذلك، فقد كانت قيم V_{max} (السرعة القصوى) فى حالة الإناث المرباه على نبات الطماطم تفوق معنوياً بمقدار ١,٣ مرة قدر الإناث المرباه على الدخان. وقد سجلت نفس الإستجابة فى حالة الذكور ولكن كانت الفروق غير معنوية فى قيم V_{max} بين مزرعتي الدخان والطماطم. وبالنسبة لكل عائل نباتي على حده، فقد أظهر إنزيم AChE فى حالة الذكور ميلاً منخفضاً للإرتباط بمادة التفاعل بالمقارنة بالإناث من نفس العائل.

كذلك تم دراسة تثبيط إنزيم الأسيتايل كولين إستيريز *in vitro* بإستخدام مبيد فوسفوري وهو الكلوربيروفوس Chlorpyrifos وآخر كاربماتي وهو الكاربارايل Carbaryl. وقد أوضحت النتائج المتحصل عليها أنه بإستثناء تأثير الكربارايل على ذكور الحشرة من مزرعة الدخان، فإن هذا المبيد كان أكثر كفاءة فى تثبيط الـ AChE فى كلا جنسي الحشرة من مزرعة الطماطم وكذلك للإناث فى مزرعة الدخان. وقد سجلت أقل قيمة للـ I_{50} (تركيز المبيد اللازم لتثبيط ٥٠% من النشاط الإنزيمي) لمبيد الكربارايل فى حالة إناث مزرعة الدخان (٠,١٣ ميكرو مولار)يتبعها تصاعدياً الإناث من مزرعة الطماطم (٠,٣٠ ميكرو مولار) ثم الذكور من مزرعة الطماطم (٠,٧١ ميكرو مولار). أما بالنسبة لمبيد الكلوربيروفوس Chlorpyrifos فقد سجلت أعلى قيمة للـ I_{50} فى حالة AChE المستخلص من ذكور الحشرات المرباه على الطماطم (٢٥١ ميكرو مولار) يتبعها تنازلياً الإناث من مزرعة الطماطم (٥٦,٢ ميكرو مولار). ومن هذه النتائج يمكن إستنتاج أن، الكلوربيروفوس أظهر تأثير تثبيطي ضعيف جداً على AChE لحشرات مزرعة الطماطم فى كل من الذكور والإناث.

أما قيمة للـ I_{50} لمبيد الكلوربيروفوس ضد الحشرات الكاملة المرباه على نبات الدخان فقد كانت منخفضة جداً (٠,٦٦٨، ٥,٩٦ ميكرو مولار) وذلك بمقارنتها بالـ AChE من حشرات مزرعة الطماطم. ومن هنا فإن النتائج المتحصل عليها أثبتت أن الكلوربيروفوس له تأثير تثبيطي قوي على الـ AChE للحشرات الكاملة المرباه على الدخان بمقارنتها بتلك المرباه على الطماطم. وهذا يعكس إختلافاً مرتبطاً بالنبات العائل بالنسبة لحساسية هذا الإنزيم للتثبيط بالكلوربيروفوس.

كذلك أثبتت النتائج أنه على مستوى العائل الواحد فقد كانت الإناث أكثر حساسية من الذكور بالنسبة لكلا المبيدين المختبرين.

هذه الدراسة أثبتت وجود ظاهرة الإختلافات المرتبطة بالعائل فى القيم الحركية لإنزيم AChE فى حشرة ذبابة القطن البيضاء وكذلك فى تثبيط هذا الإنزيم بالمبيدين المختبرين.