EVALUATION OF THREE ACARICIDES COMPARED WITH PREDATORY INSECT AGAINST *PANONYCHUS ULMI* ON APPLE TREES

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Abstract

Efficacy of three acaricides (Ortus 5% EC, Milbeknock 1% EC and Cascade 10% DC) and predatory insect; *Chrysoperla carnea* Stephens was evaluated on the European red mite *Panonychus ulmi* Koch (Acari: Tetranychidae) movable stages infested apple orchards during the two successive seasons; 2012 and 2013 at Darawa village, Ashmon, Menoufia governorate, as well as determining the biochemical changes in the treated insects. The results show that, The highest reduction percentages of *P. ulmi* movable stages during 2012 and 2013 seasons were recorded with Ortus followed by Milbecknok then the predatory insect; *C. carnea*, while the lowest reduction percentages were recorded with Cascade. The effect of the two acaricides Ortus and Milbecknok (the highest toxic acaricides) on the major biochemical components of *P. ulmi* adult females showed that, the acaricide Ortus achieved high effects on the total lipids content and the two enzymes Acetylcholinesterase (AchE) and Glutathione-S-transferase (GST). The acaricide Milbecknok recorded high effects on the total lipids and carbohydrates contents and lipase, trehalase, amylase, AchE and GST enzymes. The two acaricides Ortus and Milbecknok are effective in controlling European red mite on apple trees and this can be attributed to abnormal changes of the major biochemical components in the treated mites. The predatory insect; *C. carnea* has efficient predation against *P. ulmi* and resulting increasing in the reduction of mite populations.

Keywords: Apple, acaricides, toxicity, *Chrysoperla carnea*, *Panonychus ulmi*, biochemical changes.

INTRODUCTION

Apple *Malus domestica* Borkh. (Family: Rosaceae) is one of the most common crops in the world. It preferred by consumers and considers an economic crop that has a high rank locally. Worldwide, apple production reaches about 58 billion tons at each year. In Egypt, an average productivity of apple decreased from 557944 to 541000 tons during 2007 and 2012, respectively, (the Annual Report of Agric. Statistics Dept., Ministry of Agriculture, Egypt. Apple infested with several pests including European red mite *Panonychus ulmi* Koch (Acari: Tetranychidae) which from the most destructive mites in apple orchards (Prokopy and Croft, 1994).

The acaricides in controlling mite pests were succeeded in different orchards and field crops and also, predators are using a bio-control agent for controlling the mite pests (Jeyarani *et al.*, 2012). Many studies on the acaricides are needed to evaluate their effects on the biochemical changes of treated insects. The present work was conducted to study effect of releasing predatory insect, *Chrysoperla carnea* Stephens and spraying of acaricides; Ortus 5% EC, Milbeknock 1% EC and Cascade 10% DC to control *P. ulmi* movable stages on apple trees during two successive seasons 2012 and 2013 and evaluated the efficacy of acaricides by determining the biochemical changes in the treated adult females of the mites.

MATERIALS AND METHODS

1. Site of study:

An orchard located at Darawa village, Ashmon, Menoufia governorate was chosen to conduct these experiments during the two successive seasons; 2012 and 2013. Orchard divided as a Complete Randomized Blocks Design to 4 blocks every block contain 5 experimental units (15tree/unit). A row of trees separates between the experimental units, the distances between trees (3m×3m) every treatment contain four replicate.

2. Predator and acaricides:

Predatory insect; *C. carnea* was released as biological control agent and obtained from the Applied Center for Biological Control, Faculty of Agriculture, Cairo University, It was stored in ice box at (10°C) before one day of the application. Trade name, common name, formulation and rate of application of the three acaricides; Ortus 5% EC (Fenpyroximate), Milbeknock 1% EC (Milbermectin) and Cascade 10% DC (Flufenoxuron) are tabulated in Table (1).

3. Release of *C. carnea* and spraying of the acaricides:

The application of release was started when the population of *P. ulmi* reached 3mite/leaf. Release started on 22th April during 2012 season, and on 29th March during 2013 season (40larva/tree). The propagation of predator was in early morning on trees (40 larva/tree). The ratio between predator and prey was 1:75. The cultivated Apple trees received all normal agricultural processes without using acaricides. The experimented area was divided into four treatments and check according to complete randomized block design, each treatment was contained four replicates.

The application of acaricides was started on May 3rd at the first season and on April 9th at the second season. Check treatment was sprayed with water only. A compressor spryer (600 liters capacity) was used. Samples (20 leaves) were taken randomly from each replicate, just before spraying then weekly afterwards. Samples were carefully examined and the numbers of alive moving stages of *P. ulmi* were recorded. An additional spray was conducted after three weeks of the first spray because of the increase of *P. ulmi* population.

4. Biochemical assays:

4.1. Determination of total proteins, carbohydrates and lipids:

Protein content of *P. ulmi* samples was estimated spectro-photometrically by the method of Bradford (1976). Carbohydrates content was determined according to Dubois *et al.*, (1956). Total lipids were determined according to Knight *et al.*, (1972). Total proteins, carbohydrates and lipids contents were calculated and expressed as mg/g of mite body weight.

4,2. Digestive enzymes assays (Protease and lipase):

The proteolytic activity of *P. ulmi* was determined according to Birk, *et al.*, (1962). The proteolytic activity was determined from bovine serum albumin standard curve; as O.D. units x 10^3/ *P. ulmi* body weight. Lipase activity was determined by the method of Tahoun and Abdel Ghaffar (1986). Lipase unit was defined as the number of µg oleic acid liberated/min.

4.3. Carbohydrate hydrolyzing enzymes assays:

The activity of carbohydrate hydrolyzing enzymes (trehalase, amylase, and invertase) was determined according to method of Birk *et al.*, (1962). The optical density (OD) was measured at 550 nm using spectrophotometer.

4.4. Acetylcholinesterase determination:

Acetylcholinesterase (AchE) activity was measured according to the method of Simpson *et al.* (1964). The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

4.5. Glutathione-S-transferase (GST) determination:

Glutathione S-transferase (GST) could be detected as described by the method of Habig *et al.*, (I974). The absorbance at 340 nm was recorded against blank to determine the nanomole substrate conjugated/min/larva using a molar extinction coefficient of 9.6/mM/cm.

Statistical analysis

Level of significance using Fisher's least significant difference (LSD). Using the Statistical Analysis System. Reduction percentages of the *P. ulmi* were calculated according to Henderson and Tilton (1955).

RESULTS AND DISCUSSION

1. Reduction percentages of *P. ulmi* on apple trees during 2012 and 2013 seasons.

Data presented in Table (2) and Fig. (1) showed that, the reduction percentages of *P. ulmi* movable stages after three days of releasing *C. carnea* and spraying acaricides; Ortus, Milbecknok and Cascade were averaged 43.60, 71.40, 67.10 and 47.50 % during 2012 season, respectively; while during 2013 season, it averaged 58.90, 74.30, 74.00 and 39.20%, respectively. The reduction percentages after six days of releasing of *C. carnea* and spraying acaricides; Ortus, Milbecknok and Cascade increased to reach 65.90, 85.10, 77.70 and 60.80% during 2012 season, respectively; while during 2013 season the reduction percentages averaged 79.10, 87.40, 81.20 and 54.70% for the previous treatments, respectively. After nine days of spraying acaricides, the population of *P. ulmi* was increased in apple treated by Ortus and Milbecknok, so the reduction percentages were decreased to 82.20 and 77.00 % during 2012 season, respectively, also, the population of 2013 season increased after nine days of spraying acaricides Ortus, Milbecknok and Cascade, so the reduction percentages were decreased they recorded 75.00, 75.50 and 61.10%, respectively. It was due to new hatching of *P. ulmi* eggs.

After four days from the second spray, the highest reduction percentages of *P. ulmi* was recorded with Ortus (95.50%);while Milbecknok and Cascade were recorded 85.70 and 71.80% during 2012 season, respectively, but during 2013 season the reduction percentages of *P. ulmi* were recorded with *C. carnea*, Ortus, Milbecknok and Cascade 81.80, 87.10, 84.00 and 59.00%, respectively. The highest mean reduction percentages affected by predatory insect; *C. carnea* and three acaricides during 2012 and 2013 seasons were recorded with Ortus averaged 85.57 and 84.18 %, respectively, followed by Milbecknok averaged 80.77 and 81.63 % then the predatory insect; *C. carnea* averaged 75.19 and 78.52%, respectively; while the lowest reduction percentages were recorded with Cascade averaged 60.29 and 54.43%, respectively. Several studies have shown the effectiveness of acaricides against mites and other insects. Rana and Bhardwaj (2004) found that, the acaricides; fenpropathrin, fenazaquin and monocrotophos managed the *P. ulmi* population effectively up to 21 days after spraying. However, fenazaquin kept the mite population below economic threshold level (<5 mites per leaf) even up to 28 days after spraying. Abou El-Ela, (2014) reported that, two of acaricides, Challenger and Ortus were markedly more efficient in reducing the *Tetranychus urticae* Koch than Vertimec, Delmite and Bioca where they produced 81.55% and 80.62% reduction of *T. urticae* in average within 3, 7, 14 and 21 days after spraying at early season (2007), respectively. The similar trend of these results was observed in the second season (2008). The toxic effect of acaricides to mites depends on the chemistry of acaricides, their rates, microclimatic conditions, the development stages of mites. Larvae of *C. carnea* consumed a mean of 24.9 and 23.7 nymphs of *P. ulmi* and *T. urticae*, respectively. The mean total number of preys consumed by *C. carnea* was 396.7 and 379.4 movable stages of *P. ulmi* and *T. urticae*, respectively. *C. carnea* also consumed significant numbers of *Typhlodromus pyri* Scheuten nymphs (4.3/day) and adults (6.9/day), also, suggesting that *C. carnea* is also a hyper predator, (Khan *et al.*, 2004).

2. Biochemical assays:

The efficacy of two acaricides Ortus and Milbecknok on adult females of *P. ulmi* were investigated by estimated main energy reserve substances and seven representative enzymes.

2.1. Determination of total proteins, carbohydrates and lipids (Main energy reserves):

The obtained results in Table (3) showed that, there are significant effect of the acaricide Ortus against the total protein content of the adult females of *P. ulmi* as compared with control where it decrease the total proteins value from 35.67 to 29.93 (mg/g fresh body weight), while no significant difference (35.67 to 36.67 mg/g.b.wt) was observed in the total protein content treated with the other acaricide Milbecknok as compared with control.

The effects of Ortus and Milbecknok on total carbohydrate content were summarized in Table (3). Total carbohydrate content was significantly reduced by Milbeknock (Control value decreased from 12.93 to 7.63 mg/g.b.wt) and the other acaricide Ortus had no significant effect. Also, a significant reduction in the amount of lipid content was observed in the adult females of *P. ulmi* treated with Milbeknock (from 8.13 to 5.37 mg/g fresh body weight) as compared with control, but not significant reduction in case of Ortus (from 8.13-7.97 mg/g b.wt).

The results of the acaricide Ortus are agreement with Behroozi Moghadam *et al.*, (2011) reported that chlorfluazuron and pyriproxyfen did not affect total carbohydrate and lipid contents in white leaf borer, *Ocneria terebinthina* Strg. Megahed *et al.*, (2013) showed that the total protein of *Spodoptera littoralis* treated with certain acaricides (Emamectin benzoate "Proclaim", abamectin "Romacten" and spinosad "Tracer") is significantly decreases about five times compared to that of control. The results of the acaricide Milbecknok confirmed by Elbarky *et al.*, (2008) the effect of Radiant SC12% (Spinetoram) on larval instars of *S. littoralis* showed that, the amount of total carbohydrates and total proteins were significantly decreased. Also, Alimohamadi *et al.*, (2014) found that significant reduction in the amount of lipid content was observed in spirodiclofen-treated larvae of the ladybird beetle, *Hippodamia variegata*. The decrease of the total protein in may reflect the decrease in the enzymatic activities of various enzymes. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis are also the main causes of decreased total protein content (Nabih *et al.*, 1990). Regarding the total lipid content, a number of toxic agents have been found to cause disturbances of fats in different body organs of both vertebrate and invertebrate animals.

2.3. Determination of enzymes:

Digestive enzymes (protease and lipase)

The obtained results in Table (4), showed that, there are non significant effects of the tow acaricides Ortus and Milbeknock on protease activity of *P. ulmi* adult females treated and non treated. Acaricide Milbeknock decrease lipase enzyme activity from 46.7 to 29.17 (µm olec acid/min/g.b.wt), while the other acaricide Ortus had no significant effect. These results are agreement with Zera and Zhao (2004) showed that, methoprene caused reduction in lipase enzyme activity and lipid metabolism in *Gryllus firmus*. Spiromesifen, acts as an inhibitor of a lipid metabolism enzyme, in contrary, Spirodiclofen increases lipase enzyme activity and consequently lipid metabolism resulting decrease in lipid content Alimohamadi *et al.*, (2014). Decreasing of lipase activity tend to disturbance in the vital process of the tested mite, this is because lipids are the most suitable reserves for storage of energy. Compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight (Beenakkers *et al.*, 1985).

Carbohydrate hydrolyzing enzymes

Data illustrated in Table (5) exhibited that, non significant effect on enzyme activity of invertase, trehalase and amylase enzymes with Ortus. The other acaricide Milbeknock achieved high significant effect on trehalase and amylase activities, where it decreased the values of from 118 to 73.67 and 70 to 46.33 (µg glucose/min/g.b.wt) respectively, while, Milbeknock have non significant effect on invertase enzyme. However, activities of the carbohydrate hydrolyzing enzymes, amylase, invertase and trehalase were reduced in adult females after treatment with Milbecknok, compared with control. These data of acaricide Milbecknok, approximately are consist with results reported by Elbarky *et al.*, (2008) the effect of Radiant SC12% on larval instars of *S. littoralis* after 24 hours showed that, the hydrolyzing enzymes (invertase, trehalase and amylase), were significantly decreased compared to control.

Detoxification enzymes (AchE and GST enzymes)

The obtained results in Table (6), showed that, both of acaricides Ortus and Milbeknock have effect on the activities of two enzymes. Ortus significantly elevated activity of the AchE from 122.33 to 176.67 (µg AchBr/min/g.b.wt), but decreased the activity of GST enzyme from 162.67 to 73.33 (mmol.Sub. conjugated/min/g.b.wt). The other acaricide Milbeknock has slight increase in AchE activity from 122.33 to 136.67 (µg AchBr/min/g.b.wt), while the activity of GST enzyme decreased from 162.67 to 97.33 (mmol. Sub. conjugated/min/g.b.wt). These results confirmed by Elbarky *et al*., (2008) the activity of acetylcholineestrase larval instars of *S. littoralis* treated spinosyn was significantly increased compared to control. However, our results disagree with results obtained by Loucif-Ayad *et al*. (2008) the acaricides used to control the parasitic mite *Varroa destructor* (Acari, Varroidae) did not affect the activity of AChE, but It is possible that highly doses of acaricides will elicit AChE activity decrease.

Our results of GST enzyme are agree with the findings of Itabajara *et al*., (2004) who found that, the tested acaricides and insecticides (Ethion, amitraz, diazinon, chlorpyrifos, DDT, cypermethrin, ivermectin, deltamethrin and flumethrin) inhibit *Boophilus microplus* GST activity in 12–34% compared to control group, except for ivermectin, which does not inhibit GST and activity of GST is sensitive to various compounds used in commercial acaricides. In contrast, results of Loucif-Ayad *et al*., (2008) indicated that treatments with acaricides led to increased GST activity in the larvae and pupae of bees compared with the control.

CONCLUSIONS

The two acaricides Ortus and Milbecknok are effective in controlling European red mite *P. ulmi* on apple trees. Substantial biochemical events in adult females are involved in responding to the action of these two acaricides. The predatory insect *C. carnea* release proved to be efficient in reducing *P. ulmi* movable stages population; therefore, the study recommends the use of this predator with integrated pest management programs (IPM) in controlling *P. ulmi* to reduce the using of chemical pesticides.

Table 1. Data of three acaricides which tested against *P. ulmi* on apple trees.

Trade name	Active ingredient(s)	Chemical class of AI(s)	Rate of application
Ortus 5% EC	Fenpyroximate	Pyrazole	$50cm^3$/100L water
Milbeknock 1% EC	Milbermectin	Avermectin	$75cm^3$/100L water
Cascade 10% DC	Flufenoxuron	Benzoylurea	$60cm^3$/100L water

Table 2. Reduction percentages of *P. ulmi* movable stages on apple affected by predatory insect; *C. carnea* and three acaricides during 2012 and 2013 seasons.

Sampling date	Reduction percentages of *P. ulmi* movable stages /leaf			
	C. carnea	Ortus	Milbecknok	Cascade
06/05/2012	43.6	71.4	67.1	47.5
09/05/2012	65.9	85.1	77.7	60.8
12/05/2012	85.3	82.2	77.0	70.6
17/05/2012	84.6	95.5	85.7	71.8
22/05/2012	80.6	90.6	86.9	55.5
27/05/2012	77.6	89.0	87.7	51.7
02/06/2012	88.7	85.2	83.3	64.1
Mean	75.19b	85.57a	80.77a	60.29c
LSD at 0.05		11.4		
12/04/2013	58.9	74.3	74.0	39.2
15/04/2013	79.1	87.4	81.2	54.7
20/04/2013	81.1	75.0	75.5	61.1
25/04/2013	81.8	87.1	84.0	59.0
30/04/2013	82.4	88.9	87.4	57.7
05/05/2013	87.8	92.4	87.7	54.9
Mean	78.52a	84.18a	81.63a	54.43b
LSD at 0.05		13.5		

Table 3. Effect of two acaricides on total proteins, carbohydrates and lipids of *P. ulmi* adult females.

Acaricides	*P. ulmi* main energy reserves (mg/g.b.wt)		
	Total proteins	Total carbohydrates	Total lipids
Ortus 5%EC	29.93b	12.93a	8.13a
Milbeknock 1% EC	36.67a	11.33b	7.97a
Control	35.67a	7.63c	5.37b
F value	22.375**	73.090***	26.74***
LSD 0.05	2.650	1.100	1.038

Table 4. Effect of two acaricides on protease and lipase enzymes of *P. ulmi* adult females.

Acaricides	Enzyme activity	
	Protease O.D. units x 10^3/g.b.wt.	Lipase µg oleic acid liberated/min/g.b.wt.
Ortus 5%EC	29.93b	12.93a
Milbeknock 1% EC	36.67a	11.33b
Control	35.67a	7.63c
F value	22.375**	73.090***
LSD 0.05	2.650	1.100

Table 5. Effect of two acaricides on carbohydrate hydrolyzing enzymes of *P. ulmi* adult females.

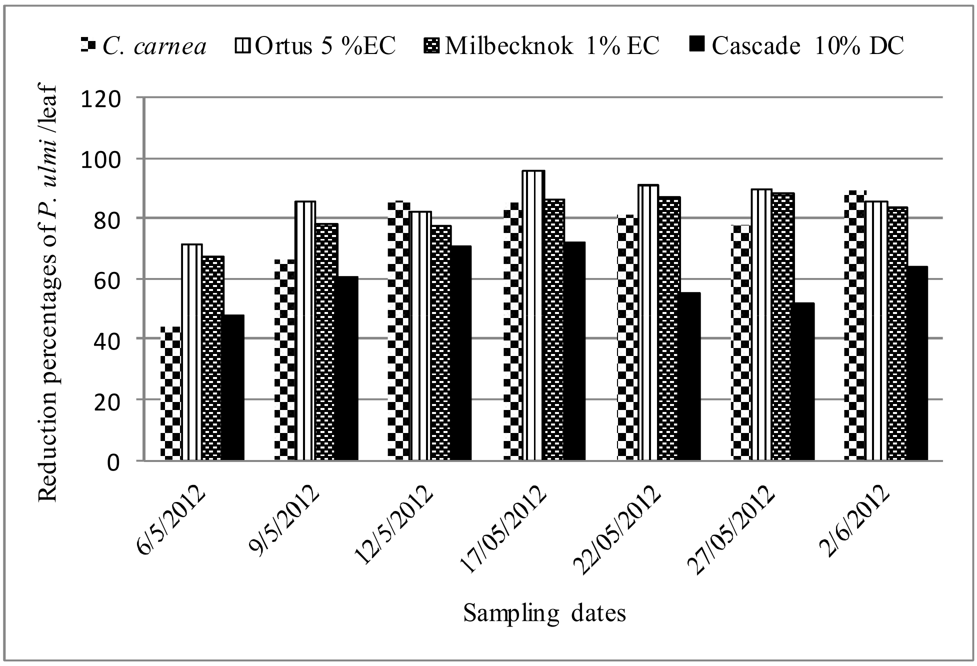
Acaricides	Enzyme activity (µg glucose/min/g.b.wt)		
	Invertase	Trehalase	Amylase
Ortus 5%EC	412.00b	128.33a	78.33a
Milbeknock 1% EC	436.33a	73.67b	46.33b
Control	424.00ab	118.00a	70.00a
F value	8.415*	84.658***	45.37***
LSD 0.05	14.514	10.923	8.528

Table 6. Effect of two acaricides on AchE and GST enzymes of *P. ulmi* adult females.

Acaricides	Enzyme activity	
	AchE (µgAchBr/min/g.b.wt)	GST (m mol.Sub.conj./min/g.b.wt)
Ortus 5%EC	176.67a	73.33b
Milbeknock 1% EC	136.67b	97.33c
control	122.33c	162.67a
F value	58.013***	137.409 ***
LSD 0.05	12.79	13.648

Means with the same letter(s) are not significantly different. (P<0.05)

LSD = least significant difference.



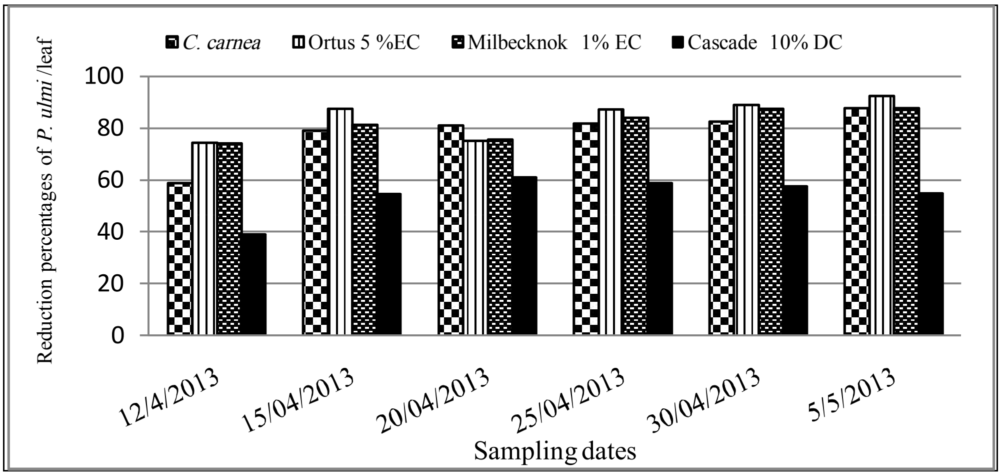


Fig. 1. Reduction percentages of *P. ulmi* movable stages on apple affected by predatory insect; *C. carnea* and three acaricides during 2012 and 2013 seasons.

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تقييم ثلاثة من المبيدات الأكاروسية وأحد المفترسات الحشرية ضد العنكبوت الأحمر الأوربي علي أشجار التفاح

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يهدف هذا البحث إلي تقييم ثلاثة أنواع من المبيدات الأكاروسية (Ortus 5% EC, Milbeknock 1% EC and Cascade 10% DC) وأحد المفترسات الحشرية (أسد المن) *Chrysoperla carnea* علي الأطوار المتحركة للعنكبوت الأحمر الأوربي (*Panonychus ulmi* Koch (Acari: Tetranychidae) الذي يصيب أشجار التفاح خلال موسمي ٢٠١٢ و٢٠١٣ في قرية داراوة مركز أشمون محافظة المنوفية وكذلك تقدير التغييرات الكيميائية الحيوية للحشرات المعاملة. أوضحت النتائج أن أعلي نسب إنخفاض في تعداد الأطوار المتحركة للعنكبوت الأحمر الأوربي أثناء موسمي ٢٠١٢ و٢٠١٣ سجلت مع مبيد Ortus يليه مبيد Milbecknok ثم المفترس الحشري *C. carnea* بينما أقل نسب إنخفاض سجلت مع مبيد Cascade.

أظهرت نتائج المبيدات الأعلي سمية (Ortus and Milbeknock) علي التغييرات في المكونات الكيميائية الحيوية لإناث العنكبوت الأحمر الأوربي، أن مبيد Ortus أحدث تغيرا كبيرا في محتوي اللبيدات الكلية والأنزيمين Acetylcholinesterase and Glutathione-S-transferase. بينما مبيد Milbecknok سجل تأثيرا عاليا علي كل من محتوي اللبيدات والكربوهيدرات الكلية وكذلك الإنزيمات التالية lipase, trehalase, amylase, Acetylcholinesterase and Glutathione-S-transferase

نستخلص من النتائج السابقة أن نوعين من الثلاثة أنواع من المبيدات الأكاروسية لهما تأثير فعال في مكافحة العنكبوت الأحمر الأوربي علي أشجار التفاح وأن تفسير هذا يمكن إرجاعه إلي التغيرات الغير الطبيعية الحادثة في معظم المكونات الكيميائية الحيوية للحشرة. أيضا مفترس الحشرات *C. Carnea* ذو كفاءة إفتراسية عالية ضد العنكبوت الأحمر الأوربي وينتج عن ذلك زيادة في إنخفاض تعداد العنكبوت.