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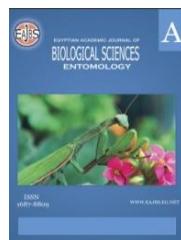
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**Disturbance of the Acetylcholinesterase Activity in Haemolymph and Fat Bodies of The Desert Locust, *Schistocerca gregaria* (Orthoptera: Acrididae) by Margosan-O® (0.3% Azadirachtin)**

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**ABSTRACT**

The desert locust *Schistocerca gregaria* is a dangerous pest for several fields and orchard crops. Great attention has been paid to use botanicals for controlling this pest. The current investigation aimed to assess the disturbing effect of margosan-O® (0.3% Azadirachtin) on the acetylcholinesterase (AchE) activity in haemolymph and fat bodies of *S. gregaria*. The penultimate (4<sup>th</sup>) instar nymphs were treated, *via* the fresh food, with two concentrations (0.9 and 0.4%) of margosan-O® and the activity of AchE was estimated in the last instar nymphs and newly emerged adults. The most important results could be shown as follows. In the present study, margosan-O® caused a predominant enhancement of AchE activity in the haemolymph of last instar nymphs and adults, regardless of the concentration. Also, the enzyme activity was induced in fat bodies of nymphs and adults, with exception of the late-aged nymphs, nymphs. In these late-aged nymphs, the AchE activity was inhibited in the fat bodies (60.8 and 65.3% decrements, at the higher and lower margosan-O® concentrations, respectively).

**INTRODUCTION**

The desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) is a dangerous pest for many crops, particularly which are used as the main food source for man and animals. In some cases, a single swarm contains 80 billion adult locusts per square kilometer of an area (Steedman, 1988). Plagues of this pest have been recognized as a threat to agricultural production in Africa and western Asia for thousands of years (Showler, 1995, 1996; Ceccato *et al.*, 2007). Each individual gregarious locust can consume roughly its own weight of foliage daily (Lindsey, 2002). Invasions of this locust are the cause of calamity because they can result in 100% crop loss (Meinzingen, 1993; FAO, 2012). Therefore, it is necessary to search and develop some effective control measures for suppressing the population density and/or inhibition of the phase transition into gregaria to avoid the formation of locust swarms. Because of the difficulty to predict locust outbreaks, the concerned countries usually apply hazardous chemical pesticides for the control (Gruys, 1993). As reported by some authors (Lecoq, 2001; Cirad, 2004), the

current control strategies against this locust are mainly based on organophosphorus pesticides.

The indiscriminate uses of many synthetic insecticides lead to the destruction of the natural enemies (like parasites, predators), allowing an exponential increase of pest populations (Naqqash *et al.*, 2016) and serious toxicological hazards to humans (Costa *et al.*, 2008; Mosallanejad and Smagghe, 2009). Also, the repeated use of a particular insecticide may result in the development of resistance in the insect (Bell *et al.*, 2001; FAO, 2003). Therefore, the desert locust remains a serious problem despite the usage of these synthetic insecticides (Ouali-N'goran *et al.*, 2013). To overcome the previously mentioned hazards of synthetic insecticides, it is important to search for new effective and safer materials with negligible effects on the ecosystem (Dubey *et al.*, 2010; Chandler *et al.*, 2011; Korrat *et al.*, 2012).

Much attention has been paid to use plant extracts or plant products that have some insecticidal effects (Schmutterer, 1990 a, b; Krall and Wilps, 1994). Plants may provide potential alternatives to currently used insecticides because they constitute a rich source of bioactive chemicals (Rembold, 1994; Cosimi *et al.*, 2009; Qin *et al.*, 2010). The botanicals are reported to be more effective, rapidly biodegradable, less expensive, low toxic to natural enemies and mammals, safe for mankind (Singh *et al.*, 1996) and more selective in action than synthetic insecticides (Keita *et al.*, 2001; Rahman and Siddiqui, 2004; Pintong *et al.*, 2020). Several plant species affect differentially the fertility, development, behaviour, and survival of *S. gregaria* (Idrissi Hassani, 2000; Abbassi *et al.*, 2004).

However, the plant compounds have been the subject of the thorough investigation for the past 40 years in an effort to discover new sources of botanical insecticides and antifeedants (Sabjan *et al.*, 2017). Therefore, botanical insecticides have been the subject of several recent books and reviews (Hedin *et al.*, 1997; Koul and Dhaliwal, 2001; Isman, 2006; Koul and Walia, 2009; Koul, 2005, 2008, 2012, 2016) and many other publications. According to Isman and Grieneisen (2014), > 20,000 papers on botanical insecticides were published from 1980 to 2012, indicating a major growth in the number of papers published annually.

The neem, *Azadirachta indica*, is one among the most well-investigated plants in this context affecting survival, growth, feeding, fecundity, fertility and some physiological and anatomical processes of insects (Mordue *et al.*, 1998; Breuer and De Loof, 2000; Sayah, 2002; Breuer *et al.*, 2003; Huang *et al.*, 2007; Senthil Nathan *et al.*, 2008; Correia *et al.*, 2009). Many entomologists proved the efficacy of extracts and essential oils derived from *A. indica*. Therefore, Neem plant was taken as a reference plant for different studies (Sultana *et al.*, 2016). Margosan-O® is a formulation of neem oil fractions (Azadirachin content is 0.3%) and reported to show toxic, repellent, oviposition-deterring and growth activities to some insect species (Meisner *et al.*, 1990, 1992; Scott and Kaushik, 1998; Ghoneim and Al-Dali, 2002). Against *Spodoptera littoralis*, Margosan-O® prolonged the developmental durations, blocked the adult emergence, caused some morphological anomalies (Haubrige *et al.*, 1994; Meisner and Nemny, 2009) and exhibited disruptive effects on some haemogram parameters (Rizk *et al.*, 2002). Also, Margosan-O® detrimentally prohibited fecundity and fertility (Amer *et al.*, 2004; Ghoneim *et al.*, 2007) and disturbed the acid phosphatase activity (Ghoneim *et al.*, 2008) of *Musca domestica* after-treatment of the early third instar larvae. In addition, Margosan-O® exhibited impairing effects on the development and morphogenesis (Al-Dali *et al.*, 2003) and drastically reduced the fecundity (Ghoneim and Al-Dali, 2002) of *Muscina stabulans*. Unfortunately, large scale production is problematic and the difficulties that face the registration of variable products will limit adoption (Meinzingen

and Kooyman, 1997). Otherwise, prior results on the effects of plant extracts on the desert locust were encouraging their implementation as an alternative measure to chemical control (Abbassi *et al.*, 2003).

Haemolymph is the only extracellular fluid in the insect body that is usually circulated by an open heart within the body cavity. It performs several functions, such as the transportation of food materials to the cells and metabolic waste products away from those cells. It, also, transports the hormones for regulation of the larval moulting, growth, metamorphosis, metabolism and other physiological processes of insects (Hietakangas and Cohen, 2009). In insects, the use of haemolymph as a medium for controlling the insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of the insect's body (Rodriguez-Ortega *et al.*, 2003; Pugazhvendan and Soundararajan, 2009). On the other hand, the fat body of insects is responsible for a wide array of different metabolic activities (Arrese and Soulages, 2010). As reported by some authors (Park *et al.*, 2006; Vivekananthan *et al.*, 2010), the insect fat body is an important organ to synthesize and store energy reserve, in addition, to regulate metabolic activities and reproduction.

Acetylcholinesterase (AchE, EC 3.1.1.7) is a key enzyme for catalyzing the hydrolysis of acetylcholine, a neurotransmitter, in the nervous system (Grundy and Still, 1985; Wang *et al.*, 2004; Zibaee, 2011). AchE is primarily responsible for the termination of cholinergic neurotransmission at synapses in both insects and human (Fournier and Mutero, 1994; Carlier *et al.*, 2008). On the other hand, AchE is known to be the target of many organophosphate- and carbamate-based insecticides which cause modifications of the active site of the enzyme leading to the inhibition of its activity and block the hydrolysis of acetylcholine (Oppenoorth and Welling, 1979). Thus, AchE activity is one of the main resistance mechanisms in various insect species against the organophosphorous or carbamate-resistant insects (Zhu and Gao, 1999; Kozaki *et al.*, 2001; Li and Han, 2002; Fournier, 2005; Yu, 2006). For medical purposes, Barbosa *et al.* (2006) reviewed 309 plants and 260 chemically defined natural molecules reported in the literature, which have been evaluated for the AchE inhibition. Some years later, a comprehensive review of cholinesterase inhibitor phytoconstituents was presented by Ahmed *et al.* (2013). In respect of pest control, AchE activity had been disturbed by some plant extracts (Senthil Nathan *et al.*, 2008). Most of the plant essential oil components inhibit the AchE activity (Lee *et al.*, 2001; Abdelgaleil *et al.*, 2009; Zapata and Smagghe, 2010). Also, essential oil from the Tunisian *Citrus aurantium* caused the death of *Bemisia tabaci* by the inhibition of AchE activity (Zarrad *et al.*, 2015). The current study was achieved aiming at the investigation of the disturbing effect of Neemazal on the AchE activities in haemolymph and fat bodies of *S. gregaria* last instar nymphs and adults.

## MATERIALS AND METHODS

### The Insect Culture:

The desert locust *Schistocerca gregaria* (Forskal)(Orthoptera: Acrididae) was used as an experimental insect in the present study. The present culture was originated by a sample of gregarious nymphs from Plant Protection Research Institute, Ministry of Agriculture, Giza. As designed by Hunter-Jones (1961) and improved by Ghoneim *et al.* (2009), insects were reared in wooden cages (60 x 60 x 70 cm). The bottom was furnished with a sandy layer (20 cm depth) provided with 10-15% humidity suitable for egg laying. An electric bulb (100 watts) was adjusted in each cage to maintain a continuous photoperiod of 12 L: 12 D as well as an ambient temperature ( $32\pm2^{\circ}\text{C}$ ). The insects were reared and handled under crowded conditions. The feces, dead locusts and

food remains were removed daily before introducing fresh food. Care was seriously taken to clean these cages at regular intervals and the sand was sterilized in a drying oven (at 140°C for 24 hours) to avoid contamination with any pathogenic microorganisms. Fresh clean leaves of clover *Trifolium alexandrinum* were provided as food.

#### **Nymphal Treatment with The Neem Extract:**

The assessed botanical in the present study was Margosan-O® (Neem seed kernel preparation with 0.3% EC Azadirachtin). It was purchased from T. Stanes & Company Ltd (Coimbatore, India). In a preliminary experiment, sublethal concentrations of Margosan-O® against *S. gregaria* were determined as 1.0, 0.9, 0.4 & 0.1%. Only two concentrations, 0.9 & 0.4%, were tested to investigate the effect of Margosan-O® on Acetylcholinesterase (AchE) activity in *S. gregaria*.

After treatment of the newly moulted penultimate (4<sup>th</sup>) instar nymphs of *S. gregaria* through the fresh food leaves of *T. alexandrinum* dipped once in each concentration of Margosan-O® for 3 minutes, the successfully moulted final instar nymphs and emerged adult females were undergone to determine the influenced AchE activity in two tissues: haemolymph and fat body. Three ages of last instar nymphs were only used: early- (1-day old), mid- (4-day old) and late-aged (7-day old) nymphs.

#### **Sampling of Tissues:**

For the determination of AchE activity in the haemolymph, it was collected from last instar nymphs and newly emerged adult females. Haemolymph was drawn into Eppendorff Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. For whole blood assays, the diluted haemolymph was frozen for 20s to rupture the haemocytes. The haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymph of two individuals were never mixed. For the determination of AchE activity in the fat body, samples were collected from last instar nymphs (of the same ages) and newly emerged adults. The fat body samples were weighed and then homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until the use for the enzymatic determination. Three replicates were used and the fat bodies from two individuals were avoided to be mixed.

#### **Determination of AchE Activity:**

The AchE activity was determined according to the method of Weber (1966) using a kit of Diamond company. The enzyme was measured at a wavelength of 405 nm by a spectrophotometer.

#### **Statistical Analysis of Data:**

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means.

## **RESULTS**

### **Effect of Margosan-O® on the Acetylcholinesterase (AchE) Activity in Haemolymph of *S. gregaria*:**

As obviously shown in Table (1), the AchE activity in haemolymph of control last instar nymphs and newly emerged adults of *S. gregaria* run in a U-shaped curve the fundus of which was found in the mid-aged nymphs ( $6648.3 \pm 678.4$ ,  $5865.0 \pm 587.5$ ,

$7430.0 \pm 675.5$  &  $8601.7 \pm 678.4$  U/L, in early-, mid-and late-aged nymphs and adults, respectively).

After treatment of the newly moulted penultimate (4<sup>th</sup>) instar nymphs with two concentrations (0.9 & 0.4%) of margosan-O®, the activity of AchE was determined in haemolymph of last instar nymphs and newly emerged adults. Depending on the data assorted in the same table, margosan-O® exhibited a predominant enhancing effect on AchE activity, since the enzyme level prevalently increased in the haemolymph of last instar nymphs and adults, regardless of the concentration. Moreover, the enhancing effect of margosan-O® was dose-dependent in all developmental stages. Also, margosan-O® displayed the strongest inducing effect on AchE activity in the mid-aged nymphs ( $10166.7 \pm 677.0$  U/L at concentration 0.9% vs.  $5865.0 \pm 587.5$  U/L of control congeners, with 73.3% increment). On the other hand, the least potent inducing effect of margosan-O® on the enzyme activity was exhibited in the same age of nymphs at the lower concentration. In other words, the maximum and minimum activities of AchE were determined in the mod-aged nymphs, after treatment with higher and lower concentrations, respectively.

**Table 1:** Effect of Margosan-O on the Acetylcholinesterase activity (U/L) in haemolymph of the desert locust, *Schistocerca gregaria*.

Conc. %		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
0.9	Mean ± SD	$10946.7 \pm 678.4$ c	$10166.7 \pm 677.0$ c	$10556.7 \pm 586.3$ c	$12120.0 \pm 675.5$ c
	Change %	+64.7	+73.3	+42.1	+40.9
0.4	Mean ± SD	$8991.7 \pm 677.0$ b	$7037.3 \pm 586.3$ a	$9775.0 \pm 675.5$ b	$10946.7 \pm 678.4$ b
	Change %	+35.2	+20.0	+31.6	+27.3
Controls	Mean ± SD	$6648.3 \pm 678.4$	$5865.0 \pm 587.5$	$7430.0 \pm 675.5$	$8601.7 \pm 678.4$

Conc.: Concentration levels, mean ± SD followed with the same letter (a): is not significantly different ( $P>0.05$ ), (b): significantly different ( $P<0.05$ ), (c): highly significantly different ( $P<0.01$ ), (d): very highly significantly different ( $P<0.001$ ).

#### Effect of Margosan-O® on the AchE Activity in Fat Bodies of *S. gregaria*:

Data of the AchE activity in fat bodies of control nymphs and adults of *S. gregaria* were arranged in Table (2). Depending on these data, the enzyme activity run in a U-shaped curve with the fundus in fat bodies of mid-aged nymphs ( $2374.4 \pm 242.3$ ,  $860.0 \pm 135.1$  and  $991.3 \pm 165.1$  U/L, in control early-, mid-and late-aged nymphs, respectively). In addition, AchE activity in fat bodies of control adults was lower than that activity in late-aged nymphs (985.7±197.5 U/L in adults, compared to  $991.3 \pm 165.1$  U/L in late-aged nymphs).

After treatment of the newly moulted penultimate (4<sup>th</sup>) instar nymphs with two concentrations (0.9 & 0.4%) of margosan-O®, the activity of AchE was determined in fat bodies of last instar nymphs and newly emerged adults. Data of the disturbed enzyme activity were listed in Table (2). With an exception of the late-aged nymphs, nymphs of other ages and adults had remarkably enhanced AchE activity in the fat bodies. In other words, The AchE activity was inhibited in the fat bodies of only late-aged nymphs (60.8 and 65.3% decrements, at the higher and lower margosan-O® concentrations, respectively). On the other hand, the most pronouncedly induced AchE activity was determined in fat bodies of the mid-aged nymphs (219.1% increment, regardless of the margosan-O® concentration). With regard to the treated adults, AchE activity was found in reverse relation to the margosan-O® concentration (98.3 & 142.2% increments, at the higher and lower concentrations, respectively).

**Table 2:** Effect of Margosan-O on the Acetylcholinesterase activity (U/L) in fat bodies of the desert locust, *Schistocerca gregaria*.

Conc. %	Last instar nymphs			Newly emerged adults	
	Early-aged	Mid-aged	Late-aged		
0.9	Mean ± SD	2407.7 ± 1039.2 a	2743.9 ± 488.1 c	391.0 ± 84.8 c	1954.8 ± 129.9 c
	Change %	+1.4	+219.1	-60.8	+98.3
0.4	Mean ± SD	2502.2 ± 312.7 a	2744.4 ± 475.0 c	344.0 ± 54.0 c	2387.8 ± 233.9 c
	Change %	+5.4	+219.1	-65.3	+142.2
Controls	Mean ± SD	2374.4 ± 242.3	860.0 ± 135.1	991.3 ± 165.1	985.7 ± 197.5

Conc., a, c: see footnote of Table (1).

## DISCUSSION

Among the detoxification enzymes, Acetylcholinesterase (AchE) is a key enzyme for catalyzing the hydrolysis of acetylcholine, a neurotransmitter, in the nervous system (Wang *et al.*, 2004; Chang *et al.*, 2004; Zibaei, 2011). AchE is primarily responsible for the termination of cholinergic neurotransmission at synapses in both humans and insects (Fournier and Mutero, 1994; Carlier *et al.*, 2008). On the other hand, AchE is known to be the target of many organophosphate- and carbamate-based insecticides which cause modifications of the active site of the enzyme leading to the inhibition of its activity and block the hydrolysis of acetylcholine (Oppenoorth and Welling, 1979).

The AchE activity in insects was reported to be disturbed by some botanicals, such as the house fly *Musca domestica* and German cockroach *Blatella germanica* by some neem compounds (Naqvi, 1986), The fall armyworm *Spodoptera frugiperda* and the Madeira cockroach *Leucophaea maderae* by *Melia azedarach* extracts (Breuer *et al.*, 2003); the American cockroach *Periplaneta americana* by azadirachtin (Shafeek *et al.*, 2004); the brown planthopper *Nilaparvata lugens* and the small brown planthopper *Leodelphax striatellus* by a mixture of insecticide carbosulfan and the plant extract wood vinegar (Kim *et al.*, 2008); *N. lugens* (Senthil Nathan *et al.*, 2008) and *S. frugiperda* (Bullangpoti *et al.*, 2012) by *M. azedarach* senescent leaf extracts. Moreover, the enzyme activity was disturbed in the diamondback moth *Plutella xylostella* and the beet armyworm *Spodoptera exigua* larvae by flupyrazofos (pyrazole organophosphorous insecticide), depending on the larval instar and the time of determination (Lee *et al.*, 2003).

However, the inhibition of AchE activity had been reported in different developmental stages of *M. domestica* by ethanol extracts of *Annona squamosa* and *Calotropis procera* seeds (Begum *et al.*, 2011). Also, ethanol senescent leaf extracts of *Jatropha gossypifolia* and *Melia azedarach* inhibited the AchE activity in *S. frugiperda* larvae (Bullangpoti *et al.*, 2012). Ghoneim *et al.* (2012) recorded a pronounced inhibition in the enzyme activity in haemolymph of early-aged nymphs and the newly emerged adults of the desert locust *Schistocerca gregaria* after nymphal treatment with *Fagonia bruguieri* extracts. The essential oil from the Tunisian *Citrus aurantium* caused the death of the Silverleaf whitefly *Bemisia tabaci* by the inhibition of AchE activity (Zarrad *et al.*, 2015).

A significant reduction in AchE activity was reported in the honey bee *Apis mellifera* after treatment with spinosad (Rabea *et al.*, 2009), in *B. germanica* after treatment with boric acid (Habes *et al.*, 2006), in *N. lugens* after treatment with azadirachtin (Senthil Nathan *et al.*, 2008) and in *B. germanica* after treatment with spinosad or indoxacarb (Maiza *et al.*, 2013). In addition, the enzyme activity significantly

decreased in the 4<sup>th</sup> instar larvae of *S. littoralis* after treatment with LC<sub>50</sub> of hexaflumuron or spinetoram (Assar *et al.*, 2016). Saad *et al.* (2018) evaluated six monoterpenes and two phenylpropenes against *Sitophilus oryzae* adults. All of the tested compounds showed AchE inhibition. According to Abdellaoui *et al.* (2019), the AchE activity in the 5<sup>th</sup> instar nymphs of the migratory locust *Locusta migratoria* was reduced after treatment with methanolic extract of olive leaves. Treatment of newly moulted 4<sup>th</sup> and 5<sup>th</sup> instar nymphs of *L. migratoria* with methanolic extract of *Pergularia tomentosa* resulted in a reduction of the activity of AchE (Miladi *et al.*, 2019). AchE activity in adults of *Sitophilus oryzae* and *Tribolium castaneum* was inhibited after treatment with the *Mentha piperita* essential oil (Rajkumar *et al.*, 2019). Among five tested plants against *M. domestica*, treatment of 2<sup>nd</sup> instar larvae with a 25% concentration of *Peganum harmala* led to a reduction in the AchE activity (Zahoor *et al.*, 2020). After treatment of the 4<sup>th</sup> larval instar of *S. littoralis* with chloroform/methanol extract of *Conyza dioscoridis* aerial parts, the acetylcholinesterase activity was remarkably reduced in the larval body homogenate (Matloub *et al.*, 2021). Results of the present study were in agreement with those reported results since the treatment of the newly moulted penultimate instar nymphs of *S. gregaria* with margosan-O® resulted in a considerable inhibition of AchE activity in fat bodies of only the late-aged nymphs.

To explicate the considerable inhibition of the AchE activity in fat bodies of the late-aged nymphs of *S. gregaria*, after treatment with margosan-O®, this inhibition might be due to the toxic potency of margosan-O® leading to accumulation of acetylcholine at the synapses, so that the post-synaptic membrane was in a state of permanent stimulation, which resulted in paralysis, ataxia, general lack of co-ordination in the neuro-muscular system, and eventually death (Aygun *et al.*, 2002; Massa *et al.*, 2008; Senthil Nathan *et al.*, 2008; Gamil *et al.*, 2011; Begum *et al.*, 2011). In addition, the prohibited AchE activity in the late-aged nymphs of *S. gregaria* might indirectly indicate the damage of their nerve cells, which induced the AchE photoactivation and death caused by the disruption of normal nerve conduction (Yin *et al.*, 2008). Also, inhibition of AchE resulted in over-accumulation of Ach and prolonged electrical activity at nerve endings, which comprises a key mechanism of toxicity for pesticides (Maiza *et al.*, 2013).

In contrast, treatment of the newly moulted penultimate instar nymphs of *S. gregaria*, in the current study, with margosan-O® resulted in a predominant enhancement of AchE activity in the haemolymph of last instar nymphs and adults, regardless of the concentration. Also, the enzyme activity was induced in fat bodies of nymphs and adults, with an exception of the late-aged nymphs. These results were, to some extent, in accordance with similar responses of different stages of *S. gregaria* to the treatments with LC<sub>30</sub> or LC<sub>50</sub> of abamectin (El-Aziz, 2010), *Nigella sativa* extracts (Hamadah, 2009). Also, AchE activity was remarkably induced in the haemolymph of the last instar nymphs and in the fat body of adults of *S. gregaria*, after treatment with *Fagonia bruguieri* extracts (Ghoneim *et al.*, 2012). Similar findings were reported, also, in the 4<sup>th</sup> instar larvae of *S. littoralis* after treatment with camphor plant oil followed by chlorpyrifos (Fetoh and Asiry, 2013). Many authors (El-Barky *et al.*, 2008; Fahmy and Dahi, 2009; Rashwan, 2013) reported a moderate induction of AchE activity of the same insect after treatment with Spinetoram or emamectin and teflubenzuron (Assar *et al.*, 2016). The activity of AchE in the 4<sup>th</sup> instar larvae of the red flour beetle *Tribolium castaneum* was significantly induced by garlic essential oil (*Allium sativum*) (El-Gizawy *et al.*, 2019).

The predominant inductive effect of margosan-O® on the AchE activity in haemolymph and fat bodies of nymphs and adults of *S. gregaria*, in the present study, could not be unfortunately explicated right now!!.. However, the increasing AchE activity in the present study on *S. gregaria* may have resulted from an increase in gene copy

number rising up to approximately 80 copies per diploid genome (Field *et al.*, 1993, 1999). Further investigation should be conducted in the future for exploring the exact inducing mechanism of margosan-O® on the activity of this enzyme in the present insect pest.

#### **Conclusion:**

The remarkable disturbance of AchE activity in *S. gregaria* haemolymph and fat bodies by Margosan-O® suggested that this neem extract may be used for the development of biopesticides in the future to control the populations of the present pest as safer, eco-friendly, and economic alternatives to the synthetic pesticides.

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### ARABIC SUMMARY

**اضطراب نشاط إنزيم أستاكوليناستيريز في الهيموليف والأجسام الدهنية للجراد الصحراوي شيسوسيركا جريجاريا (مستقيمات الأجنحة: الجراديات) نتيجة المعاملة بمستخلص مارجوزان**

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يعتبر الجراد الصحراوي آفة خطيرة للعديد من المحاصيل الحقلية والمحاصيل البستانية. وهناك اهتمام كبير في أنحاء متفرقة من العالم باستخدام المستخلصات والمنتجات النباتية لمكافحة هذه الآفة. استهدف البحث الحالي اختبار تأثير مستخلص مارجوزان (0.3% أزادراختين) في اختلال نشاط إنزيم أستاكوليناستيريز في الهيموليف والأجسام الدهنية بحوريات ويافعات الجراد الصحراوي. ومن أجل ذلك، تمت معاملة الغذاء النباتي الطازج بتركيزين من هذا المستخلص وتغذية حوريات الدور قبل الأخير (الرابع) به. ثم دراسة التأثير الاختلالي لنشاط الإنزيم في حوريات الدور الأخير وكذا في يافاعات حديثة البزوغ. وأمكن الحصول على النتائج الآتي ملخصها. أبدى مستخلص مارجوزان تأثيراً حافظاً سائداً في نشاط الإنزيم، بهيموليف حوريات الدور الأخير، بصرف النظر عن مستوى التركيز المستخدم. كما حفز المستخلص نشاط الإنزيم في الأجسام الدهنية بالحوريات واليافاعات، مع وجود حالة استثنائية لانخفاض النشاط في الحوريات متأخرة العمر، ففي هذه الحوريات، فقط، تُبطّل المستخلص نشاط الإنزيم في الأجسام الدهنية (65.3% نسبة الانخفاض) بعد استعمال التركيز الأعلى والتركيز الأسفلي، على التوالي.