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Acetylcholinesterase Activity in the Desert Locust *Schistocerca gregaria* (Acrididae) (Forsk.) as a Response to the Action of the Wild Herb *Fagonia bruguieri* DC. (Zygophyllaceae) Extracts

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ABSTRACT

The plant products are the most promising source for their biological activity against various pests. The strong inhibition of Acetylcholinesterase (AchE: EC 3.1.1.7) is the principal underlying mechanism of action of most of the potential pesticides. In the present study, the newly moulted penultimate instar nymphs of the desert locust *Schistocerca gregaria* were treated with methanolic (at concentration levels: 7.5 or 3.7%), petroleum ether or n-butanolic (at 30 or 15%) extract from the wild herb *Fagonia bruguieri.* The AchE activity was determined in the haemolymph and fat body of the last instar nymphs and newly formed adult females. Along the nymphal life, remarkably induced AchE activity was unexceptionally observed in the haemolymph, especially of the early- and mid-aged nymphs. With regard to the enzyme activity in the fat body of *S. gregaria* nymphs, pronouncedly inhibited activity in the early-aged nymphs, regardless of the extract or its concentration level. The strongest prohibiting effect was exhibited by both the petroleum ether and n-butanolic extracts. On the contrast, AchE activity was enhanced in the fat body of all nymphs other than the early-aged ones. The *F. bruguieri* extracts exerted a potent inhibitory action on the AchE activity in the haemolymph of the newly emerged adults of *S. gregaria*. Such action was reversed on the AchE activity in the fat body of adults, whatever the extract or concentration level. The present results suggest that the wild herb *F. bruguieri* may prove to be a probable candidate for the development of biopesticides to control the populations of the desert locust as safer, ecofriendly and economic alternatives to the synthetic pesticides.

Key words: *Schistocerca gregaria, Fagonia bruguieri,* Acetylcholinesterase, methanol, petroleum ether, n-butanol, haemolymph, fat body, nymph, adult.

INTRODUCTION

Needless to say that the desert locust *Schistocerca gregaria* (Forskal) is a destructive pest for several crops, particularly which are considered as the main food sources to man and animals. Therefore, it is necessary to search and develop some effective control strategies for suppressing the population density aiming to prevent the outbreak of the mobile swarms.

The plant products are reported to be more effective, less expensive, biodegradable and safe for mankind and environment, than the synthetic insecticides, which are environmentally persistent and toxic to non-target organisms including humans eliciting

many unidentified diseases after bioaccumulation (Marston and Hostettmann, 1985; Singh *et al*., 1996). Therefore, alternatives to conventional pesticides are required to be developed from the active ingredients of plant origin.

Along the late decades allover the world, so many plant species have been tested for their insecticidal activities or antifeedant, growth retarding, morphogenic impairing and reproductive disturbing effects on various insect pests. *Azadirachta indica* and *Melia azedarach* are two species among the most well investigated plants and trees in this context as alternatives of the synthetic pesticides (Schmutterer, 1995; Schmidt *et al*., 1997; Breuer and De Loof, 2000; Breuer *et al*., 2003; Senthil Nathan *et al*., 2008). On the other hand, the wild plant *Fagonia bruguieri* (Zygophyllaceae) had been assayed against the dangerous desert locust *Schistocerca gregaria* (Tanani *et al*., 2009; Hamadah *et al*., 2010; Basiouny *et al*., 2010; Aly *et al*., 2010)

Unlike vertebrates, neuro-muscular junctions in insects have shown clearly that there is no cholinesterase there and that transmission at the synapse does not include acetylcholine. However, Acetylcholinesterase (AchE, EC 3.1.1.7) is a key enzyme catalyzing the hydrolysis of the neurotransmitter, acetylcholine, in the nervous system in various organism (Grundy and Still, 1985; Wang *et al*., 2004; Zibaee, 2011). AchE is primarily responsible for termination of cholinergic neurotransmission at synapses in the central nervous system of both humans and insects (Carlier *et al.,* 2008). AChE, a serine hydrolase, catalyzes the breakdown of the neurotransmitter acetylcholine (ACh) into acetate and choline. This process involves the formation of a substrate-enzyme complex, followed by acetylation of the hydroxyl group of the amino acid serine, present within the esteratic site which is finally deacetylated (O'Brien, 1976). It facilitates nerve impulse transmission in the organisms. Its inhibition leads to paralysis and death.

Although the AchE activity is one of the main resistance mechanisms in various insect species against the organophosphrous or carbamate- resistant insects (Hemingway *et al*., 1986; Yoo *et al*., 2002), its activity was affected by some plant extracts such as an active ingredient from neem seed oil (Rahman *et al*., 1999) and azadirachtin (Senthil Nathan *et al*., 2008). The alteration of AChE was observed in the cockroach, *Periplaneta americana* L., at 4 ppm of azadirachtin (AZA), (Shafeek *et al*. 2004) and the snail, *Limnaea acuminate* Lamarck, at 40% and 80% concentrations of neem oil (Singh and Singh 2000). It was also observed that 25 g of distilled water extracts of the botanicals *Punica granatum* L., *Thymus vulgaris* L., and *Artemisia absinthium* L., significantly inhibited the AChE activity of nematodes at 100% concentrations (Korayem *et al*., 1993). Senthil Nathan *et al*. (2008) demonstrated that LC_{50} concentrations of AZA significantly inhibited the activity of AChE compared with control of the brown planthopper, *Nilaparvata lugens*. In addition, several essential oils from aromatic plants, monoterpenes, and natural products have been shown to act as inhibitors of AchE (Grundy and Still 1985, Ryan and Byrne 1988, Kostyukovsky *et.al.* 2002 and Shaaya and Rafaeli 2007). Certain essential oil monoterpenes are competitive inhibitors of AChE *in vitro* (Grundy and Still, 1985; Miyazawa *et al*., 1997), but this action may not be correlated with toxicity to insects *in vivo* (Isman, 2000).

The genus *Fagonia* includes about 35 species that are distributed in the deserts and dry areas in India, tropical Africa, Chile and South West USA. Many of these species are spiny herbs or sub-shrubs (Chopra *et al*., 1982). Some of *Fagonia* species were investigated chemically revealing the presence of flavonol glycosides, saponins, sapogenins, nahagenin and other constituents (El-Hadidi *et al*., 1988; Ansari *et al*., 1987; Al-Wakeel *et al*., 1988). Also, seven flavonol glycosides were identified from *Fagonia bruguieri* (Maksoud and El-Hadidi, 1987) in addition to erythroxan-type diterpenes (Abdel-Kader *et al*., 1994).

Fagonia bruguieri (Zygophyllaceae) is a perennial wild herb distributed in Egypt, North Africa, Kuwait, Arabia, Jordon, Iraq, Palestine, Pakistan, Afghanistan and Mediterranean area. Different extracts of *F. bruguieri* had been tested against *S. gregaria* for investigating its effects on several survival, biological and physiological processes (Ghoneim *et al*., 2009; Tanani *et al*., 2009; Basiouny *et al*., 2010; Hamadah *et al*., 2010). The present study was carried out in order to shed some light on the mode of action of this herb through its potential effect on AchE activity in nymphs and adults of *S. gregaria*.

MATERIALS AND METHODS

I) Laboratory culturing of the Experimental Insect

A gregarious stock culture of the desert locust *Schistocerca gregaria* (Forsk.) was raised by a sample from the established culture of Locust and Grasshopper Res. Division, Agric. Res. Center, Giza, Egypt. The insects were reared under crowded breeding conditions outlined by Hunter-Jones (1961) and Hassanein (1965). Newly hatched hoppers were kept in wooden cages with wire-gauze sides (40x40x60 cm) and small door in the upperside to allow the daily feeding and cleaning routine. The bottom was covered with 20 cm layer of sterilized sand. Each cages was equipped internally with 60 W electric bulb for lightening (17:7 LD) and warming (32±2 C.). The relative humidity varied from 70-80% following the introduction of fresh food plant to 60-70% several hours later. Successive generations were raised before obtaining the nymphs for the present experimental work. Fresh food plant was clover *Medicago sativa* along the period of study except few weeks every year because of the absence of this plant species. During these weeks, insects were fed on *Sesbania eagyptiaca*. All experiments were conducted with *M. sativa* only.

II) Preparation of the *Fagonia bruguieri* Extracts

In Egypt, *F. bruguieri* DC. (Zygophyllales: Zygophellaceae) in the Mediterranean area, Oasis, Gabal Elba, Sinai and some other deserts. The plant is described by Boulos (2000) as follows: Glandular-hairy low shrub or perennial herb with woody base, 15-40 cm; stems many, branched, 4-angled sulcata, bristle, internodes 0.5-2.5 cm; lower leaves 3-foliolate, the upper 1-foliolate; petiole 2-6 (-9) mm; stipular spines 0.5-2.5 cm, spreading or slightly recurved; leaflets 0.4-9 x 1.5-3 mm, oblong-lanceolate, muronate, rather fleshy; pedicel 2-5 mm, at first erect, later slightly deflexed; flowers

0.8-1.1 cm diam. At authesis; sepals 2-3 mm, persistent; petals 4-8 mm, pink; capsule 4x 3-4 mm, obconical; style 2mm; seeds 2x 1.5 mm, ovate, tuberculate, brownish.

For carrying out the present study, the aerial parts of the herb (leaves, stems and flowers) were collected from the region of Santa Catherin (Sinai) during the flowering stage. The collected samples were air-dried, powdered and kept for 6 months in tightly closed amber coloured glass containers for protecting from light, at room temperature.

The pulverized air-dried aerial parts of *F. bruguieri* (2 kg) was exhaustively macerated with methanol (7Lx3). The combined alcohol extracts were concentrated under vacuum at 500C using Rotatory evaporator (BUCHI Rotavapor ® R-210/R-215, Germany. The residue (300g) was suspended in water 600 ml, filter, filtrate was separately successively extracted with petroleum ether and n-butanol (5x 400 ml for each). Each extract was separately concentrated under reduced pressure to dryness giving (80 g and 160 g), respectively (El-Hela *et al*., 2000; Ismail *et al*., 2006).

III) Nymphal Treatments

Two concentration levels of the methanolic extract (7.5 and 3.7%), petroleum ether extract (30.0 and 15.0%) or n-butanolic extract (30.0 and 15.0%) were prepared. The newly moulted 4th (penultimate) instar nymphs (0-day old) of *S. gregaria* were adequately fed on fresh leaves of *M. sativa* after dipping (for 5 minutes) in different concentration levels of each extract. A day after treatment, all nymphs (treated and control) were provided with untreated food plant. Ten replicates (one nymph/replicate) were used for each concentration. Each individual nymph was isolated in a glass vial provided with a thin layer of sterilized sand as a floor.

IV) Enzyme Preparation and Activity Estimation

For the determination of the Acetylcholinesterase (AchE) activity in the haemolymph was collected from 5th (last) instar nymphs of 1-day old (early-aged), 4-day old (mid-aged), and 7-day old (late-aged) and newly emerged adults, after treatment the early 4th (penultimate) instar nymphs. Haemolymph was drawn into Eppendorff Pipetman containing 0.1-0.5 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.

For the determination of AchE activity in the fat body, this tissue was collected from 5th instar nymphs (of early-, mid- and late-aged) and newly emerged adults, after treatment the early 4th instar nymphs. The fat body was weighed and then homogenized in a saline solution (fat body of every 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 (one insect/replicate) were used.

Three replicates (one insect/replicate)- for haemolymph or fat body- were used. AchE activity was determined according to the method of Waber (1966) using a

kit of Diamond company. The enzyme was measured at wave length 405 nm by spectrophotometer.

V) 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 difference between means.

RESULTS

AchE activity in the haemolymph and fat body of nymphs

After treatment of the newly moulted penultimate instar nymphs of *S. gregaria* with methanolic extract (at concentration levels: 7.5 or 3.7%), petroleum ether extract or n-butanolic extract (at concentration levels: 30 or 15%) from *F. bruguieri* , The AchE activity was determined in the haemolymph of last instar nymphs at three developmental ages (early-, mid- and late-age). According to the data arranged in Table 1, remarkably induced AchE activity was unexceptionally observed. The enzyme activity in haemolymph of both the early- and mid-aged nymphs significantly increased whatever the extract or its concentration level.

Table 1. Effects of *Fagonia bruguieri* extracts on the acetylcholinesterase activity (U/L) in haemolymph of the last instar nymphs of desert locust *Schistocerca gregaria*.

Solvent	Conc. %		Last instar nymphs		
			Early-aged	Mid-aged	Late-aged
Methanol	7.5	Mean ± SD	10166.7 ± 677.0 c	8601.7 ± 678.4 c	8211.7 ± 586.3 a
		Change %	+52.9	+46.7	+10.5
	3.7	Mean ± SD	8991.7 ± 677.0 b	7038.3 ± 586.3 a	8601.7 ± 678.4 a
		Change %	+35.2	+20.0	+15.8
Petrolium ether	30	Mean ± SD	9775.0 ± 675.5 c	8211.7 ± 586.3 c	9383.3 ± 586.3 b
		Change %	+47.0	+40.0	+26.3
	15	Mean ± SD	8601.7 ± 678.4 b	7821.7 ± 677.0 b	8991.7 ± 677.0 b
		Change %	+29.4	+33.4	+21.0
n-butanol	30	Mean ± SD	10946.7 ± 678.4 c	8993.3 ± 678.4 c	9775.0 ± 896.1 b
		Change %	+64.7	+53.3	+31.6
	15	Mean ± SD	9775.0 ± 675.5 c	8211.7 ± 586.3 b	8211.7 ± 586.3 a
		Change %	+47	+40.0	+10.5
Controls		Mean ± SD	6648.3 ± 678.4	5865.0 ± 1175.0	7430.0 ± 675.5

Conc.: Concentrations; Mean ± SD followed with the letter (a): is not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01).

On the other hand, *F. bruguieri* exhibited the most potent promoting action on AchE activity in the late-aged nymphs after treatment with the petroleum ether extract (9383.3±586.3 and 8991.7±677.0 U/L at conc. Levels 30 and 15%, respectively, in comparison with 7430.0±675.5 U/L of control nymphs). In addition, the n-butanolic

extract of *F. bruguieri* pronouncedly enhanced the AchE activity in late-aged nymphs (9775.0±896.1 U/L vs. 7430.0±675.5 U/L of control congeners).

With regard to the AchE activity in the fat body of last instar nymphs, data of Table 2 show seriously inhibited enzyme activity in the early-aged nymphs, regardless of the conc. level. The strongest prohibiting effect was exhibited by both the petroleum ether and n-butanolic extracts (1094.7±135.7 and 1078.5±269.5 U/L at the high conc. Level of each, respectively, compared to 2374.4±242.3 U/L of controls). On the contrast, AchE activity was induced in the fat body of all nymphs other than the early-aged ones (for details, see Table 2).

Table 2. Effects of *Fagonia bruguieri* extracts on the acetylcholinesterase (U/L) in fat bodies of the last instar nymphs of desrt locust *Schistocerca gregaria*.

Solvent	Conc. %		Last instar nymphs		
			Early-aged	Mid-aged	Late-aged
Methanol	7.5	Mean ± SD	1486.0 ± 135.1 c	1094.7 ± 135.7 a	1329.7 ± 135.7 a
		Change %	-37.4	+27.3	+34.1
	3.7	Mean ± SD	1798.3 ± 260.8 b	860.0 ± 135.1 a	1251.3 ± 135.7 a
		Change %	-24.3	0.0	+26.2
Petrolium ether	30	Mean ± SD	1094.7 ± 135.7 c	1251.3 ± 135.7 b	1329.3 ± 270.8 a
		Change %	-53.9	+45.5	+34.1
	15	Mean ± SD	1329.7 ± 135.7 c	938.3 ± 234.5 a	1329.3 ± 135.7 a
		Change %	-44.0	+9.1	+34.1
n-butanol	30	Mean ± SD	1078.5 ± 269.5 c	1931.8 ± 169.9 d	1955.0 ± 146.5 c
		Change %	-54.6	+124.6	+97.2
	15	Mean ± SD	1379.9 ± 172.4 c	1898.1 ± 131.2 d	1278.6 ± 85.0 a
		Change %	-41.9	+120.7	+29.0
Controls		Mean ± SD	2374.4 ± 242.3	860.0 ± 135.1	991.3 ± 165.1

Conc.: Concentrations; Mean ± SD followed with the 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).

AchE activity in the Haemolymph and fat body of adults

Depending on the data distributed in Table 3, *F. bruguieri* exerted a strong inhibitory action on the AchE activity in the haemolymph of newly-emerged adult females of *S. gregaria*. Such action was reversed on the enzyme activity in the fat body of the same adults because increasing activity was estimated, regardless of the extract used or conc. level applied. However, the strongest inhibitory effect (Change %: -45.5) and the most inducing effect (Change %: +147.9) were exhibited by the n-butanolic extract (at the high conc. level) whereas the least inhibitory effect or inducing effect was exerted by the methanolic extract (at the low conc. level) of *F. bruguieri*.

Table 3. Effects of *Fagonia bruguieri* extracts on the acetylcholinesterase activity (U/L) in the newly emerged adults of desert locust *Schistocerca gregaria*.

Solvent	Conc. %		Tissue	
			Haemolymph	Fat bodies
Methanol	7.5	Mean ± SD	6648.3 ± 678.4 b	1720.3 ± 135.7 c
		Change %	-22.7	+74.5
	3.7	Mean ± SD	7430.0 ± 675.5 a	1486.0 ± 135.7 b
		Change %	-13.6	+50.8
Petrolium ether	30	Mean ± SD	6256.7 ± 678.4 b	1955.0 ± 135.1 c
		Change %	-27.3	+98.3
	15	Mean ± SD	7038.3 ± 586.3 b	1642.3 ± 234.5 b
		Change %	-18.2	+66.6
n-butanol	30	Mean ± SD	4691.7 ± 586.3 c	2443.8 ± 168.9 d
		Change %	-45.5	+147.9
	15	Mean ± SD	6256.7 ± 678.4 b	1846.8 ± 188.4 c
		Change %	-27.3	+87.4
Controls		Mean ± SD	8601.7 ± 678.4	985.7 ± 197.5

Conc.: Concentrations; Mean ± SD followed with the 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).

DISCUSSION

Determination of AchE activity have been used routinely as a biomarker of exposure to certain groups of contaminants, such as organophosphate and carbamate insecticides (Grue *et al.,* 1997). These two groups of insecticides are known as important AchE inhibitors suppressing the action of the enzyme (Lundebye *et al.,* 1997 ; Thompson, 1999). As a key enzyme in the insect central nervous system, AchE was a target for the development of inhibiting insecticides (Fournier and Mutero, 1994; Alon *et al.,* 2008). It is well known that the altered AchE activity is one of the main resistance mechanisms in many insect pests. In sensitivity of AchE observed in organophosphorous- or carbamate-resistant insects has been well documented for various insect pests (Yoo *et al.,* 2002; Wang *et al.,* 2004). AchE catalyzes the hydrolysis of the neurotransmitter, acetylcholine, at the synaptic cleft (the space between the two axonic ends of nerve cells) so that the next nerve impulse can be transmitted across the synaptic gap. Inhibition of AChE causes accumulation of AChE at the synapses, so that the post-synaptic membrane is in a state of permanent stimulation, which results in paralysis, ataxia, general lack of co-ordination in the neuromuscular system and eventual death (Aygun *et al*., 2002).

In the present study on the desert locust *S. gregaria*, the wild plant *F. bruguieri* (methanolic, petroleum ether and n-butanolic) extracts were assessed on the AchE activity in the haemolymph and fat body of nymphs and adults. Throughout the

last nymphal instar, induced AchE activity was unexceptionally observed in the haemolymph, especially of the early- and mid-aged nymphs. On the other hand, the enzyme activity in the fat bodies was pronouncedly inhibited in the early-aged nymphs, regardless to the extract or the concentration level. Such effect was reciprocally detected in the fat bodies of mid- and late-aged nymphs. Concerning with the newly formed adult females, *F. bruguieri* extracts exerted a strong inhibitory action on AchE activity in the haemolymph but promoting action on enzyme activity in the fat body, whatever the extract or its concentration level.

The inhibited AchE activity as a response to the *F. bruguieri* extracts, in the present work, comes in accordance with those results obtained by Naqvi (1986) in *Musca domestica* and *Blatella germanica* as response to neem extracts. To a great extent, similar results were reported by Khan *et al.* (2003) after treatment of *Calotes versicolor* with Biosal (neem-based pesticide), by Breuer *et al*. (2003) after treatment of *Spodoptera frugiperda* and *Leucophaea maderae* with *Melia azedarach* extract, by Shafeek *et al*. (2004) after treatment of *Periplaneta americana* with azadirachtin, by Kim *et al*. (2008) after treatment of *Nilaparvata lugens* and *Leodelphax striatellus* with a mixture of insecticide carbosulfan and the plant extract wood vinegar and by Begum *et.al.* (2010) after treatment of *M. domestica* with seed extracts of *Annona squamosa* (Annonaceae) and *Calotropis procera* (Asclepiadaceae). Results obtained by the later authors revealed that the high concentration (10%) of extreact from the seeds of *A. squamosa* exhibited maximum inhibitory effects (56%) on the AchE activity from all the three larval instars of *M. domestica*.

The inhibition of AchE activity in the haemolymph or fat bodies by some *F. brugieri* extracts at some ages of nymphs or newly emerged adults of *S. gregaria,* in the present study, indicates the presence of certain toxic components leading to accumulation of acetylcholine at the synapses, so that the post-synaptic membrane is in a state of permanent stimulation, which results in paralysis, ataxia, general lack of co-ordination in the neuro-muscular system, and eventually death (Singh and Singh, 2000 ; Aygun *et al.,* 2002 ; Massa *et al.,* 2008; Senthil Nathan *et al*., 2008). On the other hand, the prohibited AchE activity in the nymphs of some ages or adults of *S. gregaria* may indirectly indicate the damage of their nerve cells, which induce the AchE photoinactivation and death caused by the disruption of normal nerve conduction (Yin *et al.,* 2008).

However, the induced AchE activity in the fat body or haemolymph of nymphs or adults of *S. gregaria*, in the present study, agree- to some extent- with the increasing enzyme activity results in *Tribolium castaneum* after treatment with the insecticides permethrin and cypermethrin (Saleem and Shakoori, 1987). Also, the elevated AchE activity level in the present study on *S. gregaria* may be resulted from an increase in gene copy number rising up to approximately 80 copies per diploid genome (Field *et al.,* 1993, 1999).

In conclusion, the inhibitory action of some extracts of the wild herb *F. bruguieri* on the AchE activity in fat bodies of last instar nymphs or haemolymph of the newly emerged adults of the dangerous pest *S. gregaria* suggest that this herb may prove to

be a probable candidate for the development of biopesticides to control the populations of the present pest as safer, ecofriendly and economic alternatives to the synthetic pesticides.

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