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EFFECT OF THREE EGYPTIAN MEDICINAL PLANT'S EXTRACTS ON BIOCHEMISTRY OF *CULEX PIPIENS* LARVAE (CULICIDAE: DIPTERA)

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Abstract

Culex pipiens is a predominant house-resting mosquito in different countries. Third larval instar of *Culex pipiens* were exposed to sub-lethal concentration LC25of pet-ether extract for *Solenostemma* sp, *Rosmarinus* sp. and *Artemisia* sp. to study their effect on some enzymatic activities of larvae after 24 hours. AchE enzyme showed high activity in all application extracts. While, showed lower in enzyme activity of α-est, β-est in comparison with control. Glutathione S Transferase showed no difference with control sample. Mixed function oxidase appears high activity with *Solenostemma argel* and *Artemisia* sp. While appears low activity with *Rosmarinus* sp. comparing by control larvae. Finally, these extracts affect the nervous system of tested mosquitoes in different degrees and their energy system and can be used alternate to chemical insecticides in Integrated Vector Management program (IVM).

Key words: *Culex pipiens* larvae, Medicinal plant extracts, Biological control.

Introduction

Culex pipiens (*Cx. pipiens*) is a predominant house-resting mosquito of worldwide distribution (Lane and Crosskey, 1993). In Egypt, the abundance of *Cx. pipiens* was reported all-over the country (Mikhail *et al*, 2009; Abdel-Hamid *et al*, 2011). No doubt, the Egyptian filariasis (Harb *et al*, 1993), culicini mosquitoes, mainly *Cx. pipiens* transmit Rift Valley fever (El Gebaly, 1978), Sindbis virus (Wilson, 1991) and West Nile Virus (El-Bahnasawy *et al*, 2013). Apart from diseases transmission, mosquitoes can make human life miserable. Most people of all ages' particularly small children, toddlers and seniors who suffered from Skeeter syndrome experienced a very extreme reaction showed some allergic reaction levels, with itching and redness (Abdel-Motagaly *et al*, 2017)

The larval stage is the most efficient stage to control mosquito. Using the traditional method for control by chemical insecticides was causing huge hazards on public health (Ansari *et al*, 2000; Gusmäo *et al*, 2002; Bakr *et al*, 2006; 2008). Plant extracts were considered to be the rich source to produce natural safe insecticides alternative to the risky chemical ones (Kamel *et al*, 2005; Pavela, 2009; El-Maghraby *et al*, 2012; El-diasty *et al*, 2014; El-Ghaban *et al*, 2015; Kamel *et al*, 2015; Kamel and Hassan, 2015; Hassan and Kamel, 2016). The medicinal plant extracts have different uses for treating many parasitic diseases as ginger and garlic in treatment of mice infected with cryptospori diosis (Abouel-Nour *et al*, 2016), and DEET as repellent for *Cimex lectularis* (El Bahansawy *et al*, 2018), as well as *Artemisia annua* leaf chloroform extract proved to be a strong larvicides against larvae of *Anopheles stephensi* and *Aedes aegypti* (Sharma *et al*, 2014). Also, the *Solenostemma* sp. ethanolic leaves extract gave marked effect on lipid profile of albino rats which were significantly reduced ($p<0.05$) after two weeks of treatment, and high reduced hyper cholesterolemia (Osman *et al*, 2015). Besides, *Artemisia* sp. extract has effect on cardiovascular system and was used as anti-hypertensive and to prevent the cardiovascular damages (Ben-Nasr *et al*, 2013). *Artemisia* sp. has anti-diabitic effect and treated the blood glucose level with relatively very mild side effects (Dabe and Kefale, 2017). Rosemary (*Rosemarinus* officinalis L.) has been used in folk medicine to treat headaches, epilepsy, poor circulation, and many other ailments. Besides, it can be used as mild analgesic and can reduce inflammation. The Carnosic acid (CA)

component has potential antiatherosclerosis effects (Chae *et al*, 2012). Almost of natural extracts used in the control of insects have effect on the enzymatic activities of treated insects' Glutathione-S-transferases (GSTs), esterases (ESTs), mixed function oxidases (MFO) and acetylcholinesterase (AChE) (Nathan *et al*, 2005; Feyereisen, 2005; El-Kady *et al*, 2008; Dahi *et al*, 2009; Gacar and Tasksn, 2009).

The present study was carried out to evaluate the effect of three medicinal plant extracts (*Solenostemma argel*, *Rosemarinus officinalis and Artemisia* sp.) on some enzymatic activities to predict the mode of action and could alternate to risky chemical insecticides of insect-vectors control program.

Materials and Methods

Tested mosquito: Larvae of *Culex pipiens* collected from the water-body, Abo-Rowash City (Giza Governorate) and immediately transferred to the Experimental Laboratory, Department of Entomology, Faculty of Science, where self-perpetuating colonies were established and maintained under controlled laboratory conditions.

Tested compounds Tested medicinal plants (*Solenotemma argle, Rosmarinus officinalis* and *Artemisia* sp.) were collected from non-insecticidal treated cultivated fields, then washed in tap water to remove any dusts and/or dirt then lift to dry under shade in the laboratory. Dried plant (whole plant) were cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each plant were extracted with petroleum ether. The extractions were accomplished by means of a Soxhlet apparatus. The solvent extracts of each plant were evaporated and dried under vacuum using a rotary evaporator of water bath adjusted at 60-70ºC. The resulted dry crude extracts were storage at 4ºC in screw capped vials, until use.

Biochemical studies: Each of selected compounds was applied on larvae at concentration of LC_{25} level for 24 hrs. Then survived larvae post treatment were gently washed and transferred to labeled pans. Some of these survived larvae and other developmental stages emerged from treated larvae was collected and submitted to biochemical studies.

Preparation of insects for analysis: Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at -20ºC till needed. Double Beam Ultraviolet Visible Spectrophotometer (Spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

Estimation of acetylcholinesterase: AchE acetylcholinesterase activity was measured after Simpson *et al*. (1964) using acetylcholine-bromide (AchBr) as substrate. The reaction mixture contained 200µl enzyme solution, 0.5ml 0.067M phosphate buffer (pH7) and 0.5ml AchBr (3mM). The test tubes were incubated at 37ºC for exactly 30min. 1ml alkaline hydro-xylamine (equal volume of 2M hydroxyl-amine chloride & 3.5M sodium hydroxide) was added to the tested tubes. Then, 0.5ml of HCl (1 part of conc. HCl & 2 parts of ΔH_2O) was added. The mixture shaken vigorously and allowed to stand for 2 min. 0.5ml of ferric chloride solution (0.9M Fecl3 in 0.1MHCl) was added and mixed well. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

Estimation of non-specific esterases: Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined (Van Asperen, 1962) using α-naphthyl acetate or β-naphth yl acetate as substrates, respectively. The reaction mixture consisted of 5ml substrate solution (3x10-4M α-or β-naphthylacetate, 1% acetone and 0.1M phosphate buffer, pH7) and 20µl of larval homogenate. The mixture was incubated for exactly 15min at 27˚C, then 1ml of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B & 5 parts of 5% sodium lauryl sulphate)was added. The developed color was read at 600 or 555nm for α- and β-naphthol produced

from hydrolysis of the substrate, respectively. α-and β-naphthol standard curves were prepared by dissolving 20 mg α- or β-naphthol in 100ml phosphate buffer, pH7 (stock solution). Ten milliliters of stock solution were diluted up to 100ml by the buffer. Aliquots of 0.1, 0.2, 0.4, 0.8 & 1.6ml of diluted solution (equal to 2, 4, 8, 16 & 32µg naphthol) were pippeted into test tubes and completed to 5 ml by phosphate buffer. One milliliter of diazoblue reagent was added and the developed color was measured as mentioned before.

Estimation of the oxidase activity: P-nitroanisole O-demthylation was assayed to determine the mixed function oxidase activity according to the method of Hansen and (Hodgson, 1971) with slight modification. The standard incubation mixture contained 1ml sodium phosphate buffer (0.1M, pH 7.6), 1.5ml enzyme solution, 0.2ml NADPH, (Final concentration 1mM), 0.2ml glucose-6-phosphate dehydrogenase (G-6PD). Reaction was initiated by the addition of p-nitroanisole in 10µl of acetone to give a final concentration of 0.8mM and incubated for 30 min at 37ºC. Incubation period was terminated by addition of 1ml HCl (1N). P-nitrophenol was extracted with CHCl3 and 0.5 N NaOH and absorbance of NaOH solution was measured at 405 nm. An extinction coefficient of 14.28mM-1 cm-1 was used to calculate 4-nitrophenol concentration.

Estimation of Glutathione-S-transferase: Glutathione S-transferase (GST) catalyzes conjugation of the reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione was detected (Habig *et al*, I974). The reaction mixture consisted of 1ml of the potassium phosphate buffer (pH6.5),100µl of GSH & 200µl of larval homogenate. The reaction started by the addition of 25µl of the substrate CDNB solution. The concentration of both GSH and CDNB was adjusted to be 5mM and 1mM, respectively.

Enzyme and reagents were incubated at 30˚C for 5min. The increment in absorbance at 340 nm was recorded against blank containing everything except the enzyme to determine the nanomole substrate conjugated/min/larva using a molar extinction coefficient of 9.6mM/cm.

Statistical analysis: Data were analyzed by using Microsoft Excel (Mean, Stander deviation and T-test)**.** Measure activity ratio for each enzyme by equation:

$$\text{Activity ratio} = \frac{\text{Enzyme activity in treated larvae}}{\text{Enzyme activity in control}}$$

Results

The results are shown in tables (1, 2, 3, 4 & 5).

Table 1: Acetylcholinesterase activity in larvae of *Cx. pipiens* treated with medicinal plant extracts at LC_{25} compared with control:

Tested extract	*(µg AchBr/*min /mg protein) Mean ± SD	T-test At <0.05	Activity ratio
S. argel	685 ± 15.90	$5.35X10^{-13}$*	5.39
Rose marinus	172.67± 4.98	$3.81X10^{-9}$*	1.36
Artemisia sp.	649.67 ± 4.27	$2.24X10^{-19}$*	5.12
Control	127 ± 6.05		

Table 2: α-Esterases activity in larvae of *Cx. pipiens* treated with medicinal plant extracts at LC_{25} compared with control:

Tested extract	*(µg α-naphthol/*min /mg protein) Mean ± SD	T-test At <0.05	Activity ratio
S. argel	3896.22 ± 7.04	$1.65X10^{-23}$**	0.40
Rose marinus	7214.44 ± 3.59	$1.99X10^{-22}$**	0.75
Artemisia sp.	6788.44 ± 5.44	$4.27X10^{-23}$**	0.70
control	9677.22 ± 3.46		

Table 3: β-Esterases activity in larvae of *Cx. pipiens* treated with medicinal plant extracts at LC_{25} compared with control:

Tested extract	*(µg β-naphthol/*min /mg protein) Mean ± SD	T-test At <0.05	Activity ratio
S. argel	9.05 ± 0.46	$1.48X10^{-5}$*	0.74
Rose marinus	8.47 ± 0.58	$9.90X10^{-10}$*	0.69
Artemisia sp.	4.79 ± 0.18	$2.28X10^{-10}$*	0.39
control	12.2 ± 0.57		

Table 4: Glutathion-s-transferase activity in larval stage of *Cx. pipiens* treated with medicinal plant extracts at LC_{25} compared with control:

Tested extract	*(umol sub conjugated/*min /mg protein) Mean ± SD	T-test At <0.05	Activity ratio
S. argel	60.97 ± 0.82	$4.05X10^{-5}$*	0.96
Rose marinus	61.6 ± 1.04	0.02	0.97
Artemisia sp.	59.1 ± 0.70	$3.79X10^{-8}$*	0.93
control	63.23 ± 0.61		

Table 5: Mixed function oxidase activity in larval stage of *Cx. pipiens* treated with medicinal plant extracts at LC_{25} compared with control:

Tested extract	*(umol sub oxidized/*min /mg protein) Mean ± SD	T-test At <0.05	Activity ratio
S. argel	312.33 ± 2.87	$2.91X10^{-17}$**	1.35
Rose marinus	207.67 ± 2.05	$4.09X10^{-7}$*	0.90
Artemisia sp.	299.67 ± 2.05	$1.22X10^{-10}$*	1.30
Control	231.33 ± 2.62		

*= significantly different **= high significantly different

Discussion

In the present study, the highest value of AchE was 685 and the lowest one was 172.67 for *Solenostemma* sp. and *Rosmarinus* sp., respectively. The treatment for all extracts showed significant differences between treated larvae and control. These results may be explaining the mortality of larvae. The excess release of AchE which may break down any message to be sent to the receptor and then the insect become without neural orientation (Dahi *et al*, 2009). In the present study, the highly significant reduction of α-esterases in all treatment *Cx. pipiens* larvaes compared with control ranged between 3896.22 to 7214.44 for *Solenostemma sp.* and *Rosmarinus* sp., respectively. Also, β-esterases caused significant reduction. The reduction values for high and low treatment were 4.79 & 9.05 referred to *Artemisia sp*. and *Solenostemma sp.*, respectively. The reduction activities which appeared in α- and β-esterases after treatment with selected plant extracts might be attributed to no detoxification action happen by these enzymes (Abd El-aziz and El-Sayed, 2009). The variation in reduction values probably referred to difference in component of each extract and the susceptibility of *Cx. pipiens* to these extracts, the same conclusion was achieved by (Darvishzadeh and Sharifian, 2015) which stated that the esterase enzymes affected by using different insecticides in different degrees according the susceptibility of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Glutathion-s-transferase activity appeared different degrees of reduction as mentioned in table (4). *Solenostemma argel* and *Artemisia sp*. showed significant reduction, 60.97 and 59.1 respectively. *Rosmarinus* sp. give reduction in the activity but non-significant. The selected extracts inhibit the enzyme activity meaning not play role in detoxification these extracts as insecticide in different degree according their differences of component of each extract. These results agreed with Wang *et al*. (2014) who stated that the conifer bark and cone extracts and the identified allelochemicals were potent *in-vitro* inhibitors of GST in Colorado Potato-Beetle larvae. The treatment larvae by medicinal extracts showed significant increase in MFO for *Solenostemma* sp. and *Artemisia* sp., 312.33 & 299.67 respectively. *Rosmarinus* sp, decreased in the activity compared with control. The increasing of activity might be attributed to their role of detoxification effect to tested extracts by the mean of self-defense of insect to it (Terriere, 1984). Otherwise, these increasing of activity might be attributed to the fact the tested compound act as juvenile hormone analog were cytochrome P450 inducers in insect (Wilkinson, 1976). Decreasing in the activity of MFO in *Rosmarinus* sp. application might be to the absent role of detoxification of the compound.

Conclusion

The outcome data showed that the medicinal plants (*Solenostemma argel*, *Rosemarinus officinalis and Artemisia* sp.) beside their effect as medication it can be used as

insecticides alternate for traditional insecticides or as a synergist to reduce using of chemical pesticides.

The variation of component for extracts reduce the resistant arises as a result for using single component of chemical pesticide.

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