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Toxicological and Biochemical Activity of Five Plant Extracts Assayed Against Aquatic Vectors of Diseases, Culex pipiens and The Snail, *Lymnaea natalensis*

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ABSTRACT

A global health agenda that gives main concern to vector control could save many lives and avoid much suffering. Cost-effective and simple interventions like the extracts of the plants *Rosmarinus officinalis, Sorghum bicolor, Ambrosia maritime (Damsissa), Callistemon citrinus (lanceolatus) and Eucalyptus globulus* were tested in the present study against two aquatic vectors of disease *Culex pipiens* larvae and the snail *lymnaea natalensis*, the five plant extracts showed lethal effects against both *vectors*, *Cx. pipiens* larvae which showed high susceptibility to *Sorghum bicolor* extract LC50 184.37 ppm ± 24.02 ppm while the high susceptibility of *L. natalensis* snail was recorded toward Ambrosia extract LC50 =7.65 ± 1.89 ppm. The activities of the enzymes GST, GOT and ALT as well as the total proteins, carbohydrates, and lipids of *Cx. pipiens* larvae and the snail *L. natalensis* were significantly changed compared to the untreated samples. With the presence of some exceptions, most tested plant extracts generally increased GST and GOT activity in both vectors while a significant increase and decrease in the activity of ALT was reported in *Cx. pipiens and L. natalensis* respectively in most plant extracts. Also, the obtained results indicated that the total protein and carbohydrate contents in both *Cx. pipiens* larvae and the snail *L. natalensis* were significantly decreased with all tested plant extracts which are more obvious in *L. natalensis*. A slight increase in total lipid except for *Rosmarinus officinalis* which doesn't induce any change in both of them. Based on these alterations, it could be concluded that the studied plant extracts have insecticidal and molluscicidal effects on both *Cx. pipiens* larvae and *L. natalensis* respectively.

INTRODUCTION

Natural pesticides, both of microbial and plant origin, are beginning to have a significant market impact despite widespread public concern about the long-term health and environmental effects of synthetic pesticides. This will consequently create a significant market opportunity for alternative products, particularly "reduced-risk pesticides," which are preferred by the Environmental Protection Agency in the USA. In light of this, natural pesticides made from plant extracts and oils may be an alternate kind of agricultural and health protection that has reached its peak) Arthurs & Dara 2019).

In Egypt, *Culex pipiens* (Diptera: Culicidae), has been identified as a disease vector (El-Zayyat *et al*., 2017). It spreads West Nile virus, the virus that causes riparian fever (Dodson *et al*., 2017), the virus that causes Japanese encephalitis (Chancey *et al*., 2015), and the virus that causes human lymphatic filariasis, Wuchereria bancrofti accredited for transmission (Joseph *et al*., 2011& Bassal *et al*., 2017). According to Abd El-Shafi *et al*. (2016), all governorates in Egypt have recorded *Cx. pipiens* as the filarial vector. The more efficient methods of reducing disease transmission are to kill larvae in addition to avoiding *Cx. pipiens* bites (Shehata, 2019).

Another common vector-borne disease, schistosomiasis **(**Bilharzia**)**, is spread by freshwater snails, about 240 million were affected globally, Global Health Estimates (2016). *L.natalensis* snails are considered one of the most snails which play a role as intermediate hosts for *Fasciola gigantica* in Egypt. (Chitsulo *et al*., 2000). Schistosomiasis represents a major health and economic problem as it affects millions of farmers, diminishing their productivity and exerting a serious socioeconomic problem. The interruption of the lifecycle of Schistosoma in its snail host is one of the control strategies to reduce parasite transmission.

The use of plants with insecticidal and molluscicidal properties appears to be a simple and inexpensive alternative (Rodrigues *et al.,*2013). More than 1000 plant species have been screened for molluscicidal activity (Augusto & de Mello-Silva, 2018). In Egypt, screening of local plants for larvicidal and molluscicidal activity has received increasing attention (Chitsulo *et al*., 2000, Bakry, 2009, Kamaraj *et al*., 2010and*).* In recent years, new molluscicides are gaining attraction to be highly effective, less expensive and biodegradable than chemical molluscicides, readily available, and easily applied with simple techniques (Bakry *et al.*, 2016). These botanical insecticides and molluscicides are of economic importance, especially in developing countries. Also, there is a continuous need to search for new plant species with multi-benefit lethal properties (Duke *et al*., 2010).

This research introduces some plant extracts, used in the Egyptian folk treatment to be cheap and environmentally friendly in controlling two important aquatic vectors of diseases, *Culex pipiens* and *Lymnaea natalensis.*

MATERIALS AND METHODS

Plants:

The tested plants are *Rosmarinus officinalis*, *Sorghum bicolor*, *Ambrosia maritime* (Damsissa), *Callistemon citrinus (lanceolatus)* known as Lemon Bottlebrush and *Eucalyptus globulus* L. (Damsissa) (Fig.1). The fresh plants were collected, washed well and separated into leaves, rhizomes or bulbs while the Damsissa used as a whole. They were dried at 50°C in an incubator and ground into powder. Infusions from the plants were prepared by soaking 10 gm powder in 100 ml distilled water for 24 hs, then filtered to form a stock solution from which different concentrations were prepared.

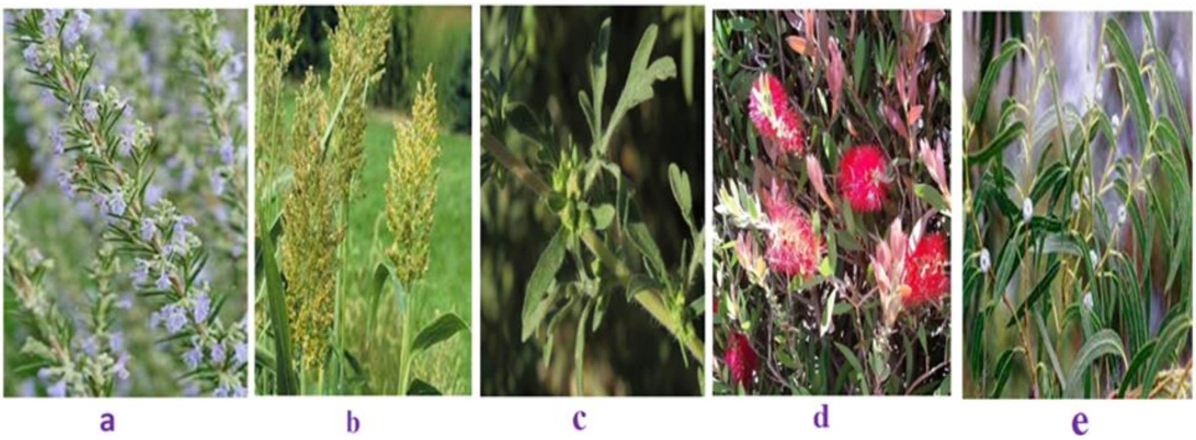

Fig.1. The tested plants are a: *Rosmarinus officinalis*,b: *Sorghum bicolor*, c: *Ambrosia maritime* (Damsissa), d: *Callistemon citrinus (lanceolatus)* and e: *Eucalyptus globulus*.

Larvicidal and Molluscicidal Evaluation:

All the plant extracts were bio assayed as aqueous different concentrations, 20 early 3rd instar larvae of *Cx. pipiens* in 50 ml distilled water received appropriate plant extract concentrations in plastic cups. Three replicates for each concentration were prepared and incubated for 24 - 48 hr at 27±2°C. Larval mortality was recorded after 24 hr. For snail bioassays, 3 replicates of each 10 snails were assayed in 100 ml distilled water for each replicate. Small pieces of Lettuce were added as food. Replicates without the addition of plant extracts were considered control experiments. Mortality readings were recorded and corrected after 24 - 48 hr. using the Abbots formula (1925). LC50 and LC90 were calculated for each plant extract by calculating the slop function of the resultant regression lines (Finney, 1971).

Measurement of Total Carbohydrate, Lipids and Protein:

Total carbohydrate content from whole body extracts was estimated using phenol- sulfuric acid reaction (Crompton & Birt 1967), while total Lipids were estimated according to Knight *et al*, (1972), total protein content has been carried out based on the method of Bradford, (1976).

Biochemical Studies:

The effect of plant extracts treatment on GST, GOT, and ALT activities were measured as an indicator of their mode of action. For enzyme assays, 3rd larval instars of *Cx. pipiens* were collected after 24 hrs post-treatment and homogenized in distilled water while snail samples were prepared by removing a small portion of the snail shell and, one gram of snails' soft tissues from each group was homogenized in 5 ml distilled water at pH 7.5, in a glass homogenizer. Homogenates were centrifuged at 5000 rpm for 15 min. The supernatant was placed in tubes as a source of biochemical assays.

GST, GOT, and ALT activities were performed as mentioned by (Assar, 2012). The activity of glutathione S-transferase (GST) was evaluated depending on Habig *et al*,(1974) method where CDNB was used as a substrate. Transaminase activity: The activities of both Aspartate aminotransferase AST (GOT) and Alanine aminotransferase ALT (GPT) were determined in the larval homogenate according to the method of Reitamn and Frankle, (1957).

The activity ratio for each enzyme was calculated according to the following equation: Activity ratio = Enzyme activity in treated larvae / Enzyme activity in control.

Statistical Analysis:

Mortality and the percentages of enzyme activation were subjected to probit analysis for calculating LC50 and LC90 (Finney, 1971), and other parameters statistics were used (LDP-line) for the goodness of fit (Chi-square test) (Duncan, 1955).

RESULTS AND DISCUSSION

All tested plant extracts alter the enzyme activities in *Cx. pipiens*. The most toxic extract of the sorghum plant increased GST, GOT and ALT activities. Treatment with *Eucalyptus glaulus* slightly increased GST, GOT and ALT activities (Figs. 1&2).

All tested plant extracts could change the enzyme levels of *L. natalensis*. The most toxic plant extract, *A. Maritima* increased the activities of GST, GOT and ALT, while *E. glaucus* decreased all tested enzyme levels (Fig. 3).

All plant extract treatments negatively changed the protein level in *Cx. pipiens* larvae and dramatically decreased the carbohydrate content but lipids recorded an increased level after treatment with all plant extracts (Fig. 4).

Protein and carbohydrate levels recorded a significant decrease ($p< 0.05$), after treatment of *L. natalensis* with all plant extracts. While lipid contents showed a sudden increase in level after all treatments (Fig.5).

Table 1: Lethal concentrations of 5 plant extracts tested against *cx.pipiens* and *l. natalensis.*

Plant extracts	*Cx.pipiens*		*l. natalensis*	
	LC50 (ppm)	LC90 (ppm)	LC50 (ppm)	LC90 (ppm)
R. officinalis	506.955±96.32	3886. 98 ± 823.3	346.79±102.2	554.2±203.2
S. bicolor	184.37±24.02	404.09±92.12	73.68±21.8	107.68±23.18
A. maritima	247.58±38.40	672.73±111.05	7.65±1.89	20.79 ± 5.32
C.citrinus	325.38±82.62	1129.99±102.20	284.91±72.80	453.22±192.6
E. glaulus	218.08±92.6	627.02±163.15	28.80 ±3.90	51.12±13.02

Table 2: GST, GOT and ALT activities in treated *Cx. pipiens* with the tested plant extracts.

Plant Extracts (LC50)	GST	GOT	ALT
Control	7.42 ± 0.077	17.23 ± 1.077	8.30 ± 0.26
R. officinalis	9.30 ± 0.08	19.30 ± 0.08	8.58 ± 0.40
***S. bicolor*-**	11.60 ± 0.38	21.60 ± 2.36	9.33 ± 0.22
A. maritima	6.58 ± 0.12	18.58 ± 0.12	7.87 ± 0.04
C. citrinus	9.95 ± 0.81	19.95 ± 0.81	10.00 ± 0.21
E. glaulus	7.02 ± 0.06	16.83 ± 0.08	8.11 ± 0.03

Table 3: GST, GOT and ALT activities in treated *l. natalensis.*

Plant Extracts (LC_{50})	GST	GOT	ALT
Control	10.88 ± 0.55	10.41 ± 0.55	12.25 ± 2.20
R. officinalis	12.43 ± 0.30	10.43 ± 0.30	12.99 ± 3.02
S. bicolor	9.89 ± 0.31	10.89 ± 0.31	10.08 ± 1.99
A. maritima	12.29 ± 0.15	11. 92 ± 0.15	13.02 ± 0.32
C. citrinus	11.02 ± 0.22	9.04 ± 0.22	12.28 ± 2.25
E. glaulus	10.44 ± 0.26	9.84 ±1.25	10.88 ± 1.66

Table 4: Changes in protein, carbohydrate and lipid content after treatment of *L. natalensis* with the tested plant extracts.

Plant Extracts	Protein		carbohydrate		Lipids	
Treatment at LC50 values	C	T	C	T	C	T
R. officinalis	40.13±1.67	38.00±2.25	14.78±0.88	11.24±2.22	26.64±3.05	26.99 ± 2.21
S. bicolor	42.46±2.60	40.62±3.55	15.34±1.03	10.99±1.35	25.05±2.11	28.34±3.02
A. maritima	41.53±0.78	38.05±1.01	16.22±2.02	12.54±2.03	26.39±3.03	29.04±2.99
C.citrinus	40.87±2.05	40.02±0.03	15.25±0.88	11.42±1.98	24.35±0.99	24.79±2.13
E. glaulus	40.40±1.05	36.89±0.12	16.38±0.63	15.22±2.34	24.32±3.04	25.48±1.82

Treatment with LC50 of each plant extract

C Control T Test {(38 – 46) / 46} ×100 = -5 % (% Change)

Table 5: Changes in protein, carbohydrate and lipid content after treatment of *l. natalensis* with the tested plant extracts.

Plant Extract Treatment at LC50 values	Protein		Carbohydrate		Lipids- glycogen	
	C	T	C	T	C	T
R. officinalis	28.90±2.13	18.45± 0.45	11.21±0.84	10.8±0.55	26.47±3.60	26.63±1.14
S. bicolor-	28.83± 0.54	19.93±0.22	14.75±0.86	12.16±1.77	25.26±1.14	26.36±1.02
A. maritima	26.83±0.54	15.58±1.56	13.99±1.61	10.12±1.20	22.45±0.67	24.14±1.72
C. citrinus	25.44± 0.66	20.11±2.0	12.82±0.88	11.02±0.88	26.25±3.2	26.82±2.36
E. glaulus	26.52±2.70	20.83±0.24	12.62±0.22	11.98±0.68	25.97±2.14	26.33±0.22

The tested five plant extracts showed larvicidal and molluscicidal activity against both *Cx. pipiens* larvae and *L. natalensis* snail. *Cx. pipiens* larvae showed high susceptibility to Sorghum bicolor extracts with the lowest LC50 value, while Lymnaea snail was highly susceptible to *Ambrosia* extract (Table 1). The toxic effect of different plant extracts against mosquitoes was detected before, (Ghosh *et al.,* 2012, and Essa *et al* .,2019) and against snails (Lo *et al*., 2018 and Atwa and Bakry, 2019)

Rosmarinus officinalis extract was tested by Yu *et al,* (2013) against a field strain of *Culex quinquefasciatus* larvae and found to have larvicidal activity with LC50 38.3 mg/liter. The major constituents of *Rosmarinus officinalis* were Eucalyptol and Camphor (Yu *et al.*, 2013). Two varieties of *Sorghum bicolor* seedlings showed significant larvicidal activities (P less than 0.05) under laboratory conditions. These plant extracts contain the organic cyanogen dhurrin and were calibrated to produce 90% mortality in 2nd instar *Cx. pipiens* larvae at 0.82 ppm and 90% mortality in 3rdinstar larvae at 1.12 ppm. (Jackson *et al*., 1990).

Plant-derived molluscicides are favorable choices for controlling snails (Kiros *et al*., 2014 and Ibrahim& Bakry, 2019). Belot *et al*, (1991) proved the toxicity of Ambrosia extract against Lymnaea sp, and Kumar *et al*, (2012) recorded the toxic effect of *Solanum nigriun* extract against the intermediate host of the liver worm, *L. natalensis*, Psilostachyin and axillary are components of Damsissa (Ambrosia sp.) proved to have high molluscicidal activity, as mentioned by Ding, (2018), who recorded high mortality after exposure of the golden apple snail to such components of Ambrosia sp.

The tested plant extracts displayed marked molluscicidal potency more than its larvicidal property, especially when comparing *Sorghum, Ambrosia and Eucalyptus* spp. activities (Table 1). This finding may be related to plant-specific differences in active gradients, differences in their mode of action, (Sakran and Bakry, 2005), or due to the higher Molecular resistant mechanism of mosquito larvae.

Eucalyptus glautus extract showed moderate toxicity for both *Cx. pipiens* and *L. natalensis* (with LC50 =218.08±92.6 and 28.80 ± 3.90 ppm, respectively. In all treatments, Lymnaea snail showed more susceptibility than Culex larvae after application of *Sorgum bicolor, Ambrosia maritimia.* and *Eucalyptus glaulus* extracts, as the most potent extracts against both aquatic invertebrates.

Plant phytochemical constructions such as flavonoids, sterols, terpenes, triterpenes, and coumarins, proved to have a key role in stress response mechanisms in plants flavonoids are self-protective components against microbial infections and as a defense against insect attack. Flavonoids, known as antioxidants or enzyme inhibitors, which involved in cellular energy transfer processes (Mierziak,2014).

Omnia *et al.* (2015) mentioned that plant extracts may alter the enzyme levels in both mosquito larvae and snails. Concerning GST activity, treatment of *Cx. pipiens* larvae with the five tested plant extracts induced variations with the enzyme levels, but this change differs from one extract to another, (Tables 2 &3). Treatment of mosquito larvae with the most toxic plant, *Sorghum sp* significantly increased the enzyme activities, while treatment with Eucalyptus slightly inhibited it. It seems that the plant extract ingredient is the limiting factor in changing GSTs levels. GST, as a group of transferases, is known to be implicated in resistance to toxic molecules and protect invertebrates from secondary toxic effects such as an increase in lipid peroxidation (Enayati, 2005*)*. The enhancement of its activity may assure its role in protection against oxidative stresses as suggested by *Yan et al.* (2012). Increasing transferase levels prevent the toxin from reaching its action site, or enhance degradation of toxic units and interfere with the response to biotoxins through changing metabolic enzyme pathways, phenomena emphasized by Hollingworth and Dong, (2008). GST is concerned with the detoxification process due to the reaction of conjugation between GSH and xenobiotics (Cummins *et al*., 2011), this may explain the elevation of such enzyme activities (Enayati, 2005).

The adverse effect of botanicals on some enzyme activity was recorded in various insects, such as *S. littoralis* (Abdel-Aal, 2003) and *A. ipsilon* (El-Sheikh, 2002). Detoxifying enzyme activity changed to a great extent, this is in accordance with the reported results of enhanced enzyme activity in different insects by various botanicals, such as *Pieris rapae* larvae by methanolic extract of *Silybium marianum* (Hasheminia *et al*., 2013), *S. gregaria* by different extracts of *Nigella sativa* extracts (Ghoneim *et al*., 2016) and *A. aegypti* larvae (Koodalingam *et al* ., 2014).

Our results are in agreement with Lin *et al*. (2007) who concluded that viral infection induced GST increase in mosquitoes. Similar results were emphasized by Boyer *et al*. (2012) who recorded increase in that enzyme activity after treating mosquitoes with Bti. Our results contradict Kamel and Hassan, (2018) who declared that glutathione S Transferase showed no change after treatment of *Cx. pipiens* with *Solenostemma, Rosmarinus and Artemisia* spp. extracts.

After the snail treatment with the most toxic extract Ambrosia sp. GST, ALT and GOT activities showed a significant increase (Table 3). Recorded increase in these detoxifying enzyme levels denoted an increasing capability of both *Cx. pipiens* and *L. natalensis* to detoxify some plant extract compounds (Hasheminia *et al*.,2013; Sharifi, *et al.*, 2013; Ghoneim *et al.*, 2016). Such enzymes are known to show great diagnostic potential to indicate the damage of invertebrate-specific tissues as a result of toxicity, **(**Hamadah, 2019).In the light of the present study, the major effects of the tested extracts were found to be stimulatory or inhibitory on the enzyme activities depending on the type of plant and the treated concentrations.

Eucalyptus as a moderate toxic extract, slightly inhibited enzyme activities in both mosquito larvae and snails. Eucalyptus contains 1, 8-cineole plus tannins, and Hydrocyanic acid in its structure. Cineole has antimicrobial properties, (Sebei *et al*., 2015) and may interfere with the beneficial gut microbiota of the gut which is important in inducing some enzymes. Some results have reported a significant decline in detoxifying enzyme activities in tissues of some mollusks in response to some molluscicides (*Bakry et al.,2002 a* & Bakry, 2004).

All tested plant extracts could suppress the protein and carbohydrate body contents after treatment of *Cx. pipiens* larvae or the *l. natalensis* snail, (Tables3 &4). The decrease in protein content explained the inability of larvae and snails to complete the growth process, this may lead us to assume that the transcriptional and translation processes were affected during such treatments. Similar results were obtained by Singh & Singh, (2004), who proved that treatment of the snail *Lymnaea acuminate* with different plant extracts led to a decrease in the protein level in its body.In order to promote energy production, *Cx .pipiens and L.natalensis* use carbohydrates, which are stored and transported to the haemolymph as glucose. It seems that the tested plant extracts, as larvicides and molluscicides greatly affected the carbohydrate metabolic activities of the target invertebrates, (Tables 4 & 5). Plant extracts interfered with some enzyme pathways chiefly those of respiration and carbohydrate metabolism (Bakry *et al*., 2002 a).

The plant extracts are supposed to impede oxygen consumption of tested invertebrates (Bakry,2004), leading to inducing a state of anoxia, which would decrease carbohydrate content in the tested vectors, It subsequently interrupts the glycolytic enzymes explaining its increase or decrease. Plant extracts were recorded by Bakry *et al*. (2002 b) to minimize ATP levels in treated snails by disturbing the enzymatic cycles concerning ATP production and hence causing depression of the snails' energy metabolism, this reduction in energy molecules slow the lipid hydrolysis leading to increasing its level in different tissues.

Conclusions

This is the first valuation of the larvicidal and molluscicidal effects of five plant extracts against two medically important aquatic vectors. The study indicated the aqueous extracts of the studied plants are effective at acceptable concentrations. The plants are widely available in most parts of Egypt and are well-known folk traditional medicines. Therefore, these plants can play role in community-based aquatic vector control activities through further investigating studies in the field condition and exploring toxic effects on non-target organisms as future prospects.

REFERENCES

Abbott, M. S. (1925). A method of computing the effectiveness of an insecticide, *Journal of Economic Entomology;* 18:265-267.

Abdel-Aal A.E. (2003). Effect of some insect growth regulators on certain biological, biochemical and histopathological aspects of the cotton leafworm, Spodoptera littoralis (Boisd.) (Unpublished). Ph.D. Thesis, Faculty of Science , Cairo University., Egypt, 119.

Abd El-Shafie, I.; Shoeib, E.; Attia, S; Rubio, J.; Edmardash, Y. and Badry, A. (2016). Mosquito identification and molecular xenomonitoring of lymphatic filariasis in selected endemic areas in Giza and Qualioubiya governorates, Egypt. *Journal of the Egyptian Society of Parasitology,* 46(1): 87-94.

Arthurs, S., & Dara, S. K. (2019). Microbial biopesticides for invertebrate pests and their markets in the United States. *Journal of invertebrate pathology,* 165: 13-21.

Augusto, R. D. C., & de Mello-Silva, C. C. C. (2018). Phytochemical molluscicides and schistosomiasis:

what we know and what we still need to learn. *Veterinary sciences, 5*(4): 94.

Assar, A.A.; Abo-El-Mahasen, M.M.; Harba, N.M, and Rady, A. A. (2012). Biochemical effects of cyromazine on Culex pipiens larvae (Diptera:Culicidae). *Journal of American Science,* 2012; 8(5): 443-450.

Atwa, M. T. & Bakry, F. A. (2019). Effect of mefloquine on biological and biochemical aspects of Lymnaea natalensis snails infected with Fasciola gigantica. *The Journal of Basic and Applied Zoology,* 80(1): 1-9.

Bakry, F.A.; Sakran, A.A.; Ismail, N.M.M. (2002a). Molluscicidal effect of fungicide, herbicide and plant extract on some biological and physiological parameters of *Biomphalaria alexandrina. Journal of the Egyptian Society of Parasitology,* 32(3): 821-835.

Bakry, F.A.; Ragab, F.M.A.& Sakran, A.M.A. (2002 b). Effect of some plant extracts with molluscicidal properties on some *alexandrina* snails. *Journal of the Egyptian German Society of Zoology,* 38(D): 101- 111.

Bakry FA. (2004). Effect of two plant extracts on susceptibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* and some enzymes of energy metabolism of these snails. *The 5th international Conference on Systems Biology*. Germany: p 149.

Bakry, F.A.; Ismail, S.M.& Abd El- Monem, S. (2004). Effect of two plant extracts on some Biological and enzymatic activities of *Bulinus truncatus* with *Schistosoma haematobium*. *Journal of Aquatic Biology and Fisheries,* 8 (4):313-446.

Bakry, F. A. (2009). Use of some plant extracts to control Biomphalaria alexandrina snails with emphasis on some biological effects. *Pesticide biochemistry and physiology,* 95(3): 159-165.

Bakry, F. A.; Eleiwa, M. E.; Taha, S. A & Ismil, S. M. (2016). Comparative toxicity of Paraquat herbicide and some plant extracts in Lymnaea natalensis snails. *Toxicology and industrial health,* 32(1): 143-153.

Bassal, R.; Shohat, T.; Kaufman, Z.; Mannasse, B.; Shinar, E.; Amichay, D.; Barak, M.; Ben-Dor, A; Bar-Haim, A.; Cohan, D.; Mendelson, E. and Lustig, Y. (2017). The sero prevalence of West Nile Virus in Palestine : A nationwide cross-sectional study. *PLOS ONE,* 12(6):1-10.

Belot, J.; Bornarel, P.; Diouf, M. & Polderman, A. M. (1991). Ambrosia maritima L: molluscicidal effects on the local snails Lymnea natalensis, Bulinus forskalii, Bulinus globosus and Biomphalaria pfeifferi from Senegal. *Plant Science, 74*(2), 167-170.

Bradford, M.M. (1976).A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-Dye Binding. *Analytical Biochemistry,* 72: 248-254.

Boyer, S.; Paris, M.; Jego, S.; Lempérière,G. and Ravanel, P. (2012). Influence of insecticide Bacillus thuringiensis subsp. israelensis treatments on resistance and enzyme activities in Aedes rusticus larvae (Diptera: Culicidae. *Biological Control,* 62 (2): 75-81p.

Chancey, C.; Grinev, A.; Volkova, E. and Rios, M. (2015). The global Ecology and epidemiology of West Nile Virus. *Bio Medical Research International,* 1-20.

Chitsulo, L.; Engels, D.; Montresor, A & Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta tropica,* 77(1): 41-51.

Crompton, M. and Birt, L.M. (1967). Changes in the amount of charbohydrates, phosphogen, and related compounds during the metamorphosis of the blow fly, Lucilia cypring. *Journal of Insect Physiology*, 13:1575-1595.

Ding, W., Huang, R., Zhou, Z., He, H., & Li, Y. (2018). Ambrosia artemisiifolia as a potential resource for management of golden apple snails, Pomacea canaliculata (Lamarck). *Pest management science*, 74(4): 944-949.

Dodson, B. L.; Kramer, L. D. and Rasgon, J. L. (2012). Effects of larval rearing temperature on immature development and west Nile virus vector competence of Culex tarsalis. *Parasites & Vectors*, 5:199.

Duke, S. O.; Cantrell, C. L.; Meepagala, K. M.; Wedge, D. E.; Tabanca, N., & Schrader, K. K. (2010). Natural toxins for use in pest management. *Toxins*, 2(8): 1943-1962.

Duncan, D. B. (1955). Multiple range and multiple F. tests. Biometrics, II, 1-42.

Habig, W.H; Pabst, M.J & Jakoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*,249: 7130–7139 .

Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.

El-Sheikh, T. A. (2002). Effects of application of selected insect growth regulators and plant extracts on some physiological aspects of the black cutworm, Agrotis ipsilon (Huf.). Ph. D. Thesis, Faculty Of Science - Ain Shams University., pp: 112-117.

El-Zayyat, E.; Solimn, M.; Elleboudy, N. and Ofaa, S. (2017). Bioefficacy of some Egyptian aromatic plants on Culex pipiens (Diptera: Culicidae) adults and larvae. *Journal of Arthropod Borne Diseases*, 11(1): 147-155.

Enayati, A. A., Ranson, H., and Hemingway, J. (2005). Insect glutathione transferases and insecticide resistance. *Insect molecular biology*, 14(1): 3-8.

Essa, E. E.; Mo'men, S. A.; Rady, M. H.; Ma'moun, S. A.; Barakat, E. M. and Salama, M. S. (2019). Eucalyptus oil nano-emulsion encapsulated in chitosan beads as a new approach in control of Culex pipiens larvae. *International Journal of Mosquito Research;* 6(5): 63-69

Finney, D.J. (1971). Probit Analysis. third ed. Cambridge Univ. Press, London, UK.

Ghosh, A., Chowdhury, N., and Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *The Indian journal of medical research*, 135(5): 581.

Global Health Estimates (2016). Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, *World Health Organization;* 2018.

Ghoneim, K.; Hamadah, K.; El-Hela, A.; MohammadA. H. & Amer, M. (2016). Efficacy of Nigella sativa (Ranunculaceae) extracts on adult performance and phase transition of the desert locust Schistocerca gregaria (Orthoptera: Acrididae). *Munis Entomology & Zoology*, *11*: 287-302.

Hamadah, K. S. (2019). Disturbance of phosphatase and transaminase activities in grubs of the red palm weevil Rhynchophorus ferrugineus (Coleoptera: Curculionidae) by certain insecticidal compounds. *The Journal of Basic and Applied Zoology*, *80*(1): 1-8.

Hasheminia SM, Sendim JJ, Jahromi Kh T, (2013). Moharramipour S. Effect of milk thistle, Silybium marianum, extract on toxicity, development, nutrition, and enzyme activities of the small white butterfly, Pieris rapae. *Journal of Insect Science*, 13(146):1-10.

Hollingworth, R. M. and Dong, K. (2008). The biochemical and molecular genetic basis of resistance to pesticides in arthropods. *Global pesticide resistance in arthropods*, 40: 89.

Ibrahim, A. M., and Bakry, F. A. (2019). Assessment of the molluscicidal impact of extracted chlorophyllin on some biochemical parameters in the nervous tissue and histological changes in Biomphalaria alexandrina and Lymnaea natalensis snails. *Invertebrate Neuroscience*, *19*(3): 1-7.

Jackson, F. L., Behkeit, S. S., eL Etr, S. M., and Quach, N. K. (1990). Larvicidal effects of grain sorghum (Sorghum bicolor) seedling extracts upon Culex pipiens larvae. *Journal of the American Mosquito Control Association*, 6(3): 500-503.

Joseph, H.; Maiava, F.; Naseri, T.; Silva, U; Lamnie, P. and MelRose, W. (2011). Epidemiological assessment of continuing transmission of lymphatic filariasis in Samoa. *Annals of Tropical Medicine and Parasitology*, 105(8):567-578.

Kamaraj, C., Rahuman, A. A., Mahapatra, A., Bagavan, A., & Elango, G. (2010). Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. *Parasitology research*, 107(6): 1337-1349.

KAMEL, O. M. H. M. and HASSAN, M. M. (2018). Effect of three egyptian medicinal plant's extracts on biochemistry of Culex pipiens larvae (Culicidae: Diptera). *Journal of the Egyptian Society of Parasitology*, *48*(2): 357-362.

Kiros, G.; Erko, B.; Giday, M. & Mekonnen, Y. (2014). Laboratory assessment of molluscicidal and cercariacidal efects of Glinus lotoides fruits. *BMC Research Notes*, *7*(1) :1-7.

Knight, J. A.; Anderson, S. & Rawle, J. M. (1972): Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. *Clinical Chemistery*,*18*:199-202

Koodalingam, A.; Deepalakshmi, R.; Ammu, M., & Rajalakshmi, A. (2014). Effects of NeemAzal on marker enzymes and hemocyte phagocytic activity of larvae and pupae of the vector mosquito *Aedes aegypti*. *Journal of Asia-Pacific Entomology*, *17*(2): 175-181.

Kumar, A.; Sagwal, S. & Rani, S. (2012). An updated review on molecular genetics, phytochemistry, pharmacology and physiology of black nightshade (Solanum nigrum). *International Journal of Pharmaceutical Sciences and Research*, 3(9): 2956-2977

Lin, C .C.; Yang , C. F.; Tu, C. H.; Huang, C. G.; Shih, Y. T.; Chuang, C. K. and Chen, W. J. (2007). A novel tetraspanin C189 upregulated in C6/36 mosquito cells following dengue.*Virus Research* , 124: 176-183.

Lo, N. C., Gurarie, D., Yoon, N., Coulibaly, J. T., Bendavid, E., Andrews, J. R., & King, C. H. (2018). Impact and cost-effectiveness of snail control to achieve disease control targets for schistosomiasis. *Proceedings of the National Academy of Sciences*, 115(4): E584-E591.

Mierziak, J.; Kostyn, K.; and Kulma, A. (2014). Flavonoids as important molecules of plant interactions with the environment. *Molecules*, 19(10): 16240-16265.

Omnia, M. H. M.; El-Ghaban, G., & Hassan, M. (2015). Evaluation of some medicinal plant extracts against mosquito culex pipiens larvae and snail biomphalariaalexandrina, International Journal of Recent

Advances in Multidisciplinary Research2. (12) : 1105-1109

Rodrigues, K. A. D. F., Dias, C. N., do Amaral, F. M. M., Moraes, D. F., Mouchrek Filho, V. E., Andrade, E. H. A., and Maia, J. G. S. (2013). Molluscicidal and larvicidal activities and essential oil composition of Cymbopogon winterianus. *Pharmaceutical biology,* 51(10): 1293-1297.

Sakran AMA and Bakry FA. (2005). Biological and physiological studies on Biomphalaria alexandrina snails exposed to different plant molluscicides. *Journal of the Egyptian-German Society of Zoology,* 48(A): 237-256.)

Shehata, A. I. (2019). Biological activity of Prunus domestica (Rosaceae) and Rhamnus cathartica (Rhamnaceae) leaves extracts against the mosquito vector, Culex pipiens L. (Diptera: Culicidae). *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control,* 11(1):65-73.

Sharifi, M.; Kosari, A. A.; Zibaee, A.; and Jalali Sendi, J. (2013). Effects of Pyriproxyfen on detoxifying and intermediary enzymes of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). *Plant Pests Research*, *3*(1): 35-44.

Singh, A. and Singh, D. K. (2004). Effect of herbal molluscicides and their combinations on the reproduction of the snail, *Lymnaea acuminata. Archives of environmental contamination and toxicology*, *46*(4): 470-477.

Sebei, K.; Sakouhi, F.; Herchi, W.; Khouja, M.L.& Boukhchina, S.(2015). Chemical composi- tion and antibacterial activities of seven Eucalyptus species essential oils leaves. *Biological Research,* 48 (1), 1–5. doi:10.1186/0717-6287-48-7.

Yan, H., Meng, F., Jia, H., Guo, X., and Xu, B. (2012). The identification and oxidative stress response of a zeta class glutathione S-transferase (GSTZ1) gene from Apis cerana cerana. *Journal of insect physiology,* 58(6): 782-791.

Yu, J.; Liu, X. Y.; Yang, B.; Wang, J.; Zhang, F. Q.; Feng, Z. L.; Wang, C. Z. and Fan, Q. S. (2013). Larvicidal activity of essential extract of Rosmarinus officinalis against Culex quinquefasciatus. *Journal of the American Mosquito Control Association*, 29(1): 44-48.

ARABIC SUMMARY

النشاط السمي والكيميائي الحيوي لخمسة مستخلصات نباتية تم اختبارها ضد ناقلات الأمراض المائية، Culex pipiens والحلزون، Lymnaea natalensis

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تعطي أجنده الصحه العالميه الاولويه لمكافحه ناقلات الأمراض بما يسهم في إنقاذ الكثير من الأرواح وتجنب الكثير من المعاناه. تم إختبار طرق بسيطه وفعاله من حيث التكلفه بتطبيق بعض المستخلصات النباتيه مثل مستخلص نبات الروزماري، الذره الرفيعه (السورغم)، الكليستمون ، الدمسيسه و الأوكالبتوس ضد أثنين من النواقل المرضيه المائيه يرقات/ *Cx. pipiens* وحلزون *L. natalensis* وقد أظهرت المستخلصات النباتيه الخمسه تأثيرات مميته ضد كل من الناقلين و أظهرت يرقات/ *Cx. pipiens* وحلزون *L. natalensis* حساسية عالية لمستخلص الذرة الرفيعة 24.02 +184.37 LC50 جزء في المليون بينما تم تسجيل الحساسية العالية لحلزون L. natalensis تجاه مستخلص الدمسيسه LC50 = 7.65+ 1.89 جزء في المليون. وقد تغير نشاط إنزيمات GST و GOTو ALTبالإضافة إلى محتوي البروتينات والكربوهيدرات والدهون الكليه بيرقات *Cx. pipiens* والحلزون *L. natalensis* المعالجه عن العينات غير المعالجة مع وجود بعض الاستثناءات ، زادت معظم المستخلصات النباتية المختبرة بشكل عام من نشاط GST و GOT في كلا النواقل بينما تم الإبلاغ عن زيادة ونقصان مهمين في نشاط ALT في *Cx. pipiens* و *L. natalensis* على التوالي في معظم المستخلصات النباتية. كما أشارت النتائج المتحصل عليها إلى أن محتوى البروتين والكربوهيدرات الكلي في كل من *Cx. pipiens* و *L. natalensis* انخفض بشكل معنوي مع جميع المستخلصات النباتية المختبرة والتي كانت أكثر وضوحا في *L. natalensis.* مع زيادة طفيفة في الدهون الكلية باستثناء مستخلص نبات الروزماري الذي لم يحدث أي تغيير في كل منهما. بناءً على هذه التعديلات، يمكن استنتاج أن المستخلصات النباتية محل الدراسه لها تأثيرات مميته ضد كل من يرقات/ *Cx. pipiens* وحلزون *L. natalensis* .