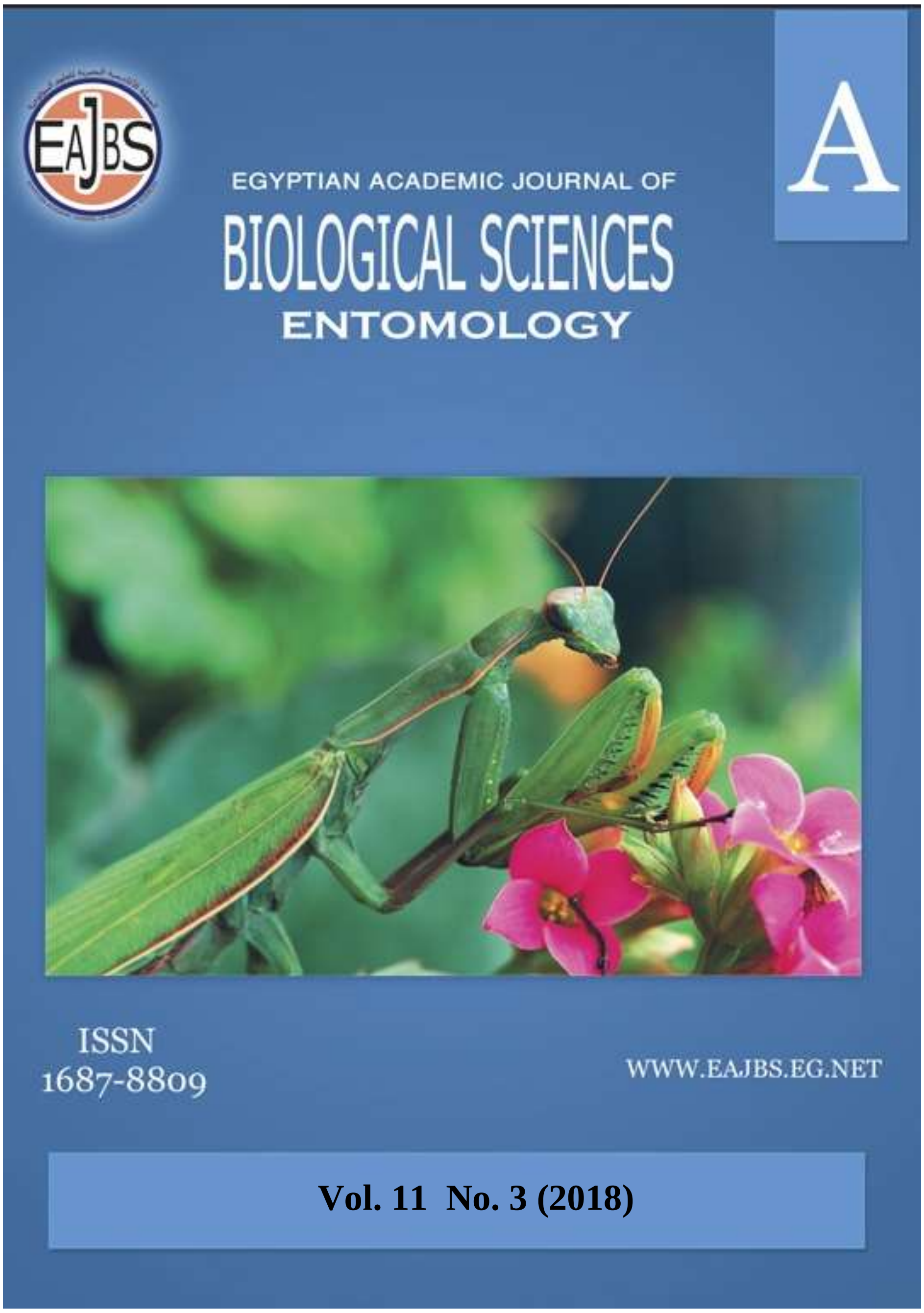


Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
Entomology Journal publishes original research papers and reviews from any entomological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution, control of insects, arachnids, and general entomology.
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 11(3)pp: 157- 172(2018)*

Egypt. Acad. J. Biolog. Sci., 11(3): 157–172 (2018)

Egyptian Academic Journal of Biological Sciences
A.Entomology

ISSN 1687- 8809
www.eajbs.eg.net

Toxicological Studies on the Effect of some Agricultural and Wild Plants Extract as Insecticidal Agent on the Common House Mosquito, *Culex pipiens* in Bisha Region, Saudi Arabia

Reda F.A. Bakr[1*]; Talal E. Dahan[1]; Hanan A.M. Bosly[2]
1-Biology Dept., Faculty of Sciences, University of Bisha, Bisha, KSA
2-Biology Dept., Faculty of Sciences, Jazan University, Jazan, KSA
Corresponding author: redabakr55@gmail.com

ARTICLE INFO
Article History
Received: 1 /6 /2018
Accepted: 1/7 /2018

Keywords:
bioassay,extraction, larva, mortality, synergist, toxicity

ABSTRACT

Culex pipiens plays a crucial role in the transmission of many vector-borne pathogens infecting humans, livestock and affecting wildlife. The present study was conducted in Laboratory of Biology Department, Faculty of Science, Bisha University, KSA, to evaluate the insecticidal activities of aqueous, ethanolic, and acetone extracts of each of nine selected wild plants, *Calotropis procera*, *Withania somnifera*, *Citrullus colocynthis*, *Mentha longifolia*, *Datura innoxia*, *Ziziphus spina christi* , *Salvadora perssica*, *Aerva javanica*, and *Punica granatum* against larvae of *Cx. pipiens* under controlled laboratory conditions (water temperature 28 ± 2 °C, 12:12 h photoperiod). After calculating the mortality percentages among treated larvae, LC_{50} values could be arranged in an ascending order as follows: acetone extract < ethanolic extract < aqueous extract of *C. procera* , *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi*, respectively. On calculating the synergistic ratio (SR), it is found that the value of SR is greater than one in all tested extracts, except of *Ziziphus spina* which was lower than one. The SR of aqueous extract of *Calotropis procera* with Triton x100 (1.39) was higher than another one .

INTRODUCTION

WHO has considered mosquitoes as "public enemy number one", since mosquitoes can transmit more diseases than other arthropods and adversely affect millions of people all over the world. They act as vectors of various diseases some of which millions of cases have been recorded as illnesses and deaths in humans and animals every year. Among these diseases, malaria, yellow fever, dengue, filariasis, and Rift Valley fever have been documented along endemic and epidemic areas in many countries (WHO, 1997; Lerdthusnee *et al*., 1995). Saudi Arabia covers the major part of vast Arabian Peninsula. In this context, the southwestern region of Saudi Arabia has unique topographic features and climatic conditions. Therefore, it was considered as the nucleus of the first protected area for a large size of diverse biota, animals and plants. Most observations on urban and sub-urban, as well as rural areas of the southwestern region, have regarded mosquito fauna very limited, except those reported by some authors (Buttiker, 1979, 1981; Zahar, 1973; Hawking, 1973; Wills et al., 1985; Harbach, 1988; Abdullah and Merdan, 1995).

For preventing or minimizing the mosquito-borne diseases, as well as to improve the environment quality and public health, the mosquito control has been as a prerequisite need. The usual measure for mosquito control is the application of conventional insecticides, such as organochlorines and organophosphates (Poopathi and Kishore, 2006; Ghosh *et al*., 2012). Unfortunately, these insecticides are not successful agents owing to various human hazards, as well as numerous technical, operational, ecological, and economic problems. In other words, use of many conventional insecticides for mosquito control has been limited due to the high costs of these insecticides, concern for environmental sustainability, hazards against human health and other non-target organisms, non-biodegradable nature in the ecosystem, beside the increasing development of insecticide resistance on a global scale (Benelli *et al*., 2016; Schorkopf *et al*., 2016).

One of the effective alternatives of insecticides is the searching for natural materials in the floral biodiversity, as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides, which are based on a single active ingredient, plant-derived active materials usually contain botanical blends of compounds which act concertedly on both behavioral and physiological processes (Ghosh et al., 2012; Dinesh *et al*., 2014; Deepalakshmi and Jeyabalan, 2017).

For the continued effective vector control management, identifying efficient bio-insecticides that adaptive to the ecological systems is necessary. Botanicals have wide range of insecticidal properties and thus they can be used as a safe weapon to fight against mosquito-borne diseases, since they have been reported environmentally safe, less hazardous to non-target biota, simple, inexpensive, and more suitable for the developing countries (Soliman and El-Sherif, 1995; El-Bokl and Moawed, 1997; Shoukry and Hussein, 1998, Massoud and Labib, 2000; Mohammed and Hafez, 2000; Mohammed *et al*., 2003).

Among mosquitoes, the common house mosquito, *Culex pipiens*, is the most common and widely distributed species in Southwestern Saudi Arabia. *Culex pipiens* plays a crucial role in the transmission of many vector-borne pathogens infecting humans, livestock and affecting wildlife including viruses (Engler et al., 2013), protozoan parasites (Santiago-Alarcon et al., 2012) and Metazoan parasites (Morchón et al., 2007). Some authors reported that adult females of *C. pipiens* feed mainly on birds, but others (Muñoz et al., 2012; Martínez-de et al., 2013; Martínez-de et al., 2015) demonstrated that mammals represent also an important source of their blood meals in some populations. Therefore, the present study was conducted to evaluate the insecticidal activities of different extracts of nine selected wild plants, as biorational agents, against *C. pipiens*. Also, a synergistic action of TritonX100 on the susceptibility of *C. pipiens* larvae to wild plant extracts was investigated in order to improve the properties of the tested materials.

MATERIALS AND METHODS

1. Study Area:

Bisha is a region in the south-western Saudi Arabian province, 'Asir'. It is located at 20°0′0″N 42°36′0″E. Bisha includes about 58 towns and 240 villages spreading out on both sides of Bisha Valley. It stands at an altitude of approximately 610 meters (2,000 ft.) above sea level. Southwestern region is located between latitude 17^{o} 30--21^{o} 00’N and Longitude 41^{o} 30--44^{o} 30E. It is entirely different from the rest of the Kingdom. Asir highland receives a variable seasonal rainfall but more than the rest of the Kingdom (300-500 mm/year as compared to 50-100 mm/year elsewhere).

According to the characteristic of the study area and its environmentally biotic and abiotic factors, it could be considered a unique study area for mosquito ecology and distribution. The area also includes variable water resources for all types of mosquito breeding sites.

2. Plant Collection:

Leaf and seed samples were collected from the valley in Bisha region during the period Augusts 2017-March 2018. The leaf and seed samples were packaged and transported to Laboratory of Biology Department, Faculty of Science, Bisha University, for processing extraction and bioassays. The collected plants were systematically identified by the co-worker Dr. T. Dahan, Bisha University. The collected plants can be described as follows (see Table 1 and Plate 1).

Table (1) The collected plants

	Plant species	**Family**	**Common name**	**Plants part**
1	***Calotropis procera***	Asclepiadaceae	Milk weed	Leafs
2	***Withania somnifera***	Solanaceae	Winter cherry	Leafs
3	***Citrullus colocynthis***	Cucurbitaceae	Bitter apple fruit	Seeds
4	***Mentha longifolia***	Lamiaceae	wild mint	Leafs
5	***Datura innoxia***	Solanaceae	Indian apple	Leafs
6	***Ziziphus spina-christi***	Rhamnaceae	Jujube	Leafs
7	***Salvadora perssica***	Salvadoraceae	tooth brush	Leafs.
8	***Aerva javanica***	Amaranthaceae	Aerva	Leafs.
9	***Punica granatum***	Myrtales	*the epic poem* Punica	Fruit rind. (peel)

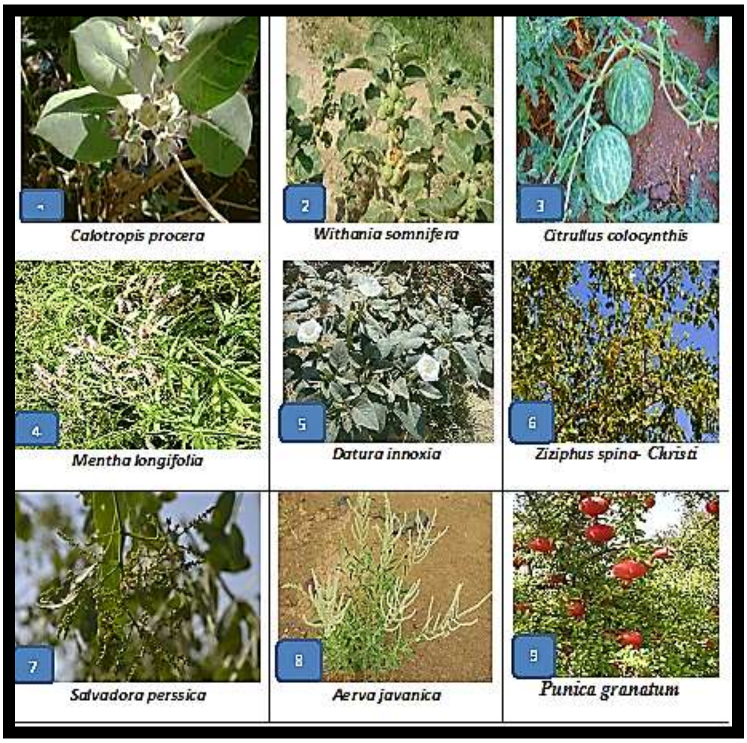


Plate (1) Showing the picture of the collected plants

2.1. ***Calotropis procera***: Common names include apple of Sodom, Sodom apple, stabragh king's crown, rubber bush, or rubber tree (Morris *et al*., 1964; Adebayo *et al*., 2015). It is a flowering plant belonging to family Apocynaceae that is native to North Africa, Tropical Africa, Western Asia, South Asia, and Indochina. The green globes are hollow but the flesh globes contain a toxic and extremely bitter milky sap that turns into a gluey coating resistant to soap.

2.2. ***Withania somnifera***: Common names include ashwagandha, Indian ginseng, poison gooseberry, and winter cherry (Stearn, 1995). It belongs to the family Solanaceae (nightshade family). However, several other species in this genus are morphologically similar to the selected species. Although those species are commonly used as medicinal herbs in Ayurvedic medicine, there is no high-quality clinical evidence that they have biological effects on insects.

2.3. ***Citrullus colocynthis*****:** Common names include colocynth, bitter apple, bitter cucumber, desert gourd, egusi, vine of Sodom, and wild gourd (Gurudeeban *et al*., 2010). It is a desert vine plant growing in sandy arid soils. It is native to the Mediterranean Basin and Asia, and is distributed among the west coast of northern Africa, eastward through the Sahara, Egypt until India, reaching to the north coast of the Mediterranean and the Caspian Seas. In addition, it grows in southern European countries, as Spain, and on islands of the Grecian archipelago. Naturally, it is an annual or a perennial plant in Indian arid zones and has a great survival rate under extreme xeric conditions (Gurudeeban *et al*., 2010). The seeds are grey and 5 mm long by 3 mm wide.

2.4. ***Mentha longifolia*****:** Common name is horsemint. It is a species in the genus *Mentha* (mint), native to Europe, western and central Asia (east to Nepal and the far west of China), and Northern and Southern (but not tropical) Africa. It is a very variable herbaceous perennial plant with a peppermint-scented aroma. It spreads *via* rhizomes to form clonal colonies (Huxley, 1992).

2.5. ***Datura innoxia*****:** Common names include prickly burr, recurved thorn-apple, downy thorn apple, Indian apple, lovache, moonflower, nacazcul, toloatzin, tolguache or toloache (Preissel *et al*., 2002). It is an annual shrubby plant whose height reaches 0.6-1.5 meters. Stems and leaves are covered with short and soft grayish hairs, giving the whole plant a grayish appearance. The elliptic smooth-edged leaves have pinnate venation. Fruit is an egg-shaped spiny capsule, about 5 cm in diameter. Fruit splits open when ripe, dispersing the seeds.

2.6. ***Ziziphus spina-christi*****:** Common name is Christ's thorn jujube. It is an evergreen plant, native to Northern and tropical Africa, Southern and Western Asia. It is native to the countries of Chad, Djibouti, Eritrea, Ethiopia, Kenya, Libya, Mali, Mauritania, Nigeria, Pakistan, Senegal, Somalia, Tunisia, Turkey and Zimbabwe (The Plant List, 2016).

2.7. ***Salvadora persica*****:** Common names include arak, *Galenia asiatica*, meswak, peelu, pīlu, *S. indica*, or toothbrush tree, mustard tree, and mustard bush. It is a species of Salvadora and has antiurolithiatic properties, and used for centuries as a natural toothbrush. Its fibrous branches have been recommended by the World Health Organization for oral hygiene use (Batwa, Mohammed; et al., 2006 ; Araya, Yoseph 2008).

2.8. ***Aerva javanica*****:** Common names include the kapok bush or desert cotton (Willis, 1985). It is a species of plant in the Amaranthaceae family. It has a native distribution incorporating much of Africa (including Madagascar), and the south-west and south of Asia, and it has become adventitious in northern Australia. The plant is herbaceous, multi-stemmed and soft-wooded. It bears broad leaves. It often has an erect habit and grows to a height of about 1.6 meters. In Western Australia, this plant tends to grow in sandy soils, especially along drainage lines.

2.9. ***Punica granatum***: Common name is pomegranate. It is a fruit-bearing deciduous shrub or small tree in the family Lythraceae growing to 5-8 m tall. It has been cultivated since ancient times throughout the Mediterranean region. Today, it is widely cultivated throughout the Middle East and Caucasus region, north and tropical Africa, South Asia, In the 20th and 21st centuries, it has become more common in markets of Europe and the Western Hemisphere (Morton, 1987; Stover and Mercure, 2007).

3. Plant Extraction Procedure:

The collected plant materials (leaves or seeds) had been thoroughly washed to avoid dust and dirt. These washed materials were then kept under shade in the laboratory for drying. Dried parts of the previously mentioned plants were cut into small pieces and ground by an electric grinder. Hundred grams of the powdered materials of each plant were exhaustively extracted with water, absolute ethanol and acetone using Soxhlet extraction technique for 4-6 h. After cooling to room temperature, the resultant extract was concentrated and stored at −20 °C until the larvicidal bioassay.

4. Mosquito Collection:

Monthly collections of mosquito larvae (Augusts 2017-March 2018) were made from selected breeding places in the Bisha area (Bisha valley, Al Hazamy, and Behind Bisha Dam). These places were variable and ranged from permanent ones to occasional water collections, which include irrigation wells and canals, seepage, surface water collections, drainage water, and stagnant water. Collection was carried out by sweeping the water surface with the long-handled larval net (WHO, 1975). In small water collections, another larval net was used with the iron ring of 10 cm diameter. During collection, the aquatic stages were washed into the nylon sieve which was then inverted and washed out in a white enamel bowl containing clear distilled non-chlorinated water. All immature mosquito stages were picked up by a pipette and transferred into a plastic bag. All samples were transported to the laboratory in thermos box. At the laboratory, pupae and 4th instar larvae were isolated, each in separate vials containing small amount of breeding site water and covered until the adult emergence. Early larval instars were transferred to breeding enamel bowls, fed tropical fish food (Tetramin) and kept at 27+/-1^{o}C. These instars were observed daily until moulting into the 4th instar.

5. Culturing of Tested Mosquito*:*

The collected *C. pipiens* larvae from Bisha Valley were used to establish a culture in the laboratory under controlled conditions (water temperature 28 ± 2 °C, 12:12 h photoperiod). The larvae were reared in large plastic pans (37 X 31 X 6 cm) containing distilled water. Larval densities ranged 200–300/pan. Each pan was supplemented with the artificial diet Tetramin® fish meal (Tetra GmbH, Melle, Germany). Each of rearing water and diet had been replaced with fresh materials every two days. Pupae were kept in plastic cups and transferred into standard 30 × 30 × 30 cm rearing cages for adult emergence. The newly emerged adults were provided with 10% sucrose solution in a cotton pad within a Petri dish. Mosquito females were blood-fed on a pigeon 4–5 days post-emergence and provided with oviposition plastic containers (11.5 cm in diameter and ~ 6.2 cm in depth) for egg laying. The egg collection was conducted 2–3 days after blood meal. These egg rafts were ready for the maintenance of the present culture.

6. Toxicological Bioassay:

A series of toxicological bioassay was carried out to evaluate the insecticidal activities of the plant extracts on the *C. pipiens* larvae. Fourth larval instars were used for this purpose. Toxicological bioassay of the selected extracts on tested mosquito larvae was carried out according to method described by Wright (1971) with some improvements. The bioassay was conducted using batches of 20 ($n = 20$) pre-fourth instar larvae of *C. pipiens* per beaker. Five replicates were used for each concentration. Five replicates of control larvae were also used beside the treated larvae. Treated and control larvae were fed on TetraMin® fish meal during the testing period. Larval mortality was observed at intervals of 24 h until the death of the last instar larvae or adult emergence. Death of larvae was checked if they remained irresponsive within a span of two minutes when gently probed with a pipette. The number of the dead larvae was calculated as average mortality percentage for each concentration relative to mortality of corresponding controls.

7. Synergistic Action of Triton x100:

Each of extract was mixed with an appropriate concentration (1 mL) of the synergist Triton x100 (0.01%) to obtain mortalities as described before. Then, data were analyzed by the probit analysis (Finney, 1971) and the synergistic ratio (SR) was calculated empirically according to Thangam and Kathiresan (1990, 1997).

SR = LC_{50} of extract alone/LC_{50} of the mixture;

value ≥ 1 indicating synergism;

≤ 1 indicating antagonism.

8. Statistical Analysis of Data:

The treatment was considered valid when it was more than 10% mortality in the control larvae (Hassan, 1989). Percent mortality was corrected using Abbot's formula (Abbott, 1925) when necessary. The lethal concentrations were deduced by extrapolation from the regression line obtained by Probit analysis (Finney, 1971).

The LC_{50} and LC_{95} values were determined at their associated 95% confidence levels as well as their slope function, according to (Finney, 1971).

RESULTS

The insecticidal activities of aqueous, ethanolic, and acetone extracts of nine tested plants (*C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, *Z. spina-christi* , *S. perssica*, *A. javanica*, and *P. granatum*) were evaluated against the 4th instar larvae of the *Cx. pipiens* in the laboratory.

The results were presented in Tables (2, 3 & 4), and the regression lines were illustrated in Figures (1- 6). The confidential limits of each of the tested nine plants were statistically calculated for LC_{50} and LC_{95} at $P = 0.05$. Extracts of these nine plants showed different toxicities except for the last three plants, *S. perssica*, *A. javanica*, and *P. granatum* which exhibited very slight toxic effects. The LC_{50} values of aqueous, ethanolic and acetone extracts were determined in 17.29, 22.22, & 25.01 ppm, respectively, of *C. procera*; 46.73, 54.11 & 59.30 ppm, respectively, of *W. somnifera*; 64.76, 84.50 & 106.81 ppm, respectively, of *C. colocynthis*; 380.38, 429.69 & 553.56 ppm, respectively, of *M. longifolia;* 560.40, 622.70 & 657.84 ppm, respectively, of *D. innoxia*; and 1667.04, 1789.17 & 1881.85 ppm, respectively, of *Z.*

spina-christi. Therefore, the toxicity of the tested plant extracts based on LC_{50} values which could be arranged in an ascending order as follows: acetone extract < ethanolic extract < aqueous extract of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi*. In addition, toxicities of the tested extracts were found in a dose-dependent course. Besides lethality of high doses, the extracts remarkably accelerated the growth of larvae into pupae.

1. Larvicidal Activity of Aqueous Extracts Against *C. pipiens* Larvae:

After treatment of mosquito larvae with aqueous extracts of the tested plant species, data of toxicity had been distributed in Table 2 and delineated in the regression lines of Figures 1 & 2. LC_{50} values of aqueous extracts of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* were recorded as 17.29, 46.73, 64.76, 380.38, 560.40 and 1667.04 ppm, respectively. As shown in the present data, there were slight differences in potency of extracts of two plants, *C. procera* and *W. somnifera*. Also, aqueous extracts of these two plants were found in parallel regression lines. This might suggest that these extracts have the same mode of action against the tested mosquito larvae .

Data of the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) to the same concentrations of aqueous extracts of all plants had been arranged in Table 2. These data revealed a considerable increase in LC_{50} and LC_{95} values, in comparison with those of each extract of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* alone. By calculating the synergistic ratio (SR), SR was greater than one in all plants except of *Z. spina-christi* which was lower than one. SR of aqueous extract of *C. procera* with Triton x100 (1.39) was higher than another one.

Table 2 Larvicidal activity of aqueous extractions of some plants against *Culex pipiens* larvae

Plant species	Part of plant	Extraction	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope function	S.R
Calotropis procera	***Leafs***	Aqueous extract	17.29 (18.96-15.76)	43.87 (52.45 -36.68)	4.072±0.109	1.393
		Aqueous + Triton x100	12.41 (13.88-11.09)	43.51 (54.83 -34.68)	3.019±0.065	
Withania somnifera	***Leafs***	Aqueous extract	46.73 (50.89-42.89)	118.86 (139.31.101.48)	4.056±0.126	1.097
		Aqueous + Triton x100	42.56 (46.47-38.97)	106.35 (123.50-91.64)	4.136±0.128	
Citrullus colocynthis	***seeds***	Aqueous extract	64.76 (70.85-59.19)	157.39 (186.46132.57)	4.264±0.119	1.278
		Aqueous + Triton x100	50.64 (56.07-45.73)	151.65 (185.04-124.46)	3.453±7.886	
Mentha longifolia	***Leafs***	Aqueous extract	380.38 (422.29-342.60)	1087.47 (1293.51-914.88)	3.605±0.116	1.138
		Aqueous + Triton x100	334.13 (373.71-298.70	928.06 (1092.43-788.72)	3.707±0.127	
Datura innoxia	***Leafs***	Aqueous extract	560.40 (602.82-520.95)	1254.63 (1449.29-1086.32)	4.699±0.166	1.118
		Aqueous + Triton x100	501.03 (541.07-463.93)	1148.85 (1321.40-999.03)	4.564±0.148	
Ziziphus spina-christi	***Leafs***	Aqueous extract	1667.04 (1829.47-1494.64)	5127.46 (7108.45 –3700.30)	3.370±0.1576	0.973
		Aqueous + Triton x100	1711.76 (1906.75-1536.83)	5025.67 (6913.73-3654.74)	3.516±0.1736	

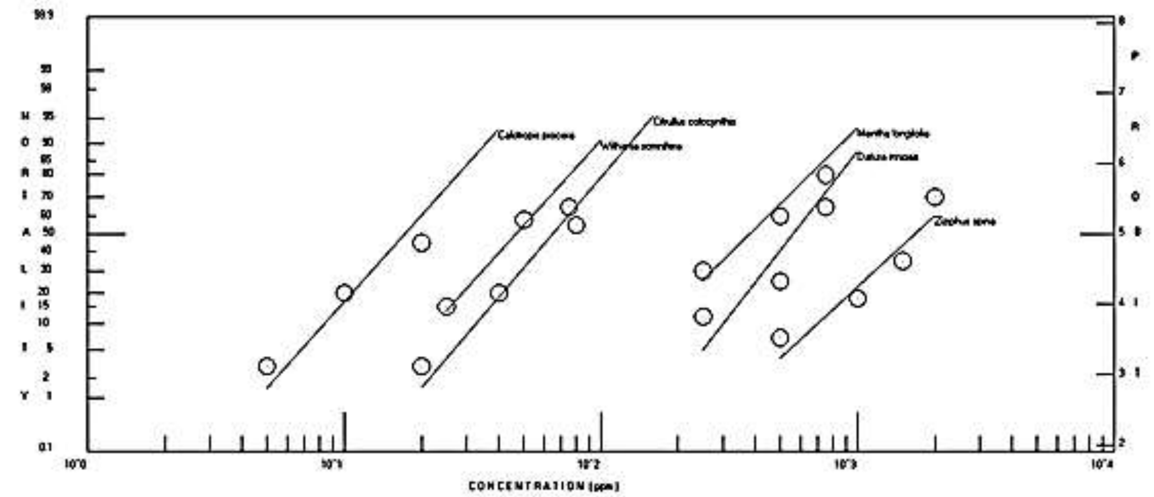
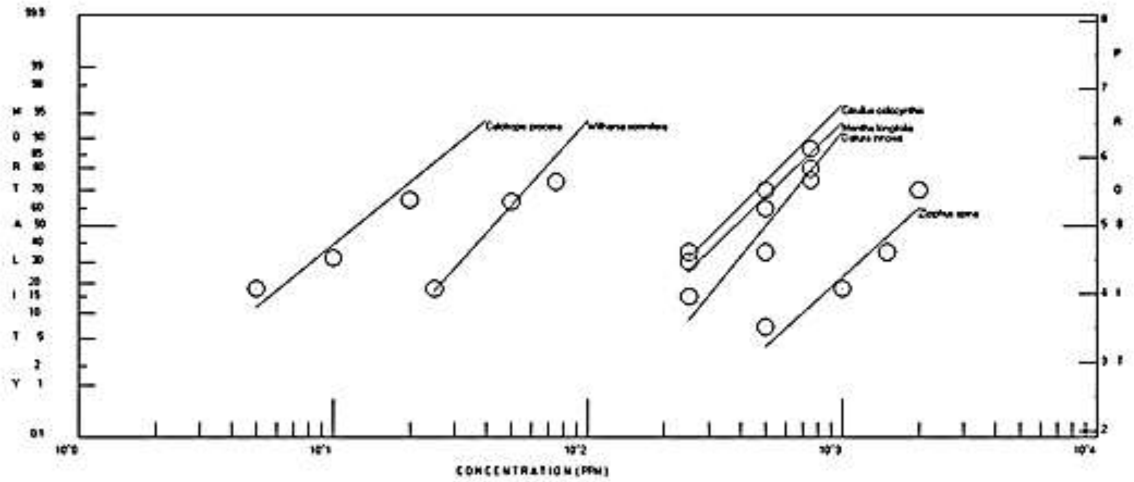


Fig.(1)Susceptibility of *Culex pipiens* larvae to aqueous extractions of selected plants



Fig,(2): Susceptibility of *Culex pipiens* larvae to aqueous extractions of selected plants with Triton x100

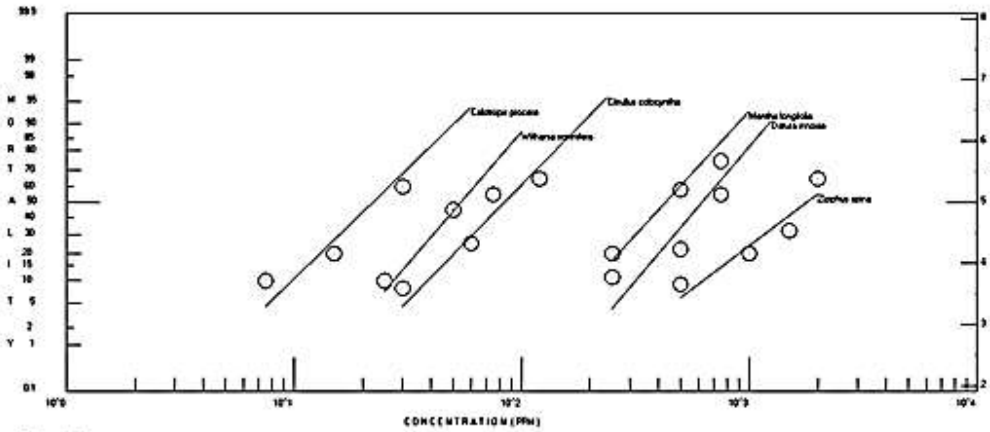
2. Larvicidal Activity of Ethanolic Extracts Against *C. pipiens* Larvae:

After treatment of mosquito larvae with ethanolic extracts, data were represented in Table 3 and the regression lines were illustrated in Figures 3 & 4. LC_{50} values of ethanolic extracts of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* were 22.22, 54.11, 84.50, 429.69, 622.70, and 1789.17 ppm, respectively. Also, these data showed the presence of differences in potency of the tested extracts.

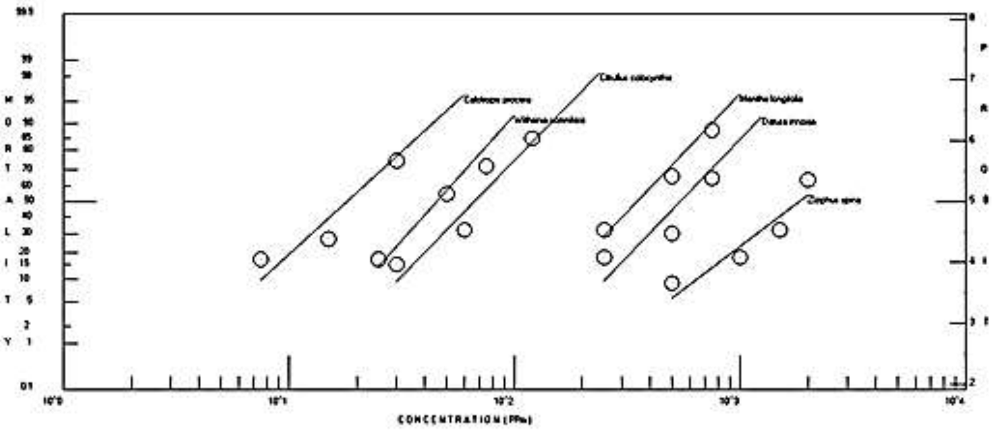
Data listed in Table 3 indicated a considerable increase in LC_{50} and LC_{95} values in comparison with those of each extract of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* alone. On calculating SR, it was greater than one in all tests except of *Z. spina* which was lower than one. SR of ethanolic extract of *C. colocynthis* with Triton x100 (1.25) was higher than another one (Table 3).

Table 3 Larvicidal activity of etanolic extractions of some plants against *Culex pipiens* larvae

Plant species	Part of plant	Extraction	LC $_{50}$ (Co. Limits)	LC $_{95}$ (Co.Limits)	Slope function	S.R
Calotropis procera	**Leafs**	Ethanolic extract	22.22 (24.53-20.12)	63.64 (77.41 -52.43)	3.599±8.375	1.22
		Ethanolic + Triton x100	18.11 (20.09-16.32)	55.54 (68.04-45.46)	3.380±7.708	
Withania somnifera	**Leafs**	Ethanolic extract	54.11 (58.47-50.06)	129.55 (151352-110.82)	4.336±0.145	1.19
		Ethanolic+ Triton x100	45.31 (49.33-41.61)	112.64 (131.07-96.86)	4.159±0.129	
Citrullus colocynthis	**Seeds**	Ethanolic extract	84.50 (93.02-76.75)	229.98 (276.54-191.43)	3.782±9.143	1.25
		Ethanolic+ Triton x100	67.41 (74.59-60.92)	190.01 (233.34-154.91)	3.655±0.102	
Mentha longifolia	**Leafs**	Ethanolic extract	429.69 (469.70-393.67	1069.93 (1279.93-940.34)	4.041±0.125	1.22
		Ethanolic+ Triton x100	351.24 (389.44-316.755)	927.72 (1082.89-795.06)	3.899±0.129	
Datura innoxia	**Leafs**	Ethanolic extract	622.70 (673.33-575.86)	1473.38 (1733.63-1252.84)	4.397±0.143	1.14
		Ethanolic+ Triton x100	542.65 (591.98-497.42)	1449.78 (1733.64-1212.80)	3.854±0.113	
Ziziphus spina-christi	**Leafs**	Ethanolic extract	1789.17 (2058.13-1555.60)	6870.86 (1068.15-4420.97)	2.814±0.134	0.98
		Ethanolic+ Triton x100	1824.04 (2104.04-1581.57)	6980.03 (10921.28-4464.78)	2.822±0.137	



Fig,(3): Susceptibility of *Culex pipiens* larvae to ethanolic extractions of selected plants



Fig,(4): Susceptibility of *Culex pipiens* larvae to ethanolic extractions of selected plants with Triton x100

3. Larvicidal Activity of Acetone Extracts Against *C. pipiens* Larvae:

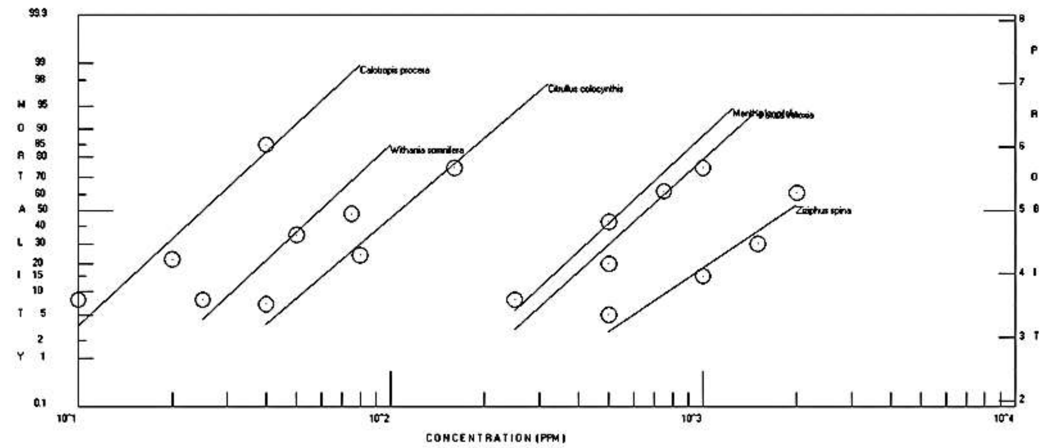
After treatment of mosquito larvae with ethanolic extracts, data were assorted in Table 4 and the regression lines are diagrammatically presented in Figures 5 & 6. LC_{50} values for acetone extracts of *C. procera*, *W. somnifera*, *C. colocynthis*, *M. longifolia*, *D. innoxia*, and *Z. spina-christi* were found 25.01, 59.30, 106.81, 553.56, 657.84, and 1881.85 ppm, respectively. These data showed the presence of differences in potency of the tested extracts.

Data of the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) with the same concentrations of acetone extract of all tested plants were distributed in Table (4). According to these data, there was a remarkable increase of LC_{50} and LC_{95} values, compared to those of each extract of *C. procera*, *W. somnifera, C. colocynthis*, *M. longifolia*, *D. innoxia,* and *Z. spina-christi* alone.

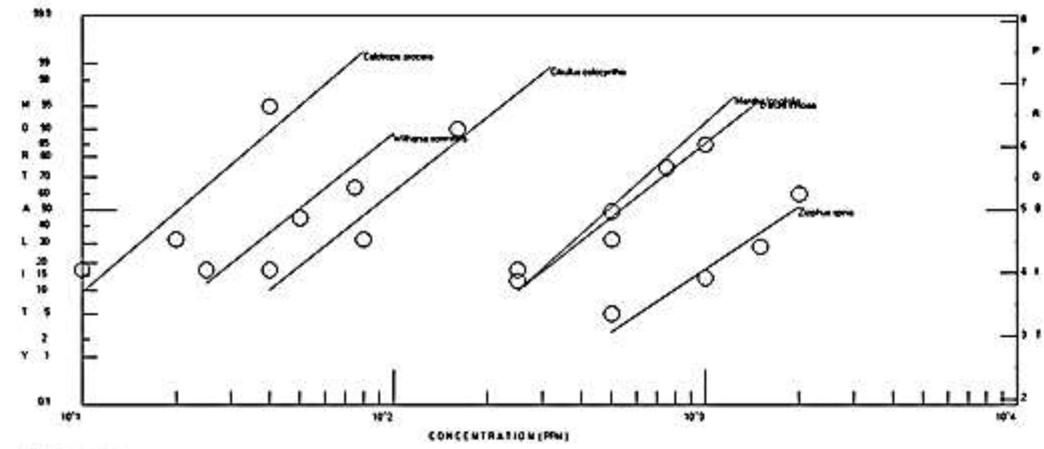
Results arranged in Tables 2, 3 &4 and graphically presented in Figures 1- 6 indicated the susceptibility of *C. pipiens* to aqueous, ethanolic, and acetone extracts of the tested plants. Besides the potency of these extracts was less than chemical insecticides, they are more safer and conversion of plant material extraction to natural beneficial insecticide.

Table 4 Larvicidal activity of acetonic extractions of some plants against *Culex pipiens* larvae

Plant species	Part of plant	Extraction	LC_{50} (Co. Limits)	LC_{95} (Co. Limits)	Slope function	S.R
Calotropis procera	**Leafs**	Acetonic extract	25.01 (27.28-22.94)	57.42 (67.28-49.06)	4.558 ±0.139	1.23
		Acetonic + Triton x100	20.30 (22.24-18.52)	49.76 (58.89-42.10)	4.224 ±0.126	
Withania somnifera	**Leafs**	Acetonic extract	59.30 (63.82-55.10)	135.73 (158.70-116.15)	4.574 ±0.168	1.20
		Acetonic + Triton x100	49.32 (53.71-45.29)	128.58 (152.26-108.66)	3.952 ±0.124	
Citrullus colocynthis	**seeds**	Acetonic extract	106.81 (116.93-97.57)	263.04 (311.09-222.56)	4.202 ±0.113	1.26
		Acetonic + Triton x100	84.25 (92.69-76.58)	221.59 (264.52-185.77)	3.916 ±0.105	
Mentha longifolia	**Leafs**	Acetonic extract	553.56 (598.08-512.35)	1269.98 (1472.88-1095.28)	4.561 ±0.146	1.13
		Acetonic + Triton x100	485.60 (526.73-447.67)	1143.86 (1325.56-987.30)	4.420 ±0.137	
Datura innoxia	**Leafs**	Acetonic extract	657.84 (715.45-605.85)	1539.34 (1781.15-1328.97)	4.458 ±0.121	1.23
		Acetonic + Triton x100	533.83 (586.17-486.15)	1416.50 (1669.69-1202.14)	3.881 ±9.507	
Ziziphus spina-christi	**Leafs**	Acetonic extracte	1881.85 (2138.23-1656.41)	5892.37 (8604.5-4037.34)	3.318 ±0.170	0.97
		Acetonic + Triton x100	1935.27 (2212.02-1693.37)	6121.41 (9080.52-4129.00)	3.289 ±0.182	



Fig,(5):Susceptibility of *Culex pipiens* larvae to acetonic extractions of selected plants



Fig,(6): Susceptibility of *Culex pipiens* larvae to acetonic extractions of selected plants with Triton x100

DISCUSSION

Economically, it is important to investigate the efficacy of plant extracts, as larvicidal agents, on the common house mosquito, *C. pipiens*.
The principal criterion in the selection of these compounds was conversion of waste materials to useful one and their production in large scale was easy and costs less (Bakr and Al-Ghramh, 2014).

Huge number of reported research works had focused on the botanical extracts of the indigenous plants of Egypt and their toxic effects on different insect species (e.g., Soliman and El-Sherif, 1995; Messeha, 1997; El-Kassas, 2001; Attiaa, 2002, Mohamed et al., 2003, Kamel et al. 2005, Bakr et al., 2006, Mansour et al., 2011,El.-Maghraby etal. 2012 ,Al-Zarog and El-Bassiouny, 2013; Elsiddig, 2015; Hosam et al., 2016; Abbas et al., 2017; Elhalawany and Dewidar, 2017; El-Rehawy, 2017 , Khater, 2017; Wahba et al., 2017; Fouda et al., 2017; Bakr and Abd El-Bar, 2017; Bakr et al., 2017, 2018). In agreement with some of those reported results, the present study revealed differences in LC_{50} and LC_{95} values of the extracts of the nine tested plants species. Also, remarkable variations in the potency of tested extracts were recorded and can be attributed to the major constituents in each plant. They were found, also, to possess parallel regression lines of nearly equal slope values. This might suggest that these extracts have the same mode of action against the tested *Cx. pipiens* larvae (Busvine, 1971). Therefore, the difference in potency of these extracts may be referred to the quantity of the extracted materials rather than the quality of such materials (Mansour et al., 1996 and Bakr et al., 2006).

In the current investigation, the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) to different concentrations of (aqueous, ethanolic, and acetone extracts) of the tested plants showed considerable increase in LC_{50} values. By estimation of SR, it was greater than one in all tested plants except of *Z. spina* which was lower than one. SR of acetone extract of *C. colocynthis* with Triton x100 (1.26) was higher than other one.

The larvicidal activity of all tested extracts on *Cx. pipiens* larvae was shown to increase greatly by adding Triton x100 which changes the surface tension of extract concentrations or dissolves the wax layer which covered the insects (Taylor and Schoof, 1967; Angus and Lutty,1971; Mkhize and Gupta,1985; Hussein, 1991; Husein et al., 2005; Kamel et al., 2005 and Mann and Koufman 2012).

Acknowledgments:

This research work was supported by the Research Sponsorship Program, University of Bisha, Kingdom of Saudi Arabia, grand Number (UB-02-1438). The authors gratefully acknowledge the contribution of laboratory technicians at Biology Department, Faculty of Science, University of Bisha, Bisha, KSA.

REFERENCES

Abbott, W.S. (1925): A method of computing effectiveness of an Insecticide. J. Econ. Entomol., 18: 265-267.

Abdullah M.A.R.and Merdan A.I. (1995): Distribution and ecology of the mosquito fauna in the South western Saudi Arabia. J. Egypt. Soc. Parasitol. 25(3) 815-837

Adebayo O. R , Efunwole O.O , Raimi M. M , Oyekanmi A. M and Onaolapo I.O (2015): Proximate, Mineral element, Antibacterial activity and Phytochemical screening of Bomubomu Leaves (Calotropis procera). International Journal of Contemporary Applied Sciences Vol.2 (9) pp 40-51.

Afaf A. Abbas; Abd-El wahab H; Neama A.Abd El-Hameid; Abd El- Gawwad, A. S. and Eman El-Said. M. Gomaa (2017): Susceptibility of Some Legume Plants to Some Leaf Miners. *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 10(1)pp: 35- 39.*

Al-Zarog, A. A. and El-Bassiouny, A. M. (2013) :Influence of some plant extracts on Varroa mite and performance of honey bee *Apis mellifera* colonies . *Egypt. Acad. J. Biolog. Sci., (F.Toxicology &Pest control) Vol.5 (2): 15 -20.*

Angus, T.A. and Lutty, P. (1971): In “microbial control of insects and mites”. (eds. M.D. Burges and N. W. Hussey) pp. 623-628 Academic Press, London & New York.

Anupam Ghosh, Nandita Chowdhury and Goutam Chandra(2012): Plant extracts as potential mosquito larvicides. Indian J Med Res 135(5): 581-598

Araya, Yoseph (2008). "Contribution of Trees for Oral Hygiene in East Africa". Ethnobotanical Leaflets. **11**: 38–44. Retrieved 2009-02-16.

Ashraf S. Elhalawany and Ahmed A. Dewidar 2017): Efficiency of Some Plant Essential Oils Against the Two-Spotted Spider Mite, *Tetranychus urticae* Koch and the Two Predatory Mites *Phytoseiulus persimilis* (A.-H.), and *Neoseiulus californicus* (McGregor). *Egypt. Acad. J. Biolog. Sci., (A. Entomology) 10(7): 135–147.*

Attiaa, S.R.A. (2002): New approaches to control filarial parasite and its vectors using selected natural extracts as safe control agents. Ph.D. Thesis Ent. Dept. Ain shams Univ. Cairo, Egypt.

Batwa, Mohammed; Jan Bergström; Sarah Batwa; Meshari F. Al-Otaibi (2006). "Significance of chewing sticks (miswak) in oral hygiene from a pharmacological view-point". Saudi Dental Journal. **18** (3): 125–133. Retrieved 2009-02-16.

Busvine, J.R. (1971): A critical review of the techniques for testing insecticides. The Commonwealth Intit. Entomol., London: 345 p.

Buttiker, W. (1979): Insects of Saudi Arabia. Diptera: Synanthropic flies.Fauna of Saudi ArabiaVolume 1, Pages 352-367

Buttiker, W. (1981): Observations on Urban Mosquitoes in Saudi Arabia. Fauna of Saudi ArabiaVolume 3, Pages 472-479.

Deepalakshmi S. and Jeyabalan D.(2017): Studies on Mosquitocidal and biological activity of endemic plants of Nilgiris Hills against filarial vector, *Culex quinquefasciatus* (Say) (Insecta: Diptera: Culicidae). International Journal of Advanced Research in Biological Sciences,Vol.4(3).

Diwakar Singh Dinesh, Seema Kumari, Vijay Kumar & Pradeep Das(2014): The potentiality of botanicals and their products as an alternative to chemical insecticides to sandflies (Diptera: Psychodidae): A review.Journal of vector borne diseases · Vol.51 pp1-7

EL-Bokl, M.M. and Moawad, H.M. (1997): Toxicity and joint action of some plant extracts against Cx. pipiens larvae. J. Union Arab Boil. 7 (A- Zoology), 449-461.

El-Kassas, N. B. (2001): Integrated effect of some mosquito larvicides and molluscicides as biological control agents against mosquitoes and snails. Ph.D. Thesis Ent. Dept. Ain shams Univ. Cairo, Egypt.

El-Maghraby, S.; Nawwar, G. A.; Bakr, R.F.A.; Helmy, N.A. and Kamel, O.M.H.M. (2012): Toxicological studies for some agricultural waste extract on mosquito larvae and experimental animals. Asian pacific J. of Trop. Biomed., 558 – 563.

Eman E. H. El-Rehawy (2017): Evaluation Studies of Some Extracted Substances From Date Palm Tissues on Its Attracting Potential of The Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Egypt. Acad. J. Biolog. Sci., (A. Entomology) 10(7): 217–220*.

Fathelrahman I. Elsiddig (2015) :Response of mosquito *(Anopheles arabiensis* patton) adult to leaves hexane extract of Rehan (*Ocimum basilicum* L.). *Egypt.Acad.J.Biolog.Sci. (F.Toxicology &Pest control) Vol.7(1)pp49-54*.

Finney, D.J. (1971): Probit Analysis (3rd ed.). Cambridge Univ. Press, 333 p. Cambridge.

Giovanni Benelli , Claire L. Jeffries and Thomas Walker (2016):Biological Control of Mosquito Vectors: Past, Present, and Future . Insects vol. 7 (52) 1-18

Gurudeeban, S.; Satyavani K.; Ramanathan T. (2010). "Bitter Apple (*Citrullus colocynthis*): An Overview of Chemical Composition and Biomedical Potentials". *Asian Journal of Plant Sciences*. **9** (7): 394–401. doi:10.3923/ajps.2010.394.401.

Harbach, RJE. (1988): The mosquitoes of the subgenerus Culex inSouthwestern Asia and Egypt (Diptera: Culicidae). Contributions of the American Entomological Institute, 24(1): 1-240.

Hawking F. (1973): The distribution of human filariasis throughout the world. WHO Geneva, WHO/FIL/73. 1/4 Mmeographed Document.

Hosam, M.K.H. El-Gepaly; Azza A. Mohamed; Aziza, M.M. Abou-Zaid and Seham A. Ezz El-Dein (2016) : Efficacy of Some Plant Extracts on the Biological Aspects of the Two Spotted Spider Mite *Tetranychus urticae* Koch (Acari: Prostigmata: Tetranychidae). *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 9(4) pp: 141- 152*.

Hussein, H.I.; Al-Rajby, D. and Al-Assiry, M. (2005): Toxicology of four pyrethroid-based insecticides and kerosene to a laboratory population of Cx.pipiens. Pakistan J. Biol. Sci., 8(5):751-753.

Hussein, M.A. (1991): Synergistic and histochemical effects of surfactants on some insecticidal activity against resistant mosquito larvae, Cx pipiens. Fourth Arab Congress of plant protection: 176-182.

Huxley A (1992). ed. New RHS Dictionary of Gardening. Macmillan ISBN 0-333-47494-5

Kamel, O.M.; Hassan, M.M.; Abd El-Baky, S.M.M.; Hafez J.A.; and Hamed, M.S. (2005): Synergistic and joint actions of some plant extracts on their larvicidal activity against the mosquito Ochlerotatus caspius (Diptera: Culicidae). J. Egypt. Acad. Soc. Environ Develop. (A. Entomology), 6 (2): 277-289.

Khater K.S. (2017):Efficacy of Some Plant Extracts on *Lucilia Sericata* (Meigen) (Diptera: Calliphoridae). *Egypt. Acad. J. Biolog. Sci. (F. Toxicology & Pest control) Vol.9(1)pp.1-7***.**

Lerdthusnee, K.; Romoser, W.S.; Faran, M.E. and Dohm, D.J. (1995): Rift Valley Fever virus in the cardia of Culex pipiens an immunocytochemical and Ultrastructural study. Am. J. Trop. Hyg., 53 (4): 331-7.

Mann, R.S. and Kaufman, P.E. (2012): Natural product pesticides: Their development, delivery and use against insect vectors. Mini-Reviews in Organic Chemi., 9(2):185-202.

Mansour, S.A.; Bakr, R.F.A.; Mohamed, R.I. and Hasaneen, N.M. (2011): Larvicidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the Housefly, Musca domestica L. The Open Toxicology Journal, 4: 1-13.

Mansour, S.A.; Messeha, S.S. and Hamed, M.S. (1996): Botanical biocides. 1. Toxicity of some plant extracts to mosquito larvae and mosquito fish in laboratory. Proc. 3rd Cong. Toxicol. Dev. Count. Cairo, Egypt. Vol.III,: 369-380.

Massoud, A.M. and Labib I. M. (2000): Larvicidal activity of *Commiphora molmol* against *Culex pipiens* and *Aedes caspius* larvae. J. Egypt. Soc. Parasitol., 30 (1): 101- 115.

Messeha, S. S. (1997): Biocidal activity of selected extracts against *Culex pipiens* (Linn.). Ph.D. Thesis Ent. Dept. Ain shams Univ. Cairo, Egypt.

Mkhize,J.N. and Gupta, A.P. (1985): The importance of formulating insect growth regulators with surfactants and their blends for the control of the rice weevil, Sitophilus oryzae L. (Coleoptera: Curculionidae). Insect Sci. Applic., 6(2): 183-186.

Mohamed, M.I. and Hafez, S. E. (2000): Biological and biochemical effects of the non volatile plant oil (Jojoba) against *Culex pipiens* (Diptera: Culicidae). J. Egypt. Ger. Soc. Zool., 13 (A): 65-78.

Mohamed, M.I.; El-Mohamady, R. H. and Mohamed, H. A. (2003): Larvicidal activity and biochemical effects of certain plant oil extracts against *Culex pipiens* larvae (Diptera: Culicidae). J. Egypt. Acad. Soc. Environ. Develop. (A. Ent.) 3 (1): 75-93.

Mohammad A. Fouda, Mostafa I. Hassan, Ahmed Z. Shehata, Ahmed I. Hasaballah and Mohammed E. Gad (2017):Larvicidal and Antifeedant Activities of Different Extracts from Leaves and Stems of *Lantana camara* (Verbenaceae) Against the Housefly, *Musca domestica* L. *Egypt. Acad. J. Biolog. Sci. (F. Toxicology & Pest control) Vol.9(1)pp.85- 98*

Mona N. Wahba, Badran A. B, Mona. I. Ammar and Hammam M. A. Nasser(2017)Control of Some Piercing Sucking Pests Infesting Cucumber by Many Botanical Oil and Synthetic Insecticides and Economically Feasibility to Control**.***Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 10(8)pp: 9- 15***.**

Morris k,K.,John,R.K. John,E.K and SaenRenauld,J.A(1964).Calotropin a cytotoxic principle isolated from Asciepias curassavica L. Science 25 December

Morton JF (1987). "Pomegranate, Punica granatum L". Fruits of Warm Climates. Purdue New Crops Profile. pp. 352–5. Archived from the original on 2012-06-21. Retrieved 2012-06-14.

Preissel, Ulrike; Preissel, Hans-Georg (2002). Brugmansia and Datura: Angel's Trumpets and Thorn Apples. Buffalo, New York: Firefly Books. pp. 117–119. ISBN 1-55209-598-3.

Reda F. A. Bakr; Hussein, M. A.; Hamouda, L. S.; Hassan, H. A. and Elsokary, Z. F. (2008): Effect of some insecticidal agents on some biological aspects and protein patterns of desert locust Schistocerca gregaria (Forskal). Egypt. Acad. Soc. Environ. Develop., 9 (2): 29-42.

Reda F. A. Bakr, , Mamdouh I. Nassar, , Nehad M. El-Barky, Mohammed S. Abdeldayem and Thorayia F. Kotb (2018) :Effect of some natural products on the vector of *Bancroftian filariasis* in Jizan, KSA˙ *Egypt. Acad. J. Biolog. Sci. (F. Toxicology & Pest control) Vol.10(1)pp.1- 11***.**

Reda F. A. Bakr, Abd Elwahab A. Ibrahim, Olfat M. El-Monairy, Yasser A. El-Sayed and Maysa Hegazy (2017):Toxicity of Taro Plant Leaves, *Colocacia esculanta*, Against the German Cockroach, *Blattella germanica . Egypt. Acad. J. Biolog. Sci. (F. Toxicology & Pest control) Vol.9(2)pp.1- 5.*

Reda F.A. Bakr; ElBermawy, S.M.; Geneidy, N.A.M.; Emara, S. A. and Hassan, H.W. (2006): Occurrence of the biological effects of some plant extracts on the cotton leaf worm *Spodoptera littoralis* (Biosd) and their physiological. Egypt. Acad. Soc. Environ. Develop., 7 (1): 109-147.

Reda F.A.bakr and Marah M. Abd El-Bar (2017): Effect of Lufenuron and *Oriza sativa* Bran Extract on Fraction Protein and Acid Phosphatase Pattern in Haemolymph of *Schistocerca gregaria.Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 10(5)pp: 21- 33.*

Reda, F. A. Bakr and Hamed A. Al-Ghrama (2014) Toxicological studies on the effect of some agricultural and plant extract as insecticidal agent on the mosquito, Culex pipiens . Egypt. Acad. J Biolog. Sci., (A EntomologyVol.7(1):11-21.

Schorkopf DLP, Spanoudis CG, Mboera LEG, Mafra-Neto A, Ignell R, Dekker T (2016): Combining Attractants and Larvicides in Biodegradable Matrices for Sustainable Mosquito Vector Control. PLoS Negl Trop Dis 10(10): e0005043. doi:10.1371/journal.pntd.0005043

Shoukry, F.I.I. and Hussein, K.T. (1998): Toxicity and biochemical effects of two plant volatile oils on the larvae of the greater wax moth *Galleria mellonella* L. (Pyralidae: Lepidoptera). J. Egypt. Ger. Soc. Zool., 27 (E): 99-116.

Soliman, B.A. and El-Sherif, L.S. (1995): Larvicidal effect of some plant oils on mosquito *Cx. pipiens* L. (Diptera: Culicidae). J. Egypt Ger. Soc. Zool., 16 (E):

161-169.
Stearn, W. T. (1995). *Botanical Latin: History, Grammar, Syntax, Terminology and Vocabulary* (4th ed.). Timber Press. ISBN 0-88192-321-4.
Stover, E.; Mercure, E. W. (2007). "The Pomegranate: A New Look at the Fruit of Paradise". HortScience. 42 (5): 1088–1092.
Subbiah POOPATHI and Brij Kishore TYAGI (2006):The Challenge of Mosquito Control Strategies: from Primordial to Molecular Approaches. Biotechnology and Molecular Biology Review Vol. 1 (2), pp. 51-65,
Taylor, R. T. and Schoof, H. F.(1967): The larvicidal activity of several liquid detergents and quaternary ammonium compounds. Mosquito News, 27:486-487.
Thangam, T. S. and Kathiresan, K. (1990): Synergistic effects of insecticides with plant extracts on mosquito larvae. Trop. Biomed., 6(2):135-137.
Thangam, T. S. and Kathiresan, K. (1997): Mosquito larvicidal activity of mangrove plant extracts and synergistic activity of Rhizophora apiculata with pyrethrum against Cx. quinquefasciatus. Inter. J. pharmacognosy, 69-71.
The Plant List ,(2016) A Working List of All Plant Species . http://www.theplantlist.org/tpl1.1/record/tro-27501041.
Wikipedia:Citation needed -Wikipedia, (2018) the free encyclopedia https://en.wikipedia.org/wiki/Wikipedia:Citation_needed.
Wills, W. M; Jakob. W. L.; Farancy. D.B.; Oerthey, R.E.; Anaml, E.; Callsher, C. H. and Monath. T. P. (1985): Sindbis virus isolations from Saudi Arabian mosquitoes. Trans. Roy. Soc. Trop Med Hyg., 79: 63-66.
World Health Orgenization (WHO) (1975): Manual on pracical entomology in Malaria Part II, Methods
World Health Orgenization (WHO) (1997): Tropical diseases: Progress in research, 1995–1996. thirteenthprogramme report. UNDP/World Bank/who special programme for research and training in tropical diseases (TDR). Geneva. Swizerland.
Wright, J.W. (1971): The WHO programme for the evaluation and testing new insecticides. Bull. Wld. Hlth. Org., 44: 11-12.
Zahar, A.K. (1973): Review of ecology of malaria vectors in the WHO Eastern Mediterranean Region, WHO. Geneva. WHO/MAL/73-808. WHO/VBO 73.453 (Mimeograph).
Zahran M.A. & Willis A.J., *(1992) :The Vegetation of Egypt*, Hong Kong , pp. 187-188 ISBN 978-0-412-31510-7.