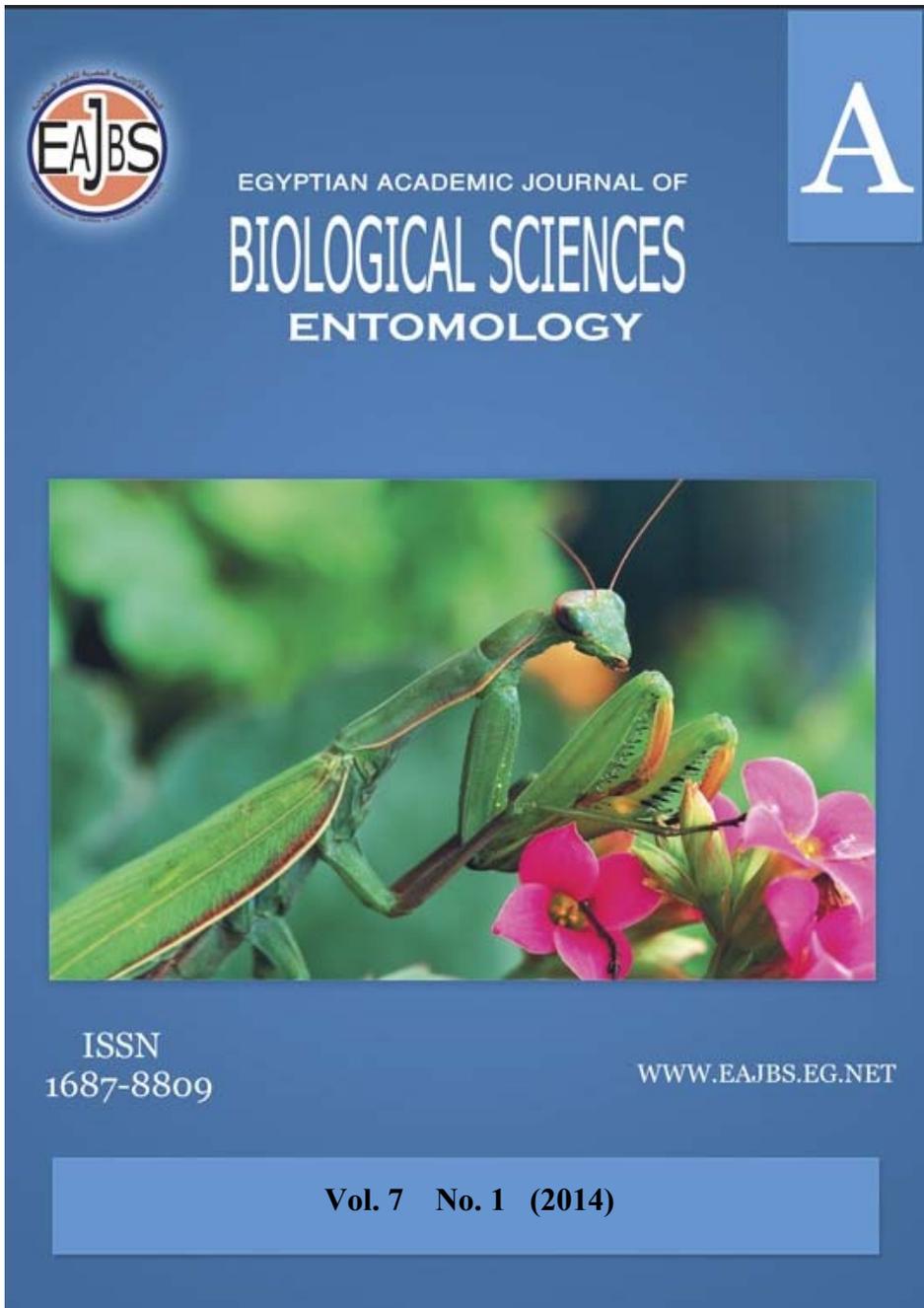


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Toxicological Studies on the Effect of Some Agricultural Waste and Plant Extract as Insecticidal Agent on the Mosquito, *Culex pipiens*

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Abstract

A mosquito larval survey was carried out in the southwestern region of Saudi Arabia. Twelve mosquito species were identified: 4 Anopheline species; *Anopheles arabinesis* Theobald, *Anopheles tenebrosus* Donitz, *Anopheles multicolor* Comboulieu, and *Anopheles sergenti* Theobald and 6 Culicine species; *Culex pipiens* Linnaeus; *Culex tritaeniorhynchus*; *Culex lutzia*; *Culex sinaiticus*; *Culex quinquefasciatus* Say; *Culex theileri* Theobald as well as one Aedine species, *Aedes caspius*, and *Culiseta subochrea*. *Culex pipiens* is the most common culicine species in the southwestern region.

The insecticidal activity of tested compounds (acetic and ethanolic extracts of agricultural waste product, rice bran and plant, extract of Milkweed) was bioassayed against the 3rd instars of the *Culex pipiens* larvae in the laboratory. Therefore, the toxicity of the tested plant extracts based on LC₅₀ values could be arranged in an ascending order as follows: ethanolic extract of milkweed leaves < acetic extract of milkweed leaves < acetic extract of milkweed flower < acetic extract of rice bran < ethanolic extract of rice bran < ethanolic extract of milkweed flower.

The SR of ethanolic extract of Rice bran with Triton x100 (1.64) was higher than other one.

Keywords: Toxicological studies, agricultural wastes, plant extract, mosquito

1. Introduction

Mosquitoes (Diptera: Culicidae) are among the most serious insect pests of medical importance. They are vectors of various disease agents some of which cause millions of cases of illnesses and deaths in humans and animals each year. Among these diseases malaria, yellow fever, dengue, filariasis, and Rift Valley fever are endemic and epidemic areas in many countries (WHO, 1991 and Lerdthusnee, *et al.* 1955). Saudi Arabia covers the major part of the vast Arabian peninsula. In this respect, southwestern region of Saudi Arabia with its unique topographic and climatic, was a nucleus for the first protected area for a large number of animal and plant species. Most of the observations made on urban and sub-urban as well as rural areas of the southwestern region regarding mosquito fauna are very limited except for those

reported by Buttiker (1979) and (1985), Za har (1973), Hawking (1973), Wills *et al.* (1985), Harbach (1988), and Abdullah and Merdan (1995).

Mosquitoes are still the world's number one vectors of human and animal diseases; and are conspicuous nuisance pests as well, even after massive efforts of eradication or control. The extensive use of chemical pesticides or insecticides resulted in inducing resistance by insect pests besides, residue contamination of human food, mammalian toxicity, and environmental pollution. These factors have created the need for environmental safe, degradable, and target specific agents for pest control purposes. Plant extracts have gained importance in insect control, being considered environmentally safe, less hazardous to non-target biota, simple, inexpensive, and can be applied effectively by using techniques more suitable for developing countries (Soliman & El-Sherif, 1995; El-Bokl & Moawed, 1997; Shoukry & Hussein, 1998, Massoud & Labib, 2000; Mohammed & Hafez, 2000 and Mohammed, *et al.*, 2003). Among mosquito species, *Culex pipiens* is the most common and widely distributed species in southwestern, Saudi Arabia. Only Bakr, *et al* (2006 & 2008) studied the effect of agricultural waste extracts (rice bran of *Oriza sativa*) on the newly moulted 5th nymphal instar of *Schistocerca gregaria*.

The present study hopefully aims that the results obtained from this survey would be of value to update the knowledge about the prevalence and distribution of mosquitoes in the southwestern region.

Therefore, the present study was undertaken to evaluate some agricultural waste extracts as insecticidal agents. Also, synergistic effects of Triton x100 on the susceptibility of *Cx. Papiens* larvae to agricultural waste and plant extracts were investigated to improve the properties of these extracts.

2. Materials and Methods

2.1. The Study Area

Southwestern region is located between latitude 17° 30'--21° 00'N and Longitude 41° 30'--44° 30'E. It is entirely different from the rest of the kingdom. The series of Al-Sarawat mountains, with its highest peak (Al-Sawdah mountain, 2800 m, near Abha 2100 m), the capital of Asir Province, divides the area into two distinct ecological regions. They are the high plateaus that slope gently eastward from the escarpment to form the highlands of Asir, and to the west the highly dissected mountains terrain to Tihamat Asir that merge westward into narrow, sandy Red Sea coastal plain, make it the hottest part of the region. The Asir highland receive a variable seasonal rainfall which is higher than the rest of the kingdom, i.e., 300-500 mm/year as compared to 50-100 mm/year elsewhere.

According to the characteristics of the study area and its biotic and abiotic factors, southwestern region with its highlands and the Tihama slopes as well as the semi-tropical Red Sea coastal area could be considered a unique study area for mosquito ecology and distribution, and water resources are variable and may include all types of water breeding sites for mosquitoes.

2.2. Mosquito Larval Collection

Monthly larval collections (October 2012--March 2013) were made from different breeding places from the highland area (Abha-Khamis Mushayt, Al-Sawdah mountain, and Al-Namas), and from moderately elevated areas (Tihama slopes and Mahayil), as well as sea level areas (Al-Shuqayq and Jizan). The selected breeding places were variable and ranged between permanent ones and occasional water collections, which include (wells used for irrigation, seepage, irrigation canals,

cesspits, surface water collections, drainage water, underground water, and stagnant water). Larvae were collected by sweeping the water surface with long handled larval net (WHO, 1975). In small water collections, another larval net was used with iron ring of 10 cm diameter. During collection, the aquatic stages were washed into the nylon sieve which were then inverted and washed out in a white enamel bowl containing clear distilled non-chlorinated water. All immature stages were collected with a pipette into a plastic bag. Each plastic bag was tightly closed and assigned a code number represented date of sampling and breeding place. 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 till adult emerging. Young larval instars were transferred to breeding enamel bowls, fed tropical fish food (Tetramin) maintained at 27±1°C and observed daily till they reached the 4th instar. Mature larvae were placed in petri-dishes, killed with hot water, and preserved in labeled specimen tubes containing 80% ethyl alcohol for mounting and identification.

2.2.1. Tested Compounds

(1) Milkweed Extract (*Calotropis procera*)

The tested plant parts (flower and leaves) were washed to avoid dusts and dirt then left to dry under shade in the laboratory. Dried parts of plant (milkweed) were cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each part were exhaustively extracted with absolute ethanol and acetone by Soxhlet apparatus.

(2) Rice Bran Extract (*Oriza sativa*)

Rice bran was exhaustively extracted with two solvents; ethanol and acetone. The extractions were accomplished by a Soxhlet apparatus. The solvent extracts were evaporated and dried under vacuum using a rotary evaporator of water bath adjusted at 40-60°C. The resulted dry crude extracts were weighted and the overall yield from 100 gm of each waste plant by each solvent was calculated before storage at 4°C in screw capped vials, until use.

2.2.2. Tested Mosquitoes

Culex pipiens (Culicidae: Diptera)

Provided by collecting from Mahayil area and transferred to the research laboratory of Biology Department, Faculty of Science, King Khalid University, where self-perpetuating colonies were established and maintained during the present study. Late third larval instars were used for toxicological studies.

2.3. Toxicological Studies

A series of toxicological bioassays were carried out to determine the insecticidal activity of the waste products and agricultural plant on the *Cx pipiens* larvae, the most common mosquito in Asir region.

2.3.1. Efficiency Agricultural Waste and Plant Extract

Preliminary, toxicological bioassay tests were carried out to the selected extracts on tested insects as a modification for the method described by (Wright, 1971). The LC₅₀ and LC₉₅ values were determined as well as their slope function, according to (Finney, 1971).

2.3.2. Synergistic Action of Triton x100

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

SR = LC₅₀ of extract alone/LC₅₀ of the mixture;
 value ≥ 1 indicating synergism;
 ≤ 1 indicating antagonism.

3. Results

During the present survey, twelve mosquito species were recorded and identified according to larval key proposed by Gad *et al.* (1964) and Harbach (1985, 1988). The encountered species belonged to 4 genera, namely; Anopheles (4 species), Culex (6 species); Aedes (One species), and Culiseta (one species).

- (1) **Anopheline Species.** Four anopheline species were collected from selected areas, namely; *Anopheles arabinesis* Theobald, *Anopheles tenebrosus* Donitz, *Anopheles multicolor* Combouliu, and *Anopheles sergenti* Theobald. The distribution of these 4 species is shown in Table 1.
- (2) **Culicine Species.** Six culicine species were encountered in the surveyed area, namely; *Culex pipiens* Linnaeus; *Culex tritaeniorhynchus*; *Culex lutzia*; *Culex sinaiticus*; *Culex quinquefascitus* Say; and *Culex theileri* Theobad. The distribution of these six species is shown in Table 1. From Table 1, it becomes clear that *Culex pipiens* is the most common culicine species in the southwestern region. It was recorded in almost all type of breeding places as well as at all altitudes.

Table 1: Distribution of the mosquito larvae in the Asir region, kSA

Species	Region	Abha	Al-Sawdah	Khanis Mus hayt	Tihanat Asir	Al-Shageq	Mahayil	Regal-Almahg	Bilasmer	Tanumah	Al-Namas	Bishah	Najran
<i>Anopheles arabinesis</i>		×	×	xx			xx		×			xx	
<i>Anopheles tenebrosus</i>		×		×	×	×	×						×
<i>Anopheles multicolor</i>							×	×				×	
<i>Anopheles sergenti</i>		×			xx	×							
<i>Culex pipiens</i>		xx	×	×	xx	xx	xxx	×	×	xx	xx	xx	xxx
<i>Culex tritaeniorhynchus</i>					×	×							×
<i>Culex lutzia</i>												×	×
<i>Culex sinaiticus</i>					×						×		×
<i>Culex quinquefascitus</i>						×						xx	
<i>Culex theileri</i>				×					×		xx		×
<i>Aedes caspius</i>		×					×						
<i>Culiseta subochrea</i>		×		×			xx		×	×	×	×	xxx

- × Low density
- xx Moderate density
- xxx High density

- (3) **Aedine Species.** *Aedes caspius* Pallas was the only recorded aedine species in the surveyed areas. This species could persist and breed at all altitudes but it was found at high densities, particularly, at wet season in low land breeding place.
- (4) **Culiseta Species.** *Culiseta subochrea* Edward was the only recorded culiseta species during the survey period. However, this species is the most dominant mosquito species in the southwestern region as compared to all other recorded species. Association of larvae of different mosquito species in the southwestern region is shown in Table 2.

Table 2: Association of larvae of different mosquito species in the South Western region of Saudi Arabia

Species	Region	An. arabinesis	An. nebrozus	An. multicolor	An. sergenti	Cx. pipiens	Cx. tritaeniorhynchus	Cx. ducta	Cx. sinaiticus	Cx. quinquefasciatus	Cx. theileri	Ae. caspius	Culiseta subochrea
<i>Anopheles arabinesis</i>		-	x			x							x
<i>Anopheles nebrozus</i>		x	-			x						x	x
<i>Anopheles multicolor</i>				-				x	x	x		x	x
<i>Anopheles sergenti</i>					-	x	x			x			x
<i>Culex pipiens</i>		x	x	x	x	-	x			x	x	x	x
<i>Culex tritaeniorhynchus</i>					x	x	-	x	x				
<i>Culex ducta</i>				x		x	x	-		x			x
<i>Culex sinaiticus</i>				x			x		-		x		
<i>Culex quinquefasciatus</i>					x	x				-		x	
<i>Culex theileri</i>		x	x			x			x			x	
<i>Aedes caspius</i>				x		x				x	x		x
<i>Culiseta subochrea</i>		x	x	x	x	x						x	-

3.1. Toxicological Studies

3.1.1. Evaluation of the Larvicidal Activity of Agricultural Waste and Plant Extracts

The insecticidal activity of tested compounds (acetic and ethanolic extracts of agricultural waste product, rice bran and plant, extract of milkweed) was bioassay against the 3rd instars of the *Culex pipiens* larvae in the laboratory. The results are presented in Tables (3&4), and the regression lines illustrated in Figures (1,2&3).

Table 3: Larvicidal activity of waste product extractions (rice bran) against *Culex pipiens* larvae.

Extraction	LC ₅₀ (Co. Limits)	LC ₉₅ (Co. Limits)	Slope function	S.R
Acetic Extract of rice bran	32.90 (36.81-29.40)	102.40 (123.93-94.74)	3.336	1.234
Acetic Extract of rice bran + Triton x100	26.65 (29.90 – 23.75)	74.83 (89.69 – 62.52)	3.669	
Ethanolic Extract of rice bran	34.59 (39.13 – 30.56)	127.37 (159.29 – 101.99)	2.90	1.64
Ethanolic Extract of rice bran + Triton x100	20.99 (24.07 – 18.29)	57.96 (69.95 – 47.92)	3.735	

Table 4: Larvicidal activity of plant extractions (apple of sodom) against *Culex pipiens* larvae.

Part of plant	Extraction	LC ₅₀ (Co. Limits)	LC ₉₅ (Co. Limits)	Slope function	S.R
Flower	Acetic Extract of apple of Sodom	33.29 (36.60-30.27)	94.99 (114.51-78.90)	3.612	1.231
	Acetic Extract of apple of sodom + Triton x100	27.03 (30.13 – 24.25)	91.09 (112.78 – 73.75)	3.117	
	Ethanolic Extract of apple of Sodom	65.71 (71.09-60.74)	138.59 (157.95-121.65)	5.07	1.49
	Ethanolic Extract of apple of sodom+ Triton x100	43.97 (40.04-39.41)	136.27 (164.81-112.82)	3.34	
Leaves	Acetic Extract of apple of Sodom	29.63 (33.01 – 27.47)	62.13 (70.90 – 54.48)	5.11	1.19
	Acetic Extract of apple of sodom + Triton x100	24.73 (26.83 – 22.79)	54.63 (62.67 – 47.67)	4.77	
	Ethanolic Extract of apple of Sodom	23.43 (25.72 – 21.34)	59.33 (71.34 – 49.42)	4.07	1.27
	Ethanolic Extract of apple of sodom+ Triton x100	18.42 (20.38 – 16.65)	53.97 (65.92 – 44.30)	3.52	

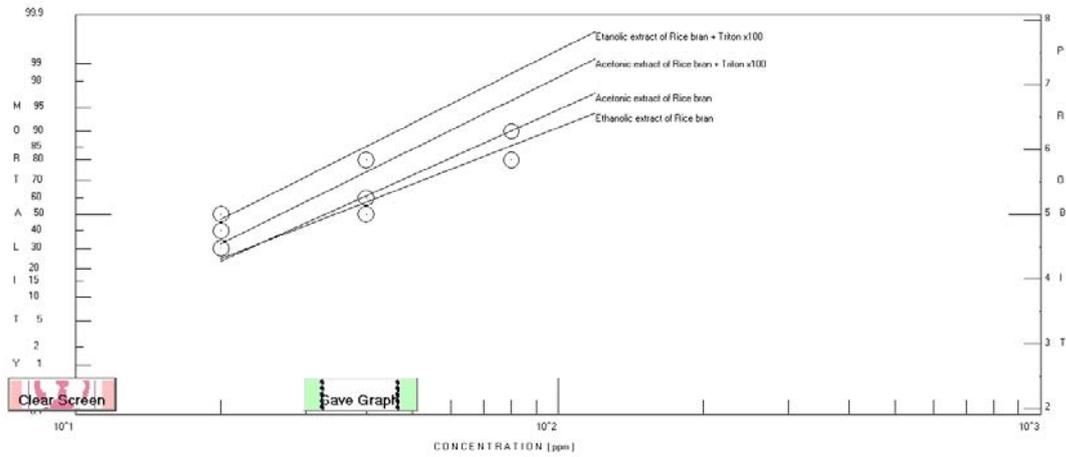


Figure 1: Susceptibility of *Culex pipiens* larvae to rice bran extracts.

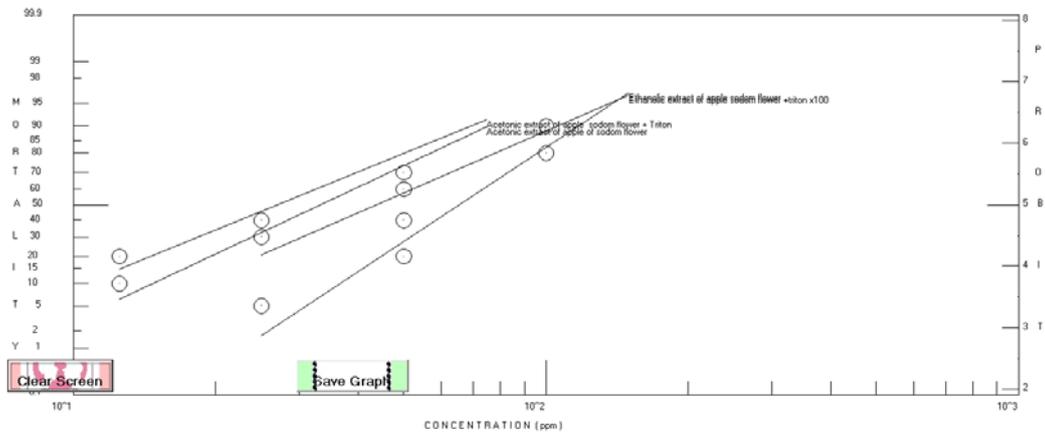


Figure 2: Susceptibility of *Culex pipiens* larvae to flower extracts of the milkweed.

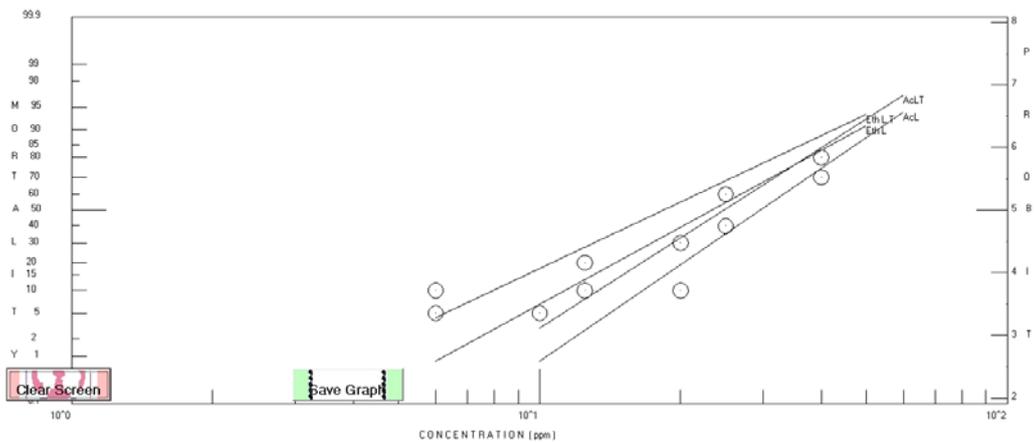


Figure 3: Susceptibility of *Culex pipiens* larvae to leaves extracts of the milkweed.

The confidential limits of each of the tested compound were statistically calculated for LC_{50} and LC_{95} at $P = 0.05$. The tested compounds showed different toxicity. The LC_{50} values of acetonic and ethanolic extract of rice bran are 32.90 and 34.59 ppm, while for acetonic and ethanolic extract of flower and leaves of milkweed are (33.24 and 65.71 ppm) and (29.63 and 23.43 ppm), respectively.

Therefore, the toxicity of the tested plant extracts based on LC₅₀ values which could be arranged in an ascending order as follows: ethanolic extract of milkweed leaves < acetic extract of milkweed leaves < acetic extract of milkweed flower < acetic extract of rice bran < ethanolic extract of rice bran < ethanolic extract of milkweed flower.

3.1.2. Larvicidal Activity of Agricultural Waste against *Cx. pipiens* Larvae

The results are represented in Table 3 and the regression lines are presented in Figure 1. The LC₅₀ values for acetic and ethanolic extract of rice bran are 32.90 and 34.59 ppm, respectively. The present data showed the presence of slight differences in the potency of the two extracts. They were found to possess parallel regression lines. This may suggest that these extracts have the same mode of action against the tested insect larvae (Busvine, 1971).

These tests were carried out to compare the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) with the same concentrations of acetic and ethanolic extracts of rice bran. Data presented in Table 3 detected a considerable increase in LC₅₀ and LC₉₅ values in comparison to those of each extract of rice bran alone (Table 3). By calculating the synergistic ratio (SR), it is found that the value of SR is greater than one in all the tests. The SR of ethanolic extract of rice bran with Triton x100 (1.64) was higher than acetic one (1.23).

3.1.3 Larvicidal Activity of Plant Extracts against *Cx. pipiens* Larvae

The results are represented in Table 4 and the regression lines are presented in Figures 2&3. The LC₅₀ values for acetic and ethanolic extract of flower and leaves of milkweed are (33.24 and 65.71 ppm) and (29.63 and 23.43 ppm), respectively. The ethanolic extract is more efficiency than acetic one.

Also, these tests were carried out to compare the synergistic action resulting from adding 1 mL of Triton x100 (0.01%) with the same concentrations of acetic and ethanolic extracts of milkweed parts (flower and leaves).

Data presented in Table 4 detected a considerable increase in LC₅₀ and LC₉₅ values in comparison to those of each extract of milkweed alone. By calculating the synergistic ratio (SR), it is found that, the value of SR is greater than one in all the tests. The SR of ethanolic extract of flower part of Milkweed with Triton x100 (1.49) was higher than the three other extracts. The SR of ethanolic extract of flower part of milkweed (1.49) slightly lower than those of ethanolic extract of rice bran (1.64).

The results showed in Tables 3&4 and Figures 1,2&3 represent the susceptibility of *Culex pipiens* to acetic and ethanolic extract of agricultural waste and plant parts, although the potency of extracts is less than chemical insecticides but they are more safe (Mann and Koufman, 2012) and conversion of waste material to natural beneficial insecticide (El-Maghraby, *et al.*, 2012).

4. Discussion

4.1. Distribution of the Mosquito Fauna in Southwestern of Saudi Arabia

The mosquitoes of Saudi Arabia were studied by several authors during the period of 1955-1995 (Mattingly and Knight (1956); Zahar (1973); Buttiker (1981); and Abdullah and Merdan (1995).

The results of the present study revealed the presence of 12 mosquito species, four Anopheline; six Culicine, one Aedes, and one Culiseta. From the results of distribution, it could be assumed that *Anopheles arabiensis* is the most dominant Anopheles species. Its wide distribution and dominance among Anopheline species may reflect its tolerance to different temperatures as well as variable altitude.

An. Sergenti and *An. Tenebrosus* were recorded before in Saudi Arabia (Wills *et al.* 1985, Buttiker, 1981). *An. multicolor* was observed and identified by Abdullah and Merdan, 1995.

Sis culicine species were also encountered during the present study. *Cx. pipiens* was the most dominant one. This dominance may be due to its wide range suitability of breeding sites, variable extremes of temperature, and different altitude. Culicine habitats during the present study gave a generalization of breeding sites that were shaded, vegetated, and had stagnant deep or shallow water, which are preferable for larval breeding.

As regards to the aedine mosquitoes, only one species was identified in the surveyed area, *Ae. caspus*. This species was previously recorded by Abdullah and Merdan, 1995.

The last mosquito species *Culiseta subochrea* was found in high density almost during all months and in a wide variety of breeding sites and altitudes.

4.2 Evaluation of the Larvicidal Activity of Some Agriculture Waste and Plant Extracts

It was thought appreciable to evaluate some agriculture waste products extract and plant extract from natural origin as larvaicide agents on mosquito, *Culex 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.

Most of the previous studies carried out mainly on the botanical extracts of indigenous plants of Egypt and their toxic effects on different insect species (Soliman and El-Sherif, 1995; Messeha, 1997; El-Kassas, 2001; Attiaa, 2002, Mohamed *et al.*, 2003, Kamel *et al.* 2005, and Bakr *et al.*, 2006). While in the present study, the use of waste product to convert the useless material to benefit one was the main aim.

The tested extracts revealed differences in LC_{50} and LC_{95} values and the slope functions of the regression lines. Also, remarkable variations in the potency of tested extracts were observed and often attributed to the major constituents of each one.

The present data showed that the differences in potency of acetic and ethanolic extracts of rice bran and flower and leaves of milkweed plant. They were found to possess parallel regression lines of nearly equal slope values. This may suggest that these extracts have the same mode of action against the tested insect 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).

The synergistic action resulting from adding 1 mL of Triton x100 (0.01%) to different concentrations of acetic and ethanolic extracts of rice bran and milkweed showed considerable decrease in LC_{50} values. The larvicidal activity of all extracts 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 Luty, 1971; Mkhize and Gupta, 1985; Hussein, 1991; Husein *et al.*, 2005, and Kamel *et al.* 2005).

Acknowledgments

This research was supported by the King Khalid University, Project No. KKU-SCI-163-33. The author gratefully acknowledges the contribution of Laboratory Technicians at Biology Department, Faculty of Science, King Khalid University, Abha, KSA.

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ARABIC SUMMARY

دراسات سمية على تأثير بعض المخلفات الزراعية والمستخلصات النباتية كمبيد حشري على بعوض كيولكس بيبينز

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تم اجراء مسح ليرقات البعوض في المنطقة الجنوبية الغربية من المملكة العربية السعودية. وقد تم تحديد اثني عشر نوعا من البعوض: اربع انواع من *Anopheline* وهم *Anopheles arabinesis* Theobald، *Anopheles sergenti*. وست انواع من *Culicine* وهم *Culex pipiens* Linnaeus، *Culex sinaiticus*، *Culex lutzia*، *Culex theileri* Theobad، *Culex quinquefascitus* Say، *Culiseta subochrea*، وكذلك نوع واحد من *Aedine* وهو *Aedes caspius* و ايضا نوع واحد من *Culiseta* وهذا النوع هو *Culiseta subochrea*. وكان بعوض كيولكس بيبينز هو الاكثر شيوعا في المنطقة، و تم عمل اختبارات حيوية لنشاط السمية لكل من المستخلصات الكحولية والاسيتونية لكل من مخلفات قش الارز وزهور واوراق نبات العشار على يرقات بعوض كيولكس بيبينز. وقد تم قياس السمية على اساس الجرعة القاتلة للنصف. وقد امكن ترتيبها تصاعديا كما يلي المستخلص الكحولي لاوراق العشار يليه المستخلص الاسيتوني لاوراق العشار ثم المستخلص الاسيتوني لزهور العشار ثم تلاه المستخلص الاسيتوني لقش الارز ثم المستخلص الكحولي لقش الارز واخيرا المستخلص الكحولي لزهور نبات العشار. وعند استخدام الترايتون لتحسين كفاءة المستخلصات كان اعلى نسبة تأزر بين المستخلص الكحولي لقش الارز مع الترايتون والذي بلغ قيمته الى 1.64