ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 02, Issue 12, pp.1105-1109, December, 2015

RESEARCH ARTICLE

EVALUATION OF SOME MEDICINAL PLANT EXTRACTS AGAINST MOSQUITO CULEXPIPIENSLARVAE AND SNAIL BIOMPHALARIAALEXANDRINA

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ARTICLE INFO

Article History:
Received 19th, September 2015
Received in revised form
05th, October 2015
Accepted 11th, November 2015
Published online 30th, December 2015

Keywords:

Evalution,
mosquitoes,
Diseases.

ABSTRACT

Mosquitoes transmit serious human diseases, causing millions of deaths every year.Snails' species are associated with transmission parasitic disease as intermediate host. The environmental problems caused by overuse of high toxicity, non-biodegradable materials of pesticides and the residue in soil, water resources and crops that affect public health. Thus, on the one hand, one needs to search the new highly selective, biodegradable pesticides and environmental friendly pesticides. The tested plant extract in present study were evaluated against *Culexpipiens* larvae and their LC50 and LC95 at P= 0.05 was calculated. The lowest LC50 is 123.05 for*Artemisia*sp, from all ethanolic extracts and39.64 for *Solenostemmaargel* from all pet-ether extracts. The lowest LC50 for ethanolic extracts and pet-ether were 32.79 and15.70 of *SolenostemmaArgel*against snail vector respectively. The binary mixtures appear synergist and additive activity when tested against *Culexpipien* and *Biomphalariaalexandrina*.

INTRODUCTION

Mosquitoes transmit serious human diseases, causing millions of deaths every year. Among these diseases, malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at endemic and epidemic areas in many countries (WHO 1991, Lerdthusnee *et al.,* 1995 and Madani *et al*., 2003). Plants may be alternative sources of mosquito control agents (Kamel *et al.,* 2005a&b; Pavela, 2009; Helmy *et al.*, 2010, Bakr *et al.*, 2010, Kamaraj *et al.*, 2011, El-Maghraby *et al.,* 2012 and Eldiasty *et al.* 2014). Snails' species are associated with transmission parasitic disease as intermediate host. Schistosomiasis is a parasitic disease that affects 200 million people in different countries (El-Ansary *et al*., 2000). Snail control with mollucicides has been one of the effective methods used for rapid and effective control of disease. Bait formulation of different molluscicides, would be an effective tool for selective killing of the snail with minimal adverse effect on the environment. The high cost of synthetic molluscicides, used in the control of the intermediate snail hosts, has resulted in renewed interest in plant molluscicides (Abdalla *et al.,* 2011 and EI-Kheir and EI-Tohami, 1997). The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years. The reasons for these problems refer to the high toxicity, non-biodegradable properties of pesticides and the residue in soil, water resources and crops that affect public health.

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Thus, on the one hand, one needs to search the new highly selective, biodegradable pesticides and environmental friendly pesticides (Kamel *et al*., 2005, Mustafa and Al-Khazraji, 2008, Bakry and Mohamed 2011, El-Maghraby *et al*., 2012 and Eldiasty *et al*., 2014). Biological control stands to be a better alternative to the chemical controls aimed against snails. The search of herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative molluscicides control (El-Sherbini *et al*., 2009, Vijay, 2010). The aim of the present study was to evaluate insecticidal and molluscicidal activities of some medicinal plants extracted by different solvents to explore full potential use of these plants as insecticides and molluscicides in future.

MATERIALS AND METHODS

Tested compounds

The tested medicinal plants (*Rosmarinus*sp, *Solenostemmaargel* And Artemisia sp.) were washed to avoid dusts and dirt then lift to dry under shade in the laboratory. Dried plant (whole plant) were cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each plant were extracted with ethanol absolute and petroleum ether. The extractions were accomplished by means of a Soxhlet apparatus. The solvent extracts of each plant were evaporated and dried under vacuum using a rotary evaporator of water bath adjusted at 60–70ºC. The resulted dry crude extracts were storage at 4 Cº in screw capped vials, until use.

Tested mosquitoes

***Culexpipiens* (*Culicidae: Diptera*)**

Provided by collecting from Tabuk area and transferred to the research laboratory of Biology Department – Science Collage – Tabuk University where self-perpetuating colonies were established and maintained during the present study, according to the method described by Kamel *et al.* (2005a). Late third larval instars were used for toxicological studies.

Efficiency of plant extracts on mosquitoes

Preliminary, toxicological bioassay tests were carried out to the selected plant extracts on tested insects as a modification for the method described by **(**Wright, 1971 and Kamel *et al.*, 2005b), their LC_{50} and LC_{95} values were determined as well as their slope function, according to Finney, 1971 and Who, 1981).

Joint action of plant extracts on mosquitoes

The selected plant extracts were mixed with each other at a level of their corresponding LC_{25} values. The tests were carried out as mentioned before. The combined action of the different mixtures was expressed as the co-toxicity factor which was estimated according to the equation given by (Kamel *et al.,* 2005a).

Tested parasite

Adult *Biomphalariaalexandrina* (Shell diameter: 12–14 mm**)** was subjected to current study. Uninfected snails, that is, those that did not show patent trematode infections, were maintained in the laboratory conditions for seven days before being used in our molluscicidal tests.

Efficiency of plant extracts on snails

Ten snails were then allocated to each of the groups and immersed in either untreated dechlorinated tap water or aqueous extract treated dechlorinated water (positive and negative controls). Preparations of the plant extracts and toxicity test protocols were adapted from those described by Brackenbury and Appleton (1997).

Joint action of plant extracts on snails

The selected plant extracts were mixed with each other at a level of their corresponding LC_{25} values. The tests were carried out as mentioned before. The combined action of the different mixtures was expressed as the co-toxicity factor which was estimated according to the equation given by Mansour *et al.* (1966).

RESULTS

Insecticidal studies

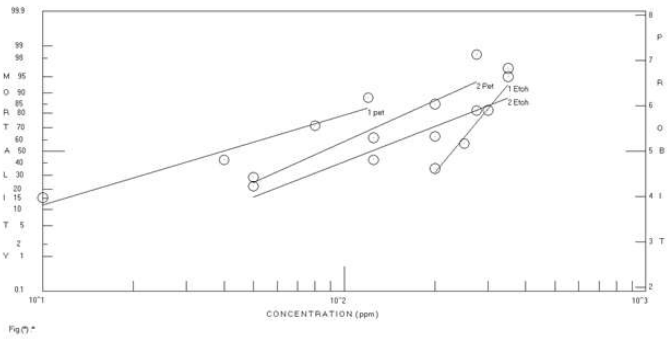
Evaluation of the larvicidal activity of some medicinal plant extracts on mosquito larvae

These experiments carried out to evaluate the potency of some medicinal plant extracts by two different solvents (pet-ether and Ethanol) against *Culexpipiens* larvae.

The results in Table (1) and Fig. (1) showed different degrees in potency according to their LC50. The confidential limits of each of the tested plant extract were statistically calculated for LC50 and LC95 at P= 0.05. The lowest LC50 is 123.05 for *Artemisia*sp for all ethanolic extracts. While, the lowest LC50 for pet-ether extracts was 39.64 for *Solenostemmaargel*.The LC50 for *Rosmarinus*sp, are 721.56 and 511.84 of ethanolic extracts and pet-ether extracts respectively as stated before by (Alghabban *et al.,* 2015).

Table 1. larvicidal activity of some medicinal plant extracts on *Culexpipiens* larvae

Plant	Solvent	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
Solenostemma argel	Pet-ether	39.64 (31.83-49.31)	267 (166.35-432.6)	1.99
	Ethanol absolute	229.32 (215.96-243.50)	368.26 (329.96-411.03)	7.9
*Artemisia*sp	Pet-ether	84.85 (71.92-100.05)	299.28 (230.66-388.97)	3.0
	Ethanol absolute	123.05 (104.88-144.30)	530.68 (390.75-722.07)	2.6



Where 1 = *Solenostemma*sp
2 = *Artemisia*sp

Fig. 1. Susceptibility of *Culexpipiens*larvae to some medicinal plant extracts

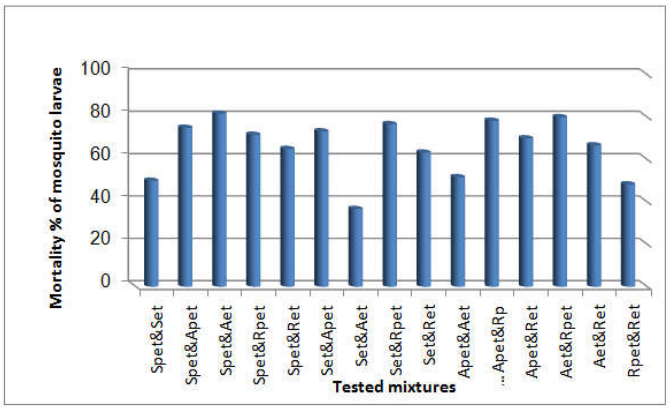
Table 2. Joint action analysis for the selected medicinal plant extracts with each other mixed at LC25 levels against *Culexpipiens* larvae

Mixture components	Observed mortality%	Co-toxicity factor	Joint action
S (pet-ether) & S(Ethanol)	50	0	Additive
S (pet-ether) & A (pet-ether)	75	50	Potentiation
S (pet-ether) & A (Ethanol)	81.7	63.4	Potentiation
S (pet-ether) & R (pet-ether)	71.7	43.4	Potentiation
S (pet-ether) & R (Ethanol)	65	30	Potentiation
S (Ethanol) & A (pet-ether)	73.3	46.6	Potentiation
S (Ethanol) & A (Ethanol)	36.67	-26.66	Antagonism
S (Ethanol) & R (pet-ether)	76.7	53.4	Potentiation
S (Ethanol) & R (Ethanol)	63.3	26.6	Potentiation
A (pet-ether) & A (Ethanol)	51.7	3.4	Additive
A (pet-ether) & R (pet-ether)	78.3	56.6	Potentiation
A (pet-ether) & R (Ethanol)	70	40	Potentiation
A (Ethanol) & R (pet-ether)	80	60	Potentiation
A (Ethanol) & R (Ethanol)	66.7	33.4	Potentiation
R (pet-ether) & R (Ethanol)	48.3	-3.4	Additive

Where: S = *Solenostemmaargel* A = *Artemisia* sp. R = *Rosmarinus*sp.

Joint action of some medicinal plant extracts on *Culexpipiens* larvae

The selected plant extracts were individually mixed with each other in a ratio of 1:1 at the level of LC25 of each. Thus, a total of 15 binary mixtures are prepared. The data in the (Table 2) and (Fig. 2) indicated that, all mixtures possess potentiation effect except *Solenostemmaargel* (Pet-ether and ethanolic extracts), *Artemisia* sp. (Pet-ether and ethanolic extracts) and *Rosmarinus*sp. (Pet-ether and ethanolic extracts) show additive effect. The antagonism effect appears only in one mixture for ethanolic extract of *Solenostemmaargel* and *Artemisia* sp.



Where Spet = *Solenostemmaargel* extracted by pet-ether
Set = *Solenostemmaargel* extracted by ethanol
Apet = *Artemisia* sp. extracted by pet-ether
Aet = *Artemisia* sp. extracted by ethanol
Rpet = *Rosmarinus*sp. extracted by pet-ether
Rpet = *Rosmarinus*sp. extracted by ethanol

Fig. 2. Joint action analysis for the selected plant extracts together at LC25 levels against *Culexpipiens* larvae

Molluscicidal studies

Evaluation of the molluscicidal activity of some medicinal plant extracts on adult snails

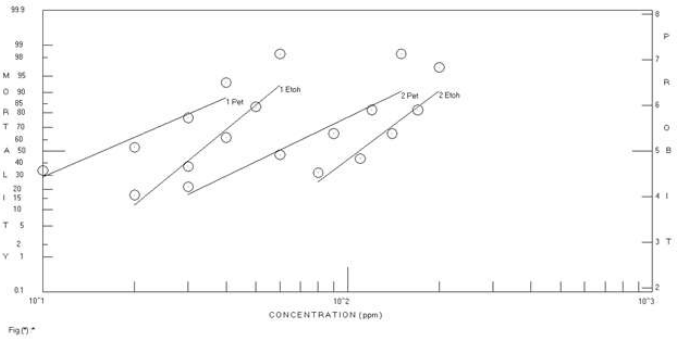
The results from evaluation test of some medicinal plant extracts against the adult snails *Biomphalariaalexandrina* are represented in Table (3) and regression lines in Fig (3). The result shows different degree of potency according to their LC50 and LC95 values and all regression lines possess nearly parallel lines.

Joint action of some medicinal plant extracts on *Biomphalariaalexandrina*

These experiments were carried out to improve the potency of plant extracts for using as molluscicidal agents.

Table 3. Molluscicidal activity of some medicinal plant extracts on *Biomphalariaalexandrina*

Plant	Solvent	LC 50 (Co. Limits)	LC 95 (Co. Limits)	Slope Function
Solenostemma argel	Pet-ether	15.70 (13.36-18.42)	58.49 (42.55-80.75)	2.9
	Ethanol absolute	32.79 (30.50-35.24)	65.01 (56.76-74.48)	5.5
*Artemisia*sp	Pet-ether	59.04 (52.05-66.94)	189.44 (150.15-239.33)	3.2
	Ethanol absolute	109.29 (100.76-118.52)	232.44 (197.88-273.11)	5.0



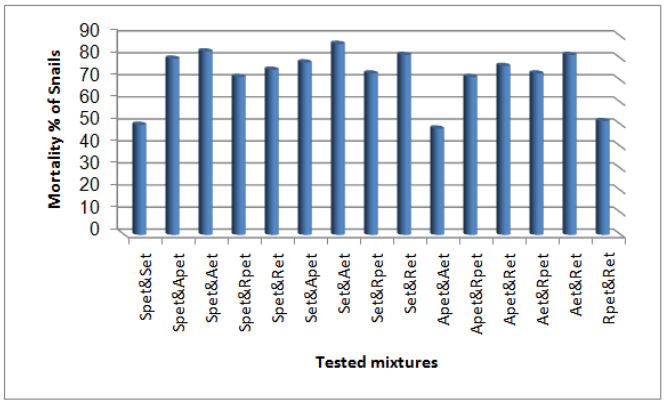
Where 1 = *Solenostemma*sp 2 = *Artemisia*sp

Fig 3. Susceptibility of *Biomphalariaalexandrina* to some medicinal plant extracts

Table 4. Joint action analysis for the selected medicinal plant extracts with each other mixed at LC25 levels against *Biomphalariaalexandrina*

Mixture components	Observed mortality%	Co-toxicity factor	Joint action
S (pet-ether) & S(Ethanol)	50	0	Additive
S (pet-ether) & A (pet-ether)	80	60	Potentiation
S (pet-ether) & A (Ethanol)	83.3	66.6	Potentiation
S (pet-ether) & R (pet-ether)	71.7	43.4	Potentiation
S (pet-ether) & R (Ethanol)	75	50	Potentiation
S (Ethanol) & A (pet-ether)	78.3	56.6	Potentiation
S (Ethanol) & A (Ethanol)	86.7	73.4	Potentiation
S (Ethanol) & R (pet-ether)	73.3	46.6	Potentiation
S (Ethanol) & R (Ethanol)	81.7	63.4	Potentiation
A (pet-ether) & A (Ethanol)	48.3	-3.4	Additive
A (pet-ether) & R (pet-ether)	71.7	43.4	Potentiation
A (pet-ether) & R (Ethanol)	76.7	53.4	Potentiation
A (Ethanol) & R (pet-ether)	73.3	46.6	Potentiation
A (Ethanol) & R (Ethanol)	81.7	63.4	Potentiation
R (pet-ether) & R (Ethanol)	51.7	3.4	Additive

Where: S = *Solenostemmaargel.* A = *Artemisia* sp. R = *Rosmarinus*sp.



Where Spet = *Solenostemmaargel* extracted by pet-ether
Set = *Solenostemmaargel* extracted by ethanol
Apet = *Artemisia* sp. extracted by pet-ether
Aet = *Artemisia* sp. extracted by ethanol
Rpet = *Rosmarinus*sp.extracted by pet-ether
Rpet = *Rosmarinus*sp.extracted by ethanol

Fig. 4. Joint action analysis for the selected plant extracts together at LC25 levels on *Biomphalariaalexandrina*

The results in Table (4) showed potentiation effect in all mixtures except *Solenostemmaargel* (Pet-ether and ethanolic

extracts), *Artemisia* sp. (Pet-ether and ethanolic extracts) and *Rosmarinus*sp. (Pet-ether and ethanolic extracts) show additive effect. These results illustrated by Fig (4). The highest value of potentiation represented in mixture of *Solenostemmaargel* ethanolic extract with *Artemisia* sp. ethanolic extract.

DISCUSSION

Insecticidal studies

The present data showed that despite the differences in potency of all extracts except ethanolic extract of *Solenostemmaargel* 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. While the cross line for ethanolic extract of *Solenostemmaargel*may be possess different mode of action from the other tested extracts.The lowering of slope function that meaning the more homogeneity of tested population (Busvine, 1971 and Kamel *et al.,* 2005b). All mixtures which tested against mosquito larvae release variation in the joint action effect. That difference of binary mixtures could be attributed to the site and mode of action of the mixture components. Therefore the potentiation and additive effects may be attributed to that the mixture components which have the same site or/and mode of actions. While, the antagonism effect may be referred to the mixture components have different site or mode of action. Several authors have used the plant extracts to synergize each other and the insecticidal activities of the traditional insecticides (El-Bokl and Moawad, 1997, Kamel *et al.,* 2005a, Abbassy *et al.,* 2009).

Molluscicidal studies

From the result obtained from evaluation some medicinal plant extracts against adult snails the potency of pet-ether extracts for all tested plants is more than potency of ethanolic extracts from the same plants this may be attributed to the difference between the extracted materials. Several authors agree with those results (Singh, *et* al., 1996, Vijay, 2010 and Bakry, *et al*., 2011). The joint action between tested plant extracts mixtures against tested snails possesses different degree of potency. The highest value of potentiation represented in mixture of *Solenostemmaargel* ethanolic extract with *Artemisia* sp. ethanolic extract. El-Zemity, 2006 stated that, the joint action effects of the four active derivatives namely; broneol, carvacrol, chlorothymol and thymol with either piperonylbutoxide or Triton–X 100 dramatically enhanced the molluscicidal activity over the standard molluscicide. Also, Radwan *et al.* 2008 evaluate some monoterpenoids mixed with piperonylbutoxide against *Biomphalariaalexandrina*.

Conclusion

The tested plant extracts can be used effectively against mosquito larvae and schistosomiasis snail vector alternate the traditional insecticides and molluscicides either alone or mixed with each other safely.

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