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LARVICIDAL EFFICACY OF LEAF EXTRACTS FROM THREE ASTERACEOUS PLANT AGAINST MOSQUITO (*Culex quinquefasciatus*)

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ABSTRACT

It is well known that the mosquito, *Culex quinquefasciatus*, badly affect individuals in endemic setting, causing filariasis. Intervention targeting the vector larva has not been given much priority. Therefore, the efficacy of ethanolic extract of *Ageratum conyzoides, Bidens pilosa L. and Spilanthes filicaulis* and a mixture of these plants in equal proportion was evaluated for larvicidal activities against filarial mosquito *Culex quinquefasciantus*. Phytochemical screening of the extracts was done by dissolving 5g each of plants in 30ml ethanol and water separately to obtain plant extracts. Samples were qualitatively screened following standard methodology for phytochemicals. Mortality of mosquito larva was examined after 12hrs, 24hr, 36hrs, and 48hrs treatment. Probit analysis for 50% Lethal Concentration was done using GraphPad Prism 9. Phytochemical screening of the plants revealed that *Bidens pilos, Spilanthes filicaulis* and *Ageratum conyzoides* contained moderated concentrations of Anthraquinones and Cardiac glycosids, Tannins, and Alkaloids respectively. Complete mortality was recorded with 0.60ml of *Bidens pilosa*, and *Spilanthes filicaulis* at 48hours and 36 hours respectively. More so, complete mortality was recorded with 0.40 and 0.60ml of the three plants in mixed proportion at 36 hours and 24 hours respectively. Larval mortality and time mortality were significantly ($p<0.05$) different. 50%Lethal Concentration for *Ageratum conyzoides L, Bidens pilosa L, Spilanthes filicaulis,* and mixture of the three plants in equal proportion are 0.33ml, 0.25ml, 0.08ml, and 0.06ml respectively. Results showed that these plant materials exhibit significant activity and could be considered as potent natural larvicidal agent.

Keywords: Asteraceous plants, *Culex quinquefasciatus,* Efficacy, Larvicide, Leaf extracts.

INTRODUCTION

The mosquito, *Culex quinquefasciatus*, (Diptera: Culicidae) is among the medical vectors, which significantly causing major health problems worldwide in millions of individuals annually, via the pathogenic agents they transmit (Maheswaran *et al.*, 2008). The vectors transmit *Wuchereria bancrofti*in the lymphatic circulation leading to the unusual swelling, severe mutilation and discomfort in addition to social stigmatization on infected individuals in many countries including Nigeria (Kalu *et al.*, 2010; WHO, 2020).The preponderance of the *Culex* mosquitoes probably exceeds other mosquito species in town and cities (Arivoli and Samuel,2001). They are common in almost any habitat that can harbor water either turbid or not. Generally, mosquitoes have been declared as "public health enemy number one" due to the various dreadful diseases they transmit including malaria, dengue, filariasis, yellow fever among others (Kalu *et al.,* 2010; WHO, 2020).According to WHO report on lymphatic filariasis in 2000, infections were common in over 120 million persons with approximately 40 million handicapped due to disfigured limb. Currently, filariasis is not only endemic in 49 countries, but over 890 million persons are globally threatened with this disfiguring and disabling disease, and as such intervention measures are required to ease the scourges (WHO, 2020).

In the late 90's when about 90 million individuals were infected worldwide, this implying that individuals at risk of infection was ten times (WHO, 1984). Today higher infection is possible if swift measures of intervention are neglected. The adoption of chemotheraphy in addition to safe medicine which involves individual use of Albendazole, diethylcarbamazine and Ivermectin has remained the acceptable in preventing the disease but with several challenges (Taylor *et al.,* 2010; WHO, 2010), and since the year 2000 about 8 million treatments have been presented to eliminate the spread of the infection (WHO 2011). The potentials of intervention targeting the vector through insecticidal efficacies cannot be overemphasized. Insecticidal control has been thriving due to their swift action and that they can be applied easily. The toxic nature of insecticides on subspecies of mosquitoes has been elaborated with its effect adversely on soil samples, water and air. However, onlya few report on developed resistance strain, ecological imbalance and harm to mammals has been done (Kaushik and Saini,2008).Nevertheless, control programmes involving mosquitoes have suffered challenges as a result of the ever-increasing issues of insecticide resistance. Hence, larvicidal trials involving biologically-active plants becomes imperative, and expectedly to lessen environmental hazards posed on living organisms by insecticides through accumulations of harmful residues in the environment (Maheswaran *et al.,* 2008). Furthermore, the present proliferation of this disease can also be ascribed to higher number of breeding sites in urban agglomeration that promote the reproduction cycle of the vector. The commercialization and use of four class of insecticides including organochlorides, organophosphate, pyrethroids and carbamate has been recommended (WHO, 2016).Controlling the larva population is much easier than the adults. This can equally reduce the overall application of pesticides needed to control mosquito population. Secondary metabolites could be found in most plants which inhibit insect growth.

Over the years, extractions of plant origin have been adopted for use as killing and repelling agent against insect pests (Chandra *et al.,* 2016; Islam *et al.,* 2017) and has proven to reduce disease transmission when effectively targeting mosquito vectors in large numbers (Allison *et al.,* 2013; Iqbal *et al.,* 2018). Kalu *et al.* (2010) reported that biologically-active materials extracted from plant can not only be a larvicidal agent, but as well act as insect growth regulators, attractants and deterrents. The potential constituent and antifungal characteristics of*Ageratum conyzoides* and *Spilanthesfilicaulis*have been reported (Ilondu *et al.,* 2014). The coexistence of insect and plants has made it possible for the development of a plethora of chemical defenses in plants which can be used against insects. Considering the fact that extracts of plants ecologically friendly to the environment, a good number of species have not only been screened but adopted for their application as bio-insecticide against mosquito species with promising effect (Kaushik *et al,* 2008). Bio-insecticides are promising in that they are active, environment-friendly, easily biodegradable, and affordable and have been locally applied to combat insect pest population in the several parts of the world (Kalu *et al.,* 2010).Therefore, this study was designed to evaluate the larvicidal efficacies of ethanol leaf extractsfrom *Ageratum conyzoides L., Bidens pilosa L., and Spilanthes filicaulis,* (*Asteraceae*) singly and in combination for the control of the larvae of filarial vector *Culex quinquefasciatus*.

MATERIALS AND METHODS

Collection and identification of plant samples

The matured fresh plants of *Ageratum conyzoides L.* (Plate 1A), *Bidens pilosa L.* (Plate 1B), *Spilanthes filicaulis* (Plate 1C), were collected from Warri, Delta State, Nigeria. Warri is located between latitude 5º 30'N and 5º 35'N and longitude 5º 29'E and 5º 48'E of the equator with mean annual temperature of 28ºc and rainfall amount of 3000mm, (Ojeh, 2011). These plants were properly identified using method of Akobundu and Agykwa (1998). The ecological attributes of these Asteraceae is shown in Table 1.

A. *Ageratum conyzoides* L. **B.** *Bidens pilosa* L.

C. *Spilanthes filicaulis*

Plate 1. Plant materials used for the larvicidal studies.

Preparation and extraction of the plant materials

The leaves were plucked and shade dried for 4 weeks at room temperature, grounded into powder with an electric blender and the weight of each plant material taken. The weight of the powdered leaves were 287.7g, 181.1g, and 178.6g, for *Ageratum conyzoides, Bidens pilosa, and Spilanthes filicaulis* respectively. The powdered plant materials were soaked with ethanol for 24 hours and the extracts were filtered with a sieve with a mesh size of 0.5mm. The extraction was monitored under laboratory condition and the residue stored in an air-tight container at 4ºc.

Test insect

The *Culex* larvae used to test for the lavicidal activity was obtained from stagnant water at medical complex site III of Delta State University, Abraka and identified at the Department of Animal and Environmental Biology prior to the experiment; the larvae were kept in plastics containing tap water to help for easy utilization.

Larvicidal bioassay

Larvicidal activity was evaluated using the method adopted from WHO (1996) with slight modifications as shown in Plate 2. The residue from the plant extracts were made into percentage by volume per volume (% v/v). One hundred (100ml) of water in plant extract formed 0.05ml, 0.10 ml, 0.20ml, 0.40ml and 0.60ml concentrations used for the treatment. Twenty (20) larvae were released into each transparent white plastic container containing these concentrations in triplicate, and untreated group served as the control. Mortality and survival rate was recorded after 12hrs, 24hr, 36hr, and 48hrs of the exposure period. Larvae were presumed dead after they failed to move while probed with aforceps at the cervical region.

Plate 2. Larvae in plant extract

Phytochemical screening:

The phytochemical screening of the aqueous extract of the plants was carried out to determine the presence of the following compounds, alkaloid, flavonoids, tannins, anthraquinones, saponins, cardiac glycosides, sterol, reducing sugar and phlobatannins using standard methods outlined by Evans and Trease (1999), and Ashafa and Afolayan (2009).

Statistical analysis

Data were entered into MS Word 2013 and checked for errors. Result was presented in percentages. Probit analysis was used for the determination of LC_{50}. Data from mortality and effect of concentrations were subjected to analysis of variance. Differences between the treatments were compared by Tukey's test at 5% level of probability ($p<0.05$). All analysis was done using GraphPad Prism 9.

RESULTS AND DISCUSSION

The efficacy of *Ageratum conyzoides, Bidens pilosa, Spilanthes filicaulis,* and a mixture of the three plants in equal proportion was determined owing that there is not much vaccine to combat the disease transmitted by *Culex quinquefasciatus* mosquito and manage the continually developing resistance to the available drugs (Arivoli *et al.*, 2011). Even with the spread of resistance, molecular analysis has been done to ascertain resistant genes (Coleman *et al.*, 2002). However, the formulation and use of pesticides of plant origin has proven to kill vector at optimum concentrations (Das *et al.*, 2007; Kamaraj *et al.*, 2009; Aarthi and Murugan, 2010; Allison *et al.,* 2013; Islam *et al.,* 2017; Iqbal *et al.,* 2018). Ecologically, the plants understudy are herbs that can be obtained annually (Table 1).

Table1: Ecological attributes of the members of Asteraceae understudy

Botanical name	Common name	Life form	Habitat of growth
Spilanthes filicaulis	Brazil cress	A	H
Bidens pilosa	Cobblers peg, hairy beggars tick, black jack	A	H
Ageratum conyzoides	Billy goat weed	A	H

Key: A=Annual H=Herb

Chemical screening of plants

In recent times, plants are being considered for their environmental safety. The insecticidal potentials of the plants under study has been carried out on other insect species (Bouda *et al.,* 2001; Chiang *et al.,* 2007; Bairwa *et al.,* 2010) Kabaru and Gichia, (2001) opined that an insecticide sometime may not necessarily cause the desired mortality on targeted species before it is accepted for use but expected to interrupt breeding of species. Phytochemicals are making way as acceptable alternatives to chemical insecticides due to their safety in use, inexpensive, biodegradable, and are readily available for use (Prabhakar and Jabanesan, 2004). At present the main threat to effective mosquito control is insecticidal resistance (Chandra *et al.,* 1998). Although a good number of plants have been reported for activity against mosquito larvae, only a few results from tested plant-based insecticides have moved from laboratory to field use which could be linked to the phytochemicals present in tested plants when compared to chemical insecticides (Arivoli *et al.,* 2011). Baseline study of chemical screening of the plant extracts is shown in Table 2. The analysis revealed the presence and absence of some of the chemicals in the plants under study. No high concentration of chemicals were recorded. However, anthraquinones and cardiac glycosids,were moderately present in *Bidens pilosa,* tannins in *Spilanthes filicaulis* and alkaloid in*Ageratum conyzoides* respectively while others were rather low in concentration or absent (Table 2).

Table2.Chemical screening of the plants understudy.

Constituent tested	Plant species		
	Bidens pilosa	*Spilanthes filicaulis*	*Ageratum conyzoides*
Alkaloids	+	+	++
Saponins	+	-	+
Phlobatanins	-	-	-
Flavonoids	+	+	+
Anthraquinones	++	-	-
Terpenoids	-	+	-
Steroids	+	+	+
Tannins	+	++	+
Cardiac glycosids	++	-	-
Reducing sugar	+	-	-

Key: + low concentration, ++ moderate concentration, +++ high concentration, -absent

Larvicidal activities

Vector control using insecticides has long been the best option proposed by Scientists in the management of mosquito caused diseases but it is facing severe threat due to the emergence of resistance in insect species to conventional synthetic insecticides, warranting either countermeasures or development of newer insecticides (Chandra *et al.,* 1998; Arivoli and Samuel, 2001). The larvicidal activities of ethanol extract of *Ageratum conyzoides L., Bidens pilosa L, Spilanthes filicaulis* and their mixture in equal proportion atvarious concentrations against *Culex quinquefasciatus,* is given in Table 3. Complete mortality was recorded with the mixture of plant extracts in 0.40 and 0.60ml, and 0.60ml of *Spilanthes filicaulis* and *Bidens pilosa* respectively whereas 0.60ml of *Ageratum conyzoides* recorded 62% mortality. Mortality ranged from 3.5 to 61.7%, 20.0 to 100%, 46.7 to 100%, and 45 to 100% in *Ageratum conyzoides, Bidens pilosa,Spilanthes filicaulis*and the mixture of the plant extracts respectively. There was significant ($p<0.05$) difference in the mortality of *Culex* mosquitoes exposed to the various treatment except with 0.10ml *B. pilosa* and 0.05ml of the mixture.

Considering the time mortality, higher mortalities was recorded in 12 hours after exposure to the treatments in single and mixed forms. A reduced larva population was observed with time showing the effectiveness of the plant extracts and the differences were significant ($p< 0.05$) as shown in Table 3.The mortality observed in this study with time was higher than the efficacies recorded with using *Tamarix aphylla*, *Jacaranda mimosifolia* and *Lavandula angustifolia* on *Cx. quinquefasciantus* at 72 hours (Mohamed *et al.,* 2020). Though, the complete mortality reported in their study corroborates with the higher concentrations of plant extracts in single forms except in *A. conyzoides* and 0.40 and 0.60ml of the mixture of extracts. Furthermore, the effect of lavicidal mortality was dependent on the concentration of leaf extract. The combination of the three plants showed a higher effect when compared to other previous works in the control of mosquito. In this present study, higher concentration killed larvae with reduced time approximately 12 and 24 hours compared to lower concentrations. Virtually all the leaf extract kill more than 50% of the larvae population tested indicating that these plant species can be adopted for the reduction of mosquito population.

Table 3: Larval mortality of the Asteraceous leaf extractsagainst *Culex quinquefasciatus*.

Treatment	Conc. (ml) /Hours	12hrs	24hrs	36hrs	48hrs	Percentage mortality
Ageratum conyzoides	0.00	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
	0.05	0.0^{a}	0.67^{a}	0.0^{a}	0.0^{a}	3.5^{b}
	0.10	1.0^{ab}	2.0^{b}	0.0^{a}	0.0^{a}	15.0^{c}
	0.20	2.67^{bc}	2.67^{b}	1.67^{b}	1.0^{b}	40.0^{e}
	0.40	5.0^{c}	4.0^{d}	2.67^{bc}	0.0^{a}	58.5^{g}
	0.60	6.0^{cd}	5.0^{e}	1.0^{b}	0.33^{a}	61.7^{h}
Bidenspilosa	0.05	2.0^{b}	1.0^{ab}	1.0^{b}	0.0^{a}	20.0^{d}
	0.10	2.7^{bc}	2.0^{b}	3.0^{c}	1.0^{b}	43.5^{ef}
	0.20	4.0^{c}	4.0^{d}	2.0^{c}	2.0^{c}	60.0^{g}
	0.40	10.0^{g}	4.0^{d}	3.7^{cd}	0.0^{a}	88.5^{k}
	0.60	15.0^{i}	$3.0b^{c}$	2.0^{c}	0.0^{a}	100.0^{n}
Spilanthes filicaulis	0.05	6.0^{cd}	2.67^{b}	0.67^{a}	0.0^{a}	46.7^{f}
	0.10	9.0^{f}	2.67^{b}	0.67^{a}	1.0^{b}	61.7^{h}
	0.20	13.0^{hi}	1.0^{ab}	0.0^{a}	0.0^{a}	70.0^{i}
	0.40	17.0^{j}	2.0^{b}	0.0^{a}	0.0^{a}	95.0^{m}
	0.60	19.33^{k}	0.67^{a}	0.0^{a}	0.0^{a}	100.0^{n}
A. conyzoides + B. pilosa + S. filicaulis	0.05	7.0^{e}	0.67^{a}	0.67^{a}	0.67^{a}	45.0^{ef}
	0.10	12.0^{h}	2.67^{b}	0.0^{a}	0.0^{a}	73.35^{j}
	0.20	16.1^{i}	$1.0a^{b}$	1.0^{b}	0.0^{a}	90.5^{l}
	0.40	19.33^{k}	0.67^{a}	0.0^{a}	0.0^{a}	100.0^{n}
	0.60	20.0^{k}	0.00^{a}	0.0^{a}	0.0^{a}	100.0^{n}

Means with the same superscript letter do not differ significantly ($p<0.05$) within column using Tukey's test.

Plants in the family *Asteraceae* has been extensively screened for their insecticide activity. Plants that showed promising larvicidal activities were *Otanthus maritmus* methanolic stem extract (LC_{50}=7.0ppm), *Artemisia campestris* methanolic stem extract (LC_{50}=23.0ppm), *Tagetes erectes* petroleum ether leaf extract (LC_{50}=100.0ppm) against *Culex quinquefasciantus* (Arivoli *et al.,* 2011). In this present study also the LC_{50} of *Ageratum conyzoides,Bidens pilosa*, *Spilanthes filicaulis,* and a mixture of the three plants in equal proportion were 0.33ml, 0.25ml, 0.08ml, and 0.06ml respectively. The result as compared to other studies showed that at the LC_{50}, these plants have higher mortality rate at a lower concentration. The probit analysis of larvicidal efficacy of leaf extracts on *Culexquinquefasciatus*is shown in Figure 1 - 4. The LC_{50} of the extractsin the order effectiveness ranged from the mixture of the extracts to *Spilanthesfilicaulis*to *Bidenspilosa* and to *Ageratum conyzoides* with the LC_{50} of 0.06ml, 0.08ml, 0.25ml and 0.33mlrespectively. The R-square value of the larvicidal efficacy showed negative model in all plants exposed to *Culexquinquefasciatus*except*Bidens pilosa.*

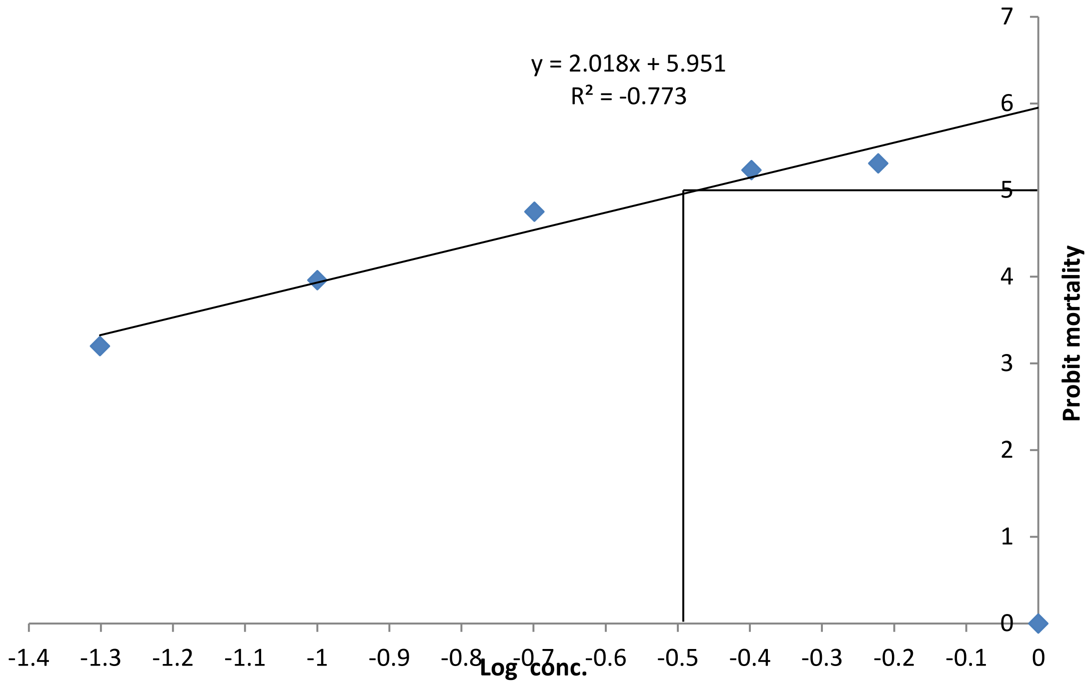

Fig.1. Larvicidal efficacy of *Ageratum conyzoides* leaf extracts on *Culex quinquefasciatus.*

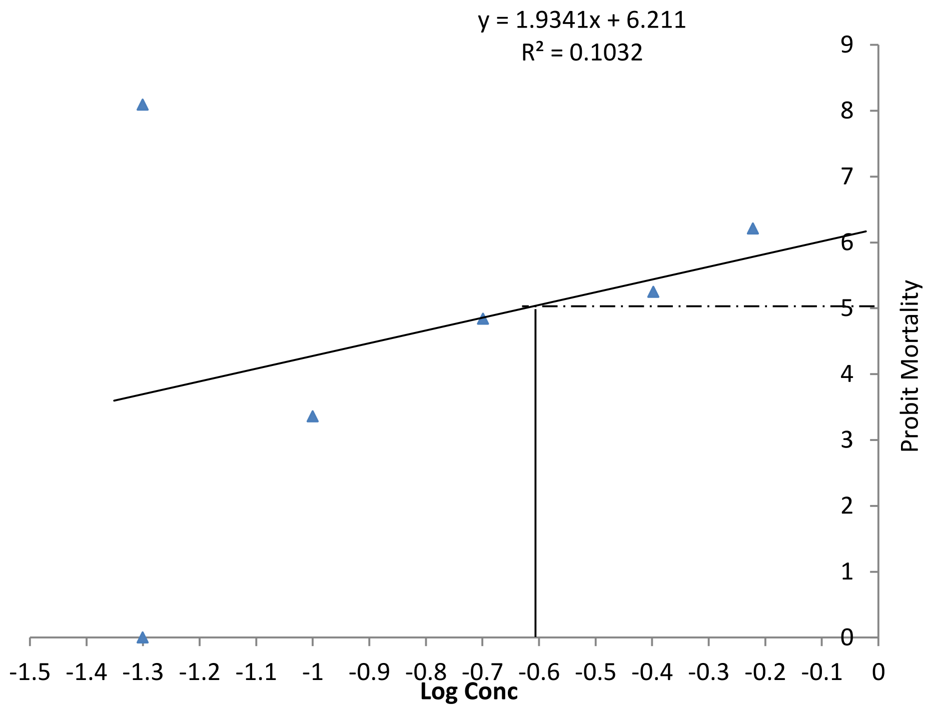

Fig. 2. Larvicidal efficacy of *Bidens pilosa L.*leaf extracts on *Culexquinquefasciatus.*

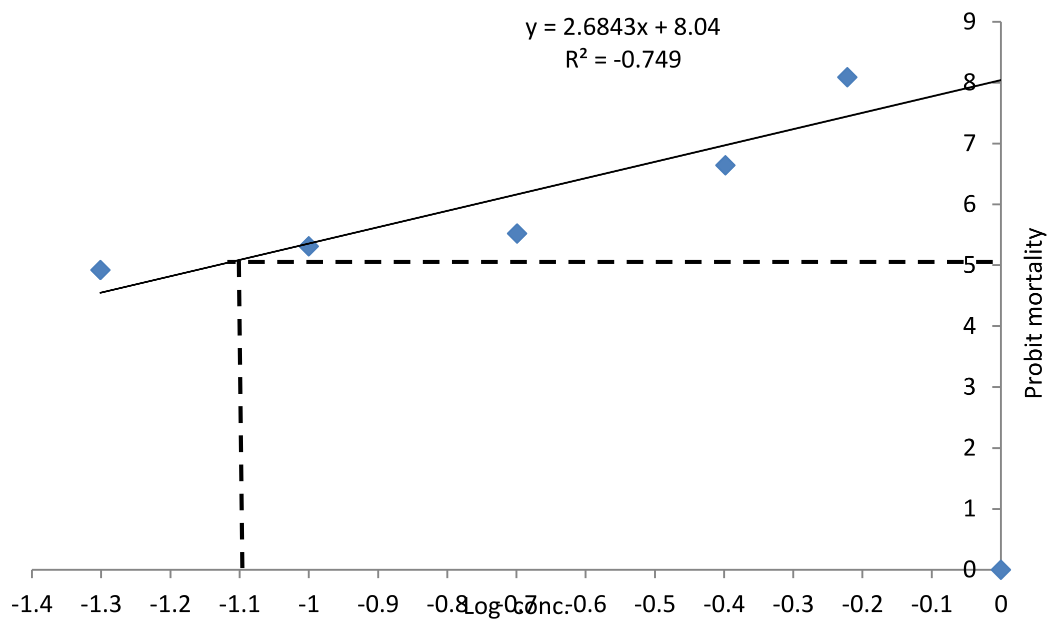

Fig. 3. Larvicidal efficacy of *Spilanthes filicaulis*leaf extracts on *Culexquinquefasciatus.*

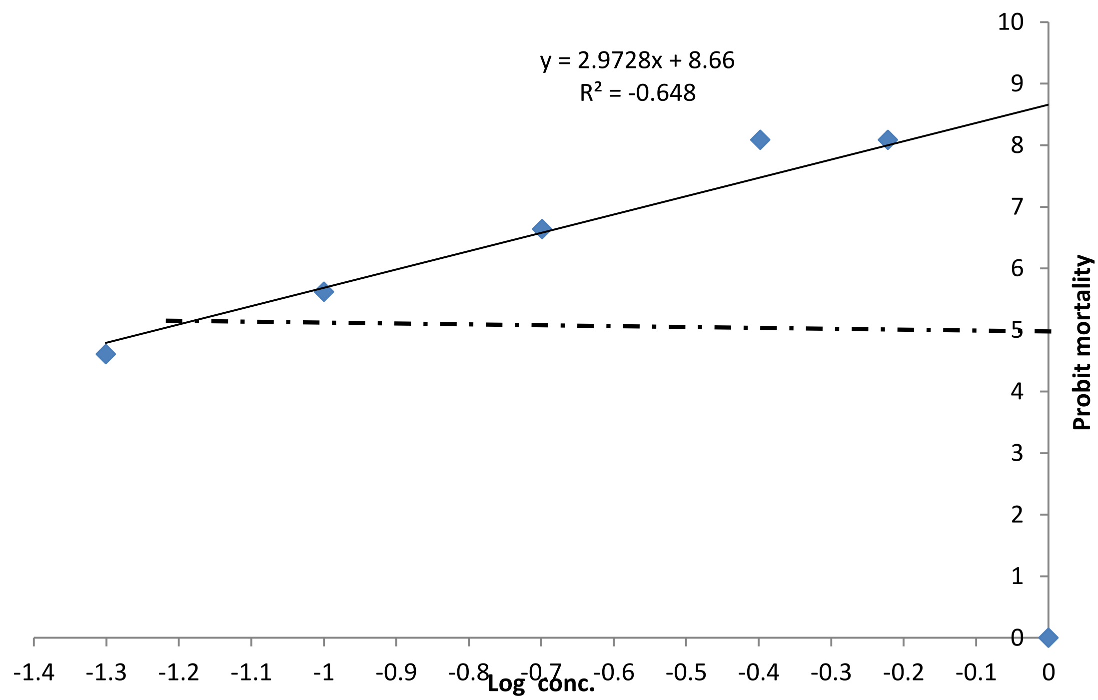

Fig 4. Larvicidal efficacy of the mixture of the plant extracts on *Culexquinquefasciatus*.

CONCLUSION AND RECOMMENDATION

This present study showed that the plant extracts understudy were effective larvicides in controlling filarial vector and hence the vector borne diseases of public health. Higher concentrations of *Ageratum conyzoides, Bidens pilosa, Spilanthes filicaulis,* and a mixture of the three plants in equal proportion, caused higher mosquito mortality. Complete mortality was recorded with the highest (0.40 and 0.60ml respectively) concentration of the three plants in combination and 0.60ml of all the plant extracts in single forms except *A. conyzoides.*

Therefore, the combination of the three extracts in equal proportion and higher concentrations of *Bidens pilosa* and *Spilanthes filicaulis* could be applied to standing water bodies, known to be breeding ground for *Culex* mosquitoes as alternative to known insecticides. Field trial of the combination of the three extracts in equal proportion and higher concentrations of *Bidens pilosa* and *Spilanthes filicaulis* is necessary in large breeding sites (abandoned ditches, ponds and puddles) and small scale (cans and leaf axils gathering water) on all stages of mosquito species.

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