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FULL LENGTH ARTICLE

Larvicidal properties of two asclepiadaceous plant species against the mosquito *Anopheles arabiensis* Patton (Diptera: Culicidae)

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Abstract Certain mosquito species are important vectors of fatal human diseases, among which *Anopheles arabiensis* is known to be associated with malaria transmission in different tropical and subtropical areas. Since chemical control of mosquitoes was linked with numerous drawbacks, like resistance development, the search for effective environmentally sound alternatives is urgently needed. Therefore, it was aimed by this study to evaluate some extracts prepared from two asclepiadaceous plants, viz., *Solenostemma argel* "Hargel" (seeds and leaves) and *Calotropis procera* "Usher" (leaves and flowers), as natural larvicides against *An. arabiensis*. The main parameters included bioassays of treatments for knockdown and residual effects, besides phytochemical analysis of the tested extracts. The results revealed variable groups of secondary metabolites in the two plants, with *S. argel* seemed to be the richest one. Hence, *S. argel* extracts caused higher larval mortalities than those of *C. procera*. This could be ascribed to some potent secondary metabolites in the former plant, which needs further studies. Almost all the high concentrations of *S. argel* extracts exerted the highest knockdown effect (90% mortality) after 24 h, which were comparable with those obtained by two standard insecticides. The highest doses of petroleum ether and water extracts of this plant also manifested significantly higher residual effects than the other extracts after three days following treatments, but were surpassed by the chemical insecticides thereafter. However, *S. argel* seed petroleum ether extract at 0.5% was the most effective of all botanicals up to three weeks of exposure. This extract needs to be evaluated under field conditions for proper exploitation as mosquito larvicide.

1. Introduction

Several mosquito species of the genera *Anopheles*, *Culex* and *Aedes* are vectors of various human diseases (Brown, 1972). *Anopheles arabiensis* is the most important species associated with the transmission of malaria disease in more than hundred countries worldwide (WHO, 2002). Therefore, one of the

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approaches for controlling mosquito borne diseases is the interruption of disease transmission through mosquito control or avoiding mosquito bites. Plant products of potentials as insecticides or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at community level (Potter and Beavers, 2005). Some botanical extracts such as nicotine obtained from *Nicotiana tabacum* leaves, alkaloidal anabasin and lupinine extracted from *Anabasis aphylla*, rotenone from *Derris elliptica* and pyrethrums from *Chrysanthemum cinererifolium* flowers have been used as natural insecticides even before the discovery of synthetic organic insecticides (Campbell et al., 1993).

Nevertheless, the discovery and use of synthetic persistent chemicals not only overshadowed the use of plant products, but also become the major tactic for mosquito control nowadays. However, chemical control of vectors had started in limited areas prior to the Second World War in 1940s (Potter and Beavers, 2005), but chemicals have managed to replace traditional control methods all over the world. Accordingly, the first chemical used against mosquitoes in Sudan during 1950–1965 was the benzene hexachloride (BHC), which was utilized as residual spray. During the period 1970–1974 temephos (Abate) was used as larvicide in limited areas (Haridi et al., 1975). In 1975 malathion (Cythion) was used extensively (Akood, 1980), but has been substituted by fenitrothion (Sumithion) since 1980. Chlorpyrifos (Dursban) and fenthion (Mercaptophos) were the two main larvicides used against the urban mosquito, and now replaced by diazinone (Alfatox) (Abdel Gadir, 1993). In the 90's malathion has been reused again in the country (Azami et al., 1996).

However, the extensive and indiscriminate uses of pesticides have resulted in serious draw backs, the most important of which was the evolution of mosquito resistant strains as well as toxicity hazards to man, livestock and wild life (Bay, 1976). Hence, a high level of adult malathion resistance in *An. arabiensis* was reported earlier from Sudan (Hemingway, 1983). A study in Ethiopia by Yewhalaw et al. (2011), proved that *An. arabiensis* was resistant to an array of insecticides, including permethrin, deltamethrin and malathion. Moreover, mosquito resistance to the four classes of insecticides was documented in Sudan and other countries (El Gadal et al., 1985; WHO, 1992; Ranson et al., 2001, 2009; Matambo et al., 2007). However, residues of some persistent chemicals in the environment have subsequently disturbed the ecosystem (Hill, 1989). Investigations in Sudan have indicated the presence of measurable amounts of organochlorines and organophosphates in surface water. Also, marine area in the Red Sea has suffered pesticide contamination as a result of desert locust control (UNESCO, 2000).

Based on the above mentioned and many other drawbacks of pesticides, researchers all over the world are working hard to find environmentally safe alternatives. They resorted again to plant extracts as potent sources of natural biocides (Ahmed et al., 1984). Botanical biocides are relatively harmless to non-target organisms and present little risks to users and consumers (Satti et al., 2004). Several botanical derivatives have shown selective actions against certain pests through a variety of biological activities, including production of behavioral modifying chemicals (insect growth regulators) such as pheromone analogs, repellents, attractants and antifeedant, besides the direct toxicant effects (Bower et al., 1976). Roark (1947) described approximately 200 plant species with insecticidal values, while Sukumar et al. (1991) listed and discussed 344 plant species that only exhibited mosquitocidal activities. In Sudan, promising results were achieved in this field, where more than twenty plant species in sixteen families, including members of Asclepidiaceae, were listed to be effective at variable levels as mosquito larvicides (Kehail and Bashir, 2004; Satti et al., 2010). However, the rich flora in Sudan as well as in other tropical countries is still waiting for thorough investigations to be exploited as natural biocides. Hence, laboratory studies were carried out to evaluate the larvicidal properties of two asclepiadaceous plant species (viz., *Solenostemma argel* and *Calotropis procera*) against the mosquito, *Anopheles arabiensis*, an important malaria vector in Sudan.

2. Materials and methods

2.1. Rearing of *Anopheles arabiensis*

Eggs of *A. arabiensis* were collected from stagnant water pools near the White River (Elozozab area), Khartoum, during the rainy season in July using a dipper. The stages of *An. arabiensis* were distinguished from those of *Culex* species due to morphological differences (Gillett, 1971; Potter and Beavers, 2005). The eggs were transferred to a glass container with clean water and brought to the laboratory to start the rearing and mass culturing according to the WHO (1975, 1992).

First instar larvae were reared in a glass container ($40 \times 40 \times 40$ cm) with tap water, and fed with a diet of Brewer's yeast and wheat flour until they reached the fourth instar. Pupae were transferred to an open glass Petri dish containing tap water and enclosed in a glass cage ($40 \times 40 \times 40$ cm), covered with muslin cloth to prevent the escape of adults. Emerging males and females were fed on 10% honey diet, which was kept in a bottle hung to the cage. In each bottle a thin cotton thread was inserted in the honey solution and extended to the tip of the bottle in order to facilitate feeding of adults. This kind of food is utilized for flight and metabolism. Since *Anopheles* females need a full blood meal for laying eggs, they were provided with Albino rats (*Rattus norvegicus*) placed in resting cages. Glass Petri dishes with 70 ml tap water covered with filter paper were placed inside the cage for oviposition. After two days, the females started to lay eggs on the surface of the wet filter paper; hence, eggs were taken from the Petri dishes to the adult rearing cage to start new culture of *An. arabiensis*. By doing so, 4th instar larvae of the second generation were provided for the different bioassay tests.

2.2. Preparation of plant materials and extracts

Different botanical parts of two asclepiadaceous plant species, viz., *Solenostemma argel* (leaves and seeds) and *Calotropis procera* (leaves and flowers), were investigated under laboratory conditions for their larvicidal effects against *An. arabiensis*. Fresh samples were collected from the Khartoum State, during autumn season, washed thoroughly with clean water and dried under room temperature. Dry samples were ground into fine powder using an electric blender. However, the powders required for each experiment were prepared in the same day of extractions (water and organic).

Regarding water extract, two hundred grams of powder from each plant sample was mixed with 1000 ml of distilled water in a conical flask. The contents were thoroughly stirred

for 8 h with a magnetic stirrer, and then filtered through a muslin cloth to obtain the stock solution. Three concentrations, 10, 5 and 2.5% (v/v), were prepared serially by distilled water. This step was done concurrently with the bioassay experiment for each plant. On the other hand, the classical procedure for obtaining plant organic chemicals through the Soxhlet apparatus was applied. Petroleum ether and ethanol solvents were used to separate apolar and polar components, respectively (Harbone, 1983). Accordingly, 200 g from each plant was extracted separately for eight hours in the Soxhlet. Such extracts were dried from solvents using the rotary evaporator, then kept in black bottles and stored in a refrigerator (at 5 °C) until being used. Whenever needed, 10 g of organic extracts was firstly dissolved in 1 ml solvent (ethanol or petroleum ether), then the volumes completed to 200 ml with distilled water to prepare stock solutions. Three concentrations (0.5%, 0.25% and 0.125%) of both ethanolic and petroleum ether extracts were prepared serially from the stock solutions.

2.3. Phytochemical analysis of the tested extracts

Phytochemical analysis of the previously prepared organic solvents (petroleum ether and ethanol) and aqueous extracts of the two plants was carried out according to Harbone (1973) and Harbone (1983). Thus, the required reagents were prepared and used for testing the different chemical groups. These mainly included; Mayer, Ninhydrin, Potassium hydroxide, ferric chloride and Vanillin reagents specific for testing alkaloids, amino acids, flavonoids, tannins and sterols/triterpenoids, respectively. Following the chemical testing procedure, different chemical groups were detected based on precipitate formation as in the case of alkaloids, or the appearance of certain colors regarding the other compounds. However, in case of saponins, simply 5 ml of water was added to 10 ml from each extract in a test tube closed with a cork and vigorously shaken. Formation of foam layer, honey comb in shapes, which remains for a minimum of thirty minutes, indicated the possible presence of saponins. Accordingly, the different classes of chemicals present in each extract were tentatively identified.

2.4. Larvicidal bioassays of treatments

2.4.1. Knockdown effects

The above prepared concentrations of extracts were subjected to biological assays against the 4th instar larvae of *An. arabiensis*, under laboratory conditions. Two standard insecticides, Malathoin 50% EC (10 ml/L) and Abate EC (1 ml/L), were included for comparisons, besides the untreated water controls. Metal plates containing 250 ml extract solutions were used to accommodate the experiment. Twenty-five larvae were introduced in each plate. Four replications were used for all treatments assigned in a Completely Randomized design (Gomez and Gomez, 1984).

Records of larval mortality were taken every 24 h for three days (i.e., 24, 48 and 72 h) to evaluate the knockdown effects of treatments. The failure of larvae to swim to the surface or their inabilities to go to the bottom in response to mechanical probing, were taken as indicators for larval mortality. The mortality percent was computed from the average of four replicates coupled with the analysis of variance and mean separations using Duncan's Multiples Range test.

2.4.2. Residual effects

To study the residual effects of botanical extracts compared with the two standard insecticides, the larvae were introduced in the treatments at variable times (3, 14 and 21 days) following preparation. Similarly to what have been applied in the previous experiment, three concentrations of aqueous (2.5%, 5%, and 10%) and organic (0.125%, 0.25% and 0.5%) extracts were tested in this experiment. However, the bioassay test for residual effects was carried out according to a standard procedure described by WHO (1975). Twenty-five 4th instar larvae of *An. arabiensis* were placed in each test solution as per the above mentioned residual intervals, replicated four times in a Completely Randomized design.

The larvae in each treatment solution were left for 24 h, after which they were transferred into distilled water for another 24 h, so as to check for any sign of recovery. New 4th instar larvae of *An. arabiensis* were added during every interval period after the removal of the previous population, in order to investigate the treatments' residual activities at the indicated durations. Finally, the sign of larval mortality was taken as mentioned previously for knockdown test. Daily records of larval mortalities were taken until the end of the study period. The recorded data were subjected to statistical analysis based on the applied design, and then the means were compared by using Duncan's Multiple Range test.

3. Results and discussion

3.1. Chemical constituents of the tested extracts

Results of phytochemical analysis are presented in Table 1. The aqueous extracts of the two plants revealed the presence of various chemical groups at variable levels. Wide ranges of polar and intermediately polar ingredients were shown. Alkaloids, saponins, flavones and amino acids were the major chemicals detected. However, ethanol extracts of these plants revealed more or less the same previous compounds, plus some traces amounts of tannins, sterols and triterpenes, but *S. argel* (seeds) was distinguished by the presence of flavonoids. On the other hand, apolar compounds achieved through the petroleum ether were mainly triterpenes and sterols. All the foregoing chemicals seemed to be relatively higher (in terms of quantity and diversity) in *S. argel* than in *C. procera*, but this remark needs to be clarified through advance techniques. Also, seeds and flowers appeared to be richer than the leaves in the two plants, respectively.

Harbone and Turner (1984) proved the role of petroleum ether and hexane solvents in the extraction of non polar compounds (e.g., triterpenes and sterols) and alcohol (ethanol) for polar components (alkaloid and flavonoids). Investigators elsewhere also showed certain biologically active chemicals in *S. argel* and *C. procera*. For instances, Debella et al. (2008) showed the presence of saponins, alkaloids, glycosides and polyphenols as the major components of *S. argel* aqueous extracts. Watt and Breyer-Brandwijk (1962) stated that the latex of *C. procera* is a mixture of triterpenes. Later on, the aerial parts of this plant were found to contain some chemicals such as alkaloids, glycosides, flavonoids, tannins, saponins, sterols and triterpenes (Giridhar et al., 1984). Despite the fact that studies on phytochemical analysis were very scanty in Sudan, more or less similar active principles were indicated in *S. argel*

Table 1 Chemical constituents of three extracts prepared from different plant parts of *Solenostemma argel* and *Calotropis procera*.

Extracts	Chemical groups detected in different plants extracts							
	Am	Sa	Al	Fl	Fn	Tn	St	Tr
Hargel leaves water extract	–	+	+	–	+	–	–	–
Hargel seeds water extract	+	+	+	–	+	–	–	–
Usher leaves water extract	–	+	+	–	–	–	–	–
Usher flowers water extract	+	–	–	–	+	–	–	–
Hargel leaves ethanol extract	–	+	+	–	+	–	–	–
Hargel seeds ethanol extract	+	–	–	+	+	+	+	+
Usher leaves ethanol extract	–	+	+	–	–	–	–	–
Usher flowers ethanol extract	+	–	–	–	+	+	+	+
Hargel leaves pet-ether extract	–	–	–	–	–	–	+	+
Hargel seeds pet-ether extract	+	–	–	–	–	–	+	+
Usher leaves pet-ether extract	–	–	–	–	–	–	+	+
Usher flowers pet-ether extract	+	–	–	–	–	–	+	+

Am = amino acids; Sa = saponins; Al = alkaloids; Fl = flavonoids; Fn = flavones; Tn = tannins; St = sterols; Tr = triterpenes; (–) = non present; (+) = present.

by some authors (El Kamali, 1991). However, according to Satti et al. (2010), Sclepiadaceae was placed among the important flora families showing several bioactive plant species in the country.

3.2. Larvicidal effects of treatments

3.2.1. Knockdown effects

Table 2 shows the mean mortality percent of the mosquito larvae at three day intervals from treatments. Significant differences were achieved among the different treatments, with Hargel (*S. argel*) extracts gave better results than those of Usher (*C. procera*) at all counts. Irrespective of the extract type, the major trend demonstrated that the mortality means increased progressively with increasing doses and exposure time. The uppermost concentrations of leaves and seeds organic extracts (0.5%) and leaves water extract (10%) of *S. argel* showed the highest significant mortality levels (90.0 ± 0.0% mortality), which came in correspondence with the results obtained by the standard insecticides (90.0 ± 0.0%). It is clear that seeds extracts were generally superior to leaves extracts in this plant. This may be due to high active ingredients content in the seeds compared to the leaves, as discussed above. Moreover, the medium dose (0.25%) of *S. argel* seeds petroleum ether extract has scored no significant difference from that of the upper dose (0.5%) of this extract. Therefore, petroleum ether extract of *S. argel* seeds was considered the best treatment regarding its knockdown effect on the larvae.

However, the ranking of the three extracts based on their current activities against the larvae of *An. arabiensis*, showed the superiority of petroleum ether extract, followed by ethanol and lastly the water extract. Similar results were obtained by Abdul Rahuman et al. (2008), Abdu Zahir et al. (2009) and Mullai and Jebanesan (2007) who reported potent mortality effects of petroleum ether extracts of some plants, having almost similar active chemicals, against *Aedes*, *Culex* and *Anopheles* species, as compared to other extracts. Considering the attained dose–mortality relationships of the adopted extracts, comparable trends were depicted by Edriss et al. (2008) and El Tayeb et al. (2009) who studied the water extracts of *S. argel* and *C. procera* as larvicides against certain mosquito species. They showed positive relationship between larval mortalities and the increase in the concentrations of extracts. Moreover, the direct proportion detected between the mortality means and exposure time from 24 to 72 h confirmed what has been reported by several investigators, who showed gradual buildup in mortalities in relation to time factor post treatments (Tonk et al., 2006; Abdul Rahuman et al., 2008; Mullal et al., 2008; Abdu Zahir et al., 2009).

As shown above, the variations among the extracts' activities were ascribed mainly to the difference in plant species and their parts used in the current test. The literature revealed that plants contain different quantity and quality of active compounds depending on the species and its habitat environment (Satti et al., 2010). Therefore, the superior mortalities manifested by *S. argel* could be attributed to the kinds of active ingredients occurred in this plant as compared to that of *C. procera*. However, the differences in kinds and concentrations of compounds among the different parts of a plant were reported by scientists (Schmutterer, 1990). Consequently, the apparent variations in mortality effects of the two studied plant parts came consistent with previous findings which reflected the preeminence of fruiting bodies (e.g., flowers and seeds) over that of the leaves (El Tayeb et al., 2009; El Kamali, 2001). According to El Tayeb et al. (2009), *S. argel* showed comparable results with certain insecticides.

The mosquito larvicidal properties of the foregoing secondary compounds, particularly saponins, alkaloids, tannins, steroids and terpenoids, were reported in different studies. Although, the actual modes of action of all ingredients were not fully elaborated, it is most likely that these chemicals interfere mainly with certain biological, ecological and physiological aspects of the insect larvae. For instance, saponin was found to interact with the cuticle membrane in a way causing its disarrangement, which was considered as the most probable reason for larval death (Lee, 2000; Wiesman and Chapagain, 2005; Khanna and Kannabiran, 2007; Chowdhury et al., 2008). Therefore, with little interest, such vast repositories of diversified biologically active compounds in plants can provide untapped sources for environmentally safe mosquitocides with manifold activities.

Table 2 Mortality percent means of *Anopheles arabiensis* 4th instar larvae, at three intervals (24, 48 and 72 h) post treatments with different Hargel (*Solenostemma argel*) and Usher (*Calotropis procera*) extracts.

Treatments	Mortality percent means (±S.E.) at three intervals		
	24 h	48 h	72 h
Hargel leaves water extract, 2.5%	$50.2 \pm 1.6^{h,i}$	$50.2 \pm 1.6^{h,i}$	$53.8 \pm 3.1^{f,g}$
Hargel seeds water extract, 2.5%	$56.2 \pm 2.4^{f,g,h}$	$56.2 \pm 2.4^{f,g,h}$	$61.4 \pm 2.6^{d,e}$
Hargel leaves water extract, 5.0%	$63.7 \pm 4.8^{d,e}$	$63.7 \pm 4.8^{d,e}$	$64.3 \pm 3.8^{c,d}$
Hargel seeds water extract, 5.0%	$64.2 \pm 4.8^{d,e}$	$64.2 \pm 1.5^{d,e}$	68.3 ± 4.4^{c}
Hargel leaves water extract, 10.0%	$69.1 \pm 4.2^{c,d}$	$69.1 \pm 4.2^{c,d}$	69.9 ± 4.2^{c}
Hargel seeds water extract, 10.0%	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Usher leaves water extract, 2.5%	1.8 ± 0.0^{p}	1.8 ± 0.0^{p}	1.8 ± 0.0^{n}
Usher flowers water extract, 2.5%	18.0 ± 5.2^{n}	18.0 ± 5.2^{n}	17.9 ± 5.2^{l}
Usher leaves water extract, 5.0%	$26.9 \pm 6.3^{l,m}$	$26.9 \pm 6.3^{l,m}$	$31.9 \pm 3.5^{i,j}$
Usher flowers water extract, 5.0%	24.3 ± 3.0^{m}	24.3 ± 3.0^{m}	25.7 ± 3.8^{k}
Usher leaves water extract, 10.0%	$62.1 \pm 1.6^{d,e,f}$	$62.1 \pm 1.6^{d,e,f}$	$62.1 \pm 1.6^{d,e}$
Usher flowers water extract, 10.0%	38.0 ± 4.9^{k}	38.6 ± 4.9^{k}	41.6 ± 2.6^{h}
Hargel leaves ethanol extract, 0.125%	$57.5 \pm 3.2^{f,g}$	$57.5 \pm 3.2^{f,g}$	58.1 ± 2.1^{e}
Hargel seeds ethanol extract, 0.125%	$64.2 \pm 1.5^{d,e}$	$64.2 \pm 1.5^{d,e}$	$64.2 \pm 1.5^{c,d}$
Hargel leaves ethanol extract, 0.25%	72.6 ± 1.9^{c}	72.6 ± 1.9^{c}	78.9 ± 7.8^{b}
Hargel seeds ethanol extract, 0.25%	80.1 ± 7.0^{b}	80.1 ± 7.0^{b}	81.3 ± 5.8^{b}
Hargel leaves ethanol extract, 0.5%	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Hargel seeds ethanol extract, 0.5%	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Usher leaves ethanol extract, 0.125%	4.2 ± 4.9^{p}	4.2 ± 4.9^{p}	4.2 ± 0.0^{n}
Usher flowers ethanol extract, 0.125%	10.3 ± 6.1^{o}	10.3 ± 6.1^{o}	11.5 ± 7.7^{m}
Usher leaves ethanol extract, 0.25%	$21.9 \pm 1.9^{m,n}$	$21.9 \pm 1.9^{m,n}$	$22.7 \pm 1.7^{k,l}$
Usher flowers ethanol extract, 0.25%	24.2 ± 3.0^{m}	24.2 ± 3.0^{m}	$26.5 \pm 2.4^{j,k}$
Usher leaves ethanol extract, 0.5%	$47.9 \pm 4.0^{i,j}$	$47.9 \pm 4.0^{i,j}$	49.1 ± 4.0^{g}
Usher flowers ethanol extract, 0.5%	31.9 ± 3.7^{l}	31.9 ± 3.7^{l}	35.6 ± 3.1^{i}
Hargel leaves pet-ether extract, 0.125%	$61.4 \pm 2.6^{e,f}$	$61.4 \pm 2.6^{e,f}$	$66.6 \pm 3.0^{c,d}$
Hargel seeds pet-ether extract, 0.125%	$65.8 \pm 3.0^{d,e}$	$65.8 \pm 3.0^{d,e}$	69.1 ± 4.2^{c}
Hargel leaves pet-ether extract, 0.25%	$67.8 \pm 6.7^{c,d}$	$70.1 \pm 4.2^{c,d}$	75.1 ± 4.2^{b}
Hargel seeds pet-ether extract, 0.25%	$84.2 \pm 6.7^{a,b}$	$84.2 \pm 6.7^{a,b}$	90.0 ± 0.0^{a}
Hargel leaves pet-ether extract, 0.5%	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Hargel seeds pet-ether extract, 0.5%	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Usher leaves pet-ether extract, 0.125%	$42.1 \pm 2.2^{j,k}$	$42.1 \pm 2.2^{j,k}$	42.1 ± 2.2^{h}
Usher flowers pet-ether extract, 0.125%	30.0 ± 1.3^{l}	30.0 ± 1.3^{l}	$30.6 \pm 1.5^{i,j}$
Usher leaves pet-ether extract, 0.25%	$46.7 \pm 3.9^{i,j}$	$46.7 \pm 3.9^{i,j}$	$53.8 \pm 3.6^{f,g}$
Usher flowers pet-ether extract, 0.25%	31.3 ± 2.5^{l}	31.3 ± 2.5^{l}	$31.3 \pm 2.5^{i,j}$
Usher leaves pet-ether extract, 0.5%	$54.4 \pm 4.7^{g,h}$	$54.4 \pm 4.7^{g,h}$	$56.3 \pm 4.2^{e,f}$
Usher flowers pet-ether extract, 0.5%	$51.4 \pm 3.5^{g,h,i}$	$51.4 \pm 3.5^{g,h,i}$	$52.0 \pm 4.6^{f,g}$
Malathion 50%, 10 ml/l	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Abate, 1 ml/l	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Water control	0.0 ± 0.0^{p}	0.0 ± 0.0^{p}	0.0 ± 0.0^{n}
C.V.%	3.5	3.5	3.3

Pet-ether = petroleum ether.
Means with the same letter (s) are not significantly different at 5% level, according to Duncan's Multiple Range test.

3.2.2. Residual effects

The results of the residual tests of organic and water extracts of *S. argel* and *C. procera*, in comparison with those of the two insecticides, are presented in Table 3. Significant differences were recorded between the different treatments at the three indicated residual intervals. Nevertheless, most of the treatments showed diminishing activities as the residual periods extended from three days to two and three weeks following treatments. During the first interval (3 days), petroleum ether (81.4 ± 7.0–90.0 ± 0.0% mortality) and water (82.7 ± 14.7–85.9 ± 8.2%) extracts of both leaves and seeds of *S. argel* reflected significantly higher mortalities than the rest of the extracts (1.8 ± 0.0–73.6 ± 1.9%), and still were comparable with the results of Malathion and Abate insecticides (90.0 ± 0.0%). However, the reasons behind the superior effects of water extracts over those of ethanol extracts, contrasting the previous experiment, were unclear. In the second (14 days) and third (21 days) inspection periods, petroleum ether extract of *S. argel* at 0.5% concentration showed the best significant results (58.1 ± 3.6% mortality) of botanical treatments, but came next in order after the synthetic insecticides (90.0 ± 0.0%). Conversely, almost all extracts of *C. procera* were not significantly different from the water control.

The above findings revealed that *S. argel* treatments, especially the highest dose of petroleum ether extract, provided satisfactory significant control of *An. arabiensis* larvae up to three weeks of exposure. The credited effect of such a treatment may be related to certain long lasting bioactive compounds embedded in the chemical classes, triterpenes and sterols, found in the plant oil. Although, very few studies were dealt with this as-

Table 3 The residual effects of different Hargel (*Solenostemma argel*) and Usher (*Calotropis procera*) extracts on mortality percent of *Anopheles arabiensis* 4th instar larvae, at different intervals from treatments.

Treatments	Mortality percent means (±S.E.) at different intervals		
	3 days	14 days	21 days
Hargel leaves water extract, 2.5%	50.6 ± 20.3^{g,h,i}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Hargel seeds water extract, 2.5%	52.0 ± 5.0^{f,g,h}	25.8 ± 1.5^{j}	15.2 ± 2.5^{h,i}
Hargel leaves water extract, 5.0%	61.8 ± 12.7^{d,e,f}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Hargel seeds water extract, 5.0%	62.7 ± 1.4^{c,d,e}	34.4 ± 2.0^{g,h}	30.9 ± 7.4^{f,g}
Hargel leaves water extract, 10.0%	82.7 ± 14.7^{a}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Hargel seeds water extract, 10.0%	85.9 ± 8.2^{a}	39.2 ± 1.9^{f,g}	33.1 ± 4.3^{e,f}
Usher leaves water extract, 2.5%	1.8 ± 0.0^{r}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher flowers water extract, 2.5%	14.0 ± 2.8^{p,q}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher leaves water extract, 5.0%	20.5 ± 6.5^{o,p}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher flowers water extract, 5.0%	21.1 ± 1.7^{n,o,p}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher leaves water extract, 10.0%	41.5 ± 4.2^{i,j,k}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher flowers water extract, 10.0%	36.9 ± 2.0^{j,k,l}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Hargel leaves ethanol extract, 0.125%	54.4 ± 4.7^{e,f,g,h}	28.7 ± 8.7^{i,j}	14.6 ± 10.0^{h,i}
Hargel seeds ethanol extract, 0.125%	55.0 ± 4.7^{e,f,g,h}	16.2 ± 3.6^{k}	1.8 ± 0.0^{j}
Hargel leaves ethanol extract, 0.25%	63.6 ± 3.0^{c,d}	40.4 ± 3.0^{f}	27.9 ± 1.6^{g}
Hargel seeds ethanol extract, 0.25%	62.1 ± 2.6^{d,e,f}	30.6 ± 1.5^{h,i}	1.8 ± 0.0^{j}
Hargel leaves ethanol extract, 0.5%	72.6 ± 1.9^{b,c}	47.3 ± 3.0^{e}	37.5 ± 2.3^{e}
Hargel seeds ethanol extract, 0.5%	73.6 ± 1.9^{b,c}	44.4 ± 6.1^{e,f}	1.8 ± 0.0^{j}
Usher leaves ethanol extract, 0.125%	1.8 ± 0.0^{r}	1.8 ± 0.0^{lm}	1.8 ± 0.0^{j}
Usher flowers ethanol extract, 0.125%	1.8 ± 0.0^{r}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher leaves ethanol extract, 0.25%	17.1 ± 2.8^{p,q}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher flowers ethanol extract, 0.25%	15.0 ± 2.8^{p,q}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher leaves ethanol extract, 0.5%	41.5 ± 4.2^{i,j,k}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher flowers ethanol extract, 0.5%	35.0 ± 1.9^{j,k,l}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Hargel leaves pet-ether extract, 0.125%	47.9 ± 4.0^{h,i}	23.3 ± 4.8^{j}	1.8 ± 0.0^{j}
Hargel seeds pet-ether extract, 0.125%	60.1 ± 2.6^{d,e,f}	31.3 ± 1.3^{h,i}	16.2 ± 3.6^{h,i}
Hargel leaves pet-ether extract, 0.25%	56.9 ± 3.2^{e,f,g}	32.5 ± 3.2^{h,i}	19.9 ± 4.8^{h}
Hargel seeds pet-ether extract, 0.25%	65.9 ± 1.5^{c,d}	41.0 ± 2.2^{e,f}	33.0 ± 1.4^{d,e,f}
Hargel leaves pet-ether extract, 0.5%	90.0 ± 0.0^{a}	49.6 ± 1.3^{c,d}	49.6 ± 1.3^{c}
Hargel seeds pet-ether extract, 0.5%	81.4 ± 7.0^{a,b}	58.1 ± 3.6^{b}	58.1 ± 3.6^{b}
Usher leaves pet-ether extract, 0.125%	32.6 ± 3.7^{k,l,m}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher flowers pet-ether extract, 0.125%	27.2 ± 1.3^{l,m,n}	1.8 ± 0.0^{l,m}	1.8 ± 0.0^{j}
Usher leaves pet-ether extract, 0.25%	39.2 ± 2.0^{j,k,l}	14.0 ± 2.8^{k}	1.8 ± 0.0^{j}
Usher flowers pet-ether extract, 0.25%	33.8 ± 3.7^{k,l,m}	7.9 ± 8.3^{l}	1.8 ± 0.0^{j}
Usher leaves pet-ether extract, 0.5%	50.2 ± 20.3^{g,hi}	28.0 ± 1.6^{i,j}	1.8 ± 0.0^{j}
Usher flowers pet-ether extract, 0.5%	46.2 ± 3.9^{h,i}	16.2 ± 3.6^{k}	1.8 ± 0.0^{j}
Malathion 50%, 10 ml/l	90.0 ± 0.0^{a}	71.9 ± 4.4^{a}	69.9 ± 2.9^{a}
Abate, 1 ml/l	90.0 ± 0.0^{a}	73.8 ± 3.6^{a}	70.8 ± 3.5^{a}
Water control	0.0 ± 0.0^{r}	0.0 ± 0.0^{l,m}	1.8 ± 0.0^{j}
C.V.%	5.8	3.1	2.8

Pet-ether = petroleum ether.
Means with the same letter (s) are not significantly different at 5% level, according to Duncan's Multiple Range test.

pect, variable residual durations were reported from some extracts of this plant. For example, El Tayeb et al. (2009) reported high efficacy of aqueous extract up to six days post treatment. Also, the methanol extract of the aerial parts of *S. argel* was found to be effective for one to seven days against mosquito larvae (El Kamali, 2001).

However, the current results of the two bioassay tests proved that the oil extract of *S. argel* was the most potent botanical treatment which showed the best knockdown and residual activities against the larvae of *An. arabiensis*. This crude oil extract should also be evaluated under field conditions so as to be exploited for practical use as the most promising and cheap alternative mosquito larvicide. Meanwhile, the product should be stressed in advanced research to enhance its potency and utility through proper formulations. Although, rapid degradation is expected in field application which may necessitate frequent treatments, the advantage is that no long term residual activities and harmful effects are likely to be occurred in the environment. Since most breeding sites of mosquitoes can easily be detected for spraying, the use of such inexpensive botanical extract for larval control can safely minimize the buildup of the vector population and its consequent transmission of the malaria disease.

4. Conclusion

The results showed that the seed petroleum ether extract of *S. argel* at 0.5% concentration induced the best significant control of *An. arabiensis* larvae under laboratory conditions, as compared with the other tested botanical extracts. Such results were comparable with those obtained by Malathion and Abate insecticides during three days of exposure, but came next in

ranking thereafter. The activities of the mentioned extract were attributed to potent secondary metabolites in the plant, which need further investigation. Since significant residual performance was manifested for up to three weeks following treatments, the extract should be evaluated under field conditions for proper utilization in larval control. Meanwhile, the product should be stressed in additional research to enhance its potency and utility through proper formulations.

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