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Evaluation of water and ethanol extracts of *Schinus molle* Linn. against immature *Culex quinquefasciatus* Say (Diptera: Culicidae)

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PEER REVIEW

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Comments

This scientific research finding contributes additional knowledge for possible exploration of *S. molle* for mosquito control program. The outcome may have great commercial application in the field of vector control by identifying and isolating novel bioactive compounds to develop ecofriendly product for human welfare.

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ABSTRACT

Objective: To evaluate larvicidal and pupicidal activities of aqueous and ethanol extract of different parts of *Schinus molle* against filarial vector, *Culex quinquefasciatus* (*Cx. quinquefasciatus*) in the laboratory.

Methods: The mortality rate of third, fourth instar larvae and pupal stages were tested at 20, 40, 60, 80 and 100 mg/L of plant extract using WHO standard protocol with modifications. The mortality rate was recorded continuously for 24, 48 and 72 h post exposure period and percentage mortality was calculated.

Results: Maximum percentage mortality of third instar was 83.3% in ethanol extract of mature fruit at 100 mg/L after 24 h exposure period. After 48 h exposure period, 93.3% percentage mortality was recorded in ethanol extract of immature fruit at 100 mg/L. After 72 h exposure period, 100% mortality was recorded in water extract of leaf at 100 mg/L. In fourth instar larvae, maximum percentage mortality of 63.3% was recorded in water extract of mature fruit and ethanol extract of immature and mature fruit at 100 mg/L after 24 h exposure period. After 48 h exposure period 86.6% mortality was recorded in ethanol extract of mature fruit at 100 mg/L. After 72 h exposure period, 93.3% mortality was recorded in ethanol extract of mature fruit at 100 mg/L. In general immature *Cx. quinquefasciatus*, percentage mortality was increased with increase in exposure time and concentration of the plant extracts tested.

Conclusions: From this laboratory study, *Schinus molle* plant parts were proved to have larvicidal and pupicidal activity against immature *Cx. quinquefasciatus*.

KEYWORDS

Schinus molle, *Culex quinquefasciatus*, Larvicidal, Pupicidal, Water, Ethanol, Extracts

1. Introduction

Southern house mosquito, *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is a cosmopolitan in distribution especially in the tropical and subtropical areas and transmits disease causing organisms of lymphatic filariasis, Saint Louis encephalitis virus and Western equine encephalitis virus[1,2]. In Western Ethiopia, lymphatic filariasis is known to be endemic in Gambella region and the prevalence rate of hydrocoele and lymphedema of lymph was estimated to be 0.8% and 3.6% respectively. Thirty four of 112 districts were found to be endemic and the prevalence rate varied, namely, >20%, 10%-20% and 5%-9% in 9, 14 and 20 districts respectively. Twenty nine of 34 endemic districts were found in three regions in which 7 from Gambella, 13 from Beneshangul-Gumuz and 9 from Southern National and Nationalities of Peoples region. The other 5 districts were 2 from Amhara region and 3 from Oromia region. Significantly higher prevalence was also noticed in known onchocerciasis endemic districts compared to non-onchocerciasis endemic

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districts[3].

Mosquito control primarily depends on application of organophosphates such as temephos and fenthion and insect growth inhibitors such as diflubenzuran and methoprene[4]. Due to negative impact of chemical pesticides, natural products of plant origin with insecticidal properties are considered as one of the alternative methods tried in recent past in order to control variety of insect pests and vectors. The highest *Cx. quinquefasciatus* larval mortality was observed in methanol extract of *Asparagus recemosus* root with ovicidal and adulticidal properties[5]. Kovendan *et al.* reported that methanol extract of *Acalypa alnifolia* was highly toxic against immature mosquitoes of *Anopheles stephensi*, *Aedes aegypti* (*Ae. aegypti*) and *Cx. quiquefasciatus*[6]. Methanol extract of *Duranta erecta* leaves was reported to be more potent against *Cx. quinquefasciatus*[7]. In addition to plant extracts, essential oil from the *Rosamarium officinalis* was proved effective against DDT susceptible, DDT resistant and field strains of *Cx. quinquefasciatus* larvae[8].

The experimental plant *Schinus molle* (*S. molle*) is traditionally used for treating variety of wound and infections due to its antibacterial and antiseptic properties. In addition, it also has been used as an antidepressant, diuretic, tooth ache, rheumatism and menstrual disorders. *S. molle* was considered as a good candidate to use as an alternative to synthetic chemicals in pest control[9]. The fruit extracts had strong ovicidal activity against first instar nymphs and eggs of Chaga's disease vector *Triatoma infestans*[10]. The ethanol and petroleum ether extracts from the leaves and fruits were reported to have significant repellent and mortality effect against *Blatella germanica*[11]. A significant repellent and insecticidal activity of fruit and leaf essential oil was reported against *Trogoderma granarium* and *Tribolium castaneum*[12]. The elm leaf beetle, *Xanthogaleruca luteola* mortality was greater than 97% with two higher concentrations (4.3% and 4.7% w/v) of ethanol extract. However, 100% feeding inhibition was reported in aqueous extract[13]. The elemol was one of the main compound found in essential oil which had repellent activity and was suggested as a fumigant for controlling *Sitophilus oryzae*[14,15]. Mossebo *et al.* reported that LC_{50} and LC_{90} value of leaf oil was 21.0 mg/L, 37.3 mg/L respectively against *Anopheles arabiensis* and 9.6 mg/L and 15.0 mg/L respectively against *Ae. aegypti*[16]. The LC_{50} value of seed extract was 26.5 mg/L and LC_{90} value was 45.4 mg/L against *Anopheles arabiensis*. The LC_{50} and LC_{90} value of 14.5 mg/L and 28.5 mg/L respectively was reported against *Ae. aegypti*. In Ethiopia, there is no much work on larvicidal and pupicidal effect of *S. molle* plant extracts against filarial vector, *Cx. quinquefasciatus*. Therefore, present study was aimed to evaluate larvicidal and pupicidal effects of aqueous and ethanol extracts of various parts of *S. molle* against immature *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Mosquito larvae collection and maintenance

The immature *Cx. quinquefasciatus* mosquito larvae were collected from the pockets of stagnant water in Kehha river, Gondar. They were brought to the laboratory and maintained in a plastic container by providing powdered dog biscuit and yeast powder (3:1 ratio) as a source of feed. After acclimatization in the laboratory conditions they were used for subsequent experiment.

2.2. Collection and processing of plant materials

Leaves, immature fruits, mature fruits of *S. molle* were collected from Maraki campus, University of Gondar and also around Gondar town, Ethiopia. The different parts of the plants were collected randomly from 10 plants and mixed together. The plant materials were thoroughly washed with water to remove unwanted debris from the natural environment and dried under shade in order to prevent denaturation of chemical substances. After drying, plant materials were powdered using electric blender and sieved through kitchen strainer to obtain fine powder and used for extraction.

2.3. Extraction process

A total of 20 g plant powder was taken in 200 mL conical flask; 100 mL of solvent such as ethanol and water was added individually and the mouth was tightly covered with cotton plug followed by aluminium foil and kept in a shaker for 12 h continuous agitation to facilitate through mixing and to get homogenous solution. The liquid part was removed by using Whatman filter paper and the residue discarded. The liquid part was kept inside the oven at 55 °C for complete evaporation of solvents. After solvent evaporation, residue was collected, weighed and stored in a refrigerator for subsequent experiment.

2.4. Preparation of concentration

Stock solution of 100 mg/L was prepared by adding 100 mg of plant residue mixed with 1 mL of acetone and made up to 1 000 mL by adding tap water. From this stock solution, 0.1% of soap powder was added for emulsification purpose.

From the stock solution, five different concentrations such as 20, 40, 60, 80 and 100 mg/L were prepared. All these concentrations were tested against third, fourth instar larvae and pupal stage of *Cx. quinquefasciatus*.

2.5. Larvicidal activity of plant extracts

Larvicidal activity of water and ethanol extract of *S. molle* was evaluated using World Health Organization method with modifications[17]. Ten third and fourth instar larvae were released in 100 mL test tube and the concentration of water and ethanol extract was maintained at 20, 40, 60, 80 and 100 mg/L. In control, except plant materials remaining all were added as mentioned in the preparation of concentration. The larval mortality rates were recorded continuously for 24 h, 48 h and 72 h post exposure period. The dead larvae were identified when they failed to move when the water disturbed. The dead larvae in three replications were counted individually and converted in to percentage mortality. The corrected percent mortality was calculated by using Abbott's formula[18].

Corrected percent mortality (%)=(% mortality in test-% mortality in control)/(100-% mortality in control)×100

2.6. Pupicidal activity of plant extracts

Pupicidal activity of the plant extracts was tested with the same concentration and methods followed as mentioned in the larvicidal activity. For pupicidal, 10 freshly emerged pupae were released in each concentration individually and the percentage of mortality was recorded continuously for 24 h, 48 h and 72 h post exposure period. The experiments were replicated three times and the percentage mortality was calculated. The corrected percentage pupal mortality was calculated based on Abbotts formula as mentioned in the larvicidal activity.

2.7. Statistical analysis

The percentage mortality values obtained from the three replications at different concentrations and also exposure period were subjected to statistical analysis. The percentage mean±SD were calculated. The significant larvicidal and pupicidal activity of *S. molle* extract was compared with Two-way analysis of variance at 5% level. The statistical analysis was carried out by using Microsoft Office Excel 2003 program.

3. Results

Table 1 indicates percentage mortality of third instar larvae of *Cx. quinquefasciatus* exposed to different concentration of the plant extracts after 24 h post exposure period. The maximum percentage mortality of 83.30% was recorded in ethanol extract of mature fruit at 100 mg/L followed by 76.60% mortality in immature fruit ethanol extract. At 80 mg/L, maximum mortality of 70.00% was recorded in ethanol extract of immature fruit. At 60 mg/L concentration, except for water extract of leaf, remaining extracts produced more than 50.00% mortality. The Two-way ANOVA results indicated that within the plant extracts results were statistically not significant (F=1.85; P>0.05). However, within the concentration, percentage mortality was statistically significant (F=48.27; P<0.05). The interaction among the samples were statistically not significant (F=0.61; P>0.05).

Table 1

Mean percentage mortality of third instar larvae of *Cx. quinquefasciatus* after 24 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	23.30±5.77	30.00±10.00	46.60±5.77	60.00±10.00	73.30±5.77
IMF water	26.60±5.77	40.00±10.00	63.30±15.27	66.60±5.77	70.00±10.00
MF water	30.00±10.00	36.60±11.54	63.30±5.77	63.30±5.77	66.60±5.77
Leaf ethanol	33.30±15.27	43.30±15.27	53.30±15.27	66.60±15.27	73.30±11.54
IMF ethanol	40.00±10.00	50.00±11.54	56.60±11.54	70.00±10.00	76.60±15.27
MF ethanol	36.60±11.54	40.00±10.00	53.30±11.54	60.00±10.00	83.30±5.77

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 2 shows percentage mortality of third instar larvae of *Cx. quinquefasciatus* exposed to different concentration of the plant extracts after 48 h post exposure period. Maximum percentage mortality of 93.30% was recorded in immature fruit ethanol extract at 100 mg/L followed by mature fruit and leaf ethanol extract. At 80 mg/L, maximum mortality of 80.00% was recorded in ethanol extract of immature and mature fruit. At 60 mg/L, all the tested extract showed above 50.00% mortality. At 40 mg/L, maximum mortality of 60.00% was observed in ethanol extract of immature fruit. The water extract of immature fruit at 40 mg/L and ethanol extract at 20 mg/L produced 50.00% mortality. The Two-way ANOVA results indicated that percentage mortality was statistically significant within the plant extracts (F=4.42; P<0.05) and within the concentration tested (F=45.63; P<0.05). The interaction among the samples were statistically not significant (F=0.63; P>0.05).

Table 2

Mean percentage mortality of third instar larvae of *Cx. quinquefasciatus* after 48 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	30.00±10.00	36.60±11.54	50.00±10.00	70.00±10.00	76.60±15.27
IMF water	33.30±5.77	50.00±10.00	60.00±10.00	63.30±15.27	80.00±10.00
MF water	40.00±10.00	43.30±15.27	63.30±5.77	66.60±11.54	80.00±17.32
Leaf ethanol	40.00±17.32	46.60±20.81	66.60±5.77	70.00±15.27	90.00±10.00
IMF ethanol	50.00±10.00	60.00±10.00	73.30±5.77	80.00±10.00	93.30±11.54
MF ethanol	36.60±5.77	53.30±5.77	50.00±10.00	80.00±10.00	90.00±10.00

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 3 depicts percentage mortality of third instar larvae of *Cx. quinquefasciatus* exposed to different concentration of the plant extracts after 72 h post exposure period. Maximum mortality of 100% was recorded in water extract of leaf at 100 mg/L followed by ethanol extract of mature fruit (96.60%), water extract of mature fruit (93.30%) and ethanol extract of immature fruit (93.30%). At 80 mg/L, maximum mortality of 90.00% was recorded in water extract of leaf and ethanol extract of mature fruit. At 60 mg/L, all the tested extract showed above 50.00% mortality. At 40 mg/L, maximum mortality of 66.6% was observed in ethanol extract of immature fruit. The Two-way ANOVA results indicated that within the plant extracts (F=4.07) and within the concentration (F=78.73) percentage mortality was statistically significant (P<0.05). The interaction among the samples were statistically not significant (F=0.96; P>0.05).

Table 3

Mean percentage mortality of third instar larvae of *Cx. quinquefasciatus* after 72 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	40.00±10.00	53.30±5.77	66.60±5.77	90.00±10.00	100.00±0.00
IMF water	36.60±11.54	40.00±0.00	56.60±15.27	70.00±10.00	86.60±5.77
MF water	40.00±10.00	46.60±11.54	63.30±11.54	80.00±10.00	93.30±5.77
Leaf ethanol	40.00±10.00	53.30±15.27	70.00±10.00	76.60±11.54	90.00±10.00
IMF ethanol	46.60±11.54	66.60±5.77	70.00±20.00	86.60±11.54	93.30±11.54
MF ethanol	40.00±10.00	56.60±5.77	70.00±10.00	90.00±10.00	96.60±5.77

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 4 indicates percentage mortality of fourth instar larvae of *Cx. quinquefasciatus* exposed to different concentration of plant extracts after 24 h post exposure period. Results revealed that maximum percentage mortality of 63.30% was recorded in water extract of mature fruit, ethanol extract of immature and mature fruit at 100 mg/L. At 80 mg/L, maximum mortality of 56.60% was recorded in water extract of mature fruit followed by water extract of immature fruit (53.30%), ethanol extract of immature (50.00%) and mature fruit (50.00%). At 60 mg/L, 53.30% mortality was recorded in water extract of mature fruit. The Two-way ANOVA results indicates that within the plant extracts (F=7.08) and within the concentration (F=38.55) percentage mortality was statistically significant (P<0.05). The interaction among the samples were statistically not significant (F=0.63; P>0.05).

Table 4

Mean percentage mortality of fourth instar larvae of *Cx. quinquefasciatus* after 24 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	16.60±5.77	20.00±10.00	33.30±11.54	36.60±5.77	40.00±10.00
IMF water	23.30±5.77	33.30±5.77	40.00±10.00	53.30±5.77	50.00±10.00
MF water	23.30±5.77	26.60±5.77	53.30±11.54	56.60±5.77	63.30±5.77
Leaf ethanol	20.00±10.00	26.60±5.77	36.60±15.27	43.30±5.77	50.00±10.00
IMF ethanol	30.00±10.00	36.60±5.77	43.30±11.54	50.00±10.00	63.30±5.77
MF ethanol	26.60±5.77	33.30±5.77	43.30±15.27	50.00±10.00	63.30±11.54

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 5 shows percentage mortality of fourth instar larvae of *Cx. quinquefasciatus* exposed to different concentration of plant extracts after 48 h post exposure period. Maximum percentage mortality of 86.60% was recorded in ethanol extract of mature fruit followed by water extract of immature fruit (76.60%) and ethanol extract of immature fruit (73.30%) at 100 mg/L (Table 5). At 80 mg/L level a maximum mortality of 73.30% was recorded in ethanol extract of mature fruit followed by immature fruit ethanol extract (70.00%). At 60 mg/L, 56.60% mortality was recorded in mature fruit water extract followed by immature fruit ethanol extract (53.30%). The Two-way ANOVA results indicated that within the plant extracts (F=7.12) and within the concentration (F=56.47) percentage mortality was statistically significant (P<0.05). The interaction among the samples were statistically not significant (F=1.23; P>0.05).

Table 5

Mean percentage mortality of fourth instar larvae of *Cx. quinquefasciatus* after 48 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	23.30±5.77	26.60±5.77	43.30±5.77	43.30±15.27	53.30±5.77
IMF water	26.60±5.77	33.30±5.77	46.60±5.77	56.60±5.77	76.60±5.77
MF water	26.60±5.77	30.00±10.00	56.60±15.27	60.00±0.00	66.60±5.77
Leaf ethanol	30.00±0.00	33.30±5.77	46.60±15.27	50.00±10.00	70.00±10.00
IMF ethanol	36.60±5.77	50.00±10.00	53.30±15.27	70.00±10.00	73.30±5.77
MF ethanol	30.00±10.00	40.00±10.00	43.30±15.27	73.30±20.18	86.60±5.77

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 6 reveals the percentage mortality of fourth instar larvae of *Cx. quinquefasciatus* exposed to different concentration of plant extracts after 72 h post exposure period. The percentage mortality of 93.30% was recorded in ethanol extract of mature fruit followed by 86.60% in water extract of mature fruit at 100 mg/L level. At 80 mg/L, maximum mortality of 90.00% was recorded in ethanol extract of mature fruit followed by immature fruit ethanol extract (73.30%). At 60 mg/L, except for water extract of leaf, remaining dose level resulted in more than 50.00% mortality. At 40 mg/L, 53.30% mortality was recorded in ethanol extract of immature fruit. The Two-way ANOVA results indicated that within the plant extracts (F=3.2) and within the concentration (F=73.01) percentage mortality was statistically significant (P<0.05). The interaction among the samples were statistically not significant (F=0.82; P>0.05).

Table 6

Mean percentage mortality of fourth instar larvae of *Cx. quinquefasciatus* after 72 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	30.00±10.00	33.30±15.27	46.60±5.77	63.30±5.77	70.00±10.00
IMF water	30.00±0.00	36.60±5.77	53.30±15.27	63.30±15.27	83.30±5.77
MF water	30.00±10.00	36.60±5.77	53.30±15.27	70.00±10.00	86.60±5.77
Leaf ethanol	33.30±5.77	40.00±10.00	60.00±10.00	66.60±5.77	80.00±10.00
IMF ethanol	36.60±5.77	53.30±20.81	53.30±15.27	73.30±5.77	76.60±5.77
MF ethanol	33.30±5.77	40.00±10.00	56.60±15.27	90.00±10.00	93.30±5.77

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 7 indicates percentage pupal mortality of the *Cx. quinquefasciatus* exposed to different concentration of plant extracts after 24 h post exposure period. Results revealed that except for water extracts remaining all extracts showed above 50.00% mortality at 100 mg/L. At 80 mg/L, maximum mortality of 50.00% was recorded in ethanol extract of immature fruit, water extract of mature and immature fruit. At 60, 40 and 20 mg/L concentration, all the extracts showed less than 50.00% mortality. The Two-way ANOVA results indicated that within the plant extracts (F=7.14) and within the concentration (F=65.26) percentage mortality was statistically significant (P<0.05). The interaction among the samples were statistically not significant (F=0.76; P>0.05).

Table 7

Mean percentage mortality of pupal stage of *Cx. quinquefasciatus* after 24 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	13.30±5.77	16.60±5.77	30.00±10.00	36.60±5.77	40.00±10.00
IMF water	20.00±0.00	26.60±5.77	36.60±5.77	50.00±10.00	56.60±5.77
MF water	23.30±5.77	26.60±5.77	46.60±11.54	50.00±10.00	60.00±0.00
Leaf ethanol	16.60±5.77	30.00±10.00	40.00±10.00	43.30±5.77	60.00±10.00
IMF ethanol	20.00±10.00	30.00±10.00	46.60±5.77	50.00±10.00	66.60±5.77
MF ethanol	23.30±5.77	33.30±5.77	36.60±5.77	40.00±10.00	63.30±11.54

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 8 shows percentage pupal mortality of the *Cx. quinquefasciatus* exposed to different concentration of plant extracts after 48 h post exposure period. The percentage mortality of 80.00% was recorded in water and ethanol extract of immature fruits at 100 mg/L followed by 76.60% in ethanol extract of mature fruit. At 80 mg/L, a maximum mortality of 73.30% was recorded in ethanol extract of immature fruit followed by mature fruit (70.00%). At 60 mg/L, 50.00% mortality was recorded in ethanol extract of immature fruit. The Two-way ANOVA results indicated that within the plant extracts (F=9.23) and within the concentration (F=93.85) percentage mortality was statistically significant (P<0.05). The interactions among the samples were statistically not significant (F=1.13; P>0.05).

Table 8

Mean percentage mortality of pupal stage of *Cx. quinquefasciatus* after 48 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	16.60±5.77	26.60±5.77	36.60±5.77	40.00±10.00	56.60±5.77
IMF water	20.00±10.00	30.00±10.00	43.30±5.77	53.30±5.77	80.60±11.01
MF water	23.30±5.77	26.60±5.77	40.00±10.00	56.60±5.77	70.00±10.00
Leaf ethanol	26.60±5.77	36.60±5.77	43.30±11.54	46.60±11.54	73.30±5.77
IMF ethanol	30.00±10.00	43.30±5.77	50.00±10.00	73.30±5.77	80.00±10.00
MF ethanol	23.30±5.77	36.60±5.77	43.30±15.27	70.00±17.32	76.60±5.77

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

Table 9 reveals percentage pupal mortality of the *Cx. quinquefasciatus* exposed to different concentration of plant extracts after 72 h post exposure period. The maximum percentage mortality of 90% was recorded in water extract of mature fruits at 100 mg/L followed by 86.60% in water extract of immature fruit and 83.30% in ethanol extract of mature fruit. At 80 mg/L, maximum mortality of 80.00% was recorded in ethanol extract mature fruit followed 76.60% in water extract of mature fruit. At 60 mg/L, 60.00% mortality was recorded in ethanol extract of mature fruit followed by ethanol extract of leaf (56.60%). The Two-way ANOVA results indicates that within the plant extracts (F=4.34) and within the concentration (F=100.64) percentage mortality was statistically significant (P<0.05). The interaction among the samples were statistically not significant (F=1.08; P>0.05).

Table 9

Mean percentage mortality of pupal stage of *Cx. quinquefasciatus* after 72 h exposure period.

Extracts tested	Concentration (mg/L)				
	20	40	60	80	100
Leaf water	23.30±5.77	30.00±10.00	40.00±10.00	56.60±15.27	66.60±5.77
IMF water	26.60±5.77	40.00±10.00	50.00±10.00	66.60±11.54	86.60±5.77
MF water	26.60±5.77	33.30±5.77	46.60±5.77	76.60±5.77	90.00±10.00
Leaf ethanol	30.00±0.00	36.60±5.77	56.60±11.54	70.00±10.00	76.60±5.77
IMF ethanol	33.30±5.77	46.60±15.27	50.00±10.00	73.30±5.77	73.30±5.77
MF ethanol	30.00±10.00	33.30±15.27	60.00±10.00	80.00±10.00	83.30±11.54

Values are mean±SD of three replications. IMF: immature fruit, MF: mature fruit.

4. Discussion

Plant products have been used traditionally in many parts of the world against vectors and other harmful species of insects. Many plant derived chemicals are non-toxic to man and domestic animals that may serve as eco-friendly resources for the development of safer and more selective mosquito insecticides[19]. Mosquitoes control in the larval stages are worthwhile to minimize the adult population and also easy to handle due to their breeding habitat. The application of chemical pesticides in the breeding places poses a threat to non target organisms and also causes environmental pollution. The pesticides derived from plants are natural which may not cause deleterious effects to environment and other beneficial organisms due to biodegradable nature. Aromatic plants which contain essential oils are highly valuable source for use as mosquito repellents. In the present study, water and ethanol extract of *S. molle* was tried in the laboratory to find out their bioactive potential against immature filarial vector, *Cx. quinquefasciatus*. *S. molle* is an evergreen ornamental tree bearing pinnately compound leaves. It produces woody seeds that turn from green to red, pink or purple. These plants are growing extensively in Ethiopian highlands and

readily available for utilization.

In the laboratory findings immature larvicidal properties of *S. molle* was varied significantly based on the solvent used for the extraction and period of exposure. The mean percentage mortality of third and fourth instar larvae was maximum at higher concentration compared to lower concentration. The mortality rate was higher in third stage larvae compared to fourth stage and pupal stage. These studies clearly demonstrate that age specific concentration and solvents have to be used is important to achieve effective control of immature mosquitoes. The solvent may determine solubility of the phytochemicals from the leaf, immature fruit and mature fruit. The variation in larvicidal and pupicidal efficacy of the plant extracts may be associated with chemical nature of the compounds. A specific compound germacrene D isolated from the leaf extracts proved to have repellent activities against cattle ticks and aphids[20,21].

The mean percentage mortality of the pupal stage was significantly varied from lower concentration to higher concentration. The overall mortality rate was greater in water extracts compared to ethanol. The variation in percentage mortality observed in the plant extracts also may be due to the nature and quantity of the chemical components and their dissolving ability in solvents. The significant mortality observed in the present study was in agreement with the report of Abdel-Satttar *et al*[12], who has reported that insecticidal and insect repellent activity of leaf and essential oil extracts against *Trogoderma granarium* and *Tribolium castaneum* was associated with monoterpene hydrocarbon and β-cymene. The oily nature of the extract blocks the respiratory system of the insects causing suffocation and larval. Another study also confirmed that ethanol extract caused greater mortality at higher concentration against elm leaf beetle, *Xanthogaleruca luteola*[13]. The elemol was another biologically active compound that have repellent activity against *Sitophilus oryzae*[14,15]. The mortality rate of immature *Cx. quinquefasciatus* in the present study was also in agreement to previous reports mentioned earlier. The results of present study, clearly indicate that water and ethanol extracts have both larvicidal and pupicidal properties against *Cx. quinquefasciatus*. Further isolation of bioactive molecules and to develop simple and effective formulation techniques may lead to the development of effective botanical pesticides that can be used to control *Cx. quinquefasiciatus* in their breeding places.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Cx. quinquefasciatus is one of the vectors need to be controlled in order to prevent wide spread transmission of filarial disease. Ecofriendly vector control is one of the challenging and important tasks to the scientific communities to avoid resistance of vectors to synthetic insecticides. Searching potential bioactive plant from the wide spread floral diversity is the road to identify novel biopesticidal compound for sustainable vector control program.

Research frontiers

The water and ethanol extracts of various parts of *S. molle* was found to be most effective against *Cx. quinquefasciatus* immature stages. The age related percentage mortality was significantly greater in higher concentration and period of exposure.

Related reports

The plant *S. molle* has been used as traditional medicine and a few reports by other workers proved to show its repellant activity against some insects. The current study demonstrated its larvicidal and pupicidal activities against immature *Cx. quinquefasciatus*.

Innovations and breakthroughs

The authors evaluated at different concentrations of water and ethanol extracts of leaves, immature fruits and matures fruits against immature stages of *Cx. quinquefasciatus*. They have recorded significantly different dose and duration depended percentage mortalities in their studies.

Applications

Vector control through ecofriendly method is important to prevent resistance development and environmental pollution. *S. molle* plant extracts are proved to have larvicidal and pupicidal activity against *Cx.*

quinquefasciatus. Future isolation of bioactive principle is useful for the development of novel biopesticide.

Peer review

This scientific research finding contributes additional knowledge for possible exploration of *S. molle* for mosquito control program. The outcome may have great commercial application in the field of vector control by identifying and isolating novel bioactive compounds to develop ecofriendly product for human welfare.

References

[1] Reisen WK, Lothrop HD, Lothrop B. Factors influencing the outcome of mark-release recapture studies with *Culex tarsalis* (Diptera: Culicidae). *J Med Entomol* 2003; **40**(6): 825-829.

[2] Jones CE, Lounibos LP, Marra PP, Kilpatrick AM. Rainfall influences survival of *Culex pipiens* (Diptera: Culicidae) in a residential neighborhood in the mid-atlantic United States. *J Med Entomol* 2012; **49**(3): 467-473.

[3] Shiferaw W, Kebede T, Graves PM, Golasa L, Gebre T, Mosher AW, et al. Lymphatic filariasis in Western Ethiopia with special emphasis in and around onchocerciasis endemic areas. *Trans Royal Soc Trop Med Hyg* 2012; **106**(2): 117-127.

[4] Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS. A piperidien amide extracted from *Piper longum* L fruit shows activity against *Aedes aegypti* mosquito larvae. *J Agri Food Chem* 2002; **50**: 3765-3767.

[5] Govindarajan M, Sivakumar R. Ovicidal, larvicidal and adulticidal properties of *Asparagus recemosus* (Willd.) (Family: Asparagaceae) root extracts against filariasis (*Culex quinquefasciatus*), dengue (*Aedes aegypti*) and malaria (*Anopheles stephensi*) vector mosquitoes (Diptera: Culicidae). *Parasitol Res* 2014; **113**(4): 1435-1449.

[6] Kovendan K, Murugan K, Vincent S. Evaluation of larvicidal activity of *Acalypa alnifolia* Klein ex Willd. (Euphorbiaceae) leaf extracts against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and bancroftian filariasis vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2012; **110**: 571- 581.

[7] Serena MM, Balasubramani MS, Rajan K, Gerald IA. Evaluation of the larvicidal activity of the leaf extracts of *Duranta erecta* Linn. (Verbanaceae) on the larvae of *Culex quinquefasciatus* (Say) (Culicidae). *J Biopest* 2010; **3**(3): 582-585.

[8] Yu J, Liu XY, Yang B, Wang J, Zhang FQ, Feng ZL, et al. Larvicidal activity of essential extract of *Rosmarinus officinalis* against *Culex quinquefasciatus*. *J Am Mosq Control Assoc* 2013; **29**(1): 44-48.

[9] Ferrero AA, Alejandra M, Cristina B, Zanettia N. Acute and subacute toxicity evaluation of ethanolic extract from fruits of *Schinus molle* in rats. *J Ethnopharmacol* 2007; **113**(3): 441-447.

[10] Ferrero AA, Werdin González JO, Sánchez Chopa C. Biological activity of *Schinus molle* on *Triatoma infestans*. *Fitoterapia* 2006; **77**(5): 381-383.

[11] Ferrero AA, Chopa CS, Gonzalez JO, Alzogarav RA. Repellence and toxicity of *Schinus molle* extracts on *Blatella germanica*. *Fitoterapia* 2007; **78**(4): 311-314.

[12] Abdel-Sattar E, Zaitoun AA, Farag MA, Gaved SH, Harraz FM. Chemical composition, insecticidal and insect repellent activity of *Schinus molle* L. leaf and fruit essential oil against *Trogoderma granarium* and *Trobolium castaneum*. *Nat Prod Res* 2010; **24**(3): 226-235.

[13] Amanda H, Italo C, Karla P, Fernando A, Jaime EA. Toxicity and repellence of aqueous and ethanolic extracts from *Schinus molle* on elm leaf beetle *Xanthogaleruca luteola*. *Crop Prot* 2010; **29**: 1118-1123.

[14] Benzi V, Stefannazzi N, Ferrero AA. Biological activity of essential oils from leaves and fruits of pepper tree (*Schinus molle* L.) to control rice weevil (*Sitophilus oryzae* L). *Chilean J Agric Res* 2009; **69**: 154-159.

[15] Upadhyay RK, Ahmad S. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. *World J Agric Sci* 2011; **7**: 527-549.

[16] Massebo F, Tadesse M, Bekele T, Balkew M, Gebre-Michael T. Evaluation on larvicidal effect of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera: Culicidae) in Ethiopia. *Afr J Biotechnol* 2009; **8**(1): 4183-4188.

[17] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 1996. [Online] Available from: http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?ua=1 [Accessed on 9th Mar, 2014]

[18] Abbott W. A method of computing effectiveness of an insecticide. 1925. *J Econ Entomol* 1925; **18**: 265-267.

[19] Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Bum. F. (Family: Malvaceae) against three important mosquitoes. *Asian Pac J Trop Med* 2010; **3**(9): 691-695.

[20] Bruce TJ, Birkett MA, Blande J, Hooper AM, Martin JL, Khambay B, et al. Response of economically important aphids to components of *Hemizygia petiolata* essential oil. *Pest Manag Sci* 2005; **61**: 1115-1121.

[21] Birkett MA, Al Abassi S, Kröber T, Chamberilain K, Hooper AM, Guerin PM, et al. Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*. *Phytochemistry* 2008; **69**: 1710-1715.