Potential larvicidal effects of *Tephrosia vogelii* leaf extract on *Culex quinquefasciatus* in Morogoro, Tanzania

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Abstract: *Tephrosia vogelii*, a local plant, was tested for its potential effects on *Culex quinquefasciatus* larvae. Two sets of experiments, one using distilled water and the other using septic tank water as diluents and control were carried out. In the first set of experiments, a total of 2700 third stage larval instars were tested against different concentrations of water/ extracts of *T. vogelli* leaves. Ten replicates were performed for each concentration. The average mortality rates were 3.3%, 16.3%, 26.3%, 71.02%, 86.0%, 91.7%, 97.7%, 99.3% and 100% at 0%, 0.01%, 0.02%, 0.05%, 0.1%, 0.5%, 1%, 2% and 4% leaf extract concentrations, respectively. In the second set of experiments where septic tank water was used, 1500 third stage larval instars were tested against different concentrations of leaf extract and five replicates were performed for each concentration. The average mortality rates were 0%, 32.0%, 44.5%, 79.7%, 99.2%, 99.2%, 100% and 100% at 0%, 0,01%, 0.02%, 0.05%, 0.1%, 0.5%, 1% and 2%, respectively. It was concluded that *T. vogelii* leaf extract has insecticidal effects against *Cx quinquefasciatus* larvae and that septic tank water diluted extracts of the material were more effective than distilled water diluted extracts. Further investigations are recommended to determine the active ingredients of the extract and on how the use of this plant extract can be integrated into mosquito control strategies.

Key words: *Tephrosia vogelli*, larvicide, *Culex quinquefasciatus*, Tanzania

Introduction

Mosquitoes are among the best-known groups of insects due to their important role as pests and vectors. Mosquitoes are economically important due to the effects they cause on man and animals. They are, a great nuisance and their bites may be severe, causing swellings and severe itching, followed by incessant scratching and the formation of pustules (Mellenby, 1946; Goma, 1966). This may be followed by restlessness, loss of sleep, nervous irritation and generalized ill health of affected animal/man. Such effects can result in reduction of animal performance.

The importance of mosquitoes is mainly on their role as major intermediate hosts and vectors of several important diseases of man and animals. These include human and avian malaria, human and animal filariases, rickettsial infections such as *Borrelia anserina* in fowl and many virus infections of man and animals including Rift Valley fever, Yellow fever, Eastern and Western Equine encephalitis, West Nile fever, St. Louis and Japanese B encephalitis and Dengue fever (Goma, 1966; Soulsby, 1982).

Several methods have been employed for controlling mosquitoes. These include the use of chemical compounds and biological agents. Biological methods are mainly used to control larvae whereby larvivorous fish (mainly *Gambusia spp*) have been used in lakes, ponds and water pools (Soulsby, 1982; Service, 1983). Chemical compounds (insecticides) have been used, both as adulticides and larvicides. These include organophosphates, organochlorines, natural pyrethrins, synthetic pyrethroids, carbamates and amitraz. These insecticides are usually applied on water bodies for larvae control and on walls, ceilings, animal bodies and other mosquito resting places for adult control. This method has a number of limitations. The insecticides are very expensive and hence, most members of communities cannot afford them. Some mosquito species have developed resistance to various insecticides, a fact facilitated by extended usage and application of sub-lethal doses (WHO, 1986, 1992). Furthermore some insecticides are highly pollutant to the environment due to their low biodegradability and accumulation in the soil, plant tissues, and/or animal bodies (Msalale, 1999).

In view of the problems associated with chemical control a search for alternative but environmentally safe means of controlling arthropod pests and vectors is desirable. Natural products including local plants are among the potential candidates being searched as alternative approaches for controlling mosquitoes. Preliminary investigations have suggested that *Ocimum americanus, Citrus sinesis* (orange), *Citrus limon* (lemon) and *Citrus aurantium* (bitter orange) peel extracts are potentially effective against mosquito larvae (White, 1973; Mwaiko *et al.*, 1989).

Tephrosia vogelii, a local plant found in many parts of Tanzania, is reportedly toxic to various organisms. This is a wild robust, soft woody, branching nodulated legume belonging to the family *Papilionaceae*. It grows to a height of up to 4m. It is reported to be adapted to a wide range of climatic conditions ranging from dry to moist tropics (500 -

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2500mm annual rainfall) (Milne-Redhead and Polhill, 1991). However, it is poorly adapted to low rainfall areas with periodic dry spells (Mugoya and Chinsembu, 1993; Otsyna *et al.*, 1995; Ngazi and Kapinga, 1998). Although its original home is Africa, it has spread to other parts of the world where it has been used for many purposes, ranging from windbreaks, ornamentals, green manure, rodenticides, pesticides and insecticides. It is for this reason that in the United States of America and Puerto Rico, the plant is currently grown on a commercial scale (Mugoya and Chinsembu, 1993).

The chemical constituents of *T. vogelii* are 2.85-4% N, 0.38% P, 1.03% K, 1.89% Ca, 0.16% Mg, 8.3-17.74% lignin, 2.37% polyphenols, 21.1% cellulose and 0.97% rotenones (Hagedorn *et al.*, 1997; Mutuo *et al.*, 1998). Based on these chemical constituents and on characterization of organic materials by Palm *et al* (1997), the biomass of *T. vogelii* is classified as high quality organic material because its N-content is >2.5% and lignin content is <15%. However, according to Gaskins *et al.* (1972) (as cited by Mugoya and Chinsembu, 1993) leaves of *T. vogelii* contain, at least four compounds that possess insecticidal properties. These are rotenone, deguelin, tephrosin and *6 a, 12 a* - dehydrodeguelin. They are generally referred to as rotenoids. Other *Tephrosia* species are rich in flavonoids, notably isopongaflavone and Tetra-hydroisopongaflavone or Tephrinone (Mugoya and Chinsembu, 1993). Unlike the synthetic organic insecticides, these compounds are well known for their low persistence in the environment (Mugoya and Chinsembu, 1993). Rotenoids also inhibit cellular respiratory metabolism by blocking electron generation from reduced nicotinamide adenine dinucleotide. As a result, oxidation of lactate, glutamate, and other substances is reduced and nerve conduction is adversely affected.

In some areas, it has been reported that the leaves of *T. vogelli* are used for poisoning fish in rivers and ponds. The plant is also grown in cassava and sweet potato farms so as to kill rats that eat its roots that grow through (rodenticide and pesticide) or across the farmer tunnels. The leaves of *T. vogelii* have also been reported to have insecticidal effects against ticks and fleas (Mugoya and Chinsembu, 1993). Similar effects of this plant against mosquitoes cannot be ruled out and a need to investigate and establish such effects is desirable. Furthermore, the use of natural and locally available products can easily be adopted and sustained by community. Thus the objective of this study was to determine the potential larvicidal effects of *T. vogelii* leaf extract on *Culex quinquefasciatus*. This was done specifically, to determine the possible toxic effects of *T. vogelii* leaf extract on larvae of *Cx quinquefasciatus,* the filariasis mosquito.

Materials and methods

Source of materials

A survey was carried out at Vibandani area in Morogoro Municipality to locate breeding sites for *Cx quinquefasciatus* mosquitoes. Broken septic tanks that were abundantly infested with Culex larvae and pupae were then located. Substantial numbers of (larvae and pupae) were collected by dipping large conical flask and/or beaker into the tank. The specimens were then taken to an insectary that was maintained at 27±2.5°C and 80±10% relative humidity. By using plastic Pasteur pipettes, all the larvae were transferred to enamel or stainless steel trays (31.5 cm x 20 cm x 2.2 cm capacity) half-filled with distilled water. Pupae were similarly transferred to 200ml glass beakers half filled with distilled water. The larval trays were put on benches in the insectary and provided with larval food. The pupae in beakers were put in mosquito cages in the same area. The specimens were observed daily, and any larvae that pupated were transferred to the cages.

The emerged adult mosquitoes were maintained on sugar solution on a piece of cotton wool and placed on top of the cage. Whenever mosquitoes were required to oviposit, a young guinea pig was partly shaved, put in a wire-mesh strainer and placed in the cage overnight for the adult female mosquito to feed on. Beakers with little distilled water were put in the same cage after the mosquitoes had fed on blood so as to provide suitable breeding facility for engorged female mosquitoes to lay eggs. The stock colonies so established were used to provide experimental larvae. The latter were used at their third instar stage, usually on the 8th - 9th day after hatching from eggs.

Green leaves of *T. vogelii* were obtained from the trees at the Rodent Research Project compound at Sokoine University of Agriculture, Morogoro. The leaves were processed and used in the experiments while fresh. Whenever the experiments were not completed on the same day, the processed leaves were kept in a refrigerator.

After harvesting, fresh leaves of *T. vogelii* were ground with motor and pestle. The resulting cake was weighed and thoroughly mixed with a known volume of distilled water or septic tank water and thus making various concentrations (w/v) of leaf extract. The mixture was left at room temperature for 24 hours and then squeezed and filtered through a 33mm wire mesh/ muslin. The filtered mixture was used as stock solution from which various concentrations were prepared and tested against mosquito larvae.

Experimental protocol

For each test, late third stage larvae of the same age (8-9 days), and size, and which were raised under the same conditions, were used. Various concentrations of the leaf extract were prepared using distilled water or septic tank water as diluent. Clean plastic cups (150ml capacity each) were numbered. Each of cups 1-3 was filled with 29ml distilled water while the rest were filled with similar amounts of various extract concentrations (Table 1). Twenty-seven clean test tubes were prepared serially. Batches of ten experimental larvae were gently taken together with 1ml of water from the rearing beaker/tray using Pasteur pipette and put into each of 27 tubes. Each batch of larvae was transferred to the plastic cup bearing corresponding number. The plastic cups were left at room temperature for 24 hours. Numbers of dead larvae in each plastic cup were counted and recorded. Moribund larvae were considered as dead. Ten replicates were done for each concentration of the extract. The whole procedure was repeated with septic tank water as diluent instead of distilled water. In the latter set of experiments five replicates were carried out. To confirm if the larvae were dead or not, the larvae were put in petri dishes containing distilled water and a thin beam of torchlight was pointed at them. Larvae that were not capable of swimming away from the light were confirmed as dead.

Data analysis

Statistical difference between proportions (percentage of larvae killed) in two sets of experiments was determined using Epi-info 6.04 Epitable programme (Coulombier *et al.*, 2001), with critical probability P=0.005.

Results

A total of 3,900 third stage larvae were tested with various concentrations of the leaf extract. Mean numbers and percentages of killed larvae were calculated and recorded as shown in Table 2 and 3. The mortalities shown were the averages of ten (10) replicates of experiments done for each concentration using distilled water as diluent and five (5) replicates using septic tank water as a diluent.

Since the control mortality in the larvae tested with septic tank water/leaf extract solution was between 5% and 20%, the data were corrected by Abbots formula (Table 3). Abbots formula takes care of deaths due to factors other than the material being tested (e.g. effects of solvent, natural deaths, effects of toxic substances from water, etc). The corrected results showed mean mortality rates as indicated in Table 3. Generally, significantly higher mortality rates of larvae were observed in leaf extract+septic tank mixture than in leaf extract+distilled water (Table 4). However the difference was not observed at ≥1% concentration.

Table 1: Aqueous concentrations of leaf extract tested against third stage Culex larvae

Weight of cake (gm)	Amount of diluent (Distilled or septic water) (ml)	% Concentration (w/v)	Plastic cup No.
0 (Control)	100	0	1-3
0.1	1000	0.01	4-6
0.2	1000	0.02	7-9
0.5	1000	0.05	10-12
0.1	100	0.1	13-15
0.5	100	0.5	16-18
1	100	1	19-21
2	100	2	22-24
4	100	4	25-27

Table 2: Effects of *Tephrosia vogelii* leaf extract on third stage Culex larvae using distilled water as diluent

Concentration of leaf extract (%) (w/v)	Total number of larvae tested (10 replicates)	Total number of larvae killed	% larvae killed
0.00	300	10	3.3
0.10	300	49	16.3
0.02	300	79	26.3
0.05	300	213	71.0
0.1	300	258	86.0
0.5	300	275	91.7
1	300	293	97.7
2	300	298	99.3
4	300	300	100

Table 3: Effects of *Tephrosia vogelii* leaf extract on third stage Culex larvae using septic tank water as diluent

Concentration of leaf extract (%) (w/v)	Number of larvae tested (5 replicates)	Number of larvae killed	Mortality rate (%) before correction	Mortality rate (%) after *correction
0	150	22	14.7	0
0.01	150	63	42.0	32.0
0.02	150	79	52.7	44.5
0.05	150	124	82.7	79.7
0.1	150	149	99.3	99.2
0.5	150	149	99.3	99.2
1	150	150	100	100
2	150	150	100	100

* Corrected by Abbots formula:

$$\text{Mortality Rate} = \frac{\%\ \text{test mortality} - \text{Control mortality}}{100\% - \%\ \text{Control mortality}} \times 100$$

Table 4: The proportion of larvae killed by *T. vogelli*+distilled water and *T. vogelli*+septic tank water

Concentration of leaf extract (%) (w/v)	Percentage of larvae killed		*P*-value
	Using distilled water as diluent	Using septic tank water as diluent	
0.01	16.3	32.0	0.00014
0.02	26.3	44.5	0.00009
0.05	71.0	79.7	0.040
0.1	86.0	99.2	0.000006
0.5	91.7	99.2	0.001
1.0	97.7	100.0	0.13

Discussion

The findings of this study suggest that *T. vogelii* leaf extract has larvicidal effects on *Cx quinquefasciatus* larvae. The mortality rates increased with increasing concentrations of the leaf extract. These observations are consistent with those observed with different arthropods elsewhere. For instance, in Zambia a 15% w/v aqueous leaf extract effectively reduced stem borer damage and lowered the severity of maize streak virus (Mugoya and Chinsembu, 1993). According to Kaposhi (1993), a 10% w/v concentration of aqueous leaf extract adequately controlled the tick *Boophilus decolaratus* larvae on cattle and provided a residual protection lasting for at least 10 days. On farm testing of *T. vogelii* extract in controlling ticks conducted in Mwagala village in Mwanza, results indicated up to 70% of the ticks were killed by less than 20% concentration of the extract (Hamza *et al.*, 1987). Furthermore, Msalale (1999), observed some insecticidal effects of *T. vogelii* on adult *Ctenocephalides felis*, the commonest livestock fleas

in Tanzania. This effect was also shown to increase with increasing concentrations of the leaf extract.

Our findings further revealed that *T. vogelii* leaf extract at lower concentrations and diluted with septic tank water is more toxic to *Cx quinquefasciatus* larvae than the same extract diluted with distilled water. This is probably due to effects caused by other toxic materials present in septic tank water. Since polluted water such as found in septic tank, is the natural breeding site of *Cx quinquefasciatus*, those observations can be justifiably interpreted to suggest that application of as low as 1% concentration of *T. vogelii* leaf extracts to polluted water, can effectively kill *Cx quinquefasciatus* larvae.

In view of our findings it can be concluded that, *T. vogelii* leaf extract is effective against *Cx quinquefasciatus* larvae, and thus it can be applied for controlling such mosquitoes especially in areas where the mosquito breeds abundantly in septic tanks, pit latrines and other polluted water bodies. However, further studies on the extract that will involve extraction and determination of the active ingredients are recommended to establish possible toxicity and other side effects on animals, man and other non-target organisms. The insecticidal/larvicidal effects of *T. vogelii* on other mosquitoes such as *Anopheles* and *Aedes*, which are important vectors of human and animal diseases and studies to establish the nature of *T. vogelii* leaf extract activity in the mosquito larvae need to be carried out.

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