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Larvicidal Activity of *Tephrosia vogelii* Crude Extracts on Mosquito Larval Stages

H. Matovu and D. Olila
Department of Veterinary Physiological Sciences, Faculty of Veterinary Medicine,
Makerere University, P.O. Box 7062, Kampala, Uganda

Abstract: The use of natural products and biological insect control methods is gaining importance because of concerns about the environment, since they are more easily biodegradable. In some parts of Uganda, organic farmers have adopted the use of *Tephrosia vogelii,* a shrubby, leguminous and woody plant for control of storage pests. However, the efficacy of *Tephrosia vogelii* crude extracts in the control of *Dipteran* insect larvae under field conditions has not been well tested. Their use for the control of insect vectors such as mosquitoes has not also been fully evaluated. *Tephrosia vogelii* plant materials were collected from two selected sites, one on a higher altitude than the other using polythene study. The material was chopped, properly labeled and air-dried in a shade for two weeks. Four solvents where used for extraction: Water, Petroleum ether, Chloroform, Methanol. The extract was dried in an oven at about 32-33°C for several days, after which it was weighed and stored in the fridge at 4°C until the time of exposing the mosquito larvae. Shoot Evening Methanol (SEM) was the most effective among methanol extracts; killing an average of 4.57 mosquito larvae in 8 min while Shoot Evening Water (SEW) was the most effective of water extracts killing an average of 2.57 mosquito larvae in 8 min; hence the SEM was considered to be nearly two times more efficacious than SEW on mosquito larvae, at a concentration of 25%: 10.8% or 2.3:1 SEM: SEW, respectively. *Tephrosia vogelii* crude extracts could potentially therefore be used to control the larval stages of mosquitoes.

Key words: Larvicidal activity, crude extracts, mosquito, larval stage, SEW, SEM

INTRODUCTION

Malaria is a leading killer disease in Africa. Its control and probable eradication revolves around the control of the mosquitoes, which is very difficult. This is because whatever option is selected should be environmentally friendly. This calls for a careful selection of suitable insecticides. The criteria for selection should put in consideration aspects such as minimizing exposure to human and other non-target harmless organisms and minimizing insecticide residues in the environment (Mohan *et al*., 2005). In addition it should be effective and affordable, should have a rapid action, should be readily available and easy too use. At the present moment control of mosquitoes using DDT has attracted a lot of interest in some circles but also a great uproar from environmentalists.

While eradication of insects is nearly impossible, with an increasing demand of environmental conservation and rapidly growing population it is prudent to investigate biological control and botanical/natural insecticides in preference for non-biodegradable synthetic insecticides (Baruah, 2004). Several studies are being done in many laboratories across the world to derive larvicidal agents against the mosquito (Chaithong *et al*., 2006; Choochote *et al*., 2006; Chansang *et al*., 2005; Amer and Mehlhorn, 2006; Bassolé *et al*., 2003).

The present study was designed to explore possibility of using *Tephrosia vogelii* extracts and mainly focused on the raw crude extracts that can be processed easily by local communities in the rural villages of Uganda with the aim of availing cheaper alternatives in the control of insects and mosquitoes in particular. Here we now report the results of larvicidal assays that screened indigenous *Tephrosia vogelii* plants as a possible remedy for mosquito control in Uganda.

MATERIALS AND METHODS

Study area: This study was carried out in Busimbi Sub County in South-Western Uganda. *Tephrosia vogelii* samples where collected from Lubanjja village. The plant was introduced there by a non governmental Organization (Rural Community In Development (RUCID))

Corresponding Author: H. Matovu, Department of Veterinary Physiological Sciences, Faculty of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda

that promotes organic farming. It was introduced for use in the control of a wide variety of insects parasites in crop, animal and households, the plant was also used to fix nitrogen in the soil.

Field data collection: A questionnaire were designed and administered in the place that was well known to have grown and or used the plant before (Lubanjja village). Homesteads in the area were given numbers from 1-50, nine pieces of papers each with a numbers from 0-9 were placed in a black polythene paper and four papers were picked at random one by one with replacement and recording the number each time to make a set of four numbers. Then the first and last numbers of the set were circled. These represented a whole number. The first ten numbers that ranged between 1-50 were chosen and these represented the homesteads that were selected to answer the questionnaire.

Plant material

Collection and pre-extraction procedure: The morning (7.00 A.M.) samples were collected near the valley and in the evening (6.00 P.M.) sample near the hilltop. The samples were collected 600 m apart on a 10% slope. All the *T.vogelii* samples where collected during the dry season.

The plant materials collected were root (radix *Tephrosia vogelii*), cortex (cortex *Tephrosia vogelii*), leaves, flowers and fruits (herba *Tephrosia vogelii*).They were put into a polythene bag; the material was then chopped into small sized pieces; root (1 cm), shoot (0.5 cm), cortex (1 cm). The chopped material was then air dried at room temperature in a shade (no sunshine).

Extraction procedure: The air-dried samples were weighed and then placed in clean conical flasks. The amount of solvent to be placed in the conical flasks was measured (the level of solvent in the conical flasks was kept at least 2 cm above the plant material). The conical flasks were covered with airtight seals and wrapped with an opaque paper to shield the mixture from light. The mixture was then left to stand for 2-7 day, shaking of the contents was done daily for about one to three hour. After 7 days the mixture was filtered into a clean container or conical flask. The solvent was then evaporated off with the help of a Rotavapor and water bath at about 60-70°C. The concentrated extract was then placed in clean, dry vials and dry in an oven at 30-31.5°C. The plant materials were mixed with the solvent as above and the mixture was left to stand for a prolonged time (about seven days) with periodic daily shaking (for about one to three hours); separation of the extract from the debris was done by filtration of the solvent. Crude extracts were stored at 0-4°C in airtight glass vials covered with aluminium foil paper to minimize any degradation by aerial oxidation and light. 0.5 g of the dry extract were weighed into a clean and dry container (vial). Two milliliters of Dimethyl Sulfoxide (DMSO) were then added to the container with the dry extract and mixed thoroughly until the solid dissolves.

Fig. 1: Mosquito larvae culture unit

Larvicidal assays

Mosquito larvae culture: A container was placed outside in a partial shade (Fig. 1). A shallow container with a large surface area was used. The container was filled with pre-conditioned freshwater obtained from rainwater collected in a low air polluted areas in an under ground concrete water tank. Fresh cow dung was added to the water as manure and was then properly mixed to form a uniform mixture and left to settle for four days to allow for slow decomposition so as to provide a source of microbes and organic matter. After four days when the water had cleared, a handful of amaranthus was added to provide the mosquitoes with a place upon which to rest while laying their eggs in the water.

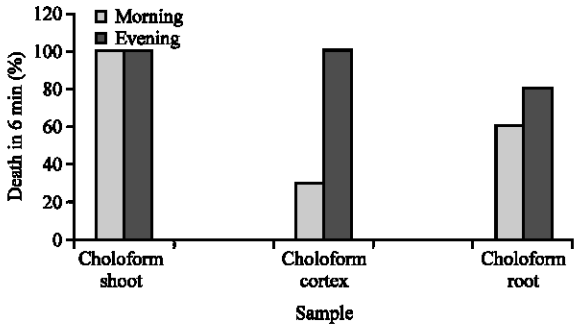

Fig. 2: Larvicidal effects of extracts obtained from morning and evening samples

Larvicidal assays: Total 0.5 mL of the concentrated crude extract were applied to ten mosquito larvae in a test tube containing 5 mL of water, (1st, 2nd, 3rd and 4th, instars mosquito larvae). The mortality of the larvae at different instars was assessed after every 2 min for 20 min, 24 and 48 h after treatment. The same number of larvae was prepared in a similar manner and treated as described in the controls. Drowning malformed larvae were recorded as being dead. The mortality was determined by counting survivors at the exposure period. The experiments were conducted at room temperature. The extract was applied directly on the water containing the larvae in a test tube. Observations were made and records were taken. Control specimens were prepared in a similar way as the test specimen. Total 0.5 mL of 12.5% m/v Amitraz was used as positive control and added to the control specimen in the same way it was done for the test specimen. Total 0.5 mL of DMSO was used as negative control and added to the control specimen in the same way it was done for the test specimen. Results were analyzed using ratios, percentages, bar graphs and t-test. Microsoft Excel and Stata 8.0 were used as tools. The t-test was used to compare the killings at the different time during exposure of the same sample.

RESULTS

Extracts and yields: Estimation of the amount of extract obtained from water extracts was difficult because it was not easy completely evaporate off all the water. However, calculating the concentration of water extracts was possible because measurements were available, sample SEW, CEW and REW had concentrations 10.8, 9 and 18.3%, respectively. Only the evening samples were used for water extracts, this was to mimic the field situation where leaves are collected after photosynthesis has started. The average extract yield of morning extracts and evening extracts were 0.04 and 0.042 g per one gram of plant raw material, respectively. The average extract production of both morning and evening extracts could therefore be considered to be the same when weighing errors are factored in. This implies that there was no significant loss of active ingredients due to plant photosynthesis or respiration.

Table 1: Mean mosquito larvae kill in eight minutes for the most effective methanol,water,evening and morning extracts

Extract		Original no. of M. larvae	Av. M.larvae death in 8 min confidence level =0.05	Slope	t-test (p-values)
Methanol	SEM (25%)	10	4.57 (3.12-6.02)	0.52	0.162
Water	SEW (10.8%)	10	2.57 (2.27-2.87)	0.11	0.000
Evening	SEC (25%)	10	8.3 (7.28-9.39)	0.85	0.192
Morning	SMC (25%)	10	9 (8.38-9.62)	1.00	0.205

The extracts from shoot, cortex and roots had an average yield of 0.06, 0.05 and 0.015 g per one gram of plant raw material, respectively. Therefore, shoot and cortex plant parts accumulate relatively high amounts of the active ingredients in *Tephrosia* compared to the roots. This could also explain why leaves (shoot) are preferred by the local farmers for effective pest control as indicated earlier in this text, in addition to maintaining the life of the plant and hence a sustainable harvest. Methanol, Petroleum ether and Chloroform yield 0.0875, 0.0142 and 0.0172 g per one gram of plant raw material, respectively, indicating a significantly valuable yield when methanol is used for extraction than any of the other two solvents or water.

Larvicidal effects: Chloroform extracts obtained from morning and Evening shoot samples show similar killing percentage (100%) of mosquito larvae in 6 min and there fore perform best, the rest of the test samples did not show consisitent killings of mosquito larvae,therefore have not been included in the graph, however, different extracts obtained from morning and evening samples show significant killings of mosquito larvae but not at a given time.

At the same concentration, SEM and SEW have the same larvicidal effect. Extracts obtained from samples collected in the morning show a higher larvicidal effect than that of extracts from samples collected in the evening.

DISCUSSION

Mosquitoes have a relatively low susceptibility to the extracts; probably because mosquito larvae are exposed within a medium of water and leading to a considerable dilution of the extract. This implies that a higher concentration of the extract is required to attain a similar efficacy approximating to that of the extract action

on ticks that we had observed earlier (results to be reported elsewhere). However, mosquito larvae showed a high susceptibility to chloroform extracts, meaning that rotenone the major active ingredient extracted by chloroform had high efficacy on them. Extracts obtained using water are second in position following chloroform extracts in showing this trend. Mosquito larvae are highly susceptible to chloroform extracts compared to petroleum ether extracts, suggesting that mosquito larvae are more susceptible to rotenone than the other rotenoids in *Tephrosia* example tephrosin and deguelin.

Mosquito larvae were more susceptible to extracts obtained from *Tephrosia* samples collected in the morning than those collected in the evening, this is emphasized by SMC the most effective sample among extract obtained from *Tephrosia* samples collected in the morning that killed an average of 9 mosquito larvae in 8 min and SEC the most effective sample among extract obtained from *Tephrosia* samples collected in the evening that killed an average of 8.3 mosquito larvae in 8 min indicating that some active ingredients were destroyed during photosynthesis or are a result of plant respiration or rotenoids were destroyed by the sun during the day.

Extracts obtained from *Tephrosia* samples collected from the shoot and cortex had similar average killing time probably indicating that similar amounts of the active ingredients were stored within these two plant parts. Unlike the extracts obtained from root samples, these require significantly more time than the shoot and cortex extracts suggesting that less of the active ingredient is stored in the roots. This explains why the local farmers and most researchers (Dorn *et al*., 2001) use leaves (shoot) for significant insecticidal effects.

Shoot evening methanol is the most effective among methanol extracts killing an average of 4.57 mosquito larvae in 8 min while SEW was the most effective among water extracts killing an average of 2.57 mosquito larvae in 8 min. Meaning that SEM is two times more efficacious than SEW on mosquito larvae, this is at a concentration of 25%: 10.8% or 2.3:1 SEM: SEW, respectively. This implies that at the same concentration, these 2 samples have the same effect. T-test values indicated that the response of mosquito larvae to *Tephrosia* extracts in susceptible tests did not have significant differences ($p>0.05$ at a 95% confidence) except for water extracts where $p<0.05$, therefore, the results where accepted.

CONCLUSION

It was therefore, concluded that *Tephrosia* crude extracts could be used to control adult/larval insects even without any other elements like ash and urine as is currently being done by the local communities. The plant's shoot and cortex accumulate the highest but similar amount of the active ingredients when compared to roots. Methanol as a solvent yields the highest amount of crude extract from *Tephrosia vogelii*. Photosynthesis and plant respiration have an effect on the production and storage of the active ingredients in *Tephrosia* with the more effective active ingredients being found in the early morning before photosynthesis has started or the sun destroys the active ingredients during the day. Chloroform crude extracts are the most effective against mosquito larvae. Water and methanol crude extracts have the same insecticidal effect against mosquito larvae. All crude extracts of *Tephrosia vogelii* extracted using Chloroform, Methanol (alcohol), Petroleum ether and water can be effectively used under field conditions to control adult and larval stages of insects if a concentration range of 12.5-25% is maintained. The concentration depends on the economic costs of extraction and marketability of the extract.

Putting environmental factors in consideration, crude extraction by local organic farmers using water as a solvent can be effectively substituted by crude extraction using alcohol that can be brewed locally by these farmers from sweet bananas, alcohol crude extracts have several advantages over water extracts and these include, economically affordable and sustainable, easy storage and preservation, has the same efficacy for insect control as the water extract, greater amounts of the extract are obtained from a single gram of plant material compared to water extracts, extracts do not persist in the environment and therefore minimize environmental contamination.

Tephrosia vogelii samples for crude extraction should be collected early in the morning before sunrise or during the rainy season so as to yield more effective extracts. *Tephrosia vogelii* should preferably be grown on gentle slopes near the valley since the morning samples that were collected close to the valley and from gentle slopes performed better than the evening samples that were collected at a higher altitude.

Rotenone (the major active ingredient in *Tephrosia* crude extracts) has widely been used as an agricultural insecticide applied to crops and livestock to control insect pests without many complications and its ability to control tumours (WHO, 1992) emphasises the use of rotenone, as a safe insecticidal agent. Fish, insects, birds and mammals have natural enzymes that will detoxify sub-lethal amounts of rotenone and therefore the concentration has to be the recommended for appropriate action.

REFERENCES

Amer, A. and H. Mehlhorn, 2006. Larvicidal effects of various essential oils against Aedes, Anopheles and Culex larvae (Diptera, Culicidae). Parasitol. Res., 99: 466-472.

Baruah, K., 2004. Laboratory bio-assay of temephos and fenthion against some vector species of public health importance. J. Commun. Dis., 36: 100-104.

Bassolé, I.H., W.M. Guelbeogo, R. Nébié, C. Costantini, N. Sagnon, Z.I. Kabore and S.A. Traoré, 2003. Ovicidal and larvicidal activity against Aedes aegypti and Anopheles gambiae complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso. Parassitologia, 45: 23-26.

Chaithong, U., W. Choochote, K. Kamsuk, A. Jitpakdi, P. Tippawangkosol, D. Chaiyasit, D. Champakaew, B. Tuetun and B. Pitasawat, 2006. Larvicidal effect of pepper plants on *Aedes aegypti* (L.) (Diptera: Culicidae). J. Vector Ecol., 31: 138-144

Chansang, U., N.S. Zahiri, J. Bansiddhi, T. Boonruad, P. Thongsrirak, J. Mingmuang, N. Benjapong and M.S. Mulla, 2005. Mosquito larvicidal activity of aqueous extracts of long pepper (Piper retrofractum vahl) from Thailand. J. Vector Ecol., 30: 195-200.

Choochote, W., U. Chaithong, K. Kamsuk, E. Rattanachanpichai, A. Jitpakdi, P. Tippawangkosol, D. Chaiyasit, D. Champakaew, B. Tuetun and B. Pitasawat, 2006. Adulticidal activity against Stegomyia aegypti (Diptera: Culicidae) of three Piper sp. Rev. Inst. Med. Trop. Sao. Paulo., 48: 33-37.

Dorn, S., I. Schmale, F.L. Wäckers and C. Cardona, 2001. Control potential of three hymenopteran parasitoid species against the bean weevil in stored beans: The effect of adult parasitoid nutrition on longevity and progeny production. Biol. Control, 21: 134-139.

Mohan, L., P. Sharma and C.N. Srivastava, 2005. Evaluation of Solanum xanthocarpum extracts as mosquito larvicides. J. Environ. Biol., 26: 399-401.

World Health Organisation (WHO), 1992. The WHO recommended classification of pesticides by hazard Organization, (unpublished document, WHO/PCS /92.14) and guidelines to classification 1992-1993. Geneva, World Health, pp: 66.