International Journal of Tropical Medicine 2 (3): 83-88, 2007
ISSN: 1816-3319

Acaricidal Activity of *Tephrosia vogelii* Extracts on Nymph and Adult Ticks

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Abstract: Botanical pesticides exist within nearly all vector disease endemic communities of the world. Natural/ botanical tick control methods offer several advantages over synthetic tick control including environmental preservation since they have shorter residual periods with rapid action. *Tephrosia vogelii* a shrubby, leguminous and woody plant is one of the potential candidates to provide affordable botanical acaricides. However, its effectiveness in the control of *Acarina* has not been fully explored in developing countries. *Tephrosia vogelii* plant materials were collected from two selected sites, one on a higher altitude than the other. The air-dry plant material was crushed into powder; and extracted with a known volume of solvent. The mixture was left to stand for seven days with daily stirring for at least 2 h. Extracts from shoot, cortex and roots have an average yield of 0.06, 0.05 and 0.015 g per one gram of plant raw material, respectively. Shoot and cortex plant parts accumulate relatively high amounts of the active ingredients in *Tephrosia* compared to the roots; probably explaining why leaves (shoot) are preferred by the local farmers for effective pest control. 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. All extracts killed 100% of the exposed ticks but variations where noted in the time taken to achieve 100% exposed tick death. Petroleum ether, chloroform, methanol and water extracts killed 100% of the ticks in an average time of 8.3, 9.7, 10.3 and 1.3 days, respectively; implying that ticks are more susceptible to the active ingredient extracted using petroleum ether relative to the other solvents. *Tephrosia* crude extracts can potentially, therefore, be used to effectively control ticks in the Ugandan animal production systems. Photosynthesis and plant respiration seem to 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.

Key words: Acaricidal activity, vector disease, choloroform, photosynthesis, plant respiration

INTRODUCTION

Tick-borne diseases (especially East Coast fever) are very important conditions, causing serious losses in cattle. The drugs and chemicals that are currently used in Uganda are very expensive. Most farmers in Uganda are resource poor. Therefore, it is important that disease control strategies should be affordable and readily available. This can not be achieved by adopting strategies that are totally dependent on imported drugs and pesticides. The need to utilize locally available natural products (especially from higher plants) has become increasingly important not only because of the high cost of imported chemicals, but also because the availability of these imported drugs and pesticides has become erratic as their importation has to compete for the meager foreign exchange with other vital imports required for industrial and social development. Furthermore, because of the ever-increasing problem of drug resistance, there is a great demand for new chemotherapeutic agents. Plants represent an almost inexhaustible source of novel pharmaceuticals (Shafy and Zayed, 2002).

Vector control is a great challenge in many parts of the world, because the majority of vectors occupy several ecological niches that are not easily accessible. Current attitudes concerning food safety and environmental quality have raised the general public's interests in using alternative (non-synthetic pesticide) pest controls methods that are not highly toxic to the ecosystem (Panella *et al*., 1997). *Tephrosia vogelii* is one of the plants with this potential.

The pesticidal properties of many plants have been known for a long time and natural pesticides based on plant extracts such as rotenone, nicotine and pyrethrum have been commonly used in pest control for some time now. The advantages of botanical insecticides include,

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among others, rapid degradation and less persistence in environment, reduced risks to non-target organisms, may be applied shortly before harvest without leaving excessive residues (Dipeslu and Ndungu, 1991).

Tephrosia vogelii is indigenous to Africa but distributed to many other parts in the tropics where it is used as shelter, cover crop, fish poison and as a pesticide. It was introduced into agriculture for the purpose of commercial production of rotenone. It is regarded as a more promising plant than *Derris* and *Lonchocarpus spp* that are also sources of the pesticide (Barnes and Freyre, 1966). In Eastern and Southern Africa *T. vogelii* and other related species have been grown in small plantations by small holders for their use in crop protection (Watt and Breyer, 1962) and improved soil fertility.

Here we now report results of an investigation designed to explore the use of *Tephrosia vogelii* as a tick control option for the local farming communities in Uganda.

MATERIALS AND METHODS

Study area: This study was done in Busimbi Sub County in Uganda. *Tephrosia vogelii* samples were collected from a village called Lubanjja where it was introduced by a non governmental Organization, Rural Community In Development (RUCID) that promotes organic farming. The plant was introduced for use in the control of a wide variety of insect parasites in crops, animals and households. It also fixes nitrogen in the soil. *Tephrosia vogelii* is mainly grown in mixed crop garden (Fig. 1).

Plant materials

Collection and pre-extraction procedure: *Tephrosia vogelii* is mainly grown on hill slopes with some considerable soil erosion hence the justification of introducing it in the area, this also means it is grown on soils with a much lower soil fertility than in the valleys. 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.

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).

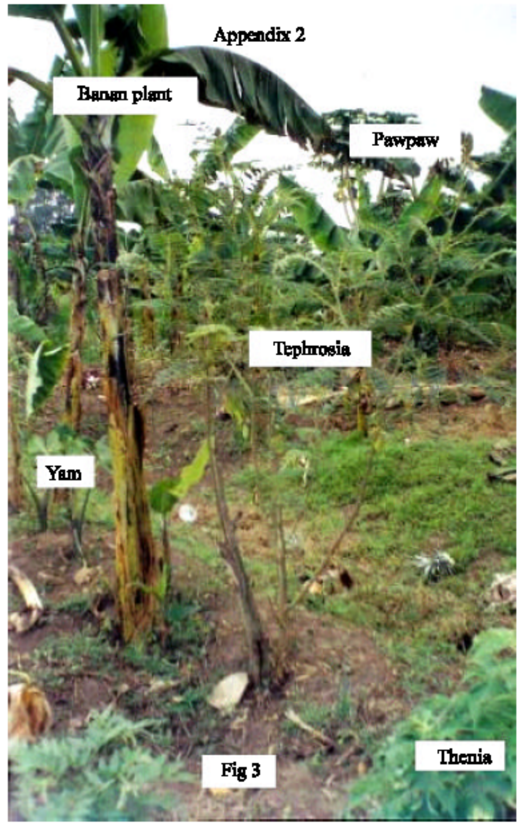

Fig. 1: Tephrosia vogelii in mixed agricultural farming systems

Extraction procedure: The solvents used were water, methanol (90%), petroleum ether and chloroform. 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 1 h. 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 dried in an oven at 30-31.5°C. 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 grams 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 to dissolve the solid.

Acaricidal assay

Tick capture: White cotton cloths were worn and pulled through the vegetation in the area of tick collection. Ticks that were questing for passing hosts clung to the cloth and were removed from both the drags and the surveyor's clothing by hand picking with fine forceps and placed in a marked vial. A T-Shaped frame was also used to hang a thick cotton cloth; the cloth was then pulled slowly over the vegetation for approximately 10 m before examination for attached ticks.

Acaricidal tests: Ticks of various genera and species were collected at various stages of development (larvae, nymph and adults). Ten ticks were placed in a specimen bottle. 0.5 mL of the crude extract per concentration was applied directly to the ticks in the specimen bottle. Ten ticks were then placed in a similar specimen bottle and used as controls. Dead ticks were then counted over the exposure time. Malformed ticks were counted as dead. All the experiments where carried out at room temperature. Control ticks were prepared in a similar way as the test specimen. 0.5 mL of 12.5 % m v^{-1} Amitraz was used as positive control and 0.5 mL of DMSO was used as negative control. 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

Indigenous knowledge: The local farmers use water as a solvent for extraction and no preservation is done, the whole extract is used after extraction and the remainder is discarded. The field findings indicate that *T.vogelii* crude extracts have reliable effects in the control of mites, ticks and fleas. The field findings also indicate that the extraction process is very brief and lasts only a few minutes; making it inefficient in extracting all the active ingredients and hence leading to wastage. However, when controlling crop pests additives example ash, urine and *Thenia* leaves are added to the *T.vogelii* crude extracts. The plant is often grown in mixed crop production systems (Fig. 1).

Comparison of the yield of extracts from different samples: Samples SEW, CEW, REW have no values in the last column because it is difficult to evaporate off the water at a temperature of 30-32°C in the oven so as to get a dry extract in addition to the solution being very difficult to filter (Table 1).

Figure 1 shows a plant of *Tephrosia vogelii* in the garden of a rural homestead, demonstrating mixed cropping.

The average extract yield of both morning and evening extracts is the same when weighing errors are considered. Shoot and cortex plant parts accumulate relatively high amounts of the active ingredient in *T.vogelii* when compared to the roots. Methanol as a solvent yields the highest amounts of the extract than any of the other two solvents (Petroleum ether and Chloroform) or water. Estimation of the amount of extract obtained from water extracts was not possible because it was difficult to completely evaporate off all the water within the limited (Table 2). However, calculating the concentration of water extracts was possible because

Table 1: Yield obtained from each sample

Sample	Solvent	Tag	Sample weight (g)	Volume of solvent (mL)	Weight of extract obtained (g)
Shoot morning	Methanol	SMM	115.0	800	13.5
Cortex morning	Methanol	CMM	55.5	450	6.6
Roots morning	Methanol	RMM	86.8	500	2.8
Shoot morning	Petroleum	SMP	112.4	800	1.8
Cortex morning	Petroleum	CMP	55.9	400	0.7
Roots morning	Petroleum	RMP	83.9	450	0.5
Shoot evening	Methanol	SEM	52.9	750	7.9
Cortex evening	Methanol	CEM	39.9	500	4.1
Roots evening	Methanol	REM	46.1	350	1.2
Shoot evening	Petroleum	SEP	56.6	600	1.1
Cortex evening	Petroleum	CEP	44.6	350	0.8
Roots evening	Petroleum	REP	48.6	350	0.6
Shoot evening	Water	SEW	59.6	550	-
Cortex evening	Water	CEW	40.5	450	-
Roots evening	Water	REW	45.6	250	-
Shoot evening	Chloroform	SEC	54.9	600	1.3
Cortex morning	Chloroform	CMC	54.0	350	0.9
Shoot morning	Chloroform	SMC	109.7	800	3.1
Roots evening	Chloroform	REC	47.3	350	0.3
Roots morning	Chloroform	RMC	75.7	350	0.2
Cortex evening	Chloroform	CEC	41.5	300	1.0

Table 2: Yield of extract per gram of plant material

Methanol		Petroleum ether		Chloroform	
Sample	Weight of extract (g)	Sample	Weight of extract (g)	Sample	Weight of extract (g)
Shoot Morning Methanol (SMM)	0.120	Shoot Morning Petroleum ether (SMP)	0.016	Shoot Morning Chloroform (SMC)	0.030
Shoot Evening Methanol (SEM)	0.150	Shoot Evening Petroleum ether (SEP)	0.020	Shoot Evening Chloroform (SEC)	0.020
Cortex Morning Methanol (CMM)	0.120	Cortex morning Petroleum ether (CMP)	0.013	Cortex Morning Chloroform (CMC)	0.020
Cortex Evening Methanol (CEM)	0.100	Cortex evening Petroleum ether (CEP)	0.020	Cortex Evening Chloroform (CEC)	0.024
Root Morning Methanol (RMM)	0.032	Root Morning Petroleum ether (RMP)	0.006	Root Morning Chloroform (RMC)	0.003
Root Evening Methanol (REM)	0.030	Root Evening Petroleum ether (REP)	0.010	Root Evening Chloroform (REC)	0.006

Table 3: Average tick death in six minutes for the most effective methanol, water, evening and morning extracts

Extract		Original no. of ticks	Av. tick death in 6 min confidence level =0.05	Slope	t-test (p-values)
Methanol	RMM (25%)	10	8 (6.99-9.01)	1.00	1.0000
Water	SEW (10.8%)	10	2.6 (0.74 –4.46)	0.63	0.0085
Evening	REP (25%)	10	8 (6.76-9.24)	2.00	1.0000
Morning	SMC (25%)	10	9.5 (9.19-9.81)	0.50	1.0000

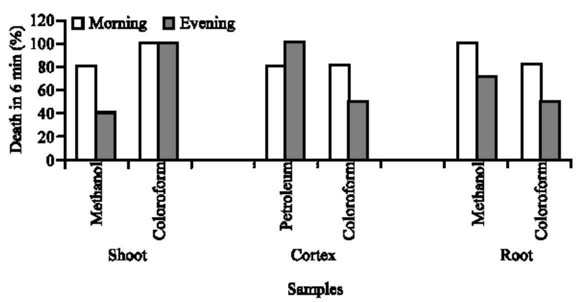

Fig. 2: Acaricidal effects of extracts obtained from morning and evening samples

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).

Acaricidal effect: Chloroform extracts obtained from morning and evening shoot samples show similar killing percentage (100%) of ticks in 6 min and there fore perform best, the rest of the test samples did not show consistent killings of ticks. However, different extracts obtained from morning and evening samples show significant killings of ticks though not at a given time (Fig. 2).

A higher concentration (32.4%) of SEW extract was required to obtain the same acaricidal effect as 25% RMM extract (Table 3). Therefore, a lot more material is required when water is used as a solvent than methanol. This implies that methanol was a more effective solvent in extracting active ingredients in *T.vogelii* with acaricidal effects than water. Extracts obtained from samples collected in the morning showed a higher acaricidal effect than that of extracts from samples collected in the evening.

DISCUSSION

The average extract yield of morning extracts and evening extracts are 0.04 and 0.042 g per one gram of plant raw material, respectively. The average extract production of both morning and evening extracts can therefore be declared the same when weighing errors are considered. This implies that there is no significant loss of active ingredients due to plant photosynthesis or respiration.

Extracts from shoot, cortex and roots have an average yield of 0.06, 0.05 and 0.015g 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 may 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.

All extracts killed 100% of the exposed ticks but variations where noted in the time taken to achieve 100% exposed tick death. Petroleum ether, Chloroform, Methanol and Water extracts killed 100% of the ticks in an average time of 8.3, 9.7, 10.3 and 1.3 days, respectively. Implying that Ticks are more susceptible to the active ingredient extracted using petroleum ether relative to the other solvents. Since rotenone is less soluble in ether, carbon tetrachloride and petroleum solvents (WHO, 1992) it may mean that ticks are more susceptible to other rotenoids such as tephrosin and deguelin found in *Tephrosia* sp. rather than rotenone.

Extracts obtained from *Tephrosia* samples collected in the Morning and evening caused an average of 100 and 90% tick death in 14 min, respectively. REP has the most effective acaricidal results among extracts obtained from *Tephrosia* samples collected in the evening since it caused 60% exposed tick death in 2 min and 100% exposed tick death in 4 min, while SMC has the most effective acaricidal results among extracts obtained from *Tephrosia* samples collected in the morning since it caused 90% exposed tick death in 2 min and 100% exposed tick death in 4 min. This is emphasized further by the average time taken to kill 100% of the exposed ticks that is 0.19 days and 8.9 min for evening and morning extracts respectively. There was slightly higher susceptibility of ticks to morning than evening extracts. It may therefore imply that some of the active ingredients with significant acaricidal effects are destroyed during photosynthesis, either they are used up during photosynthesis or are by products of plant respiration. Since samples were collected in the dry season, the sun may have destroyed rotenone and other rotenoids during the day.

Shoot, cortex and roots extracts required an average of 0.147, 0.15 and 0.29 days, respectively to attain 100% exposed tick death. Shoot and cortex extracts show similar time meaning similar efficacy while roots showed a significantly slow action requiring twice the time required by the shoot and cortex extracts.

CONCLUSION

It was therefore, concluded that roots probably store nearly half the amount of acaricidal active ingredients when compared to the shoot and cortex. The cortex and shoot parts of *Tephrosia vogelii* store similar amounts of acaricidal active ingredients. This would probably mean that acaricidal effects obtained from 1g of shoot are comparable to those obtained from 1g of cortex. But one needs nearly 4g of roots to obtain the same acaricidal effects because of the minute amounts of active ingredients stored in the roots. This may have implications from the conservation view point.

Root Morning Methanol had the most effective acaricidal effects among methanol extracts since this extract caused 100% exposed tick death in 6 min, while SEW performs best among the water extracts because it causes 90% exposed tick death in 20 min. One requires a concentration of 32.4 % of SEW extract so as to achieve the same acaricidal effect as a 25% RMM extract, this is because the active ingredients (rotenone and other rotenoids) have a low solubility in water at ambient temperature (WHO, 1992). This implies that one requires a lot more material with water extracts as compared to methanol extracts hence increase the water extract concentration. This agrees with other studies on tick larvae using Meem oils (Ismail *et al*., 2002) where acaricidal toxicity varied directly with the concentration.

It can therefore, be concluded that *Tephrosia* crude extracts can most probably be used to control *acarina* without any other elements example ash, urine and so on. The plant's shoot and cortex seem accumulate the highest active ingredients when compared to roots. Methanol as a solvent yields the highest amount of crude extract from *Tephrosia vogelii*. Methanol crude extracts have a more efficacious acaricidal effect than water crude extracts against ticks. Chloroform crude extracts are the most effective against ticks. 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.

RECOMMENDATIONS

We therefore, recommend that all crude extracts of *Tephrosia vogelii* extracted using Chloroform, Methanol (alcohol), Petroleum ether and water can be effectively used under field conditions to control ticks, if a concentration range of 12.5-25% is maintained; *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.

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