A Research Article in AJRTC (2011) Vol. 9 No. 1: Pages 30-37

Allelopathic potential of plant extracts against *Scutellonema bradys*

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Manuscript received: 06/12/2010; accepted:15/02/2011

Abstract

Experiments were carried out in the laboratory of the International Institute of Tropical Agriculture (IITA) and the Department of Agriculture, University of Reading, UK, to investigate the toxicity of some plant materials against *S.bradys*. Laboratory investigations confirmed extracts of neem, cocoyam, ginger and maize to be effective against the yam nematode and as contact nematicides since their extracts killed the nematodes within two weeks. However, the percentage mortality was directly correlated with concentrations of the extracts and exposure time. Phytochemical screening of dried neem fruit, maize root, ginger root, and cocoyam corm powder for their chemical composition revealed the presence of oils, alkaloids, saponins, and flavonoids which may be responsible for the non-host status.

Keywords: Toxicity, extracts, mortality

Introduction

The yam nematode*, Scutellonema bradys* (Fig. 1) is a migratory endoparasite present in soils, roots, and tubers. It is the cause of a decay of tubers known as "dry rot disease" (Fig. 2) and constitutes one of the most difficult pest problems encountered in the economic production of the yam crop (Obigbesan and Adesiyan 1981). Sizeable populations of the nematode can be found in soil at the beginning of the yam growing season (Adesiyan and Badra 1982).

Yams are propagated from whole tubers or pieces of tubers which are the principal means of dissemination of *S. bradys*. Comparatively low populations of the nematode (less than 20 tubers) do not produce external symptoms of damage (Bridge 1973) and thus the risk of dissemination through tubers is greater.

The objectives of the research were: to evaluate the toxicity of crude plant extracts on the yam nematode, and determine the chemical constituents of the plant(s) that significantly suppress nematode multiplication.

Materials and methods

Neem fruits lying on the ground around the trees were collected and left to dry in the sun. The dried fruits were stored in jute sacks and later pounded carefully in a mortar into a coarse powder. Granule size of the milling was 2.36 sieve aperture. Roots of maize (TZSR-W-1) and ginger (UG1) and corms of cocoyam (Tania) were rinsed in water to remove soil. They were chopped into pieces, completely air-dried and then pounded into fine powder using a hand mill. The mesh size of sieve was 500µm (Tyler equivalent 32 mesh). A different size was used from that used for the neem fruit powder because of the difference in particle size.

To detect the best medium of extraction, ethanol and distilled water were used as follows: Ten grams of neem, ginger, cocoyam, and maize root powders were weighed into heating bottles and 100 m1 of distilled water was added to each. The bottles were then heated over a water bath for an hour, allowed to cool, and the extract was sieved through Whatman No. 1 filter paper in a funnel. The same procedure was repeated using 95% ethanol. The filtrate obtained (Plate l A) was taken to be the stock solution (100,000 ppm). Serial dilutions were prepared to obtain graded extracts of 75,000 ppm, 50,000 ppm and 25,000 ppm from the 100,000 - ppm (stock) solution. Distilled water (0 ppm) was used as the control. The effects of the extracts from neem, ginger, cocoyam and maize

roots on S. *bradys* were tested in the Germplasm Health laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. Aliquots of 1ml of nematode suspension containing 20 male and female adults of *S. bradys* were dispensed into each of the 136 transparent Syracuse dishes set up for the study. A magnetic stirrer was used to ensure that every 1 ml of the aliquot contained approximately the same number of S. *bradys.* Aliquots of 1 ml, each of ethanol and water extracts (100,000 ppm, 75,000 ppm, 50,000 ppm and 25,000 ppm) and distilled water (control) were dispensed unto the 1 ml nematode suspensions in the Syracuse dishes.

The addition of 1 ml nematode suspension and 1 ml extract (treatment) in the Syracuse dishes brought the effective concentration of ethanol and water extracts to 50,000 ppm, 37,500 ppm, 25,000 ppm and 12,500 ppm. The treatments including control were left in the laboratory at room temperature (Plate l B). The Syracuse dishes were covered with glass lids to prevent evaporation. The nematodes that formed a C-shape and did not move when touched with a needle were considered dead. These were counted and picked out of the extract every 24 hr for 14 days. There were 40 treatments in all (five concentrations by four extracts by two solvents). The experiment was replicated four times, providing 160 observations. As the experiment progressed, the Syracuse dishes containing the ethanolic extracts became so turbid and greasy that it become impossible to see through the dishes and count. As a result, the experiment involving the ethanolic extracts was terminated at this point and proceeded with only aqueous extracts.

The plant materials were sun-dried and made into powder as earlier described. They were screened for their chemical composition in the laboratory at the Department of Agriculture, University of Reading, UK, using the method of Harbone, 1973. The methodology varied slightly depending on the phytochemical that was been screened.

A test for oil was achieved using the grease spot test. A dilute ether solution for each crop was made by mixing 0.5 g of each of the powders with 10-m1 ether. One drop of the solution was placed on a clean sheet of paper and allowed to dry. Observations were made for translucent spots that confirm the presence of oil.

To test for alkaloids, approximately 0.5 g of each of the powdered materials was warmed (60 °C) with 10 ml of 20% $HZSO_4$ on a Bunsen burner for 2 min and filtered. One ml portion of each was treated with a few drops of Dragendroff's reagent. Orange-red precipitate indicated the presence of alkaloids. As a confirmatory test, Thin Layer Chromatography (TLC) plates were spotted with the various plant extracts and sprayed with Dragendorff's reagent. Orange-red colour on the spots indicated the presence of alkaloids

In the test for saponins, approximately 0.5 g of each of the powdered materials was shaken with 5 ml of distilled water and heated to boiling point. Frothing indicated the presence of saponins. As a confirmatory test, the filtrates were added to 3 ml of arachis oil and thoroughly shaken to form a stable emulsion. This was left to stand for about 5 min. The presence of a stable emulsion indicated the presence of saponins.

In the test for tannins, approximately 0.5 g of each of the powdered materials was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride reagent was added to the filtrate. A dark blue or dark green solution indicated the presence of tannins.

To test for flavonoids, one milliliter of each of the aqueous extracts was dissolved in dilute sodium hydroxide solution. A yellow solution that became colorless on the addition of hydrochloric acid confirmed the presence of flavonoids. Only these few phytochemicals were tested because equipments and reagents were not available.

A toxicity evaluation study of the plant materials on *S. bradys* was carried out by introducing plant crude extracts into aliquots of nematode suspension and observing the possible influence of allelochemicals, as already described.

Data obtained were analyzed using Generalized Linear Model, Mixed Model Procedures and Repeated Measures Analysis.

Plate 1A and 1B: Aqueous and Ethanolic plant extracts (A) and Arranged Synercus dishes containing extracts and nematode aliquots (B).

Results and Discussion

The mortality rate of *S. bradys* in crude plant extracts varied at the different levels of concentration in all the test plants (Figs. 3-6). The mortality of *S: bradys* in extract from neem was greater (P=0.05) than from cocoyam, ginger, maize, or the water control (Figs. 3-6). Extracts from cocoyam and ginger induced

more mortality than maize, but the difference was not significant. At 50,000 ppm concentration of the extracts in less than 7 days, there was total mortality of the nematodes by all the plant extracts, indicating maximum inhibitory properties.

The result of the effects of neem fruit extract on *S. bradys* is shown in Fig. 3. Neem extract at all concentrations used was lethal to the nematodes. There was a general increase in percentage nematode mortality with the increase in extract concentration. There was a maximum percentage mortality of 100% by 3 days after inoculation. However, the number of nematodes killed at specified time was directly correlated with the concentration of the extract. The control (zero concentration extract of distilled water) had the significantly lowest percentage of dead nematodes (< 10% after 7 days) throughout the experiment. Mortality rate was recorded throughout the period.

The result of the effects of cocoyam extract on *S. bradys is* shown in Fig. 4. In a pattern similar to the effect of neem extract, there was a general increase in percentage nematode mortality at all the concentrations of extracts tested on *S. bradys,* with an increase in days after inoculation. At 10 days after the introduction of the extract, 100% mortality was observed in all the concentrations. However, at a zero extract concentration (distilled water) the nematode mortality recorded was about 12%. The nematode mortality rate was directly correlated with the concentration of the extract. The cocoyam extracts had complete suppression on nematode survival as 100% mortality was recorded at the end of the experiment.
The result of the effects of ginger extract on *S. bradys* is shown in Fig. 5. There was a general increase in percentage nematode mortality with the increase in extract concentration with a maximum percentage mortality of 100% at 4 days and 10 days after inoculation for the maximum and minimum extract concentrations of 50,000 and 12,500 respectively. The least mortality was recorded with extract concentration of zero (distilled water).

The result of the effects of maize root extract on the yam nematode is shown in fig. 6. The results follow the same trend as for neem, cocoyam, and ginger. Maize root extract suppressed the nematode completely as 100% mortality was recorded by day 14. Maize root extract at the lowest concentration of 12,500 ppm significantly increased the mortality of *S. bradys* compared with the control. However, mortality of between 20 and 25% was recorded with the zero concentration (distilled water).

The results obtained from screening plant materials for chemical composition are shown in Table 1. The results revealed that all the plant materials tested contained some alkaloids, as they reacted positively to Dragendorff's reagent. The specific alkaloids and their levels were not determined. Results also showed the presence of oil in all plant parts examined except maize roots. Again, the type and quality of the oil was not determined due to limitations in experimental equipment. Results showed high saponin contents in all the samples, except maize root extract. Of all materials tested, only maize root extract tested positive for flavonoids. The results of studies on the toxicity of extracts showed that there was a general increase in the percentage number of nematodes killed with increases in extract concentration. There was 100% mortality of *S. bradys* in aqueous extracts of powdered dry neem fruit at 7 days exposure time, at 10days for cocoyam corms and ginger roots, and at 13days for maize roots. Percentage mortality was directly correlated with concentrations of the extracts. At zero concentration level, there was no significant mortality rate. In this study the increased concentration of extracts had a significant faster nematode mortality rate with increased exposure time, in all the extracts. There was nematode sensitivity to extracts and neem fruit powder was the most toxic, recording the highest nematode mortality in a few days. The results showed that all the plant materials eyaluated were effective against *S. bradys*. These results corroborate earlier studies using neem and plant extracts against other nematodes. Neem fruit extract was effective against *M. javanica* under laboratory conditions (Paruthi et al 1997*)*. Preliminary *in vitro* screening of *Jawan,* a water-based formulation of neem seed extract, against populations of *Pratylenchus loosi, Radopholus similis* and *Rotylenchus reniformis* maintained in petri plates was found to be very promising, as one drop of the compound diluted 10- and 20- fold brought about 100% mortality in *2*-3 days (Gnanaprasam et al 1993).

In a laboratory screening of some oil cake extracts, it was observed that neem oil cakes at 100 ppm or 1000 ppm stock solutions made by soaking 10 g in 100 m1 water for 48hr, were effective against *Pratylenchus thornei* and gave 70-100% nematode mortality (Sebastian and Gupta 1997). Oils with nematicidal activity also immobilized more than 80% of juveniles of the root-knot nematode *Meloidogyne javanica* and

inhibited hatching at a concentration of 1,000 µ/liter (Oka et al 2000). Leaf extracts of neem, datura (*Datura var. alba* [D. mete I]) and calotropis (*Calotropis procera*) were found to be toxic to *Helicotylenchus dihystera*. The toxicity of extracts increased with an increase in their concentration as well as in the exposure time. An increase in the growth of tomato plants was found to be associated with the increasing concentration of extracts and subsequent decrease in the nematode population (Firoza and Maqbool 1996). There has not been any work in nematode control using any cocoyam, ginger, and maize extracts. However, ginger extract has been used widely in the control of plant and animal diseases, especially bacterial diseases of plants (Mar-Mar-Nyein et al 1996). Cocoyam has already been identified to contain phenolic compounds (Uhazurike and Arinze 1996) and maize to contain soluble auxin oxidases (Beffa et al 1990) and phenyl acetic acid (Anaya et al 1995). Basic phytochemical screening consists of performing simple chemical tests to detect the presence of alkaloids, tannins, saponins, anthraquinones, cardenolides, etc., in plant extracts. Confirmatory tests were, always carried out to eliminate errors from false positive reactions (Harborne 1973). *In vitro* investigation of resistant factors in neem fruit, cocoyam corm, ginger root and maize root showed that these plant materials contain alkaloids, saponins, oils, and flavonoids which may be responsible for their resistant status. The presence of alkaloids and essential oils in the extracts could therefore be said to have inhibited activities in the biosynthesis of proteins in the yam nematode. Higher concentrations of organic oils of neem and castor (*Ricinus communis*) proved effective in preventing larval penetration and gall production in the roots of tomato infected by *M. incognita* (Poornima and Vadivelu 1997). This present study showed that the extracts possibly contain some allelochemicals that may be responsible for the observed lethal effects on *S. bradys*. Nematode populations around the roots of resistant plants, sometimes decline at a more rapid rate than can be explained by starvation, and it is presumed that toxins of plant origin are responsible (Rice 1984). For example α-Terthienyl compounds isolated from some species of marigold and a glycoside from asparagus were identified as the toxic factors. The direct toxicity of neem fruit has been attributed to some of the chemicals extracted from it, such as nimbin, salannin, thronemone, aza, and nimbidine (Devakumar et al 1986) which are nematicidal. An inhibitory allelopathic potential is exerted by corn and other cereals (Shukla et al 1997). They screened oil seeds, especially maize, for natural antioxidants and observed flavonoids, tannis, alkaloids, and oil among others present. Uhazurike and Arinze (1996) identified phenolic compounds in cocoyam and phenolic compounds are supposed to be alkaloids or secondary metabolites. They observed high inhibitory activity and phenol peroxidase activities in cocoyam. The results of our study showed that cocoyam, ginger, and maize contain inhibitory properties that can reduce S. *bradys* population in the soil.

Conclusion

The tests carried out here were general (preliminary) and hence, further tests are required for specifics (types) of the various phytochemicals. In the laboratory, extracts from neem fruit, cocoyam, ginger and maize at 50,000, 37,500, 25,000 and 12,500 ppm caused 100% mortality of *S. bradys*. The level of performance of the extract was proportional to the concentration. Water was a better medium than alcohol for the extraction, because of reaction between alcohol and the oil contained in the plants which made it impossible to view the contents of the Synercuse dishes. Preliminary phytochemical screening of neem fruit powder, cocoyam corm, ginger root and maize root indicated the presence of alkaloids, oils, saponins, and flavonoids, and these are supposed allelochemicals. Based on results, we hypothesize that incorporating allelopathy into agricultural management will reduce the use of pesticides.

Table 1: Inference from preliminary phytochemical screening of extracts from plant materials

	Neem fruit	Ginger roots	Cocoyam corms	Maize roots
Alkaloids	+	+	+	+
Oil	+	+	+	_
Saponins	+	+	+	_
Tannins	_	_	_	_
Flavonoids	_	_	_	+

+ Present − absent

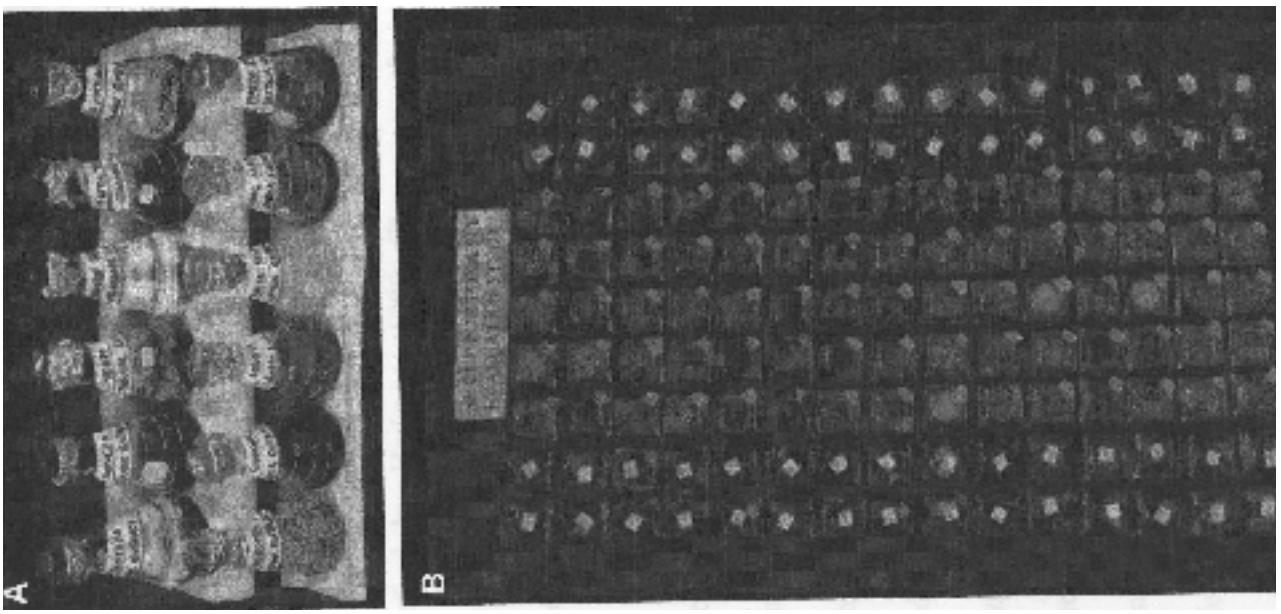

Plate 1A and 1B: Aqueous and Ethanolic (A) and Arranged Synercus dishes containing extracts and nematode aliquots (B)

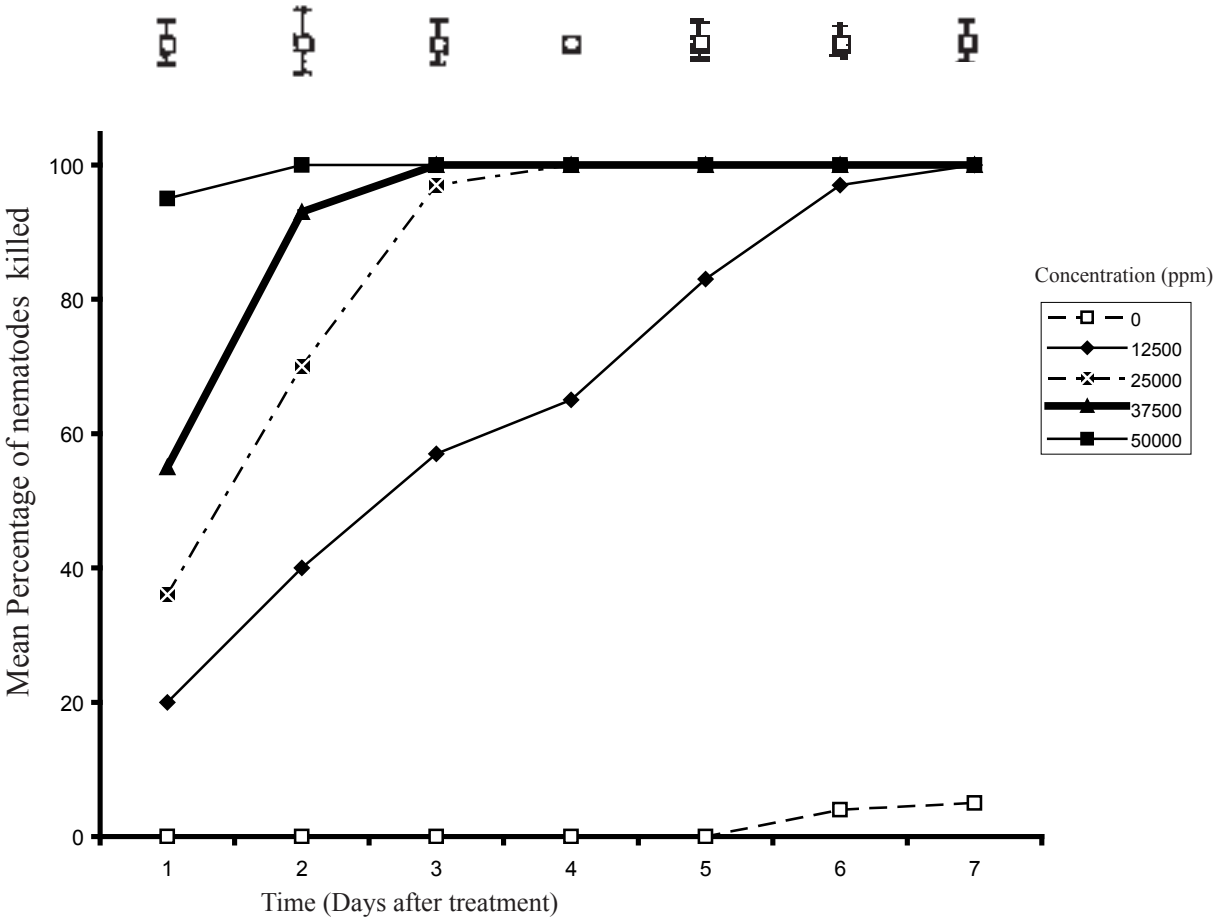

Fig 3. Percentage of *Scutellonem bradys* adults killed by specified number of days at different concentrations of aqueaous neem extract

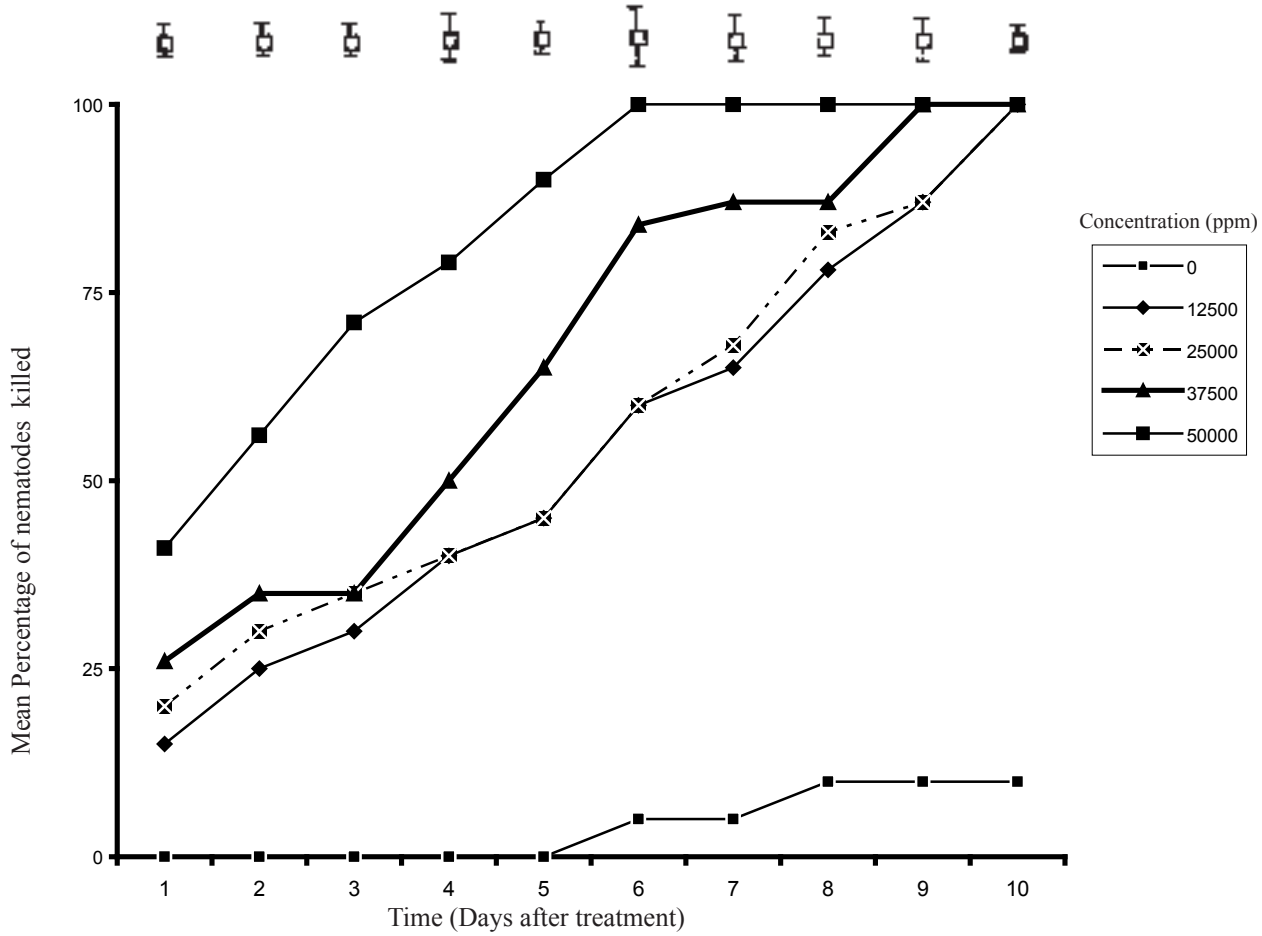

Fig 4. Percentage of Scutellonem bradys adults killed by specified number of days at different concentrations of aqueaous cocoyam extract

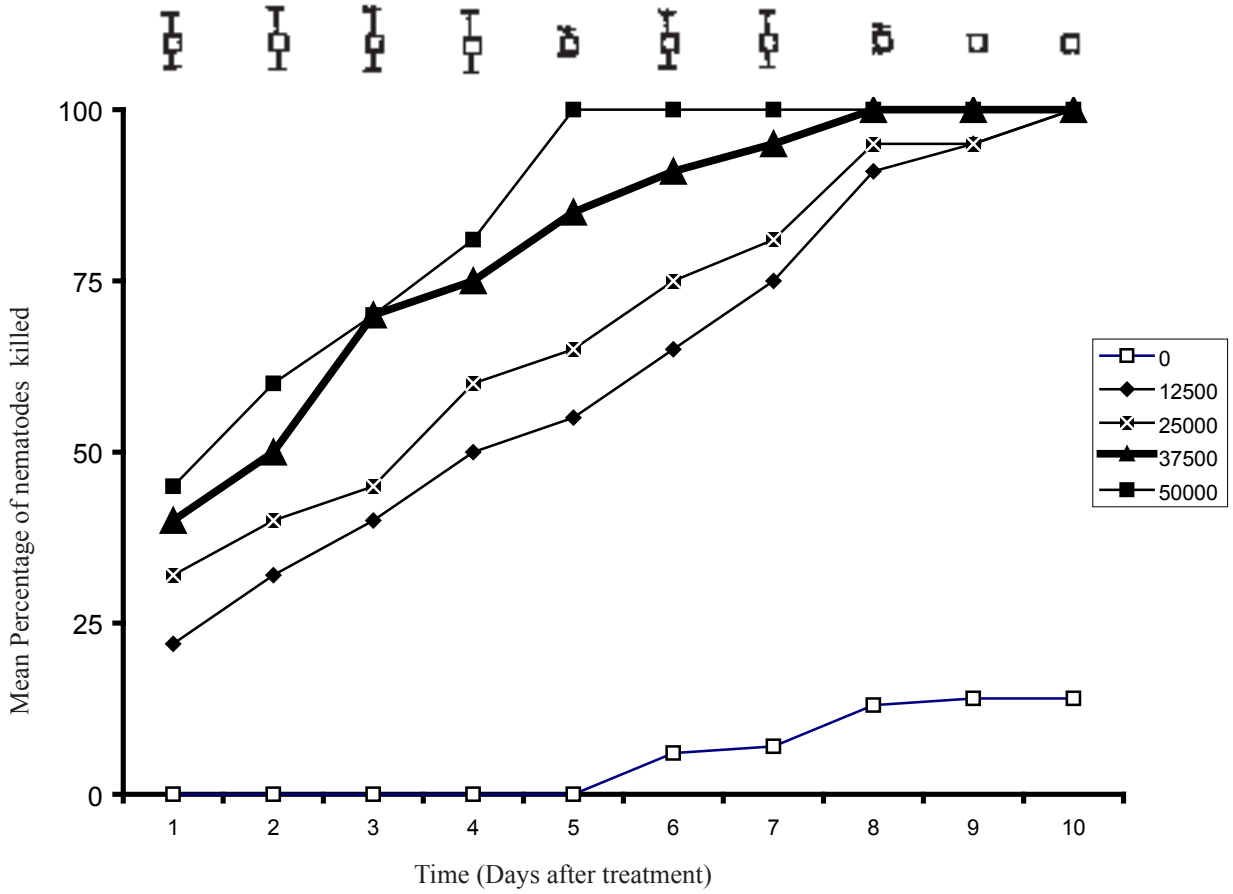

Fig 5. Percentage of Scutellonem bradys adults killed by specified number of days at different concentrations of aqueaous ginger extract

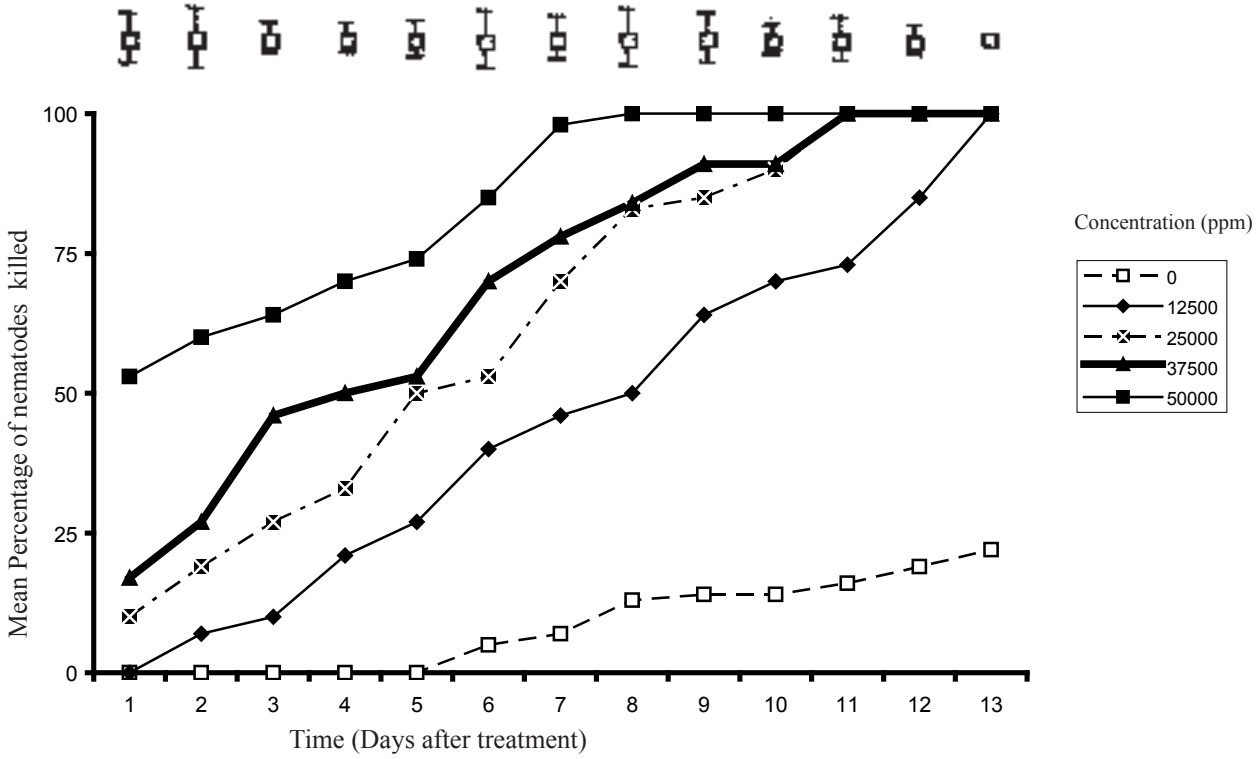

Figure 6. Percentage of Scutellonem bradys adults killed by specified number of days at different concentrations of aqueaous maize root extract

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