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Nematicidal potential of some botanicals on *Meloidogyne javanica in vivo* and *in vitro*

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ABSTRACT

Root knot nematode *Meloidogyne javanica* egg masses and larvae were separately exposed to different concentrations of leaf extract of *Euphorbia heterophylla, Richardia bransiliensis and Scoparia dulcis* contained in Petri dishes in the laboratory. Egg hatch inhibition and larval mortality were observed over a period of 96 hrs. Phytochemical analysis of each botanical was studied. A comparative study of the various extracts was carried out in the screen house potted experiment. Perforated pots were filled with 4 kg sterilised sandy loam soil. Tomato seedling EX- Gombi was transplanted in each pot and inoculated with 1000 second stage juveniles of *M. javanica* a week later. 15, 20 and 25 mL of the crude extracts was applied weekly to each pot. Distilled water serves as the control. The results of the study showed that the extracts inhibited egg hatch, caused juvenile mortality in the laboratory and reduced root knot nematodes infection on tomato in the screen house. *S. dulcis* at 25 ml showed better nematode control compared to *R. brasiliensis* and *E. Heteropylla*.

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INTRODUCTION

Root knot nematodes are one of the important root pests of different crops especially vegetable crops. The symptoms of nematode attack on crops include chlorosis, leaf and root galling, stunted growth, poor yields and sometimes total crop failure (Egunjobi, 1992). These nematodes are controlled through the use of cultural methods, chemical nematicides and resistant varieties. The use of chemical nematicides is the most effective, though; they are expensive to the average farmer. In addition environmental and health concerns are also increasing the restrictions on their use (Perez *et al*., 2003; Umar and Aji, 2013; Umar, 2013). There is therefore the need to screen out plants with naturally occurring nematicidal compounds. Many botanical extracts have been found to contain phytochemicals such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Gommers, 1981, Chitwood, 2002) which are effective against plant parasitic nematodes (Goswani and Vijayalakshmi, 1986; Adegbite, 2003). Botanicals such as *Polyaithia longifolia, Datura stramonium, Thuja orientalis, Targetes minita, Citrus medica* and *Peruvian zinna* were found to be effective on nematodes both in vivo and in vitro (Ferreira *et al*., 2013; Okeniyi *et al*., 2013; Wondimeneh *et al*., 2013; Kavita *et al* ., 2011; Chaudhary *et al*., 2013). These plant extracts have no negative effect on non-target organisms, humans and animals and are environment friendly. The present study was undertaken to evaluate leaf extracts of *Euphorbia heterophylla, Richardia bransiliensis* and *Scoparia dulcis* on eggs and larvae of *Meloidogyne javanica* invivo and invitro. These plants are common weeds found in the area of study. The availability and affordability of these plants within the local environment form the basis of this research.

Richardia bransiliensis belongs to the famiy Rubiaceae. The plant can be annual or perennial. The branching stems grow up to 40 cm and lie prostrate or upright. The leaves are oval with pointed or rounded tips. The inflorescence is a cluster of 20 flowers or more. The petals are white or rose- pink. The fruit is a hairy nutlet. In Brazil the plant is used medicinally as an antiemetic and for cure of diabetis (Wikipedia, 2014). *Scoparia dulcis* is a species of flowering plant in the coffee family. The plant may be annual or perennial which grows

from a deep root. It is found in tropics and subtropics. It is used as a medicinal plant for the treatment of ulcers, diabetis, sickle cell anemia, burns and headache. Extracts of the plant have been found to be antiglycemic, antimicrobial and antioxidant (Wikipedia, 2014). *Euphorbia heterophylla* is a hardy ruderal species, growing between 30- 70 cm in height. The leaves at the upper end of the stalk have a striking, scarlet red coloration. The leaves are mainly 2-4 lobed and 4-7 cm long and 1.5-3 cm wide. Their contrast with the lower dark green leaves gives this *Euphorbia* most of its common names. The stalks exude toxic milky white latex. This latex causes sensitive reaction on people who come into contact with them causing sometimes dermatitis and anaphylaxis. The fruits are small, segmented capsules.

Plate 1 : *Richardia bransiliensis* (right), *Euphorbia heterophylla* (centre) and *Scoparia dulcis* (left) (Source: Wikipedia 2014).

MATERIALS AND METHODS

Preparation of Extract

Fresh leaves of *Euphorbia heterophylla, Richardia bransliensis and Scoparia dulcis* were collected from farmers' plots. The leaves after collection were air - dried and ground into uniform powder using a Thomas Willey milling machine. The aqueous extract of each sample was prepared by soaking 50 g of each dried powder sample separately in 200 ml distilled water contained in 500 mL flask for 12 hrs (Edeoga *et al*., 2005). Crude extract (C) of each sample was obtained by filtering through Whatman NO 42 filter paper. The 10 ml of the crude extract was diluted with 15, 20 and 25 ml distilled water to obtain concentrations of C1, C2 and C3 respectively.

Phytochemical analysis of the extracts

Phytochemical analysis of the extracts was conducted in the laboratory using the methods described by Sofowora (1993), Trease and Evans (1989) and Edeoga *et al*. (2005).

Preparation of Eggs

Egg masses of *M. javanica* were collected from galled tomato roots raised in the nursery. Eggs were extracted using the methods described by Hussey and Barker (1973). Eggs obtained were transferred into distilled water in a 100 ml beaker forming the egg suspension.

Extraction of juveniles

Root knot nematodes second stage juveniles were extracted from galled tomato roots using the methods described by Whitehead and Hemming (1965). *M. javanica* was identified using the Head and Stylet morphology described by Eisenback *et al*. (1981). One thousand juveniles were used each for all the treatments including the control.

Effect of extract on egg hatching

Two ml of crude and diluted extract were separately dispensed into petri dishes and 3 ml of 50 nematode suspensions was introduced into each petri dish with a syringe. The control contained only nematode and distilled water. Each treatment was replicated thrice and petri dishes were arranged in a complete randomised design in the laboratory. Hatching was observed over a period of 96 hrs. All data collected were subjected to analysis of variance and means separated using Duncan's New Multiple Range Test at 5%.

Effect of extract on Juvenile mortality

Three ml of both crude and diluted extract were separately poured into petri dishes. Three ml of 1000 juvenile suspension was introduced into each petri dish with a syringe. The control received no treatment. There were four treatments replicated three times. Petri dishes were arranged in a complete randomised design in the laboratory. Juvenile mortality was observed over a period of 96 hrs. All data collected were subjected to analysis of variance and means separated using Duncan's New Multiple Range Test at 5%.

Screen house experiment

4 kg sterilised sandy loam soil was filled into 20 cm diameter perforated plastic pots of depth 30 cm. Three week old tomato seedlings EX- Gombi raised

in sterilised soil in the screen house were transplanted into each pot at one seedling/ pot. Each plant was inoculated with 1000 juveniles of *M. javanica* a week after transplanting. 15, 20 and 25 ml of the crude extract were applied weekly at the base of each plant with a syringe except the control which was not treated. Pots were treated with 0.9 g of urea fertilizer. Pots were irrigated thrice weekly. There were four treatments replicated three times and pots were arranged in a complete randomised design in the laboratory. At the end of the experiment, nematodes on roots were extracted and counted (Barker, 1985). Growth parameters and yield were also recorded during the experiment. All data collected were subjected to analysis of variance and Duncan New Multiple Range Test was used to separate means at 5 % level of significance.

RESULTS AND DISCUSSION

The result on the phytochemical analysis of the extracts indicated the presence of tannin, saponin, flavonoid and cardiac glycoside (Table 1).

Table 1. Phytochemical constituent of the extract.

Plant extract	Constituent			
	Tannin	Saponin	Flavonoid	Cardiac glycoside
E. heterophylla	+	+	+	+
R. bransiliensis	+	+	+	+
S. dulcis	+	+	+	+

+ = presence of constituent

Table 2 showed that the percentage concentrations of the phytochemicals in each plant material. The quantitative analysis of the crude extracts showed *S. dulcis* contained the highest crude percentage (Tannins -18.05%; saponin- 3.56% and flavonoid-0.98%), followed by *R. brasiliensis* and the least in *E. heterophylla*. This result was similar to those obtained by Edeoga *et al*. (2005) on the quantitative percentages of some medicinal plants.

Table 2. Percentage of crude constituents of the different plant materials

Plants	Tannin (%)	Saponin (%)	Flavonoid (%)
R. bransiliensis	11.50±0.25	2.20±0.18	0.75±0.40
E. heterophylla	16.22±0.30	0.0	0.90±0.22
S. dulcis	18.05±0.46	3.56±0.23	0.98±0.14

Figure 1 shows the effect of the different concentrations of the extracts on egg inhibition of *javanica*. The result indicated that the crude extract of *S. dulcis* gave the highest egg hatched inhibition of 87 %, followed by *R. brasiliensis* and 0% inhibition was observed

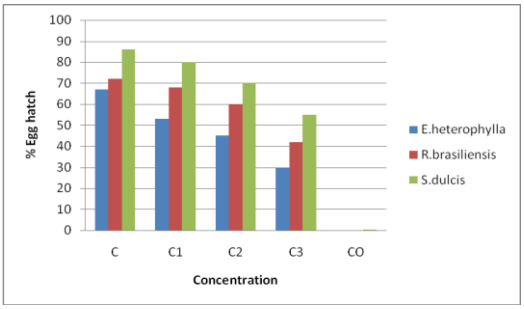

Fig. 1. Effect of extract on egg hatching.C- Crude exract, C1- Crude extract + 15 mL distilled water, C2- Crude extract + 20 mL distilled water, C3= Crude extract + 25 mL distilled water, C0- control (distilled water only)

in the control (Figure 1). The result also showed that the higher the dilution the lower the egg hatch inhibition. The control gave 0% egg inhibition because it contained only distilled water and had no effect on egg hatch. It was earlier reported by Adegbite (2003), Adegbite and Adesiyan (2005), Ranjitsingh *et al*. (2009) and Umar (2013) that botanical extracts that contained alkaloids, saponins and flavonoids either singly or in combination inhibited egg hatched of *Meloidogyne* spp. In a related work Ferreira *et al*. (2013) reported that aqueous extracts of *Zinnia peruviana* and *Wedelia* species inhibited egg hatched of *Meloidogyne incognita* when compared to the control by 92.72% and 97.48% respectively. Also Kumari *et al*. (2013) reported that plant extracts of neem, *Clitoria teratea* and *Passiflora foetida* were effective in reducing egg hatch of *M. incognita* in mulberry.

The results of juvenile mortality showed that *S. dulcis* and *R. brasiliensis* gave higher juvenile mortality than *E. heterophylla* (Figure 2). All treatments were able to kill juveniles at various concentrations. The results also showed that the higher the concentration of extracts the higher the mortality of nematodes and vice versa. The control recorded 0% mortality since it contained only distilled water. Khan *et al*. (2008) reported that extracts of some plants such as onion, garlic, tobacco, *Aloe vera*, cloves and chilli were effective against *M. incognita* larvae and caused mortality of juveniles between 82-100%. The result obtained

could be an outcome of the pesticidal content of the extract which killed nematodes. The effect of the different crude extracts of the three botanicals on the performance of tomato EX-Gombi was significantly different at 5 % level of probability.

Table 3. Effect of crude extracts of different plant species on growth parameters, yield and final nematode population on tomato EX- Gombi on control of *M. javanica* the screen house.

TRT	PH (cm)			SW (g)			RW (g)			RL (cm)			YD			FNP(mL)		
	EH	RB	SD	EH	RB	SD	EH	RB	SD	EH	RB	SD	EH	RB	SD	EH	RB	SD
25	85.2a	87.6a	90.6a	145.5a	155.5a	158.1a	20.6d	18.1d	16.8d	23.8a	28.8a	31.4a	245a	255a	258a	120d	104d	95d
20	71.6b	75.8b	78.2b	90.8b	93.3b	95.6b	26.5c	22.8c	22.5c	18.5b	20.7b	24.8b	203b	233b	238b	148c	135c	108c
15	62.3c	65.3c	66.7c	74.2c	77.8c	80.4c	33.3b	38.6b	30.7b	14.7c	16.5c	17.7c	175c	180c	183c	168b	154b	118b
Con	30.3d	29.0d	26.5d	20.5d	18.0	17.5d	55.6a	58.8a	50.4a	8.9d	7.9d	6.0c	401d	36d	30d	2600a	2400a	2520a

Means in the same column followed by same letter are not significantly different according to Duncan's New Multiple Range Test at 5 %; TRT- Treatment; EH- *Euphorbia heterophylla*; RB- *Richardia bransiliensis*; SD – *Scoparia dulcis;* Con- Control; PH- Plant height; SW- Shoot weight' RW- Root weight; RL- Root length; YD- Yield; FNP- Final nematode population, * - significant

Tomato crop treated with 25 ml of crude extract of *S.dulcis* recorded taller plants, higher shoot weight, longer roots, least root weight, higher yield and a few nematodes recovered from soil as shown in Table 4. *R. brasiliensis* recorded better growth parameters and yield as well as fewer nematodes compared to *E. heterophylla*. The control recorded the shortest tomato plants, higher root weights, shorter roots and large population of nematodes compared to the treatments. The treated plants recorded better growth parameters and fewer nematodes due to the nematicidal or nematostatic effects of the different extracts. It was reported that extracts of plants containing tannins, alkaloids and flavonoids were effective against root knot nematodes both invivo and invitro (Adegbite and Adesiyan, 2005; Anuja and Satyawati, 2007; Umar, 2013).

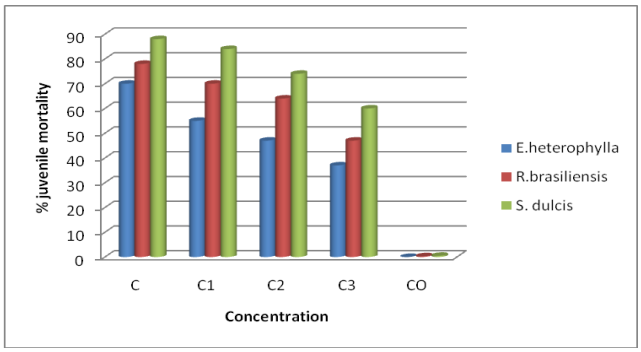

Fig. 2. Effect of extract on Juvenile mortality; C- Crude exract, C1- Crude extract + 15 mL distilled water, C2- Crude extract + 20 mL distilled water, C3= Crude extract + 25 mL distilled water, C0- control (distilled water only).

The result of the study indicated that *S. dulcis* was more effective against *M. javanica* both *in vivo* and *in vitro* and improved tomato growth and yield. Although, the other treatments were also able to reduced nematode population, they were not as effective as *S. dulcis*. It is recommended that further field study be carried out on the effectiveness of these botanicals against *M. javanica* in tomato before making any final recommendation to tomato farmers.

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