BIOLOGICAL SCIENCES

ISSN (Print): 24490954
ISSN (Online): 26364972

In- Vitro STUDIES ON THE NEMATICIDAL POTENTIAL OF EXTRACTS OF SEEDS OF SOME MEMBERS OF FAMILY-FABACEAE IN JOS

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Manuscript Received:10/06/2019 Accepted:10/12/2020 Published: March,2021

ABSTRACT

In-vitro nematicidal potentials of varying concentrations of extracts from seeds of three leguminous plants i.e. *Erythrina senegalensis*, *Leucaena leucocephala* and *Senna siamea* were investigated in juvenile mortality of the second stage larva of *Meloidogyne* species at the botanical nursery of the University of Jos, between the months of March and April, 2010. The nematodes were extracted from galled roots of tomato obtained from four different farms in Jos, while seeds of the legumes were collected dry from The University of Jos and environs. Nematicidal test was carried out with different concentrations of the extract (40g/ml, 60g/ml, 80g/ml; 100g/ml) while distilled water served as control. All the treatments were repeated three times. Live nematodes were counted every twenty-four (24) hours for six (6) days. Phytochemical screening of the seed extracts was also carried out. The results revealed that all the seeds used had nematicidal properties but to varying degrees. *Erythrina senegalensis* exhibited the highest nematicidal activity while *Leucaena leucocephala* recorded the least. The nematicidal activity of the extracts generally increased with increase in concentration and length of exposure. The results from the phytochemical screening revealed that Alkaloids and Balsam were absent in all the extracts, while Flavonoids, Saponins, Cardiac Glycosides, Terpenes and Steroids were present in the three seed extracts. The investigation showed that the seed extracts of the test plants hold promise in the control of root-knot nematodes.

Keywords: *:* Nematicidal*, Meloidogyne* species*, Erythrina senegalensis, Leucaena leucocephala and Senna siamea*

INTRODUCTION

The family Fabaceae is the third largest family of the flowering plants (after Compositae and Orchidaceae) with 650 genera and 18,000 species (Gill, 1988). In this family are *Erythrina senegalensis*, *Leucaena leucocephala* and *Senna siamea* among other plants. *Erythrina senegalensis* (coral tree), is a tree growing up to 7 m tall, with deeply fissured, corky bark. The leaves are composed of three leaflets, each measuring 5-15 × 4-10 cm and having a thorny stalk. The flowers are bright red and 4-5 cm long. The fruit is a bent, twisted and slightly hairy pod, 7-15 × 1 cm. It is constricted between the seeds, which are bright red. (Contu, 2009).

Of 700 trees now known to fix nitrogen, none is more efficient than *Leucaena leucocephala* (Brewbaker, 1987)

Senna siamea Lam. (Synonyms: *Cassia siamea Lam*., C. *florida Vahl*. S*enna sumatrana*, Roxb.) Is a non nitrogen-fixing leguminous tree in the subfamily *Caesalpinoideae* of the family *Leguminosae*. It is a good ornamental tree for planting along roadsides, and it is also used in alley cropping, intercropping, and hedgerows (Jensen, 1995).

The alkaloids and other secondary plant compounds in the leaves, flowers and pods are highly toxic to non-ruminants, such as pigs and poultry, and these animals should be kept away from S. siamea plantations.

Root-knot nematodes (*Meloidogyne* spp.) are the most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. It has been reported that root-knot nematodes either on their own or in combination with other pathogens can contribute substantially to crop losses (Okechalu and Wonang, 2004). According to Adesiyan *et-al.,* (1990), the root-knot nematodes are probably the major obstacle to the production of sufficient food and fibre crops in Nigeria and many other developing nations.

Therefore, there is urgent need to investigate different control measures for these pathogens. The present study was designed to investigate the nematicidal potentials of extracts of three leguminous plants. - E*rythrina senegalensis*, *Leucaena leucocephala* and *Senna siamea*, against root-knot nematodes and identify, if any, the Phytochemicals, present in the extracts.

MATERIALS AND METHODS

Experimental Site

This study was carried out in the research laboratory of the Department of Plant Science and Biotechnology, University of Jos.

Samples Collection

Seeds of *Erythrina senegalensis*, *Leucaena leucocephala* and *Senna siamea* were collected from university of Jos premises and at the Federal College of Forestry, Jos. The pods were plucked from trees while the seeds were handpicked from the pods. Root-knot Nematode-infected tomato plants, were collectd from farm sites at Students' Village Hostel, University of Jos, Jos Nigeria.

Extracts of Seeds

Seeds of the test plants were collected from the tree pods, removed, winnowed, ground and sifted to get the powdered-seeds. Forty, 60, 80 and 100g were weighed-out, into four dry and sterilized test tubes. Ten mililitre (ml) of distilled water was measured into each test tube using a standardized syringe with needle. All samples were properly shaken aseptically to obtain a homogenous mixture which were allowed to settle for five minutes before centrifuging at 4000 rpm for five minutes. The centrifugation separates the mixtures into two distinct layers:

(i) The upper liquid layer
(ii) The lower solid layer

The liquid layer was withdrawn into sterile syringe with needle after four minutes. This was simultaneously done for all samples. Thus, the following concentrations 40g/ml, 60g/ml, 80g/ml and 100g/ml were prepared for each of the three extracts.

Nematode extraction

Root-knot nematodes eggs were extracted from galled roots of tomato sampled from farms in Jos and environs using the modified Baermann Funnel Method as described by Southey (1970). The eggs were then kept in peri dishes containing root exudates of tomato variety UC-82-B which has been reported to be susceptible to root-knot nematodes to hatch. The eggs were left in the tomato root exudates for 72 hours.

Nematicidal Test

Freshly hatched Second stage larva of *Meloidogyne* species were incubated in different concentrations of each of the three extracts at 27 ±2°C in the laboratory, for 6 days. Distilled water served as control. Meloidogyne population was estimated in 1ml of the homogenized suspension under a dissecting microscope at ×4 magnification. This was done as follows: 1 drop of nematode suspension contains approximately 11 nematodes. There were 5 drops in one (1) mililitre, therefore, 1 ml contains approximately 55 nematodes.

One mililitre (1 ml) of the suspension containing 55 nematodes was pipetted into each of clean, dry sterile thirteen test-tubes. Approximately 0.5ml of *Erythrina senegalensis* extract of concentrations: 40g/ml, 60g/ml, 80g/ml; 100g/ml were added respectively into four of the test tubes and labelled accordingly. The same was done with *Senna siamea* and *Leucaena leucocephala* extracts (i.e. four test tubes for each extract). Also, 0.5 ml of distilled water was added as control to the remaining three test tubes. They served as controls. All the treatments were repeated three times. Live nematodes were counted every twenty-four (24) hours for six days.

Phytochemical Screening

The powdered seeds of *Erythrina senegalensis, Leucaena leucocephala* and *Senna siamea* were screened for phyto-chemical constituents. The phytochemical screening was carried out using Standard Qualitative Procedures as described by Sofowora, (1986); Trease and Evans (2002). Water extracts of the samples were used. The phytochemicals screened for were alkaloids, flavonoids, tannins, saponins cardiac glycoside, steroids and terpenes, balsam, phenols and resins.

Statistical Analysis

Data generated were analysed using mean, simple percentages and two-way analysis of variance where applicable.

RESULTS

The results of the nematicidal test revealed that all the seeds used had nematicidal properties but to varying degrees. *Erythrina senegalensis* had the highest nematicidal activity while *Leucaena leucocephala* had the least (Table1).

The nematicidal effect of the extracts generally increased with increase in concentration and length of exposure (Table2).

The extract of *Erythrina senegalensis* at concentrations: 60g/m, 80g/m, and 100g/m killed all the nematodes (100% mortality) at day 4 (96 hours) while *Leucaena leucocephala* and *Senna siamea* had a similar result on day 6 (144 hours). (Table 3).

The results from the phytochemical screening revealed that Alkaloids and Balsam were absent in all the extracts, while Flavonoids, Saponins, Cardiac Glycosides, Terpenes and Steroids were present in the three Fabaceae seed extracts.

Resin was absent in Senna siamea but present in the others, while Phenols and Tannins were found only in *Leucaena leucocephala*. (Table 4).

DISCUSSION

The three Fabaceae seed extracts had nematicidal activities on *Meloidogyne* species, suggesting that they had chemically active components that killed these nematodes. The presence of Cardiac glycosides, Flavonoids, Saponins and Terpenes & Steroids in the three extracts revealed this. The presence of these Phytochemicals in the present study also agrees with the findings of Oyedunmade *et al*.,(2008) who reported the presence of these phytochemicals in some weed extracts that had nematicidal effects on root-knot nematodes.

Saponin mixtures from *Medicago sativa* tissues have been found effective in vitro against the virus-vector nematode *Xiphinema index*, the root-knot nematode *Meloidogyne incognita* and the potato cyst parasite, *Globodera rostochiensis* (D'Addabbo, *et al.,*2010). The presence of saponin in the fabaceae leaf extracts may therefore have accounted for their nematicidal activity.

Milkweed (*Asclepias*), a new crop produced for its fibre in pillows and comforters contains, mainly, Cardiac glycosides (*cardenolides*). The defatted seed meal is an effective nematicide and pesticide for army worms (USDA, 1998). This may explain the nematicidal properties identified in the Fabaceae seed extracts since they were rich in Cardiac glycosides.

Leaf extract of *Sida acuta Burm F*., *Euphorbia hirta Linn Andropogon gayanus Kunth, Phyllanthus amarus Schum* and *Thomm and Cassia obtusifolia L.* weeds were used against *M. incognita*. and *M. incognita*, juvenile mortality rate increased with an increase in test plant extract concentrations and exposure time. Wonang *et al.,* (2006) who working on nematicidal properties of rice straw extract, reported increase juvenile mortality in nematodes with increase in concentration and exposure time. These agree with the findings in the present research. The observation may be due to increase quantity of bioactive constituents of the extracts making contact with the nematodes for a longer duration hence the increased mortality.

The investigation has revealed that the extracts of seeds of the test plants has nematicidal activity in-vitro but at varying degrees. *E. senegalensis* had the highest nematicidal activity. This may be as a result of inherent variation in the plants. It is thus concluded that seed extracts of these leguminous plants hold a promise of nematode control especially those of *E. senegalensis*

ACKNOWLEDGEMENTS

We wish to acknowledge the Chief Technologist of the Department of Plant Science and Biotechnology, Ms Kate Bassey for her assistance in the Lab., also worthy of mention are the tomato farmers in Jos and it's surrounding who allowed us took samples from their farms for nematode assay. We are indeed grateful to the Department of Plant Science and Biotechnology, University of Jos for availing us her Laboratory for this study.

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TABLE 1: MEAN ESTIMATED NUMBER OF LIVE NEMATODES AFTER EXPOSURE TO THE VARIOUS TREATMENTS.

PLANT EXTRACT	CONCERNTRATION	HOURS					
		24	48	72	96	120	144
E. senegalensis							
	40ml/g	32	28	7	1	0	0
	60ml/g	31	11	6	0	0	0
	80ml/g	30	12	2	0	0	0
	100ml/g	20	5	4	0	0	0
*L. leucocephal*a							
	40ml/g	37	28	19	10	3	0
	60ml/g	25	17	15	7	2	0
	80ml/g	27	17	11	7	0	0
	100ml/g	25	17	7	1	0	0
S. siamea							
	40ml/g	31	28	20	10	2	0
	60ml/g	30	25	17	8	1	0
	80ml/g	30	25	12	7	0	0
	100ml/g	27	22	14	1	0	0
	0ml/g	50	48	47	47	45	42

TABLE 2: MEAN NUMBER OF DEAD NEMATODES AFTER EXPOSURE TO THE VARIOUS TREATMENTS.

PLANT EXTRACT	CONCERNTRATION	HOURS					
		24	48	72	96	120	144
E. senegalensis							
	40ml/g	22	27	48	54	55	55
	60ml/g	24	44	49	55	55	55
	80ml/g	25	43	53	55	55	55
	100ml/g	35	50	51	55	55	55
L. leucocephala							
	40ml/g	18	27	36	45	52	55
	60ml/g	30	38	40	48	43	55
	80ml/g	28	38	44	48	55	55
	100ml/g	30	38	48	54	55	55
S. siamea							
	40ml/g	24	27	30	45	53	55
	60ml/g	25	30	38	47	54	55
	80ml/g	25	30	43	48	55	55
	100ml/g	28	33	41	54	55	55
	0ml/g	05	07	08	08	10	13

TABLE 3: MEAN PERCENTAGE MORTALITY OF NEMATODES AFTER EXPOSURE TO THE VARIOUS TREATMENTS.

PLANT EXTRACT	CONCERNTRATION	PERCENTAGE MORTALITY/HOURS OF EXPOSURE					
		24	48	72	96	120	144
E. senegalensis							
	40ml/g	41.81	49.09	87.27	98.18	100	100
	60ml/g	43.64	80.00	89.09	100	100	100
	80ml/g	45.46	78.18	96.36	100	100	100
	100ml/g	63.64	90.91	92.73	100	100	100
L. leucocephala							
	40ml/g	32.73	49.09	65.46	81.82	94.55	100
	60ml/g	54.55	69.09	72.73	87.27	96.36	100
	80ml/g	50.91	69.09	80.00	87.27	100	100
	100ml/g	54.55	69.09	87.27	98.18	100	100
S. siamea							
	40ml/g	43.64	49.09	63.64	81.82	96.36	100
	60ml/g	45.46	54.55	69.09	85.46	98.18	100
	80ml/g	45.46	54.55	78.18	87.27	100	100
	100ml/g	50.91	60.00	74.55	98.18	100	100
	0ml/g	9.09	12.73	14.55	14.55	18.18	23.64

TABLE: 4 PHYTOCHEMICAL RESULTS

Phytochemicals	E. senegalensis	L. leucocephala	S. siamea
Alkaloids	-	-	-
Flavonoids	+	+	+
Tannins	-	++	-
Saponins	+	+	+
Balsam	_	_	_
Cardiac glycosides	+	+	+
Terpenes and Steriods	+	+	+
Resins	+	+	_
Phenol	_	++	_

Key: + ***= Presence or Detected***

– ***= Absence or not detected***