
LAWSONIA INERMIS* IN THE CONTROL OF *MELOIDOGYNE* SPP. ON *CORCHORUS OLITORUS

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ABSTRACT

The efficacy of cypress shrub, *Lawsonia inermis* L. in the control of root – knot *nematodes*, *Meloidogyne* spp was investigated in the laboratory and the Screenhouse. In the laboratory, only the aqueous extract of the test plant (*Lawsonia inermis*) was assessed for its effects on nematode egg hatch and juvenile mortality. For the Screenhouse, the experimental design was a 2x2x3 factorial experiment, fitted into a randomized complete block design, involving two levels of nematode population (0 and 300) and two forms of test plant application (aqueous extract and powdered form). In the laboratory, the aqueous extracts of *Lawsonia inermis* caused a significant reduction ($P \leq 0.05$) in nematode egg hatched. Egg hatch was 92.0% in the untreated control (0% extract) as compared to 11.7% in 25% crude extract of *Lawsonia inermis* (L) extract. Within two days of the experiment 98.4% mortality was observed in 100% crude concentration of *Lawsonia inermis* (L), as compared with 0% mortality in the control. All concentrations of the extract caused significantly higher mortality than the control. In the screenhouse, 15% aqueous crude concentration, significantly reduced nematode population in the root and in the soil. All treated plants were also less galled compared to the untreated control.

KEYWORDS

Lawsonia inermis, *Meloidogyne* spp, *nematodes*, screenhouse.

INTRODUCTION

Jew's mallow, *Corchorus olitorius* (L) belongs to the family Tiliaceae, it is of utmost importance in the south western part of Nigeria where it is widely consumed, very popular and highly appreciated. *Corchorus olitorius* has high moisture content in its fresh form with considerable quantities of vitamins which help protect the body against diseases and contribute in no small measure to good health (Agusiobo, 1984).

Vegetables compensate for the scarce animal protein sources in rural communities in Nigeria (Oyenuga and Fetuga, 1975). The Asian vegetable Research and Development Center in its strategic plan 1991 reported that vegetable production offers great income to small scale farmers and low income earners. It provides employment opportunities for women, hence it is an important revenue source in Nigeria (AVRDC, 1991).

However, plant parasitic nematodes are recognized as one of the major pests of *C. olitorius* in the tropics. The most destructive nematodes responsible for enormous yield loss in *C. olitorius* are the root – knot nematodes, *Meloidogyne spp.* The disease inflicted by this group of nematodes is characterized by numerous pronounced swellings or galls on the root of susceptible host plant with consequent reduction in growth and yield of infected plants.

Synthetic chemicals constituted the world most effective nematode control weapon put in place by man, because of its quick efficacy. This strategy ruled the world of nematode control for more than ten decades (Johnson, 1987). However nematicides are expensive and hazardous because of the residual effect on the crop, soil and non target organisms.

As a result of the side effects and high costs associated with the use of synthetic nematicides, efforts have been made to study the nematicidal potentials of some plants on plant parasitic nematode Khan and Saxena (1997) in their experiment on the integrated management of root knot nematode, *M. javanica* infecting tomato using amendments of oil cakes, bone and horn meals, found that there was increase in growth of tomato and reduction in nematode multiplication. Kimpinski *et al* (2000) reported that *Tagetes tennifolia*, *T. patula* and *T. erecta* were found to significantly reduce the population of *Pratylenchus penetrans* on potato, Pandey (2000) observed maximum reduction in *M. incognita* populations at high doses of three Organic nematicides. Oyedunmade *et al* (2000) reported that neem leaf extract had nematicidal potential, and caused an improvement in the growth and yield of okro.

Oyedunmade (2004) also reported that African marigold, *Tagetes erecta* has nematicidal properties with positive effect on the vegetative growth of treated plant in the field. However, there are still several natural materials whose potentials remain untapped. This study was therefore conducted with the following objectives in mind.

(1) To determine the active chemical components present in *Lawsonia inermis* (2) To compare nematicidal properties of the aqueous extract and powdered form of test plant. *L. inermis* on the growth and yield of *C. olitorius* (3) To compare the effects of the different concentrations of the aqueous extract of *L. inermis* (L) on the survival of root knot nematode juveniles and eggs in the laboratory.

MATERIALS AND METHODS

Source and Preparation of Test plant extract:-

The test plant, *Lawsonia inermis* was collected from Ile apa village, close to the Faculty of Agriculture, University of Ilorin. It was air-dried thoroughly until brittle. The leaves were removed from the stem after drying and pound in mortar into powdered form.

One kilogram of powdered plant material was weighed and soaked in a liter of water for 24 hours, thereafter filtered, and the filtrate concentrated to 100 ml by heating on the water bath to remove excess water. The concentrated plant extract was taken as 100% concentration, from this, serial dilutions were made to obtain 25, 50 and 75%, extracts. Ordinary water served as the 0% extract (control).

Nematode egg hatch and juvenile mortality trials:-

Twenty (20) Petri dishes containing 8 ml of each concentration of plant extract and 5 Petri dishes containing 8 ml of water were used for the juvenile mortality assessment and egg hatch ability.

For the Juvenile mortality experiment, 300 juveniles were introduced into each Petri dish. Counting of nematodes was done under the stereo microscope at 2hours, 4hours and daily for seven days. Those juveniles that did not respond to the touch of a needle were considered dead. For the egg hatch test 300 eggs were introduced into each Petri dish. The Petri dishes were covered to prevent evaporation while count of hatched eggs was done at 24 hours intervals for ten (10) consecutive days under the stereo microscope.

Potted plant experiment:-

Soil Sterilization:- Sandy loam soil was collected behind the Faculty of Agriculture, University of Ilorin. The soil was sieved to remove gravels, stone and plant debris with a 2mm mesh sieve. The soil was put in a metal drum and then pasteurized for 4 hours at 60 °C (Gautam and Goswami, 2002). After cooling, the soil was weighed into experimental pots at 10kg per pot and the pots were placed on bricks to avoid microbial infestation in the course of the experiment.

Source and Extraction of Nematode Juveniles:

Galled roots of *Corchorus olitorius* was collected from Rice Research, Seed Multiplication and Demonstration Farm, Sango Ilorin: The roots were taken to the laboratory, washed gently under running tap to remove attached soil, small stones and other plant debris. The roots were later cut with scissors into small pieces and macerated in a warring blender for 30 seconds only. The blended mixture was then poured over a bank of sieves that had been arranged in order of mesh size viz, 10mm, 100mm, and 200mm. The contents of the 200mm mesh were carefully collected and rinsed into a beaker with little water. Whitehead and Hemming (1965) tray method of nematode extraction was used.

Planting Operation: - The seeds were planted at a sowing depth of 0.3cm, in the little holes made in the center of the sterilized soil in the experimental pots, and covered with soil. The resulting seedlings were watered daily. Germination of seeds started on the fourth day, and the plants were thinned down to a plant per pot by removing excess plants on the 10th day after germination.

Nematode inoculation and treatment application: After the 14th day of planting 300 juveniles of *Meloidogyne* spp were inoculated close to the roots of *Corchorus olitorius* plant in each of thirty pots while another set of thirty other pots were not inoculated. Seven days after inoculation, the inoculated and uninoculated pots were treated with powdered form and aqueous extract of *Lawsonia inermis*, at various rates, close to the root of the plants. The rates were 0, 15 and 30g/pot for the powdered form of treatment, while for the aqueous form, the rates used were 0, 7.5 and 15%.

Data collection and statistical analysis: - Data were collected weekly from the experimental plants to record the following parameters: Plant height, number of leaves per plant and number of branches per plant. Shoot weight, root weight, root gall index (Taylor & Sasser, 1978), nematode population per 250g soil sample and nematode population in 5g root sample were assessed at plant harvest. All data from the experiment were subjected to analysis of variance (ANOVA). Treatment means were separated using the new Duncan's Multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Phytochemical screening: Tannin, saponin, alkaloid, glucoside, anthraquinone and flavonoids were found present in the test plant (*L. inermis*)

Laboratory tests: - The effect of the different concentration of aqueous extracts of *Lawsonia inermis* on the egg hatch of *Meloidogyne* spp is shown in Table 1. The plant extracts were effective in inhibiting nematode egg hatch, as all the concentrations of *Lawsonia inermis* used were significantly more effective than the control in inhibiting egg hatch, although the lowest concentration was not as effective as the other higher concentrations. The cumulative egg hatch in the control was 92% at the end of the experiment while the highest percentage cumulative egg hatch observed was 11.7% in 25% extract treatment. The higher concentrations of 50, 75, and 100% aqueous extract of *Lawsonia inermis* did not record any egg hatch. The control experiment recorded an egg hatch value of 14% and 90.5% at day 1 and day 9 respectively.

TABLE 1:- Effects of different concentrations of aqueous extract of *Lawsonia inermis* on percentage egg hatch of meloidogyne spp.

<i>Lawsonia inermis</i>	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
0 Control	14.06	17.3c	37.3c	40.56	49.2c	62.8c	76.5c	80.0c	90.5c	92.0c
25% Crude Extract	0.6a	2.4b	2.8b	4.0b	4.9b	6.3b	9.3b	10.1b	11.1b	11.7b
50% Crude Extract	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
75% Crude Extract	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
100% Crude Extract	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Standard Error	0.179	0.190	0.536	0.553	1.460	0.298	0.190	0.378	0.104	0.162

Means in given column followed by the same letter are not significantly different at $P < 0.05$ using the new Duncan's Multiple Range Tests.

Between two and four hours of the exposure of juveniles to test plant extract, there was no significant difference in the effect of all the levels of concentration of *Lawsonia inermis* on juvenile mortality (Table 2). At six hours however, there were significant differences in juvenile mortality and the 100% concentration of extract caused the highest mortality of 26.6%. Percentage mortality ranged between 24.8 and 93% at day 1 of the experiment. The control experiment had the lowest percentage mortality during the experimental period. The first record of juvenile mortality of 2.1%

was observed in the control treatment on the 4th day of the experiment. This juvenile mortality in the control only increased to 6.0 on the 7th day of the experiment. The level of juvenile mortality increased with increase in the exposure period. At the end of the experiment, an average of 100% mortality was observed in all the concentrations of test plant extract as compared with the control experiment which had 6.0%, mortality.

Table 2:- Effects of different concentrations of aqueous extract of lawsonia inermis on percentage juvenile mortality of meloidogyne spp. Exposure time of juveniles to extract

<i>Lawsonia inermis</i>	2 Hrs	4 Hrs	6 Hrs	8 Hrs	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 7
0 Control	0.0	0.0	0.0c	0.0c	0.0c	0.0c	0.0d	2.1c	3.3c	6.0b
25% Crude extract	0.0	0.0	3.2d	3.2d	24.8d	40.8d	58.2c	73.66	89.8b	100.0a
50% Crude extract	0.0	0.0	6.7c	7.3c	48.8c	67.2c	86.2b	97.4a	100.0a	100.0a
75% Crude extract	0.0	0.0	15.2b	16.3b	78.4b	83.8b	97.6a	99.6a	100.0a	100.0a
100% Crude extract	0.0	0.0	26.6a	28.6a	93.0a	98.4a	100.0a	100.0a	100.0a	100.0a
Standard error	0.142 N.S	0.277 N.S	0.529	0.515	1.465	1.390	1.995	1.614	1.190	100.0a

Means in a given column followed by the same letter are not significantly different at $P < 0.05$ using the new Duncan's Multiple Range Test. N.S: Not Significant.

Table 3: Main effect of nematode inoculation, forms of application and level of application of lawsonia inermis, on different growth parameters of corchorus olitorius

Inoculation	Plant height			Number of leaves		Number of branches			
	7 WAP	10 WAP	13 WAP	7 WAP	10 WAP	13 WAP	7 WAP	10 WAP	13 WAP
Not inoculate(0 juvenile)	39.7a	70.6a	110.9a	49.4a	88.3a	134.7a	9.1a	15.3a	23.3a
Inoculated (300 juvenile)	25.9b	37.9b	53.4b	226.8b	46.0b	66.0b	4.8b	8.0b	11.7b
S.E	0.516	0.493	0.921	0.651	1.056	0.870	0.237	0.316	0.350
Form									
Aqueous	32.7	54.6	92.2a	40.4a	75.6a	117.1a	7.8a	13.8a	19.8a
Powder	32.9	53.8	82.1	35.8b	58.8b	83.6b	6.1b	9.5b	15.2b
S.E	0.516N.S	0.493N.S	0.921	0.651	11.05b	0.870	0.237	0.316	0.350
Level									
0 Control	28.3c	47.3b	70.4c	31.0b	51.3b	69.6c	5.1c	8.8c	14.5c
7.5 Conc 15g /10kg soil	34.1b	57.4a	86.2b	41.5a	73.6a	113.2b	6.7b	10.5b	16.4a
15% Conc 30g / 10Kg soil	36.1a	50.2b	89.8a	41.9a	76.6a	118.3a	9.2a	15.7a	21.7a
S.E	0.632	0.499	128	00.797	11.294	1.066	0.290		0.428

Means in a given column followed by the same letter are not significantly different at $P < 0.05$ using the new Duncan's Multiple Range Test. N.S: Not Significant.

Pot Experiment:

Nematode inoculation resulted in a significant reduction in the vegetative growth of *Corchorus olitorius*, as plant height, number of leaves, and number of branches per plant were significantly higher in uninoculated plants (Table 3). The test plant, *Lawsonia inermis* significantly reduced the effect of the nematodes on *Corchorus olitorius*, as treated plants had better vegetative growth than the untreated ones. The higher doses of treatment seemed to be more effective than the lower doses.

While the aqueous form of treatment was significantly better than the powdered form in increasing the plant height, number of leaves, and number of branches per plant.

Table 4:- The main effect of nematodes inoculation forms of application and level of application of *Lawsonia inermis* on root gall rating and nematode populations in soil sample or *Corchorus olitorius* roots.

Inoculation	Nematode population in 250g soil sample	Nematode population in 5g root sample	Root Gall Rating
Not Inoculation (0 Juvenile)	0.0b	0.0b	0.0b
Inoculation (300 Juveniles)	241.2a	128.4a	1.2a
S.E	0.651	3.167	0.00
Form			
Aqueous	118.7b	61.1	0.7b
Powder	122.4a	67.3	1.0a
S.E	0.651	3.167 NS	0.00
Level			
0 (Control)	355.1a	179.5a	2.0a
7.5 CONC 15g /10kg soil	6.7b	13.1b	0.5b
15% CONC 30g / 10Kg soil	0.0c	0.0c	0.0c
S.E	0.798	3.878	0.00

Means in the same column followed by the same letter are not significantly different at $P > 0.05$ using the New Duncan's Multiple Range Test. N.S – Not Significant

Furthermore, nematode inoculation resulted in a significant increase in nematode population in 250g soil sample, nematode population in root sample, and root gall rating (Table 4). The aqueous form of *Lawsonia inermis*, however significantly reduced nematode population in 5g root sample, 250g soil sample, and root gall rating. The higher doses of treatment were very effective as there was no nematode in 250g soil sample and 5g root sample, and consequently no root galling (Table 4).

DISCUSSION

The bio-active compounds (tannin, saponin, flavonoids, glucosides, alkaloids and anthraquinone present in the test plant could have been the reason for the efficacy of *Lawsonia inermis* in controlling root-knot nematodes in *Corchorus olitorius*. The observed nematotoxic effects *Lawsonia inermis* may be attributed to the presence, in the plant, of nematicide chemical compound that are injurious to the eggs and infective second stage juveniles of *Meloidogyne* spp. Deverall (1972) reported the pesticidal astringency and phyto-repellent nature of flavonoids and other bioactive compounds present in plants. Paulo (2001) reported the efficacy of the aqueous extracts of the roots and shoots of *Artemisia annua*, which at concentration of 100% resulted in 100% *Meloidogyne incognita* juvenile mortality within 24 hours of exposure in vitro.

Apart from the nematicidal properties of the plant material, it is possible for the plant powder and extract to increase the nutrient status of the soil and encourage better crop growth and yield. Gautam and Goswami (2002) reported improved yield in a specie of cowpea when neem cake was used in the treatment of *Meloidogyne incognita*. The ready availability of *Lawsonia inermis* and its effect on nematode population and plant growth suggest interests in its potentials as a biopesticidal product for the control of plant – parasitic nematode.

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