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Inhibitory effect of *Tamarindus indica* and *Carica papaya* on egg hatch larva mortality of *Meloidogyne incognita*

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Essentially, the most effective control of plant parasitic nematodes involves the use of synthetic nematicide. However, apart from their very high cost, increased concern on the environment has necessitated a reduction in the amount of nematicides used for nematode control. In view of these, this research work was conducted to evaluate the effects of different concentrations of root extract of *Tamarindus indica* and *Carica papaya* at different concentrations *S* (100% concentration), "*S*/2" (50% concentration), "*S*/10" (10% concentration) and "*S*/100"(1% concentration) in the inhibition of egg hatch and mortality of larva of root-knot nematode, *Meloidogyne incognita* .The experiment was laid out in a completely randomised design and replicated four times. Combination of equal proportion of *T. indica* and *C .papaya* root extract indicated that all the concentrations inhibited egg hatch. The standard solution *S* of all the selected botanicals were more toxic and effective at ($p \leq 0.05$) than the other concentrations. Similarly, combinations of root extracts of *T. indica* and *C. papaya* were the most effective of all the selected botanicals in the inhibition of egg hatch and larvae mortality, followed by *T. indica* root extracts and then *C. papaya* root extracts.

Keywords: egg hatch; larva mortality; *Carica papaya*; *Meloidogyne incognita*; *Tamarindus indica*

Introduction

There are many different species of root-feeding nematodes; the most important in the gardens are the root-knot nematodes (*Meloidogyne* species). Root-knot nematodes attack a wide range of plants including many common vegetables, fruit trees, ornamentals and field crops. They are difficult to control and easily spread from garden to garden through soil, on farm tools, boots, etc. and attack plant parts (Dunn 1995).

Most vegetable crops are attacked by one or more species of nematodes. Some nematodes are beneficial, feeding on bacteria, fungi or other microscopic organisms, and some may be used as biological control organisms to help manage important insect pests (Horst 1990; Ingham 1996). The infestation of the root-knot nematode results in a poor growth, a decline in quality and yield of crops, as a high-level infestation can lead to damage and total crop loss.

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Tamarind is a slow grower but can live and still remain productive for 150 years or longer. Once established, it does not need much attention, it has a very deep and extensive root system, soil do not erode easily, and can withstand very strong wind and even hurricanes (National Research Council 2008). Ripe tamarinds (*Tamarindus indica*) contain sugar (50%), whose sweet taste is, however, outweighed by up to 20% tartaric acid which has an intensively acidic taste; some cultivars decompose the tartaric acid on ripening (sweet tamarind) and can be eaten raw as fruit. The fruit pulp is rich in tartaric and citric acids, high amount of vitamin C and sugar (Spice pages … 2004). Phyto-chemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defence mechanisms against predation by many micro-organisms, insects and herbivores. This may therefore explain the demonstration of antimicrobial activity by the stem bark and leaf extracts of *T. indica* (Marjorie 1999; Bibitha et al. 2002; Doughari 2006).

In Northern Nigeria, the fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorder, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser (Doughari 2006). The plant is widely used in traditional medicines in Africa for the treatment of fever, dysentery, jaundice, gonococci and gastro intestinal disorders (Kheraro & Adam 1974; Kobayashi et al. 1996; Ferrara 2005). The foliage is common mulch for tobacco plantings. The seed coat extract is a polyphenolic flavonoid that has been shown to have anti-oxidant properties (Ferrara 2005). The seed extract possesses anti-snake venom properties (Ushanandini et al. 2006).

Research has shown that latex of pawpaw (*Carica papaya*) has anthelmintic action on Ascaris (*Entorobilis vermicularis*) and also used for rubber production. The leaves are cooked with other plants to treat fever, typhoid and malaria; in some parts of Asia, they are steamed and eaten like spinach. Also contains carpaine– an anthelmintic alkaloid (a drug that removes parasitic worms from the body) which can be dangerous in high doses. Also the juice has an anti-proliferative effect on *in vitro* liver cancer cells, probably due to its component of lycopene (Rahmat 2009).

Management of nematodes is difficult and the most reliable practices are preventive, including sanitation and choice of plant varieties. Once an area or crop is infested, damage to crop is reduced by adjusting planting and harvesting dates, and irrigation or by the use of soil amendments. (Akhtar & Malik 2000).There is a substantial evidence that the addition of organic matter in the form of compost or manure will decrease nematode pest populations and associated damage to crops (Stirling 1991; Akhtar & Alam 1993; Oka & Yermiyahu 2002; Walker 2004). Allelochemicals are plant-produced compounds (other than food compounds) that affect the behaviour of other organisms in the plant environment. For example, a Sudan-grass and sorghum contains a chemical named dhurin that degrades into hydrogen cyanide, a powerful nematicide (Luna 1993; Forge et al. 1995; Wider & Abani 2000). Various plant extracts have been evaluated for their anti-nematicidal properties against different pathogen (Tripathi et al. 2002).

In view of these, research work was conducted to observe the effect of *T. indica* and *C. papaya* root extracts on the hatching of eggs of *M. incognita.*

Materials and methods

The practical experiment was carried out in the Crop Production Department laboratory of the Federal University of Technology, Gidan-Kwano campus Minna, Niger State of Nigeria in a location between latitude 9° 37| N and longitude 6° 28| E in the southern Guinea savanna zone.

Collection and preparation of crude extract of T. indica *and* C. papaya *root*

The roots of *T. indica* were collected from a fully established tree in Maikunkele Low-cost, Minna, Niger State. The roots of *C. papaya* were collected from a fully established papaya tree in the Federal Airports Authority of Nigeria staff quarters, Maikunkele in Minna, Niger State of Nigeria.

The roots of both *T. indica* and *C. papaya* were dug out from the soil using hoe, and carefully cut out using cutlass, washed gently under running tap water and separately put into different labelled polythene bags. All the materials were transported to the Crop Production laboratory of the Federal University of Technology, Gidan-Kwano Campus, Minna. From the roots of both plants collected, about two kilogrammes (2 kg) each were weighed, chopped with knife in a tray and then crushed into smaller particles using mortar and pestle. About 200 mL of distilled water was measured from the 6 L using a measuring cylinder and added to each of the pounded roots of *T. indica* and *C. papaya* in different plastic buckets. The pastes were stirred with clean glass rod for proper mixing. Both pastes were blended in a Philip electronic blender for 2–3 min for homogeneity to be attained. They were separately poured into a plastic bowl and covered with aluminium foil paper to prevent evaporation. The crude materials were allowed to settle for 24 h. After 24 h, the remaining distilled water was added and stirred. The paste was then filtered into a clean plastic container through a Whatman No. 1 filter paper. The resultant solution was labelled standard concentration *S*. About 2–3 drops of streptomycin sulphate was added to prevent formation of bacteria growth in the concentration.

Preparation of concentration of crude extract

Four different concentration levels were prepared (*S*), (*S*/2), (*S*/10) and (*S*/100) where:

S	The undiluted standards solution
(*S*/2)	Half concentration of the standard solution
(*S*/10)	One-tenth of the standard solution and
(*S*/100)	One-hundredth of the standard solution

They were prepared as follows;

S:	The undiluted standard solution regarded as the standard stock/main concentration
(*S*/2):	To each volume of standard stock solution (*S*) equivalent volume of distilled water was added
(*S*/10):	To each volume of standard stock solution (*S*) 10 equivalent volume of distilled water was added
(*S*/100):	To each volume of standard stock solution (*S*) hundred equivalent volume of distilled water was added
C:	Distilled water/control
T_1:	Treatment 1, *T. indica*
T_2:	Treatment 2, *C. papaya*
T_3:	Treatment 3, Mixture of *T. indica* (T_1) root extracts and *C. papaya* (T_2) root extracts

Collection of inoculums

The pure culture of *Meloidogyne incognita* race one was procured from a heavily infected okra plant (*Abelmoschus esculentus*) in Gidan-Kwano village, Minna, Niger State of Nigeria. Root-infected okra plants from the culture plot were up-rooted and washed gently under running tap water to remove adhering soil and then carried to the laboratory (crop production laboratory, FUT Minna) in a polythene bag. The egg masses

were removed from the roots by cutting the roots into short pieces for easy handling, placed in a Petri dish and observed under the light microscope to locate matured egg masses. Fresh and uniform egg masses of *M. incognita* were collected using forceps for each treatment. Precaution and time were taken into consideration to inoculate all the collected egg masses within 1 h to avoid their hatching prior to inoculation. Plastic Petri dishes of 9 cm diameter were used for the study. They were arranged on the table in a completely randomised design. For each plant extract, four replicates (20 Petri-dishes) was set-up for each plant extract. The Petri dishes were lined underneath in squares for easy counting of hatched larvae and larval mortality.

For each concentration of the extract, 15 mL of solution was poured into each Petri dish using a syringe (calibrated 20 mL capacity). Two (2) egg masses freshly removed were added to each Petri dish containing the extracts and distilled water of equal volume. The ambient temperature of the laboratory ranged from 29 to 31 °C.

The effect of different concentration levels on the hatching of *M. incognita* (the total number of larvae hatched and mortality) was determined at six-observation time of 3, 6, 12, 24, 48 and 96 h intervals. The hatched larvae were counted using the light microscope.

All the data collected were subjected to one-way ANOVA (analysis of variance) using SPSS (2007) means were separated using Duncan Multiple Range test (Duncan 1997).

Results

Inhibitory response of root extracts T. indica *on egg hatch of* M. incognita

Root extract of *T. indica* delayed as well as decreased the hatching of eggs of *M. incognita* as shown in Table 1. For treatment 1 (T_1) which is *T. indica*, after 3 h there was no significant difference ($p > 0.05$) between *S* and *S*/2, but there was a significant ($p < 0.05$) difference between *S*, *S*/2 and *S*/10, *S*/100 and the control, while egg hatch increased with the increase in period in all the concentrations with the highest number of larva hatched in the control. However, as the concentration decreased, number of larva hatched increased. Table 1, (T_1) also showed that there was no significant ($p > 0.05$) difference between *S*/100 and control at 3 and 6 h, but there was a significant ($p < 0.05$) difference from 12 to 96 h, indicating that the concentrations of

Table 1. Inhibitory response of root extract of *T. indica* on egg hatch of *M. incognita* at different period.

	Period					
Treatment	3 h	6 h	12 h	24 h	48 h	96 h
S	0.00^{c}	8.00^{b}	9.00^{c}	11.00^{d}	18.00^{d}	22.00^{e}
S/2	2.00^{c}	10.00^{ab}	13.00^{b}	16.00^{c}	21.00^{cd}	27.00^{d}
S/10	5.00^{b}	12.00^{a}	14.00^{b}	19.00^{c}	26.00^{c}	33.00^{c}
S/100	8.00^{a}	13.00^{a}	16.00^{b}	24.00^{b}	37.00^{b}	41.00^{b}
C	10.00^{a}	13.00^{a}	34.00^{a}	51.00^{a}	77.00^{a}	122.00^{a}
SEM	0.90	0.60	2.05	3.23	5.06	8.51
LS	*	*	*	*	*	*

Note: Means in the same column followed by the same letter are not significantly different at ($p \leq 0.05$) according to Duncan's multiple range test.

the extracts at all concentrations inhibited egg hatch. There were significant difference between the control and all the concentrations.

Inhibitory response of root extracts of C. papaya *and on egg hatch of* M. incognita

Similar trend was observed for treatment 2 (T_2) which was *C. papaya,* egg hatch was observed after 3 h in standard solution but there was a significant difference between all the concentrations at 3 h with increase in time as shown in Table 2 below. After 6 h, there was no significant difference between *S* and *S*/2 but there was a significant difference at 48 and 96 h of observation at all times of exposure. The control showed high value of significant difference than other concentrations, egg hatch decreased with standard concentration and increased with increase in period of exposure.

Response of root extracts of C. papaya *and* T. indica *mixture on egg hatch of* M. incognita

Table 3 showed the inhibitory effects of treatment 3, on the egg hatch of *M. incognita*. Generally, egg hatch decreased with standard concentration of the root extract; for egg hatch started at 6 h, there was no significant difference between *S*/2 and *S*/10, but there was a significant difference between control which has the highest value, *S*/100, *S*/2 and

Table 2. Inhibitory response of root extracts of *C. papaya* on egg hatch of *M. incognita.*

	Period					
Treatment	3 h	6 h	12 h	24 h	48 h	96 h
S	2.00^{d}	5.00^{d}	8.00^{d}	15.00^{d}	19.00^{d}	28.00^{d}
S/2	4.00^{c}	7.00^{cd}	9.00^{cd}	17.00^{d}	26.00^{d}	35.00c
S/10	5.00^{c}	9.00^{c}	11.00^{c}	21.00^{c}	32.00^{c}	38.00^{c}
S/100	7.00^{b}	15.00^{b}	17.00^{b}	25.00^{b}	39.00^{b}	44.00^{b}
C	13.00^{a}	22.00^{a}	34.00^{a}	49.00^{a}	72.00^{a}	98.00^{a}
SEM	0.89	1.45	2.23	2.86	4.28	5.84
LS	*	*	*	*	*	*

Note: Means in the same column followed by the same letter are not significantly different at ($p \leq 0.05$) according to Duncan's multiple range test.

Table 3. Response of root extracts of *C. papaya* and *T. indica* mixture on egg hatch of *M. incognita*.

	Period					
Treatment	3 h	6 h	12 h	24 h	48 h	96 h
S	0.00^{d}	6.00^{c}	7.00^{d}	15.00^{d}	28.00^{c}	31.00^{d}
S/2	3.00^{c}	9.00^{bc}	11.00^{c}	19.00^{cd}	31.00^{bc}	40.00^{cd}
S/10	4.00^{c}	11.00^{ab}	13.00^{bc}	21.00^{bc}	34.00^{bc}	48.00^{bc}
S/100	7.00^{b}	12.00^{ab}	16.00^{b}	26.00^{b}	40.00^{b}	53.00^{b}
C	9.00^{a}	13.75^{a}	22.00^{a}	36.00^{a}	53.00^{a}	82.00^{a}
SEM	0.74	0.75	1.24	1.79	2.33	4.20
LS	*	*	*	*	*	*

Note: Means in the same column followed by the same letter are not significantly different at ($p \leq 0.05$) according to Duncan's multiple range test.

S/10 and then *S*. At 6 h there was no significant difference between *S*/10 and *S*/100 but there was a difference between *S*, *S*/2, *S*/10 and *S*/100. Egg hatch also decreased with standard concentration and increased with control over time. Treatment 3 had the highest value of egg hatch inhibition with time making it is the best treatment.

Response of root extracts of T. indica *on larval mortality of* M. incognita

The effects of *T. indica* root extracts at different concentrations and times of exposure on larval mortality rate are shown in Table 4 below. The result showed that there was high level of significance in the standard solution, as larvae mortality was highest in the standard solution and lowest in control. At 3 h, there was no significant difference between all the concentrations, larval mortality started at 6 h, *S* was more significant than the other concentrations, there was no significant difference between *S*/2 and C, *S*/10 and *S*/100. At 12 h, there was significant difference between *S*, *S*/2 and other concentrations, but there was no significant difference between *S*/10, *S*/100 and C. At 24 h, there was no significant difference between *S*, *S*/2, *S*/10, *S*/100 and C. There was also no significant difference between 6 to 96 h at *S* concentration, which means that *S* exhibited the highest larval mortality while "C" exhibited the lowest larval mortality.

Response of root extracts of C. papaya *on larva mortality of* M. incognita

Similarly, treatment 2 showed no significant difference between the concentrations at 3 h prior to treatment 1. Larva mortality started at 6 h for *S* and *S*/2 where *S*/2 was more significant. There was no significant difference between *S* and control, and also between *S*/10 and *S*/100; at 12, 24 and 48 h there was no significant difference between all the concentrations. At 96 h, *S* exhibited the highest larva mortality rate; *S*/2, *S*/10 and *S*/100 showed no significant difference between the concentrations as they were all significantly related. The control showed the lowest larva mortality rate.

Response of root extracts of T. indica *and* C. papaya *mixture on larva mortality of* M. incognita

The effects of root extracts of treatment 3 on *M. incognita* are shown in Table 6. The result showed that larva mortality started at 3 h for *S*/2 unlike other concentrations where larva mortality started at 6 h for *S*, 12 h for *S*/10 and 24 h for *S*/100. At 48 h, *S* exhibited the highest larva mortality rate; there was no significant difference between

Table 4. Response of root extracts of *T. indica* on larval mortality of *M. incognita.*

	Period					
Treatment	3 h	6 h	12 h	24 h	48 h	96 h
S	0.00	2.00[a]	6.00[a]	8.00[a]	17.00[a]	17.00[a]
S/2	0.00	0.00[c]	4.00[b]	7.00[a]	16.00[a]	16.00a
S/10	0.00	1.00[b]	2.00[c]	8.00[a]	12.00[b]	12.00[b]
S/100	0.00	1.00[b]	1.00[c]	3.00[b]	9.00[b]	9.00[a]
C	0.00	0.00[c]	1.00[c]	3.00[b]	5.00[c]	5.00[c]
SEM	0.00	0.19	0.50	0.66	1.11	1.11
LS	NS	*	*	*	*	*

Note: Means in the same column followed by the same letter are not significantly different at ($p \leq 0.05$) according to Duncan's multiple range test.

Table 5. Response of root extracts of *C. papaya on* larva mortality of *M. incognita*.

	Period					
Treatment	3 h	6 h	12 h	24 h	48 h	96 h
S	0.00	1.00^{b}	2.00^{ab}	7.00^{a}	11.00^{a}	24.00^{a}
S/2	0.00	2.00^{a}	3.00^{a}	3.00^{b}	8.00^{b}	17.00b
S/10	0.00	0.00^{c}	1.00^{b}	2.00^{b}	6.00^{ab}	14.00^{bc}
S/100	0.00	0.00^{c}	1.00^{b}	2.00^{b}	3.00^{c}	12.00^{c}
C	0.00	1.00^{b}	3.00^{a}	4.00^{b}	4.00^{bc}	7.00^{d}
SEM	0.00	0.20	0.26	0.48	0.76	1.43
LS	NS	*	*	*	*	*

Note: Means in the same column followed by the same letter are not significantly different at ($p \leq 0.05$) according to Duncan's multiple range test.

S/2, *S*/10, *S*/100 and C as they are all significantly related. At 96 h also, *S* exhibited the highest mortality rate; *S*/2, *S*/10 and *S*/100 are significantly related and have no significant difference. But the standard concentration and control have significant difference from other concentrations, with control having the lowest larva mortality rate.

Discussion

The root extracts of all the selected botanicals delayed hatching of egg of *M. incognita* (Table 1–6). The effect of different concentration levels and exposure periods on hatching of eggs varied with the different treatments at different concentrations and exposure to the extracts. For *C. papaya* root extracts, there was no significant ($p > 0.05$) difference with *S* at all periods of exposure as they all inhibited egg hatch of *M. incognita*, egg hatch was delayed to 48 h, likewise *S*/2 and *S*/10, respectively. At all the period of exposure, *S*/100 and C have the highest value of larva hatch and *S* with the lowest value.

For combined root extracts of *T. indica* and *C. papaya*, *S* and *S*/2 inhibited egg hatch, while for *S*/10, egg hatch started at 3 h, and at 12 h for *S*/100, making *S*/100 more effective than *S*/10, while control "C" also have the highest value of egg hatch and *S*, the lowest value. The inhibitory effects of all the selected botanicals could be attributed to the chemical content of their root extracts. Similar work with the use of

Table 6. Response of root extracts of *T. indica* and *C. papaya* mixture on larva mortality of *M. incognita.*

	Period					
Treatment	3 h	6 h	12 h	24 h	48 h	96 h
S	0.00^{b}	3.00^{a}	3.00^{a}	9.00^{a}	11.00^{a}	26.00^{a}
S/2	1.00^{a}	1.00^{b}	1.00^{b}	4.00^{b}	9.00^{ab}	20.00^{b}
S/10	0.00^{b}	0.00^{c}	1.00^{b}	2.00^{bc}	9.00^{ab}	18.00^{b}
S/100	0.00^{b}	0.00^{c}	0.00^{b}	1.00^{c}	6.00^{bc}	15.00^{b}
C	0.00^{b}	0.00^{c}	1.00^{b}	2.00^{bc}	2.00^{c}	6.00^{c}
SEM	0.12	0.29	0.27	0.73	0.89	1.68
LS	*	*	*	*	*	*

Note: Means in the same column followed by the same letter are not significantly different at ($p \leq 0.05$) according to Duncan's multiple range test.

leaf extracts of some other botanicals buttressed by Adegbite and Adesiyan (2005). Bello et al. (2006) reported the inhibition of egg hatch of *M. incognita* with neem extract and *Delonix regia*, *Cassia siamea*, *Cassia sieberiana* and *T. indica,* respectively. Combined root extracts of *T. indica* and *C. papaya* were found to be the most effective botanicals in delaying and controlling the hatching of eggs of *M. incognita* with highest mortality rate and high inhibition rate compared to others, followed by *T. indica* root extract and then *C. papaya* root extracts. The lowest number of hatched larvae observed with the standard solutions could be as a result of the inhibitory effects of the chemicals of the plant extracts that might have some larvicidal properties. Pharmacological investigations on *T. indica* extracts reported them to have antibacterial, antifungal (Pousset 1989), hypoglycaemic, cholesterolemic, cytotoxic (Kobayashi et al. 1996), gastrointestinal (Coutino-Rodriguez et al. 2001), hypolipomic and antioxidant activities (Ferrara 2005). The phyto-chemical examination of the methanolic extracts of the leaves of *T. indica* afforded two triterpenes i.e. lupanone and lupeol. Both compounds (metabolites) have been isolated for the first time from *T. indica* (Imam et al. 2007). The leaves of *papaya* with alkaloid have many pharmaceutical and therapeutic uses (Ghosh 1994). The increase in larvae mortality over time with increase in concentration of plant extracts indicated that the test plants could be more effective at a higher concentration level. Adegbite and Adesiyan (2005) and Bello et al. (2006) reported similar observation.

In this research work, effects of *T. indica* and *C. papaya* on egg hatch of *M. incognita,* lowest number of hatched larvae and highest mortality were recorded in the standard solutions (*S*) followed by (*S*/2) i.e. One-half of the standard solution while juvenile hatching increased with the corresponding increase in extract dilution, with the control "C" having the highest hatched larvae and lowest mortality in all the selected botanicals. This study showed the effectiveness and possibility of using the root extracts of *T. indica, C. papaya* and both combination for the inhibition of egg hatch and control of root-knot nematode, *M. incognita*. The standard solution *S* of all the selected botanicals was more toxic and effective than the other concentrations and combinations of root extracts of *T. indica* and *C. papaya* was the most effective of all the selected botanicals in the inhibition of egg hatch and larvae mortality, followed by *T. indica* root extracts and then *C. papaya* root extracts.

In conclusion, these botanicals can be used as a source of cheap and effective nematicides of root-knot nematodes. The root extracts of the test plants may be useful for root-knot nematode control which will be an economical and environmentally safe option. There is increasing hope for a bright future in identifying botanicals as biopesticides which will reduce or replace the dependence on chemical pesticides used presently which are usually expensive and non-environmental friendly. However, further study on identification of active compounds of these plant extracts is needed to classify their nematicidal efficacy, especially on *M. incognita*.

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