



Effect of *Tamarindus indica* L. Leaf Extract and *Citrus sinensis* L. Osbeck Peel Extract on *Meloidogyne* spp (Root Knot Nematodes) in Jos, Plateau State

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Authors' contributions

This work was carried out in collaboration among all authors. Author MSJ came up with the idea and designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author CTO managed the analysis and literature reviews of the study. Author AAO supervised. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The Aim of this study is to determine the effect of aqueous leaf extract of *Tamarindus indica* and peel extract *Citrus sinensis* on Root Knot Nematodes, *in vitro*.

Study Design: The experiment was carried out using a completely randomized design.

Place of Study: This study was carried out in the biology laboratory of the Department of Plant Science and Biotechnology, University of Jos, Nigeria.

Methods: Extraction of phytochemicals from *Tamarindus indica* leaf and *Citrus sinensis* peels was carried out using an aqueous solvent. *Tamarindus indica* leaves and *Citrus sinensis* peels were collected within the province of Jos, and were dried at constant room temperature to a constant weight after which pulverized, sieved and soaked in water for 24 hours. The extraction was done using the cold maceration method. Eighty (80) second-stage juveniles of *Meloidogyne* spp. were exposed to the extracts at different concentrations (100, 80, 60, 40, and 20mg/ml) for 6, 12, 18, 24, 30, 32 and 48 hours and, each treatment was replicated 5 times. The average number of dead nematodes per concentration were determined and recorded as mean \pm SEM with statistical value at $P < 0.05$ considered significant.

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Results: Results of phytochemical analysis showed that Alkaloids, Flavonoids, Tannins, Steroids, Phenols, and Cardiolglycosides were present in *T. indica* leaf extracts while *C. sinensis* peels contains Flavonoids, Alkaloids, Saponins, Tannins, Phenols, and Resins. These phytochemicals present are suspected to be responsible for the nematocidal activities of the plants. Nematode mortality in the two treatment groups (*T. indica* and *C. sinensis* extracts) was highest at 100mg/ml concentration (48 hours of exposure); It was followed by 80mg/ml in decreasing order while the least mortality rate was recorded at 20mg/ml. Hence, as the concentration of the extracts were increased with increase in time, high nematode mortality was observed. Percentage mortality of treated nematodes was statistically significant ($P < 0.05$) for all concentrations of the extracts at the different time intervals when compared to the control. Statistical analysis showed that *T. indica* had significantly higher effect on Root-Knot nematodes mortality than *C. sinensis* at varying concentrations and time of exposure.

Benefits of Results: Synthetic pesticides are the principal means used to control nematodes, but the natural products may provide a safe alternative. Hence, the active compounds (phytochemicals) in *T. indica* L. and *C. sinensis* L. can be harnessed to be used as biopesticides.

Conclusion: The study suggests that extracts of *T. indica* and *C. sinensis* can be utilized in the control of root knot nematodes and may have the potential for the production of biopesticides that can be used for the control of nematodes as it displayed nematocidal activities.

Keywords: Nematicidal potential; *Tamarindus indica*; *Citrus sinensis*; *M. Incognita*; *in vitro*.

1. INTRODUCTION

Root – knot nematodes (*Meloidogyne* spp) are one of the most economically damaging genera of plant – parasitic nematode on horticultural and field crops in all temperate and tropical areas [1]. *Meloidogyne* spp are obligate parasites that settle in the root and complete their lifecycle by feeding from the host cells [2]. Nematodes may use a combination of mechanical piercing and cell wall softening to penetrate and migrate into the roots [3,4]. Root-knot nematodes are ubiquitous in distribution and adversely affect crop productivity [5]. Among root – knot nematodes, *M. incognita* is the most prevalent and has been found infecting a wide range of crops [5,6,7]. In pulses, yield losses to the tune of 25.6% have been reported by root-knot nematodes [8]. Quantifying the amount of economic loss caused by root-knot nematodes (*Meloidogyne* spp) especially in the developing countries has been difficult as the crops are not grown as sole crops. Field studies in Nigeria have also shown drastic yield loss on different crop species due to *Meloidogyne* species [9]. Farmers loose crops and sometimes have to go through sanitary regulation involving seed inspection as a result of root knot nematode infestation and its corresponding damages to crop that plant thus increasing the cost of production and incur additional indirect costs to production due to yield loss [10], and customers suffer indirectly due to increase in cost of crops – an indirect consequence of damages this root knot nematode compound [7]. The Federal

Government of Nigeria has a search light on agriculture in the face of the present economic realities for food availability and sustainability [9]. One way of managing plant parasitic nematode is through phytotherapy and organic amendment. Plant parts, their products, extracts and certain other effective amendments have been reported to possess nematocidal properties [11]. The use of such materials has merits over other methods due to their availability, low cost, being pollution free and their capacity to improve soil fertility.

Tamarindus indica (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 [12]. Ripe tamarinds contain sugar (50%), whose sweet taste is, however, outweighed by up to 20% tartaric acid which has an intensively acidic taste; [13] 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 [13]. Phytochemical 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, insect-pests and herbivores [14]. This may therefore explain the demonstration of antimicrobial activity by the stem bark and leaf extracts of *T. indica* [14,15,16].

Citrus sinensis (Citrus) is widely grown in Nigeria and many other tropical and subtropical regions [17]. Sweet orange commonly called orange is a member of this family and a major source of vitamins, especially vitamin C, sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium [18]. Oranges form a rich source of vitamin C, flavonoids, phenolic compounds and pectins. The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin. Just one orange provides 116 % of the daily requirement for vitamin C. [18] Vitamin C is the primary water- soluble antioxidant, which prevents free radical generation in the body and damage to the tissues in the aqueous environment both inside and outside cells [18].

The Aim of this research is to determine the effect of aqueous leaf extract of *Tamarindus indica* and peel extract *Citrus sinensis* on Root Knot Nematode.

2. MATERIALS AND METHODS

2.1 Extraction of Nematodes

The extraction of root knot nematode from plant roots was done using the Modified Baerman Funnel Method [19]. The Root knot nematodes were extracted from the roots and rhizosphere of suspected host plants (particularly banana) cultivated in Jos North LGA and its environs. The host plants were carefully uprooted and roots washed gently to remove the soil. Suspected galls on the root were carefully excised and then teased with the aid of teasing pins and forceps to expose and release the nematodes from the root tissue. This was allowed for 24 hours.

Fifteen sets of test tubes were used so as to obtain enough quantity of the inoculums. The test tubes were then carefully removed and its content centrifuged at 2000RPM (Revolution per Minute) for 5 minutes to concentrate the nematode juveniles at the bottom of the test tubes.

2.2 Estimation of Nematode Population

Nematode population were estimated by counting the number of active juveniles in 1 ml of homogenised suspension of Root Knot nematodes under a binocular research light microscope at X40 magnification.

2.3 Preparation of Extracts

Fresh Tamarind leaf and Citrus peels were collected, dried at constant temperature, and

pulverized using a mortar and pestle. The powdered form was soaked in water and left to stand for 72 hours (3 days), then filtered using a white muslin cloth. The solvent in the filtrate was then evaporated using a hot plate at a temperature of 50°C. The resultant extracts were collected and used for nematicidal test.

2.4 Phytochemical Screening

Following the method described by Sofowara [20], the phytochemical screening of the extract was carried out. The presence of various secondary metabolite was screened for and recorded.

2.5 Preparation of Extracts Concentrations

Different concentration of Tamarind leaf and Citrus peels were then prepared by serial dilution method as follows:

Stock solution: 100mg/ml

- I. 0.8ml of stock solution added to 0.2ml of distilled water:
0.8ml of stock solution + 0.2ml of distilled water = 80mg/ml
- II. 0.6ml of stock solution added to 0.4ml of distilled water:
0.6ml of stock solution + 0.4ml of distilled water = 60mg/ml
- III. 0.4ml of stock solution added to 0.6ml of distilled water:
0.4ml of stock solution + 0.6ml of distilled water = 40mg/ml
- IV. 0.2ml of stock solution added to 0.8ml of distilled water:
0.2ml of stock solution + 0.8ml of distilled water = 20mg/ml

2.6 The Nematicidal Test

The nematicidal test were conducted *in vitro* using the freshly prepared extracts of *Tamarind indica* leaf and *Citrus sinensis* peels on freshly extracted nematodes. The extraction of nematodes and preparation of fresh extracts of Tamarind leaf and Citrus peels were done concurrently to ensure the use of fresh extracts and very active second stage larvae (juveniles). 1ml of each concentration of the extract was added to 1 ml of homogenized suspension of root knot nematodes extract and examined at intervals of 6, 12, 18, 24, 30, 36, 42 and 48 hours

for live and immobilized nematodes using a microscope.

The number of immobilized juveniles were counted and expressed as percentage of mortality and recorded. The immobilized juveniles were then transferred to the distilled water for 24 hours to ascertain whether or not they regain mobility. The nematode suspension to which no extract was added served as control. The test was repeated 5 times for each concentration of both fresh *Tamarindus indica* leaf and *Citrus sinensis* peels extract and average number of dead nematodes per concentration were determined and recorded.

2.7 Statistical Analysis

All data were presented as Mean ± SEM. Analysis of statistics was conducted applying a two-way analysis of variance (ANOVA). All results at $P < 0.05$ were held statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Table 1 shows that *Tamarindus indica* leaf extracts contains Flavanoids, Alkaloids, Tannins, Steroids, Phenols and Cardioglycosides, while *Citrus sinensis* leaf extracts contain various secondary metabolites including Flavanoids, Alkaloids, Saponins, Tannins, Phenols and Resins.

3.2 Nematicidal Effect of the Leaf Extract of *Tamarindus indica* on Root Knot Nematodes

The result revealed a significant increase ($P < 0.05$) in the mortality of the nematode at the

different exposure time, when compared to the control. As the time of exposure increases, the mortality Rate increases too.

3.3 Nematicidal Effect of the Leaf Extract of *Citrus sinensis* on Root Knot Nematodes

The result of the nematicidal effect of *C. sinensis* also revealed a significant increase ($P < 0.05$) in the mortality of the treated nematode when compared to the control group. As the time of exposure increases, the mortality Rate increases; with 48 hours exposed-nematodes having the highest mortality and 6 hours exposed nematodes having the least mortality.

3.4 Nematicidal Effect of the Leaf Extract of *Tamarindus indica* as Compared to the Peel Extract of *Citrus sinensis*

Results showed that both *T. indica* and *C. sinensis* possess nematicidal abilities at different concentration over a period of time of exposure. However, it also shows that *Tamarindus indica* has more effect on Root-knot nematodes compared to *Citrus sinensis*, with an average percentage difference of 10.09 over a 48hours period as seen in Fig. 3.

The root knot nematode causes a lot of damages to crops of economic importance and have been controlled over the years with synthetic nematicides which may cause environmental pollution; hence, the need for a better alternative which is eco-friendlier [21]. The result of this study shows that the extracts of *T. indica* and *C. sinensis* could be employed as nematicides to control the root-knot nematodes.

Table 1. Phytochemical constituents of *Tamarindus indica* leaf and *Citrus sinensis* peels extracts

Phytochemicals	<i>Tamarindus indica</i>	<i>Citrus sinensis</i>
Flavanoids	+	+
Alkaloids	+	+
Saponins	-	+
Tanins	+	+
Steroids	+	-
Cardioglycosides	+++	-
Phenols	+	+
Resins	-	+

Key: + = Present
 - = Absent
 +++ = Highly Present

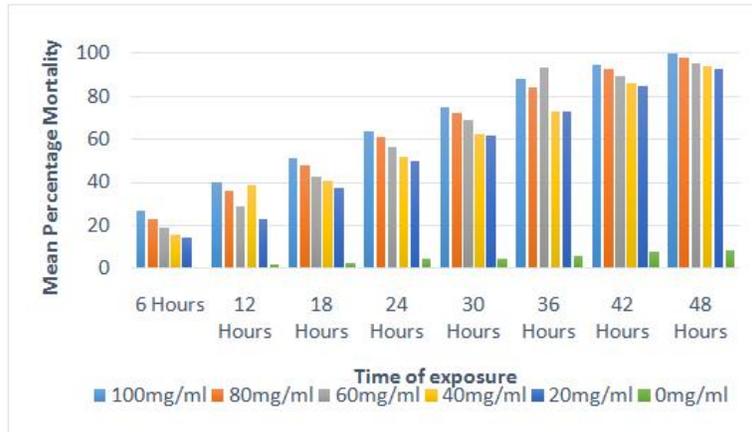


Fig. 1. Mean percentage mortality of nematode juveniles exposed to varying concentrations of *Tamarindus indica* at different time of exposure

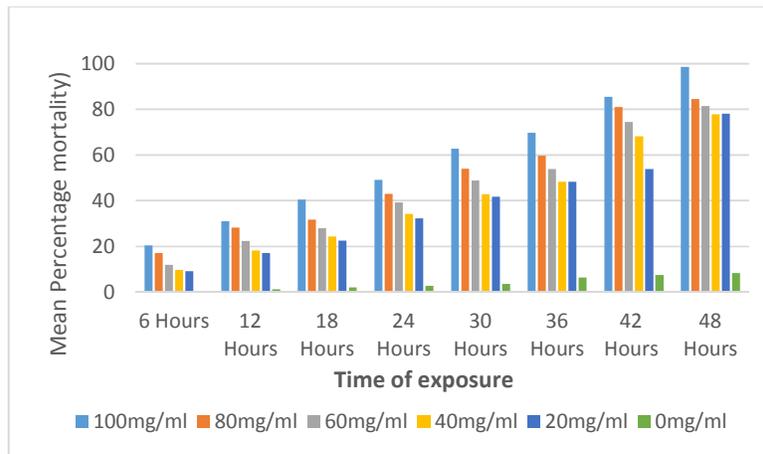


Fig. 2. Mean percentage mortality of nematode juveniles exposed to varying concentrations of *Citrus sinensis* at different time of exposure

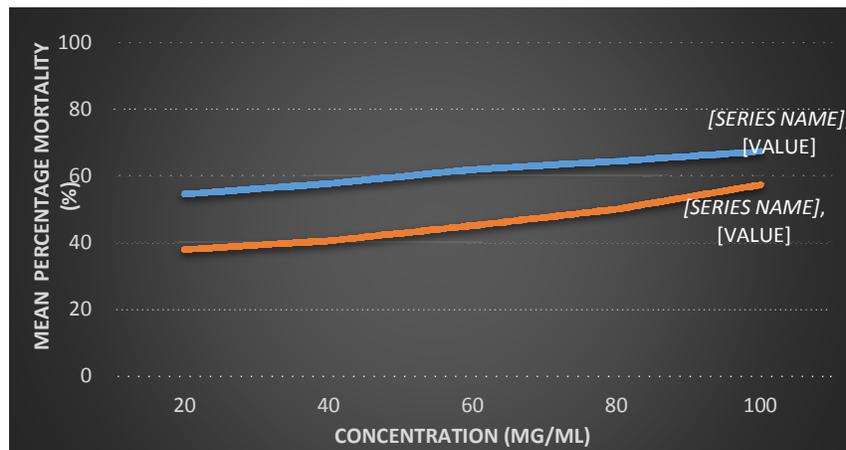


Fig. 3. Showing the (Average) nematocidal effect of the two plants' extracts on root-knot nematode over a 48 hours period at different concentrations (Data are represented as mean \pm SEM of two (2) independent variables at $P < 0.05$)

The results revealed that *T. indica* leaf extract had nematocidal activities on the root-knot nematodes. The percentage mortality increased with concentration and time exposed to the extracts as shown in Fig. 1, with 100mg/ml and 48hours being the highest concentration and time of exposure respectively. This result agrees with that of Bello et al. [22] who studied the Inhibitory effect of *T indica* on egg hatch mortality of *Meloidogyne incognita* and found out that the extract significantly inhibited the egg hatch and larvae of the nematode. Similar trend was observed for *C. sinensis*; percentage mortality was highest at 48hours time of exposure with a concentration of 100mg/ml. The results as shown in Fig. 2 also revealed that the death decreased with decrease in concentration and time of exposure. This result also supports that of Abolusoro and Olabiyi [23] who confirmed that the peel extract of *C. sinensis* reduces the multiplication rate of root-knot nematode, hence, reduction in consequent root damage of tomato. Extracts from several plants have shown certain level of inhibitory effects against nematodes and this result can be attributed to the phytochemical constituents of the extracts [24]. The extracts of *T. indica* and *C. sinensis* contained Flavonoids, Alkaloids, Saponins, Tannins, Steroids, Cardio glycosides, Phenols, and Resins. Alkaloids, glycosides, and phenolics amongst others have shown to possess nematocidal effects and they have been extracted and used as an alternative source of nematocides [25]. These phytochemicals can be assayed upon and the bioactive components be used for developing compounds that can be used to control nematode parasite.

Although, it was revealed that the different concentration of both treatments increased the mortality of the nematodes, the mortality wasn't dose-dependent all through. *T. indica* exposed nematode only showed a dose-dependency in 6, 18, 24, 42, and 48-hour exposed nematodes with 100mg/ml and 20mg/ml the having the highest and lowest mortality, respectively. Also, 6, 12, 18, 24, 30, and 42-hour *C. sinensis* treated nematodes revealed a dose-dependent increase in mortality with 100mg/ml having the highest mortality, while 20mg/ml having the lowest. So, it is evident that the effect of the extracts is based on not only the exposure time but also the concentration used. *T. indica* might be considered a better nematocide than *C. sinensis* because the result of former showed more mortality in the 48-hour exposure period. Nevertheless, both extracts could be used

independently or simultaneously, to control the nematode parasite.

4. CONCLUSION

In conclusion, *T. indica* leaf and *C. sinensis* peel extract could be used as a source of cheap and effective nematocides of root-knot nematodes. In addition, the extracts of the test's 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 bio-pesticides which will reduce or replace the dependence on chemical pesticides used presently which are usually expensive and non-environmentally friendly. However, further study on identification of active compounds of these plant extracts is needed to classify their nematocidal efficacy, especially on *M. incognita*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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