

Effect of Fig Tree (*Ficus Sycomorus*) Root Exudates on Root-Knot Nematode (*Meloidogyne Javanica*)

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Abstract: The effect of fig tree (*Ficus sycomorus*) root exudates on root-knot nematode (*Meloidogyne javanica*) was evaluated in the Laboratory of the Department of Crop Production and Horticulture/Crop Protection, Modibbo Adama University of Technology, Yola in 2008. This was to determine the egg hatch and juvenile mortality of *M. javanica* and also the nematicidal properties of the root exudates. The experimental design used was the Completely Randomized Design (CRD) consisting of six (6) treatments replicated four (4) times for both *F. sycomorus* root exudates for egg hatch and juvenile mortality. The result obtained shows that, egg hatch in 10% concentration of *F. sycomorus* root exudates at 168hours of exposure was significantly higher (318.00) followed by 8% concentration (296.00) at 168hours and the least was observed in 2% concentration of *F. sycomorus* root exudates at 24hours of exposure (97.00). Similarly, juvenile mortality at 100% concentration of *F. sycomorus* root exudates at 168hours of exposure was significantly higher (132.00) followed by 80% concentration (107.00) at 168hours and the least was reported in 0% concentration at 24hours exposure (9.00) at $p \leq 0.05$ using the least significant difference (LSD) test for variables. The result clearly shows that, egg hatch and juvenile mortality in all the treatments increased significantly with increase in time of exposure and concentration of the root exudates. 70% of juvenile mortality was recorded on the 7th day (168hours) at the highest concentration of 100%, which further stressed that, *F. sycomorus* root exudates has nematicidal properties. The chemical analysis of the root exudates also reveals that, saponins, phenols, 3.5% alkaloids and pH of 6.81 were confirmed.

Keywords: Concentration, Exudates, Eggs, Juveniles, Nematodes, Hatching, Mortality.

I. Introduction

Root-knot nematode (*Meloidogyne* spp.) is one of the most important plant parasitic nematode worldwide, causing a yield loss of 5% out of the 14% estimated to be caused by all plant parasitic nematodes (Jackson et al., 2003). It is known to cause yellowing of leaves, fruit fall, flower abortion, stunted growth and in some cases complete yield loss (Adesiyan et al., 1990; Olabiyi et al., 1992; Jackson et al., 2003). Worldwide, *Meloidogyne* spp attack a wide range of plants from vegetables (Bridge, 1972), cereals (Jada, 2001), root and tuber crops (Idowu, 1985; Jada, 2007) and tree crops (Idowu et al., 1990). Different control measures have been used to control *Meloidogyne* spp. which includes chemical control measure that is successful but costly to economically low income farmers and less friendly to the environment. Other control measures include biological, physical, cultural techniques, use of plant extracts and the use of root exudates (Baker et al., 1994). Extracts from many plants purported to have anthelmintic effects are effective against soil nematodes. Effective plants includes both annuals and perennials, range from grasses and herbs to woody trees (Prot and Kornprobst, 1983; Jada, 2001). Ferris et al. (1992) suggested that, identification of chemical stimuli released by plant roots to be used as nematicides is needed. *Ficus sycomorus* root exudates is used as medicine against whooping cough by the local people in Nigeria. This work is therefore aimed at identifying the effect of *F. sycomorus* root exudates on *M. javanica* egg hatch and juvenile mortality.

II. Materials And Methods

Collection of Root Exudates

A small pit 30cm deep and 15cm wide was dug to reach the roots. After the roots were found, a considerable portion of the root was selected and cut using an axe. To the remaining part of the root that was attached to the tree, a strong polythene bag was tied to avoid any external material from tempering with the root exudates. The cutting was done at about 5pm and the polythene was left over night and the sample was collected around 7am the following morning. The root exudates obtained was designated as 100% concentrate.

Maintenance of *M. javanica* culture

Root-knot nematodes infested roots containing egg masses were collected from the Teaching and Research Farm of the Department of Crop Production and Horticulture, Modibbo Adama University of Technology, Yola. The egg masses were then inoculated into three (3) pots with tomato growing in them in the screen house.

Preparation of Root Exudates

The root exudates collected was designated as 100% concentrate. The root exudates was prepared in concentrations by taking 2ml of the 100% concentration of the root exudates mixed with 98ml of water to give 2% concentration, 4ml of the 100% concentration of the root exudates mixed with 96ml of water to give 4% concentration, 6ml of the 100% concentration of the root exudates mixed with 94ml of water to give 6% concentration, 8ml of the 100% concentration of the root exudates mixed with 92ml of water to give 8% concentration and 10ml of the 100% concentration of the root exudates mixed with 90ml of water to give 10% concentration.

Inoculation of *M. javanica* eggs into *F. sycomorus* Root Exudates

Infected tomato was uprooted from the culture prepared and egg masses of *M. javanica* were removed from the roots using forceps and needle according to Holbrook et al. (1983). The egg masses were then introduced into 1% sodium hyper chloride to release the eggs. The eggs were washed using water and 500 eggs were counted into each dilution of the root exudates including the control (0%, 2%, 4%, 6%, 8% and 10%). Each of these were replicated four (4) times laid in a Completely Randomized Design (CRD) placed on a bench in the Laboratory of the Department of Crop Production and Horticulture/Crop Protection Modibbo Adama University of Technology, Yola in 2007.

Effect of *F. sycomorus* Root Exudates on *M. javanica* Juvenile Mortality

Two hundred (200) juveniles were counted and inoculated into the 100% concentration of the prepared root exudates. This was done when 20ml of the 100% concentration was taken and mixed with 80ml of water to give 20% concentration, 40ml of the 100% concentration mixed with 60% of water to give 40% concentration, 60ml of the 100% concentration mixed with 40ml of water to give 60% concentration, 80ml of the 100% concentration mixed with 20ml of water to give 80%, 100% concentration was taken as the last treatment.

III. Chemical Analysis

Preliminary chemical analysis of the root exudates was carried out in the Laboratory to determine the alkaloids and other constituents responsible for the nematocidal action. 2ml of the root exudates was taken in a test tube, shaken and frothing that persisted on heating confirmed the presence of saponin. In another test, 1ml of the sample was taken and three (3) drops of 1% iron chloride ($FeCl_3$) solution added indicated a colour change to green for a short time which gradually fades away and is typical of phenols. Furthermore, 1ml of the sample was taken and mixed with 3ml of picric acid, green precipitate was formed, which indicated the presence of alkaloids. The percentage alkaloids were calculated thus:

Weight (g) of filter paper = 0.7144.....weight 1

Weight (g) of filter paper and weight of sample = 0.7494.....weight 2

Weight (g) of sample = 25g

% alkaloids = $\frac{W_2 - W_1}{W_2} \times 100$

$= \frac{0.7494 - 0.7144}{25} \times 100$

$= \frac{0.035}{25} \times 100$

$= 3.5\%$

Furthermore, the pH of the sample was determined using pH model of Ph 5- 25pH meter, giving a pH of 6.81.

Data Collected

The number of hatched *M. javanica* juveniles were observed under the stereomicroscope and counted for each treatment on exposure for 24, 48, 72 and 168hours (7day) respectively. Similarly, the numbers of dead *M. javanica* juveniles were counted at 24, 48, 72 and 168hours (7days) respectively. Needle tip was used to move each juvenile three (3) times under a stereomicroscope. Those juveniles that did not respond in the form of movement were assumed dead.

Statistical Analysis

Data collected were subjected to analysis of variance appropriate to Completely Randomized Design (CRD) according to Gomez and Gomez (1984). While the means that were significant were separated using the Least Significant Difference (LSD) as described by Fisher and Hedge (1937).

IV. Results

Hatched at 24 and 48hours of exposure was recorded in 10% concentration of the F. sycomorus root exudates (245.00 and 284.00), 8% concentration (173.00 and 213.00), 6% (158.00 and 177.00), 0% (145.00 and 219.00) and the least was obtained in 2% concentration of the fig tree root exudates (97.00 and 108.000). The highest number of eggs hatched at 72 and 168hours of exposure was seen in 10% concentration of the fig tree root exudates (300.00 and 318) significantly followed by 8% (249.00 and 296.00), 0% (248.00 and 302.00), 6% (193.00 and 262.00), 4% (162.00 and 185.00) and least was observed in 2% concentration of the fig tree root exudates (121.00 and 142.00) at $P \leq 0.05$ using the least significant difference (LSD) test for variables. There was significant difference among the treatments as shown in Table 1.

Table 1: Effect of *F. sycomorus* root exudates on cumulative egg hatch of *M. javanica* for 24, 48, 72 and 168hours.

Hours of exposure Concentration (%)	24hours	48hours	72hours	168hours
0%	145.00	219.00	248.00	302.00
2%	97.00	108.00	121.00	142.00
4%	125.00	142.00	162.00	185.00
6%	158.00	177.00	193.00	262.00
8%	173.00	213.00	249.00	296.00
10%	245.00	284.00	300.00	318.00
S. E.	9.93	13.48	15.50	12.07
LSD	72.61**	98.51*	113.29*	88.18**

Note:**=highly significant ($P \leq 0.01$), *=significant ($P \leq 0.05$), LSD=least significant difference, S.E.=standard error.

The highest cumulative mortality of *M. javanica* juveniles exposed at 24 and 48hours was recorded in 100% concentration of the *Ficus sycomorus* root exudates (65.00 and 84.00) followed by 80% (53.00 and 69.000), 60% (42.00 and 53.00), 40% (31.00 and 45.00) and the least was observed in 0% concentration of the fig tree root exudates (9.00 and 15.00) respectively. Similarly, 100% concentration of *F. sycomorus* root exudates gave the highest cumulative mortality of *M. javanica* juveniles exposed at 72 and 168hours (99.00 and 132.00) followed by 80% (84.00 and 107.00), 60% (72.00 and 91.00), 40% (64.00 and 75.00), 20% (49.00 and 61.00) and the least was reported in 0% concentration of the fig tree root exudates as shown in Table 2.

Table 2: Effect of *F. sycomorus* Root Exudates on cumulative Mortality of *M. javanica* Juveniles on Exposure for 24, 48, 72 and 168hours.

Concentration (%)	Hours of Exposure			
	24hours	48hours	72hours	168hours
0%	9.00	15.00	30.00	42.00
20%	21.00	36.00	49.00	61.00
40%	31.00	45.00	64.00	75.00
60%	42.00	53.00	72.00	91.00
80%	53.00	69.00	84.00	107.00
100%	65.00	84.00	99.00	132.00
S.E.	0.63	1.10	1.19	0.96
LSD	4.59**	8.04**	8.70**	7.02**

Note: **=significant ($P \leq 0.01$) *= significant ($P \leq 0.05$), LSD=least significant difference, S.E. =standard error.

Saponin was confirmed when 2ml of the root exudates was taken in a test tube and shaken. 1ml of the root exudates was taken and 3 drops of 1% iron chloride (FeCl₃) solution added which indicates the presence of phenol. Furthermore, 1ml of the root exudates was taken and mixed with 3ml of petric acid, green precipitation was formed which indicated the presence of alkaloids of 35%. The pH of 6.81 was also determined.

Table 3: Chemical Analysis of *Ficus sycomorus* Root Exudates

Treatments	Nematicidal Action
2ml exudates + olive oil emulsion	Saponin
1ml exudates + 3 drops of 1% FeCl ₃	Phenols
1ml of exudates mixed with 3ml petric acid	Alkaloids – 3.5%
PH5-25pH meter	pH = 681

V. Discussion

This work shows that, more eggs of *Meloidogyne javanica* hatched at the highest concentration of the root exudates (10%) and with increase in the time of exposure (7days). This means that, the root exudates could not suppress or inhibit egg hatching instead stimulated them. This might be due to the fact that, the egg shell was weakened or even dissolved by the root exudates of *Ficus sycomorus*, thereby releasing more juveniles. This work is consistent with the findings by Riga et al. (2000) who reported that, root exudates increased egg hatching in nematodes by 46.6%. This invariably means that, at lower concentration of the root exudates (2%), egg hatching was low at all the time of exposure. However, the low concentration did not totally inhibit egg hatching, but rather delayed it. This study also agrees with Magnarelli and Nowierski (2004) who suggested that, the root exudates were antagonistic to cyst and root-knot nematodes as a result of the toxic metabolites in the root exudates.

The effect of fig tree (*F. sycomorus*) root exudates on cumulative mortality of *M. javanica* juveniles at different time of exposure (24, 48, 72 and 168hours) shows that, dead count was observed at all the concentration levels of the root exudates for all the time of exposure including the control. Hence, dead count increases with increase in concentration levels of the root exudates and time of exposure. This study agrees with Noling (2005), who stated that, there is a corresponding increase in juvenile mortality of *M. javanica* with increase in concentration of root exudates and also with the time of exposure. Baker et al. (1994) also validated this finding when they reported that, 100% mortality of *M. javanica* juveniles were recorded after 7days (168hours) of exposure and at higher level of root exudates concentration (100%).

Chemical analysis to determine the nematicidal constituent of the *F. sycomorus* root exudates shows that, 3.5 alkaloids, phenols, saponins and pH of 6.81 were confirmed. The presence of the above mentioned indicated that, *F. sycomorus* root exudates has nematicidal properties and also had considerable on cumulative the results obtained. This work further stressed the findings by Peterson and Clinch (1994), who suggested that, several potentially stimulating and toxic substances have been isolated from *F. sycomorus* root exudates. These includes; spectine, autofine, phenols, saponins, alkaloids, tylophonine etc.

VI. Conclusion

The study has shown that, egg hatch and juvenile mortality in *M. javanica* increased with increase in time of exposure to *F. sycomorus* root exudates. The highest egg hatch and juvenile mortality was recorded on the 7th day (168hours) at 10% concentration for egg hatch and 100% concentration of the root exudates for juvenile mortality respectively. Hence, the highest egg hatch recorded was 317 at 10% concentration level and at the longest time of exposure while the highest juvenile mortality was 132 at 100% concentration level of the root exudates and at longest period of exposure. It was also discovered that, *F. sycomorus* had no inhibitory effect on *M. javanica* eggs but had nematicidal properties that killed the juveniles. Furthermore, the chemical analysis of the root exudates reveals that, it had the following constituents, alkaloids (3.5%), phenols, saponins and a pH of 6.81. *F. sycomorus* is abundantly found in forests and savannah regions of Nigeria, the root exudates can easily be tabbed and elucidated for synthetic manufacture of the identified chemicals for uses as nematicides.

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