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Effect of Root Knot Nematode, *Meloidogyne incognita* on the Growth Characteristics of Horse Gram, *Macrotyloma uniflorum* Treated with Fruit Extract of *Aegle marmelos*

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Abstract: Plant-parasitic nematodes have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination. Root-knot nematodes, *Meloidogyne sp*., are common pathogens that parasitize vegetables and other crops and cause significant yield reductions Worldwide. They disrupt the physiology of the plant and may reduce crop yield and product quality. Therefore, the need to develop alternative methods to control the plant parasitic nematode that are cheap, environmentally friendly and not harmful to humans. The use of botanicals is one of the alternative methods suggested by nematologists for nematode control. Hence, the present investigation has been carried out by the growth characteristics, such as, root length, shoot length, fresh and dry weight of root, fresh and dry weight of shoot, leaf area, water content of the root and shoot and root gall index of *Macrotyloma uniflorum* infected with *M.incognita* and treated with fruit extract of *Aegle marmelos* were studied after 65 days of treatment. These growth characteristics, such as, root and shoot length, fresh and dry weight of root and shoot, leaf area, root gall index were found to be decreased with increasing inoculum levels of egg masses and increased with increasing concentrations of leaf extract treatment.

Key words: Ecofriendly · Inoculums · Egg Masses · Growth Changes · Leaf Area · Root Gall Index

INTRODUCTION

Agriculture is the main and basic source to human diet on the face of the earth. Many efforts were exerted to improve agricultural productions. With human development and expansion of agricultural areas, many problems emerged such as the spread of pests and diseases, prompting human to try to limit the spread of these diseases, whether fungal diseases, bacterial or even nematodes, as well as all kinds and forms of insects, weeds and snails [1]. Plant Parasitic Nematodes (PPN) infesting several growing crops, such as, vegetables and leguminous crops, oil crops, fiber crops, grain crops and fruit trees next to weeds which are the secondary host to parasitic nematodes. The most well-known species of root-knot nematode are *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* which are responsible for high economic damage to varied crops [1].

Although the application of chemical nematicides has been found to be an effective measure for the control of nematodes, the highly toxic residual effect of chemical on the environment and particularly on non-target organisms, require an urgent need to develop alternative strategies for the control of nematodes. Synthetic pesticides are the principle means of controlling nematodes but these are expensive as well as environmentally unsafe. For modernization of agriculture it has become essential that we adopt the more environmentally friendly practice. The agriculturist are taking interest in developing bio-pesticides that are pest specific, non toxic to human, less expensive and safe for the environment [2]. Many plants are known to have nematicidal properties which may be utilized as organic amendments or bio-pesticides. Many scientists have carried out the research on plant extracts for the management of root knot nematodes.

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Botanical extracts are alternative to nematicides in recent times. Some botanicals such as, *Argemone mexicana, Calotropis procera, Solanum xanthocarpum* and *Eichhornia echinulata* are already being exploited in nematode management [3]. Plants are nature's chemical factories which provide the richest sources of organic chemicals on earth [4]. Exploration of nematicidal potential of botanicals (egg hatchability and larval mortality test) and their application is on increase. Different plant species are being tested to identify the sources of nematicidal substances (analyzing photochemical using GC -MS) and many of them have shown promising results in the control of plant parasitic nematodes [5]. Hence the present study has been done to evaluate the effect of fruit extract of *A. marmelos* on root knot nematode *M. incognita* and growth characteristics of horse gram *M. uniflorum*.

MATERIALS AND METHODS

For the present study, sterilized soil mixers (River soil, Garden soil and Red soil) were used in the proportion of 2:1:1 ratio. The sterilized *M. uniflorum* seeds were shown in plastic pots of one litre capacity. The nematode egg masses were collected from the roots of infected *Acalypa indica* plants in near agricultural fields. The egg masses were isolated and separated using a compound microscope. The egg masses were inoculated at different levels (5, 10 and 15) pouring four holes in top soil of experimental plants. After inoculation the distilled water was poured for three days and plant extract were add in alternate days. The fruit extract of *A. marmelos* was prepared by soxhlet apparatus using acetone as a solvent. The different concentrations of leaf extract (5, 10 and 15 ppm) using distilled water. After 45 days of treatment, the growth characteristics, such as, root length, shoot length, fresh and dry weight of root, fresh and dry weight of shoot, leaf area, water content root and shoot and root gall index were analyzed.

RESULTS AND DISCUSSION

In the present study, various growth parameters, such as, shoot length, root length, fresh and dry weights of shoot and root, leaf area, root gall index were analyzed after 45 days of treatment with different concentrations of *A.marmelos* infected with three different inoculum levels of the root-knot nematode egg masses of *M. incognita* (5,10 and 15 egg masses). After 45 days of fruit extract treatment, the shoot and root length (cm) of horse gram, *M. uniflorum* were analyzed. The shoot and root length was found to be increased with increasing concentrations of fruit extract compared to control and its decreased with increasing inoculums levels of *M.incognita* (Table 1). The result were found to be significantly different (P<0.001). Similarly Dongre and Simon [6] reported that plant extracts were found to significantly increased shoot length and root length, root weight and root galls were reduced in high concentration. The increase in infestation as reflected by the number of galls might be the reason for the reduction of plant height [7].

Table 1: Effect of the root-knot nematode, Meloidogyne incognita and the fruit extract of Aegle marmelos on the treatments on the shoot and root length (cm) of the horse gram Macrotyloma uniflorum after 45 days treatment.

No. of	Shoot length (cm)					Root length (cm)				
Egg masses	Con	In. con	5ppm*	10ppm*	15ppm*	Con	In. con	5ppm*	10ppm*	15ppm*
5	85.13±0.25	65.06±0.64	76.73 ± 0.76	82.13 ±0.35	86.56±0.25	27.63 ± 0.49	21.36 ± 0.31	23.2 ± 0.26	25.23 ±0.35	26.9±0.61
10		58.4±0.2	73.53 ± 0.95	81.26 ±0.42	83.86±0.21		20.43 ± 0.47	22.4 ± 0.36	24.53 ±0.40	26.4±0.26
15		49.86±0.83	67.86 ± 0.50	79.33 ±0.72	80.13±0.35		19.66 ± 0.32	21.86 ± 0.25	23.36 ±0.42	25.76 ±0.45

Note: Data are the average value of three replications * Means statistically significant, P <0.001.\

Con: Control, In.con:Inoculated Control ppm: parts per million

Table 2: Effect of the root-knot nematode, M. incognita and the fruit extract of A. marmelos on the treatments on the fresh and dry shoot weight (g) of the horse gram M. uniflorum after 45 days treatment.

No. of	Fresh weight of the shoot (g)					Dry weight of the shoot (g)				
Egg masses	Con	In. con	5ppm*	10ppm*	15ppm*	Con	In. con	5ppm*	10ppm*	15ppm*
5	14.36±0.47	5.64±0.17	8.00 ± 0.35	11.30 ±0.44	15.81±0.76	6.69 ± 0.49	1.44 ± 0.06	2.06 ± 0.09	2.72± 0.09	5.43 ±0.37
10		5.06±0.24	6.92 ± 0.39	9.88±0.43	12.61±0.81		1.30 ± 0.03	1.81 ± 0.09	2.53±0.04	4.62±0.37
15		4.02±0.36	5.97 ± 0.22	9.08±0.18	11.37±0.56		1.11 ± 0.11	1.62 ± 0.09	2.25±0.11	3.58± 0.58

Note: Data are the average value of three replications * Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million

Table 3: Effect of the root-knot nematode, M. incognita and the fruit extract of A. marmelos on the treatments on the fresh and dry root weight (cm) of the horse gram M. uniflorum after 45 days treatment.

No. of	Fresh weight of the root (g)					Dry weight of the root (g)				
Egg masses	Con	In. con	5ppm*	10ppm*	15ppm*	Con	In. con	5ppm*	10ppm*	15ppm*
5	3.67±0.36	1.03±0.07	1.30 ± 0.01	2.21±0.11	2.57±0.14	1.55 ± 0.32	0.21 ± 0.03	0.34 ± 0.09	0.65± 0.09	1.32 ±0.27
10		0.87±0.05	1.17 ± 0.01	1.72±0.11	2.57±0.62		0.17 ± 0.02	0.30 ± 0.02	0.49± 0.56	1.12± 0.03
15		0.66±0.11	1.14 ± 0.02	1.48±0.14	2.36±0.53		0.13 ± 0.02	0.26 ± 0.03	0.37±0.01	0.86± 0.09

Note: Data are the average value of three replications * Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million

Table 4: Effect of the root-knot nematode, M. incognita and the fruit extract of A. marmelos on the treatments on leaf area (cm2) and root gall index of the horse gram M. uniflorum after 45 days treatment.

No. of	Leaf area (cm^2)					Root gall index				
Egg masses	Con	In. con	5ppm*	10ppm*	15ppm*	Con	In. con	5ppm*	10ppm*	15ppm*
5	22.88±0.93	11.19±0.82	12.83 ± 0.52	14.44 ±0.48	16.94±0.70	0	4.31 ±0.4	3.17 ±0.5	2.16±0.6	1.23±0.2
10		10.74±0.45	11.63 ± 0.26	13.10 ±0.40	15.08±0.90		4.64 ±0.3	2.63 ±0.6	2.03±1.3	1.07±0.1
15		8.49±0.44	10.41 ± 0.80	12.24 ±0.38	14.55±0.43		5.23 ±1.3	3.17 ±0.3	2.74±1.7	0.54±0.1

Note: Data are the average value of three replications * Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million

The fresh and dry weight of the root and shoot was significantly reduced in the increasing inoculums levels. While in the fruit extract treated plants the fresh and dry weights are increased with increasing concentrations (Table 2 & 3). The result were found to be significantly different (P<0.001). According to Hussey [8], an increase in shoot and root weights were increased, due to the uptake and transportation of water and nutrients which is dependent on the health of the roots. Couch and Staden [9] recorded significant increase in plant height and a corresponding reduction in *M. incognita* infection when *Ecklonia maxima* extract was applied as soil drench.

The leaf area of the control plants found to be 22.88 ± 0.93cm^2 and it is reduced the egg masses treated plants 11.19 ± 0.82 cm^2 (5 egg masses), 10.74 ± 0.45 cm^2 (10 egg masses) and 8.49 ± 0.44cm^2 (15 egg masses). While in the leaf extract treated experimental plants the leaf area was increased with increasing concentrations (5, 10 and 15 ppm) (Table 4). This may be probably due to the antihelminthic and nematicidal activity of the medicinal plant *A.marmelos*. Similar observations have been found by Jonathan and Rajendran [10] in the pathogenic effect of root-knot nematode *M. incognita* on leaf area were reduced in banana, *Musa* species.

The efficacy of fruit extract of *A. marmelos* on the root-knot nematode, *M.incognita* infecting the horse gram, *M. uniflorum* was elucidated individually on the root gall index and presented in the Table 4. With reference to root gall index the inoculated control plants showed increased gall index with increasing level of egg masses. The root gall index has been decreased gradually from increasing concentrations of fruit extract. The similar observation was observed Goswami and Vijalakshmi [11] they reported that the number of galls/g of root was reduced by 9 different extracts, especially with *Eclipta alba, Shorea rubuta* and *Datura metel*. Chabra *et al*., [12] also noted that leaf extracts of *Ricinus communis, Leucaena leucocephala, Populas deltoids, Azadirachta indica, Lantana camara* and *Eucalyptus hybrida* were highly toxic to juveniles of *M. incognita*. Galling is a reaction of the plant to the feeding of the root-knot nematode which may also vary in size with different applications of plant materials. Generally speaking, the total number of galls per plant was influenced by the amendment treatments. Interestingly, *Chromolaena odorata* treated plots appeared to fare better with a damage rating of moderate compared with the other two treatments? severe damage rating while the control with the highest galls/plant had a damage rating of "very severe". These findings agree with that of Amosu [13] who reported that *C. odorata* was better than the other plant materials tested against attacks of *M. incognita* attack on tomato plants.

Table 5 showed that the water content (%) on the leaves of horse gram, *M. uniflorum* infected with root knot nematode and treated with the fruit extract of *A.marmelos.* In control plants, the water content of the shoot and root was normal. While in the inoculated control plants the water content of the shoot and root was found to be decreasing with increasing levels of egg masses inoculum level. At different concentrations of *A. marmelos*, the water content of the shoot was found to be increasing with increasing concentration of the fruit extract (5, 10 and 15 ppm) treatments. Similarly Pavaraj [14] reported that

Table 5: Effect of the root-knot nematode, M. incognita and the fruit extract of A. marmelos on the treatments on water content (%) on shoot and root of the horse gram M. uniflorum after 45 days treatment.

No. of	Water content (%) in shoot					Water content (%) in root				
Egg masses	Con	In. con	5ppm*	10ppm*	15ppm*	Con	In. con	5ppm*	10ppm*	15ppm*
5	75.56±0.38	69.25±0.24	72.52 ± 0.35	73.81 ±0.16	75.03±0.18	80.18 ± 0.28	77.35 ± 0.30	81.20 ± 0.31	84.83 ±0.21	86.11 ±0.19
10		68.17±0.20	71.34 ± 0.32	73.55 ±0.32	74.23±0.23		76.17 ± 0.22	80.22 ± 0.34	82.14 ± 0.22	85.34 ± 0.25
15		65.26±0.17	68.20 ± 0.17	72.13 ±0.13	74.19±0.19		70.32 ± 0.33	73.28 ± 0.26	75.55 ±0.31	77.70 ±0.4

Note: Data are the average value of three replications * Means statistically significant, P <0.001.
Con: Control, In.con:Inoculated Control, ppm: parts per million

the, water content have been increased *Ageratum conyzoides* leaf extract treated plants. Distortion of the plant roots and nematode colonization of vascular bundles could reduce supply of nutrients to plants. The galls on the root system might disturb important root functions like uptake and transport of water and nutrients [15].

CONCLUSION

The leaf extract *Aegle marmelos* has a remarkable nemecidal property on *M. incognita* and thereby, it can be used in the control of plant root-knot nematodes. It is recommended for the control of nematode population for the small scale former.

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