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Efficacy of leaf extract of *aegle marmelos* on the biochemical changes on orse gram *macrotyloma uniflorum* infected by root-knot nematode *melodogyne incognita*

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ABSTRACT

Root-knot nematodes modify plants root tissue and decrease plant growth and ultimately the crop yield the severity of crops loss resulting from root-knot nematode attack in the field is influenced by many biological and physiological factors. Though most of the researchers have investigated that leaves, roots are valuable constituents of the plants for nematicidal activities. Hence, the present study the biochemical characteristics like phenol, nitrate reductase activity and total chlorophyll content of horse gram, *Macrotyloma uniflorum* on infected with *Meloidogyne incognita* treated with leaf extract of *Aegle marmelos*. The biochemical constituents like nitrate reductase activity and chlorophyll content were increased by increasing concentration of leaf extract treated plants and it is decreased by increasing level of egg masses (5, 10 and 15) inoculated control than non- inoculated control except phenol.

Introduction

Plant parasitic nematodes are responsible for global agricultural losses amounting to an estimated $157 billion annually (Abad *et al*. 2008). The root-knot nematodes (*Meloidogyne sp*.) seriously affect many economically important agricultural crops Worldwide. Root knot nematode is one of the most harmful pests in both tropical and subtropical agricultural production (Wesemael *et al*. 2010). Nematode not only suppresses the plant growth but also interferes in the nodulation, nitrogen fixation and adversely affects the overall yield. Root knot nematodes are obligate, sedentary parasites of vascular tissues of plant roots. Although over 4,100 species of plant-parasitic nematodes have been identified (Decraemer and Hunt, 2006) new species are continually being described while others, previously viewed as benign or non-damaging, are becoming pests due to change of cropping patterns (Nicol, 2002). However, the plant parasitic nematodes of economic importance can be grouped into relatively restricted specialized groups that either cause direct damage to their host or act as virus vectors. Root-knot disease of various plants caused by *Meloidogyne incognita* is a serious problem being encountered by large number of farmers throughout the world. (Jothi *et al*., 2001). The root-knot nematode, *Meloidogyne sp*., is one of the world's most damaging plant pests (Sasser and Carter, 1985), out of which *M.incognita* has been reported to be the most important nematode pest in India.

The symptoms of nematode infection are the formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting and mineral deficiency (Abad *et al*., 2003). Modern way of nematode control is totally based on the nematicides as higher population growth demands increase crop production. But the nematicides not only toxic to the root-knot but also accumulate in plant and often lead to environmental pollution. So, there is an urgent need for an eco-friendly substitute for nematode control. Plant parts or products proved to be the promising alternative means and showed toxicity to pest up to a certain extent and their application offers complete economic advantage. Hence, the present investigation was undertaken the root-knot nematode *M. incognita* would interfere the biochemical parameters like, NR activity, phenol and chlorophyll in the leaves of horse gram, *M. uniflorum* treated with leaf extract of *A. marmelos*.

Material and Methods

Surface sterilized *Macrotyloma uniflorum* seeds were sown in plastic pots of one litre capacity containing autoclaved sterilized river soil, garden soil and red soil (2:1:1). The egg masses of root-knot nematode, *M. incognita* were collected from the root galls infected plants of *Acalypa indica* and kept in separate embryo cups with 5, 10 and 15 egg masses. The experimental plants were inoculated with 5, 10 and 15 egg masses of the nematode by pouring into four holes and were closed with top soil. Distilled water was poured for three days after inoculation. Thereafter, the nutrient solution prescribed by Arnon and Hoagland, 1940 and plant extract were added in alternate days. Air dried *A. marmelos* leaves were prepared by extracting 25g of plant material in 200 ml acetone (55 ^{0}C) in soxhlet apparatus (Peach and Tracey, 1956). Different concentrations of plant extract, such as, 5, 10 and 15 ppm were prepared from stock solution using distilled water. After 45 days of treatment, the biochemical characteristics, such as Nitrate reductase activity of leaves Jaworski (1971), total Phenol of leaves (Bray and Thorpe, 1954) and Total chlorophyll of leaves Wellburn and Lichtenthaler (1984) were estimated.

Statistical Analysis

The efficacy of the different levels (5, 10 and 15 egg masses) of the root knot nematode, *M.incognita* and the different concentrations (5, 10 and 15ppm) fruit extract of *A.marmelos* were statistically analysed by using standard deviation and ANOVA in a computer software (www.faculty wassar.edu/lowry anova 2u).

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Results And Discussion

The nitrate reductase (NR) activity in the leaves of horse gram *M.uniflorum* infected with 5, 10 and 15 egg masses of *M. incognita* and treated with different concentration of *A.marmelos* were analyses after 45 days treatment. The NR activity of the control plants was found to be 23.31 ± 0.02. While in the inoculated control plants have low nitrate reductase activity 8.32 ± 0.03 at 5 egg masses inoculum level, 7.46 ± 0.06 at 10 egg masses inoculum level and 5.81 ± 0.03. There is increasing activity of nitrate reductase activity in the leaves of treated plants with increasing concentrations of the leaf extract of *A. marmelos* from 11.60 ± 0.05 at 5 ppm, 17.44 ± 0.08 at 10 ppm to 22.45 ± 0.04 at 15 ppm at 5 egg masses inoculum level. The same trend was observed in 10 and 15 egg masses inoculum levels. The result were found to be significantly different (P<0.001). Mohanty *et al*. (1997) reported that reduced nitrogenase activity was observed in nematode infected nodules. Similar observations made by Chahal and Chahal, (1989) in Mung bean infected by *M. incognita*. The functioning of nitrogenase is directly related to leghaemoglobin content as it regulates the diffusion of oxygen. Reduction in leghaemoglobin content might be the possible cause of low nitrogenase activity. Reduced nitrate reductase activity was observed in the present investigation in *Macrotyloma uniflorum* leaves infected by *M. incognita* at various

Inoculum levels. A similar observation has been made by James (2004) and Pavaraj (2007) reported that infected plants shows low NR activity than the experimental plants.

The total phenol content present in the leaves of *M.uniflorum* infected with 5,10 and 15 eggs masses of *M. incognita* and treated with different concentrations of *A. marmelos* were analysed after 45 days treatment. Total phenol content of the control plants was found to be 9.71 ± 0.35 (mg/g). While the phenol content in the inoculated plants increased with increasing inoculum levels of egg masses. The result were found to be significantly different (P<0.001). At different concentrations of *A. marmelos* the phenol content found to be decreasing with increasing concentrations of the leaf extract. The phenolic compounds may help in the formation of hypersensitive reactions towards the nematode infection. Therefore, the rate of production and libration of phenolics by plant tissue during post-infectional period may be one of the important biochemical events in different host parasitic relationships (Ganguly and Dasgupta, 1994).

Merely presence of phenols, which are normal metabolites in a host plant and which accumulate at sight of infection and play a role in the resistance of that host to a given parasite. Bhargava *et al*. (2007) reported that the total phenol increased with infected plants than the healthy plants. These observations are in confirmation of earlier reports (Hung and Rohde 1973) they had showed that resistant cultivar of tomato and brinjal had more total phenol than susceptible and also that greater increase in phenolic contents after infection of nematode had occurred in resistant.

The total chlorophyll content present in the leaves of horse gram, *M.uniflorum* inoculated with 5,10 and 15 egg masses of root-knot nematode, *M. inocgnita* and treated with different concentrations of *A. marmelos* were analyzed after 45 days treatment. In the total chlorophyll content of control plants has been found to be 19.02 ± 0.51 mg/g that has been reduced to 12.68 ± 0.07 at 5 egg masses inoculum level, 9.98 ± 0.23 at 10 egg masses inoculum level and 7.61 ± 0.15 at 15 egg masses inoculum level. In the treated plants the total chlorophyll content has been found to be increased with increasing concentrations of leaf extract, that is in 5 egg mass inoculum level the chlorophyll contents, has been found to be 13.83 ± 0.02 at 5 ppm, 14.74 ± 0.09 at 10 ppm to 16.06 ± 0.36 at 15ppm. The same trend was observed in 10 and 15 egg masses inoculum level. The result were found to be significantly different (P<0.001).Similar results has been found by Sitaramaiah and Prasadji (1989) in tomato plants infected with *M. javanica* for the infection of this nematode the chlorophyll content were decreased, but there is an increasing chlorophyll content was obtained due to the plant growth regulators treatment.

The same results have been obtained by Sundararaju and Cannayane (2002) by using plant extracts against *Pratylenchus coffeae* infecting banana. Ramakrishnan and Mohandas (1996) reported that the reduction of chlorophyll content in that leaves of infected plants is due to higher concentration of sugar, leads to the unavailabity of nitrogen to prevent chlorophyll formation Subramaniyam *et al*., (1976). In this present study, the total chlorophyll content present in the leaves of horse gram *M. uniflorum* have been found to be decreased in the inoculated plants, when compared to control plants. But in the treated plants the content were increased with increasing concentration of leaf extract of *A. marmelos* this is due to antinematic activity of the leaf extract.

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Table 1. Effect of the root-knot nematode, *Meloidogyne incognita* and the leaf extract of *Aegle marmelos* on the total phenol content (mg/gm) of horse gram *Macrotyloma uniflorum*

Inoculum level/ No. of Egg masses	Total Phenol content after 45 days treatment				
5	Control	Inoculated control	5ppm	10ppm	15ppm
	9.71 ± 0.35	22.40 ± 0.35	20.46 ± 0.21	16.58 ± 0.30	11.2 ± 0.07
10		26.43 ± 0.33	17.43 ± 0.28	15.50 ± 0.24	13.52 ± 0.26
15		36.57 ± 0.22	24.36 ± 0.38	19.46 ± 0.23	16.24 ± 0.08

Table 2. Effect of the root-knot nematode, *Meloidogyne incognita* and the leaf extract of *Aegle marmelos* on the Nitrogen reductase activity (NR) (M mole NO2 h^{-1},g^{-1} fr.wt) of horse gram *Macrotyloa uniflorum.*

Inoculum level/ No. of Egg masses	Nitrogen reductase activity (NR) (M mole NO2 h^{-1},g^{-1} fr.wt) after 45 days treatment				
5	Control	Inoculated control	5ppm	10ppm	15ppm
	23.31 ± 0.02	8.32 ± 0.03	11.60 ± 0.05	17.44 ±0.08	22.45 ± 0.04
10		7.46 ± 0.06	10.81 ± 0.01	15.81 ± 0.02	21.62 ± 0.04
15		5.81 ± 0.03	9.90 ± 0.08	12.50 ± 0.05	20.71 ± 0.15

Table 3. Effect of the root-knot nematode *Meloidogyne incognita* and the leaf extract of *Aegle marmelos* on the individual treatments on the total chlorophyll (mg/gm of fr.wt) content in the leaf of horse gram *Macrotyloma uniflorum* after days of treatment

Inoculum level/ No. of Egg masses	Total Chlorophyll content after 45 days treatment				
5	Control	Inoculated control	5ppm	10ppm	15ppm
	19.02 ± 0.51	12.68 ± 0.07	13.83 ± 0.02	14.74 ± 0.09	16.06 ± 0.36
10		9.98 ± 0.23	12.33 ± 0.23	13.51 ± 0.10	14.75 ± 0.09
15		7.61 ± 0.15	9.41 ± 0.06	11.57 ± 0.10	14.49 ± 0.64

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