Efficacy of leaf extract of *Aegle marmelos* on the biochemical changes on orse gram *Macrotyloma uniflorum* infected by root-knot nematode *Meloidogyne 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* 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.

**Keywords**

Meloidogyne incognita, 
Macrotyloma uniflorum, 
Aegle marmelos, 
Nitrate reductase activity.

**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 °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).
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).


References


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

<table>
<thead>
<tr>
<th>Inoculum level/ No. of Egg mass</th>
<th>Control</th>
<th>Inoculated control</th>
<th>5ppm</th>
<th>10ppm</th>
<th>15ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9.71 ± 0.35</td>
<td>22.40 ± 0.35</td>
<td>20.46 ± 0.21</td>
<td>16.58 ± 0.30</td>
<td>11.2 ± 0.07</td>
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<tr>
<td>10</td>
<td>26.43 ± 0.33</td>
<td>17.43 ± 0.28</td>
<td>15.50 ± 0.24</td>
<td>13.52 ± 0.26</td>
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</tr>
<tr>
<td>15</td>
<td>36.57 ± 0.22</td>
<td>24.36 ± 0.38</td>
<td>19.46 ± 0.23</td>
<td>16.24 ± 0.08</td>
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</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Inoculum level/ No. of Egg mass</th>
<th>Control</th>
<th>Inoculated control</th>
<th>5ppm</th>
<th>10ppm</th>
<th>15ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>23.31 ± 0.02</td>
<td>8.32 ± 0.03</td>
<td>11.60 ± 0.05</td>
<td>17.44 ±0.08</td>
<td>22.45 ± 0.04</td>
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<tr>
<td>10</td>
<td>7.46 ± 0.06</td>
<td>10.81 ± 0.01</td>
<td>15.81 ± 0.02</td>
<td>21.62 ± 0.04</td>
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<tr>
<td>15</td>
<td>5.81 ± 0.03</td>
<td>9.90 ± 0.08</td>
<td>12.50 ± 0.05</td>
<td>20.71 ± 0.15</td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Inoculum level/ No. of Egg mass</th>
<th>Total Chlorophyll content after 45 days treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Control</td>
</tr>
<tr>
<td>19.02</td>
<td>12.68 ± 0.07</td>
</tr>
<tr>
<td>10</td>
<td>9.98 ± 0.23</td>
</tr>
<tr>
<td>15</td>
<td>7.61 ± 0.15</td>
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