Nematicidal Effect of Methanolic Root Extract of *Aerva lanata* Against the Root Knot Nematode, *Meloidogyne incognita* on Bengal Gram, *Cicer arietinum*

**P. Murugeswari, C. Azhagumurugan and M.K. Rajan**

Department of Zoology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi 626-124, Tamil Nadu, India

**Abstract:** The present study has been carried out to evaluate the effect of different concentrations (5, 10, 15 ppm) of the root extract of *Aerva lanata* against the different inoculum levels (5, 10 and 15 egg masses) of root-knot nematode, *Meloidogyne incognita* on biochemical constituents of Bengal gram, *Cicer arietinum*. The results of the control and experimental plants were analyzed after 45 days, the biochemical constituents of Bengal gram such as, Protein, Amino acid and chlorophyll content were found to be decreased with increasing inoculum levels of egg masses and increased with increasing concentrations of root extract treatment except phenol content.

**Key words:** Protein · Amino Acid · Lipid · Chlorophyll · Phenol

**INTRODUCTION**

Nematodes form one of the most important groups of micro-organisms inhabiting the soil environment of the roots of plants and they frequently play a vital role in their growth and production. Rarely is any crop free of their attacks, whether in the field, orchard, the home or the green house [1]. Root knot nematodes (RKNs) affect plant growth negatively by causing small/big root galls during blocking transmission system of plant nutrients. Thus, RKNs prevent water and nutrient up from the soil and cause a reduction in the plant growth. In addition, they sensitize plants to other fungal and bacterial originated diseases by generating wounds on plant roots and access points [2].

The *Meloidogyne* genus belongs to a group of root-knot nematodes (RKN) and is represented by over 90 species that have been described so far [3]. These are ubiquitous soil organisms with a wide host range. It is ranked among the most damaging plant pathogen [4]. Infested plants shows the symptoms of stunting, yellowing, aberrant development of root system characterized by the formation of typical galls, a general unthrifty appearance and limited fruit production, estimated yield loses ranging from 28% to 68% [5].

Nematode control is necessary in order to reduce crop losses and ensure self-sufficiency in the requirements for food and industrial raw materials. Among the several ecologically-based approaches in nematode management is the use of pesticides of plant origin [6,7].

Nematicides are generally recommended for the control of nematodes. Most of the nematicides are being prohibited from use because of their harmful effect on humans and cause environmental hazards. Moreover in organic farming there should be alternative nematode control strategies, as chemical nematicides cannot be recommended. Therefore, it has become an important issue to find alternative control strategies. One of possible alternatives is the utilization of botanical toxicants as organic amendments or biopesticides [8]. Hence the present study has been carried out the nematicidal effect of root extract of *A. lanata* against the root knot nematode, *M. incognita* on Bengal gram, *C. arietinum*.

**MATERIALS AND METHODS**

The sand soils (River soil, Garden soil and Red soil) were collected and sterilized with an autoclave. The sterilization was carried out at 15 lb for 2 hours [9] to destroy various micro organisms. The sterilized soil mixers were used in the proportion of (River soil 2: Red soil 1: Garadn soil 1) ratio. The healthy seeds of Bengal gram, *C. arietinum* were chosen and their surface was sterilized.
in 0.01% (w/v) mercuric chloride solution for five minutes. They were rapidly washed well with distilled water and then soaked in distilled water for two hours. *C. arietinum* seeds were sown in mud pots of two litre capacity. The leaf extract were prepared by vacuum rotatory evaporator using acetone as a solvent and the temperature was maintained at 55°C [10] methods. The different concentrations was prepared with a 1 g of root extract is dissolved in 1000 ml of distilled water and kept as stock solution. These stock solutions were used as a preparation of different concentrations such as, 5 ppm (5 ml of stock solution with 95 ml of distilled water). The same method is followed by other concentrations. The leaf extract were applied in all experimental plants without control and egg mass inoculated control plants. The nematode egg masses were collected from the infected roots of tomato plants. The egg masses were isolated and separated using a compound microscope (45X). The average number of eggs per egg masses < 100 eggs. The collected egg masses were separated at different levels by counting (5, 10, 15, 20 and 25) and the counted egg masses were inoculated in the experimental pots (Three replicates). After inoculation distilled water was poured for three days and leaf extracts were added daily after 45 days and after analyze for various biochemical characteristics, such as Estimation of Protein content

The total soluble protein was estimated by method of Lowry’s *et al.*, (1951). Fresh leaf samples were ground in 10ml of distilled water using mortar and pestle. The homogenate was spun at 3000rpm for 5 min. The supernatant was taken and the pellet was discarded. To the supernatant 1 ml of ice cold 10% (w/v) trichloroaceticacid (TCA) was added and kept over ice for 10 minutes. The extract was centrifuged at 5000 rpm for 10 minutes. The extract consisting of total soluble proteins was dissolved in 0.1N.NaOH and was used as the test solution. An aliquot of 0.1 ml of test solution was taken in a test tube, 0.5ml of freshly diluted (1:1) folin phenol reagent and 5.5ml of alkaline copper mixture were added. Contents in the tube were vortexed immediately and left undisturbed for 10 minutes for the development of blue colour. The absorbance was measured at 650nm using a spectrophotometer with alkaline copper reagent as blank. The protein content was calculated from a standard graph of protein constructed with Bovine Serum Albumin (BSA) as market protein. (An aliquot of 100µg BSA showed 0.197 absorbance at 650nm) [11].

**Estimation of Amino Acids Content:** Free amino acids were estimated by ninhydrin assay (Jayaraman, 2000) method. The leaf material (100mg) was ground in 10ml of ethanol. The homogenate was centrifuged at 5000 rpm for 3 minutes. The pellet was discarded and the supernatant was used as the test solution. To 1ml of the test solution, 3ml of distilled water and 1ml of ninhydrin reagent were added and mixed thoroughly. After mixing, the test tube was kept in boiling water bath for 10 minutes. Then the tube was cooled down to room temperature and 1ml of 50% ethanol was added. The absorbance was measured using spectrophotometer at 550nm using proper blank. Blank solution consisted of 4ml of distilled water, 1ml of ninhydrin and 1ml of ethanol. The amino acid content was estimated from standard curve prepared with glycine as amino acid source [12].

**Estimation of Chlorophyll Content:** To extract the total chlorophyll from leaves, fresh leaves were cut into small bits. From the pooled leaf bits, a sample of 100mg was weighed. The leaf bits were homogenized with 10ml of 100% acetone using a mortar and pestle. The homogenate was centrifuged at 5000 rpm for 5 minutes at room temperature. Extraction with 100% acetone was repeated till the pellet become pale yellow or white in colour. The supernatant was measured at 662nm and 645nm for chlorophyll a and b respectively using a spectrophotometer [13].

**Estimation of Phenol Content:** The phenol was estimated using Folin Ciocalteau (FC) reagent.

1ml of alcoholic extract was taken in a test tube. 1ml of folin ciocalteau reagent and 2ml of 20% (w/v) sodium carbonate were added. The contents were boiled for 1 minute, then shaken and cooled under running tap water. The blue solution obtained was diluted to 25ml with distilled water. The absorbances of the samples were measured at 650nm by using a spectrophotometer. The amount of phenol in the sample solution was calculated from a standard graph prepared using chlorogenic acid as a source of authentic phenol [14].

**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 15 ppm) root extract of *A. lanata* was statistically analysed by using standard deviation and Analysis of variance 2 way.
RESULTS AND DISCUSSION

Total Chlorophyll Content: The chlorophyll ‘a’, ‘b’ and total chlorophyll content present in the leaves of bengal gram, *C. arietinum* inoculated with 5, 10 and 15 egg masses of root-knot nematode, *M. incognita* and treated with different concentrations of *A. lanata* was analyzed after 45 days treatment and the results are presented in the Table 1. In the total chlorophyll content of control plants has been found to be 21.97±0.01 (mg/g), that has been reduced to 14.39±0.02 at 5 egg masses inoculum level, 11.35±0.20 at 10 egg masses inoculum level and 10.96±0.03 at 15 egg masses inoculum level. In the treated plants, the total chlorophyll content has been found to be increased with increasing concentrations of root extract, that is in 5 egg mass inoculum level, the chlorophyll content, has been found to be 16.73±0.20 at 5 ppm, 17.35±0.04 at 10 ppm to 18.68±0.03 at 15 ppm. The same data was observed in 10 and 15 egg masses inoculum level. Chlorophyll is the important green pigment present in chloroplasts in all photosynthetic plant tissues. It is an essential component for photosynthesis to produce starch. It is readily isolated or extracted in solvent such as acetone and either because it is loosely bound to simple proteins [15]. Similarly Mohanty *et al.* [16] reported that the pigment chlorophyll is meant for photosynthesis, the infection of root knot nematode *M. incognita* alter chlorophyll content and reduced in the leaves of *Vigna radiate*. This is due to the formation of root galls that prevents the transport mechanism of plants, particularly in transport mechanism of plants, particularly in roots area. By the interruption of this infection chlorophyll pigment formation is affected [17].

Total Protein Content: The total protein content present in the leaves of *C. arietinum* of control and experimental plants inoculated with *M. incognita* were analysed after 45 days treatment and tabulated in Table 2. The protein content of the control plants was found to be 11.04±0.04 mg/g, while in the inoculated control plants have low protein content 9.15±0.04 at 5 egg masses inoculum level, 8.81±0.06 at 10 egg masses inoculum level and 8.66±0.07 at 15 egg masses inoculum level. There is an increasing trend of protein content in the leaves of treated plants with increasing concentrations of root extract, that is in 5 egg mass inoculated plants has been found to be 9.55±0.06 at 5 ppm, 10.03±0.03 at 10 ppm and 10.93±0.06 at 15 ppm. The same trend was observed in 10 and 15 egg masses inoculum level. Padhi and Behera [18] reported that the protein content in the leaves of tomato plants has been decreased in inoculated plants and increased in ten indigenous plant extract (Murraya koenigii (curry leaf), Jasminum sambac (Jasmine), Citrus aurantifolia (sour orange), Rauvolfia serpentina (patal garuda), Zizyphus jujuba (ber), Hibiscus rosa-sinensis (china rose) and Justicia gandurosa [J. gendarussa]) treated plants. The protein content underwent a non-significant reduction in the leaves of mung bean after inoculation with the nematode. Similarly, Oka *et al.* [19] found that, genetic and induced plant resistance at an early infection stage, tomato plants susceptible to *M. javanica* did not change the soluble protein composition of their leaves as compared with uninfected plants.

Amino Acids Content: The total free amino acid content present in the leaves of bengal gram, *C. arietinum* inoculated with different egg masses of root-knot nematode *M. incognita* and the plants were treated with different concentrations of root extract of *A. lanata* was analysed after 45 days treatment presented in the Table 3. This table clearly indicates that the total amino acid content of control plants was found to be 2.30 (mg/gm). Which reduces from (1.94 mg/gm to 1.71 mg/gm) of different egg masses inoculation and it was gradually increased (2.08 mg/gm to 2.20 mg/gm) with increasing concentration of root extract of *A. lanata* in treated plants. The same trend was observed in 10 and 15 egg masses inoculum level. Increased rates of synthesis of amino acids can be supported by the evidence of overall increase in metabolic activities of diseased tissues [20]. Overall increase in metabolism of infected tissues would not cause decrease in degradation. Nematodes contain both free and bound amino acids [21]. Since nematodes have been shown to secrete several enzymes [20] and other inorganic and organic substances, it can be assumed that they secrete amino acids also into the cells they feed upon. Similarly Rutherford *et al.* [22] explained the variable effects on amino acids encountered in nematode infections reflect the dynamic relationship between host and parasite. The importance of maintaining an osmotic balance in the insect haemolymph necessitates an efficient system of amino acids regulation. Hormones are responsible for control of excretion of nitrogenous compounds, transport of amino acids into the fat body, protein synthesis and proteolysis. A simpler alternative is that the mermethid absorbs the amino acids selectively across the cuticle.
Table 1: Effect of root-knot nematode, *M. incognita* and the root extract of *A. lanata* on the treatments on the total chlorophyll content (mg/gm of f. wt) in the leaf of Bengal gram *Cicer arietinum*

<table>
<thead>
<tr>
<th>Inoculum level/ No of Egg masses</th>
<th>Control</th>
<th>Inoculated control</th>
<th><em>5ppm</em></th>
<th><em>10ppm</em></th>
<th><em>15ppm</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>21.97±0.01</td>
<td>14.39±0.02</td>
<td>16.73±0.20</td>
<td>17.35±0.04</td>
<td>18.68±0.03</td>
</tr>
<tr>
<td>10</td>
<td>11.35±0.20</td>
<td>13.54±0.03</td>
<td>9.17±0.03</td>
<td>16.54±0.03</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>10.96±0.03</td>
<td>10.46±0.04</td>
<td>12.02±0.02</td>
<td>17.36±0.45</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are the average value of three replications * means statistically significant, P <0.01.

Table 2: Effect of the root-knot nematode, *M. incognita* and the root extract of *A. lanata* on the individual treatments on the total protein content (mg/gm) in the leaf of Bengal gram *C. arietinum*

<table>
<thead>
<tr>
<th>Inoculum level/ No of Egg masses</th>
<th>Control</th>
<th>Inoculated control</th>
<th><em>5ppm</em></th>
<th><em>10ppm</em></th>
<th><em>15ppm</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>11.04±0.04</td>
<td>9.15±0.04</td>
<td>9.55±0.06</td>
<td>10.03±0.03</td>
<td>10.93±0.06</td>
</tr>
<tr>
<td>10</td>
<td>8.81±0.06</td>
<td>9.33±0.06</td>
<td>9.82±0.05</td>
<td>10.5±0.04</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8.66±0.07</td>
<td>9.06±0.06</td>
<td>9.65±0.08</td>
<td>10.38±0.06</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are the average value of three replications * means statistically significant, P <0.01.

Table 3: Effect of the root-knot nematode, *M. incognita* and the root extract of *A. lanata* on the individual treatments on the total free amino acid content (mg/gm) in the leaf of Bengal gram *C. arietinum*

<table>
<thead>
<tr>
<th>Inoculum level/ No of Egg masses</th>
<th>Control</th>
<th>Inoculated control</th>
<th><em>5ppm</em></th>
<th><em>10ppm</em></th>
<th><em>15ppm</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.30±0.03</td>
<td>1.94±0.04</td>
<td>2.08±0.02</td>
<td>2.19±0.04</td>
<td>2.20±0.04</td>
</tr>
<tr>
<td>10</td>
<td>1.92±0.04</td>
<td>2.07±0.03</td>
<td>2.14±0.04</td>
<td>2.12±0.06</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1.71±0.07</td>
<td>1.93±0.07</td>
<td>1.91±0.07</td>
<td>2.03±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are the average value of three replications * means statistically significant, P <0.01.

Table 4: Effect of root-knot nematode, *M. incognita* and the root extract of *A. lanata* on the treatments on the total phenol content (mg/gm) in the leaf of Bengal gram, *C. arietinum*

<table>
<thead>
<tr>
<th>Inoculum level/ No of Egg masses</th>
<th>Control</th>
<th>Inoculated control</th>
<th><em>5ppm</em></th>
<th><em>10ppm</em></th>
<th><em>15ppm</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>22.64±0.12</td>
<td>27.65±0.04</td>
<td>24.67±0.04</td>
<td>23.18±0.07</td>
<td>22.56±0.07</td>
</tr>
<tr>
<td>10</td>
<td>29.68±0.07</td>
<td>26.07±0.05</td>
<td>23.77±0.06</td>
<td>22.10±0.06</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>32.12±0.06</td>
<td>28.66±0.25</td>
<td>26.89±0.02</td>
<td>20.06±0.04</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are the average value of three replications * means statistically significant, P <0.01.

**Total Phenol Content:** The total phenol content present in the leaves of *C. arietinum* infected with 5, 10 and 15 eggs masses of *M. incognita* and treated with different concentrations of *A. lanata* were analysed after 45 days treatment presented in the Table 4. Total phenol content of the control plants was found to be 22.64±0.12 (mg/g). While in the inoculated plants have high phenol content 27.65±0.04 at 5 egg masses inoculum level, 29.68±0.07 at 10 egg masses inoculum level and 32.12±0.06 at 15 egg masses inoculum level. At different concentrations of *A. lanata* the phenol content found to be decreasing with increasing concentrations of the extract from 24.67±0.04 at 5 ppm, 23.18±0.07 at 10 ppm to 22.56±0.07 at 15 ppm at 5 egg masses inoculum level. The same trend was observed in 10 and 15 egg masses inoculum level. Bhargava et al. [23] reported that the total phenol increased with infected plants than the healthy plants. These observations are in confirmation of earlier reports [24-26] 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.

A significant increase in phenolic contents in root and shoot tissues of inoculated untreated plant than that of control plant appeared to be due to host response to nematode attack [27]. A significant increase in phenolic content in root and shoot tissues of inoculated untreated plant might be due to its synthesis through shikimic pathway [28] by the enzymatic activities of glycosidase from plant or parasite. The phenomenon of resistance during pathogenesis is now known to be associated with proteins and phenols [29].

**CONCLUSION**

The present study clearly indicated that the root extract of *A. lanata* have the nematicidal against the root knot nematode *M. incognita* affecting bengal gram *Cicer arietinum*. Since the root extract of *A. lanata* has a remarkable nematicidal property on *M. incognita*.  

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REFERENCES


