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Effect of *Mimusops elengi* leaf extract on *Meloidogyne incognita* and biochemical changes of black gram

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Abstract

The present study was conducted to evaluate the effect of leaf extract of *Mimusops elengi* on the root-knot nematode *Meloidogyne incognita* infecting the black gram *Vigna mungo* with different inoculum levels of egg-masses (5, 10, 15, 20 and 25). Plants treated with the leaf extract of *M. elengi* at different concentrations (5, 10, 15, 20 and 25 ppm). The control and experimental plants were analyzed for various biochemical constituents such as, carbohydrate, protein, amino acid, lipid, proline and phenol content after 65 days of treatment. Carbohydrate and protein were found decreased with increasing inoculum levels of egg-masses and increased with increasing concentrations of leaf extract treatment and lipid, amino acid, proline and phenol content found increased with increasing inoculum levels of egg-masses and decreased with increasing concentrations of leaf extract treatment.

Keywords: *Mimusops elengi*, *Meloidogyne incognita*, *Vigna mungo*, carbohydrate, protein, amino acid, lipid, proline, phenol.

Agriculture is the main and basic source to human diet on the face of the earth; so many efforts were exerted to improve agricultural productions. Many problems were emerged to spread of pests and diseases like fungal diseases, bacterial or even nematodes, as well as all kinds and forms of insects, weeds and snails (Khalil, 2013). Plant parasitic nematodes attack and feed on plant roots of many cultivated plants (Nimbalkar & Rajurkar, 2009). The genus *Meloidogyne* comprises all root-knot nematodes. It contains over 100 described species (Karssen *et al*., 2013). Four *Meloidogyne* species viz., *M. javanica*, *M. arenaria*, *M. incognita* and *M. hapla* are major pests worldwide (Hajra *et al*., 2013). Root-knot nematode (*Meloidogyne* sp.) is an important pest all over the crops and distributed approximately 75% in agricultural soils of India.

The symptoms of nematode infection appeared as formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting and mineral deficiency, especially during periods of moisture stress and high temperature (Abad *et al*., 2003).The chemicals being used as insecticides and nematicides are costly and inherently toxic. In addition to killing pests they also have adverse effects on human beings, livestock and other living things. Insecticide resistance, environmental degradation, human health impacts, resource loss and economic concerns, thus triggered a growing interest in alternative management techniques (Dhaliwal & Arora, 2001).

Botanicals (plant-based pesticidal chemicals) have found favour as alternatives to pesticides in recent times. Some of these botanicals were already being exploited commercially in insect pest management (Agnihotri *et al*., 1999). Different plant species tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes (Abid, 1996). Hence, the present investigation has

been carried out to evaluate the effect of leaf extract of *Mimusops elengi* L., against root-knot nematode *Meloidogyne incognita* and biochemical changes of black gram *Vigna mungo* (L.) Hepper.

Materials and Methods

The sand soil mixtures (River soil, Garden soil and Red soil) were used in the proportion of 2:1:1 ratio moistened with water and sterilized in an autoclave by placing the sand-soil mixture in the containers. The sterilization was carried out at 15 lb for 2 hours (Fred & Wakesman, 1928). The healthy seeds of host plant *Vigna mungo* selected 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. *Vigna mungo* seeds were sown in mud pots of two kg capacity. The nematode egg-masses were collected from the roots of infected tomato plants from pure culture of nematode. The egg-masses were isolated and separated using a compound microscope (45x). The average number of eggs per egg-masses was < 100 eggs. The collected egg-masses were separated at different levels by counting (5, 10, 15, 20 and 25) and the counted egg-masses inoculated in the experimental pots (three replicates). After inoculation distilled water was poured for three days and leaf extracts. The leaf extract were prepared by Sohxlet apparatus using acetone as solvent, and the temperature was maintained at 55 °C (Peach & Tracey, 1956) methods. The different concentrations were prepared with a 1 g of leaf extract 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 was followed for other concentrations. The leaf extract was applied in all experimental plants without control and egg-mass inoculated control plants. After 65 days of treatment, the biochemical characteristics, such as carbohydrate Anthrone method (Jayaraman, 1981), protein Lowry *et al*., (1951), lipid Bragdon (1951), amino acid (Jayaraman, 1981), proline (Bates *et al*., 1973) and phenol content (Bray & Thorpe, 1954) were analyzed.

Statistical analysis: The data were statistically analysed by using standard deviation and ANOVA in a computer software (www.faculty wassar.edu/lowry anova 2u).

Results and Discussion

In the present study, biochemical constituents such as, carbohydrate, protein, lipid, amino acid, proline and phenol content were analyzed after 65 days of leaf extract treatment. The estimation of carbohydrates plays an important part in both pure and applied plant physiology. Carbohydrate content of the inoculated control plants was found to be 19.6 ± 0.09 (mg/g) at 5 egg-masses inoculum levels, 17.6 ± 0.85 (mg/g) at 10 egg-masses inoculum levels, 17.03 ± 0.81 (mg/g) at 15 egg-masses inoculum levels, 13.7 ± 0.08 (mg/g) at 20 egg-masses inoculum levels and 10.3 ± 0.09 (mg/g) at 25 egg-masses inoculum levels when compared with control plants 62.84 ± 0.97 (mg/g). An increasing level of carbohydrate content was observed in leaf extract treated plants with 5 egg-masses inoculum levels that has found to be 23.13 ± 0.09 (mg/g) at 5 ppm, 29.65 ± 0.17 (mg/g) at 10 ppm, 35.16 ± 0.10 (mg/g) at 15 ppm, 41.22 ± 0.17 (mg/g) at 20 ppm and 47.63 ± 0.09 (mg/g) at 25 ppm (Table 1).

The same trend was also observed in 10, 15, 20 and 25 ppm at all egg-masses inoculum levels. The results were found to be statistically significant ($P < 0.001$). The reduction in soluble, reducing and non reducing sugars, total carbohydrates in different host plants due to nematodes infection (Farahat *et al*., 2007; Parveen *et al*., 2006). One of the common features of obligate parasitism is the enhanced oxido-

reductase activity, thereby altering carbohydrate metabolism (Veech & Endo, 1970). The infection of nematode, *Aphelenchoides compositicola* reduced the biochemical compounds like protein, lipid, carbohydrate, amino acids and accumulation of enzymes in the infected plants can be observed (Grewal & Sohi, 1989) and they obtained better increase in these compounds by the use of toxicity of some plant extracts.

Table1. Effect of *Meloidogyne incognita* and the leaf extract of *Mimusops elengi* on the total carbohydrate content (mg/g) of black gram.

No. of egg-masses treated	Total carbohydrate content after 65 days treatment						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	62.84 ± 0.97	19.6 ± 0.09	23.13± 0.09	29.65 ± 0.17	35.16 ± 0.1	41.22 ± 0.17	47.63 ± 0.09
10		17.6 ± 0.85	21.2 ± 0.04	28.71± 0.14	33.73 ± 0.06	40.02 ± 0.02	46.34 ± 0.09
15		17.03 ± 0.81	21.15 ± 0.06	26.34 ± 0.12	31.68 ± 0.07	38.55 ± 0.13	44.10 ± 0.01
20		13.7 ± 0.08	20.28 ± 0.06	25.32 ± 0.1	30.08 ± 0.14	37.06 ± 0.08	43.17 ±0.08
25		10.3 ± 0.09	18.72 ± 0.03	24.15 ± 0.09	29.01 ± 0.06	36.5 ± 0.05	42.06 ± 0.06

The total protein content (Table 2) as compared with control plants (57.9 ± 0.06 mg/g), the inoculated control plants have low protein content and were decreased with increasing levels of egg-masses inoculums (22.55 ± 0.01 mg/g) at 5 egg-masses inoculum levels to 16.28 ± 0.03 mg/g at 25 egg-masses inoculum level. There is an increasing trend of protein content of the leaf extract treated plants with increasing concentrations, 30.42 ± 0.02 mg/g at 5 ppm to 54.42 ± 0.05 mg/g at 25 ppm. The same trend was also observed in other inoculum levels. The results found statistically significant ($P < 0.001$). The total protein content varied from treated and *M. javanica* infected plants (Ahmed *et al*., 2009). The protein concentration probably decreases because the giant cells utilize the amino acid pool which required for protein synthesis. Alternatively, the decrease in protein concentration was caused by the proteolysis of proteins under stress conditions. The level of proteins increased when the infection was cured. A similar result was reported by Abbasi *et al*., (2008) that *M. javanica* infection in eggplant and okra amended with *Barleria acanthoides*. Similarly, low concentrations of nucleic acids in infected plants were due to enhanced ribonuclease activity. The activity of this enzyme possibly increased in susceptible plants due to the growth and multiplication of the nematodes in the roots.

Table 3 showed that the total lipid content in control plants 3.68 ± 0.01 mg/g, while in inoculated control plants found increasing from 4.01 ± 0.01mg/g at 5 egg-masses inoculum levels to 6.2 ± 0.01 mg/g at 25 egg-masses inoculum levels. While, the leaf extract treated plants, the lipid content found decreasing with increasing concentrations (5 ppm to 25 ppm) of all the inoculum levels. The result was found to be statistically significant ($P < 0.001$). Bird (1961) noted traces of fat in syncytial cytoplasm and presence of fat besides RNA and feulgen-positive granules in hypertrophied nucleolus.

The comparison of both experimental plants (inoculated control and leaf extract treated plants) the total amino acid content decreased with increasing concentration of leaf extract in all inoculums levels (5 ppm to 25 ppm) and increased with increasing levels of inoculums (8.25 ± 0.16-13.86 ± 0.4) (Table 4). The results found statistically significant ($P < 0.001$). The possible factors for increase in amino acid levels in infected plant tissues may be i) increased rates of synthesis in diseased tissues, ii) increased rates of translocation towards diseased tissues, iii) decreased rates of translocation from diseased tissues, iv) decreased rates of degradation and v) deposition by nematodes. Increased rates of synthesis of amino acids supported by the evidence of overall increase in metabolic activities of diseased tissues. Overall increase in metabolism of infected tissues would not cause decrease in degradation. Since nematodes have been shown to secrete several enzymes (Roy, 1980) and other inorganic and organic substances, it can be assumed that they secrete amino acids also into the cells they feed upon the nematodes on plants.

The total proline content (Table 5) as compared with control plants (34.8 ± 0.19 µm/g), the inoculated control plants have low proline content and were observed increasing inoculum levels of egg-masses from 5.72 ± 0.61 µm/g at 5 egg-masses inoculum level to 9.24 ± 0.37 µm/g at 25 egg-masses inoculum levels. There is a decreasing trend of proline content observed in leaf extract treated plants with increasing concentrations (5 ppm to 25 ppm). The same trend was also observed in all the egg-masses inoculum levels. The results found statistically significant ($P < 0.001$). Proline was known as precursor of hydroxyproline an important constituent of the cell wall protein (extensin) (Lamport, 1970). Enzyme inhibition in the pathway of extension synthesis or a change in the rate of cell-wall formation might lead to proline accumulation. Hydroxyproline decreased in the wall fraction while free proline increased in the galls suggested that nematode activity interfered with the biosynthesis of the root cell walls. Proline accumulation under stress conditions may either be caused by induction or activation of enzymes of proline biosynthesis or a decreased proline oxidation to glutamate, decreased utilization of proline in protein synthesis, and enhanced protein turnover (Delauney & Verna, 1993). Similar results reported in the case of water stress, with nematode infected plants showing lower proline levels in the leaf (Saglam *et al*., 2008). Although the stress derived from nematode attacks involves mechanisms that differ from those associated with water deficit, since plant vascular cylinders are clogged when nematodes infest the root (Agrios, 1989), altering root functioning and reducing water uptake, which leads to water stress and nutrient deficit.

The phenol content was increased with increasing concentrations of leaf extract in all inoculum levels (5 egg-masses to 25 egg-masses) (Table 6). In the experimental plants treated with leaf extract, the phenol content was decreased with increasing concentrations (5 ppm to 25 ppm) of leaf extract treatment. The results found statistically significant ($P<0.001$).

The phenolic compounds are the best known factors involved in susceptible-resistant response. There is a distinct correlation between the degree of plant resistance and the phenolics present in plant tissues (Mohamed *et al*., 2010). Most phenols occur in plant tissues in bound forms as glycosides of low physiological and chemical activities. Activation requires their decompositions to free phenols (Afify *et al*., 2012). Nematodes were able to decompose glycosides by secreting β-glycosidases into host tissue (Wilski & Giebel, 1966). Hence, the present study, the leaf extract of *M.elengi* has remarkable nematicidal property on root-knot nematode *M. incognita*.

Table 2. Effect of *Meloidogyne incognita* and the leaf extract of *Mimusops elengi* on the total protein content (mg/g) of black gram.

No. of egg-masses treated	Total protein content after 65 days treatment						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	57.9 ± 0.06	22.55± 0.01	30.42 ± 0.02	37.17 ± 0.06	42.65± 0.1	48.43 ± 0.05	54.42 ± 0.05
10		20.42 ± 0.09	29.17 ± 0.06	35.07 ± 0.05	41.13 ± 0.02	46.61± 0.04	53.52 ± 0.08
15		19.63 ± 0.05	26.47 ± 0.02	34.97 ± 0.07	40.65 ± 0.02	45.22 ± 0.04	52.5 ± 0.01
20		18.27 ± 0.03	25.23 ± 0.01	33.12 ± 0.06	39.01 ± 0.03	44.36 ± 0.03	51.21 ± 0.04
25		16.28 ± 0.03	24.54 ± 0.02	32.45 ± 0.04	38.19 ± 0.04	43.25 ± 0.02	50.01 ± 0.03

Table 3. Effect of *Meloidogyne incognita* and the leaf extract of *Mimusops elengi* on the total lipid content (mg/g) of black gram.

No. of egg-masses treated	Total lipid content after 65 days treatment						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	3.68 ± 0.01	4.01 ± 0.01	3.2 ± 0.01	2.23 ± 0.12	1.81 ± 0.08	0.82 ± 0.06	0.26 ± 0.07
10		4.64 ± 0.01	3.24 ± 0.02	2.24 ± 0.06	1.84 ± 0.14	1.23 ± 0.04	0.39 ± 0.04
15		5.26 ± 0.01	3.26 ± 0.06	2.45 ± 0.09	2.01 ± 0.06	1.31 ± 0.05	0.59 ± 0.06
20		5.69 ± 0.02	3.45 ± 0.09	2.82 ± 0.06	2.11 ± 0.06	1.41 ± 0.03	0.73 ± 0.03
25		6.2 ± 0.01	3.82 ± 0.09	2.9 ± 0.06	2.18 ± 0.06	1.64 ± 0.07	0.83 ± 0.06

Table 4. Effect of *Meloidogyne incognita* and the leaf extract of *Mimusops elengi* on the total free amino acid content (mg/g) of black gram.

No. of egg-masses treated	Total free amino acid content 65 days treatment						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	5.8 ± 0.4	8.25 ± 0.16	37.7 ± 0.32	32.64 ± 0.22	23.8 ± 0.4	19.07 ± 0.16	14.86 ± 0.45
10		10.32 ± 0.49	37.93 ± 0.14	34.85 ± 0.19	23.94 ± 0.09	19.65 ± 0.28	15.42 ± 0.11
15		12.29 ± 0.12	39.37 ± 0.19	36.16 ± 0.16	28.96 ± 0.18	20.1 ± 0.43	16.34 ± 0.13
20		13.0 ± 0.45	40.69 ± 0.18	36.29 ± 0.84	30.88 ± 0.3	21.31 ± 0.15	16.84 ± 0.18
25		13.86 ± 0.4	43.52 ± 0.36	37.68 ± 0.33	31.8 ± 0.3	22.46 ± 0.42	17.68 ± 0.14

Table 5. Effect of *Meloidogyne incognita* and the leaf extract of *Mimusops elengi* on the total proline content (mg/g) of black gram.

No. of egg-masses treated	Total proline content 65 days treatment						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	34.8 ± 0.19	5.72 ± 0.61	28.13 ± 0.4	24.2 ± 0.6	21.06 ± 0.4	16.44 ± 0.37	12.59 ± 0.24
10		6.22 ± 0.42	29.39 ± 0.5	24.97 ± 0.63	21.17 ± 0.33	18.13 ± 0.4	13.77 ± 0.2
15		7.48 ± 0.51	29.48 ± 0.3	25.1 ± 0.34	21.22 ± 0.37	18.64 ± 0.4	13.88 ± 0.5
20		8.57 ± 0.46	29.51 ± 0.37	25.99 ± 0.24	22.75 ± 0.47	18.84 ± 0.66	14.28 ± 0.5
25		9.24 ± 0.37	29.99 ± 0.13	27.86 ± 0.43	23.15 ± 0.5	19.66 ± 0.41	16.04 ± 0.51

Table 6. Effect of *Meloidogyne incognita* and the leaf extract of *Mimusops elengi* on the total phenol content (mg/g) of black gram.

No. of egg-masses treated	Total phenol content 65 days treatment						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	6.2 ± 0.11	32.25 ± 0.16	25.84 ± 0.13	21.0 ± 0.11	15.2 ± 0.12	11.09 ± 0.17	7.24 ±0.11
10		33.82 ± 0.11	25.94 ± 0.07	21.06 ± 0.09	15.84 ± 0.07	11.23 ± 0.09	7.59 ± 0.18
15		34.87 ± 0.06	27.85 ± 0.14	24.66 ± 0.17	15.85 ± 0.14	13.11 ± 0.15	7.97 ± 0.17
20		36.97 ± 0.16	29.46 ± 0.11	25.22 ± 0.09	19.17 ± 0.16	13.95 ± 0.07	8.32 ± 0.12
25		38.67 ± 0.15	31.32 ± 0.08	25.84 ± 0.09	19.6 ± 0.11	15.17 ± 0.17	10.75 ± 0.21

Conclusion

The present study clearly indicated that the leaf extract of *M. elengi* have the nematicidal activity against the root-knot nematode *M. incognita* affecting black gram *Vigna mungo*. Since the leaf extract of *Mimusops elengi* has a remarkable nematicidal property on *M. incognita*, thereby it can be used in the control of plant root-knot nematodes instead of hazardous synthetic nematicides in future.

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