Effect of *Meloidogyne incognita* on the growth characteristics of *Vigna mungo* treated with leaf extract of *Mimusops elengi*

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Abstract

The present study was conducted to evaluate the effect of leaf extract of magilam, *Mimusops elengi* on the root-knot nematode, *Meloidogyne incognita* infecting the black gram, *Vigna mungo* with different inoculums levels (5, 10, 15, 20 and 25 egg-masses/plant) with different concentrations (5, 10, 15, 20 and 25 ppm). The control and treated plants were analyzed for various growth characteristics such as, root and shoot length, fresh and dry weight of root and shoot, leaf area, root gall index and chlorophyll content after 65 days. These growth characteristics found decreased with increasing inoculum levels of egg-masses and increased with increasing concentrations of leaf extract treatment and fresh and dry weight of root and root gall index 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*, inoculum levels, chlorophyll, root gall index

Plant parasitic nematodes and soilborne pathogens attack a wide range of vegetables reducing yield, quality and quantity (Nchore *et al*., 2011). Root-knot nematodes are considered among the top five major plant pathogens and the first among the ten most important genera of plant parasitic nematodes in the world (Kayani *et al*., 2013). Root-knot nematodes of the genus *Meloidogyne* are more widely distributed throughout the world than any other major group of plant-parasitic nematodes. The damage to global agricultural crops due to root-knot nematodes is estimated around US$ 157 billion annually (Abad *et al*., 2008). Infected plants showed stunt growth, swollen roots which developed into the typical root-knot galls, two or three times larger in diameter as healthy roots. Root-knot nematodes are very difficult to control because they are polyphagous, where its over 2000 plants species is a highly specialized and complex feeding relationship with their host (Hussey & Janssen, 2002). Root-knot nematodes (*Meloidogyne* sp.) are the most serious pathogens (Mukhtar *et al*., 2013). *Meloidogyne* sp., form disease complex with root rot pathogens causing major losses in vegetable production. Such problem is widely spread in controlled agricultural systems (Kago *et al*., 2013). A high level of root-knot nematode damage can lead to total crop loss. Nematode damaged roots not utilized water and fertilizers effectively, leading to additional losses. Infection of young plants may be lethal, while infection of mature plants caused yield reduction. *Meloidogyne* species constituted the major nematode problem in developing countries (Lamberti, 1997). *Meloidogyne* damage resulted in poor growth, a decline in quality and yield of the crops and reduced resistance to other stresses like drought and other diseases.

Although the application of chemical nematicides has been found as an effective measure for the control of nematodes, due to high toxic residual effect of chemicals on the environment and particularly on non-target organisms (Anastasiadis *et al*., 2008), there is an urgent need to develop alternative strategies for the control of nematodes.

Plant extracts or residues used in control of nematode have advantage of cheapness and availability over the conventional methods (Izuogu *et al*., 2012; Oyedunmade *et al*., 2011). The use of botanical extracts for controlling *Meloidogyne* is becoming appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides. Increase pressure on farmers to use non-chemical pest control methods that not contribute in environmental pollution. This emphasis the need for new methods of control such as the use of plant extracts. Efficacy of various plant extracts in nematode control has been studied (Akhtar, 1999; Netscher & Sikora, 1990). Hence the present study has been done to evaluate the effect of root-knot nematode *M. incognita* infected with growth characteristics of black gram *V. mungo* treated with leaf extract of *M. elengi*.

Materials and Methods

Sterilized soil mixers (river soil, garden soil and red soil) were used in the proportion of 2:1:1 ratio. The sterilized *Vigna mungo* seeds were shown in mud 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, 15, 20 and 25) pouring four holes in top soil of experimental plants. After inoculation the distilled water was poured for three days and plant extract were added in alternate days. The leaf extract of magilam was prepared by vacuum rotary evaporator using acetone as a solvent. The different concentrations of leaf extract (5, 10, 15, 20 and 25 ppm) using distilled water. After 65 days of treatment, the growth characteristics such as root, shoot length, fresh and dry weight of root and shoot, leaf area, water content in root and shoot, root gall index and chlorophyll content were analyzed.

Results and Discussion

In the present study, various growth parameters such as shoot, root length, fresh and dry weight of shoot and root, leaf area, root gall index and chlorophyll content were analyzed after 65 days of treatment with different concentrations of *M. elengi* infected with five different inoculum levels of the root-knot nematode egg-masses. The shoot and root length (Table 1and 2) found increased with increasing concentrations of leaf extract compared to control and decreased with increasing inoculums levels of *M. incognita* statistically significant at ($P < 0.001$). Siddiqui & Rehman (2012) reported that the shoot and root length decreased in all the inoculated plants but there is non-significant reduction in plants germinated from the plants treated with the higher concentration of leaf extract of *Mimusops elengi*. Highest plant length was recorded in untreated uninoculated plants.

Table 1. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the shoot length (cm) of black gram.

No. of egg-masses	Shoot length (cm)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	36.37±0.13	14.13±0.03	17.22±0.06	21.59±0.07	24.21±0.08	28.41±0.08	38.19±0.06
10		13.19±0.06	16.54±0.05	21.14±0.05	23.91±0.04	27.66±0.04	36.08±0.72
15		11.56±0.04	16.03±0.24	20.49±0.06	23.10±0.14	26.37±0.04	35.45±0.19
20		10.49±0.04	15.82±0.18	18.42±0.09	22.60±0.05	25.75±0.07	32.15±0.05
25		9.97±0.16	14.81±0.09	18.16±0.06	21.92±0.06	24.61±0.59	30.15±0.05

Means statistically significant, $P < 0.001$.

Table 2. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the root length (cm) of black gram.

No. of egg- masses	Root length (cm)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	18.44±0.07	10.82±0.12	15.20±0.09	18.33±0.07	24.41±0.07	27.36±0.06	31.28±0.08
10		9.64±0.08	13.29±0.07	18.11±0.09	21.85±0.12	26.60±0.26	29.80±0.11
15		9.54±0.05	12.63±0.07	17.74±0.10	20.90±0.09	26.43±0.11	28.33±0.19
20		8.92±0.09	11.27±0.08	16.57±0.13	19.66±0.54	25.23±0.05	28.31±0.17
25		7.56±0.13	10.17±0.09	16.25±0.09	17.83±0.07	24.69±0.06	28.28±0.12

Means statistically significant, P < 0.001.

The fresh and dry weight of the shoot was significantly reduced in the increasing inoculums levels. While, in the leaf extract treated plants the fresh and dry weight increased with increasing concentrations (Table 3 and 4) was significantly different (P<0.001). A positive relationship between the initial inoculum levels of *Meloidogyne incognita* and reduction in shoot, fresh and dry weight, total chlorophyll content of fresh leaves (Perveen *et al*., 2006). Neither pathogen was able to affect fresh and dry weight of shoot in comparison to the absence of either or both the pathogens together and contradicted significant variations in these parameters (Hussain & Bora, 2009). RKN infected tissues (galls) contain highly dense granular protoplasm (Trudgill, 1991). This finding agreed with Hussain & Bora (2009) and Maleita *et al*., (2012). Suppressive effect of *M. incognita* at different inoculums level on fresh and dry weight of shoots of soybean was in accordance with Robab *et al*., (2009).

Table 3. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the fresh weight in shoot (g) of black gram.

No. of egg-masses	Fresh weight in shoot (g)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	15.59±0.07	10.94±0.06	12.5±0.1	13.84±0.1	14.73±0.07	17.63±0.05	20.81±0.12
10		10.85±0.05	11.78±0.03	13.27±0.04	14.42±0.08	17.07±0.06	19.7±0.04
15		10.81±0.03	11.32±0.43	13.12±0.07	13.87±0.08	16.58±0.06	19.61±0.07
20		9.62±0.06	11.21±0.2	12.92±0.06	13.7±0.04	15.78±0.05	19.45±0.05
25		8.6±0.08	11.17±0.09	12.81±0.05	13.56±0.07	14.93±0.06	18.22±0.06

Means statistically significant, P < 0.001.

Table 4. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the dry weight in shoot (g) of black gram.

No. of egg-masses	Dry weight in shoot (g)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	8.53±0.06	4.83±0.1	6.31±0.06	7.53±0.06	9.14±0.06	11.67±0.06	13.31±0.05
10		4.33±0.06	5.8±0.09	7.28±0.03	8.57±0.06	10.79±0.10	13.04±0.07
15		4.16±0.07	5.61±0.07	7.19±0.03	8.29±0.07	10.78±0.07	12.16±0.07
20		3.54±0.06	5.06±0.04	6.83±0.07	8.13±0.07	10.12±0.08	12.12±0.04
25		3.46±0.06	4.9±0.07	6.33±0.04	7.92±0.06	9.87±0.07	11.74±0.04

Means statistically significant, P < 0.001.

The fresh and dry weight of the root was significantly increased in the increasing inoculums levels. While, in the leaf extract treated plants the fresh and dry weight decreased with increasing concentrations (Table 5 and 6) was significantly different (P<0.001). Because the root was heavily infested with the root-knot nematode to produced the galls and increased the root weight. Presence of *M. incognita* increased the weight of root in tomato plants as a result of galls produced in the roots (Olaniyi *et al.*, 2005). However, numbers of galls were less in the treated plots than the control which was an indication that applications of plant materials exerted some controlling influence on parasitic nematode; hence a reduction in the number of galls per plant as compared with the very high gall/plant resulted from the control plots. Galling was a reaction of the plant to the feeding of the root-knot nematode which also vary in size with different applications of plant materials.

Table 5. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the fresh weight in root (g) of black gram.

No. of egg-masses	Fresh weight in root (g)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	5.5±0.02	6.07±0.04	4.62±0.04	3.08±0.07	2.54±0.08	2.1±0.05	0.89±0.06
10		6.26±0.05	4.79±0.06	3.19±0.05	2.52±0.07	2.08±0.08	1.47±0.09
15		6.62±0.05	5.32±0.07	3.28±0.05	2.62±0.07	2.18±0.07	1.74±0.05
20		7.36±0.04	5.54±0.06	3.38±0.03	2.99±0.07	2.36±0.04	1.9±0.04
25		7.92±0.06	5.89±0.04	4.09±0.07	3.1±0.04	2.47±0.04	1.92±0.05

Means statistically significant, P < 0.001.

Table 6. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the dry weight in root (g) of black gram.

No. of egg-masses	Dry weight in root (g)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	4.04±0.1	5.69±0.07	4.43±0.05	3.11±0.06	2.52±0.07	2.12±0.06	0.87±0.04
10		6.21±0.09	4.63±0.07	3.2±0.05	2.58±0.1	2.14±0.06	1.6±0.11
15		6.53±0.06	5.2±0.04	3.29±0.06	2.62±0.08	2.18±0.07	1.74±0.06
20		6.74±0.06	5.6±0.07	3.41±0.06	3.06±0.05	2.43±0.05	1.86±0.10
25		6.84±0.08	5.83±0.08	4.16±0.08	3.13±0.07	2.51±0.036	1.93±0.05

Means statistically significant, P < 0.001.

The leaf area of the control plants found to be 20.54 ± 0.07 cm^2 and it reduced the egg-masses treated plants 9.55 ± 0.06 cm^2 (5 egg-masses), 8.65 ± 0.09 cm^2 (10 egg-masses) and 8.32 ± 0.08 cm^2 (15 egg-masses). While, in the leaf extract treated experimental plants the leaf area was increased with increasing concentrations (5, 10, 15, 20 and 25 ppm) (Table 7). The result were found to be significantly different (P<0.001). Plant height, girth, leaf area and numbers of leaves were reduced in the *M. incognita* on *Celosia argentea* plant (Tobih *et al.*, 2011).

Table 7. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the leaf area (cm^2) of black gram.

No. of egg-masses	Leaf area (cm^2)						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	20.54±0.07	9.55±0.06	11.39±0.11	12.42±0.09	14.37±0.07	16.86±0.06	18.72±0.10
10		8.65±0.09	11.26±0.09	12.26±0.08	14.24±0.09	16.74±0.11	17.83±0.09
15		8.32±0.08	10.73±0.07	11.9±0.08	14.09±0.04	16.65±0.09	17.38±0.61
20		7.74±0.09	10.26±0.09	11.27±0.09	13.36±0.07	15.91±0.09	17.52±0.07
25		7.32±0.07	9.16±0.09	11.13±0.08	13.22±0.13	15.28±0.06	16.82±0.07

Means statistically significant, $P < 0.001$.

The efficacy of leaf extract of *M. elengi* on the root-knot nematode, *M. incognita* infecting the black gram, *V. mungo* was evaluated individually on the root gall index and presented in the Table 8, significantly different ($P < 0.001$). 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 leaf extract. The reductions in growth parameters in susceptible cultivars were attributable to root injury due to penetration and/or feeding by the nematodes leading to impairment of the efficiency of root systems to absorb water. The induction of galls in the roots and giant cells in the stellar region by *Meloidogyne* sp. extensively disrupts xylem tissues and greatly retard absorption and upward movement of water and nutrients (Di Vito *et al*., 2004). A progressive increased in number of galls and egg-masses with the increased inoculum level of the nematode from below pathogenic level to pathogenic level (Hussain & Bora, 2009).

Table (9 and10) showed that the water content (%) in the shoot and root of black gram infected with root-knot nematode and treated with the leaf extract of *M. elengi*. 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 decreasing with increasing levels of egg-masses inoculum level. At different concentrations of *M. elengi*, the water content of the shoot found increased with increasing concentration of the leaf extract significantly different at ($P < 0.001$). Plant stress caused by *M. incognita* and increased plant growth, nodulation parameters as well as chemical components.

Table 8. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the root gall index of black gram.

No. of egg-masses	Root gall index						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	0	15.66±2.51	12.66±3.51	10.33±4.04	7.66±2.51	5.66±3.21	3.33±1.52
10		15.66±3.05	13.33±1.52	11.33±2.51	8.33±2.51	6.22±3.31	4.22±3.05
15		16.33±1.52	14.33±2.56	11.66±4.16	8.33±4.50	6.33±2.23	4.33±1.52
20		16.66±4.33	15.33±3.51	12.33±1.52	8.66±2.51	6.66±1.52	4.33±2.51
25		18.33±2.51	15.62±1.12	12.43±1.52	9.0±2.0	7.66±1.52	5.33±3.51

Means statistically significant, $P < 0.001$.

Table 9. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the water content (%) in shoot of black gram.

No. of egg-masses	Water content (%) in shoot						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	45.22±0.13	15.45±0.22	18.98±0.10	23.46±0.19	28.6±0.10	36.3±0.05	43.82±0.12
10		11.06±0.14	16.37±0.11	21.47±0.20	26.75±0.18	35.44±0.2	42.34±0.11
15		7.11±0.1	14.3±0.11	21.18±0.02	25.52±0.13	33.46±0.14	40.26±0.05
20		5.37±0.2	14.24±0.34	20.54±0.14	24.53±0.09	32.41±0.08	39.72±0.06
25		4.73±0.16	13.37±0.21	20.28±0.14	22.97±0.08	30.84±0.08	38.44±0.11

Means statistically significant, P <0.001.

Table 10. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the water content (%) in root of black gram.

No. of egg-masses	Water content (%) in root						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	26.0±0.77	4.75±0.16	8.53±0.08	13.67±0.08	17.49±0.08	20.44±0.08	24.11±0.1
10		3.62±0.04	7.2±0.05	12.55±0.1	17.49±0.06	19.22±0.04	23.79±0.08
15		3.12±0.07	6.82±0.07	11.6±0.14	16.44±0.07	19.14±0.11	22.72±0.06
20		2.67±0.17	5.16±0.10	10.17±0.05	15.67±0.1	18.05±0.16	21.7±0.1
25		1.21±0.1	4.48±0.14	9.09±0.07	14.66±0.06	17.72±0.12	21.16±0.16

Means statistically significant, P < 0.001.

The total chlorophyll content present in the leaves of black gram (Table 11) resulted significantly different at (P < 0.001). In the total chlorophyll content of control plants found 21.52 ± 0.07 mg/g that has been reduced to 7.91 ± 0.06 at 5 egg-masses inoculum level to 3.55 ± 0.06 at 25 egg-masses inoculum level. In the treated plants the total chlorophyll content found increased with increasing concentrations of leaf extract, that is in 5 egg-mass inoculum level the chlorophyll contents found 10.61 ± 0.07 at 5 ppm to 21.43 ± 0.64 at 25 ppm. The same trend was observed in 10, 15, 20 and 25 egg-masses inoculum levels.

Root-knot nematodes caused severe damage to roots and reduced the supply of water and nutrients from the soil to the upper parts of the plants by the formation of giant cells. A shortage of nutrients in the above-ground parts of the plants that may alter the biochemical processes of plants. High levels of chlorophyll may increase the photosynthetic rate and thereby increase shoot growth detected. Reduction in different growth parameters (length and weight of plant, number of pods), chlorophyll content of leaf and water absorption of roots caused by *Meloidogyne incognita* was statistically significant (Anver, 2007).

Table 11. Effect of the root-knot nematode and the leaf extract of *Mimusops elengi* on the treatments on the total chlorophyll of black gram.

No. of egg-masses	Total chlorophyll						
	Control	Inoculated control	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
5	21.52±0.07	7.91±0.06	10.61±0.07	14.78±0.06	16.76±0.08	19.67±0.13	21.43±0.64
10		6.82±0.06	10.1±0.25	14.25±0.06	16.35±0.48	19.44±0.13	20.63±0.05
15		6.37±0.08	9.39±0.06	13.9±0.05	15.79±0.06	18.49±0.07	20.46±0.06
20		4.89±0.07	8.67±0.05	13.0±0.45	15.57±0.08	18.28±0.08	19.91±0.07
25		3.55±0.06	8.43±0.07	11.85±0.07	15.25±0.06	16.87±0.08	19.72± 0.06

Means statistically significant, P <0.001.

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