

**Efficacy of Root Knot Nematode (*Meloidogyne incognita*)  
on the Growth Characteristics of Black Gram (*Vigna mungo*)  
Treated with Leaf Extract of Magilam (*Mimusops elengi*)**

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**Abstract:** The present study has been done 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 inoculum levels of egg masses (5, 10, 15, 20 and 25 egg masses) plants treated with the leaf extract of *Mimusops elengi* at different concentrations (5, 10, 15, 20 and 25 ppm). The control and treated plants were analyzed for various growth characteristics were analyzed after 65 days of treatment. All growth characteristics viz., root length, shoot length, fresh and dry weight of shoot, leaf area and chlorophyll contents were found to be decreased with increasing inoculum levels of egg masses, but increased with increasing concentrations of leaf extract treatment and fresh and dry weight of root and root gall index were also found to be increased with increasing inoculum levels of egg masses and decreased with increasing concentrations of leaf extract treatment.

**Key words:** *Mimusops elengi* • *Meloidogyne incognita* • *Vigna mungo* • Chlorophyll content • Root gall index

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## INTRODUCTION

Nematodes are microscopic multicellular round worm that inhabit marine, freshwater and terrestrial environment. Plant parasitic nematodes and soil borne pathogens also attack a wide range of vegetables reducing its yield quality and quantity [1]. 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 [2].

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 [3]. Infected plants show reduced growths, swollen roots which develop into the typical root-knot galls, are two, or three times larger in diameter as healthy root. Root knot nematodes are very difficult to control because they are polyphagous [4]. Of all the pathogens, root-knot

nematodes (*Meloidogyne* sp.) are the most serious ones [5]. *Meloidogyne* sp. form disease complex with root rot pathogens causing major losses in vegetable production. Such problem is widely spread in controlled agricultural systems [6].

A high level of root-knot nematode damage can lead to total crop loss. Nematode damaged roots do not utilize water and fertilizers effectively, leading to additional losses. Infection of young plants may be lethal, while infection of mature plants causes yield reduction. *Meloidogyne* species constitute the major nematode problem in developing countries [7]. *Meloidogyne* damage results 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 [8], 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 [9, 10]. 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. Increasing pressure is on farmers to use non-chemical pest control methods that do not pollute the environment. This emphasizes 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 [11, 12]. 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

For the present study, 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 *Acalypha 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 (*Mimusops elengi*) 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 of black gram (*Vigna mungo*) such as, root length, shoot length, fresh and dry weight of root, fresh and dry weight of shoot, leaf area, water content root and shoot and root gall index and chlorophyll content were analyzed.

## RESULTS AND DISCUSSION

In the present study, various growth parameters *viz.*, such as, shoot length, root length, fresh and dry weights of shoot and root, leaf area, root gall index and chlorophyll content of black gram (*Vigna mungo*) 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 of *M. incognita* (5, 10, 15, 20 and 25 egg masses). After 65 days of leaf extract treatment, the shoot and root length (cm) of black gram was analyzed. The shoot and root length was found to be increased with increasing concentrations of leaf extract compared to control and its decreased with increasing inoculum levels of *M. incognita* (Table 1 & 2). The results were found to be statistically significant at ( $P < 0.001$ ). Siddiqui and Rehman [13] 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 *Melia azedarach*. Highest plant length was recorded in untreated non-inoculated plants.

The fresh and dry weight of the shoot was significantly reduced in the increasing inoculum levels. While in the fruit extract treated plants the fresh and dry weights are increased with increasing concentrations (Table 3 & 4). The results were found to be significantly different ( $P < 0.001$ ). Perveen et al. [14] observed a positive relationship between the initial inoculum levels of *Meloidogyne incognita* and reduction in shoot, fresh and dry weights, total chlorophyll content of fresh leaves. 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 Hussain and Bora [15] who observed significant variations in these parameters. This is due to the fact that RKN infected tissues (galls) contain highly dense granular protoplasm [16]. This finding is in confirmation with Hussain and Bora [15] and Maleita et al. [17] Robab et al. [18] showed the suppressive effect of *M. incognita* at different inoculum levels, on fresh and dry weight of shoots of soybeans which was in accordance to our results.

The fresh and dry weight of the root was significantly reduced in the increasing inoculum levels. While in the fruit extract treated plants the fresh and dry weights are increased with increasing concentrations (Table 5 & 6). The results were found to be significantly different ( $P < 0.001$ ). Because the root was heavily infested with the root knot nematode (*Meloidogyne incognita*) to produce by the galls so increased by the root weights. Olaniyi et al. [19] reported that the presence of *Meloidogyne incognita* increased the weight of root in tomato plants as a result of galls produced in the roots. However, numbers of galls were less in the treated plots than in the control which was an indication that applications of plant materials exerted some controlling

Table 1: Effect of the root-knot nematode, *Meloidogyne incognita* on the shoot length (cm) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Shoot length (cm)						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
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

Note: Data are the average value of three replications. \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control ppm: parts per million.

Table 2: Effect of the root-knot nematode (*Meloidogyne incognita*) on the root length (cm) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Root length (cm)						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
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

Note: Data are the average value of three replications \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million.

Table 3: Effect of the root-knot nematode (*Meloidogyne incognita*) on the shoot fresh weight (g) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Fresh weight in shoot (g)						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
5	15.59±0.07	10.94±0.06	12.50±0.10	13.84±0.10	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	19.70±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±0.20	12.92±0.06	13.70±0.04	15.78±0.05	19.45±0.05
25		8.60±0.08	11.17±0.09	12.81±0.05	13.56±0.07	14.93±0.06	18.22±0.06

Note: Data are the average value of three replications. \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control ppm: parts per million.

Table 4: Effect of the root-knot nematode (*Meloidogyne incognita*) on the shoot dry weight (g) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Dry weight in shoot (g)						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
5	8.53±0.06	4.83±0.10	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	5.80±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	4.90±0.07	6.33±0.04	7.92±0.06	9.87±0.07	11.74±0.04

Note: Data are the average value of three replications \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million.

influence on parasitic nematodes, hence a reduction in the number of galls per plant compared with the very high gall/plant results from the control plots. Galling is a

reaction of the plant to the feeding of the root-knot nematode which may also vary in size with different applications of plant materials.

Table 5: Effect of the root-knot nematode (*Meloidogyne incognita*) on the root fresh weight (g) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*)

No. of Egg masses	Fresh weight in root (g)						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
5	5.50±0.02	6.07±0.04	4.62±0.04	3.08±0.07	2.54±0.08	2.10±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	1.90±0.04
25		7.92±0.06	5.89±0.04	4.09±0.07	3.10±0.04	2.47±0.04	1.92±0.05

Note: Data are the average value of three replications. \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control ppm: parts per million.

Table 6: Effect of the root-knot nematode (*Meloidogyne incognita*) on the root dry weight (g) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*)

No. of Egg masses	Dry weight in root (g)						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
5	4.04±0.10	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	3.20±0.05	2.58±0.10	2.14±0.06	1.60±0.11
15		6.53±0.06	5.20±0.04	3.29±0.06	2.62±0.08	2.18±0.07	1.74±0.06
20		6.74±0.06	5.60±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

Note: Data are the average value of three replications \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million.

Table 7: Effect of the root-knot nematode (*Meloidogyne incognita*) on the leaf area (cm<sup>2</sup>) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*)

No. of Egg masses	Leaf area (cm <sup>2</sup> )						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
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	11.90±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

Note: Data are the average value of three replications. \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control ppm: parts per million.

Table 8: Effect of the root-knot nematode (*Meloidogyne incognita*) on the shoot water content (%) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*)

No. of Egg masses	Water content (%) in shoot						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
5	45.22±0.13	15.45±0.22	18.98±0.10	23.46±0.19	28.60±0.10	36.30±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±0.20	42.34±0.11
15		7.11±0.10	14.30±0.11	21.18±0.02	25.52±0.13	33.46±0.14	40.26±0.05
20		5.37±0.20	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

Note: Data are the average value of three replications \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million.

Table 9: Effect of the root-knot nematode (*Meloidogyne incognita*) on the root water content (%) of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Water content (%) in root						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
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±0.10
10		3.62±0.04	7.20±0.05	12.55±0.10	17.49±0.06	19.22±0.04	23.79±0.08
15		3.12±0.07	6.82±0.07	11.60±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±0.10	18.05±0.16	21.70±0.10
25		1.21±0.10	4.48±0.14	9.09±0.07	14.66±0.06	17.72±0.12	21.16±0.16

Note: Data are the average value of three replications. \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control ppm: parts per million.

Table 10: Effect of the root-knot nematode (*Meloidogyne incognita*) on the root gall index of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Root gall index						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
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

Note: Data are the average value of three replications \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million.

Root gall index scale (1-6)

Index Root galls

- 1 = Nil
- 2 = 1-20
- 3 = 21-40
- 4 = 41-60
- 5 = 61-80
- 6 = 81-100

Table 11: Effect of the root-knot nematode, *Meloidogyne incognita* on the total chlorophyll of black gram (*Vigna mungo*) treated with leaf extract of magilam (*Mimusops elengi*).

No. of Egg masses	Total chlorophyll						
	Con	In. con	5ppm*	10ppm*	15ppm*	20ppm*	25ppm*
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	10.10± 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	13.90±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

Note: Data are the average value of three replications \*Means statistically significant, P <0.001.

Con: Control, In.con:Inoculated Control, ppm: parts per million

The leaf area of the control plants found to be  $22.88 \pm 0.93\text{cm}^2$  and it is reduced the egg masses treated plants  $11.19 \pm 0.82\text{cm}^2$  (5 egg masses),  $10.74 \pm 0.45\text{cm}^2$  (10 egg masses) and  $8.49 \pm 0.44\text{cm}^2$  (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). Tobih *et al.* [20] studied

that plant height, girth, leaf area and numbers of leaves were reduced in the *M. incognita* on *Celosia argentea* plant.

The efficacy of leaf extract of *M. elengi* on the root-knot nematode (*M. incognita*) infecting the black gram (*V. mungo*) was elucidated individually on the root gall index and presented in the Table 8. Results were found to be 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 are 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 [21]. A progressive increase in number of galls and egg masses with the increased inoculum level of the nematode from below pathogenic level to pathogenic level was recorded by Hussain and Bora [15]. Mukherjee *et al.* [22] showed that shoot weight of infected plants with nematodes and treated with salicylic acid were increased compared with infected plants without treatment, they also showed that numbers of root galls and eggs/g root decreased when plants treated with salicylic acid.

Table 9 & 10 showed that the water content (%) on the leaves of black gram, (*V. mungo*) 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 to be decreasing with increasing levels of egg masses inoculum level. At different concentrations of *M. elengi*, the water content of the shoot was found to be increasing with increasing concentration of the leaf extract (5, 10, 15, 20 and 25 ppm) treatments. The results were found to be significantly different ( $P < 0.001$ ). Plant stress caused by *M. incognita* and increased plant growth, nodulation parameters as well as chemical components. Morphological root changes in mycorrhiza treated plants led to increasing the absorptive surface area of the whole host root system to water and mineral nutrients supply, particularly P, so that can improve biological  $N_2$ -fixation in legumes and increase the number of nodulation sites and consequently the number of nodules per plant [23].

The total chlorophyll content present in the leaves of black gram (*V. mungo*) inoculated with 5, 10, 15, 20 and 25 egg masses of root-knot nematode, *M. incognita* and treated with different concentrations of *M. elengi* were analyzed after 65 days treatment (Table 11). The results were found to be significantly different ( $P < 0.001$ ). In the total chlorophyll content of control plants has been found to be  $21.52 \pm 0.07$  mg/g that has been reduced to  $7.91 \pm 0.06$  at 5 egg masses inoculum level to  $3.55 \pm 0.06$  at

25 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  $10.61 \pm 0.07$  at 5 ppm to  $21.43 \pm 0.64$  at 25 ppm. The same trend was observed in 10, 15, 20 and 25 egg masses inoculum levels. Root-knot nematodes cause severe damage to the roots and reduce the supply of water and nutrients from the soil to the upper parts of the plants by the formation of giant cells. This causes a shortage of nutrients in the above-ground parts of the plants that may alter the biochemical processes of plants. Chlorophyll a and b levels were higher in the treated plants than in the control plants. High levels of chlorophyll may increase the photosynthetic rate and thereby increase the shoot growth, as was 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 [24]. According to Bhuvaneshwari *et al.*, [25] chlorophyll 'a', 'b' and 'total' chlorophyll contents were found to be significantly increased in leaves of plants treated with *Cyanospray* and *Cyanopith* 500 g/sq.m.

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