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Biochemical Alterations of Black Gram, *Vigna Mungo* Infected with Root Knot Nematode, *Meloidogyne incognita* Treated with Leaf Extract of *Ervatamia Heyneana*

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Abstract: *The present study has been done to evaluate the effect of leaf extract of Ervatamia heyneana against the root knot nematode, Meloidogyne incognita infected with black gram, Vigna mungo at different inoculum levels of egg masses (5, 10, 15, 20 and 25 egg masses) and the experimental plants treated with the leaf extract of E. heyneana at different concentrations (5, 10, 15, 20 and 25 ppm). The control and experimental plants were analyzed after 65 days, the biochemical changes of root knot nematode infected plants treated with leaf extract. The biochemical changes such as, carbohydrate, protein and Nitrate reductase activity were found to be decreased with increasing inoculum levels of egg masses and increased with increasing concentrations of leaf extracts treatment except phenol content.*

Keywords: *Carbohydrate, Ervatamia heyneana, Meloidogyne incognita, Nitrate reductase activity, Phenol, Protein.*

INTRODUCTION

Nematodes are a major problem in crop production quantity and quality. Root-knot nematodes (RKN) are one of the most important nematode pests of all the crop plants and have a diverse host range. *Meloidogyne* spp are sedentary root endoparasites and are involved in the development of specialized feeding structures known as giant cells. The infective stage of the nematode is the second-stage juvenile (J_2). The J_2 penetrate the roots and go through three successive moults to become adult females or males. Several of the most important root-knot nematode species, including *M. incognita*, reproduce by obligate mitotic parthenogenesis [1]. The mechanism of feeding site development by root knot nematodes is not well understood as it is a very dynamic and complex process involving genes from both the nematode and the host plant. The secretions from the oesophageal glands of the nematode are important in initiating the development of feeding structures [2]. Root-knot nematodes (*Meloidogyne* species) are one of the major plant-parasitic nematodes causing diseases in plants all over the world [3]. Typical symptoms of root knot nematode injury include stunting, unthriftiness, premature wilting, malformed fruit ripens slowly or unevenly chlorosis (yellowing), gall formation, and non-uniform growth and reduced stand establishment [4,5] and below ground plant parts form a tight mat of short roots and galls on roots [6]. *Meloidogyne incognita* a notable species of the root-knot nematodes had been identified as a major reason for yield reduction in crops [7].

Nematodes are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing up to thousand eggs/female [8]. Synthetic pesticides, though instantaneously effective are usually prohibitively expensive, not readily available, may cause hazards to both man and livestock and inflict injury to the environment [9]. During the last decades, nematologists worldwide search the cheaper, safer and eco-friendly alternatives methods i.e. biological and cultural methods to control the plant-parasitic

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nematodes. The use of botanicals appears the most feasible especially for low income formers constitute about 98% of the farming population. Applications of these botanicals do not contribute a threat to the environment, they are easily affordable, require less skill and above all increase soil fertility [10]. Hence the present investigation has been carried out the biochemical alterations of black gram, *Vigna mungo* infected with root knot nematode, *Meloidogyne incognita* treated with leaf extract of *Ervatamia heyneana.*

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 [11] to destroy the various micro organisms. The sterilized soil mixers were used in the proportion of 2:1:1 ratio. The healthy seeds of experimental plant, *Vigna mungo* 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. *Vigna mungo* 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 55^0C [12] methods. The different concentrations was prepared with a 1 g of leaf 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. After inoculation distilled water was poured for three days and leaf extracts were added daily after 65 days and after analyze for various biochemical characteristics, such as Carbohydrate (Anthrone method [13], Protein [14], Nitrate Reductase activity [15] and phenol content [16] were analyzed.

STATISTICAL ANAYSIS

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

RESULTS AND DISCUSSION

Total Carbohydrate

In the experimental plants treated with leaf extract of *E.heyneana* the carbohydrate content of the black gram, *Vigna mungo* was analysed after 65 days of treatment (Table 1). The carbohydrate content of the control plants was observed as 76.44 ± 0.57, while in inoculated control plants, the carbohydrate content has been decreased with increasing inoculum levels of egg masses of root-knot nematode, 16.25 ± 0.06 at 5 egg masses inoculum to 7.22 ± 0.21 at 25 egg masses inoculum. At different concentrations of leaf extract, the carbohydrate content was found to be 19.18 ± 0.18 at 5 ppm to 69.23 ± 0.21 at 25 ppm at 15 egg masses inoculum level. The same trend was also observed in the plants inoculated with 5, 10, 20 and 25 egg masses and increasing with increasing

Table1
Effect of different inoculum levels of root-knot nematode, *Meloidogyne incognita* and different concentrations of leaf extract of *Ervatamia heyneana* on the total carbohydrate content (mg/g) of black gram *Vigna mungo.*

No. of Egg Masses Treated	*Total carbohydrate content*						
	Control	*Inoculated control*	**5 ppm*	**10ppm*	**15ppm*	**20ppm*	**25ppm*
5	76.44 ± 0.57	16.25±0.06	25.13±0.12	32.22± 0.20	45.13± 0.11	67.09± 0.09	72.22 ± 0.20
10		14.27 ± 0.25	22.33 ± 0.28	30.10 ± 0.09	43.14 ± 0.14	64.13 ± 0.11	70.14 ± 0.13
15		12.26 ± 0.26	19.18 ± 0.18	28.15 ± 0.15	42.13 ± 0.12	62.25 ± 0.22	69.23 ± 0.21
20		09.34 ± 0.41	17.16 ± 0.14	25.12 ± 0.11	38.09 ± 0.09	59.11 ± 0.10	68.10 ± 0.10
25		07.22 ± 0.21	15.20 ± 0.17	23.10 ± 0.09	36.10 ± 0.10	56.13 ± 0.11	65.09 ± 0.09

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

Table 2
Effect of different inoculum levels of root-knot nematode, Meloidogyne incognita and different concentrations of leaf extract of Ervatamia heyneana on the total protein content (mg/g) of black gram Vigna mungo.

No. of Egg Masses Treated	*Total carbohydrate content*						
	Control	*Inoculated control*	**5 ppm*	**10ppm*	**15ppm*	**20ppm*	**25ppm*
5	Control	Inoculated control	*5 ppm	*10ppm	*15ppm	*20ppm	*25ppm
	52.48 ± 0.37	22.00±0.10	29.18 ± 0.09	36.23 ± 0.09	41.61 ± 0.04	48.88 ± 0.07	52.25 ± 0.08
10		19.71 ± 0.27	27.30 ± 0.12	34.38 ± 0.04	40.45 ± 0.20	47.22 ± 0.06	51.48 ± 0.27
15		19.23 ± 0.09	25.48 ± 0.09	33.77 ± 0.10	39.90 ± 0.09	45.56 ± 0.05	50.82 ± 0.11
20		17.52 ± 0.26	22.75 ± 0.23	32.72 ± 0.04	38.98 ± 0.10	44.48 ± 0.09	50.11 ± 0.07
25		14.31 ± 0.13	22.35 ± 0.06	31.76 ± 0.04	37.33 ± 0.05	43.72 ± 0.06	49.45 ± 0.14

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

concentrations of leaf extracts. The results were statistically significant at p<0.01. The determination of carbohydrates in nematode infected plants. Little more than 50% reduction of free sugars was reported in galled tomato root infected with *M. incognita* over healthy ones [17]. Similarly Musabyimana and Saxena [18] reported that the carbohydrates are quick energy source compounds obtained from the vegetable plants or crops. Due to the infection of various species of root-knot nematodes, the carbohydrate content has been decreased in banana. In the use of neem seed derivations showed better improvement in carbohydrate content than this inoculum levels. Kumar [19] also found that the pathogenic effect of root knot nematode, *M. incognita* decreased the biochemical compounds of leaves of tuberose plants.

Total Protein

The experimental plant treated with leaf extract of *E.heyneana* the protein content (mg) of black gram *V. mungo* were analysed after 65 days of treatment (Table 2), the control plants showed 52.48 ± 0.37, while in inoculated control plants it was found to be decreasing from 22.0 ± 0.10 at 5 egg masses inoculum level to 14.31 ± 0.13 at 25 egg masses inoculum level. While in the experimental plants treated with *Ervattamia heyneana*, the protein content was found to be increasing from 27.30 ± 0.12 at 5 ppm to 51.48 ± 0.27 at 25 ppm at 10 egg mass inoculum level. The same results were also observed in other different inoculum levels. The results were statistically significant at p<0.01. Ramesh Kumar *et al.* [20] reported that the protein content was increased vermicompost extract treated plants (Cluster bean *Cyamposis tetragonaloba*) compared with control and inoculated control plants. The increasing concentration of vermicompost extracts shows the increasing trend of protein level. Similarly Padhi and Behera [21] reported that the protein content in the leaves of test plants has been decreased in inoculated plants and increased in 10 indigenous plant extract-treated plants. The increased concentration of protein in the inoculated untreated plants tissue might be due to the action of proteolytic enzymes from plant sources and nematode source too. It is axiomatic that protein plays a major role in defence action against the nematode [22].

Nitrate Reductase Activity

In this study the experimental plants treated with leaf extract of *E.heyneana* the nitrate reductase activity (M mol No_2 h^{-1}, g^{-1} f.wt.) of black gram, *V. mungo* were analysed after 65 days of treatment, the control plants showed (60.54 ± 0.95), while in inoculated control plants it was found to be decreasing from 19.65 ± 0.07 at 5 egg masses inoculum level to 7.48 ± 0.07 at 25 egg masses inoculum level. While in the experimental plants treated with leaf extract of *E.heyneana* the nitrate reductase activity was found to be increasing (20.42 ± 0.06 at 5 ppm to 45.54 ± 0.23 at 25 ppm at 25 egg masses inoculum level) with increasing concentration of leaf extract at different inoculum levels. The results were statistically significant at p < 0.01.

Sadasivam and Manikam [23] reported that the nitrate reductase is an enzyme that is capable of converting nitrate into nitrite compound in the presence of reduced pyridine nucleotide. Mohanty *et al.* [24] reported that reduced nitrogenase activity was observed in nematode infected nodules. Similar observations made by Chahal and Chahal [25] in Mung bean infected by *M. incognita*. The functioning of nitrogenase is directly related to leghaemoglobin content as it regulates the diffusion of oxygen.

Table 3
Effect of different inoculum levels of root-knot nematode, Meloidogyne incognita and different concentrations of leaf extract of Ervatamia heyneana on the Nitrate reductase activity (M mol No_2 h^{-1}, g^{-1} f.wt.) of black gram Vigna mungo.

No. of Egg Masses Treated	*Total carbohydrate content*						
	Control	*Inoculated control*	**5 ppm*	**10ppm*	**15ppm*	**20ppm*	**25ppm*
5	60.54±0.95	19.65 ± 0.07	29.84 ± 0.07	30.63 ± 0.07	38.66 ± 0.09	44.87 ± 0.10	48.79 ± 0.08
10		18.31 ± 0.11	27.29 ± 0.13	30.17 ± 0.09	37.85 ± 0.13	43.74 ± 0.21	47.59 ± 0.07
15		15.33 ± 0.14	25.79 ± 0.08	29.71 ± 0.14	36.50 ± 0.08	42.42 ± 0.21	47.33 ± 0.04
20		13.17 ± 0.10	23.24 ± 0.10	29.23 ± 0.11	35.43 ± 0.04	40.55 ± 0.09	46.35 ± 0.04
25		7.48 ± 0.07	20.42 ± 0.06	28.25 ± 0.13	34.12 ± 0.03	40.18 ± 0.03	45.54 ± 0.23

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

Reduction in leghaemoglobin content might be the possible cause of low nitrogenase activity.

Total Phenol

Table 4 show the experimental plant treated with leaf extract of *E.heyneana* the total phenol content (mg/g) of the black gram, *V. mungo* were analysed after 65 days, the control plants showed 5.83 ± 0.06, while in inoculated control plants it was found to be increasing from 31.88 ± 0.09 at 5 egg masses inoculum level, 32.89 ± 0.05 at 10 egg masses inoculum level, 33.89 ± 0.06 at 15 egg masses inoculum level, 35.16 ± 0.06 at 20 egg masses inoculum level and 36.16 ± 0.10 at 25 egg masses inoculum level. The experimental plants treated with leaf extract of *E.heyneana* , the phenol content was found to be decreasing (27.61 ± 0.08 at 5 ppm to 13.66 ± 0.08 at 25 ppm at 20 egg mass inoculum levels) with increasing concentration of leaf extract at different inoculum levels egg masses. The results were statistically significant at p<0.01. 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 [26]. Most phenols occur in plant tissues in bound forms as glycosides of low physiological and chemical activities. Activation requires their decompositions to free phenols [27]. Nematodes are able to decompose glycosides by secreting â-glycosidases into host tissue [28]. Similarly Bhargava *et al.* [29] reported that the total phenol increased with infected plants than the healthy plants. These observations are in confirmation of earlier reports [30,31,32] (Hung and Rohde 1973; Ganguli and Dasgupta 1979; Hasan and Saxena 1979) 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.

CONCLUSION

The present study indicated that the leaf extract of *E.heyneana* have the good nematicidal properties against the root knot nematode, *M. incognita.* Further they recommended the leaf extract of *Ervatamia heyneana* has a small scale former to control the nematodes.

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Table 4
Effect of different inoculum levels of root-knot nematode, Meloidogyne incognita and different concentrations of leaf extract of Ervatamia heyneana on the total phenol content (mg/g) of black gram Vigna mungo.

No. of Egg Masses Treated	*Total carbohydrate content*						
	Control	*Inoculated control*	**5 ppm*	**10ppm*	**15ppm*	**20ppm*	**25ppm*
5	5.83±0.06	31.38 ± 0.09	26.14 ± 0.09	20.43 ± 0.07	19.29 ± 0.09	15.23 ± 0.09	11.26 ± 0.73
10		32.89 ± 0.05	26.72 ± 0.07	20.82 ± 0.09	19.58 ± 0.10	15.81 ± 0.11	11.67 ± 0.08
15		33.89 ± 0.06	26.86 ± 0.12	21.46 ± 0.07	19.64 ± 0.05	16.09 ± 0.09	12.88 ± 0.08
20		35.16 ± 0.06	27.61 ± 0.08	22.13 ± 0.06	19.92 ± 0.06	16.86 ± 0.07	13.66 ± 0.08
25		36.16 ± 0.10	29.11 ± 0.08	22.86 ± 0.06	20.08 ± 0.08	18.12 ± 0.08	14.74 ± 0.06

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

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