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Biopotency of Oilcakes against *Meloidogyne incognita* Affecting *Vigna mungo*

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ABSTRACT

Root-knot nematode (*Meloidogyne incognita*) is a catastrophic phytonematode parasite causing enormous losses to wild as well as cultivated crops worldwide. Despite the fact that synthetic nematicides have most significant effect, limiting these phytonematodes but the inherent toxic nature of these chemicals to the environment and human beings have compelled the researchers to think for safe, alternative and eco-friendly substitutes for these chemicals. A pot trial was conducted under the greenhouse condition to evaluate the efficacy of different doses of plant oil cakes against root knot nematode infesting Black gram, *Vigna mungo*. Root knot nematode, *Meloidogyne incognita* was found to reduce plant growth and pollen fertility and yield in pulse crop. Among various treatments the Neem cake at 100 g/pot was found most effective in limiting root gall index and enhancing plant growth parameters followed by Mustard cake at 100 g. Neem cake at 50 g/pot, Castor cake at 100 g/pot and Linseed cake at 100 g/pot were statistically at par. Above studies assure an effective and commercially cheap source of controlling root knot nematode in favour of pollution free environment.

Key words: *Vigna mungo*, *Meloidogyne incognita*, neem, mustard, castor, linseed oilcakes

INTRODUCTION

Pulses are the most requisite among all the food requirements for majority of the people all around the world specifically to the vegetarian group. Blackgram (*Vigna mungo* L. Hepper), Family-Fabaceae commonly called as Urd bean has been originated in India, regarded as its largest producer and consumer in the world (Yadhu *et al.*, 2012). Pulses are special in case of maintaining the soil fertility through nitrogen fixation (Korlovich and Repyev, 1995). During the last few decades the production and quantity of blackgram has been constantly declining. A number of phyto-parasitic nematodes have been reported on pulses causing potential yield losses (Ali and Askary, 2001; Ali and Sharma, 2002). Root knot nematode (*Meloidogyne incognita* Kofoid and White) Chitwood, is one of the major constraint to the pulse production among all the other nematode (Bhat *et al.*, 2009). Root knot nematode have been responsible to cause a total yield loss of 17 to almost 23% in black gram (Khan *et al.*, 2014). Various strategies have been tried over years to eradicate the problem of root knot nematode. In the present study the effect of various oilcakes like neem, mustard, castor and linseed were investigated for the management of root knot nematode (*Meloidogyne incognita*) affecting the Black gram (*Vigna mungo*) effecting its yield.

MATERIALS AND METHODS

A pot trial was conducted under glasshouse conditions in the Department of Botany, Aligarh Muslim University, Aligarh (27°52'N latitude, 78°51'E longitude and 187.45 m altitude), U.P., India.

Host plant culture: Two hundred healthy seeds of Black gram (*Vigna mungo* cv. PU-30) were surface sterilized with 0.1% solution of HgCl₂ and washed thoroughly with distilled water. Six seeds were then sown in each clay pots (15 cm in diameter) in completely randomized block design, containing steam sterilized soil (7 clay: 2 sand: 1 farmyard manure) with pH-7.2.

Experimentation: The glasshouse experiments were established for evaluating the efficacy of different oil cakes viz., Neem, Mustard, Castor and Linseed at 50 g/pot and 100 g/pot individually against the root-knot development caused by the root-knot nematode (*M. incognita* Kofoid and White) Chitwood and their potential in enhancing the plant growth of black gram cv PU-30. The pots were regularly watered after the application of different treatments to facilitate the proper decomposition of the organic additives.

Host pathogen culture (Root knot culture): The roots of Eggplant (*Solanum melongena* L.) Family-Solanaceae showed root-knot symptoms (galls and eggmasses), were collected from the farm, Aligarh. The eggmasses were collected from the root samples. For identification, the mature females were excised from the galls of the roots. Perineal pattern of the mature female from each of the root system were prepared and examined under microscope (Eisenback, 1985).

Extraction of nematode: Collected eggmasses of *Meloidogyne incognita* were placed on a small course sieve (1 mm pore size) lined with the crossed lined tissue paper and placed in 10 cm diameter petridish containing Double Distilled Water (DDW). The petriplates were incubated in BOD incubator at 25°C for 5 days. The second stage juveniles (J₂) hatched out from eggmasses of incubated Petridishes were collected in a beaker. The inoculum was standardized in such a way that 10 mL of suspension contained 1500 freshly hatched juveniles (J₂) of nematode.

Inoculation of nematode: Inoculation was done 15 days after the germination of the seeds. A hole of 3-5 cm deep was made in the rhizosphere (1-5 cm) around the plant roots. A predetermined amount of nematode suspension containing 1500 number of second stage juveniles (J₂) of *Meloidogyne incognita* were poured into the hole using sterilized pipette. The holes were plugged gently with sterilized soil. The pots were then placed in glasshouse condition in completely randomized block manner. Necessary weeding and watering was done as per requirement.

Experiment was designed as follows:

- T1: Neem oil cake (50 g)+500 J₂
- T2: Neem oil cake (100 g)+500 J₂
- T3: Mustard oil cake (50 g)+500 J₂
- T4: Mustard oil cake (100 g)+500 J₂
- T5: Castor oil cake (50 g)+500 J₂
- T6: Castor oil cake (100 g)+500 J₂
- T7: Linseed oil cake (50 g)+500 J₂

- T8: Linseed oil cake (100 g)+500 J2
- T9: Untreated inoculated control (1500 J2)
- T10: Untreated uninoculated control

Each treatment was replicated four times. The plants were irrigated regularly. Mature plants were uprooted 60 Days after Inoculation (DAI). Roots were washed thoroughly with running tap water for the removal of other attached soil debris.

Plant growth and yield parameters: Plant growth parameters such as length (shoot and root) in centimeter, weight (fresh and dry) in grams, number of flowers, pods, number of root nodules and percent pollen fertility (Sass, 1964) were recorded.

Pathological parameter: Roots of blackgram were extracted from the soil and washed thoroughly with tap water and then air dried. Number of galls per root system, eggmass per root system and eggs per eggmasses were calculated with counter machine. Population of nematodes was calculated in 250 g of soil using Baermann Funnel technique.

Leghaemoglobin content: The root nodules were excluded from the roots and washed thoroughly. Leghaemoglobin content was determined at 539 and 556 nm against blank reagent using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) (Sadasivam and Macickam, 1992).

Chlorophyll and carotenoid content: Fresh leaf tissue was finely ground utilizing mortar-pestle in 80% acetone. The absorbance of chlorophyll was calculated at 663 and 645 nm. For the estimation of carotenoid reading was taken at 470 nm using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) (Lichtenthaler and Buschmann, 2001).

Nitrate reductase activity: The freshly chopped leaves (200 mg) were incubated for 2 h at 30°C in a mixture of 5 mL containing 2.5 mL of 0.1 M phosphate buffer+0.5 mL of 0.2 M potassium nitrate+2.5 mL of 5% isopropanol (Jaworski, 1971). Consequently, the formation of nitrite was analysed calorimetrically at 540 nm after azocoupling with sulphanilamide and naphthyl ethylenediamine. Nitrate reductase activity was expressed in $\mu\text{m h}^{-1} \text{g}^{-1}$.

Protein content: Initially seeds of blackgram were taken and washed thoroughly for removing any extra dust particles and then dried with use of oven. Fifty gram of seeds were then weighed for the protein analysis. Seeds were ground finely in 1 mL of 5% trichloroacetic acid. Samples were analyzed by taking absorbance at 660 nm using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Total content of protein was calculated by comparing the absorbance of each respective sample with that of calibrated curve plotted with known gradient concentrations of bovine serum albumin (Lowry *et al.*, 1951).

Nitrogen content: Leaves of Black gram were powdered and then digested. The peroxide digested material was passed through reaction with 2 mL of 2.5 N NaOH and 1 mL of 10% sodium silicate solution to neutralize acid and removing turbidity, respectively (Novozamsky *et al.*, 1983). Then

5 mL of sample was added with 0.5 mL Nessler's reagent. The absorbance of the sample was determined at 525 nm using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

The nitrogen content was estimated using standard graph plotted with known graded dilution of ammonium sulphate.

Statistical analysis: Data was subjected to one way analysis of variance (ANOVA) by using SPSS 16.00 Software (SPSS, Inc., 1989-2006, Chicago, IL, USA). Least significant differences (LSD) were calculated at $p = 0.05$ to test for significant differences between different treatment means.

RESULTS AND DISCUSSION

Effect of soil amendment with plant oilcakes viz., Neem, Mustard, Castor and Linseed on the root-knot development caused by *Meloidogyne incognita* and plant growth characters of Black gram (*Vigna mungo*) was evaluated.

Root knot nematode (*Meloidogyne incognita*) reduces all plant growth characters of the untreated inoculated control (T9) plants as compared to all other treated or uninoculated control plants. There was significant reduction calculated at 0.05% level in total yield observed in inoculated control (T9) plants followed by all other oil cake treated plants as compared to untreated uninoculated (T10) and Neem cake treated (T1) plants of Black gram. In untreated inoculated control, the Black gram plants suffered a severe damage by the root knot development caused by *Meloidogyne incognita* with highest increase in number of root galls (95) and reduction in number of root nodules (16).

The application of various additives significantly declined the incidence of root knot nematode, however the severity of root galls was found minimum with increase in nodule number (51 and 43) in plants treated with 100 g dose of Neem cake. The root knot galls and number of nodules of other treatments applied at the same concentrations were (56 and 40), (60 and 39) and (62 and 37) for Mustard, Castor and Linseed oil cakes (Fig. 1a-c).

The plant growth (length, fresh and dry weight) of black gram greatly improved but to varying extent depending upon the concentration of the treatment. The plants treated with Neem oil cake were found most effective in enhancing the plant growth followed by the plants treated with Mustard and Castor and at similar doses also reduces the impact of root knot development followed by Linseed oil cakes (Fig. 2a-c).

The chlorophyll content, carotenoid content, nitrate reductase activity and Leghaemoglobin content measured in (mg g^{-1} , $\mu\text{m h}^{-1} \text{g}^{-1}$ and mg g^{-1}) also increased significantly in case of higher doses of neem oil cake (2.394, 0.251, 0.259 and 3.6) when applied and followed by mustard (2.369, 0.234, 0.251 and 3.57), castor (2.348, 0.218, 0.243 and 3.48) and linseed (2.331, 0.192, 0.233 and 3.31) as compared to untreated inoculated. The lower doses also improved the plant growth character but to some lesser extent (Fig. 3a-b). Similar trends were observed in case of nitrogen and protein content (Fig. 3c).

All the treatment proved significantly effective in controlling the negative impact of root knot nematode (*Meloidogyne incognita*). Application of neem oil cake plays a crucial role in limiting the root knot infestation in Black gram. Suppression of nematode population may be due to the various nematotoxic compounds present in plant oil cakes. This could also be concluded from the present

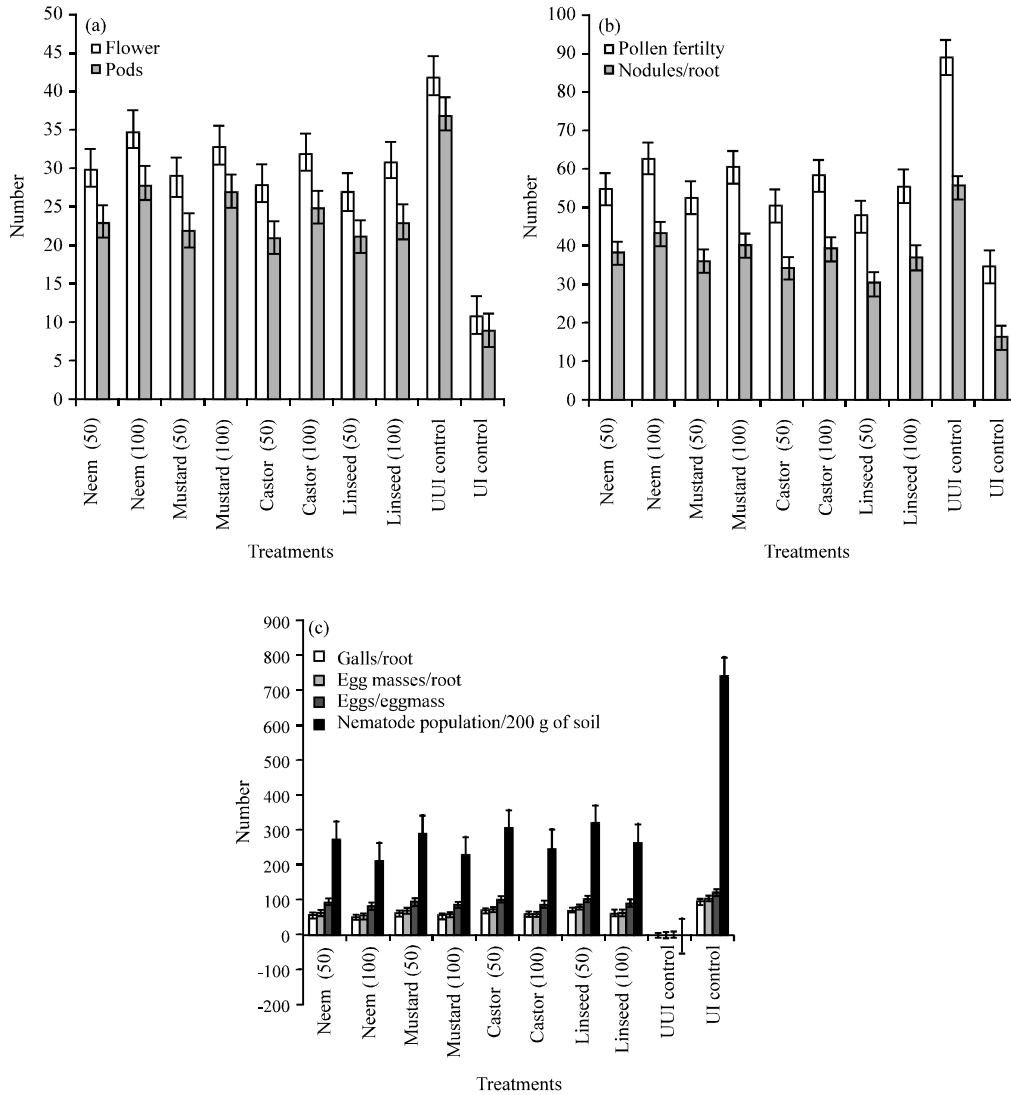


Fig. 1(a-c): Effects of different oilcakes on pollen fertility, root nodules and pathological parameters against *Meloidogyne incognita* attacking *Vigna mungo* L. cv PU-30

study that oilcake helps in stimulating various microbial activity in rhizospheric zone and some physiological alterations in plant roots to inhibit nematode. It is estimated that out of the total quality of urea applied to crops, 50-70% is lost in various forms, thereby reducing availability of nitrogen to crop. When neem products are mixed with urea and incorporated into the soil, the triterpenes retard the growth and multiplication of nitrifying bacteria resulting in delayed transformation of ammoniacal nitrogen into nitrate (Akhtar and Alam, 1993). Results obtained in the present investigations are in accordance with the results of previous researches. Zid (2002) reported the inhibition and penetration of root knot could be altered with the difference in doses applied of sesame oil seed cake in squash. Organic soil amendments utilizing manures, fresh chopped leaves of different plants and non-edible deoiled cakes of neem, mustard, castor, linseed and cotton and many others have given promising results in reducing the nematode population (Wani, 2006; Tejeda *et al.*, 2006; Rehman *et al.*, 2011; Sivakumar and Gunasekaran, 2011; Mohan,

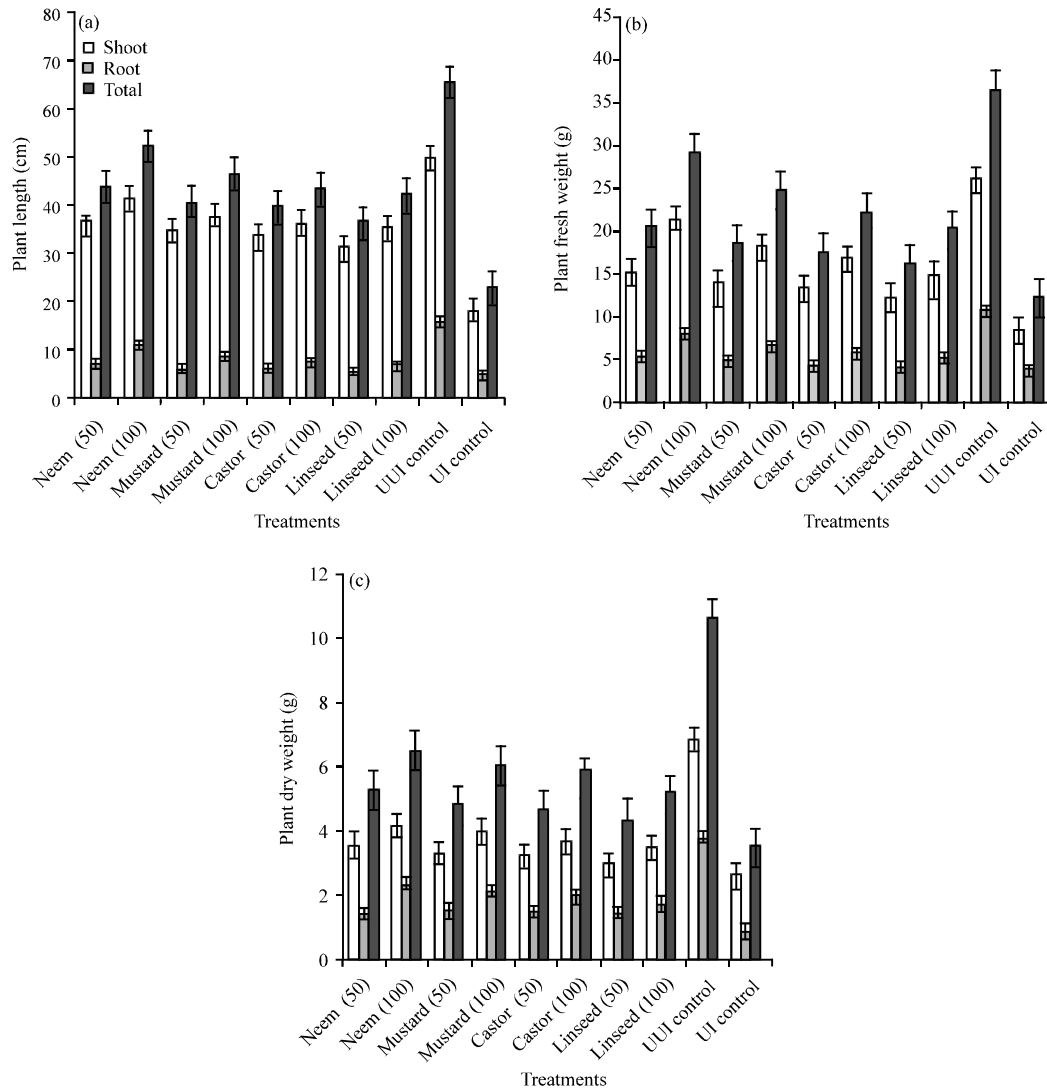


Fig. 2(a-c): Effect of different oilcakes on plant growth parameters against *Meloidogyne incognita* attacking *Vigna mungo* L. cv PU-30

2011; Deepa *et al.*, 2011; McGeehan, 2012; Parihar *et al.*, 2013; Ganai *et al.*, 2014; Asif *et al.*, 2014). Various other plant products like essential oils obtained from karanj, sunflower, mustard, chalmogra etc. have also been exploited for their growth promoting and ant-nemic properties (Leela *et al.*, 1992). The control of plant parasitic nematodes due to decomposed organic matter as suggested by scientists may be attributed to toxic compounds released during decomposition (Khan *et al.*, 1974) and the changes in physical and biological properties of soil (Ramesh *et al.*, 2009).

Incorporation of organic amendments into the soil besides suppressing the nematode population density also promote the antagonistic microbial activity and improve the fertility and organic matter status of the soil. Application of oil cakes as organic matter helped in enhancing the plant growth through the supply of many micronutrients and through the enhancement of photosynthesis due

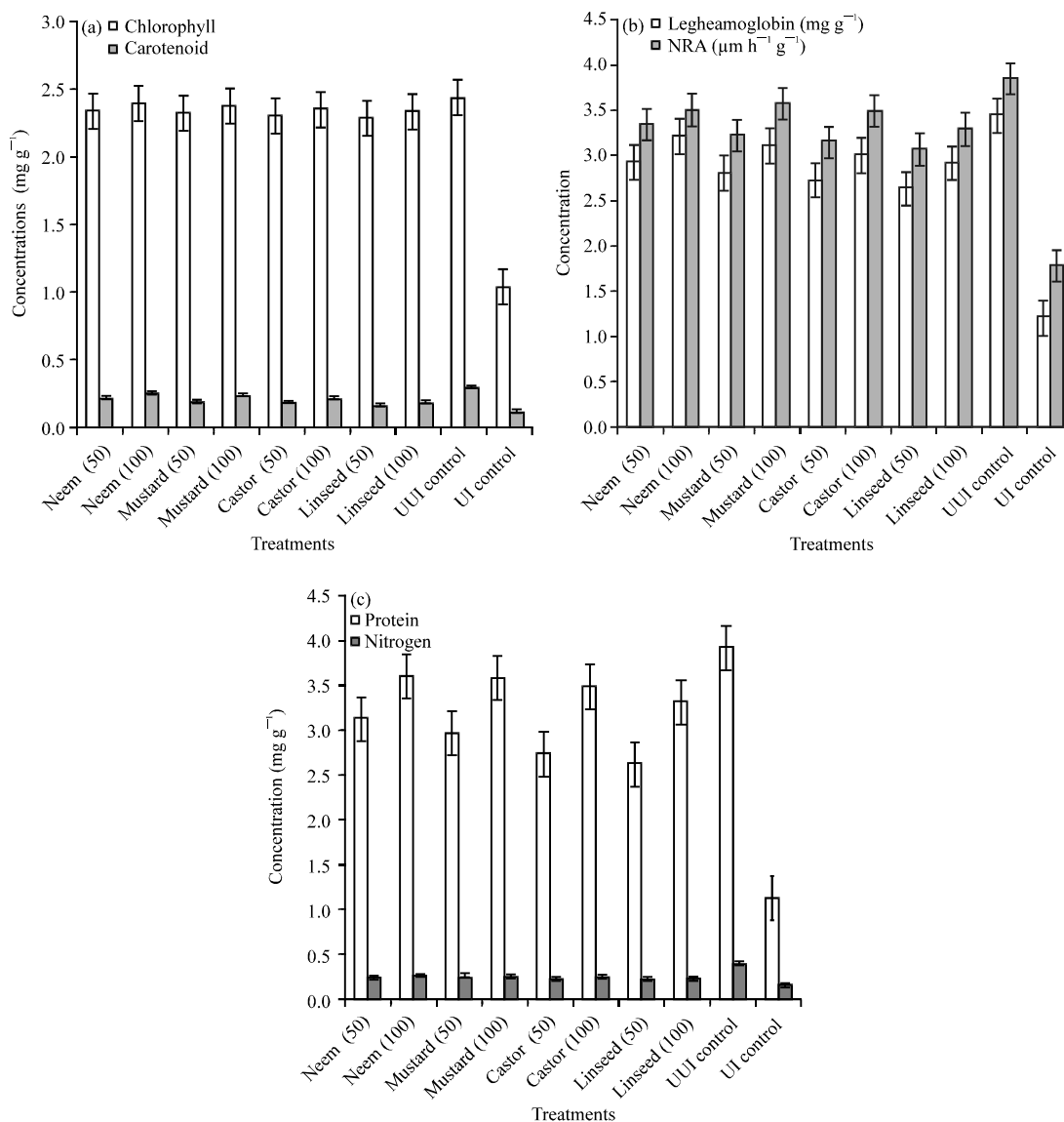


Fig. 3(a-c): Effects of different oil cakes on physiological parameters against *Meloidogyne incognita* attacking *Vigna mungo* L. cv PU-30

to which more carbohydrates and metabolites get accumulated, resulting in more biomass. The present results are in agreement with those of Barani and Anbarani (2004) and Shukla and Tyagi (2009). Organic amendments in soil provide inducing substrate (nitrate) for the enzyme nitrate reductase by stimulating the microbial activity which brings about increased conversion of N to nitrate form (Gunner, 1963) ultimately leading to increased metabolic activity of plant and thereby to increased biomass. Similarly chlorophyll content and nitrate reductase activity was also enhanced by the application of botanicals and oil cakes (Mahmood *et al.*, 2007; Rehman *et al.*, 2012, 2013; Rizvi *et al.*, 2012).

Present findings with respect to the protective action and direct toxicity of the organic amendments proves effective and paves a way which could help in developing some potential and

promising plant based nematicidal products. Above studies may go a long way in checking the development root knot nematode affecting blackgram and other economically important crops through ecologically safe and feasible approach with enhanced plant growth thus leading to sustainable agricultural productivity and food security.

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