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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 catastrophicphytonematodeparasite causing enormous losses to wild as well as cultivated crops worldwide. Despitethe fact that synthetic nematicides have most significant effect, limiting these phytonematodes but theinherent 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 conditions to evaluate the efficacy of different doses of plant oil cakes against root knot nematode infecting 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 @ 100g/pot was found most effective in limiting root gall index and enhancing plant growth parameters followed by Mustard cake @ 100g. Neem cake @ 50g/pot, Castor cake @ 100g/pot and Linseed cake @ 100g/pot were statistically at par. Above studies as surean effective and commercially cheap source of controlling root knot nematode in favour sustainable agriculture and food security.

Key words: Vigna mungo · Meloidogyne incognita · Neem · Mustard · Castor and 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 [1]. Pulses are special in case of maintaining the soil fertility through nitrogen fixation [2]. 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 [3, 4]. Root knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is one of the major constraint to the pulse production among all the other nematodes [5]causing a total yield loss of 17 to almost 23% in black gram [6]. Various strategies have been tried over years to eradicate the problem of root knot nematode. In the present study the effect of various oil cakes like neem, mustard, castor and linseedwere investigated for the management of root knot nematode, M*eloidogyne incognita* affecting the blackgram, *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 187.45m altitude), U.P., India.

Host Plant Culture: Two hundred healthy seeds of blackgram, *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 randomised 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 @ 50g/pot

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and 100g/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 showing root-knot symptoms (galls & 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 [7].

Extraction of Nematode: Collected eggmasses of *Meloidogyne incognita* were placed on a small course sieve (1mm 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 (J_2) of nematode.

Pot Soil 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 (50g) +1500 J2
- T2- Neem oil cake (100g) +1500 J2
- T3- Mustard oil cake (50g) +1500 J2
- T4- Mustard oil cake (100g) +1500 J2
- T5- Castor oil cake (50g) +1500 J2
- T6- Castor oil cake (100g) +1500 J2
- T7- Linseed oil cake (50g) +1500 J2
- T8- Linseed oil cake (100g) +1500 J2
- T9-Untreated inoculated control (1500J2)
- 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 & root) in centimetre, weight (fresh & dry) in grams,number of flowers, pods, number of root nodules and percent pollen fertility[8]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 250g of soil using Baermann Funnel technique.

Leghaemoglobin Content: The Leghaemoglobin content was estimated according to the Sadasivam and Manickam method [9]. The root nodules were excluded from the roots and washed thoroughly.Leghaemoglobin content was determined at 539 nm and 556 nm against blank reagent using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

Chlorophyll and Carotenoid Content: The chlorophyll and Carotenoid content were estimated according to Lichtenthaler and Buschmann Method [10]. Fresh leaf tissue was finely ground utilizing mortar-pestle in 80% acetone. The absorbance of chlorophyll was calculated at 663 nm and 645 nm. For the estimation of carotenoid reading was taken at 470 nm using spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

Nitrate Reductase Activity: The Nitrate Reductase activity was estimated according to Jaworski method [11].The freshly chopped leaves (200mg) were incubated for 2 hours 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. Consequently, the formation of nitrite was analysed calorimetrically at 540nm after azocoupling with sulphanilamide and naphthyl ethylemediamine.Nitrate reductase activity was expressed in $\mu mh^{-1}g^{-1}$

Protein Content: The Protein content was estimated according to Lowry's method [12]. Initially seeds of blackgram were taken and washed thoroughly for removing any extra dust particles and then dried with use of oven. 50 gram of seeds were then weighed for the protein analysis. Seeds were ground finely in 1 ml of 5% trichloroacetic acid. Samples were analysed 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.

Nitrogen Content: The Nitrogen content was estimated according to Novozamsky method [13]. Leaves of blackgram were powdered and then digested. The peroxide digested material was passed through reaction with 2 ml of 2.5N NaOH and 1ml of 10% sodium silicate solution to neutralize acid and removing turbidity respectively. 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 were 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 oil cakes viz., Neem, Mustard, Castor and Linseed on the root-knot development caused by *Meloidogyne incognita* and plant growth characters of blackgram, *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 blackgram.In untreated inoculated control, the blackgram 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&43) in plants treated with 100g dose of Neem cake. The root knot galls and number of nodules of other treatments applied at the same concentrations were (56&40), (60&39) and (62&37) for Mustard, Castor and Linseed oil cakes (Figure-2e,f).

The plant growth (length, fresh & 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 (Figure-1a,b,c).

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

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 blackgram. 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 study that oil cake 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 [14]. Results obtained in the present investigations are in accordance with the results of previous researches.

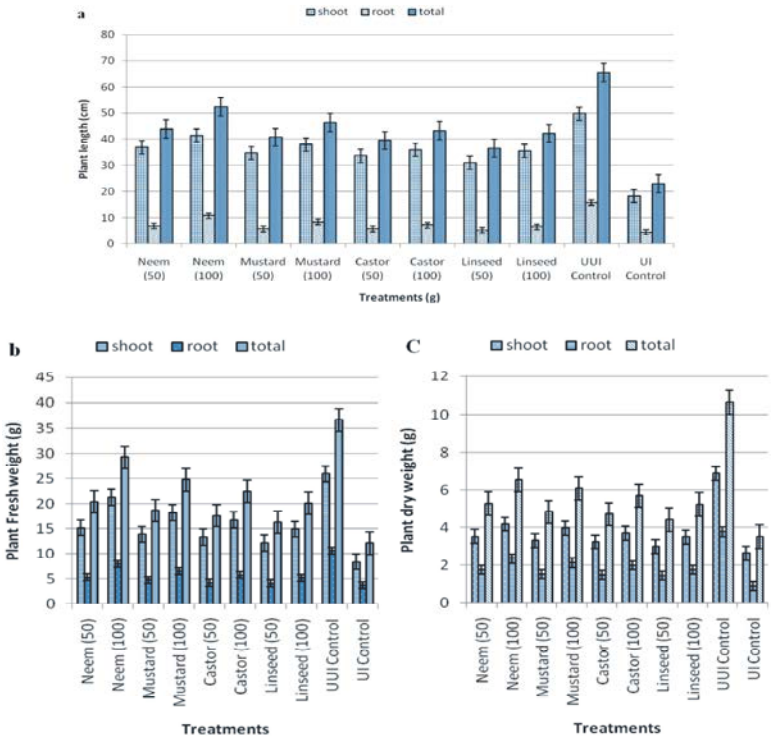

Fig 1: Graphs showing effect of different oil cakes on Plant Growth parameters against *Meloidogyne incognita* attacking *Vigna mungo* L. cv PU-30

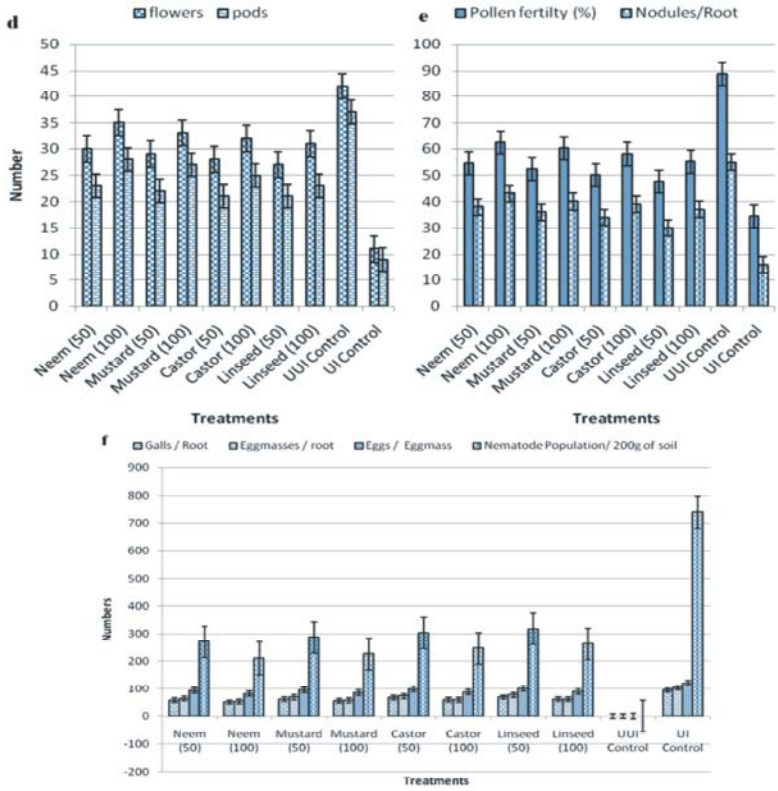

Fig 2: Ggraphs showing the effects of different oil Cakes on Pollen fertility, Root nodules and Pathological parameters against *Meloidogyne incognita* attacking *Vigna mungo* L. cv PU-30

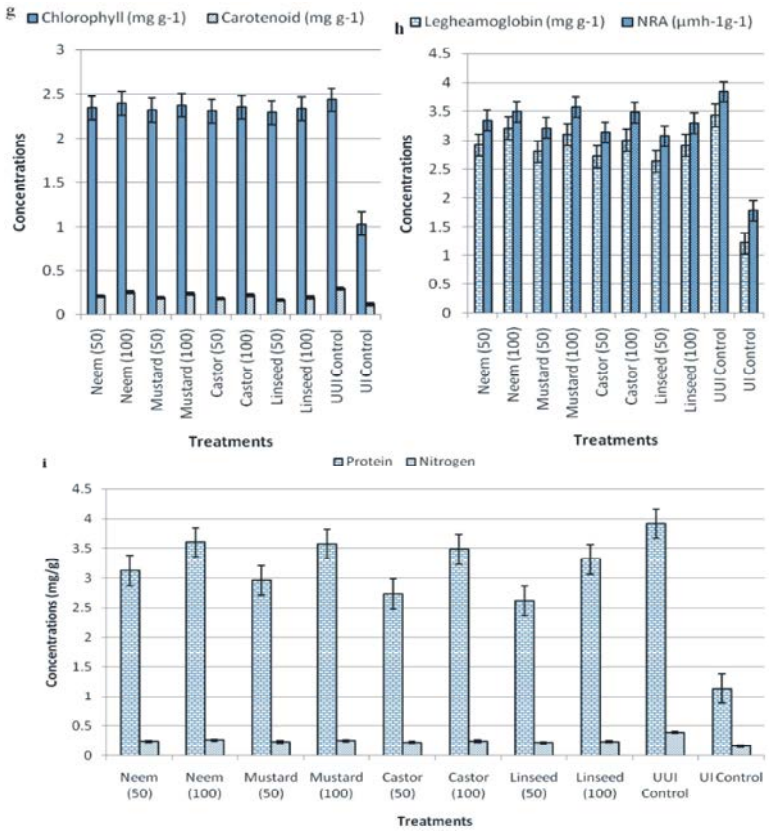

Fig 3: Graphs showing effects of different oil Cakes on Physiological Parameters against *Meloidogyne incognita* attacking *Vigna mungo* L. cv PU-30

Zid [15] 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 othershave given promising results in reducing the nematode population [16-25]. Various other plant products like essential oils obtained from karanj, sunflower, mustard, chalmogra etc. have also been exploited for their growth promoting and antnemic properties [26]. The control of plant parasitic nematodes due to decomposed organic matter as suggested by scientists may be attributed to toxic compounds released during decomposition [27] and the changes in physical and biological properties of soil [28].

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 to which more carbohydrates and metabolites get accumulated, resulting in more biomass. Our results are in agreement with those of Barani and Anbarani and also with Shukla and Tiyagi [29, 30]. 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 [31] 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 [32-35].

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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REFERENCES

1. Yadhu, S., S. Kaur, A.K. Gupta and N. Kaur, 2012. Molecular Evaluation of Genetic Variability in Blackgram Genotypes using RAPD Markers, Indian J.Agric. Biochem, 25: 36-43.
2. Korlovich, B.S. and S.I. Repyev, 1995. Theoretical Basis of Plant Breeding. In: The Gene Bank and Breeding of Grain Legume (Lupine, Vetch, Soya and Bean), Korlovich, B.S. and S.I. Repyev (Eds.). N.I. Vavilov Institute of Plant Industry, St Petersburg, Russia, pp: 438.
3. Ali, S.S. and T.H. Askary, 2001. Taxonomic status of nematodes of pulses crops. In: Nematode taxonomy concepts and recent trends, Ed. M.S. Jairajpuri and P.F. Rahman, I.Q. printers, Hyderabad, India, pp: 197-216.
4. Ali, S.S. and S.B. Sharma, 2002. Distribution and importance of plant parasitic nematodes associated with chickpea in Rajasthan state. Indian J. Pulses Res., 15: 57-65.
5. Bhat, M.Y., M. Fazal and Hisamuddin 2009. Effect of Meloidogyne incognita race -1 on the functioning of rhizobial nodules on black gram, Vigna mungo. Indian J. Nematol., 39: 59-64.
6. Khan, M.R., R.K. Jain, T.M. Ghule and S. Pal, 2014. Economic Importance and Crop Losses.Root knot Nematodes in India-a comprehensive monograph. All India Coordinated Research Project on Plant Parasitic nematodes with Integrated approach for their Control, IARI,New Delhi, pp: 3.
7. Eisenback, J.D., 1985. Detailed morphology and anatomy of second-stage juveniles, males and females of the genus Meloidogyne (root-knot nematodes). In J. N. Sasser and C. C. Carter, Eds. An advanced treatise on Meloidogyne, vol. 1. Raleigh: North Carolina State University Graphics, pp: 47-77.
8. Sass, J.E., 1964. Boatanical Microtechnique. Oxford & IBH Publishing Co., Calcutta, pp: 28.
9. Sadasivam, S. and A. Manickam, 1992. Biochemical methods, New age International Publishers (3rd edition), New Delhi, INDIA.
10. Lichtenthaler, H.K. and C. Buschmann, 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS Spectroscopy. In: R.E. Wrolstad, Editor, Current Protocols in Food analytical chemistry, John wiley& sons (New York), F4: 31-38.
11. Jaworski, E.G., 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophy. Res. Commun, 43: 1274- 127.
12. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin Phenol Reagent. J. Biol. Chem., 193: 265-275.
13. Novozamsky, V.J.G. Houba, R.V. Eck and V.W. Vark, 1983. A Novel Digestion Technique for the multi element plant analysis.Commun. Soil Sci. Plant Anal., 14: 239-248.
14. Akhtar, M. and M.M. Alam, 1993. Control of plant parasitic nematodes by 'Nimin'- a urea coating agent and some plant oils. J. Pl. Dis. Protect, 100: 337-342.
15. Zid, Asmaa, A.M., 2002. Pathological Studies on Parasitic nematodes on some economic plants. Ph. D. Thesis, Fac. Agric., Alexandria Univ., Egypt, pp: 113.
16. Wani, A.H., 2006. Management of root knot nematode, Meloidogyne incognita, on Okra and Mungbean by soil amendment with oil cakes and leaves of different Plants. Nematol. Medit., 34: 83-87.
17. Tejada, M., C. Garcia, J.L. Gonzalez and M.T. Hernandez, 2006. Organic amendment based on Fresh and composted beet vinase: Influence on Physical, Chemical and Biological properties and Wheat yield. Soil Sci. Socty Amrcn Journ., 70: 900-908.
18. Rehman, Parihar, K., B. Ganai, A. Usman and M.A. Siddiqui, 2011. Biomanagement ofRoot Knot Nematode through Non Edible Oil Seed Cakes Infesting Cicer ArietinumL. Int. J. Appl. Biol. Pharmac. Tech., 4(2): 411-414.
19. Sivakumar, M. and K. Gunasekaran, 2011. Management of root knot nematode in Tomato, Chilli and Brinjal by Neem oil Formulation. Journ. Biopest., 4(2): 198-200.
20. Mohan, K., 2011. Comparison of Inorganic and Organic nematicides on the population of soil nematodes in hybrid of Sacchrumspp. Jour. Biopest., 4(2): 201-204.
21. Deepa, S.P., S. Subramanian and S. Ramakrishnan, 2011. Biomanagement of Citrus Nematode Tylenchulussemipenetrans Cobb. On Lemon, Citrus limonia L. J. Biopest., 4(2): 205-207.

22. McGeehan, S., 2012. Impact of Waste materials and Organic amendments on soil properties and vegetative performance. Appl. Environ. Soil Sci., Article ID- 907831, 11 Pages, DOI:10.1155/ 2012/907831.
23. Parihar, K., B. Rehman and M.A. Siddiqui, 2013. Impact of Organic Additives for Sustainable Management of Root-Knot Nematode in Bottle Gourd. Biosci. Internatl., 1(4): 102-105.
24. Ganai, M.A., B. Rehman, K. Parihar, M. Asif and M.A. Siddiqui, 2014. Phytotherapeutic approach for the management of Meloidogyne incognita affecting Abelmoschus esculentus (L.) Moench. Arch. Phytopath. Plt. Protect., 47(15): 1797-1805.
25. Asif, M., K. Parihar, B. Rehman, M.A. Ganai and M.A. Siddiqui, 2014. Bio-efficacy of some leaf extracts on the inhibition of egg hatching and mortality of Meloidogyne incognita. Arch. Phytopath. Plt. Protect, 47(8): 1015-1021.
26. Leela, N.K., A.M. Khan, P.P. Reddy and E.S.J. Nidiery, 1992. Nematicidal Activity of essential oil of Pelargonium graveolens against the root-knot nematode, Meloidogyne incognita. Nematol. Medit., 20: 57-58.
27. Khan, M.W., M.M. Alam, A.M. Khan and S.K. Saxena, 1974. Effect of water soluble fractions of oil-cakes and bitter principles of neem on some fungi and nematodes. Acta Bot Indica, 2: 120-128.
28. Ramesh, P., N.R. Panwar, A.B. Singh and S. Ramana, 2009. Effect of organic nutrient management practices on the production potential, nutrient uptake, soil quality, input-use efficiency and economics of mustard (Brassica juncea). Indian J Agric Sci., 79: 40-44.
29. Barani, P. and A. Anbarani, 2004. Influence of vermicompost on growth parameters of bhindi. South Indian Hortic., 52: 351-354.
30. Shukla, L. and S.P. Tyagi, 2009. Effect of integrated application of organic manures on soil parameters and growth of mungbean (Vigna radiata). Indian J.Agric Sci., 79: 174-177.
31. Gunner, H.B., 1963. Nitrification by Arthrobacterglobiformis. Nature, 197: 1127-1128.
32. Mahmood, I., S.A. Tiyagi and M.F. Azam, 2007. Efficacy of latex bearing plants for the management of plant-parasitic nematodes and soil-inhabiting fungi on chickpea and mungbean. Environ. Biol. Conserv., 12: 23-27.
33. Rehman, B., F. Ahmad, O.O. Babalola, M.A. Ganai, K. Parihar and M.A. Siddiqui, 2013. Usages of Botanical Extracts for the Management of Root-Knot Nematode, Meloidogyne incognita in Chickpea. Jour. Pure Appld. Microbio., 7(3): 2385-2388.
34. Rehman, B., M.A. Ganai, K. Parihar, M.A. Siddiqui and A. Usman, 2012. Management of Root Knot Nematode, Meloidogyne incognita Affecting Chickpea, Cicer arietinum for Sustainable Production. Biosci. Internatl., 1(1): 01- 05.
35. Rizvi, R., I. Mahmood, S.A. Tiyagi and Z. Khan, 2012. Effect of some botanicals for the management of plant-parasitic nematodes and soil-inhabiting fungi infesting chickpea. Turk J Agric For., 36: 710-719.