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Bioefficacy of Spore Suspensions and Mass Culture of *Dactylaria brochopaga* on *Meloidogyne incognita* (Kofoid and White) Chitwood Causing Root-Knot Disease of Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

An experiment was conducted in the laboratory to study the induction of predatory rings and predacity test of isolates of *Dactylaria brochopaga* against second stage juveniles (J2s) of *Meloidogyne incognita*. Another experiment was also conducted in pots to study the effect of *D. brochopaga* (isolate D) on the management of root-knot disease of tomato. Isolate D of *D. brochopaga* showed maximum induction of predatory rings and in turn, trapped maximum number of second stage juveniles (J2s) of *M. incognita* in duel culture in laboratory test. For pot experiments, the promising isolate D among five isolates of *D. brochopaga* was grown on sorghum grains for its mass culture. The bioefficacy of spore suspensions and mass culture of *D. brochopaga* (isolate D) was studied with and without Cow Dung Manure (CDM) on root-knot, population of *M. incognita* and growth of tomato plants. The application of mass culture at 1%, its undiluted and diluted (10 times) spore suspension in soil infested with 1500 juveniles of *M. incognita* per 1000 g before planting of tomato seedlings, increased the plants growth and reduced the number of root-knots by 28.69-55.36%, of females by 25.52-49.41%, of egg masses by 20.53-54.40% and of juveniles by 30.61-54.83% in pot experiments. The bioefficacy of the fungus as nematode antagonist was enhanced when its spore suspensions and mass culture were applied with CDM which reduced the number of root-knots by 49.27-75.36%, of females by 38.87-69.32%, of egg masses by 47.04-77.20% and of juveniles by 51.87-76.09%. Furthermore, spore suspensions of the fungus also enhanced the growth of tomato plants and reduced the population of *M. incognita* significantly.

Key words: *Dactylaria brochopaga*, spore suspension, mass culture, root-knot nematode, cow dung manure

INTRODUCTION

Worldwide plant parasitic nematodes are threat in agricultural crops production and cause great economic loss (Luc *et al.*, 2005; Sasser, 1990; Sikora and Fernandez, 2005; Taylor and Sasser, 1978). Moreover, the nematodes serve as agents predisposing to the development of complex disease

with fungi, bacteria and viruses. Estimated annual yield loss of major crops due to plant parasitic nematodes in the world is about 14% in developing countries and about 12.3% in developed (Agrios, 2005). *Meloidogyne* spp. (cause root-knot diseases) are global threat in crop production (Sasser, 1990) and cause annual losses of about USD \$ 100 billion worldwide (Brand *et al.*, 2010). *Meloidogyne incognita* (Kofoid and White) Chitwood is found in tropical and sub-tropical climates (Khan, 2010) and cause extensive damage to several crops, including vegetables (Sasser, 1989). The yield of tomato, eggplants and okra suffered 46.2, 27.3 and 90.9% loss, respectively due to *M. incognita* infestation at 3-4 juveniles per gram soil under field condition in India (Bhatti, 1994). Nematicides provide immediate and effective management of plant parasitic nematodes but have been too expensive for use in developing countries, where their uses have been limited to few cash crops (Hague and Gowen, 1987). Concern over these chemicals has led to an increased interest in biological control in its widest sense, in order to achieve environmentally safe methods of reducing the nematode damage (Zareen *et al.*, 2003; Mostafa, 2001; Zaki and Maqbool, 1998).

Since long, scientists have been using nematode-trapping fungi for the control of plant parasitic nematodes (Bandyopadhyay *et al.*, 2001; Kumar, 2003, 2007; Kumar and Singh, 2006, 2010; Singh, 2003; Singh *et al.*, 2006, 2007; Stirling, 1991; Stirling *et al.*, 1998). Furthermore, it has been observed that the application of organic manures in combination with nematode-trapping stimulates the bioefficacy of these nematode-trapping fungi and consequently, lower the population of root-knot nematodes. (Bandyopadhyay *et al.*, 2001; Kumar, 2003; Kumar and Singh, 2006, 2010; Kumar, 2007; Singh, 2003; Wachira *et al.*, 2009). However, the fundamental mechanisms behind above facts were not cleared at that time and are not fully explained today.

Dactylaria brochopaga is a nematophagous fungus, which dramatically captures and kills saprophytic and parasitic nematodes *in vivo* and *in vitro* by producing three celled trapping rings. *D. brochopaga* is a common fungus in agricultural soils, decaying plant materials and old decayed root-galls (Saadabi, 2006; Bandyopadhyay, 1998; Kumar *et al.*, 2010, 2003; Kumar and Singh, 2010; Singh, 2003; Singh *et al.*, 2007). The bioefficacy of this fungus in reducing the population of *M. graminicola* was described by Singh *et al.* (2006) and recently, in reducing the population of *M. incognita* by Kumar and Singh (2010). Five isolates of *D. brochopaga* were isolated from different substrates from different parts of India were tested for their predacity against second stage juveniles (J2s) of *M. incognita in vitro*. Furthermore, among five isolates, promising isolate D of the fungus was mass cultured on sorghum (*Sorghum bicolor*) grains and tested for the control of root-knot disease of tomato plant (*Lycopersicon esculentum* Mill.) in pot experiments. The observations of the same are described in the present study.

MATERIALS AND METHODS

Isolation of isolates of *Dactylaria brochopaga*: Five isolates of *Dactylaria brochopaga* were isolated from different agricultural soils and decaying substrates from different parts of India by the method described by Duddington (1955) with slight modification (Bandyopadhyay and Singh, 2000). All the five isolates of *D. brochopaga* were purified by single spore isolation, method given by Singh *et al.* (2004) and culture of each isolate was maintained at 29±1°C on Corn Meal Agar (CMA) medium by regular subculturing at an interval of 15 days.

Collection of second stage juveniles (J2s) of *Meloidogyne incognita*: Population of second juveniles (J2s) of *Meloidogyne incognita* were obtained from tomato plant pot cultures regularly maintained in green house of the Department of Mycology and Plant Pathology, Institute of

Agricultural Sciences, Banaras Hindu University, Varanasi. Sufficient egg masses of the nematode were collected from root-knot of the tomato plants and put in cavity block for hatching at room temperature (25-30°C) for 2 days in order to get required population of J2s.

Predacity test: In order to find out the response of five isolates of *D. brochopaga* to second stage juveniles of *M. incognita*, observations were taken on the initiation of trapping rings and their numbers in duel culture. Numbers of trapping rings per microscopic field (1.6) were noted daily for 6 days under a research microscope at 100X magnification. Several observations on number of trapping rings were made from centre, middle and periphery of the Petri dishes after nematode inoculation and the average number were calculated. Observe on trapping rings formed on the surface and deep into the medium were also made. Similarly data on captured nematodes were recorded at 24 h interval for 6 days and percentage of captured nematodes was calculated. For each isolate of the fungus and nematode interaction three Petri dishes were used as replicates.

Predacity of five isolates of *D. brochopaga* against the second stage juveniles of *M. incognita* in duel culture was tested by the methods described by De Belder and Jansen (1994). Three replications were maintained for each treatment and the experiments were repeated thrice.

Mass culture: To prepare the mass culture of *D. brochopaga* (Isolate D), 20 g sorghum grains were taken separately in 250 mL conical flask and moisten with 35 mL of water. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 min. A 10 mm fungal disc was cut from the periphery of the 10 days old culture of *D. brochopaga* with a sterilized cork borer and inoculated in the centre of a substrate contained in flask with the help of sterilized inoculation needle. One disc was inoculated into each 250 mL conical flask. The inoculated flasks were incubated at room temperature (25-30°C) for 25 to 30 days.

Bioefficacy of spore suspensions and mass culture of *Dactylaria brochopaga* on root-knot nematode of tomato plant: The experiment on effects of spore suspensions and mass culture of *D. brochopaga* against root-knot of tomato plant was conducted in wire net house of the Department of Mycology and Plant Pathology, Institute of Agricultural sciences, BHU, Varanasi in 15th June, 2005 to October, 2005. Root-knot infested sick soil having approximately 1500 second stage juveniles of *M. incognita* per 1000 g soil was used for these experiments. Root-knot infested sick soil was thoroughly mixed by hand to make the uniform population of nematodes before amendments. Mass culture at 1%, its spore suspension (undiluted) and diluted spore suspension (10 times) was amended with or without 5% well decomposed Cow Dung Manure (CDM). Sick soil without mass culture and with CDM served as control. Mass culture, its spore suspension, ten times diluted spore suspension with and without CDM was uniformly mixed in sick soil before filling the pots. Root-knot infested soil and amended sick soil were filled in pots (1000 g pot⁻¹). Thirty days old seedlings of tomato plant free from root-knot infection were transplanted in pots. Each pot had a single seedling. For each treatment five pots were used as replicates. The pots were watered regularly. Observation on the plant height, root length, fresh weight of root and shoot, number of galls per plant, number of females, egg masses and second stage juveniles of *M. incognita* per root system were taken after 5 weeks of planting. The number of females and egg masses per root system, second stage juveniles were estimated by the methods described by Kumar and Singh (2006). The data was statistically analyzed using analysis of variance (ANOVA). Treatment means were compared following Duncan's multiple range test (Gomez and Gomez, 1984).

RESULTS

Predacity of five isolates of *Dactylaria brochopaga* against second stage juveniles (J2s) of *Meloidogyne incognita* in duel culture was studied in the laboratory are presented in (Fig. 1, 2). The three celled trapping rings were not recorded in 24 h after nematodes inoculation. However, after 48 h ring induction was formed in all the isolates. This clearly indicated that initiation of ring induction occurred between 24 to 48 h after nematodes inoculation. The number of rings per unit area increased with passage of time. Maximum number of rings was formed in isolate D followed by isolate C which was significantly higher than in isolates E and B (Fig. 1). These results indicated that sensitivity of the isolates of *D. brochopaga* in ring induction differed significantly. The capturing of 2nd stage juveniles (J2s) of *M. incognita* was found on day 2 in all isolates although capturing was few in number. The diameter of J2s being narrower they easily captured by the trapping rings, whose internal diameter were suitable for the entry of the nematode. The capturing of J2s of *M. incognita* was frequently at head or tail region. Occasionally, trapping rings were also seen in the middle of the nematode body. Minimum percentage of capturing was recorded in isolate E where as maximum percentage trapping was recorded in isolate D followed by isolate C (Fig. 2).

The observations on the effect of spore suspensions and mass culture *D. brochopaga* (Isolate D) with and without Cow Dung Manure (CDM) on growth parameters of tomato plant, number of root-knots and population of *Meloidogyne incognita* (Kofoid and White) Chitwood are presented in Table 1. From the data it is evident that all the growth parameters of tomato plant were significantly enhanced when seedlings were raised in soil infested with 1500 juveniles of

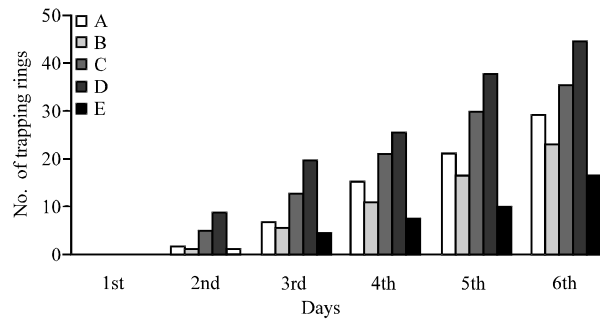


Fig. 1: Average number of trapping rings of five isolates of *Dactylaria brochopaga* induced in response to *Meloidogyne incognita* in corn meal ager (1:10) medium

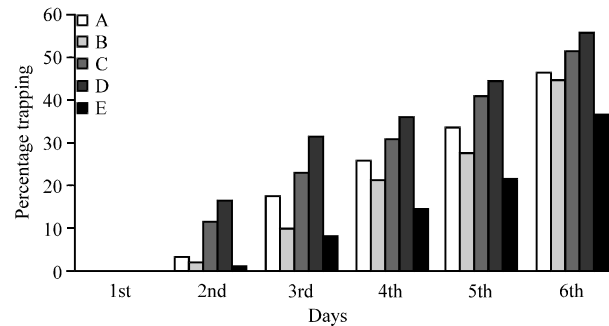


Fig. 2: Percentage trapping of second stage juveniles of *Meloidogyne incognita* by five isolates of *Dactylaria brochopaga* in corn agar (1:10) agar medium

Table 1: Effect of spore suspensions and mass culture of *Dactylaria brochopaga* (Isolate D) on growth of tomato plant, number of root-knots and population of *Meloidogyne incognita*

Parameter	Treatment							
	Control (10 ¹)	CDM (ss)	Db (10 ¹)	Db (ss)	Db (ms)	Db(10 ¹) +CDM	Db (ss) +CDM	Db (ms) +CDM
Plant height (cm)	18.5a	20.0a	28.5b	31.5b	37.5c	45.0d	49.5e	54.5f
Root length (cm)	19.0a	21.5b	22.0b	23.0b	25.5c	26.5c	28.5d	32.5e
Fresh weight of Shoot/plant (mg)	3985.0a	4025.5 a	10400.5b	14932.0c	16819.5d	15037.5c	25041.0e	29495.5f
Fresh weight of root /Plant (mg)	1623.5a	1708.5b	2839.0c	3241.5d	3782.0e	3189.0d	4470.0f	5781.5g
Knots/Plant	172.5a	168.0a	123.0b	89.5c	77.0d	87.5c	49.5e	42.5e
No. of females/plant	213.5a	206.5a	152.0b	134.5c	108.0d	130.5c	78.0e	65.5e
No. of eggs/plant	618.5a	576.0a	491.5b	348.5c	282.0d	327.5c	188.5e	141.0 f
No. of juveniles(J2s)	21008.0a	19815.5a	14576.5b	11180.0c	9489.0c	10110.5c	6813.5d	5021.5d

Data with different letters show significant difference of row data among at $p \leq 0.05$ according to Duncan's Multiple Range Test (DMRT). J2s = Juveniles; CDM = Cow dung manure; Db = *Dactylaria brochopaga*; mc = Mass culture; ss = Spore suspension; 10¹ = Ten time dilution

M. incognita per 1000 g which was amended with mass culture, spore suspensions of the fungus. Moreover, even after the dilution of the spore suspension of mass culture of *D. brochopaga* enhanced tomato plant growth significantly than control. The application of diluted, undiluted spore suspensions and mass culture without cow dung manure enhanced the plant height (33.33, 41.26 and 50.66%), root length (13.63, 21.05 and 25.49%), fresh weight of shoot (61.68, 73.71 and 76.30%) and root (42.81, 49.91 and 56.83%) of tomato whereas, diluted, undiluted spore suspensions and mass culture with cow dung manure reduced plant height (58.88, 62.62 and 66.05%), root length (33.33, 39.47 and 41.53%), fresh weight of shoot (73.49, 84.08 and 86.48%) and root (49.09, 63.68 and 71.79%) of tomato plants. Moreover, the application of diluted, undiluted spore suspensions and mass culture without CDM reduced the number of root-knots of tomato plants by 28.69, 48.11 and 55.36%, respectively, whereas, diluted, undiluted spore suspensions and mass culture with CDM reduced root-knots by 49.27, 71.30 and 75.36%, respectively. Similarly, the application of diluted, undiluted spore suspensions and mass culture reduced the number of females by 25.52, 37.00 and 49.41%, of egg masses by 20.53, 43.65 and 54.40% and of juveniles by 30.61, 46.78 and 54.83%, respectively. The performance of isolate D of *D. brochopaga* as nematode antagonist was enhanced when its diluted, undiluted spore suspensions and mass culture were applied with CDM, reduced the number females by 38.87, 63.46 and 69.32%, of egg masses by 47.04, 69.52 and 77.20% and of juveniles' by 51.87, 67.56 and 76.09%, respectively.

DISCUSSION

The differential predacity of five isolates of *D. brochopaga* to second stage juveniles of *M. incognita* may be referred to virulence of isolates and number of predatory rings formed in response to the nematode. The size of nematodes is also one of the major factors of the variation in the predacity of predacious fungi (Kumar and Singh, 2006). Isolate D captured and killed highest number of J2s of *M. incognita* seems to be related to maximum induction of rings in response to the nematode. The data on bioefficacy of spore suspensions and mass culture of *D. brochopaga* with and without CDM enhanced growth parameters of tomato plants and reduced the number of root-

knots and nematode population. The enhanced growth of tomato plants and reduced root-knots and population of *M. incognita* could be attributed to spores and fungal mycelia of *D. brochopaga* (Kumar and Singh, 2010). *M. incognita* is obviously a major constraint for plant growth in soil when harbours its pathogenic level and a serious agent predisposing to the development of the complex diseases with fungi, bacteria and viruses (Agrios, 2005; Khan, 2010; Sasser, 1990). Application of *D. brochopaga* with CDM saves plant by capturing and killing of (J2s) of *M. incognita* and in turn, enhances the growth parameters due to reduced infection. The well development roots duly protected at initial stage by capturing and killing of nematodes by this fungus supports the plant growth as well as reduction in *M. incognita* population. The findings are more or less supported by Stirling *et al.* (1998) who reported that formulation of *A. dactyloides* caused 57-96% reduction in number of root-knots and 75-80% in number of nematode per plant in tomato in pot and field experiments, respectively. The other possibilities of increased effect of *D. brochopaga* may be due to increase in population of saprophytic nematodes which after their capturing and killing possibly increase the population of the fungus. It has been noted that the plant growth increased conspicuously with better root system in pots treated with the fungus and CDM. Similarly, Kumar and Singh (2006) reported that application of *A. dactyloides* reduced the number of root-galls of tomato by 66% and increased the plant growth in pot experiments. The effect of *A. dactyloides* was enhanced when its mass culture was applied in combination with CDM. Recently, Kumar and Singh (2010) also observed that the use of *D. brochopaga* reduced the number of root-galls of eggplants and the population of *M. incognita*, which support the present work. The performance of the fungus as bio-agent was enhanced when its mass culture and spore suspensions were applied with CDM, which reduced the number of root-galls, by 48.5-74.8%, of females by 43.5-64.8%, of egg masses by 38.7-68.8% and of juveniles by 48.1-78.6%. Moreover, diluted spore suspension of the fungus also increased the growth of eggplants and reduced the population of *M. incognita* significantly.

The observations have shown that the spore suspensions and mass culture of *Dactylaria brochopaga* enhanced the growth parameters of tomato plant, reduced the number of knots and population of root-knot nematode. It has been also recorded that the bioefficacy of *D. brochopaga* was enhanced when its spore suspensions and mass culture were applied in combination with CDM. Obviously, this practice should be accepted as a good management practice for plant parasitic nematodes if we have to avoid the use of nematicides. Of course, the results would be encouraging only where the nematode population is at pathogenic level in the soil.

REFERENCES

- Agrios, G.N., 2005. Plant Diseases Caused by Nematodes. In: Plant Pathology, Agrios G.N. (Ed.). Elsevier Academic Press Ltd., London, pp: 608.
- Bandyopadhyay, P., 1998. Studies on predacious fungi with special reference to biological of plant parasitic nematodes. Ph.D. Thesis, Banaras Hindu University, Vranasi, India.
- Bandyopadhyay, P. and K.P. Singh, 2000. Effect of temperature, pH and nematode starvation of the induction of rings of *Dactylaria brochopaga* Drechsler. *J. Mycol. Plant Pathol.*, 30: 100-102.
- Bandyopadhyay, P., D. Kumar, V.K. Singh and K.P. Singh, 2001. Ecofriendly management of root knot nematode of tomato by *Arthrobotrys oligospora* and *Dactylaria brochopaga*. *Indian J. Nematol.*, 31: 153-156.
- Bhatti, D.S., 1994. Management of Phytonematodes: An Introduction. In: Nematode Pest Management in Crops, Bhatti, D.S. and R.K. Wali (Eds.). CBS Publication and Distributors, New Delhi, India, pp: 1-6.

- Brand, D., C.R. Soccol, A. Sabu and S. Roussos, 2010. Production of fungal biological control agent through solid state fermentation: A case study on *Paecilomyces lilacinus* against root-knot nematodes. *Applied Mycol. Int.*, 22: 31-48.
- De Belder, E. and E. Jansen, 1994. Capture of plant parasitic nematodes by an adhesive hyphae forming isolates of *Arthrobotrys dactyloides* and some other nematophagous fungi. *Nematologica*, 40: 423-437.
- Duddington, C.L., 1955. Notes on the technique of handling of predacious fungi. *Trans. Br. Mycol. Soc.*, 38: 97-103.
- Gomez, K.A. and A.A. Gomez, 1984. *Statistical Procedures for Agriculture Research*. 2nd Edn., John Wiley and Sons, New York, pp: 680.
- Hague, N.M.H. and S.R. Gowen, 1987. Chemical Control of Nematodes. In: *Principles and Practices of Nematode Control in Crops*, Brown, R.H. and B.R. Kerry (Eds.). Academic Press, USA., pp: 131-178.
- Khan, M.R., 2010. Current option for managing nematodes pests of crops in India. <http://www.handyman-source.com/12453/current-options-for-managing-nematodes-pest-of-crops-in-india/>.
- Kumar, D., 2003. Studies on and predacity and biocontrol potential of *Arthrobotrys dactyloides*. Ph.D. Thesis, Banaras Hindu University, Varanasi, India.
- Kumar, D. and K.P. Singh, 2006. Assessment of predacity and efficacy of *Arthrobotrys dactyloides* for biological control of root-knot disease of tomato. *J. Phytopathol.*, 154: 1-5.
- Kumar, N., 2007. Studies on and predacity and biocontrol potential of *Dactylaria brochopaga*. Ph.D. Thesis, Banaras Hindu University, Varanasi, India.
- Kumar, N. and K.P. Singh, 2010. Effect of mass culture and spore suspension of *Dactylaria brochopaga* on growth of eggplant and population of the root-knot nematode, *Meloidogyne incognita*. *Indian J. Sci. Res.*, 2: 9-14.
- Kumar, N., R.K. Singh and K.P. Singh, 2010. Occurrence and colonization of nematophagous fungi in different substrates, agricultural soils and root-knots. *Arch. Phytopathol. Plant Prot.*, (In Press).
- Luc, M., R.A. Sikora and J. Bridge, 2005. *Plant Parasitic Nematodes in Tropic and Subtropical Agriculture*. 2nd Edn., CAB International, Wallingford, Oxford, UK, pp: 871.
- Mostafa, F.A.M., 2001. Integrated control of root-knot nematodes, *Meloidogyne* spp. Infecting sunflower and tomato. *Pak. J. Biol. Sci.*, 4: 44-46.
- Saadabi, A.M.A., 2006. Survey of predacious fungi in some Saudi Arabian soils. *Res. J. Microbiol.*, 1: 285-288.
- Sasser, J.N., 1989. *Plant Parasitic Nematodes, the Farmer's Hidden Enemy*. Coop Publication, North Carolina State University, Reliegh, NC, USA., pp: 115.
- Sasser, J.N., 1990. Economic Importance of *Meloidogyne* in Tropical Countries. In: *Root-Knot News (Meloidogyne sp.) Systematics, Biology and Control*, Lamberti, F. and E. Tayler (Eds.). Academic Press, New York, pp: 477.
- Sikora, R.A. and E. Fernandez, 2005. Nematode Parasites of Vegetables. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, Luc, M., R.A. Sikora and J. Bridge (Eds.). CAB International, New York, pp: 319-392.
- Singh, K.P., D. Kumar and P. Bandyopadhyay, 2004. A new technique for single spore isolation of two predacious fungi forming trapping rings. *Mycobiology*, 32: 197-198.

- Singh, R.K., 2003. Studies on predacity and biocontrol potential of *Arthrobotrys oligospora*. Ph.D. Thesis. Banaras Hindu University, Varanasi, India.
- Singh, K.P., R.K. Jaiswal, N. Kumar and D. Kumar, 2007. Nematophagous fungi associated with root galls of rice caused by *Meloidogyne graminicola* and its control by *Arthrobotrys dactyloides* and *Dactylaria brochopaga*. *J. Phytopathol.*, 155: 193-197.
- Stirling, G.R., 1991. Biological Control of Plant Parasitic Nematodes. CAB International, Wallingford, UK., pp: 282.
- Stirling, G.R., L.J. Smith, K.A. Licastron and L.M. Eden, 1998. Control of root-knot nematodes with formulation of the nematode-trapping fungus *Arthrobotrys dactyloides*. *Biol. Control*, 11: 224-230.
- Taylor, A.L. and J.N. Sasser, 1978. Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne* species). North Carolina State University, Raleigh, NC., USA., pp: 111.
- Wachira, P.M., J.W. Kimenju, S.A. Okoth and R.K. Mibey, 2009. Stimulation of nematode-destroying fungi by organic amendments applied in management of plant parasitic nematode. *Asian J. Plant Sci.*, 8: 153-159.
- Zaki, M.J. and M.A. Maqbool, 1998. Use of biocontrol fungi with carbofuran in the control of root knot nematodes in Okra. *Pak. J. Biol. Sci.*, 1: 27-28.
- Zareen, A., M.J. Zaki and N. Javed, 2003. Nematicidal activity of ginger and its effect on the efficacy of *Pasteuria penetrans* for the control of root knot nematodes on tomato. *Asian J. Plant Sci.*, 2: 858-860.