Journal of Biotechnology
and Crop Science
4(5): 56-60, 2015

Studies on some nematophagous fungi for management of root knot diseases of tomato

Ramesh Kumar Kushwaha, BK Sarma

Received: 12 August 2015 Revised Accepted: 13 September 2015

ABSTRACT

Meloidogyne species cause root knot in Barley, Brinjal, Wheat, Rice, Banana *etc.* which can be managed by chemical and non chemical methods. The use of pesticides including nematicides has become very popular for control of pests. Isolation of nematophagous fungi for isolation of nematophagous fungi from different substrates viz., leaf litter, composts, farm yard manure (FYM) and decaying root galls, water agar, maize meal agar (MMA) and rabbit dung agar media were used. 100gm of soil sample were collected in separate polythene bag from top profile of soil from different location. The growths of plant were considerably reduced after inoculation of mass culture of *A. oligospora* even in combination with FYM. However, root wt of the plants treated with mass culture of *A. oligospora* increased maximum being in *A. oligospora* + FYM. It was interesting to know that in spite of suppressive effect on plant growth the root galls were significantly reduced in plant with *A. oligospora* + FYM. From the observation it is evident that the *A. oligospora* control root knot nematode.

Key Words: *Meloidogyne species, Mass Culture, Nematodes isolation from soils, Root knot disease*

INTRODUCTION

Plant parasitic nematodes are widely distributed in agricultural soil and are responsible for mild to heavy loss in variety of crops. It affects the root, shoot, bud, leaves. *Meloidogyne* species cause root knot in Barley, Brinjal, Wheat, Rice, Banana *etc.* which can be managed by chemical and non chemical methods. The use of pesticides including nematicides has become very popular for control of pests. Although the nematicides are quite effective in reducing the nematode population, their adverse effect such as environmental pollution, pest resistant, destruction of beneficial fungi, (Gray 1983a), loss of soil biodiversity and human health are well known. Under the prevailing situation, there is need to develop alternative control measures *i.e.* non-chemical method which includes biological control.

Ramesh Kumar Kushwaha (✉)
Central Institute of Medicinal Aromatic Plants (CIMAP) Lucknow, UP
Email: rkk.cimap@gmail.com

BK Sharma
Mycology and Plant Pathology, IAS, BHU, Varanasi-221005

Biological control (Mankau 1980) of plant parasitic nematode by predacious fungi is the most eco-friendly and natural approach provided it is effective in the field. The predacious fungi are widely distributed in agricultural and non-agricultural soils. It may occur as saprophyte in the soil or decaying plant material and may pray on saprophyte as well as plant parasitic nematodes. The capturing efficiency of predacious fungi may be influenced by the environmental conditions and nature of the soil.

The studies on predacity of *Arthrobotrys oligospora*, *A. robusta*, *A. superba*, *A. musiformis*, *A. conoides A. dactyloides* and *Dactylaria brochopaga* and *Monacosporium cianopaga* have been carried out either on saprophytic nematodes or gastro-intestinal animal parasitic nematode *in vitro*. Among the plant parasitic nematode Meloidogyne species are of global importance, therefore there is need to isolate of these predacious fungi for predacity against the economically important species. Many Scientists have worked on nematophagous fungi e.g. Dayal

1979, Woronin (1870), Drechsler (1937), Duddington (1954), Das Gupta *et al* (1964), Sachchidananda (1965), Prasad (1985), Singh (2005), Vaish and Singh (2002), Singh and Bandyopadhyay (2001). They are some of the scientists who have worked on the neamtophagous fungi.

MATERIALS AND METHODS

Isolation of nematophagous fungi for isolation of nematophagous fungi from different substrates *viz.*, leaf litter, composts, farm yard manure (FYM) and decaying root galls, water agar, maize meal agar (MMA) and rabbit dung agar media were used. 100gm of soil sample were collected in separate polythene bag from top profile of soil from different location e.g. botanical garden BHU, Daltonganj (Jharkhand), Barkachha, BHU (Mirzapur), Agriculture field, BHU. Each soil sample was thoroughly mixed before use of soil plating. Sterilized maize meal agar medium cooled near to solidification, was poured into Petri dishes to cover nearly 2/3rd area of plate. 10-15 minutes after pouring of the MMA medium melted but cooled Rabbit dung agar was poured into Petri plate to cover 2/3rd area of the plate. One gm soil of each sample was scattered over medium of a Petri dish. For each soil sample, five Petri dishes were used as replicates with adding nematodes culture. These Petri dishes were incubated at room temperature (25-30°C) in dark.

The incubated Petri dishes were routinely observed after 4 days of inoculation for nematophagous fungi under stereoscopic light microscope as well as research microscope. The fungi appearing in the Petri dishes captured the living nematode by forming the different type of capturing devices e.g. sticky hyphal branches, hyphal net, constricting ring, sticky knob, etc. were recorded. The frequency of nematophagous fungi *Arthrobotrys oligospora, A. dactyloides, A. musiformis, A. robusta, A. superba* and *D. brochopaga* and *monacosporium* etc was recorded. Isolates of different nematophagous fungi were cultured, Purified and maintained separately on maize meal agar medium. Purification, single spore culture and maintenance of culture. The nematophagous fungi producing spore were isolated by picking spores of individual fungus with the help of a sterilized needle and inoculating the same on maize meal agar medium. For identification of different nematophagous fungi, size of hyphae, conidia and conidiophores were measured and compared with relevant literature (Drechsler 1937 and Cooke and Godfrey 1964).

Mass culture on some substrate: Substrates such as barley (*Hordeum vulgare*) and sorghum (*Sorghum bicolor*) were taken separately in 250 ml conical flasks for studying the growth of *A. oligospora*, *A. dactyloides* and *D. brochopaga* for its mass culture. The substrate and water were taken as given below:

Sorghum grain 20g + 35 ml water, Barley grain 20g + 35 ml water

The substrate was taken into 250 ml conical flasks and moistened with water as mentioned above. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. In another set, the boiled grain of sorghum were mixed with CaCO3 @ 10g per kg grain and CaCO3 + CaSo4 each @g 10 per kg grain and taken into 250 ml flasks. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. 10 mm fungal disc was cut from the periphery of the 10 day old culture of D. Brochopaga by a sterilized cork borer and inoculated in the center of a substrate contained in a flask with the help of sterilized inoculation needle. One disc was inoculated into each 250 ml flask. The inoculated flasks were incubated at room temperature (25-30˚C). Visual ratings were made to asses the growth of D. brochopaga after 20 days of inoculation.

Performance of mass culture of D. brochopaga against root knot of rice: For evaluation of efficacy of mass culture of *D. brochopaga*, nematode infested soil containing juveniles of *M. graminicola* was used. The experiment was conducted in a glass house. The infested soil was thoroughly mixed to homogenize

the nematode inoculum. Mass culture @ 1% i.e. 10 gm mass culture of *D. brochopaga*, containing 106 colony forming unit (CFU) per kg soil, were amended with or without 5% well decomposed FYM and thoroughly mixed to homogenize the soil separately under various treatments. Nematodes infested soil with fungal inoculum served as a control. Pots were then filled @ 1kg soil under various treatments. Thirty sprouted rice seeds (variety MUT-7029) were sown in each pot on the day. In second experiment, the rice seeds (unsprouted) were used with similar treatments as described above.

Performance of mass culture of A. dactyloides and A. Oligospora against root knot of tomato: For evaluation of efficacy of mass culture of *A. dactyloides* and *A. oligospora*, nematode infested soil containing juveniles of *M. inconita* was used. The experiment was conducted in a glass house. The infested soil was thoroughly mixed to homogenize the nematode inoculum. Mass culture@ 1% i.e. 10gm mass culture of *A. dactyloides*, containing 106 colony forming unit (CFU) per kg soil, were amended with or without 5% well decomposed CDM and thoroughly mixed to homogenize the soil separately under various treatments. Nematodes infested soil with fungal inoculum served as a control. Pots were then filled @ 1kg soil under various treatments. For each treatment five replication were used. Observation on number of root galls, shoot and root length, fresh weight of shoot and root were recorded 40 days after sowing. Also the population of eggs, juveniles and females were recorded. For determination of final population of eggs, j2 and females, root were stained in boiling 0.1% (w/v) acid fuschin in lactic acid, glycerol and distilled water (1:1:1) by the method described by Bridge *et al* (1981). Stained root were macerated in distilled water and number of eggs, J2S and females were counted.

RESULTS AND DISCUSSION

Mass culture of A. oligospora, A. dactyloides and D. brochopaga: For mass culture of *A. oligospora* and *D. brochopaga* sorghum grain was used which supported excellent growth of both the fungi. For the mass culture of *A. dactyloides* barley grains were used, which also supported excellent growth of the fungus. The mass culture was ready in 20 days and the grains were fully colonized by these fungi.

Performance of mass culture of A. oligospora on root knot and growth of tomato: Application of mass culture of *A. oligospora* in pot showed suppressive effect on the growth of the tomato plant in the initial stage. The growths of plant were considerably reduced after inoculation of mass culture of *A. oligospora* even in combination with FYM. However, root wt of the plants treated with mass culture of *A. oligospora* increased maximum being in *A. oligospora* + FYM. It was interesting to know that in spite of suppressive effect on plant growth the root galls were significantly reduced in plant with *A. oligospora* + FYM. From the observation it is evident that the *A. oligospora* control root knot nematode.

Performance of mass culture of A. dactyloides on root knot disease in tomato plant: Observation on growth parameter of 40 days old plant under various treatment show significant difference in plant height, fresh shoot wt and root wt of tomato plant. Maximum plant height recorded for the plant treated with *A. dactyloides* + cow dung manure (CDM) followed by CDM alone. Although plant treated with *A. dactyloides* alone were slightly taller than those in control, however difference was not significant. The application of mass culture of *A. dactyloides* alone and in combination with CDM significantly reduced the number of root knot, however *A. dactyloides* + CDM was more effective in controlling the nematode.

Effect of mass culture of D. brochopaga on root knot disease of rice caused by M. graminicola: The result on growth parameter of shoot length, root length, shoot & root wt and number of root knot under the influence of various treatments, clearly indicated that the number of root knot was significantly lower after treating the root knot

infested soil with mass culture of *D. brochopaga* in combination with FYM.

The application of FYM did not affect the number of root knot; however growth parameter of rice plant were increased. Mass culture of *D. brochopaga* in combination with FYM not only control nematode but also increases growth parameter of rice plant significantly more than the FYM alone. The results from the current experiments revealed that there is large range of diversity of occurrence of nematophagous fungi in different location.

Application of mass culture of A. oligospora in tomato plants decreased root knot disease. The reduction in root knot and nematode population by FYM + mass culture of A. oligospora may only be attributed to increased predacious activity of the fungus in presence of FYM. Ali (1990) evaluated the effect of A. oligospora and organic amendment by pigeon dropping in green house experiment for biological control of M. incognita in tomato. The colonization of A. oligospora in soil amended with FYM is conspicuously increased, which certainly might have increased predacity of the fungus and thereby significantly reduced the number of root knots and nematode population.

Similar results on control of root knot diseases of plants have been made by several workers (Bandyopadhyay 1998, Singh 2003, Singh et al 2007). Root-knot infested soil when thoroughly mixed with mass culture of A. dactyloides in soil with 5% cow dung manure (CDM) significantly reduced root knots, when observed after 40 days in tomato. A. dactyloides in combination with CDM, suggested that role of CDM is for the multiplication of the fungus and sustenance of its population in the soil.

The plant growth of tomato was also increased conspicuously with better root system in pots treated with A. dactyloides + CDM again confirming the role of organic matter in the form of CDM for increase in population of A. dactyloides leaving no scope for development of the root-knot pathogen due to higher parasitization by the nematophagous fungus. Prasad *et al*(1985) observed that inoculation of substrate with the *A. oligospora* and *A. dactyloides* reduced the population of M. incognita in tomato plant. Kumar and Singh 2006, Singh *et al* 2007 also reported similar observation on control of root knot of tomato and rice.

REFERENCES

Gray NF (1983a) Ecology of nematophagous fungi: distribution and habitat. Ann Appl Biol 102: 501-509.

Mankau R (1980) Bio control: Fungi as nematodes control agent. Journal of Nematology 12(4): 244-252.

Dayal R, Srivastava SS (1979) Three catenulate conidial Phycomycetes destructive to soil amoebae. Indian Phytopathology 32: 409-412.

Woronin MS (1970) Sphaeria lemaneae, Sordaria coprophila, Arthrobotrys oligospora. Beitr Z Morphol U Physiol D Pilze III Abhandal. Senkenb Nat Ges 7: 325.

Drechsler C (1937) Some hyphomycetes that prey on free living terrricolous nematodes. Mycologia 29: 447-552.

Duddington CL (1954) Nematode destroying fungi in Agricultural soils. Nature 173 (4402): 1-79.

Das Gupta SN, Shome U, Shome SK (1964) Preliminary report of predacious fungi in India. Curr Sci 33: 380-381

Sachchidananda J, Swarup G (1965) Nematophagous fungi in Delhi soil. Indian Phytopath 19: 279-285.

Prasad G, Singh PR, Dayal R (1985) Studies on nematophagous fungi VII. Two new records from India. Proc Nat Acad Sci India 54: 163-166.

Singh K, Gupta RC, KP Singh (2005) Distribution of predacious fungi in agricultural soil in Eastern Uttar Pradesh. Indian J Pl Patholo 23 (1&2): 119-121.

Vaish SS, Singh KP (2002) Distribution of Catenaria anguillulae Sorokin, a facultative endoparasite of nematodes in soils from different locations of India. World J Micrrobiology and Biotechnology 18: 65-67.

Singh KP, Bandyopadhyay P (2000) Effect of temperature, pH and nematode starvation on the induction of rings of *Dactylaria brochopaga* Dreschseler. J Myco Pl Patholo 30(1): 100-102

Cooke RC, Godfrey BES (1964) A key to the nematode destroying fungi. Trans Brit Mycol Soc 51: 555-561.

Ali AHH (1990) The use of nematodes trapping fungi and organic amendments to control root-knot nematodes. Bulletin of Faculty of Agriculture. University of Cairo 41: (3) Suppl 1: 1001-1012.