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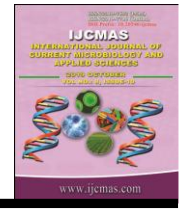

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Effect of *Beauveria bassiana* with Botanicals on Root Knot Nematode Population (*Meloidogyne graminicola*) in Rice Seedlings

P. Niveditha*, Sobita Simon, R. V. Neeraja Reddy and Abhilasha A. Lal

Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, India

**Corresponding author*

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An experiment was conducted in Laboratory of the Department of Plant Pathology, SHUATS, Prayagraj, and U.P. during 2018-19 to observe the effect of *Beauveria bassiana* (white muscardine fungus) with botanicals on root knot nematode (*Meloidogyne graminicola)* population in rice seedlings. In vitro compatibility of *Beauveria bassiana* was carried out with ten selected botanical extracts. The leaf extracts of Neem, Ashoka, Chrysanthemum, Marigold, Guava, Chilli, Castor, Hibiscus, Curry leaves and *Lantana camara* extracts were taken @ 10% for the compatibility of *Beauveria bassiana* by the food poison technique. After 4, 7 and 10 days of incubation the radial growth (mm) of *Beauveria bassiana* was recorded. Out of the selected 10 botanicals neem leaf extract shows significantly increased growth rate of *Beauveria bassiana* as 11.17, 14.50 and 18.00 mm/plate. A pot experiment was conducted in green house in combination of selected botanicals with *Beauveria bassiana* to see the effect on Root-knot nematode (*Meloidogyne graminicola)* population in rice seedlings. In the pots the selected botanicals were amended @ 10 ml each with *Beauveria bassiana* 10 ml (1×10^5 cfu) per pot infested with *Meloidogyne graminicola* @ 2 J/gm of soil.

Introduction

Rice (*Oryza sativa* L.) belongs to family Poaceae. It is the staple food in developing countries. China and India are two major rice producing countries. India stands first in rice cultivated area and second in its production, after China (Rai, 2006). About 90.0% of world's total rice is grown in Asian countries alone (FAO, 2014). In India, rice is grown in almost all the states. Rice is an important cereal in source of calories for more than one-third of the world population. Rice is consumed after cooking with water. Other edible uses include rice flakes, puffed rice, rice wafers and canned rice. It is also used in starch and brewing industries. Rice straw is a good cattle feed besides being used in making hats, mats and ropes. It is also grown successfully in humid to sub-humid region under subtropical and temperate climate. Rice is cultivated in almost all type of soil with varying productivity. Many biotic and abiotic stresses are responsible for reducing the production of rice. Among the biotic stresses, many fungi, bacteria, virus and nematodes are causing serious losses in rice production. More than 200 species of Plant-Parasitic Nematodes (PPN's) have been reported to be associated with rice worldwide. Among these nematodes, root-knot nematode (*Meloidogyne graminicola*) is considered as the major problem in rainfed, upland and lowland rice production regions, whereas rice root nematode (*Hirschmanniella* spp.) is a problem on lowland rice only in South and Southeast Asia Root-knot nematode is an important problem in rice based production systems (Prot, 1994).

Beauveria bassiana is the most widely used entomopathogen. It has been reported to control many crop pests such as stem borers, beetles, aphids, mites, termites, white flies, mealy bugs, thrips etc. (Biswas *et al.*, 2012). The major species *B. bassiana* and *Beauveria brongniatii* are often used in crop and forest pest prevention. Species of the genus *Beauveria* have been reported to produce the secondary metabolites bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein (Strasse *et al.*, 2000; Quesada and Vey, 2004). Currently, there are few reports on the application of *Beauveria* in the control of nematode diseases. Junxianke, a fermentation product using a fungal isolate Snef907 *(B. bassiana)*, is lethal to *Ditylenchus destructor*, *Heterodera glycines* and *Meloidogyne incognita* (Liu *et al.*, 2007). Thus, *Beauveria* fungi can be potentially applied in prevention of plant parasitic pests and nematodes. Liu *et al.*, (2008) reported that the biological control of PPN's can be carried out by soil drenching with culture filtrates of *B. bassiana* and achieved significant reduction in nematode population densities in soil and in the roots and subsequent gall formation and egg-mass production by *M. hapla* under glasshouse conditions. The interaction of microbial control agents could be additive, synergistic or antagonistic. Synergistic interactions would enhance the effectiveness of the microbial control agent while reducing the adverse effects of pesticides (Islam and Omar, 2012).

Keeping this view, present studies were under taken on compatibility of *B. bassiana* with selected botanical leaf extract viz., *Bougainvillea glabra*, Chilli (*Capsicum frutescens*), Neem (*Azadirachta indica*), *Lantana camara*, *Chrysanthemum indicum*, Ashoka (*Saraca asoca*), Guava leaves (*Psidium guajava*), *Hibiscus rosa sinensis*, Marigold (*Tagetes erecta*) and Curry leaves (*Murraya koenigii*) against *Meloidogyne graminicola* in rice.

Materials and Methods

Isolation of the pathogen

An infected *Inderbella quadrinotata* larva due to *Beauveria bassiana* was collected from guava orchard at SHUATS, and the sample was isolated and cultured on Potato Dextrose Agar (PDA) medium. *Beauveria bassiana* was identified by referring relevant literature (Dube, 2018). The selected botanicals (Neem, Ashoka, Chrysanthemum, Marigold, Guava, Chilli, Castor, Hibiscus, Curry leaves and *Lantana camara*) fresh leaves were washed under the tap water and grounded in a pestle and mortar by using 100 gm of leaf and 100 ml of sterile distilled water. The extracts were filtered through double layered muslin cloth followed by centrifugation 3000 rpm for 10 min and made to the required volume by adding sterile water (Gurjar *et al.*, 2012). In PDA media 10% of each botanical extracts was mixed separately. The compatibility of *Beauveria bassiana* and botanicals were checked by using poison food technique adopted by (Vincent, 1947; Mohana, D.C., 2007).

Poisoned Food Technique

Five mm diameter of culture disc of *Drechslera oryzae* was taken with the help of cork borer and was kept at the centre of each petriplate containing botanical extracts of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 27^0C for ten days and colony diameter was recorded. Percent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100$$

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C = Mycelium weight in control.

T= Mycelium weight in treatment.

Application of botanicals and *Beauveria bassiana* in pots

The results of the compatibility test revealed that fresh leaf extract of neem and chrysanthemum significantly and supporting the growth of the test fungus as such these were selected for the pot experiment. Fresh leaves of neem and chrysanthemum @ 200gm (w/v) per each were grounded in a pestle and mortar by using 200 ml of sterile distilled. The ten days old culture of *Beauveria bassiana* was taken and conidia were harvested, using a sterilized inoculation loop, attached to 1ml borosilicate pipette at the angle of 45°. Scraped material was shifted into sterilized Petri dishes and stored at 4°C in refrigerator. Serial dilutions were prepared and the number of conidia was measured by a haemocytometer to achieve the concentrations of $1{\times}10^5$ cfu ml^{-1} (Kepenekci *et al.*, 2017). Then 50 ml of each extract were added in each pot infested by *Meloidogyne graminicola*@ 2J/gm of soil, one treatment of infested pots of *Meloidogyne graminicola* were maintained as control. Twenty seed of rice per sown in each pot, replicated four times. Observations were recorded after 35 days of germination.

Results and Discussion

In the current study, *in-vitro* the compatibility of *Beauveria bassiana* with different ten botanicals indicated significantly increases the growth of *Beauveria bassiana* in neem (11.17, 14.50 and 18.00 mm) and chrysanthemum (10.67, 13.67 and 18.00 mm). In pot experiment the extract of neem and chrysanthemum are used in the combination of *Beauveria bassiana* against root gall nematode (*Meloidogyne graminicola)* population. The result occurred in neem + *Beauveria bassiana*, Neem and *Beauveria bassiana* significantly reduced the population of root gall as comparison to chrysanthemum, chrysanthemum + *Beauveria bassiana* and control (Nematode alone).

Diepieri *et al.*, (2005) reported the compatibility of aqueous extracts of neem leaves and seeds with *B. bassiana* at 1.5% and 15% concentrations. The difference in inhibition rates of mycelial growth of *B. bassiana* by aqueous neem leaf extract and Neem Seed Kernel Extract (NSKE) was due to the variability in the amount of terpenoids, phytoalexins, sulfurade compounds and triterpenoids. Ambethgar *et al.*, (2009) has also reported that NSKE (5%) exhibited minimum inhibition of *B. bassiana*. Similarly, the compatibility of *B. bassiana* with ANLE and NSKE was also observed in present study at all the concentrations (Lipa *et al.*, 2017). The compatibility of *Beauveria* isolated was evaluated with different botanicals such as Aqueous Neem Leaf Extract (ANLE) (1% w/v), Aqueous Garlic Extract (AGE) (1% w/v) and Neem Seed Kernel Extract (NLKE) (5% w/v) at three different concentrations. The isolate of *Beauveria* showed maximum growth in Neem Leaf Extract (74.75 mm) and Neem Seed kernel Extract (70.75 mm), whereas, the least growth was observed in Aqueous Garlic extract AGE (60.50 mm) consists of high concentration of sulphur (aliin, allicin etc.), amino acids, enzymes (flavonoids, saponins), phenol content of 3.57 ± 0.49 mg/g and antioxidants. The antimicrobial activity of AGE is due to the interaction of allicin with cysteine undergoin biosynthetic pathway and inhibition of RNA synthesis. Compatibility of AGE with biocontrol agents e.g., *Trichoderma* spp. was also studied by Maheshwari (2014). Usha *et al.,* (2014) isolated of *Beauveria bassiana* compatibility assessment was made with botanical at three concentrations (0.1X, 0.5X and 1X) in the laboratory based on the recommended dose for field application by food poison technique and their effect on conidial germination, vegetative growth and sporulation. All the botanicals were compatible to the isolates.

Fungal natural products are very promising potential sources of new chemicals to manage plant-parasitic nematodes (Anke and Sterner, 1997). Culture filtrates of many fungi possess activity against nematodes, and the nematicidal action of these culture filtrates may involve the production of toxic metabolites by the fungi (Caroppo *et al.,* 1990; Liu *et al.,* 2008; Lin *et al.,* 2009). As a classic fungal biocontrol agent, *Beauveria* possesses great potential for the control of sucking insect pests (Feng *et al.,* 2004, Hatting *et al.,* 2004; Jean *et al.,* 2008). Although there have been numerous reports of toxicity of *B. bassiana* to insects, similar investigations with plant-parasitic nematodes have been very limited. Mayer (1995) reported that beauvericin produced by *B. bassiana* had weak nematicidal activity against *M. incognita*. Chen *et al.,* (1996) found that *B. bassiana* showed little parasitism of nematode eggs but reduced hatch of *Heterodera glycines*.

The maximum mean radial growth of *Beauveria bassiana* at 4,7 and 10 days after inoculation was recorded in T_5 - Neem (11.17, 14.50 and 18.00 mm, respectively) followed by T_6 - Chrysanthemum (10.67, 13.67 and 18.00 mm, respectively), T_3 - Marigold (10.33, 13.67 and 20.67), T_9 - Curry leaves (10.17, 13.00 and 20.33), T_{10} - Guava (9.50,12.50 and 20.17), T_8 - Castor (9.00, 12.33 and 19.00), T_7 - Chilli (9.00, 12.33 and 17.67), T_2 - Lantana (8.83, 11.67 and 17.17), T_4 - Ashoka (8.83, 10.17 and 16.83), T_1 - Hibiscus (8.67, 9.67 and 16.50) and T_0 - Control (12.67, 15.33 and 23.17).

The results on the effect of botanicals on *Meloidogyne graminicola* at 35 days old seedlings indicated that the population of *Meloidogyne graminicola* in T_3 (Neem + *Beauveria bassiana* - 11), T_2 (Neem-11), T_1 (*Beauveria bassiana* - 12), T_6 (Chrysanthemum+ Neem - 12.67) and T_5 (Chrysanthemum + *B. bassiana* - 13) which are significantly reduced the root gall population as compared to T_4 (Chrysanthemum - 19) and T_0 (Control - 46.33). Maximum percentage of reduction over control was obtained is T_2 (Neem- 76.26%) T_3 (Neem + *Beauveria bassiana* - 76.26%) followed by T_1 (*Beauveria bassiana* - 74.10%), T_6 (Chrysanthemum + Neem - 72.65%), T_5 (Chrysanthemum + *B. bassiana* - 71.94%), T_4 (Chrysanthemum - 58.99%).

The present studies clearly indicated that Neem and *Beauveria bassiana* were found effective treatments against *Meloidogyne graminicola* recorded with maximum reduction of root galls population in rice seedlings (Table 1 and 2).

Table.1 *In vitro* studies on compatibility of *Beauveria bassiana* and selected botanicals at different days of interval

Treatments no.	Treatment name	Mean radial growth (mm) of the three replicates					
		4 Days	Inhibition %	7 Days	Inhibition %	10 Days	Inhibition %
T_0	Control	12.67		15.33		23.17	
T_1	Hibiscus	8.67	31.58	9.67	36.96	16.50	28.78
T_2	Lantana	8.83	30.26	11.67	23.91	17.17	25.90
T_3	Marigold	10.33	18.42	13.67	10.87	20.67	10.79
T_4	Ashoka	8.83	30.26	10.17	33.70	16.83	27.34
T_5	Neem	11.17	11.84	14.50	5.43	18.00	9.35
T_6	Chrysanthemum	10.67	15.79	13.67	10.87	18.00	9.35
T_7	Chilli	9.00	28.95	11.67	23.91	17.67	23.74
T_8	Castor	9.00	28.95	12.33	19.57	19.00	17.99
T_9	Curry leaves	10.17	19.74	13.00	15.22	20.33	12.23
T_{10}	Guava	9.50	25.00	12.50	18.48	20.17	12.95
S. Ed. (±)		0.665		1.500		0.816	
C. D. (5%)		1.410		3.180		1.730	

Table.2 Effect of botanicals on root galls population of *Meloidogyne graminicola* in rice seedlings

S.NO.	TREATMENTS	*Meloidogyne graminicola* population at 35 days after germination of rice	
		Mean of the three replicates	% reduction over control
T_0	Control	46.33	100
T_1	*Beauveria bassiana* 10 ml (1×10^5 cfu)	12.00	74.10
T_2	Neem (10 ml)	11.00	76.26
T_3	Neem (10 ml) + *Beauveria bassiana* 10 ml (1×10^5 cfu)	11.00	76.26
T_4	Chrysanthemum (10 ml)	19.00	58.99
T_5	Chrysanthemum (10 ml) + *Beauveria bassiana* 10 ml (1×10^5 cfu)	13.00	71.94
T_6	Chrysanthemum(10 ml) + Neem (10 ml)	12.67	72.65
S. Ed. (±)		2.418	
C. D (5%)		5.126	

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