Original Research Article

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

**Abstract**

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.

**Keywords**

*Beauveria bassiana*, *Meloidogyne graminicola*, Botanical extracts, Compatibility

**Article Info**

Accepted: 04 September 2019
Available Online: 10 October 2019

**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 S nef907 (*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 undertaken on compatibility of *B. bassiana* with selected botanical leaf extract viz., *Bougainvillea glabra*, Chill i (*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°C for ten days and colony diameter was recorded. Percent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

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

C = Mycelium weight in control.

T = Mycelium weight in treatment.

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 (alilin, 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 T5 - Neem (11.17, 14.50 and 18.00 mm, respectively) followed by T6 - Chrysanthemum (10.67, 13.67 and 18.00 mm, respectively), T3. Marigold (10.33, 13.67 and 20.67), T9 - Curry leaves (10.17, 13.00 and 20.33), T10 - Guava (9.50,12.50 and 20.17), T8. Castor (9.00, 12.33 and 19.00), T7 - Chilli (9.00, 12.33 and 17.67), T2 . Lantana (8.83, 11.67 and 17.17), T4 - Ashoka (8.83, 10.17 and 16.83), T1 . Hibiscus (8.67, 9.67 and 16.50) and T0 . 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 T3 (Neem + *Beauveria bassiana* - 11), T2 (Neem-11), T1 (*Beauveria bassiana* - 12), T6 (Chrysanthemum+ Neem - 12.67) and T5 (Chrysanthemum + *B. bassiana* - 13) which are significantly reduced the root gall population as compared to T4 (Chrysanthemum - 19) and T0 (Control - 46.33). Maximum percentage of reduction over control was obtained is T2 (Neem-76.26%) T3 (Neem + *Beauveria bassiana* - 76.26%) followed by T1 (*Beauveria bassiana* - 74.10%), T6 (Chrysanthemum + Neem - 72.65%), T5 (Chrysanthemum + *B. bassiana* - 71.94%), T4 (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 % |
| T₀             | Control          | 12.67               | 15.33               | 23.17               |
| T₁             | Hibiscus         | 8.67                | 31.58               | 9.67                | 36.96               | 16.50               | 28.78               |
| T₂             | Lantana          | 8.83                | 30.26               | 11.67               | 23.91               | 17.17               | 25.90               |
| T₃             | Marigold         | 10.33               | 18.42               | 13.67               | 10.87               | 20.67               | 10.79               |
| T₄             | Ashoka           | 8.83                | 30.26               | 10.17               | 33.70               | 16.83               | 27.34               |
| T₅             | Neem             | 11.17               | 11.84               | 14.50               | 5.43                | 18.00               | 9.35                |
| T₆             | Chrysanthemum    | 10.67               | 15.79               | 13.67               | 10.87               | 18.00               | 9.35                |
| T₇             | Chilli           | 9.00                | 28.95               | 11.67               | 23.91               | 17.67               | 23.74               |
| T₈             | Castor           | 9.00                | 28.95               | 12.33               | 19.57               | 19.00               | 17.99               |
| T₉             | Curry leaves     | 10.17               | 19.74               | 13.00               | 15.22               | 20.33               | 12.23               |
| T₁₀            | 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 |
| T₀    | Control                     | 46.33                       | 100                       |
| T₁    | *Beauveria bassiana* 10 ml (1×10⁵ cfu) | 12.00                       | 74.10                      |
| T₂    | Neem (10 ml)                | 11.00                       | 76.26                      |
| T₃    | Neem (10 ml) + *Beauveria bassiana* 10 ml (1×10⁵ cfu) | 11.00                       | 76.26                      |
| T₄    | Chrysanthemum (10 ml)       | 19.00                       | 58.99                      |
| T₅    | Chrysanthemum (10 ml) + *Beauveria bassiana* 10 ml (1×10⁵ cfu) | 13.00                       | 71.94                      |
| T₆    | Chrysanthemum(10 ml) + Neem (10 ml) | 12.67                       | 72.65                      |

S. Ed. (±)  2.418
C. D (5%)  5.126
References

Ambethgar, V., Swamiappan, M., Rabindra, R. and Rabindran, R. (2009). Biological compatibility of Beauveria bassiana (Balsamo) Vuillemin isolate with different insecticides and neem formulations commonly used in rice pest management. Journal of Biological Control. 23(1):11-15.

Anke, H. and Sterner, O. (1997). Nematicidal metabolites from higher fungi. Curr. Org. Chem. 1:361-374.

Biswas, C., Dey, P., Satpathy, S. and Satya, P. (2012). Establishment of the fungal entomopathogen B. bassiana as a season long endophyte in jute (Corchorus olitorius) and its rapid detection using SCAR marker. BioControl, 57:565-571.

Caroppo, S., Perito, B. and Pelagatti, O. (1990). In vitro evaluation of nematicide activity by several fungi against Meloidogyne incognita eggs, Redia 73: 451 - 462.

Chen, S. Y., Dickson, D. W. and Mitchell, D.J. (1996). Pathogenicity of fungi to eggs of Heterodera glycines, J. Nematol. 28(2):148-158.

Depieri, R. A., Martinez, S.S. and Menezes, Jr. A. O. (2005). Compatibility of the Fungus Beauveria Bassiana (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. Neotropical Entomology. 34(4):601-606.

Dube, H.C. (2018). A Text Book of Fungi Bacteria and Viruses, Student Edition.

Feng, M. G., Chen, B. and Ying, S.H. (2004). Trails of Beauveria bassiana, Paecilomyces fumosoroseus and imidacloprid for management of Trialeurodes vaporariorum (Homoptera: Aleyrodidae) on greenhouse grown lettuce, Biocontrol Sci. Technol. 14:489-496.

Food and Agriculture Organization of the United Nations (FAO), 2014.

Gurjar, M. S., Ali, S., Akhtar, M., and Singh, K. S. (2012). Efficacy of plant extracts in plant disease management, Agricultural Sciences, 3: 425-433.

Hatting, J. L., Wraight, S. P. and Miller, R.M. (2004). Efficiency of Beauveria bassiana (Hyphomycetes) for control of Russian wheat under weld condition, Biocontrol Sci. Technol. 14:459-473.

Islam, M.T. and Omar, D.B. (2012). Combined effect of Beauveria bassiana with Neem on virulence of insect in case of two application approaches, The Journal of Animal & Plant Sciences, 22(1): 77-82.

Jean, P. K., Les, S., Peter, K. and Bruce, B. (2008). Optimal concentration of Beauveria bassiana vectored by bumble bees in relation to pest and bee mortality in greenhouse tomato and sweet pepper, Biocontrol 53:797-812.

Kepenekci, I., H. D. Saglam, E. Oksal, D. Yanar and Y. Yanar (2017). Nematicidal activity of Beauveria bassiana (Bals.-Criv.) Vuill. Against Root-Knot Nematodes on Tomato Grown under Natural Conditions, Egyptian Journal of Biological Pest Control, 27 (1): 117-120.

Lin, D., Dehai, L. and Tianjiao, Z. (2009). New alkaloids and diterpenes from a deep ocean sediment derived fungus Penicillium sp., Tetrahedron 65(5):1033-1039.

Lipa, Deb., Rajesh, T., Majumdar, D. and Tombisana, R.K. (2017). Evaluation of biological compatibility of Beauveria bassiana with fungicides and botanicals, Journal of Pharmacognosy and Phytochemistry, SP1: 1120-1124.

Liu, T., Li, Y.F., Chen, L.J. (2007). The research on the Junxianke that can control north root knot nematode
disease, *J. Changjiang Vegetables*, 2:48-49.

Liu, T., Wang, L., Duan, Y.X., Wang, X. (2008). Nematicidal activity of culture filtrate of *Beauveria bassiana* against *Meloidogyne hapla*, *World J. Microbiol. Biotechnol.*, 24:113-118.

Maheshwari M. (2014) Compatibility study of isolates of *Trichoderma* spp. with plant extracts. *Asian Journal of Biological Science*. 9(2):242-245.

Mayer A (1995) PhD Thesis, University of Kaiserslautern, Kaiserslautern, Germany.

Mohona, D.C. and Raveesha, K. A. (2007). Antifungal evaluation of some plant extracts against some plant pathogenic field and storage fungi, *Journal of Agriculture Technology*, 4(1): 119-137.

Prot, J.C., Soriano, I.R.S. and Matias, D. (1994). Major root-parasitic nematodes associated with irrigated rice in the Philippine, *Fundam. Appl. Nematol.*, 17:75-78.

Quesada, M.E. and Vey, A. (2004). Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*, *Mycol. Res.*, 108:441-452.

Rai, M. (2006). Rice - the cereal that feeds billion. *Indian Farming*, 576(7):4-9.

Strasse, H., Vey, A. and Butt, T. (2000). Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Sci. Technol.*, 10:717-735.

Usha, J., Naren Babu, M. and Padmaja, V. (2014). Detection of Compatibility of Entomopatho -genic fungus *Beauveria bassiana* (Bals.)Vuill. with pesticides, fungicides and botanicals, *International Journal of Plant, Animal and Environmental Sciences*, 4(2): 613-624.

Vincent, J.M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*. 150: 850.

**How to cite this article:**

Niveditha, P., Sobita Simon, R. V. Neeraja Reddy and Abhilasha A. Lal. 2019. Effect of *Beauveria bassiana* with Botanicals on Root Knot Nematode Population (*Meloidogyne graminicola*) in Rice Seedlings. *Int.J.Curr.Microbiol.App.Sci.* 8(10): 442-448.

doi: [https://doi.org/10.20546/ijcmas.2019.810.047](https://doi.org/10.20546/ijcmas.2019.810.047)