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Integration of Bioagent and Bioproduct for the Management of Root-Knot Nematode, *Meloidogyne Incognita* on Eggplant

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Abstract:

Efficacy of bioagent (*Paecilomyces lilacinus*) and the bioproduct (Radiant) in various combinations was assessed on the reproduction of *Meloidogyne incognita* on eggplant. The influence of *P. lilacinus* and Radiant was determined on egg hatching and second stage juvenile (J_2) mortality under in vitro conditions. The concentrations of 1% and 100% of Radiant and *P. lilacinus* respectively both alone and in combined application caused significant mortality and reduction in egg hatching at all time intervals. The interaction of *P. lilacinus* and Radiant was determined individually, concomitantly, and sequentially on reproduction of *M. incognita* on eggplant under greenhouse. The reproduction of *M. incognita* was significantly reduced in the concomitant treatment consisting of both *P. lilacinus* and Radiant followed by sequential and individual treatment of Radiant and the plant growth parameters incresed significantly. Our findings suggest that *P. lilacinus* and Radiant have the ability to regulate nematode population and may serve as nematicides.

Keywords: *P. lilacinus*, Radiant, Egg hatching, Mortality, Concomitant, Sequential

1. Introduction

Eggplant (*Solanum melongena* L.) is an important cheap summer vegetable with medicinal properties as well as containing water, protein and vitamin A (Tindall 1978). In Pakistan, it is cultivated on an area of 8673 ha having annual production of 88148 tonnes (FAO 2009). The average harvested yield is very low due to various pests such as insects, fungi, bacteria, viruses and nematodes. In Pakistan, root-knot nematode, *M. incognita* caused severe damage to eggplant and other vegetables and their host range include more than 3000 plant species (Anwar *et al.* 2009). High temperature is suitable for endurance and reproduction of nematodes. Among the root knot nematodes, *M. incognita* and *M. javanica* are of wide spread and economically most important (Anwar & McKenry 2010).

Nematode control is far more complex than any other kind of pathogens because nematodes mainly attack under ground parts of plants (Sikora & Fernandez 2005). A range of management strategies studied, including crop rotation, soil amendments and nematicides that could be collectively used to enhance the activity of naturally occurring biological control methods (Sikora 1992). Even though chemical nematicides have a great promise for the control of nematodes but restricted due to health hazards involved. Different practices are used in the integrated pest management (IPM) but the biological control would be the most enviable. Culture filtrates from various fungi such as species of *Paecilomyces*, *Verticillium*, *Fusarium*, *Aspergillus*, *Trichoderma*, *Myrothecium* and *Penicillium* were tested against juvenile mortality and on egg inhibition of nematodes. These filtrates proved to be toxic due to the release of antibiotics or enzymes (Dorcas *et al*. 2010). The potential of *B. thuringiensis* var. *Berliner*, *Saccharopolyspora spinosa*, *Fusarium* spp. and *Trichoderma* spp. against *G. rostochiensis* on potato was evaluated. The plant growth and yield was improved by all the treatments. Significant inhibitory effect was caused by bioagents on the reproduction of the nematode (Trifonova 2010).

Keeping in view the importance of biological control of root knot nematodes, it was planned to manage *M. incognita* by the interaction of *P. lilacinus* and Radiant. The objective of the present study was to assess the effectiveness of *P. lilacinus* and the Radiant (active ingredient; Spinetoram; 11.7%) for the management of *M. incognita* on eggplant. Radiant was not yet used for the management of root-knot nematode and it was also integrated for the first time with *P. lilacinus* for the control of *M. incognita*.

2. Materials and Methods

2.1 Nematode inoculum

Seeds of tomato cv. Money maker were planted in soil sterilized with formalin. Transplanting of seedlings was done after three weeks in earthen pots. Seedlings were inoculated with 5000 J_{2s} per pot by making holes around the plant rhizosphere.

2.2 Fungal inoculum

Pure culture of *Paecilomyces lilacinus* was obtained from the culture collection of Arid Agriculture University, Rawalpindi. One week old cultures of bioagent maintained on PDA slants at 28 ± 1 were used for the present study. Culture filtrates of *P. lilacinus* were obtained by growing one week old culture of *P. lilacinus* on Potato Dextrose Broth (in 250 ml flasks containing 100 ml broth media). Flasks were autoclaved at 121 ^{O}C and 15 psi for 15 minutes. Each flask was inoculated with three scoops of 5mm diameter from pure culture containing petri dishes and incubated at 25^{O}C for 15 days. After incubation the cultures were filtered through Whatman filter paper No.1 to remove the mycelial mats. These filtrates thus obtained were considered as standard (100% concentration).

2.3 Influence of P. lilacinus and Radiant on juvenile mortality and egg hatching of M. incognita

Radiant (Spinetoram; 11.7%) was obtained from the local market to check its efficacy against ***M.*** *incognita*. Different concentrations viz. 1%, 0.75%, 0.50% and 0.25% were prepared by adding requisite amount of sterilized distilled water. Different concentrations of culture filtrates of *P. lilacinus* viz. 75%, 50% and 25% were prepared from standard (100% concentration) by adding requisite amount of sterilized distilled water. The population of *M. incognita* maintained on the roots of tomato cv. Money maker in the glass house of Department of Plant Pathology, University of Agriculture, Faisalabad was used. Eggs were isolated from egg masses and nematodes were extracted (Hussey & Barker 1973). The effect of different concentrations of Radiant and culture filtrates of *P. lilacinus* were investigated on juvenile mortality and egg hatching of *M. incognita*. Three ml of each concentration of Radiant and *P. lilacinus* were pipetted into Petri dishes. Fifty freshly hatched Juveniles of *M. incognita* in 0.5ml distilled water were added to each Petri dish. Fifty eggs of *M. incognita* were picked and placed into each dish and kept at 28^{0}C. Eggs and juveniles placed in distilled water served as control. Each treatment was replicated five times. Data on juvenile mortality and egg hatching was recorded after 24, 48, 72 hours.

2.4 Interaction of P. lilacinus and Radiant on reproduction of M. incognita on egg plant individually, concomitantly and sequentially in green house

Three weeks old eggplant seedlings were transplanted into 20 cm diameter earthen pots containing formalin sterilized soil. Concentrations 1% and 100% of Radiant and *P. lilacinus* respectively were used because these concentrations caused maximum juvenile mortality and reduction in egg hatching during in vitro investigation. There were six treatments and five replications for each treatment.

2.4.1 Treatments (T)

T1 =*P. lilacinus*, T2 = Radiant, T3 = *P. lilacinus* + Radiant, T4 = *P. lilacinus* then Radiant, T5 = Radiant then *P. lilacinus*, T6 =Check (Nematode).

Each pot received 20 ml of 100% concentration of *P. lilacinus* containing 1x 10^{7} spores/ml and 20 ml of 1% concentration of Radiant except check plants according to the treatment scheme individually, concomitantly and sequentially. A period of one week was followed in the sequential treatments. Freshly hatched 1000 J_{2s} of *M. incognita* per pot were used as inoculum by making 3-4 holes (3 cm deep) around

each plant with the help of pointed wood. After inoculation pots were kept in greenhouse (25-30^{0}C) in completely randomized design. Plants were allowed to grow for 60 days. The data was recorded on number of galls, number of egg masses per root system by staining the roots with Phloxine B solution for 5 minutes (Holbrook *et al*. 1983), eggs per egg mass, number of females by staining the roots with acid fuchsin-lactophenol, number of J_2/100 cm^{3}of soil and number of J_2/g of root were determined by processing the soil with the help of Cobb's sieving and decanting method along with Baermann's funnel technique (Southey, 1986). The reproduction factor (Rf) was calculated by dividing the final population (pf) by initial population (pi), root weight (g), root length (cm), shoot weight (g), shoot length (cm) and number of leaves. Experiment was repeated to confirm the results and thus the data was subjected to analysis of variance and the significant difference in treatment means were separated by using Duncan Multiple Range Test at (P= 0.05) and Bartlett's test at (P= 0.05).

3. Results

3.1 Influence of P. lilacinus and Radiant on Juvenile mortality and egg hatching of M. incognita

Mortality of juveniles of *M. incognita* was affected by concentrations and exposure time in all treatments (Table 1). The J_{2s} treated with Radiant and combine application of Radiant and *P. lilacinus* had significant more mortality as compared to *P. lilacinus* alone at all time intervals. The combined application of Radiant and *P. lilacinus* showed the maximum mortality at all concentrations after all time intervals. Egg hatching of *M. incognita* was inversely proportional to the concentrations of Radiant and culture filtrates of *P. lilacinus*. Radiant at 1% concentration had significant (P = 0.05) more effect on mortality (50.0) after 72 h from its 0.5% (48.2) and 0.25% (40.2) concentrations. *Paecilomyces lilacinus* at 100% concentration caused significant greater mortality (40.2) as compared to all other concentrations at all time intervals. At 75% concentrations *P. lilacinus* caused significantly high mortality (31.6) from its 50% concentration (24.4) after 72 hours of exposure. The combined application of Radiant and *P. lilacinus* (1 + 100%) showed significant results of J_2 mortality (50.0) as compared to the alone application of Radiant and *P. lilacinus* at all time intervals. At 0.75+75% concentration results (50.0) were not significantly different from 1+100% concentration (50.0) after 72 h of exposure but different from its 0.5+50% (48.4) and 0.25+25% (40.2) concentrations.

Egg hatching of *M. incognita* was significantly reduced with the duration of exposure (Table 2). Egg hatching was significantly decreased in Radiant and in combined application of Radiant and *P. lilacinus* than treated with *P. lilacinus* alone at all time intervals. The combined application of Radiant and *P. lilacinus* exhibited the maximum reduction in egg hatching of *M. incognita* at all concentrations after all time intervals. At 1% concentration, Radiant had significant reduction (14.6) in hatching over all other concentrations at all time intervals. Hatching of *M. incognita* was significantly (P = 0.05) reduced in 0.75% (23.8), 0.5 % (31.0) and 0.25% (39.6) concentrations of Radiant after 72 h of exposure as compared to control (49.00). *Paecilomyces lilacinus* at 100% concentration caused significant reduction (17.80) in hatching over all other concentrations at all time intervals. At 75% concentration *P. lilacinus* caused significant reduction (26.2) in hatching from its 50% (31.8) and 25% (41.4) concentrations at all time intervals. Radiant in combination with *P. lilacinus* showed more significant reduction in egg hatching than in alone application at all concentrations after all time intervals. The combined application at of Radiant and *P. lilacinus* at 1+100% concentration caused significantly more reduction (10.4) in egg hatching from all other concentrations at all time intervals. Egg hatching was also significantly reduced at 0.75+75% (20.8), 0.5+50% (29.4) and 0.25+25% (38.2) concentrations as compared to control (49.0) after 72 h of exposure.

3.2 Interaction of P. lilacinus and Radiant on reproduction of M. incognita on egg plant individually, concomitantly and sequentially in green house

The interaction of *P. lilacinus* and Radiant on plant growth parameters and on reproduction of *M. incognita* on eggplant was studied individually, concomitantly and sequentially in greenhouse. The plant growth parameters varied significantly, there was significant (P= 0.05) increase in root length, shoot weight, shoot

length, number of leaves and decrease in root weight of eggplant was observed in concomitant treatment consisting both bioagent and bioproduct (Table 3). An increase in growth parameters of eggplant treated with *P. lilacinus* alone, followed by Radiant and sequential treatments of Radiant and *P. lilacinus* was observed. The results revealed that root weight was maximum in the check plants treated with *M. incognita* only due to the presence of galls on the roots. The reproduction of *M. incognita* was significantly reduced in the concomitant treatment consisting of both *P. lilacinus* and Radiant followed by sequential and individual treatment of Radiant (Table 4). Maximum reduction in number of galls (15.0), number of egg masses (4.2) counted by staing the roots with phloxine, number of eggs per egg mass (75.4), number of females (16.2), J_2/100 $cm^3$of soil (75.8), J_2/g of root system (87.8) was observed in concomitant treatment. There was significant reduction in the reproduction parameters of *M. incognita* in plants treated with Radiant in individual treatment. Reproduction of *M. incognita* was also reduced in the sequential treatments of Radiant and *P. lilacinus* followed by the individual treatment of *P. lilacinus.* Maximum reproduction of *M. incognita* was observed on the roots of check plants. Reproduction factor was (0.6) in concomitant treatment, (0.8, 0.8) in sequential treatments and (2.2, 0.8) respectively in individuals treatments of *P. lilacinus* and Radiant as compared to check (13.4).

4. Discussion

Root- knot nematodes are the most prevalent and very important group of plant parasitic nematodes occurring all over the world but found more the in areas with warm climate. Root knot nematodes reduce the market value of infected plants by depriving their nutrients. Losses are profound at seedling stage and results in devastation of the crop. Root knot nematodes are difficult to control because of their high reproductive potential. Biological management of nematodes has become one of the most feasible alternatives to the nematicides. Different practices are used in the integrated pest management but the biological control would be the most enviable because most of the successful nematicides have been restricted in agriculture due to health hazards involved (Veremis & Roberts 1996).

The present investigation revealed that Radiant and the culture filtrates *P. lilacinus* possessed the nematicidal activity. Nematicidal effects of culture filtrates of various fungi on egg hatching and J_2 mortality of root knot nematodes have been investigated by many workers (Cayrol *et al.* 1989; Hallmann & Sikora 1996; Zaki & Maqbool 1991). In our study, the effectiveness of *P. lilacinus* and Radiant was assessed on J_2 mortality of *M. incognita* under in vitro conditions. The findings suggested that J_2 mortality of *M. incognita* was directly propotional to concentratins and duration of exposure. Our findings are in conformity with the previous studies (Ahmad *et al.* 2010; Ayatollahy & Fatemy 2010; Goswami & Mittal 2002; Satyandra & Mathur 2010). Another in vitro experiment was conducted to assess the nematicidal effects of *P. lilacinus* and Radiant on egg hatching of *M. incognita*. Egg parasitization of *M. incognita* increased with the increase in concentration of Radiant and *P. lilacinus* and also with duration of exposure. Maximum parasitization was assessed after 72 h. Antagonist fungi possessed larvicidal and ovipocidal properties against root knot nematode. Most of the antagonistic fungi are egg parasitizing but a few culture filtrates of these fungi produced the antibiotics, glioviren and gliotoxin, (Lumsden *et al*. 1992; Howell *et al*. 1993). The pathogenesity of *P. lilacinus* on the eggs of the *Meloidogyne* spp. was tested (Bonants *et al*. 1995). The enzyme in culture filtrates entirely bound with the nematode eggs. Immature eggs were more susceptible to protease treatments, while the mature eggs have the resistance (Mukhtar *et al*. 2003; Siddique & Haque 2000; Ashoub *et al*. 2009).

Our results confirmed that integration of *P. lilacinus* and Radiant reduced the population of *M. incognita* and also improved the plant growth parameters. Filtrates of *P. lilacinus* strains related to their capability to produce leucinostatins. It was also observed that root weight was directly propotional to the number of galls which were maximum in the check plants. The growth parameters were improved by biological control agent *P. lilacinus* whereas it also reduced *M. javanica* reproduction on simultaneous and sequential inoculation of *P. lilacinus* with *M. javanica* (Ganaie & Khan 2010). The efficacy of *Serratia* 214 *marcescens*, three different *T. harzianum* isolates and carbofuran against *M. incognita* was 215 evaluated in the glasshouse and the results showed that chemical nematicide could be replaced 216 to some extent by

microbial antagonists viz. *Serratia marcescens* and *T. harzianum* isolates as 217 they are environmentally safe (Mahfouz *et al*. 2010). The integration gave better results than the alone application that is also proved from our results which are in conformity with (Anjos *et al.* 2010; Park *et al.* 2004; Rao *et al*. 1998; Sharma & Pandey 2009). It was assessed from the present investigation that plant growth parameters were enhanced significantly and also the reproduction of *M. incognita* was decreased due to the application Radiant and *P. lilacinus*. Our results revealed that the bioagent; *P. lilacinus* and the bioproduct; Radiant have the potential to regulate the population of root-knot nematode, *M. incognita* by reducing egg hatching and killing the J_{2s} and if applied in a nematode infested field before planting.

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