Section-IV
Effect of *Rhizobium* and *Paecilomyces lilacinus* on the Growth and Root-rot Disease Complex of Lentil.

**INTRODUCTION**

During the last few decades, plant disease control has been based largely on the use of chemicals. However, because of environmental toxicity and cost of these chemicals, alternative means of control have been investigated. Apart from disease causing organisms, a number of beneficial microorganisms also live in the soil environment. Many species of soil bacteria are reported to promote plant growth by enhancing the availability of nutrients to plants besides control of soil-borne plant pathogens (Weller, 1988).

One of the methods to overcome the nematodes, fungi, and the disease complexes formed as a result of root-knot nematode, *Meloidogyne incognita* and fungi, is to combine the disease-suppressive activity of two or more beneficial microbes in a biocontrol preparation. Such combinations have great potential for more extensive colonization of the rhizosphere and antagonism to a larger number of plant pathogens than individual applications. Conversely, microbes applied in combination also may have antagonistic interactions with each other. The complexity of interactions involved in the application of multiple organisms for biological control has slowed the progress towards development of successful formulations. However, this approach has a potential for overcoming the efficacy problems for *Meloidogyne* spp. and fungi in concomitance that occurs with application of individual biocontrol agents.

Presences of *Rhizobium* sp. Jordan in the rhizosphere improved the soil quality and promoted plant growth by symbiotic nitrogen fixation in the bacterial nodules (Tilak, 1991). The establishments of *Rhizobium* protected the host roots of leguminous plants from the pathogens and reduced the damage caused by them (Ehteshamul-Haque and Ghaffar, 1993; Siddiqui and...
Mahmood, 1995).

The fungus *Paecilomyces lilacinus* (Thom.) Samson has been found to be a potential biocontrol agent (Jatala, 1986; Khan and Williams, 1998), which colonizes the reproductive structures of nematode and cause destruction of females and eggs thus increasing the crop yield by lowering the population of *M. incognita* juveniles (Noe and Sasser, 1985; Cardona and Leguizamon, 1997).

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood and the root-rot fungus, *Macrophomina phaseolina* (Tassi) Goid. (*Rhizoctonia bataticola* (Taub.) Butler) co-infect the lentil under field conditions and develop disease complexes, resulting in much higher losses (Tiyagi *et al.*, 1988; Singh *et al.* 2008). The antagonistic interaction of these pathogens was also reported on other leguminous and non leguminous plants (Agarwal and Goswami, 1973; Siddiqui and Husain, 1991; Zahid *et al.* 2002).

In the present study an attempt was made to use *Rhizobium* sp. and *P. lilacinus* alone and in combination for the biocontrol of two pathogens (*Meloidogyne incognita* and *Macrophomina phaseolina*) alone and the root-rot disease complex formed as a result of their combination on lentil.

**MATERIAL AND METHODS**

**Raising and Maintenance of Test Plants:**

Sandy loam soil collected from experimental plots of the Department of Botany, A.M.U., Aligarh, was passed through 20 mesh sieve. The soil was prepared by mixing sand and organic manure in the ratio of 3:1:1 and 15 cm earthen pots were filled with 1 kg of the soil mixture. A little amount of water was poured into each pot to wet the soil surface before sterilization at 137 kPa for 25 min. Autoclaved pots were allowed to cool at the room temperature for use.
Lentil (*Lens culinaris* Medik.) seeds, cv. K-75 were surface sterilized with 0.1% sodium hypochlorite and rinsed with distilled water (Koenning and Barker, 1985). Three seeds were sown in each clay pot containing steam sterilized soil. After 1 week of germination thinning was done to maintain a single seedling per pot.

**Preparation of Nematode Inoculum:**

*Meloidogyne incognita* collected from lentil fields was cultured on egg plants using single egg mass. These egg masses were washed in distilled water and then poured into 10 cm diameter 15 mesh coarse sieves containing crossed layered tissue paper. The sieves were kept in Petri plates containing distilled water just touching the lower surface of the sieves. Second-stage juveniles hatched from egg masses were collected regularly after every 24 h and stored in a beaker. The concentration of *M. incognita* in water suspension was adjusted so that each ml contained 200 + 5 nematodes.

**Preparation of Fungal Inoculum:**

*Macrophomina phaseolina* was isolated from the infected root samples of lentil. The root bits from root-rot affected plants were surface sterilized with 1% sodium hypochlorite (NaOCl) for 3 minutes and washed twice with sterilized distilled water. For isolation of the fungus the roots were placed on potato dextrose agar (PDA) in Petri plates containing 1 drop of streptomycin each, and incubated at 24ºC for a week. After incubation, the pathogen was purified by single hyphal tip method (Riker and Riker, 1936). The preparation method for PDA is being given in detail in experiment 3.

The biocontrol fungus, *Paecilomyces lilacinus* was isolated from rhizosphere soil by dilution plate technique, (Warcup, 1950) as has been stated in experiment 3. Pure culture of *M. phaseolina* isolated from the infested plants and *P. lilacinus* from rhizosphere soil infested with root-knot nematode was
maintained on potato dextrose agar (PDA) slants. For mass production of pure culture, the fungi individually were inoculated, under aseptic conditions, into 500 ml Erlenmeyer flasks containing Richard’s liquid medium given by Riker and Riker, 1936 (preparation given in experiment 3). The flasks were incubated in a BOD incubator for 10 days at 28± 2°C, to ensure proper growth of fungal mycelium on the liquid medium. Mycelial suspension was prepared by blending 100 g of mycelium mat in 1000 ml distilled water so that 10 ml of suspension contained approximately one gram mycelium for inoculation.

**Preparation of Rhizobial Inoculum:**

One hundred gram commercial *Rhizobium* (lentil strain) was suspended in 1000 ml distilled water so that 10 ml of suspension contain 1 g of bacterial inoculum.

**Inoculation Technique:**

After establishment of the seedlings, feeder roots of one week old lentil plants, just before inoculation, were exposed without damaging the roots. Inoculation with the nematode was done @ 1000 J₂ per pot and with the root-rot pathogen @ 1 g per pot. One gram (equivalent to 10 ml) of rhizobial and one g *P. lilacinus* (10 ml mycelial suspension) were applied to control the root-knot nematode, fungus and root-rot disease complex. The inoculum of pathogens and biocontrol agents was poured uniformly all around the exposed roots using sterilized pipette. The roots were covered immediately with the soil.

**Experimental Design:**

The experiment was designed according to the following treatment scheme:

Four experimental sets i.e.

(a) C – Uninoculated control.
(b) MI – *Meloidogyne incognita* alone.
(c) MP – *Macrophomina phaseolina* alone.
(d) MI+MP – *M. incognita* and *M. phaseolina* simultaneously.

The three variable inoculated plant sets (MI, MP and MI+MP) were treated with:

1. *Rhizobium*
2. *Paecilomyces lilacinus* and
3. *Rhizobium* + *P. lilacinus*.

In all, there were 4 + (3 × 3) = 13 treatments and there were five replicates for each treatment arranged in completely randomized block design fashion on a glass house bench. Watering was done regularly as and when needed. The plants were harvested 90 days after inoculation.

**PARAMETERS STUDIED**

**Plant Growth and Yield:**

Data on plant length was measured by a meter scale; plant weight (fresh and dry) was taken in grams. Number of flowers, number of pods and seed weight were recorded.

**Nodulation:**

Number of nodules per root system was counted by visual observation after harvesting and washing the roots of the plants gently.

**Chlorophyll Contents:**

Chlorophyll content was estimated by the method described by Mac Kinney (1941) as has been stated in Experiment No. 2.

**NPK Estimation:**

Nitrogen, phosphorus and potassium (NPK) contents were estimated per g fresh leaf weight. Potassium content was estimated by Flame photometer; phosphorus content by Fiske and Subbarow (1925); and nitrogen content by Linder (1944) methods (All methods were being given in Experiment No. 2).

**Nematode Population Estimation:**
Nematodes from soil were extracted by Cobb’s sieving and decanting technique followed by Baermann funnel; and from roots by macerating the root tissues in a warring blender (Southey 1986), and counted in a counting dish as suggested by Doncaster (1962).

**Reproduction Factor:**

Reproduction factor (RF) was calculated by dividing final nematode population (PF) by initial population (PI) i.e. \( RF = \frac{PF}{PI} \).

**Rate of Population Increase:**

Rate of population increase RPI was calculated by \( \frac{(PF - PI)}{PI} \) (Oostenbrink, 1966).

**Root-Knot Index:**

Root-knot index (RKI) was determined by scoring the number of galls on scale ranging from 0 (no galls) to 5 (more than 100 galls) (Taylor and Sasser, 1978).

**Root-Rot Percentage:**

The root-rot percentage was calculated by measuring the infected portion in relation to total length of root pieces (Biermann and Lindermann, 1981).

**Root-Rot Index:**

Root-rot index (RRI) was determined by scoring the extent of disease on the scale ranging from 1, (less than 10%) to 5, (76 – 100%) severe rot (Batten and Powell, 1971).

**Statistical analysis:**

Data were analyzed statistically and the significance was calculated at \( p=0.05 \) and \( p=0.01 \) probability levels.
RESULTS

Plant Growth

Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-knot nematode, *Meloidogyne incognita*:

The data (Table 5 A) revealed that the root-knot nematode inoculated (MI) lentil plants exhibited significant (p=0.01) reductions in plant growth parameters (plant length, fresh weight and dry weight of the roots and the shoots) in comparison to uninoculated control (Fig. 1).

On incorporation of root-nodule bacteria, *Rhizobium* (MI+R) and egg parasitic fungus, *Paecilomyces lilacinus* (MI+PL) alone and in combination (MI+R+PL) along with nematode significantly (p=0.01) increased plant growth characters. The highest enhancement in plant growth parameters was recorded where both the biocontrol agents were applied in combination with nematode (MI+R+PL), followed by single application of *Rhizobium* with nematode (MI+R) and individual inoculation of *P. lilacinus* (MI+PL) with root-knot nematode. Moreover the differences in plant growth characters were non-significant (P=0.05) among the individual applications of both the biocontrol agents with nematode (Table 5 A, Fig. 1).

The single treatments with *Rhizobium* and *P. lilacinus* showed significant (p=0.05) but less improvement in plant growth parameters i.e. plant length, fresh weight and dry weight of the roots and the shoots, when compared with uninoculated control (C). The improvement in plant growth was much higher, where both the bio-agents were applied into the soil along with nematode inoculation (MI+R+PL) in lentil seedlings. The combination of biocontrol agents showed overcoming in plant growth parameters, which were slightly higher but non-significant as compared to control (Table 5 A and Fig. 1, 2, 3).

Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-rot fungus, *Macrophomina phaseolina*. 
Significant (p=0.01) decrease in plant growth characters i.e. plant length, fresh weight and dry weight of the roots and the shoots, were noticed in fungus (MP) inoculated plants when compared with uninoculated control (C) (Fig. 1).

The growth parameters significantly (p=0.01) increased with the addition of biocontrol agents i.e. *Rhizobium* and *P. lilacinus*, singly and in combination, in fungus (*M. phaseolina*) inoculated lentil plants. The plant growth was found to be maximum where both the biocontrol agents were applied in combination along with *M. phaseolina* (MP+R+PL), followed by *Rhizobium* (MP+R) and *P. lilacinus* (MP+PL) inoculations (Table 5 A, Fig. 1).

Data presented in table 5 A showed that, the individual treatments of biocontrol agents with fungus encountered significant (p=0.05) but less improvement in plant growth parameters in comparison to control (C). A slight increase in growth parameters over control (C) in lentil plants was observed with *Rhizobium* and *P. lilacinus* (MP+R+PL) along with fungus, but the differences were non-significant (Fig. 1, 2, 3).

**Application of *Rhizobium* and *Paecilomyces lilacinus** in lentil plants inoculated with the combination of *Meloidogyne incognita* and *Macrophomina phaseolina*.

Significant decrease (p=0.01) in plant growth characters (plant length, fresh weight and dry weight of the roots and the shoots) were noticed in the concomitant (MI+MP) inoculation of pathogens, when compared with the individual treatments of nematode (MI) and fungus (MP). When compared with uninoculated control (C), the combination of pathogens resulted in significant (p=0.01) and much higher losses in plant growth parameters i.e. plant length, fresh weight and dry weight of the roots and the shoots (Table 5 A and P-53).

Data (Table 5 A) revealed a significant (p=0.01) increase in plant growth parameters in *Lens culinaris*, by the application of biocontrol agents singly and
in combination with the concomitant inoculations of both the pathogens (P-53). Maximum growth in lentil plants were being reported where both the bio-agents were applied with the combination of pathogens (MI+MP+R+PL), followed by single inoculation of Rhizobium (MI+MP+R) and then when P. lilacinus was applied individually with pathogens (MI+MP+PL) (Fig. 1, 2, 3).

The improvement in plant growth characters were higher where the biocontrol agents were applied simultaneously along with the combination of nematode and fungus, but significantly (p=0.05) less, in comparison to uninoculated control (Table 5 A and Fig. 1, 2, 3).

Yield

Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the root-knot nematode, Meloidogyne incognita:

The data presented in Table 5 A, revealed that plant yield in terms of number of flowers and pods per plant were significantly (p=0.01) reduced when the plants were inoculated with M. incognita (Fig. 4).

The presence of Rhizobium and P. lilacinus individually and concomitantly increased the number of flowers and pods significantly (p=0.01) in the nematode inoculated plants. Highest and more significant enhancement in plant yield (number of flowers and pods) over M. incognita inoculated plants were observed where both the biomanagement organisms were applied together with the nematode (MI+R+PL) followed by individual application of Rhizobium (MI+R) and P. lilacinus (MI+PL) (Table 5 A, Fig. 4).

The data (Table 5 A) showed a significant (p=0.01) increase in the yield characters in terms of number of flowers and pods per plant, in the single inoculation of biocontrol agents in nematode inoculated lentil plants and was less than uninoculated control (C). The improvement in yield parameters were significantly (p=0.01) higher, as compared to control (C), when both the bio-
agents were incorporated along with the nematode inoculation (MI+R+PL) (Fig. 4).

**Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-rot fungus, *Macrophomina phaseolina*.

The data (Table 5 A) revealed a significant (p=0.01) loss in the yield characters i.e. number of flowers and pods per plant, when the lentil plants were inoculated with the root-rot fungus (*M. phaseolina*).

Significant (p=0.01) increase in plant yield in terms of number of flowers and pods in *M. phaseolina* inoculated lentil plants were observed, where the biocontrol agents were applied alone (MP+R and MP+PL) as well as in simultaneous (MP+R+PL) inoculations along with the pathogenic fungus. Highest and more significant enhancement being noted where the bio-agents were applied in combination followed by *Rhizobium* and *P. lilacinus* individually with *M. phaseolina* (Table 5 A, Fig. 4).

The data presented in table 5 A, showed significantly (p=0.01) less improvements in the yield parameters of lentil plants where the biocontrol agents were applied individually to *M. phaseolina* inoculated plants i.e. MP+R and MP+PL, when compared to uninoculated control (C). The increase in yield, however significantly (p=0.01) more where the lentil plants were treated with the combination of biocontrol agents along with the root-rot pathogen (MP+R+PL), in comparison to control (C) (Fig. 4).

**Application of *Rhizobium* and *Paecilomyces lilacinus* in the lentil plants inoculated with the combination of *Meloidogyne incognita* and *Macrophomina phaseolina*.

Data presented in table 5 A revealed significant (p=0.01) and much higher losses in yield (number of flowers and pods) in the lentil plants, inoculated with both the pathogens simultaneously (MI+MP), when compared
with control (C) and the individual inoculations of root-knot, nematode (MI) and root-rot fungus (MP) (Fig. 4).

Data (Table 5 A) showed a significant increase (p=0.01) in plant yield (number of flowers and pods) where the biocontrol agents individually and concomitantly were applied with simultaneous inoculation of pathogens. Highest increase in plant yield was noticed where both the bio-agents were applied with concomitant inoculation of the pathogens (MI+MP+R+PL) followed by Rhizobium (MI+MP+R) and then when P. lilacinus were inoculated with the root-knot and root-rot pathogens (MI+MP+PL) (Fig. 4).

In comparison to control (C), the increase in the number of flowers and pods per plant (yield) were found significantly (p=0.05) less where both the biocontrol agents were applied along with the pathogens (MI+MP+R+PL) inoculated lentil plants (Table 5 A, Fig. 4).

**Weight of Seeds**

**Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the root-knot nematode, Meloidogyne incognita:**

The data presented in table 5 A, revealed that seed weight showed significant (p=0.01) reductions in M. incognita inoculated lentil plants (MI), when compared with uninoculated control (C) (Fig. 6).

Seed weight increased significantly (p=0.05) where the biomanagement organisms i.e. Rhizobium and P. lilacinus individually as well as concomitantly incorporated into the soil with the nematode. Highest increase in seed weight of nematode inoculated plants was observed where both the bio-agents were applied together i.e. MI+R+PL, followed by Rhizobium (MI+R) and P. lilacinus (MI+PL) alone (Table 5 A, Fig. 6).

The improvement in seed weight was significantly (p=0.05) less as compared to control (C), when the nematode inoculated lentil plants were treated with individual inoculations of Rhizobium (MI+R) and P. lilacinus
(MI+PL). Seed weight was found slight less, and non-significant differences were found over the control, where both the biomanagement agents were applied along with the nematode inoculations i.e. MI+R+PL (Table 5 A, Fig. 6).

**Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the root-rot fungus, Macrophomina phaseolina.**

The data (Table 5 A) showed a significant (p=0.01) decrease in seed weight in *M. phaseolina* inoculated (MP) lentil plants, as compared to uninoculated control (Fig. 6).

A significant (p=0.01) increase in seed weight was noticed where the fungus inoculated plants were also treated with the *Rhizobium* and *P. lilacinus* alone and in combination. Higher seed weight was observed where both the bio-agents were inoculated with root-rot fungus, *M. phaseolina* (MP+R+PL), followed by *Rhizobium* (MP+R) and *P. lilacinus* (MP+PL) individually (Fig. 6).

The increase in seed weight was more but non-significantly less over control, in the treatments where combined application of biocontrol agents (MP+R+PL) and *Rhizobium* alone was done along with fungus (MP+R). But the improvement in seed weight was significantly (p=0.05) less, where biocontrol fungus, *P. lilacinus* was applied with *M. phaseolina* i.e. MP+PL (Table 5 A, Fig. 6).

**Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the combination of Meloidogyne incognita and Macrophomina phaseolina.**

The data (Table 5 A), revealed that seed weight reduction was highest and significant (p=0.01) in lentil plants which were concomitantly inoculated with the root-knot and root-rot pathogens (MI+MP), when compared with control (C). This decrease in seed weight was also found significant (p=0.05),
when the comparisons were made with the individual inoculation of nematode (MI) and fungus (MP) (Fig. 6).

Soil application with biomanagement agents simultaneously along with both the pathogens (MI+MP+R+PL) treated lentil plants significantly (p=0.01) increased the seed weight followed by the root-nodule bacteria, *Rhizobium* (MI+MP+R) and *P. lilacinus* (MI+MP+PL) individually along with the pathogens (Table 5 A, Fig. 6).

In comparison to uninoculated control (C), significantly (p=0.01) less increase in seed weight was recorded in plants with individual application of *Rhizobium* (MI+MP+R) and *P. lilacinus* along with the pathogens. Higher but significantly (p=0.05) less increase in seed weight was noticed, by the incorporation of both the bioagents simultaneously in pathogens inoculated plants i.e. MI+MP+R+PL (Table 5 A, Fig. 6).

**Nodulation**

**Application of *Rhizobium* and *Paecilomyces lilacinus* in the lentil plants inoculated with the root-knot nematode, *Meloidogyne incognita*:**

The data presented in table 5 A, showed that inoculation of lentil plants with *M. incognita* significantly (p=0.01) reduced the number of nodules per root system in comparison to control plants (Fig. 5).

Application of *Rhizobium* and *P. lilacinus* in nematode inoculated plants significantly (p=0.01) increased the number of nodules per root system. In comparison to *M. incognita* inoculated plants (MI) the highest and greater enhancement in nodulation was noted in the treatments where both the bioagents were applied concomitantly with nematode inoculated plants (MI+R+PL) followed by where root nodule bacteria were applied with the nematode (MI+R) and lastly where the egg parasitic fungus was inoculated with the nematode (MI+PL). Individually, *Rhizobium* caused more increase in
nodulation than caused by \textit{P. lilacinus} in \textit{M. incognita} inoculated plants (Table 5 A, Fig. 5).

When compared with control (C), the individual applications of biocontrol agents in nematode inoculated lentil plants exhibited significantly (p=0.05) less increase in the nodulation per root system. \textit{Rhizobium} alone along with nematode inoculated plants (MI+R) showed significantly (p=0.05) more increase in nodule number per root system as compared to the \textit{P. lilacinus} alone with the nematode (MI+PL). Incorporation of both the biocontrol agents simultaneously with nematode (MI+R+PL), resulted in higher increase in the number of nodules per root system, which was significantly (p=0.01) more as that of control (Table 5 A, Fig. 5).

\textbf{Application of \textit{Rhizobium} and \textit{Paecilomyces lilacinus} in lentil plants inoculated with the root-rot fungus, \textit{Macrophomina phaseolina}.}

Data (Table 5 A), revealed that in comparison to control (C), significant (P=0.01) reductions in the number of nodules per root system were noticed in lentil plants, inoculated with the root-rot fungus, \textit{M. phaseolina}. The decrease in nodulation in the fungal inoculated plants were also found significant (p=0.05) as compared to \textit{M. incognita} lentil plants (MI) (Fig. 5).

Soil application of both the biomanagement organisms significantly (p=0.01) increased the number of nodules per root system in \textit{M. phaseolina} inoculated plants. Highest increase in nodulation in fungus inoculated plants (MP) was observed in the plants which were inoculated with \textit{Rhizobium} and \textit{P. lilacinus} concomitantly (MP+R+PL) followed by individual application of \textit{Rhizobium} (MP+R) and \textit{P. lilacinus} (MP+PL) with \textit{M. phaseolina} (Table 5 A, Fig. 5).

\textit{Rhizobium} alone with \textit{M. phaseolina} (MP+R) significantly (p=0.05) increased the number of nodules, as compared to when \textit{P. lilacinus} alone was treated with fungal inoculations (MP+PL). Both the biomanagement organisms,
resulted in higher overcome in the number of nodules which was slightly less, but non-significant when compared with control (Table 5 A, Fig. 5).

Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the combination of *Meloidogyne incognita* and *Macrophomina phaseolina*.

Significant (p=0.01) and highest reductions in the number of nodules per root system were encountered in the lentil plants inoculated with both the pathogens concomitantly (MI+MP), when compared with control (C), and then when compared with the individual inoculated plants with nematode (MI) and the fungus, *M. phaseolina* (Table 5 A, Fig. 5).

Data (Table 5 A) revealed that biocontrol agents, individually as well in combination, significantly (p=0.01) increased the number of nodules per root system in lentil plants also treated with both the pathogens i.e. MI+MP+R+PL. Highest number of nodules, were noted where both the bioagents were applied with the pathogens (MI+MP+R+PL), followed by individual applications of *Rhizobium* (MI+MP+R) and *P. lilacinus* (MI+MP+PL) along with the pathogens, with respect to control. *Rhizobium* again showed better results than *P. lilacinus* individually in the enhancement of nodulation with the pathogens (Fig. 5).

The number of nodules per root system in individually treated biocontrol agents with both the pathogens showed significantly less improvement as compared to control, but the combined application of *Rhizobium* and *P. lilacinus* in pathogen inoculated plants resulted in higher increase which is slight less, but non-significant in comparison to control (Table 5 A, Fig. 5).
Total Chlorophyll Contents

Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-knot nematode, *Meloidogyne incognita*:

Data (Table 5 B) revealed that in comparison to control (C), significant (p=0.05) reductions in total chlorophyll content (chlorophyll a and b) was observed in the plants inoculated with the nematode, (MI) individually (Fig. 7).

Inoculation of *Rhizobium* and *P. lilacinus* alone and in combination increased the total chlorophyll content of nematode inoculated plants. The highest and significant (p=0.05) increase in chlorophyll contents, were observed where both *Rhizobium* and *P. lilacinus* were applied with *M. incognita* inoculation (MI+R+PL), followed by where nematode is inoculated with individual inoculations of *Rhizobium* (MI+R) and *P. lilacinus* i.e. MI+PL (Table 5 B, Fig. 7).

The data presented in the table 5 B, showed that the single inoculation of *Rhizobium* and *P. lilacinus* in nematode inoculated plants, encountered an increase in chlorophyll contents, as compared to control. The *Rhizobium* alone (MI+R), resulted in significantly (p=0.05) more increase than *P. lilacinus* alone. The bio-agents simultaneously showed significantly (p=0.05) higher increase in total chlorophyll content of lentil plants in comparison to control (Fig. 7).

Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-rot fungus, *Macrophomina phaseolina*.

In comparison to control (C), significant (p=0.05) decrease in the total chlorophyll contents were observed in the lentil plants inoculated with the root-rot fungus, *M. phaseolina* (Table 5 B, Fig. 7).

Significant (p=0.01) increase in total chlorophyll contents in fungus inoculated plants was noticed when the *M. phaseolina* inoculated plants were
treated with the bio-agents singly and concomitantly. Highest and more significant (p=0.01) increase in the chlorophyll content was encountered where both the biocontrol agents i.e. *Rhizobium* and *P. lilacinus* were inoculated as soil applications along with pathogenic fungus, *M. phaseolina* (MP+R+PL) and than in the individual treatment of the biomanagement organisms with the root-rot fungus i.e. MP+R and MP+PL. The root-nodule bacteria, *Rhizobium* alone (MP+R) resulted in more increase in the total chlorophyll contents than *P. lilacinus* alone (MP+PL) (Table 5 B, Fig. 7).

The data (Table 5 B), revealed that in comparison to control, the individual inoculation of *Rhizobium* and *P. lilacinus* in the *M. phaseolina* inoculated lentil plants exhibited significant (p=0.05) increase in the total chlorophyll contents. *Rhizobium* alone showed better results than *P. lilacinus* in overcoming the total chlorophyll contents in fungus inoculated lentil plants. The combination of the bio-agents in *M. phaseolina* inoculated plants, resulted in highest increase in the total chlorophyll contents which was significantly (p=0.01) more as that of control (Fig. 7).

**Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the combination of Meloidogyne incognita and Macrophomina phaseolina.**

The root-knot nematode, *M. incognita* and root-rot fungus, *M. phaseolina* in combined inoculations (MI+MP), resulted in significantly (p=0.01) higher loss in the total chlorophyll contents, as compared to control (C) and then when compared with the individual inoculations of nematode (MI) and fungus MP (Table 5 B, Fig. 7).

Data presented in table (5 B) revealed that *Rhizobium* and *P. lilacinus* individually and concomitantly increased total chlorophyll content significantly (p=0.01) in pathogen inoculated plants. Greater and more (significant) increase in the plants were noticed where both the bio-agents were also present along
with both the pathogens i.e., *M. incognita* and *M. phaseolina* (MI+MP+R+PL) as soil applications followed by where only *Rhizobium* was inoculated with the combination of pathogens (MI+MP+R) and then *P. lilacinus* inoculation along with *M. incognita* and *M. phaseolina* (MI+MP+PL) (Fig. 7).

The combined application with both the biocontrol agents in pathogen inoculated plants (MI+MP+R+PL) showed significantly (p=0.05) more increase in the chlorophyll contents, when compared with the control and the individual application of bio-agents with the combination of nematode as well as fungus (Fig. 7).

**NPK Contents**

**Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-knot nematode, *Meloidogyne incognita*:**

Inoculation of *M. incognita* caused significant (p=0.05) reductions in nitrogen, phosphorus and potassium (NPK) contents over uninoculated control (Table 5 B, Fig. 8).

Data (Table 5 B) revealed that inoculation of *Rhizobium* and *P. lilacinus* alone with nematode caused a significant (p=0.05) increase in NPK contents. Root nodule bacteria alone with *M. incognita* (MI+R) caused more increase in nitrogen, phosphorus and potassium contents than *P. lilacinus* in nematode inoculated (MI+PL) lentil plants. Moreover, inoculation of lentil plants with *Rhizobium* and *P. lilacinus* together, significantly (p=0.01) increased the NPK contents of *M. incognita* inoculated plants (MI+R+PL) (Fig. 8).

The data (Table 5 B) revealed that the individual applications of the *Rhizobium* and *P. lilacinus* in *M. incognita* inoculated plants, observed an increase in the nitrogen, phosphorus and the potassium contents, as compared to control. The *Rhizobium* alone caused more increase in NPK contents than caused by *P. lilacinus*. The concomitant presence of both the biocontrol agents with nematode showed higher increase in the NPK contents of the plants. The
NPK contents were found to be significantly (p=0.05) more in comparison to control plants, where both the bio-agents were applied along with the root-knot nematode, *M. incognita* (Fig. 8).

**Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-rot fungus, *Macrophomina phaseolina*.**

Significant (p=0.05) decrease in the NPK contents was noticed in lentil plants inoculated with the root-rot fungus, *M. phaseolina* (MP), when compared with control i.e. C (Table 5 B, Fig. 8).

Application of *Rhizobium* and *P. lilacinus* alone also caused a significant (p=0.05) increase in NPK contents of *M. phaseolina* inoculated lentil plants, but root nodule bacteria individually (MP+R) caused more increase in the nitrogen content than *P. lilacinus* alone in fungus inoculated (MP+PL) plants. Both the biocontrol agents together (MP+R+PL), significantly (p=0.01) improved the NPK contents of fungus inoculated plants i.e. MP+R+PL (Table 5 B, Fig. 8).

The data presented in table 5 B, showed that individual application of the *Rhizobium* and *P. lilacinus* in fungus inoculated plants exhibited more but non-significant increase in NPK contents as compared to control. The rhizobia alone again showed better performance in overcoming the NPK contents than *P. lilacinus* in *M. phaseolina* infected plants. The concomitant presence of the biocontrol agents in fungus inoculated lentil plants, resulted in higher increase in the NPK contents, which were significantly (p=0.01) more as compared to control plants (Fig. 8).

**Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the combination of *Meloidogyne incognita* and *Macrophomina phaseolina*.**

The root-knot nematode (*M. incognita*) and the root-rot fungus (*M. phaseolina*) in combined inoculations (MI+MP) resulted in significantly
highest loss to nitrogen, phosphorus and potassium contents, in comparison to control and also when compared with the individual inoculations of nematode (MI) and fungus i.e., MP (Table 5 B, Fig. 8).

Data presented in table 5 B showed that inoculation of *Rhizobium* and *P. lilacinus* alone significantly (p=0.05) improved the nitrogen, phosphorus and potassium contents in the plants where both the pathogens were inoculated together. *Rhizobium* alone with the combination of pathogens (MI+MP+R) showed better overcome in nitrogen content than inoculation of *P. lilacinus* along with both the pathogens together (MI+MP+PL). Moreover, in combined inoculation, both the biocontrol agents together with the pathogens (MI+MP+R+PL) significantly (p=0.01) increased NPK contents when compared with the plants which were inoculated only with *M. incognita* and *M. phaseolina* in combination (MI+MP) (Fig 8).

The combined treatment with the *Rhizobium* and *P. lilacinus* in pathogens inoculated plants resulted more increase in NPK contents, but non-significantly (p=0.05) less as compared to control (Fig. 8).

**Galling and Egg Mass Production**

**Application of *Rhizobium* and *Paecilomyces lilacinus* in lentil plants inoculated with the root-knot nematode, *Meloidogyne incognita*:**

Data presented in table 5 C, revealed that galling had occurred only in *M. incognita* inoculated plants. The number of galls and egg masses were high in nematode inoculated *Lens culinaris* plants. In presence of root-rot pathogen, *M. phaseolina* root galling and egg mass production was found to be significantly (p=0.01) reduced (Fig. 9).

Inoculation of *Rhizobium* and *P. lilacinus* alone and in combination caused more significant (p=0.01) reduction in root galling and egg mass number in nematode inoculated plants. In comparison to the *M. incognita*
inoculated plants, highest decrease in galling and egg mass production was recorded where the nematode inoculated plants were also inoculated with the combination of Rhizobium and P. lilacinus (MI+R+PL) followed by where the biocontrol organisms were inoculated individually along with nematode i.e. MI+R and MI+PL (Table 5 C, Fig. 9).

Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the combination of Meloidogyne incognita and Macrophomina phaseolina.

Data (Table 5 C) revealed that in comparison to lentil plants inoculated with both root-knot and root-rot pathogens concomitantly (MI+MP), a significant (p=0.01) decrease in gall and egg mass number per root system was observed in plants which were also inoculated with Rhizobium (MI+MP+R) and P. lilacinus alone (MI+MP+PL) along with the combination of both pathogens. However, the inoculation of both the biomanagement organisms along with the combination of pathogens (MI+MP+R+PL) further reduced galling as well as the egg mass production as compared to the plants which were inoculated with the combination of pathogens (MI+MP) as well as the individual applications of the bio-agents with M. incognita and M. phaseolina (P-53 and 54, Fig 9).

Nematode Multiplication

Application of Rhizobium and Paecilomyces lilacinus in lentil plants inoculated with the root-knot nematode, Meloidogyne incognita

Data presented in table 5 C, showed that total nematode multiplication, reproduction factor (RF) and rate of population increase (RPI) was high in plants where M. incognita was inoculated alone (MI). Presence of root-rot fungus, M. phaseolina (MI+MP) significantly (p=0.01) reduced the nematode multiplication and development (RF and RPI) (Fig 10).
Inoculation of *Rhizobium* and *P. lilacinus* alone and in combination further reduced the nematode population to a great extent. The greater and significant reduction in nematode multiplication (RF and RPI) was observed where both the bio-agents were applied with nematode inoculation i.e. MI+R+PL, followed by individual inoculations of *Rhizobium* (MI+R) and *P. lilacinus* (MI+PL) when compared to the nematode inoculated plants i.e. MI (Table 5 C, Fig. 10).

**Application of Rhizobium and Paecilomyces lilacinus in the lentil plants inoculated with the combination of Meloidogyne incognita and Macrophomina phaseolina**

The nematode population and multiplication (RF and RPI) were found to be further reduced in plants where *M. incognita* plus *M. phaseolina* were also inoculated with the bio-agents alone and together. Inoculation of *Rhizobium* and *P. lilacinus* significantly (p= 0.01) reduced the nematode population and multiplication in pathogens inoculated plants (MI+MP+R+PL) followed by rhizobial (MI+MP+R) and *P. lilacinus* (MI+MP+PL) alone (Table 5 C, Fig. 10).

**Root-Knot Index (RKI)**

Root-knot index was 4 when the plants were inoculated with *M. incognita* (MI) alone and in combination with root-rot fungus, *M. phaseolina* (MI+MP). The Index was reduced to 3 when the nematode inoculated plants were further inoculated with the individual application of rhizobia (MI+R) and *P. lilacinus* i.e. MI+PL. Root-knot index was further reduced 2 when the *M. incognita* inoculated plants were treated with both the biomanagement organisms together i.e. MI+R+PL (Table 5 C). Data (Table 5 C) showed that the index was found to be 3 when *M. phaseolina* plus *M. incognita* plants were treated with *Rhizobium* or *P. lilacinus*. Index reduced to 2 when pathogen
inoculated plants were treated with *Rhizobium* plus *P. lilacinus* (MI+MP+R+PL).

**Root-Rot Index (RRI)**

Data presented in table 5 C showed that root-rot index was 2 where the lentil plants were inoculated with *M. phaseolina* only. Index was increased to 4 when the plants were inoculated with root-rot fungus plus *M. incognita* (MI+MP). The Index was reduced to 1 when *M. phaseolina* inoculated plants were also inoculated with *Rhizobium* (MP+R) as well as *P. lilacinus* (MP+PL). Index was reduced to 0 when the fungus inoculated plants were treated with the bio-agents in combination (MP+R+PL). Index was 3 when *M. incognita* plus *M. phaseolina* were treated with either *Rhizobium* or *P. lilacinus* alone. Root-rot index was reduced to 1 when pathogen inoculated plants were also treated with the combination of both biocontrol organisms i.e. MI+MP+R+PL (Table 5 C, Fig. 11).

**DISCUSSION**

Inoculation of lentil seedlings with *M. incognita* and *M. phaseolina* alone and in combination caused significant reductions in plant growth and yield parameters, nodulation per root system, and chlorophyll as well as NPK contents of plant leaves when compared with the uninoculated control (C). Simultaneous inoculation of both the pathogens (MI+MP) increased the severity of root-knot and root-rot disease complex, resulting in great reductions in all the parameters studied, as compared to the individual inoculation of nematode (N) and fungus (MP).

Application of root-nodule bacteria, *Rhizobium* and egg parasitic fungus, *P. lilacinus* alone and in combination significantly overcame the plant growth and yield parameters in nematode and fungus inoculated *L. culinaris* plants singly and concomitantly (MI+MP). Highest and more significant enhancement in plant growth and yield characteristics in pathogen inoculated plants (MI,
MP, and MI+MP) were recorded when both the biocontrol agents were inoculated simultaneously along with the pathogens singly (MI, MP) as well as in the combination (MI+MP). Similarly the total chlorophyll and NPK contents of pathogen treated lentil plants increased by the incorporation of biocontrol fungus and bacteria. *Rhizobium* treatments resulted in reduced damage and enhanced the growth and yield characters of lentil plants might be attributed to the root-nodule bacteria that produced anti-pathogenic substances affecting nematode multiplication deleteriously (Siddiqui and Mahmood, 1994). Nitrogen fixation by the *Rhizobium* improve the nitrogen status of soil and also had greater colonization and siderophore production which showed high potential in suppressing the root-knot nematode, *Meloidogyne* sp. or produced metabolites that were toxic to the nematodes (Siddiqui *et al.*, 2007).

It is evident from the results that the galling and egg mass production in *M. incognita* inoculated plants were reduced due to the presence of root-rot pathogen *M. phaseolina*. Presence of *Rhizobium* and *P. lilacinus* alone and in combination caused enhanced reductions in root galling and egg mass number in nematode inoculated plants. Moreover, nematode reproduction, multiplication, and development reduced with the concomitance of *M. phaseolina*. Treatment with *Rhizobium* and biocontrol fungus *P. lilacinus* alone and in combination further reduced the nematode population development and root-knot index (RKI) to a great extent. Root-rot index (RRI) was found increased in fungus inoculated plants by the addition of root-knot nematode juveniles. Incorporation of both the biocontrol agents in pathogen (MP) and pathogens (MI+MP) inoculated plants reduced the severity of root-rot disease complex. Barker and Huisingh (1970), observed necrosis in nodular tissue following invasion by nematodes, this in part, account for reduced nematode multiplication and development. The root-nodule bacteria, *Rhizobium* also produced an antibiotic namely, Bacteriocin which had an adverse affect on
root-knot severity, egg hatching and disease development (Roslycky, 1967). Reduced galling and nematode multiplication by the *Rhizobium* also reported by Siddiqui *et al.*, (2007).

*Rhizobium* instead of nitrogen fixation, produced some toxic metabolites, possessed antifungal affects (Haque and Ghaffar, 1993). Rhizobitoxine secreted by rhizobia also contains antifungal substances which decreased the root-rot severity of all fungal inoculated plants thereby improving the plant growth, yield parameters, total chlorophyll content, and nitrogen, phosphorus and potassium (NPK) contents of lentil as has also been reported by Chakraborty and Purkayastha, (1984). Rhizobia significantly inhibited the growth of root-rot fungus, *M. phaseolina*, thus reducing the root-rot percentage and root-rot index (RRI), that had been reported on *Rhizoctonia solani* and *Fusarium solani*, in leguminous and some non-leguminous plants (Ehteshamul-Haque *et al.*, 1992; Ehteshamul-Haque and Ghaffar, 1993 and Omar and Abd-Alla, 1998). This might be attributed due to the influence of rhizobial, antifungal compounds on fungal colonies (Khokhar *et al.*, 2001). *Rhizobium* present in the rhizosphere of lentil plants presumably prevent the contact of pathogenic root infecting fungus by covering hyphal tips and by parasitizing it, and the antibiotics produced by rhizobia resulting in lysis of the fungal hyphae (Tu, 1978; Malajezuk *et al.*, 1984). *Rhizobium* has also been reported to produce growth regulators such as auxins, cytokinins and gibberellins that enhance plant growth (Triplett *et al.*, 1981).

The biocontrol fungus *Paecilomyces lilacinus* also checked multiplication and development of root-knot nematode, *Meloidogyne incognita*, checked their population and ultimately enhanced growth of affected lentil plants. The growth and yield characters of *L. culinaris* were found increased when nematode infected plants were treated with biocontrol fungus, *P. lilacinus*. Incorporation of *P. lilacinus* incorporation into the soil improved the
plant growth, increased nodulation and seed yield. In nematode infected lentil plants, the biocontrol fungus \textit{(P. lilacinus)}, developed around the eggs, inside the roots and inside the mature females. The hyphae of the fungus colonized on the outer surface of root and penetrated into the inner tissue. Reduced egg mass number which might be attributed to comparatively fewer number of juveniles penetration into the roots, or due to suppression of secondary infection, or due to parasitization of mature females by \textit{P. lilacinus}. The biocontrol fungus, \textit{P. lilacinus} has also been reported damaging the eggs and egg masses by Niyaz and Hisamuddin, (2009) and Bhat \textit{et al.}, (2009). Khan and Williams (1998) found \textit{P. lilacinus} entering into the body of mature females of \textit{M. incognita} through natural openings. A check in the root knot-disease on tomato by \textit{P. lilacinus} has also been reported (Cabanillas and Barker, 1989; Goswami and Sharma 2001) and Dhawan \textit{et al.}, (2004) in okra. Significant reduction in root-knot nematode population in various plants by \textit{P. lilacinus} was reported by Walia \textit{et al.}, (1991) and Khanna (2000).

The fungus \textit{P. lilacinus} show diverse modes of habits. Basically it is a saprophyte (Domusch \textit{et al.}, 1980) and can easily be grown on artificial culture media. At one time it acts as an epiphyte and grows on the surface of plant roots (Cabanillas \textit{et al.}, 1988) and at other times it grows inside the root tissue and behaves as an endophyte without causing any damage to the plant. Still at other times it parasitizes eggs and egg masses of \textit{Meloidogyne} sp. and destroys them. Because of this property the fungus, \textit{P. lilacinus} has been used, by several workers, as a biocontrol agent against root-knot and other nematodes (Morgan Jones and Rodriguez-Kabana, 1984).

In our studies the biocontrol fungus, \textit{P. lilacinus} which is used to parasitize the eggs and egg masses of root-knot nematodes was also found to inhibit the growth of root-rot pathogen, \textit{M. phaseolina} by reducing the root-rot severity and thus improving plant growth and yield characters. This has been
previously observed in an *in-vitro* study (Shahzad and Ghaffar, 1989) and also in the field experiments on sunflower, okra, soybean and mungbean (Ehteshamul-Haque *et al*., 1990).

The biocontrol fungus, *P. lilacinus* has a great competitive sporophytic ability and the fungus can be found up to a depth of 30 – 40 cm. (Saksena, 1955). It is possible that bio-agent which ultimately reached to soil and multiplied around root zone and thus inhibit and reduce pathogenic fungi.

Mostly single biocontrol agent was used by various worker as antagonist against single or combination of pathogens, but single biocontrol agent is not likely to be active in all soil environments in which they are applied or against all pathogens that attack the host plant. Consequently, application of a mixture of introduced biocontrol agents would more closely imitate the natural situation and might broaden the spectrum of biocontrol activity, thus enhancing the efficacy and reliability of control.

Integration of two or more biocontrol agents against single or combination of pathogens provides better results than their separate use. Rhizobia which are known to produce rhizobitoxin have shown promising results in the control of the root-rot fungus *M. phaseolina* and the root-knot nematode, *M. incognita* as reported earlier on mungbean (Siddiqui *et al*., 1998) and on okra (Siddiqui *et al*., 2000).

*Rhizobium* and *P. lilacinus* singly and concomitantly showed promising results in the control of root-knot and root-rot pathogens in individual (MI, MP) and combined (MI+MP) inoculations. All these results might be attributed to biocontrol agents which have different mechanisms involving in the suppression of different plant diseases and the disease complexes as a result of root-knot and root-rot infection i.e. inhibition of pathogens by antimicrobial substances (antibiosis) (El-Mehalawy, 2004); or production of diverse microbial metabolites like siderophore and rhizobitoxin (Deshwal *et al*., 2003);
competition for nutrients supplied by seeds and roots and colonization sites; induction of plant resistant mechanisms; inactivation of pathogen germination factors present in seed and root exudates and degradation of pathogenicity factors of the pathogen/s such as toxins; parasitism that may involve production of extracellular cell wall degrading enzymes, i.e. chitinase that can lyses pathogen cell wall (El-Mehalawy, 2004), or plant growth enhancement through IAA and other growth regulators (Deshwal et al., 2003).

From this study it can be deduced that the root-nodule bacterium, *Rhizobium* alone was proved to be the effective biocontrol agent as that of *P. lilacinus* on lentil plants. The *Rhizobium* showed more antagonistic effect against *M. incognita* juveniles and *M. phaseolina* when added with the biocontrol fungus *P. lilacinus*. *Rhizobium* and *P. lilacinus* in the rhizosphere of *Lens culinaris* represent a potential reservoir of biological control that can be used to challenge nematode multiplication and root infecting fungi. The compatibility of these bio-agents needs to be evaluated for using such combination in the field.

Biological control is considered a new and efficient method for controlling plant parasitic nematodes as well as pathogenic fungi with an aim to decrease the extent of environmental degradation and effect of excessive toxic nematicides and fungicides.