ROOT COLONIZATION AND PERSISTENCE OF *Purpureocillium lilacinum* IN RHIZOSPHERE AS INFLUENCED BY SOME CROP SPECIES AND *Meloidogyne incognita*

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BY

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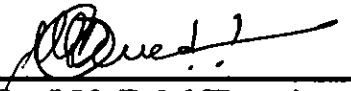

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CERTIFICATE

This is to certify that the thesis entitled, **"ROOT COLONIZATION AND PERSISTENCE OF *Purpureocillium lilacinum* IN RHIZOSPHERE AS INFLUENCED BY SOME CROP SPECIES AND *Meloidogyne incognita*"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of *bona fide* research work carried out by **MD. MOSTAQUR RAHMAN** bearing **Registration No. 09-03692** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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Dated: 26.05.2016

Place: Dhaka, Bangladesh

Dedicated To

My

Beloved Parents

List of Abbreviations of Technical Terms and Symbols

Abbreviation	Full Word
et al.	and others (at elli)
BARI	Bangladesh Agricultural Research Institute
Cm^3	Cubic Centimeter
Cm^2	Square Centimeter
Cm	Centimeter
μgcm^{-2}	Microgram/cm^2
^oC	Degree Centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
g	Gram
PDA	Potato Dextrose Agar
HSD	Honest Significant Difference
%	Percent
CRD	Completely Randomized Design
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Viz.	Namely
BCA	Bio Control Agent
DAI	Days After Inoculation

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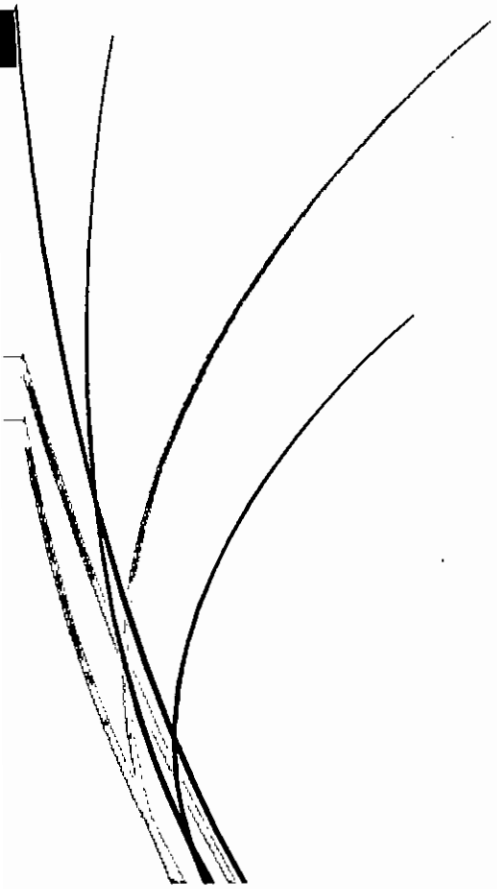
ROOT COLONIZATION AND PERSISTENCE OF *Purpureocillium lilacinum* IN RHIZOSPHERE AS INFLUENCED BY SOME CROP SPECIES AND *Meloidogyne incognita*

ABSTRACT

The effect of ten crop species along with two different rooting media viz. coco dust and soil on the root colonization ability of the fungal BCA *Purpureocillium lilacinum* and its persistence in rhizosphere of the ten crop species along with the presence or absence of nematode were evaluated through a test tube experiment in laboratory and a pot experiment in shade house. *P. lilacinum* resulted in root colonization in all of the crop species in varying percentage depending on the rooting media and the crop species themselves. A constant 100% and an average of 45.30% root colonization were obtained in coco dust and in soil, respectively. In soil, a maximum and a minimum root colonization was observed in cucumber (67.17%) and chickpea (30.55%), respectively; whereas maize (55.50%), potato (50%), brinjal (48.14%), cabbage (44.28%), rice (44.26%) and tomato (43.45%), wheat (37%), soybean (32.07%) gave statistically similar result to cucumber and chickpea, respectively. Population dynamics of the fungus showed no significant difference between soil without crop species and soil from the root zone of majority of the test crop species. Overall, 8 out of 10 crop species showed higher densities compared to soil. Of them, 3 (maize, brinjal, soybean) showed significantly higher CFU/g of soil whereas the rest 5 (rice, wheat, potato, cucumber, chickpea) showed insignificantly higher CFU/g of soil and conversely, 2 (tomato and cabbage) showed significantly lower CFU/g of soil in the rhizosphere compared to soil. Both the crop species (in most cases) and the nematode population did not exert significant effect whereas time was the factor to have an obvious effect on the population densities of *P. lilacinum*. The reduction in CFU/g of soil compared to initial densities ranged from 6.5% in brinjal to 14.6% in cabbage, 9.8% in brinjal to 21.8% in cabbage and 17.3% in maize to 32% in tomato at 10, 20 and 30 DAI.

Chapter 1 Introduction



Introduction

The fungus *Purpureocillium lilacinum* is more widely known as *Paecilomyces lilacinus*, having undergone a recent taxonomic revision (Luangsa-ard *et al.*, 2011). *Purpureocillium lilacinum* Samson is an egg pathogenic fungus, attacking mainly sedentary stages of root-knot and cyst nematodes and considered one of the promising and practicable biological control agents for the management of plant parasitic nematodes (Jatala, 1986; Siddiqui and Mahmood, 1996). Promising report of its biocontrol capacity against root-knot, cyst and burrowing nematode was reported under field condition (Stirling, 1991). It has also the potential of using as endophytes for the biological control of aphids and other herbivores considering it as entomopathogenic (Castilla *et al.*, 2014). *P. lilacinum*, a saprophytic soil fungus has drawn many research attentions due to its promising effect in parasitizing and controlling population of phytonematodes (Jatala, 1986; Dube and Smart, 1987; Hewlett *et al.*, 1988; Freitas *et al.*, 1995; Nagesh *et al.*, 1997; Khan *et al.*, 2006; Kiewnick and Sikora, 2006 a; Brand *et al.*, 2010). It has a high frequency of occurrence in the tropics and subtropic (Morgan *et al.*, 1984; Chen *et al.*, 1996, Atkins *et al.*, 2005) and can be found in most of agricultural soils (Brand *et al.*, 2010). It has been recognized as a common egg pathogenic fungus of root-knot and cyst nematodes (Rumbos and Kiewnick, 2006). Besides, *P. lilacinum* has high adaptability in its life strategy, making it competitive in a broad spectrum of range adaptability. It can tolerate wide range of soil pH and able to grow well at 15 -30°C. Cabanillas *et al.* (1989) observed maximum growth of *P. lilacinum* at temperature from 24-30°C and reported its ability to grow and compete for a wide range of common substrate in soil.

Nematodes were noted early in human history because some serious human diseases are caused by relatively large vertebrate-parasitic nematodes. Some of these nematodes were first described in the ancient Chinese scientific literature as early as 2700 B.C. (Maggenti, 1981). Since plant parasitic nematodes often are small and subterranean, there are not many ancient references to phytoparasitic nematodes. One interesting observation suggests that phytoparasitic nematodes were known in antiquity (235 B.C.) because the ancient Chinese symbol for a soybean root-infesting organism resembles in shape an adult female soybean cyst nematode (Noel, 1992). The first described plant parasitic nematodes were discovered in wheat seeds by Needham (1743).

Plant-parasitic nematodes (PPN) cause high yield losses annually worldwide in many important crops like rice, wheat, barley, maize, banana and vegetables (Ferraz and Brown, 2002). Rice, wheat, maize, potato, soybean, chickpea, tomato, brinjal, cabbage and cucumber- all are important crops to meet up the nutrient requirements of the people of Bangladesh and in Bangladesh some of them (Rice, wheat, maize, potato) are notified crops as well. So a successful production of these crops is very important especially for the notified crops. However, all these crops are infected by PPNs and cause significant yield losses. Rice can be infected by the white tip nematode (*Aphelenchoides besseyi*), the rice stem nematode (*Ditylenchus angustus*), the root nematode (*Hirschmanniella* spp.) and the rice cyst nematode (*Heterodera oryzae*). In wheat, the nematode species causing diseases are cereal cyst nematode, (*Heterodera avenae*), the seed gall or ear-cockle nematode (*Anguina tritici*), root-knot and the lesion nematodes. In maize, the most economically important nematode species are the corn cyst nematode (*Heterodera zeae*). In potato, potato cyst nematodes (*Globodera* spp.) are most important and other nematode species are root-knot (*Meloidogyne* spp.), false root-knot (*Nacobbus*), bulb and stem (*Ditylenchus dipsaci*), potato-rot (*Ditylenchus destructor*) and lesion nematodes (*Pratylenchus*). In soybean, the soybean cyst nematode is the most

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serious pest of soybean throughout the world. In tomato and brinjal, the root-knot nematode (*Meloidogyne* spp.) is the most serious pest. For chickpea, the cyst nematode (*Heterodera ciceri*), also known as chickpea cyst nematode, is one of the most aggressive parasites of food legumes, such as chickpea and other nematode species are the dirty root or reniform nematode (*Rotylenchulus reniformis*), root-knot nematodes (*Meloidogyne* spp.), root lesion nematode (*Pratylenchus* spp.). In cabbage, the important nematode species are the cyst nematodes (*Heterodera* spp.), lesion nematode (*Pratylenchus pratensis*), pin nematode (*Paratylenchus* spp.), root-knot nematodes (*Meloidogyne* spp.) and sting nematodes (*Belonolaimus* spp.). In cucumber and other cucurbits, the most important nematode species is the root-knot nematode (*Meloidogyne* spp.) (Zafar, 1998).

The losses of crops due to pathogens ranges between 16 and 18% (Oerke, 2006). Of this, on a worldwide basis, annual crop losses due to nematode damage have been estimated to average 12.3 percent (Sasser and Freckman, 1987), amounting to some US$77 million annually. The estimated losses for vegetable crops due to nematode-related disease complexes in Egypt amounted to some 15 percent in 1986, with losses for field crops ranging from 5 to 20 percent (Eissa, 1988). There was an observation of a 23% yield losses ranged from 2% for cabbage to 45% for squash, which is 35%, 80%, and 46% higher compared to developed countries, USA, and India, respectively (Anwar and McKenry, 2012). Root-knot nematodes cause annual losses of about USD $100 billion worldwide (Brand *et al.*, 2010).

The use of soil fumigation to reduce nematode populations and increase crop yields in the 1940's (Carter) demonstrated that nematodes were significant crop pathogens and ushered in the "chemical era" for nematode management in production agriculture (Maggenti, 1981). But the loss of important non-fumigant nematicides and the phase out of the fumigant methyl bromide, which until recently have been considered panacea for nematode control, has led to more holistic integrated nematode management approaches (Sikora *et al.*, 2005). Nematicides, fumigants or

non-fumigants, are combined with physical and cultural methods, like solarization and soil heating, flooding, fallow, crop rotation with non-hosts, tolerant or resistant cultivars, incorporation of organic amendments to suppress nematode densities (Sikora and Fernandez, 2005). Public demand for more environment friendly approaches in agriculture has provided a strong impetus for the exploitation of microbial agents for the biological control of plant-parasitic nematodes (Sikora, 1992; Kerry, 2000). Soil microorganisms, like bacteria, fungi, actinomycetes, viruses and rickettsia are a valuable resources of microbial control agents of PPNs that has only been partially exploited and needs to be further investigated. Nematophagous fungi are natural enemies of nematodes, an essential component of the nematode antagonistic microflora and comprise many potential biological control agents of PPNs. They consist of three main groups of fungi: i) the nematode-trapping or predatory fungi, ii) the endoparasitic fungi and iii) the facultative parasites or opportunistic fungi (Siddiqui and Mahmood, 1996). Among them *P. lilacinum* as a BCA for nematodes is promising and practicable one.

However, understanding dynamics of a BCA is important for predicting the success of biocontrol (Bidochka, 2001). Plants have a profound effect on the impact of this microflora on the regulation of nematode populations by influencing both the dynamics of the nematode host and the structure and dynamics of the community of antagonists and parasites in the rhizosphere (Lynch, 1990; Kerry, 2000). In general, those organisms that have a saprophytic phase in their life cycle are most affected by environmental conditions in the rhizosphere, but effects on obligate parasites have also been recorded. Although nematodes influence the colonization of roots by pathogenic and beneficial microorganisms, little is known of such interactions with the natural enemies of nematodes in the rhizosphere. As nematodes influence the quantity and quality of root exudates, they are likely to affect the physiology of those microorganisms in the rhizosphere; such changes may be used as signals for nematode antagonists and parasites.

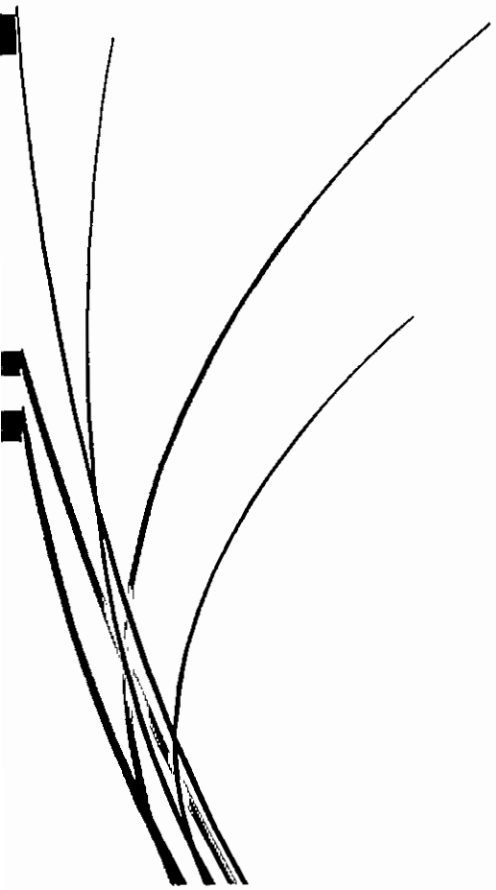
Successful biological control strategies will depend on a thorough understanding of these interactions at the population, organismal, and molecular scale (Kerry, 2000). Many BCAs require a certain population density to control pests or diseases (Paulitz, 2000). For a *Purpureocillium* strain PL251, it has been demonstrated that a threshold level of 10^6 cfu g^{-1} of soil is needed for biocontrol (Kiewnick and Sikora, 2003), consequently it is critical to determine the factors that influence the survival of the biocontrol agent. Better knowledge of the fate and behavior of an introduced microorganism in the environment is also critical for the appropriate assessment of potential side effects (Vestergaard *et al.*, 2004). Establishment of the biocontrol agent in the applied area only for the period necessary for the control of the pathogen or pest is desirable in order to minimize the risks that may derive from its application. Longer persistence of the biocontrol agent would increase the exposure and the possibility of unwanted side effects (Cook *et al.*, 1996). In the case of *P. lilacinum*, controversial studies have been presented concerning its interaction with the host plant. Cabanillas *et al.* (1988) reported that a particular strain of *P. lilacinum* was able to colonize the root tissue epiphytically and endophytically when excised tomato roots were challenged with the fungus. However, Holland *et al.* (2003) did not detect any hyphae of a *P. lilacinum* strain PL251 within roots of eight crops.

Limited information is at present available concerning the environmental fate of *P. lilacinum* if applied as an inundation BCA to the soil and even less about the parameters that have an impact on its survival. More importantly, interactions of *P. lilacinum* with the host plant are poorly understood. Therefore, present study was carried out to determine the biotic and abiotic factors that affect the population dynamics of the fungus with the following objectives:

I. To determine the root colonization ability of *Purpureocillium lilacinum* as influenced by some crop species and rooting media.
II. To determine the persistence of *Purpureocillium lilacinum* in rhizosphere as influenced by some crop species and *Meloidogyne incognita*.

Chapter 2

Review of Literature



Review of Literature

Cabanillas *et al.* (1988) examined histological interactions of the fungus *Paecilomyces lilacinus* and *Meloidogyne incognita* race 1 on tomato roots. Few to no galls and no giant-cell formation were found in roots dipped in a spore suspension of *P. lilacinus* and inoculated with *M. incognita*. Numerous large galls and giant cells were present in roots inoculated only with *M. incognita*. *P. lilacinus* colonized the surface of epidermal cells as well as the internal cells of epidermis and cortex and gives protection of plant surfaces against root-knot nematodes.

Hewlett *et al.* (1988) evaluated the efficacy of the nematode parasite *Paecilomyces lilacinus*, alone and in combination with phenamiphos and ethoprop, for controlling the root-knot nematode *Meloidogyne javanica* on tobacco and the ability of this fungus to colonize in soil under field conditions for 2 years in microplots. Plants with *M. javanica* alone or in combination with *P. lilacinus* had galling indices of 5.0 both years; the latter produced lower yields than all other treatments during both years of the study. Nevertheless, the average soil population densities of *P. lilacinus* remained high, ranging from 1.2 to 1.3 x 10^6 propagules/g soil 1 week after the initial inoculation and from 1.6 to 2.3 x 10^4 propagules/g soil at harvest the second year. At harvest the second year the density of fungal propagules was greatest at the depth of inoculation, 15 cm, and rapidly decreased below this level.

Cabanillas *et al.* (1989) conducted laboratory and microplot experiments to determine the influence of carrier and storage of *Paecilomyces lilacinus* on its survival and related protection of tomato against *Meloidogyne incognita*. Spores of

P. lilacinus were prepared in five formulations and observed that fungal viability was high in wheat and granules, intermediate in pellets, and low in soil and chitin-amended soil stored at 25 ± 2° C. In 1985 *P. lilacinus* in field microplots resulted in about a 25% increase in tomato yield and 25% gall suppression, compared with nematodes alone. The greatest suppression of egg development occurred in plots treated with *P. lilacinus* in pellets, wheat grain, and granules. In 1986 carryover protection of tomato against *M. incognita* resulted in about a threefold increase in tomato fruit yield and 25% suppression of gall development, compared with plants treated with nematodes alone. Higher numbers of fungus-infected egg masses occurred in plots treated with pellets (32%) than in those treated with chitin-amended soil (24%), wheat (16%), granules (12%), or soil (7%). Numbers of fungal colony-forming units per gram of soil in plots treated with pellets were 10-fold greater than initial levels estimated at planting time in 1986.

Cabanillas and Barker (1989) conducted microplot experiments to evaluate the effects of inoculum level and time of application of *Paecilomyces lilacinus* on the protection of tomato against *Meloidogyne incognita.* The best protection against *M. incognita* was attained with 10 and 20 g of fungus-infested wheat kernels per microplot which resulted in a threefold and fourfold increase in tomato yield, respectively, compared with tomato plants treated with this nematode alone. The greatest protection against this pathogen was attained when *P. lilacinus* was delivered into soil 10 days before planting and again at planting. Yield was increased twofold compared with yield in nematode-alone plots and plots with *M. incognita* plus the fungus. Percentages of *P. lilacinus*-infected egg masses were the greatest in plots treated at midseason or at midseason plus an early application, compared with plots treated with the fungus 10 days before planting and (or) at planting time.

Gaspard and Ferris (1990) determined population densities of *Meloidogyne incognita* and the nematophagous fungi, *Paecilomyces lilacinus* and *Verticillium chlamydosporium*, in 20 northern California tomato fields over two growing seasons. *Paecilomyces lilacinus* was isolated from three fields, *V. chlamydosporium* was isolated from one field, and both fungi were isolated from 12 fields. *Verticillium chlamydosporium* numbers were positively correlated with numbers of *M. incognita* and *P. lilacinus*. *Paecilomyces lilacinus* numbers were positively correlated with *V. chlamydosporium* numbers, but they did not correlate with *M. incognita* numbers. The correlation coefficients were low ($R < 0.5$) but significant ($P < 0.05$). All *P. lilacinus* and *V. chlamydosporium* field isolates parasitized *M. incognita* eggs *in vitro*. In a greenhouse study, numbers of *V. chlamydosporium* and *P. lilacinus* increased more in soils with *M. incognita*-infected tomato plants than in soil with uninfected tomato plants. After 10 weeks, the Pf/ Pi of second-stage juveniles in soils infested with *P. lilacinus*, *V. chlamydosporium*, and *M. incognita* was 47.1 to 295.6. The results suggested that *V. chlamydosporium* and *P. lilacinus* were not effectively suppressing populations of *M. incognita* in California tomato fields.

Frans (1991) investigated the potential of three *Verticillium chlamydosporium* isolates as biological control agents against *Meloidogyne arenaria* on tomato plants under glasshouse conditions. All three isolates survived well in soil but showed marked differences in their ability to colonize uninfected roots, nematode galls and nematode eggs. Significant population reductions of >80% after the first nematode generation, were achieved with one isolate, which resulted in significant damage control, but not population control, in subsequent generations. Establishment of *V. chlamydosporium* in soil was significantly greater if the fungus was introduced without a foodbase, i.e. as hyphal fragments and chlamydosprore rather than colonized sand-bran. The fungus did not invade the root cortex and there were no adverse effects of the fungus on plant growth.

Gomes Carneiro and Cayrol (1991) studied relationship between inoculums density of *Paecilomyces lilacinus* against *Meloidogyne arenana* on tomato. They incorporated five doses (0.01 - 0.1 - 1 - 10 and 100 g/m^2) of *Paecilomyces lilacinus* isolated from eggs of *Meloidogyne incognita* that applied in a powder formulation (1011 spore/g of product) in a glasshouse pot experiment against large infestations of *Meloidogyne arenana.* The trial was conducted over eleven months on three successive tomato crops. Results showed that the number of fungal propagules in the soil was correlated to the initial dose applied and decreased progressively through the time with increased dose. Populations of *M. arenaria* were significantly reduced by the fungus at 10 and 100 g of spores/m^2 in the second and third nematode generations. The number of colonized egg masses and the number of non-viable eggs increased at a density of 106 spore/g of soil. In the highest level of control (100% colonized egg masses) only 50% of the eggs were parasitized. Twenty-three percent of the larvae remained which constitutes an important residual inoculum potential. This fact and a rapid decrease in fungal density in soil below the acceptable control levels, limit the use of this fungus as a biological control agent.

Manhong *et al.* (1998) showed that *Paecilomyces lilacinus* could colonize in soybean rhizosphere, when it was used as a seed-coat. The number of propagules observed in endorhizosphere in sterilized soils was 1/1000 of the coated fungi at the 1st week. it increased over 10-fold in the 2nd week, and began to decrease after 4 weeks planting. The fungus could also endoparasitize a few in soybean root. In the soils which were sensitive to soybean cyst nematode. The strain multiplied greatly in the 3rd week. The various micro-organisms in soybean rhizosphere were influenced by *P. lilacinus* when it was introduced in natural soils. The numbers of fungi, bacteria and actinomyces reduced by 16%, 27% and 27%, respectively in the 1st week. But after a longer period (4 weeks), all the microbes were as same as the control group.

The experiment was conducted by Siddiqui *et al.* (2000) under laboratory and field conditions to evaluate the efficacy of *Pseudomonas aeruginosa* alone or in combination with *Paecilomyces lilacinus* in the control of root-knot nematode and root-infecting fungi. In field experiments, biocontrol fungus and bacterium significantly suppressed soilborne root-infecting fungi including *Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani* and *Meloidogyne javanica*, the root-knot nematode. *P. lilacinus* parasitized eggs and female of *M. javanica* and this parasitism was not significantly influenced in the presence of *P. aeruginosa. P. aeruginosa* was reisolated from the inner root tissues of tomato, whereas *P. lilacinus* did not colonize tomato roots.

Holland *et al.* (2003) stated that *Paecilomyces lilacinus* is generally considered to be both a soil fungus and a nematode-egg parasitic fungus, it has been reported that it can also colonize roots and protect the root surface from root-knot nematode attack. When eight crop plant species were challenged with *P lilacinus* strain Bioact251, fungal hyphae were never detected within roots, though occasionally colonies arose from the root surface. Examination of the behavior of *P lilacinus* hyphae on root and nematode egg surfaces were compared and found to be very different, with *P. lilacinus* behaving like a parasitic fungus when growing on a nematode egg but not when on a root surface.

Jacobs *et al.* (2003) studied the efficacies of three nematophagous fungi, *Paecilomyces lilacinus, Plectosphaerella cucumerina* and *Pochonia chlamydosporia*, for controlling potato cyst nematodes (PCN) as part of an Integrated Pest Management (IPM) regime. The compatibility of the nematophagous fungi with commonly used chemical pesticides and their ability to compete with the soil fungi *Rhizoctonia solani, Chaetomium globosum, Fusarium oxysporum, Penicillium bilaii* and *Trichoderma harzianum* were tested *in vitro*.

Paecilomyces lilacinus was the most successful competitor when the ability to grow and inhibit growth of an opposing colony at both 10 and 20°C was considered. *P. lilacinus* also showed potential for control of the soil-borne fungal pathogen *R. solani*, *Pochonia chlamydosporia* was least susceptible to growth inhibition by other fungi at 20°C *in vitro*, but the isolate tested did not grow at 10°C. Treatment with *P. lilacinus* significantly reduced the symptoms of *Rhizoctonia* disease on potato stems in a pot trial. The effectiveness of *P. lilacinus* and *P. cucumerina* against PCN was also tested *in situ*. Three application methods were compared; incorporating the fungi into alginate pellets, Terra-Green inoculated with the fungi and applying conidia directly to the tubers. Both formulations containing *P. lilacinus* and formulation mixtures alone, particularly alginate pellets, significantly reduced multiplication of PCN in soil.

Kiewnick *et al.* (2003) conducted dose response experiments with the root-knot nematode *Meloidogyne incognita* on tomatoes using the new *P. lilacinus* WDG formulation. The results revealed a clear correlation between rate applied and the degree of control concerning the reduction in damage to the root and multiplication of the nematode. Best control was achieved by applying the biological nematicide at rates of 2 to 4 times 10^9 conidia per plant as a soil treatment one week before planting. Monitoring the *P. lilacinus* population in the rhizosphere showed a decline after 2 to 3 month which can lead to insufficient control over a full growing season. Repeated application to maintain the antagonist population at a sufficient level could be used to secure long term control of root-knot nematodes.

Brand *et al.* (2004) made attempts to select low-cost substrates for spore production of a strain of *Paecilomyces lilacinus* with known nematicide capacity. Coffee husks, cassava bagasse, and defatted soybean cake were utilized as substrates, and sugarcane bagasse was used as support. Fermentations were carried out in flasks covered with filter paper at 28°C for 10 days. The products obtained by SSF were evaluated for their nematicide activity in pot experiments containing one seedling of the plant Coleus inoculated with the nematode *M. incognita*. The plants were evaluated 2 months after inoculation. Fermented products showed a reduction in the number of nematodes. The best results were obtained with defatted soybean cake, which showed 100% reduction in the number of nematodes; the reduction with coffee husk was 80% and with cassava bagasse was about 60%.

Kiewnick *et al.* (2004) conducted greenhouse experiments with the root-knot nematodes *Meloidogyne incognita* and *M. hapla* on tomato. *P. lilacinus* was incorporated into soil inoculated with root-knot nematode eggs prior to transplanting susceptible tomato cultivar. Furthermore, soil treatments were combined with seedling treatments 24 hours before transplanting and a soil drench 2 weeks after planting, respectively. Seedling and post planting treatment was also combined with a soil treatment at planting. All single or combination treatments decreased the gall index and the number of egg masses compared to the untreated control 12 weeks after planting. However, the combination of the seedling treatment with a pre- or at-planting application of *P. lilacinus* was necessary to achieve higher levels of control. Additional post plant drenching resulted in only a slight increase in efficacy. In field experiment, it could be demonstrated that the above mentioned combination of pre-planting application plus the seedling and one post plant drench gave the best control and resulted in a significant fruit yield increase in concurrence with a decrease in number of galls per root.

Mendoza *et al.* (2004) conducted dose response and form of application experiments with burrowing nematode, *Radopholus similis*, on banana using a commercial water dispersible granulate formulated *P. lilacinus* (strain 251) product. The results revealed that nematode activity decreased in the presence of this fungus. An important correlation between rates of application and the degree of control of *R. simnilis* penetration and banana root weight was observed. The best control was achieved in the treatment when plantlets and soil were pre-inoculated with *P. lilacinus* and reinoculated during transplantation. The results showed that the biocontrol agent *P. lilacinus* is an excellent candidate for an IPM program against nematodes such as *Radopholus similis*.

Goswami *et al.* (2006) investigated the effect of two fungal bioagents along with mustard oil cake and furadan against root-knot nematode *Meloidogyne incognita* infecting tomato under greenhouse condition. Bioagents viz., *Paecilomyces lilacinus* and *Trichoderma viride* alone or in combination with mustard cake and furadan promoted plant growth, reduced number of galls/plant, egg masses/root system and eggs/egg mass. The fungal bioagents along with mustard cake and nematicide showed least nematodes reproduction factor as compared to untreated infested soil.

Gulsar Banu *et al.* (2006) mass multiplied *Paecilomyces lilacinus* in both solid substrates and liquid media under *in vitro* condition. Among solid substrates tried, sorghum grains encouraged maximum spore production (327.78×10^6 cfu /g at 20 Days After Inoculation) followed by coconut oil cake. When various durations of incubation were tried, maximum spore production was observed at 20 DAI (Days After Inoculation) followed by 30 DAI.

Kiewnick and Sikora (2006 b) established significant dose–response relationships, when conidia of *Paecilomyces lilacinus* strain 251 were applied to soil either with or without the glucose-based formulation. The effective concentration 50 (EC50) values for the commercially formulated product ranged between 0.097 g and 0.08 g/500 cm^3 soil, equivalent to an EC50 of 1.29×10^6 and 9.88×10^5 colony forming units (CFU)/g soil for the parameters gall index and final population per root, respectively. For the number of egg masses per root the EC50 was 0.007 g product or 2.64×10^5 CFU/g soil. Similarly, EC50 values for conidia applied without formulation were 0.068 g or 0.103 g/500 cm^3 soil (EC50 of 8.10×10^5–1.40×10^6 CFU/g soil) for gall index and final population per root. In contrast, the EC50 was 0.096 g (EC50 of 1.28×10^6 CFU/g soil) for the number of egg masses per root. They demonstrated that a single pre-plant application at a concentration of 1×10^6 CFU/g soil is needed for sufficient biocontrol of *M. incognita* by PL251.

Rumbos and Kiewnick (2006) investigated the effect of 12 plant species on the persistence of *Paecilomyces lilacinus* strain 251 in soil. They incorporated formulated conidia into non-sterile soil followed by transplanting different test plants, the population dynamic of the fungus was determined over 100 days. The fungal population in the planted soil was compared to the density of *P. lilacinus* in the rhizosphere and the percent increase or decrease was calculated for each crop. In addition, the potential of *P. lilacinus* strain 251 to colonize roots endophytically was investigated. Comparison of the slopes describing the population dynamics of the fungus showed no significant differences between soil without plants and soil from the root zone of the majority of the test plants. Bean was the only plant species consistently exerting a negative effect on the persistence of *P. lilacinus* strain 251 in the soil. For the first time, *P. lilacinus* strain 251 was isolated in significant numbers from healthy root tissue of barley plants.

Rumbos *et al.* (2006) observed the interactions of *Paecilomyces lilacinus* strain 251 with the arbuscular mycorrhizal fungus *Glomus intraradices* against *Meloidogyne incognita* on tomato in greenhouse experiments. Application of *P. lilacinus* had no effect on the frequency and intensity of tomato root colonization by *G. intraradices*. Likewise, the decline of the nematophagous fungus densities after single application in soil was not affected by the presence of the mycorrhizal fungus.

Shanmuga and Kumar (2006) conducted an experiment under glass house conditions to establish the optimum dose of the fungus, *Paecilomyces lilacinus* against *Meloidogyne incognita* in tomato. The fungal suspension was inoculated a $1x10^6$, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$ and $8x10^6$ spores per 3 kg soil. Four ml of fungal suspension ($8x10^6$ spores) of *P. lilacinus* per 3 kg soil was found to be the optimum dose for effective reduction of *M. incognita* population in soil, egg masses and number of galls and increased the plant growth parameters.

Kiewnick (2007) examined the efficacy of *Paecilomyces lilacinus* 251 (PL251) for controlling root-knot nematode. In addition, it was found that rhizosphere competence is not the key factor for the efficacy of PL251. Co-application of PL251 with other soil antagonists increased biocontrol efficacy against root-knot nematodes. The efficacy of PL251 depended strongly on the ratio between application rate and inoculum density in soil. As pre-plant treatment, a rate from 1.5 to 7.5×10^5 CFU/g soil resulted in significant control at inoculum densities of 100 to 400 root-knot nematode eggs/100 ml soil. However, due to the rapid decline of PL251 in soil, repeated applications are needed to maintain a sufficient density of PL251 for season-long protection.

Alexander *et al.* (2009) investigated the biological control efficacy of single or multiple applications of the mutualistic endophyte *Fusarium oxysporum* strain 162, the egg pathogen *Paecilomyces lilacinus* strain 251 and the antagonistic bacteria *Bacillus firmus* toward *Radopholus similis* in pot trials with banana under glasshouse conditions. *R. similis* was controlled substantially in single and combined applications of *F. oxysporum* with *P. lilacinus* or *B. firmus*. The combination of *F. oxysporum* and *P. lilacinus* caused a 68.5% reduction in nematode density whereas the individual applications reduced the density by 27.8% and 54.8% over the controls, respectively. Combined application of *F. oxysporum* and *B. firmus* was the most effective treatment in controlling *R. similis* on banana (86.2%), followed by *B. firmus* alone (63.7%). The compatibility of the biocontrol agents, as well the capacity of *F. oxysporum* to colonize banana roots in the absence or presence of *P. lilacinus* was also investigated. *P. lilacinus* did not adversely affect endophytic colonization by *F. oxysporum*.

Aminuzzaman (2009) observed the egg and juvenile parasitism of *Meloidogyne* spp. by nematophagous fungus *Paecilomyces lilacinus* in soil tube test and it has been found that egg and juvenile parasitism depends on the fungal population density but not on nematode population density.

Franco-Navarro *et al.* (2009) taken one hundred and six soil samples and examined for the presence of native isolates of the fungus *Pochonia chlamydosporia*. Samples were collected from locations with different land uses (i.e. natural forest, secondary forest, pasture fields and maize fields) and were processed using a selective medium to isolate the fungus. Two varieties of the fungus (alone or in combination), were found in a total of 30 soil samples: *P. chlamydosporia* var. *chlamydosporia* was present in 25 samples and *P. chlamydosporia* var. *catenulate* in 10 of the samples. Six isolates were present in pasture fields, 4 in maize fields, 13 in secondary forest

and 12 in natural forest. All isolates were tested on maize for their ability to colonize roots, and to parasitize eggs of the nematode *Nacobbus aberrans*. There were highly significant differences in the proportion of eggs parasitized by the different isolates (Tukey, ≤ 0.01). Eight isolates parasitized >80% of the eggs, 16 parasitized between 70-80%, and 11 parasitized <70%. Root colonization ranged from 75-100%: 12 isolates colonized all root segments, 14 colonized 90-99% of them, six colonized 80-89% and three colonized only 75%.

Kiewnick (2009) tested the facultative BCA *Paecilomyces lilacinus* strain 251 (PL251) to control nematodes. PL251 demonstrated efficacy in reducing root-knot, cyst and free living plant-parasitic nematodes on a range of crops. However, to better understand the multitrophic interactions of PL251 with host- or non-host plants, nematodes, mutualistic fungal endophytes, and mycorrhiza studies were conducted to determine their importance for biological efficacy. In none of the studies conducted, adverse effects on mutualistic fungal endophytes, mycorhiza, fungal antagonists or entomopathogenic nematodes were observed. Conversely to other nematophagous fungi, rhizosphere competence seems not a key factor for the efficacy of PL251. Monitoring the persistence of PL251 under field conditions using dilution plating techniques and nested PCR revealed a rapid decline of the fungal density in soil over time. Although detection of PL251 in soil was still possible two years after application, the overall suppressive of egg pathogenic fungi towards cyst nematodes was not affected.

Siddiqui *et al.* (2009) assessed the effects of antagonistic fungi *Aspergillus niger*, *Paecilomyces lilacinus* and *Penicillium chrysogenum* and plant-growth-promoting rhizobacteria (PGPR) *Azotobacter chroococcum*, *Bacillus subtilis* and *Pseudomonas putida* with cattle manure on the growth of tomato and on the reproduction of *Meloidogyne incognita*. Application of antagonistic fungi and

PGPR alone and in combination with cattle manure resulted in a significant increase in the growth of nematode-inoculated plants. The highest increase (79%) in the growth of nematode-inoculated plants was observed when *P. putida* was used with cattle manure, followed by use of *P. lilacinus* plus cattle manure. *Paecilomyces lilacinus* resulted in a high reduction in galling and nematode multiplication, followed by *P. putida, B. subtilis, A. niger, A. chroococcum* and *P. chrysogenum.* The combined use of *P. lilacinus* with cattle manure resulted in a maximum reduction in galling and nematode multiplication.

Singh *et al.* (2009) used 1, 2 and 3 g substrate of *P. lilacinus* (19.59×10^8 spores/g substrate) per kg soil in pot experiments on plant growth and nematode multiplication on tomato. There was a significant increase in growth characters and reduction in nematode population in the treatments receiving 1 g of *P. lilacinus* and this effect increased further with the increase in *P. lilacinus* levels. A significant enhancement in all the plant growth parameters and reduction in nematode populations were recorded in the treatments where *P. lilacinus* was used in combination with carbofuran. The eggs parasitized by *P. lilacinus* were found to be fungus density dependent and parasitization increased with the increase in level of fungus in the soil.

Ganate and Khan (2010) studied the biocontrol potentiality of *P. lilacinus* was examined *in vitro* conditions against the *Lycopersicon esculentum* root-knot nematode *Meloidogyne javanica*. The parameters were measured on plant height, fresh weight, dry weight and number of leaves per plant. The number of galls, number of egg masses, infection of eggs and final nematode population was also evaluated. *Paecilomyces lilacinus* significantly improved the growth of tomato plants inoculated with 2000 juveniles of *Meloidogyne javanica*. The plant length, fresh weight, dry weight and number of leaves per plant significantly improved

whereas, number of galls, egg masses, eggs per egg mass and final nematode population greatly reduced on simultaneous and sequential inoculation of *P. lilacinus* and *M. javanica.*

Jahan (2011) assessed the microflora on egg and egg masses of *Meloidogyne* spp. collected from galled plant roots in Bangladesh. A total of 69 fungal isolates belonging to 15 genera were obtained from 42 galled root samples. Of them, 50 isolates were subjected to pathogenicity tests *in-vitro*. *Paecilomyces* spp. were accounted for 2.90% of total fungal isolates with egg parasitic rate 13.33 to 19.11% and juvenile mortality rate 15.63 to 29.81%.

Kiewnick *et al.* (2011) studied potentiality of *Paecilomyces lilacinum* (PL251), for against root-knot nematode *Meloidogyne incognita* on tomato at varying application rates and inoculum densities. They demonstrated that a preplanting soil treatment with the lowest dose of commercially formulated PL251 (2×10^5 CFU/g soil) was sufficient to reduce root galling by 45% and number of egg masses by 69% when averaged over inoculum densities of 100 to 1,600 eggs and infective juveniles/100 cm^3 of soil. To determine the role of colonization of *M. incognita* egg masses by PL251 for biocontrol efficacy, a real-time quantitative polymerase chain reaction (PCR) assay with a detection limit of 10 CFU/egg mass was used which revealed a significant relationship between egg mass colonization by and the dose applied to soil but no correlation was found between fungal density and biocontrol efficacy or nematode inoculum level. The results demonstrated that rhizosphere competence is not the key mode of action for PL251 in controlling *M. incognita* on tomato.

Yan *et al.* (2011) isolated endophytic fungi from cucumber seedlings and screened for their potential as seed treatment agents against *M. incognita*. Among the 294 isolates screened, 23 significantly reduced galls. The 10 most effective isolates were

Fusarium (5), *Trichoderma* (1), *Chaetomium* (1), *Acremonium* (1), *Paecilomyces* (1), and *Phyllosticta* (1). Their control efficacies were repeatedly tested and their colonization as well as *in vitro* activity against *M. incognita* were studied. They reduced the number of galls by 24.0%-58.4% in the first screening and 15.6%-44.3% in the repeated test, respectively. *Phyllosticta* Ph511 and *Chaetomium* Ch1001 had high colonization on both the roots and the aboveground parts of cucumber seedlings. *Fusarium* isolates had higher capacity to colonize the roots, ranging from 20.1% to 47.3% of the total root area. *Trichoderma* Tr882, *Paecilomyces* Pa972, and *Acremonium* Ac985 had low colonization on both the roots and the aboveground parts.

Mitu (2012) evaluated impact of *Paecilomyces lilacinus* application time on plant growth and suppression of root-knot nematode (*Meloidogyne incognita*) in some selected vegetables. The application of *P. lilacinus* at the rate of 36×10^7spore of *P. lilacinus*/plant reduced number root galling, egg mass production, number of juvenile (J_2)/g soil and reproduction factor (R_f) as compared to plants inoculated with nematode alone. In application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting gall index, egg mass/root, J_2/g soil and R_f were 0.50, 3.25, 104.4 and 8.41 in comparison to 5.63, 43.31, 438.8 and 36.46 in *M. incognita* inoculated plants. Similar reduction was observed, when *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting sequentially, where gall index, egg mass/root, J_2/g soil and R_f were 0.25, 1.00, 71.88 and 5.77.

Rao *et al.* (2012) evaluated bio-efficacy of a bio-nematicide, IIHR - *Paecilomyces lilacinus* (*Purpureocillium lavendulum* Luangsa-Ard) for the management of *Meloidogyne incognita* (Chitw.) on tomato (*Solanum lycopersicum* L.). The trials were conducted in two different agro-climatic regions using Farm Yard Manure (FYM) enriched with *P. lilacinus* (1%) W.P. Seed treatment at the rate of 20g/kg,

nursery bed treatment at the rate of 50g *P. lilacinus* /m^2 and application of FYM (5 tons) enriched with 5kg of *P. lilacinus*/ha proved to be significantly effective in the management of *M. incognita* and these treatments increased the yield of tomato significantly.

Shammi (2012) assessed the biocontrol potency of *Paecilomyces lilacinus* by evaluating the BCA and chemical nematicide fosthiazate against root-knot nematode *M. incognita* in eggplant. The gall index reduction (78.4 to 81.25%) and yield (14.05 to 14.90 t/ha) were highest when *P. lilacinus* was applied in combination with fosthiazate. However, the effect of *P. lilacinus* alone and *P. lilacinus* in combination with fosthiazate resulted in statistically similar results in case of most of the parameters suggesting the application of *P. lilacinus* alone or in combination with fosthiazate to control root-knot nematode.

Aminuzzaman *et al.* (2013) used alginate pellets of *Paecilomyces lilacinus* YES-2 and *Pochonia chlamydosporia* HDZ-9 for controlling of *M. incognita* on tomato in a greenhouse by adding them into a soil with sand mixture at rates of 0.2, 0.4, 0.8 and 1.6% (w/w). *P. lilacinus* pellets at the highest rate (1.6%) reduced root galling by 66.7%. *P. chlamydosporia* pellets at the highest rate reduced the final nematode density by 90%. The results indicate that *P. lilacinus* and *P. chlamydosporia* as pellet formulation can effectively control root-knot nematodes.

Udo *et al.* (2013) conducted a greenhouse experiment to investigate the single and combined effects of different arbuscular mycorrhizal fungi (AMF) and bioformulated *Paecilomyces lilacinus* against *Meloidogyne incognita* race 1 on tomato. Three applications of the bionematicide were combined with five species of AMF plus an un-inoculated control. The results indicated that *Glomus etunicatum* and *G. deserticola* were most efficient species and differed significantly ($p \leq 0.05$)

in their efficacy of gall and egg mass inhibition, tomato root colonization rate, growth and fresh fruit yield enhancement. Two applications of the bio-nematicide more significantly ($p \leq 0.05$) reduced galling and egg production than a single application. Individual combinations of two AMF (*G. etunicatum* and *G. deserticola*) with a double application of the bionematicide, resulted in the greatest gall and egg mass inhibition and consequently the greatest growth and fresh fruit yield enhancement.

Mokbel *et al.* (2014) evaluated the effectiveness of different bacterial and fungal genera against *M. javanica* on eggplant under laboratory and greenhouse conditions. Treatment with *Arthrobotrys conoides*, *A. oligospora*, *Paecilomyces lilacinus* and *Saccharomyces cerevisiae* caused significant reductions (69.5-89.5%) in the number of nematode root galls, egg-masses/root system and number of J_2/250 cc soil and showed 53.7-60.9% increase in root and shoot dry weights of eggplant. The potential of *P. lilacinus* in colonization *M. javanica* egg masses and eggs formed on eggplant roots ranged from 45.2- 99.2%, compared to the control treatment.

Yu *et al.* (2015) investigated the effects of BCA *Paecilomyces lilacinus* strain PL1210 on ammonia-oxidizing microorganisms and fungal community composition of tomato rhizosphere. Real-time quantitative polymerase chain reaction (qPCR) detected stable colonization of *P. lilacinus* in the tomato rhizosphere and significant inhibition of ammonia-oxidizing bacteria (AOB) and archaea (AOA), which could be responsible for the decrease of NO_3^--N content in soil. PCR-denaturing gradient gel electrophoresis (DGGE) analysis demonstrated no significant difference in soil fungal community composition associated with the application of *P. lilacinus*. Cluster analysis showed that the composition of rhizosphere fungal community was more significantly influenced by time-related differences than by the inoculation of biocontrol agents.

Chapter 3

Materials and Methods

Materials and Methods

Laboratory and pot experiments were conducted to study the effect of some crop species on the root colonization ability and persistence of a BCA, *Purpureocillium lilacinum*. The materials used and the methods followed in the study are presented in this chapter.

3.1. Experimental site and experimental period

The experiments were carried out during the period from January 2015 to April 2016 in the laboratory and in the shade house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka -1207.

3.2. Environment of experimental site

All the experimental plants were kept in the shade house where the temperature was 28 ± 2° C during the "day" and 23 ± 2° C during "night" with an average temperature of 26± 2° C. In the laboratory, the temperature was 25 ± 2° C during the "day" and 20 ± 2° C during "night" with an average temperature of 23± 2° C.

3.3. Crop species and their varieties

The crop varieties used in this experiment are given below in Table 1.

Table 1. List of crop species and their varieties

Name of crop species	Variety
Rice	BR4
Wheat	BARI Gom 27
Maize	BARI Bhutta 7
Potato	BARI TPS-1
Brinjal	BARI Begun 8
Tomato	BARI Tomato 14
Cabbage	BARI Bandha Kopi 1 (Provati)
Cucumber	BARI Sasha 1
Chickpea	BARI Chhola 5
Soybean	BARI Soybean 5

3.4. Collection of seeds

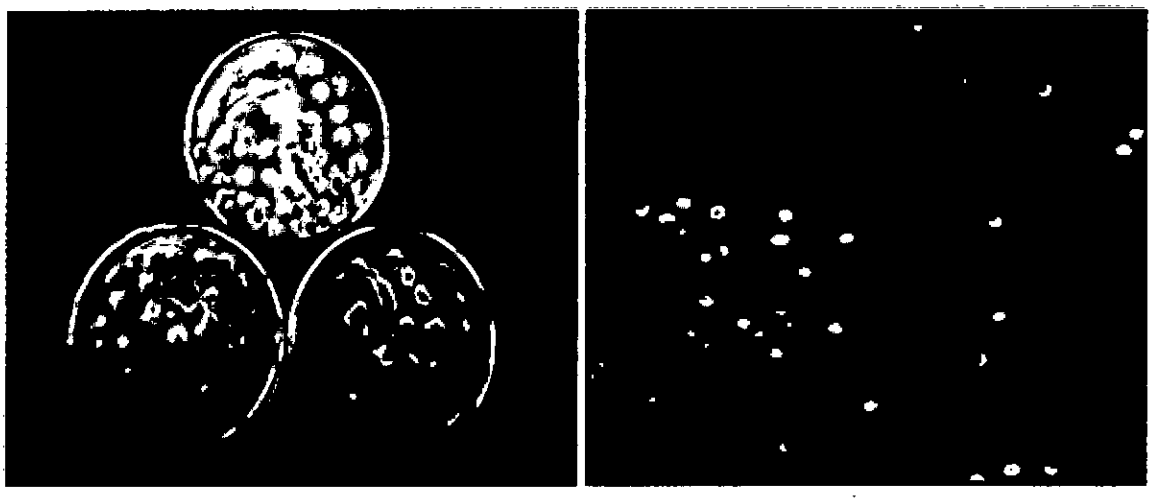
All the crop seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur except rice seeds which were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

3.5. Fungal isolate

The isolate of *Purpureocillium lilacinum* isolated from Mymensingh, Bangladesh, previously shown to have high biocontrol ability against root-knot nematode was used in this study (Aminuzzaman and Liu, 2011).

3.6. Culture of *Purpureocillium lilacinum*

Purpureocillium lilacinum was grown on Potato Dextrose Agar (PDA) medium for 8-10 days (Aminuzzaman and Liu, 2011) (Plate 1).



A B

Plate 1. Pure culture of *Purpureocillium lilacinum* on PDA media (A) and Conidiophore and conidia of *Purpureocillium lilacinum* under compound microscope (X400) (B).

3.7. Surface sterilization of seeds

Seeds of the ten selected crop species were surface sterilized with 0.1% $HgCl_2$ in a mechanical shaker for 1 minute. Then the seeds were washed in sterile distilled water for 3 minutes 3 times to wash out the residual $HgCl_2$. At the end the seeds were kept in blotter paper to remove the excess water from the seeds.

3.8. Seed placement on petri dishes for germination

At first, required number of sterilized plastic petri dishes were taken and two moistened blotter papers were placed in each of the petri dishes. Then 10 seeds were set on the moistened blotter paper in an orderly manner. At 27°C, seeds of all selected crop species were germinated after a varying duration of 4-9 days. All the activities were performed in laminar air flow chamber (Plate 2).

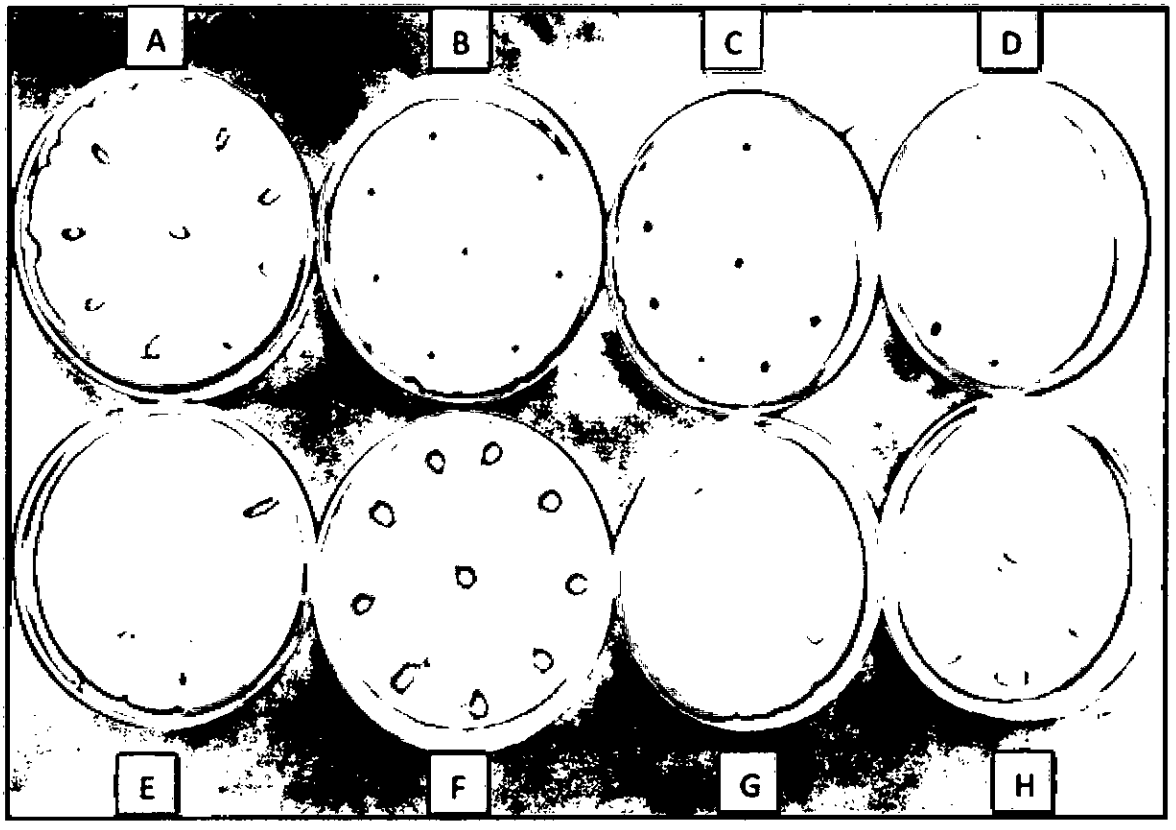


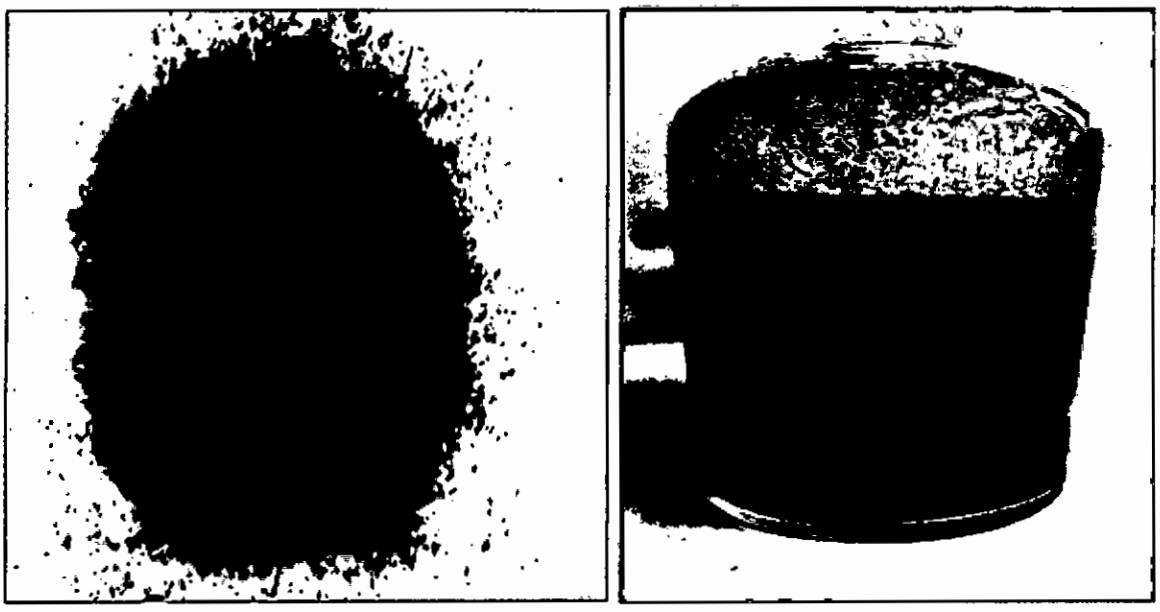
Plate 2. Seed placement on petri dishes for germination; Rice seeds (A), Cabbage seeds (B), Brinjal seeds (C), Tomato seeds (D), Cucumber seeds (E), Chickpea seeds (F), Wheat seeds (G) and Maize seeds (H).

3.9. Collection and sterilization of rooting media

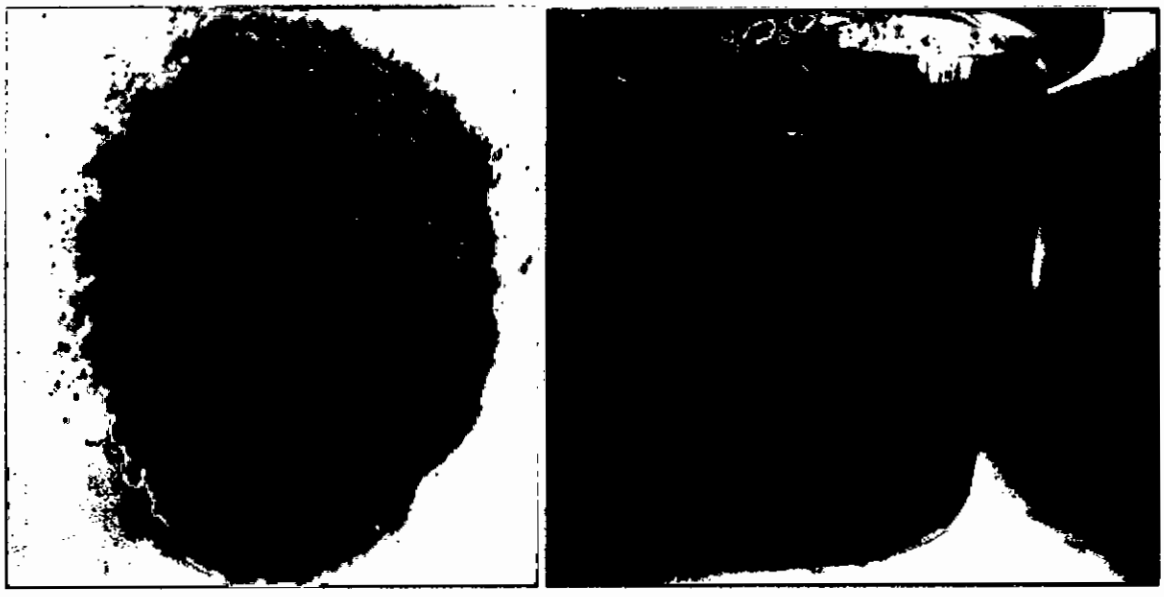
In this experiment two media were used, one was coco dust and another one was soil and sand mixture. The coco dust was collected from the nursery besides Sher-e-Bangla Agricultural University. The soil and sand mixture was prepared by mixing a required amount of soil and sand at a proportion of 1:1 (v/v). These two media were autoclaved at 121°C, 15 psi for 15 minutes (Plate 3). Detailed of soil properties presented below in Table 2.

Table 2. Physicochemical characteristics of soil

Soil type	pH		Organic matter (%)	Total N (%)	Particle size			P (µg/g soil)
					Sand (%)	Silt (%)	Clay (%)	
Loam	5.2		2.29	0.114	48	41	11	210.08
Exchangeable cations (meq/100g soil)			B	S	Mn	Fe	Cu	Zn
Ca	Mg	K	µg/g soil					
17.50	21.25	3.76	10.40	523.70	8.60	23.82	1.68	8.05



A



B

Plate 3. Preparation of rooting media; Unsterilized and sterilized coco dust (A), Unsterilized and sterilized soil (B).

3.10. Test tube experiment for assessment of root colonization ability of *Purpureocillium lilacinum*

3.10.1. Design and layout of the experiment

This experiment was laid out in a Completely Randomized Design (CRD) with three replications per treatment.

3.10.2. Treatments of the experiment

The experiment of root colonization ability was conducted by considering each of the crops as a treatment in two media.

Media: 1. Coco dust and 2. Soil

Crops:

T_1 =Rice

T_2 = Wheat

T_3 = Maize

T_4 = Potato

T_5 = Brinjal

T_6 = Tomato

T_7 = Cabbage

T_8 = Cucumber

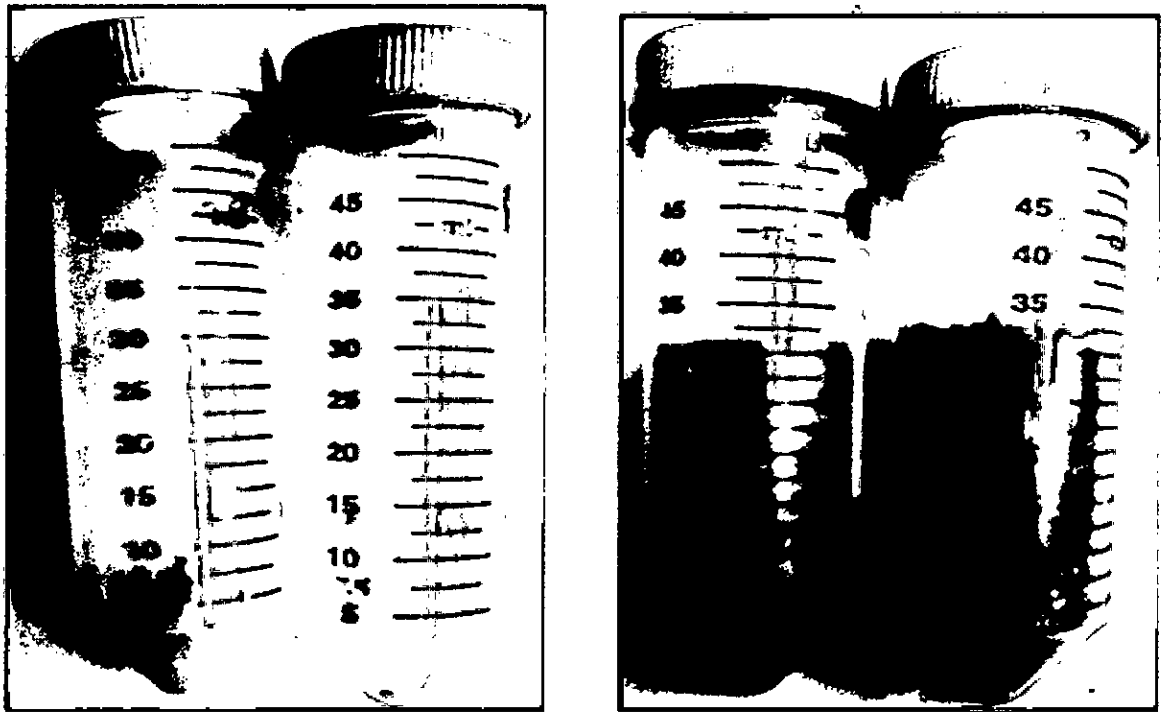
T_9 = Chickpea

T_{10} = Soybean

All treatments were replicated three times.

3.10.3. Preparation of test tubes

Required numbers of test tubes (Falcon tube) were cleaned, washed and dried up. Then they were autoclaved at 121°C, 15 psi for 15 minutes. When cooled, they were arranged in a surface sterilized tube holder inside of laminar flow according to selected experimental design (Plate 4).



A B

Plate 4. Preparation of test tube; Sterilized empty test tube (A) and Sterilized test tube filled with rooting media (B).

3.10.4. Placing germinated seeds

The test tubes were filled with the two different autoclaved media up to two-third of the test tube. Then five 1 cm plugs of the fungus *P. lilacinum* were inserted just below the surface of the media and two surface sterilized germinated seeds of each crop species were placed on top of the fungus (Hidalgo-Diaz *et al.*, 2000). Tubes were sealed with cotton plug and lid. Then the tubes were incubated at 27°C in the dark of 1 week. There were three replications for each of the crop species (Plate 5).

3.10.5. Root harvesting

After incubation for a week the roots were harvested by shaking followed by washing with sterile distilled water to make them free of the media. Then the roots were placed on blotter to remove the water.

3.10.6. Plating of root segments on PDA

The harvested roots of each crop were cut into 1 cm segments and kept separately. Then they were placed on PDA media and left incubated at 27°C for 5 days.

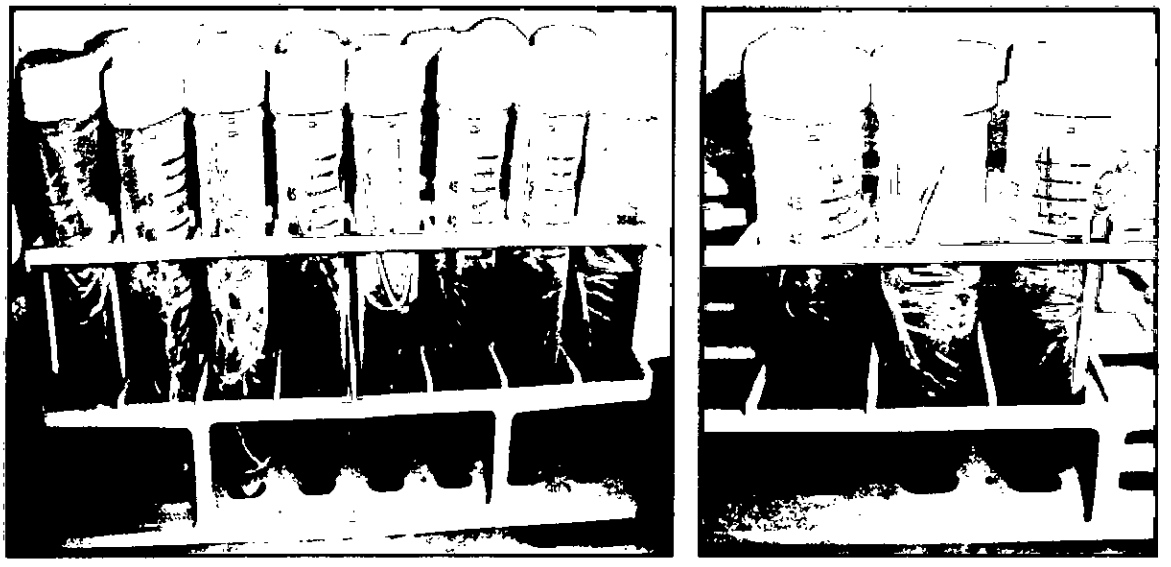
3.10.7. Data recording

After 5 days of incubation period the root segments colonized by the fungus were counted. Growth of *P. lilacinum* colonies was confirmed after further incubation for 5 days and these data were recorded for further analysis (Plate 6 and 7).

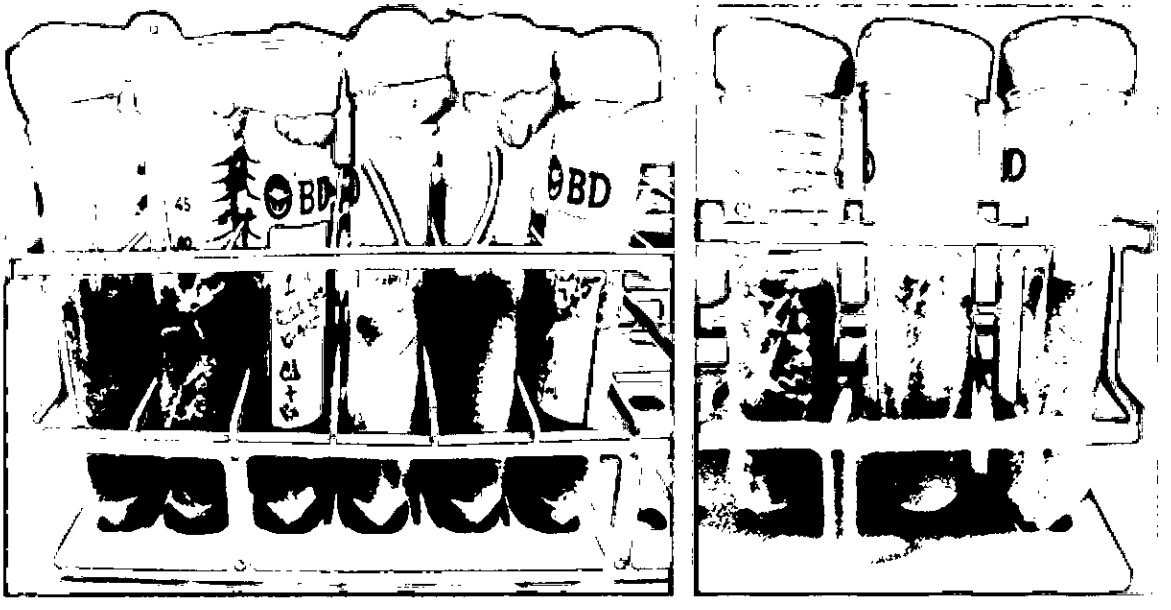
3.10.8. Data recorded

3.10.8.1. Number of root segments colonized

In two different media, the number of root segments colonized by the fungal BCA was counted carefully and recorded for each treatment and each replication.



A



B

Plate 5. Test tube experiment for root colonization ability of *P. lilacinum* showing crop roots spreading in two media; Coco dust (A) and Soil (B).

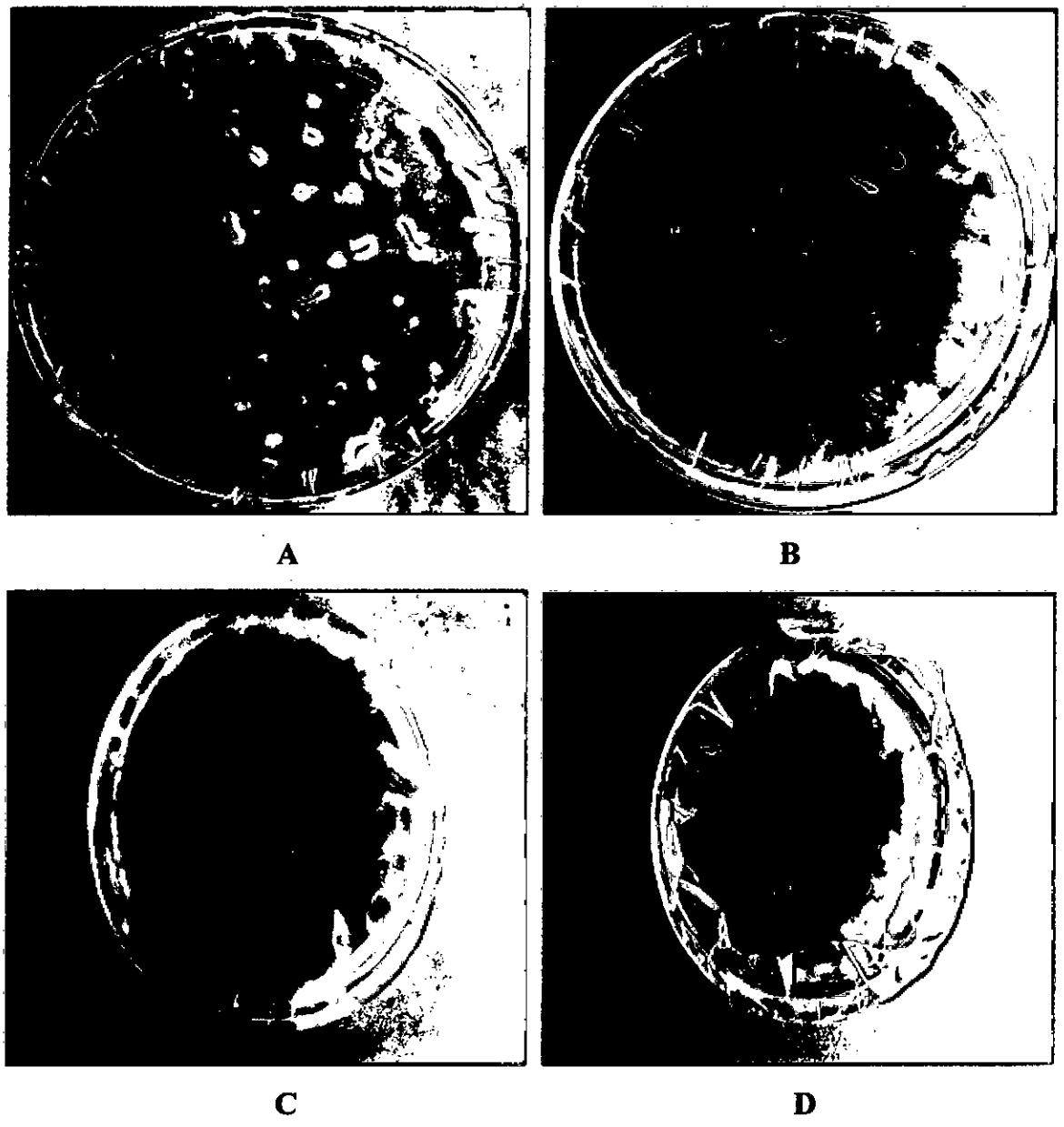


Plate 6. Root colonization (coco dust as rooting medium) by *P. lilacinum* on PDA; Maize (A), Rice (B), Wheat (C) and Soybean (D).

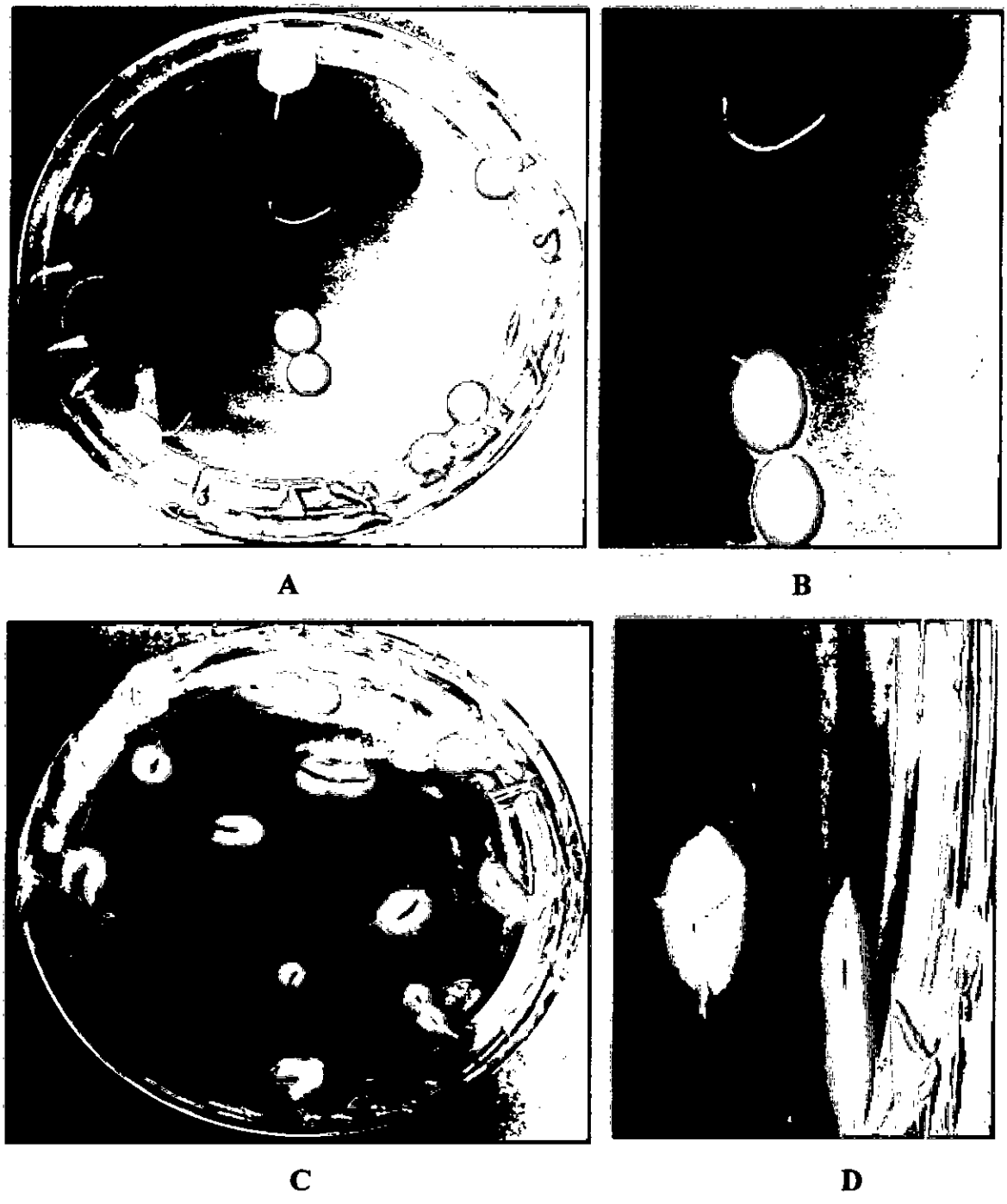


Plate 7. Root colonization (soil as rooting medium) by *P. lilacinum* on PDA; Rice (A), Rice (close view) (B) and Maize (C), Maize (close view) (D).

3.10.8.2. Percent root colonization

The percent root colonization was determined by dividing the number of root segments colonized by the fungal BCA by the total number of root segments plated on PDA.

$$\% \text{ Root colonization} = \frac{\text{No.of root segments colonized by the fungal BCA}}{\text{Total no.of root segments plated on PDA}} \times 100$$

3.11. Persistence of the BCA *Purpureocillium lilacinum* in soil

3.11.1. Design and layout of the experiment

This experiment was laid out in a Completely Randomized Design (CRD) with three replications per treatment.

3.11.2. Treatments of the experiment

For each of the crop species, the experiment was conducted according to the following treatment scheme:

T_1 = Soil (Control)

T_2 = Soil + *Purpureocillium lilacinum* (Control)

T_3 = Soil + *Meloidogyne incognita* + *Purpureocillium lilacinum*

T_4 = Soil + Crop root + *Purpureocillium lilacinum*

T_5 = Soil + Crop root + *Meloidogyne incognita* + *Purpureocillium lilacinum*

All treatments were replicated three times.

3.11.3. Mass production of *Purpureocillium lilacinum*

Purpureocillium lilacinum was grown on Potato Dextrose Agar (PDA) medium for 8-10 days (Aminuzzaman and Liu, 2011). Within 8-10 days the fungus was transferred on chick pea for mass production. For mass production one hundred grams of chick pea seed free of any pesticide treatment was placed in 250 ml conical flasks and soaked in lukewarm water for 3-4 hours. Then the water was drained off, and each flask was closed with a cotton plug and covered with brown paper in two-layer paper. Then flasks were placed in an autoclave for 15 minutes at 15 psi. After the flasks and contents cooled, *P. lilacinum* as a mycelial mat growing on PDA was added aseptically to one flask and shaken for better distribute of the fungus; the other flask served as an un-inoculated control. The flasks were incubated at 25-30°C for 20 days (Plate 8).

3.11.4. Harvesting and preparing spore suspension of *Purpureocillium lilacinum*

After incubation the sterile distilled water was added into the conical flask and the spore masses scraped away with sterile brush within laminar air flow chamber. The harvested spores were filtered through sterilized cheesecloth. The spore was harvested from each conical flask and spore was counted with a haemocytometer (Plate 8).

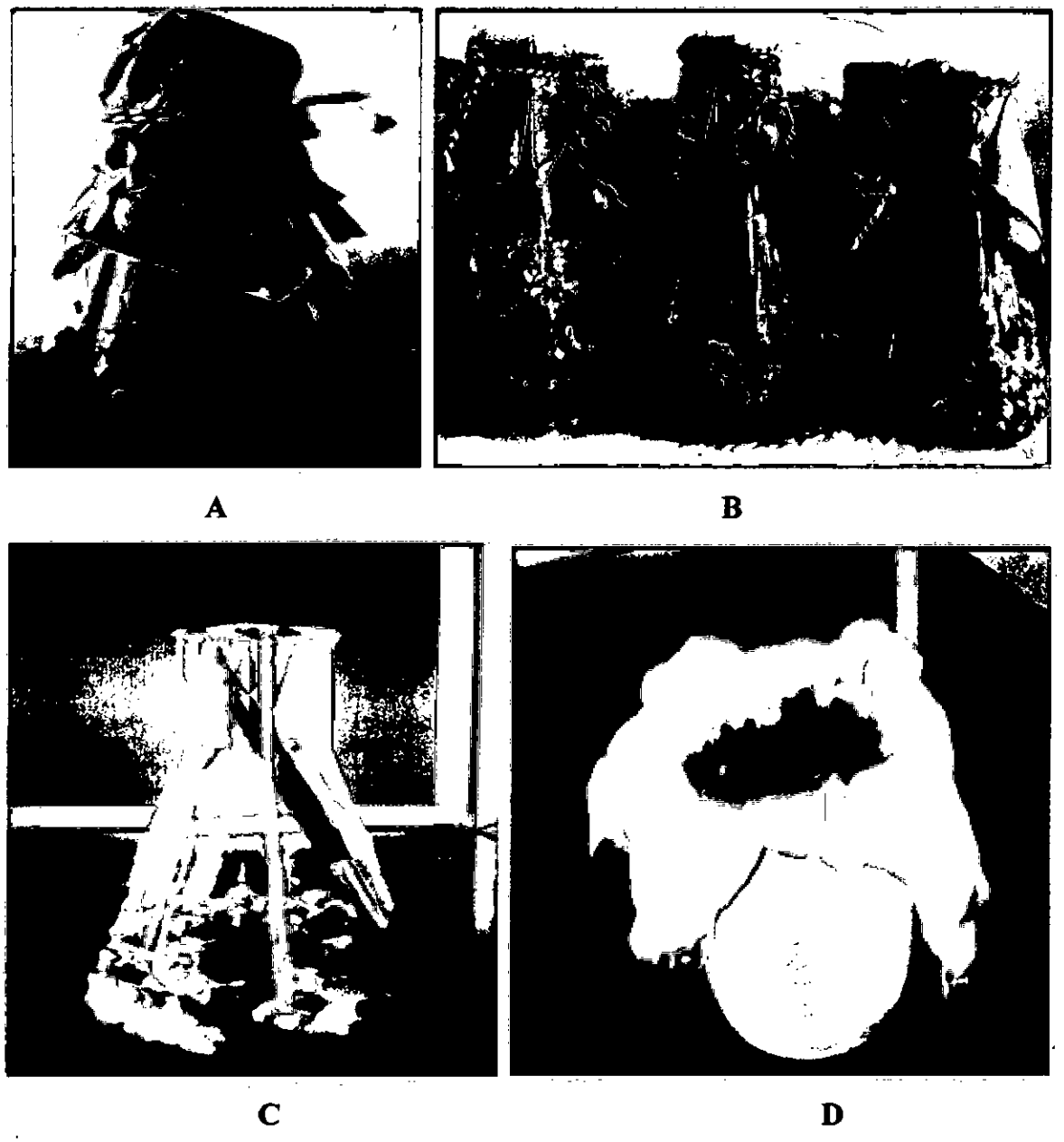


Plate 8. Mass multiplication of and harvesting of *P. lilacinum*; Sterilized chickpea without inocula (A), Mass production of *P. lilacinum* on chick pea (B), Harvesting of spore from conical flask (C) and Sieving of spore (D).

3.11.5. Preparation of pots

Plastic pots of 1000 cm^3 were cleaned, washed and dried up. Then the pots are filled with previously sterilized and fertile soil which was used in root colonization experiment. Each pot contained 830 g of soil. Then the pots were arranged according to selected experimental design (Plate 9).

3.11.6. Application of *Purpureocillium lilacinum* into soil

After preparation of pot in the shade house, spore suspension of *Purpureocillium lilacinum* was carefully mixed into soil. 28 ml of spore suspension was added to each pot (Plate 9) to achieve an initial population of 5.23×10^5 $CFUg^{-1}$ soil as determined by soil dilution plate method.

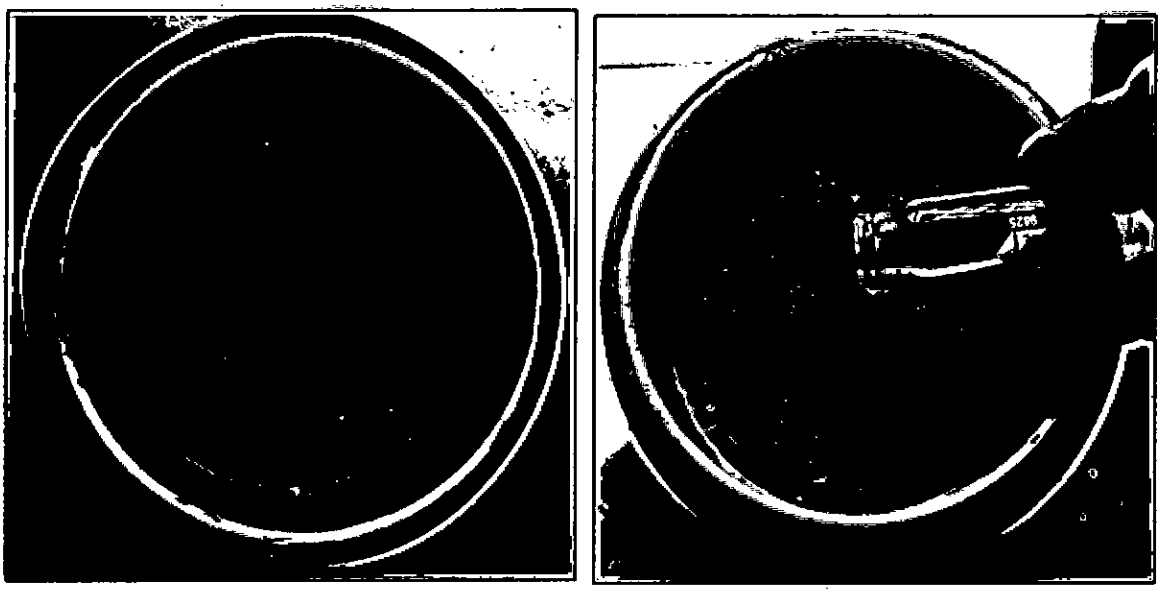


Plate 9. Preparation of pot and application of *Purpureocillium lilacinum* into soil.

3.11.7. Placing of germinated crop seeds

After application of *Purpureocillium lilacinum* into soil of each pot, one germinated seed of each crop was placed in the middle of the pot. The small roots of the seeds are placed carefully in the middle of the pot by making a hole and then covered with surrounding soil (Plate 10).

Plate 10. Pots in the shade house containing seedlings of crop species for the experiment of persistence of *P. lilacinum* in rhizosphere.

3.11.8. Collection and application of nematode egg masses

Egg masses of *Meloidogyne incognita* was collected from Bangladesh Agricultural Research Institute and five egg masses were applied in the pots two days after the placement of germinated crop seeds according to the treatment scheme of the experiment.

3.11.9. Intercultural operations

After placement of germinated crop seeds in the pots and final experiment set up weeding and irrigation were regularly done as per necessity. General sanitation was maintained throughout the growing period.

3.11.10. Harvesting and data recording

After termination of the experiment at selected time interval, 1 g of rhizosphere soil was collected from 3 different depths of one replicate of each treatment at 15, 30 and 45 days of interval. The collected soil samples were then subjected to determine the persistence of *Purpureocillium lilacinum in* soil using the soil dilution plate method.

3.11.11. Data recorded

3.11.11.1. Rhizosphere colonization by *Purpureocillium lilacinum* ($CFUg^{-1}$ soil)

Samples of 1 g soil from each treatment were collected after harvest of the crop around the root zone. The number of colony forming unit per gram soil ($CFUg^{-1}$ soil) was determined using the soil dilution plate method. In detail, 1 g of soil (wet weight) was transferred to a test tube containing 10 ml of sterile distilled water and shaken. Then 1 ml of soil suspension was taken with a micro pipette and transferred to another a test tube containing 9 ml of sterile distilled water. This process was continued once more to obtain a 1:1000 dilution of the soil suspension. After preparation of appropriate dilution cfu were determined by plating onto PDA media. There were three plate replicates for each soil sample. Following incubation for 5 days at 25 ± 2° C the fungal colonies are counted. After further incubation for 5 days the number of *Purpureocillium lilacinum* colonies per plate was confirmed and the cfu number per gm of soil was calculated. Identification of *Purpureocillium lilacinum* was based on morphological characteristics (Plate 11).

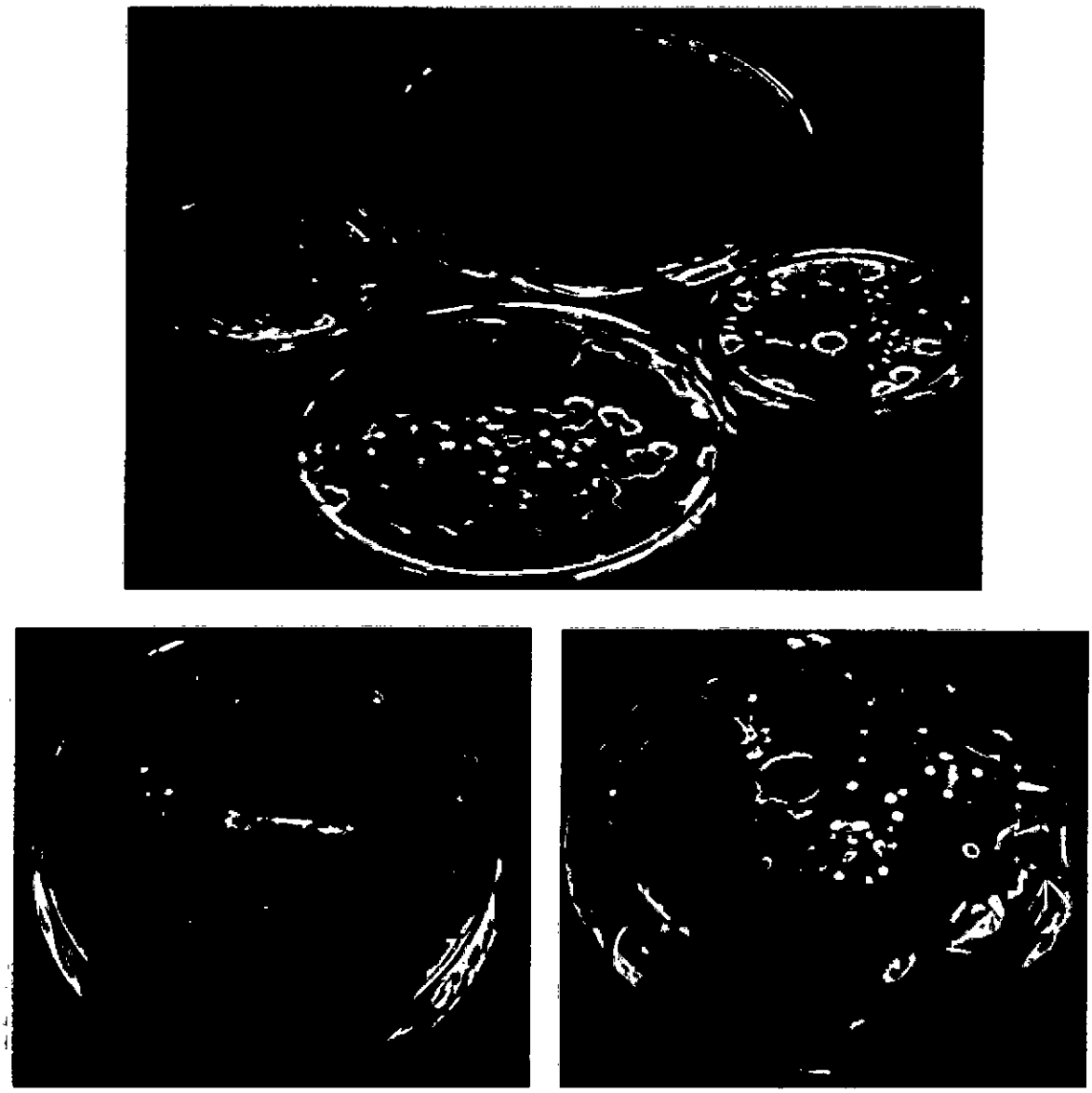
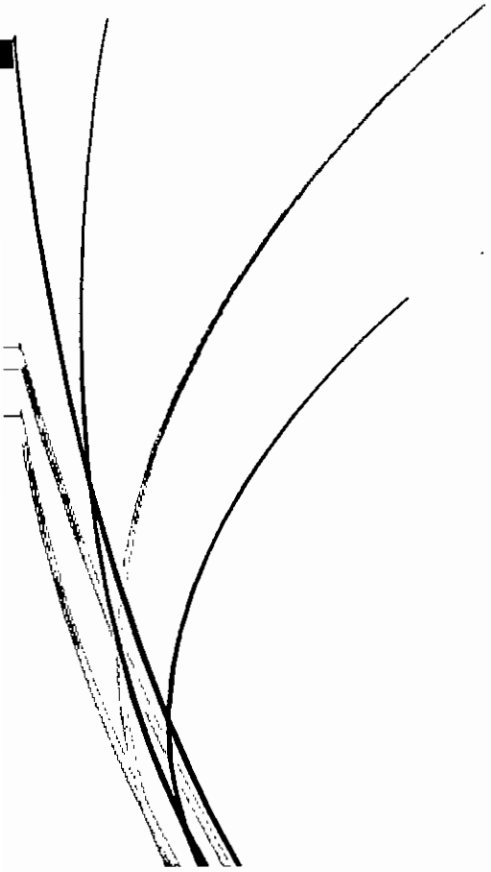


Plate 11. Colony growth of *P. lilacinum* on PDA media by soil dilution plate technique.

3.11.11.2. Analysis of data

In both experiments, the data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments. Treatment means were compared by Tukey HSD. Data were analyzed by Statistix 10 statistical package program.

Chapter 4 Results and Discussion



Results and Discussion

4.1. Root colonization ability of the BCA *Purpureocillium lilacinum*

4.1.1. Effect of crop species on the colonization ability of the BCA *Purpureocillium lilacinum*

The effect of crop species on the colonization ability of the fungal BCA *Purpureocillium lilacinum* was observed in two different rooting media, coco dust and soil. In coco dust, the percent root colonization by the fungus *Purpureocillium lilacinum* obtained from all crop species was constant. In coco dust, 100% root colonization was obtained from all crop species. In soil, there are 3 groups (a, b and c) in which the percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was not significantly different from one another. The percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was varied in some crop species. The varying effect of crop species on the colonization ability of the BCA *Purpureocillium lilacinum* was shown in Table 3.

The maximum percent root colonization was emanated from Cucumber (67.17). The percent root colonization followed by cucumber was obtained from Maize (55.50), Potato (50.00) and Brinjal (48.14). Following them Cabbage (44.28) and Rice (44.26) showed almost the same percent of root colonization which was followed by Tomato (43.45). They all were statistically similar to each other and also similar to Cucumber. The minimum percent root colonization was emanated from Chickpea (30.55) which was preceded by Soybean (32.07) and Wheat (37.00), which were statistically similar to each other and also similar to Maize, Potato, Brinjal, Rice, Cabbage and Tomato but statistically different only from Cucumber. However, Chickpea statistically showed dissimilarity with maize only.

Table 3. Effect of crop species on the colonization ability of the BCA *Purpureocillium lilacinum*

Crop Species (Treatments)	Percent Root Colonization	
Rice (T_1)	44.26	abc
Wheat (T_2)	37.00	bc
Maize (T_3)	55.50	ab
Potato (T_4)	50.00	abc
Brinjal (T_5)	48.14	abc
Tomato (T_6)	43.45	abc
Cabbage (T_7)	44.28	abc
Cucumber (T_8)	67.17	a
Chickpea (T_9)	30.55	c
Soybean (T_{10})	32.07	bc
CV (%)	18.97	
Tukey HSD	24.81	
Level of Significance	**	

** (Significant at 1% level)

The first experiment was conducted to investigate the effect of 10 crop species on the colonization ability of the BCA *Purpureocillium lilacinum* in the laboratory through a test tube experiment. The effect of crop species on the colonization ability of the fungal BCA *Purpureocillium lilacinum* was observed in two different rooting media, coco dust and soil. The results revealed that all of the crop species were colonized by *Purpureocillium lilacinum* in varying percentage depending on the rooting media and the crop species themselves.

In this experiment, it was observed that in coco dust, the percent root colonization by the fungus *Purpureocillium lilacinum* obtained from all crop species was constant. In coco dust, 100% root colonization was obtained from all crop species. So there was no exertion of crop species' effect on root colonization ability of *Purpureocillium lilacinum* observed. In an experiment conducted by Franco-Navarro *et al.* (2009), it was observed that all isolates *Pochonia chlamydosporia* were tested on maize for their ability to colonize roots and root colonization ranged from 75-100%. This observation could explain the ability of *Purpureocillium lilacinum* to colonize all of the roots of a crop.

However, in soil the percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was varied but not significantly different from one another. The percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was varied significantly in some of the crop species tested. In the experiment with soil, we observed maximum percent root colonization in Cucumber (67.17) which was followed by Maize (55.50), Potato (50.00) and Brinjal (48.14). Following them Cabbage (44.28) and Rice (44.26) showed almost the same percent of root colonization which was followed by Tomato (43.45). However, they all were statistically similar to each other and also similar to Cucumber whereas we observed minimum percent root colonization in Chickpea (30.55) which was preceded by Soybean (32.07) and Wheat (37.00), and they were statistically similar to each other and also similar to Maize, Potato, Brinjal, Rice, Cabbage and Tomato but

statistically different only from Cucumber. Among the crop species only chickpea statistically showed significant dissimilarity with maize.

The result of the experiment revealed root colonization ability of the BCA in a diversified crop species with a varying percentage. The root colonization ability of *Purpureocillium lilacinum* in various crops was also examined by many researchers which validates our results. Rumbos *et al.* (2006) examined effect of 12 crop species on the colonization ability of the BCA *Paecilomyces lilacinus.* They found 5 out of 10 crop species in which roots were colonized by the BCA *Paecilomyces lilacinus* though for the first time they isolated *P. lilacinus* in significant numbers from healthy root tissue of barley plants only. Among the 12 crops they examined, there were 7 same crop species which we examined in this experiment. However, we did not conduct our experiment to observe the colonization ability whether it was colonized epiphytically or endophytically. Manhong *et al.* (1998) showed that *Paecilomyces lilacinus* could colonize in soybean rhizosphere as well as endoparasitize a few in soybean roots, when it was used as a seed-coat.

Yan *et al.* (2011) isolated *Paecilomyces lilacinus* (*Paecilomyces* Pa972) and some other endophytic fungi from cucumber seedlings and observed that *Paecilomyces lilacinus* can colonize on both the roots and the aboveground parts which also validates our results. But they showed *Paecilomyces* had low colonizations on both the roots and the aboveground parts of cucumber seedlings which contradicts to our results whereas we found the highest of 67.57 % root colonization in cucumber among the crop species we tested. This difference may be due to the race of *Paecilomyces* or the statement of low colonization of *Paecilomyces* was found in comparison to other endophytic fungi. These contrasting findings could in part be due to differences in the capacity of diverse isolates of Nematophagous fungi to colonize roots. Bourne *et al.* (1994) as well as Persson and Jansson (1999) demonstrated this effect for different isolates of *V. chlamydosporium* and nematode

trapping fungi respectively. However, further investigation is needed to find out the cause behind the difference.

The potential of *Purpureocillium lilacinum* to endophytically colonize root tissue has been a matter of controversy. In an examination of histological interactions of the fungus *Paecilomyces lilacinus* and *Meloidogyne incognita* on tomato roots and Cabanillas *et al.* (1988) showed that *P. lilacinus* colonized the surface of epidermal cells as well as the internal cells of epidermis and cortex of tomato. But Holland *et al.* (2003) reported that *Paecilomyces lilacinus* colonize roots and protect the root surface from root-knot nematode attack. When eight crop plant species including tomato, potato, banana, pepper, barley and wheat were challenged with *P lilacinus* strain Bioact251, fungal hyphae were never detected within roots, though occasionally colonies arose from the root surface. Again, a similar report from Siddiqui *et al.* (2000) who evaluated the efficacy of *Pseudomonas aeruginosa* alone or in combination with *Paecilomyces lilacinus* in the control of root-knot nematode and root-infecting fungi under laboratory and field condition showed that *P. lilacinus* was reisolated from the inner root tissues of tomato, whereas *P. lilacinus* did not colonize tomato roots.

These reports revealed that *Purpureocillium lilacinum* have the potentiality or ability to colonize the roots of various crops which validates our results. But these reports of the researchers have one contradictory finding about whether *Purpureocillium lilacinum* could colonize epiphytically or endophytically. However, our experiment was executed to find out the colonization ability of *Purpureocillium lilacinum* in various crops whether it is epiphytically or endophytically.

4.1.2. Effect of the rooting media on the colonization ability of the BCA *Purpureocillium lilacinum*

To determine the effect of the rooting media on the colonization ability of the fungal BCA *Purpureocillium lilacinum,* two different rooting media were used viz. coco dust and soil. The percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was significantly different from one another due to the two different rooting media. The varying effect of these rooting media on the colonization ability of the BCA *Purpureocillium lilacinum* was shown in Table 4.

Table 4. Effect of the rooting media on the colonization ability of the BCA *Purpureocillium lilacinum*

Rooting media (Treatment)	**Percent Root Colonization**	
Coco dust (T_1)	100.00	A
Soil (T_2)	45.30	B
CV (%)	10.37	
Tukey HSD	7.079	
Level of Significance	**	

** (Significant at 1% level)

The maximum percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was obtained in coco dust (100), as a constant value of 100% root colonization was obtained from all crop species, whereas the minimum percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was obtained in soil medium (45.30).

4.1.3. Combined effect of the crop species and rooting media on the colonization ability of the BCA *Purpureocillium lilacinum*

To determine the combine effect of on the colonization ability of the fungal BCA *Purpureocillium lilacinum,* two different rooting media were used viz. coco dust and soil for 10 different crop species. The percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was significantly varied due to the two different rooting media but not very significantly varied due to crop species except a few crops. The varying effect of the crop root and rooting media on the colonization ability of the BCA *Purpureocillium lilacinum* was shown in Table 5, 6 and 7.

Table 5. Combined effect of the crop species and rooting media on the colonization ability of the BCA *Purpureocillium lilacinum* in Rice, Wheat and Maize

Rooting Media	Percent Root Colonization					
	Rice		Wheat		Maize	
Coco Dust	100.00	a	100.00	a	100.00	a
Soil	44.26	b	37.00	b	55.50	b
CV (%)	13.55		5.72		9.41	
Tukey HSD	22.17		8.90		16.60	
Level of Significance	**		**		**	

** (Significant at 1% level)

In case of Rice, Wheat and Maize the maximum but constant percentage of colonization of root by the BCA *P. lilacinum* was observed in coco dust (100), whereas the percent root colonization of Rice, Wheat and Maize in soil was 44.26, 37 and 55.50 respectively which all are significantly different from coco dust.

Table 6. Combined effect of the crop root and rooting media on the colonization ability of the BCA *Purpureocillium lilacinum* in Potato, Brinjal, Tomato and Cabbage

Rooting Media	**Percent Root Colonization**			
	Potato	Brinjal	Tomato	Cabbage
Coco Dust	100.00 a	100.00 a	100.00 a	100.00 a
Soil	50.00 b	48.14 b	43.45 b	44.28 b
CV (%)	11.79	3.06	6.18	5.05
Tukey HSD	20.06	5.15	10.06	8.26
Level of Significance	**	**	**	**

** (Significant at 1% level)

In case of Potato, Brinjal, Tomato and Cabbage the maximum but constant percentage of colonization of root by the BCA *P. lilacinum* was observed in coco dust (100), whereas the percent root colonization of Potato, Brinjal, Tomato and Cabbage in soil was 50.00, 48.14, 43.45 and 44.28 respectively which all are significantly different from coco dust.

Table 7. Combined effect of the crop root and rooting media on the colonization ability of the BCA *Purpureocillium lilacinum* in Cucumber, Chickpea and Soybean

Rooting Media	Percent Root Colonization		
	Cucumber	Chickpea	Soybean
Coco Dust	100.00 a	100.00 a	100.00 a
Soil	67.17 b	30.55 b	32.07 b
CV (%)	9.32	6.70	6.70
Tukey HSD	17.68	9.93	6.40
Level of Significance	ns	**	**

** (Significant at 1% level)

ns (Not Significant)

In case of Cucumber, Chickpea and Soybean the maximum but constant percentage of colonization of root by the BCA *P. lilacinum* was observed in coco dust (100), whereas the percent root colonization of Cucumber, Chickpea and Soybean in soil was 67.17, 30.55 and 32.07 respectively. In soil root colonization in cucumber was insignificantly different from coco dust whereas root colonization in chickpea and soybean were significantly different from coco dust.

Table 7. Combined effect of the crop root and rooting media on the colonization ability of the BCA *Purpureocillium lilacinum* in Cucumber, Chickpea and Soybean

Rooting Media	Percent Root Colonization					
	Cucumber		Chickpea		Soybean	
Coco Dust	100.00	a	100.00	a	100.00	a
Soil	67.17	b	30.55	b	32.07	b
CV (%)	9.32		6.70		6.70	
Turkey HSD	17.68		9.93		6.40	
Level of Significance	ns		**		**	

** (Significant at 1% level)

ns (Not Significant)

In case of Cucumber, Chickpea and Soybean the maximum but constant percentage of colonization of root by the BCA *P. lilacinum* was observed in coco dust (100), whereas the percent root colonization of Cucumber, Chickpea and Soybean in soil was 67.17, 30.55 and 32.07 respectively. In soil root colonization in cucumber was insignificantly different from coco dust whereas root colonization in chickpea and soybean were significantly different from coco dust.

In this experiment, it was also observed the effect of crop species along with rooting media on the colonization ability of the BCA *Purpureocillium lilacinum.* The effect of rooting media on the colonization ability of the fungal BCA *Purpureocillium lilacinum* was observed using two different rooting media, coco dust and soil. The result revealed a very significant effect of two different rooting media on the colonization ability of the BCA *Purpureocillium lilacinum.*

In case of all crops, the coco dust rooting media caused a constant and maximum percentage of root colonization (100) whereas the minimum percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was obtained in soil medium (45.30). In a combined effect of crop species and rooting media, in all crop species the coco dust resulted in 100 % root colonization whereas the soil resulted in a maximum of 67.17 % of root colonization in cucumber which was insignificantly different from coco dust and the minimum of 30.55 % root colonization in chickpea.

The result of 100 % root colonization in coco dust might be due to the colonization of the coco dust itself by *Purpureocillium lilacinum*. Because the nature or the composition of the media have effect on sporulation of fungus. This finding can be explained by the experiment conducted by Gulsar Banu *et al.* (2006). They mass multiplied *Paecilomyces lilacinus* in both solid substrates and liquid media under *in vitro* condition. Among solid substrates tried, sorghum grains encouraged maximum spore production (327.78×10^6 cfu /g at 20 Days after inoculation). Conversely, the reports of Cabanillas (1988), Manhong *et al.* (1998), Siddiqui *et al.* (2000), Holland *et al.* (2003), Rumbos *et al.* (2006), and Yan *et al.* (2011) which were mentioned earlier also validate the results of root colonization ability of *Purpureocillium lilacinum* in soil medium.

4.2 Effect of crop species and nematode on the persistence of *Purpureocillium lilacinum* in rhizosphere soil

The effect of crop species and nematode on the persistence of *Purpureocillium lilacinum* in rhizosphere soil varied due to some crop species. Out of 10 crop species 8 crop species showed higher population density than in soil with BCA and soil with BCA and nematode and in contrast, out of 10 crop species 2 crop species showed lower population density than in soil with BCA and soil with BCA & nematode but they are not significant. Furthermore, there was no effect of nematode population observed on the persistence of the BCA *P. lilacinum*. The varying effect of effect of crop species and nematode on the persistence of *Purpureocillium lilacinum* in rhizosphere soil was shown in Table 8 to 17.

Table 8. Effect of rice crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil ($\times 10^3$)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	b	0.00	b	0.00	b
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	a
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	468.33	a	438.33	a	395.00	a
T_5=Soil + Crop + N + BCA	468.00	a	443.00	a	392.67	a
CV (%)	1.06		2.04		2.82	
Tukey HSD	10.59		19.26		23.92	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of rice, at 10 DAI the highest CFU/g of soil (468.33 × 10^3) was observed in T_4 (Soil + Crop + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_5 (Soil + Crop + N + BCA). At 20 DAI the highest CFU/g of soil (443 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_4 (Soil + Crop + BCA). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA).

Table 9. Effect of wheat crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	b	0.00	b	0.00	b
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	a
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	467.67	a	438.67	a	395.67	a
T_5=Soil + Crop + N + BCA	466.67	a	435.67	a	392.33	a
CV (%)	1.12		2.48		2.67	
Tukey HSD	11.21		23.37		22.59	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of wheat, at 10 DAI the highest CFU/g of soil (467.67 × 10^3) was observed in T_4 (Soil + Crop + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_5 (Soil + Crop + N + BCA). At 20 DAI the highest CFU/g of soil (441.67 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA).

Table 10. Effect of maize crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	c	0.00	d	0.00	c
T_2=Soil + BCA (Control)	465.00	b	437.67	c	391.67	b
T_3=Soil +N + BCA	466.33	b	441.67	bc	396.33	b
T_4=Soil + Crop + BCA	483.67	a	458.00	ab	422.33	a
T_5=Soil + Crop + N + BCA	484.33	a	463.00	a	424.00	a
CV (%)	0.79		1.85		2.62	
Tukey HSD	8.06		17.93		22.99	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of maize, at 10 DAI the highest CFU/g of soil (484.33 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_4 (Soil + Crop + BCA) but different from T_3 (Soil + N + BCA) and T_2 (Soil + BCA), which showed the lowest CFU/g soil (465 × 10^3). At 20 DAI the highest CFU/g of soil (463 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_4 (Soil + Crop + BCA) but different from T_3 (Soil + N + BCA) and T_2 (Soil + BCA), which showed the lowest CFU/g soil (437.67 × 10^3). At 30 DAI the highest CFU/g of soil (424 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_4 (Soil + Crop + BCA) but different from T_3 (Soil + N + BCA) and T_2 (Soil + BCA), which showed the lowest CFU/g soil (391.67 × 10^3).

Table 11. Effect of potato crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	b	0.00	b	0.00	b
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	a
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	467.67	a	436.00	a	394.00	a
T_5=Soil + Crop + N + BCA	466.67	a	439.00	a	393.00	a
CV (%)	1.08		2.18		2.51	
Tukey HSD	10.81		20.56		21.28	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of potato, at 10 DAI the highest CFU/g of soil (467.67 × 10^3) was observed in T_4 (Soil + Crop + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_5 (Soil + Crop + N + BCA). At 20 DAI the highest CFU/g of soil (441.67 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA).

Table 12. Effect of brinjal crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	c	0.00	c	0.00	c
T_2=Soil + BCA (Control)	465.00	b	437.67	b	391.67	b
T_3=Soil +N + BCA	466.33	b	441.67	ab	396.33	b
T_4=Soil + Crop + BCA	484.67	a	460.33	a	419.00	a
T_5=Soil + Crop + N + BCA	486.67	a	462.33	a	425.00	a
CV (%)	0.75		2.24		2.12	
Tukey HSD	7.69		21.68		18.61	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of brinjal, at 10 DAI the highest CFU/g of soil (486.67 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_4 (Soil + Crop + BCA) but different from T_3 (Soil + N + BCA) and T_2 (Soil + BCA), which showed the lowest CFU/g soil (465 × 10^3). At 20 DAI the highest CFU/g of soil (462.33 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_3 (Soil + N + BCA) and T_4 (Soil + Crop + BCA) but different from T_2 (Soil + BCA), which showed the lowest CFU/g soil (437.67 × 10^3). At 30 DAI the highest CFU/g of soil (425 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_4 (Soil + Crop + BCA) but different from T_3 (Soil + N + BCA) and T_2 (Soil + BCA), which showed the lowest CFU/g soil (391.67 × 10^3).

Table 13. Effect of tomato crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	c	0.00	c	0.00	d
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	ab
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	454.67	b	418.67	b	364.33	c
T_5=Soil + Crop + N + BCA	450.00	b	419.00	b	369.67	bc
CV (%)	0.72		1.98		2.83	
Tukey HSD	7.08		18.31		23.19	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of tomato, at 10 DAI the highest CFU/g of soil (466.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA) but different from T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA), which showed the lowest CFU/g soil (450 × 10^3). At 20 DAI the highest CFU/g of soil (441.67 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA) but different from) T_5 (Soil + Crop + N + BCA) and T_4 (Soil + Crop + BCA), which showed the lowest CFU/g soil (418.67 × 10^3). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA) but different from T_4 (Soil + Crop + BCA), which showed the lowest CFU/g soil (364.33 × 10^3).

Table 14. Effect of cabbage crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	c	0.00	c	0.00	d
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	ab
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	453.33	b	415.67	b	369.00	bc
T_5=Soil + Crop + N + BCA	450.33	b	416.67	b	367.00	c
CV (%)	0.77		2.19		2.90	
Tukey HSD	7.60		20.12		23.75	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of cabbage, at 10 DAI the highest CFU/g of soil (466.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA) but different from T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA), which showed the lowest CFU/g soil (450 × 10^3). At 20 DAI the highest CFU/g of soil (441.67 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA) but different from T_5 (Soil + Crop + N + BCA) and T_4 (Soil + Crop + BCA), which showed the lowest CFU/g soil (415.67 × 10^3). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA) but different from T_5 (Soil + Crop + N + BCA), which showed the lowest CFU/g soil (367 × 10^3).

Table 15. Effect of cucumber crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	b	0.00	b	0.00	b
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	a
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	465.67	a	437.33	a	395.00	a
T_5=Soil + Crop + N + BCA	467.67	a	440.00	a	395.67	a
CV (%)	0.99		2.50		2.70	
Tukey HSD	9.91		23.61		22.93	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of cucumber, at 10 DAI the highest CFU/g of soil (468.33 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_4 (Soil + Crop + BCA). At 20 DAI the highest CFU/g of soil (441.67 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA).

Table 16. Effect of chickpea crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	b	0.00	b	0.00	b
T_2=Soil + BCA (Control)	465.00	a	437.67	a	391.67	a
T_3=Soil +N + BCA	466.33	a	441.67	a	396.33	a
T_4=Soil + Crop + BCA	467.00	a	442.00	a	392.33	a
T_5=Soil + Crop + N + BCA	468.00	a	440.67	a	395.00	a
CV (%)	0.90		2.50		2.41	
Tukey HSD	9.02		23.64		20.40	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

In case of chickpea, at 10 DAI the highest CFU/g of soil (468 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_4 (Soil + Crop + BCA). At 20 DAI the highest CFU/g of soil (442 × 10^3) was observed in T_4 (Soil + Crop + BCA) which was statistically similar to T_2 (Soil + BCA), T_3 (Soil + N + BCA) and T_5 (Soil + Crop + N + BCA). At 30 DAI the highest CFU/g of soil (396.33 × 10^3) was observed in T_3 (Soil + N + BCA) which was statistically similar to T_2 (Soil + BCA), T_4 (Soil + Crop + BCA) and T_5 (Soil + Crop + N + BCA).

Table 17. Effect of soybean crop and nematode on the persistence of *P. lilacinum* in the rhizosphere soil at 10, 20 and 30 days after inoculation (DAI)

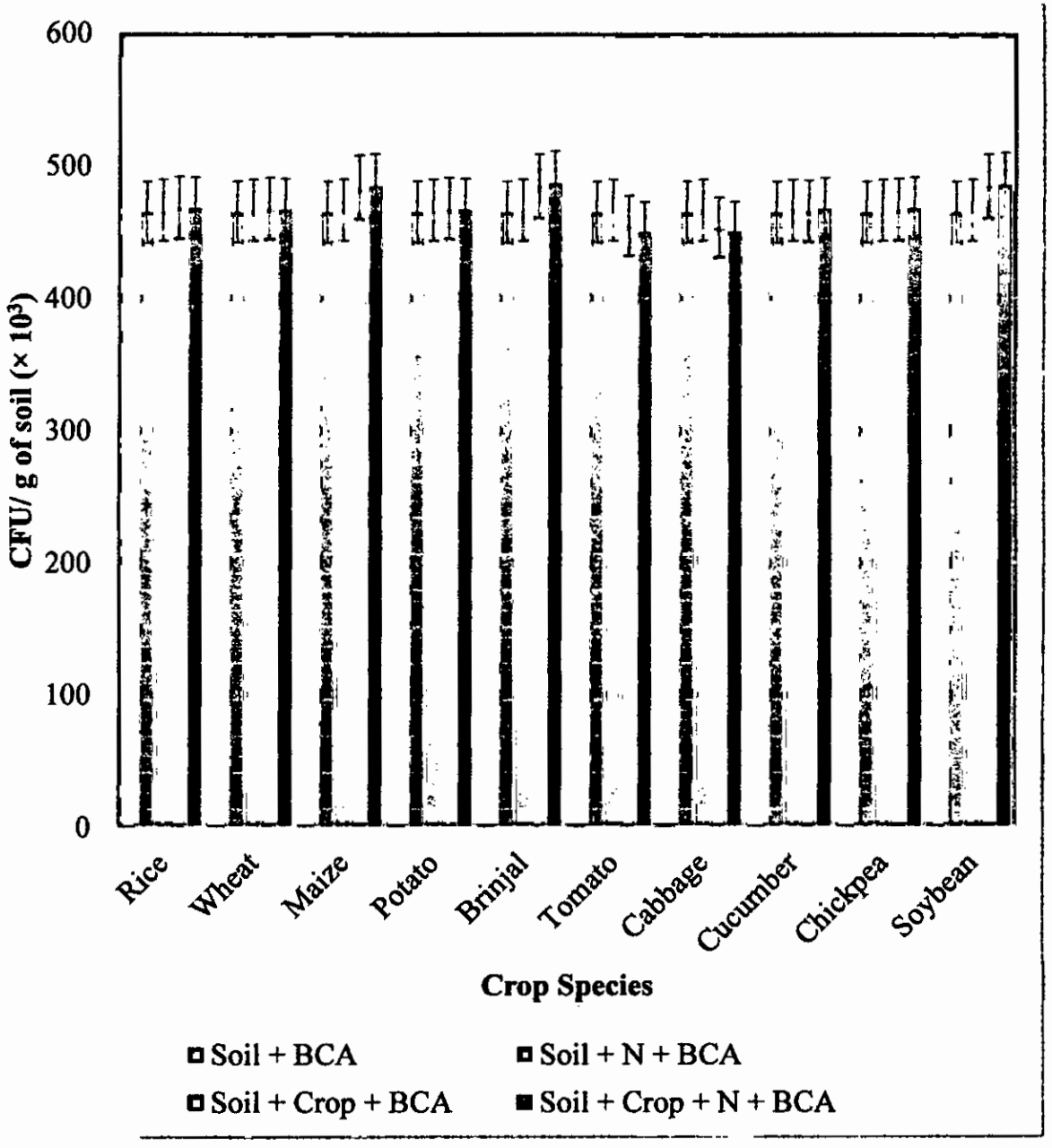
Treatments	CFU/g of soil (× 10^3)					
	10 DAI		20 DAI		30 DAI	
T_1= Soil (Control)	0.00	c	0.00	c	0.00	c
T_2=Soil + BCA (Control)	465.00	b	437.67	b	391.67	b
T_3=Soil +N + BCA	466.33	b	441.67	ab	396.33	ab
T_4=Soil + Crop + BCA	484.33	a	458.67	ab	418.33	a
T_5=Soil + Crop + N + BCA	485.67	a	460.33	a	416.67	a
CV (%)	0.75		2.18		2.52	
Tukey HSD	7.69		21.03		22.01	
Level of Significance	**		**		**	

N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

** (Significant at 1% level)

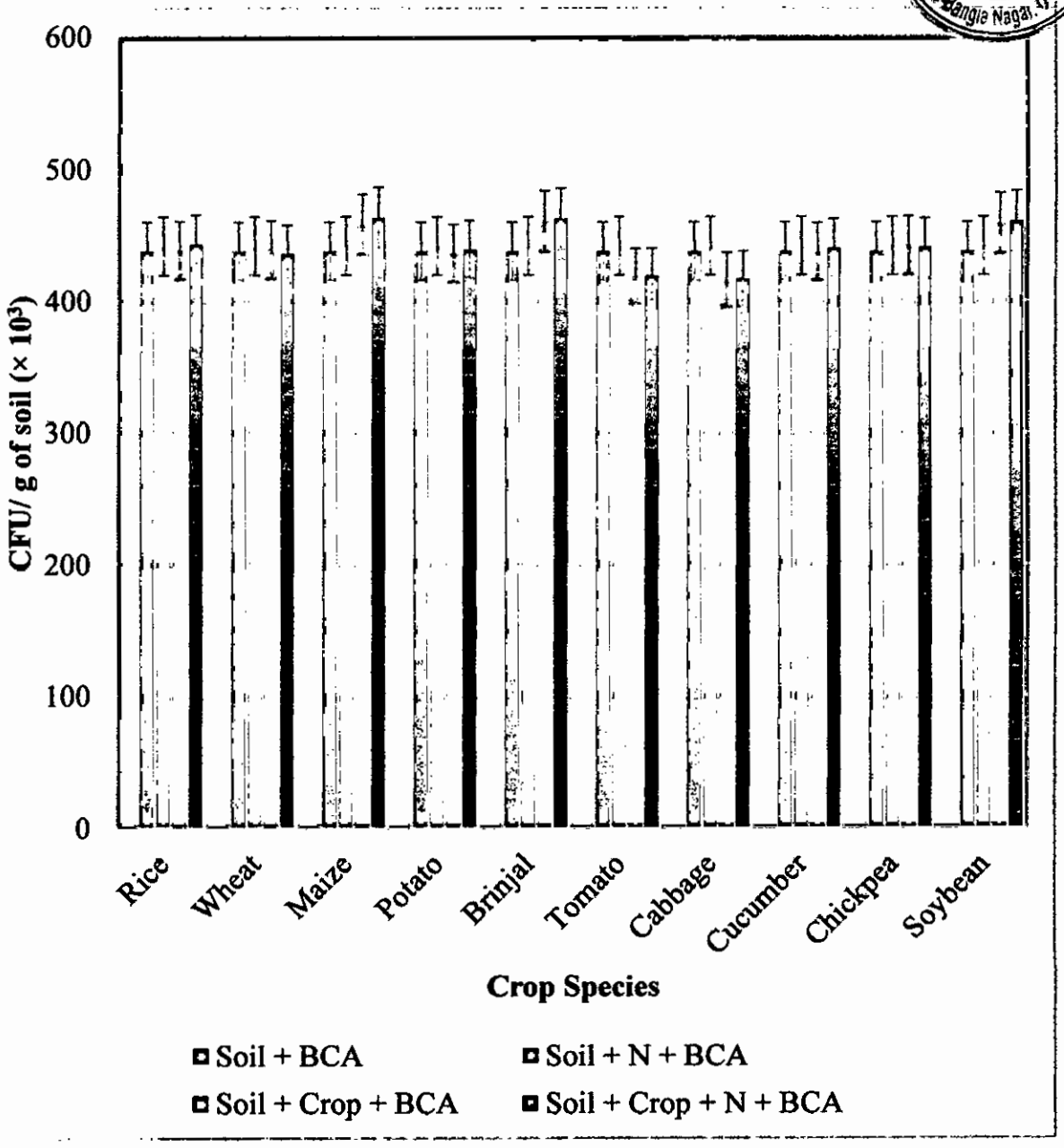
In case of soybean, at 10 DAI the highest CFU/g of soil (485.67 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_4 (Soil + Crop + BCA) but different from T_3 (Soil + N + BCA) and T_2 (Soil + BCA), which showed the lowest CFU/g soil (465 × 10^3). At 20 DAI the highest CFU/g of soil (460.33 × 10^3) was observed in T_5 (Soil + Crop + N + BCA) which was statistically similar to T_3 (Soil + N + BCA) and T_4 (Soil + Crop + BCA) but different from T_2 (Soil + BCA), which showed the lowest CFU/g soil (437.67 × 10^3). At 30 DAI the highest CFU/g of soil (418.33 × 10^3) was observed in T_4 (Soil + Crop + BCA) which was statistically similar to T_3 (Soil + N + BCA) and T_5 (Soil + Crop + N + BCA) but different from T_2 (Soil + BCA), which showed the lowest CFU/g soil (391.67 × 10^3).



N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

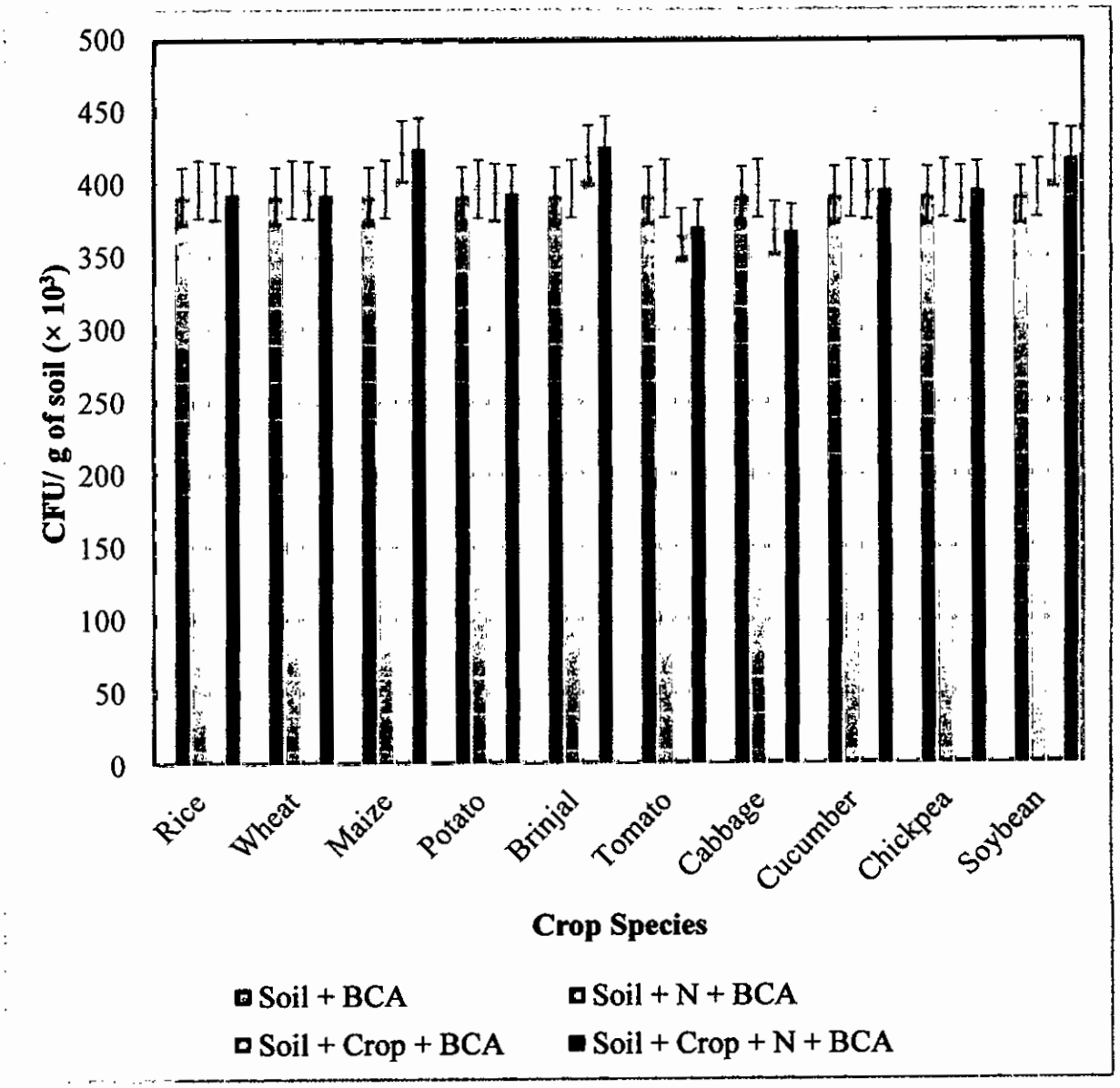
Figure 1. Effect of crop species and nematode on the persistence of *Purpureocillium lilacinum* in rhizosphere soil at 10 days after inoculation (DAI).



N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

Figure 2. Effect of crop species and nematode on the persistence of *Purpureocillium lilacinum* in rhizosphere soil at 20 days after inoculation (DAI).



N= Nematode (*Meloidogyne incognita*)

BCA= Bio Control Agent (*Purpureocillium lilacinum*)

Figure 3. Effect of crop species and nematode on the persistence of *Purpureocillium lilacinum* in rhizosphere soil at 30 days after inoculation (DAI).

In general, population density of *Purpureocillium lilacinum* in planted and non-planted soil did not increase rather decreased after inoculation. In this study, a density between 486.67 × 10^3 in brinjal to 450 × 10^3 in tomato at 10 DAI, 463 × 10^3 in maize to 415.67 × 10^3 in cabbage at 20 DAI and 425 × 10^3 in brinjal to 364.33 × 10^3 in tomato at 30 DAI was found after 10, 20 and 30 DAI whereas the initial population was 5.23×10^5. The reduction in CFU numbers per gm of soil compared to initial densities ranged from 6.5 % in brinjal to 14.6 % in cabbage at 10 DAI, 9.8 % in brinjal to 21.8 % in cabbage at 20 DAI and 17.3% in maize to 32 % in tomato at 30 DAI. This indicates that multiple applications of *Purpureocillium lilacinum* are essential to maintain a high density for sufficient, long-term control.

In the experiment, the CFU/ g of soil in the rhizosphere of 3 out of 10 crop species i.e. maize, brinjal, soybean was significantly higher compared to soil. Conversely, in the experiment, 2 out of 10 crop species i.e. tomato and cabbage showed significantly lower CFU/g of soil in their rhizosphere compared to soil. Overall, it was found that, 8 out of 10 crop species tested showed higher densities compared to soil.

In the present study, we observed that rice, wheat, potato, cucumber and chickpea did not show insignificantly higher population densities compared to soil at all three time intervals. It was shown that at 10, 20 and 30 days after inoculation, in these crops the highest CFU/ g of soil was obtained from Soil + N + BCA or Soil + Crop + BCA or Soil + Crop + N + BCA. In all cases the CFU/g of soil obtained from these treatments were statistically similar to Soil + BCA and also to each other. These findings revealed that both the crop species and nematode population did not exert significant effect on the population densities or population dynamics of *Purpureocillium lilacinum* whereas time was the factor to have an obvious effect on the persistence of BCA *Purpureocillium lilacinum* as the CFU/g of soil was becoming lower in course of time. The population density of *Purpureocillium*

lilacinum was rapidly declined from the initial population densities at 10, 20 and 30 days after inoculation.

This study also revealed that maize, brinjal and soybean showed a significantly higher population density compared to soil at all the three time intervals. At 10, 20 and 30 days after inoculation it was showed that the highest CFU/ g of soil was obtained from Soil + Crop + BCA or Soil + Crop + N + BCA. The CFU/g of soil obtained from these treatments were statistically similar to each other but they showed higher population densities than in Soil + BCA. These findings also explored that the population densities of *Purpureocillium lilacinum* were not affected by nematode population rather time was the factor to decline the population densities of BCA *Purpureocillium lilacinum* as the fungal population density also decreased here in course of time at 10, 20 and 30 days after inoculation. But here it might have some effects of these crop on the persistence ability of the BCA *Purpureocillium lilacinum* in rhizosphere.

Furthermore, the experiment also explored that tomato and cabbage showed significantly lower CFU/g of soil in their rhizosphere compared to soil at 10, 20 and 30 days after inoculation. The lowest CFU/g of soil in both of them was found in Soil + Crop + BCA or Soil + Crop + N + BCA which were statistically similar to each other whereas the highest CFU/g of soil was found in Soil + BCA or Soil + N + BCA which were also statistically similar to each other. These findings also explored that the population densities *Purpureocillium lilacinum* were not affected by nematode population but likewise maize, brinjal and soybean there might have some effect of crop species on the population densities of *Purpureocillium lilacinum*. Here also in course of time at 10, 20 and 30 days after inoculation the results showed a declining population densities of *Purpureocillium lilacinum*.

Studies of several researchers reported that the antagonistic effect of the fungal BCA *Purpureocillium lilacinum* mainly depends on its population density in soil. For instance, Aminuzzaman (2009) observed the egg and juvenile parasitism of *Meloidogyne* spp. by nematophagous fungus *Paecilomyces lilacinus* in soil tube test and it has been found that egg and juvenile parasitism depends on the fungal population density. In another experiment, Aminuzzaman *et al.* (2013) used alginate pellets of *Paecilomyces lilacinus* YES-2 and *Pochonia chlamydosporia* HDZ-9 for controlling of *M. incognita* on tomato in a greenhouse by adding them into a soil with sand mixture at rates of 0.2, 0.4, 0.8 and 1.6% (w/w) and found that *P. lilacinus* pellets at the highest rate (1.6%) reduced root galling by 66.7%. In an experiment conducted by Singh *et al.* (2009), they used 1, 2 and 3 g substrate of *P. lilacinus* (19.59×10^8 spores/g substrate) per kg soil in pot experiments on plant growth and nematode multiplication on tomato. They also found that the eggs parasitized by *P. lilacinus* were fungus density dependent and parasitization increased with the increase in level of fungus in the soil.

In the experiment, in most of the crop species the population density of BCA *Purpureocillium lilacinum* declined at 10, 20 and 30 days after inoculation. Many reports of researchers validate the results. Hewlett *et al.* (1988) evaluated the efficacy of the nematode parasite *Paecilomyces lilacinus*, alone and in combination with phenamiphos and ethoprop, for controlling the root-knot nematode *Meloidogyne javanica* on tobacco and the ability of this fungus to colonize in soil under field conditions for 2 years in microplots and observed that the average soil population densities of *P. lilacinus* remained high, ranging from 1.2 to 1.3 x 10^6 propagules/g soil 1 week after the initial inoculation and from 1.6 to 2.3 x 10^4 propagules/g soil at harvest the second year which indicates rapid decline of the fungal population density. Cabanillas *et al.* (1989) conducted laboratory and microplot experiments to determine the influence of carrier and storage of *Paecilomyces lilacinus* on its survival and related protection of tomato against

Meloidogyne incognita and observed that the fungal viability was high in wheat and granules, intermediate in pellets, and low in soil. In an experiment, Gomes Carneiro and Cayrol (1991) studied relationship between inoculums density of *Paecilomyces lilacinus* against *Meloidogyne arenana* on tomato. Results showed that the number of fungal propagules in the soil was correlated to the initial dose applied and decreased progressively through the time with increased dose. This fact and a rapid decrease in fungal density in soil below the acceptable control levels, limit the use of this fungus as a biological control agent. Kiewnick *et al.* (2003) conducted dose response experiments with the root-knot nematode *Meloidogyne incognita* on tomatoes using the new *P. lilacinus* WDG formulation. Monitoring the *P. lilacinus* population in the rhizosphere showed a decline after 2 to 3 month which can lead to insufficient control over a full growing season. They suggested repeated application to maintain the antagonist population at a sufficient to secure long term control of root-knot nematodes. In another experiment, Kiewnick *et al.* (2004) conducted greenhouse experiments with the root-knot nematodes *Meloidogyne incognita* and *M. hapla* on tomato. They found that due to the decline in fungal population a combination of pre-planting application plus the seedling and one post plant drench of *P. lilacinus* gave the best control. Mendoza *et al.* (2004) conducted dose response and form of application experiments with burrowing nematode, *Radopholus similis*, on banana using a commercial water dispersible granulate formulated *P. lilacinus* and observed that the best control was achieved in the treatment when plantlets and soil were pre-inoculated with *P. lilacinus* and reinoculated during transplantation as the fungal density declined rapidly. Rumbos *et al.* (2006) investigated the effect of 12 plant species on the persistence of *Paecilomyces lilacinus* strain 251 in soil and observed no significant differences between soil without plants and soil from the root zone of the majority of the test plants. They observed that bean was the only plant species consistently exerting a negative effect on the persistence of *P. lilacinus* strain 251 in the soil. In another experiment, Rumbos *et al.* (2006) studied the interactions of *Paecilomyces lilacinus* strain 251 with the arbuscular

mycorrhizal fungus *Glomus intraradices* against *Meloidogyne incognita* on tomato and observed the decline of the nematophagous fungus densities after single application in soil. Kiewnick (2007) and Kiewnick *et al.* (2011) evaluated the fungal biocontrol agent, *Paecilomyces lilacinus* strain 251 (PL251), for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato and the results demonstrated that rhizosphere competence is not the key mode of action or the key factor for the efficacy of PL251 rather the efficacy of PL251 depended strongly on the ratio between application rate and inoculum density in soil. They concluded that due to the rapid decline of PL251 in soil, repeated applications are needed to maintain a sufficient density of PL251 for season-long protection. The experiments conducted by Sarven (2013) and Faria (2013) also validates the results of declining *Paecilomyces* population in rhizosphere.

The results also revealed that the fungal population density was declined in course of time irrespective of crop species or nematode population. These findings could be validated by the experiment of Zhen Yu *et al.* (2015) who investigated the effects of root-knot nematode biocontrol agent *Paecilomyces lilacinus* strain PL1210 on ammonia-oxidizing microorganisms and fungal community composition of tomato rhizosphere and cluster analysis showed that the composition of rhizosphere fungal community was more significantly influenced by time-related differences than by the inoculation of biocontrol agents. In another experiment, Kiewnick (2009) tested the facultative egg-pathogenic fungus *Paecilomyces lilacinus* strain 251 (PL251) for control of plant-parasitic nematodes and monitoring the persistence of PL251 under field conditions using dilution plating techniques and nested PCR revealed a rapid decline of the fungal density in soil over time. Although detection of PL251 in soil was still possible two years after application.

Some studies could contradict to the results as they showed an influence of crop species on the persistence of biocontrol agent but that could not as the biocontrol agent was other than *Purpureocillium lilacinum*. Mahdy *et al.* (2001) studied the influence of plant species on the antagonistic activity of the rhizosphere bacterium *Rhizobium etli* G12 towards the root-knot nematode *Meloidogyne incognita* and the results indicated a clear influence of plant species on the ability of *R. etli* G12 to reduce nematode infection. This indicated that plant species might have some impact on the persistence of *R. etli* G12. Jash and Pan (2007) evaluated antagonistic activity and root colonizing behavior of 10 *Trichoderma* isolates and observed that the colonization of non-rhizospheric soil by *Trichoderma* isolate was very low compared to that of rhizosphere.

The results of the experiment revealed that the inoculation of nematode did not have any significant effect on the persistence of the *Purpureocillium lilacinum* whether it was in planted or non-planted pot. This result could be supported by the experimental findings of Mitu (2012) who evaluated impact of *Paecilomyces lilacinus* application time on plant growth and suppression of root-knot nematode (*Meloidogyne incognita*) in some selected vegetables and showed that in application of *P. lilacinus* and inoculation of *M. incognita* simultaneously at planting or *P. lilacinus* was applied at planting and *M. incognita* at 7 days after planting sequentially similar reduction of *M. incognita* population was observed. This revealed that the persistence of the fungus was not influenced by the nematode population as nematode parasitism depends on fungal population density. (Aminuzzaman, 2009, Singh *et al.* 2009, Aminuzzaman *et al.* 2013). However, some studies contradict to this finding of having no effect of nematode population on the persistence of *Purpureocillium lilacinum* rather the nematode infection increased the fungal population density in soil. In an experiment, Gaspard and Ferris (1990) determined population densities of *Meloidogyne incognita* and the nematophagous fungi, *Paecilomyces lilacinus* and *Verticillium chlamydosporium* in

soil and found that the numbers of *V. chlamydosporium* and *P. lilacinus* increased more in soils with *M. incognita*-infected tomato plants than in soil with uninfected tomato plants.

According to the data derived, the presence of crop species rice, wheat, potato, chickpea, cucumber did not exert any significant effect on the dynamics of the fungus in the soil. It was observed that soil planted with 3 crops viz. maize, brinjal and soybean showed higher CFU/g of soil than non-planted soil and on the contrary, 2 crops viz. tomato and cabbage showed lower CFU/g of soil than non-planted soil. The experiment conducted by Rumbos *et al.* (2006) clearly supports these findings of our result. They investigated the effect of 12 plant species on the persistence of *Paecilomyces lilacinus* strain 251 in soil and observed that corn, barely, bean and brinjal showed higher population densities of *Purpureocillium lilacinum* compared to soil whereas tomato and cabbage showed lower population densities of *Purpureocillium lilacinum* compared to soil. A similar effect was observed by Aziz *et al.* (1997) who showed that bean exudates reduced the biocontrol efficacy of *Trichoderma lignorum.* In this study, the decline of *Purpureocillium lilacinum* population density in pots containing cabbage and tomato was higher compared to soil. In an experiment, Manhong *et al.* (1998) showed that *Paecilomyces lilacinus* could colonize in soybean rhizosphere, when it was used as a seed-coat. The number of propagules observed in endorhizosphere in sterilized soils was 1/1000 of the coated fungi at the 1st week. It increased over 10-fold in the 2nd week, and began to decrease after 4 weeks planting. These findings support the result as well. But it was assumed that there might have some effects of these crops on the persistence of BCA *Purpureocillium lilacinum* in rhizospheric soil. However, in order to clarify whether these effects are due to root morphology or root exudates, further investigation is needed.

The data presented demonstrate that the density of *Purpureocillium lilacinum* in the rhizosphere in most of the crop species was higher than in soil. This effect is probably due to the saprophytic nature of *Purpureocillium lilacinum* and higher nutrient availability in the rhizosphere. For *Verticillium chlamydosporium,* an intensively studied nemmatophagous fungus, it has been shown that rhizosphere colonization is a prerequisite for the control of nematode by this antagonist (De Leij and Kerry, 1991). In addition, Mauchline *et al.* (2002) showed the potential of *Verticillium chlamydosporium* to proliferate in soil and on the roots of the host plants and suspected that changes in the root exudation caused by inoculation with plant parasitic nematodes resulted in different population densities of the fungus. In contrast to these findings, *Purpureocillium lilacinum* was never found to proliferate or to establish in the soil and rhizosphere. Furthermore, previous studies have shown that the decline of *Purpureocillium lilacinum* densities was not altered by the presence of nematode infected tomato roots (Kiewnick *et al.* 2004). These results indicate that unlike *V. chlamydosporium*, in the soil and root environment, a different behavior is shown by *Purpureocillium lilacinum.*

However, the significance of these findings for the biocontrol activity of *Purpureocillium lilacinum* needs further investigation.

Chapter 5

Summary and Conclusion

Summary and Conclusion

Experiments were conducted to evaluate the root colonization ability in 10 crop species along with 2 different rooting media and the persistence of the fungal BCA *Purpureocillium lilacinum* in rhizosphere soil of the 10 crop species along with the presence or absence of nematode population.

In the first experiment, ten crops viz. Rice, Wheat, Maize, Potato, Brinjal, Tomato, Cabbage, Cucumber, Chickpea and Soybean were tested in a test tube experiment using two different rooting media viz. coco dust and soil rooting media to observe their effect on root colonization ability. This experiment was laid out in a Completely Randomized Design (CRD) with three replications per treatment. The test tubes were filled with two different autoclaved media up to two-third of its volume and five 1 cm plugs of the fungus *P. lilacinum* were inserted just below the surface of the media and two surface sterilized germinated seeds of each crop species were placed on top of the fungus and then sealed and incubated at 27°C in the dark of one week. After incubation for a week the roots were harvested and cut into 1 cm sections and placed on PDA media and incubated at 27°C for 5 days. After 5 days of incubation period the root segments colonized by the fungus were counted and the percent root colonization was determined.

In the second experiment, the same ten crop species were tested with or without nematode in pot experiment to observe their effect on the persistence of the fungal BCA *Purpureocillium lilacinum* in rhizosphere. This experiment was also laid out in a Completely Randomized Design (CRD) with three replications per treatment. Five treatments viz. T_1 = Soil (Control), T_2 = Soil + *Purpureocillium lilacinum*

(Control), T_3 = Soil + *Meloidogyne incognita* + *Purpureocillium lilacinum*, T_4 = Soil + Crop root + *Purpureocillium lilacinum* and T_5 = Soil + Crop root + *Meloidogyne incognita* + *Purpureocillium lilacinum* were used. After mass multiplication spore suspension of *Purpureocillium lilacinum* was applied to each pot to achieve an initial population of 5.23×10^5 $CFUg^{-1}$ soil and one germinated seed was placed. Five egg masses were applied in the pots two days after the placement of germinated crop seeds. 1 g of rhizosphere soil was collected from each treatment at 10, 20 and 30 days of interval and the number of colony forming unit per gram soil ($CFUg^{-1}$ soil) was determined using the soil dilution plate method to determine the persistence of *Purpureocillium lilacinum* in rhizosphere.

The results of the first experiment revealed that *Purpureocillium lilacinum* resulted in root colonization in all of the crop species in varying percentage depending on the rooting media and the crop species themselves. In this experiment, a constant 100% root colonization was obtained from all crop species observed in coco dust which excluded the effect of crop species on root colonization ability of *Purpureocillium lilacinum.*

On the contrary, in soil, the percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was varied but not significantly different from one another except some of the crop species tested. The maximum percent root colonization was observed in Cucumber (67.17) which was followed by Maize (55.50), Potato (50.00) and Brinjal (48.14). Following them Cabbage (44.28) and Rice (44.26) showed almost the same percent of root colonization which was followed by Tomato (43.45). However, they all were statistically similar to each other and also similar to Cucumber whereas we observed minimum percent root colonization in Chickpea (30.55) which was preceded by Soybean (32.07) and Wheat (37.00). Only chickpea statistically showed significant dissimilarity with maize in the root colonization ability of *Purpureocillium lilacinum.*

In case of the effect of rooting media, the coco dust rooting media caused a constant and maximum percentage of root colonization (100) whereas the minimum percent root colonization of the crops by the fungus *Purpureocillium lilacinum* was obtained in soil medium (45.30). But in a combined effect of crop species and rooting media, in all crop species the coco dust resulted in 100 % root colonization whereas the soil resulted in a maximum of 67.17 % of root colonization in cucumber and the minimum of 30.55 % root colonization in chickpea. Despite finding out the colonization ability of *Purpureocillium lilacinum* in various crops whether it is epiphytically or endophytically the results of the experiment revealed root colonization ability of the BCA in a diversified crop species in two different media with a varying percentage. However, the colonization of roots may influence the persistence and establishment in the environment and needs to be considered for an appropriate assessment of the risks involved by applying biocontrol agents on a large scale.

The results of the second experiment revealed that population density of *Purpureocillium lilacinum* in planted and non-planted soil did not increase rather decreased after inoculation. In this study, a density between 486.67×10^3 in brinjal to 450×10^3 in tomato at 10 DAI, 463×10^3 in maize to 415.67×10^3 in cabbage at 20 DAI and 425×10^3 in brinjal to 364.33×10^3 in tomato at 30 DAI was found after 10, 20 and 30 DAI whereas the initial population was 5.23×10^5. The reduction in CFU numbers per gm of soil compared to initial densities ranged from 6.5 % in brinjal to 14.6 % in cabbage at 10 DAI, 9.8 % in brinjal to 21.8 % in cabbage at 20 DAI and 17.3% in maize to 32 % in tomato at 30 DAI.

The result indicated that the density of *Purpureocillium lilacinum* in the rhizosphere in most of the crop species was higher than in soil. The CFU/ g of soil in the rhizosphere of 3 out of 10 crop species i.e. maize, brinjal, soybean was significantly higher compared to soil. Overall, it was found that in this experiment, 8 out of 10 crop species tested showed higher densities compared to soil. Whereas, in the

experiment, 2 out of 10 crop species i.e. tomato and cabbage showed significantly lower CFU/g of soil in their rhizosphere compared to soil.

Among the crop species five out of ten crop species i.e. rice, wheat, potato, cucumber and chickpea showed insignificantly higher population densities compared to soil at 10, 20 and 30 days after inoculation. In these crops the highest CFU/ g of soil was obtained from the treatment Soil + N + BCA or Soil + Crop + BCA or Soil + Crop + N + BCA. But maize, brinjal and soybean showed a significantly higher population density compared to soil where the highest CFU/ g of soil was obtained from Soil + Crop + BCA or Soil + Crop + N + BCA. The CFU/g of soil which were statistically similar to each other. On the contrary, tomato and cabbage showed significantly lower CFU/g of soil in their rhizosphere compared to soil and the lowest CFU/g of soil in both of them was found in Soil + Crop + BCA or Soil + Crop + N + BCA which were statistically similar to each other whereas the highest CFU/g of soil was found in Soil + BCA or Soil + N + BCA which were also statistically similar to each other.

All the findings of this experiment concluded that both the crop species (in most cases) and nematode population did not exert significant effect on the population densities or population dynamics of *Purpureocillium lilacinum* whereas time was the factor to have an obvious effect on the persistence of BCA *Purpureocillium lilacinum* in soil. The population density of *Purpureocillium lilacinum* was rapidly declined from the initial population densities at 10, 20 and 30 days after inoculation.

For the nematophagous fungus *Purpureocillium lilacinum,* the presented data lead to the conclusion that only a low persistence in the environment is to be expected. This fact and a rapid decrease in fungal density in soil below the acceptable control levels, limit the use of this fungus as a biological control agent. Based on this fact, many researchers suggested a repeated application of antagonist population with a combination of pre-planting application plus the seedling and one post plant drench

of *P. lilacinus* to secure long term control of root-knot nematodes and to leave the pest control.

From an environmental perspective, decline of an antagonist after application is a positive feature. Due to large doses applied, it is important to know that the biocontrol agent does not persist so that its potential for adverse environmental impacts is low (Westergaard et al., 2004). Concerning the importance of this finding for biological control efficacy, the most critical point is to maintain the necessary population density of the biocontrol agent long enough to protect the plant (Pandit, 2000). For *Pochonia chlamydosporia* (PL251), a concentration of 10^6 CFU/g of soil or higher is required to achieve a sufficient reduction in root-knot nematode damage (Kiewnick and Sikora, 2003, 2004).

In conclusion, *Pochonia chlamydosporia* showed a diversified ability to colonize roots in a wide range of crop species. Irrespective of the presence or absence of nematode, some crop species showed insignificantly higher and some showed significantly higher or lower population densities of *Pochonia chlamydosporia* compared to soil. But the fact was *Pochonia chlamydosporia* was not found to persist long in soil. This indicates that applying this biocontrol agent will have a minimal impact on the environment and possible effects will be limited to the season of application. Furthermore, it could be demonstrated that the host plant and nematode was not the primary factor affecting the persistence of the fungus *Pochonia chlamydosporia*. More research is needed to investigate the importance of this findings for appropriate assessment of this fungus to colonize crop roots, persist and establish in the environment.

Chapter 6 References

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