DISCUSSION

The strains/isolates of *Trichoderma harzianum*, *Trichoderma virens*, *Pochonia chlamydosporia*, *Bacillus subtilis* and *Pseudomonas fluorescens* used in the study showed varied degree of antagonism against *Fusarium udum* in preliminary *in-vitro* tests. All isolates of *T. harzianum* and *T. virens* overgrew the pathogen in the dual culture test, but to a varied extent. *Trichoderma* isolates possess diversity in virulence and growth patterns (Dennis and Webster, 1971a; Gao et al., 2002). In the dual culture, hyphae of some isolates Th00, Th5 and Tv00 coiled around the hyphae of *F. udum* as soon as the two colonies came in contact. While in other, coiling did not occur until the *Trichoderma* hyphae had penetrated far into the host fungus colony. Other researchers have reported similar pattern of mycoparasitism by *Trichoderma* isolates on various pathogenic fungi (Lim and Teh, 1990; Nigam et al., 1997; Gao et al., 2001). The tested isolates also produced certain volatile compounds and suppressed the colonization by *F. udum*. The standard strains of *T. harzianum* (Th00) and *T. virens* (Tv00) followed by the isolate Th05 of *T. harzianum* and Tv01 of *T. virens* caused maximum reduction in radial growth of *F. udum*. Effectiveness of the volatile compounds produced by *Trichoderma* spp. varied with the age of culture. Two day old cultures of both *T. harzianum* and *T. virens* caused greatest reduction in radial growth of *F. udum* being greater with former species. With the age, the cultures lost ability to produce volatile metabolites and consequently the ability to suppress fungal growth with a maximum colonization by *F. udum* with 10 day old culture of *Trichoderma* species. In a classical study Dennis and Webster (1971c) investigated the production of volatile compounds by different isolates and species groups of *Trichoderma* against plant pathogenic fungi, *Fusarium*, *Sclerotium*, *Pythium*, *Rhizoctonia* etc. These compounds play an active role in the suppression of soil borne plant pathogens (Lim and Teh, 1990; Angappan, 1992; Gao et al., 2001; Gao et al., 2002).

Suppressive effects of culture filtrates (nonvolatile compounds) of *Trichoderma* spp. on the radial growth of *F. udum* were found to be concentration dependent. Higher concentration of the filtrates (50, 75 and 100%) were more
inhibitory than lower concentration (25%). Dennis and Webster (1971b) reported the production of nonvolatile compounds by *Trichoderma* spp. which were inhibitory to various pathogens. Gliotoxin or viridin were not produced by the mycoparasite, but other chloroform-soluble antibiotics, including trichodermin and peptide antibiotics were detected in culture filtrates. Other researchers have also reported the production of nonvolatile compounds by *Trichoderma* spp. and resulted inhibition in the radial growth of pathogens (Deshmukh and Pant, 1992; Dwivedi, 1992).

Culture filtrates of *B. subtilis*, *P. fluorescens* and *P. chlamydosporia* suppressed egg hatch and induced mortality to the juveniles of *Meloidogyne incognita*. *Pochonia chlamydosporia* colonized egg masses of the nematode and decreased the egg hatch. The culture filtrates of *P. fluorescens* and *B. subtilis* also inhibited hatching and caused death to larvae. *In-vitro* antagonism through the culture filtrate of *B. subtilis* (Siddiqui and Mahmoud, 1995), *P. fluorescens* (Siddiqui et al., 2003; Siddiqui and Shaukat, 2004) and *P. chlamydosporia* (Saifullah, 1996 a. b. c) against plant parasitic nematodes is well documented, but degree of effectiveness vary with the isolate. In the present study, isolates of *P. chlamydosporia*, *P. fluorescens* and *B. subtilis* induced varied degree of inhibition in hatching and mortality to juveniles of *M. incognita*.

The integration of biocontrol agents along with fungicides for effective management of soil borne diseases is present day’s need. The tolerant strains of biocontrol agents against pesticides need to be identified and integrated in managing the diseases. Attempts have been made to develop tolerant strains against carbendazim (Yang Qian and Zhao, 1996) and some other fungicides (Pecchia, 1994; Cuevas et al., 1994; Migheli et al., 1995; Mrinalini and Lalithakumari, 1998). In the present study, compatibility of *Trichoderma harzianum*, *T. virens*, *P. chlamydosporia*, *B. subtilis* and *P. fluorescens* with two systemic and three nonsystemic fungicides was examined. The concentrations of 60 μg carbendazim/ml, 1050 μg metalaxyl/ml, 160 μg captan/ml and 225 μg mancozeb/ml seem to be safe tolerance limit (ED$_{90}$) for *T. harzianum* whereas the corresponding values for *T. virens* were 40 μg carbendazim/ml, 1000 μg metalaxyl/ml, 125 μg captan/ml and 177 μg/ml (mancozeb). The ED$_{90}$ values of thiram for growth of *T. harzianum* and *T. virens* were 150 and
Whereas, 25 and 9 \( \mu \text{g/ml} \) concentrations seem to be safe tolerance limit (ED\(_{50}\)) for \textit{T. harzianum} and \textit{T. virens}, respectively. Similar results have been obtained by other workers. Sharma \textit{et al.} (2001) found 0.1% metalaxyl and 0.0065% carbendazim as safe tolerance limit (ED\(_{50}\)) for \textit{T. harzianum}. Similar results were obtained for carbendazim and benomyl (Papavizas \textit{et al.}, 1982; Jayaraj and Radhakrishnan, 1997; Viji \textit{et al.}, 1997) and for metalaxyl (Mukhopadhyay \textit{et al.}, 1986; Mukherjee \textit{et al.}, 1989). Different workers have reported chlorothalonil, captan and captafol as tolerant for \textit{T. harzianum} even at higher concentrations upto 2000 \( \mu \text{g/ml} \) in spore germination tests (Abdel Moity \textit{et al.}, 1982; Papavizas \textit{et al.}, 1982). More researches are needed on the integration of \textit{Trichoderma} spp. with chemical approaches to improve effectiveness of the disease control module. \textit{P. chlamydosporia} showed less tolerance to the five fungicides tested than \textit{Trichoderma} spp., whereas the biocontrol bacteria were more tolerant than the fungi. Among the bacteria, \textit{P. fluorescens} was found to be more compatible with fungicides than \textit{B. subtilis}, the MTC for the former being 2500 \( \mu \text{g} \) Thiram/ml, 1600 \( \mu \text{g} \) mancozeb/ml and 50,000 \( \mu \text{g/ml} \) for captan and carbendazim. Researchers have shown that some bacteria can use pesticides as nutrients, and hence can tolerate higher concentrations of the chemicals (Kishore and Jacob, 1987; Aislabie and Jones, 1995).

\textit{In vitro} studies were also carried out to study the fermentation and biochemical characteristics of the isolates of \textit{B. subtilis} and \textit{P. fluorescens}. All the isolates differed in the fermentation behaviour and biochemical properties. Similar variations in biochemical and fermentation behaviour of soil isolates of \textit{B. subtilis} and \textit{P. fluorescens} have been reported by other researchers (Satar and Gaur, 1989; Gaind and Gaur, 1991; Mahmood, 1999).

All six strains each of \textit{P. fluorescens} and \textit{B. subtilis} solubilized phosphorus evidenced by the zone of solubilization on agar plates; diameter of the zone was wider with \textit{P. fluorescens} than \textit{B. subtilis} and varied with the isolate. \textit{P. fluorescens} and \textit{B. subtilis} are well known for phosphate solubilization (Gaur, 1990; Dave and Patel, 1999). Production of organic acids by the bacteria is one of the important determinants of phosphorus solubilization (Gaur, 1990; Satpul and Kapoor, 1992;
Singal et al., 1994; Illmer and Schinner, 1995; Dave and Patel, 1999). In the present study a direct inverse relation was observed between pH value and phosphorus solubilization in vitro. It is an established fact that \( P. \) fluorescens and Bacillus subtilis produce growth promoting substances (phytohormones) like indole acetic acid (IAA), gibberellic acid etc. (Pal et al., 2001; Gracia de Salamone et al., 2001). However, amount of IAA production depends on the species and strains. The present results showed that \( P. \) fluorescens and \( B. \) subtilis produced IAA in Luria Bertani broth (LBB) supplemented with tryptophan. Other researchers have also detected IAA production by \( P. \) fluorescens and \( B. \) subtilis in the supplemented LBB (Oberhansli et al., 1991; Glickman et al., 1998; Kawaguchi and Syono, 1996).

The wilt fungus, \( Fusarium \) udum at the inoculum level of 2 g/kg soil caused considerable wilting in pigeonpea and suppressed the growth and yield of plants grown in the pots or field. The wilt symptoms on pigeonpea can appear 4 to 6 weeks after sowing (Upadhayay and Rai, 1992). The initial visible symptoms are loss of turgidity in leaves, and slight interveinal clearing. The foliage shows slight chlorosis and sometimes becomes bright yellow before wilting (Reddy, 1990). In the present study, pigeonpea responded similarly to the inoculation with the fungus and developed typical symptoms of the disease. The first sign of the disease was mild chlorosis and stunted growth that appeared at seedling stage. Some of the stunted seedlings succumbed to the infection. The percent wilting in pigeonpea seedlings and their mortality was greater in pots than microplots. Under field conditions, recognizable wilt symptoms developed when plants were 8-10 weeks old. The seedlings, which escaped early infection exhibit stunted growth and leaf chlorosis at one month age. At a later stage, leaves/branches wilted, drooped or dried. The mycelium of \( F. \) udum grows profusely in vascular tissue especially in xylem tissue and causes browning of vessels from the root to the stem (Agrios, 2000). As a result black streaks gradually develops in xylem tissue whereas brown to black bands appear on the stem surface of partially wilted plants and extend upward from the base. When the bark of such bands is peeled off, browning or blackening of the wood beneath can be seen (Upadhayay and Rai, 1992). In the present study, transverse sections of root and stem revealed the presence of the fungus in xylem tissue. The fungus also colonized
on PDA by inoculating the surface sterilized root and stem pieces of infected pigeonpea plants. The isolated fungus was then reinoculated in the pigeonpea plants to establish its pathogenicity. Nene et al. (1990) have reported that *F. udum* can be isolated from any part of the infected plant from lateral fine roots to pedicel and pod hull.

The used pigeonpea cultivar UPAS 120 was found to be highly susceptible to wilt and exhibited 24-38% yield loss. The annual monetary loss in pigeonpea production due to the wilt has been estimated to be $36 million in India (Kannaiyan et al., 1984). In the present study, Rs. 4162/ha monetary loss was estimated due to wilt in pigeonpea. Application of biocontrol agents either through soil application or seed treatment considerably decreased the pathogenic effect of the wilt fungus in pot experiments. As a result plant growth and yield of pigeonpea increased significantly. Seed treatment with *T. harzianum* decreased the wilt incidence by 49%, whereas its soil application resulted to a 47% check in the disease. *T. virens* was also equally effective in controlling the disease. *Trichoderma* spp. are the established antagonists of soil borne fungal pathogens (Pappavizas, 1985). The satisfactory control of the wilt indicates that the used strain of *T. harzianum* and *T. virens* (strain) suppressed the pathogen effectively.

The suppression in fusarium wilt due to application of *Trichoderma* spp. may have resulted through competition for nutrients that has been found to be involved in the antagonism exerted by the fungus against *F. oxysporum* (Sivan and Chet, 1989). *Trichoderma* spp. produce antibiotics and other metabolites which are potentially capable of inhibiting the colonization by *Fusarium* spp. *in-vitro* (Dwivedi, 1992). The antifungal activity of the mycoparasite was ascertained in dual culture and culture filtrate tests. The *Trichoderma* spp. produced volatile compounds which inhibited the growth of pathogenic fungus (Whipps, 1987; Lim and Teh, 1990). *Trichoderma* spp. are efficient mycorasites and react violently with hyphae of *Fusarium* spp. (Gao et al., 2001). Hyphae of *Trichoderma* attach to the hyphae of pathogenic fungus, either by running parallel or coil around and cause degradation of the host wall by producing lytic enzymes like chitinase and glucan 1, 3 β-glucosidase (Tronsmo et al., 1993). In the present study *T. harzianum* and
T. virens effectively controlled the fusarial wilt; the former was, however, found relatively more aggressive. In the pot experiment also T. harzianum decreased the wilt severity greater than T. virens. In literature, numerous evidences exist which reveal effectiveness of T. harzianum, T. hamatum and T. virens against soil borne fungi (Khan and Gupta, 1998; Khan and Akram, 2000; Bunker and Mathur 2001; Singh and Singh, 2003; Prasad et al., 2003; Singh et al., 2003; Wani, 2005).

Soil application of P. fluorescens and B. subtilis also provided satisfactory decrease in the wilt severity in pigeonpea plants growing in pots. Soil application with P. fluorescens was effective against the wilt as was T. harzianum but its treatment decreased the wilting greater than the mycoparasite. Numerous strains of P. fluorescens and B. subtilis have been found suppressive against soil borne fungal pathogens (Bull et al., 1991). The possible mechanism involved in the suppression may be the competition and rhizosphere colonization (Berger et al., 1996). Antibiosis is the other mechanism by which the biocontrol bacteria would have suppressed F. udum. Antibiotics such as pholoroglucinols (Mazzola et al., 2002), pyrolintrin (Burkhead et al., 1994) and phenazin (De-Souza, 2002) produced by P. fluorescens and agrocin-84 (Kim et al., 1997), bulbiformin (Brannen et al., 1995) etc. produced by B. subtilis have been reported to be fungicidal in nature. Siderophore producing strains of P. fluorescens possess ability to inhibit the germination of chlamydospores of F. oxysporum (Scher et al., 1988; Gamliel and Katan, 1993). The dual culture tests with P. fluorescens and B. subtilis conducted in the present study have shown 52-67% and 49-62% inhibition in the growth of F. udum during 2-6 days, respectively. Both the bacteria have also been found to induce systemic resistance in the plants against fusarial wilt (Leeman et al., 1995; Podile and Laxmi, 1998). In the present study, P. fluorescens was found more effective than B. subtilis against the wilt fungus. Similar observations have been made earlier (Khan and Khan, 2002). Carbendazim treatment resulted to significantly greater decrease in the wilting (P<0.05).

The three biopesticides namely, Biowilt-X (T. harzianum), Bionem-X (P. chlamydosporia) and Biocomp-X (P. fluorescens) prepared on fly ash based carrier contained 10^{8-10} CFUs of biocontrol fungi and 10^{12-13} CFUs of biocontrol
bacteria/g formulations. Highest CFU load was recorded during 6-12 weeks of inoculation. Increase in CFU count during storage indicates that the biocontrol agents utilized the nutrients present in fly ash-molasses mixture. Bacteria and fungi can degrade and utilize the essential elements of fly ash (Fakoussa and Hofrichter, 1999; Schmidt and Noack, 2000) and can utilize the sugars present in molasses (Maneerat, 2005). Molasses contains 48-56% total sugar (mainly sucrose), 9-12% non-sugar organic matter, 2-4% protein, 1.5-5% potassium, 0.4-0.8% calcium, 0.06% magnesium, 0.6-2.0% phophorus, 1.0-3.0 mg/kg biotin, 15-55 mg/kg pantothenic acid, 2500-6000 mg/kg inositol and 1.8 mg/kg thiamine (Curtin, 1983; Makkar and Cameotra, 1997). These nutrients can serve as a good source of carbohydrates for sustenance and multiplication of microorganisms (Patel and Desai, 1997; Maneerat, 2005). Because of low cost of ingredients used to prepare present commercial formulation, cost of the biopesticides came much lower than their contemporaries available in Indian market. The shelf life test has revealed that the present biopesticides contained CFU load of biocontrol agents on or above the standard level of 10^8/g formulation (Tilak, 1993). The highest CFU counts were recorded at room temperature (22-37°C) or 25°C during 6-14 weeks of formulation and packaging. These temperatures and durations well suit Indian condition as ambient temperature normally remains 20-35°C except during January-February and May-July. A six week period is sufficient for transportation and distribution of the biopesticides to local pesticide dealers. Normally it takes 8-12 weeks in the procurement of a biopesticide by a farmer and its application in field. Hence it is well expected that when the biopesticide will reach in the field it will contain the standard CFU load of biocontrol agents.

Effectiveness of the newly prepared biopesticides was evaluated against the target diseases on pigeonpea under field condition. Inoculation with *F. udum* resulted to 54% wilt incidence with an average severity of 3.5 on 0-5 scale. Application of the biocontrol agents singly or in combination provided varied disease control. Greatest decrease in the wilt was recorded with the combination of *T. harzianum* and *P. fluorescens* applied through crude formulation (bagasse-soil molasses mixture). Application of their commercial formulations, Biowilt-X and Biocomp-
X checked the wilt incidence by 53 and 44%, and the wilt severity by 42 and 35% over control. The combination of Biowilt-X and Biocomp-X was more effective than former alone and checked wilt severity and incidence by 63 and 50%, respectively. Effect of carbendazim was almost equal to Biowilt-X. Other researchers have also shown that efficacious biofungicides can provide disease control equal to fungicides (Khan, 2005).

Root-knot nematodes are sedentary obligate endoparasites and they induce certain structural, physiological and biochemical changes in the host plant (Huang, 1985; Hussy, 1989). Second stage juveniles enter into young lateral roots, and after getting a suitable site for feeding become sedentary with their heads inserted in vascular tissue and body in the cortical region of the root. As a result of nematode pathogenesis, a few cells around the head region preferably in primary phloem are transformed into multinucleate giant cells. These cells are permanent feeding sites of the nematodes. Giant cells, which act as transfer cells or nurse cells provide nutrition to the sedentary females throughout their life span. Whereas cells around the female body become hyperplastic dividing repeatedly by mitosis resulting to enlargement of the tissue which is commonly called as gall or knot (Bird, 1972). Galled roots become short, thick and deshaped. Root growth suppression disturbs the root/shoot ratio resulting in appearance of water stress symptoms in foliage, especially during periods of moisture stress and high temperature (Wilcox and Loria, 1987). Absorption of water and minerals and their conduction are impaired (Wallace, 1987). Photosynthesis decreases but transpiration rate increases (Wilcox Lea and Lorea, 1987) accompanied by greater allocation of photosynthates to roots particularly infected tissue and the giant cells (Wallace, 1987). Cumulatively the infection leads to poor growth and reduced yield of the host. Infected plants show nutrient deficiency symptoms which can be recognized in field as patch of plants. In the present study the pigeonpea cv. UPAS 120 inoculated with 2000 J/\text{kg} soil showed poor shoot growth and mild leaf chlorosis, and discernible galls formed on the roots. The galls were, however, small in size. On an average 84 galls and 71 egg masses/root system were formed in the pot trial whereas in field grown plants 97 galls and 83 egg masses developed per root system. Application of biocontrol agents or their commercial formulation (biopesticides) suppressed the gall formation and
egg mass production. Application of *P. chlamydosporia* and *P. fluorescens* or their biopesticides caused the highest decrease in the number of galls and egg masses per root system followed by nemacur.

*Pochonia chlamydosporia* is an important parasite of root-knot nematodes (Stirling, 1991). The fungus is also known to produce some exoenzymes that help in disintegration of egg shell (Seggers *et al.*, 1996). The used strain of the antagonist demonstrated strong nematicidal activity *in vitro* evidenced by 100% mortality in the juveniles of *M. incognita* due to 50, 75 and 100% culture filtrate. When the egg masses were incubated in the suspension of *P. chlamydosporia*, the fungus colonized them and their hatching was inhibited to a great extent. Strains of *P. chlamydosporia* and *T. harzianum* have been found to parasitize the eggs of *G. rostochiensis*, *G. pallida* and *Panagrellus redivivus* leading to significant decline in their soil populations (Saifulah, 1996a, b, c; Viaene and Abawi, 2000).

Treatments with *P. fluorescens* suppressed the galls, egg masses and soil population of *M. incognita* but it was 8-10% less than *Pochonia chlamydosporia* in both pot or field trial. *Pseudomonas fluorescens* is not a parasite of plant nematodes, but the bacterium may have suppressed nematode infection through other action (Siddqui and Shaukat, 2004). The suppression of nematode infection may result through (i) production of antibiotics such as phenazin (Schoonbeck *et al.*, 2002), pyrrolintrin (Burkhead *et al.*, 1994), phloroglucinol (Mazzola *et al.*, 2002) and siderophores (Perez *et al.*, 2001) which may affect egg hatch and oriented movement of larvae, (ii) degradation of specific root exudates which control nematode behaviour and movement and/or (iii) induction of systemic resistance in plants (Sikora and Hoffmann-Hergarten, 1993). The combined application of *P. fluorescens* with *Pochonia chlamydosporia* or *T. harzianum*, however, caused greater reduction in the galls or *M. incognita* population than *P. fluorescens* alone. Similar effect of combined treatment with the same two microorganisms on root-knot nematodes were also reported by Siddiqi and Shaukat (2004).

Pant and Pandey (2002) reported a significant reduction in the *M. incognita* galls when *T. harzianum* was applied. However, in the present study a discernible adverse effect of strains of *T. harzianum* or *T. virens* on *M. incognita*
was not noticed in field. In pots the fungus, however, suppressed the galling. *Trichoderma* spp. are active colonizers in soil and preferably grow on organic material. The fungus may have colonized larvae and subsequently adults, eggs or egg masses, and resulted to significant decline in galls and soil population of *M. incognita* in the pots. This was probably due to the organically rich soil and the confined space in pots which may have supported greater multiplication of the antagonist. *Trichoderma* spp. produce certain enzymes/metabolites such as gliotoxin (Weindling, 1941), hydrolytic enzymes (Schermbock et al., 1994), endochitinase and chitobiosidase (Larito et al., 1993), which may have been involved in the antagonism against root-knot nematodes. Sharon et al. (2001) have reported a significant decrease in galling with the application of *Trichoderma* species.

The major advantage with RAPD-PCR is that the technique can discriminate population of pathotypes even when large number of species or strains of bacteria and fungi are to be dealt under natural condition (Williams et al., 1993). RAPD-PCR has been used successfully to differentiate *F. oxysporum* (Manulis et al., 1994). *Trichoderma* spp. (Fujimori and Okuda, 1994; Schlick et al., 1994), *B. subtilis* (Istock et al., 2001) and *P. fluorescens* (Dirk et al., 1999) at intraspecific, strain and isolate level. In the present study DNA templates of 8-9 colories of *F. udum, T. harzianum, T. virens, P. chlamydosporia, B. subtilis* and *P. fluorescens* recovered from the soil resembled with that of the applied strains. On this basis, it can be concluded that populations of the wilt fungus and biocontrol agents recovered by the dilution plate method were upto 80-90% of the origin of applied strains.

Soil population of the pathogens significantly decreased with the treatments that checked the disease severity. Nemacur (Johnson, 1985) and carbendazim (Nene and Thapliyal, 1993) are efficacious pesticides and their application usually resulted to considerable decline in the severity of wilt and root-knot and soil populations of *Fusarium* and *Meloidogyne* spp. (Neophytou et al., 2002; Saleh et al., 2002; Agarwal et al., 2003). The biocontrol agents may have suppressed the nematode population with the actions as explained for decrease in galls and egg masses. An inverse relationship was recorded between the soil populations of a pathogen and biocontrol agent. Soil population of the biocontrol agents increased proportionately with the
decrease in disease severity and pathogen population. Greatest increase in the population of biocontrol agents was recorded for *T. harzianum* in the wilt fungus infested plots. The antagonist parasitizes *F. udum* (Singh et al., 2002) and draw nutrition from mycelium to grow and sporulate (Pandey and Upadhayay, 2000). Likewise, *P. chlamydosporia* is a parasite of root-knot nematodes (Kerry, 2000) and its population increased in the pots/plots inoculated with *M. incognita*. The fungus multiplies efficiently on eggs and egg masses of *Meloidogyne* spp. (Stirrling, 1991; Khan et al., 2005). The fungus population was greater in the plots that had nematode alone than together with *F. udum*. The wilt fungus had suppressed galling and egg mass production. Due to this less availability of preferred substrate (egg masses) the *P. chlamydosporia* population could not increase as much as with *M. incognita* alone. *P. fluorescens* is not a parasite of *M. incognita*, but greater increase in its population in pathogen infested soil indicates that presence of pathogens facilitated the bacterial multiplication. This may have occurred through host, especially the root exudation. Root exudates contain carbohydrates and other nutrients which serve as energy source for multiplication of rhizobacteria including *P. fluorescens* (Scher et al., 1988). Penetration and infection by *M. incognita* might have caused greater root exudation than *F. udum* resulting to 5-12% greater increase in the soil population of *P. fluorescens* in the nematode infected soils.

Synergistic interaction between root-knot nematodes (*Meloidogyne* spp.) and wilt inducing fungi (*Fusarium* spp.) is well established on a number of crops including legumes like, alfalfa (Griffin, 1986), cowpea (Thomason et al., 1959), pea (Davis and Jenkins, 1963), beans (Riberio and Ferraz, 1984), mungbean (Khan et al., 2002), chickpea and pigeonpea (Khan, 2005). The interaction between these two pathogens have been found to be generally synergistic. In such interactions the fusarial wilt has become severe in presence of the nematodes resulting to significantly greater crop damage. In the present study also, *M. incognita* and *F. udum* interacted synergistically and caused greater suppression in plant growth and yield parameters of pigeonpea cv. UPAS 120. Severity of wilt symptoms was enhanced when both the pathogens were present together in comparison to *F. udum* alone. Root-knot nematode infection leads to alteration in root exudation of the
infected plants. Root exudates of nematode infected plants contain greater concentration of Ca, Mg, Na, K, Fe and Cu, and during first fourteen days of infection, carbohydrates are the major organic constituents of root exudates and nitrogenous compounds predominate afterwards (Van Gundy et al., 1977). According to Powell (1971) and Webster (1985) root-knot nematode infection predisposes the host plant to wilt fungus. During development of giant cells, infected roots of host plant exhibit decrease in cellulose and lignin, and considerable increase in the amino acids, hemicelluloses, lipids, minerals, nucleotides, organic acids, proteins, DNA and RNA (Khan, 1993). These biochemical changes enrich the medium, which is the cause for rapid growth and colonization of the wilt fungus (Francl and Wheeler, 1993). The greatest host-predisposing capability of Meloidogyne spp. has been observed at this stage which results in synergistic interaction with Fusarium spp. (Webster, 1985). Giant cells being rich nutritionally, serve a good site for the colonization of the fungus. The giant cells, located in vascular tissue serve as launching pad for the fungus for spread in xylem tissue and also to transport toxins. Wilt fungus produce fusaric acid and other toxins that contribute in the development of wilting symptoms (Bell and Mace, 1981; Glick, 1995).

The concomitant inoculation with M. incognita and F. udum in the present study, exacerbated the fusarial symptoms but galling and egg mass production were significantly decreased compared to M. incognita inoculated control. Fusarium spp. have shown strong affinity to feeding sites of sedentary endoparasites. The giant cells formed by Meloidogyne spp. are rapidly invaded by the wilt fungus utilizing its contents (Webster, 1985) as a result developing nematode females starve to death. In addition, the metabolites produced by F. udum may suppress hatching of eggs and induce mortality to larvae (Ciancio et al., 1988). The sedentary endoparasites such as Meloidogyne spp. show decreased populations in soils infested with Fusarium or Verticillium (Hassan, 1989; Fazal et al., 1994). In the present study, greatest decrease in the wilt symptoms of concomitantly inoculated plants was recorded due to P. fluorescens or T. harzianum. The combinations of these biocontrol agents also provided promising control.

Nodule formation on pigeonpea was quiet good and it further increased due to application of biocontrol agents. P. fluorescens alone or in combination with
other biocontrol agents significantly promoted the nodulation. Infection by *F. udum* and *M. incognita* singly or concomitantly decreased the number of functional and total nodules/root system in comparison to the control. Decrease in nodulation by concomitant inoculation was significantly greater than the individual effects of the two pathogens. Suppressive effects of *Fusarium* spp. on root nodules has been observed in a number of studies (Twng-Wah and Howard, 1969; Sawada, 1982 and 1983), but the mechanism involved is not properly understood. It looks plausible that the fungus infected roots due to physiological and/or structural modifications caused by the wilt fungus render the roots unsuitable for the infection by the rhizobium and development of root nodules. The suppression may also be due to competition between the two microorganisms at initial stage of the infection. Fusaric acid produced by the *Fusarium* spp. (Toyoda and Utsumi, 1991) may also be involved in inhibiting the rhizobium. Wilt causing fusaria are known to cause less infection on nodulated roots than non-nodulated roots (Zombolim and Schenk, 1984).

Antagonistic interaction between root nodule bacterium and root-knot nematode was recorded resulting to decrease in the nodulation. Mutual antagonism between *Meloidogyne* and *Rhizobium* evidenced by decreased number of nodules has been well documented (Huang and Barker, 1983; Verdejo *et al.*, 1988; Khan *et al.*, 2000). Various explanations including competition for space and nutrition between the two organisms have been offered for this kind of mutual inhibitory effects (Taha, 1993). The suppression of root nodulation in pigeonpea may have occurred due to nutritional interference, particularly carbohydrates or physiological changes brought about by the nematode infection and/or competition for infection site (Taha, 1993). Number of functional nodules were found to be decreased but non functional nodules increased significantly as a result of infection by *M. incognita*. This may be due to invasion of nodules by the nematode and causing histological changes in the nodular tissue (Taha and Raski, 1969; Barker and Hussey, 1976) thereby rendering them nonfunctional.

In the present study the used strain of *P. fluorescens* was found to be an efficient plant growth promoter. Its application resulted to significantly greater dry matter and yield of pigeonpea. *Pseudomonas fluorescens* produces phytohormones.
solubilizes minerals and produces other compounds like vitamins that are directly responsible for growth enhancement. *P. fluorescens* also produces siderophores that can solubilize and sequester iron from the soil and provide it to plants cells (Glick, 1995). It has been evidenced that the plants have an ability to incorporate Fe$^{3+}$ of siderophores into their biomass (Reid *et al.*, 1984; Barker *et al.*, 1985). Strains of *P. fluorescens* are well reported to produce various phytohormones like cytokinin (Gracia de Salamone *et al.*, 2001), IAA, gibberellins and zeatin (Meng *et al.*, 1998) or vitamins (Marek-Kazaczok and Skorupsks, 2001) that may directly contribute in growth and yield promotion. Application of biocontrol agents or their commercial formulations checked the suppressive effect of the pathogens on pigeonpea leading to significant increase in the dry matter production and yield. Pigeonpea plants infected with *M. incognita* and *F. udum* singly or concomitantly produced significantly greater dry matter and grains with *P. fluorescens* treatments compared to other treatments. The bacterial treatments enhanced the plant growth and yield greater than other biocontrol agents used. Although decrease in the disease severity with *P. fluorescens* treatment was less than other treatments but yield enhancement was greater. Apparently *P. fluorescens* may have acted through two ways. Yield enhancement partly occurred due to plant growth promotion and partly due to disease suppression. *B. subitlis* and *Trichoderma* spp. are also reported to solubilize phosphorus (Kole and Hajra, 1997; Sharma, 2003) and produce antibiotics (Brannen, 1995; Haggag and Mohamed, 2002) but they are not so efficient as *P. fluorescens*. Treatments with nemacur or carbendazim checked the disease much greater than *P. fluorescens* but yield enhancement in pigeonpea was less than the bacterial treatment. The pesticide treatments improved the yield of infected plants by suppressing the pathogens.

Overall seed treatment with biocontrol agents was relatively more effective than soil application. The amount of biocontrol agents added to a microplot through soil application (40 g/microplot) was much greater than that carried by the seeds (2 g/kg seed). But with soil application, the CFU’s dispersed in a greater area giving rise to a much smaller CFU count/g soil or seed, whereas with seed treatment the microorganisms remained concentrated on or around the seed and later on in the root zone. The germinating seeds attract rhizobacteria (Scher *et al.*, 1985) and are
rapidly colonized due to profuse exudation of a wide range of amino acids, carbohydrates, organic acids (Hayman, 1969, Lynch, 1978). Hence chemotaxis towards nutrient-rich seed exudates may represent a competitive advantage for certain bacteria. The biocontrol agents thus received greater nutrients through exudates of germinating seeds to full root growth and also have faced less competition and exerted more because of their aggregation in a limited area in close vicinity of roots, as a result the applied strains multiplied with a greater pace evidenced by the higher CFUs/g soil observed during crop growth in the microplots which received biocontrol agents through seed treatment. Because of greater CFU/g soil, phosphate solubilization, hormone production and/or pathogen suppression by the biocontrol agents would have been greater in the root zone and reflected into better disease control, plant growth and yield of pigeonpea.