INTRODUCTION

In the pursuit of faster economic growth, there has been over exploiting of natural resources without serious consideration of the ecological consequences. In the quest to improve food availability and its quality, advancement made in the crop production and other related operations have caused environment degradation in terms of salinity, water logging, soil erosion, air and water pollution and poor soil health. This situation warrants a conscious effort to improve crop production through the use of environment friendly and bioincentive practices such as organic farming and biological control instead of using synthetic fertilizers and pesticides. This approach may bring sustainability in agricultural production. A crop growing in a soil fertilized by a rich mix of organically derived compounds (manures and compost) will contain a wider range of nutrients and microflora.

Application of synthetic fertilizers and pesticides have caused elimination of several beneficial microorganisms from soil and allowed resurgence of pests and pathogens. Repeated applications of pesticides have generated a kind of tolerance in pathogens as a result crops suffer heavy damage and yield loss inspite of pesticide application. Besides, chemicals cause several other hazards to such an extent that their use in crop production is now being disregarded. A situation has arisen which has forced scientists and planners to necessarily explore and use nonchemical methods of pest and disease management, and to understand the role of soil microorganisms in sustainable crop production. The biological control offers a method of improving crop production within existing resources, without a risk of environmental degradation and pest resurgence. Biological control strategies are highly compatible with the sustainable agricultural practices and conserve natural resources.

Biological control is based on the natural principle that each living entity has its several adversaries. Hence suppression in the disease severity is achieved through the activity of natural enemies (parasites and predators) of the disease causing organism. Technically biological control is reduction in the population or disease/damage causing activity of a pest or a pathogen in its active or dormant state.
by one or more organisms that occur naturally or through manipulation of the environment or by mass introduction of antagonist(s). Biological control of soil borne pathogens by introduced microorganisms has been studied for several decades. Recent researches, however, have been carried out with greater systematic approach and practical utility, and have demonstrated potential of biological control in the present agriculture. Various microorganisms viz., bacteria, fungi, mycorrhizae etc. have been tested for their ability to suppress plant diseases. In addition to conventional parasites and predators, plant growth promoting microorganisms can also be used in the biomanagement of diseases. *Pseudomonas fluorescens* is the most versatile plant growth promoter and possesses great antagonistic potential against plant pathogens (Marek-kazaczuk and Skorupska, 2001). Leeman *et al.* (1995) demonstrated that *P.fluorescens* can induce systemic resistance in radish against *Fusarium oxysporum* f. sp. *raphani* where as the bacteria can also trigger a part of systemic acquired resistance against the fungus (Hoffland *et al.*, 1995). Leemanceau *et al.* (1993) demonstrated that application of a nonpathogenic strain of *F. oxysporum* and fluorescent *Pseudomonas* spp. significantly decreased the suppressive effect of pathogenic *Fusarium*. Bacterization of chickpea seeds with a siderophore producing fluorescent pseudomonad reduced the number of chickpea wilted plants in a wilt sick soil by 52% (Kumar and Dubey, 1992). Other *in vitro* tests have demonstrated antifungal activity of certain strains of *P. fluorescens* against *F. oxysporum* f. sp. *ciceri* and *F. udum* (Kumar *et al.*, 1997; Goudar *et al.*, 2000).

*Bacillus subtilis* is another potential suppressant of plant pathogens (Kim *et al.*, 1997). Two isolates of *B. subtilis* were found antagonistic to *F. oxysporum* f. sp. *ciceri* *in vitro* (Dhedhi *et al.*, 1990). *B. subtilis* produced a wide zone of inhibition against *F. udum* and completely inhibited spore germination at 8 x 10^7 cells/ml. Seeds germinated better than untreated seeds and produced longer roots and shoots when sown in either wilt infested or sterile soil (Sumitha and Gaikwad, 1995). Seed treatment of pigeonpea with *B. subtilis* and *Trichoderma* spp. effectively controlled pigeonpea wilt and enhanced the yield considerably (Nakkeeran and Renukadevi, 1997). Seed bacterization with *Bacillus* spp. reduced the number of wilted chickpea plants in sick plots infested with *F. oxysporum* f. sp. *ciceri* (Kumar, 1996). Various
other studies have also shown antagonistic effects of *P. fluorescens* and *B. subtilis* on *Fusarium* spp. and enhancement in the plant growth and yield (Khan and Khan, 2001, 2002; Jahagirdar *et al.*, 2002; Dhar, 2003; Naik, 2003; Khan *et al.*, 2004).

*Trichoderma* spp. are established mycoparasites and can antagonize numerous pathogenic fungi (Hanson and Howell, 2004). In a classical study, Dennis and Webster (1971a) isolated several species of *Trichoderma* and found *T. harzianum* most suppressive against *Rhizoctonia solani*, *Pythium debyranum*, *Fusarium oxysporum*. *T. harzianum* produced a wide zone of inhibition against *F. udum* and suppressed mycelial growth by 89%. Spore germination of the wilt fungus was completely inhibited. Seed dressing with *T. harzianum* resulted to a lowest wilt incidence (20%) in sick plots infested with *F. udum* (Jayalakshmi *et al.*, 2003). Prasad *et al.* (2002) recorded 4-5% wilt incidence in the sick plots applied with *T. harzianum* compared to the control plot where wilt incidence was 16%. Wilt of pigeonpea caused by *F. udum* was effectively managed with the application of *T. harzianum* and *T. virens* (Chaudhary and Prajapati, 2004).

*Pseudomonas* and *Trichoderma* species can also antagonize plant parasitic nematodes (Khan and Khan, 1998). Siddiqui and Shaukat (2004) recorded 28% and 25% decrease in the soil population of root-knot nematode, *Meloidogyne* spp. in tomato treated with *T. harzianum* and *P. fluorescens*, respectively compared to inoculated control. Meyer *et al.* (2000) found that the number of eggs and second stage juveniles of *M. incognita* per gram root of bell pepper were significantly lower following combined application of *Pseudomonas* isolates and *T. virens* than the untreated control. Antagonistic effect of *B. subtilis* and *P. fluorescens* on the development of *Meloidogyne* spp. have also been reported by other researches (Kerry, 2000; Khan and Akram, 2000; Khan and Kounsar, 2000; Khan *et al.*, 2002; Khan *et al.*, 2005a, b).

*Pochonia chlamydosporia* (=*Verticillium chlamydosporium*) is one of the most important pathogen of root-knot nematodes (Stirrling, 1991) that parasitizes the eggs and adult females of plant parasitic nematodes (Kerry, 2000). The fungus forms a branched mycelial network in close contact with the smooth egg-shell (Morgan-Jones *et al*., 1983; Lopez-Llorca and Duncan, 1988) and produces an
appressorium that adheres to the egg shell by mucigens to invade the egg shell (Seggers et al., 1996). It has been observed that the fungus causes disintegration of the egg shell’s vitelline layer and also causes partial dissolution of the chitin and lipid layers, possibly due to the activity of exoenzymes. Effects of *P. chlamydosporia* on root-knot disease have been examined. Application of *P. chlamydosporia* at 5000-10000 chlamydospores/cm³ to *Meloidogyne* infested soil resulted in the colonization of egg masses by the fungus that varied from 16-43% and reduced the penetration of roots by second stage juveniles (Nicole and George, 2000). De Leij et al. (1993) have recorded 95% decrease in the soil population of *M. hapla* due to *P. chlamydosporia* treatment. Khan et al. (2002 and 2005) demonstrated that application of *P. chlamydosporia* through root-dip or seed treatment effectively controlled the root-knot of tomato and mungbean and significantly improved the yield of infected plants.

Commercial formulations of bioagents have been developed by various private and governmental agencies and are being marketed with different trade names (Singhal, 2004). These formulations are prepared on inert materials like talc powder, vermiculite, perlite, sand etc. (Singh et al., 2003). Tiwari et al. (2004) evaluated grains of sorghum, wheat, pear millet, wheat bran, rice bran and sugar cane bagasse for mass multiplication of *T. viride*. Colonization by the fungus was greatest on sorghum grains with 8 x 10⁹ spores/g grains and 93% spore viability after 15 days of incubation at 5°C. Kerry et al. (1984) used oat seeds to rear *P. chlamydosporia* for field application. Soil application of the colonized oat kernels @ 0.5% and 1.0% (w/w, soil:seed) considerably reduced the population of root-knot and cyst nematodes (Godoy et al., 1983; Rodriguez-Kabana et al., 1984). Encapsulation of the liquid suspension of spores and hyphae of *P. chlamydosporia* by sodium alginate containing 10% (w/v) kaolin or wheat bran has been done successfully (De Leij and Kerry, 1991). On soil application, the fungus proliferated in soil from only those granules which contained wheat bran as energy source. In another study, Kerry (1988) estimated approximately 9x10⁷ and 4x10⁷ spore load of *P. chlamydosporia* g soil after 1 and 12 weeks of application of granules, respectively. De Leij and Kerry (1991) found that chlamydospores when introduced through
sand/bran, powdered grain or aqueous suspension, established successfully in soil with out any food base. Lewis et al. (1995) reported that among the different carriers tested, the shelf life of Bacillus subtilis in soybean flour increased up to three months. Vidyasekaran et al. (1997) developed powder formulations of Pseudomonas fluorescens using talc powder, peat, vermiculite, lignite and kaolinite. The freshly prepared powdered formulations were effective in controlling pigeonpea wilt, but their efficacy decreased with storage duration. Talc formulations were effective even after 6 months of storage. A talc-based formulation of P. fluorescens with 7x10^5/g CFUs was found effective in decreasing root-knot nematode population, egg masses and galls in tomato and brinjal plants (Anita and Rajendran, 2002). Khan et al. (2004, 2005a) developed commercial formulations of T. harzianum, P. chlamydospora and P. fluorescens based on fly ash-sawdust mixture.

Wilt and root-knot are important diseases of pigeonpea and occur almost in every pigeonpea growing area in India. The incidence of fusarium wilt has been recorded 1-10% (UP), 10-20% (Bihar) and 20% or more (Maharashtra) (Kannaiyan et al., 1984). In India, the annual monetary loss to pigeonpea due to the wilt has been estimated worth of US $ 36 million (Kannaiyan et al., 1984). The yield loss depends on the stage at which the plant wilts; the loss can be 100% if disease develops at pre-pod stage, about 67% and 30% when wilt occurs at maturity and pre-harvest stage, respectively (Kannaiyan and Nene, 1981). Root-knot nematode, Meloidogyne spp. is another important pathogen of pigeonpea and is widely distributed in India. The yield loss to pigeonpea due to the nematode infection may depend on the infestation level (Bridge, 1981). Khan (2003) have reported 10-18% yield loss to two commonly grown varieties of pigeonpea, Bahar and UPAS 120. The disease complex involving species of Fusarium and Meloidogyne is one of the most commonly occurring and destructing diseases of vegetables and pulse crops (Francl and Wheeler, 1993). Many researchers have demonstrated definite role of root-knot nematodes, especially M. incognita and M. javanica in the wilt of pigeonpea (Salam and Khan, 1986). Association of root-knot nematodes not only aggravates the wilt severity but also breaks the resistance of cultivars against the fungus (Webster, 1985). Dwivedi et al. (1992) reported that in pigeonpea plants
inoculated with *M. incognita* and *F. udum* simultaneously, the plant growth was suppressed greater than the plants inoculated with *F. udum* alone. Marley and Hillocks (1996) reported that wilt resistant cultivars of pigeonpea became susceptible to *F. udum* in the presence of *M. incognita* and *M. javanica*.

Critical analysis of the evidences presented above have revealed that *Bacillus subtilis, Pseudomonas fluorescens, Pochonia chlamydosporia, Trichoderma harzianum* and *T. virens* can effectively suppress *Fusarium* and *Meloidogyne* resulting in promotion of growth, vigour and yield of plants. Commercial formulations of such organisms are essentially needed to be prepared that could carry the bioagent in active and viable form to a field. Most of the formulations presently available only support survival of the bioagent but not the multiplications. If other immobilizing substrates are explored and developed in which an organism could survive as well as multiply may lead to a formulation with a much greater CFU load and consequently a better control of the disease. The present study was undertaken with an objective to develop suitable formulation of bioagents to achieve an effective and satisfactory control of wilt (*Fusarium udum*), root-knot (*Meloidogyne incognita*) and disease complex of pigeonpea (*F. udum* and *M. incognita* concomitantly). To attain the objective following studies were undertaken:

1. Isolation and characterization of *Trichoderma harzianum, T. virens, Pochonia chlamydosporia, Bacillus subtilis* and *Pseudomonas fluorescens*, and *in vitro* evaluation for antagonism against *Fusarium udum* and *Meloidogyne incognita*.

2. Screening of various industrial and agricultural materials to mass culture biocontrol bacteria and fungi (crude formulations).

4. Re-evaluation for efficiency of selected microorganisms (T. harzianum, P. chlamydosporia and P. fluorescens) singly and jointly to control wilt, root-knot and wilt disease complex of pigeonpea under field condition.

5. Screening of different materials for immobilization of T. harzianum, P. chlamydosporia and P. fluorescens.


7. Field evaluation of the biopesticides developed for effectiveness against wilt, root-knot and disease complex of pigeonpea.