INTRODUCTION

Leguminous crops occupy very important position in Indian agriculture and the main source of protein (Ali and Kumar, 2006) for the predominantly large vegetarian population of the country. These crops are included in the cropping system patterns to fix atmospheric nitrogen with the help of nitrogen fixing bacteria to make soil productive. In India, these crops are being cultivated in 22.47 million hectares with an average production of 13.38 million tones (Ali and Kumar, 2006).

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is the second most important pulse crop (after chickpea), cultivated mainly as Kharif crop (summer crop). In Asia, pigeon pea is grown in an area of 4.3 million ha and production of 3.3 million tones. India has the largest area under pigeon pea, followed by Myanmar, Kenya, China, Malawi, Uganda, Mozambique, Tanzania and Nepal. Pigeon pea, occupies an area of 3.58 million hectares in India and contributing 3.29 million tones to the pulses bowl of the country (Anonymous, 2006). Pigeon pea is a perennial erect bush, 0.5 to 4 m tall, and has strong stem. It has pubescent trifoliate leaves. Flowers are present in axillary or terminal pedunculate or almost sessile racemes. Its fruit is a pod, linear-oblong, apex obtuse or acute, compressed, bi-valved, depressed between the seeds with transverse lines. Its seeds are reniform to sub-orbicular and shiny. It has deep, strong, woody tap root with well developed lateral roots in the superficial layers of the soil. It is nodulated and most nodules may vary from 2mm to 2 cm, and the shape may be spherical, oval, elongate, or branched. Pigeon pea is part of many farming systems throughout the tropics and sub-
tropics. An average annual rainfall between 600 and 1,000 mm is most suitable. However, it can be grown in humid areas, even over 2,500 mm of rainfall and is renowned for its drought tolerance.

Pigeon pea is a major source of ‘dal’ an important constituent in the food habit of Indian people. Availability of 20-21% protein in pigeon pea is an important source for supplementing the energy rich cereal diet (Anonymous, 2006). Additionally, pigeon pea crop improves the soil characteristic and fertility status ensuring better growth to succeeding crop. Long duration pigeon pea could fix up to 200 kg N ha⁻¹ and has a beneficial effect on subsequent crops equivalent to about 40 kg ha⁻¹ (Anonymous, 2006). The leaf drop helps improving soil structure. Its stalks are sources of fuel and used for other socio-economic purposes in rural areas.

Unfortunately, there are several constraints in increasing production of pigeon pea. It has been estimated that this crop suffers about 13.2 % worldwide yield loss by plant parasitic nematodes (Sasser, 1989). Although large number of plant parasitic nematodes have been found associated with pigeon pea but Rotylenchulus reniformis, Hoplolaimus seinhorsti, Heterodera cajani and Root-knot nematodes Meloidogyne arenaria, M. incognita, M. javanica are of major importance in India (Gill, 1989). Similarly, most common fungal pathogens known to parasitize pigeon pea include blight caused by Alternaria alternate, anthracnose by Colletotrichum cajani, gray mold by Botrytis cinerea, leaf spot by Cercoseptoria cajanicol, collar rot by Sclerotium rolfsii, wilt by Fusarium udum and powdery mildew by Leveillula taurica (Rangaswami and Mahadevan, 1999). However, important bacterial disease includes bacterial leaf spot and stem canker caused by Xanthomonas campestris pv. cajani.
Survey of cyst forming nematodes of Uttar Pradesh, India has revealed that *Heterodera cajani* is widely distributed (Husain *et al.*, 1989). Some plants were simultaneously galled due to presence of root-knot nematodes. These two nematodes were also found associated with wilt fungus *Fusarium udum*, which is destructive to this crop in certain states of northern India (Singh, 1983). These pathogens together are highly destructive causing a wilt disease complex which is a major constraint in the successful cultivation of pigeon pea in India (Edward and Singh, 1979; Hasan, 1984; Singh *et al.*, 1993; Siddiqui and Mahmood, 1996a, 1999a).

The growing concern about the toxic effects of chemical pesticides has created a need to develop suitable non-toxic and eco-friendly alternative methods for the management of plant diseases. The rhizosphere provides a front line defense against pathogen attack to root (Weller, 1988). Plant growth promoting rhizobacteria (PGPR) present in the rhizosphere have the ability to improve plant growth by colonizing the root system and preempting the establishment or suppressing harmful rhizosphere microorganisms (Schroth and Hancock, 1982; Glick, 1995; Siddiqui and Mahmood, 1999b; Kloeper, 2003). Rhizosphere microorganisms provide biocontrol through mechanisms such as the production of antibiotics (Hebbar *et al.*, 1992; Bender *et al.*, 1999), iron sequestering compounds siderophores (Loper and Buyer, 1991; Dwivedi and Johri, 2003; Siddiqui, 2006), extra cellular hydrolytic enzymes (Fridlender *et al.*, 1993), other secondary metabolites like hydrogen cyanide (HCN) (O' Sullivan and O' Gara, 1992; Dowling and O'Gara, 1994; Bagnasco *et al.*, 1998), and by inducing systemic resistance (Liu *et al.*, 1995).
The effects of *Pseudomonas* spp. in plant growth promotion have been observed (Lemanceau, 1992; Digat, 1994). The beneficial effects of *Pseudomonas* spp. have been attributed to their ability to promote plant growth and also to protect the plant against pathogenic microorganisms. The fluorescent pseudomonads are involved in the natural suppressiveness of some soils to fusarium wilts, and have been applied successfully to suppress fusarium wilts of various plant species (Lemanceau and Alabouvette, 1993). *Pseudomonas aeruginosa* 78 produce a polar substance, heat labile, sensitive to extreme pH values causing in vitro juvenile mortality of *Meloidogyne javanica*, (Ali et al., 2002). *P. fluorescens* CHAO produces several bioactive compounds giving it one of the broadest spectra of potential biocontrol and growth-promoting mechanisms of known PGPR (Weller and Thomashow, 1993). Many strains of pseudomonads can indirectly protect the plants by inducing systemic resistance against various pests and diseases (Van Loon et al., 1998; Ramamoorthy et al., 2001; Zehnder et al., 2001).

*Bacillus* spp. are able to form endospores that survive for long time in the soil and under adverse environmental conditions. *Bacillus* species have been reported to promote the growth of a wide range of plants (De Freitas et al., 1997; Kokalis-Burelle et al., 2002) and are very effective in biological control of many plant microbial diseases. Jetiyanon et al. (2003) observed that a mixture containing *B. amyloliquefaciens* and *B. pumilus* induced systemic resistance against southern blight of tomato, anthracnose of long cayenne pepper and mosaic disease of cucumber. *Bacillus subtilis* synthesizes an antifungal compound inhibiting *Fusarium oxysporum* f. sp. *ciceris* (Kumar, 1999) and strain RB14 produces the cyclic lipopeptides antibiotics iturin A and surfactin active against several phytopathogens. The best isolates to inhibit *Fusarium roseum* var.
*Sambucinum* belongs to *B. cereus*, *B. lentimorbus* and *B. licheniformis* (Sadfi et al., 2001).

The selection of effective strains of particular bacteria is of prime importance for the biocontrol of plant pathogens. Isolation of bacteria from pathogen suppressive soils may increase the chances of finding effective strains (Cook and Baker, 1983). To get effective isolates, isolation of bacteria should be made from the same environment in which they will be used (Weller et al., 1985). The ability to colonize roots and resistance against antibiotics are the other parameters to screen effective strains (Siddiqui et al., 2005). In general, a single biocontrol agent is used to control a plant disease by a single pathogen (Wilson and Backman, 1999). This may sometimes account for the inconsistent performance by the biocontrol agent, because a single agent is not active in all soil environments or against all pathogens that attack the host plant. On the other hand, mixtures of biocontrol agents with different plant colonization patterns may be useful for in controlling different plant pathogens via different mechanisms of disease suppression. Moreover, mixtures of biocontrol agents with taxonomically different organisms that require different optimum temperatures, pHs, and moisture conditions may colonize roots more aggressively, improve plant growth and efficacy of biocontrol. Therefore, I tried to isolate *Bacillus* and *Pseudomonas* spp. from pathogen suppressive soils. These isolates were tested alone and in combination with root-nodule bacterium *Rhizobium* sp. to achieve biocontrol of wilt disease complex of pigeon pea. Rhizobacteria found best under pot condition were further tested for their biocontrol potential under field condition. Attempts were made to achieve following objectives.
Objectives of the study

1. Survey of pigeon pea fields of Uttar Pradesh, India were conducted for wilt disease complex of pigeon pea caused by root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood, pigeon pea cyst nematode *Heterodera cajani* Koshy and wilt fungus *Fusarium udum* Butler.

2. Isolation of rhizobacteria especially fluorescent pseudomonads and *Bacillus* spp. were made from pathogen suppressive soils.

3. Isolates of *Pseudomonas* and *Bacillus* were screened through green house assay and their biocontrol potential was tested in pots both in mono and multi-pathogenic conditions.

4. Siderophores, HCN and IAA productions was estimated from *Pseudomonas* and *Bacillus* isolates. Phosphate solubilization and effect of different rhizobacterial isolates on seedling growth was also studied.

5. Effect of root-nodule bacterium *Rhizobium* sp. alone and in combination with efficient isolates of *Bacillus* and *Pseudomonas* was studied under pot condition for the management of wilt disease complex.

6. Potential isolates *Pseudomonas* and *Bacillus* obtained through greenhouse assay and pot test were further tested for the biocontrol of wilt disease complex of under field condition both in the presence and absence of root-nodule bacterium *Rhizobium* sp.