1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most important commercial crops grown in India. It is a crop of tropical and sub-tropical regions and requires a warm humid climate. There are more than fifty chilli varieties grown in India (Rajur *et al.*, 2008). Chilli is an indispensable condiment of Indian cuisine because of its pungency, colour, flavour and aroma. It is used in the daily diet in one form or the other. It is a rich source of vitamin A [292 IU/100 g] and C [111 mg/100g] and thiamine [0.19 mg per 100 g] (Pugalendhi *et al.*, 2010; Marin *et al.*, 2004). Two chemical groups produced by chilli are capsaicinoids and carotenoids. A large number of carotenoids provide high nutritional value and colour to chilli (Perez-Galvez *et al.*, 2003; Hornero-Mendez *et al.*, 2002).

India is the world’s largest producer of chillies, accounting for about 50% of production and exports about 20% of its production (Ali, 2006). In India, chilli is grown over an area of 0.654 million hectare with production of 1.0146 million tonnes, with productivity of 1551 kg/ha (Anonymous, 2005). India accounts for about 1.1 million of annual production followed by China [around 0.4 million tonnes], Mexico and Pakistan [around 0.3 million tonnes each]. The most important chilli states in India are Andhra Pradesh (51%), Madhya Pradesh (11%), Karnataka (9%), Orissa (4%), Maharashtra (4%), Rajasthan (3%) and Tamilnadu (3%) (Jagtap *et al.*, 2012). The mean productivity of chilli in Karnataka was 1.57 t/ha [1990-2004] (Rajur *et al.*, 2008). Major chilli growing districts are Gadag, Haveri [Haveri, Byadagi taluks] and Dharwad [Dharwad, Hubli taluks]. The varieties cultivated in south Karnataka are Byadagi Kaddi, Byadagi Dabbi, Sankeshwar, Ariskere, Annigeri and Jwala (Rajur *et al.*, 2008). India is the largest exporter of chilli in
the world. India exports chilli to USA, UK, Russia, Canada, Italy, Netherlands, Singapore, Saudi Arabia, UAE and Germany in the form of dry pods, chilli powder and oleoresin.

Chilli is known to be affected by as many as 83 different diseases, of which more than 40 are caused by fungi (Rangswami, 1988). Among the fungal diseases, anthracnose (fruit rot) and root rot caused by *Colletotrichum* spp. and *Rhizoctonia solani* are the most serious, causing loss to growers throughout the chilli growing regions of India.

Anthracnose of chilli caused by *Colletotrichum gloeosporioides* is one of the most devastating disease of the crop (Than *et al*., 2008b). It causes severe damage to fruits both in field and storage. The disease is very severe in humid weather and spreads rapidly causing extensive losses (Pandey and Pandey, 2006). Heavily infected fruits may lose their normal red colour and turn pale white. The pathogen also causes necrosis of tender twigs and the entire branch (Mesta *et al*., 2007). Chilli anthracnose can cause fruit yield losses upto 50% (Than *et al*., 2008b; Pakdeevaraporn *et al*., 2005). Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli. In some cases, the lesions are brown and then turn black, due to the formation of setae and sclerotia. Fruit rot reduces dry weight, and capsaicin and oleoresin content of affected fruits (Mistry *et al*., 2008), leading to reduction in the medicinal properties of chilli.

Among the fungal diseases, damping-off disease of seedlings as well as root and stem rot in young transplants incited by *Rhizoctonia solani* is a major constraint in the production of chilli. *R. solani* is essentially a soil-borne pathogen which inflicts heavy losses under conditions favourable to disease expression (Seema and Devaki, 2010). Depending on which stage of this pathogen is active, it may cause different symptoms and diseases. The anamorph stage causes damping off, root rot, crown blights and fruit rots.
However, it causes leaf blights when it is in its teleomorph stage and when it has the right environmental conditions. Root rot affects the plant on the stem near the soil; from this it moves into the stem and the taproot causing sunken brown necrotic lesions which contribute to delayed emergence and lower yield (Gonzalez et al., 2011). In chilli, it causes seed decay, pre- and post-emergence damping-off, wirestem, root rot, and necrotic spots on the hypocotyl or tap root (Sherf and MacNab, 1986). Maulani (2005) reported 1-35% pre-emergence damping-off, 12.6-51.0% post-emergence damping-off and disease severity ranged from 10.9-47.8%, respectively in chilli plants. The management of this disease is difficult owing to long saprophytic survival ability of pathogen in soil (Dey, 2005).

Conventional agricultural practice depends largely on the use of chemical inputs such as pesticides and fertilizers, to control plant pathogens and to enhance crop yield (Kitzes et al., 2008). During the 1990s, the world use of pesticides increased by 4.4% annually (Oerke and Dehne 2004). Every year pesticides and fungicides corresponding to 768,000 tonnes of active ingredient are used world-wide, which of course leads to an additional strain on the environment (U.S. Environmental Protection Agency, 2008). However, increasing use of chemical inputs have led to several negative effects, i.e., decrease in biodiversity of the soil-inhabiting microorganisms; hazardous effects of pesticide runoff on the aquatic systems, development of resistance in pathogen and their negative environmental impacts (Urban and Lebeda 2006; Ma and Michailides 2005), compaction and increased salinity in the agricultural soil, leaching of inorganic nitrate, and contamination of water resources (Kibblewhite et al., 2008).
Increasing health concerns, environmental hazards and stringent regulations and restrictions on the usage of hazardous chemicals, have led to a burgeoning demand for alternative safer methods of disease management.

Biological control is being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Welbaum et al., 2004; Postma et al., 2003). Interest in biological control of plant pathogens has increased considerably over the past years, partly as a response to public concern about the use of hazardous chemical pesticides, and also because it provides control of diseases that cannot, or only partially, be managed by other control strategies (Compant et al., 2005).

A large number of microorganisms present in rhizosphere have been considered to be important in sustainable agriculture because of their biocontrol potential and ability to promote plant growth. Bacteria that inhabit the rhizosphere and are beneficial to plants are termed Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper et al., 1980). PGPR exhibit direct and indirect mechanisms as plant growth promoters and biological control agents. Direct mechanisms by PGPR include enhanced nutrient mobility, nitrogen fixation, denitrification, siderophore and phytohormone production (Babalola, 2010; Glick et al., 2007). Indirect mechanisms of PGPR include production of antibiotics, viz. 2,4-Diacetyl phloroglucinol (DAPG), phenazine, pyoluteorin and pyrrolnitrin against pathogenic fungi and bacteria, reduction of iron available to phytopathogens in the rhizosphere, synthesis of fungal cell wall-lysing enzymes (Jalili et al., 2009; Ramamoorthy et al., 2001).

*Azospirillum, Azotobacter, Bacillus, Enterobacter, Paenibacillus, Pseudomonas* and *Streptomyces* are recorded as the potent genera of rhizobacteria acting against wide range of pathogens. Fluorescent *Pseudomonas* is common rhizosphere inhabitants and
ideal biocontrol antagonists because of their adaptive metabolism and ability to produce an array of inhibitory compounds. *Pseudomonas* spp. produces wide varieties of antibiotics, which confer a competitive advantage and microbial fitness to survive in most environments (Paulsen et al., 2005). Due to their ability to produce variable metabolites and to utilize several organic compounds, *Pseudomonas* are not specific for one pathogen or plant species only, but have a wide host range and suppress several pathogens.

To be an effective biocontrol agent, microbial inoculants have to meet several important criteria, such as effective and competitive colonizer of the host plant (e.g. rhizosphere, spermosphere), Stimulation of plant defense by Induced Systemic Resistance (ISR) and/or Systemic Acquired Resistance (SAR), Direct antagonistic effects on the pathogen e.g., by antibiosis or by inactivation of virulence factors of the pathogen, and expression and/or production of the antagonistic traits need to occur at the right time and place (Lugtenberg and Bloemberg, 2004). Combining all of these traits into a single strain or mixture of strains is likely to produce a more consistent and effective level of plant protection (Haas and Keel, 2003). In this context, efficient exploitation of these bacteria in agriculture and horticulture requires more fundamental knowledge of traits that enhance their ecological performance.

The major problem of biological control is its lack of consistency due to variable efficacy of the biological control agent depending on the soil environment, method of application, the host plant or the pathogen species. Therefore, even though several strains have been reported to show good performance *in vitro*, in specific trials only a few have proved effective in biocontrol under different field situations (Kiely et al., 2006). As a result only a few get to the market, and the estimated number of biocontrol products in the market constitute only 1% of agrochemical sales (Fravel, 2005). Development of better
formulations to ensure survival and activity in the field and compatibility with chemical agents and thorough understanding of the mechanisms of interaction between biocontrol agents and their pathogenic counterparts can aid in improving the efficacy and consistency of biological control.

Therefore, there is need to search for more reliable and consistent biocontrol agents to meet up the increasing demand for chemical residue-free agricultural products. With this supportive background information and keeping all the considerations in view, the present study was undertaken to understand the mechanism of action of PGPR against *C. gloeosporioides* and *R. solani*, with following objectives to develop an efficient bioformulation.

**Objectives of the study**

- Isolation, identification and characterization of antagonistic *Pseudomonas* from rhizospheric soil samples against *C. gloeosporioides* and *R. solani*

- Screening and study of factors influencing plant growth promoting traits (IAA, Phosphate and siderophore) of FP6

- Elucidation of the antifungal mechanism involved in control of *C. gloeosporioides* and *R. solani*

- Evaluation of carrier material (talc and lignite) for the formulation of an effective bioinoculant with the biocontrol strain *Pseudomonas aeruginosa* FP6

- Assessment of bioformulation efficacy, alone and in combination with fungicide for biocontrol and plant growth promoting efficiency of chilli by pot experiments