2. LITERATURE REVIEW

Chilli (*Capsicum annuum L.*) one of the most important commercial crop of India belongs to the Solanaceae family which represents a diverse plant group. The genus name *Capsicum* derived from the latin word ‘capsa’ meaning chest or box because of the shape of fruit which encloses seeds very neatly, as in the box (Berke and Shieh, 2000). Chilies are cultivated mainly in tropical and sub-tropical countries like India, Japan, Mexico, Turkey, United States of America and African countries (Panda, 2010). Genus *Capsicum* represents a diverse plant group which contains approximately 30 species, 5 of which *C. baccatum, C. annuum, C. chinense, C. frutescens* and *C. pubescens* are domesticated and cultivated in different parts of the world. Among the five species of *Capsicum* cultivated, *C. annuum* is one of the most common cultivated crop worldwide (Tong and Bosland, 1999) followed by *C. frutescens* (Ince *et al.*, 2010; Wang and Bosland, 2006).

Chillies are known from pre-historic times in Peru and believed to have originated in the tropical America. It is also said that chillies have originated in the Latin American regions of the New Mexico and Guatemala as a wild crop around 7500 BC, as per the remains of the pre-historic Peru. Columbus carried chilli seeds to Spain in 1493. The cultivation of chilli spread rapidly from Spain to Europe. The Portuguese brought capsicum from Brazil to India during the year 1584. Chillies became popular in the whole of Asia rapidly and native Asians started cultivating this crop as well (Raju and Luckose, 1991). The south Asian climate suited this crop, and since its introduction in the 16th century chilli has been increasingly cultivated in south Asia. Chillies are the cheapest spices available in India and are eaten across all groups (Shinoj and Mathur, 2006).
2.1 International scenario

The world area and production of chilli is around 1.5 million ha and 7 million tonnes respectively. In Asia, India, China, Pakistan, Indonesia, Korea, Turkey and Sri Lanka; Nigeria, Ghana, Tunisia and Egypt in Africa; Mexico, United States of America in North and Central America; Yugoslavia, Spain, Romania, Bulgaria, Italy and Hungary in Europe and Argentina, Peru and Brazil in South America are the major chilling growing countries. India is the world leader in chilli production followed by China and Pakistan (Hussain and Abid, 2011). The bulk share of chilli production is held by Asian countries.

India, China, Mexico, Thailand, United States of America, United Kingdom, Germany and Sweden (Hanamashetti et al., 2009) are the major chilli consumers in the world. The major chilli exporting countries with their percentage share in world total exports are India (25 %), China (24 %), Spain (17 %), Mexico (8 %), Pakistan (7.2 %), Morocco (7 %) and Turkey (4.5 %). The world trade in chilli account for 16 % of the total spice trade in the world. United Arab Emirates, European Union, Sri Lanka, Malaysia, Japan and Korea (Thampi, 2004) are major chilli importing countries.

2.2 National scenario

India is not only the largest producer but also the largest consumer of chilli in the world. Chilli is the most common spice cultivated in all States and Union Territories of India contributing about 36% to the world total production. Andhra Pradesh is the largest chilli producer in India contributing about 26% to the total area, followed by Maharashtra (15%), Karnataka (11%), Orissa (11%), Madhya Pradesh (7%) and other states contributing nearly 22% to the total area under chilli cultivation (Jagtap et al., 2012). “Naga Jolokia” the world’s hottest chilli is cultivated in the hilly terrain of Assam in a
small town, Tezpur in India (Goudappa et al., 2012). The crop is a significant source of income making India the world’s single largest producer and exporter to the USA, Canada, UK, Saudi Arabia, Singapore, Malaysia, Germany and many more countries across the world (Chandra Nayaka et al., 2009).

India is not only largest producer but also exporter of chilli in the world, after China. In India, it is grown practically all over the country. In India, chilli is cultivated over an area of 0.81 million ha during 2010-11 with an annual production of 1.22 million tonnes green chillies (Anonymous, 2011). Chilli occupies number one position in export of spices with 2,09,000 metric tonnes volume worth Rs. 1097 crores (Anonymous, 2009). Another important export component of the Indian spice export is value added production like oils and oleoresin with Rs. 563 crores for which the major share of the raw material used is chilli and hence in the recent year, chilli is gaining greater importance in global market (Saideswara Rao, 2008). India is the largest exporter of chilli and about 2.5 to 3.0 % of country's total production is exported.

Andhra Pradesh and Karnataka account for 75% of the country's net area under chilli and its production. At present, Karnataka ranks second in area (0.1322 million ha) and production of chilli (0.148 million tonnes) and is being extensively cultivated in Dharwad, Haveri, Belgaum, Gadag, Bellary, Gulbarga, Chikkamagalur and Raichur (Goudra et al., 2011). Karnataka state currently possesses 10 to 15 varieties and produces about 10 varieties of chillies which contain different ranges of colour, pungency, size and shape. The main varieties grown in Karnataka are namely Byadagi Kaddi, Byadgi Dabbi, Guntur and NP-46A (Jwala) (Rajur and Patil, 2013).
2.3 Cultivation

Chilli is an annual herbaceous crop that reaches a height of one meter and has glabrous or pubescent lanceolate leaves with white flowers and fruits with varied, colour and pungency. Chilli plant requires a warm and humid climate for its best growth and dry weather during the maturation of fruits. Chilli grows best at 20 -30°C. It can be grown in higher altitude up to 2000 metres above the sea level. It can be grown successfully as a rain-fed crop in areas receiving an annual rainfall of 850-1200 mm and a soil pH of 4.3-8.7. Capsicum species are cold sensitive and generally grow best in well-drained, sandy/ silt-loam soil. Plantings are established by seeding or transplanting. Usually flowering usually occurs three months after planting. High temperature associated with low relative humidity at flowering increases the transpiration resulting in shedding of buds, flowers and small fruits (Rammohan et al., 2001).

2.4 Growth phases in chilli

The crop duration of chilli is about 150-180 days depending on variety, climate, fertility and water management. The growth of chilli consists of vegetative and reproductive phases. Vegetative phase in chilli extends to 75-85 days followed by 75-95 days of reproductive phase. The vegetative phase is characterized by increase in plant height with branching. Flowering starts from 80-85 days of the crop or 40-45 days after transplanting. Chilli plant is an often cross pollinated crop with 50% of natural crossing. For fruit development and maturity about 40 days time is required after anthesis and pollination (Rajput and Paraluke, 1998).
2.5 Economic importance of chilli

Chilli has been used since ancient times, traditionally in the form of spice. It is also used as a natural flavour and colorant in food industry (Vinaya et al., 2009) as well as raw material for the pharmaceutical industry. Chilli is nutritious crop, every 100 gm of green and dry chilli yield about 229 and 297 calories of energy (Table 2.1). It is mainly cultivated for three constituents of fruits viz., capsaicin, capsanthin and oleoresin (Amusa et al., 2004).

It is grown for its pungent fruits which are used both as green and ripe to impart pungency and flavour to the food. Pungency, one of the important attributes of Capsicum species is due to the presence of alkaloid ‘capsaicin’ in the fruit. It is used primarily in the flavouring of pickles, meats, barbecue sauces, ketchup, cheese, snack food, dips, chilli cake, salads, and sausages (Pugalendhi et al., 2010).

As a medicinal plant, the Capsicum species has been used as a carminative, stomachic, stimulant, rubefacient and tonic. It prevents heart diseases by dilating blood vessels. Chilli stimulates saliva and gastric juices and aids in digestion. Oleoresin of capsicum is used in pain balms and vapour rubs. Chilli extracts are used in wide range of medicines against tonsillitis, loss of appetite, flatulence, intermittent fever, sore throat, swellings and hardened tumours (Bosland and Votava, 2003). Chilli leaves are used as a dressing for wounds and sores and the leaf sap is squeezed into eyes against headache (Molnar et al., 2004). Chilli leaves are also used to treat toothache (Medvedeva et al., 2003).
Table 2.1 Nutritive value of chilli

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dry chillies (per 100 gm)</th>
<th>Green chillies (per 100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>31.60 gm</td>
<td>3.00 gm</td>
</tr>
<tr>
<td>Proteins</td>
<td>15.00 gm</td>
<td>2.90 gm</td>
</tr>
<tr>
<td>Fats</td>
<td>6.20 gm</td>
<td>0.60 gm</td>
</tr>
<tr>
<td>Minerals</td>
<td>6.10 gm</td>
<td>1.00 gm</td>
</tr>
<tr>
<td>Fibre</td>
<td>30.20 gm</td>
<td>6.80 gm</td>
</tr>
<tr>
<td>Calcium</td>
<td>160.00 mg</td>
<td>30.00 mg</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>370.00 mg</td>
<td>80.00 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>2.30 mg</td>
<td>4.40 mg</td>
</tr>
<tr>
<td>Moisture</td>
<td>10.00 gm</td>
<td>85.70 gm</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotene</td>
<td>345.00 µg</td>
<td>175.00 µg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.93 mg</td>
<td>0.19 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.43 mg</td>
<td>0.39 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>9.50 mg</td>
<td>0.90 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>50.00 mg</td>
<td>111.00 mg</td>
</tr>
<tr>
<td><strong>Minerals &amp; trace elements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>14.00 mg</td>
<td>-</td>
</tr>
<tr>
<td>Potassium</td>
<td>530.00 mg</td>
<td>-</td>
</tr>
<tr>
<td>Phytin phosphorous</td>
<td>71.00 mg</td>
<td>7.00 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>-</td>
<td>272.00 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>-</td>
<td>1.40 mg</td>
</tr>
<tr>
<td>Manganese</td>
<td>-</td>
<td>1.38 mg</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>-</td>
<td>0.07 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>-</td>
<td>1.78 mg</td>
</tr>
<tr>
<td>Chromium</td>
<td>-</td>
<td>0.04 mg</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>-</td>
<td>67.00 mg</td>
</tr>
<tr>
<td>Caloric values</td>
<td>229</td>
<td>297</td>
</tr>
</tbody>
</table>

(Source: The National Institute of Nutrition, Hyderabad, Gopalan et al., 2004)
2.6 Diseases of chilli

Plant diseases are an ongoing limiting factor in crop production. Diseases of crops lead to yield losses and are gaining importance with an increase in world population. Several abiotic and biotic stresses affect the productivity of chilli crop worldwide. Fifty one different pathogens have been reported to cause diseases on various parts of chilli (Saha and Singh, 1988) (Table 2.2). Out of them, thirty nine belong to the fungi of classes Mastigomycotina, Ascomycotina and Deuteromycotina. Fungal diseases are still an obstacle to the economic production of chilli. The most serious disease for agriculturist cultivating chilli are anthracnose and root rot (Vudhivanich, 2003). Anthracnose disease caused by Colletotrichum species, root rot caused by Rhizoctonia solani are the most serious destructive diseases of chilli (Isaac, 1992).

2.6.1 Chilli anthracnose

Anthracnose, derived from a Greek word meaning ‘coal’, is the common name for plant diseases characterized by very dark, sunken lesions, containing spores (Isaac, 1992). Anthracnose of chilli was first reported from New Jersey, USA, by Halsted in 1890 who described the causal agents as Gloeosporium piperatum and Colletotrichum nigrum (Halsted, 1891). These taxa were then considered as synonyms of Colletotrichum gloeosporioides by von Arx (1957).

Anthracnose of chilli is one of the most economically important disease reducing marketable yield from 10% to 80% (Poonpolgul and Kumphai, 2007; Than et al., 2008b). Colletotrichum is capable of causing disease on virtually all parts of the chilli plant during any stage of plant growth. However, fruit lesions are the most economically important
aspect of anthracnose disease (Phoulivong et al., 2011). Under favourable conditions of disease development, up to 50% of the fruits can be damaged. Fruit rot takes place at 28°C and 95.7% relative humidity (Hyde et al., 2009).

Table 2.2. Some of the important diseases of chilli in India

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the disease</th>
<th>Causal organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Fungal diseases</strong></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Anthracnose/ Die back and fruit rot</td>
<td><em>Colletotrichum gloeosporioides</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Colletotrichum capsici</em></td>
</tr>
<tr>
<td>2.</td>
<td>Root rot</td>
<td><em>Phytophthora capsici</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rhizoctonia solani</em></td>
</tr>
<tr>
<td>3.</td>
<td>Cercospora leaf spot</td>
<td><em>Cercospora capsici</em></td>
</tr>
<tr>
<td>4.</td>
<td>Damping off</td>
<td><em>Pythium aphanidermatum</em></td>
</tr>
<tr>
<td>5.</td>
<td>Powdery mildew</td>
<td><em>Leveillula taurica/ Oidiopsis taurica</em></td>
</tr>
<tr>
<td>6.</td>
<td>Fusarium wilt</td>
<td><em>Fusarium oxysporum</em></td>
</tr>
<tr>
<td>7.</td>
<td>Southern blight, collar rot</td>
<td><em>Sclerotium rolfsii</em></td>
</tr>
<tr>
<td>8.</td>
<td>Gray leaf spot</td>
<td><em>Stemphylium solani</em></td>
</tr>
<tr>
<td>9.</td>
<td>Gray mold</td>
<td><em>Botrytis cinerea</em></td>
</tr>
<tr>
<td>10.</td>
<td>Phytophthora Blight</td>
<td><em>Phytophthora capsici</em></td>
</tr>
<tr>
<td>11.</td>
<td>Verticillium wilt</td>
<td><em>Verticillium alboatrum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Verticillium dahlia</em></td>
</tr>
<tr>
<td></td>
<td><strong>Bacterial diseases</strong></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Bacterial wilt</td>
<td><em>Ralstonia solanacearum</em></td>
</tr>
<tr>
<td>13.</td>
<td>Bacterial leaf spot</td>
<td><em>Xanthomonas vesicatoria</em></td>
</tr>
<tr>
<td>14.</td>
<td>Bacterial Soft Rot</td>
<td><em>Erwinia carotovora</em></td>
</tr>
<tr>
<td></td>
<td><strong>Viral diseases</strong></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Leaf curl</td>
<td><em>ChiLCV (chilli leaf curl virus)</em></td>
</tr>
<tr>
<td>16.</td>
<td>Chilli mosaic</td>
<td><em>Cucumber mosaic virus, Potato virus Y</em></td>
</tr>
<tr>
<td></td>
<td><strong>Nematode disease</strong></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Root-Knot Nematode</td>
<td><em>Meloidogyne incognita</em></td>
</tr>
</tbody>
</table>

(Source: Pepper disease: a field guide, Black et al., 1991)
Typical anthracnose symptoms on chilli fruit include water soaked, 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. The lesion may increase to 2-3 cm in diameter on larger fruits (Fig. 2.1). The attack commences from the growing point of the flower bud, the tops of the affected branches wither and turn brown. The infected plants bear fewer fruits of low quality (Voorrips et al., 2004). Fruit rot reduces dry weight, capsaicin and oleoresin content of affected fruits (Mistry et al., 2008), leading to reduction in the medicinal properties of chilli.

Figure 2.1. Anthracnose of chilli

Anthracnose is mainly a problem on mature fruits, causing severe loss due to both pre and postharvest fruit decay exhibiting the phenomenon of quiescence in which symptoms do not develop until the fruit ripens (Bosland and Votava, 2003). Appressoria formed on immature fruits may remain quiescent until the fruits mature or ripen. The biotrophic life strategies adopted by Colletotrichum sp. may also contribute to their prominence as symptomless endophytes of living plant tissues (Yuan et al., 2011; Rojas et al., 2010).
2.6.1.1 Causal agent of chilli anthracnose – *Colletotrichum gloeosporioides*

Anthracnose disease caused by *C. gloeosporioides* belongs to the Kingdom Fungi, Phylum Ascomycota, Class Sordariomycetes; Order Glomerellales and Family Glomerellaceae (Agrios, 2005). Sixty-six species of *Colletotrichum* has been recently described by Hyde et al. (2009) to cause plant diseases. *C. gloeosporioides* Penz is so far the most predominant *Colletotrichum* sp. and can attack about 470 different host genera (Cannon et al., 2008).

Many plant pathologists had recorded anthracnose disease throughout the world. *C. acutatum, C. gloeosporioides* and *C. capsici* were considered to be major causal agents of chilli anthracnose. Anthracnose of chilli has been shown to be caused by *C. capsici* and *C. gloeosporioides* in India, Indonesia, Korea, Thailand (Sharma et al., 2005; Pakdeevaraporn et al., 2005; Voorrips et al., 2004); *C. acutatum* in Australia and Indonesia (Nirenberg et al., 2002).

Yield loss up to 50% in Thailand, 21-47% in Sri Lanka, 15% in Korea and 50% in Malaysia has been reported (Kumaran et al., 2013; Than et al., 2008b). In India, the disease was first reported by Sydow in 1913 from Coimbatore of Madras Presidency (Sydow, 1928). Bansal and Grover (1969) during their studies on *C. frutescens* Linn. reported 10-35% fruit loss due to anthracnose disease in 1966 and 20 to 60% fruit loss during 1967 in six districts of Punjab and Haryana. Thind and Jhooty (1985) reported fruit loss of 66-84% in Northern Karnataka.

Kim et al. (2004) reported that different species *Colletotrichum* cause diseases of different organs of the chilli plant, *C. acutatum* and *C. gloeosporioides* infect chilli fruits at
all developmental stages, but not the leaves or stems, which are damaged by *C. coccodes* and *C. dematium*.

The primary source of inoculum is seed and can survive well in soil by growing saprobically on dead plant fragments, and spreads via water-splash dispersal of conidia and air transmission of ascospores from the sexual morphology. Anthracnose usually appears after a rain or prolonged dew period. The initial infection processes of *Colletotrichum* spp. involves the attachment of conidia to plant surfaces, conidial germination, adhesive appressoria production and penetration of plant epidermis, plant tissue colonization and production of acervuli and sporulation (Prusky *et al*., 2000).

*Colletotrichum* species can survive in and on seeds as acervuli and micro-sclerotia. *Colletotrichum* may also be introduced into fields by infected transplants or it may survive between seasons in plant debris or on weed hosts. Microsclerotia are naturally produced by *Colletotrichum* sp. to allow the fungus to lie dormant in the soil during winter or under stressed conditions. Microsclerotia can survive for many years even after 2 or 3 years of crop rotation. *Colletotrichum* sp. may also persist on alternative hosts such as other Solanaceous or legume crops (Pernezny *et al*., 2003).

The fungi produces 17-18 x 3-4 µm, colourless, one-celled, ovoid, cylindrical and sometimes curved or dumb bell shaped conidia in acervuli (Agrios, 2005). The acervulus is saucer-shaped and surrounded by stiff, black, unbranched hairs, which are typically referred to as setae. The conidiogenous cells form a closely packed palisade of phialides. The curved elongated phialoconidia are produced in slimy droplet which is held in place by stiff dark setae surrounding the acervulus. The phialides are very small in size, producing phialoconidia at their apex. The conidia are aseptate, fusoid, and somewhat
curved or sickle-shaped (Webster and Weber, 2007). They are often hyaline or slightly pink-coloured with rounded ends. The conidia germinates by producing 1 to 4 (typically 2) germ tubes to give rise to new mycelium. The mycelium has richly-branched, septate hyphae, which are hyaline in the beginning, later turning to dark at maturity (Sharma, 2005).

Asalmol et al. (2001) reported both seed borne and air borne transmission of the diseases. Kumudkumar et al. (2004) in a study on infected chilli seeds showed C. dematium presence in the seed coat of 31.25 % infected seeds and reported that the pathogen was transmitted to young seedling through infected seeds by local contact giving a ratio of 8:1 in seed infection and seed transmission. Chilli seed samples collected from different chilli growing districts of Northern Karnataka revealed C. capsici to be most predominant fungi (Vinaya et al., 2009).

Sharma et al. (2005) reported the existence of 15 pathotypes of Colletotrichum capsici from the Himachal Pradesh area of northern India based on quantitative differences in lesion development on inoculated fruit of C. annuum genotypes. Than et al. (2008b) showed pathotype differences within C. acutatum isolates from infected strawberry and chilli fruit.

2.6.2 Rhizoctonia root rot

Rhizoctonia solani Kuhn is a common soil borne pathogen with worldwide distribution and great diversity of host plants (Thomton et al., 2004). Species of Rhizoctonia infect over 500 plants, mainly in the family’s Compositae, Gramineae, Leguminosae, Solanaceae and Cruziferae (Ogoshi, 1996). The most widely recognized
species of *Rhizoctonia* was originally described by Julius Kuhn on potato in 1858 (Ceresini, 1999).

The genus *R. solani* belongs to Form Class Deuteromycetes that does not make vegetative spores and can be present as mycelium, sclerotia or basidiospores. It produces shade of brown, thread-like growth called hypha. It is characterized by the diameter of vegetative hyphae (8-12 µm), constriction at the point of branching, and right angle branching of matured hyphae (Parmeter and Whitney, 1970).

*R. solani* is found in agricultural soils and survives on plant residues as microsclerotia (Laemmlen, 2004). Once *R. solani* is in the soil or seed it moves quickly through the seedlings, the plant tissues become water-soaked and mushy. It attacks seeds (turning brown) and plants on the lower stem near the soil. As the fungus moves up and down the stem, the tap root rots and the developing lesions turn reddish-brown. These reddish-brown lesions are a diagnostic characteristic for this disease (Dorrance, 2003). Diseased plants often produce an abundance of secondary roots above the rotted tap root, but wilting, stunted growth and death of plants scattered throughout the field are the most noticeable symptoms of Rhizoctonia root rot. Once a plant is infected, vigour is greatly reduced and production is poor and the result is a poor stand that is mistakenly ascribed to poor seed quality or seed maggots rather than to the presence of a disease (Harikrishnan and Yang, 2004).

*R. solani* is the imperfect state of the basidiomycete fungus that does not produce any asexual spores (called conidia) and only occasionally produce sexual spores (basidiospores). In nature, *R. solani* exists primarily as vegetative mycelium and/or sclerotia. Depending on active stage of this pathogen, 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 (Carroll, 2004).

2.6.2.1 Lifecycle of *Rhizoctonia solani*

The disease cycle of this pathogen consists of two stages; a rarely observed 
basidiomycetous perfect stage where the teleomorph is known as *Thanatephorus 
cucumeris* and an imperfect stage known as *Rhizoctonia solani*, where the fungus survives 
in the soil as a sterile mycelium (Parmeter and Whitney, 1970).

2.6.2.1.1 The perfect state - *Thanatephorus cucumeris*

When exposed to certain environmental conditions, some isolates of *R. solani* will 
produce the teleomorph, *T. cucumeris*, which is characterised by the production of 
basidiospores. The hymenium consists of basidia produced on mats of interwoven 
branched hyphal cells, most often located on the lower aerial parts of the host plant near 
the soil surface (Sneh et al., 1991). Sterigmata are produced from the basidia, with 
numbers ranging from 1 to 7 observed, and basidiospores produced at the tips of the 
sterigmata (Sneh et al., 1991).

2.6.2.1.2 The imperfect state - *Rhizoctonia solani*

In general, *R. solani* hyphae can be identified by pale/dark brown hyphal 
pigmentation, branching near the distal septum of young hyphal cells and the constriction 
of branch hyphae at the origin, where the branch attaches to the main hyphae. Septum 
formation occurs near the origin of hyphal branches, with the presence of dolipore septa 
and multinucleate cells in young, actively growing hyphae characteristic of *R. solani*
(Laroche et al., 1992). Branch hyphae form at 45° and 90° angles to the main hyphae. Other features often present in isolates of *R. solani*, but not universal, include a rapid growth rate, the presence of monolloid cells and sclerotia, with many strains exhibiting varying levels of host specificity and pathogenicity. Monolloid cells, also called barrel-shaped cells, are formed from buds or at the ends of pre-existing cells, and can form into infection cushions and sclerotia. Characteristics never present in a fungus belonging to the genus *Rhizoctonia* include clamp connections, conidia, rhizomorphs and sclerotia that are differentiated into a rind and medulla (Sneh et al., 1991).

### 2.7 Conventional control of *C. gloeosporioides* and *R. solani*

Although controlling plant diseases with chemicals is common method, some plant diseases such as anthracnose and root rot lack effective chemicals to manage the disease. The fungicide traditionally recommended for anthracnose management in chilli is manganese ethylenebisdithiocarbamate (Maneb) (Smith, 2000), although it does not consistently control the severe form of anthracnose on chilli fruit. The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have recently been used for the control of anthracnose of chilli (Lewis and Miller, 2003). Hegde (1998) reported that among the non-systemic fungicides mancozeb (DM-45) was found to be highly effective in inhibiting growth and germination of conidia of *C. capsici* at 3000 ppm, whereas among systemic fungicides carbendazim was found effective at 1500 ppm. The efficacy of Bavistin against the fruit rot pathogen was reported by several workers (Voorrips et al., 2004).

Kumudkumar et al. (2004) reported that among 8 fungicides tested as seed treatment for the management of dieback and anthracnose of chilli, Companion, JKstein
and bavistin + thiram were found significantly superior in eliminating the infection from the seed. Sitara and Hasan (2011) showed that out of 8 fungicides tested, chilli seed treated with fungicide ridomyl gold at 0.15 and 0.25% inhibited the growth of all fungi *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. alternata*, *Drechslera hawaiensis*, *Fusarium moniliforme*, *F. oxysporum* and *F. solani*. Study on efficacy of nine fungicides against *C. gloeosporioides* showed that Bavistin (0.05 and 0.1%), thiophanate methyl (0.05 and 0.1%), emisan (0.15 and 0.25%), propiconazole (0.05 and 0.1%) and hexaconazole (0.2%) completely inhibited mycelial growth (Nandoskar, 2001).

Mehendale (1994) studied efficacy of eight fungicides against *C. gloeosporioides* causing anthracnose of ‘Bakul’ and reported that mancozeb (0.20% and 0.25%), bordeaux mixture (1%), benlate, carbendazim and thiophanate at 0.1 and 0.15 % concentrations were very effective. The application of the azoxystrobin and prothioconazole completely contained fungal growth when applied at the full recommended rate under the conditions tested.

Fungicide fludioxonil was found to be effective in controlling Rhizoctonia stem canker and black scurf in potatoes (Bains *et al.*, 2002). Foliar application of azoxystrobin effectively reduced 64% sheath blight disease incidence and increased 60% rice yield (Sundravadana *et al.*, 2007). A new combination fungicide having azoxystrobin (1.25ml/l) and difenoconazole (1.0ml/l) was found effective against sheath blight recording least disease incidence of 9.36% and 16.43% respectively (Bhuvaneswari and Krishnam Raju, 2012).

Although chemical compounds have been used to diminish crop and yield loss caused by plant pathogens and pests, there are numerous reports of negative effects of
using chemicals which include decrease in biodiversity of the soil-inhabiting microorganisms, hazardous effects of pesticides/ fungicides runoff on the aquatic systems (Johnston, 1986); contamination of environment and water resources, reduction or elimination of beneficial organisms, development of fungicidal resistant pathogen, and contamination of non target vegetation and acute health problems resulting from exposure of farmers to chemical pesticides (Arcury and Quandt, 2003). Furthermore, the increasing cost of pesticides, particularly in low-income countries of the world (Gerhardson and Wright, 2002) is also a limitation. Health concerns and environmental hazards associated with the use of chemical fungicides have resulted in an increasing interest in the use of microbes to control plant diseases which is an environment-friendly approach.

With an objective to reduce residual levels of these chemicals on food, soil and water resources, restrictions were imposed on a number of registered chemical fungicides and efforts have been put towards research for alternative or complementary control methods. Alternative control methods include stimulation of plant defences, cultural practices and biological control.

2.8 Biological control

Biocontrol organisms offer environmentally friendly alternatives to chemical control methods to manage plant diseases or pests. Biological control agents could be used where chemical pesticides are banned (organ chlorines) or being phased out (methyl bromide) or where pests or pathogens have developed resistance to conventional pesticides or to grow organic food to satisfy consumer perception (Butt et al., 2001).

According to Cook and Baker (1983) ‘‘Biological control is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or
through one or more organisms other than man.’” Biological control method has potential to control crop diseases while causing no or minimal detrimental environmental impact. Controlling plant disease with biocontrol microorganisms will lead to reduction of environmental pollution and resistance development as compared to chemical methods. This is because they produce degradable chemical in low amounts at targeted locations. This approach fits well in the worldwide strategy to grow healthy plants in a sustainable way and, therefore produce high quality food (Haggag et al., 2007).

Baker and Paulitz (1996) outlined three strategies for biological control 1) protection of infection courts, 2) reduction of inoculum potential in sites not necessarily associated with the infection court, and 3) induction of host resistance. Over the years, many bacterial isolates have been evaluated as potential biocontrol agents against soil borne fungal phytopathogens. However, few of them are ultimately successful after evaluation in field trials.

2.9 Rhizosphere and PGPR

The rhizosphere can be defined as any volume of soil specifically influenced by plant roots and/or in association with roots and hairs and plant-produced material. This space includes soil bound by plant roots, often extending few mm from the root surface and includes the plant root epidermal layer. The root rhizosphere is the place of an intense microbial life with a high microbial activity (Mohamed, 2009).

During plant growth, various substances are deposited by roots into the rhizosphere is referred to as rhizodeposition, which are divided into two main groups. First group comprises a wide variety of water-soluble compounds including sugars, amino acids, organic acids, fatty acids, vitamins and enzymes (Brimecombe et al., 2001) while second
group comprises sloughed-off root cap cells and other debris and mucilage (polysaccharide) originating from the root cap or from lysates released during autolysis. Most of the bacterial colonization occurs in the areas of maximum root exudation viz. the elongation zone junctions between epidermal cells, on root hairs, and at lateral root emergence sites (Fig. 2.2).

![Zones of the root](image)

**Figure 2.2 Rhizodeposition root zones in the rhizosphere** (Gobat *et al.*, 2004)

Rhizosphere is relatively rich in nutrients due to the loss of as much as 20-40% of plant photosynthesis from the roots (Lugtenberg *et al.*, 2001). Thus, root exudates *viz.* carbohydrates, amino acids, organic acids and mucilage-derived nutrients attract deleterious rhizobacteria as well as beneficial and neutral bacteria allowing them to colonize and multiply in the rhizosphere (Walker *et al.*, 2003). PGPR have to be highly competitive to colonize the root zone successfully (Compant *et al.*, 2010).
Most rhizosphere organisms occur within 50 mm of root surface and populations within 10 mm of root surface may reach $10^9$-$10^{12}$ microbial cells/g of soil. Despite large numbers of bacteria in the rhizosphere, only 7-15% of the total root surface is generally occupied by microbial cells (Gray and Smith, 2005).

Root colonization is influenced by many biotic factors (genetic traits of the host plant and the colonizing organism) and abiotic factors (growth substrate, soil humidity, soil and rhizosphere pH, and temperature). To colonize the rhizosphere during an extended period characterized by strong microbial competition and to exert plant growth promoting traits, soil bacteria need to be rhizosphere competent (Whipps, 2001). Root colonization and rhizosphere competence are heavily influenced by the plant changes in the physical and chemical composition of the rhizosphere soil compared to the bulk soil. These differences are manifested by changes in water potential, partial pressure of O$_2$, and other physical and chemical characteristics due to plant exudations (Vessey, 2003).

2.10 Plant Growth Promoting Rhizobacteria

Microorganisms that colonize the rhizosphere are classified as beneficial, deleterious and neutral groups on the basis of their effects on plant (Dobbelaere et al., 2003). Beneficial microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents. Kloepper and Schroth (1981) termed these beneficial rhizobacteria as **Plant Growth Promoting Rhizobacteria (PGPR)**. PGPR are defined by three intrinsic characteristics: (i) they must be able to colonize the root (ii) they must survive and multiply in micro-habits associated with the root surface, in competition with other micro-biota at least for the time needed to express their plant growth promotion/protection activities (iii) they must promote plant growth. Plant growth promoting
rhizobacteria are thus free-living, soil-borne bacteria which when applied to seeds/soils or crops, enhance the growth of the plant directly by providing nutrients to plants or indirectly by reducing the damage from soil borne plant pathogens (Klopper et al., 1994).

Plant growth promoting rhizobacteria have first been used for agricultural purposes in the former Soviet Union and India in the early 20th century and are now being tested worldwide. In general, PGPR can be divided into two categories (i) Extracellular PGPR (ePGPR, free living) existing in the rhizosphere on the rhizoplane or in the spaces between cells on the root cortex and (ii) intracellular PGPR (iPGPR, symbiotics) which exist inside root cells (Gray and Smith, 2005). Generally, iPGPR include the members of the family Rhizobiaceae, capable of forming nodules on the root systems of leguminous plants (Figueiredo et al., 2011). Among ePGPR Pseudomonas and Bacillus are the most commonly described genera (Bhattacharyya and Jha, 2012). Based on their mechanism of action, PGPRs can be classified into three general forms viz. biofertilizer, phytostimulator and biopesticide (Table 2.3).

Recent investigations on PGPR revealed that they promote plant growth by [1] producing ACC deaminase to reduce the level of ethylene in the roots of developing plants (Dey et al., 2004) [2] producing plant growth regulators like indole acetic acid (IAA), gibberellic acid, cytokinins (Castro et al., 2008) and ethylene (Saleem et al., 2007) [3] asymbiotic nitrogen fixation (Ardakani et al., 2010) [4] exhibition of antagonistic activity against phytopathogenic microorganisms by producing β-1,3-glucanase, chitinases, antibiotics, siderophores and cyanide (Pathma et al., 2011) and [5] solubilization of mineral phosphates and other nutrients (Hayat et al., 2010). PGPR may use more than one of above mechanisms to enhance plant growth as experimental evidence suggests that the plant growth stimulation is the net result of multiple
mechanisms that may be activated simultaneously (Martinez-Viveros et al., 2010) (Fig. 2.3).

Table 2.3. PGPR forms and their mechanisms of action stimulating plant growth

<table>
<thead>
<tr>
<th>PGPR form</th>
<th>Definition</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Biofertilizer | A substance which contains live microorganisms which when applied on the seed, plant surface or the soil, colonizes the rhizosphere or the interior of the plant and promotes growth through increased supply of primary nutrients of the host plant | -Biological N₂ fixation  
-Utilization of insoluble forms of phosphorous | Fuentes-Ramirez, and Caballero-Mellado, 2006  
Vessey, 2003 |
| Phytostimulator | Microorganisms with the ability to produce growth regulators such as indole acetic acid, gibberellic acid and cytokinins and ethylene | -Production of phytohormones | Lugtenberg et al., 2002  
Somers et al., 2004 |
| Biopesticide  | Microorganisms that promote plant growth by controlling phytopathogenic agents | -Production of antibiotics (siderophores, HCN, antifungal metabolites)  
-Production of enzymes that degrade the cell wall of the fungi  
-Competitive exclusion  
-Acquired and induced systemic resistance | Somers et al., 2004  
Chandler et al., 2008 |
In addition, PGPR have great adaptation to harsh environments including drought stress, salt stress, high temperatures, dryness or heavy rainfall in tropical countries, and contaminated environments (Arzanesh et al., 2011; Dell Amico et al., 2008; Mayak et al., 2004), indicating that they could contribute to ameliorate plant crops in areas with poor agricultural potential. The first reports of PGPR on potato noted that growth promotion was associated with a reduction of total fungal propagules on the rhizoplane (Kloepper and Schroth, 1981). This suggested that the select PGPR strains could also be used to reduce pathogen populations in the root zone.

2.11 *Pseudomonas* as PGPR

Members of the genus *Pseudomonas* (γ-Proteobacteria subclass, Pseudomonadales order, Pseudomonadaceae family) are non-sporulating rods with Gram-negative reaction, motile (one or several polar flagella), and a high genomic G+C content 58–69% (Palleroni, 2008). They are catalase and oxidase positive and chemo organotrophic, with a strictly respiratory metabolism (using oxygen and in some cases nitrate as terminal electron
acceptor). The fluorescent Pseudomonads include all *Pseudomonas* species with the ability to produce fluorescent pyoverdine siderophore(s), noticeably *Pseudomonas aeruginosa*, *P. syringae*, *P. putida* and *P. fluorescens* (Bossis *et al.*, 2000). The generation time of *Pseudomonas* spp. in the rhizosphere was found to be 5-14 h, whereas it was found to be 77 h in the bulk soil (Bowen and Rovira, 1973).

There are many species such as *P. fluorescens*, *P. putida*, *P. aeaureofasciens* and *P. chloraphis*, which may act as plant beneficial bacteria by antagonizing plant pathogens and through the production of traits that directly influence plant disease resistance and growth (Ortiz-Castro *et al.*, 2009). Pseudomonads have been mostly studied for protection of crop plants from phytopathogenic oomycetes (*Pythium* spp.) and fungi (*F. oxysporum, Gaeumannomyces graminis, R. solani*, etc.), and to a lesser extent bacteria (*Pectobacterium carotovorum*) and nematodes (*Meloidogyne* spp.) (Table 2.4). Disease suppression by these bacteria often entails inhibition of phytopathogens in soil or on roots, by competition and/or antagonism (Haas and Defago, 2005). Plant protection may also result from direct interactions with the host plants, especially in the case of Induced Systemic Resistance (ISR) (Bakker *et al.*, 2007). Several lines of evidence indicate that siderophore production when iron is limited is responsible for the antagonism of some strains of *P. aeruginosa* against *Pythium* spp., the causal agents of damping-off and root rot of many crops (Charest *et al.*, 2005, Mavrodi *et al.*, 2012).

*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 most biocontrol pseudomonads are not specific for one
pathogen or plant species only, but have a wide host range and can suppress several pathogens (Fig. 2.4).

Table 2.4. Disease suppressed by *Pseudomonas* biocontrol species

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host</th>
<th>Pathogen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf29A</td>
<td>wheat</td>
<td><em>G. graminis var. tritici</em></td>
<td>Barret <em>et al.</em>, 2009</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBW25</td>
<td>pea</td>
<td><em>Pythium ultimum</em></td>
<td>Sanguin <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR54</td>
<td>sugarbeet</td>
<td><em>R. solani</em></td>
<td>Sanguin <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2P24</td>
<td>wheat</td>
<td><em>R. solanacearum, F. oxysporum, R. solani,</em></td>
<td>Sanguin <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><em>P. chlororaphis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA-23</td>
<td>canola</td>
<td><em>S. sclerotiorum</em></td>
<td>Fernando <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf-5</td>
<td>cotton</td>
<td><em>R. solani</em></td>
<td>Loper <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><em>P. aureofaciens</em></td>
<td></td>
<td></td>
<td>Jung <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2-87</td>
<td>wheat</td>
<td><em>G. graminis var. tritici</em></td>
<td>Weller <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP10</td>
<td>Banana</td>
<td><em>F. oxysporum f.sp cubense</em></td>
<td>Ayyadurai <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7NSK2</td>
<td>Rice</td>
<td><em>Magnaporthe grisea</em></td>
<td>De Vleesschauwer <em>et al.</em>, 2006</td>
</tr>
</tbody>
</table>
2.12 PGPR traits of fluorescent Pseudomonads

2.12.1 Phosphate solubilization

Phosphorus is second important mineral nutrient after nitrogen required for plant development and growth making up about 0.2% of plant dry weight (Vikram and Hamzehzarghani, 2008). It is also involved in photosynthesis, signal transduction, macromolecular biosynthesis, and respiration (Fernandez et al., 2007). Although Phosphate (P) is abundant in soils in both inorganic (originating mainly from applied P fertilizer) and organic forms (derived from microorganisms, animals and plants), it is still one of the major plant growth limiting nutrients. About 10-25% of P fertilizer is required by the plants (Saha and Biswas, 2009) for promoting their functions. The low availability of P to plants is because majority of soil P is found in insoluble forms. Most of the P is
found in form of iron, calcium or aluminum phosphates (Fe-P, Ca-P, or Al-P). Plant roots can only absorb P in two soluble forms, the monobasic (H$_2$PO$_4^-$) and the diabasic (HPO$_4^{2-}$) ions (Vessey, 2003). To circumvent the problem, chemical phosphate fertilizers are used. However, due to its high reactivity, almost 75 to 90% of added P fertilizer is precipitated by Al, Fe, and Ca complexes present in the soils, creating a demand for suitable alternatives to mobilize this fixed fraction of the important bio-element (Glick, 2012).

Organisms with phosphate solubilizing activity are called phosphate solubilising microorganism (PSM), may provide the available forms of P to the plants and are viable substitute to chemical phosphatic fertilizer. Members of the genus *Azotobacter, Bacillus, Enterobacter, Erwinia, Pseudomonas, Rhizobium* and *Serratia* are reported as the most significant phosphate solubilising bacteria (Bhattacharyya and Jha, 2012).

Several species of fluorescent pseudomonads such as *P. aeruginosa* (Ahemad and Khan, 2011a), *P. putida* (Ahemad and Khan, 2012a), *P. fluorescens* (Shaharoona et al., 2008), *P. chlororaphis* (Liu et al., 2007), *P. jessenii* (Rajkumar and Freitas, 2008) were reported as phosphate solubilizers.

Solubilization of inorganic phosphorus occurs as a consequence of the action of low molecular weight organic acids which are synthesized by various soil bacteria (Zaidi et al., 2009). Conversely, the mineralization of organic phosphorus occurs through the synthesis of a variety of different phosphatases by catalyzing the hydrolysis of phosphoric esters (Glick, 2012). Importantly, phosphate solubilization and mineralization can coexist in the same bacterial strain (Tao et al., 2008).

Several mechanisms have been proposed to explain the microbial solubilization of P compounds. The mechanisms include: (1) release of organic acids produced during
organic residue decomposition (Hameeda et al., 2006), (2) excretion of protons due to NH$_4^+$ assimilation by microorganisms (Whitelaw, 2000), (3) formation of complexes between organic acids/ anions with cations [Al$^{3+}$, Fe$^{3+}$, Ca$^{2+}$] (Welch et al., 2002) and (4) desorption of P sorbed onto soil clay and/or oxides (Osorio, 2008). Nitric and sulfuric acid produced by Nitrosomonas and Thiobacillus species, respectively, have also been reported to dissolve P compounds (Azam and Memon, 1996). Equally, P compounds may be solubilized by carbonic acid formed as a result of organic matter decomposition (Memon 1996) (Fig. 2.5).

Figure 2.5. Microbial contribution to plant phosphorous nutrition (Richardson, 2009).

Kumar et al. (2008) isolated metal tolerant, plant growth-promoting bacteria (Enterobacter sp.) which decreased the pH of the growth medium from 7 to 2, thereby achieving the maximum solubilization of P (229 mg/l). Chen et al. (2006) also showed that
the P solubilizing activity of isolated strains was related to the release of organic acids and the subsequent pH reduction in the medium.

Hameeda et al. (2006) reported that phosphate solubilizing bacteria with cellulolytic activity enhanced the mineralisation and decomposition of crop residues. Pratibha and Arvind (2009) found that phosphate-solubilising fluorescent *Pseudomonas* strains producing 2-ketogluconic acid, gluconic acid, oxalic acid, succinic acid, lactic acid, formic acid, citric acid and malic acid in the culture filtrates during the solubilisation of tricalcium phosphate. De Werra et al. (2009) concluded that the ability of fluorescent *Pseudomonas* strain CHAO to acidify its environment and to solubilise mineral phosphate is strongly dependent on its ability to produce gluconic acid.

Phosphate-solubilizing microbes are reported to reduce the toxicity of metals and protect the plants against the toxic effects of these metals and consequently enhance the growth and yield of plants in contaminated soils (Wani et al., 2007). Attia et al. (2009) reported that inoculation of phosphate-solubilizing bacteria with mineral phosphorus increased the efficiency of P fertilizer and decreased the required P rate to plants with enhanced vegetative growth and fruit quality.

### 2.12.2 Siderophore production

Iron is a vital nutrient for almost all forms of life. In the aerobic environment at physiological pH, the reduced ferrous (Fe$^{2+}$) form is unstable and is readily oxidized to oxidized ferric (Fe$^{3+}$) which normally occurs as poorly soluble iron hydroxides and oxyhydroxides, basically unavailable to biological systems (Rajkumar et al., 2010).

Siderophores are low molecular weight molecules (400-1000 daltons) which have high affinity for iron and thus bind ferric ions available in the soil. Many PGPR strains
like *Pseudomonas, Bacillus, Acinetobacter, Serratia* known to produce siderophores, thus improves the availability of iron to plants. Indirectly they control the pathogens by scavenging the limited amount of ferric ions available in the rhizosphere and thus inhibiting the pathogens in their immediate vicinity (Yu *et al.*, 2011; Sarode *et al.*, 2009).

In both Gram positive and Gram negative rhizobacteria, Fe$^{3+}$ - siderophore complex on bacterial cell membrane is reduced to Fe$^{2+}$ which is released into the cell from the siderophore via a gating mechanism linking the inner and outer membranes (Rajkumar *et al.*, 2010). Based on the structural features, functional groups and types of ligands, bacterial siderophores are classified into four main classes namely, carboxylate, hydroxamates, phenol catecholates and pyoverdines (Crowley, 2006). Most of the bacterial siderophores are catecholates, and few are hydroxamates and carboxylates, whereas most fungal siderophores are hydroxamates (Schalk *et al.*, 2011).

Siderophores are reported to form stable complexes with other heavy metals such as Al, Cd, Cu, Pb, Zn (Chamongkolpradit *et al.*, 2008). Siderophore production by various bacteria in response to iron deficiency normally occurs in neutral to alkaline pH soils, due to low iron solubility at elevated pH have been reported (Sharma and Johri, 2003).

Of the several mechanisms used to facilitate plant growth, siderophore synthesized by microbes including rhizobia and species of *Bacillus, Pseudomonas* and *Azotobacter* (Ahmad *et al.*, 2008) is well documented due to their iron sequestration ability from the soil. Rane *et al.* (2008) showed that biocontrol ability of *P. aeruginosa* ID 4365 against groundnut (*Arachis hypogaea*) phytopathogens was due to production of pyoverdin and pyochelin siderophores. Application of cadmium- resistant plant growth-promoting *P. aeruginosa* exhibiting siderophore production, when used as inoculant for blackgram
(Vigna mungo L.) plants grown in soil treated with a gradient of CdCl$_2$ concentration, reduced the toxicity of metal to plants (Ganesan, 2008).

Wani et al. (2008b) and Tripathi et al. (2005) suggested that plant chlorosis due to heavy metals can be prevented by providing them with siderophore producing bacterium that supplements sufficient amounts of iron to the plant. Vansuyt et al. (2007) have reported increased plant growth in Arabidopsis thaliana due to intake of Fe-pyoverdine complex synthesized by P. fluorescens C7. Charest et al. (2005) have demonstrated the contribution of siderophore towards inhibitory potential of P. aeruginosa towards Pythium spp. in an iron-depleted medium. Siderophore mediated suppression of rice fungal pathogens R. solani and Pyricularia oryze in an in-vitro assay on Kings-B medium has been reported by Battu and Reddy (2009).

2.12.3 Indole Acetic Acid

Many important plant-microbial interactions center on the production of auxins. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites. IAA is produced by PGPR by using the rich supplies of substrates exuded from the roots and release of auxin in the rhizosphere as secondary metabolites. Several PGPR are reported to produce IAA which stimulates cell division, seed and tuber germination, increase the rate of xylem and root development (lateral and adventitious root formation) and thereby play a significant role in increasing the root surface area and number of root tips in many plants (Bhattacharyya and Jha, 2012). A greater root surface area and length enables the plant to access more nutrients from soil and thus contribute to plant growth promotion (Tsavkelova et al., 2007).
Tryptophan has been identified as a main precursor for IAA biosynthesis pathways in bacteria (Zaidi et al., 2009). Tryptophan inhibits anthranilate (reduces IAA synthesis) formation by a negative feed-back regulation on anthranilate synthase, resulting in indirect induction of IAA production. The identification of intermediates led to the identification of five different pathways for IAA synthesis using tryptophan as a precursor for IAA (1) IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in a majority of bacteria like Erwinia herbicola; saprophytic species of the genera Agrobacterium and Pseudomonas; certain representatives of Bradyrhizobium, Rhizobium, Azospirillum, Enterobacter and Klebsiella (2) Conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway in which tryptamine is formed (pseudomonads and azospirilla) and (3) IAA biosynthesis via indole-3-acetamide formation is reported for phytopathogenic bacteria Agrobacterium tumefaciens, P. syringae, and E. herbicola; saprophytic pseudomonads like P. putida and P. fluorescens (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in the cyanobacterium (Synechocystis sp.) and (5) the tryptophan-independent pathway, which is common in plants and also found in azospirilla and cyanobacteria (Fig. 2.6).

A positive correlation between auxin production and growth-promoting activity of diverse PGPR has been also reported in Brassica juncea and wheat (Khalid et al., 2004). Species of Bradyrhizobium and Rhizobium produced a substantial amount of IAA under in vitro conditions (Wani et al., 2008a; Ahmad et al., 2008). Among other PGPR strains, Pseudomonas, Bacillus, Agrobacterium sp., Alcaligenes piechaudii and two strains of Comamonas acidovorans secreted IAA (Rajkumar et al., 2006). In another study, numerous bacterial isolates recovered from wheat (Triticum aestivum) rhizosphere
demonstrated the production of auxins (ranging from 1.1 to 12.1 mg/l) under *in vitro* conditions. However, when the medium was supplemented exogenously with tryptophan, it significantly enhanced the auxin biosynthesis which was confirmed by high performance liquid chromatography (HPLC) analysis (Khalid et al., 2004).

![Diagram of different pathway to synthesize IAA in bacteria](image)

**Figure 2.6. Overview of different pathway to synthesize IAA in bacteria**

(Spaepen et al., 2009)

Tryptophan increased production of IAA in *B. amyloliquefaciens* FZB42 (Idris et al., 2007). IAA production, even in the culture without tryptophan supplementation has also been reported (Wahyudi et al., 2011). It has also been reported by Patten and Glick (2002) that the enzyme indolepyruvic decarboxylase (IPDC) is the principal enzyme which determines IAA biosynthesis and stimulates the development of the root system of the host plant. It has been reported that IAA production by PGPR can vary among different species and strains and is also influenced by growth stage, culture condition and substrate availability (Ashrafuzzaman et al., 2009).
Production of IAA has been shown in species of *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum* and *Glucanoacetobacter*. It functions as an important signal molecule in the regulation of plant development and indirectly by influencing bacterial amino cyclopropane-1-carboxylate (ACC) deaminase activity (Wahyudi *et al.*, 2011; Saharan and Nehra 2011).

### 2.12.4 Volatile organic compounds (VOC)

Volatile organic compounds (VOCs) are defined as compounds that have high enough vapour pressures under normal conditions to significantly vaporize and enter the atmosphere. This class of chemicals includes compounds of low molecular weight (<300 g/mol), such as alcohols, aldehydes, ketones and hydrocarbons (Dunkel *et al.*, 2009; Vespermann *et al.*, 2007).

The role of VOCs on antibiosis and the biocontrol of plant pathogens mechanism has received much attention in the last decade (Fig. 2.7). There are numerous reports showing that volatiles produced by bacteria such as ammonia, butyrolactones, HCN, phenazine-1-carboxylic acid, alcohols may have *in vivo* activity in different fungal species (Trivedi *et al.*, 2008).

Volatile can diffuse through the air-filled pores and depending on the type of mineral, texture and particle architecture, they may in fact be trapped in the soil (Aochi and Farmer, 2005). Volatiles, besides other compounds, may be involved in fungistasis (Zou *et al.*, 2007). Soil-dwelling organisms and roots were shown to synthesize, excrete and perceive volatiles (Wenke *et al.*, 2010), and may travel short and long distances below ground.
Cyanide is a volatile secondary metabolite produced during the early stationary growth phase by several PGPR, notably *Pseudomonas* spp. and *Bacillus* (Ahmad *et al.*, 2008) and *Rhizobium* spp. (Wani *et al.*, 2008a) by oxidative decarboxylation pathway using glycine, glutamate, or methionine as precursors. Hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However, producer microbes, mainly Pseudomonads, are reported to be resistant (Bashan and de-Bashan 2005). HCN produced by *Pseudomonas* in the rhizosphere inhibits the primary growth of roots in *Arabidopsis* due to the suppression of an auxin responsive gene (Rudrappa *et al.*, 2008).

*P. fragi* CS11RH1, a psychrotolerant bacterium produced hydrogen cyanide (HCN) and seed bacterization with the isolate significantly increased the germination percentage, rate of germination, plant biomass and nutrient uptake of wheat seedlings.
HCN producing fluorescent *P. aeruginosa* GRC\(_1\) and *P. aeruginosa* GRC\(_2\) inhibited *M. phaseolina* and *S. sclerotium* respectively (Bhatia *et al.*, 2003).

### 2.12.5 Production of lytic enzymes

One of the major mechanisms used by biocontrol agents to control soilborne pathogens involves the production of cell wall degrading enzymes such as chitinase, β-1,3-glucanase, peroxidase, protease, and lipase. Chitinase and β-1,3-glucanase degrade the fungal cell wall and cause lysis of fungal cell. Furthermore, chitin and glucan oligomers released during degradation of the fungal cell wall by the action of lytic enzymes act as elicitors that elicit various defense mechanisms in plants (Karthikeyan *et al.*, 2005).

Trivedi *et al.* (2008) explained production of HCN, siderophores, ammonia, lipase and chitinase by *Pseudomonas corrugate* to be the contributing factor for its antagonistic activity against *Alternaria alternate* and *Fusarium oxysporum*. Nihorimbere *et al.* (2013) studied the relationship between hydrolytic enzymes and antagonistic potential in *Bacillus* strains and concluded that antagonistic potential was related to protease, chitinase and lipase production.

*P. fluorescens* CHA0 produces four extracellular enzymes; protease, phospholipase C, lipase, and alkaline protease. Mutation of *aprA* gene encoding for protease resulted in reduced biocontrol in *P. fluorescens* CHA0 which substantiated the antagonistic effect of protease (Siddiqui *et al.*, 2005).

Mycoparasitism by *P. fluorescens* using scanning electron microscope, revealed that attachment of *Pseudomonas* to fungal hyphae, causes deterioration of fungal mycelium and cell wall (Ziedan and El-Mohamedy, 2008), possibly due to the secretion of
extracellular mycolytic enzymes. Dunne et al. (2000) showed that overproduction of extracellular protease in the mutant strains of Stenotrophomonas maltophilia W81 resulted in improved biocontrol of Pythium ultimum (Mavrodi et al., 2012).

2.12.6 Quorum sensing

Many bacteria regulate diverse cellular processes in concert with their population size, a process referred to as quorum sensing (QS) (Reading and Sperandio, 2006). Bacterial cell-to-cell communication utilizes small diffusible signals, which the bacteria produce and perceive. N-acyl-homoserine lactones (AHLs), are the most commonly reported type of quorum sensing signals, and production of this molecule is more common among plant- associated Pseudomonas spp. than in soil borne species, confirming the importance of quorum sensing in plant associated bacterial communities (Elasri et al., 2001).

In Gram-negative bacteria, the quorum-sensing molecule most commonly present is N-acyl-L-homoserine lactones. AHLs are composed of a homoserine lactone residue linked to an acyl-side chain. The specificity derives from the length of the acyl chain (4-18 carbon atoms), substitution at the C3 position and saturation level within the acyl chain (Raffa et al., 2004). AHLs can be broadly classified as long, medium or short-chained depending on whether their acyl moiety consists of more than eight, between eight-to-twelve or less than twelve carbon atoms, respectively (Ortiz-Castro et al., 2008). These molecules are freely diffused through the bacterial membrane and distribute within the rhizosphere (Schuhegger et al., 2006).

AHLs orchestrate important processes of many beneficial rhizosphere colonizing bacteria. Deletion of the gene pco1 responsible for the production of the AHLs 3-oxo-C6-
HL and 3-oxo-C8-HL in _P. fluorescens_ 2P24 caused the mutant to be defective in colonization of wheat rhizosphere and biocontrol ability against wheat take-all, while complementation of _pco1_ restored the biocontrol activity to the wild-type level (Wei _et al._, 2006).

The presence of AHL-producing bacteria in the rhizosphere of tomato induced the salicylic acid and ethylene-dependent defense responses, which activated systemic resistance in plants and conferred protection against the fungal pathogen _Alternaria alternata_ (Schuhegger _et al._, 2006).

### 2.12.7 Antibiotics

Among the variety of _Pseudomonas_ species inhabiting the rhizosphere, certain strains of fluorescent pseudomonads have received particular attention because of their potential to control seed and soilborne pathogenic fungi through the secretion of a diverse array of antimicrobial metabolites (Haas and Keel, 2003). Members of pseudomonads are known to produce six general categories of antibiotics which include phenazines, phloroglucinol, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (all of which are diffusible) and hydrogen cyanide (volatile) (Haas and Defago, 2005).

Among the antimicrobial compounds released by plant-beneficial pseudomonads, 2,4-diacetylphloroglucinol (DAPG), pyoluteorin (PLT), phenazines (Phz), hydrogen cyanide (HCN) have received particular attention for their major contribution in biocontrol of root diseases that are caused by agronomically important fungal and oomycete pathogens including _Gaeumannomyces, Rhizoctonia, Thielaviopsis, Fusarium_, and _Pythium_ species (Raaijmakers _et al._, 2006, Mavrodi _et al._, 2012).
In plant beneficial bacteria, biosynthesis of secondary metabolites required for biocontrol activity such as 2,4-DAPG, Phz, PLT, and PRN, HCN, chitinase, and exoproteases are directly regulated by the GacA/GacS system which was first described in *P. fluorescens* CHA0 (Selin *et al.*, 2010). Antibiosis and antagonistic activities of PGPR recovered from wheat (*T. aestivum*) and rice (*Oryza sativa*) seeds, corn (*Zea mays*) plants, and potato have been suggested as possible mechanisms of growth inhibition of various phytopathogens (Rosenblueth and Martinez-Romero, 2006).

### 2.12.7.1 Phenazines

Phenazines are an extensive group of pigmented, heterocyclic, nitrogen-containing secondary metabolites with broad-spectrum activity (Kavitha *et al.*, 2005). Currently, more than 50 naturally occurring phenazines compounds have been described and are exclusively produced by bacteria, such as *Pseudomonas, Streptomyces, Nocardia, Sorangium, Brevibacteriu, Pantoea* and *Burkholderia* species (Marvodi *et al.*, 2006) (Fig. 2.8).

Phenazines are redox active compounds, and thus, the mechanism for their action is assumed to be due to their ability to engage in redox cycling in the presence of various reducing agents and molecular oxygen, resulting in the accumulation of toxic superoxide ions like hydrogen peroxide (H$_2$O$_2$) which are harmful to the cell or can lead cell death (Mavrodi *et al.*, 2012).

Bacterization of wheat seeds with *P. fluorescens* strains 2-79 producing the antibiotic PCA resulted in 60% suppression of take-all diseases at field trials (Weller, 2007). *P. chlororaphis* PCL1391 strain, isolated from roots of tomato plants, synthesized phenazine-1-carboxamide, which is able to release soluble iron from insoluble
ferric oxides at neutral pH, indicating phenazines might contribute to iron mobilization in soils (Haas and Defago, 2005). Phenazine producing *P. aeruginosa* ID 4365 showed the role of phenazine-1-carboxylic acid in control of phytopathogens *Aspergillus niger*, *F. oxysporum*, *S. rolfsii*, *C. falcatum* (Rane *et al.*, 2007).

![Chemical structure of some antibiotics produced by Pseudomonas spp.](image)

**Figure 2.8. Chemical structure of some antibiotics produced by Pseudomonas spp.**

Morohoshi *et al.* (2013) demonstrated that the multiple quorum-sensing system play an important role in PCA production and antifungal activity in StFRB508 against *F. oxysporum*. *P. chlororaphis* strain PCL1391 effectively suppressed *C. lindemuthianum* in bean plant, antibiotic effect was considered to be due to its ability to produce phenazine-1-carboxamide (Bardas *et al.*, 2009). *Pseudomonas* strain LBUM223 inhibited *in vitro* growth of *Phytophthora cactorum*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* and it carried phenazine biosynthetic genes involved in production of phenazine-1-carboxylic acid (Paulin, 2009).
2.12.7.2 Phloroglucinols

Phloroglucinols are phenolic compounds produced by bacteria, algae and plants (Jimenez- Escrig et al., 2001). More than 60 derivatives of phloroglucinol have been reported, of which only 3 are known to be produced by *Pseudomonas* species namely, mono acetylphloroglucinol, 2, 4-DAPG and tri-acetylphloroglucinol. Phloroglucinols are known to induce systemic resistance in plants, thus serving as a specific elicitor of phytoalexins and other similar molecules (Dwivedi and Johri, 2003).

2, 4-Diacetylphloroglucinol (2, 4-DAPG) is a polyketide compound which has received particular attention because of its broad-spectrum antiviral, antifungal, antibacterial, antitumor activity and phytotoxic properties (Haas and Keel, 2003) (Fig. 2.8). The phenolic metabolite 2,4-DAPG is an important component for the natural suppressiveness of certain agricultural soil with take-all disease of wheat and black root of tobacco, and is also the active ingredient of many of the key biocontrol strains of *P. fluorescens* (Rezzonico et al., 2007). 2,4-DAPG is synthesized by several plant-associated fluorescent pseudomonads plays a key role in the suppression of a wide variety of soil-borne diseases (Kang, 2012).

2.12.7.3 Polyketides

Pyoluteorin (PLT) is an antifungal compound with a resorcinol ring linked to a bichlorinated pyrrole moiety (Bender et al., 1999) (Fig. 2.8). It is produced by several *Pseudomonas* species and its inhibitory activity against oomycetes fungi, including the plant pathogenic *Pythium ultimum* has been documented. Hassan et al. (2011) showed that pyoluteorin-producing bacteria *P. putida* strain NH-50 significantly reduced red rot disease severity on both sugarcane varieties Co-1148 and SPF-234, irrespective of fungal
inoculation. Study by Mauhofer et al. (1994) reported that pyoluteorin production plays a role in suppression of damping-off by *P. fluorescens* CHA0 strain. Howell and Stipanovic (1980) suggested that treatment of cottonseed with pyoluteorin producing *P. fluorescens* pf-5 at the time of planting in *P. ultimum* infested soil increased seedling survival from 28 to 71% respectively.

*P. fluorescens* Pf-5 secretes secondary metabolites which are toxic to plant pathogens causing seed and root rot diseases. Evidences strongly indicated that pyoluteorin serves as an autoregulator, positively influencing production of *P. fluorescens* Pf-5 which is responsible for biocontrol action and exogenous application of pyoluteorin repressed 2,4-DAPG in *P. fluorescens* Pf-5 (Brodhagen et al., 2004).

Pechy- Tarr et al. (2005) in his study showed that loss of sigma factor RpoN function led to the marked reduction in PLT production and *plt* gene expression whereas increased production of DAPG and high level expression of *phlA* gene suggesting that RpoN may control the balance of antibiotics DAPG and PLT in *P. fluorescens* CHA0 strain.

### 2.12.7.4 Pyrrols

Pyrrolnitrin (PRN) (3-chloro-4-(2’- nitro-3’-chlorophenyl) pyrrole) is a broad spectrum antifungal metabolite produced by strains of *Enterobacter agglomerans*, *Myxococcus fulvus*, *Corallococcus exiguous*, *Cystobacter ferruginus*, *Serratia* spp. and several strains of *Pseudomonas* and *Burkholderia* (Hammer et al., 1999) (Fig. 2.8). The antibiotic was first isolated from *Pseudomonas pyrocinia* (Hammer et al., 1999). PRN functions by inhibiting fungal respiratory chain (Tripathi et al., 1969). PRN are active against wide range of, ascomycete, basidiomycete and deuteromycete fungi.
*Pseudomonas* strains CMR5C and CMR12a exhibited excellent biocontrol activity *in vivo* against *Pythium myriotylum*. These strains produced phenazines and surfactants. Strain CMR5C formed pyoluteorin and pyrrolnitrin also (Perneel *et al.*, 2007). Strain *P. chlororaphis* PA23 strain producing phenazine and pyrrolnitrin provided effective protection to canola and sunflower against *S. sclerotiorum* (Athukorala *et al.*, 2010).

**2.12.8 Biosurfactants**

Biosurfactants are amphiphilic compounds possessing both hydrophilic and lipophilic properties. These surface-active metabolites are produced by several *Pseudomonas* spp. include rhamnolipids and several types of cyclic lipopeptides (CLPs), both of which may possess antifungal properties (Raaijmakers *et al.*, 2006). Biosurfactants are capable of affecting cell surface of plant pathogenic fungi and also have the ability to act on lipids creating pores on the membrane layer (Raaijmakers *et al.*, 2006).

*In vitro* and in soil microcosm antifungal properties of biosurfactants (viscosinamide, tensin and amphisin) against *R. solani* have been described by Andersen *et al.* (2003). Perneel *et al.* (2008) showed that simultaneous production of rhamnolipid-type biosurfactants and phenazines is crucial for biological control of *Pythium* spp. by *P. aeruginosa* PNA1. Daes *et al.* (2011) in his study showed that involvement of phenazines and CLPs in the disease reduction of Rhizoctonia root rot of bean by *Pseudomonas* CMR 12a. Biosurfactant from *P. koreensis* strain 2.742 inhibited the motility of *Phytophthora infestans* zoospores (Hultberg *et al.*, 2010). Hultberg *et al.* (2011) study indicated that biosurfactant producing strains can be used for the management of diseases caused by zoospore producing oomycetes.
2.13 PGPR in stress agriculture

Agricultural crops are exposed to many stresses that are induced by both biotic and abiotic factors. These stresses invariably affect plant growth and crop yield depending on the type and intensity of stress. Under stress conditions, such as salinity, drought, waterlogging, heavy metals and pathogenicity, the production of ethylene in plants at substantially accelerated rates is a very common feature, which adversely affects the root growth and the development of the plant. Recently, several authors have documented profound effects of inoculation with PGPR on plant growth under stress conditions.

*P. mendocina* inoculated plants showed significantly greater shoot biomass, higher K concentration of foliar than the control plants at low and high salinity levels (Kohler *et al.*, 2009). Inoculation with *Pseudomonas* sp containing ACC deaminase significantly improved fresh biomass under water-deficient field conditions (Zahir *et al.*, 2008). Inoculation with *Pseudomonas* sp counteracted the Cd-induced inhibition of nutrient uptake by roots in pea plant (Safronova *et al.*, 2006). Inoculation with *P. asplenii* resulted in normal plant growth under high levels of Cu$^{2+}$ (Reed *et al.*, 2005).

2.14 Bioformulation

The use of antagonist with different mode of action may improve biocontrol efficacy under wide range of environmental conditions (Grosch *et al.*, 2011), however the success of biological control of plant diseases depends upon the availability of effective formulations of biocontrol agents, survivals as well as rapid multiplication during storage and colonization after inoculation (Ashofteh *et al.*, 2009). Ardakani *et al.* (2010) results indicate that organic and inorganic carriers may efficiently be used for improving the
stability and effectiveness of biocontrol-active microorganisms in controlling plant diseases.

Carrier material formulations are widely used in biological control such as a solid form (granules), flour and suspension (Ardakani et al., 2009). Jayaraj et al. (2005) showed that use of talc or bentonite combined with bacterial antagonist may improve its efficacy in suppressing tomato damping off disease. Anitha and Rabeeth (2009) pointed out that talc-based formulation were also stable up to 3 month with cell viability of $122 \times 10^7$ cfu/g.

Suryadi et al. (2013) showed that talc-A8 based formulation was stable at period of storage showing no viability lost. Talc-A5 (B. firmus E65, P. aeruginosa C32b) formulation was effective against sheath and bacterial leaf blight but showed lower effect on neck blast disease in the field. Shelf life evaluation of P. fluorescens PfT-8 strain in seven different carrier formulations (lignite-based powder, talc-based powder, peat based powder, lignite + fly ash-based powder, wettable powder, bentonite-paste and polyethylene glycol at room temperature showed peat, lignite, lignite + fly-ash and bentonite paste based formulations maintained higher propagule number than others and also showed greater biocontrol potential (Jayaraj et al., 2007).

P. fluorescens strain 134 delivered as both dry and liquid formulations was able to colonize cotton root at a population density of about $10^8$ CFU/g fresh root, 15 days after sowing (Russo et al., 2005). Seed treatment, followed by foliar application of talc formulated bio-control agent triggered systemic resistance in watermelon. In response to ISR, the activities of defense related enzymes viz., phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and $\beta$-1-3-glucanase were enhanced and the accumulation of phenols were also noticed in the watermelon upon challenge inoculation.
with *A. alternata*, the causal agent for leaf blight in watermelon (Chandrasekharan *et al.*, 2009).

Gandhi and Saravanakumar (2009) found that vermicompost carrier prepared from vegetable waste was found to be better carrier for bioinoculants like *A. lipoferum*, *B. megaterium* and *P. fluorescens*. Bharathi *et al.* (2004) in evaluating the efficacy of 13 PGPR strains against chilli fruit rot and dieback incited by *C. capsici* observed that *P. fluorescens* pf1 and *B. subtilis* were effective in increasing seed germination and seedling vigor, and that a mixed bioformulation (pf1+*B. subtilis*+neem+chitin) was effective in reducing fruit rot incidence of chilli besides increasing plant growth and yield under both greenhouse and field conditions.

Peat formulation supported the survival of both *P. fluorescens* Pf 51 and *B. subtilis* B45 up to 270 days with a viable population of $4.3 \times 10^7$ cfu/ g and $6.2 \times 10^7$ cfu/ g respectively. Combined application (rhizome bacterization and soil application) of antagonists resulted in 54.0% reduction in rhizome rot over control as compared to single method such as rhizome bacterization (43.0%) or soil application (39.0%) (Sivakumar *et al.*, 2012). Ramamoorthy and Samiyappan (2001) reported that seed and soil application of talc based bioformulation of *P. fluorescens* pf1 effectively reduced disease incidence under greenhouse conditions.

### 2.15 Effect of PGPR on plant growth and yield

The potential of PGPR for improving growth and yields of various crops has been extensively documented (Table 2.5). However, most of the studies have been conducted
Table 2.5. Plant crop response to PGPR inoculation under different experimental conditions

<table>
<thead>
<tr>
<th>Plant</th>
<th>PGPR inoculant</th>
<th>Plant growth parameter</th>
<th>Assay condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichoke</td>
<td><em>P. putida</em></td>
<td>Increase in radical and shoot length, shoot weight, seedling vigour index</td>
<td><em>In vitro</em></td>
<td>Jahanian <em>et al.</em>, (2012)</td>
</tr>
<tr>
<td>Mustard plant</td>
<td><em>Pseudomonas</em> sp. A3R3</td>
<td>Increase in biomass</td>
<td>Pot</td>
<td>Ma <em>et al.</em>, (2011)</td>
</tr>
<tr>
<td>Green gram</td>
<td><em>Pseudomonas</em> sp. PS1</td>
<td>Increase in plant dry weight, nodule number, root and shoot P nitrogen, root and shoot</td>
<td>Pot</td>
<td>Ahemad and Khan (2012b)</td>
</tr>
<tr>
<td>Maize</td>
<td><em>P. putida</em> R-168</td>
<td>Increase in plant height, seed weight, shoot dry weight</td>
<td>Field</td>
<td>Gholami <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>Chickpea</td>
<td><em>Pseudomonas</em> sp.</td>
<td>Enhanced fresh and dry weight of plant even at 2Mm nickel concentration</td>
<td>Pot</td>
<td>Tank and Saraf (2009)</td>
</tr>
<tr>
<td>Oat</td>
<td><em>Pseudomonas</em> sp. (ChO9)</td>
<td>Root length, Root area, Shoot dry weight, Total N</td>
<td>Green house</td>
<td>Yao <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Indian mustard,</td>
<td><em>P. aeruginosa</em></td>
<td>Stimulated plant growth and reduced Cd uptake</td>
<td>Pot</td>
<td>Sinha and Mukherjee (2008)</td>
</tr>
<tr>
<td>Pumpkin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato, Okra</td>
<td><em>P. aeruginosa</em></td>
<td>Increase in dry biomass</td>
<td>Green house</td>
<td>Adesemoye <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Sweet cherry cv. 0900</td>
<td><em>Pseudomonas</em> BA-8</td>
<td>Fruit weight, fruit diameter, shoot length, shoot diameter</td>
<td>Field</td>
<td>Esitken <em>et al.</em>, 2006</td>
</tr>
</tbody>
</table>
under controlled environments rather than under natural field conditions. Success of biological control depends on the mode and dose of application. In fields it is usually not economically feasible to apply the BCA evenly at the dose needed for efficacy. In non-sterile soils where there is competition with the resident flora and predation by protozoa and nematodes, bacterial populations declines rapidly by orders of magnitude per week until the population reaches equilibrium with its environment which accounts for inconsistent performance that are observed in lab and greenhouse studies of PGPR inoculation are much more (Shaharoona et al., 2008).

Seed coating is seen as the best approach, as it enables the biological control agent to be positioned close to the seedlings and good colonization of the rhizosphere of the plant can be expected (Bennett & Whipps, 2008). The success of biological control depends on the activity of the BCA at the root level, in the rhizosphere. Rhizosphere competence is a criterion that must be taken into account early during the screening process.

Praveen Kumar et al. (2012) showed seed bacterization of sorghum with Pseudomonas sp. P17 strain enhanced the uptake of essential macro and micro-nutrients resulting in overall increase of plant growth. Kourosh et al. (2011) reported enhanced uptake of nutrients in black pepper and sweet basil due to seed bacterization with Pseudomonas spp.

Shaterabadi et al. (2011) showed that double inoculation (seed inoculation + spray) was the most effective application method and increased dry forage yield by 39.79% and 70.85% compared with seed inoculation and spray, respectively. Ngullie et al.
(2010) revealed that spraying with *P. fluorescens* was effective against *C. gloeosporioides* against fruit rot disease of chilli in field experiment.

Jeger and Jeffries (1988) also stressed the possibilities of biological control of post-harvest fruit diseases by using *P. fluorescens*. Intanoo and Chamswarng (2007) reported that antagonistic bacterial strains (DGg13 and BB133) effectively controlled *C. capsici*, the major anthracnose pathogen in Thailand.

Tariq *et al.* (2009) reported that *P. aeruginosa* strains showed positive impact on plant growth by increasing the plant height and fresh shoot weight and were found to produce indole-acetic acid at varying degree.

Akhtar and Siddiqui (2009) reported that phosphate solubilising microorganism (*P. putida*, *P. aeruginosa* [Pa28], *P. alcaligenes*) and *Rhizobium* sp. improved the growth, nodulation, yield and reduced root-rot disease complex of chickpea under field condition.

Saravanakumar *et al.* (2007) reported that among the bioformulations tested, foliar application of *P. fluorescens* Pf1 at 7-day intervals consistently reduced the disease incidence of blister blight for two seasons, almost comparable with that of chemical fungicide. In addition to disease control, it also increased tea yield significantly compared to the untreated control. This finding revealed the probable influence of plant growth promotion and induced systemic resistance (ISR) in enhancing the disease resistance in tea plants against blister disease by PGPR bioformulations.

Ekbote (2005) reported that treatment of 40 day old chilli seedlings with *P. fluorescens* solution (1%) reduced the incidence of dieback and fruit rot and increased the yield of chili compared to the control. Kloeper *et al.*, (2004) observed that the PGPR
(plant growth-promoting rhizobacteria) are root-colonizing bacteria that benefit plants by increasing plant growth or reducing disease.

According to Hegde et al. (2002) *P. fluorescens* showed higher antagonistic activity against *C. capsici* under *in vitro* conditions. Under greenhouse conditions *P. fluorescens* sprayed plants showed significantly less mortality compared control plants.

### 2.16 Integrated pest management

Over use or over dependence on chemical control or any other single control method is not sufficient to manage chilli pathogens *C. gloeosporioides* and *R. solani*. A systemic control approach uniting all disease management options may produce better pathogen management. Integrated disease management is a broad-based ecological plant pathogen control approach, combining all the available disease control methods with each method compensating the deficiencies of others (Kumar *et al.*, 2009). It reduces the emphasis on fungicides by including other disease control methods. Integrated disease management is an environmentally sensitive approach and is gaining popularity worldwide.

Khan and Gangopadhyay (2008) reported that carboxin, chlorothalonil and carbendazim were least toxic to *P. fluorescens* strain PFBC-25, while captan was inhibitory *P. fluorescens* strain PFBC-25. Laha and Venkataraman (2001) also reported the compatibility of *P. fluorescens* with carbendazim while studying sheath blight management in rice.

Sultana *et al.*, (2006) reported that an application of *P. aeruginosa*, a plant growth promoting rhizobacterium alone or with crustacean chitin, fungicides (benlate/captan) or
*Paecilomyces lilacinus* (a biocontrol agent) significantly suppressed *M. phaseolina*, *R. solani*, *F. oxysporum* and *F. solani* attacking roots of chilli.

In another study, Khorshidi *et al.* (2011) showed that application of fertilizers with *P. fluorescens* and *Azospirillum lipoferum* had a significant effect on rice yield in Iran. In two field trials, Pf1 tested in combination with azoxystrobin was highly efficient in management of *C. capsici* and *L. taurica* disease of chilli. Combination of the biological control agent Pf1 with reduced concentration of fungicide azoxystrobin was as effective as the standard fungicide alone. Application of *P. fluorescens* along with azoxystrobin significantly increased the survival of Pf1 in the phylloplane of chilli (Anand *et al.*, 2010).

Malathi *et al.* (2002) study on compatibility of biocontrol agent *P. fluorescens* with fungicide thiophanate methyl showed maximum plant survival of 81.67%.

*P. fluorescence* Pf4 isolate showing high efficacy in biocontrol and growth promotion maintained maximum shelf-life viability in talc formulation at room and refrigerated temperature up to 360 days of storage. *P. fluorescens* Pf4 showed compatibility with carbendazim and thiram fungicides when used as an integral component for management of insect pests in irrigated chilli ecosystem, increased the yield 5000 kg more in fungicides and *P. fluorescence* Pf4 integrated plots compared to non integrated plots (Patil *et al.*, 2013). Kumar *et al.* (2008b) reported the compatibility of *P. fluorescens* with imidachloprid and carbofuran.

Naik *et al.* (2013) reported that foliar spray and seed treatment of *P. fluorescens* and *T. viride* consortia with fungicides (carbendazim, hexaconazole and propiconazole) reduced pesticide sprays in chilli thereby reducing 30 % cost of protection.