Chapter 1
1.1 Introduction

In India, agriculture contributes 21% to our national GDP and over 100 million families of farmers manage 142 mha. Nearly 22% of our population is still living below poverty line. Agriculture needs special attention to sustain our national economy and alleviate rural poverty. There is an urgent need to get over the constraint of current low yield levels of crops. The available land in India as of 2010 being 169 mha is expected to go down to 100 mha by 2020. Thus the priority is to increase the crop yield/ha considerably to meet the food requirements of our ever growing population. This is possible only with the adoption of modern technology in agriculture.

Varied agro-climatic conditions in India make it possible to grow a wide variety of vegetable crops all the year round in one part of the country or another. India can claim to grow the largest number of vegetable crops compared to any other country of the world and as many as 61 annual and 4 perennial vegetable crops are commercially cultivated. Some of the important vegetable crops grown are: tomato, chilli, capsicum, brinjal, cabbage, cauliflower, knolkhool, onion, garlic, okra, longmelon, muskmelon, snapmelon, watermelon, cucumber, pumpkin, summer squash, bitter gourd, bottle gourd, pointed gourd (parwal), ridge gourd, round gourd, snake gourd, sponge gourd, wax gourd (ash gourd), carrot, radish, turnip, broad bean, cluster bean, cowpea, dolichos bean, french bean, peas, amaranthus, fenugreek, spinach, lettuce, drumstick, curry leaf, agathi, etc. India is the second largest producer of vegetables in the world constituting around 11 to 12% next only to China. It is estimated that the area under vegetables would be around 4.5 million hectares with the production approximately 45 million tonnes. But the per capita consumption is very low working out only 120g.

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop next to potato grown around the world. Easy adaptability to a wide ranges of climatic and soil conditions enable its worldwide cultivation. Present world production is about 100 million tonnes fresh fruit produced on 3.7 mha. Tomato production has been reported from 144 countries (FAOSTAT Database, 2004). An estimated area of tomato production across the world is about 46,43,957 Ha and production is about 12,99,42,416 tonnes and the average yield is about 2,79,809 Hg/ha (FAOSTAT Database, 2010). The top four leading fruit-producing countries are the China, United States, Turkey and India. The world production of tomato sums up to 129 mt during 2009.
and is cultivated on approximately 85 mha of land. China the largest producer and accounted for about one quarter of the global output followed by United States and Turkey, India stands at 4th position in the global tomato production of the world. In India, Bihar is at the leading position followed by Uttar Pradesh and Orissa in terms of area under tomato crop. The maximum production of tomato occurs in Uttar Pradesh followed by Karnataka, Punjab, West Bengal and Assam (Science reporter, 2010).

Table 1.1. Tomato production around the world and in India during 2010

<table>
<thead>
<tr>
<th>Country</th>
<th>Area harvested (Ha)</th>
<th>Yield (hg/Ha)</th>
<th>Production (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>619800</td>
<td>193283</td>
<td>11979700</td>
</tr>
<tr>
<td>World</td>
<td>4643957</td>
<td>279809</td>
<td>129942416</td>
</tr>
</tbody>
</table>

In India, the estimated area and production of tomato is about 599100 Ha and about 1,11,48,800 tonnes, respectively. The average productivity (yield) of tomato in our country is merely 1, 86,092 Hg/Ha. India stands at fourth position in tomato producing countries of the world. Bihar is at the leading position followed by Uttar Pradesh and Orissa in terms of area under tomato crop. The maximum production of tomato occurs in Uttar Pradesh followed by Karnataka, Punjab, West Bengal and Assam (Science report, 2010). In Karnataka, Kolar is at the leading position in tomato production. Total tomato production in Karnataka is estimated about 2,65,094 tonnes which was grown in area of 31,261 hectares with an average yield of 8480 kg Ha⁻¹ (Directorate of Economics and Statics, Bangalore, 2010).

In Karnataka, tomato is mainly grown in southern districts in an area of 24 thousand hectares with an annual production of 0.3 million tonnes with an average yield of 11,386 kg/ha. This crop is grown in three seasons Kharif, Rabi and summer, with more than 60 % of the total crop in the kharif.
1.2 Tomato

Tomato is classified under,
Kingdom : Plantae
Division : Spermatophyta
Order : Solanales
Family : Solanaceae
Genus : Lycopersicon
Species : Lycopersicon esculentum Mill.

Tomato (Lycopersicon esculentum Mill.) is a vital component of our daily food which we are consuming regularly in different forms. The tomato is native to South America having a secondary centre of origin in highlands of Peru. Tomato is now grown worldwide for its edible fruits with thousands of cultivars having been selected with varying fruit types. Tomato is an annual herbaceous crop and it appears rich in diversity for size, shape, colour and quality.

The common names for the tomato are: Tomato (India), tomate (Spain, France), tomat (Indonesia), faanke’e (China), tomati (West Africa), tomatl (Nahuatl), jitomate (Mexico), pomodoro (Italy), nyanya (Swahili) (Hammerschmidt, D; M Franklin (2005)).

1.3 Agronomical conditions for the cultivation

Tomato is suitable for growing in tropical, subtropical and temperate regions of India and it requires a long, warm growing season for successful production. Loamy soil with little sand and temperature between 24-31°C is desirable for the high production. The pH of the soil should be six to seven. Tomato is being grown in all the three seasons in India viz., summer, Kharif and rabi season.

1.4 Temperature and light

Tomato requires a relatively cool, dry climate for high yield and premium quality. However it is adapted to a wide range of climatic conditions from temperate to hot and humid tropical. The plants can survive a range of temperatures, but the plant tissues are damaged below 10°C and above 38°C.
1.5 Plant structure

Tomato is an herbaceous, usually sprawling plant in the *Solanaceae* or night shade family that is typically cultivated for the purpose of harvesting its fruits for human consumption. Tomato plants typically reach 1-3 meters in height, and have a weak, woody stem that often vines on other plants. The leaves are 10-25 cm long, odd pinnate, with 5-9 leaflets on petioles, each leaflet up to 8 cm long, with a serrated margin, and both the stem and leaves are densely glandular-hairy. The flowers are across, yellow, with five pointed lobes on the corolla, they are borne in a cyme of 3-12 together. The fruit is a berry with fleshy placenta and many small kidney shaped seeds covered with short stiff hairs. Tomato is used as salad, conserves, pickles, ketchup, sauces, soup and other products. It is a perennial often grown outdoors in temperate climates as an annual.

1.6 Cultivation practices

The land is prepared for transplanting to a fine tilt by plugging and three to four harrowing followed by leveling. Seed beds should be prepared of about 18-20 cm high from the ground level, 200 cm wide rows two-four cm apart. Twenty five such beds will raise enough seedlings to plant one hectare.

Twenty five tonnes of well-decomposed farmyard manure may be applied at the time of soil preparation. At the time of transplanting 175-300 kg of superphosphate, 175-200 kg of ammonium sulphate, 100-175 kg of potassium sulphate should be applied per hectare. Top dressing will be done 15 days after transplanting and the second at flowering time with fertilizers. Tomato seedlings, when 7.5-10 cm in height should be transplanted during evening time and irrigated immediately. Spacing of tomato seedlings will be about 75×60 cm between rows and plants.

Fort night irrigation during winter and weekly during summer should be followed for tomato cultivation. Two to three times weeding is necessary to keep the field free from weeds and off-type plants will be roughed. Harvesting will be done when fruits are fully developed or depending upon the purpose for which they are growing.
Table 1.2. Nutritional quality of Tomato fruit

<table>
<thead>
<tr>
<th>Constituents Gross constituents (g)</th>
<th>Contents (Per 100 g edible portion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>93.76 g</td>
</tr>
<tr>
<td>Fat</td>
<td>0.33 g</td>
</tr>
<tr>
<td>Protein</td>
<td>0.85 g</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>1.1 g</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.9 g</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Minerals (mg)</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>223 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>24 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>11 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>6 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.2 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>5mg</td>
</tr>
<tr>
<td>Organic acids (g)</td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.43 g</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.08g</td>
</tr>
<tr>
<td>Vitamins (mg)</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>19 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>6231 U</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.38 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.628 mg</td>
</tr>
<tr>
<td>Thiamin</td>
<td>10mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>100mg</td>
</tr>
</tbody>
</table>

(Source: USDA Nutrient Base, 2008)

1.7 Planting schedules

A two crop season is the common practice in the northern latitudes in order to avoid the cold low light intensity months (January and February). Plants are set in late August and harvesting of fruit continuous through December, and the plants are set in March and harvesting of fruit continuous into June or July. In the lower latitudes where the period of cold and light intensity is less, a single crop is commonly used with plants set in September and the crop is carried through until June or July. Variations of these various planting schedules are practiced based on consumer demand and price obtained for fruit.
1.8 Nutritional value

Tomatoes constitute an excellent food and as a natural diet. It is considered as a protective food because of its nutritive value. Tomatoes contain a series of elements very appropriate to detoxify the organism and to prevent the appearance of many illnesses. The first of them is lycopene, a component to which owes its red colour, has anticancerous properties; lycopene seems to reduce the probabilities of many cancers such as those affecting the prostrate, lungs, stomach, bladder and uterus (Barone 2003). Tomato is rich in Vitamin A and C, which are beneficial components to detoxify the organism. It is a very rich source of potassium, calcium, minerals that maintain good state of nerves, heart and the muscles (Frusciante et al., 2007).

1.9 Diseases

Vegetables are threatened by both non-infectious and infectious diseases. Non-infectious disorders are caused by extremes of light, temperature, soil moisture, soil pH and nutritional imbalances. Unfavourable oxygen relations, atmospheric pollutants and damage due to herbicides and other pesticides. Bacteria, fungi, viruses, nematodes and insects cause infectious disorders.

Diseases are often a factor that limits production. Worldwide estimates of losses of fruits and vegetables have ranged as high as 50%. Despite the most sophisticated marketing system in the world, the United States still suffers enormous losses in the marketing of horticultural crops.

Among various disease causing organisms, seed-borne fungi are posing a major problem to vegetables cultivation than any other group of pathogens. Seed-borne fungi not only reduce the germination and vigour of the seedlings but may also act as a source of inoculums for development of disease in the field. Laying down the health standards against seed-borne diseases of vegetables needs information on mycoflora associated with the seed, their nature and the extent of damage in natural as well as in controlled conditions. Seed industries are now interested in populizing the vegetables by introducing hybrids.

Several fungi found on the market seeds of vegetables are known to cause considerable damage either directly to the seeds that carry them to the crops that are raised from contaminated seed blocks. The nature of damage consists of total failure of germination of poor development
of crops. Some seed-borne fungi of vegetables cause drastic reduction in germination and seedling vigour, seeds collected from the infected fruits are carriers of diverse saprophytic fungi compared with the seeds of healthy fruits which have decreased level of mycoflora.

1.10 Diseases of tomato

Plant diseases become limiting factor in tomato production in many parts of world. Many diseases affect the plants which belong to the Solanaceous family.

1.10.1 Fusarium wilt

Soil and seed borne pathogens are important biotic constraints in sustainable crop production systems because the complexity of the soil environment makes their control with chemical difficult. Many crops throughout the world are host plants for Fusarium species causing soil-borne diseases of great economic importance. Fusarium wilts of tomato caused by Fusarium oxysporum f. sp. lycopersici (Sacc.) Synder and Hans occur worldwide and lead to high losses of tomatoes. It is primarily a soil inhabiting pathogen, once introduced, it remains in the soil for very long time and attacks tomato at all stages of its growth starting from nursery to fruiting. In the nurseries, it causes rotting of germinating seeds as pre-emergence damping off, rotting at collar region and dyeing of young seedlings as post-emergence damping off (Varma, 1954). This pathogen invades through wounds on roots. Infected plants become stunted, chlorotic and wilt (Jones et al., 1991). Wilting is a result of impaired water translocation due to clogging of vessels by mycelium, spores, gels, gums and tylosis. The fungus also produces toxins such as fusaric acid and lycoparasmin which chelate with iron and cause iron deficiency in plants and consequently yellowing of leaves (Gaumann, 1951).
Table 1.3. Major diseases of Tomato

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Causal organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungal diseases</strong></td>
<td></td>
</tr>
<tr>
<td>Alternaria stem canker</td>
<td><em>Alternaria alternata f. sp. lycopersici</em></td>
</tr>
<tr>
<td>Anthracnose</td>
<td><em>Colletotrichum coccodes, Colletotrichum dematium, Colletotrichum gleosporoides</em></td>
</tr>
<tr>
<td>Didymella stem rot</td>
<td><em>Didymella lycopersici</em></td>
</tr>
<tr>
<td>Early blight</td>
<td><em>Alternaria solani</em></td>
</tr>
<tr>
<td>Fusarium wilt</td>
<td><em>Fusarium oxysporum f. sp. lycopersici</em></td>
</tr>
<tr>
<td>Powdery mildew</td>
<td><em>Leveillula laurica</em></td>
</tr>
<tr>
<td>Rhizoctonia damping-off and fruit rot</td>
<td><em>Rhizoctonia solani,,Thanatephorus cucumeris</em></td>
</tr>
<tr>
<td>Verticillium wilt</td>
<td><em>Verticillium dahlia, Verticillium albo-atrum</em></td>
</tr>
<tr>
<td><strong>Bacterial diseases</strong></td>
<td></td>
</tr>
<tr>
<td>Bacterial canker</td>
<td><em>Clavibacter michignesis sub sp. Michiganesis</em></td>
</tr>
<tr>
<td>Bacterial wilt</td>
<td><em>Ralstonia solanacearum</em></td>
</tr>
<tr>
<td>Bacterial spot</td>
<td><em>Xanthomonas campestris pv. vesicatotia</em></td>
</tr>
<tr>
<td>Bacterial speck</td>
<td><em>Xanthomonas syringe pv.tomato</em></td>
</tr>
<tr>
<td><strong>Viral diseases</strong></td>
<td></td>
</tr>
<tr>
<td>Common mosaic of tomato</td>
<td>Tomato mosaic virus</td>
</tr>
<tr>
<td>Tomato mosaic</td>
<td>Tomato mosaic virus</td>
</tr>
<tr>
<td>Tomato mottle</td>
<td>Tomato mottle Gemini virus</td>
</tr>
<tr>
<td>Tomato necrosis</td>
<td>Alfa alfa mosaic virus</td>
</tr>
<tr>
<td><strong>Nematode diseases</strong></td>
<td></td>
</tr>
<tr>
<td>Root-knot</td>
<td><em>Meloidogyne spp.</em></td>
</tr>
</tbody>
</table>

(Source: http://www.apsnet.org)
1.10.2 Symptoms

*Fusarium oxysporum* and its various formae speciales have been characterized as causing the following symptoms: vascular wilt, yellows, corm rot, root rot, and damping-off. The most important of these is vascular wilt. The vascular wilt-causing *Fusarium oxysporum* is the most important species (Agrios, 1988; Smith *et al*., 1988). Strains that are rather poorly specialized may induce yellows, rot, and damping-off, rather than the more severe vascular wilt (Smith *et al*., 1988).

Since fusarium wilt is the most important disease caused by *F. oxysporum*, the focus of this section will be on this symptom. In general, fusarium wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988). Browning of the vascular tissue is strong evidence of fusarium wilt. If the main stem is cut, dark brown streaks may be seen running lengthwise through the stem. This discolouration often extends far up the stem and is especially noticeable in a petiole scar. The browning of the vascular system is characteristic of the disease and generally can be used for its identification (Cerkauskas, 2005). Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Smith *et al*., 1988).

1.10.3 *Fusarium oxysporum* f. sp. *lycopersici* Schlect. Emend. Synder and Hansen

*Fusarium oxysporum* produces sparse to abundant growth, covering part or whole part of seed. Mycelium can be white to cream coloured. Slimy masses of conidia which are seen along the hyphae are characteristic of the species. Pale orange, very slimy pionnotes, full of macroconidia can also be produced. Microconidia are generally produced in abundance, they vary a lot in size and are oval shaped, elliptical or reiform, usually non septate but 1 septate conidia may be found. Microconidia can also be formed in false-heads on short monophialides. Macroconidia are hyaline, thin walled, 3-5 septate, falcate to almost straight. Chlamydospores intercalary or terminal on short lateral branches (Mathur and Kongsdal, 2003).
Fusarium oxysporum f. sp. lycopersici is classified under,

Kingdom :  Fungi  
Division :  Ascomycota  
Class :  Sodarimycetes  
Order :  Hypocreales  
Family :  Nectriaceae  
Genus :  Fusarium  
Species :  Fusarium oxysporum f.sp. lycopersici

1.1.0.4 Distribution

Overall, the distribution of Fusarium oxysporum is known to be cosmopolitan. However, the different special forms (f.sp.) of F. oxysporum often have varying degrees of distribution.

1.1.0.5 Biology

In solid media culture, such as potato dextrose agar (PDA), the different special forms of F. oxysporum can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colours - ranging from violet to dark purple - according to the strain (or special form) of F. oxysporum. If sporodochia are abundant, the culture may appear cream or orange in colour (Smith et al., 1988).

F. oxysporum produces three types of asexual spores: microconidia, microconidia, and Chlamydospores (Agrios, 1988). Microconidia are one or two celled, and are the type of spore most abundantly and frequently produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macroconidia are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochia like groups. Chlamydospores are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in microconidia. These spores are either one or two celled (Agrios, 1988).
1.10.6 Disease cycle

*F. oxysporum* is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al.*, 1988). Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium, or as any of its three different spore types (Agrios, 1988).

Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant either with its sporangial germ tube or mycelium by invading the plant's roots. The roots can be infected directly through the root tips, through wounds in the roots, or at the formation point of lateral roots (Agrios, 1988). Once inside the plant, the mycelium grows through the root cortex intercellulary. When the mycelium reaches the xylem, it invades the vessels through the xylem's pits. At this point, the mycelium remains in the vessels, where it usually advances upwards toward the stem and crown of the plant. As it grows the mycelium branches and produces microconidia, which are carried upward within the vessel by way of the plant's sap stream. When the microconidia germinate, the mycelium can penetrate the upper wall of the xylem vessel, enabling more microconidia to be produced in the next vessel. The fungus can also advance laterally as the mycelium penetrates the adjacent xylem vessels through the xylem pits (Agrios, 1988).

Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly (Agrios, 1988). The resulting spores can then be used as new inoculum for further spread of the fungus.

1.10.7 Epidemiology

*F. oxysporum* is primarily spread over short distances by irrigation water and contaminated farm equipment. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and
contaminate its seed, the spread of the fungus by way of the seed is very rare (Agrios, 1988). It is also possible that the spores are spread by wind.

1.11 Management

Since 1960s, the public became aware of environmental pollution and increasing amounts of toxic chemicals in nature’s food chains. Earlier public opinion was for the cheap and rapid control of pathogens and insect pests by chemicals, and then they moved towards the opposite extreme of imposing serious restrictions on their use. Most pesticides have only a temporary effect and therefore, require repeated applications.

Use of resistant cultivars in the management of disease does not have long time success due to appearance of new races of the pathogen that overcome plant resistance (Beckman, 1987). The increasing resistant on the use of chemical pesticides associated with high cost and environmental hazards encourage alternative strategies of disease control. Currently many fungicides such as, benomyl, thiram, thiabendazole, carbendazim are used to manage this fungus. But these fungicides adversely affect the useful soil microorganisms and environment. Soil fumigates such as methyl bromide (bromo methane) that have a broad spectrum of activity have been used extensively to protect high-value crops from soil-borne pathogens. Scientific assessment of Ozone depletion by the world meteorological organization (1998) has assigned methyl bromide an ozone-depleting substance. Crop-wise market share of pesticide usage in India indicates highest use pattern to the extent of 45% in cotton followed by 22% in rice, 9% in vegetables, 7% in plantations, 4% each in wheat and pulses, and 9% constituting others. Compared with usage of chemical pesticides, biopesticides constitute around 2% in the country.

Therefore, it is imperative to develop a holistic system of tackling pests to make it more eco-friendly, economically viable and socially acceptable for the farmers. In this regard to tackle the pests and diseases of major crops, integrated management practices have been adopted since 1985 and in the recent years biotechnological approaches have been more perceptible. Use of bio-control agents and bio-pesticides are increasingly gaining acceptance with farmers. The beneficial and eco-friendly microorganisms like fungi, bacteria, virus and protozoan’s capable of killing specific disease causing microbes, nematodes and insect pests and also those promoting plant growth are being considered as potential biological alternative in eco-friendly agriculture.
Bio-pesticides are likely to have a greater impact on pesticide sector. Presently, bio-pesticides represent approximately 4.5% of the world insecticide sales. The growth rate for biopesticides over the next ten years has been forecasted at 10-15% per annum.

Biological control of seed-borne vegetable diseases can offer a potential alternative to chemical fungicides. Suppression of root diseases by Plant growth promoting rhizobacteria (PGPR) is attributed to its ability to colonize the host root, compete for space and nutrition and inhibit the growth of the fungus by producing antibiotics or siderophores. Apart from a direct antagonistic effect on soil-borne pathogens, some PGPR strains are also able to reduce disease in above-ground plant parts through ISR. PGPR-mediated ISR has been demonstrated in many plant species, e.g. Bean, Carnation, Cucumber, Radish, Tobacco, Tomato and the model plant Arabidopsis, against fungi, bacteria and viruses (Van Loon et al., 1998). Biological control often works by gentler, more suitable methods by suppressing the pathogen (Cook and Baker, 1983).

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, support large and metabolically active groups of bacteria (Villacieros et al., 2003) are known as PLANT GROWTH PROMOTING RHIZOBACTERIA (Kleopper et al., 1980). These PGPR are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Kleopper, 1994; Glick, 1995; Cleyet et al., 2001). Specific mechanisms involved in pathogen suppression by PGPR vary and include antibiotic production, substrate competition, and induced systemic resistance in the host (Van loon et al., 1998). Growth promotion results mainly from suppressing soil-borne pathogens and other deleterious micro-organism (Schippers et al., 1987), but direct effects on plant growth have also been reported (Lynch, 1976; Van Peer and Schippers, 1989), includes the provision of bioavailable phosphorous for plant uptake, nitrogen fixation, for plant use sequestration of iron for plants by siderophores, production of plant hormones like auxins, cytokinins and gibberlins and lowering of plant ethylene levels (Glick, 1995; Glick et al., 1999). PGPR activity has been reported in strains belonging to several other genera, such as Azotobacter, Anthrobacter, Bacillus, Clostridium, Hydrogenophaga, Enterobacter, Serratia and Azospirillum (Rovira 1969; Kleopper et al., 1986 and 1989). There are several reports that PGPR have promoted the growth and reproductive parameters of plants ranging from cereals, pulses, ornamentals, vegetable crops, plantation crops and even in tree species. Treatment with PGPR has increased germination
percentage, seedling vigour, emergence, plant stand and root growth, shoots growth, total biomass of the plants, seed weight, early flowering, increased grain, fodder, fruit yields etc. (Vanloon et al., 1998; Ramamoorthy et al., 2001a; Nezarat and Gholami, 2009). Bacteria with additional traits of producing antibiotics, siderophores, HCN, phytohormones and phosphate solubilizing activity are known to serve as substitute for chemical fertilizers and pesticides (Pandey et al., 2005). Naturally occurring rhizosphere microbial community plays an important role in improvement of root health and biological control of plant pathogens. Particularly fluorescent pseudomonad and rhizobia have revolutionized the field of biocontrol of soil borne plant pathogenic fungi (Arora et al., 2001; Gupta et al., 2002).

The rhizosphere is the front-line between plant roots and soil-borne pests. Therefore it seems logical that microorganisms that colonize the same niche could be ideal candidates for sustainable agriculture (Weller, 1988). In the rhizosphere, bacteria are the most abundant microorganisms (Antoun & Prévost, 2006). Rhizobacteria are rhizosphere-competent bacteria that aggressively colonize plant roots; they are able to multiply and colonize all the ecological niches found on the roots at all stages of plant growth, in the presence of a competing microflora (Antoun & Kloepper, 2001). Rhizobacteria can have a neutral, detrimental or beneficial effect on plant growth. Deleterious rhizobacteria are presumed to adversely affect plant growth and development through the production of undesirable metabolites (phytotoxins) or through competition for nutrients or inhibition of the beneficial effects of mycorrhizae (Sturz & Christie, 2003).

The PGPR are defined by three intrinsic characteristics (Barea et al., 2005): (i) they must be able to colonize the root, (ii) they must survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities, and (iii) they must promote plant growth. The PGPR are known to participate in many important ecosystem processes. They were first used for agricultural purposes in the former Soviet Union and India and are now being tested worldwide (Lucy et al., 2004).

Beneficial free living soil bacteria that enhance the plant growth are usually referred as plant growth promoting rhizobacteria (PGPR) (Kleopper et al., 1989) or yield increasing bacteria (YIB). PGPR, originally defined (Kleopper and Schrath, 1978) as root colonizing bacteria
(rhizobacteria), cause either growth promotion or biological control of plant diseases. The large scale application of PGPRs to crop as inoculants would be attractive as it would substantially reduce the use of chemical fertilizers and pesticides, which pollute the environment. In addition, the application of PGPRs would increase crop yield, thereby helping to feed the growing world population. The ways by which PGPR can influence plant growth directly may differ from species to species as well as strains to strains. The beneficial bacteria, i.e., plant growth promoting rhizobacteria (PGPR), are widely studied by microbiologists and agronomists because of their potential in plant production (Barea et al., 2004).

Though, by definition PGPR are free-living plant associated bacteria, few Rhizobium that colonize the roots of non-legume plants such as Gramineae and crucifers and promote root growth by mechanisms other than biological N\textsubscript{2} fixation, are also considered as PGPR (Antoun et al., 1998). Subsequent experiments also demonstrated that potential application of root endophytes as PGPR (Vessy, 2003).

1.12 Mechanisms of action: Overview of PGPR traits

Figure 1.1 Interaction of biocontrol plant growth promoting rhizobacteria, plant, pathogens and soil. These elements interact with one another through biotic and abiotic signals. Many of these elements still unknown.
1.12.1 Phytohormones

Plant growth stimulating phytohormones produced by PGPR within the root zone stimulate the density and length of root hairs. The increase in root surface area improves the plant uptake potential of water and mineral nutrients from a large volume of soil (Volkmar and Bremar, 1998). If such rhizobacteria also possess the ability to solubilize the complex form of nutrients in rhizosphere to simple forms that can be utilized by plants, such plants have the advantage of growing well and being healthy. Indole acetic acid (IAA) is a quantitatively important phytohormone produced by PGPR, and treatment with auxin-producing rhizobacteria increased the plant growth (Vessy, 2003). A positive correlation was observed between L-tryptophan dependent auxin production by different PGPR strains and their ability to increase the grain yield, and number of branches and pods per plant in Brassica spp. (Asghar et al., 2002).

Bacteria like Azospirillum and Pseudomonas spp. produce cytokinins and gibberellins (gibberellic acid), in addition to IAA (Gaudin et al., 1994). A few PGPR strains were reported to produce cytokinins (Vessy, 2003) and gibberellins (Glick et al., 1998). Ethylene, a gaseous photohormone commonly induced by wounding in plants, causes root growth inhibition. Studies focused on indirect promotion of root growth by ethylene inhibition revealed that PGPR produced ACC deaminase cleaves ACC, the immediate precursor molecule of ethylene in plants, with a net result of increase in root growth (Glick et al., 1998).

1.12.2 Solubilization of Phosphate

Phosphorus is the second most plant nutrient available in soil after nitrogen. Though soils usually contain high amount of total phosphorous, most of the phosphorous occur in insoluble form as iron and aluminum phosphates in acidic soils and calcium phosphates in alkaline soils. Some of them appear after the application of chemical fertilizers. Seventy five percent of the soluble phosphate fertilizers added to crops may be converted to sparingly soluble form by reaction with the free Ca$^{2+}$ ions in high pH soils or with Fe$^{3+}$ or Al$^{3+}$ in low pH soils (Goldstein, 1986). Plants are unable to utilize precipitated form of phosphorous. However, organic matter, on the other hand, is an important reservoir of immobilized phosphate that accounts for 20–80% of soil phosphorous (Richardson, 2001) and only a small portion (~0.1%) is available to plants. Conversion of the insoluble forms of phosphorous to a form accessible by plants, like
orthophosphate, is an important trait of Phosphate solubilizing rhizobacteria in increasing growth and yield of crop plant.

The ability of phosphate solubilizing microorganisms to solubilize phosphorous complexes has been attributed to their ability to reduce pH of the surroundings, either by releasing organic acids or protons. Organic acids such as citrate, lactate, succinate etc. secreted contribute for phosphate solubilization in the rhizosphere. The organic acids secreted can either directly dissolve the mineral phosphates as a result of anion exchange or can chelate both Fe and Al ions associated with phosphate (Bajpai and Rao, 1971). Finally, the insoluble form of phosphorous is converted into soluble monobasic ($H_2PO_4^-$) and dibasic ($HPO_4^{2-}$) ions, a process referred to as mineral phosphate solubilization. This leads to an increase in the availability of phosphorous to plants and in turn the plant uptake (Gyaneshwar et al., 2002).

The enzyme phytase, belongs to a special class of phosphomono esterases (myo-insitol hexakisphosphate 3-phosphorylase, EC 3.1.3.8 and Myo-inositol hexakisphosphate 6-phosphorylase, EC 3.1.3.26) is known to secreted by several soil microorganisms. It is involved in the stepwise degradation of phytase to lower phosphate esters. Plants are known to produce phytase (Greiner and Alminger, 2001), which display low activity in roots and other plants organs. This suggests that plant roots poorly or may not possess an innate ability to acquire phosphorous directly from soil phytase.

**1.12.3 Increased uptake of iron**

Fig.1.2. Competition for iron between microorganisms in the rhizosphere: a plant growth promoting rhizobacterium (PGPR) deprives a harmful microorganism (HMO) of iron by secreting siderophores (SID), which can (+) or cannot (-) also be used by plant roots (Bakker, 1989).
Iron is an essential micronutrient of plants as it serves as a cofactor of many enzyme activity. A large portion of iron in soil is in the highly insoluble form of ferric hydroxide, thus iron acts as a limiting factor for plant growth even in iron rich soils. The availability of iron in soil solutions is $10^{-18}$ M, a concentration which even cannot sustain the microbial growth. Several soil microorganisms produce siderophores, low molecular weight iron chelating compounds that bind Fe$^{3+}$ with very high affinity and helps in iron uptake. It is possible for the rhizosphere microorganisms to use siderophores provided they contain the appropriate uptake protein (Raaijmakers et al., 1995). A great deal of evidence exists that a number of plant species can absorb bacterial Fe$^{3+}$siderophore complexes, and this process is vital in absorption of iron by plant, especially in calcareous soils (Wang et al., 1993; Masalha et al., 2000

### 1.12.4 Volatiles in growth promotion

Plant growth promotion by volatiles produced by PGPR is the most recently identified mechanism. Ryu et al., (2003) demonstrated that PGPR strains release different volatile blends and the difference in these volatile blends stimulate the plant growth. Volatiles produced by *Bacillus subtilis* and *B. amylo liquefaciens* stimulated the growth of *Arabidopsis thaliana* in *in vitro* experiments as observed by an increase in the total leaf surface area compared to control. Two compounds 3-hydroxy 2-butanone (acetoin) and 2, 3-butanediol, were produced by both the bacterial strains whereas they are not produced by bacterial strains that did not affect the plant growth. Ryu et al. (2004) identified that exposure of *A. thaliana* seedlings to the volatile blends from *B. subtilis* and *B. amylo liquefaciens* reduced the disease severity by the bacterial pathogen *Erwinia carotovora* subsp. *carotovora*. The level of disease protection was directly related to the production of volatile organic compounds by *B. subtilis*. It is also observed that the signaling pathway activated by volatiles from *B. subtilis* is dependent on ethylene and independent of the salicylic acid or jasmonic acid signaling pathways.

### Indirect plant growth promotion

### 1.12.5 Antibiosis

Antibiosis is the inhibition or destruction of one organism by a metabolite produced by another organism. Antagonists may produce powerful growth inhibitory compounds that are effective against a wide array of microorganisms. Such compounds are referred as s broad-
spectrum antibiotics. On the other hand, some metabolites, such as bacteriocins, are effective only against a specific group of microorganisms. The bacterial antagonist *Agrobacterium radiobacter* K84 produces agrocin 84, a bacteriocin that is effective only against bacteria that are closely related to *A. radiobacter*, such as the crown gall pathogen *A. tumefaciens*. Antagonists that produce antibiotics have a competitive advantage in occupying a particular niche and securing substrates as food sources because their antibiotics suppress the growth or germination of other microorganisms.

Antibiosis can be an effective mechanism to protect germinating seeds. The rhizosphere is the thin layer of soil that adheres to the root after loose soil has been removed by shaking and is directly influenced by substances that are exuded by the root into soil solution. As the seed germinate, the bacteria multiply in the rhizosphere, using exudates from the roots as a food source. For example Hariprasad and Niranjana, 2009, in their studies demonstrated that phosphate solubilizig rhizobacteria which showed inhibition to *Fusarium oxysporum* growth under *in vitro* conditions also found to reducing the Fusarium wilt incidence under greenhouse conditions when seed treatment was done.

### 1.12.6 Competition for space and nutrition with other deleterious organisms

The root surface and surrounding rhizosphere are significant carbon sinks (Rovira, 1965). Photosynthate allocation to this zone can be as high as 40% (Degenhardt *et al.*, 2003). The roots of the plant release soluble exudates components, mucilage and sloughed off cells. Compounds detected in plant root exudates include amino acids, proteins, fatty acids, flavonoids, hormones, organic acids, polysaccharides, organic phosphorus compounds, purines, pyrimidins, sterols, sugars including oligosaccharides, vitamins and unidentified compounds which inhibits or stimulate fungi, bacteria, protozoa and nematodes. The amount and composition of exudates is largely affected by multiple factors such as plant species, root region, plant age, pH, temperature and surrounding microbes (Meharg and Killham, 1995). Despite fact that sugar has often been reported as the major carbon source in exudates, the ability to use specific sugar does no play a major carbon source in exudates, the ability to use specific sugar does not play a major role in tomato root colonization (Lugtenberg *et al.*, 1999).
Plant growth promoting rhizobacteria compete with deleterious microorganisms and pathogens for limited available nutrients in root exudates and suitable colonization niches, and finally out number them. Populations of PGPR established on the plant roots could act as a sink for the available nutrients and limit the nutrient availability for pathogen stimulation and its subsequent root colonization. This mechanism is most often used by fluorescent pseudomonads due to their nutritional versatility, and because of their high growth rates in the rhizosphere (Walsh et al., 2001). Apart from root colonization, the PGPR should be able to compete for nutrients with native microbial populations in the rhizosphere for successful elimination of the pathogens. Siderophore production at the rhizosphere by PGPR also contributes for disease suppression.

1.12.7 Parasitism or lysis

Parasitism of pathogenic fungi by PGPR is facilitated through the production of hydrolytic enzymes that degrade the fungal cell walls. Chitinases, among the hydrolytic enzymes, are of prime importance since chitin, a linear polymer of β- (1,4)-N-acetylglucosamine is a major cell wall constituent in majority of the phytopathogenic fungi. Purified chitinases of Bacillus subtilis AF1 (Manjula et al., 2004), Serratia marcescens (Kishore et al., 2005b; Ordentlich et al., 1988) and S. plymuthica (Frankowski et al., 2001) were highly antifungal. Another important group of hydrolytic enzymes, glucanase degrade the β 1-3 glucanase producing strain of P. cepacia inhibited the rhizosphere proliferation of various phytopathogenic fungi including Rhizoctonia solani, S. rolfsii and Pythium ultimum (Fridlender et al., 1993).

1.12.8 Inhibition of pathogen produced enzymes or toxins

Pathogenic fungi produce extracellular hydrolytic enzymes, which degrade the polymers present in plant cell walls and facilitate the fungal infection by polymers present in the plant cell walls and facilitate the fungal infection by disintegrating the cell wall. These hydrolytic enzymes include pectolytic enzymes (exo and endo – polygalacturonases, pectin lyases), cellulose and cutinase. Bacillus megaterium B 153-2-2 inhibited the activities of extracellular enzymes, like cellulose, pectin lyase and pectinase produced by R. solani, by producing an extracellular endoproteinase (Bertagnolli et al., 1996).
1.12.9 Induced systemic resistance

Induced systemic resistance occurs when the plant’s defense mechanisms are stimulated and primed to resist infection by pathogens. Once natural plant-resistance mechanisms are activated, increase defensive capacity is maintained for prolonged periods against multiple pathogens (Tuzun and Kuc, 1987). Resistance to pathogen(s) is associated with the accumulation of several factors like enzymes, antibiotics and inhibitors etc. The level of defense related enzymes are known to play a crucial role upon the degree of host resistance (Shivakumar et al., 2000). Plant growth promoting and bioprotecting bacteria triggered ISR fortifies plant cell wall strength and alters host physiology and metabolic responses, leading to an enhanced synthesis of plant defense chemicals upon challenge by pathogens and/or abiotic stress factors. The type of bacterized plant response induced after challenge with a pathogen resulted in the formation of structural barriers, such as thickened cell wall papillae due to the deposition of callose and the accumulation of phenolic compounds (Benhamou et al., 1996). Biochemical or physiological changes in plants include induced accumulation of pathogenesis-related proteins such as PR-1, PR-2, chitinase and some peroxidases. However, some PGPR do not induce PR-proteins but rather than increase accumulation of peroxidase, phenylalanine ammonia lyase, phytoalexin, polyphenol oxidase (Ramamoorthy et al., 2002).

![Figure 1.3. Signaling in Arabidopsis thaliana leading to rhizobacterium-mediated induced systemic resistance (ISR) or to pathogen-induced systemic acquired resistance (SAR). JA–Jasmonate 4; PRs pathogenesis-related proteins; SA – Salicylate (Van Loon et al., 1998).](image-url)
1.12.10 PGPR in management of abiotic stress

Most agricultural and agronomic practices are designed to optimize crop growth by avoiding or reducing abiotic stress. Plant breeding is designated, among other things, to develop genetically stable crop cultivars that are more resilient under stress. However, application of plant growth promoting rhizobacteria is an alternative approach in the management of Abiotic stress in crop production. The most successful strategy for managing the Abiotic stress in plants is lowering the level of stress ethylene production by using PGPR that capable of producing ACC deaminase (Glick et al., 1998; Mayak et al., 2004; Belimov et al., 2005).

1.12.11 Effect of PGPR on seed germination

Arora et al. (2001) observed enhanced seed germination, seedling biomass and nodule weight with reduced disease incidence in groundnut crop. Increased seed germination by 47% and 53%, biomass by 16.5% and 22%, and fresh nodule weight by 36% and 56.6% were obtained with respective inoculation of Sinorhizobium meliloti RMP3 and S. meliloti RMP5. Gupta et al. (2002) found reduced disease incidence, better vegetative growth parameters and ultimately enhanced grain yield in peanut by the addition of Pseudomonas aeruginosa GRC2 in M. phaseolina-infested field soil.

1.12.12 Microbial interactions in Rhizosphere

For any disease suppressive mechanism to be effective it is important that the antagonist first be able to efficiently establish itself in the rhizosphere of that particular plant (Kleopper et al., 1980). Many workers documented that inadequate colonization leads to decreased PGPR activities (Antounn et al., 1998). Pseudomonas sp. was prominent in the rhizosphere, rhizoplane and ectorrhizosphere (Curl and Truelove, 1986) because of its siderophore complexes (Baker et al., 1986) and production of antibiotic compounds (Howell and Stipanovic, 1980).

1.12.13 Pseudomonads

Members of genus Pseudomonas are rod shaped Gram negative bacteria characterized by metabolic versatility, aerobic respiration (some strains also have anaerobic respiration) (Holt et al., 1994). The term” Pseudomonads” (Pseudomonas like bacteria) is often used to describe strains for which the taxonomic affiliation has not been established in detail. Fluorescent
Pseudomonads produce the fluorescent pigment pyoverdin (Pvd) (also known as pseudobactin). This large and heterogenous group comprises most of them notably, *P. aeruginosa*, *P. putida*, *P. fluorescens* and *P. syringae* (recently reviewed by Kumar *et al.*, 2005).

*Pseudomonas* spp. is particularly suitable for application as agricultural biocontrol agents since they can use many exudates as a nutrient source, which is indicative of their adaptive potential (Lugtenberg *et al.*, 2004). *Pseudomonas* possesses diverse mechanisms by which they can exert inhibitory activity towards phytopathogens and thereby mediate crop protection. They have a high growth rate relative to many other rhizosphere bacteria, are easy to grow *in vitro*, and can subsequently be reintroduced into the rhizosphere by seed bacterization. In most of the investigations, the efficacy of individual biocontrol strain or combination of different strains is assessed by screening these agents against a single isolate of the target pathogen. Fluorescent *pseudomonads* spp. rapidly and aggressively colonizes the root system and suppressive pathogenic microorganisms, thus improving plant growth and grain yield (Schippers *et al.*, 1987; Weller, 1988; Gupta *et al.*, 2002). Fluorescent pseudomonads are reported for the biological control of different fungal species of various genera such as *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Pythium*, *Erwinia*, *Macrophomina phaseolina*, etc. (Defago *et al.*, 1990; Gupta *et al.*, 2001; Garbeva *et al.*, 2004). Pseudomonads and Rhizobia are also reported to direct plant growth promoting activities leading to yield promotion (Arora *et al.*, 2001; Gupta *et al.*, 2002; Deshwal *et al.*, 2003).

### 1.13 Challenges in selection and Characterization of PGPR

One of the challenges in developing PGPR for commercial application is ensuring that an effective selection and screening procedure is in place, so that the most promising organisms are identified and brought forward. Effective strategies for initial selection and screening of rhizobacterial isolates are required. It may be important to consider host plant specificity or adaptation to a particular soil, climatic conditions or pathogen in selecting the isolation conditions, and screening assays (Bowen *et al.*, 1999, Chanway *et al.*, 1989). One approach for selection of organisms with the potential to control soil borne phytopathogens is to isolate from soils that are suppressive to that pathogen (Weller *et al.*, 2002). Other approaches involve selection based on traits known to be associated with PGPR such as root colonization (Silva, et
al., 2003), 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Cattelan, 1999; Glick, 1995), and antibiotic (Giacomodonato, 2001) and siderophore production (Cattelan, 1999).

1.14 Challenges in field application of PGPR

The application of PGPR for control of fungal pathogens in greenhouse systems shows considerable promise (Paulitz and Belanger, 2001), due in part to the consistent environmental conditions and high incidence of fungal disease in greenhouses. Achieving consistent performance in the field where there is heterogeneity of abiotic and biotic factors and competition with indigenous organisms is more difficult. Knowledge of these factors can aid in determination of optimal concentration, timing and placement of inoculant, and of soil and crop management strategies to enhance survival and proliferation of the inoculant (Bowen et al., 1999; Mc Spadden Gardener and Fravel, 2002). The concept of engineering or managing the rhizosphere to enhance PGPR function by manipulation of the host plant, substrates for PGPR, or through agronomic practices, is gaining increasing attention (Bowen et al., 1999; Mansouri et al., 2002). Development of better formulations to ensure survival and activity in the field and compatibility with chemical and biological seed treatments is another area of focus; approaches include optimization of growth conditions prior to formulation and development of improved carriers and application technology (Bashan 1998; Bowen et al., 1999; Date 2001; Mathre et al., 1999; Yardin 2000). Formulation development must consider factors such as shelf life, compatibility with current application practices, cost, and ease of application. Health and safety testing may be required to address such issues as non-target effects on other organisms including toxigenicity, allergenicity and pathogenicity, persistence in the environment, and potential for horizontal transfer.

1.15 Scope of the study

Tomato is an important and valuable crop for low income farmers in the tropics. Tomato production all over the world struggles with the threat of diseases. Many of the pathogens are spread worldwide and cause enormous yield losses ever year. Tomato production is often limited by the fusarium wilt. Fusarium wilt has a worldwide economic importance since the disease is devastating to a large number of important crops and causes great losses in tomato. According to
literature survey made regarding this disease there are scanty reports about disease management using PGPR. With this background, the present work was taken up with following objectives,

1. **Field survey of major tomato growing areas of Karnataka to assess fusarium wilt disease incidence.**

2. **Isolation and characterization of *Pseudomonas fluorescens*.**

3. *Pseudomonas fluorescens* mediated induced systemic resistance in tomato.

4. **Development of suitable formulations of *Pseudomonas fluorescens* and field studies.**

**Objective 1. Field survey of major tomato growing areas of Karnataka to assess Fusarium wilt disease incidence.**

This chapter includes field survey, Collection of diseased plant samples, and seed samples from different places of Karnataka, assessment of Fusarium wilt incidence in major tomato growing field of tomato and also screening for incidence of seed mycoflora in tomato seeds. Field survey plays an important role in assessing the relation of pathogen with weather condition depending upon soil variety and to estimate the crop loss in field conditions, which is very important in agriculture. Field survey can provide information about the status, location of the disease and economic loss. Quality of the seeds is very much important for seed purpose as well as consumption point of view. Seed-borne fungi reduce the seed germination, seed viability, crop yield, nutritional and market value. Infection of seeds by fungi can have a marked negative effect on seed and nutritional qualities. For seed producers and farmers the seed quality is very much important. In addition to that infected seeds that germinate will produce blighted seedlings, which also further reduce planting value of seeds. Seed-borne fungi not only reduce the quality and quantity of fruits but also infect and transmit the disease through seeds. Therefore the main objective of this chapter was to survey the tomato growing regions of Karnataka state. Prevalence of Fusarium wilt of tomato in Karnataka could be established by undertaking a field survey in all tomato-growing areas of Karnataka at different seasons and Screening for incidence of seed mycoflora in tomato seeds.
Objective 2. Isolation and characterization of *Pseudomonas fluorescens*.

The rhizosphere or the zone of influence around roots harbors a multitude of microorganisms that are affected by both abiotic and biotic stresses. Among these are the dominant rhizobacteria that prefer living in close vicinity to the root or on its surface and play a crucial role in improving soil health and plant growth. With the currently available tools, the microbial community structure can be examined at several levels. The simplest analysis is by isolating each and every bacterial isolates colonizing the rhizosphere and their identification by traditional biochemical methods. Use of 16S rRNA sequencing provides greater discrimination than earlier studies and better characterization of an isolate. Therefore this chapter involves isolation of rhizobacteria from rhizosphere soil samples of different agroclimatic regions of tomato growing areas of Karnataka and their characterization up to their genus level by conducting physiological and biochemical tests and also conducting tests to characterization to their PGPR traits. In addition, selected strains were identified by 16S rRNA sequencing.

Objective 3: *Pseudomonas fluorescens* mediated induced systemic resistance in tomato

This chapter includes the study of defense–related enzymes during the host pathogen interaction. Plants are continually exposed to vast number of potential pathogens and as a result they have evolved intricate defense mechanisms to recognize and defend themselves against a wide array of these disease causing agents by including a set of defense responses that can defeat the invading pathogens. These mechanisms include pre-existing physical and chemical barriers, as well as inducible defense responses in the form of induction of defense-related enzymes that become activated upon pathogen infection. The interaction between the pathogen and host plant induce some changes in cell metabolism, primarily in the enzyme activities, including that of phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, lipoxygenase, β-1, 3-glucanase *etc*. So this chapter includes the study of role of phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and phenolics in the host pathogen interaction.
Objective 4: Development of suitable formulations of *Pseudomonas fluorescens* and field studies.

This chapter includes the control of fusarium wilt disease using *Pseudomonas fluorescens*. Plant diseases need to be controlled to maintain the quality and abundance of food, feed and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Many practices, such as crop rotation, seed certification, resistant cultivars, chemical fungicides, soil fumigation *etc.*, are used to control pathogens, especially soil-borne pathogens. However there are many problems associated with controlling pathogens with long-term persistent survival structures due to difficulties in reducing pathogen inoculums and lack of good sources of plant resistance. Plants have endogenous mechanisms that can be induced in response to attack by pathogens. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant’s own defense mechanisms by prior application of biocontrol bacteria is thought to be a novel plant protection strategy. Use of plant growth promoting bacteria for controlling soil-borne diseases has been well documented. Once when the organism is screened, evaluated and improved, it becomes essential to formulate and to develop a suitable delivery system. The design of the delivery systems varies depending on organism, crop and mode of application. Hence, the development of feasible and efficient delivery system is an important component of the biocontrol research. Though the PGPR have a very good potential in the management of pests and diseases, it could not be used as cell suspension under field conditions. Hence, the cell suspensions of PGPR should be immobilized in certain carriers and should be prepared as formulations for easy application, storage, commercialization and field use. Therefore, in this chapter fresh suspensions of *Pseudomonas fluorescens* bacteria as well as its different formulations were prepared and used to improve plant growth and management of fusarium wilt in tomato plants.