Chapter 1: Introduction

Introduction

Plants make up the majority of the earth’s living environment as trees, grass, flowers and so on. Directly or indirectly, plants also make up all the food on which humans and animals depend. The agents that cause disease in plants include pathogenic microorganisms, such as viruses, bacteria, fungi, protozoa and nematodes and unfavorable environmental conditions, such as lack or excess of nutrients, moisture and light, and the presence of toxic chemicals in air or soil. Pathogenic microorganisms, i.e., the transmissible biotic (living) agents cause disease in plants by disturbing the metabolism of plant cells through enzymes, toxins, growth regulators, and other substances they secrete and by absorbing nutrients from the host cells for their own use. Some pathogens may also cause disease by growing and multiplying in the xylem and phloem vessels of plants, thereby blocking the upward transportation of water or the downward movement of sugars, respectively, through these tissues (Agrios 2005).

Plant diseases: A threat to global food security:

The past two decades have witnessed an increasing number of virulent infectious diseases in both animals and plants. Pathogenic fungi have long been known as a widespread threat to plant species. An unprecedented number of fungal and fungal-like diseases have recently caused some of the most severe die-offs and extinctions ever witnessed in wild species, and are threatening food security. Human activities are modifying natural environments further intensifying fungal disease dispersal and thus creating new opportunities for evolution (Fisher et al., 2012). Emerging infectious diseases (EIDs) caused by fungi are increasingly recognized as presenting a global danger to food security (Anderson et al., 2004). The risk of plant disease has not declined; in fact it is heightened by resource-rich farming practices and exaggerated in the landscape by microbial adaptation to new ecosystems, brought about by trade and transportation, and by climate fluctuations (Grunwald et al., 2008; Brown and Hovmoller 2002).

Vascular wilt disease:

Vascular wilts are widespread, highly destructive and threatening diseases of cultivated crops or indigenous plant species. The disease results in heavy yield losses
and the impossibility of growing the crop without effective control of the disease. The disease appears as more or less rapid wilting, browning, and dying of leaves and succulent shoots of plants followed by death of the entire plant. The presence and activity of the pathogen in the xylem vessels of the tomato plant results in the occurrence of the wilt disease. Entire plant may die within a matter of weeks, although in perennials, death may occur until several months or years after infection. Wilt-causing fungi remain in the xylem tissues and a few surrounding cells as long as the infected plant is alive. Only when the infected plant is killed by the disease do these fungi move into the other tissues and sporulate at or near the surface of the dead plant (Agrios 2005).

Vascular wilts are caused by four genera of fungi: Ceratocystis, Ophiostoma, Fusarium and Verticillium. All vascular wilts have certain characteristics in common. The leaves of infected plants lose turgidity, become flaccid and lighter green to greenish yellow, droop and finally wilt, turn yellow, then brown, and finally die. Wilted leaves may be flat or curled. Young, tender shoots also wilt and die. Discoloured brown areas as a complete or interrupted ring consisting of discolored vascular tissues are seen in cross sections of infected stems and twigs. Mycelium and spores of the casual fungus may be present in the xylem vessels of infected stems and roots. Some of the vessels may be clogged with mycelium, spores, or polysaccharides produced by the fungus. Gels and gums formed by the accumulation and oxidation of breakdown products of plant cells attacked by fungal enzymes further increase the clogging. The oxidation and translocation of such breakdown products seem to also be responsible for the brown discolouration of affected vascular tissues. In newly infected young stems, the number of xylem vessels formed is reduced and their cell walls are thinner than normal. Often the parenchyma cells surrounding xylem vessels are stimulated by secretions of the pathogen to divide excessively, and this, results in a reduction of the diameter or complete collapse of the vessels. In some hosts, balloon-like tyloses are produced by parenchyma cells adjoining some xylem vessels. The protrusion of the tyloses into the vessels also contributes to their clogging. Toxins secreted by the wilt-causing fungus in the vessels are carried to the leaves, in which they cause reduced chlorophyll synthesis along the veins (vein clearing) and decrease photosynthesis, disrupt the permeability of the leaf cell membranes and their ability to control water loss through transpiration, and thereby result in leaf epinasty, wilting,
intervenial necrosis, browning and death. The first wilt toxins defined were Lycomarasmin, N-(hydroxypionic acid)-glycylasparagine and Fusaric acid (5-butylpicolinic acid). The fungal wilt pathogens produce cellulosytic and pectinolytic enzymes along with other enzymes helping them to attack the cell walls of the host tissues. The action of these enzymes facilitates the penetration and migration of the pathogen in host tissues as well as the hydrolytic breakdown products may be used as nutrients. In fungal wilt diseases, dissemination of the pathogen occurs by movement of infected plants, plant debris or infested soil. (Agrios 2005; Green 1981).

**Phytopathogenic Fusaria:**

Fungi are an important component of the world's microbiota and are responsible for many of the key steps in a wide range of ecosystem processes (Anderson et al., 2007). They occupy a broad range of roles in the environment, functioning as pathogens, symbionts, and decomposers, and are a very diverse group with estimates of 1.5M species distributed globally (Hawksworth 2001). Fungal plant pathogens play an important role in our lives as they can threaten food security, economic prosperity and natural environments. Fungal pathogens of plants particularly cause serious damage and extensive losses to agriculture and forestry. *Fusarium* species are among the most diverse and ubiquitous plant-pathogenic fungi with far reaching social and economic impacts (Summerell et al., 2010). They are saprophytes and many species of the genus *Fusarium* are devastating plant pathogens causing economically important blights, vascular wilts, leaf spots, root rots, fruit rots, cankers, dieback (Nelson 1981). They produce a wide range of secondary metabolites and are mycotoxin-producing contaminants of human and animal food (Moss and Smith, 1984). Some species, such as *F. graminearum* and *F. verticillioides*, have a narrow host range, infecting predominantly the cereals. By contrast, *F. oxysporum*, has a remarkably broad host range, infecting both monocotyledonous and dicotyledonous plants (Armstrong and Armstrong 1981) and is an emerging pathogen of immunocompromised humans (O’Donnel et al., 2004) and other mammals (Ortoneda et al., 2004). Apart from their differences in host specificity, Fusarium species also vary in reproductive strategy. Some, such as *F. oxysporum*, are asexual, whereas others are both asexual and sexual with either self-fertility (homothallic) or obligate out-crossing (heterothallic) (Ma et al., 2010).
Chapter 1: Introduction

Of all the plant diseases caused by members of the genus Fusarium, the most important is the vascular wilt disease caused by *Fusarium oxysporum*. *Fusarium oxysporum* is a common soil-borne plant pathogen with a worldwide distribution. It is an anamorphic species characterized by a series of morphological features including shape of macroconidia, structure of microconidiophores and the formation and disposition of chlamydospores. The genome size of *F. oxysporum* has been estimated to be 59.9Mb and the genome assemblage has 15 chromosomes. Approximately 3.98% of the genome is constituted by transposable elements (Ma *et al*., 2010). *Fusarium oxysporum* causes vascular wilt disease in a wide variety of economically important crops (Beckman 1987). As a species, it probably causes more economic damage to agricultural crops than any other plant pathogen. Within the species, however, there is a high level of host specificity described as formae speciales and races capable of causing vascular wilt diseases of many agricultural crops (Armstrong and Armstrong 1981). *Fusarium oxysporum* species complex comprises ubiquitous soil-borne plant pathogens with more than 150 host-specific forms or formae speciales (Baayen *et al*., 2000). Historically, strains of *F. oxysporum* have been divided into formae speciales on the basis of virulence on a particular host or group of hosts. Further subdivisions of formae speciales into races are made based on virulence to a particular set of differential host cultivars that vary in disease resistance (Correll 1991). A gene for gene relationship has been proposed for the interaction between *F. oxysporum* races and host cultivars, based on dominant monogenic resistance traits against known races (Di Pietro *et al*., 2003).

**Fusarium oxysporum**: Species and Subspecific Categories

*Fusarium* species were traditionally differentiated into groups and sections exclusively on the basis of morphology (Toussoun and Nelson 1976). Although morphology of asexual reproductive structures had been used to define species, these characters were unstable and considerable variation occurred in these features. The species was placed in the section (Gruppe) Elegans by Wollenweber and Reinking (1935) along with nine other species in three subsections (Untergruppen). However, the small morphological divisions within section Elegans, and highly variable features were subject to environmental influence (Nelson 1991). For this reason, Snyder and Hansen (1940) described a simplified taxonomic system by collapsing section Elegans into a single species: *F. oxysporum*. Further, they also recognized true variants within the species,
with respect to host plant species specialization. Although many or most isolates of the fungus may be nonpathogenic soil inhabitants (Appel and Gordon 1994), the concept of form or specialized form (forma specialis) arose to delineate morphologically similar or indistinguishable isolates having the ability to cause disease on different plants (Kistler 1997).

*Fusarium oxysporum* is characterized by a series of morphological features including shape of macroconidia, structure of microconidiophores and the formation and disposition of chlamydospores. The colonies of *F. oxysporum* on Potato Sucrose Agar (PSA) are floccose in texture and appear whitish cream from obverse and pale to bluish violet from reverse of the plate. The macroconidia are borne on urn-shaped monophialides in false heads oval to reniform and are aseptate. The macroconidia are borne on monophialides, falcate to straight, apical cell somewhat pointed, basal cell foot shaped, macroconidia usually 3 septate. Chlamydospores are abundant, usually single on hyphae. The distinguishing characters of *F. oxysporum* are the macroconidia are on false heads on short monophialidic conidiophores, false heads often clumping, chlamydospores formed singly on hyphae (Kulwant Singh *et al.*, 1991). When identifying strains of *Fusarium*, spore type and morphology are commonly viewed as the most important pieces of data, but the diagnostic process can be simplified if additional information such as host plant, disease symptoms, and observations made in the isolation and recovery process are included (Summerell *et al.*, 2003).

**Forma Specialis:**

*Fusarium oxysporum* has received considerable attention from plant pathologists because of its ability to cause vascular wilt or root rot diseases on a wide range of plants with host specialization of individual isolates. Snyder and Hansen (1940, 1941, 1945) integrated this diversity of host specificity within a single species into the forma specialis (f. sp.) system; each forma specialis within a species exhibited high level of virulence on a particular host species (or a group of species) but not on others (Suga and Hyakumachi 2004). The majority of the isolates causing vascular wilts are specific strains that infect only a small number of host plants and have been differentiated from each other using the subspecific term formae speciales (f. sp.). Isolates with the same or similar host ranges are assigned to a forma specialis (Kistler 1997), and more than 150 formae speciales have been described (Baayen *et al.*, 2000). For example, the
strains commonly attacking banana are *F. oxysporum* f. sp. *cubense*; cotton, *F. oxysporum* f. sp. *vasinfectum*; watermelon, *F. oxysporum* f. sp. *niveum* and tomato, *F. oxysporum* f. sp. *lycopersici*. These strains are morphologically identical, and they also cannot be differentiated from nonpathogenic or saprophytic strains, of which there is a huge diversity, especially in soil. From a diagnostic point of view, the separation of this species into formae speciales has important diagnostic and quarantine implications. Identification of these strains has traditionally involved pathogenicity testing with sets of host differentials appropriate for the formae speciales in question (Summerell et al., 2003). Most often, host range is restricted to a few plant species. For example, although many plants may be symptomless carriers of *F. oxysporum* f. sp. *lycopersici* (Katan 1971), the fungus causes disease only in plants of the genus *Lycopersicon* (tomato) (Rowe 1980). However, some formae speciales such as *F. oxysporum* f. sp. *radicis-lycopersici*, have broader host ranges causing disease on hosts from several other plant families as well as tomato (Menzies et al., 1990, Rowe 1980). Therefore, host specificity is not limited to tomato in this forma specialis. The assumption often made in the categorization of strains by host range is that isolates with shared host range, and thus within the same forma specialis, are more similar genetically than isolates with other host specificities. The evolutionary interpretation resulting from this assumption is that formae speciales are monophyletic and that isolates with a shared host range are likely derived from a single, particularly successful pathogenic genotype (Kistler 1997). The advent of molecular phylogenetic analyses have significantly contributed to the better understanding of the concept of formae speciales and the evolution of pathogenic specificity in *F. oxysporum*.

**Vegetative Compatibility Groups (VCG):**

Puhalla (1985) first attempted to distinguish and classify strains of *Fusarium oxysporum* purely by genetic means, rather than by morphology and host range. Vegetative compatibility was used as a basis for subdivision within formae speciales of *F. oxysporum*. Auxotrophic mutants unable to use nitrate as a sole nitrogen source were developed. These *nit* mutants, affected at different steps in the pathway involved in nitrogen reduction, were paired on minimal medium. If pairs of isolates fused to produce a heterokaryon, complementation resulted in wild-type growth. Pairs of isolates that can undergo hyphal fusion to produce heterokaryons belong to the same VCG. Vegetative compatibility is thought to result from the action of alleles at several
distinct loci and to be vegetatively compatible, a pair of isolates need to have the same alleles at all loci governing compatibility (Leslie 1993). By inference, only isolates of *F. oxysporum* very similar genetically would be members of the same VCG. Therefore, VCG determination may reflect genetic similarities among isolates of the species and about the reproductive strategy and, ultimately, the population structure of the fungus (Kistler 1997). Relationships between VCG and formae speciales of *F. oxysporum* have been extensively studied in the species. The concept of genetic similarity and relatedness between members of same VCG has been confirmed by molecular genetic analyses.

**Pathogenic Races:**

Isolates from a particular forma specialis can be further subdivided into races showing a characteristic pattern of virulence on differential host cultivars. Subdivision of the formae speciales into races is on the basis of virulence to a particular set of cultivars of the host that vary in disease resistance (J. C. Correll 1991). In some instances, races correspond to cultivar-level specificity of the host genotype which is often determined by single genes in the host (Kistler 1997). Formae speciales and races differ in symptomatology, epidemiology and cultivar susceptibility and can be differentiated by pathogenicity tests with appropriate host cultivars (Roncero *et al.*, 2003). A gene-for-gene relationship has been proposed for the interaction between *F. oxysporum* races and host cultivars, because monogenic, dominant resistance traits against known races have been described. In the case of the tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici*, where the tomato resistance gene *I*-2 confers resistance to *F. oxysporum* f. sp. *lycopersici* race 2 (Ori *et al.*, 1997), expressing the putative avirulence gene *avrI*-2 (Mes *et al.*, 1999). (e.g., races 1 and 2 of *F. oxysporum* f. sp. *lycopersici* and the corresponding *I* and *I*-2 genes in tomato (Jones and Woltz 1981; Stall and Walter 1965).

**The host: Tomato**

Tomato belongs to the large and diverse Solanaceae family also called Nightshades which includes more than three thousand species (Bauchet and Causse 2012). Tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum* Mill.) is one of the most widely cultivated vegetable crops in the world. Tomato has its origin in the South American Andes. In the sixteenth century, the cultivated tomato was brought to
Europe by Spanish conquistadors and later introduced from Europe to southern and eastern Asia, Africa and the Middle East. Later, wild tomato has been distributed into other parts of South America and Mexico. Common names for the tomato are: tomate (Spain, France), tomat (Indonesia), faan ke’e (China), tomati (West Africa), tomatl (Nahuatl), jitomate (Mexico), pomodoro (Italy), nyanya (Swahili) (Shankara et al., 2005).

Tomato is a vital component of daily food and is consumed as fresh fruits and various types of processed products (Giovanni et al., 2004). It is used as a fresh vegetable and can also be processed and canned as a paste, juice, sauce, puree, powder and as pickle (Barone and Frusciante, 2007). It is used in various ways, including in salads, and processed into ketchup or as soup. It is a diet rich vegetable with special nutritive value and a rich source of micronutrients. The popularity of tomato is rising among consumers, not only because of its good taste, but also because it contains high levels of vitamin C, lycopene, and betacarotene, which are natural anti-oxidants that promote good health. The enormous health benefits of tomatoes and tomato products are often attributed to the carotenoid lycopene, whose antioxidant properties have raised interest in tomato as a food with potential anticancer properties (Giovannucci, 1999). Tomatoes can make people healthier and reduce the risk of conditions of many types of cancer and cardiovascular diseases (Bhowmik et al., 2012).

**Tomato production in India and the world:**

Tomato is one of the most widely cultivated crops in the world. It is an important cash crop for smallholders and medium-scale commercial farmers (Shankara et al., 2005). Tomato, ranking 2nd in the world for vegetables, tops the list of canned vegetables. Total global area under tomato production is 4.58 million hectare with an estimated production of 150,513,813MT and an average productivity 32.8 MT/HA. China is the largest tomato producer followed by India which ranks second with 11% of global tomato production. In India, tomato is the second largest grown vegetable crop after potato and is followed by onion, brinjal and other vegetable crops. In India tomato is grown in an area of 0.86 million HA; production of 16,826,000 MT; with a productivity of 19.5 MT/HA. The estimated area under tomato cultivation is 0.86 million hectares which is 11.5% of the production share of major vegetable crops of the country. The average productivity of tomato in our country is merely 19.5MT/HA.
verses 32.5MT/HA of world productivity, while its productivity in USA is 81MT/HA and 48.1MT/HA in China. A total of 68,183.7 MT (worth 114,806.6 lakhs) tomatoes were exported from India to various countries such as United Arab Emirates, Bangladesh, Saudi Arabia, Oman, Pakistan, Nepal, Maldives, Bahrain, Kuwait, Qatar and other countries in the year 2010-11 (FAO 2012, Indian Horticulture Database 2011).

Tomato is grown in a wide range of climatic conditions across states of Andhra Pradesh, Orissa, Karnataka, Maharashtra, West Bengal, Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh, Assam and Chhattisgarh. The main varieties grown are Arka Saurabh, Arka Vikas, ARTH 3, ARTH 4, Avinash 2, BSS 90, CO3, HS 101, HS 102, HS 110, Hisar Anmol, Hisar Arun, Hisar Lalima, Hisar Lalit, Krishna, KS 2, Matri, MTH 6, NA 601, Naveen, Pusa 120, Punjab Chuhara, Pant Bahar, Pusa Divya, Pusa Early Dwarf, Pusa Hybrid 1, Pusa Hybrid 2, Pusa Hybrid 4, Pusa Ruby, Pusa Sheetal, Pusa Uphaar, Rajni, Rashmi, Ratna and Rupali. At present, Karnataka is the second leading producer of tomato in the country and contributes 10% of national production (Fig. 1.1). In Karnataka tomato is grown in an area of 0.51 million HA with the production of 1,756,700 MT and a productivity of 34.3 MT/HA. The major tomato producing belts of Karnataka include Kolar, Bangalore Rural and Urban, Bellary, Belgaum and Dharwad (Indian Horticulture Database 2011).

**Agronomy of tomato:**

Tomato is adapted to a wide range of climatic conditions from temperate to tropical. However, it requires a relatively cool, dry climate for high yield and best quality. A long, warm growing season favours its successful production. Tomato is a moderate season crop and does not tolerate frost but different optimum temperature is required for different stages of tomato. The plants can survive a range of temperatures; however, the optimum temperature for most varieties lies between 21 and 24 °C. Below 10 °C and above 38 °C the plant tissues are damaged. Tomato can be grown in many types of soil from light sandy to heavy clay. Most mineral soils that have proper water holding capacity and aeration, and are free of salt are best suited for growing tomatoes. Good texture of the soil is of primary importance. A deep, well-drained, sandy loam soil is ideal for a good crop of tomato.
Table 1.1. State wise production of tomato in INDIA (Area and Productivity).

<table>
<thead>
<tr>
<th>State</th>
<th>Area in 000' HA</th>
<th>Production in 000' MT</th>
<th>Productivity in MT/HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andhra Pradesh</td>
<td>296.3</td>
<td>5926.2</td>
<td>20.0</td>
</tr>
<tr>
<td>Karnataka</td>
<td>51.2</td>
<td>1756.7</td>
<td>34.3</td>
</tr>
<tr>
<td>Orissa</td>
<td>96.6</td>
<td>1367.2</td>
<td>14.1</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>52.0</td>
<td>738.0</td>
<td>14.2</td>
</tr>
<tr>
<td>West Bengal</td>
<td>54.1</td>
<td>1063.7</td>
<td>19.6</td>
</tr>
<tr>
<td>Bihar</td>
<td>46.8</td>
<td>1056.2</td>
<td>22.6</td>
</tr>
<tr>
<td>Gujarat</td>
<td>38.8</td>
<td>978.4</td>
<td>25.2</td>
</tr>
<tr>
<td>Chhatisgarh</td>
<td>42.9</td>
<td>627.9</td>
<td>14.6</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>27.2</td>
<td>580.6</td>
<td>21.4</td>
</tr>
<tr>
<td>Jharkhand</td>
<td>22.3</td>
<td>401.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Others</td>
<td>136.6</td>
<td>2330.0</td>
<td>17.1</td>
</tr>
<tr>
<td>Total</td>
<td>864.9</td>
<td>16286.4</td>
<td>19.5</td>
</tr>
</tbody>
</table>

Source: (Indian Horticultural Database 2011)

Fig. 1.1. Tomato Producing States in INDIA (2010-11). Indian Horticulture Database 2011.
Chapter 1: Introduction

The upper layer needs to be permeable. Soil depth of 15 to 20 cm is needed to grow a healthy crop. Deep ploughing allows better root penetration in heavy clay soils. Tomato is moderately tolerant to a wide range of pH (level of acidity), but the crop prefers soils with a pH of 5.5 – 6.8 with adequate nutrient supply and availability. Addition of organic matter is, in general, favourable for good growth. Soils with very high organic matter content, like peat soils, are less suitable due to their high water holding capacity and nutrient deficiencies. The choice of the tomato variety to be cultivated depends on the local conditions and the purpose of growing. Continuous processes of selection of plants have resulted in the development of local varieties (land-races) and improved (or commercial) varieties. The selection criteria are not only based on characteristics such as type of fruit, shape of plant, vitality and resistance (have an in-built resistance) to pests and diseases, but also on factors related to climate and management. Farmers select varieties that perform best under the local conditions (Shankara et al., 2005).

Diseases of Tomato:

The high demand for tomato makes it a high value crop that can generate much income for farmers. Tomato is best adapted to warm and dry environments. Tomato crop is highly sensitive to environmental stress caused by high temperature and low soil moisture. In fact, temperature is rising in the tomato growing locations and high temperatures can cause poor fruit-setting, lower the quality of fruits as well as many severe disease problems. In addition, the episodes of insect pests and diseases dependent on prevailing weather play a significant role in determining quantity and quality of harvest (NICRA 2012). Diseases are the most serious constraints in tomato production causing severe losses. Environmental factors and intensity of abiotic stresses may influence the incidence of the diseases, e.g. yield losses due wilt and root rot diseases increase under drought and high temperature situation in the country (Gurha et al., 2003). Due to nutritive and succulent nature of the plant, a large number of plant pathogens have been found associated with tomato.

Fusarium wilt of tomato

Fusarium wilt affects and cause severe losses on most vegetables and flowers; several field crops, such as cotton and tobacco; plantation crops such as banana, plantain, coffee and sugarcane; and a few shade trees. Fusarium wilts are most severe under warm soil conditions and in greenhouses (Agrios 2005).
Table 1.2. Major microbial diseases of Tomato.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causal organism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungal diseases</strong></td>
<td></td>
</tr>
<tr>
<td>Alternaria stem canker</td>
<td><em>Alternaria alternata</em> f. <em>lycopersici</em></td>
</tr>
<tr>
<td>Anthracnose</td>
<td><em>Colletotrichum coccodes, Colletotrichum dematium, Colletotrichum gloeosporoides</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>Late blight</td>
<td><em>Phytophthora infestans</em></td>
</tr>
<tr>
<td>Fusarium wilt</td>
<td><em>Fusarium oxysporum</em> f. <em>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</em> sub sp. <em>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. vesicatoria</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>Tomato Leaf Curl</td>
<td>Tomato Leaf Curl Virus</td>
</tr>
</tbody>
</table>

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

Tomato is one of the world’s most widely cultivated vegetable crops for consumption as fresh fruits and various types of processed products (Giovanni et al., 2004). Low yield of tomato is attributed to its susceptibility to several pathogenic fungi, bacteria, viruses and nematodes which are major constraints to tomato cultivation (Barone et al., 2007). Fusarium wilt caused by the soil borne fungus, *Fusarium oxysporum* Schlectend.: Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen, is one of the
major diseases of tomato (Sudhamoy et al., 2009). Fusarium wilt of tomato was initially described by G. E. Massee in England in 1895 (Walker, 1971) and according to Jones and Woltz (1981), the disease occurs in most of the tomato growing regions of the world. It is a warm weather disease and causes most severe symptoms at 28°C. It affects greenhouse and field grown tomatoes in warm vegetable production areas. The disease is characterized by yellowed leaves and wilted plants with minimal or absent crop yield. There may be a 30 to 40% yield loss due to the disease (Kirankumar et al., 2008).

Fusarium wilt of tomato is one of the most prevalent and damaging diseases wherever tomatoes are grown intensively. The disease is most destructive in warm climates and warm, sandy soils of temperate regions. The disease causes great yield losses, especially on susceptible tomato varieties and when soil and air temperatures are high during much of the season. Infection results in stunting followed by wilt and finally death of the plant. Occasionally, the entire fields of tomatoes are killed or damaged severely before the crop is harvested (Agrios 2005).

The pathogen invades the root epidermis and extends into the vascular tissue. It colonizes the xylem vessels producing mycelium and conidia. The characteristic wilt symptoms appear as a result of severe water stress, mainly due to vessel clogging (Beckman 1987). Lower leaves turn yellow. Yellowing often begins on one side of the plant and progresses upwards. Infected leaves curl downward, followed by browning and drying. Vascular browning is evident in stems and leaf petioles. Young plants when infected, are severely stunted (NICRA 2012). The pathogen can survive extended periods in infested soils as thick walled chlamydospores in the absence of the host (Agrios 2005).

**Symptoms:**

The first symptoms of the disease appears 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 Fusarium oxysporum may wilt and die soon after symptoms appear. Older plants in the field may wilt and die suddenly if the infection is severe and if the weather conditions favour the pathogen. 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 whole plant. 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. The brown ring is evident at the region of the vascular bundles. This discoloration often extends far up the stem and is especially noticeable in a petiole scar. The upward extent of the discoloration depends on the severity of the disease. The browning of the vascular system is characteristic of the disease and generally can be used for its identification (Agrios, 2005, Cerkauskas, 2005) (Fig. 1.2). Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Smith et al., 1988).

The Pathogen:

*Fusarium oxysporum* Schlectend.: Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen

*Fusarium oxysporum* f. sp. *lycopersici* is classified under,

**Kingdom**: Fungi

**Division**: Ascomycota

**Class**: Sordariomycetes

**Order**: Hypocreales

**Family**: Nectriaceae

**Genus**: *Fusarium*

**Species**: *Fusarium oxysporum* f. sp. *lycopersici*
Fig. 1.2. (A). Yellowing of lower leaves of Tomato plants affected with Fusarium wilt

Fig. 1.2. (B). Vascular browning in Tomato plants affected with Fusarium wilt.

Fig. 1.2. (C). Tomato plants affected with Fusarium wilt

Fig. 1.2. Fusarium wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. 
Distribution:

Overall, the distribution of *Fusarium oxysporum* is known to be cosmopolitan. It occurs chiefly as a soil saprophyte and is typically one of the most common and prevalent fungus of cultivated soils. Strains of the species are serious wilt pathogens of many crop plants, and most of the strains are morphologically indistinguishable (Booth, 1971; Nelson 1981). However, the different special forms (f. sp.) of *F. oxysporum* often have varying degrees of distribution.

Morphology:

The aerial mycelium of *Fusarium oxysporum* f. sp. *lycopersici* initially appears colourless, but turns cream-colored, pale yellow, pale pink or purplish with age. If sporodochia are abundant, the culture may appear cream or orange in colour (Smith et al., 1988). *Fusarium oxysporum* produces three types of asexual spores: microconidia, macroconidia, and Chlamydomspores. 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. Microconidia are generally produced in abundance, they vary a lot in size and are oval shaped, elliptical or reinform, usually non septate but 1 septate conidia may be found. Microconidia can also be formed in false-heads on short monophilialides. Macroconidia, the typical spores of the genus *Fusarium*; 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. Chlamydomspores may be one or two celled, round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia. All the three types of spores are produced when the fungus is cultured and probably in the soil. Only the chlamydomspores are capable of survival in the soil for long periods in the absence of the host (Agrios, 2005).

Epidemiology:

*Fusarium 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, 2005). It is also possible that the spores are spread by wind. Dissemination of the pathogen can occur via seeds, transplants, soil or other means (Booth 1971).

**Disease cycle:**

*Fusarium 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 *Fusarium 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 (Fig 1.3). 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 plants 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, 2005).

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.
Fig. 1.3. Disease cycle of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. (Source: Agrios 2005)

**The process of vascular infection and disease development:**

The process of vascular infection by *Fusarium oxysporum* is a complex phenomenon and the sequential steps involved in the infection process are (1) root recognition through host-pathogen signals, (2) attachment to the root surface and hyphal differentiation, (3) penetration of root cortex, (4) invading the vascular tissue and proliferation within the xylem vessels, (5) secretion of virulence factors and other toxins. Colonization of the vessels leads to disease development and the characteristic wilting of the host plant (Di Pietro et al., 2003).

As a typical soilborne pathogen *Fusarium oxysporum* can survive extensively in the soil as dormant propagules (chlamydospores). Between crops it survives in infected plant debris in the soil as mycelium and in all its spore forms, most commonly as chlamydospores. It spreads over short distances by means of water and contaminated farm equipment and over long distances primarily through infected transplants or in soil carried with them. In the presence of the host, the root proximity induces the
germination of the chlamydospores. When healthy plants grow in contaminated soil, the germ tube of the spore or the mycelium penetrates the roots at the point of formation of lateral roots or through wounds. The infection hyphae adhere to the root surface followed by penetration. The mycelium invades the root cortical cells intercellularly and enters the vascular system particularly through the xylem pits. Subsequently, the fungus displays a unique mode of infection were it remains exclusively within the xylem vessels using them as avenues to rapidly colonize the host. Within the vessels, the mycelium branches and produces microconidia, which on detachment are carried laterally upwards in the sap stream. Further, germination of microconidia lead to mycelial penetration to the upper vessels. The typical wilt symptoms develop as a result of vessel clogging and severe water stress caused by a combination of host pathogen activities such as accumulation of fungal mycelium, toxins and host defence responses including production of gels, gums, tyloses etc. which are responsible for the breakdown of the water economy of the infected plant. The characteristic disease symptoms, such as vein clearing, leaf epinasty, wilting and defoliation appear eventually leading to death of the host plant. When the leaves transpire more water than the stem and root can transport to them, the stomata close leading to the wilting and death of leaves, followed by death of the rest of the plant. At this stage, the vascular wilt fungus invades all tissues of the plant extensively, sporulates abundantly on the plant surface. Dissemination of the pathogen can occur via seeds, transplants, soil or other means. Sometimes, when the temperature is relatively low and soil moisture is high, infected plants may produce good yields. At this stage, the fungus may reach the fruit and contaminate the seed (Booth 1971; Agrios 2005).

The ultrastructural aspects of the *F. oxysporum* and tomato plant interaction has been investigated based on light, fluorescence and electron microscopy. Scanning electron microscopy of transverse and longitudinal sections through the dried stems of tomato plants colonized by *F. oxysporum* revealed microconidia were largely associated with the xylem vessels which germinated and the mycelium entered the vessels and cortex 14 days after inoculation. However, hyphae within the vessels were thicker in diameter (1.5-2µm) and grew through the pits in the walls of the vessels. No mechanical barrier in the vessels could control the longitudinal spread of the microconidia. Uncolonized vessels appeared granular while the colonized vessels
appear smooth. Distribution of hyphae showed that the lower regions of the stem were more intensively colonized than the upper regions (Gbaja I. S. 1982). In tomato plants, when the vascular elements get infected with \textit{F. oxysporum}, the contact parenchyma cells ensheathing the vessels develop callose containing deposits. These contact parenchyma cells play a crucial role in governing the exchange between vascular elements, storage, developmental and defense related functions. Light and Transmission Electron Microscopic examination of callose deposits in the vascular parenchyma cells of infected tomato plants showed wall appositions were associated with vesiculation and blebbing of the plasmalemma and generally contain globular bodies. In later stages of development, they exhibit a striated or marbled appearance. The callose deposits were found only in inoculated plants (Mueller and Beckman 1988). The marbled appearance of the deposits that form in response to infection may be due to the incorporation of lignins and their phenolics precursors with callose (Taylor and Zucker 1966). Hutson and Smith 1980 examined the vascular colonization and tylose production in tomato plants infected with \textit{F. oxysporum}. In resistant plants larger number of vessels with tyloses were seen compared to susceptible plants suggesting that tyloses may play a role in blocking or preventing the upward movement of conidia. Thus, creating an environment within the vessels were the lytic enzymes and other antifungal compounds can act effectively (Tjamos and Smith 1975).

Chantal \textit{et al.}, (2006) used transformed strains of \textit{F. oxysporum} expressing reporter genes such as green fluorescent protein (GFP) and DsRed2. Confocal laser scanning microscopy was used to visualize the colonization of tomato root by the pathogenic and non-pathogenic strains. Most of the germinated conidia were found in the region of the soil explored by the root hairs. The hyphae that reached the root surface created small networks. Fungal colonization was found limited to the length of the taproot and lateral roots and was never observed in the apical zones. The region behind the apex is the main zone of root exudation (Rovera \textit{et al.}, 1974). At later stages penetration of the epidermal cells was observed.
Virulence genes and Molecular requirements for pathogenicity of *F. oxysporum* f. sp. *lycopersici*:

Over the years numerous studies have been performed to get a better insight of the molecular mechanisms involved in pathogenesis *Fusarium oxysporum* f. sp. *lycopersici*. Soil borne phytopathogenic fungi must possess appropriate signalling mechanisms which enable them to respond by changes in gene expression leading to host recognition, root penetration, overcome the host defence mechanism, proliferate within the host tissue and disease establishment (Di Pietro *et al.*, 2003).

The cAMP-PKA (cyclic AMP-protein kinase A) cascade and Mitogen-Activated Protein Kinase (MAPK) cascade are two major signal transduction pathways regulating fungal development and virulence (Lengeler *et al.*, 2000). Di Pietro *et al.*, (2001) suggested that the MAPK- and cAMP-PKA cascades also operate in *F. oxysporum* f. sp. *lycopersici* and may control a number of key steps during the infection process. The targeted inactivation of gene encoding a mitogen-activated protein kinase (*fmk1*), produced mutants that were unable to penetrate the roots of tomato plants and failed to produce any disease symptoms. Fluorescence microscopy with the mutant strains expressing the green fluorescent protein, revealed their inability to attach to tomato roots unlike the infection hyphae of wild-type strain which firmly attached to the root surface prior to penetration. Interestingly, the Δ*fmk1* mutants also showed decreased production of polygalacturonase and pectate lyase, the pectinolytic enzymes involved in cell wall degradation during pathogenesis.

The *chsV* gene encodes a class V chitin synthase that are membrane-associated enzymes involved in biosynthesis of chitin, a major structural component of fungal cell wall (Roncero 2002). A mechanism of tolerance to plant defence compounds was identified by Madrib *et al.*, (2003) using a non pathogenic mutant of *F. oxysporum* obtained through random insertional mutagenesis. The mutant showed complete loss of virulence and characterization of the insertion site revealed the inactivation of *chsV* gene. In addition, *F. oxysporum* *chsV* mutants exhibited morphological abnormalities, reduced growth in the presence of aqueous extracts from tomato tissues and were hypersensitive to *H₂O₂* and α-tomatine, two different classes of plant defence compounds. This suggests that *chsV* gene is necessary to resist the defence compounds, a prerequisite for pathogenicity (Roncero 2003).
Several genes of *F. oxysporum* f. sp. *lycopersici* (Fol) were identified whose protein products are secreted into the host during infection (Rep et al., 2004; Houterman et al., 2007). Two of these genes, *SIX1* and *SIX2* (proteins secreted in xylem 1 and 2), are located within 8 kb of each other, present on one of the smallest chromosomes of approximately 2 Mb (Rep et al., 2004). *SIX1* matches the *I*-3 resistance gene in tomato. The *SIX1* product is a small cysteine-rich protein was shown to be required for full virulence, (Rep et al., 2005). In addition, eight more fungal proteins were identified from xylem sap of infected plants, the genes of which lie on the same chromosome as *SIX1* and *SIX2* (Houterman et al., 2007). This chromosome also harbors a homolog of *SIX1*, called *SIX1-H*, encoding a salicylate hydroxylase homolog and another gene *SIX3* for a small, in xylem secreted protein. *SIX1, SIX2, SIX3* and (*SHH1*), were unique to *F. oxysporum* f. sp. *lycopersici* isolates. Despite their polyphyletic origin, all the *F. oxysporum* f. sp. *lycopersici* isolates contained an identical genomic region of at least 8 kb comprising the genes *SIX1, SIX2* and *SHH1* that was absent in other *forma* *speciales* and non-pathogenic isolates. The virulence factor encoded by *SIX1* as well as the Six2 and Six3 proteins secreted into the xylem sap of infected tomato plants may function in colonization and positively contribute to virulence. These genes, by themselves or as part of a larger region of the genome may contribute to the development of vascular wilt disease in tomato (Houterman et al., 2007).

To identify the molecular requirements for pathogenicity of *F. oxysporum* f. sp. *lycopersici*, Caroline et al., 2009, used the *Agrobacterium*-mediated insertional mutagenesis approach to generate more than 10,000 transformants of *F. oxysporum* f. sp. *lycopersici* and further screened them for loss or reduction of pathogenicity. Cellular processes involving amino acid and lipid metabolism, cell wall integrity, protein translocation and protein degradation, seemed to be crucial for the pathogenicity of *F. oxysporum* f. sp. *lycopersici* based on the functional categorization of the potential pathogenicity genes. Several genes, such as those encoding chitin synthase V (*chsV*), developmental regulator (*flhA*) and phosphomannose isomerase were identified which play a putative role in primary or secondary metabolism and the pathogenicity of *F. oxysporum*. In addition, complementation and gene knock-out experiments confirmed that proteins involved in cell wall integrity such as the glycosylphosphatidylinositol-anchored protein, proteins involved in peroxisome
biogenesis, a transcriptional regulator, and a protein with unknown function are required for full pathogenicity and play a crucial role during infection of tomato by \textit{F. oxysporum}.

\textbf{Disease resistance/ genetics of host resistance}

Chemical treatments and soil solarisation in the fields usually fail to control the vascular wilt fungus. Use of resistant cultivars is the most reliable method of disease prevention. Cultivar resistance may vary by location, therefore selection of an appropriate cultivar also requires a thorough understanding of the form and race of the pathogen emerging in the field (Yasushi and Tsutomu 2006). Developing resistant varieties involves crossing resistant wild types and existing cultivars for their properties like good taste, shape and colour. Breeding of resistant cultivars, an alternative approach to chemical treatment, limits environmental and consumer risks. A molecular marker linked to resistance would be useful for tomato improvement programmes (Staniazsek \textit{et al.}, 2007).

The interaction between \textit{F. oxysporum} f. sp. \textit{lycopersici} and tomato is race cultivar specific. Resistance to all three races of \textit{F. oxysporum} f. sp. \textit{lycopersici} has been identified in wild \textit{Lycopersicon} spp. species and introgressed into commercial tomato cultivars. The \textit{I} and \textit{I-1} genes (Sarfatti 1991) conferring resistance to Fol race 1, originate from accession 160 of \textit{L. pinnipelliformis} and LA716 of \textit{L. pennellii}, respectively. The accession PI126915 (\textit{L. esculentum} × \textit{L. pinnipelliformis} hybrid), was found resistant against both races 1 and 2 (Alexander and Hoover 1955; Cirulli and Alexander 1966). The dominant \textit{I2} gene in tomato, governing resistance against race 2 FOL, originates from the wild tomato species \textit{Lycopersicon pinnipelliformis} (Stall and Walter, 1965). The \textit{I2} gene was found in tomato (\textit{Lycopersicon peruvianum}) resistant to both r1 and r2 (Hirano and Arie 2006). Resistance to race 3, controlled by a single dominant gene \textit{I-3}, was described in \textit{L. pennellii} accessions PI414773 and LA716 (McGrath \textit{et al.}, 1987; Scott and Jones 1989).

According to the gene for gene hypothesis, the dominant race specific resistance genes (\textit{R} genes) in tomato species would respond to the products dominant avirulence (\textit{Avr}) genes of the pathogen (Hemming \textit{et al.}, 2004). The \textit{I-2} gene, conferring resistance to Fol race 2, would respond to the dominant avirulence gene (\textit{AvrI-2}), present in race 2 of Fol and the activation of plant defense responses (Mes \textit{et al.}, 2004).
1999). The \( I \) genes have been identified and mapped to chromosomes 11 and 7 (Bohn and Tucker 1939; Paddock 1950; Sarfatti et al., 1991). \( I-2 \) lies within a cluster of seven similar genes on the long arm of chromosome 11 (Laterrot, 1976). The \( I-3 \) locus has been mapped to the long arm of chromosome 7 (Bournival et al., 1989). Two members of a multigene family, were isolated from the \( I2 \) Fol race 2 resistance locus in tomato and designated complex \( I2C \) (Ori et al., 1997). The members of the \( I2C \) family were mapped to five genomic positions. Two of these are clusters of several genes, both located on chromosome 11. These genes encode cytoplasmic proteins containing a nucleotide binding site motif and leucine-rich repeats (LRRs). Alignments between the various members of the \( I2 \) gene family reveal two important variable regions within the leucine-rich repeat region, one or both of these leucine-rich repeats may be involved in Fusarium wilt resistance with \( I2 \) specificity (Simons et al., 1998).

**Challenges in managing Fusarium wilt of Tomato:**

Management of soil-borne diseases such as Fusarium wilt has always been problematic. The best ways of eliminating soil borne pathogens are by use of synthetic fungicides in seed treatment, soil solarization/disinfection, crop rotation and mixed cropping. However, the use of synthetic chemicals for disease control is economically and environmentally undesirable. The most effective alternative approaches to control wilt disease is the use of resistant plant varieties. But, the failure of resistance due to high variability in the pathogen population, restricts the usefulness of many resistant cultivars only a few years. Thus there is a need to develop alternative strategies to provide durable resistance over a broad geographic area.

**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. 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. Fusarium wilt is one of the major constraints to tomato production in Karnataka causing enormous yield losses. Chemical treatments and soil solarisation in the fields usually fail to control the pathogen. Using resistant tomato cultivars is the most reliable method of disease
prevention. The selection of an appropriate cultivar requires a thorough understanding of the form and race of the pathogen emerging in the field. Genetic diversity and phylogenetic analysis within local populations of the pathogen help in elucidating the emergence and evolutionary relationships between the pathogenic and non-pathogenic strains and also may provide information of the pathogen dispersal from other geographical areas.

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, isolation and identification of *Fusarium oxysporum*.

2. Pathogenicity testing of the *Fusarium oxysporum* strains on susceptible tomato cultivars and determination of extracellular enzyme production.

3. Molecular characterization of pathogenic *Fusarium oxysporum* f. sp. *lycopersici* and non pathogenic strains based on ISSR, ITS-RFLP and ITS Sequencing and detection of toxigenic strains.

4. Characterization and bioactivity of secondary metabolite from *Fusarium oxysporum* f. sp. *lycopersici*.