Chapter-1

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most economically important vegetable crops and it is cultivated worldwide for its fresh fruits. Tomato is a short duration, high yielding crop and belongs to family Solanaceae. Cultivation of tomato has been increased over the years due to its popularity and economic importance (Elphinstone *et al.* 1996). Tomato is a rich source of minerals, vitamins, organic acids, essential amino acids and dietary fibres. Tomato also contains lycopene and beta carbonate pigments (http://www.indiagronet.com/tomato/resources). In India, the most promising states for cultivation of tomato are Bihar, Uttar Pradesh, Orissa, Karnataka, Punjab, West Bengal and Assam. Previously tomatoes were grown only once in growing season, but presently tomato are grown round the year.

Like many other crop plants tomato also suffer from several diseases. Most of the diseases caused by fungi but bacterial wilt of the plants also cause a substantial loss. Wilt is a disease where loss of turgor in a plant or plant part is happened (Windhan and Alan, 2003). Bacterial wilt can cause severe yield reduction, in the range 30-80%, however, it is geographically restricted (Clark and Moyer, 1988). The causal organism of bacterial wilt is *Ralstonia solanacearum. R. solanacearum* is supposed to be a soil born bacterium originating from the tropics, subtropics and warm temperate regions (Hayward, 1991). *R. solonacearum* is a serious pathogen causing bacterial wilt in solanaceous vegetables in India, such as tomato, potato, banana, eggplants and some ornamental plants (Hayward, 1994).

In case of wilt disease the vascular tissues in the lower stem of plants show a dark brown discoloration. This symptom is similar to that of some fungal diseases. A cross section of the stem of a plant with bacterial wilt produces white, milky strands of bacterial cells in clear water. This ooze distinguishes the wilt caused by bacterium from that caused by fungal pathogens (Leppal *et al.* 2004). On all infected plants, bacterial streaming (ooze) may occur upon placing cut main stem material in a test tube with water. If vascular tissue is collapsed, sunken skin lesion will also appear (Swanson *et al.*, 2005).

The pathogen *Ralstonia solanacearum* has been classified in five biovars according to carbon source utilization and six races based on host range (Hayward *et al.* 1990). There is no general correlation between races and biovars, however biovar 2 strains are almost always race 3 (and vice versa). The five races of *R. solanacearum* have different host ranges and geographic distributions. Race 1 is a poorly-defined group with a very wide host range and is endemic to the southern United States. Race 2 principally attacks bananas, and is found mainly in Central America and Southeast Asia. Race 3 is distributed worldwide and has primarily been associated with potato. Race 4 affects ginger in much of Asia and Hawaii, and race 5 affects mulberries in China. Recently a more phylogenetically meaningful system has classified *R. solanacearum* into four phylotypes roughly corresponding to geographic origin (Fegan and Prior, 2005).

Bacterial wilt of tomato caused by *R. solanacearum* is a major disease in winter season. A specific and sensitive PCR detection method that uses primers targeting the gene coding for the flagella subunit (*fliC*) was established. Based on the first *fliC* gene sequence of *R. solanacearum,* strain K60 is available at GenBank, the Ral_*fliC* PCR primer system was designed and this system yielded a single 724 bp product with the DNAs of all *R. solanacearum* strains tested (DeShar *et al.* 1997; Hales *et al.* 1998; Shah *et al.* 2000).

Bacterial wilt pathogenesis is incompletely understood, genetic and molecular studies have shown that contributing factors are controlled by environmentally responsive regulatory cascades and that disease development depends on the action of the Type II and Type III protein secretion system. Secreted proteins are central to the pathogenesis of pathogenic bacteria (Schell, 2000 and Boucher, 2004).

Many plant species produce volatile essential oil compounds. These oils are considered to play a role in host defence mechanisms against plant pathogens. Essential oils and their components, usually from medicinal plants, have been recognized as having antifungal effects, but their efficacy

as a biofumigant on *R. solanacearum* has not been studied widely. However, some greenhouse experiments were conducted to determine the effectiveness of plant essential oils as soil fumigants to manage bacterial wilt in tomato (Kucharek, 1998; Mihaliak *et al.* 1991 and Wisniewski, 1997). Preliminary *in vitro* and greenhouse experiments conducted with several plant essential oils and their components showed the significant efficacy against *R. solanacearum* and against several soilborn fungi of tomato (Rich, 1999 and Mitchell, 2000).

With the above informations it was considered worthwhile to study the interactions of the *Ralstonia solanacearum* and *Lycopersicon esculentum* in sub-Himalayan north Bengal, where large quantity of tomato is produced. It was observed, during the present study, that bacterial wilt was a major constraint for successful cultivation of tomato in north Bengal (Fig 1.1). In addition it was also considered to suggest some control measures against the pathogen by using botanicals and biocontrol agents if any. Hence, the following objectives have been taken into consideration for the present study.

With the above information it was considered worthwhile to study the interactions of the *Ralstonia solanacearum* and *Lycopersicon esculentum* in sub-Himalayan West Bengal, where large quantity of tomato is produced. It was also considered to suggest some control measures against the pathogen by using botanicals and biocontrol agents if any. In addition, management of bacterial wilt disease (caused by *Ralstonia solanacearum*) by some abiotic inducers have also been taken into consideration. Hence, the main objectives of this study are as follows.

Objectives:

- Isolation of *Ralstonia solanacearum* from soils of infected plants of North Bengal.
- Pathogenesity test of selected isolates and assessment of disease.
- Morphological studies of selected Bacteria and Electron microscopy.
- Biochemical characterization and identification of the isolates.

- Molecular identification of selected isolates following 16S rRNA.
- PCR amplification and sequencing of *fliC* gene of some selected isolates.
- Isolation of antagonistic bacteria and their identification.
- Control of the disease by biocontrol agents and botanicals.
- Induction of defense related enzymes by some abiotic inducers.

Fig. 1.1: a) Infected young plants in farmers field b) Infected plants in farmers field.