Chapter-2

LITERATURE REVIEW

Major factor for limiting the production and market value of horticultural crops is their susceptibility to diseases. Tomato plants also face similar limitations of yield and commercial value due to diseases caused by several pathogens. To control the diseases of tomato, it is necessary to understand the different aspects of pathogens and hosts along with their interactions. Recent advanced initiatives in plant pathology have paved the way for development of new innovative techniques to control crop diseases. Molecular events of pathogenesis and induced systemic resistance have been recognized as the present era of plant pathology (Vidyasekharan, 1988). In addition to induced systemic resistance, biological control and control of diseases by botanicals have also gained much importance due to recent worldwide awareness on negative impact of chemical fungicides. Therefore, present research works of disease control of plants have also been focused towards eco-friendly mode of control that is benign to environment.

At the onset of the present research work, it was considered worthwhile to review the research findings of the previous researchers in a selective manner rather than in a comprehensive way. The observations of recent past have been presented briefly in the following paragraphs. For convenience, the observations have been grouped into some aspects. The different aspects of this literature review are as follows:

2.1: Pathogen (*Ralstonia solanacearum*)

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2.8.3: Biological Control.

2.8.4: Antagonists.

2.1.1: Taxonomy of causal organism with remarks

Kingdom- Bacteria	
Phyllum- Proteobacteria	
Class- Betaproteobacteria	One of four classes of 'purple bacteria'
Order- Burkholderiales	_
Family- Burkholderieriaceae	Group has nine genera.
Genus- *Ralstonia*	Genus has five species.
Species- *R. solanacearum*	Species has five races. All the five races are plant pathogenic. Some with wide host range and some with narrow host range.
Race 3	Race 3 is found in India and attack tomato plants. The genus was earlier known as *Pseudomonas solanacearum*

Tomato bacterial wilt is caused by *Ralstonia solanacearum*, formerly known as *Pseudomonas solanacearum.* The pathogen has different races, each of them unique and each of them attacking different plants. Tomato bacterial wilt is mostly caused by race 1 strain, which has a wide host range and can survive in the soil for a long period of time. Race 1 strains are highly variable in

their genotype and aggressiveness on tomato. Some highly aggressive strains can cause severe symptoms, even on “resistant” varieties. Fortunately, such strains are not predominant.

Taxonomic groups should follow the natural divisions that are apparent after characterizing the phenotype and genotype of related organisms and determining their relationship to known taxa. Until recently, however, the knowledge necessary to discern natural groups was often unavailable and many groups were incorrectly classified in phylogenetic terms. This was certainly true for the organisms now considered members of the *Burkholderia* group, which were long classed as nonfluorescent *Pseudomonas* species (Anzai *et. al.,*2000;). However, recent genetic analyses have revealed many new relationships and prompted renaming many bacteria in this and other groups (one Web site with official nomenclature is http://www.bacterio.cict.fr/).

2.1.2: Dissemination of *Ralstonia solanacearum*

R. solanacearum has been found to be one of the main constraints in the field of agriculture as it causes wilt disease by blocking the water transportation path i.e. the xylem. A large number of crops and some ornamental plants are being affected globally though bacterial wilt caused by the most devastating phytopathogen called *Ralstonia solanacearum* (Elphinstone, 2005; Hayward, 1994; Fegan and Prior, 2005). *Ralstonia solanacearum* mostly affects solanaceous crops (Alvarez et al. 2008).

R. solanacearum bacterium is transmitted through water and soil, mostly through waterways (Elphinstone, 2005). It colonizes the vascular system after entering the plant through root, resulting in the occurrence of severe symptoms (Hayward, 1992; Kelman, 1953). Disease outbreaks occur due to presence of *R. solanacearum* phylotype (ph) II biovar (bv) 2 in European waterways (Caruso et al., 2005; Van Elsas et al., 2000, 2001). This bacteria can survive for prolonged period in sterile water, deficient of plant materials (Alvarez et al., 2008;

Kelman,1956; Van Elsas et al., 2001; Van Overbeek et al.,2004; Wakimoto et al.,1982).

Starvation survival state is completely different from that of active growth state (Heim et al., 2002). Under prolonged starvation condition, the change of shape of bacteria to round cell and reduction in its size are considered as their strategies to survive in oligotrophic environment (Novitsky and Morita, 1976; Rollins and Colwell, 1986; Ruiz et al., 2001). The induction of viable and non-culturable state named as the VBNC state is one of the adaptation in the oligotrophic state (Oliver, 2005; Roszak and Colwell, 1987). *R. solanacearum* acquires VBNC state after being exposed to copper (Grey and Steck, 2001).

R. solanacearum is a soil borne microbe and transmits through soil-routes. It infects the host plant through their roots (Xue et al., 2020). The invading bacteria causes wilting by multiplying in the xylem and producing huge amount of exopolysaccharides that leads to the blockage in the vessels affecting the water conductance (Saile et al., 1997). Studies have shown that this pathogen can survive in soil for considerable amount of time without any host. There are many theoretical reasons for this bacterial behavior such as association with plant debris or several weed hosts which are symptomless carriers (Genin and Boucher, 2002). Infection in the plants mainly occurs though root-to-root transmission and movement of the contaminated soil (Gopalakrishnan et al., 2014). Swimming motility of *R. solanacearum allow*s it to find suitable host and disperse effectively (Kersten et al., 2001)

The long distance transmission of the pathogen is mainly through infected propagation materials such as potato seed tubers (Abdurahman et al., 2019). The wilt disease cause by *Ralstonia solanacearum* has broad geographical distribution and the dissemination through infected potato seed tubers occurs at a very high rate, contributing to the pathogen dispersal (Elphonstone, 2005; Mansfield et al., 2012; Buddhenhagen 1986; Janse 1996; Janse et al. 2004). *R. solan*acearum lie dormant in propagative organs like

tubers, suckers, seeds or rhizomes and infected plant debris of crops. Infected potato tubers are the main source of dissemination of this pathogen (Choudhary et al., 2018). The widespread of this disease is due to the transmission of these phytopathogen through latently infected ginger rhizome and limited crop rotation due to decreasing land holdings (Kurabachew and Ayana, 2016).

The bacterium can also be transmitted through insects as bacteria can survive on insects for several days resulting in the initiation of crop infection. There is a clear chance of fast disease transmission as insects can move from one plant to another plant within a short period of time. Insects are particularly attracted to plant wounds which form the main infection site (Wolf and Boer, 2007).

Ramirez et al., (2019) reported that the natural spread of most races of *Ralstonia solanacearum* bacteria is slow although he reported an exceptional case where one race that causes Moko disease in banana and has a potential to spread naturally at faster rate. The association of nematode (*Meloidogyne* sp.) and *R. solanacearum* contributes to the spreading of the diseases (Hayward, 1991).

2.1.3: Detection and Identification

Ralstonia solanacearum causes lethal diseases in most of the economically important crop plants thus affecting the agricultural field to a greater extent. So there is a need to characterize this bacterium through detection and identification. Several techniques have been developed so far for the detection of *R. solanacearum* to control bacterial wilt disease (Machmud and Suryadi, 2008).

Grover et al. (2009) performed multiple displacement amplification PCR amplification (MDA-PCR) for detection. Detection and identification are conceptually and methodologically intertwined processes. He performed this

technique on pure cell lysates and soil samples. DNA of pure cell lysates of *R. solanacearum* and soil sample DNA was used as template in MDA reaction. Sample buffer, reaction buffer and enzyme mix were need in MDA procedure. In study of pathobiology and epidemiology of *R. solanacearum,* it is usually necessary to first detect its presence (based on a tentative identification) before isolation and rigorously identifying a strain.

Substantial international effort has been focused on developing better detection methods, because soil and water samples typically have low populations of *R. solanacearum* and not all cells may grow *in vitro* (Anon., 2004; Alvarez, 2005; Denny *et al.*, 2001; Elphinstone *et al.*, 1996; Saddler, 2000; Seal and Elphinstone, 1994). Pure cultures of *R. solanacearum* are not difficult to identify. Cultural and physiological tests can quickly rule out related organisms (Anon., 2004). There are also commercially available fatty acid methyl ester (FAME) analyses and BIOLOGTM kits (Black and Sweetmore, 1993; Janse, 1991; Li and Hayward, 1993; Stead *et al.*, 1992)). Several nucleic acid and serological based methods are also present (Alvarez, 2005; Seal et al., 1994).

One of the effective serological techniques used for detection and identification of bacterial plant pathogen is the Enzyme–Linked Immunosorbent Assay. This technique is rapid, cost–efficient, and practical for field application and does not require sophisticated equipment (Machmud and Suryadi, 2008). In Indonesia, many workers use this technique to detect plant diseases (Machmud et al., 1996). Techniques of ELISA basically involve reaction between antigen (Ag) and antibody (Ab). The technique needs substrates and enzymes to label the reaction that produces colour which can be observed either by our naked eyes or using ELISA Reader (Converso and Martin, 1990; Mc Laughlin and Chen, 1990). The ELISA technique had been modified to increase its effectiveness and had given different names, such as Direct ELISA, Indirect ELISA, Double Antibody Sandwiched ELISA (DAS – ELISA) (Canale et al.,1983 ; Stobbs and Barker ,1985 ; Yadi et al., 1998).

Modification of the ELISA includes the production of Polyclonal antibodies and components of ELISA kits which is needed for the detection of some viral and bacterial plant pathogens including *R solanacearum* (Machmud et al., 1996, 1997, 1998, 1999,).

Lemessa and Zeller (2006) collected eighty one isolates of *Ralstonia solanacearum* like bacteria from different crops like tomato, potato and pepper. Out of those eighty one isolates, sixty two strains were detected and identified as *R. solanacearum* based on tomato pathogenecity bioassay, carbon source utilization patterns and PCR based assay. Out of these 62 strains which have been identified as *R. solanacearum*, 19 were identified as biovar I and 43 strains were identified as biovar II by Hayward's classification method which is based on carbon source utilization patterns. They also performed PCR with the isolates of *R. solanacearum* which produced wilt symptoms in tomato pathogenicity bioassay. They Extracted genomic DNA using the "DNeasy Tissue" Kit and PCR-amplified in a thermal cycler.

Ramirez et al. (2019) have assesed the phylogenetic relationship, diversity and pathogenecity of *R. solanacearum* involved in the production of moko disease in Colombia. They performed multiplex PCR of the 65 isolates obtained from the growing regions of banana / plantain to determine genetic diversity of the isolates. They analysed partial sequences of egl, mutS and rplB genes. From their assessment, they concluded that all the strains belonged to *R. solnacearum* phylotype II, sequevers 4 and 6.

2.1.4: Conditions favourable for development of *Ralstonia solanacearum*

R. solanacearum is an aerobic obligate phytopathogen (Tahat and Sijam, 2010). It survives well in high-drained soil having high moisture content. Its survival in the soil is basically dependent on temperature (Choudhary et al., 2018). Bacterial population has been shown to be reduced when exposed to high day temperature of 40°C for more than four hours (van Elsas et al., 2000). Increase in the disease incidence of bacterial wilt on host, such as tomato and

increase in the ambient temperature were found to be correlated as stated by Hayward, (1991).

Wung and Lin (2005) described the favourable conditions such as high temperature and moist soil are required for the development of bacterial wilt in tomato. They found that the development of bacterial wilt in tomato slowed down on reducing the soil temperature below 20° C or in the reduced soil moisture content.

The pathogen growth is found to be suppressed in some soil types as the soil moisture determines the level of some antagonistic population, which is a competitor of *R. solanacearum*. The association of nematode (*Meloidogyne* sp.) and *R. solanacearum* contributes to the spreading of the diseases (Hayward, 1991). In the hill regions of India, the wilt disease is highly predominant throughout the year where the soil is acidic (Velma et al., 2014)

2.1.5: Distribution and host range of *Ralstonia solanacearum*

The host range of *R. solanacearum* is wide. *R. solanacearum* is an economically important pathogen as it is known to infect large number of crop plants. It causes disease in potato *(Solanum tuberosum)*, tomato (*Lycopersicon esculentum*), peppers (*Capsicum annum*), egg plant (*Solanum melongena*), ginger (*Zingiber officinale*) and a few weed species bitter sweet (*Celastrus orbiculatus)*, stinging nettle (*Urtica dioica*) and night shade (*Solanum karsense*). Earlier strains of *Ralstonia solanacearum* were classified into five races based on the host range. The five races of *R. solanacearum* have different host ranges and wide distribution. Southern United States as well as Africa, Asia and South America are the places where Race I was endemic. Race I was reported to infect a wide range of crop plants. Race 2 was reported to causes wilt disease mainly in bananas and was endemic to the Central America and South East Asia. Race 3 was distributed worldwide and attacks mostly potato. Race 4 infects ginger in Hawaii and Asia. Race 5 is known to infect mulberries in China (Tahat and Sijan, 2010).

Ralstonia solnacearum was divided into six biovars based on its ability to metabolize three diasaccharides and three sugar alcohols (Hayward, 1964; 1991 and 1994).

Bacterial wilt disease caused by *R. solanacearum* generally occurs in lowlands in tropical and subtropical regions but Race 3 biovar 2 (R3bv2), a sub-group of *R. solanacearum* was exceptionally present at high altitudes and temperate regions (Elphinstone, 2005). R3bv2 was known to cause brown rot of potato in highland tropics of Latin America, Africa and Asia (Elphinstone, 2005). It was found that Biovar 3 had the widest host range (Hassan et al., 2016). Chandrashekhar and Prasann, 2010 found two new host plants called davana (*Artemisia pallens*) and coleus (*Coleus forkohlii*) which showed typical wilting symptoms after infection with *R. solanacearum*. Both the crops were important in aromatic and medicinal industries.

2.2.1: Diseases caused by *R. solanacearum*

Gopalkrishnan et.al (2014) Bacterial wilt reduces the production of eggplant in India. The occurrence of the disease has been reported to be much more pronounced in tropical, subtropical and some warm temperate regions of the world. The disease was known to spread fast due to its soil-borne nature and occurs in a wide range of hosts. The disease is transmitted through insects, root-to-root transfer, and dissemination of farm implements and movement of the soil. The conditions like high temperature and poor drainage also trigger the development of the disease resulting in the loss of productivity of crops during summer in India.

Yadessa et.al, (2010) reported that *Ralstonia solanacearum* causes wilt disease in several economically important crops like tomato, potato, egg plant, pepper and tobacco in Ethiopia. Tomato was the most widely grown crops of Ethiopia. Conditions like high temperature, dry days and cooler nights, altitudes between 700 and 2000metres in Ethiopia favoured the production of tomato plants. However, bacterial wilt was the common hindrance in the

production of tomato plants there. Incidence of bacterial wilt in tomato in Ethiopia was very high (55%).

Coutinho et al. (2000) stated that first report of *Ralstonia solanacearum* was found in late 1980s in Brazil. Similarly he also stated that in 1997, bacterial wilt on Eucalyptus in South Africa was found. In the affected plants, the vascular tissue was discoloured and bacterial exudation was produced from cut surfaces.

Mepharishvili et al. (2012) stated that in June, 2010, wilt disease of tomato seedlings in western Georgia was confirmed for the first time. The disease caused drastic loss in the productivity. They also reported presence of infection of some egg plants and sweet pepper plants in some *R. solanacearum* infected regions. The symptoms like wilting and vascular tissue discolouration were observed.

2.2.2: Fusarium Wilt

The other most economically important pathogen group is *Fusarium oxysporum* species which comprises a group of strains that are known to cause vascular wilt disease known as *Fusarium* wilt in most of the economically important crops throughout the world. Variation in the pathogen is due to the horizontal gene transfer. Sexual reproduction is not known in this pathogen. Development of the disease depends on several factors like the structure of the root cortex, capacity of the host to recognize and response to the growth of the pathogen and composition of root exudates (Gordon, 2017).

Fusarium wilt is one of the most devastating diseases in plants that cause huge loss to the agriculture. The causal organism of this disease is *Fusarium oxysporium or Fusarium solani.* Tomato plants are mostly affected by this disease thoughout the world, especially in the upland areas. More than 100 *Fusarium* vascular wilt diseases have been reported worldwide (Ajilogba and Babalola, 2013).

Fusarium wilt was first reported in India by Butler in 1918 and Later Padwick in 1940 correctly determined its etiology (Jimenez-Diaz et. al., 2015). They also reported Chickpea had a significant role in farming system. But *Fusarium* wilt was the main hindrance in the path of production of chickpea. *Fusarium* wilt was one of the most economically important diseases affecting chickpea worldwide. Symptoms of the disease appear at any stage of plant growth. The infected plants formed group as patches. Symptoms were flaccidity of individual leaves, dessication, dull green discolouration, collapse of the whole plant. Fusarium wilt reduced chickpea production by reducing both seed weight and seed yield.

2.3: Loss caused by *Ralstonia solanacearum*

Knap et al. (2004) reported that the bacterial wilt was one of the most destructive plant diseases affecting most of the economically important crops including plants of solanaceae as main target of the pathogen in Ethiopia. In the solanaceae family the most affected plants were tomato, potato, eggplant and pepper. They also reported that those crops were economically important as Ethiopian farming community primarily depended on those crops as sources of their income and food security. They reported that substantial yield loss of different solanaceous crops by the bacteria were found to occur from time to time in different parts of the country.

Seleim et al. (2014) isolated and identified fifteen *Ralstonia solanacearum* isolates from Egypt and confirmed their pathogenicity which showed that all isolates were pathogenic to tomato plants and their wilting capacity varied from 52 to 97%. They found whitish ooze from the cut stems of symptomatic plants when submerged in water.

Chilli pepper (*Capsicum annum)* is one of the most important vegetables from the family solanaceae because of its use as spices and condiments (Aslam et al., 2017). Bacterium *Ralstonia sola*nacearum is one of the most economically important parasite that cause huge loss in the yield of Chilli

pepper (Elphinstone 2005; Wicker et al. 2007). It had a devastating effect on crops worldwide especially in the tropical regions (Artal et al. 2012).

2.4: Symptoms caused by *Ralstonia solanacearum* in tomato and allied crops:

Symptoms of the wilt disease appear after few days of infection (Buddenhagen and Kelman, 1964; Haywards, 2000). *R. solanacearum* makes its way to the plant through roots having wounds that are made by insects or certain nematodes, transplanting, cultivation or natural wounds where the secondary root arises (McCarter, 1991). After entering inside the host, the bacterium establishes itself in the vascular tissue, where it multiplies rapidly, blocking the water transportation, which causes wilting (Hayward, 1991; Wang and lin, 2005). Symptoms, such as, wilting, dwarfing, later yellowing is observed. Death of the plant occurs due to the filling of host cell with bacterial cells and exopolysaccharaide slime (Buddenhagen and Kelman, 1964; Hayward, 2000). Later, discolouration of the vascular system from pale yellow to dark brown occurs. Further symptoms represent exudation of droplets of milky bacterial ooze from infected tissue (McCarter, 1991).

Infection of the host plant by *R. solanacearum* may express all or none of the symptoms. This observed condition is known as Latency where the plant infected with pathogen expresses all or none of the symptoms, even under ideal conditions (Tahat and Sijam, 2010).

There may be some variation in the symptoms of *R. solanacearum* among the wide range of host (Garcia et al., 2018). The wilt is located on one side of the plant i.e. unilateral (Denny, 2006)

Younger tobacco (*Nicotiana tabacum*) leaves are the first to get wilted at the early stages of infection (Denny 2006; Echandi ,1991). In the next stage, the leaves turn yellow and followed by scorch –like symptoms between the veins and at the margins. Total collapse of the plant occurs at the later stages

(Denny, 2006). Echandi, 1991 reported that plants that do not die show leaf distortion and stunting like symptoms. Primary and secondary roots may rot.

Wilting occurs specifically on the younger leaves of tomato (Momol and Champoiseau ,2009). The first visible symptoms are chlorosis and wilting located at the tip of branches of potato (Denny 2006 ; Stevenson et al.,2001). Petiole epinasty is the another symptom that occur on potato (Denny 2006; Stevenson et al.,2001)

In June, 2010, farmers in Chkhorotsku region, Western Georgia reported wilt disease infecting tomato plants, causing plant loss up to 100%. Farmers in Kutaisi region also observed similar symptoms of bacterial wilt, such as, wilting and discolouration in tomato plants (Mepharishvili et al., 2012).

Depending upon the diverse nature of the pathogen infecting various host plant, there exist several bacterial wilt symptoms. some bacterial wilt symptoms occur as flabby appeareance on the youngest leaves of tomato, usually at the hottest time of the day, few days after inoculation (Vasse et al.,1995).

2.5: Virulence, pathogenesis and Fli C gene

Virulence is a special ability of an organism to cause disease by involving some virulence factors. These factors are either cytosolic, membrane associated or secreatory in nature. There are several regulatory pathways that control the expression of several virulence factors in *Ralstonia solanacearum* (Brito et al., 2005).

Trans-Kerstein et al. (2001) reported that *R. solanacearum* in planta was essentially nonmotile, but it was highly motile in culture. To determine the role of pathogen motility in this disease, they cloned, characterized, and mutated two genes in the *R. solanacearum* flagellar biosynthetic pathway. The genes for flagellin, the subunit of the flagellar filament (*fliC*), and for the flagellar motor switch protein (*fliM*) were isolated based on their resemblance to these proteins

in other bacteria. As is typical for flagellins, the predicted FliC protein had well-conserved N- and C-terminal regions, separated by a divergent central domain. The predicted *R. solanacearum* FliM closely resembled motor switch proteins from other proteobacteria.

He et al. (2012) reported FliC, as a flagellar filament structural protein, and hypothesized to be involved in the pathogenesis of infection in case of *Edwardsiella tarda*, a flagellated Gram-negative bacterium which causes edwardsiellosis in fish. They studied, a *fliC* in-frame deletion mutant of a virulent isolate of *E. tarda* was constructed through double crossover allelic exchange by means of the suicide vector pRE112, and its virulence-associated phenotypes and pathogenicity were tested. It was found that the deletion of *fliC* significantly decreased the diameter of flagella filaments. In addition, the mutant showed reduced pathogenicity as well as showed impaired bacterial growth, reduced motility, decreased biofilm formation and reduced levels of virulence-associated protein secretion involved in the type III secretion system (TTSS). The phenotypic characteristics of the *fliC* deletion mutant uncovered in this investigation suggest that *fliC* plays an essential role in normal flagellum function, bacterial growth, protein secretion by TTSS and bacterial virulence.

Haiko and Westerlund-Wikstrom (2013) stated that flagellin or FliC, The major subunit, of the flagellum plays a well-documented role in innate immunity and as a dominant antigen of the adaptive immune response. Importantly, flagella have also been reported to function as adhesins. Whole flagella have been indicated as significant in bacterial adhesion to and invasion into host cells. In various pathogens, e.g., *Escherichia coli*, *Pseudomonas aeruginosa* and *Clostridium difficile*, flagellin and/or the distally located flagellar cap protein have been reported to function as adhesins. They also reported that FliC of Shiga-toxigenic *E. coli* was shown to be involved in cellular invasion via lipid rafts. They studied flagellar adhesive and invasive properties, especially focusing on the flagellum as a potential virulence factor.

Tans-Kersten et al (2004) reported that motility was a virulence trait of *Ralstonia solanacearum* and they showed that motility allows the bacterial wilt pathogen *Ralstonia solanacearum* to efficiently invade and colonize host plants. They showed in culture, flhDC expression depended on PehSR, a regulator of early virulence factors; and, in turn, FlhDC was required for fliC (flagellin) expression. They also showed that fliC gene was expressed in planta at cell densities where motile bacteria were not observed, as well as in a nonmotile flhDC mutant. Thus, expression of flhDC and flagellin itself are uncoupled from bacterial motility in the host environment, indicating that additional signals and regulatory circuits repress motility during plant pathogenesis.

Brito et al., (2005) reported that PhcA is a part of regulatory network that express several virulence factors. It controls several virulence factors either directly or indirectly (via some intermediatory regulatory genes). PhcA is involved in the bacterial motility, plant cell wall degrading enzymes and expression of extracellular polysaccharide (EPS) (Huang et al.1995).

Pathogenicity of *R. solanacearum* is also determined by hrp-encoded Type III secretion system (TISS). TISS translocate effector proteins into plant dells (Genin et Boucher, 2004). Disease development occurs due to *hrp* gene. For induction of a defensive hypersensitive response (HR), gene *hrp* is required. Once pathogen gets recognized, this hypersensitive response is triggered. Growth environment strongly influences the *hrp* gene expression in several Gram–negative bacteria (Arlat, 1992; Huynh et al. 1989).

Many cellular processes like virulence, stress tolerance etc. are related to cold shock proteins (Liu et al., 2020). Cold–shock proteins (Csp) are small and highly conserved proteins. They comprises cold–shock domain, the RNA binding domain (Timonon et al., 2016; Chaikam et al., 2010; Eshwar et al., 2017). Research on the Csps of *R. solanacearum* is scanty. Many works based on the genomic data have been done to know the role of Csps in virulence of *R. solanacearum*. The role of HrpG and XpsR have been correlated with the host-

bacterium interactions and expression of extracellular polysaccharide (EPS) related genes (Genin, 2010; Genin et Denny, 2012).

2.6: Host Resistance

The most effective disease control method is producing resistant cultivars against bacterial wilt. This is considered as the most economical, eco- friendly method of disease control. The most economically important crops such as potato, tomato, pepper, peanut, tobacco and eggplant have been selected for developing resistant cultivars against bacterial wilt disease though breeding (Boshou, 2005). Some factors such as temperature, soil moisture, pathogen strains, host–pathogen interaction, presence of root-knot nematodes, genetic linkage between resistance and breeding methodology highly control the stability of resistant varieties (Boshou, 2005; Elphinstone 2005; Wang and Lin, 2005).

Getachew et al. (2009) assessed all the tomato plants affected by the disastrous strain of *R. solanacearum* originated from Ethiopia. They found that six were resistant, eleven moderately resistant whereas most of the genotypes including all tomato cultivars commonly grown in Ethiopia were found highly susceptible. Colonization and invasion of *R. solanacearum* on resistant plants makes them more tolerant to diseases. Nakaho et al. (2004) reported that multiplication of bacteria was suppressed in the stems of resistant tomato plants due to restricted pathogen movement between the xylem tissues basically from the protoxylem to other xylem tissues. Furthermore, Dahal et al. (2010) used proteomic approach to explain the molecular interactions in the cell walls of resistant and susceptible plants infected with *R. solanacearum*.

2.7: Disease control

R. solanacearum causes huge yield loss on different economically important crops worldwide (Kurabachew and Ayana, 2016). It causes 50-100% loss in potato yield in Kenya (Muthoni et al.,2012), 70% on potato in India

(APS, 2005) and 88% on tomato in Uganda (Kataflire et al., 2005). It causes considerable economic loss every year (Elphinstone, 2005). Thus, control of the disease is required to minimize crop loss (USDA, 2003). Management of bacterial wilt disease is really difficult because of its wide host range, long survival in sterile water and soil, several ways of transmission, genetically diverse strains (APS, 2005; EU, 2003). There is a need to standarise different management programs to control bacterial wilt disease (Kurabachew and Ayana, 2016). Collective application of all management programs is needed (Lemessa and Zeller ,2007; Bekele and Berga, 2001). Different methods such as Agronomic practices (Bekele and Berga, 2001; Janiver et al., 2007), host resistance (Boshou, 2005), chemical control (Fortnum and Martin, 1998; Santos et al., 2006; Edward Jones, 2008). Biological control (Bias, 2004; Whips, 2001; Whipps et al., 2007; Kurabachew et al., 2007; Lemessa and Zeller, 2007; Alyie et al.,2008; Kurabachew and Wydra, 2013; Ciampipanno et al.,1989 and Intregated disease management (Yuliar et al., 2015; Kinyua et al. 2001).

2.7.1: Agronomic practices

Cultural practices, if used properly, can efficiently decrease the incidence and severity of Bacterial wilt disease. Soil borne bacterial population can be reduced by the application of crop rotation with nonsusceptible crops (Bekele and Berga, 2001). Identification and use of “non-host break crops” and “exact rotation period” should be taken into consideration. Plantation time should be shifted to cooler periods of the year to escape the disease. Particular phyto-pathogenic population is established on continuous cropping with same susceptible host, therefore crop rotation with change in varieties is also need to be considered to reduce the disastrous effect of soil–borne pathogen (Janiver et al., 2007). Katafire et al., (2005) discovered that the wilt disease incidence was reduced by 64% to 94%, after rotating cultivation of potato with sorghum, millet, sweet potato, wheat, carrots or phaseolus beans.

Soil amendment has also been used in many areas to control bacterial wilt disease and was found to restrict the growth of pathogens in the soil (Michel and Mew, 1998). Application of organic amendments to soil improvises the chemical, physical and biological properties of soil, which directly increases crop productivity (Bailey at al., 2003). Survival of the pathogen is highly affected by the degradation of organic matter present in the soil, which restricts available nutrients in the soil (Bailey et al., 2003). Degradation of organic matter releases inhibitory chemical substances that enhances the activity of antagonistic pathogen in the soil (Bailey et al., 2003; Akathar and Malik, 2000).

Lemaga et al. (2001) reported that application of organic materials (*Leucaena diversifolia* and *Sesbania sesbana*) in soil either singly or combined with inorganic fertilizer decreased wilt disease incidence, thus yield of potato tuber was increased.

Getachew et al. (2011) reported that the combined effect of silicon fertilizer and sugarcane bagasse (alternatively used silicon source) on wilt incidence and reported that tomato fruit yield was increased.

Bacterial wilt incidence could be reduced significantly by 81% by the application of soil amendments combined with coco peat farmyard manure (FYM) compost in the soil (Yedessa et al., 2010)

Lemaga et al. (2005) reported that the bacterial wilt was reduced by 29% and 50% on the application of Nitrogen(N) + Phosphorus(P) + Potassium(K) and Nitrogen(N) + Phosphorous(P) respectively.

2.7.2: Chemical control

Several fumigants such as metal sodium, 1,3-dichloropropene and chloropicrin and pesticides such as 3-[3 –Indolyl] butanoic acid have been worked efficiently against bacterial wilt disease. Several plant activators, such as, Validamycin A and Validoxylamine, which trigger plant systemic resistance have been used in tomato to control bacterial wilt disease. The combination of

metal sodium with choloropicrin significantly reduced the disease incidence from 72% to 100% and increased tobacco and tomato yield in the field. The yield of tomato was 1.7 to 2.5 fold higher when treated with pesticides (Fortnum and Martin, 1998, Santos et al., 2006).

Edward–Jones (2008) reported that pesticide offered a significant reduction in the bacterial wilt disease in many fields, but its effect on plants may differ depending on the way it has been used. If farmers use pesticides without proper knowledge and care, some amount of it may remain in the soil for many years (Gadeva and Dimitrov, 2008) ,become contaminated in groundwater and soil (Acero et al.,2008) and become poisonous (Dasgupta et al., 2007).

Chlorine dioxide (ClO_2), Chloro –oxide (CaClO) and organic acids such as acetic acids and lactic acids are effective in controlling bacterial pathogens (Janse ,2002; Choudhary et al., 2018).

Pradhanang et al. (2002) reported the use of essential oils as soil fumigants to control bacterial wilt disease. Treatments with thymol, lemongrass oil and palmarosa oil gave positive results on the control of the *R.solanacearum* population. The fungicidal effect of essential oils extracted from medicinal plants is well known (Wilson et al., 1997).

Systemic resistance is induced by the application of Acibenzolar –S-methyl (Hacisalihoglu et al., 2007; Pradhanang et al., 2005; Yuliar et al., 2014). The combination of thymol and Acibenzolar – S- methyl (ASM) suppressed the disease incidence and increased tomato yield, but thymol and ASM alone could not do the same (Hong et al., 1996)

Silicon (Dannon and Wydra, 2004; Kurabachew and Wydra, 2014; Wydra and Dannon, 2006) and Chitosan (Kirkegaard et al., 1996) are known to induce induced resistance , thus helps in the reduction of the incidence of the bacterial wilt disease

Bacteriostatic action with Phosphoric acid solution can be used to control infection caused by the bacterial wilt pathogen (Norman et al.,2006).

The use of antibiotics such as streptomycin increased the bacterial wilt in Egypt, thus questioned the application of antibiotic in controlling plant diseases as it is responsible for the development of resistant strains of bacteria (Farag et al., 1986; OEPP/EPPO, 2004).

Amzalek and Cohen (2007) examined the efficacy of four inducers (AABA, BABA, GABA AND NaSA) to control rust infection in sunflower plants caused by *Puccinia helianthi* . BABA was found to be the most effective inducer and sodium salicylate (NaSA) was the least effective in controlling rust infection caused by *Puccinia helianthi* in sunflower plants.

SA, AABA, GABA, BABA and BTH are known to act as elicitors which has the capacity to induce natural defense system of plants (Gomez –Vasquez et al., 2004). Silue et al.,(2002) reported the efficacy of BABA as inducer in inducing defensive response in Couliflower (*B. oleracea* var.botrytis) against *Peronospora parasitica*, the causal agent of downy mildew.

Sharma and Sohal (2016) reported the effects of GABA in inducing defensive system in Indian mustard (*Brassica juncea* var. RLM619). GABA may help in triggering the level of antioxidant content in Indian mustard, thus conferring its resistance against various fungal diseases (Sharma and Sohal, 2016).

2.7.3: Biological Control

The use of biological control plant disease has been increased in recent times due to public concerns about the effect of using chemicals in the fields (Whips, 2001). The reason behind this is the self –sustaining nature of biological control agents (Whipps et al., 2007). This are many other promising characteristics of the biological control agent's such as: long-term disease management in an eco- friendly manner, potentially spread on their own after

initial establishment, reduced use of nonrenewable resources (Whipps et al., 2007).

The rhizosphere resident microbial antagonist is considered as a favourable approach in the management of several plant diseases. The rhizosphere is a place where several biologically important processes and interactions occurs (Bias, 2004). Several studies have shown that the use of various antagonistic species have helped in controlling bacterial wilt. Major antagonistic species are *Bacillus subtilis, B. cereus, B. pumilis, Pseudomonas putida, P. fluorescens, Paenibacillus macerans* and *Serratia marcescens.* These were collected from potato and tomato rhizosphere from Ethiopia (Kurabachew et al.2007, Kurabachew and Wydra, 2013; Lemessa and Zeller, 2007; Alyie et al., 2008). Ciampi Panno et al. (1989) have proved the role of antagonistic bacteria to manage *R. solanacearum* population under field condition.

Potential biological agents include genetically engineered antagonistic bacteria (Kang et al., 1995), avirulent mutant of *R. solanacearum* (Dong et al., 1999) and some naturally occurring antagonistic rhizobacteria such as *Bacillus subtilis*, *B. cereus*, *B. pumilis, Pseudomonas fluorescens, P. putida, Serratia marcescens* and *Paenibacillus macerans* (Alyie et al., 2008; Lemessa and Zeller, 2007; Kurabachew et al., 2007; Kurabachew and Wydra, 2013).