IMPORTANCE, SOURCE AND CONTROL OF BACTERIA WILT DISEASE IN GREENHOUSE TOMATO (*Solanum lycopersicum L.*) IN SOUTHERN GHANA

BY

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DECLARATION

I, Sarfo Nana Yaw, declare that with the exception of materials used and referenced which have been duly acknowledged, this work submitted to College of Agriculture & Consumer Sciences (CACS) of the Department of Crop Science for the award of a Master of Philosophy degree in Crop Science (Plant Pathology) is the result of my own research and has not been presented anywhere for the award of a degree.

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ABSTRACT

Bacterial wilt of tomato is a devastating disease in greenhouse in southern Ghana where most greenhouses are sited. This study was conducted to assess farmer's knowledge and experiences on the prevalence, detection, spread and control of the disease in greenhouses in Volta, Eastern, Central and Greater Accra regions of Ghana. The disease incidence and severity was also determined and the causal organism of the disease isolated from diseased tissues and characterized using morphological, biochemical, and molecular techniques. Sources of the bacteria inoculum in greenhouses were identified and some tomato cultivars were screened for resistance to the bacterial wilt disease. Questionnaires were administered to (50) greenhouse farmers purposefully selected using a database of greenhouse producers in southern Ghana provided by West Africa Agricultural Productivity Programme (WAAPP) between August and December 2017. The questionnaire investigated the background of greenhouse farmers and their knowledge and experiences on the prevalence, detection, spread and control of the disease in greenhouses. Frequency data was analyzed using descriptive statistical analysis (means and percentages) by means of EPI INFO version 7.2 software. Disease incidence was determined and severity scored using a 0-4 rating scale. Gram strain reaction test, potassium hydroxide solubility test, tobacco hypersensitivity test, bacteria colony morphology and reaction on 2, 3, 5-triphenyl tetrazolium chloride (TZC) were used to identify the causal organism. Multiplex polymerase chain reaction was used to determine phylotypes of the bacterial isolates. Plant samples, growth substrate, seeds and irrigation water collected from surveyed greenhouses were screened for the presence of the causal organism using polymerase chain reaction. Five tomato accessions (WACC1, WACC2, WACC3, WACC4 and WACC5) and a high yielding variety preferred by greenhouse farmers (EVA F1) were screened for resistance against the bacterial wilt disease in a screen house at Nungua Livestock breeding station. Seedlings were transplanted in a naturally infested coco peat and inoculated with 5 ml of a 10^8 CFU/ml bacterial suspension using the stem puncture technique. Disease severity was observed after inoculation and the area under disease-progressive curve (AUDPC) calculated for each variety. Results from survey indicated that 28% of greenhouse farmers knew the test for detection of the disease with 64% of greenhouse farmers having no knowledge of how the disease spreads. Majority (62%) used roughing and burying of the infected plant to control the disease. Out of the 54 greenhouses (domes) surveyed, only 12 had the infestation from the disease. Disease incidence and severity within greenhouses ranged from (5.9%-18.5%) and (0.5 – 1.2) respectively, with Greater Accra (18.5%) and the Eastern region (8.2%) with highest disease incidence. Central and Volta regions had no infestation of the disease in their greenhouses. *Ralstonia solanacearum* was identified as the causal organism of the disease and Phylotype I (Asian origin), Phylotype III (African origin) and Phylotype IV (Tropical origin) strains were identified from the study. The source of bacteria inoculum in the greenhouses were irrigation water and the growth media (substrate) used. All varieties tested were susceptible to the bacterial wilt disease, however, WACC 5 showed the least susceptibility to the bacterial wilt disease. Greenhouse farmers had little knowledge on the spread, detection and control of the bacterial wilt disease of tomato. The disease was detected in Greater Accra and Eastern regions disease incidence of 18.5% and 8.2% respectively. The causal organism was confirmed as *Ralstonia solanacearum* and the other sources of inoculum was irrigation water and the growth medium. Research on the use of resistant rootstocks in grafting and the screening of more varieties for resistance to the disease is recommended.

DEDICATION

I dedicate this work to the Glory of the Almighty God, Mr. & Mrs. Quainoo-Arthur and my late uncle Mr. John Obeng Debrah.

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CHAPTER ONE

INTRODUCTION

Tomato (*Solanum lycopersicum* L) is the second most cultivated vegetable worldwide after potato, and the most important vegetable in terms of value (Hanson *et al*., 2001). The global land area for crop production in 2012 was 4,803,680 ha, with a production volume of 161,793,834 tons amounting to US$59,108,521 (FAOSTAT, 2013). Tomato production in recent times, has observed a growth of about 10% due to demand for its rich source of vitamins, minerals and antioxidants with greenhouse cultivation becoming significant (FAOSTAT, 2009; HE *et al*., 2009; Shalaby and El-Banna, 2013). Nutritionally, tomato fruits and products are described as “protective foods”, owing to the presence of lycopene and other anticarcinogenic and antioxidant compounds (Alam *et al*., 2007). A positive correlation between the consumption of tomato fruits and incidence of some chronic and degenerative diseases, such as cardiovascular diseases and aging condition has been observed (Ishida and Chapman, 2004; Rao and Rao, 2007).

The total land area under tomato production in Ghana in 2017 was 47,932 ha yielding 321,000 tons (FAOSTAT, 2017). Tomato is regarded as a high-value crop cultivated mainly by smallholder farmers in all the agro-ecological zones in Ghana (Diao, 2010) and the monetary expenditure on tomatoes exceeds any other vegetable in the country (Osei *et al*., 2010). In Ghana, the crop is prone to a myriad of fungal, virial, bacterial, nematodes and insect pests (Sasser *et al*., 1983), and over 30 diseases have also been reported (Oduro, 2000; Offei *et al*., 2008). Diseases and other constraints on production of the crop in the country has resulted in a high yearly importation from neighbouring countries especially Burkina Faso (Robinson and Kolavalli, 2010).

The Government of Ghana in collaboration with relevant stakeholders, launched the Export Marketing and Quality Awareness Project (EMQAP) under the Ministry of Food and Agriculture (MOFA) in January, 2007 to enhance productivity and ensure a secure and sustained supply of tomatoes and other vegetables throughout the year (Harry Bleppony, personal communication, June 5, 2017). The project included the setting up of greenhouses at Vakpo (Volta region), Kade (Eastern region), Okyereko (Central region) and Amrahia (Greater Accra Region) to address the constraints and to increase export earnings from non-traditional agricultural produce. Similarly, the Forest and Horticultural Crops Research Centre, Kade, under the Envirodome project, University of Ghana in 2012 established some domes at the Centre, and later in other parts in the country (Dr. Stephen Torkpo, personal communication, July 4, 2018).

In 2016, the World Bank also donated over USD$2 million to the West African Agricultural Productivity Program (WAAPP) in support of a Greenhouse Project to boost vegetable production in Ghana. This project supported the construction and operation of over 260 greenhouses across the country to grow vegetables under fully controlled environmental conditions for optimum growth and productivity (Ali Azara, personal communication, June 9, 2017).

Unfortunately, the greenhouses of EMQAP and WAAPP alongside with private ones established in the country have reported high incidence and severity of a devastating bacterial wilt disease of tomato. The disease is expressed by an overnight irreversible wilting without leaf yellowing or necrosis and the browning or discoloration of xylem tissue, with visible bacterial ooze (streaming) in water upon preliminary investigation. Wilting symptoms typically occurs first on the top young leaves and fits that of the bacterial wilt disease of tomato caused by *Ralstonia solanacearum* (Smith, 1984; Agrios 2005). The disease was reported in tomato fields in the Ashanti (Agogo, Akumadan) , Northern (Vea, Tono, Pwalugu), and Brong Ahafo (Tanoso, Tuobodom) regions of Ghana in 2012 (Subedi *et al.*, 2014).

Genin (2010) revealed that the modus operandi of the bacterium is the invasion of the xylem vessels of infected plant through the rhizosphere, and subsequent high rate of multiplication and spread to above-ground parts of infected host plants culminating in wilting and eventual death. The bacterial disease of tomato is the most difficult to control due to the extreme aggressiveness, extensive host range, wide geographical distribution, broad genetic diversity, multiple virulence factors and the ability of the causal organism to persist in soils and water (Hayward, 1991; Prior *et al.*, 1994; Elphinstone, 2005; Denny, 2006). Mansfield *et al*. (2012) ranked the bacterium worldwide as the second most scientifically and economically important phytopathogenic bacterium in 2012, after *Pseudomonas syringae*. This ranking was voted for by plant bacteriologists associated with the journal Molecular Plant Pathology worldwide.

The suspected pathogen, *Ralstonia solanacearum*, is an A2 quarantine pest classified by the European Plant Protection Organization (EPPO, 2004) and so endangering the export of Ghanaian tomatoes in international trade. There was, therefore, a critical need to identify the causal organism of the bacteria wilt disease of greenhouse tomato in southern Ghana to aid in the development of control measures.

Several attempts by farmers to control the disease with synthetic chemicals has been ineffective and moreover, the use of organo-synthetic pesticides and fumigants in managing soil-borne pathogens of vegetables has been made difficult by the growing regional and international legislation and restrictions, such as the Montreal Protocol and the Clean Air Act 1987. This is due, mainly to their toxicity to users, high persistence in the environment and pesticide residue in food crops (Asante and Ntow, 2009). However, the use of resistant varieties to control bacterial wilt has been observed to be cost-effective, eco-friendly and an effective control strategy (Sharma *et al*., 2006; Kim *et al*., 2016). Varietal trials identified Cherry Jaspur genotype of tomato to be of high resistance, ATL- 01-19, Pant T-10 and CO-3 as moderately resistant against the bacterial wilt disease and other tomato lines as highly susceptible to the bacterial wilt disease (Dutta and Rahman, 2012; Tiwari *et al*., 2012).

The objectives of the study were therefore to:

1. Conduct a questionnaire survey to assess farmer's knowledge and experiences on the prevalence, spread, detection and control of a bacterial wilt disease of greenhouse tomato in Volta, Eastern, Central and Greater Accra regions of Ghana.
2. Determine the incidence and severity of the bacterial wilt disease in greenhouse tomatoes in the four regions of Ghana.
3. Identify and characterize the causal organism of the bacterial wilt disease in southern Ghana.
4. Confirm the source of inoculum for the bacterial wilt disease in greenhouses.
5. Screen six tomato hybrids (WACC 1, WACC 2, WACC 3, WACC 4, WACC 5 and Eva F1) for resistance/tolerance to the bacterial wilt disease.

CHAPTER TWO

LITERATURE REVIEW

2.1 Greenhouse tomato production in Ghana

The tomato sector in Ghana has failed to achieve its potential in terms of attaining yield as compared to other countries such as Burkina Faso (Bortey and Osuman, 2016) . Some major challenges faced by farmers include high input cost, poor accessibility to market, high perishability, competition from imports and pests and diseases (Robinson and Kolavalli, 2010).

To address some of these constraints in the Ghanaian horticultural sector and to increase export earnings of non-traditional agricultural produce through enhanced productivity, the Government of Ghana established four demonstration farms including greenhouses in each of four project regions to serve as centers of excellence to disseminate modern technologies in horticulture. This was under the Export Marketing and Quality Awareness Project (EMQAP) of the Ministry of Food and Agriculture (MOFA) and received donor support from the African Development Bank. These centers have houses for the packaging and storage of vegetables and fruits for export (Kasalu-Coffin *et al.*, 2005).

The West African Agricultural Productivity Programme (WAAPP) with support and funding from the World Bank also launched the 'Greenhouse Project' with the aim of boosting vegetable production in Ghana. This project allowed the construction and development of over 260 greenhouses across Ghana to grow vegetables such as tomato, sweet pepper and cucumber under fully controlled environmental conditions for optimum growth and productivity (Ali Azara, personal communication, June 9, 2017).

The advantages of the greenhouse technology, as indicated by Juárez-Maldonado *et al*. (2014) includes the provision of a barrier between the crops and the external environment, creating a near optimal microclimate condition for crop growth by controlling abiotic factors such as temperature, radiation, relative humidity, CO_2 concentration and biotic factors such as pests and diseases.

Despite its high initial capital outlay, greenhouse farming technology has witnessed a steady adoption by some private vegetable farmers, especially in southern Ghana. Presently, a seemingly insurmountable challenge facing greenhouse tomato producers is the incidence and upsurge of tomato bacterial wilt (TBW) disease in the country. Nearly all greenhouses (government and private) in the country have reported the occurrence of the TBW, reducing output significantly.

2.2 Pests and diseases of greenhouses tomato in Ghana

The Greenhouse technology was to provide protection to high-value crops against harsh weather conditions and pests and pathogens which may cause losses in crop yield (Elings *et al*., 2015). However, pests and pathogens are able to invade these greenhouses due to poor management practices and other factors leading to the low output in production of greenhouse crops such tomato in Ghana (Elings *et al*., 2015).Tomatoes are attacked by different pathogens including; fungi, viruses, nematodes and bacteria (Chaudhry and Rashid, 2011). Information on various diseases and pests affecting tomato in Ghana is presented in Table 2.1.

Table 2.1: Diseases and pest of tomato in Ghana and their causal agents

Disease type	Name of Disease	Pathogen	Affected plant parts
Fungal	Leaf Mold	*Cladosporium herbarum*	Leaf
		Fulvia fulva	Leaf, fruit
	Early blight*	*Alternaria solani*	Leaf, stem
	Late blight*	*Phytophthora infestans*	Leaf
	Anthracnose	*Colletotrichum lindemuthianum*	Leaf, fruit
	Septoria leaf spot	*Septoria lycopersici*	Leaf
	Verticillium Wilt	*Verticillium albo-atrum*	Whole plant
	Pythium damping off*	*Pythium* spp.	Seedling
	Powdery Mildew	*Leveillula taurica*	Leaf
	Fusarium Wilt*	*Fusarium oxysporum*	Whole plant
Viral	Tobacco mosaic	*Tobacco mosaic virus*	Leaf
	Tomato yellow leaf	*Tomato yellow leaf virus*	Leaf
Bacterial	Bacterial canker*	*Clavibacter michiganensis*	Whole plant
	Bacterial spot*	*Xanthomonas vesicatoria*	Leaf, fruit, stem
	Bacterial speck*	*Pseudomonas syringae*	Leaf, flower, fruit
	Bacterial wilt*	*Ralstonia solanacearum*	Whole plant
Nematode	Root-knot	*Meloidogyne* spp.	Root
		Tylenchus spp.	Root
		Helicotylenchus multinctus	Root
Pest	Whitefly infestation*	*Whiteflies*	Leaf
	Mite invasion	*Mite*	Leaf
	Tomato boring	*Fruit borers*	Fruit
Physiological (Abiotic)	Blossom end rot*	*Calcium Deficiency*	Fruit
	Growth crack	*Noninfectious moisture related*	Fruit

Modified after Oduro (2000) **Important Disease, Pests and Disorders*

2.2.1 Bacterial diseases of greenhouse tomato

Field tomatoes and greenhouse tomato suffer severely from bacterial diseases causing much stress and destruction to affected plant. There may be total crop losses in greenhouse cultivation because it is an intensive system of production, and once there is a favourable environment in which the pathogens to thrive, their control becomes difficult (Tsitsigiannis *et al.*, 2008). Bacteria are the second most important phytopathogenic organism after fungi causing yield losses on tomatoes in both field and greenhouse production (Srinivasa *et al.*, 2012).

Ralstonia solanacearum, *Pseudomonas syringae*, *Xanthomonas vesicatoria* and *Clavibacter michiganensis* are the four main bacteria that damage tomatoes worldwide causing significant destruction and losses (Chaudhry and Rashid, 2011).These pathogens also parasitize other plants in the solanaceae family spreading easily through any suitable growth medium (Damicone and Brandenberger, 2015).

Xanthomonas vesicatoria and *Pseudomonas syringe* damages above ground parts of tomato plants and with spots (Zitter, 2001).Their optimum growth temperature is 18°C – 24°C and 24°C – 30°C respectively, and may be misdiagnosed for the other if not critically examined.

Bacteria wilt and canker of tomato are caused by *Ralstonia solanacearum* and *Clavibacter michiganensis* respectively (Tsitsigiannis *et al*., 2008). The presence of a heavy bacterial streaming from a cut stem placed in water helps to differentiate bacteria wilt from the other diseases (Mansfield *et al*., 2012). *Ralstonia solanacearum* is gram negative and aerobic whilst *Clavibacter michiganensis* is gram-positive and aerobic (Tamura *et al*., 2005).
Clavibacter michiganensis is a seed-borne bacterium and critical investigations have shown that it affects vulnerable tomato plants at both their young and mature stage but their symptoms and the intensity of infection differ for young transplants and older plants (Liu, 2015).

Bacteria diseases can affect crops either by systemic or foliar infection; systemic infection originates from infected seeds, diseased transplants, plant debris and vascular infection whilst foliar infection can come from splashing water and contaminated farm tools and machinery (Tsitsigiannis *et al*., 2008). Some control methods for the disease include the use of certified disease-free seeds, soil sterilization, disinfection of tools, rouging infested plants and good management practices.

2.3 Description of the bacteria wilt pathogen, *Ralstonia solanacearum*

Ralstonia solanacearum (RS) (Smith, 1896) formerly known as *Pseudomonas solanacearum*, is the biotic causal agent of tomato bacterial wilt (TBW) disease. It is rod-shaped, Gram-negative, non-sporing, a non-capsulate and monotrichous bacterium of about 0.5-0.7 µm x 1.5-2.0 µm (Sneath *et al*., 1986; Couthino *et al*., 2000; Champoiseau *et al*., 2009) that accumulates poly-β-hydroxylbutyrate intracellularly (Denny, 2006). It is oxidase positive and arginine dihydrolase negative, with minimum, optimum and maximum growth temperatures of 10°C, 35°C and 40°C respectively (Kelman, 1953; Denny, 2007). No growth of the organism has been observed at 40°C or 4°C (EPPO, 2004). Additionally, strains from the tropics worldwide have a high optimum growth temperature of 35°C.

Ralstonia solanacearum (RS) is an aerobic obligate organism and a raft of media is used to identify the bacteria. The media 2, 3, 5-triphenyltetrazolium chloride (TZC) (Kelman and Person, 1961) is the most commonly used semi-selective medium for its culturing (Tahat and Sijan, 2010). The growth medium groups *Ralstonia solanacearum* (RS) into virulent and avirulent morphological strains. The virulent strains are typified by unstable fluidal or mucoid colonies with white, pink centers whereas the avirulent strains are characterized by stable non-fluidal or non-mucoid colonies with dark red pigmentation (Kelman, 1954; Denny, 2001). HE (1983), who observed fluidal, central pinkish or light red colonies by virulent strains of the organism after 48h, confirmed this distinction of the strains of RS on TZC medium. In Ghana, Subedi *et al.* (2014), investigated the first report of bacterial wilt caused by *Ralstonia solanacearum* (RS) and discovered that bacterial ooze plated on TZC medium were fluidal, irregularly round, white with pink centers, gram-negative and oxidase positive.

Semi-selective Medium, South Africa (SMSA) is another universal significant medium for culturing RS colony. The characteristic of RS on this medium is similar to that observed on

TZC. Kinyua *et al.* (2014) reported RS appearing as mucoid, whitish colonies after 48h, and upon subsequent incubation, the colonies develop blood red whorls in the center. Same observations of RS on SMSA were made by Makari-Hanumanthappa *et al.* (2013). SMSA is preferred for isolating RS from soils (Englebrecht, 1994; Elphinstone *et al*. 1996) because it contains antibiotics and fungicides that inhibit the growth of competing saprophytic bacteria or fungi (Champoiseau *et al*., 2010).

On nutrient agar (NA), bacterial colonies become visible after 36h to 48h of culturing, and colonies of the normal or virulent type are white or cream-colored, irregularly shaped, highly fluidal and opaque. Colonies of RS isolated from eucalyptus in South Africa on nutrient agar were creamy in colour, fluid, smooth and elevated (Coutinho *et al*., 2000). Zehr (1970) reported circular, convex with irregular margins, fluid, grayish-white or brownish-white colonies on nutrient agar, with 2-3 mm in diameter. Makari-Hanumanthappa *et al.* (2013) also showed white-cream colonies of RS on NA. On potato-dextrose agar plus 1% peptone (PDPA), Zehr (1970), noted a greater variation in colony pigmentation of RS isolates. After 48h, colonies were 3.5 mm in diameter and very fluid with pinkish-buff whilst others were white and opaque and some grayish-white and translucent.

Ralstonia solanacearum (RS) is usually inhibited in acid media but favoured in alkaline conditions (Kelman, 1954). It can grow in 1% NaCl liquid media but little or none in 2% NaCl (EPPO, 2004). At room temperature, it can be stored in sterile de-ionized water. Long-term storage and survival can be achieved at -80^{o}C in liquid culture broth amended with 40% glycerol. Nonetheless, the pathogen has a high rate of loss of virulence and viability. Continuous transfer of RS on agar plates and storage of plates at 4^{o}C tend to hamper the pathogen's virulence and viability respectively (Champoiseau *et al*.,2009).

2.3.1 Taxonomy of *Ralstonia solanacearum*

Ralstonia solanacearum historically was first discovered in tobacco in Granville County, North Carolina, USA in the 1880's and was morphologically classified as *Pseudomonas solanacearum* by Erwin F. Smith in 1896 (Kelman, 1954). Since then the bacterium's genera have been variously classified as *Bacillus* (Smith 1908; Kelman 1954), *Bacterium*, *Phytomonas*, *Xanthomonas*, *Pseudomonas* (Kelman, 1954), Burkholderia (Yabuuchi *et al*., 1992) and finally, *Ralstonia* based on its evolutionary and polyphasic phenotypic studies (Yabuuchi *et al*., 1995). It belongs to the rRNA homology group II and β-subdivision of the Proteobacteria of the family Burkholderiales (Buddenhagen and Kelman, 1964; Stackebrandt *et al*., 1988).

2.3.2 Strain differentiation of *Ralstonia solanacearum*

The bacteria is described as a species complex due to its extreme phenotypic, genetic, and ecological variability among strains (Allen and Ort, 2001; Fegan and Prior, 2005). Traditionally, two (2) different systems 'race and biovar' are commonly used to differentiate RS strains (Buddenhagen *et al.*, 1962; Buddenhagen and Kelman, 1964; Hayward, 1964).

Under race differentiation, which is poorly defined and taxonomically unreliable, the bacterium is grouped into five (5) races based on host range (Buddenhagen *et al.*, 1962). The Race 1 strains mainly attacks tobacco and other solanaceous crops; Race 2 strains are attack musaceous species including *Heliconia* sp. and triploid banana; Race 3 strains primarily attack potato; Race 4 strains are particularly virulent on ginger; and Race5 strains affect and cause disease on mulberry tree and are only found in China (HE, 1983).

Biovar characterization, on the other hand, is biochemical in nature and is based on the different strains' abilities to oxidize and utilizes some disaccharides (cellobiose, lactose and maltose) and hexose alcohols (dulcitol, mannitol, and sorbitol). By this classification, RS strains are divided into five biovars (Hayward, 1964). Biovar 1 strains metabolize none of the sugars; biovar 2 strains only metabolize disaccharides; biovar 3 strains metabolize all of the sugars; biovar 4 strains metabolize only hexose alcohols; and biovar 5 strains metabolize all of the sugars except dulcitol and sorbitol (Hayward, 1964; He, 1983). New RS isolates from the Amazon basin were differentiated from biovar 2 strains using ribose and trehalose (Hayward and Hartman, 1994) and named as biovar 2-Tor biovar N2. As a result, a new name was given to the original biovar 2 strains as 2-A. Meng (2013a) established that there was no association between biovars and races, except for biovar 2-A, which most of the time corresponds to race 3, and biovar 5, which is identical to race 5.

Seleim *et al.* (2014) confirmed the first incidence of bacterial wilt disease caused by RS biovar 2 race 1 on tomato in Egypt. All strains isolated by Adebayo and Ekpo (2006) in Nigeria utilized the three disaccharides and three hexose alcohols, falling into biovar 3 race 1, (Fajinmi and Fajinmi (2010)). Pradhanang *et al.* (2005) also found race 1, biovar 1 as the most important strain of tomato in the southeastern United States. Based on biochemical tests and utilization of sugar and sugar alcohols, RS isolates were grouped under biovar III by Maji and Chakrabartty (2014). Chakraborty and Sadhu (1994) reported the occurrence of *Pseudomonas* (*Ralstonia*) *solanacearum* biovar 3 in tomato, eggplant and potato in India. In Ghana, strains of RS were found to be biovar 1 and biovar 3 (based on the utilization of cellobiose, lactose, maltose, dulcitol, mannitol, and sorbitol) (Subedi *et al.*, 2014).

Currently, strains of RS are differentiated based on ancestral and geographical relatedness. The phylogeny of RS reveals it is a heterogeneous group of related but evolutionary distinct strains (Allen and Ort, 2001; Fegan and Prior, 2005; Mansfield *et al.*, 2012). RS is grouped into four phylotypes, which matches the geographical origin of the strains. It is also based on phylogenetic analysis of sequence data from the highly conserved 16S-26S internal transcribed spacer region and several gene regions related to pathogenicity, such as the endoglucanase locus (egl gene), the hrpB gene, and the mutS gene (Poussier *et al*., 2003; Allen and Ort., 2001; Fegan and Prior, 2005). Phylotype I primarily originates from Asia, phylotype II originates from America, phylotype III originates from Africa and surrounding islands in the Indian Ocean, and phylotype IV originates from Indonesia (Allen and Ort., 2001; Fegan and Prior, 2005). Phylotype differentiation of RS strains is achieved by multiplex polymerase chain reaction (PCR) amplification with universal primer pairs and phylotype specific primers (Opina *et al*., 1997; Fegan and Prior, 2005). Subedi *et al*. (2014) reported a band size of 280 bp amplicon using RS-specific primers for all test strains when working on tomato and pepper plants exhibiting symptoms of wilt symptoms in Ghana. The diversity among RS isolated from the southeastern United States had a 372 bp band (phylotype II) for some strains on tomatoes and 144 bp band (phylotype I) on pepper (Hong *et al*., 2012). Phylotype identification of 15 strains of RS from potato in India using multiplex PCR showed 144 bp amplicon belonging to phylotype I (Sagar *et al*., 2014)

2.3.3 Disease cycle and epidemiology of the *Ralstonia* wilt disease of tomato

Ralstonia solanacearum is a soil and water-borne bacterium which invades and inhabits the vascular tissue of host plants from the soil via root wounds, natural openings, and sites of secondary root emergence (Genin, 2010). Following vascular invasion, the pathogen induces colonization of the intercellular space of the root cortex and vascular parenchyma (Yao and Allen, 2006; Turner *et al.*, 2009), by producing and secreting copious amounts of extracellular products, cell wall-degrading enzymes and exopolysaccharides (EPS) controlled by a regulatory network that uses PhcA (Schell, 2000). There is a commensurate increase in the pathogen cell density (usually surpassing 10^9 CFU/g) and the accumulation of EPS in the vascular bundle, pith and cortex, clogs the water conduction system. This manifests in the characteristic wilting, chlorosis, stunting and the rapid death of infected plants (Genin and Denny, 2012; Jacobs *et al*., 2012; Monteiro *et al*., 2012). Severe infection of host plants is reported to be favoured by temperatures between 24°C and 35°C and decreases in aggressiveness when temperatures exceed 35°C or fall below 16°C (Hayward, 1991). After the death of infected hosts, large numbers of cells of RS are shed from the roots, providing a pathway for the bacterium to return to the soil and initiate new infections (Hayward, 1991). Infected and colonized plant cells show both external and internal symptoms.

Internal symptoms portray a progressive brown discoloration of the vascular tissue, mainly the xylem, at initial stages of infection. As the disease develops, the internal discoloration extends to portions of the pith and cortex and in course, caves into necrosis (Kelman, 1953). Slimy viscous ooze typically appears on the transverse-sectioned stems at the points corresponding to the vascular bundles (Smith, 1896). The most common external symptoms of the bacterial wilt disease are; irreversible wilting, stunting and leaf chlorosis (Kelman, 1953; Smith, 1944). Dwarfing and stunting are typical external symptoms of infected plants, which do not show wilting (Kelman, 1953; Smith, 1944). The pathogen also develops latent infection in asymptomatic hosts, with high populations of bacterial cells.

The spread of RS usually occurs through contaminated irrigation water, infected soil surface and planting materials, field supplies and equipment (Louws *et al*., 2010). The survival of RS in soil and aquatic habitats is greatly influenced by environmental (mainly temperature and soil moisture) factors and biological (such as antagonist microorganisms). Bacterial population decreases rapidly at low temperatures (< 4°C). In natural habitats, different strains can survive moderate winters in host weeds or in the rhizosphere of non-host plants, acting as inoculum reservoirs (Champoiseau *et al*., 2009). Elphinstone (1996) discovered that *Solanum dulcamara*, a semi-aquatic weed, is an important survival refuge and source of inoculum in Europe. In a similar study by Nortj (2015) in South Africa the major sources of inoculum were through infected plant material and infested soil. Phenotypic conversion is another survival strategy exploited by the bacterium to persist in the environment (Denny *et al.*, 1994 and Poussier *et al.*, 2003).

Some researchers have observed that the invasion and spread of RS is aided by flagellation, chemotaxis and aerotaxis (Tans-Kersten *et al*., 2001; Yao and Allen, 2007). In chemotaxis, root exudates of host plants attract RS in the soil (Yao and Allen, 2006a) whereas aerotaxis or energy taxis confers the ability to the pathogen to locate and interact with the host (Kelman and Hruschka, 1973; Yao and Allen, 2007). Flagellation, also bestows swimming motility to the bacterium to move from plant to plant under saturated soil conditions (Tans-Kersten *et al*., 2001).

2.3.4 Pathogenicity factors

Genomic sequence analyses of RS have shown that the causal organism has multiple virulence factors and effectors purported to overcome host defenses (Poueymiro and Genin, 2009). RS requires scores of gene products and factors to induce successful infection and disease development in its host.

Virulence or pathogenicity factors of RS are the hrp genes, which regulate disease development and hypersensitive reaction (HR) (Boucher *et al.*, 1992). Hrp mutants are unable to induce symptoms in susceptible host plants, resistant hosts, or non-hosts (Boucher *et al.*, 1992). The hrp genes are clustered on the megaplasmid (Genin and Boucher, 2004), and encode components of a T3SS and effector proteins. T3SSs contribute to pathogenesis by secreting effector proteins translocated inside host cells and accessory proteins supporting the translocation called translocators (Genin and Boucher, 2004; Meyer *et al*., 2006). This protein acts in the invasive stages of infection by overcoming plant defenses (Boucher *et al*, 2001; Meyer *et al*., 2006).

Ecological fitness or survival and virulence of RS are augmented by both flagella-driven swimming motility and type IV pili-driven twitching motility (Liu *et al.*, 2001; Kang *et al.*, 2002; Tans-Kersten *et al.*, 2004). While swimming motility does aid virulence only in the early stage of colonization and host invasion (Tans-Kersten *et al*., 2004; Meng *et al.*, 2011), the type IV pili and twitching motility are relevant for most developmental steps of wilt disease (Liu *et al*., 2001; Kang *et al*., 2002).

2.3.5 Host range and distribution

Thomas Jonathan Burrill in Japan reported the first incidence of bacterial wilt disease caused by RS on tuber rot of potato in 1890 (Gota, 1992). The disease has since seen a worldwide spread and is endemic in tropical, subtropical and temperate regions, due to the causal bacterium's capacity to adapt successfully to such regions (Elphinstone, 2005; Denny, 2006). In terms of host range, the bacterium has an unusually wide host range, as it has been isolated from over 200 plant species in 53 different botanical families (Kelman, 1953; Hayward and Hartman, 1994; Elphinstone, 2005).

The description of new host is uncommon due to continuous expansion of wide host range (Alvarez *et al*., 2010). Banana (*Musa paradisiaca*), plantain (*Musa sapientum*), eggplant (*Solanum melongena*), groundnut (*Arachis hypogaea*), *Heliconia* spp., potato (*Solanum tuberosum*), tobacco (*Nicotiana tobacum*), and tomato (*Solanum lycopersicum*), constitute the most essential widespread agronomic hosts (EPPO, 2004; Vailleau *et al.*, 2007; Genin and

Denny, 2012). The race and phylotype classification of RS offers enough information about the host range and distribution of bacterial wilt disease (Patil *et al*., 2012).

2.3.6 Economic importance of *Ralstonia solanacearum*

The global relevance of the disease is due to its destructive nature, wide host range and geographical distribution of the pathogen (Pradhanang *et al*., 2005). The colossal crop losses caused by the pathogen has been enormous in many parts of the world and with the limited information on the effect especially to subsistence agriculture makes it difficult to estimate the economic damage (Elphinstone, 2005; Hayward, 1991).

The bacterium causes severe damage and loss to farmers engaged in the greenhouse production of crops susceptible to the bacterium, especially tomatoes (Kinyua *et al*., 2014). The disease on tomatoes is called bacterial wilt; on potatoes, it is known as brown rot; on tobacco, it is named Granule wilt and on geranium, it is referred to as southern wilt.

Ralstonia solanacearum (RS) was ranked second among the top 10 scientifically / economically important phytopathogenic bacteria worldwide in molecular plant pathology in 2012, after *Pseudomonas syringae* (Mansfield *et al.*, 2012). The pathogen is grouped as a quarantine pest in Europe because of its huge economic losses to potatoes, notably the strain race 3 biovar 2 (Madden and Wheelis, 2003). It is listed as a bioterrorism select agent under the Agricultural Bioterrorism Protection Act of 2002 in the United States (Lambert, 2002).

The pathogen's wide host range has assessed its economic losses difficult to undertake (Nion *et al*., 2015). The host, cultivar, climate, soil type, cropping pattern, and strain are the determinants of the degree of crop losses (Kinyua *et al*., 2014). The extent of damage is commonly expressed on a crop-by-crop basis. Fields of potato and tomato in the southeastern United States were completely wiped out by RS (Kelman, 1953). Commercial tomato production, particularly in Southern Mississippi, southern Alabama and parts of Florida in the United States, were totally terminated by RS, worsening the socio-economic conditions of hundreds of farm families. In Ghana, Subedi *et al*. (2014) recorded a less than 20% incidence of symptomatic plants (tomato and pepper).

2.4 Management of bacterial wilt disease of tomato in greenhouse

2.4.1 Chemical control of bacterial wilt disease

Chemicals for tomato bacterial wilt (TBW) control in both greenhouse and open fields include Actigard (acibenzolar-S-methyl) and phosphorous acid (Pradhanang *et al*., 2005, Norman *et al*., 2006, Ji *et al*., 2007). Pesticides such as algicide (3-[3-indolyl] butanoic acid), fumigants (metam sodium, 1, 3-dichloropropene and chloropicrin) and plant activators that confer systematic resistance to tomato (validamycin A and validoxylamine) have been used to control TBW (Nion *et al*., 2015). A combination of methyl bromide, 1, 3-dichloropropene with chloropicrin had been reported to significantly reduce bacterial wilt of tomato in the field from 72% to 100% (Nion *et al*., 2015).

Effective control of TBW can be attained by seed treatment with Thiram 75 WDP before sowing, followed by 10 minutes dipping of seedling roots in 0.3% solution of Carbendazim 50 WP before transplanting, (Ajay and Shashi, 2012). The drenching of plant roots with copper oxychloride [$Cu(OH)_2$] 50 WP at 0.3% solution + 0.01% streptomycin solution, a month after transplanting has been observed to control the disease.

2.4.2 Biological control of bacterial wilt disease

There are ongoing intense investigations into a number of soil bacteria and plant growth promoting rhizobacteria (PGPR) for their prospects in the control of RS in small-scale experiments, whose commercialization and large-scale efficacy control trials are yet to be determined (Hass and Defago, 2005; Aliye *et al*., 2008; Xue *et al*., 2009; Champoiseau *et al*., 2010; Nguyen and Ranamukhaarachchi, 2010). Some disadvantages limiting commercialization and large scale is due to their poor performance and inconsistency in colonization on such a scale (Whipps *et al*., 2007). Other notable factors also include the degree of suppression by the BCAs which maybe too low or require high rates of application to be commercially acceptable (Whipps *et al*., 2007). *Bacillus* spp. and *Pseudomonas fluorescens* (Anuratha and Gnanamanickam 1990; Xue *et al*., 2009), *Stenotrophomonas maltophilia* (Messiha *et al*., 2007), *Streptomyces setonii* (Lemessa and Zeller, 2007) are among the antagonistic microorganisms used to control the *R. solanacearum* effectively. Ramesh and Phadke (2012) screened strains of Rhizobacteria for their antibacterial activities against RS and identified species of *Pseudomonas* and *Bacillus* to be most effective whilst, Kurabachew and Wydra (2013) observed inhibitory effect of *Pseudomonas* spp., *Serratia marcescens*, and *Bacillus cereus* on RS on tomato in an *in vitro* antibiosis.

A number of fungal biological control agents (BCAs) have also been noted to manage RS (Nion, *et al*., 2015). In pot cultures, Zhu and Yao (2004) found populations of RS in the rhizosphere, on root surfaces, and in the xylem of tomato plants decreased by 26.7, 79.3 and 81.7% respectively, following inoculation with *Glomus versiforme*. This induced systemic resistance was due to the increased contents of soluble phenols and cell-wall bound-phenols in the root tissue brought about by colonization of host by both RS and *G. versiforme.* Hass and Defago, 2005 also concluded that *Pythium oligandrum,* through the activation of ethylene-dependent signaling pathway and cell wall proteins, has the potential to control RS. *Shiitake mycelia* *leachate* was ascertained by Pacumbaba *et al*. (1999) in *in-vitro* studies to have an antibacterial property on RS. *Gigaspora margarita*, *Glomus mosseae*, and *Scutellospora* sp. which are endomycorrhizal fungi (Tahat *et al*., 2012) and *Parmotrema tinctorum*, a lichen has been identified as BCAs against *R. solanacearum* (Gomes *et al*., 2003).

2.4.3 Application of organic matter and plant residue to control bacterial wilt disease

The viability and survival of RS can be hindered by the decomposition of organic matter in the soil, by the restriction of available nutrients and release of secondary metabolites with varying degrees of inhibitory activities (Bailey and Lazarovits, 2003). During organic matter decomposition, the carbon released enhances soil antagonistic microbial populations and their predatory and inhibitory effects (Bailey and Lazarovits, 2003). Addition of organic amendments to soil has also been found to promote the activities of antagonistic microbes, and that they contain biologically active molecules and toxins which can affect soil pathogens (Akhtar and Malik, 2000).

Different organic matter sources have been shown to control RS (Nion *et al*., 2015). The efficacy of simple organic compounds, including amino acids, sugars and organic acids on BW of tomato has been evaluated. Application of lysine to a pumice culture medium (0.25mg g^{-1}) and soil (2.5mg g^{-1}) reduced BW in tomato by 85 to 100% (Nion and Toyota, 2008) and by 58 to 100% (Posas *et al*., 2007). Riboflavin, on the other hand, protected tobacco against RS through the induction of a series of defense responses and secondary metabolism in cell suspensions (Ling *et al*., 2006). Methyl gallate had been shown to exhibit strong bactericidal effects on RS (Fan *et al*., 2014).

2.4.4 Use of physical method to control bacterial wilt disease

Hot water treatment and soil solarization have been confirmed to be effective against RS (Nion *et al*., 2015). The use of transparent plastic mulches for 60 days prior to the planting of tomatoes has been reported to reduce the incidence of bacterial wilt appreciably (Vinh *et al.,* 2005). A reduction of overall bacterial population by 60%-97% and bacteria wilt incident by 50-75% was reported using heat treatment (Kongkiattikajorn and Thepa, 2007). Islam and Toyota (2004) discovered that lower moisture conditions (20–30% maximum water holding capacity) and pre-incubation at lower temperatures (4°C) reduced the incidence of the disease and interfered negatively with the survival of the pathogen.

The survival period of RS on geranium was 6 months at a constant temperature (°C), but declined rapidly in repeated winter temperature cycles of 2 days at 5°C followed by 2 days at −10°C (Scherf *et al.*, 2010). Investigating the mechanisms of soil solarization in controlling BW in the tomato (Baptista *et al.*,2006) observed that soil solarization reduced soil pH, K, Na, B, and Zn, microbial biomass, and microbial respiration in the soil, but soil chemical properties was not significantly affected.

2.4.5 Phytosanitation and cultural practices to control bacterial wilt disease

Farm sanitation, crop rotation, multiple cropping, and soil amendment, when used in association with other control measures, have the potential to drastically reduce TBW. Farm sanitation reduces inoculum volume and the spread of the disease from infected to non-infected plants. Continuous cropping with the same susceptible host plants however, lead to the build-up of the pathogen's populations, crop rotation avoids this detrimental effect (Kurle *et al.*, 2001; Janvier *et al*., 2007). Adhikari and Basnyat (1998) affirmed that when a susceptible tomato variety was grown after corn, lady's fingers, cowpea, or resistant tomato, the onset of BW was delayed by one or three weeks and that wilt severity was reduced by 20-26%. The production of potato crop rotated with sweet potato, wheat, carrots, maize, millet, sorghum, or phaseolus beans reduced the incidence of BW by 64% to 94% while the yield of potatoes was 1- to 3- fold higher than that of monoculture potatoes (Katafiire *et al*., 2005). In multi-cropping, the root exudates of Chinese chive (*Allium tuberosum*) minimized the infection of tomato plants of RS by 60% (Yu and Komada, 1999).

The combined effect of rock dust and commercial organic fertilizer was revealed to have reduced the incidence of BW in tomato. Amendment with rock dust was reported to be effective in lowering the incidence of BW in tomatoes (Li and Dong, 2013).

2.4.6 Use of host resistance by grafting to control bacterial wilt disease

The host-plant resistance of some solanaceous crops to RS has been previously reported (Lebeau *et al.*, 2011). In tomato, resistance to RS is mainly polygenic and demonstrated to be strain-specific (Thoquet *et al.*, 1996), with the resistance derived from intra- or interspecific sources (Wang *et al*., 1998; Lafortune *et al*., 2005). A variety of eggplant rootstocks by grafting, have been used to manage BW in tomato production, such as *Solanum melogena, S. marcrocarpon, S. aethiopicum* (gilo, shum, kumba group) and *S. torvum* (Gisbert *et al*., 2011). Also, wild relatives of tomatoes (*Solanum lycopersicum*, such as *S. habrochaites* is reported to be a good rootstock against soil-borne phytopathogens (Venema *et al.*, 2008).

2.4.7 Screening of resistant varieties to control bacterial wilt of tomatoes

The use of resistant varieties to control bacterial wilt of tomato has been observed to be cost-effective, eco-friendly and an effective control strategy (Kim *et al*., 2016). In recent times, there is much attention on breeding of resistant hybrids against bacteria wilt for crops in the solanaceous family and this has been affected by several factors which include the availability of resistant varieties, pathogen strain differentiation and the mechanism of plant-pathogen interaction (Nion *et al*., 2015). This makes the screening of tomato for resistance important in developing new breeds and the control of bacterial wilt.

The multiplication of the bacteria in resistant tomato plant stems was reduced drastically due to constrained movement of the pathogen from a xylem tissue to another (Nakaho *et al*., 2004). In a research involving the screening of 35 tomato lines against bacterial wilt, Hawaii 7996 expressed the highest level of resistance to bacterial wilt with other lines which include;BF-Okitsu 101, Hawaii 7997 and CRA 66 reporting high levels of resistance (Wang *et al.*,1998a).

In another study, Kim *et al*. (2016) determined the resistance of accessions of tomato hybrids, out of which four accessions (IT 201664, IT 201662, IT 201659 and IT 173773) were found to be resistant with IT 201664 having the highest resistance at a mean disease severity of 1.11 and two other accessions (K177647 and IT 173830) were moderately resistant to the pathogen. The study revealed an invasion and accumulation of bacteria cells in the xylem tissues of both susceptible and resistant varieties. Bacteria populations were low in the resistant varieties than in the susceptible varieties because of the thickness of the pit membrane of parenchyma cells and vessels of the resistant plants.

Marta (2017), also conducted an experiment on sixteen varieties of tomato to study their resistance against bacteria wilt and out of the total number, five varieties; Rewako F1, Amelia, Kaliurang, Melinda, and Servo were moderately resistant. Rewako F1 showed the highest resistance with a disease intensity of 7%.

Screening of tomato varieties for resistant against *Ralstonia solanacearum* and breeding of new resistant varieties is still ongoing in order to reduce the destruction and losses caused by the pathogen in tomato production all over the world.

2.4.8 Ozone water application on bacteria

Ozone is a natural gas composed of three oxygen atoms (O_3) having a strong odour and also blue in colour (Sunday, 2004). Ozone is differentiated from oxygen by one oxygen atom (O_2) and can be generated as gas from oxygen in air or concentrated oxygen. Decomposition of ozone is harmless because it does not produce chemical hazards but it leads to the production of oxygen (Sunday, 2004).

Ozone is considered as a safe disinfectant in the world and 5 times stronger than chlorine as a disinfectant. It is used for water treatment, food processing and storage, irrigation in agriculture and the destruction of microorganisms (Jaramillo, 2017). It can be applied as either ozone gas or ozone water (ozone dissolved in water) depending on the preference, application medium and conditions present. The use of ozone water in agriculture for irrigation protects the plants against diseases and kills pathogens which are difficult to control using alternative methods. Ozone has been observed to have an effect on bacteria cells by preventing growth and causing the death of both gram negative and positive bacteria (Thanomsub *et al*., 2002; Sunday, 2004).

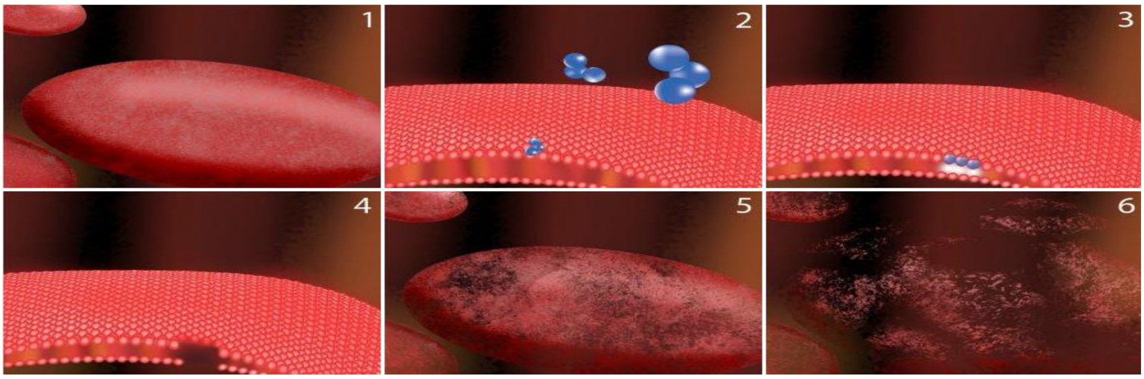

Figure 2.1: Effects of ozone on bacteria

1. A bacterium cell which has already found its way into your greenhouse via substrate or irrigation water.
2. The ozone when introduced first attaches its molecule to the cell wall of the bacteria cell (RS).
3. The contact causes a reaction called oxidative burst.
4. This causes small holes in the cell wall of the bacterium. The cell wall is an essential part of the bacteria in maintaining the shape of the organism.
5. The bacterium cell hence loses its ability to maintain it shapes and dies as illustrated from the figure above (ozonesolutions.com, 2014).

Ozone has been confirmed effective for controlling common pathogens in greenhouses without any phytotoxic effect on the plants at low doses (Zheng *et al.* 2014). More investigations and developments have to be made in the agricultural sector on the use of ozone as a disease control method and to promote its use as it is beneficial.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Selection of study area for assessment of greenhouse famers' knowledge and experiences on the prevalence, spread, detection and control of bacterial wilt of greenhouse tomato

The list of greenhouses visited and assessed and the number of respondents interviewed in respective communities in southern Ghana can be seen in (Table 3.1.) A total of 54 greenhouses were sampled and surveyed in Greater Accra, Eastern, Central and Volta Regions of Ghana. Greenhouses in each region were purposefully selected using a database acquired from the West African Agricultural Productivity Programme (WAAPP), which had records of all greenhouse operators in respective regions unevenly clustered into groups, and with the help of Agricultural Extension Agents (AEA's). The number of greenhouses selected for the survey was based on greenhouse operators known to produce tomatoes.

A survey was conducted between August and December 2017 in four regions - Volta, Greater Accra, Eastern and Central regions, to obtain information on prevalence, spread and control of bacterial wilt disease of greenhouse tomatoes. Fifty (50) greenhouse farmers were selected from the study area and a semi-structured questionnaire (Appendix 1) was designed, pre-tested and administered to elicit data on their knowledge and experiences on the prevalence, spread, detection and control of a bacterial wilt disease of tomato. Data collection, analysis and descriptive statistical analysis (means and percentages) was done using EPI INFO version 7.2.

Table 3.1: Regions, districts and communities selected for the survey, and the number of greenhouses in tomato production at the time of the survey and respondents interviewed

Region	District	Communities	No. of Greenhouse	No. of respondents
Greater Accra	Ningo Prampram	Dawhenya	5	7
	Adenta Municipal	Amrahia (Dairy farms)	1	1
	Tema-West	Borteyman	5	10
	Ashiaman Municipal	Adjei Kojo	13	3
Central	KEEA*	Nsadwir	4	8
Eastern	New Juabeng	Akwadum	10	10
	Nsawam Adoagyiri	Praso	1	2
	Denkyembour	FOHCREC*	1	1
	Shai Osudoku	Natriku	5	6
Volta	South Dayi	Adidome	2	1
		Dzemeni	2	1
	North Dayi	Vakpo	4	-
Total			54	50

*Forest and Horticultural Crops Research Centre (FOHCREC), Kade
*Komenda Edina Eguafo Abirem District (KEEA)

3.2 Determination of incidence and severity of bacterial wilt disease of tomato in greenhouse in the study area.

In each community, disease incidence and severity were assessed between August and December 2017. All plants in each greenhouse were assessed. Prior to the administration of the questionnaire to the farmers, a quick diagnosis of diseased tomato plants was carried out using wilting of leaves without foliar yellowing, streaming and an examination of vascular tissue of plant for vascular discolouration to distinguish between bacterial wilt and other wilts. Stream of milky exudates from the bacteria stream test was used as a confirmation for the presence of the bacterial (Goszczynska *et al*., 2000).

Disease incidence and severity assessment were carried out immediately after the interview and questionnaire administration within each greenhouse visited. Disease incidence was determined using the formula:

$$\textbf{\textit{Dome incidence}} = \frac{Total\ number\ of\ domes\ affected}{Total\ number\ of\ domes\ assessed} \times 100$$

$\textbf{\textit{Disease incidence in greenhouse}}$

$$= \frac{Number\ of\ diseased\ plant\ in\ greenhouse}{Total\ number\ of\ plants\ in\ greenhouse} \times 100$$

Disease severity was scored using a 0 – 4 rating scale modified after Horita and Tsuchiya (2001) and severity index determined with the formula:

$$\textbf{\textit{Disease severity in greenhouse}} = \frac{Sum\ of\ all\ ratings}{Total\ rating} \times Maximum\ disease\ grade$$

3.3 Isolation and identification of causal organism of the bacterial wilt disease of tomato

Tomato plants showing symptoms of the bacterial wilt were obtained from the various greenhouses during the survey and transported to the Plant Pathology Laboratory Department of Crop Science, University of Ghana. Stem segments (about 10 cm in length) from the collar region of wilted plants were washed thoroughly under running tap water to remove soil and debris. The cut tissues were surfaced sterilized with 70% ethanol for 60 seconds and rinsed with sterile distilled water under a laminar flow chamber. Disinfected stems were cut longitudinally and suspended in a beaker containing sterile distilled water (SDW) for five minutes. A loop full of the turbid stream suspension was then streaked on a 9 cm Petri dish containing nutrient agar (NA) using disposable inoculation loops and incubated for 2-3 days at 20-30°C. Daily observations were then made for colony growth.

The bacteria isolates were sub-cultured on nutrient agar and 2, 3, 5-triphenyl tetrazolium chloride medium (Casamino acid (casein hydrolysate), 1g; peptone, 10g; Glucose, 5g; Bacto-

Agar, 17 g per litre of distilled water, autoclaved at 121˚C for 20 minutes and 5 ml of 2, 3, 5-triphenyl tetrazolium chloride added after allowing to cool) to obtain pure colony forming units (CFU) of the bacterium. Pure isolates were kept in slants on nutrient agar and stored at 4˚C in a refrigerator until needed.

3.3.1 Potassium hydroxide solubility test on the pathogen causing bacterial wilt disease

Potassium hydroxide solubility test was carried out to ascertain the bacterium as plant pathogenic by placing a drop of 3% KOH solution on a 48-72 old pure culture of the bacterium on a slide glass. A disposable inoculation loop was used to stir for about 10 seconds and the loop raised to observe the formation of slime threads (Suslow *et al*., 1982).

3.3.2 Hypersensitivity reaction (HR) test on tobacco (*Nicotina tabacum*)

Bacteria isolates from affected plant tissues collected from various greenhouses were tested for hypersensitivity on tobacco (*Nicotina tabacum*). Tobacco seeds were nursed in pots using a soil starter (Fertiplus ® potting mix) and watered frequently. The seedlings were transferred to a screen house after four days of germination. Two test plants (potted seedlings) of tobacco at 3 weeks were selected for the hypersensitivity test. One seedling was inoculated with all bacteria isolates and the other potted plant was used as a check (inoculated with sterile distilled water).The test was conducted using an aqueous suspension of the bacterium from a 48 h bacteria cultures grown on TZC medium. A 5 ml sterile hypodermal syringe without a needle was used to inject the bacterial suspension into the underside of four tobacco leaves of the test plant. Sterile distilled water was injected in the underside on the control plant. The plants were kept in a screen house at 25-30°C and observed for leaf yellow chlorosis or necrosis (Lelliott and Stead, 1987; Schaad *et al*., 2001; Wick, 2010).

3.3.3 Gram staining on the pathogen causing bacterial wilt disease

Gram staining was conducted on all isolates. A loop full of 48-hour pure culture of all bacterium isolates were each smeared on sterile glass slide and fixed by slightly heating over a flame. The smear was then flooded with aqueous crystal solution (0.5%) for 30 seconds and then washed under running tap water for 4-6 sec. The fixed bacterium isolates were deluged with iodine for 1 minute and rinsed under tap water. The slides were blotted with tissue paper and decolorized with 95% ethanol for 10 seconds. The specimens were then washed with water and then counterstained with safranin for about 10 sec. Final washing of the stained specimens was done, air-dried and then the specimens observed under a microscope (OPTECH, B5P X1000). Pinkish or reddish cell wall confirmed Gram negative bacteria (Schaad, 1988).

3.3.4 Catalase oxidase test on the pathogen causing bacterial wilt disease

A pure culture of the bacterium (18-24 hr.) was fixed on a glass slide using a disposable inoculation loop. Few drops of hydrogen peroxide (3% H_2O_2) were added. The production of gas bubbles was observed by the naked eye and under a stereomicroscope 25X (Leica Biosystems, Germany) (Schaad, 1988).

3.3.5 Colony growth characteristics on 2, 3, 5-triphenyl tetrazolium chloride (TZC medium)

Bacteria colonies from all isolates cultured on nutrient agar were subculture onto a 9 cm Petri dish containing TZC medium using a sterilized disposable inoculation loop under a laminal flow chamber. Cultures were incubated for 2 to 5 days at 28ºC (Champoiseau, 2008).

Identification of bacteria isolates and growth was observed based on colony morphology and colour characteristic of *Ralstonia solanacearum* on TZC medium as described by Kelman (1954).

3.3.6 Molecular identification of the pathogen causing bacterial wilt disease

3.3.6.1 DNA extraction

Genomic DNA was extracted from bacteria isolates using a modified CTAB (cetyl trimethylammonium bromide) protocol described by William *et al*. (2012). A pure 24 h bacterial colony cultured on nutrient agar was used to inoculate 5 ml of CPG broth (Bacto casamino acids, 1.0 g; Bacto peptone, 10 g; and glucose, 10 g in 1 liter of water) in falcon tubes. The cultures were then grown at 28 $^{\circ}$C for 48 h in a shaking incubator at 200 resolutions per min (rpm) and harvested by centrifugation at 10,000 rpm for 5 minutes and the supernatant discarded. The resulting cell pellets were suspended and lysed in 740 µl of TE buffer (10mm Tris-HCL, 1 mm EDTA, pH 8.0) by vigorous pipetting. A 20 µl lysozyme (conc. 100 mg/ml) was added to the mixture and incubated for 30 min at 37 $^{\circ}$C. Sodium dodecyl sulfate (10%) and 18 µl of proteinase K (20 mg/ml) were added to the mixture and incubated for one hour. A 100 µl of 5 M NaCl solution and 100 µl of 10% CTAB solution in 0.7 M NaCl were added and thoroughly mixed to remove most protein and cell debris. The ensuing suspension was incubated for 10 min at 65 $^{\circ}$C and then kept on ice for 15 min. The mixture was then centrifuged at 10000 rpm for 10 min after the addition of 0.5 ml chloroform: isoamly alcohol (24:1). Isopropanol of 0.6 ml was added and incubated at -20 $^{\circ}$C for 2 hours and centrifuged at 10000 rpm for 15 min at 4 $^{\circ}$C. The DNA pellets were washed with cold 500 µl of 70% ethanol, air dried at room temperature for about three hours and re-dissolved in 50-µl TE buffer. DNA quality and quantity was checked by running 7 µl of extracted DNA on 1% agarose gel and concentration was adjusted to get 100 ng of DNA per µl of sterile distilled water. The extracted DNA was finally stored at -20 $^{\circ}$C until needed.

3.3.6.2 Multiplex polymerase chain reaction (PCR)

Phylotype identification, described by Fegan and Prior (2005) was used for strain identification of isolates. Phylotype specific multiplex PCR (Pmx-PCR) was carried out in a 25 µl final volume of reaction mixture, containing 1xTaq Mastermix (PCR buffer,1.5mM $MgCl_2$, 200 mM of each dNTP, 50 mM KCL, 10 mM Tris-HCL and 1.25U of Taq DNA polymerase) (England Biolabs INC., U.K), 6 pmoles of primers Nmult:21: 1F, Nmult: 21: 2F, Nmult: 22: InF,18 pmoles of the primer Nmult: 23: AF, Nmult: 22: RR and 4 pmoles of the primers 759 and 760 (Sagar *et al*., 2014) (Table 3.2).

The following cycling conditions were used in a thermocycler (BIO-RAD, Icycler, USA): 96˚C for 5 minutes; then 30 cycles of 94˚C for 15 seconds, 59˚C for 30 seconds and 72˚C for 30 seconds, followed by a final extension at 72˚C for 10 minutes (Kumar *et al*., 2004; Fegan and Prior, 2005)

Table 3.2: Primers used for phylotype strain identification of *Ralstonia solanacearum*

Primer Name	Primer Sequence	Expected Band Size (bp)	Phylotype category
Nmult:21:1F	5’-CGT TGA TGA GGC GCG CAA TTT-3’	144	Phylotype 1 (Asia)
Nmult:21:2F	5’-AAG TTA TGG ACG GTG GAA GTC-3’	372	Phylotype 2 (America)
Nmult:23:AF	5’-ATT ACG AGA GCA ATC GAA AGA TT-3’	91	Phylotype 3 (Africa)
Nmult:22:InF	5’-ATT GCC AAG ACG AGA GAA GTC-3’	213	Phylotype 4 (Tropical)
Nmult:22:RR	5’-TCG CTT GAC CCT ATA ACG AGA GTA-3’		Amorce reverse unique
759F 760R	5’-GTC GCC GTC AAC TCA CTT TCC-3’ 5’-GTC GCC GTC AGC AAT GCG GAA TCG-3’	280	*R. solanacearum* universal primers

Source: Sagar et al., 2014

3.3.6.3 Gel Electrophoresis

Polymerase chain reaction products or amplicons were separated by electrophoresis on 1.5% agarose gel stained with 5% ethidium bromide (5 µl). A 1.5% agarose gel was prepared by weighing 1.71 g of agarose powder into 114 ml of 1× TBE buffer in a conical flask. The content was heated in a microwave to dissolve. Upon cooling for five minutes, 5 µl ethidium bromide was added to the heated agarose mixture, swirled gently to reach a uniform mixture, and then poured into a casting tray to solidify. The taped ends of the casting tray were removed with the tray placed in an electrophoresis tank (containing 1× TBE buffer) and the combs removed after 30 minutes. Subsequently, a 10 µl aliquot of each PCR products (amplicons) was mixed with a loading dye and loaded into the wells of the gel using a sterilized pipette. The electrodes were then connected by closing the tank lid and the leads attached to the volt regulator set at 100V for 2 hours. The resulting bands were viewed on a UV trans-illuminator (BioDoc Imaging System, U.K)

3.4 Establishing the source of inoculum of bacterial wilt disease of greenhouse tomato in southern Ghana

In establishing the source of origin of the causal bacterium of the wilt disease, samples of plant growth media, tomato seeds and irrigation water were collected from each greenhouse during the disease survey. Samples were labelled and sealed in sterile transparent polythene bags, kept in an ice box and transported to the Plant Pathology Laboratory, Department of Crop Science, University of Ghana. Deoxyribonucleic acid (DNA) was isolated from of 2-3 days old cultures collected from the various sources and numerically coded for further confirmation using multiplex polymerase chain reaction and data was presented positive (+ve) or negative (-ve) for the presence of the bacterium. The universal primers 759 and 760 were used in amplification (Table 3.2).

3.4.1 Isolation of bacteria from tomato seeds affected by bacterial wilt disease

The method used by Umesha and Avinash (2015) was adapted for the isolation of the bacterium from the seed samples. Tomato seed samples collected from greenhouses during the survey were surface sterilized with 70% ethanol for 60 seconds and rinsed with sterile distilled water under a laminar flow chamber. Liquid assays of the collected seeds were prepared by macerating 5 g of the seeds using sterile mortar and pestle in 10 ml of sterile distilled water (SDW). One ml of the resultant supernatant was mixed into 9 ml of SDW to obtain a dilution of 10^{-1} which serially diluted to 10^{-5}. A loop full of each dilution was streaked on nutrient agar and incubated for bacteria isolation and purification.

3.4.2 Isolation of bacteria from plant growth media

A magnetic shaker was used to thoroughly mix 20 g of the nutrient agar in 100 ml SDW in 250 ml Erlenmeyer flasks for 10 minutes, and five-fold serial dilutions made from the resultant supernatant. A loop full of each dilution was aseptically streaked on NA media and the plates were incubated for colony observation and purification (Nguyen and Ranamukhaarachchi, 2010; Umesha and Avinash, 2015).

3.4.3 Isolation of bacteria from irrigation water

The collected water samples were transferred to sterile falcon tubes (50 ml) and serial dilutions of up to 10^{-5} of each water sample were made. Each dilution was then streaked on nutrient agar and observed for colony growth and further characterization.

3.5 Screening of tomato varieties for resistance/tolerance to the bacterial wilt disease of greenhouse tomatoes

The experiment was conducted in a screen house at the Nungua Livestock breeding station (WAAPP Nungua greenhouse cluster) at Borteyman in November 2017 using six tomato hybrids. Five out of six tomato varieties (WACC 1, WACC 2, WACC 3, WACC 4 and WACC 5) were acquired from the West African Centre for Crop Improvement (WACCI) at the University of Ghana, Legon, and Eva F1 variety was supplied by Dizengoff Ghana LTD. The five WACC tomato varieties were reported to be resistant to bacterial wilt of tomatoes (Dr. Agyemang Danquah, personal communication, September 4, 2017) and Eva F1 is a preferred variety of greenhouse farmers in Ghana (high yield) but reported as susceptible to the BWD and was used as a check.

3.5.1 Plant growth conditions and establishment

Seedlings were sown in a 72 seed-cell trays with a soil starter (Fertiplus® potting mix) and conventional nursery management practices were observed. Seedlings were transplanted after 2 weeks into a 12 L pots containing naturally infested coco peat and incubated in a screen house. The pots were arranged in a completely randomized design (CRD) with six treatments (variety) replicated 10 times. A daily application of NPK 19:19:19 soluble fertilizer (Poly-feed, Haifa chemicals®) at a rate of 200g / 15L of water was applied through fertigation using a drip irrigation system. Another nutrient supplement, (Agriful, Haifa chemicals®) was applied at the rate of 20 ml/15L every 5 days. A foliar application of Mix and Max at a rate of 20 ml/15L was sprayed on the plants every 5 days using a 15L knapsack sprayer. An application of Ca-B (Calcium oxide + Boron) at 65 ml/15L gallon was applied once every week for preventing calcium deficiencies. Multi K at 200 ml/15L was also applied after the third week.

The plants were sprayed with Agrithane® 80 WP (Mancozeb) at a rate of 2.7 g/L to control fungal diseases, and 2 ml/L lambda cyhalothrin insecticide to control insects using a 15L manual knapsack sprayer.

3.5.2 Inoculation of plant varieties

Bacteria cultures were suspended in sterile distilled water to make a suspension containing about of ($1x10^8$ CFU/mL) and used to inoculate transplants using a stem puncture technique (Winsted and Kelman, 1952). This involved the puncturing of stems of plants with a sharp needle through which a 5mL of bacterial suspension was injected into the axil of the second or third expanded leaf below the stem apex using a sterile syringe. The plants were monitored for wilt symptoms and disease incidence and severity determined. Re-isolation was done from symptomatic plants to confirm the presence of the pathogen during the trail.

Disease severity was scored on a 0-4 disease assessment scale (Table 3.3) modified after Horita and Tsuchiya (2001) as described by Shenge *et al*. (2007). Plants were evaluated at 0, 7, 14, 21, 28, 35, and 42 days post inoculation following the severity scale.

Table 3.3: Assessment scale for bacterial wilt disease of tomato

Index	Qualitative rating	Pictorial rating
0	No symptom/healthy plants	
1	Top young leaves wilted	

2	Clear typical symptoms of wilting	
3	Total wilt of plant	
4	Plant death/Plant roughed	

Source: Modified after Horita and Tsuchiya (2001)

The experiment was terminated on the 42nd day after inoculation and area under the disease-progressive curve (AUDPC) calculated for each variety after Shaner and Finney (1977) using the formula:

$$AUDPC = \sum^{n} [(X_{i+1} + X_i)/2]\,[t_{i+1} - t_i]$$

Where, Xi = the proportion of the host tissue wilted at i^{th} day

t_i= the time in days after the appearance of the disease at i^{th} day

n = the total number of observations

Data generated in the course of the study were analyzed using analysis of variance (ANOVA). Where significance differences existed, least significant difference procedure at 5% level of significance was used to separate means, using GenStat® (12^{th} Ed).

CHAPTER FOUR

RESULTS

4.1 Background of greenhouse farmers in Southern Ghana

In all the four regions under study, greenhouse tomato farms were mainly owned and operated by males (70%) with females representing 30%.

Majority (60%) of all greenhouse tomato farmers from the survey had attained tertiary education with 26% attaining senior high education and a few with no formal education (Table 4.1).

Table 4.1: Educational level of greenhouse tomato farmers in the study areas

Level of education	**Percentage %**
No formal Education	8.00
Primary	4.00
Junior High (JHS)	2.00
Senior High (SHS)	26.00
Tertiary	60.00
Total	**100**

4.1.1 Greenhouse use intensity and the proportion of greenhouses in operation in the study areas during the survey.

Most greenhouse tomato farmers (86%) have been engaged in greenhouse tomato production for 1-3 year whilst a few (8%) were in production for 4-6 years, and 6% had been in production for 7-9 years (Figure 4.1).

In Central region, only 20% of greenhouses visited were in operation whilst 52% were in operation in the Eastern region. The Greater Accra region had the highest number of greenhouses with (90%) in operation whilst the Volta region recorded no greenhouse activities (Fig. 4.2).

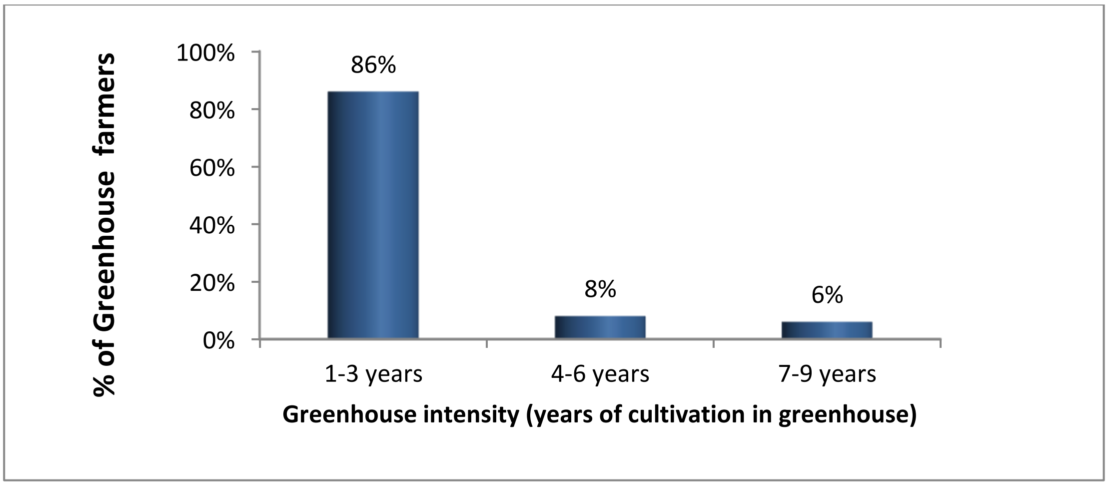

Figure 4.1: Greenhouse use intensity by farmers in tomato production in the study areas

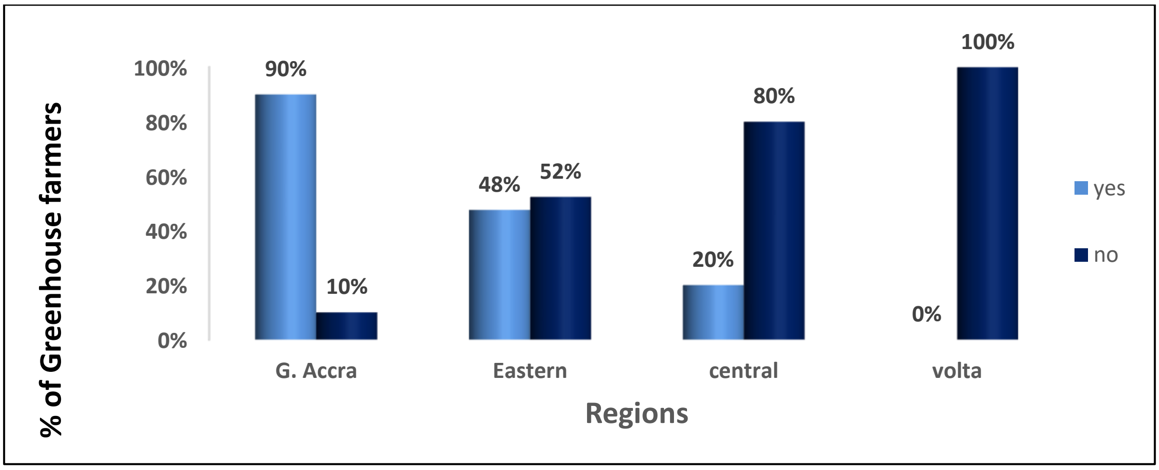

Figure 4.2: Percentage of greenhouses in operation categorized by regions

4.1.2 Cropping frequency of greenhouse farmers

Most greenhouse farmers (80.0%) in the Central region mentioned grew tomatoes once in a year with (20.00%) growing twice in a year. In the Eastern region, 66.67% of greenhouse farmers mentioned growing once a year with 33.33% growing twice a year.

Greater Accra region had a few (5.26%) greenhouse farmers growing tomatoes once a year with 68.42%, 10.53% and 15.79% of farmers growing twice, thrice and all year round respectively. Greenhouse tomato farmers (33.33%) in the Volta region grew tomatoes once a year with (66.67%) growing twice a year (Table 4.2).

Table 4.2: Cropping frequency of greenhouse farmers in the region surveyed

Cropping frequency/Year	G. Accra Region (%)	Eastern Region (%)	Central Region (%)	Volta Region (%)
Once	5	67	80	33
Twice	68	33	20	67
Thrice	11	0	0	0
Year Round	16	0	0	0
Total	100	100	100	100

4.1.3 Sources of plant material used by greenhouse tomato farmers.

The sources of planting material used by most greenhouse tomato farmers in the study areas were seeds bought from Dizengoff Gh. Ltd. (92%) and Agrimat Gh Ltd. (26%). Other sources of planting material were East-West Gh Ltd. (12%), Imported (8%), local market (8%) and other Agro dealers (14%) (Table 4.3).

Table 4.3: Source of planting materials for greenhouse tomato production in the study areas

Source of seed	Frequency	Percentage of farmers (%)
Dizengoff Gh Ltd.	46	92.00
Agrimat Gh Ltd.	13	26.00
East West Gh Ltd.	6	12.00
Imported	4	8.00
Local market	4	8.00
Local agro inputs	7	14.00

Farmers had multiple responses

4.1.4 Major pests and diseases affecting greenhouse tomatoes in the study areas.

The survey indicated that whitefly was the most prevalent pest (94%) infesting greenhouses in the study area, followed by Fusarium wilt disease (76%), blossom endrot (70%). Leaf curl, leaf miner and powdery mildew followed were similar with 46%, 46%, 44%, respectively. Bacterial wilt disease was reported by 44% of greenhouse farmers. Nematode (26%), spider mites (10%), aphids (10%), worms (8%), fruit cracking (4%) and caterpillar (2%) were other, pests and disorders reported (Table 4.4).

Table 4.4: Diseases, insect pests, and disorders affecting greenhouse tomatoes in the study areas

Disease/Pest/Disorder	Frequency	Percentage (%)
Whitefly	47	94
Fusarium wilt	38	76
Blossom end rot	35	70
Leaf curl	23	46
Leafminer	23	46
Powdery mildew	22	44
Bacterialwilt	22	44
late blight	21	42
Nematodes	13	26
Spider mite	5	10
Aphids	5	10
Worms	4	8
Fruit cracking	2	4
Caterpillar	1	2

Farmers had multiple responses

4.1.5 Sources of irrigation water used by greenhouse tomato farmers

Fifty percent (50%) of greenhouse farmers use borehole as a source of irrigation water. This was followed by the use of pipe-borne water (28%), river source (20%) and dams (2%), which are mostly pumped to poly-tanks and distributed via irrigation pipes within greenhouse (Fig. 4.3).

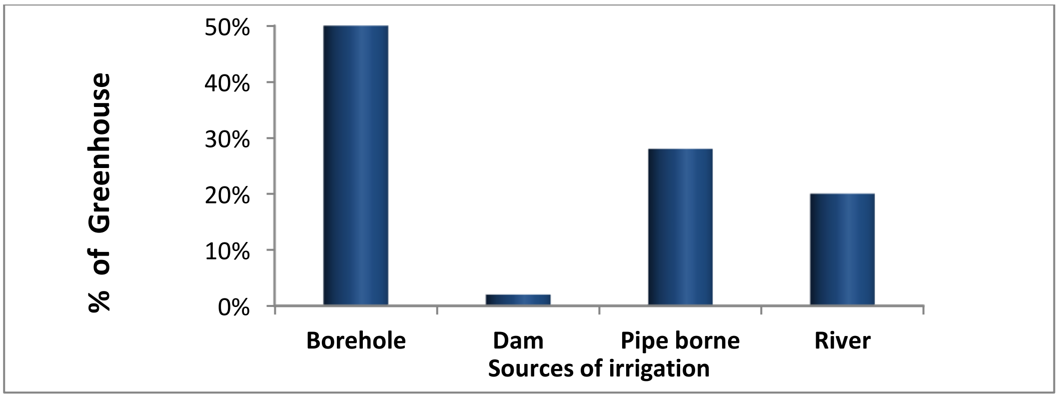

Figure 4.3: Sources of irrigation water used by greenhouse farmer's in the study areas

4.1.6 Tomato varieties commonly grown in greenhouse and their susceptibility to bacterial wilt disease

The most cultivated tomato varieties in greenhouse across all regions under study were Eva, Nimo necta, Tatiana, Pectomech, Anna and Padma (Table 4.5). Most greenhouse farmers (54%) did not know which varieties were susceptible to tomato bacterial wilt. However, Eva tomato variety, which was mostly grown by greenhouse farmers, was mentioned to be the most susceptible (28%). This was followed by Tatiana (14%), Anna (10%), Pectomech (10%), Nimo-Necta (8%), Roma (8%), 2013-4 (6 %,) ABM-152 (6%), 8014 (6%) and Jinping (2%) (Table 4.6).

Table 4.5: Tomato varieties grown by greenhouse farmers in the study areas

Varieties grown in Greenhouse	Frequency	Percentage (%)
Eva	44	88
Nimo Necta	30	60
Tatiana	27	54
Pectomech	23	46
Anna	17	34
Padma	11	22
Napoli	6	12
Roma	5	10
8014	3	6
2013-4	3	6
ABM 152	3	6
Jingping	2	4
Big guy	2	4
Martima	1	2
Jaguar	1	2
Money maker	1	2
Platinum	1	2
NKansah HT	1	2
Inlay	1	2

Farmers had multiple responses

Table 4.6: Greenhouse farmers' indication of susceptibility of tomato varieties grown in greenhouses to the bacterial wilt disease

Susceptible varieties	Frequency	Percentage farmers (%)
Dont know	27	54
Eva	14	28
Tatiana	7	14
Anna	5	10
Pectomech	5	10
Nimo Necta	4	8
Roma	4	8
2013-4	3	6
ABM 152	3	6
8014	3	6
Jingping	1	2

Farmers had multiple responses

4.1.7 Perception and experiences of greenhouse farmers' concerning prevalence, symptoms and awareness of bacterial wilt disease of tomato

Half of greenhouse farmers (50%) had heard about the bacterial wilt of tomato with 44% encountering the disease in their greenhouse. The remaining half of the greenhouse farmers interviewed had not heard about the bacteria wilt disease with 56% not encountering the disease in their greenhouses.

From the (Table 4.7), some greenhouse farmers (46%) interviewed had observed sudden wilt of tomatoes as symptoms of the disease in their greenhouses with 44% not knowing the symptoms of bacterial wilt of tomatoes. Some greenhouse farmers (40%) had observed low yield in infected plants. Stunting of tomato plant (26%), discoloration of vascular tissue and (6%) sudden death (14%) were other notable symptoms of the BWD known to greenhouse farmers during the survey.

Table 4.7: Knowledge of greenhouse tomato farmers on symptoms of bacterial wilt disease

BWD symptom criterion mention by Greenhouse farmers	Frequency	Percentage (%)
Sudden wilt of plant	23	46
Dont know	22	44
Low production	20	40
Stunting of plants	13	26
Sudden death	7	14
Discoloration of vascular bundle	3	6
Green and appears cooked	3	6
Oozing of whitish substance	0	0

Farmers had multiple responses

4.1.8 Growth stage of greenhouse tomato at which bacteria affect tomatoes.

From the survey, 60% of greenhouse farmers did not know the growth stage of tomato at which the bacteria wilt affects tomatoes. However, 22% had observed bacteria wilt on their tomatoes in all the growth stages with 10% and 8%, observing the disease in the flowering stage and vegetative stages of the crop (Figure 4.4).

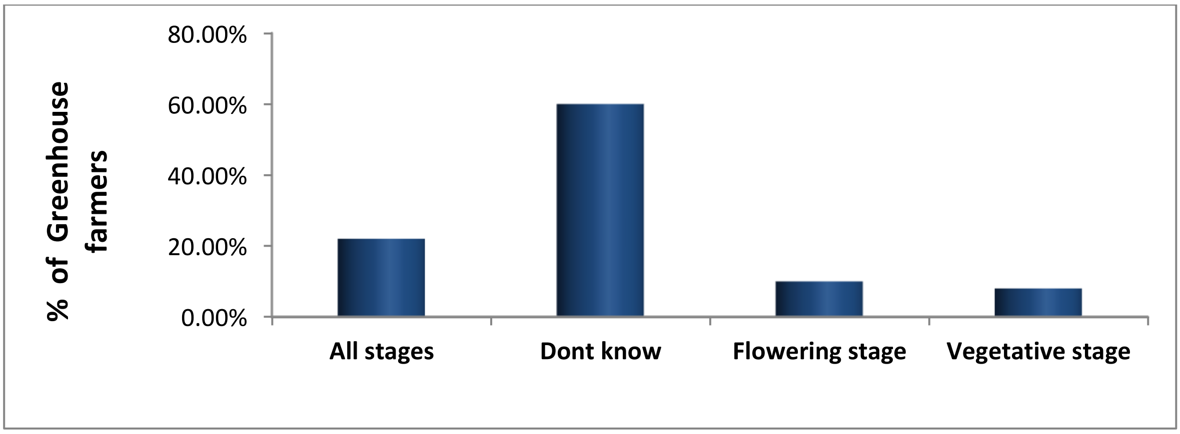

Figure 4.4: Perception of greenhouse farmers on growth stages of the plant where the disease occurs.

4.1.9 Seasonal prevalence of bacterial wilt of tomatoes in greenhouses

Generally, greenhouse farmers (60%) were not aware of the season in which the disease occurred or was prevalent. However, (26%) of them were of the view that the disease occurred during the wet seasons or when humidity were high. Some of the greenhouse farmers (14%)

stated that the disease occurred in both seasons (wet season and dry season) and no farmer observed the disease in the dry season.

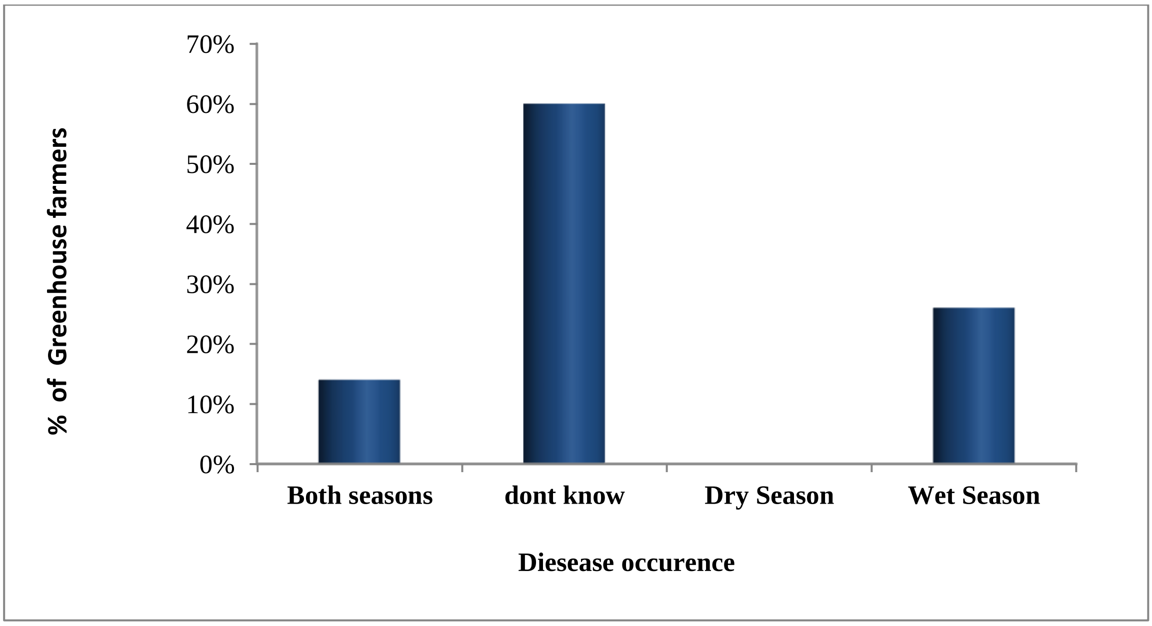

Figure 4.5: Greenhouse farmers' knowledge on season on when bacterial wilt of tomato occurs in the study area.

4.1.10 Proportion of greenhouse tomatoes affected by bacterial wilt disease.

Fig. 4.6 shows the proportion of tomato affected by the bacterial wilt disease in greenhouses surveyed. About 45.45% of greenhouse farmers observed 25%-50% of their tomatoes were affected by the bacterial wilt disease. Others (18.18%) observed 50%-75% of their tomatoes affected by the disease and about (36%) also had less than 25% of tomato plants affected by the disease.

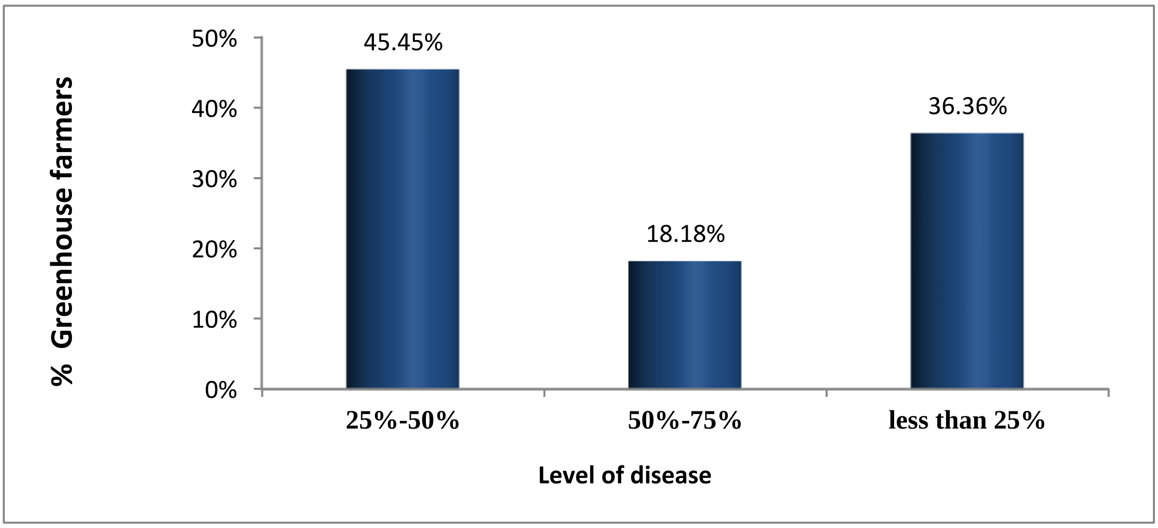

Figure 4.6: Incidence of bacterial wilt disease in greenhouse tomatoes in the study area

4.1.11 Awareness of greenhouse tomato farmers about the bacterial wilt disease of tomato

About 46% of greenhouse farmers interviewed mentioned they had not heard about the bacteria wilt of tomatoes. Some farmers 30% mentioned they had heard about the BWD from school whilst a few 16% mentioned they had heard the disease from extension officers who usually come to visit their greenhouse site. A minority of greenhouse farmers 2% had heard it from NGO/Workshop and others 2% from their own experience.

Table 4.8: Greenhouse farmers' source of information on BWD of tomato

Sources of information on BWD	**Frequency**	**Percentage famers (%)**
Ignorant of disease	23	46.00
Extension Officers	8	16.00
Fellow farmers	2	4.00
NGO/Workshop	1	2.00
Own experience	1	2.00
School	15	30.00

Farmers had multiple responses

4.2 Knowledge, perception and experiences of greenhouse farmer's concerning the detection of BWD

The survey showed that 28% of greenhouse farmers in the study area knew the test for detection of the bacterial wilt of tomato while 72% were ignorant about detection of the disease.

From the survey, 66% of the greenhouse farmers had no knowledge on detection of the disease. Thirty percent (30%) used wilting of plants to detect the bacteria wilt, 16% detected using the streaming test technique and 10% used low production as a key indicator. Other farmers mentioned sudden wilt 6%, stunting of plants 6%, oozing of whitish substance 6%, green with cooked appearance 4% and a brown discoloration of vascular tissues 4% (Table 4.9).

Table 4.9: Percentage of greenhouse farmers' responds to the detection of TBW

Criterion for detection of TBW by farmers.	Frequency	Percentage of farmers (%)
no idea	33	66
Wilting of plants	15	30
Streaming test	8	16
Low production	5	10
Sudden death	3	6
Stunting of plants	3	6
oozing of whitish substance	3	6
Green and appears cooked	2	4
Brown discolouration of tissues(CS)	2	4

Farmers had multiple responses

4.2.1 Greenhouse farmers' sources of information on the detection of BWD.

Tables 4.10, below shows sources of information on detection of bacteria wilt of tomatoes by farmers. Most farmers (66%) knew how to detect the BWD with 10% learning its detection from school. A few farmers (6%) were taught to detect the disease by extension officers, 2% colleague farmers, (2%) from NGO/Workshop and 8% through personal experience.

Table 4.10: Source of information on detection of bacterial wilt disease of greenhouse farmers

Source of information	Frequency	Percentage of farmers (%)
Don't know	33	66
Extension Officer	6	12
Fellow farmers	1	2
NGO/Workshop	1	2
Own experience	4	8
School	5	10
Total	50	100

4.3 Perception of greenhouse farmers on the causes, spread and control of bacterial wilt disease.

Greenhouse farmers in the study area attributed BWD disease to various causes (Fig. 4.7). Most of the greenhouse farmers (70.83%) attributed the cause of the disease to bacteria, while (29.17%) were ignorant of the cause of the disease. No farmer attributed the cause to fungi, nematode and insects.

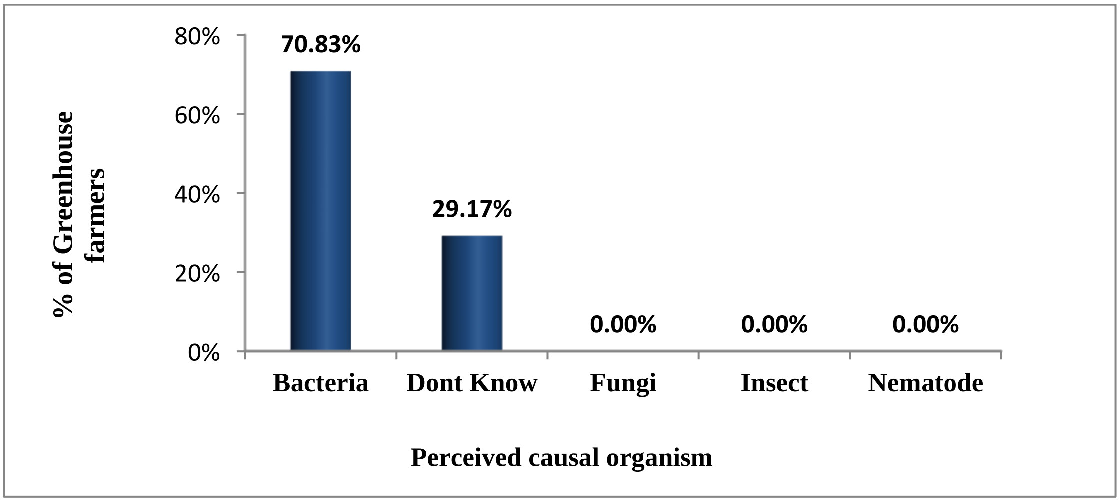

Figure 4.7: Perception of greenhouse farmers' on the causal agent of TBWD

4.3.1 Knowledge of greenhouse farmers on spread under study area.

About 36% of greenhouse farmers knew how the disease spreads whilst 64% were ignorant. Some farmers attributed the spread to poor sanitation (36%), infected seeds (22%), contaminated tools (20%) and irrigation water (18%). Others (6%) attributed the transmission to insects whiles a few (4%) mentioned extreme temperatures and dust (2%) as means of spread (Table 4.11).

Table 4.11: Perception of greenhouse farmers' on spread of BWD

TBW spread criterion mentioned	Frequency	Percentage farmer (%)
Don't know	30	60
Poor sanitation	18	36
Infected seeds	11	22
Infected tools	10	20
Infected-irrigation water	9	18
Insect	3	6
Extreme temperatures	2	4
Dust	1	2
Wind	0	0

Farmers had multiple responses

4.3.2 Greenhouse farmers' source of information on spread of BWD

Table 4.12 shows the various sources of information from which greenhouse farmers learned the spread of bacterial wilt. Majority of the greenhouse farmers (62%) ignorant, 8% mentioned extension officers as a source of their information, 2% each stated that their source of information was from fellow farmers and NGO/Workshop with 14% and 12% learning from their own experience and school, respectively.

Table 4.12: Greenhouse farmers' source of information on the spread of BWD

Source of information on spread	Frequency	Percentage farmers (%)
Dont know	31	62.00
Extension Officers	4	8.00
Fellow farmers	1	2.00
NGO/Workshop	1	2.00
Own experience	7	14.00
School	6	12.00

Farmers had multiple responses

4.4 Greenhouse farmer's knowledge on the control of BWD

Majority of the greenhouse farmers (86%) perceived bacterial wilt of tomatoes as controllable whilst 12% did not know how the disease is controlled. A few greenhouse farmers (2%) believed BWD could not be controlled.

About 62% roughed and buried infected plant as a way to control the disease. Others (58%) practiced crop rotation, 36% sterilized their growth medium, (36%) drenched medium with bactericides and fungicides, (36%) planted resistant varieties, (20%) disposed of infected medium, (14%) does nothing, (10%) used healthy seeds and (6%) chlorination of irrigation water (Table 4.13).

Table 4.13: Greenhouse farmers' responses to control measures of BWD

TBW control criterion mentioned	Frequency	Percentage (%)
Roughing and burying of infected plant	31	62
Crop rotation	29	58
Sterilizing medium	18	36
Drenching with bactericides or fungicides	18	36
Planting resistant variety	18	36
disposing of infected medium	10	20
Does nothing/ Don't know	7	14
Using healthy seeds	5	10
Chlorination of irrigation water	3	6

Farmers had multiple responses

4.4.1 Greenhouse farmers' source of information on control of BWD.

Greenhouse farmers sources of information on how to control BWD shown in (Table 4.14) 12% of greenhouse farmers attributed their source of information to extension officers whilst (2%), (6%), (32%) and (48%) sourced information from fellow farmers, NGO/Workshop, own experience and school respectively.

Table 4.14: Greenhouse farmers' source of information on control of BWD

Source of information on control	Frequency	Percentage of farmers (%)
Extension Officers	6	12
Fellow farmers	1	2
NGO/Workshop	3	6
Own experience	16	32
School	24	48
Total	50	100

4.5 Challenges faced by greenhouse farmers in the production of tomatoes

There were various challenges faced by greenhouse farmers in greenhouse production of tomatoes during the survey. Pest and disease-related problems (98%) were noted as the most challenging with fire outbreaks (2%), wind destruction (2%) and cattle invasion (2%) respectively (Table 4.15).

Table 4.15: Some challenges faced by greenhouse farmers in the study area

Challenges faced by greenhouse farmers mentioned.	**Frequency**	**Percentage (%)**
Pest and disease problems	49	98
High maintenance cost	38	76
Lack of capital	22	44
High temperatures	21	42
NO power source	20	40
Lack of irrigation water	19	38
Theft	13	26
Flooding	8	16
fire outbreak	1	2
wind destruction	1	2
Cattle invasion	1	2

Farmers had multiple responses

Figure 4.8: Challenges faced by greenhouse tomato farmers in southern Ghana. (A) Unroofed greenhouse caused by a fire outbreak, (B and C) Abandoned greenhouses in the Volta region (D) Disease outbreak in a greenhouse in the Eastern region

4.6 Incidence and severity of bacterial wilt disease of tomato in the study area

Fifty-four (54) domes were assessed for incidence of BWD of tomato and out of these, 12 domes had experienced TBWD across the study area (Table 4.16). Disease incidence within greenhouses from the study ranged from (5.9%-18.5%) and severity ranging from (0.5-1.2) using a severity scale of 1-4. In all four regions, the Central region and Volta regions did not record of the disease in domes; with Greater Accra and Eastern region recording 22% dome incidence.

Communities such as Dawhenya in the Greater Accra region had disease incidence and severity of 18.5% and 1.2, respectively. Borteyman also recorded disease incidence and severity of 7.7% and 0.6 respectively. Kade in the Eastern region recorded an incidence of 8.2% with a 0.8 severity whilst Natriku had 5.9% incidence with a severity of 0.5 (Table 4.17).

Table 4.16: Dome incidence and severity of the BWD in the study area

Region	District	Communities	Number of Domes Assessed	Number of Domes Affected
Greater Accra	Ningo prampram	Dawhenya	5	1
	Adenta Municipal	Amrahia (Dairy farms)	1	0
	Tema-West	Borteyman	5	5
	Ashiaman Municipal	Adjei Kojo	13	0
Central	KEEA	Nsadwir	4	0
Eastern	New Juabeng	Akwadum	10	0
	Nsawam Adoagyiri	Praso	1	0
	Denkyembour	FOHCREC*	1	1
	Shai Osudoku	Natriku	5	5
Volta	South Dayi	Kpeve	1	0
		Dzemeni	4	0
	North Dayi	Vakpo	4	0
Total			54	12

*Forest and Horticultural Crops Research Centre (FOHCREC), Kade

*Komenda Edina Eguafo Abirem District (KEEA)

Table 4.17: Incidence and severity within greenhouses in the study area

Region	District	Community	Number of affected Domes	Incidence (%)	Severity (1-4)
Greater Accra	Ningo prampram	Dawhenya	1	18.5	1.2
	Tema West	Borteyman	5	7.7	0.6
Eastern	Denkyembour	FOHCREC*	1	8.2	0.8
	Shai Osudoku	Natriku	5	5.9	0.5
Mean				10.0	0.7

*Forest and Horticultural Crops Research Centre (FOHCREC), Kade

4.7 Identification of pathogen causing bacterial wilt disease of tomato using various tests

4.7.1 Gram stain reaction on bacterial isolates

Bacterial isolates from samples showing symptoms of the TBW disease revealed red short rod-shaped bacteria when subjected to Gram stain reaction indicating bacteria as Gram-negative- a characteristic of *Ralstonia solanacearum*.

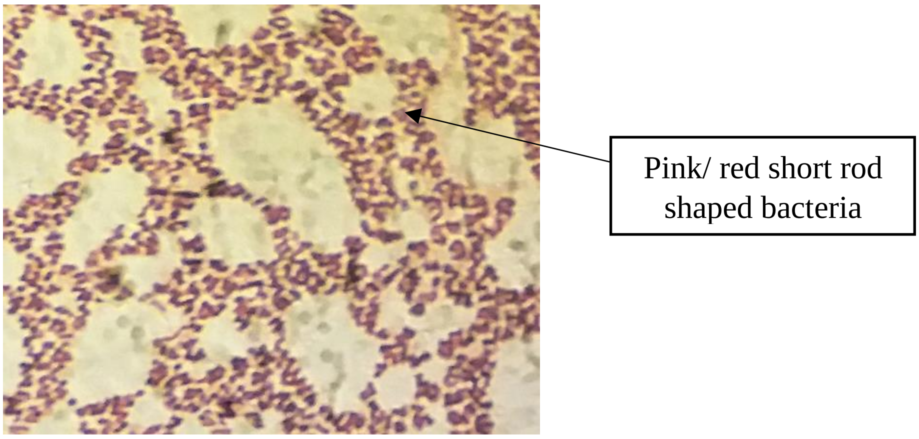

Figure 4.9: Micrograph of bacterial cells showing Gram-negative short rod shape bacteria isolates from irrigation water

4.7.2 Potassium hydroxide (KOH) solubility test on bacterial isolates

The production of mucoid and slimy threads was observed when bacterial colonies from diseased samples were mixed with 3% KOH, stirred and picked with a disposable inoculation loop. This observation also infers the bacteria isolates as plant pathogenic, which is also a characteristic of *Ralstonia solanacearum* (Fig. 4.11). This test was reported by Suslow *et al*. (1982) as a faster way to differentiate between Gram-negative and Gram-positive bacteria. The 3% KOH breaks down of the outer wall membrane of Gram-negative bacteria leading to the formation of slimy viscous thread whilst the Gram-positive bacteria tends to resist the break down due to its thick cell wall.

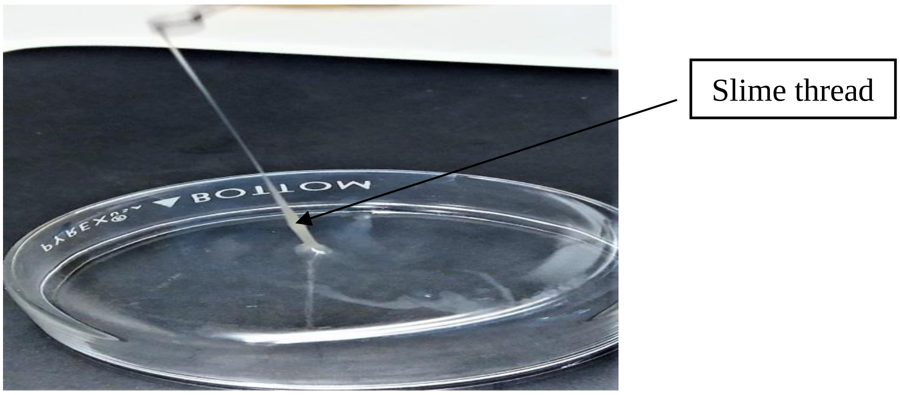

Figure 4.10: Mucoid slime thread observed after treatment of bacterial colony with 3% potassium hydroxide solution

4.7.3 Bacteria colony morphology on TZC medium on isolates

Bacterial cultures from diseased samples grown on TZC medium at 25-30^{o}C for 2-5 days, showed characteristic red center and whitish margin which are fluidal after 24 hours of incubation. This morphology characterizes them as virulent isolates (Fig. 11).

Figure 4.11: Colony morphology of bacteria (*R. solanacearum*) from irrigation water on TZC medium

4.7.4 Hypersensitivity test of bacterial isolates

Bacteria isolates from symptomatic plant tissue collected from the study areas during the survey indicated a positive hypersensitive reaction on tobacco 4 days after inoculation using a sterile syringe. The site of inoculation exhibited yellow chlorotic or necrotic symptoms which turned brown after 7 days (Fig. 4.12).

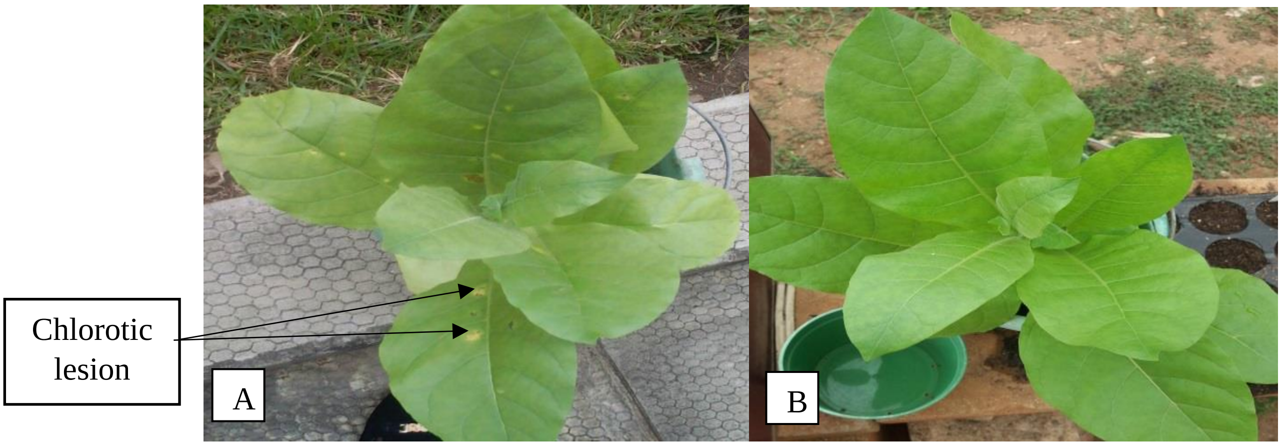

Figure 4.12: Hypersensitive reaction on tobacco plant (*Nicotina tabacum*) (A) Hypersensitive reaction on leaves 6 days after inoculation with the bacterium (B) Symptomless tobacco plant 7 after treatment with sterile distilled water (SDW); Control.

4.7.5 Molecular identification of bacterial isolates

4.7.5.1 Multiplex PCR amplification using *Ralstonia solanacearum* genus-specific primer

Result from phylotype specific multiplex PCR (Pmx- PCR) showed that *R. solanacearum* strains from Southern Ghana were Phylotype I (280 and 144 bp amplicon), Phylotype III (280 and 91 bp amplicon) and Phylotype IV (280 and 213 bp amplicon). Phylotype I were from Asia, and were characterized by 280 and 144bp amplicon, Phylotype III were from Africa characterized by 280 and 91 bp amplicon, Phylotype IV were from Tropics (Indonesia, Japan and Australia) characterized by 280 and 213 bp amplicon (Table 18). All samples were negative for Phylotype II, which is from America and characterized by 280 and 372 bp amplicons.

Table 4.18: Phylotype categorization of *Ralstonia solanacearum* from samples collected from communities in the study area.

Communities	Phyloptype I (Asia- 144bp)	Phyloptype II (America- 372bp)	Phyloptype III (Africa- 91bp)	Phyloptype IV (Tropical- 213bp)	Universal RS (280bp)
Dawhenya					
1. Plant samples	+	-	-	-	+
2. Wet substrate	+	-	+	+	+
3. Dry Substrate	+	-	+	+	+
4. Irrigation (Mains)	-	-	-	-	-
5. Dust	+	-	-	+	+
6. Irrigation (drip)	+	-	-	+	+
7. Seed	-	-	-	-	-
8. Seed	-	-	-	-	-
9. Seed	-	-	-	-	-
Amrahia (Diary farm)					
10. Plant tissue	-	-	-	-	-
11. Wet Substrate	-	-	-	-	-
12. Dry substrate	-	-	-	-	-
13. Irrigation	-	-	-	-	-
14. Seed	-	-	-	-	-
15. Seed	-	-	-	-	-
Nungua (Agric. farms)					
16. seed	-	-	-	-	-
17. seed	-	-	-	-	-
18. seed	-	-	-	-	-
19. Irrigation	-	-	-	-	-
20. Dry substrate	+	-	+	+	+
21. Plant sample GH1	+	-	+	+	+
22. Plant sample GH2	+	-	+	+	+
23. Plant sample GH3	+	-	+	+	+
24. Plant sample GH4	+	-	+	+	+
25. Plant sample GH5	+	-	+	+	+
26. Wet substrate GH1	+	-	+	+	+
27. Wet substrate GH2	+	-	+	+	+
28. Wet substrate GH3	+	-	+	+	+
29. Wet substrate GH4	+	-	+	-	+
30. Wet substrate GH5	+	-	+	-	+
Akwadum					
31. Wet substrate	-	-	-	-	-
Kade					
32. Plant tissue	+	-	+	+	+
33. Irrigation	-	-	-	+	+
34. Wet substrate	+	-	-	+	+
35. Seed	-	-	-	-	-

+ = Present
- = Absent

Table 4.19(Cont'd): Phylotype categorization of *Ralstonia solanacearum* from samples collected from communities in the study area.

Communities	Phyloptype I (Asia- 144bp)	Phyloptype II (America- 372bp)	Phyloptype III (Africa- 91bp)	Phyloptype IV (Tropical- 213bp)	Universal RS (280bp)
Nsadwir					
36. Dry Substrate	-	-	-	-	-
37. Irrigation	-	-	-	-	-
Praso					
38. Plant samples	-	-	-	-	-
39. Irrigation water	-	-	-	-	-
40. Substrate (in-use)	-	-	-	-	-
Vakpo					
41. Dry Substrate	-	-	-	-	-
Natriku					
42. Irrigation water	+	-	-	+	+
43. Dry Substrate	-	-	-	-	-
44. Plant Sample GH1	+	-	-	+	+
45. Plant Sample GH2	+	-	-	+	+
46. Plant Sample GH3	+	-	-	+	+
47. Plant Sample GH4	+	-	-	+	+
48. Plant Sample GH5	+	-	-	+	+
49. Wet Substrate GH1	+	-	-	+	+
50. Wet Substrate GH2	+	-	-	+	+
51. Wet Substrate GH3	+	-	-	+	+
52. Wet Substrate GH4	+	-	-	+	+
53. Wet Substrate GH5	+	-	-	+	+

+ = Present
- = Absent

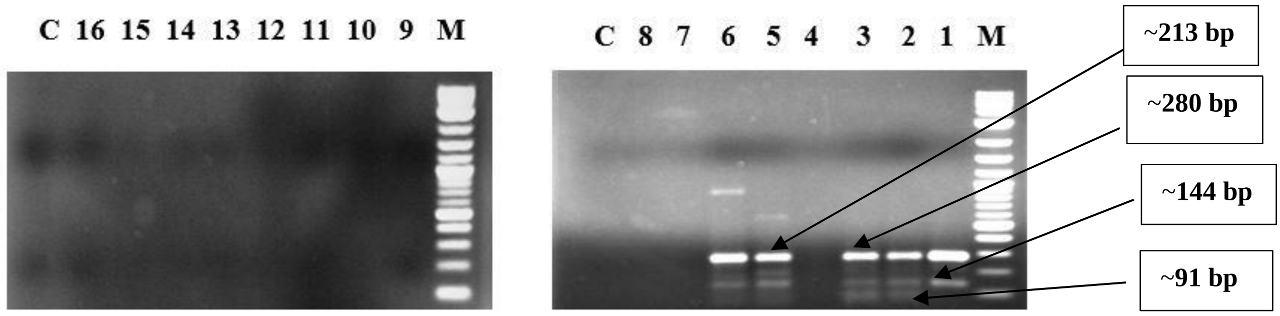

Figure 4.13: Phylotype specific multiplex PCR of (16) isolates from collected samples (Lane M = 1 kb ladder, lane 1-16 = suspected strains of *R. solanacearum*, lane C = control)

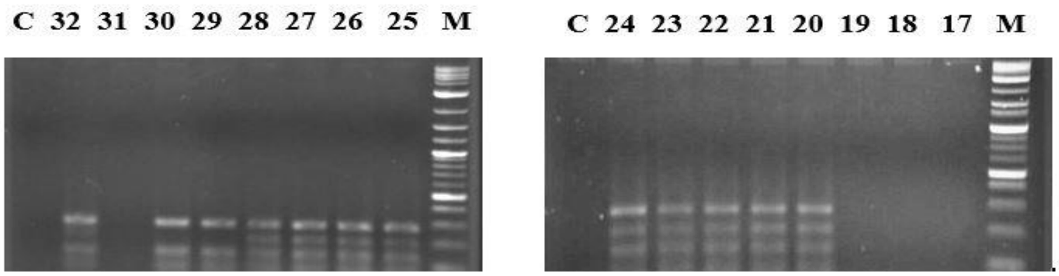

Figure 4.14: Phylotype specific multiplex PCR of (16) isolates from collected samples (Lane M = 1 kb ladder, lane 17-32 = suspected strains of *R. solanacearum*, lane C = control).

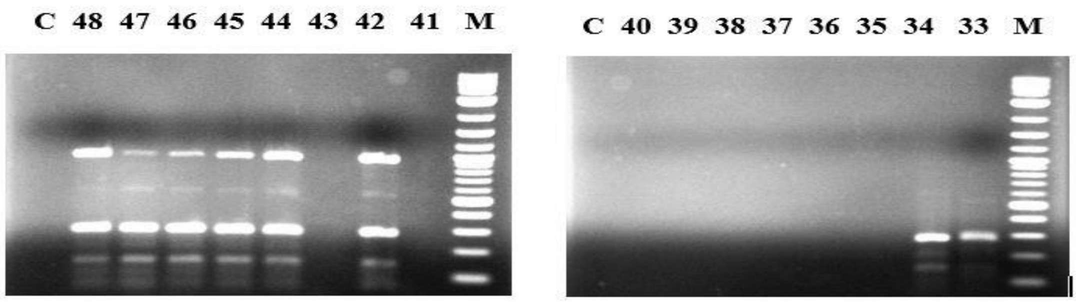

Figure 4.15: Phylotype specific multiplex PCR of (16) isolates from collected samples (Lane M = 1 kb ladder, lane 33-48 = suspected strains of *R. solanacearum*, lane C = control).

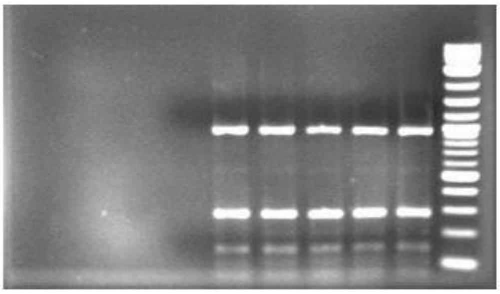

Figure 4.16: Phylotype specific multiplex PCR of (5) isolates from collected samples (Lane M = 1 kb ladder, lane 49-53 = suspected strains of *R. solanacearum*, lane C = control, X =empty wells)

4.8 Sources of inoculum for bacteria wilt disease of tomato in greenhouses.

The survey revealed sources by which the bacterium enters the greenhouses under study (Table 4.19). Samples collected from Dawhenya community which includes plant samples, wet substrate, dry substrate, dust sample and irrigation water were positive for RS but seed samples collected from the community tested negative (-ve) for the bacterium. In Borteyman community, samples collected which included plant tissues, wet substrate and dry substrate in all domes or greenhouses were positive (+ve) whereas irrigation water and seed samples were negative (-ve) for the bacterium.

At Kade, plant tissue, wet substrate and irrigation water samples were RS-positive but seed samples were RS-negative (-ve). All samples collected from Natriku were positive (+ve) for the bacterium (Table 4.19). Samples from communities such as Nsadwir, Vakpo, Praso, Akwadum and Amrahia were all negative for the bacterium.

Table 4.20: Sources of inoculum for bacterial wilt disease in greenhouse in Southern Ghana

Communities	Universal RS (280bp)	Communities	Universal RS (280bp)	Communities	Universal RS (280bp)
Dawhenya		**Amrahia (Dairy farm)**		**Vakpo**	
1. Plant samples	+	11. Plant tissue	-	44. Dry Substrate	-
2. Wet substrate	+	12. Wet Substrate	-		
3. Dry Substrate	+	13. Dry substrate	-		
4. Irrigation (Mains)	-	14. Irrigation	-		
5. Dust	+	15. Seed	-		
6. Irrigation (drip)	+	16. Seed	-		
7. Seed	-	17. control	-		
8. Seed	-				
9. Control	-				
10. Seed	-				
Nungua (Agric. farms)		**Akwadum**		**Natriku**	
18. seed	-	34. Wet substrate	-		
19. seed	-			45. Irrigation water	+
20. seed	-	**Kade**		46. Dry Substrate	+
21. irrigation	-	35. Plant tissue	+	47. Plant Sample GH1	+
22. Dry substrate	+	36. Irrigation	+	48. Plant Sample GH2	+
23. Plant sample GH1	+	37. Wet substrate	+	49. Plant Sample GH3	+
24. Plant sample GH2	+	38. seed	-	50. Plant Sample GH4	+
25. Plant sample GH3	+			51. Plant Sample GH5	+
26. Plant sample GH4	+	**Nsadwir**		52. Wet Substrate GH1	+
27. Plant sample GH5	+	39. Dry Substrate	-	53. Wet Substrate GH2	+
28. Wet substrate GH1	+	40. Irrigation	-	54. Wet Substrate GH3	+
29. Wet substrate GH2	+			55. Wet Substrate GH4	+
30. Wet substrate GH3	+	**Praso**		56. Wet Substrate GH5	+
31. Wet substrate GH4	+	41. Plant samples	-		
32. Wet substrate GH5	+	42. Irrigation water	-		
33. Control	-	43. Substrate (in-use)	-		

+ = Present
- = Absent

4.9 Screening of tomato varieties for resistance/tolerance to the bacterial wilt disease of tomato in greenhouses

All six varieties (WACC 1, WACC 2, WACC 3, WACC 4, WACC 5 and Eva F1) screened were susceptible to the bacterial wilt disease after they were inoculated with bacteria suspension of *Ralstonia solanacearum* (Figure 4.17 and Table 4.20). Symptoms of the disease were observed at different times (7, 14, 21, 28, 35 and 42 days after inoculation), and the most susceptible variety was the Eva variety with WACC 5 being the least susceptible.

The area under disease progressive curve (AUDPC) of the disease for all varieties increased steadily with time. Significant differences (P < 0.05) in disease severity (AUDPC) were observed amongst the varieties with WACC 2, WACC 4, WACC 5 having similar disease severity, but significantly higher than WACC 1 and lower than WACC 3 and Eva varieties (P < 0.05).

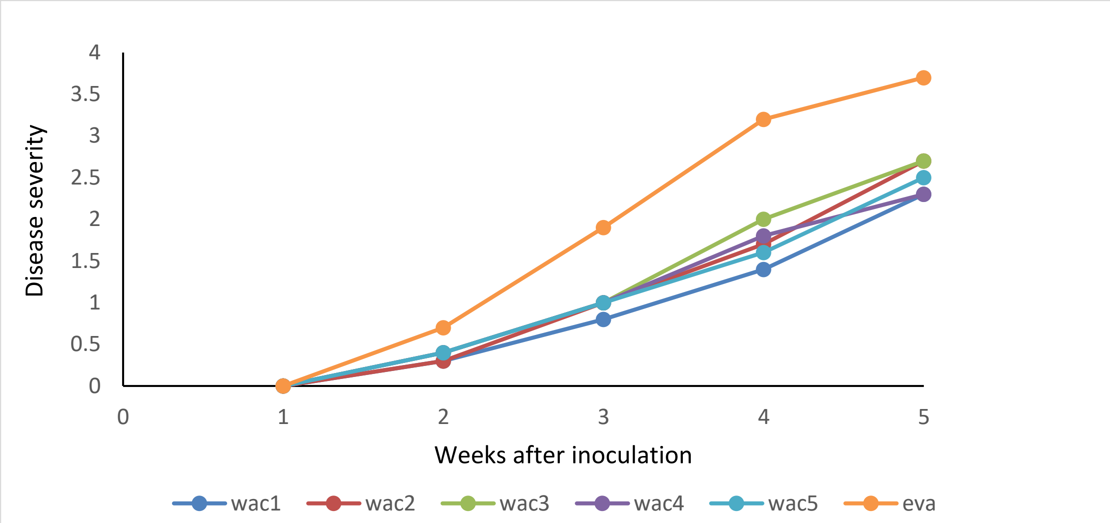

Figure 4.17: Disease progressive curve of bacterial wilt disease on six greenhouse tomato varieties

Table 4.21: Severity of the tomato bacteria wilt disease in six tomato varieties artificially inoculated with *Ralstonia solanacearum*

Tomato variety	AUDPC*
WACC 1	24.50
WACC 2	29.40
WACC 3	31.85
WACC 4	29.05
WACC 5	28.35
EVA	51.10
LSD(p<0.05)	1.50

*AUDPC (area under the disease progress curve) for tomato was calculated using the formula of Shaner and Finey (1977).

CHAPTER FIVE

DISCUSSION

This study confirmed *Ralstonia solanacearum* as the causal organism of bacterial wilt disease affecting greenhouse tomatoes in southern Ghana. The bacteria has been found to cause wilt of tomato both in greenhouses and open fields by other works (Zitter, 1985; Hayward, 1991; Subedi *et al*., 2014).

Majority of greenhouse farmers in the study area had been in greenhouse production for less than four years with tomato being the predominant crop grown, and then cucumber. Although tomatoes has a high capital input, high returns could be easily achieved due to high market demands for good and quality crops (Elings *et al*., 2015). The sources of seeds by majority of greenhouse farmers were from certified agro-dealers such as Dizengoff Gh. Ltd. (also suppliers of the Amiran greenhouse farmer's kit), Agrimat Gh. Ltd, East-West Gh. Ltd with a few greenhouse farmers sourcing their planting materials from the local market and hence had no traces of the disease from seeds. In contrast, some famers in Malawi had no available source of seeds with most using seeds from their own production. These seeds however, were latent sources of the bacterial wilt disease (Kagona, 2008).

Majority of greenhouse farmers had heard about the bacterial wilt disease but had little knowledge on its spread, detection and effective control. Similar observations were made by Onduso (2014), who noted that farmers had less knowledge about the disease especially with the poor disease identification of the bacterial wilt disease. This can lead to wrong disease management practices and further yield losses.

The causal organism was perceived to be a bacterium and this could be due to the high literacy rate of the farmers, which was a criterion for the selection and distribution of the greenhouse kits by WAAPP and EMQAP projects.

Most greenhouse farmers attributed the spread of the bacteria wilt disease to poor sanitation practices in greenhouses. A high incidence of the disease was also observed in greenhouses in Kenya due to poor hygiene practices (Onduso, 2014). *Ralstonia solanacearum* is a soil-borne bacterium and survives in soil, water, and plant materials for prolonged periods (Lopez and Biosca, 2004) and thus to prevent pathogens spread amongst greenhouses, it is important to use clean seeds, soil, water and tools (Meng, 2013).

The detection of bacterial wilt by greenhouse farmers was mostly by visual observation of wilt symptoms, with few farmers using bacteria stream for detection. Similar detection method were used by farmers in Ethiopia and Kenya (Kagona, 2008; Teyika *et al*., 2016). Bacterial stream test can be an effective diagnostic tool in detecting bacterial wilt disease in fields (Champoiseau *et al*., 2009). De Boer and Lopez. (2012) recommended the use of immunostrips for rapid and accurate identification of disease-causing organism in the field.

Greenhouse farmers also had inadequate information on the management and control of the disease, and so practiced the removal and burning of infected plants with few farmers adopting sterilization of substrate before use and crop rotation. Farmers in Kenya also controlled the bacterial wilt disease by uprooting, burying or burning infected plants (Kagona, 2008).

There was varying levels of disease incidence and severity amongst greenhouses in the study area. Out of the 54 greenhouses (domes) surveyed, 12 had an incidence of the bacterial wilt disease. The highest incidence was recorded in Dawhenya, followed by Borteyman, Kade and Natriku. Incidence and severity were low at the time of survey but progressed with time, with greenhouses in Dawhenya experiencing total crop loss two weeks after assessment was made. This could be attributed to the lack of knowledge on how to detect and also in management of the disease.

These findings were in line with Vanitha *et al*., (2009) who reported disease incidence of 34% in Mysore and up to 9% in Mandya district, 24% and 39% in Bangalore and Kolar districts respectively in India.

Onduso (2014) also reported the occurrence of the bacterial wilt disease on tomato in four counties in Kenya with Kiambu county, leading with a disease incidence of 37.4%, and as higher as 65% incidence recorded in a number of greenhouses. This he attributed to conditions in the green house that favoured pathogen multiplication.

Most farmers in the study area had totally abandoned their greenhouse or resorted into the cultivation of other crops such as cucumber due to the upsurge of the bacterial wilt disease.

The sudden spread and prevalence of the disease could be attributed to the high temperature and moisture in the greenhouses, which creates thriving conditions for the growth of the pathogen (Tsitsigiannis *et al*., 2008).

Strains from southern Ghana were identified as Phylotype I from Asia, Phylotype III from Africa and Phylotype IV from Tropics. Fegan and Prior (2005) described the Phylotypic classification system consisting of four phylotypes however, the Phylotype II strains from America was not identified in the study area. Subedi *et al*., (2014) identified Phylotype I and Phylotype III on tomatoes in Ghana. This is the first report of phylotype IV (Tropics) in Ghana. The exchange of infected planting materials such as substrate and seeds by RS strains may occur amongst countries and this can explain the introduction of phylotype IV in the country (Garcia *et al*., 2013).

Phylotyping is considered important for the strategic management and control of diseases as a variety said to be resistance to a phylotype can be susceptible to another phylotype (Sagar *et al*., 2014). There are several sources of inoculum of the bacterium in greenhouses and knowledge of these sources could aid in the control of the disease. Bacteria inoculum where found in irrigation water in communities such as Kade and Natriku. Waiganjo *et al.* (2006) also reported the spread of the disease through contaminated irrigation water. In Dawhenya, the source was contaminated substrate and Borteyman community had bacteria inoculum in substrate and plant tissues. Seed samples collected from all the greenhouses were not contaminated with the bacterium. However, in other studies, seeds have been noted to be a potential source of the disease (Zitter *et al.*, 1985; Abdurahman *et al*., 2017). The use of infected seedlings was among the most important means by which the disease spread (Nyangeri *et al.*, 1984; Ajanga, 1993). The adoption of good sanitation practices including the use of disinfectants in footbath at entrance of greenhouses, sterilization of substrates and the cleaning of equipment before and after use has been reported to reduce spread of diseases (Dudek, 2008; Meng, 2013).

The use of resistant tomato varieties for control of bacterial wilt disease has been observed to be eco-friendly, cost-effective and an effective means of control (Sharma *et al*., 2006; Kim *et al*., 2016). Tomato varieties acquired from the WACCI and EVA variety, which is preferred by the greenhouse farmers for its high yield, were all susceptible to the bacterial wilt disease. Surprisingly, the WACCI accessions were bred for resistance to the bacterial wilt disease Dr. Agyemang Danquah, personal communication, June 5, 2017). The susceptibility for the WACCI accessions could be attributed to breakdown in resistance in Ghana since they were bred in the United States of America outside the region in which it was developed (Zitter, 1985). In other studies, the use of resistance tomatoes accessions A4-7-1-1-5, THBW104, and THBW109 has shown high levels of resistance to the bacterial wilt disease (Techawongstein *et al*., 2007). More studies should be done on screening and developing of resistant varieties. The use of resistant rootstock in grafting for control of the bacterial wilt disease should be explored.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 CONCLUSION

- Majority of greenhouse farmers attributed the cause of the tomato wilt disease to a bacterium. Famers had little knowledge on how the disease was detected. The means of spread of the disease was unknown to the farmers and majority of farmers (86%) used roughing and burying of infected plants as means of controlling the disease.
- Of the four regions selected for study, bacterial wilt of tomato was observed to be prevalent in only the Greater Accra and the Eastern regions of Ghana with a total dome incidence of 22%.

  For the Greater Accra region, the highest incidence was recorded at Dawhenya (18.5%) followed by Borteyman community (7.7%) and in the Eastern Region, the highest incidence was recorded at Kade (8.2%) followed by Natriku (5.9%).
- The causal organism of bacterial wilt of tomatoes was identified as *Ralstonia solanacearum.*
- Sources of inoculum for the bacterial wilt disease in greenhouses were irrigation water and growth media.
- The five WACCI accessions (WACC1, WACC2, WACC3, WACC4, and WACC5) and Eva variety screened were all susceptible to the bacterial wilt disease.

6.2 RECOMMENDATION

- More samples of the causal organism should be isolated from other parts of the country for strain identification to aid in the testing of resistant varieties.
- Studies should be conducted on the use of botanicals for the effective control of bacterial wilt disease.

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APPENDICES

Appendix 1. Survey on farmer's knowledge, perception and experiences concerning prevalence, control, spread and detection of bacterial wilt of tomato in Greenhouse.

Questionnaire No..................................

A. Background of farmer

1. Name of Interviewee: ....
2. Gender: Male [ ] Female [ ]
3. Age: ....
4. Level of Education: No formal education [ ] Primary [ ] Secondary [ ] Tertiary [ ]

   Other (specify)...
5. Name of District
6. Name of community: ....
7. Major occupation or profession (aside greenhouse farming)?

   ....

B. Greenhouse use and intensity

8. What crops do you grow in your Greenhouse

   ☐ Tomato ☐ Okro ☐ Carrot ☐ Green pepper

   ☐ Cucumber ☐ Lettuce ☐ Cauliflower ☐ Sweet pepper

   ☐ Egg Plant ☐ Cabbage ☐ Chili pepper

   Region:

   Central Region [ ] Greater Accra Region []

   Eastern Region [ ] Volta Region [ ]

   In operation: Yes [ ] No [ ]

9. What is the size of your Greenhouse?

10 × 50m (500 m^2)	11.3 × 24m (271.2 m^2)	56 × 66m (3696 m^2)
60 × 11.3m (678 m^2)	8 × 15m (120 m^2)	

10. Type of Greenhouse

Environdome	Even Span	Gothic Arch	Quonset
Ridge and Furrow	Sawtooth	Unevenspan	

11. How long have you been in greenhouse farming (Years)? ………………………

12. What type of varieties do you grow in your Greenhouse?

☐ Eva	☐ Pectomech	☐ Tatiana	☐ Napoli	☐ Nemo Neta
☐ Inlay	☐ Nkansah	☐ Bigguy	☐ Chilibi	☐ Platinum
☐ Anna	☐ Jingping	☐ Jaguar	☐ Mongal	☐ Maya
☐ Padma	☐ Roma	☐ Martima	☐ Legon 18	☐ Momotaro

13. Where do you get your planting material?

☐ Agrimat	☐ Local markets	☐ Other Agro shops
☐ Dizengoff	☐ Friends/Farmers	
☐ East West	☐ Imported	

14. What diseases and pests affect tomato in your greenhouse? ……………………………

15. What your source of water?

Borehole [ ] Dam [ ] Lake [ ] Pipe borne [ ]

River [ ] Well [ ]

16. How many times do you grow in a season?

Once [ ]   Thrice [ ]   Twice [ ]   All Year Round [ ]

What substrate do you use in your greenhouse?

- [ ] Perlite
- [ ] Biochar
- [ ] Cocopeat
- [ ] Compost
- [ ] Saw dust
- [ ] Carbonated Rice Husk

17. Have you rotated your tomato crop before?   Yes [ ]   No[ ]

18. If Yes, What crop did you rotate with?

- [ ] Carrot
- [ ] Chilli pepper
- [ ] Eggplant
- [ ] Cucumber
- [ ] Sweet pepper
- [ ] Lettuce
- [ ] Green pepper
- [ ] Okro
- [ ] Cabbage

C. Farmer's knowledge, perception and experiences concerning prevalence

19. Have you heard about the bacterial wilt disease of tomato in greenhouse? Yes[ ] No [ ]

20. Have you encountered the bacterial wilt disease in your greenhouse? Yes [ ]   No [ ]

If Yes what are the symptoms of BWD

- [ ] Wilting of plants
- [ ] Green and appears cooked
- [ ] Low production
- [ ] Discoloration of xylem tissues (CS)
- [ ] Stunting of plants
- [ ] Oozing of whitish substance
- [ ] Sudden death
- [ ] Do not know

21. What percentage of your tomato did BWD affect?

Less than 25% [ ]   25-50% [ ]   50-75% [ ]   75-100% [ ]   100% [ ]

22. At what stage of growth does the disease affect tomatoes?

Flowering stage [ ]   Fruiting stage [ ]   Seeding stage [ ]   Vegetative stage [ ]

All stages [ ]   Do not know [ ]

23. What time of the year is BWD common?

Dry Season [ ] Wet Season [ ] Both Seasons [ ] Do not know [ ]

24. Which of the varieties are susceptible to BWD? (Arranging from least susceptible to most susceptible)

…………………………………………………………………………….

25. Where did you hear about BWD?

Extension officers [ ] Fellow farmers [ ] Newspaper [ ] NGO/Workshop [ ]

Posters [ ] School [ ] Television [ ] Brochures [ ]

Own Experience [ ] Parents [ ] Radio [ ] Do not know [ ]

26. When did it first happen in your greenhouse? ……………………………………….

27. What do you think is the cause of Bacteria Wilt?

Fungi [ ] Insect [ ] Nematode [ ] Bacteria [ ] Do not know [ ]

D. Farmer's knowledge, perception and experience concerning Detection

28. Do you know how to detect bacterial wilt? Yes [ ]No [ ]

29. If yes, how do you detect it?

☐ Streaming test ☐ Brown discolouration of tissues ☐ No idea

☐ Low production ☐ Oozing of whitish substance ☐ Stunting of plants

☐ Wilting of plants ☐ Green and appears cooked ☐ Sudden death

30. How did you learn to detect?

Extension officers [ ] Fellow farmers [ ] Newspaper [ ]NGO/Workshop [ ]

Posters [ ] School [ ] Television [ ] Brochures [ ]

Own Experience [ ] Parents [ ] Radio [ ] Do not know [ ]

E. Farmer's knowledge, perception and experience concerning Spread

31. Do you know how BWD spreads? Yes [ ] No [ ]

32. How did you learn its spread?

Extension officers [ ] Fellow farmers [ ] Newspaper [ ] NGO/Workshop [ ]

Posters [ ] School [ ] Television [ ] Brochures [ ]

Own Experience [ ] Parents [ ] Radio [ ] Do not know [ ]

33. How does the bacteria wilt spread?

☐ Insect ☐ Infected seeds ☐ Extreme temperatures

☐ Dust ☐ Infected tools ☐ Infected irrigation water

☐ Wind ☐ Poor sanitation ☐ Do not know

34. What crop will you grow if tomato fails due to BWD?

☐ Chili pepper ☐ Lettuce ☐ Cauliflower

☐ Green pepper ☐ Egg Plant ☐ Okro

☐ Cucumber ☐ Cabbage ☐ Carrot

Why? ☐

F. Farmer's knowledge, perception and experience concerning Control

35. Can the bacterial wilt be prevented? Yes [ ] No [ ]

36. Do you know how to control BWD? Yes [ ] No [ ]

37. If yes how do you think it can be prevented or controlled?

Planting resistant variety [ ] Disposing infected medium [ ] Selection of healthy seeds [ ]

Drenching with bactericides [ ] Roughing and burning [ ] Sterilization of medium [ ]

Chlorination of irrigation water [ ] Done nothing [ ]

38. How did you learn to control the BWD?

Extension officers [ ] Fellow farmers [ ] Newspaper [ ] NGO/Workshop [ ]

Posters [ ] School [ ] Television [ ] Brochures [ ]

Own Experience [ ] Parents [ ] Radio [ ] Do not know [ ]

39. What have you done or what will you do to control BWD in your greenhouse tomato?

Planting resistant variety [ ] Disposing infected medium [ ] Selection of healthy seeds [ ]

Drenching with bactericides [ ] Roughing and burning [ ] Sterilization of medium [ ]

Chlorination of irrigation water [ ] Done nothing [ ]

40. Has tomato production in greenhouse been profitable? Yes [ ] No [ ]

41. What are some of the challenges you face in greenhouse production?

☐ Lack of capital ☐ Lack of irrigation water ☐ No power source

☐ Cattle invasion ☐ High maintenance cost ☐ Pest and disease problems

☐ Theft ☐ Flooding ☐ High temperatures in GH

☐ Wind destruction ☐ Sabotage (Fire)

Appendix 2. Analysis of Variance table for area under disease of six tomato varieties screened for resistance to bacterial wilt**.**

Variate: AUDPC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
accessions	5	3.522	0.704	0.67	0.65
Residual	18	18.798	1.044		
Total	23	22.32			