TOMATO RESISTANCE TO *FUSARIUM* WILT DISEASE: EFFECT OF GRAFTING COMBINATION AND MOLECULAR CHARACTERISATION OF *FUSARIUM* ISOLATES

BY

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DEDICATION

I dedicate this work to my beloved father, Mr Awu-Etsey Agbezorli (of blessed memory) and my mother, Dora Anane Sakpaku

DECLARATION

I, Eleagbe Jennifer Awu, hereby declare that except for references cited, which have duly acknowledged this thesis, " Tomato resistance to *Fusarium* wilt disease: effect of grafting combination and molecular characterisation of *Fusarium* isolates" is the result of my research. This thesis has never been presented either in part or in whole for the award of a degree in another Institution or University.

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ABSTRACT

Recently, tomato cultivation in the Berekum West District of Ghana faced a severe yield loss due to *Fusarium* wilt disease. In this study, a survey was conducted to assess farmers' knowledge on prevalence, spread, control, economic importance, and the use of grafted tomato to manage tomato wilt disease. Six communities were selected to determine the incidence and severity of the disease and identify the causal organism of the wilt using molecular markers. Based on molecular identification using Internal Transcribed Spacer (ITS) region sequencing did not only identify *Fusarium oxysporum* isolate but in addition, *Fusarium equiseti* isolate was identified as the causal organism of the wilt disease in Berekum West District. Out of several Solanum plants screen for resistance against *Fusarium* wilt, the best two, *Solanum macrocarpon* and *Solanum torvum* were selected as rootstock for grafting onto *Solanum lycopersicum* (Petomech). Plant growth, yield improvement and disease management of both grafted plants, non-grafted plant were evaluated under artificial inoculation condition (4.4 x 10^6 spores suspension) in a pot experiment and under a naturally infected open field condition at Berekum. There was significant interactive effective between the rootstocks and *Fusarium* inoculum, where grafting was effective in reducing the disease severity and incidence on the field and in the pot experiment. There was also a significant interactive effect on the growth, photosynthetic activity and yield but the fruit quality traits of either grafted and non-grafted plants showed similar or no significant differences. For the pot experiment, Petomech grafted onto *Solanum macrocarpon* produced significantly higher yield 453.1 g per plant compared to Petomech grafted onto *Solanum torvum* (350.3 g) and non-grafted plant (205 g). However, under the open field experiment (naturally infected field), Petomech grafted onto *S. torvum* produced the highest fruit per plant (158 g), Petomech grafted onto *S. macrocarpon* (148.7 g) and non-grafted petomech produced 51.4 g per plant. Overall, grafting was effective tool used to improve the yield and growth of tomato plants in a *Fusarium* infected field.

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CHAPTER ONE
1.0 INTRODUCTION

Tomato (*Solanum lycopersicum*) is a precious and popular crop of the Solanaceae family worldwide (Pritesh & Van Der Knaap, 2011). The present-day tomato originated from the South America Andes, growing up in the wild at the foot of a mountain (Naika *et al*., 2005). Mass migration by early explorers and the traditional blending of culture facilitated the spread of tomato throughout the world (Paran *et al*., 2007). According to Norman, the Portuguese introduced tomatoes in Ghana in the 16th century (Norman, 1992).

Tomato is a high valued crop in Ghana and the most consumed vegetable by people from diverse backgrounds. It is central to most Ghanaian diets in its raw and processed forms and remains a regular ingredient in our local soups, stew, sauces and dishes (Maršić & Osvald, 2004). It is an essential dietary component that contributes to better nutrition and living conditions for rural and urban populations (Waiganjo *et al*., 2006). Tomato is rich in vitamins A, C & E and has become extremely important because it contains lycopene, a food component known to reduce the incidence of prostate cancer, heart and age-related diseases (Sravanthi & Rao, 2015). Tomato cultivation cuts across most regions in Ghana due to its adaptability to a wide range of soil and climate conditions. In addition, it is a short duration vegetable crop, so most farmers find it convenient to cultivate. Ashanti (Akumadan and Ejura), Brong Ahafo (Wenchi, Tanoso, Derma and Berekum), Volta, Upper East and Upper West regions are among the leading tomato producers in Ghana (Robinson & Kolavalli, 2010)

In Ghana, tomato production is predominantly a small-scale activity and seasonal with a few large-scale irrigation sites (FAO, 2005). In 2017, Ghana was ranked 49th worldwide in producing 371,811 tonnes of tomatoes on a land area of 47,932 hectares. Average yields in the country are estimated at 395755 tonnes (FAO, 2019), which is far below the imported, 8000

tonnes (Comtrade, 2019). The tomato production in Ghana has not been able to reach its full capacity to attain yields that can meet its demand in the country (Robinson & Kolavalli, 2010). This is due to several challenges in its production, including pest and diseases, limited availability of improved varieties, access credit for production, inability to get extension services and unavailable source of water for irrigation. Consequently, huge tonnes of tomatoes are imported from neighbouring countries (Burkina Faso and Togo) into the country to make up for the market and consumer demands.

Pest and diseases cause extensive loss to tomato production in both field and greenhouse, making it difficult for farmers to meet market demands. The estimated loss in tomato production due to pest and diseases is about 34.4%. Without crop protection losses could increase to 77.7% (Zalom, 2002). Among the pathogens that affect tomato crop, soil-borne fungal pathogens, including *Fusarium, Pythium, Rhizoctonia*, and *Verticillium* causes root rot, damping-off or wilts to reduce plant growth and fruit yield (Verdejo-Lucas *et al*., 2013).

There are multiple unknown *Fusarium* species causing crop losses in tomato production. *Fusarium* species such as *F. oxysporium, F. equiseti* and *F. verticilioides* are associated with wilt (Rozlianah *et al*., 2010). *Fusarium oxysporum* is a species complex comprised of 120 formae speciales (ff. spp.) based on host specificity (Michielse and Rep, 2009; Arie, 2010). The different ff. spp. show considerable genetic diversity and have a polyphyletic origin (O'Donnell *et al.*, 1998; Nirmaladevi *et al*., 2016). Among these pathogens, *F. oxysporum* f. sp. *lycopersici* (FOL) which is strictly host-specific is a highly destructive pathogen that causes significant economic loss in both greenhouse and field-grown tomatoes worldwide.

In Ghana, *Fusarium* wilt incidence recorded in three regions ranged between 24%- 46% (Opoku, 2014). A survey conducted in four farming communities in the central region of Ghana

identified three different *Fusarium* spp. namely; *F. equiseti*, *F. acuminatum*, *F. monilliforme* causing wilt on their farms (Opoku-Asiama, 2003).

Diversity studies on *Fusarium* species have been done using Internal Transcribed Spacer (ITS) region and elongation factor α (EF-1α) genes sequencing (Nirmaladevi *et al*. 2016; Lievens *et al*., 2009). However, in Ghana, there is limited knowledge of the diversity of species infecting tomato in Ghana. There is the need to know if all wilts are caused by the same species using morphological and molecular tools.

Sveral studies currently have increased the understanding of the fusarium-tomato pathosystem and it's management. The indiscriminate use of fungicides to control *Fusarium* wilt has led to the development of more virulent strains of the causal agent and also increased the biological fitness of these species (McGovern, 2015). There is a global effort to reduce the use of soil fumigants for a more environmentallly friendly practice such as grafting (Gao *et al*., 2016). A study done, shows that the use of wild resistant *Solanum* species (*Solanum torvum*, *Solanum xanthocarpum*, *Solanum khasianum* and *Solanum surathense*) as graft rootstocks represent the best strategy to manage tomato bacterial wilt diseases. In the said study *Solanum torvum* grafted onto *Pusa Shyamala* had the lowest disease infection (12.2%) and the highest graft compatibility of 81.85% (Kumar *et al*., 2017). Agyeman (2021) grafted *Solanum macrocarpon* and *Solanum aethiopicum* onto *S. lycopersicum* to control nematode and had grafted plants with greatly reduced root-knot galls compared to non-grafted plants and also obtained 93% and 94% graft success respectively. *S. macrocarpon* offered resistance against nematodes. Maxifort was used as a rootsock to control *Fusarium* wilt in heirloom tomato and had a low level of disease severity (29%) (Rivard, 2007). Therefore there is a need to graft *S. lycopersicum* onto two selected wild *Solanum* species (*S. torvum* and *S. macrocarpon*) against *Fusarium* spp. and thereof increase the agronomic performance of the plant, improve yield, fruit quality attribute and reduce the level of disease infection.

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The specific objectives of the study were to:

1. Assess the knowledge, perception and experience of farmers in Brekum West District on *Fusarium* wilt of tomato and its management
2. Determine whether different *Fusarium* species are involved in the wilt disease aetiology.
3. Assess the potential of grafted tomato plants in reducing disease incidence, severity, and enhanced agronomic performance in *Fusarium* infested fields.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, history and botany of tomato

Tomato (*Solanum lycopersicum*) a flowering plant cultivated for its edible fruit belongs to the nightshade family *Solanaceae*, which contains more than 3000 species of plants with high economic importance (Weese and Bohs, 2007).. It is believed that wild species of tomato originated in the Andes Mountains of South America, mainly in Peru and Ecuador. It derived its name from the Náhuatl (Aztec) word tomato (Naika *et al*., 2005). Over many years, it was cultivated as an ornamental because it was considered poisonous until the Europeans first accepted it as a vegetable in the 16th century (Peralta and Spooner 2007). Its history of domestication is indefinite, whether it was first domesticated in Peru or Mexico. Consequently, through mass migration now tomato is present in all tropical and temperate continents (Weese and Bohs, 2007) and hence has contributed to wild tomato diversity (Nakazato and Housworth, 2011). Today, tomato is extensively cultivated across Africa especially Burkina Faso, Ghana and Nigeria (De Lannoy, 2001). Now it is a critical and ubiquitous part of African dishes, used for stew, soup, salad, kebab and sauces.

Tomatoes are grown in temperate and tropical climate either in an open field or under a greenhouse. It is a perennial herbaceous plant however, it is often cultivated as an annual crop (Nuez, 2001). A tomato plant can grow to a height of 2-4 meters depending on the growth habit, which influences a variety to grow as an annual or a perennial. The growth habit varies from determinate to indeterminate (OECD, 2017; Dam *et al*., 2005). *Solanum lycopersicum* usually takes 45 days from germination to flowering stage and 90 to 100 days to reach fruit ripening (Nuez, 2001). It is mostly self-pollinated but also sometimes cross-pollinated (*Dam et*

al., 2005). The stem of a tomato plant is blunt, lined with hairy and granular trichrome that gives a distinct scent. Its leaves vary in shape from lobed to compound. The compound leaves consist of five to nine leaflets which has a petiole and are dented. From the botanical perspective, tomato (*Solanum lycopersicum*) is regarded as a fruit berry and not a vegetable. It comes from a vineyard and has similar features to berries; a simple fleshy fruit containing light-brown hairy seeds in a pulp. It takes seven to nine days for the fruit to develop after fertilisation (OECD, 2008). The characteristic pigmentation of red tomato fruit is due to the deposition of lycopene, which is the predominant carotenoid present in tomato fruit. The presence of beta-carotene in tomato causes the change of colour from green to red (https://www.geochembio.com/biology/organisms/tomato/). Tomato fruit is rich in minerals (iron and phosphorus), vitamins (A, B and C), sugars, essential amino acids, dietary fibre, lycopene and beta-carotene. These compositions of the fruit contribute to a healthy and balanced meal (Wilcox, 2003).

2.2 Climatic and Soil requirement for tomato growth

Tomatoes need a relatively warm, cool, and dry climate for growth and high yield. Due to the influence of temperature required at each growth stage (Table 1), tomato plants can withstand a wide range of climatic conditions. The optimum temperature for cultivation ranges between 21 and 26 ºC during the day and 12 to 15 ºC at night (Green *et al*., 1989). Plants require minimum temperatures above 18 ºC for vegetative growth but can survive at lower temperatures (12 ºC). Fruit set colour and leave colour are affected by light intensity. Temperatures above 31 ºC reduce the rates of flower fertilisation, plant development and fruit ripening (Geisenberg and Stewart 1986).

The preferred type of soil for growing tomato should be sandy-loam soil, deep, well-drained but must be capable of holding water, aeration and salt-free. However, in clayey soil, deep ploughing should be employed to enhance and ease root movement. It grows well in soil with

a pH range of 5.5-6.8. Nevertheless, it can moderately tolerate a wide range of pH (Rice *et al*., 1993).

Table 1: Temperature requirement for different stages of tomato

	Temperature (ºC)		
Stages of plant development	Minimum	Optimum range	Max
Seed germination	11	16-29	34
Seed growth	18	21-24	32
Fruit set	18	20-24	30
Red colour development	10	20-24	30

Source: Garza and Molina (2008); Nieves-García, van der Valk and Elings (2011).

2.3 Crop Production

Amongst all vegetables, tomato is known to be one of the most important because it generates revenue through export. It is produced on a highly commercial basis due to its numerous functions. Favourable areas considered to be good for tomato are located in the temperate and warm regions. Globally, tomato production amounts to 180 million tonnes per annum with a total area of 5 million hectares. Asia produces 112,104,020 tonnes of tomatoes annually which is 62% of the world's tomato production, 13.2% from America harvesting 23,786,872 tonnes, 12.6% from Europe (22,806.866 tonnes), 12% from Africa (21,664,774 tonnes) and, 0.2% from Oceania harvesting 406797 tonnes per annum (FAOSTAT, 2019). Approximately, 40 million tonnes of tomatoes are processed every year in factories that belong to the greatest labels of the global food industry (http://www.tomatonews.com/en/background). In 2019, the world's top 10 countries' in tomato production tomato were China-mainland, United States of America, India, Turkey, Egypt, Italy, Iran (Islamic Republic of), Sapin, Brazil and Mexico (FAO, 2019).

2.4 Tomato production in Ghana

Tomato is the most important vegetable in Ghana compared to others but, its production in Ghana is highly seasonal due to the irregularity and inconsistency of rainfall patterns, it is mostly rain-fed (GSSP, 2010). Tomato is cultivated all over the country due to its tolerance to a wide range of soil and climatic conditions (Tampoare *et al*., 2013). The leading producing regions are Ashanti (Akumadan and Ejura), Brong Ahafo (Wenchi, Tanoso, Derma and Berekum), Volta , Upper East and Upper West regions (Obeng-Ofori *et al*., 2007). These regions supply tomatoes throughout the year in the country (Adu- Dapaah & Oppong-Konadu, 2004). Though Ghana has the natural potential to stand a better opportunity to produce tomato all year round, it is the second-largest importer of tomato paste, consuming about twenty-five thousand tonnes per annum (Aryeetey, 2006). The country has not been able to meet the demand of the market. Hence, middlemen cross the border to purchase fresh produce from Burkina Faso to meet their target. In 2019, Ghana cultivated 92045 hectares of land and harvested about 395755 tonnes of fresh tomato (FAO, 2019) which is low to meet both International and local demand.

A study conducted identified some major problems that caused low yield. These were: non-availability of quality seed, the infestation of diseases and insect-pests, indiscriminate use of pesticides and non-availability of quality pesticides, imbalanced use of fertilisers, monoculture, lack of irrigation, lack of availability of critical inputs, limited credit facilities, lack of proper technical know-how, poor investment capacity and non-availability of insurance cover (Shukla, 2007).

Most varieties in Ghana used for commercial purposes are introduced varieties that are well adapted to the local environment. The local varieties cultivated are ‘Power’ ‘Ankoma’, ‘Caterpillar’, ‘Burkina’, ‘Cocoaba’, ‘Ashanti’ and ‘Rando’ (Khor, 2006). Recommended varieties of tomato include Petomech, Petomech VF, Roma VF, Wosowoso, Rio Grande, and

Lauranno 70 (MOFA, 2009). Petomech is most preferred by consumers and for processing (Robinson and Kolavalli, 2010).

2.5 Pests and Diseases of tomato

Prevention and control of pest and disease infestation are very important since it is one of the major causes of low production of tomato cultivation (Elings *et al*., 2015). It affects both large and small-scale farming. Tomatoes are highly susceptible to various pathogens and these include fungal, bacteria, viruses and nematodes (Chaudhry & Rashid, 2011). Fungi, bacteria and nematode mostly cause rots, stem and foliar disease. Whiles viruses often cause to stunted growth and decreased production (Shankara *et al*., 2005). The outcome of these pathogenic attacks results in diseases such as tobacco mosaic, *Fusarium wilt*, and blight.

As aforementioned, there are other organisms whose activities in both the soil and environment have also led to severe losses and damages of tomato. A few ones commonly associated with the cultivation of tomatoes include aphids, whiteflies, cutworms and flea beetles (Freedman *et al*., 2008). Some are known to be the vectors that transmit/spread disease. Information on various diseases and pests affecting tomato in Ghana is presented in Table 2.

Table 2: Diseases and pest associated with tomato in Ghana and their causal agents

Type of Disease	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.	Whole plant
		Tylenchus spp.	Leaf, fruit, stem
		Helicotylenchus multinctus	Leaf, flower, fruit
Pest	Whitefly infestation*	Whiteflies	Leaf
	Mite invasion	Mite	Leaf
	Tomato boring	Fruit borers	Fruit
Physiological (Abiotic)	Blossom end rot*	Calcium Deficiency	Fruit
	Growth crack	Non-infectious moisture related	Fruit

Source: Sarfo (2018) *Important Disease, Pests and Disorders

2.6 *Fusarium oxysporum*

The genus *Fusarium* collectively is one of the most important group of fungi among plant pathogens. It is a common pathogen associated with several economic importance to many *Solanum* plant species (Ma *et al*., 2010). *Fusarium* is a significant contributor pathogen to tomato vascular wilt. Members of *Fusarium* species that cause wilt are ubiquitous soil-borne pathogens with a wide range of host (about 150) and specific formae speciales that cause

destructive vascular wilts, rots, and damping-off diseases (Singh *et al.*, 2015 & Bodah, 2017). *Fusarium* spp. is capable of causing losses before or during harvest (Chandra *et al*., 2011). *Fusarium oxysporum*, *Fusarium verticillioides*, and *Fusarium equiseti* are the most common identified species of *Fusarium* causing wilt of tomato (Cherick, 2016). *Fusarium oxysporum* is well known for its phylogenetic diversity and hence serves as key model organism for biological and evolutionary research (LeBlanc *et al*., 2017). Strains are classified into forma specialis (f. sp.) based on specialisation to parasitize specific hosts. Also, strains of *Fusarium oxysporum* could be saprophytic or non-pathogenic (Kumar *et al*., 2010). However, the phytopathogenic strains cause destructive vascular wilt disease. (Servin *et al*., 2015; Shahzad *et al*., 2017). *Fusarium oxysporum* uses different ways to survive for a long period under extreme conditions. *F*. *oxysporum* live as a saprophyte feeding on dead decay organic matter in the soil, it also forms mycelium to survive in the rhizosphere, in spore form and commonly as chlamydospores (Schippers & van Eck, 1981).

2.6.1 Biology and life cycle of *Fusarium oxysporum*

Fusarium oxysporum produces asexual spores. The life cycle of *F*. *oxysporum* starts with a saprophytic phase when the fungus survives in the soil as chlamydospores (Beckman & Roberts 1995). Chlamydospores are produced from modified vegetative hyphal segments, formed on senescent host tissues or old cultures. They are formed in singles or pairs or either appear in chains or clusters. Chlamydospores remain dormant until it receives a favourable condition. Consequently, a thallus is produced from which conidia are formed. The roots of the host are then invaded by penetrating the epidermal cells of the host plant and hence the occurrence or development of systemic vascular disease. At the advanced stage of the disease, the fungus grows out of the vascular system into close tissues and cells producing several conidia and chlamydospores. It also sporulates abundantly on plant surfaces like stem and leaf.

Dissemination of the pathogen can occur via seeds, transplants, soil or other means (Agrios 1997; McGovern, 2015; Renu Joshi, 2018).

2.6.2 *Fusarium* wilt disease of tomato

Tomato wilt is one devastating disease of tomato normally caused by *Fusarium oxysporum* f. sp. *lycopersici* (Borisade *et al*., 2017). When the pathogen penetrates the epidermis of the root, it then spreads through the vascular tissue and inhabits the plant xylem vessels, which causes the vessel to block, and results in water stress which leads to plant wilt (Singh *et al*., 2017). The presence of the disease is identified by leaf epinasty, vein clearing, wilting, wilted plants bearing yellow-coloured leaves with minimal or absent crop yield, defoliation, and eventually plant death. The dormant chlamydospore of *F.oxysporium* in infested soil can survive indefinitely in the absence of a host (Khan *et al*., 2017; Cha *et al*., 2016). A cross-section of the roots and stem of infected roots shows browning of the xylem tissues or the vascular tissues with brown streaks along the length of the roots or stem (Wong, 2003). Signs appearing as mycelial layers can be seen on dead plants usually during wet weather (Singh, 1985).

Symptoms of *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *Lycopersici* can sometimes be confused with bacterial wilt caused by *Ralsotonia solanacearum* since both diseases occur and begin with the yellowing of the leaves. However, the first observed indication of the disease is the yellowing of the lower or older leaves which coincides with fruit maturity. Also, bacterial wilt of tomato is a top-down wilt whereas *fusarium* shows symptoms begin at the bottom of the plant (Roberts *et al*., 2001).

2.7 Characterisation of *fusarium*

2.7.1 Morphological characteristics of *Fusarium oxysporum*

In a culture, *Fusarium* produces cotton-like flat spreading colony with a colour ranging from cream, white, light pink to yellow with the colour beneath the culture ranging from colourless,

dark brown, brown to tan (De Hoog *et al*., 2000). The pathogen produces macroconidia, microconidia, and chlamydospores. Spores may be produced abundantly. Macroconidia are short to medium in length, falcate to almost straight, thin-walled and usually 3-septa whilst the microconidia are abundant in aerial mycelia. It is sometimes oval, elliptical, or kidney-shaped and usually has no septate. Chlamydospores usually form in singles or in pairs, but also may be found in clusters or short chains. It may be either terminal or intercalary in aerial, submerged, or surface hyphae. Its cell wall appears with either smooth or rough surface (Leslie *et al*., 2006). Morphological identification of the Fusarium species is the primary, but most difficult, step in the detection procedure. However, for the species that cannot be reliably recognized by morphological characterization, additional analysis, such as DNA sequencing and species-specific polymerase chain reaction (PCR) assays, must be performed.

2.7.2 Molecular identification of *Fusarium oxysporum* causing wilt of tomato

Traditionally, identification of *Fusarium o*xysporum is based on the combination of diagnostic symptoms on the host and the visible presence of the fungus at the affected plant part/tissues (Baayen *et al*., 2000). However, the use of this approach has become a challenge to appropriately detect pathogens that infect the same host. *Fusarium oxysporum* has several forma specialis with a particular host, along with non-pathogenic strains, which are also common soil and rhizosphere inhabitants (Edel *et al*., 2000). The traditional method of identification has now been replaced by molecular techniques which are more reliable, faster, specific and sensitive. Some molecular techniques that can be used to identify a large number of isolates in a single assay are; microarrays, whole-genome sequencing, DNA barcoding and metagenomics. Plant pathologists now prefer to use sequencing and metagenomics tools to detect and identify plant pathogens. The use of molecular techniques reveals the characteristics of the pathogen and its nature of pathogenicity hence making it easy to manage the disease (Saikai, 2010).

Isolates able to cause tomato wilt do not have a single common ancestor within the *Fusarium oxysporum* species complex. Studies have shown that some formae speciales (*F*.sp. *cubense*, *gladioli* and *lycopersici*) are composed of multiple vegetative compapatibity groups (VCGs) and do not form a monophyletic group within the species (O'Donnell *et al*., 1998; Baayen *et al*., 2008).

Work done by Van Der Does *et al*., (2008) shows that despite the polyphyletic origin of *Fusarium oxysprium*, isolates belonging to f. sp. *Lycopersici* contains an identical genomic region of at least 8kb that is absent in other formae speciales and non-pathogenic isolates and comprises the genes *SIX1*, *SIX2* and *SHH1*. These genes encode a virulence factor towards tomato, which secrete small proteins into the xylem during infection of tomato plants (Houterman *et al*., 2007). *SIX1* and *SIX2* are unique to f. sp. *lycopersici*, and are present in the entire polyphyletic group of f. sp. *lycopersici* isolates. Van Der Does *et al*., (2008) reported that these genes may be part of a larger, dispensable region of the genome that confers the ability to cause tomato wilt and has spread among clonal lines of *F.oxysporum* through horizontal gene transfer.

Vega-Gutiérrez (2019) also assessed the aggressiveness of different isolates obtained from the vascular tissues of tomato plants showing symptoms of *Fusarium oxysporum*. The pathogenic isolates were evaluated through phylogenetic analysis of the TEF-1α gene and ITS region, morphological markers and pathogenicity tests. The isolates showed differences in aggressiveness.

2.8 Management of *Fusarium* wilt of tomato

Several attempts have been made to control *fusarium* wilt with a non-significant success story due to the emergence of new pathogenic races (Juliano *et al*., 2005). Integrated Disease Management (IDM) is one of the most environmentally friendly strategies and cost-effective. It is harmless to the environment, plants, useful microbes, animals and human lives (Bawa, 2016).

2.8.1 Chemical control of *Fusarium* wilt disease

In 2017 Nel *et al*., reported some chemicals that are effective for the control of *fusarium* wilt of tomato. These agrochemicals include propiconazole, prochloraz, thiabendazole, carbendazim, benomyl, thiophante, fuberidazole and all of the benzimidazoles. Treating seeds with fungicides before sowing has been shown to minimise the incidence of *Fusarium* wilt. Soils can also be disinfected with methyl bromide, chloropicrin, or metham sodium. Despite the promising results shown by using synthetic chemical treatments in controlling the pathogens, the chemical releases residues and toxins which pollute the environment and also cause health hazards to humans (Janahiraman *et al*., 2016). Pathogens can develop resistance to agrochemicals due to excessively which makes its management difficult (Recycled Organics Unit, 2006).

2.8.2 Biological control of *Fusarium* wilt disease

This management strategy makes use of an antagonistic pathogen to control *Fusarium oxysporum*. This reduces environmental pollution and health hazards. Biological agents could either be fungi or bacteria which interacts with the soil pathogen. Alwathnani *et al*. (2012) reported the use of biological agents was quite effective to control Fusarium wilt disease on tomato. *Trichoderma harzianum*, *Aspergillus niger*, *Penicillium citrinum* and *Penicillium sp*. inhibited the radial colony growth of the pathogen.

Widnyana *et al*. (2013) reported that three isolates of rhizobacteria obtained from the rhizosphere of plants from the families *Solanaceae* and *Leguminoseae* namely KtS1, TrN2 and TmA1 identified as *Pseudomonas alcaligenes* and exhibited antagonistic effect against *Fusarium oxysporum* f. sp. *lycopersici*. *Pseudomonas alcaligenes* effectively reduced the incidence of wilt disease on tomatoes under greenhouse experiment.

2.8.3 Physical control of *Fusarium* wilt

Physical treatment involves techniques that use heat to inactivate or weaken pathogens. This includes steaming, solarisation, hot water treatment, and composting. This technique can reduce the population density of the pathogen *Fusarium oxysporum* in the soil. Challenges to physical treatment include the uneven distribution of heat applied to the soil and hence does not completely inactivate the pathogen (McGovern; McSorley, 2012).

2.8.4 Application of organic matter and plant residue

It was observed that the decomposition of organic matter in the soil plays a role in inhibiting the activity of *Fusarium oxysporum* in the soil. Also, the addition of organic amendments to soil was found to promote the activities of antagonistic microbes, which contained biologically active molecules and toxins which can affect soil pathogens (Bailey & Lazarovits, 2003)

An experiment conducted by Kimaru *et al*. (2004), revealed that neem cake powder contains ingredients that have fungistatic effects against Fusarium wilt of tomatoes. Simlly Coventry *et al*. (2001), as the neem extracts were found to possess antimicrobial activity with notable effects on some fungal pathogens. Hanaa *et al*. (2011) investigated the effect of aqueous extracts of Azadirachta indica (Neem) and Salix babylonica (Willow) 10 % on *Fusarium* wilt disease in tomato and revealed that the percentage of disease incidence was reduced to of 25.5 % and 27.8 % after 6 weeks of infection respectively.

2.8.5 Cultural control

This involves the correct implementation of cultural practices and manipulation of farms/cultivation to make the environment unfavourable for the growth of the pathogen (Saikai, 2010). However, managing soil-borne diseases like *Fusarium oxysporum* with cultural method has not been easy since *Fusarium oxysporum* can survive in the soil between crop cycles and plant debris, making it difficult to control. Crop rotation, intercropping, soil amendment and farm sanitation, when used in association with other control measures can reduce the growth of *F. oxysporum*.

2.8.6 The use of host resistance plant

The most effective, less costly and environmentally friendly strategy to control *Fusarium* wilt is the use of resistant cultivars (Sheu *et al*., 2006). The use of resistant varieties reduces and save the cost associated with chemical control of the disease and enhances cultivation of previously infested field (Singh, 2005).

Agyeman, *et al*. (2021) evaluated the resistance and agronomic performance of *Solanum lycopersicum* (Tomato) grafted onto four *Solanum* spp. rootstocks against soil-borne disease (root-knot nematode). In his research, he found out that the grafted plant did not only show resistance to root-knot nematode but also offered resistance to the spread of *Fusarium oxysporum f. sp. lycopercici* which is host-specific to tomato compared to the non-grafted tomato. This is an indication that grafting has multiple functions in crop protection.

2.9 Grafting as a technique to control soil-borne diseases

Grafting is the ability to join together two living plant parts (scion and rootstock), to produce a living single plant (Janick, 1986). Vegetable grafting has become a potential tool in boosting the production of fruiting vegetables of *Solanaceae* and *Cucurbitaceae* families in many countries (Lee *et al*., 2010). Grafting allows a grower to join a scion possessing desirable fruit

traits with a rootstock that can tolerate or resist a lot of biotic and abiotic stresses. This process aims at enhancing the productivity of the plant (Petran *et al*., 2014). Vegetable grafting began in the 1920s using resistant rootstock to control soil-borne diseases. Rootstocks of grafted plants exude allelopathic chemicals that inhibit fungal growth (Davis *et al*., 2008). It helps to increase the uniformity and vigour of plants as well as enhances fruit qualities (Ofori, 2015 & Yassin *et al*., 2015). It is commonly practised in Asia, some parts of Europe, the United States and the Middle East. This method is rarely deployed in Africa to control soil-borne diseases. Old records on vegetable grafting can be found in Chinese, Korean and Japanese writings (Melnyk and Meyerowitz, 2015).

To obtain a good graft take, several grafting techniques have been adopted based on the kind of crop, age of the plant, available facilities and tools, number of plants, environmental conditions, healing chamber and preference of the grafter (Sakata *et al*., 2007). Grafting can be done manually (using your hands), using grafting machines or robots. Grafting methods or techniques commonly used worldwide are; Clef graft, Offset clef graft, Saddle graft, Side graft, Side veneer graft, Epicotyl graft, Splice graft and Whip and tongue graft (Lee and Oda, 2003). The splice or tube grafting and cleft grafting are often used for grafting vegetables (3-5week old seedlings) with ease and flexibility (Lee *et al*., 2010).

Effective and successful graft take involves different stages which include (1) Selection of the rootstock and scion species, (2) Making a graft union by physical method, (3) Healing of the union, and (4) Adaptation of the grafted plant before planting them out onto the field (Lee *et al*., 2010).

2.9.1 Rootstock selection/scion compatibility for Tomato grafting

Vegetables with certain environmental susceptibilities could have grafting-compatible relatives within the same genus that possess a natural resistance to a specific stress. Grafting could be used to broaden rootstock diversity against environmental pressures (Venema *et al*., 2008 & King *et al.,* 2008). Several solanum plants have been screened and selected as graft compatible with *Solanum lycopersici* and resistant to soil-borne diseases (Oda, 2002; Lee, 2003; Kubota *et al*., 2008).

A study conducted by Hoover (2014), shows that Pusa Hybrid-6 grafted onto *Solanum torvum* recoredthe highest survival rate of 81.85% and highest graft compatibility (10days after grafting) among all other graft combinations. It was also recorded that *Solanum torvum* rootstock was found resistant to wilt disease whilst the other *Solanum* species (Pusa Shyamala and Pusa Hybrid-6) were found susceptible. Nonetheless, non-grafted plants recorded the highest infection rate (71.35%) to the grafted plants. The lowest infection rate was recorded in *Solanum torvum* × *Pusa Shyamala* (12.22%). Based on the mean performance of the grafted plants, Hoover (2014), selected *Solanum torvum* and *Solanum khasianum* as the best rootstock to use against soil-borne disease (bacteria).

Agyeman *et al*. (2021) evaluated graft success of *S. lycopersici* (Petomech) grafted onto *Solanum macrocarpon*, *Solanum aethiopicum*, and *Solanum lycopersici* 'Mongal' in root-knot infected soil. S. *macrocarpon* and *S. aethiopicum* gave the highest graft success (93% and 94%) compared to the graft success of *Solanum lycopersicum* "Mongal (0%). Furthermore, grafted plants produced higher yields, good fruit qualities and longer shelf life compared to the non-grafted plants. In his findings, it was observed that grafted rootstocks also offered resistance to the spread of *Fusarium oxysporum* f. sp. *lycopersici* compared to the non-grafted petomech. Davis *et al*. (2008) reported that some rootstock exudes allelopathic chemicals that inhibit fungal spore germination and mycelium growth.

Currently, hybrids (F1) or interspecific hybrids are preferred for commercial grafting. Hybrids are produced by crossing selected tomato varieties with other wild Solanum species which have the genetic ability to offer resistance to specific diseases and pathogen like nematodes, *Ralstonia* wilt, *Verticilium* wilt and *Fusarium* wilt (Verdejo-Lucas *et al*., 2013). However in developing countries, grafting with other *Solanum* species like *Solanum torvum*, and *Solanum macrocarpon* and *Solanum aethiopicum* is preferred over tomato hybrids as rootstocks due to the cost involved in accessing hybrid seeds (Kubota *et al*., 2008).

2.9.2 Methods of Grafting

There are different methods of grafting which varies from each other based the type of crops being grafted (both scion and rootstock), time of sowing the scion and rootstock since the interval of seed emergence varies for each crop. However, the common methods used for grafting fruit and vegetables are the splice or tube, cleft and tongue grafting method (Lee *et al*., 2010).

Splice method is also known as top grafting, tube grafting, and slant-cut (45°) grafting. It is the most popular technique used by growers for tomato and eggplant graft (Johnson *et al*., 2011). A slant cut is made at an angle of 45˚C, on the scion and the rootstock. The cut surfaces are carefully attached, ensuring the cambium layer of the scion and the rootstock are properly aligned. The two plants (the scion and rootstock) are held together or supported with a grafting tube (Black, 2003; Lee *et al*., 2010).

Cleft grafting is also called apical grafting or the wedge grafting. This technique is done by making a horizontal cut slightly below the cotyledon of the rootstock, leaving the lower part and discard the top of the plant. A longitudinal cut 0.5- 1cm is made into the centre of the rootstock. The scion is also cut slightly above the cotyledon discarding the lower portion of the plant with the root. The scion is then cut between 0.5-1cm long in a wedge (tapered) shape

which will insert into the vertical incision in the rootstock. The two plants are aligned and held with a grafting tube (Hassen *et al*., 2015).

Tongue-approach grafting also known as side-by-side grafting. Rootstock cut is made in a downward direction and the scion cut in an upward direction usually at 30^0- 40^0 to the perpendicular axis, and deep enough to allow the fusion of as many vascular bundles as possible. Clips are then placed to keep the graft union in position. (Khankahdani *et al*., 2012).

2.9.3 Graft healing process

The newly grafted plants are immediately placed into a healing chamber. The healing period is the most significant phase during grafting (Kleinheinz *et al*., 2013). It takes an average of 5–8 days after grafting for the rootstock and scion (top of the grafted plant) to establish a vascular connection and 14 days for the graft union to fully heal. During the first week after grafting, the scion is unable to receive water from the rootstock. It is therefore important to maintain proper environmental conditions to prevent water loss from the scion and promote rapid formation of the graft union (Black, 2003)

A healing chamber is a small covered structure, built with materials that are capable of maximising humidity and limit the entry of light to allow grafted plants to heal. The main purpose of the healing chamber is to minimise transpiration (water loss) from the scion. The size and design of the healing chamber depend on the number of plants to be grafted (Johnson *et al*., 2017). The scion may develop adventitious roots if the humidity in the healing chamber is too high. Also, if grafted plants are left to stay in the chamber for too long, plants may begin to show physiological disorders (Johnson *et al*., 2017).

Furthermore, to achieve a successful graft union, environmental element such as temperature and relative humidity must be taken into consideration. During the period of graft union formation, environmental conditions can alter the connection of vascular bundles of the scion

and the rootstock. Too much or low humidity in the chamber creates conditions necessary for the development of disease and wilts (Alai, 2014). A lot of explanations of graft incompatibility are centred on biological and natural processes. Other reasons associated with graft incompatibility include; taxonomic variations, cellular recognition and the presence of diseases (Andrew and Marquez, 1993).

A study done by Fan *et al*. (2015) ed that between 7 to 24 days after grafting, vascular bundle bridges appeared within 11-14 days after grafting (DAG) and at 22 days after grafting the scion and rootstock fully connected. The graft union between rootstock and scion is mainly enhanced by the environmental conditions to which the plant is exposed (Fan *et al*., 2015). It is therefore important to create a conducive environment with optimum relative humidity and temperature to enhance the healing of the graft union (Kleinheinz *et al*., 2013).

2.10 Cost implications of using grafted tomato transplants

Farmers and consumers are looking for sustainable alternatives or measures that are effective and economical compared to the use of synthetic chemicals. Currently, the high demand for organically produced fruits and vegetables has created the need to use an environmentally friendly approach to control/manage soil-borne diseases . Grafting is an environmentally friendly approach to use (Barrett *et al*., 2012). Many field trials have found dramatic net economic return increases relative to non-grafted scion cultivars (Grieneisen *et al*., 2018).

Fusarium wilt normally becomes devastating during the latter stages of production when farmers have incurred a lot of cost in production. Taylor *et al*. (2008), estimated about 40,000 kg/ha of grafted and non-grafted plant to receive a price of $0.19/kg and $0.13/kg respectively at the same yield. However, in an instance where a field has a history of *Fusarium* wilt, there is a higher chance of losing all or almost the crop after production costs have been incurred.

This then encourages farmers to evaluate the best alternative to manage the disease based on costs versus probable revenues (Taylor *et al*., 2008).

A study conducted by Barrett *et al*. (2012), to determine if grafting with a resistant rootstock could be cost-effective to overcome root-knot nematodes (RKN) (*Meloidogyne*p.) revealed that under critical disease severity, grafting was best economical measure to control RKN which also helped to maintain a profitable production given that the risk of economic crop losses due to RKN outweighed the higher cost of grafted transplants. A positive or negative net return is mainly dependent on the cost of producing the grafted plants and the prevailing market price for the tomato fruits that will be produced. Falling tomato prices coupled with high input cost for raw materials needed for grafting may result in some negative net returns.

According to Djidonou *et al*. (2013), yield improvement obtained from grafted plants (thus grafted plants producing 1890 to 2166 25-lb cartons per acre whereas non-grafted plants produced 1457 to 1526 25-lb cartons per acre), had the highest market price. It was also stated that an increase in production cost is based on the type of rootstock used and the growing season.

Grafting is tested as a valuable tool to obtain a good yield of tomato, good fruit qualities with a good market price, and above all a successful measure to control soil-borne disease (Lee & Oda 2003; Lee *et al*., 2010)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Selection of experimental sites in Berekum West District in the Bono Region of Ghana for a survey on tomato wilt disease

Farmers and agricultural extension agents (AEAs) in Berekum West District in the Bono Region of Ghana reported the prevalence of tomato wilt disease in their district. A questionnaire was designed to assess farmers' knowledge, perceptions, and experiences concerning the occurrence and management of tomato wilt disease on their farms. One hundred (100) farmers were selected randomly to fill out a structured questionnaire and respond to the interview with the help of the team and the AEAs. The communities selected from the district for the survey were; Jinijini, Fatenta, Mantukwa, Kwasi Mensah kura, Forest Nkwanta and Pruso.

Figure 1: Questionnaire administration to farmers (A & B), Illustration of grafting to farmers (C)

3.2 Determination of disease incidence and severity of *Fusarium* wilt disease of tomato in Berekum West District

Immediately after the interview and questionnaire administration, 16 farms were randomly selected and visited by the research team. On each farm, disease assessment was carried out to determine the level of disease incidence and severity of *Fusarium* wilt based on these characteristic; yellowing of leaves, wilting and vascular discolouration of stem without any

root rot. This was done to confirm a report received from AEAs and farmers of Berekum West District about the occurrence/presence of the disease and its effect on them. The sampling pattern was random, with an x-shaped path covering the entire field which included both healthy and infected plants. GPS coordinates of these farms were also recorded (Table 3).

Disease incidence was obtained by counting the number of diseased plants out of the total number of plants observed as follows,

$$\textbf{Incidence \%} = \frac{\text{Number of diseased plant}}{\text{Total number of plants}} \times 100$$

The disease severity was deterimed based on the method described by Akber *et al* (2018) wth a 0-4 indexing scale, where 0= No symptoms; 1= initiation of wilt symptoms; 2 = Vascular discolouration in the stem, stunting, wilting with or without yellowing of the leaves (up to 50%); 3 = plants wilted, yellow-brown discolouration more pronounced, and entire plants started dying (75%); 4= completely dead plant. The severity was calculated as follows:

$$\textbf{Wilt Severity (\%)} = \frac{\text{Sum (rating number x number of plants infected)}}{\text{Total number of plants x highest rating}} \times 100\%$$

Table 3: GPS coordinates of 16 farms from which *Fusarium* wilt disease plants were sampled

FARM NUMBER	COMMUNITY	GPS
1	Mantukwa	7° 29' 25" NL, 2° 42' 42" WL
2	Mantukwa	7° 29' 27" NL, 2° 42' 41" WL
3	Mantukwa	7° 29' 28" NL, 2° 42' 41" WL
4	Mantukwa	7° 29' 29" NL, 2° 42' 47" WL
5	Mantukwa	7° 29' 3" NL, 2° 42' 57" WL
6	Kwasi Mensah Kura	7° 30' 17" NL, 2° 46' 35" WL
7	Kwasi Mensah Kura	7° 30' 16" NL, 2° 46' 37" WL
8	Kwasi Mensah Kura	7° 30' 17" NL, 2° 46' 39" WL
9	Forest Nkwanta	7° 29' 41" NL, 2° 42' 57" WL
10	Forest Nkwanta	7° 29' 39" NL, 2° 42' 57" WL
11	Forest Nkwanta	7° 29' 45" NL, 2° 42' 52" WL
12	Prosu	7° 31' 36" NL, 2° 43' 16" WL
13	Prosu	7° 31' 54" NL, 2° 42' 53" WL
14	Prosu	7° 35' 20" NL, 2° 47' 51" WL
15	Fatenta	7° 31' 38" NL, 2° 43' 34" WL
16	Jinijini	7° 26' 13" NL, 2° 38' 8" WL

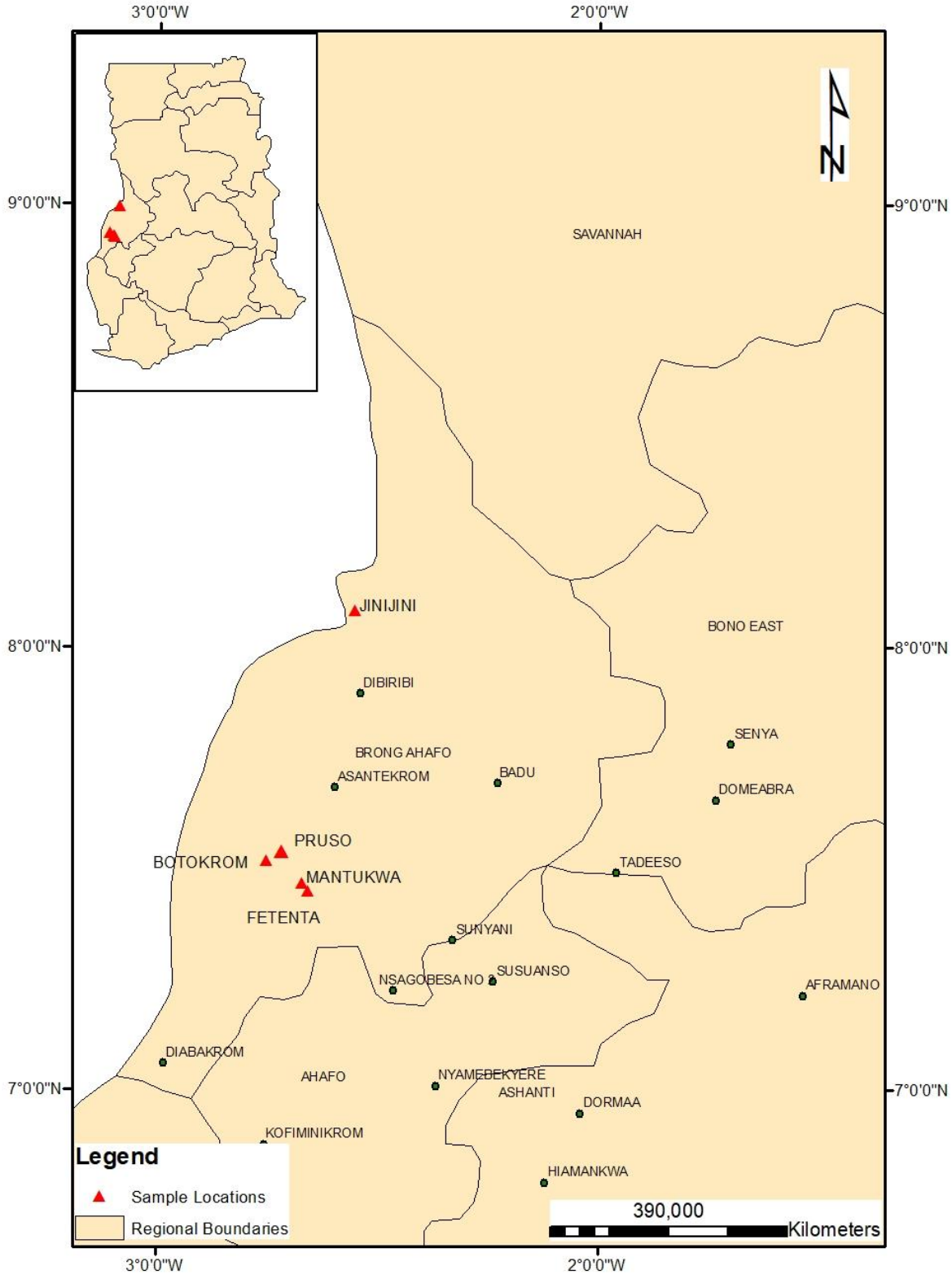

Figure 2: Map of Bono Region showing the locations of tomato farms, where *Fusarium oxysporum* isolates were collected from tomato plants

3.3 Experiment 1: Isolation and Identification of *Fusarium* spp. Causing Tomato Wilt at Berekum West

3.3.1. Sampling and isolation of *Fusarium* spp. infecting tomato

Tomato plants with suspected *Fusarium* infection that exhibited signs and symptoms were sampled randomly from sixteen (16) farms in the district (Table 3). Root, stem, leaf, fruit and soil of tomato plants of different varieties were picked from the farms for isolation. Each sample taken was placed in plastic zip-lock bags, which were then kept in a cooler filled with ice to keep the temperature at or below 4˚C. The samples stored in the cooler were transported from Berekum West district for eight hours drive to the plant pathology laboratory of the department of Crop Science at the University of Ghana for further study.

, Plants and root samples were thoroughly washed under running water to get rid of soil, debris and other contaminants. Plant tissue (from the root, stem and leaf) were cut at the advancing edge or border of the diseased and healthy portion of the plant at a size of ~3 mm to 6 mm. The cut tissues were surfaced sterilised by immersing in 1% Sodium hypochlorite (NaOCL) for 1 min, rinsed in sterile distilled water and blotted dry on sterilised tissue paper. The cut tissue was then plated onto water agar, prepared at a rate of 3 g of agar powder to 100 ml of distilled water, autoclaved at a pressure of 1.05 Kg/cm^2 and 121˚C for 15 min. The plates were incubated at 26˚C ± 1˚C. On day three after incubation, all plated samples showed mycelia growth. The mycelia were cut and placed onto a plated Potato Dextrose Agar (PDA) that was prepared at a rate of 4 g to 100 ml and autoclaved. Penicillin was added to the sterile PDA before pouring into the plate to impede bacteria growth. The plate was incubated at 26˚C ± 1˚C and consistently observed for mycelia growth. The incubated culture showed a lot of fungal and bacteria growth. The preferred fungal colonies were sub-cultured to obtain a pure culture. Also, isolation from rhizosphere soil samples was done using the serial dilution method. Soil dilutions were plated

on PDA and incubated at 28± 2°C for 5 days until it was ready for morphological characterization. This isolation process employed was based on. (Nelson *et al*., 1983)

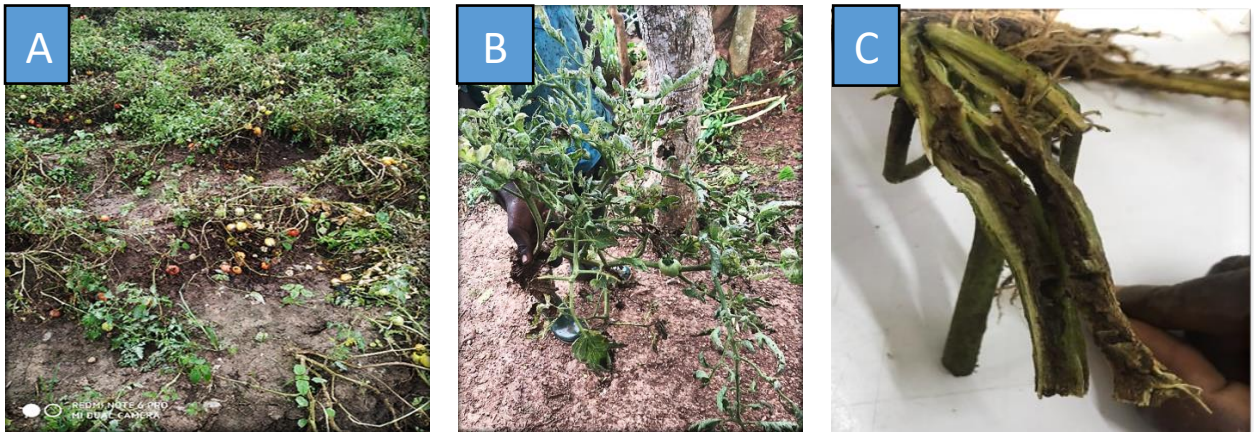

Figure 3: *Fusarium* infected field (A), Wilted plant (B), Browning of vascular bundle (C)

3.3.2 Morphological Characterisation

The morphological characterisation was carried out as described by Summeral *et al*., (2003) and Leslie *et al*., (2006). y, Single spore isolates were cultured on PDA and incubated at 26˚C ± 1˚C for 14 days. During this period, cultural characteristics such as colony colour, texture and growth rate were observed. Micro-morphological characters (shape, size, colour, septation etc.) of macroconidia, microconidia and chlamydospores were observed in water mount, under a compound microscope (AmScope, United Scope LLC UK). Spore length and width measurements (n=10 spores per isolate), and micrographs were taken using the AmScope imaging software (Version 3.7). Isolates were coded for easy identification

Isolates sampled from the rhizosphere soil were identified as *Trichoderma* spp. It produced pure cultures of *Trichoderma* spp.

OBSERVATION: Even though all sampled rhizosphere soil produced pure culture of *Trichoderma* spp. some wilted plant part from the same field produced *Fusarium* isolates.

Table 4. Isolates and their codes, numbers and source of origin

Code	Isolate number	Source (Host/Habitat)
1	F08P	Stem & Leaf.
2	F09C	Leaf
3	F03C	Stem
4	F04C	Fruit
5	F05C	Leaf
6	F07C	Fruit
7	F11C	Fruit
8	SF	Soil
9	F01C	Soil
10	F03P	Soil
11	F10P	Soil
12	F02C	Soil
13	F3C	Soil
14	F08P	Soil
15	F13C	Stem
16	F13C	Leaf
17	F16	Leaf
18	F10 P	Stem
19	F07C	Stem
20	F07A	Stem
21	F11C	Leaf
22	F11C	Stem
23	F4C	Stem

3.3.3 Extration of DNA

DNA isolation was done at the Biotechnology Centre of University of Ghana, Legon. Pure cultures, specifically selected for molecular identification were grown for seven (7) days. In all, 23 isolates were selected (Table 4). Extraction of DNA from the selected isolates was carried out using the Genomic DNA (gDNA) isolation protocol described by Fulton *et al.* (1995). Fungal mycelia obtained from a seven-day-old culture were scrapped and harvested with a sterile toothpick into an autoclaved frozen mortar. It was ground into a fine powder and placed into an eppendorf tube (1.5 mL). Fresh microprep buffer was prepared and kept at room temperature. Tris-EDTA buffer (750 µ1) was added to the grounded mycelia, vortexed lightly and incubated at 54˚ C in a water bath for 15 min. The tube was filled with chloroform/isoamyl (24:1), vortexed and vigorously shaken to mix. The tube was centrifuged at 13,000 rpm for 5 minutes and the supernatant w as pipetted into a new microfuge tube. Cold isopropanol (2 /3 to 1 times the volume) was added to precipitate the gDNA. The tube was immediately spun at 10,000 rpm for 5 min and the isopropanol decanted and DNA pellets washed with 70% ethanol. The DNA pellets were air-dried by leaving tube upside down on paper towel for 1 hr, re-suspended in 50 µ1 of Tris-EDTA buffer (10 mM Tris HCI, pH 7.5, 1 mM EDTA) at 65 ˚C for 15 min and spanned 10 min at 10,000 rpm. Extracted DNA was stored at 4 ˚C in a refrigerator until it was needed for polymerase chain reaction (PCR)..

3.3.4 Polymerase chain Reaction (PCR)

Polymerase chain Reaction (PCR) was conducted using a 25 µl reaction mixture which consisted of 7.50 µl of nuclease-free water, 12.50 µl of 2x Quick-Load Master Mix with Standard Buffer (Zymo Research Inc., UK), 1.0 µl of each primer and 3.0 µl of template DNA. The primers listed in Table 5 were used to detect the presence of genes which are unique to *Fusarium oxysporum* f.sp. *lycopersici* in each of the isolate.

3.3.5 Gel electrophoresis

A 1.5% agarose gel was prepared for a 114 cm electrophoresis tank by weighing 1.71 g of agarose powder into 114 mL IX TAE buffer in a conical flask. The volume of the solution was weighed after which the agarose mixture was heated in a microwave till the agarose dissolved. The solution was reweighed after heating and water added to make up the original volume of the solution. Five microliters (5 µL) of ethidium bromide was added to the final solution and the conical flask swirled gently to mix the solution. The gel was then poured after cooling into a casting electrophoresis tank with wells set in place and left to set. The combs were removed after 35 min and the gel placed in an electrophoresis tank such that wells were oriented at the anode and filled with IX TAE. DNA samples (5 µL) were mixed with loading dye and gently loaded into the wells using a sterile pipette. The electrophoresis tank was closed, electrical leads were then attached to the tank and the DNA samples run at 80 V for 90 min after which the gel was removed and visualised using the gel documentation system (SynnGene Inc, UK).

Table 5: List of Primers used for genomic amplification

Name of primer	Sequence 5'-3'	Target	Annealing temperature (^{0}C)	Reference
SIX2-F2	CAACGCCGTTTGAATAAGCA	SIX2	50	Van Der Does
SIX2-R2	TCTATCCGCTTTCTTCTCTC	SIX2		*et al*., 2008
SIX3-F1	CCAGCCAGAAGGCCAGTTT	SIX3	55	Van Der Does
SIX3-R2	GGCAATTAACCACTCTGCC	SIX3		*et al*., 2008
EF11	GTGGGGCATTTACCCCGCC	EF-1 α	54	O'Donnell
EF22	AGGAACCCTTACCAGCTC	EF-1α		*et al*., 1998
MS1	CAGCAGTCAAGAATATTAGTCAATG	mtSSUrDNA	50	White *et al*.,
MS2	GCGGAATTATCGAATTAAATAAC	mtSSUrDNA		1990
MS21	CTCTCCTCCTCAAGTACTGC	mtSSUrDNA	54	White *et al*.,
MS11B	GCAGTACTTGAGGAGGAG	mtSSUrDNA		1990
ITS1	TCCGTAGGTGAACCTGCGG	rDna	54	White *et al*.,
ITS4	TCCTCCGCTTATTGATATGC	rDNA		1990

The PCR thermocycler was programmed for 5 min at 95°C followed by 35 cycles for 45s at 94 °C, 1 min at respective annealing temperatures (Table 5), 3 min at 72 °C and a final extension at 72 °C for 7 min. All PCR experiments included a negative control (distilled water without DNA) and a positive control consisting of DNA of *Fusarium* spp. isolate

3.3.6 Sequencing, Nucleotide Alignment and Phylogenetic Analysis

Amplified PCR products were sequenced at Inqaba Biotec and the same primers utilized for the PCR amplification were used for sequencing. PCR products (sequences) were assembled using SeqMan Pro assembler (DNA Star) to produce contigs. These were further aligned using the CLUSTAL W method (Bio-Edit). The sequences were compared to those in GenBank (http://www.ncbi.nlm.nih.gov/) using Mega BLAST. The BLAST analysis was performed with full length ITS sequences as queries to detect homologies to sequence gene bank. Sequences from this study, and those downloaded from GenBank were used in phylogenetic analysis and phylograms constructed using MEGA 7 (Tamura *et al*., 2011). Out group sequence was *Phytophthora infestans* isolate Soil 0441.

3.4 Experiment 2: Effect of grafted *Solanum lycopersicum* onto two selected rootstock; *Solanum Macrocarpon* and *Solanum Torvum* (Pot Experiment)

3.4.1 Study Area

A pot experiment conducted at the University of Ghana school farm. University of Ghana is sited at Legon in the Greater Accra Region, the capital of Ghana. The farm lies at NL 5˚39' 34" and WL 0˚11'31"

3.4.2 Selection of Scion and rootstock

. Two solanum species; *Solanum macrocarpon* 'gboma' and *Solanum torvum* 'abedru' were selected as rootstock based on their ability to tolerate drought, flooding, salinity and soil-borne diseases. *Solanum lycopersicum* (Petomech) was selected as the scion.

Seeds of *solanum macrocarpon* and *solanum torvum* were obtained from University of Ghana farms whilst petomech seed was purchased from Agriseed limited. Germination test showed over 90% germination for *Solanum lycopersicum* (petomech) and *Solanum torvum* whilst *Solanum macrocarpon* (gboma) had the lowest germination rate which is 60%.

In May 2020, seeds of *Solanum torvum* were sown 3 weeks earlier than *Solanum macrocarpon* which was sown on the 25th May 2020. Seeds of petomech (the scion) were sown two weeks after sowing *Solanum macrocarpon*, on the 8th June 2020. This was done to obtain similar stem diameters during grafting since both rootstocks and scion have uneven emergence and seedling growth. *Solanum lycopersicum* (petomech) used as a control was sown two weeks after sowing the rootstock to maintain a uniform age of all plants for the experiment since the grafted plants will under go a period of acclimatisation.

All seeds were nursed in peat-moss-potting soil (made up of partially decomposed sphagnum moss plants, which were harvested from peat bogs) filled in a seventy-two (72) cell seed tray. The second week after the emergence of the scion *Solanum lycopersicum* (Petomech), seedlings suffered from damping-off disease caused by Pythium. Ridomil Gold Copper fungicide was used to control *Pythium* spp. However, damping-off was not observed on *Solanum torvum* and *macrocarpon.*

3.4.3 Fertilizer application

Two weeks after *Solanum torvum* and *solanum macrocarpon* had emerged, NPK 19:19:19 was applied at a rate of 5g per 1L once in a week, until they reached a sizable girth for planting. The scion, petomech received the same starter (N.P.K 19:19:19) once in two weeks.

3.4.5 Grafting procedure

Grafting of scion onto the rootstock was carried out when the *S. lycopersicum, S. macrocarpon* and *S. torvum* plants were five (5) week old, seven (7) week old and nine (9) weeks old respectively. The difference in selected ages of the of the seedlings wase to ensure similar girth diameter for grafting to increase cambial union and graft compatibility. All seedlings at this stage had initiated their third true leaf. Seedlings were well watered in the late afternoon, a day before grafting.

The working bench, blade, and grafting clips were sterilised with 5% sodium hypochlorite. A paper towel was used to blot the blade and clips before use. Grafting was done using the cleft graft method. At a maximum of two (2) cm above the growing media, a cut was made on the rootstock. Just above the cotyledon, a cut was also made on the scion. The 1-2 true leaves of the scion (petomech) were pruned to minimise moisture loss from the lower leaves to support the growth and development of upper leaves. Two sides of the lower stem of the scion, parallel to each other were cut at a slant angle to make a 'V-shape or a tapered wedge. The wedge (V) shaped scion was carefully placed in the slightly split rootstock. A grafting clip was used to hold the union to align the cambium layer of both the scion and the rootstock. This was done to aid in graft take.

Subsequently, the grafted plants were then placed in a humidity chamber. The healing chamber measured 1.4 × 1.0 × 0.4 m and was made from wawa wood, plastics and iron rods. The base of the chamber was made with wooden slab and the inner portion lined with a black plastic sheet. The apical portion was covered with a double layer of plastic sheet (one black and a transparent sheet) to control light and humidity in the chamber.

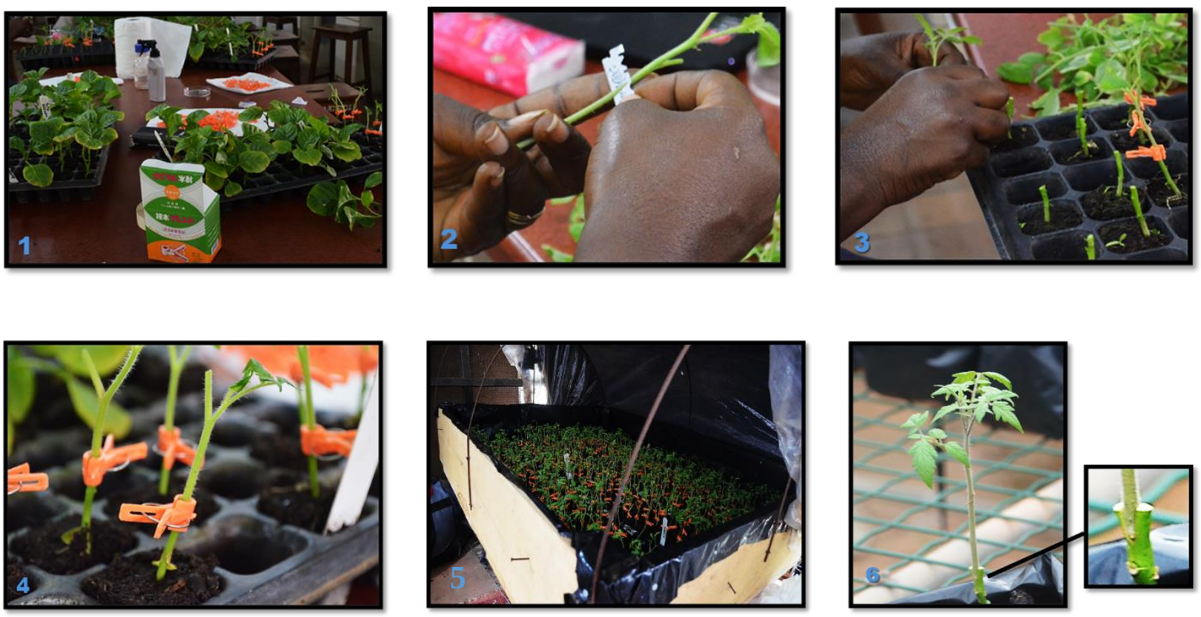

Figure 4: Grafting process using the cleft grafting method. 1- Roots stocks; **2**- Severing the scion below the cotyledon into a wedge form after defoliation of leaves; **3**- making a vertical slit in the middle of the rootstock (~ 4 mm deep) after it has been severed; **4**- A wedge-shaped scion gently inserted into the vertical slit on the rootstock with a supporting clip; **5**- grafted plants placed in a healing chamber; **6**- A grafted plant showing a successful union between the scion and the rootstock.

3.4.6 Healing of grafted plant

The grafted plants were allowed to heal under 98% humidity, 26 ^{0}C and complete darkness in the healing chamber. Humidity level was achieved at 98% by misting the chamber with water and covered with a black plastic sheet to prevent light from entering for 2 days. From day three onwards the black plastic sheet was removed, leaving the transparent sheet for partial entry of light. Besides this, the chamber was opened partially at noon for 30 minsto allow ventilation and to reduce the build-up of heat in the chamber. This was repeated for four days before opening the chamber fully under a shade structure. Grafts that showed mouldy growth or appeared weak or toppled were discarded to prevent contamination during the healing process.

3.4.7 Acclimatisation of successful grafts

The plants were acclimatised by exposing them to sunlight and ambient temperature daily for seven days. The acclimatisation was done gradually; the plants were exposed to ambient conditions for 30mins on the first day, 1 hr on day 2, 4 hrs on day 3, 7 hrs on day 5 and 6, and a full day on day 7 and 8. The plants were kept hydrated by applying water to just the base of the rootstock. At this stage side shoots on the rootstocks were removed by pinching them off whiles those on the scion were kept to allow for new growth. The media was regularly checked and fertigated with N.P.K 19:19:19 at 3g/L by capillary action when necessary. As part of the hardening process, the grafts were brought out of the chamber for 1, 3 and 6 hours from the 10^{th} to 14^{th} day.

3.4.8 Transplanting of grafted plants

Topsoil was collected from University of Ghana farms to fill plastic pots for the experiment. The soil was sterilised by using hot water (at boiling point) . This was done by fetching the soil in bits onto a slab and subsequently added the boiled water and stirred thoroughly until all the soil was sterilised. The sterilised soil was heaped and covered with a plastic bag for two days to keep and contain the heat in the soil to kill all soil pathogens. After two days, the sterilised soil was filled into plastic bags (9 x 14cm) and watered. In the late afternoon, two hundred and sixteen (216) seedlings were transplanted into the pots and watered after transplanting. Plastic pots with seedlings were carried to a prepared experimental field.

3.4.9 Experimental design (Pot Experiment)

A 3 x 2 factorial treatment structure was used. This was laid in a Completely Randomized Design (CRD) design with three replicates.

Factor one consisted of three rootstocks:

- ✓ *Solanum lycopersicum* grafted onto *Solanum macrocarpon*
- ✓ *S. lycopersicum* grafted onto *S. torvum*
- ✓ Control (ungrafted *S. lycopersicum*, Petomech)

Factor two was the inoculum

- ✓ 4.4 x 10^6 spore suspension of *F. oxysporum*
- ✓ Distilled water was inoculated as the control

The treatments used are as follows;

PI_o= Control- ungrafted petomech + distilled water

PI_i= ungrafted petomech + 4.4 x 10^6 spores suspension *F. oxysporum*

P/SMI_o = Petomech grafted onto *S. macrocarpon* + distilled water

P/SMI_i= Petomech grafted onto *S. macrocarpon* + 4.4 x 10^6 spores suspension *F. o*

P/STI_o= Petomech grafted onto *S. torvum* + distilled water

P/STI_i= Petomech grafted onto *S. torvum* + 4.4 x 10^6 spores suspension *F. oxysporum*

There were 18 plots with each plot containing 12 plants, three (3) replicates and six treatment in each replicate. This made a total land size of 121.52 m^2. The planting distance of the potted plants was 0.70m x 0.50m. Distance between plots was 0.5 meters and 1meter between blocks. Plots were well labelled according to treatment.

3.4.10 Cultural practices

A day after transplanting, crop starter was applied to the grafted and non-grafted seedlings at a rate of 2 g/L. Plants were watered every morning (6:30 am) and early evening each day (4:00 pm). Five days after transplanting N.P.K. 19-19-19 (Polyfeed, Haifa chemicals limited) water-soluble fertiliser was applied at the rate of 5 g/L. Ammonium sulphate (5 g) was used as a side dress before flower bud initiation. This was done every two weeks until full blossom. Calcium nitrate was also applied at fruit set at the rate of 5 g/seedlings. Weeds were cleared and an integrated pest management approach was used to control the pest. Each plant was staked for support.

3.5 Preparation of *Fusarium oxysporum* f. sp. L*ycopersici* inoculum

A pure culture was with morphological characteristics of *Fusarium oxysporum* f. sp. *lycopersici* was plated on PDA and incubated at 25-27˚C in light/dark condition for two weeks. The mycelia was scraped from the Petri dishes into a blender and topped up with 1000 ml of distilled water. The concentration of the spore suspension was adjusted to 4.4×10^6 spores/5 ml using a hemocytometer under an optical microscope. Each selected seedling was inoculated with the conidial suspension at 4.4×10^6 spores/ 5ml of distilled water. Control seedlings were inoculated with only sterile distilled water.

3.6 Data collection

Data were collected on :

1. Percentage graft take (%)
2. Disease incidence and severity
3. Plant height, Stem girth ,Number of leaves per plant, Chlorophyll content
4. Photosynthetic rate
5. Yield per plant (Number of fruit per plant , fruit weight, girth and length per plant)
6. Fruit quality (Fruit firmness, Total soluble solids (TSS), Pericarp thickness, Number of locules, pH)
7. Fresh shoot and root weight
8. Dry shoot and root

3.6.1 Percentage graft take (%)

This was taken by dividing the total number of plants that survived (established graft union) after the healing process of the grafted plants by the total number of grafted plants for each graft combination, expressed as a percentage.

3.6.2 Disease incidence and severity

Disease incidence was obtained by counting the number of plants showing wilt symptoms out of the total number of plants observed, multiplied by 100 (Michel *et al*., 2006)

The disease severity rating was deterimed using a scale of 1-6, where 1= No symptoms; 2= Chlorosis and wilting of the first branches; 3 = Chlorosis above second and third branches; 4 = Chlorosis above third, second and third branches may be lost; 5= Chlorosis and partial desiccation; 6= completely dead plant (Marley & Hilocks, 1996)

3.6.3 Method of measuring plant height (cm)

A rule was used to measure the length of each record plant (6). This was done by taking the height of the plant from the soil level to the highest leaf of the plant. Data of the plant height was taken two weeks after transplanting and repeated attwo weeks interval for three times.

3.6.4 Method of measuring stem grith (mm)

A Vernier calliper (Neiko, China) was used to take the diameter of the recordable plants in millimetres. Data was taken from the fourth week at two weeks interval till the tenth week after planting to the field. The girths of the grafted plants were taken at four different points along the stem at 5 cm above the soil with an electronic calliper and the average taken.

3.6.5 Number of leaves per plant

The number of leaves per plant was determined by counting branches produced by the plant. Similarly, this is done four weeks after transplanting and repeated three times at two weeks interval.

3.6.6 Chlorophyll content

The Chlorophyll meter (Apogee Instruments, USA) was used to measure the Chlorophyll Content Index (CCI) of the six recordable plants for each treatment. Four weeks after the tomato plants were transplanted, the chlorophyll content was taken and then every two weeks for three more times.

3.6.7 Photosynthetic rate

A portable photosynthetic (PP) system was used to measure the photosynthetic rates in the plant. The PP system operates in a closed system mode, where a leaf is enclosed in a chamber with no exchange of air with the outside. Transpiration will cause the humidity in the chamber to rise, and photosynthesis will cause carbon dioxide (CO_2) concentration to fall. The conductance and photosynthetic rate are calculated from the rates of change of these quantities.

It measures the change in carbon dioxide (C_i) concentration (µmol mol^{-1}), stomal conductance (mmol H_2O m^{-2} s^{-1}), leaf area metre (mol m^{-2} s^{-1}), vapour pressure deficient (VDP) in mBar, water use efficiency (WUE) and transpiration.

3.6.8 Number of fruit per plant and yield per plant

Fruits were hand-harvested, counted and weighed with an electronic digital scale, per plant from each experimental plot. The fruits were harvested at the full ripped stage. Fruit length and girth was measured using the Vernier calliper.

3.6.9 Fruit firmness

The firmness of the tomato fruit samples was determined using a hand penetrometer with an 11 mm plunger; the plunger was pressed against the flesh of the fruit until it reached a marked fixed depth on the piston. The display value, which represents the resistance offered by the pericarp of the fruit to the plunger, was recorded. Two readings were taken from each fruit and the average recorded in Newton.

3.6.10 Total soluble solids (TSS)

Totalsolublesolids was determined using a digital hand refractometer (Hanna© refractometer 96801) at room temperature. A drop of distilled water was first placed on the illumination plate and zeroed, after which the water was wiped off the illumination plate. A drop of tomato juice was squeezed out of the tomato samples and placed on the illumination plate, and the percent Brix was then read from the LCD monitor on a scale of 0 to 85% Brix. The process of zeroing was repeated preceding each sample reading.

3.6.11 Number of locules and Pericarp thickness

Samples of the tomato fruits were transversely cut open, and the number of locules in one half of the fruit counted. With the same cut fruit, the Vernier calliper is used to measure the pericarp thickness of the fruit.

3.6.12 pH

The pH of the tomato fruits was determined using a 3330 pH meter, which was buffered at a pH of 7. Samples of the tomato fruits were blended and sieved to obtain the juice, and the pH determined by dipping the pH meter into the juice.

3.6.13 Fresh shoot and root

The record plants were gently uprooted from the soil. The lower part of the plant (the roots) was cut to be separated from the upper part of the plant. The roots were washed to get rid of sand then weighed.. Whereas the shoot of the plant wass also weighed and recorede

3.6.14 Dry shoot and root

This was determined by chopping up the upper portion of the plant above the soil level and drying it in an electric oven at 80 ^{0}C for 72 hr to constant weight. The root of the plants were also dried in an electronic oven at 80 ^{0}C for 72 hours and then weighed. .

3.7 Statistical analysis

Data collected in the questionnaire survey were coded and subjected to descriptive statistical analysis using Statistical Package for Social Sciences (SPSS) version 20 and Microsoft Excel 2016.

Agronomic data was subjected to Analysis of variance (ANOVA) with Genstat statistical package (v.12). Where there were significant differences, the Least Significant Difference test (L.S.D.) was used to determine differences among the means for the various parameters studied. Significance was defined at $P<0.05$ and where necessary data was transformed for normality.

3.8 Experiment 3: Performance of *Solanum lycopersicum* onto two selected rootstock; *Solanum Macrocarpon* and *Solanum Torvum* cultivated on a *Fusarium* infected field at Berekum (Field Experiment)

3.8.1 Planting materials used and raising of grafted seedlings

Solanum macrocarpon and *Solanum torvum* seeds were obtained from the University of Ghana farms and *Solanum lycopersicum* (Petomech) purchased at Agriseed Limited.

Lycopersicum (Petomech) was used as scion and *macrocarpon*, and *torvum* used as rootstocks. *Solanum torvum* seeds were sowed a week earlier than *macrocarpon.* Two weeks after, *lycopersicum* (Petomech) were also sown. A fertiliser solution was formulated by dissolving 5g of N.P.K. 19:19:19 in 1L of water and applied to the seedlings once weekly after emergence until they were ready for grafting.

On the fourth week of nursing, petomech had obtained a sizable girth (≈ 2.7mm) for grafting. *S. macrocarpon* obtained its sizable girth (≈ 3.00mm) at week six of nursing whereas *S.torvum* was ready for grafting (girth ≈ 2.9) of on the seventh week.

3.8.2 Grafting of seedlings

The seedlings were grafted using the cleft method. Grafted plants were misted with water and placed in a chamber. The chamber, containing the grafted plants was managed at a constant humidity (95%-100%) and temperature (21˚C- 25˚C) to facilitate healing of the graft union. At this stage, grafted plants were susceptible to wilt hence regular misting was done, and the chamber opened slightly to allow aeration.

The grafted plants were acclimatized for two weeks and then hardened by exposing the plants to ambient conditions. The plants were potted in polybags, placed in styrofoam boxes and transported to Berekum West District for the field experiment.

3.8.3 Experimental site

The experimental field was located at Fatenta in Berekum west district in the Bono region of Ghana. The experiment was conducted on a farmers field that is found along latitude 7˚ 30' 16" N and longitude 2˚ 46' 37" W. The land was cleared to remove trees, stumps, weeds and stone and was levelled to give it a good tilt. It was then lined and pegged into three main plots measuring 16 m x 2.5 m with a pathway of 0.5 m. Each main plot was subdivided into four mini-plots measuring 3.5 m x 2.5 m

3.8.4 Experimental design

The experiment design used for this experiment was a randomised complete block design with four replicates. The treatments were: P/SM= Petomech grafted onto *Solanum macrocarpon* 'Gboma', P/ST= Petomech grafted onto *Solanum torvum* and P= Petomech ungrafted as control. Each of the three treatments was replicated four times consisting of twenty-five (25) seedlings in an experimental unit.

3.8.5 Cultural management

A starter solution was formulated by dissolving 60 g of N.P.K 15:15:15 per 13 L of clean water. Two weeks after transplanting, 3.0 g of sulfate of ammonia was applied by side dressing. Supplemental calcium in the form of Calcium Nitrate was also applied at week five after transplanting at a rate of 5 g per plant. Weeds were controlled by hoeing. An integrated crop protection and management approach was used to control all major pest and diseases. Plants were staked with wooden sticks one month after transplanting to give support to the plant. The experiment was mainly rain-dependent however, in extreme cases water was fetched from the community for irrigation.

3.9 Statistical analysis

Data were subjected to Analysis of variance (ANOVA) with Genstat statistical package (v.12). Where there were significant differences the Least Significant Difference test (LSD) was used to determine differences among the means for the various parameters studied. Where necessary data was transformed for normality.

CHAPTER FOUR

4.0 RESULTS

4.1 Farmers knowledge and perception on *Fusarium* wilt of tomato

The results obtained from the interviews and questionnaire administered to 100 tomato farmers from Berekum West district on the prevalence, spread, economic impact and control of *Fusarium* wilt disease of tomato are presented below.

4.1.1 General background of respondents

Out of the 100 respondents 81% were males and 19% were females. About 5% had no formal education whilst 95% had formal education including Junior High School/ Middle School, Senior High School/ Technical/Vocational and the Tertiary level (Table 6). Most of the farmers (42%) have attained education to the Senior High/Middle school level. Most farmers (72.6%) in Berekum West are into small scale farming with land size less than 4 acres whilst 28.4% `were large scale farmers with land size more than 5 acres (Table 7).

Table 6: Level of education of tomato farmers in Berekum West District

Level of education	Number of farmers	Percentage (%)
No formal education	5	5
Primary	18	18
MSCL/JHS	42	42
SHS/Technical/Vocational	29	29
Tertiary	6	6
Total	**100**	**100**

4.1.2 Period of land use for tomato cultivation

Most farmers (76%) produced tomato on the same land between 1-3 years whilst a few (18%) were in production for 4-6years, and 6% had been in production for 7-10 years (Table 7).

Table 7: Years of tomato production on the same land

Number of Years	Number of farmers	Percentage (%)
1-3	76	76
4-6	18	18
7-10	6	6
Total	**100**	**100**

4.1.3 Source of planting materials used by tomato farmers in Berekum West

Majority of farmers obtained their planting materials from the local market in 2016 (4.4%), family members in 2017 (37%) and 2018 (35.5%). A few (7.8%) of the farmers obtained their seed from their own farm or agro-input dealers in 2016. In 2017 and 2018, few farmers obtained their seeds from agro-input dealers (6.2% and 4.4%) respectively). (Table 8).

Table 8. Source of planting material

SOURCE	Percentage of Farmers		
	2016(%)	2017(%)	2018(%)
Own farm	7.8	21	34.4
Family member	40.3	37	35.5
Local Market	44	35	25.5
Agro-input dealers	7.8	6.2	4.4

4.1.4 Variety of tomato commonly grown in Berekum West and area under cultivation

The most cultivated tomato varieties in Berekum West are Petomech (76%), Cocoa (76%), Power (74%) whiled the least cultivated is the local variety (55%). Most farmers cultivate tomato on a land size from 1-5 acres whilst few cultivate above 5 acres (Table 9).

Table 9. Variety and land area under cultivation

Varieties	< 1 acre	1-2 acres	3-5 acres	>5 acres	Total
Power	28	40	6	0	74
Cocoa	28	36	5	7	76
Petomech	26	38	8	4	76
Local	26	27	2	0	55

4.1.5 Farmers' perception of the presence and symptoms of the wilt on their farm

Majority (85%) of farmers confirmed the presence of the diseases on their farms based on the symptoms observed while 15% of farmers did not observe anything about the disease. Most of the farmers (95%) indicated that the leaves of the tomato plants yellowed, whilst 85% of farmers reported their plant wilted as well. Most of the farmers (78%) also indicated that they had poor fruit set. Other symptoms indicated by the farmers included stunted growth and discolouration of vascular tissue (Table 10).

Farmers observed some varieties were more susceptible to the disease and hence showed the above symptoms. Farmers had multiples responses for the varietal susceptibility to the disease. They rarely observed the symptoms of the *Fusarium* wilt on the local variety. However, some farmers (49%) mentioned Petomech as the most susceptible to the disease while others (34%) mentioned Power as the second most susceptible. The rest (17%) mentioned cocoa as the most susceptible (Table 11).

Table 10. Symptoms observed by farmers on diseased plants

Symptoms	Yes	No
Wilting	85	15
Yellowing of leaves	95	5
Poor fruit set	78	22

Table 11: Farmer's view on the most susceptible variety to *Fusarium* wilt

Variety	Frequency (%)
Petomech	49
Power	34
Cocoa	17

4.1.6 The growth stage of the tomato plant at which the symptoms were observed

Majority of the farmers (64%) observed the presence of *Fusarium* wilt on their field at the fruiting stage. Whilst 2% of farmers observed it at the at the seedling stage. The rest observed the symptoms either at the vegetative stage (27%) or the flowering stage (7%). t (Fig. 5)

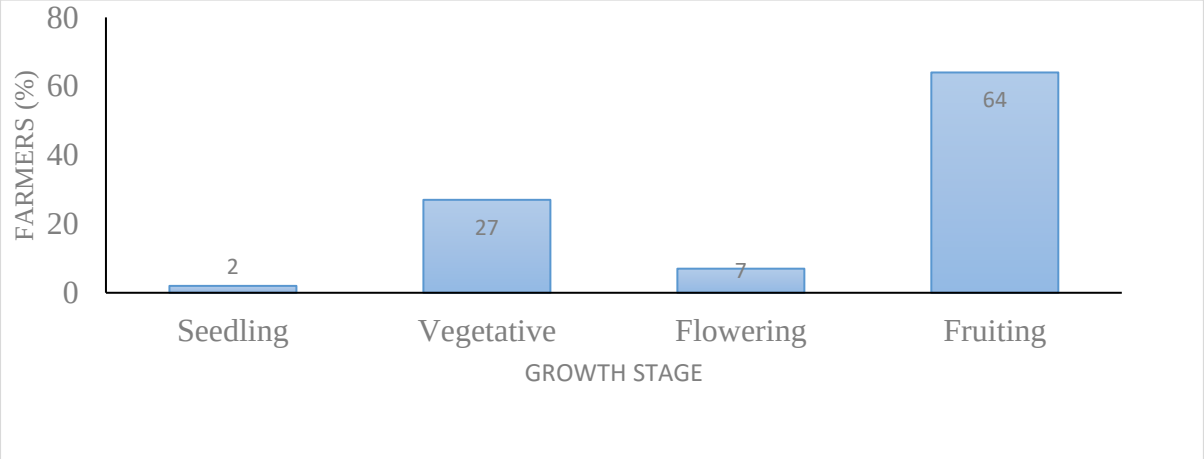

Figure 5. Farmers' perception on the growth stages of plant when the disease is observed

4.1.7 Methods used by farmers to manage/control *Fusarium* wilt of tomato on their farm

Thirty-six percent (36%) of the farmers indicated that they adopted crop rotation to control disease and four percent (4%) also sterilized their soil to control the soil-borne disease. Others (55%) also responded that they used organic or inorganic fertilizer to manage *Fusarium* wilt.

Thirty-two percent of farmers practised land rotation to get rid of the disease, whilst majority (72%) used synthetic fungicide (Table 12). Most farmers (84%) reported that the disease incidence is high during the raining season whilst sixteen (16) percent also reported to notice a high disease incidence during the dry season.

Table 12: Ways to control/manage *Fusarium* wilt disease of tomato

Control Method	Yes	No
Crop rotation	36	64
Solarisation	4	96
Organic/inorganic fertilizer	55	45
Land rotation	32	68
Use of synthetic fungicide	72	28

4.1.8 Awareness on the use of grafted tomato plants

Farmers in Berekum west were not aware crops such as tomato could be grafted, as they were only familiar with tree crop grafting. Farmers had little knowledge about tomato graft. Eighty-nine (89) percent of the farmers were not aware that grafting could be used to manage soil-borne diseases and also, could reduce the effect on the use of fumigants to manage soil pathogen. Also, ninety percent (90%) were not aware grafting could be used to improve fruit quality and crop response to abiotic stress (Table 13)

Table 13: Farmers' knowledge and perception on the use of grafted plants and its benefits

Importance of grafted plants	Farmers view (%)	
	Yes	No
Manages/Control soil-borne diseases	11	89
Improves fruit quality and yield	10	90
Increases tolerance to abiotic stressors	10	90

4.1.9 Perception of farmers on economic importance of *Fusarium* wilt disease of tomato

The survey was conducted on expected yield and actual yield obtained by farmers for 2018 to 2019. In 2018, 11 farmers expected to harvest between 2000- 3400 kg/ha, 22% expected to produce 2700-3375 kg/ha, 59% of the farmers expected 3500-4400 kg/ha whilst 21% expected 4200-4875 kg/ha at the beginning of the season. However, at the end of the season 23% of the farmers obtained 2000-3400 kg/ha of tomatoes, 46% also had between 3500-4400 kg/ha, 31% harvested between 4500-6400 kg/ha whilst none of the farmers harvest above 6500 kg/ha (Table 14).

In the year 2019 about 50% of the farmers expected to harvest between 3500-4400 kg/ha but there was an increase to 70% of the farmers making a harvest within that range. This reduced the number of hoping to make a greater harvest with 4500-6400 from 40% to 9%. Only 2% of the farmers were able to meet their target to produce more than 6500 kg/ha of tomatoes (Table 14).

Table 14: Impact of *Fusarium wilt* on expected and actual yield obtained by in 2017-2019 growing season

Yield of tomato per hectare	2018		2019	
	Expected yield (% of farmer)	Actual yield (% of farmer)	Expected yield (% of farmer)	Actual yield (% of farmer)
2000-3400	11	23	13	19
3500-4400	22	46	50	72
4500-6400	59	31	40	9
>6500	6	0.0	2	0.0

4.1.10 Incidence and Severity of *Fusarium* wilt disease at Berekum West District

Kwasi Mensah Kura had the highest disease incidence and severity of 53.3% and 2 respectively. Mantukwa also recorded disease incidence and severity of 18.3% and 0.8 respectively. Forest Nkwanta recorded 38.3% of disease incidence and 1.5 of severity . Pruso also had an incidence of 13.4% with a severity of 1.5 whereas Fatenta and Jinijini recorded an incidence of 36.7% and 15% respectively with a severity of 1.8 and 1 respectively (Table 15).

Table 15: Incidence and severity of *Fusarium* wilt disease of tomato in 6 communities at Berekum West District

Community	% Disease incidence	Severity index (1-4)
Prosu	13.4	0.8
Jinijini	15.0	1.5
Mantukwa	18.3	1.0
Fatenta	36.7	1.8
Forest Nkwanta	38.3	1.5
Kwasi Mensah Kura	53.3	2.0
Mean	22.8	1.4

Disease incidence and severity was calculated using the formula of Akbar *et al*., 2018

4.2 Morphological and Molecular identification of the causal agent of *Fusarium* wilt disease

4.2.1 Morphological identification of pathogen causing *Fusarium* wilt disease of tomato on 16 farms

Six isolates with varying cultural and morpjological features were identified as *Fusarium oxysporum*. These were labelled as F02, F03, F04, F05, F07 AND F011. These isolates were obtained from 6 different farms with each being isolated from one farms. The cultural and morphological features of the isolates are as follows:The isolates of the fungi obtained from farm 2 (F02) produced white colonies with yellow pigmentation and appeared light yellow at the reverse side. Within 10 days, the culture attained a growth of 90 mm in Petri dish. Isolates from farm F02 had sparse macro and micro conidia. Macroconidia were observed to have 4 septat with an epical cell that is hook-shaped whilst the basal cell is distinctly notched. It had two-celled oval microconidia (single septa). It also produced abundant chlamydospores in the aerial mycelia. Chlamydospores were produced in chains and were spherical.

Isolate F03 produced whitish pink colonies on the upper view, pink to brown colouration at the base and attained a mycelia growth of 90 mm on day 10th after incubation. Microscopic observation revealed that, Isolate F03 produced abundant macroconidia with 3 septat, which were straight to slightly curve in shape. It tapered at the basal cell and curved at the epical cell. Microconidia were sparse, oval in shape and with no septation.

Isolate F04 initially produced white colonies which gradually turned to light orange coloured at agar base. Spores of isolate F04 contained both abundant macroconidia and sparse macroconidia. Septation of macroconidia ranged from 2 to 3 but were predominantly three septate. The epical cell of the macroconidia is slightly curved and tapered whilst the basal cells are distinctly notched. Microconidia were oval in shape, mostly non-septate and rarely two-celled.

Isolate F04 produced macro and micro conidia in abundance. Macroconidia were slightly curved in shape and usually have three septate and rarely 2 septat. The epical cells of the macroconidia are slightly hooked, more hyaline and smooth wall. The dorsal side is more curved than the ventral side. The microconidia were two-celled and oval in shape. There were no chlamydospores.

Pure culture of isolate F05 appeared pinkish on the upper view and pink at the agar base, produced mycelia which filled the 9 mm plate in 10^{th} days after incubation . It produced no chlamydospores and macroconidia but produced microconidia in abundance. Microconidia were oval in shape with no septation.

Colonies of Farm 7 (F07) appeared whitish brown and brown at the agar base, attaining 9 mm mycelia growth on the 10^{th} day of incubation. Microscopic observation revealed few macroconidia with four to five septat, straight to slightly curve hooked epical cell and barely notched basal cell. Microconidia and chlamydospores were absent.

Culture of Farm 11 (F11) isolates appeared white and light yellow at the agar base. It had abundant macroconidia with two septate. Relatively small in size and slightly curved. The epical cells slightly hooked and barely notched basal cell. Microconidia were also in abundant, oval shaped with no septation (Table 16).

Table 16: Morphological characteristics of *Fusarium* species from Berekum west district

ID NO	MACROCONODIA					MICROCONDIA			Chlamydo spores	Culture colour Upper/lower
	Shape	**MSL(µm)**	**Apical**	**Foot**	**No. of Septat**	**Shape**	**MSL(µm)**	**No of Septat**		
F02	Dorsal side more curved	26.4± 4.4	Hooked	Distinctly notched	4	Two-celled oval		1	Spherical, formed in chains with rough wall	White & yellowish pigmentation/ brown
F03	Straight to slightly curved	24.9± 4.2	Curved	Tapered	3	Oval	8.7± 2.8	0	No	Whitish pink/ pinkish brown
F04		19.8± 3.9	Slightly curved	Distinctly notched	2-3	Oval	8.6± 2.9	0	No	White/ light orange
F04L	Hyaline and slightly curved	23± 4.3	Slightly hooked with smooth wall	It is more curved at the dorsal side than the ventral	Predominantly 3 and rarely 2	Two-celled Oval	8± 2.5	1	No	White/ light orange
F05	No	No	N	No	No	Abundance and oval shaped	7.3± 3.7	0	No	White/ light yellow
F07	Verucose and straight to slightly curved	28± 4.6	Hooked	Barely notched	4-5	No	No	No	No	Whitish brown /brown
F11	Falcate	22.4± 3.1	Slightly hooked	Barely notched	2	Oval	6.3± 2.6	0	No	White/ light yellow

MSL = mean spore length of macro and microconidia No =not observed

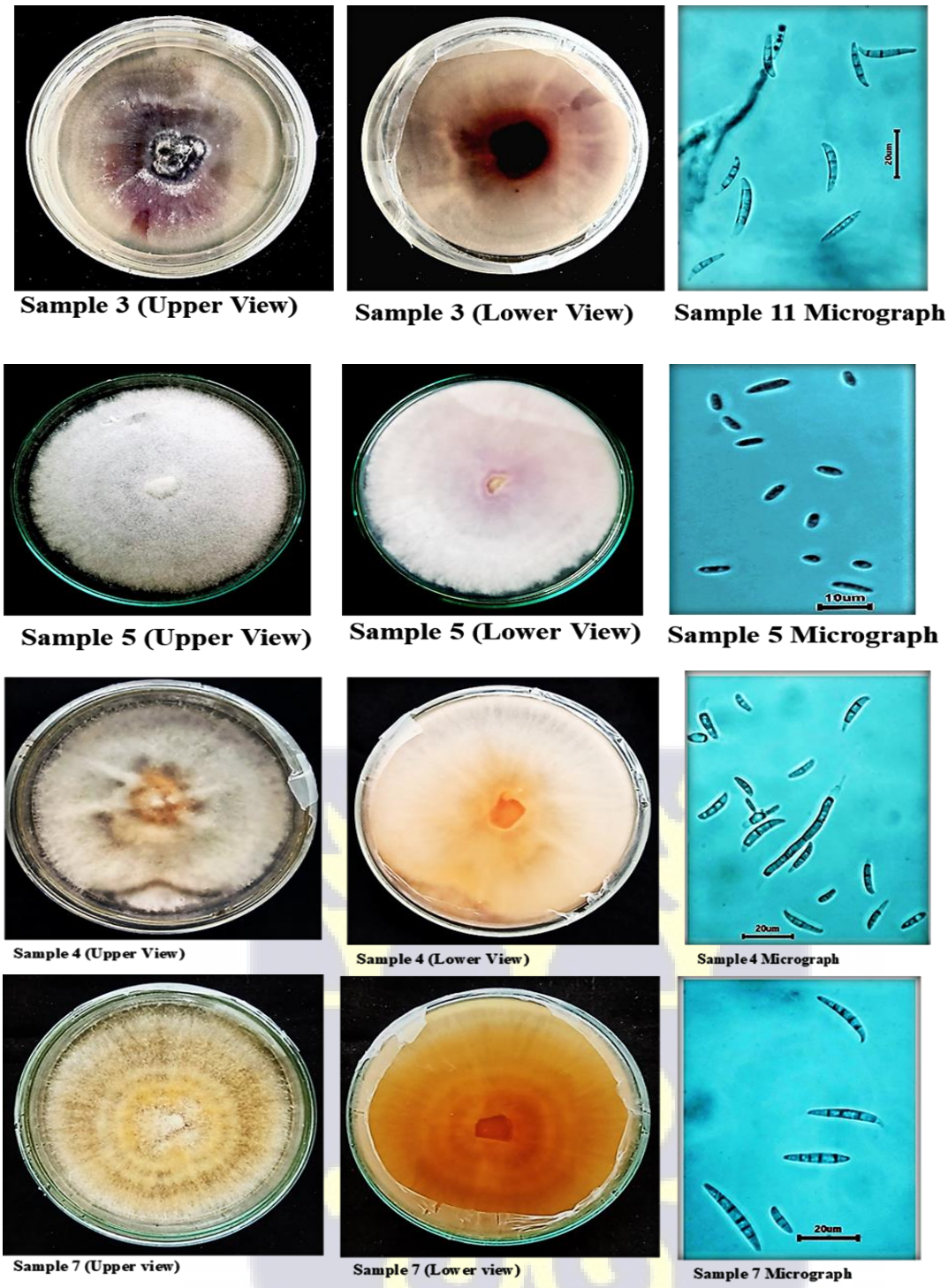

Figure 6: Culture and spores of *Fusarium* spp. Isolated from infected fields at Berekum

4.2.2 Molecular identification of *Fusarium* isolates

PCR on genomic DNA of 23 isolates, using primers SIX1, SIX2, SIX3, EF11-EF22, MS1-MS2, and MS21- MS11B did no amplify but primer pair ITS1-ITS4 amplified ITS region of 11 isolates of the expected band size (Figure 7).

Eight (8) isolates were identified using DNA sequence of ITS. All sequence showed 98%-100% sequence homology with the reported *Fusarium* species isolate. Phylogenetic analysis revealed that isolates clustered along with other *Fusarium* isolates in the NCBI GeneBank which were *Fusarium oxysporum* f. sp. *lycopersici* strain, *Fusarium oxysporum* isolate, and *Fusarium equiseti* isolate. *Phytophthora infestans* isolate used as a group formed a different cluster in the Dendrogram. Isolates were group into three based on their level of virulence. Group 1 includes F04 Fruit, F03 Stem, F05 Leaf and F07 Stem as moderately virulent isolate. FO11 as highly virulent isolate in Group 2. Isolates with unknown virulence were clustered in Group 3 which includes FO13 Leaf, FO4 Stem and FO7 Fruit (Figure 8).

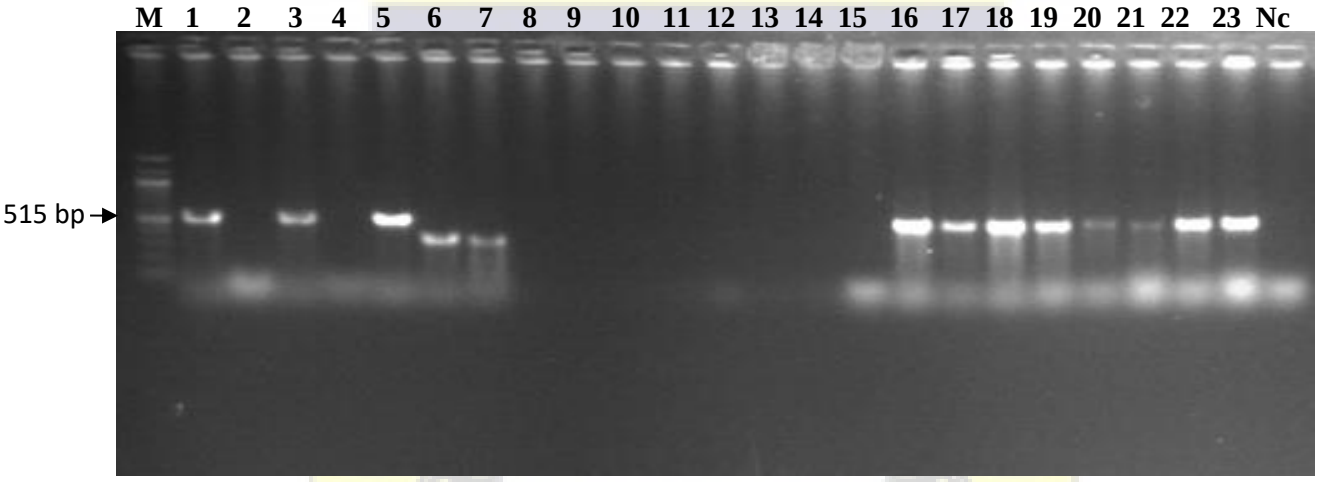

Figure 7: Gel electrophoresis showing the PCR amplifications of the ITS region in the different isolates of Fusarium spp. Lane M- 1Kb DNA ladder; Lane- 1,3,5,16,17,18,19,22,23 amplified ITS regions for 23 isolates of *Fusarium* spp.; Lane Nc- negative control

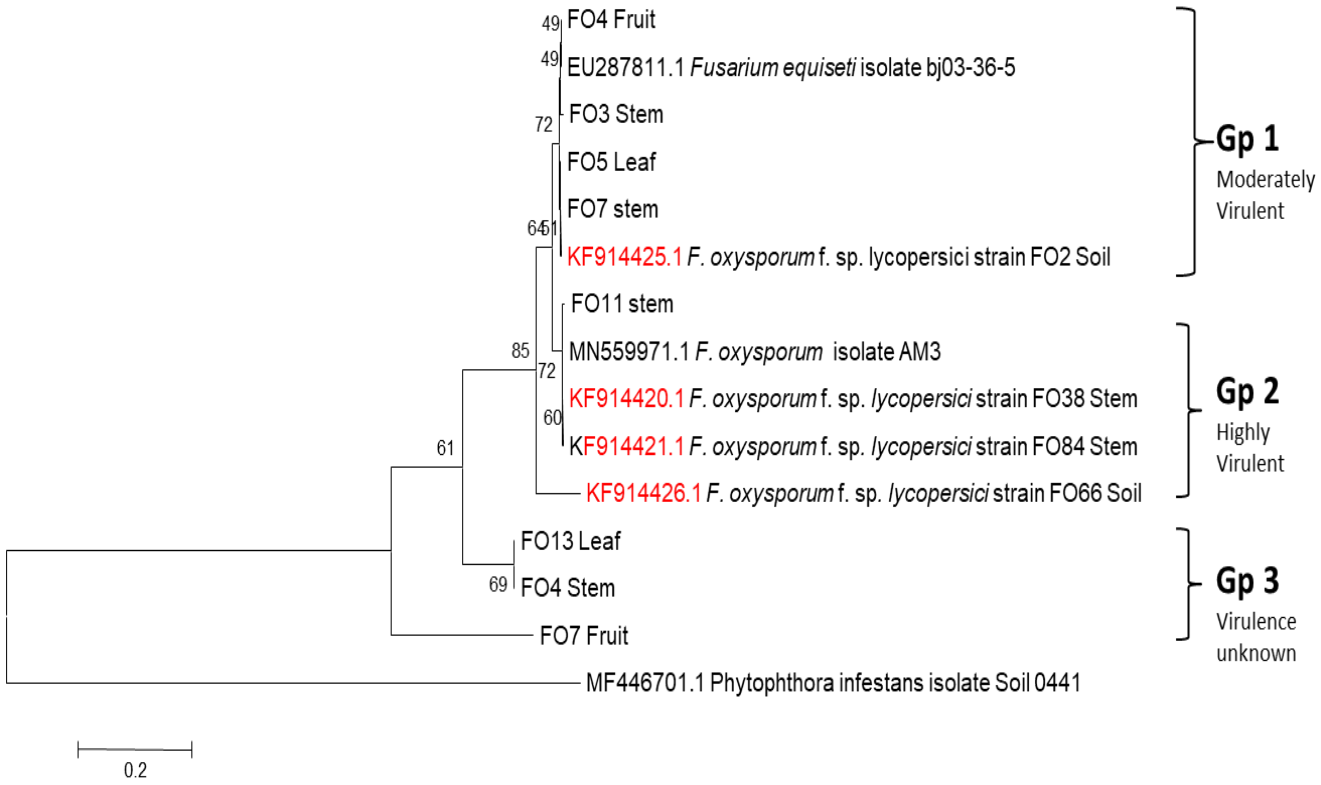

Figure 8: Dendrogram generated by ITS sequence data predicting similarity and evolutionary relationship between the isolates.

4.3 Results of Grafting *Solanum Lycopersicum* (Petomech) Onto *Solanum Macrocarpon* & *Solanum Torvum* To Manage *Fusarium* Wilt

4.3.1 Optimum environmental conditions for enhancing graft take

The widest range of temperature recorded over a seven day period during the wet season in the wooden chamber was 24.0°C - 29.5 °C and a corresponding humidity range of 78.5-100% (Fig. 9A). On the other hand, the environmental conditions recorded outside the wooden chamber (in the shed house) gave a temperature range of 24.1°C - 30.4 °C and 67.3% - 90.7% of humidity (Fig. 9B). During the dry season temperature range recorded in the wooden chamber was 23.9 °C -30.5 °C and a corresponding humidity of 76.7% -100.0% (Fig. 9C). Whereas the external environment recorded 24.1 °C -30.2 °C temperature range and a humidity of 65.4% - 88.5% (Fig. 9D).

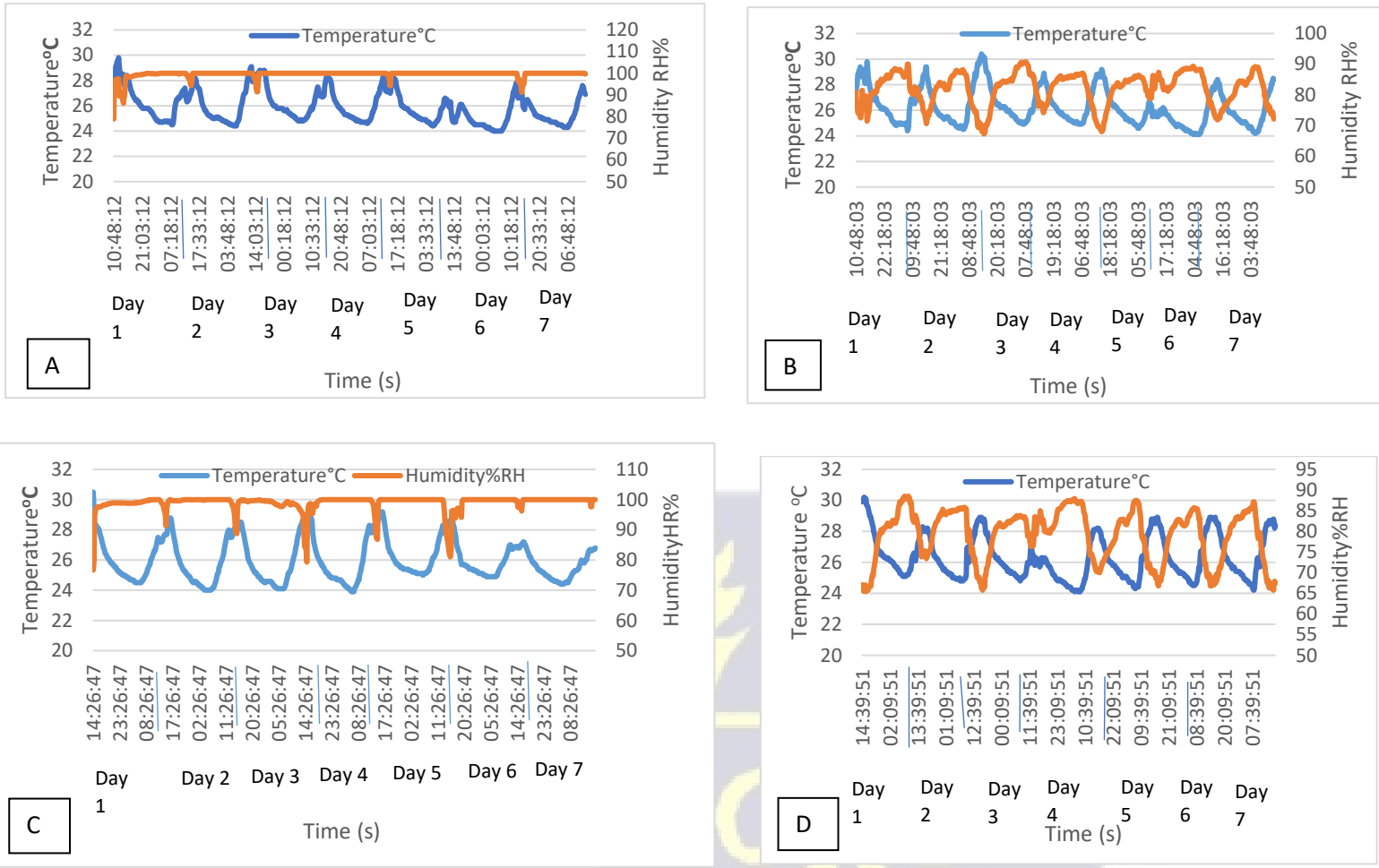

Figure 9: Temperature and Humidity conditions of the grafted plants during the wet and dry season; A) Within healing chamber during wet season; B) Outside healing chamber during wet season; C) Within healing chamber during dry season; D) Outside healing chamber during dry season

4.3.2 Graft success

Petomech grafted onto *S. torvum* had the highest graft success during dry season and rainy season with 72% and 91% graft take respectively. In contrast, Petomech grafted onto *S. macrocarpon* had the lowest percentage of graft success during the dry season (68%) and the rainy the season (83%).

Plants grafted during the rainy season for the field experiment showed the highest graft success of 83%-91% graft take whereas plants grafted for the pot experiment during the dry season showed the lowest graft success of 58%-72% (Table 17).

Table 17: Percentage graft success 14days after grafting *Petomech* onto *Solanum torvum* (P/ST) and *Petomech* onto *Solanum macrocarpon* (P/SM) during the wet and dry season

	Dry season		Wet season	
Treatment	Number of Grafted plant	Percentage graft success	Number of Grafted plant	Percentage graft success
P/ST	100	72	120	91
P/SM	100	68	120	83

4.3.3 Transplanting Grafted Plants and Non-Grafted Plants Inoculated with *Fusarium Oxysporum* and Sterile Distilled Water (Control) In a Pot Experiment at Legon School Farm

4.3.3.1 Influence of grafting combinations and *Fusarium oxysporum* inoculum on the vegetative growth and photosynthetic rate of tomato (petomech) plant

There was a significant interactive effect ($P<0.05$) between the inoculum and the graft combinations on plant height at week 2 after inoculation. *Solanum lycopersicum* (Petomech) grafted onto *Solanum torvum* with *Fusarium* inoculum (P/ST x I) produced the tallest plants (49.9cm) followed by Petomech grafted onto *Solanum macrocarpon* rootstock inoculated with *Fusarium* (P/SM x I) produced the taller plants (46.1cm) whilst Petomech ungrafted (control) with *Fusarium* inoculum (P x I) had the least height (42.3cm). However at 4 weeks after

inoculation, no significant difference ($P<0.05$) were found among the various treatment for plant height (Table 18).

There was a significant interactive effect of the inoculum and the rootstock on stem diameter of the plant. The girth of P/ST x I at week 2 (10.19mm) and week 4 (13.0 mm) after inoculation were significantly thicker than the girth of P/SM x I at 2weeks (9.06 mm) and at week 4 (9.70 mm) after inoculation. P x I had the thinnest girth at week 2 (9.20 mm) and week 4 (9.93 mm) after inoculation. The girth of P/SM x I was not significantly different from the girth of P x I

At week two (14 days) after inoculation there was a significant difference ($P<0.05$) in the number of leaves among the various rootstock. P/ST x I produced most leaves (24) and P x I produced the least number of leaves (19). But at week 4 (28 days) after inoculation, no significant difference was found among the various treatment for their number of leaves (Table18).

The chlorophyll content recorded for all treatment shows a significant interaction ($P<0.05$) between *Fusarium oxysporum* inoculum and the rootstocks. P/SM x I recorded the highest chlorophyll content, 57.22 µmol m^{-2} and 49.49 µmol m^{-2} at 2 week and 4 week after inoculation respectively. Where P x I recorded the least chlorophyll content with 37.6 µmol m^{-2} and 25.18 µmol m^{-2} at week 2 and 4 after inoculation respectively.

The net photosynthetic rate of all treatment at week 2 (14 days) showed a significant interactive effect ($P<0.05$) between *Fusarium oxysporum* inoculum and the rootstock combinations and control plants. P/ST x I had the highest photosynthetic rate 9.59 µmol$^{-2}s^{-1}$, P/SM 7.83 µmol$^{-2}s^{-1}$ and P x I recorded the least photosynthetic rate 7.64 µmol$^{-2}s^{-1}$. However, there was no significant interactive effect at week 4 (28 days) after inoculation but there was a steadily increase of photosynthetic rate for both rootstock combinations; P/ST x I (10.01µmol$^{-2}s^{-1}$) and

P/SM x I (8.03 µmol^{-2}s^{-1}) and a decrease for the control plant P x I (6.83µmol^{-2}s^{-1}) on the 4th week (28days) (Table 18).

Table 18: Influence of graft combinations and *Fusarium oxysporum* inoculum on Vegetative parameters and photosynthesis of grafted Petomech tomato plants at 2 weeksand 4 Weeks after Inoculation

Scion/Rootstock	Plant Height (cm)		Plant Girth (mm)		Number of Leaves		Chlorophyll content (µmol m^{-2})		Photosynthetic rate (µmol $^{-2}s^{-1}$)	
	2WAI	4WAI	2WAI	4WAI	2WAI	4WAI	2WAI	4WAI	2WAI	4WAI
P (Control)	43.3 a	49.6 a	8.86 a	10.21 a	21 a	27 b	40.78 a	33.79 a	7.90 a	7.03 a
P/SM	45.6 b	50.2 a	9.64 b	10.65 a	22 1	25 a	53.83 b	42.17 c	11.32 b	9.04 b
P/ST	48.1 c	53.1 b	10.06 b	12.22 b	24 b	26 ab	45.66 a	38.95 b	8.93 a	10.14 b
Inoculum (I)										
Non-Inoculated	45.1 a	50.0 a	9.56 a	11.18 a	22 a	26 a	47.49 a	39.85 b	10.41 b	9.18 a
FOL Inoculated	46.1 a	51.9 b	9.48 a	10.88 a	22 a	26 a	46.02 a	36.76 a	8.36 a	8.29 a
Interaction (R x I)										
P/SM x U	45.1 b	49.7 a	10.22 c	11.60 bc	23 b	23 a	50.44 bc	34.86 b	14.81 c	10.04 a
P/SM x I	46.1 b	50.6 a	9.06 ab	9.70 a	22 a	26 ab	57.22 c	49.49 d	7.83 a	8.03 a
P/ST x U	46.3 b	51.0 a	9.92 bc	11.44 bc	23 b	26 ab	48.06 b	42.28 c	8.27 ab	10.26 a
P/ST x I	49.9 c	55.2 a	10.19 c	13.01 c	24 c	26 ab	43.26 ab	35.63 b	9.59 b	10.01 a
P x U	44.2 ab	49.4 a	8.52 a	10.50 ab	23 b	29 c	43.96 ab	42.40 c	8.15 ab	7.23 a
P x I	42.3 a	49.8 a	9.20 ab	9.93 ab	19 a	25 ab	37.60 a	25.18 a	7.64 a	6.83 a

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA. WAI indicates weeks after inoculation, P indicates Petomech ungrafted, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST indicates Petomech grated onto *Solanum torvum*, R= Rootstock, I indicate Inoculated plants, U indicate non-inoculated plants and R indicate rootstock.

4.3.3.2 The influence of graft combinations and *Fusarium oxysporum* inoculum on fruit quality and yield of Petomech tomato plants

There was no significant interactive effects (P< 0.05) between *Fusarium oxysporum* inoculum and the rootstocks; Petomech (P), Petomech grafted onto *Solanum macrocarpon* (P/SM), and Petomech grafted onto *Solanum torvum* (P/ST) on fruit qualities which are total soluble solids, fruit firmness, number of locules, pericarp thickness, power of hydrogen, fruit girth, fruit length and also number of fruit per plant. (Table 19)

There were significant interaction effects (P< 0.05) between *Fusarium oxysporum* inoculum and the grafted and non-grafted plants on the yield per plant. Petomech grafted onto *Solanum macrocarpon* inoculated with *Fusarium oxysporum* (P/SM x I) recorded the highest yield (465.9 g/plant) while non-grafted Petomech inoculated with *Fusarium oxysporum* (P x I) produced the least yield (157.7 g/plant) and the difference were significant (P< 0.05). Generally, grafted rootstock inoculated with *Fusarium* produced significantly higher yield compared to the non-grafted inoculated Petomech. P/SM x I yielded 465.9g/plant, P/ST x I also yielded 252.5g/plant and the least P x I 157.7g/plant yielded (Table 19).

Table 19: Influence of graft combinations and *Fusarium oxysporum* inoculum on Fruit quality parameters and Yield of grafted (Petomech) tomato plants

Scion/Rootstock	TSS (%)	FF (Lbs)	NOL	PT (mm)	Ph	FG (mm)	FL (mm)	NF/Plant	Yield/Plant (g)
P (Control)	4.99 a	2.67 a	2 a	5.85 a	3.87 a	34.91 a	40.63 a	6 a	205.1 a
P/SM	5.00 a	2.68 a	2 a	6.70 b	4.00 b	44.32 b	53.78 b	6 a	453.1 c
P/ST	4.94 a	3.02 a	2 a	7.43 c	4.03 b	44.73 b	53.27 b	7a	350.3 b
Inoculum (I)									
Non-Inoculated	5.07 a	2.81 a	2 a	6.52 a	3.94 a	43.18 b	51.28 b	6 a	336.8
FOL Inoculated	4.89 a	2.76 a	2 a	6.80 a	3.99 a	39.46 a	47.17 a	6 a	335.6
Interaction (R x I)									
P/SM x U	4.83 a	2.58 a	2 a	6.83 a	3.99 a	46.98 a	56.60 a	6 a	440.4 de
P/SM x I	5.17 a	2.77 a	2 a	6.57 a	4.00 a	41.65 a	50.95 a	7 a	465.9 e
P/ST x U	4.98 a	2.98 a	2 a	7.06 a	3.97 a	45.05 a	54.20 a	6 a	317.3c
P/ST x I	4.91 a	3.05 a	2 a	7.81 a	4.10 a	44.41 a	52.34 a	7 a	383.2 d
P x U	5.41 a	2.88 a	2 a	5.67 a	3.85 a	37.49 a	43.05 a	7 a	252.5 b
P x I	4.58 a	2.46 a	2 a	6.03 a	3.88 a	32.33 a	38.21 a	5 a	157.7 a

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA. P indicates Petomech P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST indicates Petomech grated onto *Solanum torvum*, R= Rootstock, I= Inoculated plants, U= non-inoculated plants and TSS = Total Soluble Solids; FF = Fruit Firmness; NOL = Number of Locules; PT = Pericarp thickness; pH = Power of Hydrogen; FG =Fruit Girth; FL = Fruit Length; NF = Number of fruits.

4.3.3.3 The influence of grafting combinations and *Fusarium oxysporum* inoculum on the shoot and root biomass of Petomech tomato plants

The fresh and dry (shoot and root) biomass results show a significant interactive effect (P<0.05) between the graft combinations and its control and *Fusarium oxysporum* inoculum. P/ST x I had the highest fresh root (27.89 g) as well as the highest fresh shoot (74.37). On the other hand, P/SM x I had the least fresh root (14.93 g) whilst P x I also recorded the least fresh shoot (53.07 g).

There was a significant difference between the fresh root weight of the inoculated plants (20.04g) and non-inoculated plants (26.88g) as well as the inoculated dry shoot weight (21.50g) and non-inoculated dry shoot weight (17.15g) (Table 20).

Table 20: Influence of graft rootstock and *Fusarium oxysporum* inoculum on the shoot and root biomass of grafted Petomech tomato plants

Scion/Rootstock	Fresh Root Weight (g)	Fresh Shoot Weight (g)	Dry Root Weight (g)	Dry Shoot Weight (g)
P (Control)	25.89 b	60.17 a	5.15 b	17.24 a
P/SM	16.02 a	68.70 b	4.00 a	20.96 b
P/ST	28.45 b	70.09 b	6.58 c	19.77 ab
Inoculum (I)				
Non-Inoculated	26.88 b	68.5	4.89	21.50 b
FOL Inoculated	20.04 a	64.1	5.59	17.15 a
Interaction (R x I)				
P/SM x U	17.12 a	72.53 b	4.63 ab	21.31 b
P/SM x I	14.93 a	64.87 b	3.37 a	20.61 b
P/ST x U	29.02 b	65.82 b	5.57 b	20.05 b
P/ST x I	27.89 b	74.37 b	7.59 c	19.49 b
P x U	34.50 c	67.27 b	4.46 ab	23.14 b
P x I	17.29 a	53.07 a	5.83 b	11.33 a

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA. P indicates Petomech ungrafted, P/ST= Petomech grafted onto *Solanum macrocarpon*, P/ST indicates Petomech grated onto *Solanum torvum*, R= Rootstock, I indicate Inoculated plants, U indicate non-inoculated plants and R indicate rootstock.

4.3.3.4 Disease incidence and severity of grafting combinations and ungrafted plants after inoculation

The area under disease progressive curve (AUDPC) of the grating combinations and the non-grafted plants increased steadily with time. There were significant differences between the grafting combinations and the non-grafted plants. However, no significant difference was observed between the grafting combinations P/SM and P/ST. P/ST showed the lowest AUDPC. Using the severity scale of 1-6, P/SM recorded 2, P/ST had 3 and P recorded 5.4. At week six after inoculation non-grafted had 100% of its population show symptoms of the disease. P/ST recorded 77.8% incidence of the disease and also P/SM recorded 66.7% incidence (Figure 10).

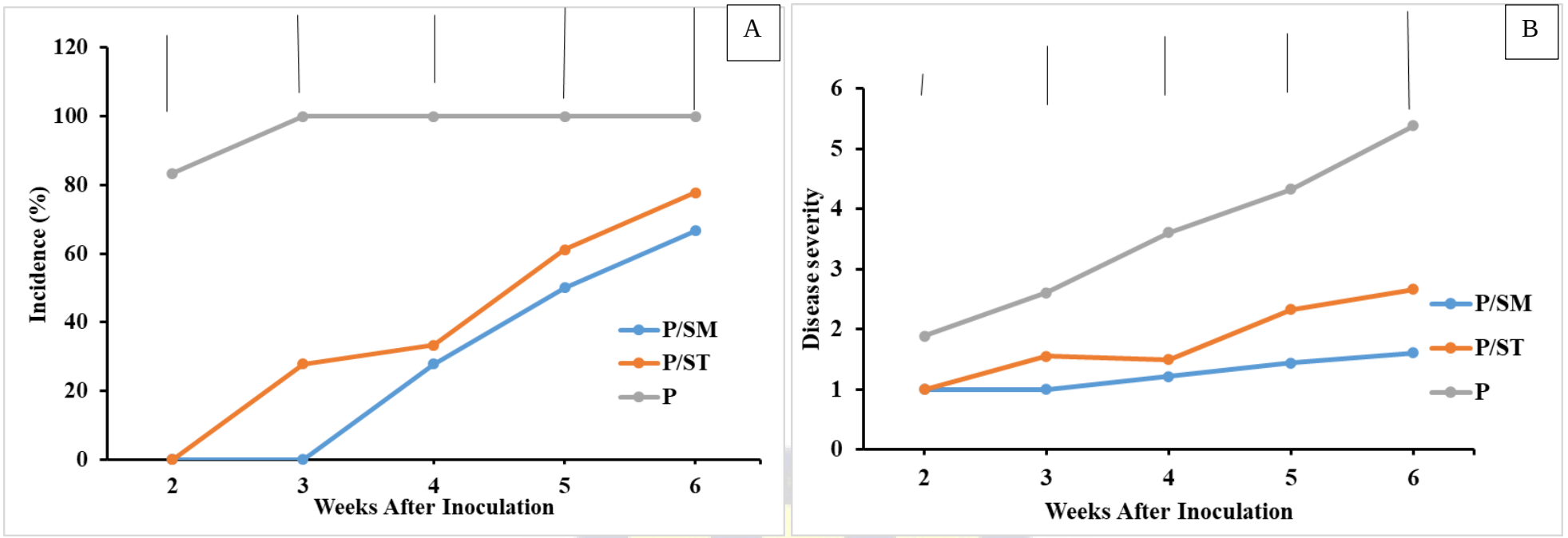

Figure 10: Disease incidence (A) and Disease severity (B) of grafted and non-grafted tomato plants inoculated with Fusarium at 2, 3, 4, 5 and 6 weeks after transplanting [vertical bars represent LSD (p<o.o5)]

4.3.4 Results of Cultivating Grafted Plants in a Naturally Infected *Fusarium* Farm at Jinijini in Berekum West District

4.3.4.1 Influence of grafting on plant height, plant girth, number of leaves, chlorophyll content and photosynthetic rate of the plant in a *fusarium* infected field

There was significant difference (P<0.05) in plant height among the grafting combinations (P/ST= 41.5cm, P/SM= 38.0cm) and the non-grafted plants (control) (P=33.6) at 3 weeks after transplanting. At six weeks after transplanting, no significant differences were observed for plant height in all treatments (Table 21).

There was no significant difference among grafting combinations and non-grafted plant with regards to plant girth at week three after transplanting. However, a significant difference (P<0.05) was observed at 6 weeks after transplanting, where P/ST produced the thickest girth (9.48 mm) followed by P/SM (9.00 mm) whereas the non- grafted had the least mean girth at 8.41mm (Table 21). The highest number of leaves was recorded for non-grafted plants (23) and the least was P/ST (20) at week six after transplanting. There were no significant difference (P<0.05) in the number of leaves at 3WAT.

The graft combinations recorded the highest chlorophyll content (P/SM= 64.93 µmol/cm^3, P/ST= 64.92 µmol/cm^3) at three weeks after transplanting while non-grafted plants recorded the least chlorophyll content (53.85 µmol/cm^3). The chlorophyll content of all treatments steadily decreased with time at week six after transplanting (Table 21).

There was no significant effect on the net photosynthetic rate among rootstock combinations and the control (non-grafted plant) during the first three weeks after transplanting. P/SM recorded the highest photosynthetic rate (15.68µmol$^{-2}s^{-1}$), followed by P/ST (13.30µmol$^{-2}s^{-1}$), and the least was non-grafted plants (12.45µmol$^{-2}s^{-1}$). At week six it was observed that, there was a decrease in photosynthetic rate. P/ST (5.51 µmol$^{-2}s^{-1}$) was significantly higher (P < 0.05) than P/SM (4.16 µmol$^{-2}s^{-1}$) and P (3.40 µmol$^{-2}s^{-1}$) (Table 21).

Table 21: Effect of graft rootstock on the height and girth of Petomech tomato plants in a field naturally infected with *Fusarium oxysporum*

Scion/Rootstock	Plant height (cm)		Plant girth (mm)		Number of leaves		Chlorophyll content ($\mu mol/cm^3$)		Photosynthetic rate ($\mu mol^{-2}s^{-1}$)	
	3 WAT	6 WAT	3 WAT	6 WAT	3WAT	6WAT	3 WAT	6 WAT	3WAT	6WAT
P (Control)	33.6 a	49.4 a	5.85 a	8.41 b	12 a	23 c	53.85 a	41.67 a	12.45 a	3.40 a
P/SM	38.0 b	46.4 a	6.00 a	9.00 b	11 a	22 b	64.93 b	57.39 a	15.68 a	4.16 a
P/ST	41.5 b	46.3 a	5.62 a	9.48 a	13 a	20 a	64.92 b	53.19 a	13.30 a	5.51 b

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA. Weeks After Transplanting

4.3.4.2 Effect of grafting on yield and fruit qualities of tomato (petomech) plants in *Fusarium* infected field

The grafting combinations P/SM and P/ST significantly increased the yield per plant, fruit girth and fruit length. The grafted rootstock P/ST recorded the highest yield per plant and number of fruit (153 g and 7) which was significantly higher compared to the control or the ungrafted petomech plants which produced the least yield and number of fruits per plant (51.4g and 5). P/SM obtained the highest mean for fruit girth (42.67mm) and length (49.12mm) followed by P/ST (41.08mm and 46.64mm) whilst the ungrafted plants produced fruits with smaller girth and length with a mean of 35.84mm and 43.11 respectively (Table 22).

There was no significant difference among grafting combinations and ungrafted plants for fruit brix, pericarp thickness, number of locules and pH. Fruit firmness was significantly higher for both grafting combination P/ST and P/SM (4.12 KgN^{-1} and 4.07 KgN^{-1}) in comparison to that of the ungrafted plants (2.80 KgN^{-1}) (Table 23)

Table 22: Influence of grafting on fruit girth, fruit length, number of fruit per plant and yield of grafted Petomech tomato plants

Scion/Rootstock	Yield/plant (g)	Number of fruit /plant	Fruit girth (mm)	Fruit length (mm)
P (Control)	51.4 a	7 a	35.84 a	43.11 a
P/SM	148.7 b	5 a	42.67 b	49.12 b
P/ST	153.0 b	7 a	41.08 b	46.64 b

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA.

Table 23: Influence of grafting on fruit girth, fruit length, number of fruit per plant parameters and yield of grafted Petomech tomato plants

Scion/Rootstock	Brix	Fruit firmness (KgN^{-1})	Pericarp thickness (mm)	Number of locules	pH
P	5.76 a	2.80 a	5.39 a	2 a	3.94 a
P/SM	6.37 a	4.07 b	4.86 a	2 a	4.13 a
P/ST	5.99 a	4.12 b	5.12 a	2 a	3.99 a

Means followed by the same letter in a column do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA

4.3.4.3 Effect of grafting on plant biomass of tomato (petomech) plants in *Fusarium* infected field

There was no significant difference in the fresh shoot and root weight of the grafted plants and non-grafted plants. P/ST recorded the highest dry shoot weight (17.58 g) and fresh shhot weight (38.50 g) whilst P recorded the highest dry root weight, 2.22 g (Table 24).

Table 24: Influence of graft rootstock on Fresh and Dry Biomass of grafted Petomech tomato plants

Rootstock (R)	Fresh Shoot Weight	Fresh Root Weight	Dry Shoot Weight	Dry Root Weight
P (Control)	21.00	7.39	12.13 a	2.22 b
P/SM	29.40	4.48	11.81 a	1.07 a
P/ST	38.50	8.17	17.58 b	2.16 b

Means followed by the same letter do not differ statistically from each other by the Fisher's Protected LSD test at 5% probability. NS indicate lack of statistical significance (P = 0.05) in data after ANOVA.

4.3.4.3 Disease incidence and severity of grafted tomato (petomech) onto *S. torvum*, grafted petomech onto *S. macrocarpon* and non-grafted petomech in *Fusarium* infected field

There was significant difference ($P < 0.05$) in *Fusarium* wilt incidence between the grafting combinations and non-grafted plants at week 2, 3, and 4 after transplanting. At week 5 and 6 after transplanting, it was observed that, there was no significant difference in *Fusarium* wilt incidence among all treatment. P/SM recorded the lowest *Fusarium* wilt incidence (%) from week two to four (0%, 13.89%, 25%, and 63.89%). On the contrary, P (ungrafted plants) and P/ST had 100% of its plant population showing symptoms of *Fusarium* wilt at week 2 and 3 respectively after transplanting. Non-grafted plants however, had the highest *Fusarium* wilt incidence (%) (Figure 11 A).

There was significant differences ($P < 0.05$) in *Fusarium* wilt severity between the grafting combinations and the non-grafted plant. On the severity rating scale of 1-6, the highest disease severity was observed in the non-grafted petomech plants (P) with disease rating 5.45 at week six after transplanting followed by petomech grafted onto *Solanum torvum* (P/ST) with disease severity rating 3.5 while the lowest disease severity rating was observed in petomech grafted onto *Solanum macrocarpon* (P/SM) with disease rating 3.2 at week six after transplanting (Figure 11 B).

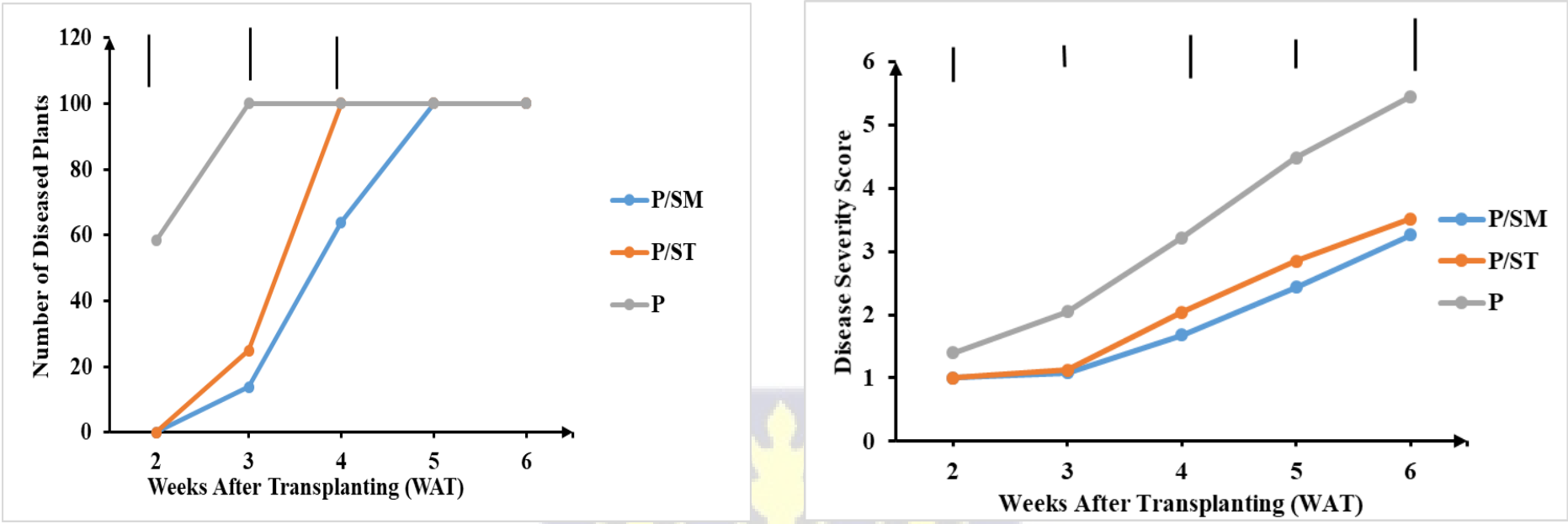

Figure 11: (A) Disease incidence and (B) Disease severity of grafted and non-grafted tomato plants in *Fusarium* infected field at 2, 3, 4, 5 and 6 weeks after transplanting [vertical bars represent LSD (p<o.o5)]

CHAPTER FIVE

5.0 DISCUSSION

5.1 A survey on farmers knowledge and perception on *Fusarium* wilt of tomato

Tomato is mostly cultivated by men in the Berekum West region. A recent report by Hortifresh (2020) showed similar trends in major tomato farming communities in Akumadan, Afrancho and Tuobodon in region of Ghana. Usman and Bakari (2013) reported that women were involved in tomato production but to a small extent. In typical tomato farming communities, men mostly own lands whiles women tend to be engaged in cultural practices and farm management. Constraints such as pest and disease infestation, lack of credit and irrigation facilities, and access to ready market were concerns raised by farmers in the study area . Karuku,(2017) reported that farmers in Kenya encountered similar constraints which include access to credit for production, inability to get extension services and unavailable source of water for irrigation.

In this present study, it was observed that ninety-five (95%) of tomato farmers sampled had received formal education which may have enabled them to read and find solution to manage *Fusarium* wilt disease on their farm and also comprehend the use of safe approaches to control diseases. As reported by Moranga (2016), a farmer who has received some form of formal education makes good choices and is more inclined to adopt improved technologies that will increase productivity. This study showed that majority of farmers (90%) of farmers who are actively involved in tomato production falls within the range of 30-40 years and this could be translated to increase in productivity because most farmers are strong and energetic to work effectively. This finding is consistent with Usman and Bakari (2013) who reported that tomato production was predominantly by adults. Ayerh (2015) also recorded the youth group as the highest and the widest age range between 36-46years in tomato growing communities at

Ashanti region of Ghana. This also means that youth in Berekum West District indulge less in rural-urban migration and are focused in making a livelihood in their community.

This study confirmed *Fusarium oxysporum* as the causal pathogen of tomato wilt in Berekum West District. Most (85%) of the respondents were able to identify the disease through symptoms such as yellowing of leaves, discolouration of vascular tissue, stunted growth and wilting of plant. In all six (6) sampled communities, it was observed that *Fusarium* wilt disease incidence varied. This could be attributed to the type of soil, environmental conditions, source of seed, management practices and previous crop cultivated on the land. A survey conducted in four tomato growing areas in the Central region of Ghana indicated tomato wilt disease was caused by one or more species of *Fusarium* (Opoku-Asiama *et al*., 2013).

The majority of farmers (84.3%) obtained their seeds from unaccredited sources which could be associated with high *Fusarium* wilt outbreak disease in the study areas. Seeds obtained from unapproved sources could carry *Fusarium* spore or other pathogenic fungi which will be transferred to the next growing season. This findings confirms Adu-Dapaah *et al*., (2002) report that 95% of farmers in Ghana used seeds mainly from their own selections from previous crops and also exchanged amongst themselves. ().

Farmers resorted to several ways to manage *Fusarium* wilt on their tomato farm. In a bid to control the disease, most farmers (72%) misapplied synthetic agrochemicals. They either increased the application rates or under applied agrochemicals which could not manage the disease which was unsafe for human health. These synthetic fungicides cause a huge environmental pollution which are detrimental to human health and other organisms. The excessive or indiscriminate use of agrochemicals could cause the target pathogen to develop resistance against fungicides/pesticides. It was also observed that majority (57%) of the respondents practice mono-culture which caused the build of *Fusarium* spores. Few farmers

adopted other control methods like crop rotation, solarisation, land rotation and the use of organic and inorganic fertilizers. Onion farmers used farm management strategies in combating *Fusarium* wilt disease which includes crop rotation, farm sanitation, weeding and solarisation (Alidu, 2013). In a study by Mwangi (2015), it was observed that 40.6% of tomato farmers uprooted the infected plants and 21.8% managed Fusarium wilt disease by applying Ridomil Gold while others also used resistant varieties, irrigation, and crop rotation as management strategies. Farmers sampled in Berekum West had never used grafted tomato plants as a means of managing *Fusarium* wilt. They had little idea of vegetable grafting as compared to tree graft. Ninety percent (90%) were not knowledgeable about tomato grafted onto a desirable rootstock. This finding is similar to the results by UCDAVIS (2021), that though 52% of tomato farmers used grafted plants of various crops, only 2% used grafted tomato.

In this study, *Fusarium* wilt reduced yield of tomato by 1000-2000 kg per 0.4 hectare. Farmers incurred high cost of production in making efforts to manage the disease and could not achieve their expected yield. Similar findings have been reported in the Kwahu South District of Ghana, where onion famers recorded significant loss of income due to *Fusarium* infections (Alidu, 2013).

5.2 Morphological and molecular identification of *Fusarium*

5.2.1 Morphological identification of the pathogen causing *Fusarium* wilt disease of tomato from 16 farms

Diseased plant samples collected on the basis of symptomatology from sixteen field in six communities of Berekum West District had lesions and brown discolouration of the stem, yellowing of leaves, low crop yield, weakening/wilting and death of the plants. The incidence and severity of the *Fusarium* wilt disease was significantly higher (53.3%) at Kwasi Mensah kura compared to the other five sampled areas. The results from this study are similar to the findings of Opoku-Asiama (2003) who reported through a survey on the incidence and severity

of *Fusarium* in the Central region of Ghana and Kwapro obtained the highest wilt incidence and severity of 58.3% and 1.02 respectively out of four tomato growing communities.

Morphological identification of the fungi isolated from the diseased tomato plants was based on the micro and macroconidia, chlamydospores and colour of culture. Cultural characteristics of isolates varied greatly. Some appeared white to pink, purple tinge, white to yellow and white to brown. Microconidia were in abundance and were oval shaped and mostly non-septate or rarely single septate. The mean spore length of microconidia ranged from 6.3-8.7 µm x 2.6-3.7 µm. Macroconidia appeared straight to slightly curved or boat shaped, few were hyaline, 2-4 septate but most had 3 septation. Macroconidia were sparse to abundant and ranged between 22.4 µm -28µm x 3.1-3.6 µm. Chlamydospores were only identified from one isolate. Generally, chlamydospores were spherical, formed in chains with rough walls. The result of this study confirmed the findings of Nirmaladevi *et al*. (2016) & Abkar *et al*., (2018) who reported that isolates displayed a high level of diversity in their morphological and cultural characteristics. Eight isolates (FO3 Stem, FO4 Fruit, FO5 Leaf, FO7 Stem and FO11 Stem, FO13 Leaf, FO4 Stem and FO7 Fruit) were identified as *Fusarium* species. The absence of *Fusarium* spores from the other sampled sites and diseased plant part could be influence of *Trichoderma* spp. found in the soil when the isolation was done was done. Carmona *et al.* (2020) also reported that out of 105 isolates, 120 were grouped into *F. oxysporum* species complex.

5.2.2 Molecular identification of *Fusarium* isolates

Identification of organisms based on their morphology should be complemented with molecular characterisation (Saikai, 2010). In this study, the rDNA Internal Transcribed Spacer (ITS) region was used to identify the isolates to species level. The expected band size of the ITS1 and ITS4 region of the isolates were amplified at 515bp. Singah *et al*. (2016) also used

ITS1 and ITS4 region to identify *Fusarium* species on tomato plants and observed a band size of 515bp. Results obtained from ITS sequencing showed that, the wilt disease was associated with different *Fusarium* species. Isolates obtained from this study clustered with closely related *Fusarium* species which were; *Fusarium equiseti* isolate, *Fusarium oxysporum* f. sp. *lycopersici* strain, and *Fusarium oxysporum* isolate. This view is supported by a study in the Central region of Ghana where *F. equiseti*, *F. moniliforme* and *F. acuminatum* were associated with tomato wilt in the tomato growing communities (Opoku-Asiama, 2003). This also confirmed the genetic diversity of *Fusarium* spp. Isolates were found to have different levels of virulence. FO11 was highly virulent isolate whilst F04 Fruit, F03 Stem, F05 Leaf, and F07 Stem were moderately virulent . The findings of this study and that reported by p Opoku-Asiama, (2003) agree with the observations made by Nirmaladevi *et al*. (2016) that the pathogenicity of *Fusarium* spp. towards tomato had evolved several times independently.

5.3 Effect of grafting and *Fusarium* inoculum on tomato plant

5. 3.1 Optimum environmental conditions for enhancing graft take during the dry and wet season

In this study, the temperature and humidity observed in the grafting chamber (Pot experiment) for 7 days were 24.0 °C - 29.5 °C and 78.5-100 % RH respectively for the dry season. While 24.0 °C - 29.5 °C and 78.5-100% RH reported for the wet season . During the dry season , high (30%) graft failure was observed. This could be attributed to fluctuating environmental conditions. However, during the wet season , graft failure was low (13%). This finding confirms the results of Vu (2013) who stated that increasing relative humidity in grafting chambers significantly increased percent of diseased plants. And also, a constant high relative

humidity (90%) for first 2 to 3 days, and afterwards reduced low relative humidity (70%) at 23 °C condition during healing and acclimatization promoted the graft-take and quality of grafted tomato seedlings (Lee *et al*. (2010). In this present study, it was observed that graft take ratio was not dependent on the type of rootstock used as there were no significant differences among the two grafting combinations (Petomech grafted onto *Solanum torvum* and Petomech grafted onto *Solanum macrocarpon*).

Increasing graft success does not only depend on the scion and rootstock compatibility or a close contact between the scion and rootstock vascular bundles. Proper environmental conditions such as temperature and humidity are also required to facilitate rootstock and scion union (graft take) during the healing and acclimatization period (Davis *et al*., 2008).

5.3.2 Effect of grafting combinations and *Fusarium oxysporum* inoculum on plant growth and photosynthetic rate of *Solanum lycopersicum* 'Petomech' (pot experiment).

There was significant interaction between rootstocks (grafting combinations and non-grafted) and inoculum after treatment application on plant height. The inoculated graft combinations produced significantly taller plants compared to the non-grafted plants inoculated (42.3 cm-49.8 cm). Among the grafting combinations, Petomech grafted onto *Solanum torvum* x inoculum (P/ST x I) were taller (49.9 cm-55.2 cm) than Petomech grafted onto *Solanum macrocarpon* x inoculum (P/SM x I) (46.1cm-50.6cm). This finding confirmed findings by Jabnoun-Khiareddine *et al*. (2019), a significant interactive effect of rootstock used and the fungal treatment on plant height. Further observed that generally, all pathogens combined with grafting, had higher plant heights compared to the non-grafted.

Petomech grafted onto *S. torvum* treated with *F. oxysporum* inoculum at 2 and 4 weeks after transplanting had a thicker plant girth. However, P/SM x I and P x I had no significant difference at week 2 but at week 4, P x I developed thicker girth than P/SM x I. The results of this study is similar to a study by Leonardi & Giuffrida (2006) and Buller *et al*. (2013) where

scions grafted onto *S. torvum* in soil infected with *Verticillium* wilt had thicker girth (Buller *et al*., 2013). Musa (2020), also conducted a similar study and attributed increase in girth of the scion due to the vigorous root system of the rootstock. Leonardi & Giuffrida (2006) also reported that non-grafted eggplants had same or thinner plant girth as the grafted plants which differed from this current study.

In this study, P/SM x I and P/ST x I had significantly higher chlorophyll content than P x I. However, P/SM recorded the highest chlorophyll content in this study. This does not differ from the photosynthetic rate of the treatments as chlorophyll content influences photosynthetic activity of the plant. Although P/SM x I had the highest chlorophyll content, P/ST x I recorded the highest photosynthetic rate at 2 and 4 weeks after transplanting. Liu *et al*. (2011) reported that the chlorophyll content of grafted tomato plants was significantly higher than non-grafted plants.

5.3.3 The influence of graft combinations and *Fusarium oxysporum* inoculum on fruit quality and yield of Petomech tomato plants (Pot experiment)

There was no significant interaction among grafting combination, non-grafted plants and *F. oxysporum* inoculum on the fruit quality attributes measured. Similar results were reported by Jabnoun-Khiareddine *et al*. (2019), who found that fruit weight and other fruit qualities on *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) inoculated plants were not significantly different for grafted and non-grafted plants. A study conducted by Agyeman *et al*. (2021). to evaluate tomato (Petomech) grafted onto *S. macrocarpon* and *S. aethiopicum* against root-knot nematode (*Meloidogyne incognita*) also reported no significant difference for fruit diameter, total soluble solids, titrable acidity, and pH among the grafted and non-grafted plants This could be that, the qualities attributed by the fruit are dependent on the scion and not the rootstocks.

A significant interaction was observed between the factors; rootstock and *F. oxysporum* inoculum on the total plant yield. Grafted plants yielded better compared to non-grafted plants. *Fusarium oxysporum* inoculated P/SM had significantly higher yield than *Fusarium oxysporum* inoculated P/ST. The result of this study was consistent with report by Schwarz *et al*. (2010) who found plants grafted onto Maxifort rootstock produced 63% higher yield than the non-grafted control. In this study, it was observed that both graft combination (P/SM and P/ST) inoculated with *F. oxsporum* yielded better than graft combinations without inoculum. Also, statistically, all rootstock combined with *F. o* and those without *F. o* when pooled showed no significant difference for yield per plant. As reported by Ibrahim *et al*. (2001) and Marsic and Osvald (2004), the increase in yield may depend on rootstock used which may provide higher yield through added vigour and plant growth. The result of this study contradicts Jabnoun-Khiareddine *et al*. (2019), reported that all cultivars and grafting treatments combined, fruit yield was higher for non-inoculated control plants followed by that of FORL inoculated plants.

5.3.4 Effect of grafting and *F. oxysporum* inoculum on fresh root weight, dry root weight, fresh shoot weight and dry root weight (Pot experiment).

P/ST x I recorded the highest fresh shoot, fresh root, dry shoot and dry root. Jabnoun-Khiareddine *et al*. (2019) reported that grafted plants and *Fusarium oxysporum* f. sp. *lycopersici* race-2 inoculum combined showed a significant increase in fresh root weight over the non-grafted plants but it contradicts the findings of this study because grafted P/SM inoculated with *F. oxysporum* recorded the least fresh root weight.

5.3.5 Effect of grafting on disease incidence and severity in the dry season (Pot experiment)

Grafting delayed the development of *Fusarium* wilt disease and also reduced the rate at which symptoms were expressed compared to the non-grafted plants. P/SM showed a high level of resistance to *F. oxysporum* inoculum. The delay in disease development as observed in this

current study indicated that the tomato plant grafted onto resistant rootstocks could tolerate *Fusarium* wilt disease over a period of time along the disease development cycle. Similar findings were reported by Rivard and Louws (2008), who found that Maxifort offered a complete control of *Fusarium* wilt when Heirloom tomatoes were used as scions. Grafting against root-knot nematodes using *S. macrocarpon* as rootstock did not only offer resistance to root-knot nematodes but *Fusarium* wilt as well (Agyeman *et al*., 2021).

5.3.6 The effect of grafting on plant growth and yield of *S. lycopersicum* (Petomech) under infected *Fusarium* field at Berekum

Under natural infected field, it was observed that both graft combinations recorede a significant increase in plant growth parameters compared to the non-grafted plants at 3 weeks after transplanting. However, eventually , all treatments showed similar or no significant difference in plant growth at 6 weeks after transplanting. This indicates that, grafted plants might be able to manage *Fusarium* wilt better at the early stage of the disease development to build the vigour of the plant but declines as the plant matures. The inconsistency in grafted plants might be due to differences in rootstock-scion combinations and growing conditions (Guan *et al.*, 2012).

In this study grafting had a significant effect on the growth and yield of tomato plants in fields infected with *Fusarium.* Similarly, Gisbertet *et al*. (2011) recorded a higher yield for Black Beauty grafted onto *Solanum torvum* (6.4kg/plant) compared to those grafted onto *S. macrocarpon* and the control.

Grafted plants recorded the highest mean for fruit girth, fruit length, brix, firmness and pH. This contradicts the findings of Nkansah *et al*. (2013), who observed no significant difference in fruit firmness and brix of grafted and non-grafted plants. Fruits with lower pH value tends to have a longer shelf-life. Friut from the non-grafted plants have a chance of storing longer shelf-life than those from grafted plants because they have a lower pH which enables them to

resist microbial attack (Manashi, 2011). This current study shows that non-grafted plants recorded the thickest pericarp. Fruit with thicker pericarp also have longer shelf life (Kumar *et al*., 2017).

P/ST recorded significant higher fresh shoot weight, fresh root weight and dry shoot weight compared to the non-grafted and the grafted P/SM. However, the dry root weight of the non-grafted was significantly higher than the grafted plants. Mohammed *et al*. (2009) reported that dry root weight of Cecillia F1grafted onto Beaufort and He-man rootstocks was significantly higher than the non-grafted one and this findings were not consistent with the current study.

5.3.7 Effect of grafting on disease incidence and severity of *S. lycopersicum* under infected *Fusarium* field

In this study, the disease development assessment over six weeks reflected a similar trend as observed in the pot experiment, where the grafted rootstocks offered high to moderate level of protection to the scion (*S. lycopersicum* 'Petomech') against *Fusarium* wilt. P/SM and P/ST delayed the onset of the disease from week one to week three after transplanting but this was not observed for non-grafted plants. There was a significant difference between the graft combinations and the non-grafted plants. Rivard *et al*. (2008) reported that Maxifort treatments had the lowest AUDPC values, and Robusta had an intermediate value, and the non-grafted plants had the highest AUDPC value however, there were no significant differences between the two rootstocks.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

- This research revealed that, *Fusarium* wilt disease of tomato was widespread within tomato growing areas in Berekum West District, a suburb of Bono region in Ghana. *Fusarium* wilt could result from the use of infected seed and mono-culture which could promotes build-up of inoculum. Also, indiscriminate use of fungicides may cause the pathogen to develop resistance. Most farmers in the styudy area Jinijini, Fatenta, Pruso, Kwasi Mensah kura, Mantukwa and Forest Nkwanta in Berekum West districts could identify the disease by its symptoms but did not have enough knowledge of the causal agent and therefore appropriate way of controlling the disease. The deployment of grafted plants to manage *Fusarium* wilt disease was new to most of tomato farmers (89%). Farmers were ready to accept the new technology to help manage the disease because it caused a drastic yield loss and affected their livelihood.

- Based on morphological and molecular characterization, different species of *Fusarium* were identified from the disease tomato plants, in Berekum West District of Ghana.

- From the present study, grafting *Solanum lycopersicum* (Petomech) onto *Solanum torvum* showed a higher graft success (91%). There was a significant interaction between the graft combinations (*S. lycopersicum* onto *S. torvum* and *S. lycopersicum* onto *S. macrocarpon*) and non-grafted plants combined with *Fusarium oxysporum* inoculum under the pot experiment. It was that both graft combinations enhanced the resistance of the scion (petomech) against *Fusarium oxysporum* which enhanced the growth and yield of fruit per plant. Petomech grafted onto *S. macrocarpon* produced the highest fruit per plant (465.9 g) and also showed the lowest disease incidence and severity. However, under natural infected field condition, Petomech grafted onto *S.*

Torvum produced the highest yield (153.0 g) but those grafted onto *S. macrocarpon* showed the least disease incidence and severity. The significant yield difference between the pot experiment and the field experiment could be due to variation in time of planting, climatic conditions and inoculum density.

6.2 Recommendations

- Grafting resistant rootstock against *Fusarium* wilt of tomato should be repeated in the rainy season at Berekum and other tomato growing areas in the country.
- Additional molecular markers should be used in characterizing *Fusarium* isolates to ascertain their diversity.
- Further study should be done on molecular identification of indigenous *Trichoderma* spp. under tomato soil rhizosphere and its potential as a biocontrol agent against FOL.

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APPENDIX

Questionnaire

A survey of farmers' knowledge, perceptions and experiences concerning the occurrence and management of *Fusarium* wilt using grafted plants

QUESTIONNAIRE NO: …….

INTRODUCTION

Name of Enumerator ……..... Date of interview…../……./ 20…..
District ……..
Community/Village/Town: …….
Name of farm/farmer: ……

Telephone number:

A. Demographic characteristics

Q1. Gender of farmer: 01 = Male [ ] 02 = Female [ ]
Q2. Age: <20 = 1 [ ] 21-30 = 2 [ ] 31-40 = 3 [ ] 41-50 = 4 [ ] >50 = 5[ ]
Q3. Marital Status: 01 = Single [ ] 02 = Married [ ] 03 = Divorced [ ] 04 = Separated [ ] 05 = Widowed [ ]
Q4. Religion: 01= Christian [ ] 02 = Muslim [ ] 03 = Traditionalist [ ] 04 = Others (Specify)…….
Q5.What is your level of education? 01 = No Schooling [ ] 02 = Primary school [ ] 03 = MSCL/JHS [ ] 04 = SHS/Technical/Vocational [ ] 05 = Tertiary [ ] 06 = Others (Specify)……..
Q6. Years of farming: ……....

B. Land use intensity

Q7. Size of farm, year of cultivation and source of planting materials

Year of cultivation	Size of farm(Acre)	Source of planting materials
2016		
2017		
2018		
2019		

Codes: Size of farm: 0-1 acre [1], 1-2 acres [2], 3-4 acres [3], 5-8 acres [4], more than 8 acres [5]

Source of planting materials: own farm [1] family member [2], market [3], others (specify)……

Q8. What are the varieties of tomato grown in your farm?

Variety	**Proportion of farm**

Code for proportions: <1 acre [1], 1-2 acres [2], 3-5 acres [3], >5 acres [4]

Q9. How long have you cultivated this land? 1-3 years =1 [ ] 4-6 = 2[ ] 7-10years = 3 [ ] 11-15 years = 4 [ ] more than 15 years = 5 [ ]

Q10. what type of crop have you planted on this piece of land in the past 5 years?
Tomato = 1 [ ] Yam = 2 [ ] Cassava = 3 [ ] Maize = 4 [ ] Others (specify)…….

Q11. How long have you planted tomatoes on this piece of land? 1-3 years = 1 [ ] 4-6 years = 2 [ ] 7-10 years = 3 [ ] more than 10 years = 4 [ ]

Q12. Have you done crop (tomato) rotation on your land before? Yes = 1 [ ] No = 2 [ ]

Q13. If yes, with what crop(s)?.....

Q14. What was your period of rotation? 1-2 years = 1[ ] 3-5 years = 2 [ ] more than 5 years = 3 [ ]

Q15. Have you practiced land fallow in the past? Yes = 1 [ ] No = 2 [ ]

Q16. If yes, how long was the fallow period? 1 year = 1[ ] 2-4 years = 2 [ ] 5-10 years = 3 [ ]

C. Farmers' knowledge, perception and experiences concerning occurrence of fungi on the farm and in storage.

Q17. Do you experience any pest and disease problems in your farm? Yes = 1 [ ] No = 2 [ ]

Q18. Do you know what fungi are? Yes = 1 [ ] No = 2 [ ]

Q19. Do you see symptoms of fungal infections? Yes = 1 [ ] No = 2 [ ]

Q20. Which of your varieties do you observe these symptoms more? .....

Q21. Which of these symptoms do you observe in the field?

Symptom	Yes [1] No [2]
Necrotic streaks or lesions on stems	
Wilting	
Yellowing of leaves	
Poor fruit set	
Other (Specify)	

Q22. Has the disease affected your yield and fruit quality? a. Yes= 1[ ] b. No=2[ ]

Q23. What time of the year do you experience symptoms of fungi damage?
a. Rainy season [ ] b. Dry season [ ] c. Other [ ] (Please specify)……

Q24. At what stage in the development of your crops are these symptoms pronounced? a. Seedling [ ] b. Vegetative [ ] c. Flowering [ ] d. Fruiting [ ] e. Others (specify)……..

Q25. What are the conditions of the soil in your field in which these symptoms/signs are prevalent?

a. Lowland (waterlogged) [ ] b. Well drained soil [ ] c. Sandy –clayey [ ] d. Sandy soil [ ] e. Loamy soil [ ] f. Clayey soil [ ]

D. Farmers' knowledge, perception and experiences concerning control/management

Q26. Source of irrigation water. a. well = 1 [ ] b. borehole = 2 [ ] c. stream = 3[ ] d. river =4 [ ] e. dam = 5 [ ]

Q27. Do these symptoms spread fast within the farm? a. Yes=1 [ ] b. No= 2 [ ]

Q28. Do these symptoms spread to other farms? a. Yes = 1 [ ] b. No = 2 [ ]

Q29. Are you able to manage / control *Fusarium* infestation? a. Yes = 1 [ ] b. No = 2 [ ]

Q30. If yes, what are some of the management practices used in your farm?

Management Practice	Yes[1] No [2]
1. Crop rotation	
2. Use of fungicides	
3. Land rotation	
4. Use of organic/ inorganic fertilizers	
5. Solarization	
6. Others (Specify)	

Q31. If no, give reasons. a. No reason = 1 [ ] b. I have no knowledge about their control = 2 [ ] Other (specify) ……

Q32. If yes, what do you think can be done to control the fungi?

What can be done?	Who should do it?	Why it should be done?

E. Farmers knowledge and perception about grafting

Q33. Are you aware grafting can be used to manage soil-borne diseases?

a. Yes=1[ ] b. No =2[ ]

Q34. Are you also aware grafting can be used to improve fruit quality and crop response to a variety of abiotic stress? a. Yes=1[ ] b. No =2[ ]

Q35. Have you cultivated any grafted tomato plant on your field? a. Yes=1[ ] b. No =2[ ]
Q36. If yes, where the grafted plant purchased or they were self-made?
……..
Q37. If no, would you like to manage Fusarium wilt by using grafted tomato?
a. Yes=1[ ] b. No =2[ ]

E. Farmers knowledge, perception and experiences concerning economic importance of fungi

Q38. What are your reasons for farming tomatoes? .....

Q39. What are your expected and actual yields obtained over the years?

Year	Farm size	Estimated yield (Crates/Ha)	Actual yield (Crates/Ha)	Reasons for difference (If any)
2019				
2018				
2017				
2016				

Q40. Were actual yields higher than expected yield? Reasons: a. No reason = 1 [ ] b. Favorable rainfall = 2 [ ] c. Use of inorganic/ organic fertilizers = 3 [ ] High soil fertility = 4 [ ] Others (Specify)…….
Q41. Please explain the effect of fungal parasitism on the following:
I. Money to pay children's school fees……
II. Money to repay loans…..……
III. Money to buy assets…….
IV. Money to pay medical bills……..
V. Your relationship with extension officers……..……...
VI. Your relationship with your spouse and neighbours……..……

Other comments……