Integrated Management Strategies for Tomato Fusarium Wilt

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Fusarium wilt is caused by the fungal pathogens, Fusarium oxysporum or Fusarium solani. It is a devastating disease that affects many important food and vegetable crops and a major source of loss to farmers worldwide. Initial strategies developed to combat this devastating plant disease include the use of cultural, physical and chemical control. None of these strategies have been able to give the best results of completely ameliorating the situation except for the cultural method which is mainly preventive. A good knowledge of the nature, behaviour and environmental conditions of growth of the disease agent is very important to controlling the disease development in that case. Biological control has been shown to be an environmentally friendly alternative. It makes use of rhizospheric and endophytic microorganisms that can survive and compete favourably well with the Fusarium wilt pathogen. They include plant growth-promoting rhizobacteria (PGPR) such as Bacillus spp. and Pseudomonas spp.. For PGPR to control or inhibit the growth of the Fusarium wilt pathogen, they make use of mechanisms such as indole acetic acid production, siderophore production, phosphate solubilization, systemic resistance induction and antifungal volatile production among others.

Key words : Biological control / Disease development / Pathogen / Plant growth-promoting rhizobacteria.

INTRODUCTION

Fusarium is one of the more troublesome genera of fungal plant pathogens, causing devastating diseases like Fusarium wilt and Fusarium root/stem rot in numerous economically important crops. It is one of the most serious diseases affecting tomato plants throughout the world, especially in upland areas (Charoenporn et al., 2010). Fusarium wilt is caused by Fusarium oxysporum Schlecht or Fusarium solani. Formae speciales of F. oxysporum can usually only cause Fusarium vascular wilt on one plant host species. The predominant hosts for F. solani are vegetable crops but some strains may be infectious to man. There are more than 100 Fusarium vascular wilt diseases worldwide (Burgess et al., 2008). Apart from causing diseases, they colonize outer cells of roots as harmless endophytes after the pathogen has killed the root tissues and others live as saprophytes in soil (Burgess et al., 2008). Some strains of F.oxysporum are not pathogenic and may even antagonize the growth of pathogenic strains and can be used as biological control agents (Fravel et al., 2003).

To provide an environmentally friendly Fusarium disease control system, the use of antagonistic microorganisms represents an alternative disease management strategy (Lugtenberg and Kamilova, 2009). This review will summarize the knowledge available for the use of native Bacillus spp. with biocontrol potential to combat Fusarium wilt of tomatoes. The origin, taxonomy and morphology of the Fusarium wilt pathogen will be introduced together with the pathogen and the disease. The initial management strategies will be reviewed and then the use of biological control will be discussed.
Origin, taxonomy and morphology of *Fusarium*

*F. solani* comprise a complex group of species that are widely distributed worldwide in soils. It causes tuber, root, and stem rots of plants. It includes at least 50 subspecies lineages (Schollenberger et al., 2005). Taxonomically *F. solani* belongs to the kingdom Fungi, phylum Ascomycota, subphylum Pezizomycota, class Sordariomycetes, subclass Hypocromycetidae, order Hypocreales, family Nectriaceae and genus *Fusarium* (Fry, 2004).

Distribution and environmental conditions

Weather conditions such as temperature and humidity normally affect the distribution of *F. solani* (Kosiak et al., 2004). *Fusarium* spp. is found to be widely distributed in nature in various environments. Toxic *Fusarium* spp. can cause disease in both plant and animals, including humans. The fungi are mostly present in subtropical regions of the world (McGovern et al., 2001). The optimum temperature for infection is between 27-31°C, but the disease can develop at a temperature that is lower, and soil moisture levels range from 28 to 75% with a pH of between 6-8 (Glen et al., 2003). However, the highest damage occurs in fields where the moisture content is low.

*Fusarium* wilt can remain viable in the soil for up to 30 years (Thangavelu et al., 2003). It is destructive to plants and progresses swiftly. By the time a plant shows any outward sign of infection, it is already too late, and the plant wilts and dies.

The disease and the infection behaviour of the pathogens in planta

After conidial germination in soil, entry of the pathogen into the plant system is through the root tips and is facilitated by the presence of wounds, which can be due to nematode feeding or through vascular wounds which can be sustained by the plant when cuttings are procured for planting or when leaves are detached from the stems. In the tomato- *F. oxysporum* f.sp. radicis-lycopersici (FORL) pathosystem, the pathogen multiplies abundantly through much of the root tissues, causing severe damage including cell disorganization through the Fusarium hyphae colonizing the roots and cell wall alterations (M'Piga et al., 1997) which may be due to the production of plant chitinases. In addition, during pathogen ingress in the outer root cortex there is elaboration of callose-enriched wall appositions at sites of attempted fungal penetration. However, penetration of the callus that has grown over the wounds caused by the fungus is impossible. Enzymes may also facilitate Fusarium penetration into plant host (Babalola, 2010a). It was earlier proposed that there might be value to transforming biocontrol agents to overproduce hydrolytic enzymes (Babalola, 2007).

Each pathogen then grows intercellularly through the root cortex, and, subsequently rapidly grows into the water-conducting tissue (xylem) (Beckman, 1990). The microconidia produced invade the xylem of the pseudostem where it obstructs the upward movement of water and nutrients. Sieve cells in the stem hinder the conidia from further spread, and, as this happens the spores germinate and grow through the sieve cells where they continue to germinate until the whole xylem system is blocked. As the vessels are plugged and collapse, the water supply to the leaves is blocked, thus causing wilt symptoms. In most cases, the first sign of wilt appears on one and a half month old plants and intensifies gradually. Wilting is seen on the lower leaves first and this later extends to the upper leaves. Leaves, twigs or even the whole plant turns brown and later dies and dries up (Khan and Khan, 2002). Death of the tomato plant is brought about by the failure of the infected xylem to meet the water requirements of the plant (Burgess et al., 2008). As the plant dies, the spores grow into the surrounding tissues where they form chlamydospores that are released back into the soil (Jones, 2000). Chlamydospores can be in the soil until there is enough moisture in the soil for them to germinate and the process is set into motion again. Due to the presence of antagonistic microorganisms, insufficient moisture and alkalinity, germination can be impaired in certain soils.

The disease or infection can be transmitted through infected plant material and through contaminated soil. Other means of spreading the disease is through human movement around the infected field, or the use of irrigation water and implements previously used on an infected crop.

Symptoms of *Fusarium* wilt

Plants affected with *Fusarium* wilt first develop stunted seedlings and yellowing of the lowest leaves that is often restricted to one side of the plant or a single shoot and later show the defoliation of older leaves. The affected leaves wilt and die. Wilting progresses up the stem until the foliage is killed and the stem decays (Lund et al., 1998). For this disease, brown vascular discoloration can be observed in stem tissue cross sections near the soil line (Burgess et al., 2008) even though these stems remain firm and green on the outside. In some cases, affected plants that do not die are often stunted and their yields are quite reduced. Where the inoculum pressure is high, seedlings may damp off as they emerge from the soil.

The general symptoms of many diseases of the root and stems are yellowing, wilting and stunting while a key symptom of pathogens which cause vascular
Integrated Management of Tomato Fusarium wilt disease, including *Fusarium* wilt pathogens, is the browning of the internal stem (vascular) tissue (Burgess et al., 2008). Reddish brown discolouration of the xylem vessels is visible inside the stem as lines (if the stem is cut open lengthways) or dots (if it is cut across). On the outside of affected stems, white, pink or orange fungal growth can be seen especially in wet conditions.

**Isolation of the pathogens from soil and infected plants**

Generally, the fungus is isolated from infected plant tissues, which are disinfected by soaking each sample in 1% sodium hypochlorite for 2 min. The tissues are placed on Potato Dextrose Agar and left for incubation at room temperature (25°C) until the growth of colonies became visible (5 days). Mopshological identification of *F. oxysporum* isolates is based on characteristics of the macroconidia, phialids, microconidia, chlamydospores, and colony growth traits. The *forma specialis* of FORL is identified using pathogenicity tests (Hibar et al., 2007). Bimonthly infection and reisolation of the pathogen from host plants is essential to maintain virulence (Babalola, 2007).

**CULTURAL, PHYSICAL AND CHEMICAL CONTROL METHODS**

According to Borrero et al. (2006), Elmer (2006) and L’Haridon et al. (2011), *Fusarium* wilt is a difficult disease to control. Some of the initial management strategies include cultural control, physical control and chemical control.

**CULTURAL CONTROL**

Cultural control involves practices and farming techniques that will help to increase the quality and quantity of the yield and also reduce the influence of pests and diseases. It involves manipulation of the environment in non-mechanical ways to control plants pests and diseases. It includes altering farming practices to make the environment unfavourable for the growth of disease pathogens and pests (Islam, 2001). It is also the purposeful manipulation of a garden or farm in the growing, planting and cultivation of plants to reduce plant disease, pest damage and numbers of pests. It has been shown that the correct implementation of cultural methods to control soilborne pathogens yields improved soil structure and consequently decreases disease incidence (Neshev, 2008). These methods are mainly preventive and a good knowledge of the nature, behaviour and environmental conditions of the growth of the disease agent is very important to controlling the disease development. Some of the methods used in the cultural control of *Fusarium* wilt of tomato are shown in Figure 1.

**Crop rotation**

Crop rotation is the practice of growing a series of crops that are not similar in the same area season after season. It is an easy way to control insects and diseases at little or no cost. For example, cabbage, tomatoes, or cauliflower planted in the same farm area each year will actually encourage disease-causing organisms to multiply in the soil. Plants affected by disease should be removed and disposed of as soon as possible, and then the soil should not be cultivated with the affected plant type for at least 4-5 years. The contaminated soil should be replaced with fresh soil from another part of the garden. Disinfection of soil by steaming or soil solarization also helps to reduce the disease load. Crop rotation helps to reduce the amount of pathogens in the soil. For it to effectively control a soilborne pathogen, the pathogen must be completely eliminated along with the plant residues from the farmland (Neshev, 2008).

**Hygiene (sanitation control)**

Diseased tomato plants and plant parts, weeds and residues should be removed and destroyed. They should not be kept in a compost pile. Burning them is the best, but they can also be placed inside plastic bags and disposed as garbage or treated with a disinfectant. Rapid crop destruction at the end of the season is crucial for managing tomato yellow leaf curl. Tools that have been used on the farm should always be disinfected and cleaned before reuse. The use of sanitized foot wear and clothing while working on the farm helps to prevent the carrying of infected soils from one plot of the field to another.
Fallowing
Fallowing is when a piece of land is left idle without cultivation throughout the growing season. This will reduce disease agents in the soil. Fallowing during the summer months is helpful when soil temperatures are high. The soil is kept dry and free of plant growth by constantly ploughing. Exposure of the soil to high temperature and excessive drying by ploughing helps to destroy exposed soil-borne disease organisms, such as nematodes. Other benefits of fallowing are insect and weed control. Factors that encourage fallowing include high temperature and low rainfall.

Mulching
Mulching is the addition of a thick layer of mulch on the soil surface to help control weed, optimise soil moisture and keep the soil cooler than 80°F. It helps in disease control by standing as a barrier between the plant parts above the ground and plant pathogen in the soil. Since it helps to control weeds, it also helps in altering the environment for these pathogens thereby creating unfavourable conditions for them and controlling diseases (Raid, 2011). In order to avoid splashing soil borne diseases on tomato leaves during watering, mulching of the plant is advised (Francis, 2012). Combination of sun solarization and two layers of mulching decrease the rate of fungal infection in plants (Garibaldi and Gullino, 1991).

Avoid overhead irrigation
Overhead irrigation aggravates disease problems; this is because if the foliage is wet, fungi and bacteria are easily spread. For example leaf spot diseases develop rapidly when leaves are moist. Overwatering should also be avoided because excessively wet conditions increase soil-borne fungal diseases like root rots and wilts (Raid, 2011). Drip drainage is recommended (Labrada, 2008).

Plant spacing
Plants should be properly spaced. Air circulation is reduced with overcrowded plants and this enhances leaf spot diseases like Septoria leaf spot on tomato. On the other hand, with curly top virus disease, overcrowding tomato plants reduces the disease incidence as the insect vector finds the plants less attractive in their crowded state. Also in a situation of disease outbreak, overplanting of plants where possible will ensure adequate production.

Weed control
Weeds harbor insects and serve as hosts for many viral diseases. For most viruses to survive they must remain in a living organism whether it be a host plant or insect. Destroying weeds in and around the garden may eliminate potential overwintering host plants. Destroying susceptible diseased weeds helps to reduce the incidence of transfer of pathogens like Fusarium spp. to plants.

Use of disease resistant cultivars
Cultivars resistant to or tolerant to Fusarium wilt are available for some plants, e.g. peas and China aster. However a cultivar that is resistant to a particular forma specialis may not be resistant to the other races of the same forma specialis (Burgess et al., 2008).

Excessive handling of plants
This includes tying, thinning, and pruning. Wounds on tomato plants as a result of thinning and pruning increase their susceptibility to Fusarium wilt. Some diseases such as bacterial spot, tomato mosaic, and bacterial speck can be spread while carrying out any of these actions. Do not prune roots while cultivating leaves because roots with cuts can create openings for root rot and wilt fungi to enter and reduce plant vigour.

Grafting
Grafting of vegetables is available, where the rootstock is resistant or not susceptible to Fusarium, for example, tomatoes. Young plants, seeds and cuttings from disease-free stocks can also be obtained from reliable sources.

Planting on raised beds
Planting seeds on raised beds help to improve drainage. Warmth in raised beds is higher than on level soil and this increases the rate of emergence of the seedlings, thus reducing their susceptibility to disease compared to the case of slow emergence.

Choosing the best planting and harvesting times
When conditions are not favourable for the disease or pest, crops may be planted and harvested and full yield can still be achieved. In order to achieve this full yield and get a head start growing, plant seeds can be protected by using cold frames or hot caps. The crops then have a competitive edge over the disease or pest. In the case of pests, the farmer must have a good knowledge of the emergence times and life cycle of the pest to be controlled while in the case of diseases, the farmer must have a good knowledge of the environmental conditions that enhance the emergence of the disease and its causal agent.

Taking advantage of alleloparthy and intercropping
Alleloparthy is a natural chemical interaction among plants. Some plants may release toxins; decaying plant
tissues with the help of microorganisms can also release toxins. In some cases, nontoxic compounds can be modified into toxic compounds by microbial activities. For example, the growth of winter annual weeds may be inhibited by black walnut trees and Johnson grass and may also offer some control of root knot nematodes.

**Intercropping**

Intercropping is the cultivation or planting of two or more crops together on the same farm land or field at the same time. It helps to maintain crop yield, soil fertility, reduce risks, control weeds, and reduce the incidence of pests and diseases.

For example, tomato and maize can be intercropped. The tomato gets shade from the maize and is produced out of season, thereby increasing the farmer’s yield and income. In Cuba this method increased annual yield by 5-6 tonnes/ha (Wolfswinkel, 2010).

**PHYSICAL CONTROL**

This includes soil solarization and soil disinfection

**Soil solarization**

This is done by spreading a clear plastic sheet over the soil for several weeks. This helps to trap solar energy which in turn inhibits soilborne disease, nematodes, insects and many weed seeds. This is usually done during summer when the air temperature is high and there is intense radiation (Ploeg and Stapleton, 2001).

**Soil disinfection using heating and steam.**

This is usually done as a preplanting method. Hot water can be used according to Tetaya (2001) and can keep the soil sterilized for up to three years. Steam can also be used especially in greenhouse conditions (Gullino, 2001). Soil disinfection helps to keep the soil sterile and free from disease causing pathogens.

**CHEMICAL CONTROL**

Chemical control is the use of chemicals to control plant diseases (fungal, viral, bacterial and nematicidal), pest infestation and weeds. Some of these chemicals include prochloraz, propiconazole, thiabendazole, carbendazim, benomyl, thiophante, fuberidazole and all of the benzimidazoles. In 2007, Nel et al reported that benomyl was partly effective against *F. oxysporum* f.sp cubense using the root dip treatment method. This method was applied to using carbendazim on tomato seedlings infected with *Fusarium* wilt and it led to about 24% increase in yield (Khan and Khan, 2002). Prochloraz has shown to be very effective against *Fusarium* wilt of tomato and banana (Song et al., 2004; Nel et al., 2007). Fungal diseases may be controlled through the use of fungicides and other agriculture practices; however, new races of fungi often evolve that are resistant to various fungicides (Sierotzki and Ulrich, 2003). Apart from this, fungicides adversely affect other useful soil microorganisms, affect human health and also pollute the environment (Gerhardson, 2002). Chemical control measures create imbalances in the microbial community, which may be unfavorable to the activities of the beneficial organisms and may also lead to the development of resistant strains of pathogens. Pollution of the soil by overuse of chemicals has also led to harmful effects on humans (Asaka and Shoda, 1996).

Chemical control of the disease is not satisfactory because generally the disease is controlled by preplant soil fumigation with methyl bromide. In addition to other potential health, safety and environmental risks, methyl bromide was classified as an ozone-depleting compound and has been banned from use (Larkin and Fravel, 1998). There are no effective fungicides for the control of *Fusarium* wilt (Burgess et al., 2008).

The lack of reliable chemical control agents, the occurrence of fungicide resistance in pathogens, and the breakdown or circumvention of host resistance by pathogen populations are among the key factors underlying efforts to develop other control measures (Weller et al., 2002). The search for alternative strategies also has been stimulated by public concerns about the adverse effects of soil fumigants such as methyl bromide on the environment and human health (Weller et al., 2002). Since the earliest observations of antagonist disease suppressing soil microorganisms more than 70 years ago, plant pathologists have been fascinated by the idea that such microorganisms could be used as environmentally friendly biocontrol agents both in the field and greenhouses. Thus biological control of *Fusarium* wilt can offer a potential alternative to chemical fungicides (Alwathnani et al., 2012).

**BIOLOGICAL CONTROL AND BIOLOGICAL CONTROL AGENTS (BCAS)**

Biological control offers a better alternative to the use of chemicals. It is the use of natural antagonistic organisms to combat pests or suppress plant diseases (Lugtenberg and Kamilova, 2009). Since PGPR (plant growth-promoting rhizobacteria) is known for growth promotion and disease reduction in crops, biocontrol by use of PGPR represents a potentially attractive alternative disease management method (Jeyiyayan and Kloepper, 2002).

According to Jacobsen et al. (2004), *Bacillus-
based BCAs have great potential in integrated pest management (IPM) systems (plant diseases included). Multiple Bacillus and Paenibacillus spp. can promote crop health in a variety of ways. Some populations suppress plant pathogens and pests by producing antibiotic metabolites, while others may directly stimulate nutrient uptake by plants, either promoting rhizobial and mycorrhizal symbiosis or by fixing atmospheric nitrogen directly (Gardener, 2004). Specific strains of the species B. amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides, and B. sphaericus elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts. Elicitation of Induced Systemic Resistance (ISR) by these strains has been demonstrated in greenhouse or field trials on tomato (Kloepper et al., 2004; Choudhary and Johri, 2009).

**Biocontrol by PGPR**

PGPR can be defined as free-living rhizosphere-occupying bacteria that enhance plant growth and can also be classified as biocontrol agents, biofertilizers or biopesticides depending on their activities/abilities (Labuschagne et al., 2010). It can also be defined as root-colonizing bacteria (Rhizobacteria) that exert beneficial effects on plant growth and development, for example, in seedling emergence, colonizing roots, stimulating overall plant growth, mineral nutrition, and water utilization, as well as disease suppression (Choudhary and Johri, 2009). As they control plant pathogens, and promote plant growth, they are also involved with rhizomediation by degrading xenobiotic compounds and enhancing the efficiency of fertilizers (Kloepper et al., 2004). Some of the reasons for the sustained interest in biocontrol by means of PGPR include the following:

- huge crop losses sustained due to diseases including soilborne diseases.
- increasing production costs, especially fertilizer costs
- the global trend toward the use of more environmentally friendly production methods (Labuschagne et al., 2010).

**Bacillus as PGPR**

PGPR such as Bacillus spp. have received much attention as biocontrol agents. They are able to produce several broad spectrum antibiotics and have a longer shelf life as a result of their ability to form endospores (Cavagneri et al., 2005). This allows them to resist adverse environmental conditions and permit the easy formulation and storage of the commercial products (Schallmey et al., 2004; Francis et al., 2010). In addition to their antibiotic properties, Bacillus spp. exhibit antagonistic behaviour against fungal pathogens by competition or exploitation, which leads to predation and direct parasitism.

**Bacillus**

Bacilli are Gram-positive, rod-shaped bacteria and a member of the faranack Firmicutes. They can be facultative anaerobes or obligate aerobes. They can be free-living and pathogenic and test positive for the enzyme catalase. Under stressful environmental conditions, the cells produce oval endospores that can remain dormant over a long period of time under stressful and harsh environmental conditions until favorable conditions are restored. These endospores may remain viable for a long period of time and are resistant to sunlight, heat and chemicals.

**Origin, taxonomy and morphology of Bacillus**

Ferdinand Cohn, a contemporary of Robert Koch in 1872, recognized and named the bacterium B. subtilis. This organism is described as Gram-positive, and it can grow in the presence of oxygen, forms endospores in its resting phase and represents what has become a large and diverse genus of bacteria named Bacillus, in the family Bacillaceae. It was in 2004 that the genus Bacillus was split into several families and genera of endospore-forming bacteria, based on ssRNA analysis.

The taxonomic hierarchy of Bacillus according to Bergey’s Manual 2004 is kingdom: Bacteria; phylum: Firmicutes; class: Bacilli; order: Bacillales; family: Bacillaceae (genus: Bacillus); family: Paenibacillaceae (genus: Paenibacillus); family: Planococcaceae (genus: Sporosarcina).

**Bacillus distribution and environmental conditions**

Bacillus spp. are widely distributed in nature, primarily in soil (Babalola and Akindolire, 2011), from which they invade dust particles. Some are in water while others are in the air. They are so numerous and include: B. alcalophilus, B. alvei, B. aminovorans, B. amyloliquefaciens, B. aneurinolyticus, B. anthracis, B. aquaemaris, B. atrophaeus, B. boroniphilus, B. brevis, B. caldolyticus, B. centrosporus, B. cereus, B. circulans, B. coagulans, B. firmus, B. flavothermus, B. fusiformis, B. globigii, B. infernus, B. larvae, B. laterosporus, B. lentus, B. licheniformis, B. megaterium, B. mesentericus, B. mucilaginosus, B. mycoides, B. natto, B. pantothenticus, B. polymyxia, B. pseudoanthracis, B. pumilus, B. schlegelii, B. sphaericus, B. sporothermodurans, B. stearothermophilus, B. subtilis, B. thermoglucosidasius, B. thuringiensis, B. vulgaris and B. weihenstephanensis.

In extremely harsh conditions, B. subtilis has the ability to form tough protective endospores. This
ability to withstand harsh conditions makes *B. subtilis* a perfect candidate for probiotics. *B. cereus* is mesophilic, and is capable of adapting to wide and varying environmental conditions, widely distributed in nature and commonly found in nature as a saprophyte. It grows optimally at temperatures between 20°C and 40°C (Vilain et al., 2006). Since it is a soil bacterium, it can easily enter different food plants or food made from animals (eggs, meat, and dairy products) as they feed on the plants. It causes about 25% of food-borne diseases. This occurs when food has been left without refrigeration before being served, and toxins secreted like emetic toxins and enterotoxins into the food will lead to food poisoning (USFDA, 2007).

**Bacillus industrial and health significance**

Some *Bacillus* spp. are pathogenic to plants, animals, humans and other organisms while some are very important and useful industrially and medically.

For example, some of them secrete large amount of enzymes that are important like the enzyme Barnase secreted by *B. amyloliquefaciens*. Barnase, a ribonuclease is important as it helps to kill plant cells that have been infected by pathogenic fungi in other to stop the spread of the infection. *B. amyloliquefaciens* also is the natural source of amylase which is used in starch hydrolysis. Subtilisin, a protease widely used in commercial products, for example, in synthetic organic chemistry research, laundry and dishwashing, skin care ointments, detergents, food processing, cosmetics and contact lens cleaners, can also be secreted by *B. amyloliquefaciens*. Also the BamH1 restriction enzyme used in DNA research is secreted by *B. amyloliquefaciens*.

Useful antibiotics are also produced by several *Bacillus* spp like bacitracin produced by *B. subtilis* and polymyxin produced by *B. polymyxa*. Part of the genome of *B. thuringiensis* incorporated into corn and cotton crop resulted in genetically modified plants that are resistant to some insect pests (Bt cotton).

**Bacillus as biopesticides, biofertilizers and bioinoculants**

Biopesticides are formulations containing microorganisms mixtures that can safely control insect pests. For example *B. thuringiensis* has been formulated into target specific and environmentally friendly products that help to control insects and pests (Poopathi and Abidha, 2009). *B. subtilis* QST 713 has also been formulated into biopesticides (USEPA, 2006).

Biofertilizers are selected strains of beneficial microbes cultured in the laboratory and packaged in such a way that they can be applied for seed treatment (Muraleedharan, 2010). They are preparations containing cells of microorganisms which may be nitrogen fixers, phosphorus solublizers, sulphur oxidizers or organic matter decomposers.

Bioinoculants are microbial inoculants or soil inoculants. They are microbes or beneficial endophytes that have been prepared into microbial products to promote plant health. Their mechanism involves the microbes forming symbiotic relationship with the crop or plant in question and both of them benefitting from the association. Applied microbial inoculants help to improve plant nutrition, and promote plant growth by stimulating the production of plant hormone (Sullivan, 2001).

**Utilization of non-pathogenic *F. oxysporum* as biological control**

| TABLE 1. Some antibiotics produced by *Bacillus* as Biological Control Agents |
|---------------------------------|-------------------|-----------------|-----------------|-----------------|
| Bacillus                        | Plant pathogen    | Disease in plant | Metabolites produced | References       |
| *B. subtilis* RB14              | *Rhizoctonia solani* | Damping off tomato | Iturin A and Surfactin | Asaka and Shoda, 1996 |
| *B. subtilis*                   | *Fusarium oxysporum* f.sp ciceris | Fusarium wilt of chickpea | Subtilin | Karimi et al., 2012 |
| *B. subtilis* AU195             | *Aspergillus flavus* | Aflatoxin contamination | Bacillomycin D | Moyné et al., 2001 |
| *B. amyloliquefaciens* FZB42    | *F. oxysporum*     | Wilt              | Bacillomycin and fengycin | Kousmoutsis et al., 2004 |
| *B. subtilis* QST713            | *Botrytis cinerea* and *R. solani* | Damping off | Iturin A | Kloepfer et al., 2004 |
| *B. subtilis* BBG 100           | *Pythium aphani dermatum* | Damping off | Mycosubtilis | Leclere et al., 2005 |
| *B. cereus* UW85                | *Phytopthora medicagins* | Damping off | Zwittermycin A, Kanosamine | Silo-Suh et al., 1994; Milner et al., 1996 |
The vast majority of the research conducted to date on the biological enhancement of plants with fungal endophytes involves the use of non-pathogenic strains of *F. oxysporum*. For an example, *F. oxysporum* Fo47 was originally isolated from disease suppressive soils from the Chateaurenard region of France and is well-known for its biological control properties (Fravel et al., 2003). Non-pathogenic strains of *F. oxysporum* have the capacity to protect plants against wilt induced by pathogenic strains of *F. oxysporum*. Among the mechanisms involved in this protection, the induced systemic resistance and priming of the plant defense reaction could be the mode of action of Fo47 (Aimé et al., 2008).

**MECHANISMS ADOPTED BY BIOLOGICAL CONTROL AGENTS**

This is the interaction that takes place between the pathogen and antagonist organism and also with the crop or particular plant, and this interaction could be direct, mixed or indirect (Pal and Gardener, 2006). Direct antagonism can include parasitism and predation while indirect can include competition. Mixed path antagonism can include amenalism (a situation when an organism inhibits or suppresses the growth of the other organism by producing metabolites without itself being affected), i.e., antibiosis and lysis.

**Parasitism and predation**

Microbial parasitism involves the pathogen being attacked directly or killed. This could take the form of mycoparasitism, in which case the parasite kills the host fungus by secreting cell wall degrading enzymes. This could help to create holes into the host’s hyphae and cause the death of the host by absorbing its nutrients. The death of the host can also be as a result of close contact between the two fungi. In some instances, the fungi penetrate, infect and damage the host plant (Babalola, 2010a, Babalola, 2010b, Babalola, 2010c).

**Competition**

As a result of the limited resources in the rhizosphere, there is competition among the microbes for nutrients. An example is seen with regard to the limited amount of iron in the rhizosphere. This leads to the production of siderophores in some organisms to sequester iron from the environment. When this happens, iron is made available to the organism that produces the siderophores while depriving the other organism; this can lead to disease suppression of several fungal pathogens.

**Antibiosis**

Antibiosis is a situation where the microbial toxins or metabolites secreted by the antagonist organism inhibit the growth of the pathogen or completely kill it while the antagonist organism is not affected (Pal and Gardener, 2006). Some antagonist organisms produce only one metabolite while others are known to produce multiple metabolites so they can be effective against one or more pathogens as shown above.

**Phosphate solubilization**

Phosphate solubulization involves liberating soluble phosphorus from insoluble calcium phosphate (Ca (PO₄)₂). Some bacteria are able to dissolve the mineral phosphate and make it available for plants (Babalola, 2010d). Solubilization of phosphate by microbes is of practical importance in today’s agriculture. Application of the phosphate-solubilizing microbes, *Bacillus*, *Pseudomonas*, *Agrobacteria*, *Aspergillus*, *Enterobacter* and *Trichoderma* around the roots of plants, in soils, and in fertilizers release soluble phosphate, promote plant growth and protect plants from pathogen infection.

**Hydrogen cyanide production**

Cyanide is a volatile inhibitor of microbial growth (Bakker and Schippers, 1987). HCN is a low molecular weight metabolite, a biocide with antifungal activity (Babalola, 2010d). Volatiles produced by bacteria can influence both fungal mycelial growth rate, enzyme activity (Fiddaman and Rossall, 1995) and inhibit spore germination in a variety of fungal species.

**Induced systemic resistance**

ISR refers to the ability of the plant to induce defense responses as a result of some biotic or abiotic inducing agent from pathogens. When plants and pathogens interact, it could result in a response that is compatible to both of them and lead to infection. On the other hand it could also result into a response that is not compatible to both organisms and lead to resistance from the plant. The resistance can be localized or systemic depending on the response from the plant. When the response is incompatible, the resistance from the plant can be an oxidative outburst that will lead to the eventual death of the pathogen (localized). It could also lead to production of antimicrobial products such as phytoalexins and pathogenesis related proteins (systemic) (Heil and Bostock, 2002).

Kloeper et al. (2004) reported that some strains of *B. cereus*, *B. sphaericus*, *B. subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. pasteurii* and *B. mycoides* elicited induced systemic resistance in plants like the tomato.

**Soil suppressiveness**
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Soils harbour several PGPR, some of which produce siderophores. Suppressive soils are those in which a specific pathogen does not persist or cause disease despite favorable environmental conditions though it could establish itself. In some other cases, the disease occurs but gradually is reduced in severity with the continuous growing of the same crop. This suppressiveness can be inducible and durable for years. Nonpathogenic *F. oxysporum*, isolated from *Fusarium* wilt-suppressive soils have been effective against *Fusarium* wilt of several different crops (Minuto et al., 1995).

CONCLUSION

Each strategy used in the control of tomato *Fusarium* wilt is unique. Integrated disease management strategies are been advocated for in such a way that will not be harmful to the environment, useful microbes, plants, animals and human lives.

Apart from looking at the fact that these strategies are working in different ways, efforts should also be on examining and developing the efficacy, efficiency and durability of these strategies on the fields and not just in the greenhouse. The different formulations of microbial products that will give the most efficient result should also be considered very important in biological control while education of farmers on the appropriate use of cultural methods and their integration into other strategies should also be an area of interest.

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