Chapter 3
Fungi as an Alternative to Agrochemicals to Control Plant Diseases

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3.1 Introduction

Majority of the populations living in developing countries are actively engaged in agriculture with a good percentage being small scale farmers, however, the turn out of their farm produce are low owing to crops crippling diseases. In Nigeria, smallholder farmers produce crops such as cocoa, cereals, potato, tomato, vegetable, yam, cassava, plantain, banana, orange, which are the raw materials for local industries and also contribute to the nation's economic development as foreign exchange earners (Oloruntoba 1989). Plant diseases account for considerable losses in crop production and storage. Currently, growers, particularly in developing countries like Nigeria still rely heavily on agrochemicals to prevent and/or control these crops threatening diseases. Despite the high effectiveness and ease of utilization, these agrochemicals can result in environmental contamination and pesticide residue presence on food, contributing to additional social and economic problems. Varieties of causal agents such as fungi, bacteria, viruses, nematodes amongst others have been implicated in plant diseases with an enormous reduction in crop yields globally. In most developing countries, Nigeria inclusive, crop losses are usually higher than their developed counterparts (FAO 2004).

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D. Purchase (ed.), *Fungal Applications in Sustainable Environmental Biotechnology*, Fungal Biology, DOI 10.1007/978-3-319-42852-9_3

Diseases caused by Oomycetes fungi of the order perenosporales present major problems world-wide. Important foliar diseases include late blight on potatoes, blue mould of tobacco, grape downy mildew, plus a wide range of other foliar blights and downy mildew on cereals, fruits, and vegetables (Coffey et al. 1984). In addition, *Phytophthora* and *Pythium* species are responsible for many pre- and post-harvest problems of fruits and vegetables including late blight of potato tubers (Barak et al. 1984), brown rot of citrus (Cohen 1981), and black pod of cocoa (McGregor 1984). Bacterial wilt of potato, tomato, eggplant, tobacco, groundnut and banana is caused by *Pseudomonas solanacerum* (Wheele 1969).

A serious shoot disease of *Amaranthus* spp. causing a blight of the young shoot which can result in a total crop failure, and associated with *Choanephora cucurbitarum* has been reported in Benin City, Nigeria (Ikediugwu 1981; Ikediugwu et al. 1994; Emoghene and Okigbo 2001). Evidence on the disease of crops such as fruits and vegetables and their known control measures are well documented in literature. A good example is strains of *Rhizoctonia* causing damping-off on a wide range of cultivated plants. These include cereals, potato, root and fodder crops, legumes, vegetables and ornamentals (Moore 1959). Sclerotia of *Rhizoctonia solani* are frequently found on potato tubers. *Botrytis cinerea* often cause damping-off of lettuce in association with *Pythium* and *Rhizoctonia*. *Uromyces appendiculatus* attack bean, *Puccinia asparagi* on asparagus, *Puccinia alli* on onion, leek and garlic, *Puccinia methae* on peppermint (Wheele 1969). A number of control measures which have been adopted include: (i) inspection and quarantine procedures, (ii) cultural methods and (iii) fungicide applications. Organomercurials such as methylmercury dicyandiamide (Panogen) and the compound tetramethythiuram disulphide (Thiram) are amongst those chemicals which have been commonly used. Similarly, copper fungicides including Bordeaux and Burgundy mixtures and some of the dithiocarbamates such as maneb and organic tin compounds have been applied to manage fungal plant pathogens. Other important classes of systemic fungicides such as carbamates, cymoxamil, acylanilides and alky phosphate have also been used in the control of crop diseases (Cohen and Coffey 1986).

The above control methods are effective but have their disadvantages. Fungicides may not be the most desirable means of disease control for several important reasons. Fungicides are heavily regulated and vary from country to country in their use and registration (Jones 1985). In addition, they are expensive, can cause environmental pollution, and may induce pathogenic resistance. They can also cause stunting and chlorosis of young seedlings (Jones 1985). Cultural methods can injure plants, are labour intensive, and are less attractive to commercial growers (Rytter et al. 1989).

The use of microorganisms to control crop pests and diseases is an exciting and rapidly advancing branch of biotechnology. Novel methods have been established by different researchers to control plant pests and plant diseases. For instance, Emoghene and Futughe (2011) reported a more sustainable control measure using soil solarization to control *Amaranthus viridis* shoot disease caused by *Choanephora cucurbitarum*. Biological control, a term first coined by Smith (1919) to denote insect pest control by the use of natural enemies, is another sustainable example. Biological control when effective is usually more enduring than any other control methods as reported by Baker and Cook (1982). Successful applications of biological control with the use of microorganisms against plant pathogens began with the control of crown gall with *Agrobacterium radiobacter* K84 (Kerr 1980), and seedling blight caused by *Pythium* and *Rhizoctonia* with *Trichoderma harizanum* (Harman and Bjorkman 1998). Ikediugwu et al. (1994) reported the biological control of the shoot disease of *Amaranthus viridis* caused by *Choanephora cucurbitarum* with *Bacillus subtilis*.

3.2 Agrochemicals

Agrochemicals including pesticides and fertilizers are considered the result of modern technology that depends on inorganic processes. Pesticide according to FAO (1989) is any substance or mixture of substances intended for preventing, destroying, or controlling any pest including vectors of human or animal diseases, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. Chemicals employed such as growth regulators, defoliants, desiccants, fruit thinning agents, or agents for preventing the premature fall of fruits, and substances applied to crops either before or after harvest to prevent deterioration during storage or transport are also included in the term. However, it excludes such chemicals used as fertilizers, plant and animal nutrients, food additives and animal drugs. The term pesticide is also defined by FAO in collaboration with UNEP (1990) as chemicals designed to combat the attacks of various pests and vectors on agricultural crops, domestic animals and human beings. The above definitions suggest that, pesticides are toxic chemical agents (basically organic compounds) that are intentionally introduced to attack crop pests and disease vectors in the environment. Pesticides are chemically synthesized compounds, devise or organisms that are applied routinely in agriculture in order to mitigate, destroy, attack or repel pests, pathogens and parasites. They include both organic and inorganic moieties and may be classified into different groups depending on their chemical compositions. Examples of these agrochemicals include organochlorines, organophosphates, carbamates, formamidines, thiocyanates, organotins, denitrophenols, synthetic pyrethroids and antibiotics (Bohmont 1990). Upon application, the fate of these agrochemicals in the soil and the transport processes that take place are dependent on: (i) the cumulative effects of the physicochemical characteristics such as adsorptivity, solubility, volatility and degradation rate; (ii) the soil's characteristic; (iii) application methods and (iv) the site condition (Jeong and Forster 2003).

3.3 Effect of Agrochemicals Usage

Over 15,000 metric tons of agrochemicals are applied in Nigeria annually, comprising about 135 pesticide chemicals marketed locally under 200 different produce brands and formulation, thereby making Nigeria one of the largest agrochemicals users in sub-Sahara African (Osibanjo and Adeyeye 1995). According to Kamrin (1997) the benefits of agrochemicals cannot be overemphasized, however, their uses are a source of environmental, human and other animal concerns. It has been estimated that over 98 % of sprayed insecticides and 95 % herbicides get to a destination other than their intended target, in addition to non-target species, air, water and soil (Miller 2004). Runoff of agrochemicals into aquatic environment is one of the causes of water pollution, while they can be air-borne and drifted to other fields, grazing areas, human settlements and undeveloped areas which can potentially affect other species. Repeated application can cause persistent resistance and sources of soil contamination. Agrochemicals poisoning incidence may occur as a result of misuse, storage near consumable food stuff or farm produce and the use of agrochemical containers for domestic purposes, such as the case of Iraq 1970 as reported by WHO (1990). People exposed to agrochemicals either accidentally or occupationally include: manufacturers, vendors/seller, mixers, transporters, loaders, operators of application equipment, growers, pickers and clean-up workers, and consumers of farm produce with pesticide residues. It has been estimated by WHO and UNEP that about 3 million workers in agriculture from developing countries suffer severe poisoning from agrochemicals each year with about 18,000 deaths (Miller 2004). As many as 25 million workers in developing countries may be affected with mild pesticide poisoning yearly (Jeyaratnam 1990; WHO 2006). Just recently, according to the Punch newspaper (2015) the deaths of 18 people in south-western Nigeria were attributed to strange disease probably associated with agrochemical poisoning.

3.4 Mechanisms of Fungi-Based Biocontrol of Plant Pathogens

Plants and fungi have different interactions resulting in different mechanisms of action. The most common mechanisms for fungi-based biocontrol of plant pathogens are: (i) parasitism, (ii) mutualism, (iii) predation, (iv) competition, (v) induced resistance and (vi) the production of antimicrobial substances. In order to interact, fungi must have some form of direct or indirect contact with the plant and/or plant's pathogen and often, several mechanisms act together to give the most effective biocontrol. Direct fungal-based biocontrol result from physical contact and a high-level of specificity for the plant's pathogen. In hyperparasitism, the plant's pathogen is directly attacked by a selective fungi-based biocontrol agent that destroys it or its propagules. Several fungal hypoparasites have been implicated in addition to those attacking the sclerotia e.g. *Coniothyrium minitans*, others attacking pathogenic fungal hyphae such as *Pythium oligandrum*. However, cases abound where a single fungal pathogen can be attacked by multiple hyperparasites. A good example is powdery mildew pathogens which are susceptible to different hyperparasites such as *Acremonium alternatum*, *Acrodontium crateriforme*, *Ampelomyces quisqualis*, *Cladosporium oxysporum* and *Gliocladium virens* (Milgroom and Cortesi 2004). Fungi predation, unlike hyperparasitism, is a more general, non-specific and less predictable levels of plant disease biocontrol. Some Fungi such as *Trichoderma* sp. exhibit predatory behaviour under nutrient-limited (e.g. cellulose) conditions by synthesizing a range of enzymes e.g. chitinase that are directed against pathogenic fungi cell walls like *Rhizoctonia solani* (Benhamou and Chet 1997). Genes encoding for cell wall degrading enzyme (CWDES) such as chitinolytic, glucanolytic, and proteolytic enzymes have been isolated and applied to enhance fungi-based biocontrol capabilities of *Trichoderma* strains (Elad et al. 1982; Chet et al. 1993; Lorito et al. 1993).

Indirect fungi-based biocontrol, in contrast, results from activities that do not involve targeting a plant's pathogen by a biocontrol active fungus. Reports have demonstrated that some lytic enzyme activity may induce indirect efficacy against plant pathogens e.g. oligosaccharides from fungal cell walls can stimulate plant host defences (Howell et al. 1988). According to Van Loon et al. (1998) and Ryu et al. (2004), substantial number of fungi products such as transglutaminase, elicitins and a-glucan in Oomycetes; chitin and ergosterol in all fungi; and xylanase in *Trichoderma* have elicited plant host defences. Stimulation and improvement of plant host defence mode of action by non-pathogenic fungi such as mycorrhizae is the most indirect form of 'antagonism' (Kloepper et al. 1980; Maurhofer et al. 1994; Lafontaine and Benhamo 1996).

Mycorrhizae are formed due to a mutualistic symbiosis between plants and fungi. A resting spore germinates upon perception of exudates from root of host plant resulting to an induced hyphal branching which heightened the tendencies of a direct symbiotic contact as illustrated in Fig. 3.1. This interaction enables ubiquitous root colonists assisting plants to take up nutrients especially phosphorus and micronutrients. Arbuscular mycorrhizal fungi also known as vesicular arbuscular mycorrhizal fungi start to form by continuous dichotomous branching of fungal hyphae about two days after its root penetration inside the cortical cell of the host plant. It is believed that arbuscules is the site of communication between the host plant and the fungus (Biermann and Linderman 1983). Arbuscular mycorrhizal fungi can prevent root infection during colonization by reducing the access sites and stimulating plant host defence. Linderman (1994) reported that arbuscular mycorrhizal fungi reduced root-knot nematode incidence. There are also various mechanisms allowing arbuscular mycorrhizal fungi to increase host plant's stress tolerance. One of such mechanisms includes the intricate network of fungal hyphae around the roots which prevent pathogen infection. Catska (1994) inoculated apple-tree seedlings with arbuscular mycorrhizal fungi *Glomus fasciculatum* and *Glomus macrocarpum* and observed suppressed apple replant disease caused by phytotoxic myxomycetes. Arbuscular mycorrhizal fungi also protect the host plant

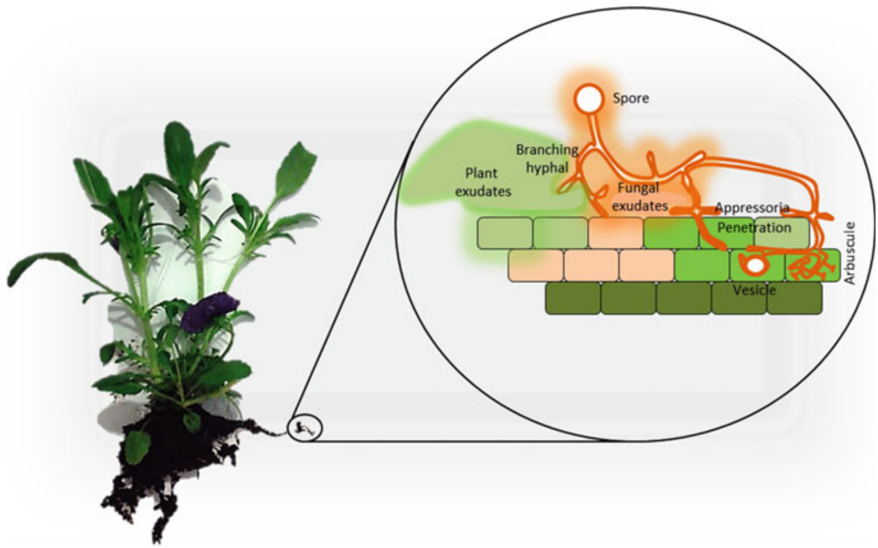

Fig. 3.1 Fungi-plant symbiotic relationship-mycorrhizea

against root-infecting pathogenic bacteria as reported by Garcia-Garrido and Ocampo (1989) where the damage on tomato caused by *Pseudomonas syringae* was reduced significantly as a result of mycorrhizal colonization of the tomato plant. The mechanisms include physical protection, chemical interactions and indirect effects (Fitter and Garbaye 1994). Enhanced nutrition to plant; morphological changes in the root by increased lignification; changes in the chemical composition of plant tissues such as antifungal chitinase, isoflavonoids are other mechanisms employed by arbuscular mycorrhizal fungi to indirectly suppress host plant pathogens (Morris and Ward 1992). Alleviation of abiotic stress and changes in the microbial content in the mycorrhizophere are also implicated mechanisms as reported by Linderman (1994).

Proliferation of ectomycorrhizae outside the root surface as against arbuscular mycorrhizal fungi, form a sheath around the root by the combination of mass of root and hyphae known as mantle. Multiple mechanisms in addition to antibiosis, fungistatic substances produce by plant roots in response to mycorrhizal infection and a physical barrier of the fungal mantle around the plant by ectomycorrhizal fungi give disease protection to the host plant (Duchesne 1994). According to Ross and Marx (1972) ectomycorrhizal fungi such as *Paxillus involutus* controlled effectively root rot caused by *Fusarium oxysporum* and *Fusarium moniliforme* in red pine. Inoculation of sand pine with *Pisolithus tinctorius*, another ectomycorrhizal fungus, controlled disease caused by *Phytophthora cinnamomi*. Literatures abound demonstrating post-harvest disease control by applying antagonistic microbes especially fungi (Table 3.1).

Table 3.1 Successful biocontrol of post-harvest diseases

Crop	Disease	Antagonist	Mechanism	Reference
Birch	Decay	*Trichoderma* sp.	Competition for nutrient and space	Shields and Atwells (1963)
Lemon	Green mold	*Trichoderma* sp.	Competition for nutrient and space	de Matose (1983)
Citrus	Green mold and Blue mold	*Trichoderma* sp. *and Bacillus* sp.	Competition for nutrient and space and antibiosis	Futughe (2007)
	Green mold	*Candida famata*	Induction of resistance	Arras (1996)
	Green mold	*Debaryomyces hansenii*	Competition for nutrient and space	Taqarort et al. (2008)
	Blue mold	*Cryptococcus laurentii*	Competition for nutrient and space	Liu et al. (2010)
	Green mold	*Wickerhamomyces anomalus*	Antibiosis	Platania et al. (2012)
Pine	Penicillium rot	*Trichoderma* sp.	Competition for nutrient and space	Lindgren and Harvey (1952)
Pineapple	Decay	Attenuated strains of *Penicillium* sp.	Competition for nutrient and space	Lim and Rorhbach (1980)
Potato	Soft rot	*Pseudomonas putida*	Antibiosis	Colyer and Mount (1984)
Stone fruit	Brown rot	*Bacillus subtilis*	Antibiosis	Pusey and Wilson (1984)
Straw berry	Botrytis rot	*Trichoderma* sp.	Competition for nutrient and space	Tronsmo and Dennis (1983)

3.5 Examples of Fungal-Based Biocontrol of Plant Pathogens

It is clear that there are a number of advantages in using fungal-based biocontrol against plant pathogens, including:

(i) Prevents environmental pollution of soil, air and water.
(ii) Maintains healthy biological control balance by avoiding adverse effects on beneficial organisms.
(iii) Less expensive than agrochemicals and devoid of resistance problems.
(iv) Fungi-based biocontrols are self-maintaining in simple application while agrochemicals need repeated applications.
(v) Very effective for soil-borne pathogens where agrochemical approach is not feasible.
(vi) Eco-friendly, durable and long-lasting.

(vii) Very high control potential by integrating fungicide resistant antagonists.
(viii) Helps in inducing system resistance among the crop species e.g. *Trichoderma* sp. resistant to fungicide such as Benomyl and Metalaxyl among others.

We present two examples to illustrate the potential of fungal-based biocontrol.

3.5.1 *Against* **Fusarium** *Wilt of Tomato* **(Licopersicon Esculentum** *Mill***)** *by* **Trichoderma** *Species*

Tomato (*Licopersicon esculentum* Mill) is a very important fruit vegetable that is used extensively for salad, soups and stews. Industrially, ripe tomato fruits are processed into puree, sauce and juice (Purseglore 1977). Many countries around the world have large scale production of tomato with the United States, Italy, Spain and Bulgaria as the leading producers (Simons and Sobulo 1975; Purseglore 1977). Tomato has been cultivated almost all over Nigeria for decades with the most predominant area being the North and South Western regions (Erinle 1979; Denton and Swarup 1983). Crop crippling diseases are serious limitations to tomato production (Wheele 1969; Simons and Sobulo 1975; Erinle 1979; Adelana and Simons 1980; Denton and Swarup 1983). Bacterial and fungal wilts are the most commonly known field diseases of tomato. *Ralstania* (*Pseudomonas*) *solanacearum* and *Fusarium oxysporum* f. sp *lycopersici* are the most devastating in many growing belts of the world (Wheele 1969; Walker 1971; Prior et al. 1990), Nigeria inclusive (Erinle 1977; Osuinde and Ikediugwu 1995). *F. oxysporum* f. sp *lycopersici* and *Ralstania* (*P*) *solanacearum* which are causative agents of tomato wilt disease are soil inhibiting microorganisms and survive saprophytically in soil (Walker 1957, 1971; Park 1959). *Fusarium* wilt of tomato caused by *F. oxysporum* f. sp *lycopersici* is a serious economic problem in Southern Western Nigeria (Erinle 1977). Tomato wilts, like most soil-borne diseases of plant have proved extremely difficult to control by the application of agrochemicals which are expensive and hazardous to man and the environment. Currently, research into alternative sustainable control measures to agrochemicals is getting global attention. Efforts have been made in some parts of the world towards genetic (by using resistant cultivars) and biological control (biotechnology). However, the use of resistant cultivars has been complicated by the occurrence of more than one species of some wilt pathogens (Walker 1957), resulting in costly loss of resistance in the field, thereby, making biological control mostly favourable as it has attracted a growing market base with more diversified biotechnological products (Ardakani et al. 2009). A common form of biological control such as the use of fungi encourages the growth of microorganisms (e.g. fungi) antagonistic to the pathogen in the environment of the crop plant to the detriment of the pathogen (Alexander 1977; Baker and Cook 1982).

Trichoderma species, a fungus, has been used as an alternative to agrochemicals to control *Fusarium* wilt disease of tomato. Its potential was previously found to be antagonistic to *F. oxysporium* f. sp. *Lycopersici* in vitro. Seedlings of tomato inoculated with the pathogen (*F. oxysporium* f. sp. *Lycopersici*) alone revealed mild wilt symptoms by the following day and by the fourth (4th) day; plant sagged and wilted completely (Table 3.2). In contrast, fungi control of the plant disease was observed with the *Trichoderma* spp., depending on the concentration of spores and method of application (whether root-dip or direct soil inoculation), on whether the pathogen was applied simultaneously with antagonist, and on how long spores of the pathogen was allowed to grow ahead of the spores of antagonist (Osuinde et al. 2002). When the pathogen and antagonist were applied simultaneously, the result depending on the spores concentration and method of application: 10^3 spores/mL delayed symptom expression only for one day (Table 3.2). Mild wilt symptoms which affected 40 % and 80 % plants in root-dip and direct soil inoculation methods respectively was observed from day 2 to day 4. However, when 10^6 spores/mL of antagonist was applied by root-dip method, there was no wilt development at all as plants were healthy throughout the study period. But in the direct soil inoculation method, mild wilt was observed in 60 % of the seedlings by the 2nd and 3rd day. When the spores of the pathogen were allowed to grow one day (24 h) ahead of spores of antagonist, the result also depended on the concentration of the spores and method of application. When 10^3 spores/mL of antagonist was used, mild wilt was observed the following day in 80 and 100 % of seedling up to the 3rd day in root-dip and direct soil inoculation methods respectively. Nevertheless, when 10^6 spores/mL of antagonist was applied there was no wilt symptoms in plants in the root-dip method, while 40 % of plants developed mild wilt by 2nd day up to 4th day in the direct soil inoculation methods (Tables 3.2 and 3.3) (Osuinde et al. 2002). There was no effect on progress of wilt upon germination of spores of the pathogen after 2 day (48 h) ahead of spores of antagonist irrespective of the spore concentrations of antagonist and application methods. All the plant (100 %) were completely wilted by the following day and died two days later. All the plants, however, fair better and look healthier compared to plants treated with pathogen and antagonist. When antagonist alone was applied, there were no wilt symptoms whatsoever (Osuinde et al. 2002).

It was also observed that roots of tomato seedlings treated with antagonist and pathogen showed root rot (necrosis) depending on the concentration of the antagonist, method of application, and how long the pathogen was allowed to germinate ahead of antagonist. Roots of plants inoculated with antagonist by root-dip method had lower level of necrosis than those inoculated by direct soil inoculation method. All the roots of the plant (100 %) treated with pathogen alone had severe necrosis. In contrast, roots of plants inoculated with antagonist alone had no necrosis at all, rather, were better than those inoculated with antagonist and pathogen (Table 3.4) (Osuinde et al. 2002).

Table 3.2 Wilt disease development in tomato plants with time after inoculation with *Trichoderma* (antagonist) in *F. oxysporum* f. sp. *Lycopersici* (pathogen) infested soil

Wilt development with time (days)							
	1	2	3	4	5	6	7
Treatments—root dip method							
1a. Pathogen alone (control)	+	+	+	++	++	++	++
2a. Antagonist alone (control)	−	−	−	−	−	−	−
3a. Pathogen + 10^3 spores/mL antagonist simultaneously	−	+	+	+	−	−	−
4a. Pathogen + 10^6 spores/mL antagonist simultaneously	−	−	−	−	−	−	−
5a. Pathogen incubated 24 h + 10^3 spores/mL antagonist	+	+	+	−	−	−	−
6a. Pathogen incubated 24 h + 10^6 spores/mL antagonist	−	−	−	−	−	−	−
7a. Pathogen incubated 48 h + 10^3 spores/mL antagonist	++	D	D	D	D	D	D
8a. Pathogen incubated 48 h + 10^6 spores/mL antagonist	++	D	D	D	D	D	D
Treatment—direct soil inoculation							
1b. Pathogen alone (control)	+	+	+	++	++	++	++
2b. Antagonist alone (control)	−	−	−	−	−	−	−
3b. Pathogen + 10^3 spores/mL antagonist simultaneously	−	+	+	+	−	−	−
4b. Pathogen + 10^6 spores/mL antagonist simultaneously	−	+	+	−	−	−	−
5b. Pathogen incubated 24 h + 10^3 spores/mL antagonist	+	+	+	−	−	−	−
6b. Pathogen incubated 24 h + 10^6 spores/mL antagonist	−	+	+	+	−	−	−
7b. Pathogen incubated 48 h + 10^3 spores/mL antagonist	++	D	D	D	D	D	D
8b. Pathogen incubated 48 h + 10^6 spores/mL antagonist	++	D	D	D	D	D	D

Source Osuinde et al. (2002)
\+ = Partial (Mild) wilt
++ = Complete wilt
− = No wilt
D = Plant death

The antagonist and pathogen were re-isolated from root segments of tomato plants after 7 days growth in the greenhouse study. The frequency of re-isolation of the antagonist and the pathogen differ greatly. The frequency of re-isolation of *Trichoderma* from treated plants was 60–100 % while that of *F. oxysporum* f. sp. *Lycopersici* was 30–50 % in root dip; 40–80 and 50–60 % in direct soil inoculation method respectively. Re-isolation of *Trichoderma* spp. and *F. oxysporum* f. sp. *Lycopersici* in the control plants was 100 % in the separate treatment. Colonies of antagonist (*Trichoderma* spp.) was far more numerous than the pathogen (*F. oxysporum* f. sp. *Lycopersici*) in the root washes (Osuinde et al. 2002) and this agrees with several reports that the high competitive ability, antibiosis and mycoporasitism of *Trichoderma* spp. made them persist on the rhizoplane (root-surface) and rhizosphere of plants and thus out-number other soil microorganisms especially the plant pathogens (Harman et al. 1980; Chet and Henis 1987; Sivan et al. 1987)

Table 3.3 Tomato plants (%) affected by wilt disease with time after inoculation with *Trichoderma* species in *F. oxysporum* f. sp. *Lycopersici* infested soil

Wilt development with time (days)							
	1	2	3	4	5	6	7
Treatments—root dip method							
1a. Pathogen alone (control)	100	100	100	100	100	100	100
2a. Antagonist alone (control)	0	0	0	0	0	0	0
3a. Pathogen + 10^3 spores/mL antagonist simultaneously	0	40	40	40	0	0	0
4a. Pathogen + 10^6 spores/mL antagonist simultaneously	0	0	0	0	0	0	0
5a. Pathogen incubated 24 h + 10^3 spores/mL antagonist	80	80	80	0	0	0	0
6a. Pathogen incubated 24 h + 10^6 spores/mL antagonist	0	0	0	0	0	0	0
7a. Pathogen incubated 48 h + 10^3 spores/mL antagonist	100	100	100	100	100	100	100
8a. Pathogen incubated 48 h + 10^6 spores/mL antagonist	100	100	100	100	100	100	100
Treatment—direct soil inoculation							
1b. Pathogen alone (control)	100	100	100	100	100	100	100
2b. Antagonist alone (control)	0	0	0	0	0	0	0
3b. Pathogen + 10^3 spores/mL antagonist simultaneously	0	80	80	80	0	0	0
4b. Pathogen + 10^6 spores/mL antagonist simultaneously	0	60	60	0	0	0	0
5b. Pathogen incubated 24 h + 10^3 spores/mL antagonist	100	100	100	0	0	0	0
6b. Pathogen incubated 24 h + 10^6 spores/mL antagonist	0	40	40	40	0	0	0
7b. Pathogen incubated 48 h + 10^3 spores/mL antagonist	100	100	100	100	100	100	100
8b. Pathogen incubated 48 h + 10^6 spores/mL antagonist	100	100	100	100	100	100	100

Source Osuinde et al. (2002)

3.5.2 *Against Post-harvest Blue Mould of Oranges* (Citrus Sinensis) *by Screened Microbial Antagonist*

Orange (*Citrus sinensis*) ranks among one of the most important fruits produced in Nigeria. The principal orange-producing region in Nigeria is the southern part of the country, from where it is exported to various markets all over Nigeria and even abroad. Orange accounts for over 90 % of total fruit production in the region (Lateef et al. 2004), however, post-harvest losses associated with fungal diseases

Table 3.4 Tomato plant (%) affected by wilt disease with time after inoculation with *Trichoderma* species in *F. oxysporum* f. sp. *Lycopersici* infested soil

(%) Plants wilt root necrosis and severity of root necrosis					
	4	3	2	1	0
Treatments—root dip method					
1a. Pathogen alone (control)	100	–	–	–	–
2a. Antagonist alone (control)	–	–	–	–	100
3a. Pathogen + 10^3 spores/mL antagonist simultaneously	–	–	40	60	–
4a. Pathogen + 10^6 spores/mL antagonist simultaneously	–	–	20	80	–
5a. Pathogen incubated 24 h + 10^3 spores/mL antagonist	–	–	40	60	–
6a. Pathogen incubated 24 h + 10^6 spores/mL antagonist	–	–	20	80	–
7a. Pathogen incubated 48 h + 10^3 spores/mL antagonist	100	–	–	–	–
8a. Pathogen incubated 48 h + 10^6 spores/mL antagonist	100	–	–	–	–
Treatment—direct soil inoculation					
1b. Pathogen alone (control)	100	–	–	–	–
2b. Antagonist alone (control)	–	–	–	–	100
3b. Pathogen + 10^3 spores/mL antagonist simultaneously	–	20	40	40	–
4b. Pathogen + 10^6 spores/mL antagonist simultaneously	–	–	60	40	–
5b. Pathogen incubated 24 h + 10^3 spores/mL antagonist	–	–	80	20	–
6b. Pathogen incubated 24 h + 10^6 spores/mL antagonist	–	–	80	20	–
7b. Pathogen incubated 48 h + 10^3 spores/mL antagonist	100	–	–	–	–
8b. Pathogen incubated 48 h + 10^6 spores/mL antagonist	100	–	–	–	–

Source Osuinde et al. (2002)
4 = Very severe necrosis all secondary root dead
3 = Considerable root necrosis, with little root regrowth above dead region
2 = Moderate root necrosis
1 = Very slight necrosis limited mainly to tips of a few secondary root
0 = Root system well developed and no visible lesions

are a major limiting factor of its shelf-life. Post-harvest blue and green moulds caused by *Penicillium italicum* and *Penicillium digitatum* respectively are among the most economically important post-harvest diseases of citrus globally. At below 10 °C, *P. italicum* grows faster than *P. digitatum* as a result; blue mould incidence becomes more important when citrus fruits are kept under cold storage over a long period of time (Palou et al. 2001). Agrochemicals such as imazalil, sodium ortho-phenyl phenate, or thiabendazole have been commonly used to control these diseases (Yildiz et al. 2005; Torres et al. 2007). These synthetic fungicides have been applied for many years with few or limited success owing to resistance development by the fungal pathogens (Holmes and Eckert 1999; Zamani et al. 2006). Moreover, the accumulation of these hazardous agrochemicals in the environment has generated public concern about their impact on human health, thus, creating an opportunity for sustainable alternative methods to control post-harvest diseases without harming either man or his environment. Biological control such as the used of antagonist fungi has been proposed as an alternative to agrochemical and considerable success has been recorded by utilizing antagonistic microorganisms for post-harvest disease control (Wilson et al. 1993). The use of fungi as an alternative to synthetic fungicides has other benefits such as reducing environmental pollution, effectively controlling post-harvest diseases and producing high quality and safe food (He et al. 2003).

Screening for potential antagonistic microorganisms to *P. italicum* from the phylloplane and soil in the orchard was carried out to investigate their efficacy in controlling post-harvest blue mould of orange fruit under in vitro and in vivo conditions. Three fungi genera, *Trichoderma*, *Aspergillus*, *Penicillium*; two yeasts of the genus *Saccharomyces* and a bacterium, *Pseudomonas* were isolated from the phylloplane of leaves, healthy orange fruits and from the orchard soil. The result varied from treatments when pathogen, *P. italicum* was allowed 24–48 h growth ahead of each antagonist depending on its exhibited mechanism against the pathogen. *Trchoderma* sp., a fast grower and good competitor of nutrient showed the best level of antagonism than the others as it stands out to be the best fungal-base biocontrol agent (Tables 3.5 and 3.6) (Emoghene et al. 2011). As can be observed in Table 3.5 antagonism of the pathogen by each of the antagonist varied. Biocontrol of the pathogen placed after 24 h, was significantly higher than that of 48 h. *Penicillium* sp. showed a gradual and steady control rate of the *P. italicum* than *Trichoderma* sp. which demonstrated a better control outcome in a markedly sharp increase in its antagonism (Emoghene et al. 2011). From Table 3.6, it was deduced that the later the antagonists were introduced after *P. italicum*, the better the antagonism. When the pathogen had growth prior to the inoculation of *Trichoderma* sp., inhibition of mycelial growth by mycoparasitism, hyphae interference or antifungal (antibiosis) production was highest than that of *Aspergillus niger* and *Penicillium* sp. (Emoghene et al. 2011).

The bacteria or yeast antagonists seeded on potato dextrose agar (PDA) plate with mycelial extension growth of *P. italicum*, inhibited growth at varying degrees within 6 days of measurement depending on inoculation time. It was observed that mycelial extension growth was best inhibited by *Pseudomonas* sp. followed by yeast when *P. italicum* was inoculated after one day. However, the reverse was the case after two days, with the mycelial extension growth best inhibited by Saccharomyces sp. followed by *Pseudomonas* sp. (Emoghene et al. 2011).

Biocontrol of *P. italicum*, the causative agent of post-harvest rot of orange fruit by *Trichoderma* sp. *Penicillium* sp., *Aspergillus niger*, *Pseudomonas* and *Saccharomyces* sp. showed different levels of antagonistic control efficacy. *Trichoderma* sp. showed a superior biocontrol efficacy and its antagonistic effect on different pathogens is well documented (Grondona et al. 1997; Kucuk and Kivanc 2005; Shaigan et al. 2008). *Trichoderma* sp. grows tropically toward hyphae of other fungi, coil around them in a lectin-mediated reaction and degrade cells of the target fungi. This mycoparasitism process limits growth and activity of most plant pathogenic fungi (Carsolio et al. 1999; Shaigan et al. 2008). *Trichoderma* spp. grows more rapidly than *P. italicum* in mixed culture and this gives it an

Table 3.5 Effects of inoculation time of *Penicillium italicum* on orange fruit rot controlled by fungal antagonist

Mycelial inhibition with time (mm/days)								
Antagonists	Inoculation time (hour)	0	1	2	3	4	5	6
Control	24	0.00 ± 0.0	15.5 ± 1.5	20.7 ± 7.7	29.0 ± 18.7	37.1 ± 16.6	40.5 ± 16.8	43.9 ± 16.1
Penicillium sp.	48	0.00 ± 0.0	0.00 ± 0.0	15.5 ± 1.7	19.7 ± 7.6	27.2 ± 18.2	35.3 ± 14.5	37.5 ± 17.7
		0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	14.3 ± 0.0	24.2 ± 1.1	31.9 ± 1.1	32.6 ± 10.0
Control	24	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	12.0 ± 0.7	13.3 ± 1.4	16.3 ± 0.6	24.6 ± 1.8
Aspergillus	48	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	7.2 ± 10.1	18.8 ± 8.8
niger		0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	16.7 ± 0.0	14.3 ± 0.0
Control	24	0.00 ± 0.0	0.00 ± 0.0	13.4 ± 1.6	27.3 ± 18.3	42.0 ± 11.5	48.3 ± 10.6	54.5 ± 8.4
Trichoderma	48	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	12.5 ± 17.7	27.2 ± 18.2	41.7 ± 11.8	46.9 ± 9.8
sp.		0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	8.4 ± 11.8	8.4 ± 11.8	22.7 ± 8.4	32.5 ± 10.6

Source Emoghene et al. (2011)

Table 3.6 Effects of inoculation time of fungal antagonists on orange fruit rot control

Mycelial inhibition with time (mm/days)								
Antagonists	Inoculation time (hour)	0	1	2	3	4	5	6
Control *Penicillium* sp.	24 48	0.00 ± 0.0 0.00 ± 0.0 0.00 ± 0.0	15.5 ± 1.5 5.6 ± 7.8 15.0 ± 7.1	20.7 ± 7.7 5.6 ± 7.8 13.8 ± 5.4	29.0 ± 18.7 5.6 ± 7.8 12.9 ± 5.4	37.1 ± 16.6 9.6 ± 0.6 24.1 ± 1.3	40.5 ± 16.8 5.6 ± 5.0 27.5 ± 6.2	43.9 ± 16.1 24.1 ± 1.3 30.2 ± 2.3
Control *Aspergillus niger*	24 48	0.00 ± 0.0 0.00 ± 0.0 0.00 ± 0.0	0.00 ± 0.0 0.00 ± 0.0 5.0 ± 7.1	0.00 ± 0.0 21.2 ± 17.1 14.9 ± 11.6	12.0 ± 0.7 21.8 ± 19.1 21.7 ± 7.1	13.3 ± 1.4 26.7 ± 26.0 26.2 ± 7.2	16.3 ± 0.6 26.7 ± 26.0 26.2 ± 7.2	24.6 ± 1.8 33.8 ± 19.5 30.2 ± 7.3
Control *Trichoderma* sp.	24 48	0.00 ± 0.0 0.00 ± 0.0 0.00 ± 0.0	0.00 ± 0.0 0.00 ± 0.0 10.8 ± 2.4	13.4 ± 1.6 24.3 ± 6.1 24.5 ± 3.2	27.3 ± 18.3 24.3 ± 6.1 24.8 ± 3.6	42.0 ± 11.5 22.5 ± 3.5 32.4 ± 8.6	48.3 ± 10.6 27.2 ± 3.1 38.4 ± 11.8	54.5 ± 8.4 35.1 ± 2.5 52.3 ± 3.2

Source Emoghene et al. (2011)

important advantage in the competition for space and nutrients with plant pathogenic fungi (Barbosa et al. 2001). It can be deduced above that, introduction of the pathogen after the inoculation of the antagonist resulted to a better antagonism. This could give the antagonists enough time to grow, reproduce and sporulate using the available nutrient in a competitive manner, in addition to secreting enough antagonistic substances which affect the establishment of the pathogen. Therefore, colonization of the host could be prevented by early application of fungi antagonist to prevent infection and plant diseases, even though there was no significant difference in the time of application of either the pathogen or antagonist ($p > 0.05$) (Emoghene et al. 2011).

3.6 Conclusion

Control of fungal pathogen of plant diseases is based on the application of agronomic practices and pesticides; however, widespread use of agrochemicals inundates the agroecosystems with hazardous substances that impact the balance of the natural food chain. Coupled with the selection of resistant and more virulent plant pathogens resulting to escalation in the quantity of pesticides used. Researches are ongoing to develop new, alternative and sustainable methods to integrate or substitute the application of agrochemicals in an attempt to reduce ecological impact and financial cost of plant disease control. Antagonistic microorganisms especially fungi have been investigated in depth and considered as an attractive alternative to agrochemicals in the control of plant diseases. Fungi-based biofungicides have yielded successful and consistent results as depicted above; however, its application has been delayed owing to the poor relative understanding of the plant-microbe and microbe-microbe interactions in the antagonistic processes amongst others. Diverse microorganisms may have been used for biocontrol of plant diseases, but the most widely applied and researched are on isolates of genera of *Trichoderma*, *Bacillus* and *Pseudomonas* with *Trichoderma* being the most studied fungal biocontrol agent. The mechanism of action of *Trichoderma* spp. as effective biofungicides is well documented. Fundamental discoveries show that *Trichoderma* and other mycoparasites have developed a vast array of molecular technique to enhance their parasitic behaviour. It is agreed that *Trichoderma* produces different types of lytic enzymes that target the cell wall of fungi resulting to their death. Since fungal-biocontrol of plant pathogens are very diverse with different plant hosts, it is therefore very imperative to look for new and novel biocontrol fungi with different mechanisms. The greatest hope for fungi as alternative to agrochemicals lies in understanding its mechanism(s) of action as biofungicides and the pathogenesis of the pathogens. It is anticipated that this knowledge will open up new possibilities and innovative approaches for controlling plant diseases as agrochemicals usage is on the increase, particularly in developing countries like Nigeria and is no longer sustainable owing to adverse environmental effect and loss of human life.

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