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Control of Fusarium Wilt of Tomato (*Lycopersicon esculentum* Mill) by *Trichoderma* species

M. I. OSUINDE[1*], E. I. ALUYA[1] and A. O. EMOGHENE[2]

[1]Department of Microbiology, Ambrose Ally University, Ekpoma, Edo State, Nigeria
[2]Department of Microbiology, University of Benin, Benin City, Nigeria

Trichoderma species isolated from soil was investigated for ability to control wilt disease of tomato seedlings in sterile potted soil. The roots of four weeks old tomato seedlings were treated with different spores concentration (10^3/10^6 spores/ml) of *Trichoderma* species and placed into sterile soil inoculated with 10^6 spores/ml) of *Fusarium oxysporum* f. sp. *lycopersici.* The different spores concentration (10^3 and 10^6 spores/ml) of *Trichoderma* species prevented wilt disease development in the tomato seedlings in varying degrees. Tomato seedlings were effectively protected from infection when the pathogen *F. oxysporum* f. sp. *lycopersici* and *Trichoderma* species were iniculated simultaneously. Control of wilt disease was less effective when spores of *F. oxysporum* f. sp. *lycopersici* were allowed to grow one day ahead of spores of *Trichoderma* species. And there was no protection when the spores of *F. oxysporum* f. sp. *lycopersici* were allowed to grow two days ahead of *Trichoderma* species.

Keywords: *Fusarium* wilt, *Trichoderma* species, tomato wilt.

Tomato (*Lycopersicon esculentum* Mill) is a very important fruit vegetable used extensively for salats, soups and stews. Industrially, ripe tomato fruits are processed into puree, sauce and joice (Purseglore, 1977). Large-scale tomato production takes place in many countries of the world. United States of America (U.S.A.), Italy, Spain and Bulgaria are among large-scale producers (Simons and Sobulo, 1975; Purseglore, 1977).

Tomato has been in cultivation almost all over Nigeria for a very long-time, the most important areas being the North and South Western parts of the country (Erinle, 1979; Denton and Swarup, 1983). Diseases constitute a serious limiting factor to tomato production (Wheeler, 1969; Simons and Sobulo, 1975; Erinle, 1979; Adelana and Simons, 1980; Denton and Swarup, 1983). Of the known field diseases of tomato, bacterial and fungal wilds most commonly caused by *Ralstania (Pseudomonas) solanacearum* and *Fusarium oxysporum* f. sp. *lycopersici* are the most devastating in many growing belts of the world (Wheeler, 1969; Walker, 1971; Prior et al., 1990), Nigeria inclusive (Erinle, 1977, 1981; Osuinde and Ikediugwu, 1995).

The aetiological agents of tomato wilt disease (*F. oxysporum* f. sp. *lycopersici* and *Ralstania* (P) *solanacearum*) are soil inhibiting organism and survive saprophytically insoil (Park, 1959; Walker, 1957, 1971). Tomato wilts, as indeed most soil-borne diseases of plants have proved extremely difficult to control by used of chemicals which are expensive and hazardous to man and the environment. In most part of the world, efforts

*Corresponding author

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have therefore been made towards genetic (by use of resistant cultivars) and biological control. Use of resistant cultivars has, however, been complicated by the occurrence of more than one race of some wilt pathogens (Walker, 1957) often leading to costly loss of resistance in the field. Thus biological control is now being highly favoured though litle has been achieved in this direction (Papavizas, 1973). One common form of biological control involves the encouragement of the growth of microorganism(s) antagonistic to the pathogen in the environment of the crop plant, to the detriment of the pathogen (Alexander, 1977; Baker and Cook, 1982).

In the present study, we investigated the potential of an isolate of *Trichoderma* species which was previously found to be antagonistic to *F. oxysporum* f. sp. *lycopersici in vitro* to control Fusarium wilt disease of tomato.

Materials and Methods

Isolation of Trichoderma *species from soil*

Trichoderma species was isolated from soil obtained from Botanical garden of Ambrose Alli University, Ekpoma, Edo State, Nigeria. A 10^{-1} dilution (weight/volume) of the soil was thoroughly mixed into sterile water. Samples of (0.1 ml) of a 10^{-6} dilution in water of soil were spread on potato dextrose agar (PDA) and incubated at room temperature (28±2 ºC) for up to 48 hours during which time characteristic colonies of *Trichoderma* developed. The isolate was subjected to preliminary microbiological analysis, based on colony morphology and was further identified using standard procedures (Rifai, 1969).

Isolation of Fusarium oxysporum *f. sp.* lycopersici *from wilted tomato plants*

Five millimetre rooth segments off partially and completely wilted tomato seedlings were surface – sterilized in 70% ethanol for 1 min, rinsed in sterile water and inoculated (4 segments per plate) into potato dextrose agar (PDA). The plates were incubated at room temperature (28±2 ºC) for up to 14 days during which time the fungi growing from rooth segments were isolated and identified based cultural and morphological characterictics according to Booth (1971).

Inoculation of tomato seedlings with F. oxysporum *f. sp.* lycopersici *and* Trichoderma *species*

The test plants were four-week old seedlings of "Ame" a widely grown local cultivar of tomato highly susceptible to *F. oxysporum* f. sp. *lycopersici* (Osuinde and Ikediugwu, 1995). The seedlings were raised from seeds sown in wooden trays containing garden soil earlyer sterilized in an autoclave at 1.1 kg pressure for 30 minutes.

Inoculum and inoculation metods

For the pathogen, *Fusarium oxysporum* f. sp. *lycopersici,* spore suspension containing 10^6 spores/ml in potato dextrose broth (PDB) was prepared from 5 days old PDA

culture at room temperature (28±2 ºC). Five millilitres of the spore suspension was mixed with 50 g of sterilized rice-husks which were then mixed with 2 parts of sterile soil in the potting cup (25 ml). In other set-ups, after mixing the spores suspension with sterile rice husks, the mixture were incubated for 24 hours and 48 hours, respectively, before mixing with sterile soil. Two different spores concentrations, 10^3 spores/ml and 10^6 spores/ml, respectively of the antagonist, *Trichoderma* species were prepared in potato dextrose broth (PDB) from 4-day-old PDA culture at room temperature (28±2 ºC). Two methods were used to inoculate test plants. There were 5 replicate plants for the control, and antagonist organism for each treatment. Seedlings were incubated in the greenhouse for each teathment. Seedlings were incubated in the greenhouse for up to 7 days during which observations were made daily for wilt disease symptoms.

Rooth dip method

The roots of seedlings were carefully washed in sterile distilled water to remove the adherent soil withouth damage to the roots. The root system of the seedlings was dipped in the inoculum suspension of the antagonist; *Trichoderma* species and the excess liquid allowed to run off before seedling was transplanted into the pathogen infested soil in the polycup (1 seedling per cup). Two seds of control experiments were done. The first set of tomato seedlings were root-dipped into sterile PDB and placed in infested soil (pathogen only). The second set of tomato seedling that were root-dipped into antagonist were placed in sterile soil without pathogen (antagonist only).

Direct soil inoculation method

Five millilitres of either of 10^3 spores/ml and 10^6 spores/ml of the antagonist was poured separately into the planting furrow of the pathogen infested soil in the potting cup. One seedling was plated per cup. One set of seedling were planted into infested soil withouth antagonist (pathogen only) while the second set had antagonist poured in the planting furrow in sterile soil withouth pathogen (antagonist) served as control.

Isolation of rhizosphere soil mycoflora

To estimate the population of *Trichoderma* species and *F. oxysporum* f. sp. *lycopersici* on the rhizosphere of tomato plants, root samples were obtained from tomato plants root-dipped into 10^6 spores/ml of *Trichoderma* species before planting into infested soil, after growing plant for 7 days in the greenhouse. The rhizosphere soil was obtained by shaking roots of tomato plants in sterile water (Clark, 1949). Decimal dilutions were then prepared and aliquot plated out on potato dextrose agar (PDA) plates incubated at room temperature (28±2 ºC) for 48 hours for fungal count. Final counts were based on the number of organisms per millilitre of wash water.

Re-isolation of pathogen and antagonist

Five millimetres root segments from control and experimental plants were surface-sterilised in 70% ethanol for 1 min rinsed in sterile water and inoculated (5 segments per

plate) into PDA. The plates were incubated at room temperature for 14 days during which fungi growing from the root segments were isolated and identified. The frequency of isolation of each organism was also recorded.

Results

Trichoderma species was effective in controling fusarion wilt of tomato in greenhouse. The tomato seedlings inoculated with the pathogen (*F. oxysporum* f. sp. *lycopersici*) alone showed mild wilt symptoms by the following day and by the fourt (4th) day plant sagged and wilted competely *(Table 1).* In contrast, control of the disease was observed with the *Trichoderma* species, depending on the concentration of spores method of aplication (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 spores of antagonist *(Tables 1 and 2).*

Table 1

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
Treatments – 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

\+ = Partial (mild) wilt
++ = Complete wilt
– = No wilt
D = Plant death

Table 2

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
Treatments – 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

When the pathogen and antagonist were applied simultaneously, the result depended on the spores concentration an method of application: 10^3 spores/ml delayed symptom expression only for one day *(Table 1).* 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 *(Tables 1 and 2).*

When 10^6 spores/ml of antagonist was applied by root-dip method, there was no wilt development at all, all plants were healtly all through the study period *(Tables 1 and 2);* but in the direct soil inoculation method mild wilt was observed in 60% of the seedlings by the 2th and 3rd day *(Tables 1 and 2).* When the spores of pathogen was allowed to grow one day (24 hours) ahead of spores of antagonist the result also depended on the spore concentration and method of application *(Tables 1 and 2).* When 10^3 spores/ml of antagonist was used, mild wilt was observed the following day in 80% and 100% of seedlings up to the 3rd day in root-dip and direct soil inoculation methods, respectively *(Tables 1 and 2).* However, when 10^6 spores/ml of antagonist was applied there was no wilt symptoms in plants in the root-dip menthod, while 40% of plants developed mild wilt by 2th day up to 4th day in the direct soil inoculation method *(Tables 1 and 2).*

There was no effect on progress of wilt when the spores of the pathogen was allowed to grow two days (48 hours) ahead of spores of antagonist irrespective of spore concentration of antagonist and method of applications *(Tables 1 and 2).* All the plants (100%) were completely wilted by the following day and died two days later *(Tables 1 and 2).* When antagonist alone was applied to plants, there was no wilt symptoms, rather the plants looked better an than those treated with pathogen and antagonist *(Tables 1 and 2).*

Roots of tomato seedlings treated with antagonist and pathogen showed root rot (necrosis) depending on the concentration of the antagonist, method of application and on how long the pathogen was allowed to graw ahead of antagonist *(Table 3).* Roots of plants inoculated with antagonist by root-dip method had lower level of necrosis than those inoculated by direct soil inoculation method *(Table 3).* All the roots of the plants (100%) treated with pathogen alone had severe necrosis *(Table 3).* In contrast roots of plants inoculated with antagonist alone had no necrosis at all; they were better than those inoculated with antagonist and pathogen *(Table 3).*

Table 3

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

	(%) plants with 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	–	–	–	–
Treatments – 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	–	–	–	–

4. = Very Severe necrosis all secondary 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

Table 4

Frequency (%) of reisolation of *Trichoderma* species and *F. oxysporum* f. sp. *lycopersici* from treated plants after 7 days

	Organisms	
	A	P
Treatments – Root dip method		
1a. Pathogen alone (control)	0	100
2a. Antagonist alone (control)	100	0
3a. Pathogen + 10^3 spores/ml antagonist simultaneously	80	40
4a. Pathogen + 10^6 spores/ml antagonist simultaneously	100	30
5a. Pathogen incubated 24 h + 10^3 spores/ml antagonist	60	40
6a. Pathogen incubated 24 h + 10^6 spores/ml antagonist	60	50
7a. Pathogen incubated 48 h + 10^3 spores/ml antagonist	ND	ND
8a. Pathogen incubated 48 h + 10^6 spores/ml antagonist	ND	ND
Treatments – Direct – Soil – Inoculation		
1b. Pathogen alone (control)	0	100
2b. Antagonist alone (control)	100	0
3b. Pathogen + 10^3 spores/ml antagonist simultaneously	60	50
4b. Pathogen + 10^6 spores/ml antagonist simultaneously	80	60
5b. Pathogen incubated 24 h + 10^3 spores/ml antagonist	50	60
6b. Pathogen incubated 24 h + 10^6 spores/ml antagonist	40	60
7b. Pathogen incubated 48 h + 10^3 spores/ml antagonist	ND	ND
8b. Pathogen incubated 48 h + 10^6 spores/ml antagonist	ND	ND

ND = Not done
A = *Trichoderma species* (Antagonist)
P = *F. oxsporium* f. sp. *lycopersici* (Pathogen)

Table 5

Population of *Trichoderma* species and *F. oxysporum* f. sp. *lycopersici* on the rhizosphere of tomato plants after 7 days

Mycoflora	Viable count (x 10^5 CFU/ml)	
	1st wash	5th wash
Trichoderma sp.	7.5	5.5
F. oxysporum f. sp. *lycopersici*	2.5	0.5

The antagonist (*Trichoderma* species) and pathogen (*F. oxysporum* f. sp. *lycopersici*) were re-isolated from root segments of tomato plants after 7 days growth in the greenhouse *(Table 4).* 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; and 40–80% and 50–60%,

respectively, in direct soil inoculation method *(Table 4).* In the control plants, the re-isolation of *Trichoderma* species and *F. oxysporum* f. sp. *lycopersici* was 100% in the separate treatments *(Table 4).* In the root washes, colonies of *Trichoderma* species was far more numerous than *F. oxysporum* f. sp. *lycopersici (Table 5).*

Discussion

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is a serious economic problem in South Western Nigeria, causing up to 25% loss of crop plants in the fileld (Osuinde and Ikediugwu, 1995) and in Northern Nigeria (Erinle, 1977; 1981). Biocontrol of soilborne plant pathogens with antagonist is little known in Nigeria, and much less practiced despite its tremendous advantages and potentials.

In this study, *Trichoderma* species effectively controlled Fusarium wilt of tomato in greenhouse. This result agreed with earlier reports (Sivan and Chet, 1987; Elad et al., 1983; Sivan and Chet, 1989) on the biocontrol potentials *Trichoderma* spp. The antagonist (*Trichoderma* sp.) was more effective when applied using the root-dip method than the direct soil inoculation method. Also spore concentration of 10^6 spores/ml was better in preventing wilt disease than 10^3 spores/ml. Chet and Baker (1980) had earlier reported that the minimal effective amount of *Trichoderma* is about 1×10^6 (CFU/gram) of soil.

Trichoderma species also caused a reduction in the root necrosis (root-rot) of tomato seedlings. This result agreed with the report of Sivan and Chet (1987) that *Trichoderma harzianum* T-35 caused up to 80% reduction of crown rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis – lycopersici* under greenhouse conditions.

In the root washes colonies of *Trichoderma* species were far more numerous than *F. oxysporum* f. sp. *lycopersici* indicating that the antagonist dominated the mycloflora of root-surface. Also most of the root-segment inocula yielded on agar plates only the growth of *Trichoderma* on the contrary, few of the root segments yielded *F. oxysporum* f. sp. *lycopersici* as the only colonizer. Several workers (Harman et al., 1980; Chet and Henis, 1987; Chet, 1987; Sivan and Chet, 1989) have reported the high competitive ability, antibiosis and mycoporasitism of *Trichoderma* spp., which they suggested made them perist on the rhizoplane (root-surface) and rhizosphere of plants and thus out-number other soil microorganism especially the pathogens.

The technique of root-dipping tomato seedlings into spores *Trichoderma* species before transplanting to pathogen infested soil clearly has potential to be applied by tomato growers in Nigeria and elsewhere.

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