

Biocontrol of *Fusarium* wilt of cucumber with *Trichoderma longibrachiatum* NGJ167 (Rifai)

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Authors' contributions

This work was carried out in collaboration between all authors. Author TKK designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors OEU and OOA supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research investigated the use of *Trichoderma longibrachiatum* NGJ167 (Rifai) as a biocontrol agent of *Fusarium* wilt in cucumber varieties (Ashley and Marketmoor) both in the screenhouse and on the field.

Study Design: The screenhouse experiment was laid down in a Completely Randomized Design (CRD) while Randomized complete block design (RCBD) was used for the field experiment.

Place and Duration of Study: The study was conducted at the National Horticultural Research Institute, Ibadan between 2012 and 2013.

Methodology: Soils were inoculated with mycelial plugs of *T. longibrachiatum* NGJ167 before planting while the control soil was mock-inoculated with agar plugs of Potato dextrose agar (PDA). Two weeks after planting, *F. oxysporum* was inoculated into the soils in the screenhouse while natural infection was allowed to occur on the field. The biocontrol abilities of *T. longibrachiatum* NGJ167 on *F. oxysporum* was observed on disease incidence and severity and the fruit yield. The presence of *T. longibrachiatum* NGJ167 genes was detected in the treated cucumber fruits to ensure consumers' safety.

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Results: The control plants had higher incidence and severity of *F. oxysporum* than the *T. longibrachiatum*-treated plants. The *T. longibrachiatum* NGJ167-inoculated Marketmoor had higher fruit weight value of 200g in the greenhouse when compared with the control which had a fruit weight value of 133.33 g. On the field, *T. longibrachiatum*-treated Marketmoor produced the highest fruit weight of 220 g while the control had a mean weight of 120.6 g. Results also revealed that *T. longibrachiatum* DNAs were absent in the inoculated cucumber fruits.

Conclusion: The use of *T. longibrachiatum* NGJ167 as a biocontrol agent indicates its potentials in improving plant health in agriculture. The absence of *T. longibrachiatum* NGJ167 in the treated cucumber indicated that the consumption of such fruits will have no adverse effect on consumers' health.

Keywords: Biocontrol; Cucurbitaceae; disease incidence; polymerase chain reaction; yield.

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a popular vegetable crop of the family Cucurbitaceae comprising 70 genera and 750 species [1]. The nutritional composition of cucumber fruit per 100 g edible portion is 3% carbohydrate, 1% protein, 0.5% total fat and 1% dietary fibre [2]. However, cucumber is susceptible to many pathogens and pests [3]. *Fusarium oxysporum* is one of the most important phytopathogens causing *Fusarium* wilt disease in more than a hundred species of plants [4]. Cucumber *Fusarium* wilt disease is one of the most serious fungal diseases in cucumber production in the world [5,6]. Generally, it caused cucumber yield losses of ~10% to 30% and poor quality products resulting in severe economic losses [7]. Cucumber *Fusarium* wilt disease may occur at all growth periods of the cucumber plant [8]. The pathogen can survive as durable spores for many years with or without plant debris in soil, and it retains the ability to infect cucumber plants causing pre- or post-emergence damping-off, vascular discoloration of roots and stems, and eventually the entire plants wilt or die. The disease management of *Fusarium* wilt usually consists of soil fumigation, seed treatment, use of disease resistant varieties and biocontrol bacteria to reduce infection and disease severity [8,9]. Some antagonists show potential to suppress this disease, such as mycorrhizal fungi and *Trichoderma* [10], *Penicillium* [11], *Streptomyces* [12] and *Bacillus* [13].

Trichoderma spp. are among the most frequently isolated soil fungi and present in plant root ecosystems [14]. These fungi are opportunistic, avirulent plant symbionts, and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases. Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages: (i) colonization of the

rhizosphere by the fungus ("rhizosphere competence") allowing rapid establishment within the stable microbial communities in the rhizosphere (ii) control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms (iii) improvement of the plant health and (iv) stimulation of root growth [14]. Chemical control agents are implicated in ecological, environmental and human health problems and pathogens can develop resistance to them. However, the use of biological control agents such as *T. longibrachiatum* are non-toxic to human health and once colonized by the plant roots, may last for several years. Therefore, the objectives of this work are (1) to determine the effectiveness of *T. longibrachiatum* NGJ167 as a biocontrol agent of *F. oxysporum* in cucumber both in the greenhouse and on the field and (2) to detect the presence of *T. longibrachiatum* NGJ167 genes in the cucumber fruits using Polymerase chain reaction.

2. MATERIALS AND METHODS

2.1 Source of Seeds

Two varieties of *Cucumis sativus* L. (Cucumber) seeds; Ashley and Marketmoor were obtained from the Vegetable Improvement Programme of National Horticultural Research Institute, Ibadan, Oyo State, Nigeria.

2.2 Sources of Fungal Isolates

Trichoderma longibrachiatum NGJ167 (Rifai) was obtained from the Pathology Laboratory of International Institute of Tropical Agriculture (IITA), Ibadan while *Fusarium oxysporum* was isolated from cucumber plant rhizosphere by weighing 10g of soil sample into a conical flask containing 90 mls of sterile distilled water. The suspension was shaken vigorously and serially diluted. Aliquots of 1 ml each from the serial

dilutions were placed into sterile Petri-dishes and molten PDA was poured on them. The plates were swirled to obtain homogenous mixtures on the inocula and PDA. Plates were incubated at 25°C for 72 hrs. The plates were observed for the appearance of *Fusarium* in the mixed cultures. *Fusarium oxysporum* was identified in the laboratory using cultural, microscopic and molecular methods. The two organisms were maintained in the laboratory by periodic transfer onto PDA slant and kept in the refrigerator at 4°C until when required.

2.3 Pathogenicity test for *Fusarium oxysporum*

Pathogenicity test was confirmed using dipping method [15]. Conidial suspension of *F. oxysporum* was harvested by flooding the culture plate with sterile distilled water and gently scraped with spatula. Thereafter, the conidia were filtered through three layers of cheese-cloth and adjusted to a final concentration of 10⁶ microconidia/ml using hemocytometer. Cucumber leaves were dipped in spore suspensions for 5 minutes while leaves dipped in sterile distilled water served as the control. Both inoculated and control treatments were incubated for 7 days at room temperature for disease development. To fulfil Koch's postulate, re-isolation of the pathogenic fungus was done and compared with the original isolate.

2.4 PCR-based Assay for the Identification of *F. oxysporum*

Genomic DNA was extracted from 72 h broth culture of *F. oxysporum* using Zymo Research (ZR) fungal/bacterial DNA MiniPrep™ kit. The total DNA extracted from *F. oxysporum* was used as template in polymerase chain reaction (PCR) using primers, Forward (5'- ATG GGT AAG GAA GAC AAG AC -3') and Reverse (5'- GGA GGT ACC AGT GAT CAT GTT -3'), which have been designed to amplify approximately 700bp from the translational elongation factor (TEF) gene region of *F. oxysporum* [16].

2.5 Dual Culture of *Fusarium oxysporum* and *Trichoderma longibrachiatum*

The identified *F. oxysporum* was co-cultured on duplicate plates of PDA with the *T. longibrachiatum* NGJ167 (Rifai) obtained from IITA, Ibadan in order to determine the biocontrol ability of *T. longibrachiatum*. This was achieved by preparing conidial suspension of

T. longibrachiatum NGJ167 and 1.0 ml (10⁶ microconidia/ml) of this suspension was put into a sterile Petri-dish. Molten PDA (45 °C) was poured into the plate. The plate was swirled to ensure the mixing of *T. longibrachiatum* NGJ167 and the agar. After the agar had solidified, 1.0 ml (10⁶ microconidia/ml) of *F. oxysporum* conidial suspension was inoculated into the plate and a sterile glass spreader was used to spread the pathogen on the surface of the plate.

2.6 Soil Treatment and Planting Operations

The experiment was conducted both on the field and in the greenhouse. The greenhouse experiment was conducted between July, 2012 and September, 2012. The top soil for planting was collected from a depth of 3-5cm with a disinfected hand trowel. The soil was sterilized at 100°C for 3 h to eliminate pathogenic microorganisms. Mycelial plugs from actively growing *Trichoderma longibrachiatum* NGJ167 on Potato dextrose agar (PDA) were inoculated into the soil and treatments were carried out as follows: (i) seedlings and *T. longibrachiatum* in the same 3 cm deep hole (T1) (ii) seedlings in 3 cm deep hole with *T. longibrachiatum* placed 3 cm below the seedlings (T2) (iii) seedlings in 3 cm deep hole with *T. longibrachiatum* placed on one side of the seedlings (T3) (iv) seedlings in 3 cm deep hole with *T. longibrachiatum* placed 3 cm on both sides of the seedlings (T4) (v) seedlings in 3 cm deep hole with agar plug of PDA without *T. longibrachiatum* (control). In the greenhouse, two weeks after the treatment, 100 ml spore suspension (10⁸ cfuml⁻¹) from 8-day old *F. oxysporum* culture was used to inoculate the soil in which the treated seedlings and the control were grown. However, *F. oxysporum* was not inoculated into the soil on the field; natural infection was allowed to set in. Plants were watered regularly. The first data was taken two weeks after pathogen inoculation and afterwards data were at 2 weeks interval on fungal incidence, severity and fruit weight.

2.7 Field Experiment

The field experiment was conducted at the vegetable field of National Horticultural Research Institute, Ibadan, Nigeria (Latitude 70 54'N, and Longitude 30 54'E, 213 meters above the sea level) between October and December, 2013. Ibadan is in the rain forest-savanna transition ecosystem of South-West Nigeria. Randomized complete block design (RCBD) was used for the

field experiment. The randomization was generated on the computer using random table generator. There were four replicates and each replicate represented a block. In each block there were 5 treatments as stated above and each treatment represented a plot. For the two varieties, each plot was separated from the adjacent one by a distance of 1 m while plants within each plot were spaced 50 cm x 75 cm. Similar data taken in the greenhouse were also taken on the field.

2.8 Measurement of Disease Incidence

Disease incidence was estimated by counting the number of symptomatic plants and expressing it as a percentage of the total plants sampled. Recording of disease incidence was carried out 4 weeks after transplanting (WAT). The incidence of *F. oxysporum* wilt disease was recorded by visual symptom observation such as necrosis, wilting and plant death characteristic of the infection. The visible symptoms of the disease were critically observed and the infected plants were identified according to Givord et al. [17]. Disease incidence was estimated by counting the number of symptomatic plants and expressed as a percentage of the total plants sampled.

2.9 Measurement of Disease Severity

Disease severity was assessed using a 1–5 scoring scale, where (1= no visible symptoms; 2= symptoms on less than 25% of the plant; 3= symptoms cover 50% leaf area; 4= symptoms on entire leaf area and 5= stunting, deformation and death of plant [18]. This was carried out at 4 weeks after planting (WAT).

2.10 Measurement of Fruit Weight

The effect of the different treatments on fruit weight was determined by using a sensitive weighing balance (Mettler Toledo) to determine the weight of fruits in grams (g).

2.11 Evaluation of Cucumber for the Presence of *Trichoderma longibrachiatum* (Rifai) Genes

The extraction of *T. longibrachiatum* DNAs from cucumber fruits was achieved by using the cetyltrimethylammonium bromide (CTAB) procedure as described by Abarshi et al. [19]. The total DNAs extracted from the fruits were used as templates in PCR using the following universal PCR primers: Forward (5'- TCC GTA

GGT GAA CCT GCG G -3') and Reverse (TCC TCC GCT TAT TGA TAT GC -3') [20] for the amplification of the internal transcribed spacer (ITS1 and ITS2) regions of *T. longibrachiatum* [20].

2.12 Statistical Analyses

Data obtained were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Scientists (SPSS) version 16.0 and means were compared using Duncan's Multiple Range Test at $P < 0.05$.

3. RESULTS

3.1 Characteristics of Isolated *Fusarium oxysporum*

Mixed fungal culture was obtained during the isolation of *F. oxysporum*. The fungi that were present in the mixed culture include *Fusarium oxysporum*, *Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum*, *P. notatum* and *Rhizopus* spp. The 5-day old pure culture of *F. oxysporum* obtained from the mixed fungal culture revealed that the colonies had pink colour with cottony surface texture. The reverse side of the agar had pink pigmentation. The colony margin was smooth with semi raised elevation (Fig. 1). The morphological characteristics revealed that the hyphae were septate. They had conidiophores which were not well differentiated from the hyphae. Both macro- and micro-conidia were present. They had brown chlamydospores which were solitary (Fig. 2). The isolate was identified as *Fusarium oxysporum* after further identification using PCR.

3.2 Pathogenicity Test

When the isolated *F. oxysporum* was inoculated into cucumber leaves, necrotic lesions were observed indicating the presence of *F. oxysporum* in the plant. However, the cucumber leaves inoculated with sterile distilled water did not show any symptom.

3.3 PCR-based Assay for the Identification of *F. oxysporum*

The gel electrophoresis result of the *F. oxysporum* Translation Elongation Factor (TEF) gene showed that the gene responsible for *Fusarium* wilt disease in plants was extracted from the isolate identified as *F. oxysporum*. The PCR amplification product of the TEF gene gave a product size of 650 bp (Fig. 3).



Fig. 1. Pure culture of *Fusarium oxysporum* on Potato Dextrose Agar

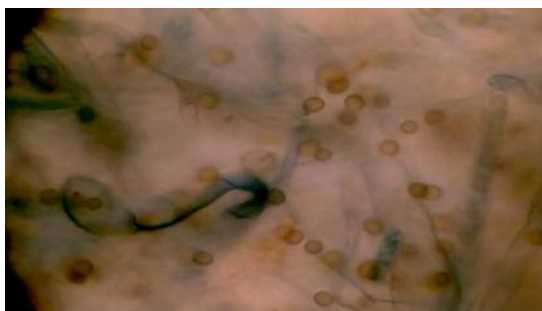


Fig. 2. Micrograph of *Fusarium oxysporum* showing the spores

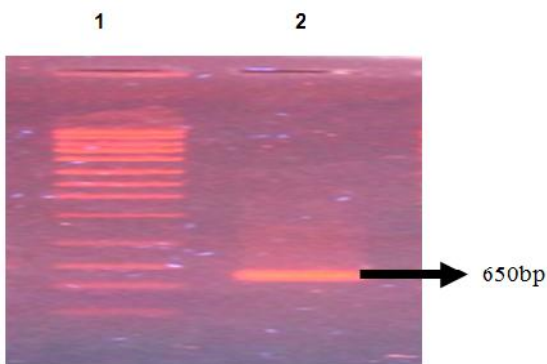


Fig. 3. Agarose gel electrophoresis of PCR products of the TEF gene in *F. oxysporum* using species-specific primer Lane 1; DNA sizer marker; Lane 2; *F. oxysporum* band

3.4 Dual culture of *Fusarium oxysporum* and *Trichoderma longibrachiatum*

The antagonistic *T. longibrachiatum* suspension was able to grow faster than *Fusarium oxysporum* and made contact with colonies of *F. oxysporum* after 48 h of co-culturing.

Trichoderma longibrachiatum overgrew colonies of the pathogen and formed green clusters on *F. oxysporum* (Fig. 4).

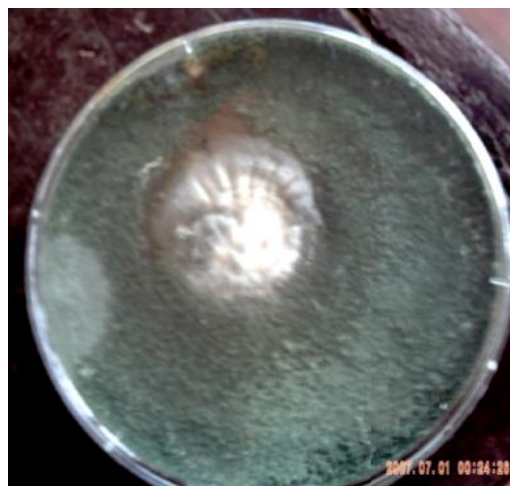


Fig. 4. Dual culture of *Trichoderma longibrachiatum* and *Fusarium oxysporum*

3.5 Incidence of *Fusarium oxysporum* in Cucumber Grown in the Screenhouse

The control of the Ashley cucumber had the highest incidence of 68.33% while T2 had a value of 53.37% and the lowest incidence in Ashley was recorded in T1 with a value of 38.58%. However, 63% was the highest incidence recorded for the Marketmoor cucumber and this value was from the control plant. In the *T. longibrachiatum*-treated Marketmoor cucumber, T2 had an incidence value of 44.29% while it was less in T4 with a value of 25% (Fig. 5).

3.6 Severity of *Fusarium oxysporum* in Cucumber Grown in the Screenhouse

The control of Ashley had a severity score of 4 indicating that the plants were severely infected with *F. oxysporum* while the *T. longibrachiatum*-treated plants were moderately infected with severity score of 2. With the exception of T1 which had a score of 3. The severity of infection of *F. oxysporum* on the control of Marketmoor was high with a score of 4.5 which indicated that the plants had combinations of leaf yellowing, necrosis and wilting. The *T. longibrachiatum*-treated Marketmoor had severity scores which varied from 2.5 to 3 with T1 and T4 having the least score (Fig. 6).

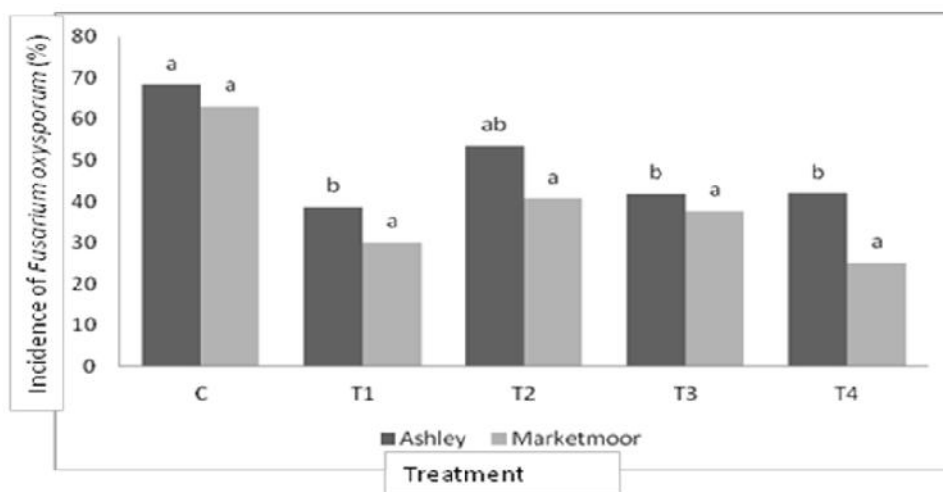


Fig. 5. Incidence of *Fusarium oxysporum* infected cucumber grown in the screenhouse
For each variety, bars sharing the same letter are not significantly different according to the Duncan multiple range test ($P \leq 0.05$).

C- control; T1- seedling and *T. longibrachiatum* in the same 3 cm deep hole; T2- seedling in 3 cm deep hole with *T. longibrachiatum* placed 3 cm below the seedlings; T3- seedling in 3 cm deep hole with *T. longibrachiatum* on one side of the seedlings; T4- seedling in 3 cm deep hole with *T. longibrachiatum* on both sides of the seedlings.

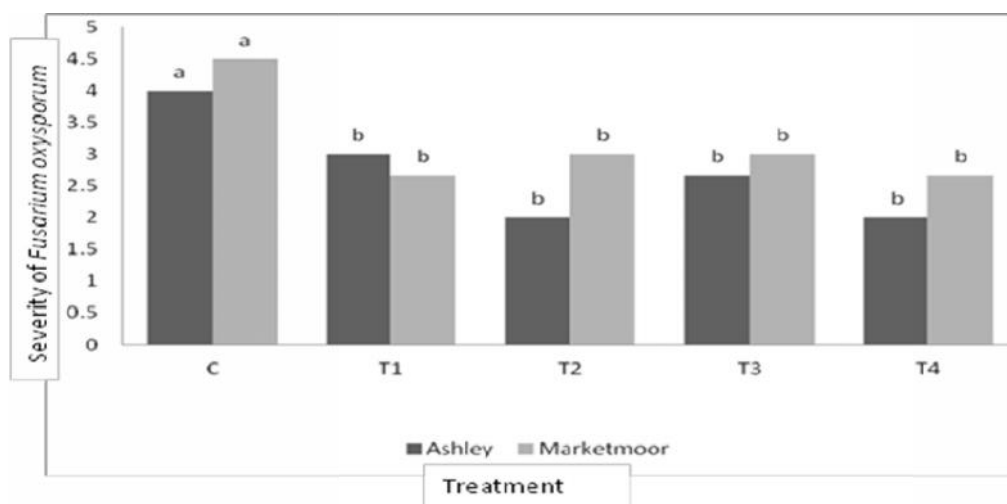


Fig. 6. Severity of *Fusarium oxysporum* infected cucumber grown in the screenhouse
For each variety, bars sharing the same letter are not significantly different according to the Duncan multiple range test ($P \leq 0.05$).

C- control; T1- seedling and *T. longibrachiatum* in the same 3 cm deep hole; T2- seedling in 3 cm deep hole with *T. longibrachiatum* placed 3 cm below the seedlings; T3- seedling in 3cm deep hole with *T. longibrachiatum* on one side of the seedlings; T4- seedling in 3 cm deep hole with *T. longibrachiatum* on both sides of the seedlings.

3.7 Incidence of *Fusarium oxysporum* Infected Cucumber Grown on the Field

The incidence of *F. oxysporum* in the control of Ashley cucumber was 63% and this was highest

incidence. Of all the *T. longibrachiatum*-treated Ashley cucumber, T2 had an incidence of 50% while T4 had an incidence of 40.95% (Fig. 7). The result obtained from Marketmoor cucumber revealed that 53.3% of the control plants were susceptible to *Fusarium wilt* infection.

The infection of the *T. longibrachiatum*-treated Marketmoor cucumber ranged between 18.3% and 26.7% (Fig. 7).

3.8 Severity of *Fusarium oxysporum* Infected Cucumber Grown on the Field

The severity of *F. oxysporum* in the control of Ashley cucumber was 3.33 which was the highest while the least score was 1.33 by T4. In Marketmoor cucumber, the control had a severity score of 3. Apart from T2 which had a severity score of 2, the other *T. longibrachiatum*-treated plants had severity score of 2.33 (Fig. 8).

3.9 Effect of *Fusarium oxysporum* and *Trichoderma longibrachiatum* on Fruit Weight of Cucumber Varieties

The control of Ashley cucumber variety grown in the screenhouse had mean fruit weight value of 75 g which was significantly different ($P < 0.05$) from the mean values of the *T. longibrachiatum*-treated plants. Treatments T1 and T2 had the highest significant values of 201 g and 190 g respectively followed by T4 and then T3. Similarly, the control of Marketmoor cucumber grown in the screenhouse had the least significant fruit weight value of 133.33 g while the

treated plants had higher values with T2 having the highest value of 200 g (Table 1).

The fruit weights of Ashley cucumber grown on the field showed that the control had the least fruit weight of 76.67 g. The fruit weights of the *T. longibrachiatum*-treated Ashley were not significantly different at ($P < 0.05$) with mean values ranging between 120 g and 128.33 g, except T2, which had an average fruit weight of 106.67 g. The highest significant fruit weight of Marketmoor cucumber grown on the field was observed in T2 with an average weight of 220 g while the least weight of 120.6 g was observed in the control (Table 1).

3.10 Evaluation of Plants for the Presence of *Trichoderma longibrachiatum* (Rifai) Genes

The result of the agarose gel electrophoresis indicated that *T. longibrachiatum* DNAs were not present in the plant materials (Fig. 9). This implies that *T. longibrachiatum*-treated crops are safe for consumption.

4. DISCUSSION

In this study, *Fusarium oxysporum* was isolated from the rhizosphere of cucumber plant. The presence of this pathogen in the soil can be

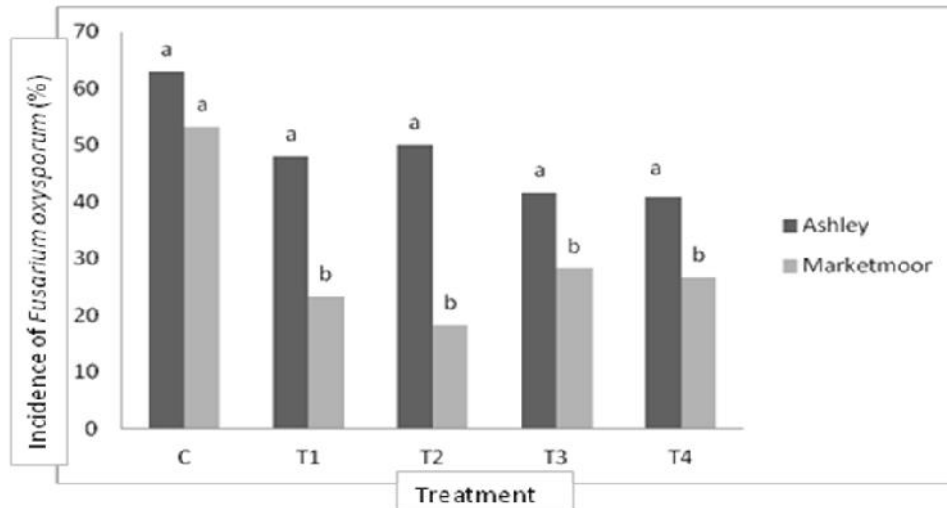


Fig. 7. Incidence of *Fusarium oxysporum* infected cucumber grown on the field

For each variety, bars sharing the same letter are not significantly different according to the Duncan multiple range test ($P \leq 0.05$).

C- control; T1- seedling and *T. longibrachiatum* in the same 3 cm deep hole; T2- seedling in 3 cm deep hole with *T. longibrachiatum* placed 3 cm below the seedlings; T3- seedling in 3cm deep hole with *T. longibrachiatum* on one side of the seedlings; T4- seedling in 3 cm deep hole with *T. longibrachiatum* on both sides of the seedlings.

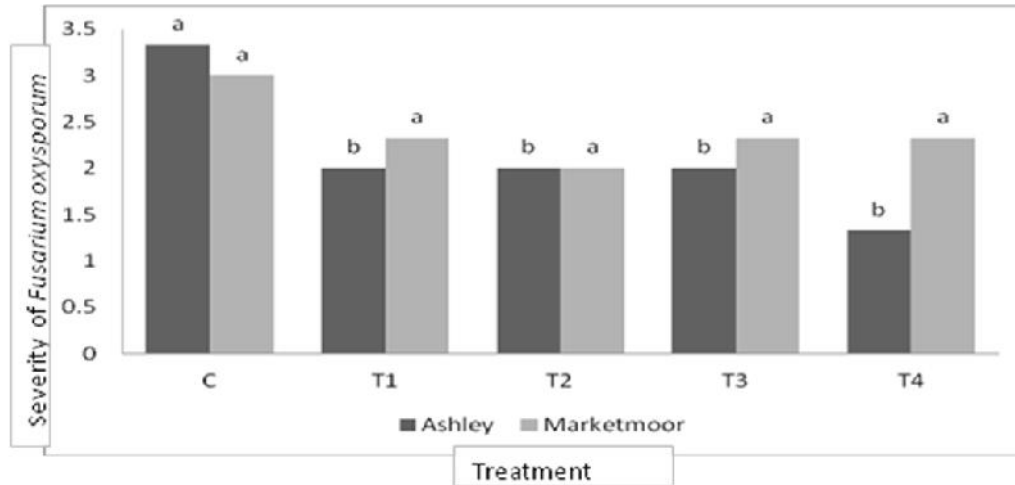


Fig. 8. Severity of *Fusarium oxysporum* infected cucumber grown on the field

For each variety, bars sharing the same letter are not significantly different according to the Duncan multiple range test ($P \leq 0.05$)

C- control; T1- seedling and *T. longibrachiatum* in the same 3 cm deep hole; T2- seedling in 3 cm deep hole with *T. longibrachiatum* placed 3 cm below the seedlings; T3- seedling in 3cm deep hole with *T. longibrachiatum* on one side of the seedlings; T4- seedling in 3 cm deep hole with *T. longibrachiatum* on both sides of the seedlings.

Table 1. Effect of *Fusarium oxysporum* and *Trichoderma longibrachiatum* on the fruit weight of cucumber varieties

Treatment	Screenhouse cucumber		Field cucumber	
	Weight of ashley (g)	Weight of marketmoor (g)	Weight of ashley (g)	Weight of marketmoor (g)
Control	75.00± 5.00 ^d	133.33± 16.67 ^b	76.67± 8.82 ^b	120.60± 25.14 ^b
T1	201.00± 1.53 ^a	193.90± 26.10 ^{ab}	128.33± 12.41 ^a	168.30± 16.92 ^{ab}
T2	190.00± 10.00 ^a	200.00± 17.32 ^a	106.67± 13.02 ^{ab}	220.00± 28.16 ^a
T3	100.00± 0.00 ^c	160.00± 5.77 ^{ab}	126.67± 17.64 ^a	176.67± 14.53 ^{ab}
T4	124.67± 12.78 ^b	195.00± 13.23 ^{ab}	120.00± 7.64 ^a	141.66± 10.14 ^b

Means ± S.E.M followed by the same letter along the column are not significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

T1- seedling and *Trichoderma* in the same 3 cm deep hole; T2- seedling in 3 cm deep hole with *Trichoderma* put 3 cm below the seedlings; T3- seedling in 3 cm deep hole with *Trichoderma* put 3 cm below the seedlings and in other 3 cm hole on one side of the seedlings; T4- seedling in 3 cm deep hole with *Trichoderma* placed in hole 3 cm below and 3cm on both sides of the seedlings.

linked to the fact that plant rhizospheres are rich in photosynthates which are utilized by microorganisms. There are several studies on the isolation of *F. oxysporum* from agricultural soils. Leslie and Summerell [21] reported that the genus *Fusarium* is a ubiquitous soil saprophyte and has been isolated from debris and roots, stems and seeds of a wide variety of plants.

The pathogenicity tests carried out between *F. oxysporum* and cucumber revealed that Koch's postulate was established. This is because of the necrotic lesions and yellowing induced on the healthy plant after inoculation with *F. oxysporum*. Boughalleb and El Mahjoub [22]

confirmed Koch's postulate by re-isolating *F. oxysporum* from watermelon seed and confirming it to be responsible for vascular wilt of the seedlings.

The genomic DNA extracted from the morphologically identified *F. oxysporum* was identified to be *F. oxysporum* using PCR. The result obtained proved that the primer pair allowed a fast, reliable and specific identification of *Fusarium oxysporum* isolate and could be suitable for early diagnosis of *Fusarium* wilt of in soil. The use of species specific primers for the identification of *Fusarium* species has been described in many literatures [23,24].

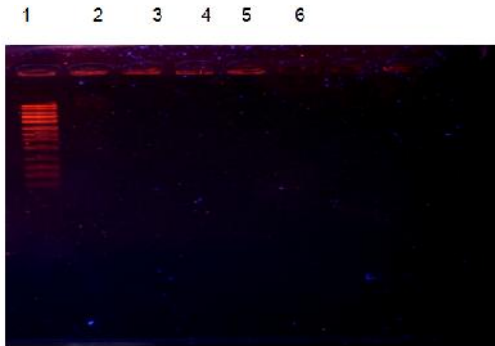


Fig. 9. Agarose gel electrophoresis of PCR products of the ITS1 and ITS2 genes of *T. longibrachiatum* using universal primer to detect the presence of *T. longibrachiatum* in cucumber fruits

Lane 1=DNA sizer marker, Lane 2= T1- seedling and *Trichoderma longibrachiatum* in the same 3 cm deep hole; Lane 3=T2- seedling in 3 cm deep hole with *Trichoderma longibrachiatum* put 3 cm below the seedlings; Lane 4=T3- seedling in 3 cm deep hole with *Trichoderma longibrachiatum* placed on one side of the seedlings; Lane 5=T4- seedling in 3 cm deep hole with *Trichoderma longibrachiatum* placed on both sides of the seedlings; Lane 6=control

When *T. longibrachiatum* cell suspension was grown in dual culture with *F. oxysporum*, the growth of the pathogen was inhibited by *T. longibrachiatum*. This could be attributed to the antagonistic ability of *T. longibrachiatum*, thereby inhibiting the growth of *F. oxysporum*. Also, competition between the two microorganisms for space and nutrients could be responsible for the growth inhibition of the pathogen. Viterbo et al. [25] reported that *T. harzianum* IT-35 was able to control *Fusarium* species on various crops via competition for nutrients and rhizosphere colonization.

The incidence and severity of *F. oxysporum* were reduced in the *T. longibrachiatum* NGJ167-inoculated cucumber plants when compared with the control plants. This could be attributed to the ability of *Trichoderma* species to induce systemic resistance in plants. It is well documented that the interaction of *Trichoderma* strains with the plant may promote growth, improves crop yield, increase nutrient availability and enhance disease resistance [26]. Smolińska et al. [27] in their experiments conducted in a greenhouse reported that *T. harzianum* strain PBG decreased incidence of damping-off of cucumber caused by *R. solani*.

The fruit weights were higher in the *T. longibrachiatum* NGJ167-inoculated cucumber

plants. This is because *Trichoderma* species was able to increase nutrient uptake in roots through maximum exploitation of soils. This conforms to the report of Altintas and Bal [28] who stated that *T. harzianum* significantly increased yield both in leafy vegetable crops and fruit bearing vegetables such as cucumbers. Moreso, Harman [29] demonstrated that *Trichoderma* increased root development and crop yield, the proliferation of secondary roots, and seedling fresh weight and foliar area.

The result on the safety of *T. longibrachiatum*-treated food showed that *T. longibrachiatum* was not present in the cucumber fruits obtained from the *T. longibrachiatum*-treated crops. The reason for this is that *Trichoderma* was not transported to the aerial parts of the plant. It was the hormones and chemicals that were communicated to the aerial parts. Benitez et al. [26] reported that the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists.

5. CONCLUSION

The use of *T. longibrachiatum* as a biocontrol agent is promising in agricultural setting as it increases crop yield and improves plant health with no adverse effect on the environment and the consumers at large. The use of biocontrol agents should be a suitable alternative because of the adverse effects associated with chemical control agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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