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Assessing the Biocontrol Potential of *Trichoderma* species on Sclerotia rot disease of Tomato Plants in Chile Island (Makurdi)

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Abstract: *An assessment of the biocontrol potential of Trichoderma species on sclerotia rot disease of tomato plants in Chile Island (Makurdi) was conducted. Soil samples were collected for the isolation of Trichoderma species while tomato plants showing symptoms of sclerotia rot disease were collected for isolation of Sclerotium rolfsii. Two Trichoderma species; Trichoderma harzianum and Trichoderma viride including Alternaria species and Aspergillus niger were isolated from the soil samples. Sclerotium rolfsii grew rapidly on PDA and the colony colour was white. For the evaluation of the antagonistic potential of Trichoderma species in vitro, Trichoderma harzianum gave the highest inhibition of 74.50% while Trichoderma viride gave an inhibition o f 68.75%. The reduction in radial growth of Sclerotium rolfsii by Trichoderma harzianum did not differ significantly (P>0.05) from that of Trichoderma viride. The antagonistic potential of the Trichoderma isolates against Sclerotium rolfsii on the tomato cultivars in bioassay showed significant difference (P<0.05) with respect to parameters evaluated such as number of leaves, branches and height. Trichoderma harzianum and Trichoderma viride share great success in several parameters evaluated with respect to biological control and can be considered for field applications in the biocontrol of soil borne pathogenic fungi.*

Keywords: *Biocontrol potential, Sclerotia rot, Chile Island, Assessing, Trichoderma.*

I. Introduction

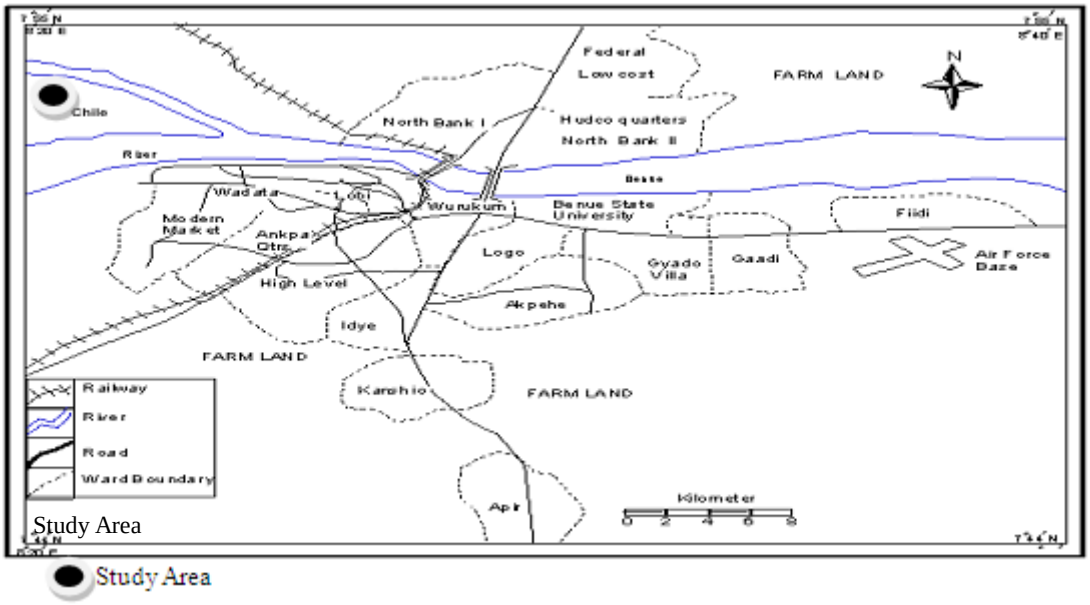
The term "biological control" and its abbreviated synonym "biocontrol" implies the use of microbial antagonists to suppress disease as well as the use of host specific pathogens to control weed populations [1]. The organism that suppresses the pest or pathogen is referred to as the Biocontrol Agent (BCA). Interactions between antagonistic microorganisms and plant pathogens are widespread in nature [2]. These interactions can be highly effective especially with hyperparasitizing potentials of antagonists such as Trichoderma on pathogenic fungi.

Trichoderma is a genus of fungi present in all soils. Many species in this genus have been developed as biocontrol agents against several plant pathogenic fungi. The genus has attracted considerable scientific attention and gained immense importance since last few decades due to its biological control ability [1]. Therefore, this present study was proposed to assess the biocontrol potential of Trichoderma species on sclerotia rot disease of tomato plants in Chile Island (Makurdi), Nigeria.

II. Materials and Methods

2.1 Description of Study Area

This study was carried out in Chile, and Island which lies in the middle of the Benue River on the Northern part of Makurdi, which is a major tomato producing area in the Benue State capital. The Island experiences a tropical climate with two distinct seasons: the wet or rainy season and the dry season. The rainy season lasts from April to October. The dry season begins in November and ends in March with dry north easterly winds being experienced, especially in the harmattan months of November to February. Temperatures fluctuate between 23^{o}C and 32^{o}C in the year. The vegetation of the Island consists of bamboo trees and tall grasses with trees that are generally of average height. These together with its location and a favourable rainfall pattern account for its support for a wide variety of crops.



Source: Google Maps

Map of Makurdi showing the Study Area

2.2 Soil Sample Collection

Soil samples were collected in polyethylene bags at a depth of 2 – 3cm from the rhizosphere of tomato plants and six different points on the field and pooled together. These were conveyed to the Botany laboratory of the Benue State University for isolation of Trichoderma species.

2.3 Collection of Diseased Plant Samples

Tomato plants showing symptoms of sclerotia rot disease were harvested, put in polyethylene envelops and taken to the Botany laboratory of the Benue State University for isolation and identification of Sclerotium rolfsii.

2.4 Media preparation

The medium used for the isolation of fungi was Potato Dextrose Agar (PDA). This was prepared according to manufacturer's instruction. About 39.6g of powdered PDA medium was dissolved in 1 liter of sterile distilled water and sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool before pouring carefully into sterile Petri dishes.

2.5 Isolation of Trichoderma Species from Soil Samples

For isolation of Trichoderma species, a serial dilution technique was employed and a 10^4 dilution of the soil sample was prepared. In this method, a stock suspension was prepared by adding one gramm of the soil samples to 9mls of sterile distilled water in a sterilized glass tube. After shaking, one millimeter of dilution level 10^4 was dispersed in a 9cm diameter Petri dish after which about 15-20 mls of sterilized molten Potato Dextrose Agar (PDA) was added. The agar and the Inoculum was swirled gently and allowed to set. Culture plates were incubated at 25-28^{o}C for 1 week. The plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). Individual colonies were isolated from the same plates and transferred to plates containing freshly prepared PDA. Pure cultures were maintained on PDA slants in sterile McCartney bottles and stored at 4^{o}C for further use.

2.6. Identification of Trichoderma Isolates

Two techniques, visual observation on Petri dishes and micro-morphological studies in slide culture were used to identify Trichoderma species. For visual observation, isolates were grown on PDA for 5 days. Growth rates, changes in medium colour and colony appearance were examined every day. These characteristics are regarded as taxonomically useful characteristics for Trichoderma [3]. For micro-morphological characteristics, observations were made for morphology of conidiophores and conidia after which identification was done using the recommendations given by [4], [5], [6] and other relevant electronic documentations on the genus Trichoderma.

2.7. Isolation and Identification of Sclerotium rolfsii

The infected stems of tomato plants were cut into small sections of 0.5-1.0cm long bits. The bits were surfaced sterilized by dipping in 5% sodium hypochloride (NaOCI) solution for 2 minutes. The treated plant bits were rinsed 2 times in sterilized distilled water. The excess water on the surface of the tissues was removed by blotting on sterile blotting paper. The sterilized tissues were placed on Petri dishes containing Potato Dextrose Agar (PDA) and incubated for 7 days at 25- 30^{O}C. The pathogen was identified based on mycelia and Sclerotia characters [7] and maintained on PDA for further studies.

2.8. Experiment One

2.8.1 Evaluation of the Antagonistic Potential of Trichoderma species in vitro

In vitro tests were conducted to evaluate the antagonistic effect of Trichoderma species against Sclerotium rolfsii on PDA medium by dual culture technique [8]. One mycelia disc (5mm) of individual isolates of Trichoderma species and one mycelia disc (5mm) of the test pathogen were placed simultaneously 1cm from the edge of each Petri dish plate at opposite directions. Three replications were used for each Trichoderma isolate and the test pathogen. The plates were arranged on laboratory desk following Complete Randomized Design. The plates that received only mycelia disc of Sclerotium rolfsii served as control. Plates were incubated in the laboratory having ambient temperature of 25 - 28^{o}C. Thereafter, percentage inhibition of Sclerotium rolfsii was calculated using the formula;

Inhibition of growth (%) = $\frac{(R_1 - R_2)}{R_1}$ X 100

Where R_1 = Mycelia growth of the pathogen without Trichoderma (control).

R_2= Mycelia growth of the pathogen in the presence of Trichoderma.

2.9. Experiment Two

2.9.1Evaluation of the Antagonistic Potential of Trichoderma Isolates in Biological Assay (Biocontrol Experiment)

Trichoderma isolates that showed signs of antagonistic activity in in vitro bioassays were used for the biocontrol experiment. The isolates were grown on PDA at 25-30^{o}C for seven days. After 7 – 10 days of incubation, conidia were harvested from cultures by flooding the plates with 10mls of sterile distilled water then removed by agitation with a sterile glass rod. These were poured into sterile test tubes and agitated for 30 seconds. The resulting suspensions was filtered through a layer of sterile filter papers. The conidia concentration in the suspensions was determined using a haemacytometer and sterile distilled water was added to bring the concentration to 3 x 10^{6} conidia/ml.

Four millimeters of each suspension was added to 0.5kg of sandy loam soil previously sterilized at 82.2^{o}C for 30 minutes. The inoculated Sandy loam was incubated for 5 - 7 days at 25-30^{o}C and then mixed with 1 gramm Sclerotia of Sclerotium rolfsii.

The pots were then seeded with 3 cultivars of tomatoes and laid out in complete randomized design. There were four pots per cultivar and controls were pots containing seeds inoculated with Sclerotium rolfsii only.

2.10. Data analysis

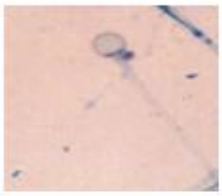
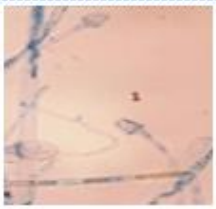
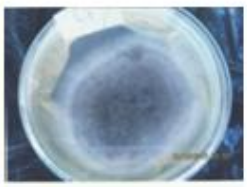
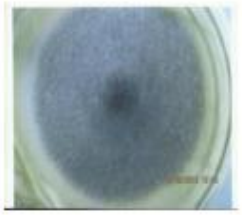
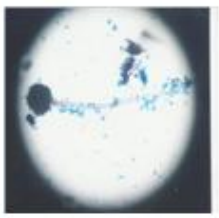
Data generated from the study was analyzed using Analysis of Variance (ANOVA) and the Fishers Least Significant Difference (FLSD) was used to separate the means at 5% level of significance.

III. Results

3.1Trichoderma Species Isolated from Soil Samples

A total of two Trichoderma species; Trichoderma harzianum and Trichoderma viride were isolated from the soil samples. Other fungi isolated include Aspergillus niger and Alternaria species as shown in Table1.

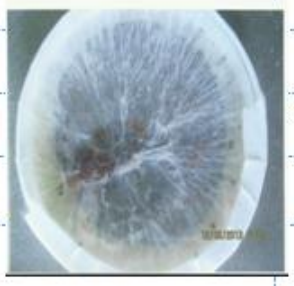
Table 1: Characterization and identification of fungal isolates from soil Samples on Potato Dextrose Agar

Micro/Macroscopic Characteristics	Appearance on PDA	Photo micrograph	Probable Organism
Initial colour of the colony was whitish (1-2 days), which turned globose dark green in the centre then dull green with compact and wooly conidiophores throughout the Petri plates. Mostly spherical, smooth and hyaline conidia produced on conidiophores.			*Trichoderma harzianum*
Initial colour of the colony was whitish (1-2days), then turned light green and watery in the centre. Conidiophores were erect, compact, wooly and pencillately branched. Conidia were hyaline, sub-globose to curve shaped like an oval and smooth walled.			*Trichoderma viride*
Colony is wooly, have a black reverse and a gray white surface which becomes greenish brown with a light border as the colony ages. Conidium is light brown with a club-shaped configuration and is divided by transverse and longitudinal septations.			*Alternaria spp.*
Growth rate is rapid and surface colony colour is initially white becoming black to deep brown with conidial production while the reverse is pale yellow or uncolored. Conidiophores are hyaline, smooth-walled with length ranging from 400-3,000um long, becoming darker at the apex and terminating in a globose vesicle with size of 30-75 um in diameter.			*Aspergillus niger*

3.2 Isolation and Identification of Sclerotium rolfsii on PDA.

The fungus grew rapidly on Potato Dextrose Agar (PDA). The colony was white. Sclerotia were produced within 3 – 4 weeks and were found to be round and white but turning brown with age and produced in large numbers over the entire colony surface. Primary hyphae showed clamp connections at the septa. Aerial mycelia usually formed many narrow hyphae strands that were between 4.2 – 8.4 um wide. Based on morphological and cultural characteristics, the isolate was identified as Sclerotium rolfsii as shown in Table 2.

Table 2: Characteristics of Sclerotium rolfsii on Potato Dextrose Agar (PDA)

S/No	Parameters	Characteristics	Appearance on PDA	Probable Organism
1	Colony colour	White		*Sclerotium rolfsii*
2	*Sclerotium* size (mm)	1-3		
3	*Sclerotium* colour	White to brown		
4	*Sclerotium* shape	Spherical		
5	Hyphae diameter (µm)	4.2-8.2		

3.3 Evaluation of the Antagonistic Potential of Trichoderma species In vitro

Simultaneous pairing of Trichoderma isolates with S .rolfsii gave rise to growth reductions of S. rolfsii. T. harzianum gave the highest inhibition of 74.50%, while T. viride gave an inhibition of 68.75% as shown in Table 3. The reduction in radial growth of S. rolfsii by T. harzianum did not differ significantly ($P > 0.05$) from that of T. viride as shown in Table 4.

Table 3: Percentage Growth inhibition of S. rolfsii paired with Trichoderma species.

Trichoderma species	Day 1	Day 2	Day 3	Day 4	Day 5
T. harzianum	33.33%	52.08%	60.27%	70.52%	74.50%
T. viride	15.78%	42.00%	55.84%	61.11%	68.78%

Table 4: Analysis of Variance in the growth inhibition of S. rolfsii by Trichoderma species

Trichoderma Species	Day 1	Day 2	Day 3	Day 4	Day 5
T. harzianum	33.33a	52.08a	60.27a	70.52a	74.50a
T. viride	15.78a	42.00a	55.84a	61.11a	68.75a
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**

Footnote: means tagged with the same letters in each column are not significantly different at P = 0.05
NS - No significance

3.4 Evaluation of the Antagonistic Potential of Trichoderma Isolates in Bioassay

The Trichoderma Isolates inhibited the growth of S.rolfsii on all cultivars based on the growth parameters evaluated such as numbers of leaves, number of branches and height. Analysis of Variance (ANOVA) revealed significant difference ($P < 0.05$) in the inhibition of S.rolfsii on all cultivars with respect to their controls as shown in Tables 5 and 6.

Table 5: Antagonistic Potential of T. harzianum on Sclerotium rolfsii in Bioassay

Variety	Number of leaves	Number of Branches	Height
Hoozua	12.00a	3.00a	7.00a
Control	2.00b	1.00b	1.00b
LSD (0.05)	**(8.88)**	**(1.87)**	**(3.89)**
Shase	15.00a	4.00a	8.00a
Control	2.00b	1.00b	1.00b
LSD (0.05)	**(12.49)**	**(2.38)**	**(4.65)**
UTC	16.00a	3.33a	7.00a
Control	2.00b	1.00b	1.00b
LSD (0.05)	**(13.24)**	**(2.23)**	**(4.32)**

Footnote: Means tagged with different alphabets within each variety are significant at P = 0.05

Table 6: Antagonistic Potential of T. viride on Sclerotium rolfsii in Bioassay

Variety	Number of leaves	Number of branches	Height
Hoozua	13.00a	3.00a	7.00a
Control	1.60b	1.00b	1.00b
LSD (0.05)	**(9.27)**	**(1.09)**	**(5.56)**

Shase	9.00a	3.00a	6.00a
Control	2.00b	1.00b	1.00b
LSD (0.05)	**(6.89)**	**(1.09)**	**(3.21)**
UTC	12.00a	3.00a	6.00a
Control	2.00b	1.00b	1.00b
LSD (0.05)	**(8.50)**	**(1.96)**	**(3.16)**

Footnote: Means tagged with different alphabets within each variety are significant at P = 0.05

IV. Discussion

In this study, a total of two Trichoderma species; Trichoderma harzianum and Trichoderma viride including Aspergillus niger and Alternaria species were isolated from the soil samples. Similar results were observed by [9] who reported that Trichoderma species are ubiquitous saprobes, common in soil and root ecosystems. Also [10] reported that Trichoderma species are easily isolated from soil, decaying wood and other organic material.

For the Isolation and Identification of S. rolfsii on PDA, the fungus grew very rapidly on PDA and the colony colour was white. The white mycelium formed many narrow mycelia strands in the aerial mycelium and they measured 4.2-8.4um in width. This mycelium showed characteristic clamp connection structure. The sclerotia were formed between 18-21days and were small and globoid. They were white at first but became dark brown after maturity and ranged from 1-3mm. This observation is similar to that of [11] who reported that growth of S.rolfsii on all organic-based and inorganic synthetic media is accompanied by forming spherical, white to brown coloured sclerotia measuring 0.3 to 3.0m in diameter. In similar studies carried out, [12] reported that the colony of S.rolfsii was white on PDA with the hyphae ranging from 4.5 – 9.0 um in diameter. He also reported that sclerotia were spherical, brown and ranging from 1-2mm in size. Also [13] and [14] reported characteristics of S.rolfsii that was almost with the Isolate in this study. The pattern of mycelia growth on medium, aerial mycelium and clamp connection structure are considered as the decisive characteristics of S. rolfsii [15].

In another set of experiments, Isolated Trichoderma Strains were simultaneously paired with S.rolfsii in a dual culture test for a total duration of five days to examine and compare the ability of Trichoderma species to compete with the test fungi for space and nutrients and to observe patterns of antagonism in dual culture. This is in agreement with [16] who stated that due to the variable antagonistic potential of individual isolates, the first screening is to select the most active antagonist against that particular pathogen before a species or particular isolate of Trichoderma can be considered as a biocontrol agent. From the results, T. harzianum had the highest overall inhibition of S.rolfsii (74.50%) followed by T. viride (68.75%). The results revealed that Trichoderma Isolates T. harzianum and T. viride parasitized the hyphae of S. rolfsii. Also, the penetration and growth of the Trichoderma Isolates inside the hyphae of S.rolfsii was observed. This is similar to observations made by [17] who reported same for T. harzianum and Sclerotinia sclerotiorum interaction. Also according to [18], T. harzianum and T. viride are fast growing soil fungi that parasitize the mycelia of other fungi. [19] reported that the parasitic activity of T. viride is mediated by its excretion of a variety of enzymes including cellulases, chitinases and antibiotics such as gliovirin. In a study on the antagonism of Trichoderma harzianum on soil borne plant pathogenic fungi, [20] also reported that T. harzianum had considerable antagonistic effect on the mycelia growth of the pathogens S. rolfsii and R. solani. Also similar to the observation made in this study, a work carried out by [21] reported that T. viride was identified as a mycoparasite against S. rolfsii. When grown near the pathogen, T. viride was seen entwining around the pathogen mycelium and was stimulated to produce branches that grew directly on the pathogen mycelia. They concluded that the antagonism by T.viride was a multifaceted process that required the synergistic contribution of several mechanisms including entwining hyphae, spores attachment to its host, growing inside host conidia, and subsequently death of host conidia. The inhibition of S. rolfsii in the dual culture test as reported in the present study can be attributed to the faster growing ability of Trichoderma species and the secretion of toxic extra cellular compounds such as antibiotics and cell wall degrading enzymes such as B- 1,3-glucanases, chitinases and proteases [22]. During mycoparasitic activity, these enzymes Iyse pathogen hyphal cell walls [23] and [24].

In another set of experiments, it was observed that T.harzianum and T. viride application as conidial suspensions inhibited the growth of S. rolfsii on all cultivars in bioassay. There was significant difference ($P < 0.05$) in the inhibition of S.rolfsii on all cultivars with respect to their controls on the parameters evaluated such as number of leaves, branches and height. This observation is similar to that made by [25] who reported that Trichoderma species are well documented as effective biological control agents of plant disease caused by soil borne fungi. Also application of T. harzianum to pea seeds has been reported to reduce the incidence of pre-

emergence damping off caused by Pythium species [26]. Recently, [27] reported on the use of T. viride as seed treatment to control Pythium species, the causal agent of damping off of Chinese kale seedling. In a recent work also by [28], it was reported that isolates of T. harzianum gave higher reduction in occurrence of damping off disease of tomato induced by S. rolfsii than the conventional fungicide. Also, according to [29], after several years of screen testing a large number of potential biocontrol agents, a strain of T. viride was identified which destroyed S. cepivorum sclerotia and reduced Allium white rot disease. The ability of T. harzianum and T. viride to inhibit the growth of S. rolfsii disease as reported in this study can be attributed to the antagonistic properties of Trichoderma, which involves parasitism and/or competition for limiting factors in the rhizosphere manly iron and carbon [30]. Another mechanism has been suggested by [31] and is related to Trichoderma induced resistance in host plants to fungal attack.

V. Conclusion

Results obtained from this study show that the genus Trichoderma comprises antagonistic species that are able to inhibit the growth of S. rolfsii. The ability of T. harzianum and T. viride to inhibit the growth of S. rolfsii can be explained in the light of their ability to compete and exhibit mycoparasitism. These observations agree with the reports of [22]; [32]. T. harzianum and T. viride share great success in several parameters evaluated with respect to biological control and can be considered for field applications in the biocontrol of soil borne pathogenic fungi.

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