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THE GROWTH AND SPREAD OF *TRICHODERMA HARZIANUM* ON SOME DOMESTIC FOOD WASTES

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ABSTRACT

The growth and spread of *Trichoderma harzianum* on some foodwastes were investigated as a prelude to considering the use of these wastes for *Trichoderma harzianum* growth, formulation and delivery base for biocontrol purposes. The wastes, ripe and unripe plantain, cassava, yam, sweet potato, orange and cocoyam peels were oven dried and powdered. The powder was used for culture media preparations in plates and as soil amendment in pots. Radial growth and colony counts on recovery from the pots were used to determine the extent and rate of growth of the fungus. The result showed that growth occurred, with varying rates, on all the seven foodwastes. Optimum growth and spread were observed on orange peel (reaching 4.5 cm radius in the plate and 9.70×10^6 colony forming units per gramme of soil in the pot on the 6th day of inoculation) and least growth and spread were observed on sweet potato (reaching 1.2 cm radius on the 6th day in the plate) and on unripe plantain peel (reaching 5.0×10^5 colony forming units per gramme of soil on the 15th day in the pot). Orange peel is the preferred waste for consideration for growth, formulation and delivery base of *T. harzianum*.

KEY WORDS: ***Trichoderma harzianum*, food wastes, growth.**

INTRODUCTION

Merriman and Russell (1990) had observed that current interests are high on the use of *Trichoderma spp* for control of plant diseases as indicated by a computer search showing more than 30 papers being annually published over the past half decade. The bane of this current interest is the gap that exists, as observed by Chet (1987), in the further development of the antagonism of *Trichoderma*, its application in biocontrol systems etc., because of the lack of efficient wide-spectrum isolates, the know-how to scale up fungal growth and appropriate and economic techniques for formulation and delivery.

Different workers have used different materials and methods for the growth and formulation of *Trichoderma harzianum* (TH) for application as a biocontrol agent (Chet *et al.*, 1979; Dunn *et al.*, 1983), but consistency in efficacy has failed to be established. The food wastes employed in this study have not been tried anywhere for attempted *Trichoderma* growth medium or formulation and delivery base.

Trichoderma harzianum is known to have very high competitive saprophytic ability (CSA). The height of the CSA of any organism is not only determined by how fast or well an organism utilizes a given carbon source, but also by how many different carbon sources it is able to degrade or utilize. TH is also known to utilize diverse plant materials for saprophytic activities (Baker and Cook, 1982). No evidence was found in the literature, of the experimental evaluation of these wastes for enhancement of the growth of TH or any other organism; neither was it found that they were used for soil amendments nor for preparing culture media for micro-organisms, except the latter (Godwin-Egein, 1991). This investigation aimed to use these food wastes for the preparation of culture media and as soil amendments for the study of the growth and spread of TH in the plate and pot, as a prelude to considering the wastes for *Trichoderma harzianum* growth, formulation and delivery base for biocontrol purposes.

MATERIALS AND METHODS

SOURCE OF THE ORGANISM AND ISOLATION: The organism used for the investigation was *Trichoderma harzianum* Rifai (TH) which was isolated from the soil of the Botanical Garden of the University of Port Harcourt. The soil was loamy, dark brown with high organic matter. Soil samples were taken from the first 15 cm depth of the soil. *Trichoderma* Selective Medium (TSM) developed by Papavizas and Lumsden (1982) was used for the isolation. It was isolated using the standard dilution plate method (Harley and Prescott, 1993); subcultures were made until pure axenic cultures were raised and maintained on potato dextrose agar (PDA) medium.

FOOD WASTES: Seven food waste types were used, viz: unripe and ripe plantain (*Musa paradisiaca* L.) peels; cassava (*Manihot esculentus* Crantz) peels; yam (*Dioscorea rotundata* Poir) peels; orange (*Citrus sinensis* (L.) Osbeck) peels; sweet potato (*Ipomea batatas* (L.) Lam.) peels; and cocoyam (*Xanthosoma saggittifolium*) peels. The peels were used as (1) the carbon source in growth media and (2) soil amendments.

MEDIA PREPARATION: Yam tuber, cocoyam cormel, sweet potato tuber, cassava tuber, orange hesperidium and plantain finger were first of all surface sterilized by washing in 2% sodium hypochlorite solution before peeling. The peels were chipped and soaked in a fresh solution of sodium hypochlorite for 4 hr. The chips were then dried in a drying

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oven at 60°C for 24 hr. The dry crispy food waste were comminuted in a Moulinex grinder-mill (France) to powder form. The powder was sifted to remove coarse particles before use. Seven different media were prepared based on the 7 food wastes - ripe plantain peel dextrose agar (RPPDA); unripe plantain peel dextrose agar (UPPDA); cassava peel dextrose agar (CPDA); yam peel dextrose agar (YPDA); sweet potato peel dextrose agar (SPPDA); orange peel dextrose agar (OPDA); and cocoyam peel dextrose agar (CoPDA). They were composed as follows:- 200 g peel powder; 20 g dextrose; 20 g agar powder and 1000 ml distilled water.

GROWTH ON THE MEDIA BASED ON THE FOOD WASTES: Three millimetre core of the organism on PDA was plated on each of the media based on the food wastes, in 9 cm Petri dishes. The cores were obtained with 3 mm diameter sterile cork borer. The plates had 4 replicates and incubated at 25°C. Daily measurement of the radial growth of the organism was taken for 15 days. Control plates had only agar discs.

GROWTH AND SPREAD OF THE ORGANISM IN STERILE SOIL AMENDED WITH THE FOOD WASTES: Food wastes for soil amendment were also prepared as above and amendment was at the rate of 20% weight by weight. Soil (which was heat sterilized in the oven at 110°C for 24 hr) and waste powder were weighed (0.5 kg and 100 g respectively) into sterile transparent polythene bags (obtained from General Plastics (Nig.) Ltd., No 7 Kaduna Street, D/Line, Port Harcourt), and thoroughly mixed before placing in the pots. The use of the transparent polythene bags gave 3 advantages:- (a) made for easy manipulation of the soil and amendemnt when mixing, without risk of contamination (b) protected the amended soil from the walls of the pot and (c) provided mulch. Amended soil was then watered with sterile distilled water. The soil was generously watered for best results for *Trichoderma harzianum* growth as reported by Liu and Baker (1980). Holes were made through the polythene bags corresponding to the holes already in the bottom of the pots, to allow easy drainage of excess water in the soil. Inoculation of the organism into the soil and recovery procedures were as follows:- One centimetre cubes were consructed with aluminium foil. The cubes were filled with the organism on PDA. Central holes (one each) were made on the 6 faces of the cube. A cube each was then buried, after surface sterilization with 70% ethyl alcohol, 4 cm deep in the pots. A semi-circular plastic disc was constructed, such that the disc had the same diameter as the pots. Five concentric semi-circles were then marked on the disc at regular intervals (see Fig. 1).

Soil samples were then taken at 3 days interval for 15 days using a 4 mm diameter cork borer as a "micro-auger". Soil samples of the 3rd day were taken at the innermost semi-circle on the plastic disc placed on the soil in the pot; that of the 6th day was taken from the 2nd semi-circle; that of the 9th dayu from the 3rd; that of the 12th day at the 4th; and that of the 15th day at the 5th semi-circle. Soil samples were taken through a 5 cm-depth and randomly round the circles at 4 points. The 4 samples from each collection were

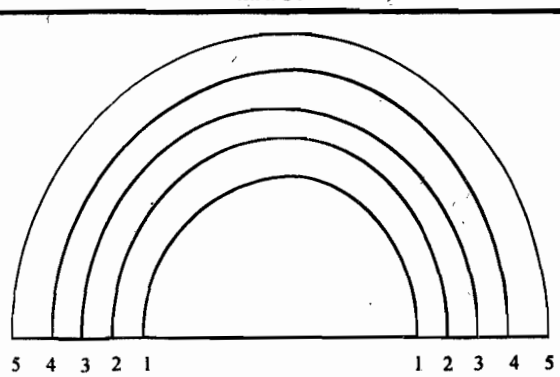

Fig. 1 Plastic semi-circular disc with 5 concentric semi-circles.

thoroughly mixed together to form a composite sample. From the composite sample, 1 g of soil was taken for a dilution series. Dilutions at the 5th degree (10^{-5}) were plated on TSM. On the 2nd day of plating, the plates were observed and colony forming units (CFU) counted. Each treatment was replicated 4 times. Control pots had PDA cubes without the organism.

INOCULATION WITH SPORE SUSPENSION: Spore suspension of the organism was obtained by flooding 14 day old cultures on PDA, with sterile distilled water and the spores dislodged with inoculation needle. The spores were washed three times by centrifugation at 1500 g for 1 min with changes of sterile distilled water and the concentration was adjusted to 1×10^9 conidia with a haemocytometer. The soil was then inoculated at the rate of 1×10^9 CFU/g soil (Chet and Baker, 1980). The organism was reisolated using TSM on the 3rd, 6th, 9th, 12th and 15th days. Data on the recovery rate of the organism was collected. Control Pots were inoculated with distilled water.

RESULTS

GROWTH ON THE MEDIA BASED ON THE FOOD WASTES: Results are as shown on Table 1.

Table 1. Growth of *Trichoderma harzianum* on the various food wastes based media Incubated at 25°C for 15 days in Petri dishes.

FOOD	GROWTH (CM) DAY				
WASTE	3RD	6TH	9TH	12TH	15TH
RPPDA	1.7	5.2	8.1	8.1	8.1
UPPDA	5.2	8.4	8.4	9.0	9.0
CPDA	4.4	9.0	9.0	9.0	9.0
SPPDA	1.7	2.4	2.4	2.4	2.4
YPDA	7.2	9.0	9.0	9.0	9.0
OPDA	5.2	9.0	9.0	9.0	9.0
CoPDA	5.4	8.4	8.4	8.4	8.4
ConDA	-	-	-	-	-

Growth was observed on all media and was at various rates. Growth was fastest on YPDA, reaching a diameter of 7.2 cm on the 3rd day, on which day UPPDA, CPDA, OPDA and CoPDA showed 5.2, 4.4, 5.2 and 5.4 cm diameter respectively. On CPDA and YPDA, TH grew and covered

the plates by the 6th day, while on CoPDA, it was on the 9th day and on the 12th day on UPPDA.

Trichoderma harzianum stagnated as from the 6th day at 2.4 cm on SPPDA and as from the 9th day at 8.1 cm on RPPDA. So growth and spread was slowest on SPPDA.

GROWTH AND SPREAD OF THE ORGANISM IN STERILE SOIL AMENDED WITH THE FOOD WASTES: Figure 2 shows the result of the growth and spread of TH. Growth was observed in all amended soil. The growth and spread of TH was measured by the colony counts on recovery. On the 3rd day recovery was from the 1st (Fig. 2) and 2nd (Fig. 2) rings. UPP (Fig. 2) showed no recovery in the first ring and it was only YP and OP (Fig. 2) that showed recovery. On the 6th day recovery was made from all five rings (Fig. 2): in the 1st ring (Fig. 2) no colony counts in UPP; in the 2nd ring no colony counts in UPP and SPP (Fig. 2); in the 3rd ring (Fig. 2) no colony counts in UPP, SPP and CoP; in the 4th ring (Fig. 2) colony counts were only in YP and OP; and in the 5th ring (Fig. 2) recovery was from only OP.

On the 9th day no recovery was made from UPP in all rings (Fig. 2); no recovery was made from SPP in the 2nd 5th rings (Fig. 2); no recovery was made from CP in the ring (Fig. 2). On the 12th day, no recovery was made fr UPP in the 2nd-5th rings (Fig. 2); and no recovery from S in the 3rd to 5th rings. On the 15th day recovery from U was made only in the 1st and 2nd rings; and from SPP, recovery was made only in the 1st ring. No recovery v made in the control pots. The highest growth was obser in OP and the least was observed in UPP as shown on Ta 2.

Colony counts increased from the 3rd to the 15th day in the amended soils except in OP amended soil, where decline was observed on the 15th day (Table 2). Tl phenomenon was observed in all the rings except in the ring (Fig. 2). The decline was observed as from the 12th d in the 1st and 2nd rings and on the 15th day in the 3rd a 4th rings. In YP amended soil the colony counts stagnat as from the 9th day. Fastest spread was observed in C

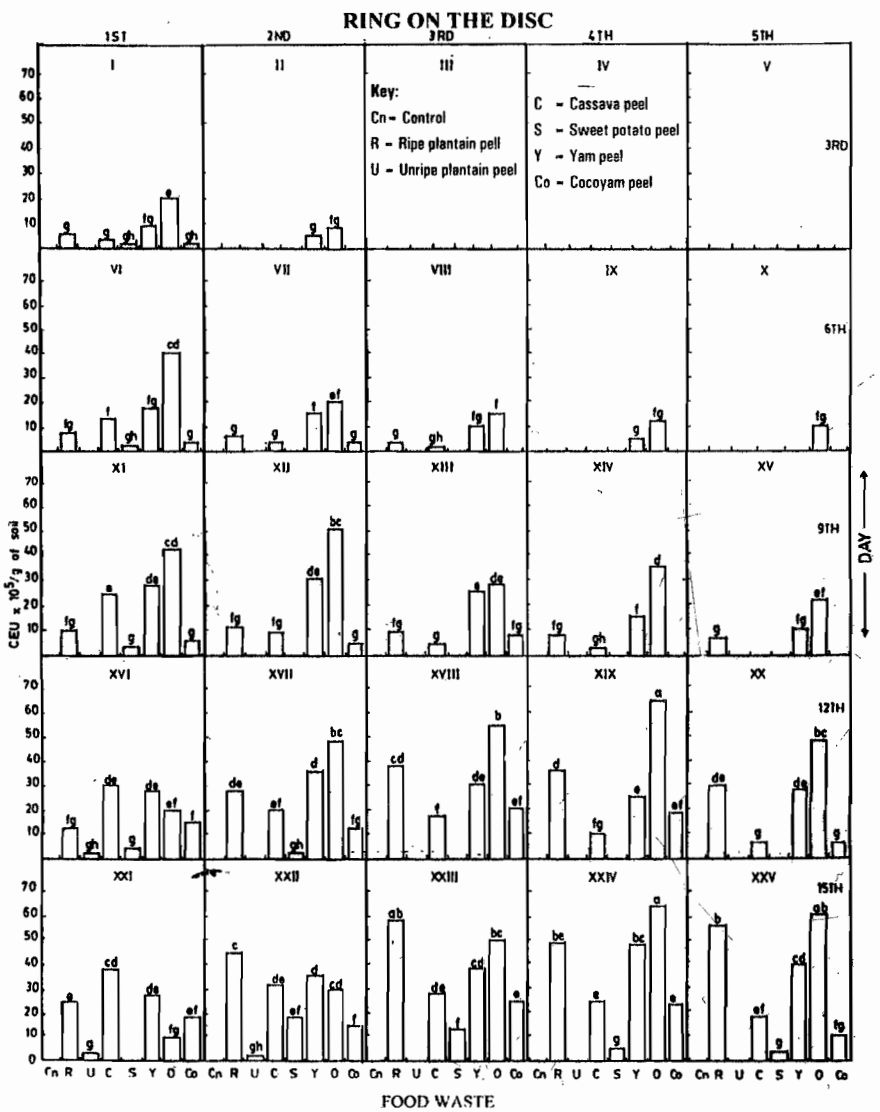

Fig 2. Growth and spread of *Trichoderma harzianum* in sterile soil amended with the food wastes.
Figures show colony counts on the dilution plates from soil samples collected from the experimental pots. Bars with the same letter(s) are not significantly different at P = 0.05

Table 2. **Growth of *Trichoderma harzianum* in sterile soil amended with the food wastes. Figures show total colony counts (1x 10^5) through the 5 rings on the various days when recovery was made.**

FOOD WASTE	DAY					CUM
	3RD	6TH	9TH	12TH	15TH	
RPP	6	17	45	143	233	444
UPP	-	-	-	2	5	7
CP	3	17	39	83	140	282
SPP	1	2	3	6	38	50
YP	13	47	108	147	190	505
OP	28	97	175	242	213	755
CoP	1	6	18	71	89	185
Con	-	-	-	-	-	-

amended soil and slowest spread was observed in UPP amended soil as shown in Table 3;

Table 3. **Spread of *Trichoderma harzianum* in sterile soil amended with the food wastes through the rings on the plastic semi-circular disc.**

FOOD WASTE	SPREAD/RING DAY				
	3RD	6TH	9TH	12TH	15TH
RPP	1st	3rd	4th	5th	5th
UPP	-	-	-	1st	2nd
CP	1st	3rd	4th	4th	5th
SPP	1st	1st	1st	2nd	5th
YP	2nd	4th	5th	5th	5th
OP	2nd	5th	5th	5th	5th
CoP	1st	2nd	3rd	4th	5th

the spread did not go beyond the 2nd ring.

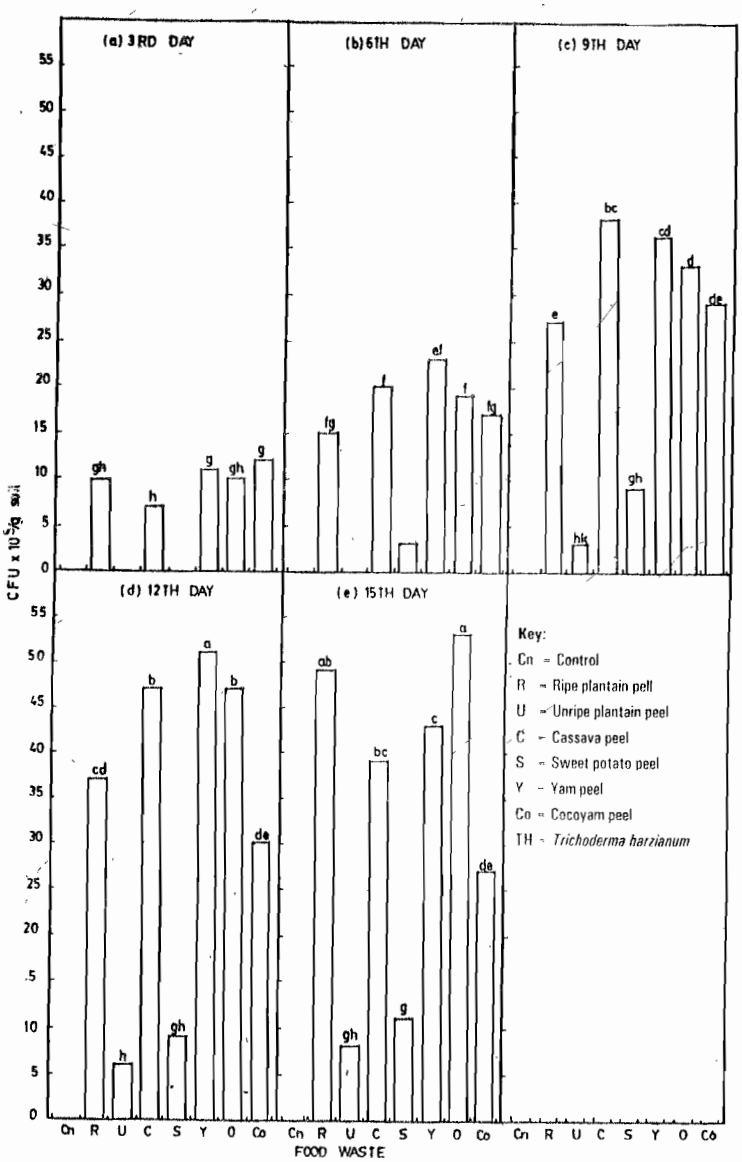

Fig. 3 Recovery of TH from Soil amended with the food wastes and TH. The bars represent colony counts. Bars with the same letter(s) show no significant difference at P = 0.05.

INOCULATION WITH SPORE SUSPENSION: Fig. 3 shows the results of the recovery of TH from the pots. No recovery was made from the control pots. The general recovery pattern was the same as observed above. Colony counts increased with time of incubation. Least recovery was made from UPP and this was as from the 6th day (Fig. 3b-e). Highest recovery was made from OP, but the decline phenomenon was not observed (Fig. 3c-e). The decline phenomenon was instead observed in YP (Fig. 3d and e).

DISCUSSION

Trichoderma harzianum is known to have very high CSA and utilizes diverse plant materials for saprophytic activities as reported by Baker and Cook (1982). This statement has been corroborated by the findings of this investigation as *T. harzianum* grew on all the seven food wastes, both in the Petri plates and in the soil. The difference in rate of utilization of and hence growth on the various food wastes may be as a result of what Weinhold and Bowmann (1968) and Godwin-Egein (1991) had observed - that growth of organisms is dependent on the availability of usable nutrients in the media. It can be speculated that the apparent difference (in growth performance of *T. harzianum)* between ripe plantain and unripe plantain peel may be as a result of the presence or absence of some protein and/or mineral ions in one or the other waste because it is known that the difference between them is the presence or absence of these proteins and/or mineral ions (Oyenuga, 1978). The exceptional growth performance *T. harzianum on ripe plantain,* yam and orange peels (Table 2), particularly in the soil, presents them as likely candidates for their consideration as growth, formulation and delivery vehicles for biocontrol and/or soil amendements for the enhancement of TH growth. Conversely sweet potato and unripe plantain peels can be considered as soil amendments for the suppression of *T. harzianum* as they supported least growth and spread of the fungus. Since orange peel supported optimum growth and spread of them all, it is the preferred waste for consideration

for growth, formulation and delivery base of *T. harzianum* for biocontrol purposes.

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