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EFFECTS OF SOLUBILISED ORANGE PEELS ON THE OCCURRENCE OF SOME FUNGAL SOIL-BORNE PATHOGENS OF *ZEA MAYS* (L.)

***C. W. Worlu, A. B. Nwauzoma, E. C. Chuku and M. G. Ajuru**

Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University, Port Harcourt, Rivers State, Nigeria.

Corresponding author: *C. W. Worlu
Email : cworlu1@gmail.com

ABSTRACT

This work examined the use of *Trichoderma* solubilised orange peel to inhibit the occurrence and growth of some fungal pathogens associated with soil-borne diseases of maize (*Zea mays*). The study was conducted in the Department of Plant Science and Biotechnology demonstration plot, Rivers State University, Port Harcourt, Nigeria. *Trichoderma* species were isolated from contaminated mushroom substrates at Dilomat Farms and Services Limited, Rivers State University. Sweet orange (*Citrus sinensis*) peels were collected from a local market at the Port Harcourt Metropolis, air-dried for seven days and ground to powder. The treatments were *Trichoderma harzianum+orange peel, T. koningii+orange peel, orange peels only, T. harzianum only, T. koningii only, T. harzianum+T. koningii* and Soil only. The layout was a completely randomized design with three replications and concentrations at 5g/15ml, 10g/15ml and 15g/15ml which were applied to the soil at the interval of two weeks for 10 weeks. *Pythium spp, Fusarium spp, Rhizoctona spp* and *Phytopthora spp* were isolated and identified at three weeks and ten weeks of treatments. Average number of individual colonies of soil mycoflora in the various treatments at the three concentration levels varied relatively and the total number and frequency of occurrence decreased as the number of weeks of treatments increased. There was relatively high occurrence of soil-borne pathogens of maize at 5g/15ml concentration, but 10g/15ml concentration recorded less whereas 15g/15ml had the least occurrence. However, the frequency of occurrence was very high on the control experiment. Frequency of soil-borne fungal pathogens of maize decreased with increase in concentration as well as *Trichoderma spp* occurrence in the soil across the treatments when compared to their controls. The treatments had higher inhibitory effects on all the soil-borne pathogens of maize at 15g/15ml at 10 WAP.

KEYWORDS

Trichoderma harzianum, Trichoderma koningii, Solubilised orange peels, Zea mays, Soil-borne fungal pathogens, Soil mycoflora.

INTRODUCTION

The natural soil is the home to innumerable communities of microbial flora that interact with plants, on constant bases resulting to harmful or useful effects (Mukerji *et al*., 2006). The soil soil microflora do not only play important role in decomposition of organic matter, but also are responsible for the prevalence of diseases in the crop fields. Research on fungal diversity and percentile contribution and their periodic occurrence are useful to farmers, agronomists, researchers and microbiologists for future activities in view of role in the conservation of soil ecosystem and microbial diversity and sustainable agriculture (Gaddeyya *et al.*, 2012). However, the imbalance in the soil microbial community structure is the main cause of soil-borne diseases and its diversity is an important indicator to measure soil properties…. Essentially, the way nutrient-pathogen interact and eventual occurrence of diseases are not fully understood due to their intricate nature, which depends on a number of environmental factors (Brown *et al*., 2002; Bondada and Syvertsen, 2005; Abiodun *et al.*, 2017).

Reports have shown that a more favourable environment for disease development may be created due to plant nutrient deficiency or toxicity and this disease susceptibility would be influenced and enhanced due to change in the plant metabolic activities (Strange and Scott, 2005; Huber *et al.*, 2005). However, when a plant is infected by a particular pathogen(s), it alters the plant's physiology directly or indirectly, particularly with regards to mineral nutrient uptake, assimilation, translocation, and utilization (Spann and Schumann. 2009; Shuping and Eloff, 2017). Although fungal pathogens may immobilize mineral nutrients in the soil and in infected plant tissues they may also truncate translocation or utilization of nutrients thereby resulting in nutrient deficiencies or toxicities to plant (Stangoulis and Graham. 2007; Abiodun *et al.*, 2017)

Plant roots are commonly prone to soil borne pathogens; this however brings about damaging effect on the roots, thereby reducing the plant's ability to absorb and take up water and nutrients from the soil (Bové, 2006; Kavitha and Satish, 2011) and this might lead to secondary infections which may reflect on the foliar parts of the plant due to nutrients deficiencies around the rhizosphere and rhizoplane (Dordas, 2008; Duru and Onyedineke, 2010). It is a known fact that plant pathogens can infect vascular system of plant and impair uptake of nutrients and water, assimilation, translocation and utilization of some photosynthetic products (Marschner, 1995; Abiodun *et al.*, 2017) resulting in root starvation, wilting, and plant death, even when the fungi are not toxic to the plant ((Huber and Graham, 1999; Shuping and Eloff, 2017)

However, *Trichoderma* inoculation into the soil has been reported to increase the effective nutrient content and the soil enzyme activity to repair soil and promote plant growth. The Trichoderma genus also has the feature of hyperparasitism; it secretes cell wall-degrading enzymes such as chitinases, cellulases, xylanases, glucanases, and proteinases, *Trichoderma spp.* absorb nutrients through degrading soil microbial cells, leading to the change of the soil microbial community structure, The introduction of Trichoderma into the soil increases nutrient content and microbial biomass, in addition to improving the soil microbial community structure (Hi *et al*., 2016).

Fusarium, *Pythium*, *Sclerotium, Phytopthora, Verticillium*, *Rhizoctonia Spp* and *Diplodia* are the common fungal pathogens that are associated with soil borne diseases of corn seed and result in seedling diseases. Literatures have shown that *Pythium* and *Rhizoctonia* do not have potential to solubilize mineral nutrients but can be suppressed easily by the high competitive activity of *T. harzianum* through the uptake of phosphorus (Altomare *et al.*, 1999).

Furthermore, long-term residual effects of organic manure coupled with slow release pattern of its nutrient also contributed to its ability to supply nutrient in *Talinum trangulare* during the growth period (Isitekhale *et al*., 2014). Also, Reyes *et al*., (2002) reported that phosphate solubilizing *Penicillium* was able to stimulate the growth of maize plants as indicated by 3.6 to 28.6% increase in dry matter yields under greenhouse trials, *Trichoderma* sp., which have often been reported as a well-known biological control agent against several phytopathogens was also tested for its mineral nutrients and especially phosphate (P) solubilizing potential (Utkhede *et al.* 1996; Anil and Lakshmi, 2010). However, increase in growth and yield parameters of chickpea

grown in P-deficient soil amended with insoluble rock phosphate due to *Trichoderma* inoculation, as compared to uninoculated controls under both glass house and field conditions has been reported by (Rudresh *et al*., 2005).

Apart from P solubilization, simultaneous synthesis and release of pathogen suppressing metabolites like siderophores, phytohormones and lytic enzymes, by Phosphate Solubilisng Microorganisms have been proven useful in the provision of mineral nutrients to plants Reyes *et al*., (2002). Anil and Lakshmi, 2010 has also reported that the disappearance of Tricalcium Phosphate (TCP) within 72 h which indicated the high potential of *Trichoderma* for solubilization of inorganic bound phosphate (TCP). Several researches revealed that there was a gradual decrease in pH values up to four days followed by a gradual rise during P-solubilization by *Penicillium* and *Pseudomonas* in liquid cultures which also agrees with the findings of (Illmer and Schinner 1992). It has been said that microorganisms which tend to decrease the pH of the medium during growth are efficient P-solubilizers (Kpomblekou and Tabatabai, 1994).

As observed by Anil and Lakshmi (2010) that decrease in pH for individual cultures up to 48 h and then acquiring constancy makes the soluble phosphate concentrations continue to increase after 48 h. This clearly suggests that pH drop is not the sole factor for P-solubilization. An initial increase in phosphate concentration followed by a gradual decrease in culture filtrate as observed has also been well documented by other workers (Nautiyal, 1999). Decrease in phosphate concentration at 120 h might be correlated with its sequestration in *Trichoderma* mycelium, to be released in a readily available form, in close proximity to the roots after lysis of mycelium with age. The aim of this work is to test the efficiency of *Trichoderma* solubilised orange peel to inhibit the occurrence and growth some soil-fungal pathogens responsible for soil-borne diseases of maize.

Materials and Methods

Study Area and Sample Collection

This work was carried out in the Department of Plant Science and Biotechnology demonstration plot, at the Faculty of Science, Rivers State University, Port Harcourt, Nigeria, which lies within latitudes 4^0 43'0743'07'' and 4^0 54'3254'32'' N and longitudes 6^0 56'0456'04 and 7^0 03'2003'20''E. The mean annual rainfall of the area is 2000mm and mean temperature of 29^0 C (Tubonimi and Udonna 2015).

Collection of Orange Peels

Sweet orange fruits were purchased from a local market at Rumuekini in Obio-Akpo Local Government Area, Port Harcourt, Nigeria in January 2020 and were identified at the department of Plant Science and Biotechnology, Rivers State University. The fresh oranges were washed with running water and thereafter, the peels were separated from the entire fruit and cut into smaller pieces followed by shade drying for 7 days. Dried peels were ground to coarse powder with the aid of pestle and mortar and stored in a plastic container for further use.

Isolation of Fungal Pathogens from Soil Sample

The isolation of fungal pathogens from the soil sample was done following the methods of Dong *et al.*, (2004) and Aina *et al.*, (2011). Hence, 1g of soil from each treatment (*T. harzianum+orange peel*, *T. koningii+orange peel*, *orange peels only*, *T. harzianum only*, *T. koningii only*, *T. harzianum+T. koningii* and *Soil only*) was suspended in 10ml of sterile distilled water. Then serial dilutions of 10^{-3}, 10^{-4} and 10^{-5} were made and used to isolate fungi so as to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Petri dishes containing (PDA) medium in triplicates of each dilution. The dilution was supplemented with 1% streptomycin solution to prevent bacterial growth. The plates were then incubated at 28±2ºC for 5-7 days. Organisms were isolated from the surface colonies formed and sub-cultured to obtain pure cultures (Ratna *et al.*, 2015). These isolations were done at 3 WAP and 10 WAP to determine the causal agent of soil-borne diseases of maize and the consistency of occurrence in the soil.

Isolation and Identification of Soil Fungal Pathogens of Maize

Inoculating needle was sterilized over the burning Bunsen burner. A small portion of the mycelium growth on the culture plate was transferred onto the slide using needle, and a drop of lacto phenol cotton blue was also

added on the slide for visualization of fungal structures (Nagamani *et al.*, 2006). The specimen was teased carefully using inoculating wire loops to avoid squashing and over-crowding of the mycelium (Aina *et al.*, 2011). The specimen was observed under the microscope and identified based on morphological characteristics of the colony with the aid of microscopic examinations (Gilman, 2001; Nagamani *et al.*, 2006; Diba *et al.*, 2007; Ratna *et al.*, 2015). The fungi were compared and identified with the help of standard procedure and relevant literatures (Gilman, 2001; Nagamani *et al.*, 2006; Ratna *et al.*, 2015).

Statistical Analysis

Population density was expressed in terms of Colony Forming Unit (CFU) per gram of soil with dilution factors while the percent contribution of each isolate was calculated thus:

$$\% \text{ Contribution} = \frac{\text{Total No. of CFU of an individual Sps}}{\text{Total No. of CFU of all sps}} \times 100$$

Results

Frequency and Percentage Occurrence of Mycoflora in various Bio-inoculants in PDA (3 WAP)

The frequency of mycoflora at three weeks after planting, that is, the total number of colonies and average number of individual colonies (*Pythium spp, Fusarium spp, Rhizoctonia spp, Phytopthora spp and Trichoderma spp*). is shown in Tables 1, 2 and 3. There was relatively high occurrence of soil-borne pathogens of maize (Table 1), but Table 2 recorded few organisms whereas Table 3 had the least occurrence. However, a critical study of the tables revealed that there was a varying occurrence in the average number of individual colonies amongst the treatments at different concentrations when compared to the control. Here, the inhibition which reflected on the degree and extent of average number of individual colonies occurrence was treatment and concentration dependent.

Table 1. Frequency of Mycoflora in the various bio-inoculants at 5g/15ml concentration on PDA medium (3 WAP)

Treatment	Total no. of colonies	Average number of individual colonies						
		Pythium spp	*Fusarium spp*	*Rhizoctonia spp*	*Phytophtora spp*	*T. harzianum*	*T. koningii*	Unknown
T. harzianum+Orange peel	27	4	4	3	3	4	2	7
T. koningii+Orange peel	24	3	2	2	4	3	3	5
Orange peel	30	4	4	4	5	2	1	10
T. koningii+T. harzianum	24	2	3	2	2	5	7	3
T. harzianum	18	3	2	1	1	6	2	2
T. koningii	17	3	2	2	1	2	5	3
Soil only	33	6	8	4	5	1	1	8
Total	173	25	25	18	16	23	21	38
% Contribution		15	15	13	9	13	12	21

Table 2. Frequency of Mycoflora in the Various Bio-inoculants at 10g/15ml concentration on PDA medium (3 WAP)

Treatment	Total No. of colonies	Average number of individual colonies						
		Pythium spp	*Fusarium spp*	*Rhizoctonia spp*	*Phytophtora spp*	*T. harzianum*	*T. koningii*	Unknown
T. harzianum+Orange peel	22	3	4	2	2	5	1	4
T. koningii+Orange peel	24	2	3	3	1	2	6	3
Orange peel	22	3	3	4	4	1	1	6
T. koningii+T. harzianum	22	2	3	2	2	4	3	3
T. harzianum	19	2	2	3	2	5	2	3
T. koningii	17	2	2	2	3	2	4	2
Soil	33	6	8	4	5	1	1	8
Total	159	20	25	20	19	20	18	29
% Contribution		13	16	13	12	13	11	18

Table 3. Frequency of Mycoflora in the various Bio-inoculants at 15g/15ml concentration on PDA medium (3 WAP)

Treatment.	Total No. of colonies	Average number of individual colonies						
		Pythium spp	*Fusarium spp*	*Rhizoctonia spp*	*Phytophtora spp*	*T. harzianum*	*T. koningii*	*Unknown*
T. harzianum+Orange peel	20	3	2	2	1	7	2	3
T. koningii+Orange peel	19	2	3	2	2	2	6	2
Orange peel	23	4	2	3	3	3	2	6
T. koningii+T. harzianum	30	4	7	3	2	5	4	5
T. harzianum	16	2	2	1	2	4	2	3
T. koningii	13	1	2	1	1	2	4	2
Soil only	33	6	8	4	5	1	1	8
Total	154	22	26	16	16	24	21	29
% Contribution		14	17	10	10	16	14	13

Frequency and Percentage Occurrence of Mycoflora in various Bio-inoculants in PDA (10 WAP)

The frequency of mycoflora in maize cultivated soil at 10 WAP is presented in Tables 4, 5 and 6. All the treatments had varying occurrences of soil-borne fungi (*Pythium spp, Fusarium spp, Rhizoctonia spp, Phytophtora spp*) of maize at the various concentrations which were lower compared to their controls.

Interestingly, the test fungi (*Trichoderma spp*) increased arbitrarily amongst the treatments though higher than the control. This means that *Trichoderma spp* had a suppressive effect on the soil-borne fungi of maize. However, the inhibitory impacts of the treatment at 10 WAP were better than the earlier weeks (3 WAP). This might be as a result of the contact period between the treatments and soil-borne fungal pathogens of maize in the soil. That is, the longer the contact period, the more inhibitory effect exhibited by the treatments against the pathogens.

Table 4. Frequency of Mycoflora in the various Bio-inoculants at 5g/15ml concentration on PDA medium (10WAP)

Treatment	Total No. of colonies	Average number of individual colonies						
		Pythium spp	*Fusarium sp spp*	*Rhizoctonia spp*	*Phytophtora spp*	*T. harzianum*	*T. koningii*	Unknown
T. harzianum+Orange peel	13	1	0	1	1	6	3	3
T. koningii+Orange peel	14	1	1	0	0	2	8	2
Orange peel	11	1	1	2	1	1	1	4
T. koningii+T. harzianum	15	0	0	1	0	7	6	2
T. harzianum	14	0	0	0	1	8	2	2
T. koningii	11	0	0	0	0	3	7	1
Soil	43	8	8	6	7	1	1	12
Total	121	11	10	10	10	28	28	26
% Contribution		9	8	8	8	23	23	21

Table 5. Frequency of Mycoflora in the various Bio-inoculants at 10g/15ml concentration on PDA medium (10 WAP)

Treatment	Total No. of colonies	Average number of individual colonies						
		Pythium spp	*Fusarium pp*	*Rhizoctonia spp*	*Phytophtora sp*	*T. harzianum*	*T. koningii*	Unknown
T. harzianum+Orange peel	14	1	0	1	1	1	1	2
T. koningii+Orange peel	10	1	1	1	0	2	3	2
Orange peel	8	0	0	1	1	1	1	4

T. koningii+T. harzianum	12	0	0	1	0	4	4	3
T. harzianum	13	1	0	0	0	7	2	2
T. koningii	11	1	0	1	0	2	5	2
Soil	43	8	8	6	7	1	1	12
Total	111	12	9	11	9	18	16	27
% Contribution		11	8	10	8	16	14	24

Table 6. Frequency of Mycoflora in the various Bio-inoculants at 15g/15ml concentration as on PDA medium (10WAP)

Treatment	Total No. of colonies	Average number of individual colonies						
		Pythium spp	*Fusarium spp*	*Rhizoctonia spp*	*Phytophtora spp*	*T. harzianum*	*T. koningiii*	Unknown
T. harzianum+Orange peel	12	1	0	1	0	6	2	2
T. koningii+Orange peel	14	1	0	0	1	3	8	2
Orange peel	11	2	0	1	1	2	1	4
T. koningii+T. harzianum	18	0	5	1	1	4	3	4
T. harzianum	10	0	0	1	1	5	1	2
T. koningii	7	0	0	0	0	1	3	3
Soil	43	6	8	6	7	1	1	12
Total	115	10	13	10	11	22	18	27
% Contribution		9	11	9	10	19	16	24

Discussion

Soil fungal pathogens act as important factors in determining the incidence, severity and spread of plant diseases. They also influence plant growth and productivity. Soil fungal pathogens associated with soil-borne diseases of maize were observed, isolated and identified. *Pythium spp, Fusarium spp, Rhizoctona spp* and *Phytopthora spp* were isolated and identified as soil-borne fungal pathogens responsible for soil-borne diseases of maize. This is in accordance with several literatures that *Fusarium, Pythium, Sclerotium, Phytopthora, Verticillium, Rhizoctonia Spp* and *Diplodia* are the common fungal pathogens that are associated with soil borne diseases of corn seed and result in seedling diseases.

The average number of individual colonies of soil fungi in the various treatments at the three concentration levels varied relatively and the total number and frequency of occurrence decreased as the number of weeks of treatments increased. This might be as a result of long-term residual effects *Trichoderma* solubilised orange peel on the soil where the treatments gradually released that eventually exhibited suppressive effects on soil-borne fungal pathogens of maize. The above observation was also made by Isitekhale *et al*., (2014) who reported that long-term residual effects of organic manure coupled with slow release pattern of its nutrient also contributed to its ability to supply nutrient to the waterleaf plant throughout the growth period. Altomare *et al.*, (1999) however submitted that the phytopathogenic fungi like *Pythium* and *Rhizoctonia* do not have potential to solubilize mineral nutrients but can be suppressed easily by the high competitive activity of *T. harzianum* through the uptake of Phosphorus.

There was relatively high occurrence of soil-borne pathogens of maize at 5g/15ml concentration, but 10g/15ml concentration recorded fewer colonies of fungi whereas 15g/15ml had the least occurrence however, the

frequency of occurrence was very high on the control experiment. This indicated that there was varying occurrence in the average number of individual colonies of soil-borne fungal pathogens of maize amongst the treatments at different concentration levels when compared to the control. The decrease in occurrence might be as a result of changes in the physicochemical properties of the soil due to *Trichoderma* solubilised orange peel. This agrees with the works of (Gaddeyya *et al.*, 2012) who stated that fungal population and diversity (distribution of mycoflora) is affected by environmental factors such as the soil pH, moisture, temperature, organic carbon and NKP.

Similarly, frequency of soil-borne fungal pathogens of maize decreased with increase in concentration as well as *Trichoderma spp* occurrence in the soil and solubilised mineral elements across the treatments when compared to their controls. This finding agrees with Utkhede *et al.* (1996) who reported that mineral element is one of the factors that influence and determine the incidence and severity of disease in a plant. This corroborates the reports of Also, Anil and Lakshmi, (2010) that phosphorus in the soil reduced the severity of take-all disease of barley caused by *Gaeumanomyces graminis* and potato scab caused by *Streptomyces scabies.*

Conclusion

The degree and extent of average number of individual colonies occurrence was found to be treatment and concentration dependent where *T. koningii* had the highest inhibitory impact on all the pathogens followed by *T. koningii+orange peel*, *T. harzianum+orange peel*, *orange peel* and *T. koningii+T. harzianum* when compared to the control that had no inhibitory effect on the pathogens. However, the treatments had higher inhibitory effects on all the soil-borne pathogens of maize at 15g/15ml at 10 WAP.

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