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Biological Control of *Ceratocystis paradoxa* Causing Black Seed Rot in Oil Palm Sprouted Seeds by *Trichoderma* species

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Abstract: *Trichoderma viride*, *T. polysporum, T. hamatum* and *T. aureoviride* were used as potential biological agents for seed treatments against *Ceratocystis paradoxa* causing black seed rot in oil palm sprouted seeds *in vitro*. *T. polysporum* significantly reduced the percentage infection to 13 and 10%, 14 days post inoculation. *T. viride* 18.3 and 11.7%, *T. hamatum* 22 and 15%, *T. aureoviride* 23.7 and 13%, benlate solution 21.7 and 18.3% and control 80 and 30% for wounded and unwounded seeds, respectively. *In vivo,* the emergence (survival) and the growth of oil palm seedlings from *Trichoderma* species treated oil palm sprouted seeds were significantly higher than the emergence and the growth observed from the control treatment. *C. paradoxa* infested seeds had 63 to 71% survival and average seedling heights of 18.4 to 25.1 cm. Seeds without pathogen had 100% survival with average seedling heights of 38.2 to 46.5 cm. The effects of the bioagents were similar or high than the one obtained from benlate solution.

Key words: *Trichoderma, Ceratocystis paradoxa*, seed rot, oil palm sprouted seed

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is said to be indigenous to West Africa (Zeven, 1965; Corner, 1966). The oil palm industry provides direct employment to about four million Nigerian people in about twenty oil palm growing states in Nigeria and indirectly to other numerous people involved in processing and marketing (Ahmed, 2001).

Despite the enormous potential of the oil palm, there is problem with soil borne fungus, *Ceratocystis paradoxa* causing black seed rot disease in oil palm sprouted seeds. The cause of dry basal rot in adult palm, conducted by Robertson (1962) was shown to be due to the Ascomycete, *C. paradoxa* of which the imperfect stage is known as *Thielaviopsis paradoxa.* It is a soil inhabitant, widely distributed throughout the tropics of Africa and Asia and causes disease of several other crops (Rajagopalan, 1965).

An outbreak of *C. paradoxa* in 1982 caused black rot of plumule and radicle of sprouted seeds in a seed store at Nigerian Institute for Oil Palm Research (NIFOR). About 109,000 sprouted seeds (72.7%) of the total stock were affected resulting in a huge financial loss to the institute (Omamor, 1985). Although, binomial is recommended for the treatment of *C. paradoxa* causing seed rot of sprouted seeds of the oil palm (Omamor, 1985). However, fungicide seed treatment is not desirable for disease control due to some adverse effects on the environmental and ecosystem, such as harm to non target organisms, animals and plants. This results in residues in soil and contamination of the water and food chains. Fungicides may induce pathogen resistance, making their effects variable and short lived (Cook and Baker, 1983). In addition, fungicides are expensive in comparison with the relatively low commodity price of sprouted seeds of the oil palm. Hence, there is a need for an improved control management system with reduced fungicide use.

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulilitz, 1996; Cook and Baker, 1983). Harman *et al.* (1980) and Nelson *et al.* (1988) reported the use of *Trichoderma hamatum* for the control of *Pythium* seed rot and *Rhizoctonia* root rot in pea. Hwang and Chakravarty (1992, 1993) reported findings of *Bacillus subtilis* and *Gliocladium virens* (syn. *Trichoderma virens*) as

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bioagents against *Rhizoctonia* root rot when employed with a fungicide.

Since the bioagents are effective and has been pursued against seed and root rot pathogens with success, biological control against *C. paradoxa* causing seed rot of sprouted oil palm seeds has not been pursued. The objective of the present study was to examine the efficacy of *Trichoderma* species on oil palm sprouted seeds for the control of *C. paradoxa* and compare with commercial benlate fungicide.

MATERIALS AND METHODS

Sprouted seeds of the oil palm used in these investigations were raised from germinated improved seed (Tenera hybrid) produced at the Nigerian Institute for Oil Palm Research (NIFOR) near Benin City, Edo State. The seeds were sprouted by the heat treatment method (Anonymous, 1965). Before the seeds were sprouted, they were soaked in 90% (vol./vol.) ethanol for 2 min and washed five times with sterile distilled water. Treated seeds were transferred to sterile filter paper to absorb excess water and then disinfected in 2% (wt./vol.) sodium hypochlorite (Sigma-Aldrich Chenire) for 2 min (Yedidia *et al*., 1999). The disinfected seeds were washed five times in sterile distilled water and excess water was removed with sterilized filter paper under asceptic conditions and left in sterile polythene bags for sprouting. The studies were conducted in 2005 under glasshouse conditions at botanical garden and botany and microbiology laboratories, University of Lagos, Nigeria.

A total number of 720 oil palm sprouted seeds were used for *in-vitro* seed treatments, 360 wounded (stapped with sterile inoculating needle) and 360 unwounded sprouted seeds, all showing presence of radicles and plumules. One hundred and twenty seeds of which 60 wounded and 60 unwounded (20 wounded and 20 unwounded replicated 3 times) were used for each treatment. The fungal cultures used in this investigation were previously isolated by the authors from diseased sprouted seeds of the oil palm collected from the above-mentioned location (NIFOR) in 2004. They are *Trichoderma viride*, *T. hamatum*, *T. polysporum*, *T. aureoviride* and the pathogenic fungus *Ceratocystis paradoxa*, the causal agent of black seed rot of sprouted oil palm seeds.

Sprouted seeds, selected for the uniformity (length of radicle and plumule) were placed in each *Trichoderma* species suspension (25×10^6). For the benlate solution (1 g 100 mL), sprouted seeds were soaked for 10 min. They were incubated for 24 h. The sprouted seeds were challenged by (radicle and plumule) root dipping in a conidia suspension of *C. paradoxa* (25×10^6). Non-challenged control sprouted seeds were dipped in sterile distilled water in the same condition. The treatment were as follows:

- *Trichoderma* sp. or benlate/*C. paradoxa*
- Control/sterile distilled water

They were maintained in sterile petri dish plates and incubated at 28° C. Percentage infection was determined on 1, 3, 7 and 14 days post inoculation.

***In vivo* oil palm sprouted seed treatments:** A total number of 864 oil palm sprouted seeds (432 *C. paradoxa* infested seeds and 432 seeds without pathogen) were used in glasshouse. Twenty four seeds replicated 3 times were used for each treatment. The sprouted seeds were planted with their plumules the right way up at a depth of 2.54 cm in black polythene bags filled with top soil (pH 5.5 to 6.5). Soil was sterilized at 121°C for 1 h for two successive days to destroy weeds, seeds, pests and microorganisms. Each sprouted seed was inoculated using sterile syringes with the same spore suspension and soak method of *in-vitro* earlier mentioned. The polythene bags had holes at their bottoms to allow excess water to drain away. The control experiment had sterile distilled water in the place of fungal cultures or benlate solution. The treatments were as follows:

- *Trichoderma* sp. or benlate/*C. paradoxa*
- *Trichoderma* sp. or benlate/sterile water
- Control/sterile distilled water

The seedlings were watered as at when required. Oil palm seedlings heights and survivals were taken six months after planting. Both the *in vitro* and *in vivo* experiments were repeated twice.

RESULTS

Sprouted seeds of the oil palm treated with *Trichoderma* species and benlate solution reduced infection of *Ceratocystis paradoxa*, but the reduction varied. There were no infections recorded on the 1st day post inoculation. Infection was noticed on the 3rd day with *Trichoderma polysporum*, *T. viride* and benlate having significant ($p<0.005$) reductions than the other *Trichoderma* species (Table 1). On the 7th day *T. polysporum*, *T. viride* and benlate were significantly ($p<0.005$) effective and similar in reduction of *C. paradoxa*. But more consistence was *T. polysporum* with 13.0 and 10.0% for wounded and unwounded sprouted seeds on the 14th day. However, the

Table 1: Effects of *Trichoderma* species and benlate solution on sprouted seeds of the oil palmagainst *Ceratocystis paradoxa*

Days post inoculation	Seed treatments	Percentage infection of oil palm sprouted seeds: Wounded	Unwounded
1	*T. viride*	0.0	0.0
	T. hamatum	0.0	0.0
	T. polysporum	0.0	0.0
	T. aureoviride	0.0	0.0
	Benlate (1 g/100 mL)	0.0	0.0
	Control: (Sterile water)	0.0	0.0
3	*T. viride*	6.7*	1.7*
	T. hamatum	8.3	1.7*
	T. polysporum	3.3*	0.0*
	T. aureoviride	3.3*	1.7*
	Benlate (1g/100 mL)	3.3*	0.0*
	Control (Sterile water)	15.0	10.0
7	*T. viride*	11.7*	5.0*
	T. hamatum	13.3*	6.0
	T. polysporum	8.3*	3.0*
	T. aureoviride	15.0	8.3
	Benlate (1 g/100 mL)	8.3*	6.7
	Control (Sterile water)	25.0	10.0
14	*T. viride*	18.3*	11.7*
	T. hamatum	22.0*	15.0*
	T. polysporum	13.0*	10.0*
	T. aureoviride	23.7*	13.0*
	Benlate (1 g/100 mL)	21.7*	18.3
	Control (Sterile water)	80.0	30.0

*Mean values followed by are significant ($p<0.05$) according to one-way analysis of variance as compared to the control. Values are average of three replicates

Table 2: Effects of *Trichoderma* species and benlate solution on sprouted seeds of the oil palm against *Ceratocystis paradoxa* 6 months after planting in glasshouse conditions

Seed treatment	*C. paradoxa* infested seeds: Survival (%)	Height (cm)	Seeds without pathogens: Survival (%)	Height (cm)
T. viride	71*	23.8*	100*	43.6*
T. hamatum	67*	20.4*	100*	46.5*
T. polysporum	71*	21.2*	100*	38.3
T. aureoviride	63	18.4	100*	38.2
Benlate (1 g/100 mL)	71*	25.1*	100*	40.6*
Control:(sterile water)	33	14.6	100*	23.6

*Mean values followed by are significant ($p<0.05$) according to one-way analysis of variance as compared to the control. Values are average of three replicates

consistence of benlate dropped having 21.7 and 18.3% also on the 14th day (Table 1). Effect of *T. polysporum* was more significant compared with the control treatment (80 and 30%) but not much significant different from other *Trichoderma* species and benlate.

Trichoderma species and benlate solution treatments on sprouted seeds of the oil palm increased emergence (percentage of survival) and reduced percentage of infection (Table 2). Both emergence and the growth (height) of oil palm seedlings from antagonists treated seeds were significantly ($p<0.005$) higher than the emergence and the growth observed from *C. paradoxa* infested seeds and the control. *T. viride* and *T. polysporum* increased emergence and growth of oil palm seedlings by 71% and 23.8 cm and 71% and 21.2 cm, respectively (Table 2). These are not significantly different from the one obtained from benlate (71% and 25.1 cm). The effects of reductions were significantly higher when compared with the control but not significantly different among *Trichoderma* species.

DISCUSSION

Trichoderma sp. are active ingredients in a variety of commercial biofungicides used to control a range of economically important aerial and soil borne fungal plant pathogens (Harman, 2000; Harman and Kubicek, 1998). The observations that *Trichoderma* species applied to sprouted seeds of the oil palm significantly reduced infection of *C. paradoxa* suggested that the bioagents have the substantial potential to control many soilborne fungal pathogens involved in seed decay. However, the reason for the dropped in consistence of benlate solution on the 14th day post inoculation (Table 1) was not given in this study.

The emergence and height of oil palm seedlings from antagonists treated seeds (Table 2), suggested active growth. This active growth may have been supported by the production of extracellular enzymes capable of releasing cell wall components that provide nutrients and/or further stimulated host colonization (Lorito and Woo, 1998; Woo *et al*., 2000).

The unwounded (new developing radicle and plumule) sprouted seeds were more susceptible while the older ones (with ticker radicle and plumule) were resistant to attack by *C. paradoxa*. Infection was high with wounded sprouted seeds when compared with the unwounded. *C. paradoxa* has been reported as pineapple disease of sugarcane setts, entering the seed piece through cut ends, destroys the buds, thus causing germination failure (Yin and Hoy, 1997, 1998). Although germination failure was observed more with control treatments, treated with *C. paradoxa* infested seeds while seeds treated with *Trichoderma* species had significant reduction in germination failure.

The results of this investigation have confirmed the efficacy of *Trichoderma* species to control *C. paradoxa* with similar or high effects when compared with benlate fungicide. Because of the remarkable properties and potentials of *Trichoderma* species, more studies of these bioagents should be encouraged.

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REFERENCES

Ahmed, S.A., 2001. Agriculture and Food Security in Nigeria. Paper prepared for a forum with Mr. President on Agriculture and Food Security. Council chambers, Presidential Villa, Abuja. January 2001.

Anonymous, 1965. Germination of oil palm seed by dry heat treatment. Nigerian Institute for Oil Palm Research Advisory Sheet No. 12. Revised Edition.

Baker, R. and T.C. Paulitz, 1996. Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens In: Principles and Practice of Managing Soilborne Plant Pathogens. R. Hall (Ed.). The American Phytopathological Society. St. Paul, MN., pp: 50-79.

Cook, R.J. and K.F. Baker, 1983. The Nature and Practice of Biological Control of Plant Pathogens. America Phytopathology Society, St. Paul, Minnesota.

Corner, B.J.H., 1966. The Natural History of the Palms. Wiedenfeld and Nicholson. pp: 202.

Harman, G.E. and C.P. Kubicek, 1998. *Trichoderma* and *Gliocladium*, Taylor and Francis Ltd. Vol. 1 and 2. London, United Kingdom.

Harman, G.E., 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis., 84: 377-393.

Harman, G.E., I. Chet and R. Baker, 1980. *Trichoderma hamatum* effects on seed and seedlings disease induced in radish and pea by *Pythium* sp. or *Rhizoctonia solani*. Phytopathology, 70: 1167-1172.

Hwang, S.F. and P. Chakravarty, 1992. Potential for the integrated control of Rhizoctonia root rot of *Pisum sativum* using *Bacillus subtilis* and a fungicide. J. Plant Dis. Prot., 99: 626-636.

Hwang, S.F. and P. Chakravarty, 1993. Integrated biological and chemical control of *Rhizoctonia* root rot of field pea by *Gliocladium virens* and a fungicide. J. Plant Dis. Prot., 100: 308-316.

Lorito, M. and S.L. woo, 1998. Advances in Understanding the Antifungal Mechanism(s) of Trichoderma and New Applications for Biological Control In: Molecular Approaches in Biological Control. Duffy, B., U. Rosenberger and G. Defago (Eds.), IOBC WPRS Bulletin/Bulletin OILB SROP, Dijon, Franc, 21: 73-80.

Nelson, E.B., G.E. Harman and G.T. Nash, 1988. Enhancement of *Trichoderma* induced biological control of *Pythium* seed rot and pre-emergence damping of peas. Soil Biol. Biochem., 20: 145-150.

Omamor, I.B., 1985. Black rot of sprouted seeds of the oil palm. Trans. Brit. Mycol. Soc., 84: 159-161.

Rajagopalan, K., 1965. NIFOR. First Annual Report. 1964-5, pp: 88-89.

Robertson, J.S., 1962. Dry Basal Rot, a new disease of oil palms caused by *Ceratocystis paradoxa*. (Dade) Moreau. Trans. Br. Mycol. Soc., 475: 478.

Woo, *et al*., 2000. Host-derived molecules that activate the mechanism of biocontrol in *Trichoderma* sp., In: Proceedings of the Meeting on Methods to Microbial Inoculants to Improve their Success, Wageningen, The Netherlands, pp: 25.

Yedidia, L., N. Benhamou and I. Chet, 1999. Induction of defence responses in cucumber palnts (*Cucumis sativa* L.) by the biocontrol agent *Trichoderma harzianum*. Applied Environ. Microbiol., 65: 1061-1070.

Yin, Z. and J.N. Hoy, 1997. Effect of stalk desiccation on sugarcane red rot. Plant Dis., 81: 1247-1250.

Yin, Z. and J.N. Hoy, 1998. Effect of oxygen deprivation and *Pythium* root rot on sugarcane red rot. Plant Dis., 82: 1237-1243.

Zeven, A.O., 1965. The origin of the oil palm (*Elaeis guineensis* Jacq.) a summary. J. Nig. Inst. Oil Palm Res., 4: 218-225.