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SOIL AND SEED TREATMENT WITH *Bacillus Thuringiensis* AND AVOCADO (*Persea Americana*) SEED POWDER AGAINST *FUSARIUM* ROOT ROT OF OKRA

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ABSTRACT

Disease management strategies using synthetic chemicals are expensive, hazardous and environmentally unfriendly and have necessitated the search for alternatives in biological agents with antimicrobial properties. This research was aimed at assessing the effectiveness of *Bacillus thuringiensis* and avocado seed powder applied either as soil amendments and/or seed coatings on *Fusarium* root rot of potted okra. *Fusarium oxysporum* isolated from diseased okra plants was used to inoculate the pots. The experiment was a completely randomized design (CRD) replicated eight times. Data were collected on percentage germination, disease incidence and plant growth parameters such as shoot weight, root weight, shoot length and root length. Results showed significant (P = 0.002) difference among treatments with the hybrid seeds having the highest percentage germination of 94% followed by soil treated with avocado seed powder (85%) and their effect differed significantly from the control. In terms of disease incidence, hybrid seeds, seed coating or soil treatment with avocado and soil treatment with Bt all controlled the disease giving incidence values of 26%, 25%, 18% and 16%, respectively, which differed from the control pots (41%). Shoot length was significant (P = 0.049 and P = 0.007) at both assessments where hybrid seeds constantly maintained the tallest plants. Since the treatments suppressed the incidence of root rot disease, the use of these treatments is advocated for effective management of *Fusarium* root rot disease.

***Key words*:** Hybrid, *Bacillus thuringiensis, Fusarium oxysporum, Abelmoschus esculentus, Persea americana,* root rot

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is affected by root rot disease caused by *Fusarium* spp and this has contributed significantly to low yields and death of plants (Rahim *et al*., 1992; Ahmad *et al.*, 2014). *Fusarium* is a soil inhabiting pathogen that attacks a large number of host plants (Ahmad *et al*., 2012; Waheed *et al*., 2013; Ahmad *et al.*, 2014). Plant materials and biological control agents have been explored to their potentials against these soil-borne pathogens with huge success. Avocado *(Persea americana*) has an edible fruit, which has been used as phytotherapic source and has been traditionally used in mycoses and parasitic infections. They contain various classes of natural products such as, phytosterols, triterpenes, fatty acids and furanoic acids some of which are related with antifungal activity and larvicidal effects (Leite *et al*, 2009). The secondary metabolites produced by *Bacillus* spp. have also been found to show antibacterial and/or antifungal activity against plant pathogens and food borne pathogenic organisms (Dawar *et al*., 2008 and Alvindia, and Natsuaki, 2009). Results of *in vitro* studies using *Bacillus* spp as bio-control agents showed positive responses against foliar and soil-borne pathogens. Asaka and Shoda, (1996) associated the inhibitory effect of *B. thuringiensis* on plant pathogenic fungus with enzyme production that act against the fungal cell wall. Mojica-Marín *et al*., (2008) showed that *B. thuringiensis* proved to be efficient in the control of *R. solani* of chilli pepper in *in vitro* assays and antibiosis seems to be the principal mode of action (Lee *et al*., 2003 and Ligon *et al*., 2000). Waheed *et al*., (2013) also reported a significant reduction in disease severity of tomato seedlings treated with *B. thuringiensis* strain 199 against *Fusarium* wilt and advocated the need to develop inoculum for commercial application at field level. It has also been shown that where *Bacillus* was applied at the time of transplanting, plants had an increase in height, flowering and fruiting compared to traditional

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crop management. Szczech and Shoda, (2006) also reported that the application of some *Bacillus* strains to seeds or seedlings was found to be effective in the suppression of soil-borne diseases and successfully induced systemic resistance in the treated plants. Pollution caused by excessive use and misuse of agrochemicals results in several complications, which include the development of pesticide resistance by pathogens, elimination of natural enemies and have caused adverse effects on the soil health and other environmental components (Diwedi and Diwedi, 2007). It is in this context that the application of biological control as an alternative and environmentally friendly measure for phytopathogens becomes handy (Akram *et al.,* 2013). Due to constant increase in the cost of pesticides and the environmental hazard associated with their use, the need to use more environmentally friendly ways to control soil-borne pathogens has become necessary (Agrios, 2006). This research was therefore aimed at assessing the effectiveness of *B. thuringiensis* and avocado seed powder applied as soil amendments and/or seed coatings on *Fusarium* root rot of potted okra.

MATERIALS AND METHODS

The experiment was conducted at the Screen House of the Department of Crop and Soil Science, University of Port Harcourt, Port Harcourt Nigeria (6.55^{o} 0.2' N latitude, 4.54^{o} 10.02' E longitude).

Experimental materials

Okra (*A. esculentus* L.) hybrid cultivar (Madison F1) was procured from Agri-Tropic Nig. Ltd., Rivers State; a sub-branch of Technisem Company, Lounge-Jumelles (France), while the local variety was procured from the open market in Choba (the University town). Avacado (*P. americana*) fruit were sourced from the local market and the seed extracted, air-dried and ground to powder. A commercially produced (*B. thuringiensis* (Bt) Biothor 120g) was purchased from Tratamientos Bio-Ecology, S.A san Javier Spain.

Seed viability test

Fifty seeds each from the hybrid and local okra seeds were soaked overnight in 100 ml of sterile distilled water and plated in 90 mm Petri dishes containing sterile Whatman No. 1 filter paper at 10 seeds/plate and incubated at 25 ± 2^{o}C for five days. Germinated seeds per plate were recorded.

Collection of infected okra and isolation of fungal pathogen

Infected okra plants were collected from the Teaching and Research Farm of the Department of Crop and Soil Science to the laboratory. The roots were washed thoroughly with tap water and chopped into pieces (5 mm). They were surface sterilized with 2% sodium hypochloride (NaOCl) and rinsed in 3 changes of water. The plant parts were placed on filter paper and dried in an inoculation chamber. The plant parts were plated in PDA (200 g potato + 20 g glucose + 20 g agar in 1 litre of water) in 9 mm Petri dishes at the rate of 4 four pieces per plate and kept in the incubation chamber at 25 ± 2^{o}C and observed for fungal growth. Subcultures were made from emerging colonies repeatedly until pure cultures were obtained. The fungal isolates were further checked for identity, viability and purity using standard biochemical test as described by Cheebrough (2000). Identification was done using Illustrated Genera of Imperfect Fungi by Barnett and Hunter (1987). Pathogenicity test was carried out to ascertain the pathogenicity of the isolates.

Soil sterilization

Soil used in the experiment was obtained from the experimental plots of the Faculty of Agriculture Teaching and Research Farm, University of Port Harcourt. The soil was passed through 2 mm sieve to discard particles. It was transferred into a drum covered tightly and heated at 120^{o}C for 4 hours. The soil was left for 48 hrs to cool. 5 kg of the sterilized soil was transferred into 22.5 cm (diameter) pots perforated at the bottom.

Inoculum production and pot inoculation

Pure-cultures of *Fusarium oxysporum* isolate used in the experiment was sub-cultured into forty (40) potato dextrose agar (PDA) plates modified with 0.5 g of streptomycin. 100 ml sterile distilled water was added to each plate and spores were dislodged by scraping the sur-

face of the plate. The spore suspension obtained was filtered through two layers of cheese cloth to remove mycelia. The volume of the suspension was made up to 4000 ml by adding distilled water. 100 ml of the spore suspension was used to drench each pot and left for 7 days to colonise the pot before treatment application.

Treatment preparation and application

Seeds were treated by scooping 100 g of the local variety into 500 ml conical flask containing 10 g of avocado seed powder + 20 ml of sterile distilled water to form a coat on the seeds.

For soil treatment, 5 g of either the avocado seed powder or *B. thuringiensis* was spread on the soil surface of the pot containing 5 kg soil and covered with 1 kg of soil.

Sowing of seeds

Before sowing, the treated pots were filled with water and left overnight to drain out. Five seeds of the local variety were sown per pot, treated with either avocado seed powder or *B. thuringiensis* powder. Avocado coated seeds and hybrid seeds served as two treatment where 5 seeds each were sown per pot. The control pots had only local okra variety sown in *Fusarium* inoculated pots.

Data collection

Number of germinated seeds was evaluated 10 days after sowing and percentage germination derived from the formula below;

$$Germination\ percentage = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown} \times 100$$

Incidence of root rot was evaluated at 21 days after sowing by uprooting one plant per pot and percentage root rot derived using the formula below;

$$Root\ rot\ percentage = \frac{Number\ of\ infected\ roots}{Total\ number\ of\ roots\ per\ plant} \times 100$$

At 30 and 45 days after sowing, okra plants at one plant/pot was uprooted, taken to the laboratory and washed thoroughly. Plant growth parameters in terms of root length, shoot length, fresh weight of roots and fresh weight of shoots were measured. The number of fruits per plant was counted at 45 days after sowing.

Experimental design and data analysis

The experiment was laid out in completely randomized design (CRD) replicated eight times. Data were analysed using analysis of variance (ANOVA) using Genstat (13 Edition) and means separated using the least significant difference (LSD) at 5% probability levels.

RESULTS AND DISCUSSION

The pathogenicity test confirmed *F. oxysporum* as the causal organism. Results of the seed viability test showed that the percentage seed germination of hybrid seeds ranged from 95-100% while that of the local variety was 90%, thus confirming that the seeds used in the experiment were viable. Table 1 shows percentage germination of okra seeds assessed at 10 days after sowing. Results showed significant (P = 0.002) difference among treatments with the hybrid seeds having the highest percentage germination, followed by soil treated with avocado seed powder and their effect differed significantly from the control which had the least. *Fusarium* root rot disease incidence showed significant (P = 0.01) difference among treatments (Table 1). Control pots had the highest *Fusarium* root rot incidence, the effect of which was different from the other treatments. Hybrid seeds, seed coated with avocado powder, soil treatment with either avocado or *B. thuringiensis* all reduced the incidence of *Fusarium* root rot. Results on growth parameters assessed 30 days after sowing showed that root weight (P = 0.016) and shoot length (P = 0.049) were significantly different. Hybrid seeds and pots treated with avocado seed powder had higher root weights. However, highest shoot length was observed in pots where hybrid seed were sown (Table 2). Assessment at 45 days after sowing showed significant (P = 0.007) effect on shoot length. Hybrid seeds maintained the tallest shoot length and the effect was statistically different from the control plants (Table 3). Root weight (P = 0.24), root length (P = 0.36),

shoot weight (P = 0.8) and number of fruit per plant (P = 0.29) showed no effect of treatments. Seed and soil treatment are striking methods for introducing biocontrol agents into the soil root environment (rhizosphere). They protect the seed from seed-borne and soil-borne pathogens and enable the seed to germinate and become established as healthy seedlings.

The antagonist and avocado powder applied to the seeds and soil may have had the opportunity to be the first colonizer of the roots. Similarly, control of soil-borne pathogens by the addition of antagonistic microorganisms to the soil is a potential non-chemical means for plant disease management. Soil amendments with microbial antagonist *B. thuringiensis* have been effective in the control of root rot fungi and have been extensively studied (Krebs *et al*., 1998; Dawar *et al*., 2008 and Mojica-Marin *et al.,* 2008). The current work corroborates other research findings that *Bacillus spp* have significant inhibitory activity against many plant pathogens including *Ceratocystis ulmi* (Gregory *et al.,* 1986), *Puccina pelargonii-zonalis* (Rytter *et al*., 1989), *Euthypa lata* (Ferreira *et al*., 1991), *Fusarium moniliforme* (Agarry *et al*., 2005), *Phythopthora capsici* (Jo, 2005), *P. cinnamon* (Aryanta and Guest, 2006), *Colletotrichum gloeosporoides*, *Botrytis cinerea*, *Monilinia laxa*, *Sclerotium rolfsii* (Prapagdee *et al*., 2008), *Colletotrichum musae* (Alvinda and Natsuaki, 2009) and *F. oxysporum* (Nikam *et al*., 2011). The possible mode of action may be the production of antibiotics and competition or fungicidal action as a result of the secretion of lytic enzymes (Pukall *et al*., 2005). The reduced seed germination observed in pots amended with Bt was contrary to the works of Sheikh *et al*., (2006) and Dawar *et al*., (2008). The authors observed increased seed germination, shoot length and weight, root length and weight when *Bacillus thuringiensis* was applied as seed dressing. However, the significant increase in seed germination and reduced incidence of root rot associated with seed coating with avocado is in line with the works of Lall *et al*., (2006). The authors showed that avocado seed extracts possess antifungal properties against seed pathogens such as *Aspergillus niger* and *Cladosporium cladosporioides*. Previous phytochemical studies on avocado seeds have also identified various classes of natural products such as phytosterols, triterpenes, fatty acids, furanoic acids, flavonol dimers, proanthocyanidins and abscisic acid and these products are connected with antifungal activities (Leite *et al*., 2009).

CONCLUSIONS

The use of biological agents such as microbial antagonist *B. thuringiensis* or plant powders with antifungal properties has been successful in the control of soil-borne pathogens, thus providing a useful option for sustainable dis-

Table 1: Effect of soil and seed treatments on percentage seed germination and incidence of *Fusarium* root rot disease of okra

Treatment	*% Germination*	*% Disease incidence*
Control	58.0	41.2
Hybrid	94.0	26.2
Sc + Av	75.0	25.0
St + Av	85.0	17.5
St + Bt	68.0	16.2
LSD	17.6	14.2
P.value	0.002**	0.01*

Where; Sc + Av = seed coated with avocado, St + Av = soil treatment with avocado, St + Bt = soil treated with *B. thuringiensis* (Bt).

ease management program in agriculture. In this study, the effective control of root rot disease by the treatments was evident. For this reason, it is suggested that *B. thuringiensis* or avocado seed powder could be incorporated into the soil for effective management of *Fusarium* root rot diseases of okra at the seedling stage. Avocado seed powder could also be used to treat the seeds before planting since there was no evidence from the study that the powder adversely affected germination of okra seeds. The use of hybrid seeds should also be encouraged for effective integrated disease management.

Table 2: Effect of avocado seed powder and *B. thuringiensis* on *Fusarium* root rot of okra at 30 days after sowing

Treatment	*Growth parameter*				
	Leaf/plant	*Root length (cm)*	*Root weight (g)*	*Shoot length (cm)*	*Shoot weight (g)*
Hybrid	4.0	7.11	0.33	32.0	5.38
Sc+Av	5.0	7.44	0.33	26.4	4.72
St+Av	5.0	5.84	0.34	29.2	5.94
St+Bt	5.0	7.56	0.30	28.1	4.69
Control	5.0	5.89	0.30	28.5	5.42
LSD	0.47	2.4	0.02	3.5	2.11
P.value	0.22^{ns}	0.092^{ns}	0.016*	0.049*	0.716^{ns}

Where; Sc + Av = seed coated with avocado, St + Av = soil treatment with avocado, St + Bt = soil treated with *B. thuringiensis* (Bt).

Table 3: Effect of avocado seed powder and *B. thuringiensis* on *Fusarium* root rot of okra 45days after sowing

Treatment	*Growth parameter*				
	Root length (cm)	*Root weight (g)*	*Shoot length (cm)*	*Shoot weight (g)*	*Fruit/plant*
Hybrid	7.55	0.73	36.61	5.55	2.0
Sc+Av	7.87	1.64	33.62	5.03	2.0
St+Av	10.56	0.94	35.16	6.56	2.0
St+Bt	9.79	1.53	34.30	6.20	2.0
Control	8.26	0.58	29.51	6.40	1.0
LSD	3.47	1.30	3.7	2.94	1.1
P.value	0.24^{ns}	0.36^{ns}	0.007**	0.80^{ns}	0.29^{ns}

Where; Sc + Av = seed coated with avocado, St + Av = soil treatment with avocado, St + Bt = soil treated with *Bacillus thuringiensis*

Further research using a combination of hybrid seeds and soil treatment with either *B. thuringiensis* or avocado seed powder in the field is recommended. The fungicidal compounds in the avocado seeds should also be identified and characterized.

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