Rice Husk Extract is Potentially Effective as a Phytopesticide against Root-/Soil-borne Fungal Pathogens of Cowpea

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Abstact: Phytopesticide produced from rice husk extract (RHE) was evaluated, in the laboratory and in the glasshouse as a potential biocontrol agent for controlling root- and soil-borne fungal pathogens isolated from field-grown cowpea in the northern Guinea savanna of Nigeria. The pathogenicity test was carried out in the glasshouse on the fungal species isolated from infected plants in cowpea field trials conducted in 2006 and 2007 cropping seasons. Five root- and soil-borne fungal pathogens: *Fusarium verticilloides*, *F.equiseti*, *F.solani*, *F. oxysporum* and *Rhizoctonia solani*, were the major highly virulent fungal pathogens which caused severe problems including damping off, root rot, reduction in nodulation, vascular wilt/discoloration, chlorosis, necrotic lesions, leaf blight, complete defoliation, seedling mortality, and death in cowpea. Plants from the glasshouse experiments (on the microbial antagonism study) were examined for disease incidence and severity symptoms. *In–vitro* and *in–vivo* studies revealed that RHE significantly (P<0.05) inhibited all the five fungal pathogens at 1.5% concentration. However, at 1% concentration of RHE did not inhibit mycelia radial growths of *F. verticilloides*, *F. equiseti* and *F. oxysporum* after 7 days incubation *in-vitro*. The RHE was phytotoxic on cowpea seedlings at 2% concentration. The RHE can thus be regarded as a potential bioprotectant as an alternative to chemical pesticides which are known to be environmentally unsafe for the management of common root- and soil–borne fungal pathogens of cowpea.
[A.S. Killani, R.C. Abaidoo and A.K. Akintokun. **Rice Husk Extract is Potentially Effective as a Phytopesticide against Root-/Soil-borne Fungal Pathogens of Cowpea.** Nature and Science 2011;9(3):72-79]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Phytopesticides; pathogenicity; bioprotectant; antagonism; fungal pathogens

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is highly susceptible to a number of root-/soil-borne fungal diseases, causing great losses in yield and seed quality (Lichtenzveig et al. 2006). A critical appraisal of why farmers in sub-Saharan Africa particularly, in Nigeria are becoming skeptical about growing cowpea unlike other legumes showed that the number one reason was the increasing yield losses that root-/soil-borne fungal pathogens have been causing over the years. Despite the technological changes in cowpea research across the globe which had resulted in yield increases over-time, diseases and pests have been identified as major production constraints (Singh et al. 1989), limiting high productivity and accounting for more than 80% yield losses in cowpea (Komarwa et al. 2002). In Africa, diseases and pests are often responsible for 100% losses of cowpea yield if not controlled. Yield losses to pests in northern Nigeria of 78% and 80% in southern Nigeria have been reported (Booker, 1965).

Cowpea is attacked by more than 35 major diseases caused by viruses, bacteria, fungi and nematodes (Emechebe and Soyinka, 1985). Lichtenzveig et al. (2006) reported that root-/soil-borne fungal pathogens are causal agents of legume diseases of increasing economic importance, such as root rots, seedling damping-off, and vascular wilts. As agricultural production intensified over the years, farmers became increasingly dependent on application of agrochemicals as a method of crop protection and conservation without considering safer and environmentally friendly biological control agents (Newsham et al. 1995). The increasing trend in environmental awareness has prompted efforts towards finding environmentally and toxicologically safe and efficacious integrated disease management options (Mukerji and Ciancio, 2007). Likewise, the increasing incidence of resistance by pests to pesticides and environmental impact associated with the use of agrochemicals for crop protection contributed immensely to the search for safer and environmentally friendly pest control measures. In this respect, natural products are considered to be potential sources of developing biodegradable pesticides.

Plants are known to produce a variety of secondary metabolites, which are bioactive and thus may have inhibitory effects on bacteria, fungi, insects and other microorganisms (Odebode et al. 2004). The objective of this study was to evaluate the effect of (RHE) as a phytopesticide against root-/soil-borne fungal pathogens isolated from cowpea in northern Guinea savanna of Nigeria

2. Materials and Methods

2.1 Laboratory Experiments

2.1.1 Isolation and Identification of Root-/soil-borne Fungal Pathogens

Root-/soil-borne fungi were isolated in the laboratory from naturally infected roots/stems of cowpea plants and their rhizosphere soil collected from the experimental plot site. Infected plant tissues were surface sterilized in 3% sodium hypochlorite (NaClO) for 3 min, rinsed in three changes of sterile distilled water and then blotted dry with a sterile paper towel pad. Approximately 2mm × 7mm tissue sections were cut from the advancing portion of the lesion of surface sterilized tissue using a sterile scalpel blade. The sections were plated on specific *Fusarium* spp. medium [Peptone Pentachloronitro-benzene Agar (PPA) modified by Nash and Snyder (1962). The PPA contained the following: Difco agar powder (15 g l^{-1}), peptone (15 g l^{-1}), KH_2PO_4 (1 g l^{-1}), $MgSO_4.7H_2O$ (0·5 g l^{-1}). The medium was autoclaved at 120°C for 20 min. The medium was then amended with Chloramphenicol (0·05 g l^{-1}), Pentachloronitrobenzene (0·75 g l^{-1}), Chlorotetra-cycline (0·5% in water) (10 ml l^{-1}) as suggested by Ros et al. (2005).

For *R. solani*, full strength Difco Potato Dextrose Agar (PDA), prepared according to manufacturer's specifications was employed. The plates were incubated at 28°C in an incubator (Model Gallenkamp Cooled Incubator) for 7 days. Young active growth of different fungal mycelia from each isolate on the plated tissue was sub-cultured onto PDA and *Fusarium* spp. isolates were purified using single spore technique on PPA. The pour plate method was used for the isolation of fungi from the soil. Fungal isolates were characterized and identified based on their colonial morphology and microscopic characteristics using different identification keys and methods developed by Domsch et al. (1980); Nelson et al. (1983); and Summerell et al. (1993). The phytopesticide **(**concentrated RHE) was obtained from Gernplasm unit, IITA, Ibadan.

2.1.2 *In-vitro* Phytopesticide and Pathogens Antagonistic Interaction

One litre of PDA (39 g/l) was prepared in media bottles and dispensed at varying volumes of 99 ml, 98.5 ml, 97.5 ml, 95 ml and 100 ml (control) into 250 ml sterile media bottles. Bottles and its contents were then sterilized in the autoclave at temperatures of 121^{o}C for 15 min at 1.2 bar. The medium was allowed to cool to 45^{o}C. Thereafter, 1 ml, 1.5 ml, 2 ml, 2.5 ml, and 5 ml of RHE were aseptically measured with a 5 ml sterile syringe into the sterilized medium to represent concentration of 1%, 1.5%, 2%, 2.5% and 5% (v/v). Each bottle was rolled in the palm to allow a homogenous mixture of medium and the extract. Fifteen millilitres of this mixture was poured aseptically into 9 cm sterile disposable Petri dishes and allowed to solidify at room temperature inside the laminar flow. With a sterile 5 mm cork borer, mycelia discs of young actively growing cultures of each pathogen were cut separately and inoculated right at the centre of the already prepared plates containing the mixtures and the control plates. There were three replicates for each pathogen, both on the cultured plates with RHE and the control. The plates were incubated at 28°C for 9 days and periodically observed for antagonist-pathogen interactions. Laboratory data were collected at 3, 6, and 9 days. The mycelial growth diameter (cm) of each pathogen was measured and the percentage growth inhibition was calculated according to Awuah (1989) and Odebode et al. (2004) as follows:

Percentage of growth inhibition = $(D_c - D_t) / D_c \times 100$.

Where D_c= Diameter of pathogen in the control plates, and D_t = Diameter of the pathogen in the treatment plates

2.2 Glasshouse Experiments

These experiment were conducted in the glasshouse of IITA, Ibadan, Nigeria (7^{o}30'N, 3^{o}5'E) using 3 kg (dry weight) of sterilized sub–soil and acid washed ocean sand (1:1) with the following physico–chemical properties: soil texture: sand, 80%, silt, 10%, clay, 10%, pH, 6.0, organic C, 0.39%, N, 0.036%, Ca, 1.546 cmol+/kg, Mg, 0.38 cmol+/kg, K, 0.08 cmol+/kg, Na, 0.81 cmol+/kg, Exch. Acidity, 0.00 cmol+/kg, ECEC, 2.82 cmol+/kg, Zn, 0.41 ppm, Cu, 1.43 ppm, Mn, 95.84 ppm, and Fe, 53.77 ppm.

2.2.1 Pathogenicity Test

This *in-vivo* assay was conducted in the glasshouse employing the method developed by Koch (1891) and modified by Ros et al. (2005). Twelve root-/soil-borne fungal isolates were randomly selected based on their degree of occurrence and virulence in the field from the 22 fungi isolated from diseased roots/stems of cowpea and soil collected from their rhizosphere in the NGS agroecology for the glasshouse pathogenicity test. The objective was to select the most virulent strains among several isolates. Isolates R101A, R105E, R126F, S106B, S103C, S102D, S126F, S117J, S109I, R110J, R113K, and S112L, were sub-cultured on Difco PDA and incubated at 28°C for 7days. Spores of each fungal isolate were harvested and suspended in sterile distilled water. The fungal spores' suspension was re-adjusted and standardized spectrophotometrically to approximately 10^7–10^8 spores/mL (CFU mL^{-1}) with SDW (Optical Density (OD) of 1.0–1.3 at 600 nm using Spectro- UV-VIS AUTO UV 2602 Labo. Med. Inc.).

For non-spore formers (*R. solani*), mycelia bits were use as inoclum. The counting of the mycelia bits was done and re-adjusted with haemocytometer to 3.3 x 10^6

mycelia bits/ml. Each inoculum suspension was immediately inoculated to each pot containing a mixture of sterile sub-soil and acid washed ocean sand (1:1) at planting to reach the pathogenic level around 10^4 CFU g^{-1} inside sterile 3 kg plastic pots. Seeds of healthy cowpea genotype (IT90K-277-2) were surface sterilized in 1% sodium hypochlorite for 3 min to remove surface contaminants. The seeds were rinsed immediatelly in three changes of distilled sterile water, and allowed to air dry in the laminar flow. Four seeds were simultaneously planted in the 50 ml of fungal inocula and RHE mixture at a depth of approximately 1 cm. The control pots were inoculated with 50 ml of sterile distilled water. The development of signs and symptoms of pathogenicity were observed weekly and records were taken. Re–isolation was carried out to confirm isolates identity at 8 weeks after planting (WAP).

2.2.2 Effect of Interaction between Phytopesticide (RHE) and Fungal Pathogens on Cowpea.

Materials used were two clean cowpea genotypes, one susceptible IT90K-277-2 (Gen 1) and one resistant IT97K-340-1(Gen 2). The two genotypes were selected based on their natural field reactions to root-/soil-borne fungal pathogens. Seeds were surface sterilized with 1% sodium hypochlorite for 3 min to remove surface contaminants and rinsed immediately in three changes of distilled sterile water. The water holding capacity of the mixture of sterile sub-soil and sand were determined. Four seeds of each cowpea genotypes were then planted. Experiments were in completely randomized designs, and in three replicates.

The quantitative estimation of the pathogenic fungi used in this study i.e. *Fusarium* spp. spores suspension ($3{\cdot}3 \times 10^5$ CFU ml^{-1}) and *R. solani* (3.3×10^6 mycelia bit / ml) of the root/soil-borne fungal pathogen inocula were inoculated simultaneously with 1.5% concentration of RHE to reach the soil pathogenic level of around 10^4 CFU g^{-1} (Ros et al. 2005). Plants were watered daily for 2 weeks and thereafter twice every other day until harvesting. The data for glasshouse *in-vivo* antagonistic experiment were collected weekly from 3 WAP for a period of 5 weeks (8 WAP).

2.3 Assessment of Disease Occurrence

Disease incidence (DI) for each root/soil borne fungal pathogen at 8 weeks after planting (8 WAP) was calculated using the equation proposed by Cooke (2006) as follows:

$$DI = \frac{\text{No. of infected plant units}}{\text{Total no. of plant units assessed}} \times 100$$

The disease severity (DS) was assessed according to alternative rating scale index proposed by Ros et al. (2005) as follows: 1- all leaves green (plant without symptoms), 2- 25-49 % lower leaves yellow (very slight browning of hypocotyls), 3- 50-75 % lower leaves dead and some upper leaves yellow (some wilting of plant), 4- 75-99 % lower leaves dead and upper leaves wilted (wilting of entire plant) and 5- 100% plant dead. Nodule number, nodule dry weight (wt), biomass dry weight, biomass nitrogen uptake and phosphorus accumulation were also determined using method developed by Olsen et al. (1954) and IITA (1982).

2.4 Statistical Data Analysis

All statistical analyses were performed using General Linear Modeling (GLM) procedure with Duncan Multiple Range Test (DMRT) using SAS® (2009) System for Windows Version 9.1 software, SAS Institute, Cary, North California, USA, to compare different states with respect to disease incidence and severity. In all the observational laboratory, and greenhouse bioassay experiments, dependent variables were subjected to analysis of variance (ANOVA). The least square means (LSM) test at 0.05 level of significance was used to compare treatment means for each measured parameter. Standard error (SE) and Coefficient of variation (CV in %) were also computed.

3. Results

3.1 Pathogenicity test

In-vitro tests on the 12 selected fungal isolates revealed that only five root-/soil-borne fungi were virulent, *F. verticilloides* (R105E), *F. equiseti* (R126F), *R. solani* (S102D), *F. solani* (R108H) and *F. oxysporum* (R103C). Isolates R105E and R108H were significantly (P<0.05) different from the rest of the isolates. Isolate R103C was significantly (P<0.05) different compared with the control (C100M). The isolates, R103C, R105E, and R108H, were highly virulent compared with the others and control. Cowpea plant height, total biomass and nodulation were greatly affected by the root-/soil-borne fungal pathogens, although there were no significant (P>0.05) differences in nodule number, and nodule dry weight between the treatments compared with the control (Table 1). However, root formation and architecture were affected by the devastating effect of the fungal pathogens, particularly by isolates R103C, R105E, and R108H. The root and shoot biomass dry weight showed significant (P<0.05) differences between the treatments and the control (Table 1).

3.2 Phytopesticide and Pathogens Antagonistic Experiment

The minimum *in-vitro* inhibitory concentration of RHE on the root-/soil-borne fungal pathogens of cowpea was 1.5 % after 9 days incubation at 28°C (Plate 1A-E). All the five fungal pathogens, *F. verticilloides, F. equiseti, F. solani, F. oxysporum,* and *R. solani*, were

significantly (P<0.05) inhibited at 1.5% RHE concentration. The mycelial radial growths of the five fungal pathogens were completely inhibited compared with the control. However, at 1% RHE concentration, the mycelial radial growths of *F. verticilloides*, (R103C), *F. solani* (R108H) and *R. solani* (S102D) were not inhibited completely, whereas the mycelial radial growth of *F. equiseti* (R126F) and *F. oxysporum* were completely inhibited, compared with the control (Plate 1A-E).

3.3 Chemical Composition of Phytopesticide

Chemical analysis of diluted RHE used in this study showed that it contains: PO_4, 9.33 ppm; NH_4, 62.15 ppm: NO_3, 42.87 ppm; Pb, 0.16 ppm Cd, 0.04 ppm; Cr, 0.017 ppm; Co, 0.24 ppm; Ni, 1.24 ppm; Mg, 0.06 ppm; K, 2.007 ppm; Na, 1.28 ppm; Mn, 0.02 ppm; Fe, 1.41 ppm; Cu, 0.011 ppm; Zn, 0.12 ppm; Ca- hardness, 3.108 mg/l; Mg- hardness, 0.246 mg/l; Total- Hardness, 3.355 mg/l; Electrical conductivity, 15 uS; and has pH of 3.2. Pyroligeneous acid concentration of the diluted RHE was not determined due to lack of facilities. However, Yoshida et al. (2000) reported that the concentrated RHE contains 0.25 g/liter of pyroligeneous acid.

3.4 Effect of Interaction between Phytopesticide (RHE) and Fungal Pathogens on Cowpea.

The *in-vivo* antagonistic study between the five fungal pathogens and 1.5% RHE concentration showed greater reduction in the development of disease signs and symptoms. There were significant (P<0.05) effect of genotypes, treatment, and genotypes × treatment interactions in the number of dead plants compared with the control. However, no significant (P<0.05) effects of genotypes, treatments and genotypes × treatment interactions were observed in the number of infected plants, disease incidence, and severity scores compared with the control (Table 2).

The plant heights at 8 WAP showed significant (P<0.05) difference in both genotypes. In genotype 1 RHE+P5 had highest mean value of 21.09 cm followed by RHE+P1, with 17.09 cm, and RHE+P2 with 8.50cm compared to the control with 11.63 However, in genotype 2 the control had the highest plant height at 8 WAP with high level of significant (P<0.05) difference compared to the treatments (Table 2).

Root dry weight, and shoot dry weight showed significant (P<0.05) differences between treatments compared with the controls. Regarding nodulation, RHE+ *F. oxysporum* was significantly (P<0.05) different from the rest of the treatments and the controls but there were no significant (P<0.05) differences between positive control (+), RHE+ *F. verticilloides*, and RHE+ *R. solani* compared to other treatments (Table 2). Moreover, RHE+ *F. verticilloides* and RHE+ *F. oxysporum* were significantly (P<0.05) higher in shoot biomass production than the rest of the treatments. Percentage N in shoot biomass was significantly (P<0.05) higher among many of the treatments relative to the control. However, percentage biomass phosphorus showed significant (P<0.05) difference between genotypes but not significant (P<0.05) differences between the treatments and treatment × genotype interactions (Table 2) were recorded.

4.0 Discussion

Biological control of plant diseases is one of the viable alternatives in sustainable agriculture because it is safe and environmentally friendly (Newsham et al. 1995). However, little work has been done on the potential use of phytopesticides such as RHE as biocontrol agent against cowpea fungal pathogens. The present preliminary findings suggest that natural products from the RHE have high potential for the control of root/soil-borne cowpea fungal pathogens with little or no environmental hazard.This in agreement with the reported work of Abiala et al. (2010) on the use of rice husk extract in the laboratory to control mycelial growth of *Mycosphaerella fijiensis* causing black sigatoka diseases of banana and plantains. Yoshida et al. (2000) also reported that RHE contains pyroligeneous acid which is bioactive secondary metabolite that has inhibitory effects on fungi.

The RHE used in this study completely inhibited the mycelial growth of the five root/soil-borne fungal pathogens *F. verticilloides*, *F. equiseti*, *R solani*, *F. solani* and *F. oxysporum in-vitro* and *in-vivo*. This is in agreement with the result of Peluola (2005) that Neem extract at lower concentration inhibited some fungal pathogens of cowpea in both laboratory and greenhouse experiments. Odebode et al. (2004), had previously confirmed this observation but only for an *in–vitro* experiment. The basic chemical analysis of RHE carried out in this study showed that it is an acidic compound with pH 3.2 which agreed with the report by Yoshida et al. (2000) that RHE contains pyroligeneous acids which completely inhibited the growth of fungal mycelia of *Thanatephorus cucumeris* (MAFF305844) at the concentration of 1/80 in and *F. solani* (MAFF306358) in 1/20 concentration.

The results of the bioassay tests both *in-vitro* in the laboratory and *in-vivo* in the glasshouse suggested that the RHE is a source of naturally occurring bioactive compounds that have antifungal properties which inhibited radial mycelial growth of the five root-/soil-borne cowpea fungal pathogens used in this work.Odebode et al. (2004) reported similar result from the bioassay test carried out on the two annonaceous plants *Isolona cualifora* Verdc and *Cleistochlamys krikii* Benth (Oliv); the crude extract and pure compounds isolated from both plants inhibited both bacterial and fungal pathogens tested.

Colonization of cowpea root by the five root/soil-borne fungal pathogens, i.e., *F. verticilloides*, *F. equiseti*, *R solani*, *F. solani* and *F. oxysporum* were completely inhibited by RHE at 1. 5% leading to reduction in diseases incidence and severity as well as increased total plant biomass accumulation. Our observations are in agreement with the reports of Gharib et al. (2008) that confirmed the effectiveness of extract of aqueous compost in controlling fungi diseases leading to increases in fresh and dry weight of *Majorana hortensis*. In this study, nodule dry weight in of the RHE treatment plants significantly increased compared to that of controls. This might be the result of RHE effectiveness in the control of root borne pathogens. The percentage nitrogen (%N) in biomass in both cowpea genotypes was also affected by the fungal pathogens; nitrogen uptake was higher in IT90K-227-2 compared to IT90K-340-1 however percentage phosphorus (%P) accumulation in dry biomass was not significantly affected.

Killani (2010) observed a linear relationship between the *in-vitro* laboratory experiments and *in-vivo* glasshouse experiments when phytopesticide was used against *F.verticilloides*, *F.equiseti*, *R. solani*, *F.solani* and *F. oxysporum*. Therefore, phytopesticides tested in this present study is a promising potential biocontrol agents as well as bioprotectants against major root-/soil-borne fungal pathogens isolated from the northern Guinea savannan (NGS) cowpea based cropping system.

Table 1**.** Pathogenicity of Root and Soil-borne Fungal Pathogens on Cowpea Variety IT 90K-227-2 *in-vivo*

Isolate Identity	**Isolates Code**	**Plant Height 8WAP (cm)**	**Nodule Number**	**Nodule Dry Weight (mg)**	**Root Dry Weight (g)**	**Shoot Dry Weight (g)**
F. equiseti	R101A	17.13ab	2b	1.00b	1.07cd	2.80c
F. oxysporum	S106B	17.63ab	3b	4.00b	0.94d	3.38bc
F. oxysporum	R103C	7.75c	0b	0.00b	0.04e	0.16d
R. solani	S102D	15.25b	3b	5.00b	1.31a	3.00bc
F. verticilloides	R105E	0.00d	0b	0.00b	0.00e	0.00d
F. equiseti	R126F	16.38b	3b	3.00b	1.25ab	3.51b
F. oxysporum	S117G	16.63ab	3b	2.00b	1.12bc	3.25bc
F. solani	R108H	0.00d	0b	0.00b	0.00e	0.00d
F. oxysporum	S109I	17.25ab	2b	2.00b	1.27ba	3.09bc
Fusarium spp	R110J	17.75ab	4b	24.00b	1.07dc	2.76c
R. solani	S113K	17.75ab	2b	4.00b	1.17abc	3.08bc
F. oxysporum	S112L	18.00ab	1b	2.00b	1.14abc	3.27bc
Control	C100M	19.88a	24a	13.50a	1.15abc	4.49a
CV (%)		7.80	79.07	121.71	10.05	13.00

*Means within column followed with same letter are not significantly different at 95% confidence interval (P<0.05) analyzed using GLM procedure with DMRT test, CV (%) = Coefficient of variation in percentage; 8WAP = 8 Weeks After Planting.

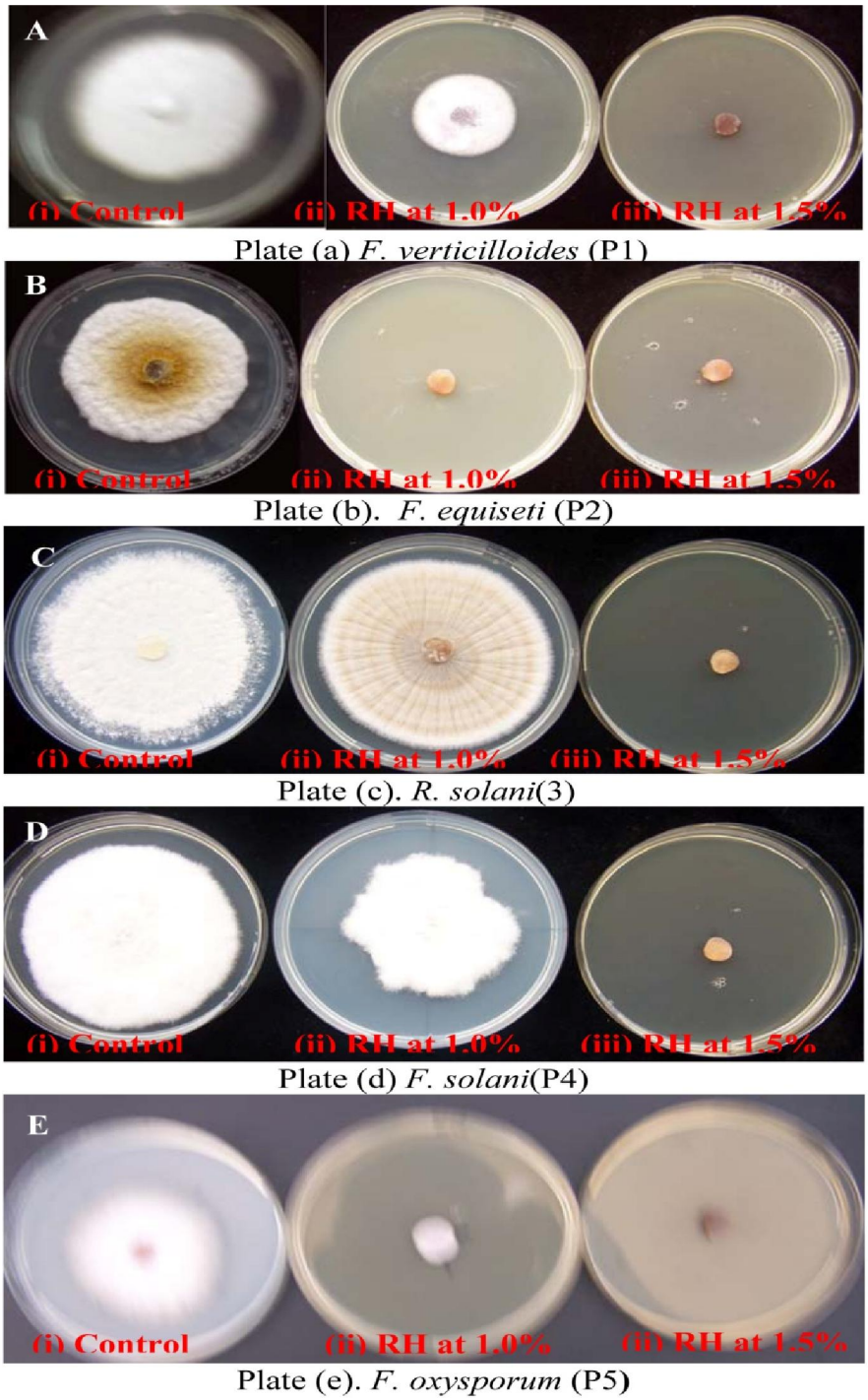

Plate 1A-E. Inhibitory effect of RHE on the mycelial growth of A = *F. verticilloides*, B = *F. equiseti*, C = *R. solani*, D = *F. solani*, E = *F. oxysporum* at two concentration after 7 days of incubation at 28°C ± 2°C

Table 3: Effect of interaction between phytopesticide (RHE), five fungal pathogens on cowpea *in-vivo*

Treat	gen	no dpl	no ipl (%)	dis incs (%)	dis sev (%)	plt ht (cm)	rt dwt (g)	sh dwt (g)	N (%)	P (%)
RHE+P1	1	0.33	0.00	0.00	0.00	17.76	2.04	6.27	1.09	0.13
RHE+P2	1	2.67	0.00	0.00	0.00	8.50	1.10	3.19	0.77	0.14
RHE+P3	1	1.00	0.00	0.00	0.00	14.89	1.63	4.63	1.29	0.10
RHE+P4	1	2.00	0.00	0.00	0.00	13.13	0.76	3.70	1.81	0.10
RHE+P5	1	0.33	0.00	0.00	0.00	21.09	2.35	6.52	1.13	0.10
Control	1	0.00	0.00	0.00	0.00	11.63	0.63	2.04	4.71	0.08
Means		1.06	0.00	0.00	0.00	14.50	1.42	4.39	1.80	0.11
RHE+P1	2	0.33	0.00	0.00	0.00	13.88	1.71	4.39	1.16	0.14
RHE+P2	2	0.33	0.00	0.00	0.00	13.88	1.83	5.13	1.30	0.12
RHE+P3	2	0.00	0.00	0.00	0.00	18.40	1.80	5.62	1.46	0.10
RHE+P4	2	0.00	0.00	0.00	0.00	15.95	1.35	4.93	1.36	0.13
RHE+P5	2	0.67	0.00	0.00	0.00	14.90	1.91	4.94	1.58	0.13
Control	2	0.00	0.00	0.00	0.00	20.65	3.26	5.51	1.36	0.15
Means		0.22	0.00	0.00	0.00	16.28	1.98	5.09	1.37	0.13
S.E.										
Genotype		0.13	0.00	0.00	0.00	0.38	0.10	0.23	0.07	0.005
Treatment		0.24	0.00	0.00	0.00	0.67	0.19	0.44	0.13	0.009
Genotype*treatment		0.35	0.00	0.00	0.00	0.94	0.27	0.62	0.18	0.013
F value										
Genotype		***	ns	ns	ns	**	***	**	***	**
Treatment		**	ns	ns	ns	***	**	*	***	ns
Genotype*treatment		**	ns	ns	ns	***	***	***	***	*

Treat = Treatment, S.E. = standard error, ns = non significant at (P≤0.05), * = significant at (P≤0.05), ** = significant at (P≤0.01), ***= significant at (P≤0.001), gen = genotype; 1 = IT90k-277-2; 2 = IT97K-340-1, nodpl = number of dead plants; noipl = number of infected plants; disincs = disease incidence score; dissev = disease severity score; pltht = plant height; rtdwt = root dry weight; shdwt = shoot dry weight; N% = percentage nitrogen uptake in dry shoot; P % = percentage phosphorus accumulation in dry shoot; RHE = rice husk extract, P1 = *F. verticilloides*, P2 = *F. equiseti*, P3 = *R. solani*, P4 = *F. solani*, P5 = *F. oxysporum*.

Conclusion

Application of phytopesticide (RHE) in this study for the control of root/soil-borne fungal pathogens of cowpea is a promising control strategy for the management of cowpea fungal pathogens in the field. The RHE also exhibited great phytoprotectant capability due to its good *in- vitro* and *in-vivo* inhibitory performance in the control and the reduction of the root/soil-borne fungal cowpea pathogens.

Aknowledgement

The authors are grateful to IITA for supporting this research study.

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References

[1] Abiala, M.A, Ogunjobi, A.A, Odebode, A.C and Ayodele, M.A. Microbial Control of Mycosphaerella fijiensis Morelet A Notable Pathogen of Bananas and Plantains, Nature and Science 2010;8(10):299-305.
[2] Awuah, R.T. Fungitoxic effects of extract from some West African plants. Annals of Applied

Biology. 1989;115:451-54.
[3] Booker, R.H. Pests of cowpea and their control in northern Nigeria. Bulletin of Entomology Research 1965;55:63-72.
[4] Cooke, B.M. Disease Assessment and Yield Loss. In: Cooke, B.M., D.G. Jones and B. Kaye (eds.), The Epidemiology of Plant Diseases, 2nd edition, Springer, The Netherlands 2006;43-80..
[5] Domsch, K.H., Gams, W. and Anderson, T.H. Compendium of Soil Fungi.: Academic Press, London 1980;840-841.
[6] Emechebe, A.M. and Shoyinka, S.A. Fungal and bacterial diseases of cowpeas in Africa. In: Cowpea Research, Production and Utilization. Singh, S.R. and Rachie, K.O. (Eds.). John Wiley & Sons Ltd. Great Britain1985;173-97.
[7] Gharib, F.A., Moussa, L.A. and Massoud, O.N., Effect of compost and bio-fertilizers on growth, yield and essential oil of sweet marjoram (*Majorana hortensis*) plant. International Journal of Agriculture and Biology 2008,10:381–87
[8] IITA (International Institute of Tropical Agriculture). Automated and semi-automated methods of soil and plant analysis manual series, IITA 1982;7:33.
[9] Killani**,** A. S. Biological Control of Root-/Soil-Borne Fungal Pathogens of Cowpea Isolated in the Northern Guinea Savannah of Nigeria. PhD submitted to University of Agriculture, Abeokuta, Ogun State, Nigeria 2010. 234pp.
[10] Koch, R. Uber bakteriologische Forschung Verhandlung des X Internationalen Medichini-schen Congresses, Berlin, 1890, 1, 35. August Hirschwald, Berlin. (In German.). Xth International Congress of Medicine 1891.
[11] Komarwa, P.M., Manyong, V.M. and Chianu, J.N. Cowpea demand and supply patterns in West Africa: The case of Nigeria. In: Challenges and opportunities for enhancing sustainable cowpea production. Edited by Fatokun, C.A., Tarawali, S.A., Singh, B.B., Komarwa, P.M., and Tamo, M. 2002;376-86.
[12] Lichtenzveig, J., Anderson, J., Thomas, G., Oliver R., and Singh, K. Inoculation and growth with soil-borne pathogenic fungi *M. truncatula* handbook 2006;10.
[13] Mukerji, K. G. and Ciancio, A. Mycorrhizae. In: The Integrated Pest and Disease Management: General Concepts in Integrated Pest and Disease Management section 2007;2:245-266.
[14] Nash, S.M. and Snyder, W.C. Quantitative estimations by plate counts of propagules of the bean root rot of *Fusarium oxysporum* and strains of *Pseudomonas putida.* Canadian Journal of Microbiology. 1962;33:349-353.
[15] Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. Fusarium species: an illustrated manual for identification. The Pennsylvania State University Press, University Park 1983;193.
[16] Newsham, K.K., Fitter, A.H. and Watkinson, A.R. Arbuscular mycorrhiza protect an annual grass from from root pathogenic fungi in the field. Journal of Ecology 1995;83:991–1000.
[17] Odebode, A.C., Madachi, S.J.M., Joseph, C.C., and Irungu, B.N. Antibacterial activities of constituents from *Isolona cualifora* Verdc and *Cleistochlamys krikii* Benth(Oliv) (Annonaceae). Journal of Agricultural Sciences 2004;49(1): 109-16.
[18] Olsen, S.R, Cole, C.V., Watanabe, F.S. and Dean, L.A. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Development Agency Circular 1954; 939.
[19] Peluola, C.O. Evaluation of botanicals and microbial bioagents for the control of some fungal pathogens of cowpea (*Vigna unguiculata* (L) Walp.). PhD submitted to University of Ibadan, Ibadan, Oyo State, Nigeria 2005.
[20] Ros, M., Hernandez, M.T., Garcia, C., Bernal, A. and Pascual, J.A. Biopesticide effect of green compost against Fusarium wilt on melon plants. Journal of Applied Microbiology 2005;98(4): 845–54.
[21] SAS (Statistical Analysis System). SAS/STAT Guide for Personal Computers, Version 9.2 (TSIMO) Edition, SAS Institute Inc. Cary, North California; USA, 2008;1028pp.
[22] Singh, S.R., Jackai, L.E.N., Cardwell, K., Singh, B.B., Tare, B.N.; Rossel, H.W, Thottapilly, G., Ng, N.G., Hossien, M.A., Padulosi, S., and Mayers, G. Cowpea research at International Institute of Tropical Agriculture, Ibadan Nigeria. GLIP Monograph 1989;1:19.
[23] Summerell, B.A., Rugg, C.A. and Burgess, L.W. Mycogeography of Fusarium: survey of *Fusarium* species associated with forest and woodland communities in north Queensland, Australia. Mycological Research 1993;97:1015-019.
[24] Yoshida, T., Terao, H., Tsuzuki, E. and Kamiunten, H. Effect of components of pyroligeneous acid on the several plant pathogenic fungi. Japan Journal of Crop Science 2000; 69(2):196-97.

Submission date: October 15, 2010