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Biocontrol agents in combination with *Moringa oleifera* extract for integrated control of *Sclerotium*-caused cowpea damping-off and stem rot

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Abstract

Damping-off and stem rot disease-causing *Sclerotium rolfsii* has been reported as a destructive soil-borne pathogen of numerous crops, especially in the tropics and subtropics. Trials were conducted to test the efficacy of biocontrol agents alone or combined with *Moringa oleifera* leaf extracts for the control of the disease. In the laboratory, PDA was amended with *Moringa* leaf extract, and mycelial growth of *S. rolfsii* was measured. In the greenhouse and field, *Trichoderma* Kd 63, *Trichoderma* IITA 508 and *Bacillus subtilis* were evaluated as seed treatments, soil drench or sprinkle, separately or combined with *Moringa* leaf extracts. Percentage disease incidence, severity and control were recorded. In the laboratory, the higher the extract concentration the less the mycelial growth and no mycelial growth occurred on extract at 15 or 20 g leaves 10 ml^{-1} water. In the greenhouse, the highest disease control was observed at a *Moringa* extract concentration of 15 kg leaves 10 l^{-1} water (w/v). Seed treatments using *Trichoderma* Kd 63, and soil sprinkle using *Trichoderma* IITA 508 had a significantly ($P = 0.05$) higher effect on a disease incidence than *Bacillus*. Disease severity followed the same pattern. *Moringa* seed treatment combined with *Trichoderma* soil sprinkle resulted in significantly more than 94% and 70% disease control in the greenhouse and field, respectively, with significant yield increase in the field. This is the first report of *Moringa* leaf extract combined with *Trichoderma* as an integrated control for *Sclerotium* damping-off and stem rot of cowpea in the field.

Introduction

Sclerotium rolfsii (teleomorph: *Athelia rolfsii*) is a soilborne plant pathogenic fungus that causes diseases in over 500 plant species (Punja, 1985) including cowpea, *Vigna unguiculata* (Adandonon et al., 2003). Control of *Sclerotium* damping-off and stem rot may be achieved by cultural means or by applying fungicides (Punja, 1985). In Benin, the only registered fungicide used on edible crops, such as cowpea, is Super-Homai 70% PM (active ingredient: methylthiophanate 35%, thiram 20%, and diazinon 15%) (SPV, Benin). Unfortunately, there is a problem regarding the efficacy of this product (Kakpo-Zannou, Pers. comm.). Despite the effectiveness of synthetic fungicides, there are potential harmful effects on human health and the environment (Demoz and Korsten, 2006). There is then a need to examine possible non-synthetic chemical approaches for disease management.

Research has demonstrated that biological control (Widyastuti et al., 2003; Jacobsen et al., 2004) is a potentially feasible alternative to the use of pesticides (Lewis et al., 1993; Madi et al., 1997).

Micro-organisms used as biological control agents (BCAs) include *Trichoderma harzianum* against soilborne pathogens (Widyastuti et al., 2003), and *Bacillus* against root rot (Jacobsen et al., 2004) and postharvest diseases (Demoz and Korsten, 2006; Govender and Korsten; 2006). Wokocha (1990) and Tu (1997) indicated that disease control is enhanced when BCAs are integrated with minimum use of pesticide dosage. *Trichoderma* combined with chemicals such as metalaxyl (Howell et al., 1997) has been effectively tested for control of soilborne diseases. However, not only do chemical residues remain after treatment with synthetic fungicides, but some fungicides are also toxic to BCAs (Papavizas et al., 1982). As alternative to synthetic fungicides, plant extracts with fungicidal properties could be used (Stoll, 1988; SIBAT, 1993; Obagwu et al., 1997). *Moringa oleifera* leaf extracts have been successfully used as a seed treatment against some soilborne fungi in cereals (Stoll, 1988). The difficulties with plant extracts are that the active ingredients break down easily, thereby reducing persistence of the compound. Stoll (1988) proposed addition of kerosene to the extracts to improve active ingredients extraction and reduce their degradation. So far, there are no or few reports on using plant extracts integrated with BCAs to control *Sclerotium* damping-off and stem rot in the field.

The main objective of this study was to evaluate different formulations of *T. harzianum*, *B. subtilis* and *Moringa* extracts (as a plant-based fungicide), and their performance either on their own or in combination, for integrated control of *Sclerotium* damping-off and stem rot of cowpea in the greenhouse and field.

Material and methods

Pathogen culture and inoculum

Sclerotium rolfsii strain IITA 409 was isolated from a diseased cowpea plant collected in 2001 from the Ouémé valley. The fungus was sub-cultured and maintained on potato dextrose agar (PDA) slants at 4 °C. Fungal inoculum was prepared using the millet seed inoculum technique (Weideman and Wehner, 1993). Five 5 mm diam. discs cut from a *S. rolfsii* colony on PDA were used to inoculate 50 g of sterilised millet seed. The inoculated millet seed was incubated for 21 days at 27 °C, and subsequently air-dried in a paper bag, lightly ground with mortar and pestle and passed through nested sieves with 3 mm diameter openings. Sandy loam soil was pasteurised by aerated steam (60 °C for 30 min) and stored for 14 days before inoculation with the isolate.

Cowpea plant material

Two susceptible cowpea cultivars, Tchawé kpayo (local from the Ouémé valley, Benin,) and IT89-KD-374-2 (IITA) were used. Before planting, the seeds were surface-sterilized in 1% NaOCl for 2 min and rinsed in sterile distilled water (SDW).

Biological control agents (BCAs) and formulation

Trichoderma IITA 508 (isolated from a diseased cowpea stem, Cotonou, Benin), *Trichoderma* Kd 63 (Dr M. Morris, Plant Health Products, Pietermaritzburg, South Africa) and *B. subtilis* (Prof P. L. Steyn, Stimuplant CC, Mooiplaats, Pretoria, South Africa) were used. *Trichoderma* Kd 63 and *B. subtilis* were each in powder formulation with 10^9 colony forming units (cfu) g^{-1} powder. *Trichoderma* IITA 508 had shown *in vitro* inhibitory action against *S. rolfsii* in a previous study (Adandonon et al., 2003) and was used in the current study in millet seed inoculum formulation. *Trichoderma* Kd 63 or *B. subtilis* were used at 5 g powder per litre distilled water, as recommended by the manufacturer.

In vitro *effects of* Moringa *leaf extracts on mycelial growth of* S. rolfsii

Fresh *Moringa* leaves were collected and 5, 10, 15 and 20 g samples were weighed, surface-sterilised and rinsed in SDW and separately crushed into a pulp using sterilised porcelain mortars and pestles. The pulp of each sample was suspended in 10 ml of SDW. Three different volumes (0.1, 0.2 and 0.3 ml) of kerosene were separately added to each of the four extract suspensions to yield final concentrations (of kerosene) of 1, 2 and 3% v/v. The extract-kerosene suspensions were agitated for 2 min, stored overnight at 26–28 °C, and filtered through sterile cotton cloth into another sterile Erlenmeyer flask to yield the final extract. Based

on a preliminary study (Adandonon, unpublished), 30 ml of the extract was added to 300 ml cooled PDA amended with chloramphenicol (25 mg l^{-1}) and agitated to allow for proper mixing of extract and media. Therefore, the final concentration in agar medium was 10%. Twenty-millilitre aliquots of the amended media were dispensed into 90 mm Petri dishes. Once the amended agar had solidified, 2 mm discs cut from 3 day-old colonies of *S. rolfsii* on PDA were placed in the centre of each plate. The controls consisted of the pathogen grown on unamended PDA and kerosene amended PDA, respectively. Five replicates of four plates per replicate per concentration were used. Plates were incubated at 25 ± 1 °C and the radial growth of the colonies was measured after 5 days of incubation.

In vivo *effects of* Moringa *leaf extracts,* Trichoderma *Kd 63,* Trichoderma *IITA 508,* B. subtilis *and their combination on damping-off and stem rot incidence in the greenhouse*

For the *in vivo* tests of *Moringa* leaf extract, 0.5, 1.0, 1.5 and 2.0 kg of *Moringa* leaves were weighed out, ground and the pulp suspended in 1 l of SDW. These quantities of the crude leaf extracts were in the same proportions as those used in the *in vitro* experiment. In the *in vitro* experiment, results (Table 1) showed no significant difference in colony growth among the three different doses of kerosene (plus *Moringa* extracts) tested. Therefore, only 1% kerosene (v/v) was included in the *in vivo* tests. Treatments included: *Moringa* extract combined with kerosene (1% v/v) and Marseilles soap (0.1% w/v), *Moringa* extract with 1% kerosene only, *Moringa* extract with 0.1% soap only, *Moringa* extracts alone, water alone, 1% kerosene alone, 0.1% soap alone, Super-Homai 70% PM (synthetic fungicide) and untreated control. The use and dosage of Marseilles soap in this experiment was based on earlier results with other plants (Stoll, 1988).

In the powder application experiment, three dosages (0.05, 0.1 and 0.15 g powder) of each commercial product (*Trichoderma* Kd 63 and *B. subtilis*) were tested. Each quantity was mixed with 5 g disinfected soil and sprinkled into the planting hole at planting. Three controls were included: pasteurised soil mixed with 10 g millet seed inoculum, pasteurised soil without millet seed inoculum and seed treatment with the fungicide Super-Homai applied at the rate of 40 g powder for 10 kg cowpea seeds.

In the comparative effect experiment, there were 11 treatments: seed treatment, soil drench, and soil sprinkle of *Trichoderma* Kd 63 or *B. subtilis*, seed treatment of *Moringa* leaf extracts, soil sprinkle of *Trichoderma* IITA 508, seed treatment of *Moringa* leaf extracts + soil sprinkle of *Trichoderma* Kd 63, seed treatment of *Moringa* leaf extracts + soil sprinkle of *B. subtilis* and seed treatment of *Moringa* leaf extracts + soil sprinkle of *Trichoderma* IITA 508. Suspensions of *Trichoderma* Kd 63 and *B. subtilis* (5 g l^{-1} water) were soil-drenched at 3 ml solution per planting hole shortly after cowpea seed planting. In the comparative effects of different concentrations of *Moringa* extracts, 15 or 20 kg leaves 10 l^{-1} SDW yielded the highest significant effect. Therefore, extract concentration of 15 kg leaves 10 l^{-1} SDW (plus kerosene (1%) and soap (0.1%)) was evaluated. Before planting, seed treatments consisted of soaking cowpea seeds in *Trichoderma* Kd 63 or *B. subtilis* cell suspension (5 g l^{-1}) and in *Moringa* leaf extract solutions for

Table 1. Effect of *Moringa* leaf extract on the mycelial growth of *Sclerotium rolfsii* on PDA

Treatments[a]	Colony diameter (mm)
*Moringa*5 + K1%	33.6 c[b]
*Moringa*5 + K2%	36.5 c
*Moringa*5 + K3%	38.7 c
*Moringa*10 + K1%	14.4 b
*Moringa*10 + K2%	17.8 b
*Moringa*10 + K3%	12.3 b
*Moringa*15 + K1%	0.0 a
*Moringa*15 + K2%	0.0 a
*Moringa*15 + K3%	0.0 a
*Moringa*20 + K1%	0.0 a
*Moringa*20 + K2%	0.0 a
*Moringa*20 + K3%	0.0 a
*Moringa*5	54.1 d
*Moringa*10	37.2 c
*Moringa*15	20.6 b
*Moringa*20	13.7 b
K1% alone	75.3 e
K2% alone	69.0 e
K3% alone	70.4 e
Unamended PDA (control)	88.1 f

[a]*Moringa* extract at 5, 10, 15 and 20 g 10 ml^{-1} SDW mixed with kerosene (1, 2, 3%, v/v) to amend PDA at the rate of 10% (v/v).
[b]Each value is a mean of five replicates. Values not followed by the same letters are significantly different ($P = 0.05$) according to Student Newman Keuls.

5 min. The seeds were left to dry for 2 min at air temperature and then planted. In the powder application experiment, there was no difference ($P < 0.05$) among all three tested dosages of *Trichoderma* whereas a *Bacillus* dosage of 0.1 or 0.15 g powder per 5 g soil yielded less diseased plants than that of 0.05 g per 5 g soil. The *Trichoderma* Kd 63 and *Bacillus* powder dosage of 0.05 g and 0.1 g, respectively, per 5 g soil per hole were evaluated further. The *T. harzianum* IITA 508 millet seed inoculum (prepared as described for *S. rolfsii)* was sprinkled at the rate of 0.1 g per 5 g soil per hole. This inoculum dosage of *T. harzianum* IITA 508 was based on a preliminary study (Adandonon, unpublished). In the integrated control evaluation, seeds were first treated with *Moringa* leaf extract and planted before the soil mixed with BCAs was sprinkled into the planting hole.

Soil inoculation was done 2 days before planting by mixing 10 g of the *S. rolfsii* millet seed inoculum with 1 kg steam-pasteurized sandy loam soil in a pot. Surface-sterilised seeds of cowpea cultivars Tchawé kpayo and IT89-KD37457 were treated respectively and then planted at four seeds per pot (one replicate). Treatments were arranged in a randomised block design with four replicates. Pots were kept in the greenhouse at temperatures varying between 23 and 30 °C.

The number of damping-off seedlings and stem rot plants was visually recorded 2 days after planting and everyday thereafter until 30 days after planting. The percentage disease control per treatment was calculated as follows:

$$\mathrm{DR}\% = [1 - (\mathrm{DT}/\mathrm{DC})] * 100$$

where DR: disease control or reduction; DT: disease incidence on the treatment unit; DC: disease incidence on the control unit (zero treatment).

The symptoms on plants were rated using a scale of 0–6 to determine the disease severity (Adandonon et al., 2003) as follows: 0: no visible symptoms; 1: leaves did not wilt; plants fell over on the ground after the 4th day; 2: leaves wilted on 3rd day; plants fell over on the ground on 4th day; 3: leaves wilted on 3rd day; plants fell over on the ground on 3rd day; 4: leaves wilted on 2nd day; plants fell over on the ground on 2nd or 3rd day; 5: leaves wilted on 1st day; plants fell over on the ground on 2nd day and; 6: leaves wilted; plants fell over on the ground within 24 h.

To fulfil Koch's postulates, dying seedlings were removed at each observation, and at least one plant from each pot was assayed to verify the presence of the appropriate fungal species. The reisolated fungus was cultured on PDA and colony characteristics were recorded and compared to the original isolates.

Field experiment

The experiment was conducted between 2002 and 2003 in the Ouémé valley, Benin to test the performance of the treatments and correlate greenhouse and field experiment results. In the field, the soil was known to be naturally infected with the pathogen. The air temperature and relative humidity recorded in the field between 08.00 h and 14.00 h varied between 25.7 to 27.8 °C and 62 to 97%, respectively. Each treatment was assigned to a plot of 200 m^2. Two cowpea cultivars, namely Tchawé kpayo and IT89-KD-374-57, were used. The experimental design was a randomised block design including all treatments tested in the greenhouse experiment, four replicates and two controls: plot planted with untreated seeds and plot planted with Super-Homai-treated seeds. The plots were planted and weeded by the farmers themselves using traditional cultural practices. The plant intervals were those of farmers. The number of damping-off seedlings and stem rot plants was visually recorded 7 days after planting and at 7-day intervals thereafter until 30 days after planting.

Statistical analysis

The percentage data were arcsine (Y1/2) transformed. The analysis of variance was performed using the general linear model (GLM) procedure in the SAS System (SAS, 1997) and mean separations were done using the Student Newman Keuls (SNK) option.

Results

In vitro *effects of* Moringa *leaf extracts on mycelial growth of* S. rolfsii

The recorded mean fungal radial growth (mm) for each treatment is presented in Table 1. In all cases

where PDA was amended with the extract alone or mixed with kerosene, it resulted in significantly less mycelial growth than that of kerosene alone-amended PDA or unamended PDA (control) (Table 1). Extracts combined with kerosene, showed less mycelial growth than did respective extracts alone. At a given extract concentration, there was no significant difference among the kerosene doses. The effect of extract was more pronounced at higher concentrations: the higher the extract concentration the less the mycelial growth and no colony mycelium was formed on PDA amended with extracts at 15 and 20 g leaves 10 ml^{-1} water. There was no significant difference ($P < 0.05$) between extracts at 15 and 20 g leaves 10 ml^{-1} water (Table 1).

In vivo *effects of* Moringa *leaf extracts,* Trichoderma *Kd 63,* Trichoderma *IITA 508,* B. subtilis *and their combination on damping-off and stem rot incidence in the greenhouse*

The disease incidence recorded with the *Moringa* leaf extracts, alone or mixed with kerosene and soap, regardless of extract concentration was the least, compared to either control, kerosene alone, soap alone, water or Super-Homai (Table 2). At a given concentration, extract mixed with both kerosene and Marseilles soap yielded disease incidences less than that of extract mixed with either kerosene or soap. No or 0.1% disease incidence was recorded with an extract concentration of 15 or 20 kg leaves 10 l^{-1} water mixed with kerosene and soap. The treatment effect trend in terms of disease incidence was similar for both cowpea cultivars used (Table 2). The lowest disease severity was recorded, in both cowpea cultivars, when seeds were treated with *Moringa* extract concentration of 15 or 20 kg 10 l^{-1} water combined with kerosene and soap.

In the powder dosage experiment, percentage diseased plants recorded were significantly less ($P < 0.05$) in soil treated with *Trichoderma* Kd 63, compared to other treatments (Table 3). There was no significant difference ($P < 0.05$) among all powder dosages of *Trichoderma* Kd 63 tested. *Bacillus* powder dosage of 0.1 or 0.15 per 5 g soil per hole yielded significantly ($P < 0.05$) less diseased plants than that of 0.05 g soil per hole. No significant difference ($P > 0.05$) was detected between the *B. subtilis* dosage of 0.05 g per 5 g soil per hole, Super-Homai and the untreated, infested control. Disease severity followed a similar pattern (Table 3).

In the comparative effect experiment of *Moringa* leaf extracts and biological control treatment (soil drench, seed treatment or soil sprinkling), *Trichoderma* treatment effects were significantly higher than those of *Bacillus*. Furthermore the BCA (*Trichoderma* Kd 63 or *B. subtilis*), seed treatments yielded the lowest percentage diseased plants, followed by the soil sprinkling and soil drench treatments. Results presented in Table 4 show that seed treatments of *Moringa* leaf extracts, seed treatments of *Trichoderma* Kd 63 and soil sprinkle of *Trichoderma* IITA 508 had significant ($P < 0.05$) effects on disease incidence, when compared to other treatments, untreated *Sclerotium*-inoculated pasteurised soil (control) and Super-Homai (fungicide) treatments. A remarkable improvement in the performance of the antagonists was observed when *Moringa* seed treatments were combined with *Trichoderma* or *Bacillus* soil sprinkles (Table 4).

Field experiment

The disease incidence recorded with the untreated control was the highest (16.8%) compared to all treatments. The trend of disease incidence for the treatments in the field was similar to that recorded in the greenhouse (Table 4). Seed treatment with *Moringa* leaf extracts and *Trichoderma* Kd 63, and soil sprinkle of *Trichoderma* IITA 508 millet seed inoculum yielded the lowest percentage of diseased plants and the highest percentage of disease control, regardless of the cultivars. The disease control trend of the field treatments was similar to but less than that in the greenhouse (Table 4). At harvest, all BCAs or *Moringa* extracts, when applied alone, performed significantly better than the untreated control in terms of recorded yields in the field. However, the highest yields were recorded when the *Moringa* extract seed treatment was combined with soil sprinkles of *Trichoderma* Kd 63 or *Trichoderma* IITA 508 or *B. subtilis*. The trend was similar for both cultivars (Table 4). Disease incidence, control and yield were less in the second year (2003) experiment in the field.

Table 2. Effect of seed treatment with *Moringa* leaf extracts in various combinations with kerosene and Marseille soap applied on the severity, incidence and control of *Sclerotium rolfsii* damping-off and stem rot of cowpea in the greenhouse

Treatments[a]	Disease severity[b]		Disease incidence (%)		Disease control (%)	
	Tchawé kpayo	IT89-KD 374-57	Tchawé kpayo	IT89-KD 374-57	Tchawé kpayo	IT89-KD 374-57
*Moringa*5 + KS	3.0 bcde[c]	4.3 hi	35.9 f	32.9 de	64.1 e	66.7 ef
*Moringa*10 + KS	1.2 ab	0.9 b	11.0 b	9.0 b	89.0 i	90.9 h
*Moringa*15 + KS	0.0 a	0.1 a	0.0 a	0.0 a	100.0 j	100.0 i
*Moringa*20 + KS	0.0 a	0.0 a	1.1 a	0.0 a	98.9 j	100.0 i
*Moringa*5 + K	3.9 cdef	4.1 ghi	45.9 h	50.9 f	54.1 c	48.5 d
*Moringa*10 K	3.1 bcde	3.7 fg	32.8 ef	30.0 d	67.2 ef	69.7 f
*Moringa*15 + K	2.6 bcde	3.1 de	24.9 de	19.0 c	75.1 fg	80.8 g
*Moringa*20 + K	1.9 abc	2.9 d	16.1 bc	17.9 c	83.9 hi	81.8 g
*Moringa*5 + S	4.4 ef	3.1 de	48.2 h	51.9 f	51.8 c	47.5 d
*Moringa*10 + S	3.2 bcde	4.5 ij	30.9 ef	31.9 de	69.1 ef	67.7 ef
*Moringa*15 + S	2.0 abc	2.9 c	22.0 cd	16.1 c	78.0 gh	83.8 g
*Moringa*20 + S	2.2 bcd	2.3 c	19.0 cd	20.0 c	81.0 gh	79.8 g
*Moringa*5	4.4 ef	4.6 ij	60.0 i	57.9 g	40.0 b	41.4 c
*Moringa*10	4.3 def	3.5 ef	44.9 gh	46.9 f	55.1 cd	52.5 d
*Moringa*15	3.2 bcde	3.5 ef	37.2 fg	36.0 e	62.8 de	63.6 e
*Moringa*20	2.6 bcde	3.8 fgh	33.9 f	30.0 d	66.1 e	69.7 f
K	4.3 def	4.2 ghi	94.9 j	97.8 i	5.1 a	1.0 a
S	5.5 f	4.1 ghi	97.0 j	100.0 i	3.0 a	0.0 a
Water	4.5 ef	4.6 ij	100.0 j	99.1 i	0.0 a	0.0 a
Super-Homai	3.3 bcde	3.5 ef	66.0 i	72.8 h	34.8 b	26.3 b
Untreated control	5.7 f	5.0 j	100.0 j	98.9 i	0.0 a	0.0 a

[a]*Moringa* extracts at 5, 10, 15 or 20 kg leaves 10 l^{-1} SDW mixed with Kerosene (1%) and Marseille soap (0.1% w/v). K = kerosene; S = Marseille soap.
[b]Severity was rated on a scale of 0–6. Each value is a mean of four replicates.
[c]In the same column, means followed by the same letter are not significantly different ($P = 0.05$) according to the General Linear Model using the Student Newman Keuls option.

Table 3. Effect of different dosages of *Trichoderma* Kd 63 and of *B. subtilis* on *Sclerotium rolfsii* damping-off and stem rot incidence and severity on cowpea in the greenhouse

Treatments[a]	Diseased plants (%)	Disease severity[b]
Trichoderma Kd 63, (0.05)	20.8 b	1.3 b[c]
Trichoderma Kd 63, (0.10)	16.7 b	1.1 b
Trichoderma Kd 63, (0.15)	21.0 b	1.0 b
Bacillus (0.05)	83.3 de	3.8 d
Bacillus (0.10)	56.7 c	2.1 c
Bacillus (0.15)	59.2 c	1.9 c
Non-treated inoculated control	95.8 e	4.9 e
Uninoculated control	0.0 a	0.0 a
Super Homai	80.4 d	3.4 d

[a]Powder inoculum of *Trichoderma* Kd 63 and *B. subtilis* at a rate of 0.05, 0.1 and 0.15 g inoculum per 5 g soil per hole.
[b]Severity was rated on a scale of 0–6. Each value is a mean of four replicates.
[c]In the same column, means followed by the same letter are not significantly different ($P = 0.05$) according to the General Linear Model using the Student Newman Keuls option.

Discussion

The use of plant extracts and BCAs is seen as a viable method for controlling plant diseases (Stoll, 1988; SIBAT, 1993; Howell et al., 1997; McLean et al., 2005). Results from the present study show that *Moringa* leaf extracts are effective against *Sclerotium* mycelial growth on PDA. No growth

Table 4. Effects of *Moringa oleifera* leaf extracts, *Trichoderma* Kd 63, *Trichoderma* IITA 508 and *Bacillus subtilis* on *Sclerotium* damping-off and stem rot of cowpea seed yield in the greenhouse and field in 2002

Treatments	Diseased plants (%)			Disease severity[a]	Disease control (%)			Seed yield (kg ha^{-1})	
	Field		Greenhouse	Greenhouse	Field		Greenhouse	Field	
	Tchawé kpayo	IT89-KD-374-57	Tchawé kpayo	Tchawé kpayo	Tchawé kpayo	IT89-KD-374-57	Tchawé kpayo	Tchawé kpayo	IT89-KD-374-57
Trichoderma Kd 63 soil drench	12.2 ef[b]	13.9 f	38.9 f	2.7 g	29.48 de	17.26 b	60.14 de	679.2 bc	658.1 c
Bacillus soil drench	14.4 gh	15.8 fg	49.8 g	3.9 h	16.76 bc	5.95 ab	48.97 cd	641.1 ab	650.0 c
Moringa extract sead treatment (MEST)	8.3 cd	6.1 bc	5.5 abc	1.1 cde	52.02 fg	63.69 ef	94.36 hij	791.4 de	776.4 f
Trichoderma Kd 63 seed treatment	9.1 d	9.8 de	13.9 bc	1.3 de	47.40 f	41.67 cd	85.76 ghi	775.2 d	764.2 f
Bacillus seed treatment (BST)	12.8 efg	11.7 e	32.7 de	2.7 g	26.01 cde	30.36 c	66.50 ef	683.5 c	690.1 d
Trichoderma IITA 508 millet seed inoculum soil sprinkling (TiSS)	6.9 bc	7.8 cd	15.3 cd	1.5 ef	60.12 gh	53.57 de	84.32 gh	804.7 de	810.3 g
Trichoderma Kd 63 Soil sprinkling (TkSS)	11.3 e	10.9 e	25.1 de	1.9 f	34.68 e	35.12 c	74.28 fg	698.1 c	724.5 e
Bacillus soil sprinkling (BSS)	13.5 fg	14.7 fg	56.8 g	3.2 g	21.97 cd	12.50 ab	41.80 c	665.2 bc	644.2 bc
MEST + TiSS	2.5 a	1.8 a	1.2 a	0.5 ab	85.55 i	89.29 g	98.77 j	965.3 g	987.3 i
MEST + TkSS	5.1 b	4.9 b	2.5 a	0.7 bc	70.52 h	70.83 f	97.44 ij	858.4 fg	835.4 h
MEST + BSS	7.4 cd	6.5 bc	4.3 ab	0.8 bcd	57.22 fg	61.31 ef	95.59 hij	821.6 ef	843.6 h
Untreated control	17.3 i	16.8 g	–		0.00 a	0.00 a		625.8 a	590.5 c
Sclerotium-inoculated pasteurised soil	–	–	97.6 i	5.4 i	–	–	0 a	–	–
Uninoculated pasteurised soil	–	–	0 a	0 a	–	–	100 j	–	–
Super-Homai	15.9 hi	14.5 fg	79.7 h	3.8 h	8.09 ab	13.69 b	18.34 b	640.5 ab	627.4 b

[a]Severity was rated on a scale of 0–6.

[b]Each value is a mean of four replicates. In the same column, means follow by the same letters are not significantly different ($P = 0.05$) according to the General Linear Model using the Student Newman Keuls option.

was recorded at an extract concentration (original crude) of 15 or 20 kg 10 l^{-1} solution. These results might indicate that the treatment affected the mycelial growth and any further development of the pathogen. This confirms the antifungal activities exerted by *Moringa* extracts against fungal pathogen mycelium (Stoll, 1988; SIBAT, 1993).

In the greenhouse, significant disease control was recorded when *Moringa* extracts of 15 or 20 kg 10 l^{-1} water were applied in a mixture with kerosene and soap, indicating that a concentration of 15 kg 10 l^{-1} of water is adequate for *Sclerotium* disease control in the greenhouse. Extracts from plants such as garlic (*Allium sativum*) (Obagwu and Korsten, 2003), neem (*Azadirachta indica*) (Obagwu et al., 1997) and pawpaw (*Carica papaya*) (Stoll, 1988) have been tested on many other soilborne fungi. There are, however, few references on the use of *Moringa* extracts to control plant pathogens. Stoll (1988) reported the fungicidal effect of *Moringa* leaf extracts on some soilborne fungi such as *Rhizoctonia*, *Pythium* and *Fusarium*. In the current study, *Moringa* extracts were also shown to be effective against *S. rolfsii*.

When applied in powder form under greenhouse conditions, *Trichoderma* Kd 63 and *B. subtilis* significantly reduced *Sclerotium* damping-off and stem rot incidence of cowpea. The antifungal activities appeared to be dose-dependent.

Notably, with the biocontrol treatments, seed treatments were more effective than the powder formulation, which, in turn, performed better than the soil drench treatments. In concurrence with a previous report (McLean et al., 2005), the current study shows that the type of the biological formulation affects the efficacy of the agent. *Trichoderma*, used as a seed treatment, was reported to protect many vegetable crops from damping-off diseases induced by *Pythium* spp., *Rhizoctonia solani* and *S. rolfsii* (Hornby, 1990). *Trichoderma harzianum* Kd 63 seed treatment, *Trichodema* IITA 508 millet seed inoculum soil sprinkle and *Moringa* leaf extract seed treatment yielded significant disease control and were more effective than the *Bacillus* treatments in the greenhouse. The trend was similar for all treatments and cowpea varieties in the field. This confirms early findings which indicated that biocontrol efficacy depends on the agent used (Widyastuti et al. (2003). *Bacillus subtilis* was previously reported as a BCA against root diseases (Asaka and Shoda, 1996; Wulff et al., 2003; Jacobsen et al., 2004). However, for field evaluation, *B. subtilis* is said to be often very variable with very different results in different locations, or even different parts of a season in the same location (Campbell, 1989). This might explain the low efficacy of *B. subtilis* observed in the current work.

Results in the current study indicated that *Moringa* extracts not only suppress *S. rolfsii* growth in the laboratory, but also control the disease caused by the fungus both in the greenhouse and field. Furthermore, *Moringa* combined with *Trichoderma* (and to some extent with *Bacillus*) resulted in the best disease control in the field, although less effective than under greenhouse conditions. Probably, large soil volume and leaching effect in the field was the cause of lower efficacy. When seeds were treated with *Moringa* leaf extracts, the fungicidal active ingredients in the extracts might be toxic to the pathogen, as shown in the *in vitro* assay. As reported with some plant extracts (Stoll, 1988), *Moringa* extracts might act systemically, and therefore protect the seedlings against attack from the pathogen. *Moringa oleifera* leaf extracts were reported to inhibit the *in vitro* growth of some fungal pathogens and reduce *Pythium* damping-off incidence in legumes, vegetables (SIBAT, 1993) and cereals (Stoll, 1988). However, this is the first report of the effect of the *Moringa* extract on *S. rolfsii* mycelium growth on PDA in the laboratory and on disease caused by the pathogen in the greenhouse and field. *Moringa oleifera* leaves contain some crystalline alkaloids, fatty acid, proteins, glycosides and niazirin, said to be responsible for antimicrobial activities (SIBAT, 1993). This might explain the results obtained in the present study. This action might have been reinforced when *Moringa* was combined with a soil treatment with *Trichoderma*. The effects of *Trichoderma* on many pathogens are well documented (Weidman and Wehner, 1993; Howell et al., 1997; Madi et al., 1997; Widyastuti et al., 2003; McLean et al., 2005) and its efficacy was reported to improve when integrated with synthetic chemicals such as metalaxyl (Howell et al., 1997) or other methods (Tu, 1997). The effectiveness of *Trichoderma* was thought to lie in a combination of competition, antifungal metabolites, toxic antibiotic and mycoparasitism (Madi et al., 1997; Mukherjee and Raghu, 1997; Howell, 2003). In the current study, *Moringa* extracts and antibiotics

produced by *Trichoderma* might be toxic to *S. rolfsii* and the future growth of the pathogen would have been suppressed by the mycoparasitism from the BCA. This might explain the efficacy of *Trichoderma* observed in the present study. As a result of this combination, the synergistic effect of *Trichoderma* and *Moringa* protected the further growth of the plant against infection by the pathogen. This is the first report of *Moringa* (plant extract-based) seed treatments combined with *Trichoderma* soil treatments for integrated control of damping-off and stem rot of cowpea in the field.

Moringa is native to India but has been planted and naturalised in many areas around the world (Davis, 2000). A previous study showed that *Moringa* leaves are relatively easy to crush for extraction, compared to papaw and neem (Adandonon, unpublished). Furthermore, *Trichoderma* IITA 508 was collected from *Sclerotium*-infected fields in the valley, so it is in its native ecosystem. These proven effects of the combined *Moringa* extracts and *Trichoderma* in the field are quite promising since biological or plant based-product controls offer durable, safe and cost-effective alternatives to soil-applied chemicals (Hornby, 1990). Farmers could use the combination to reduce the yield losses inflicted by the diseases to the crop, and therefore increase their income. The present results are of interest since they point to the high possibility of plant extract-based and biological control of *S. rolfsii* in the field. Further work is required to increase the efficacy of *Moringa* extracts in the field and also to determine the biologically active ingredient present in extracts as well as its mode of action.

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