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Pathogenicity of *Sclerotium rolfsii* Isolates Causing Stem and Root Rot Disease of Cowpea (*Vigna unguiculata* (L.) Walp) and Management Using *Trichoderma* Species

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ABSTRACT

Sclerotium rolfsii is a soil-borne pathogen causing stem and root rot disease with concomitant reduction in growth and yield of cowpea. Therefore, this study evaluated pathogenicity of *S. rolfsii* isolates and the management of root and stem rot disease using *Trichoderma* species. The isolates were obtained from cowpea rhizosphere and pathogenicity was determined using a susceptible cowpea genotype ITI0K–815-5 in an experiment arranged in a completely randomized design with four replications. *In vitro* and *in vivo* trials of *Trichoderma* spp. against a selected virulent isolate *Sclerotium rolfsii* (SR06) were conducted in the laboratory and screenhouse, respectively. All nine *S. rolfsii* isolates were pathogenic to the cowpea genotype, while isolate SR 06 was the most virulent with 85.56% infectivity rate. Treatments with *T. virens* and *T. atroviride* application reduced the disease incidences to 22.2% and 25.3%, respectively, compared to synthetic funguforce fungicide. Plants that were inoculated and treated with *T. atroviride* had significantly higher yield of 435 kg/ha than other treatments ($p<0.037$). Findings from this research encourages the incorporation of *Trichoderma* species in the integrated management of *S. rolfsii* pathogen and the incidence of stem and root rot disease in cowpea.

INTRODUCTION

Cowpea is cultivated as a major legume worldwide, particularly in the dry savanna of tropical Africa where over 200 million people depend on it as a source of food, livelihood and income (Kumari et al., 2015). It contributes to the sustainability of cropping systems through fixation of nitrogen to succeeding crops in rotation on poor fertility soils. Nigeria is the largest world producer of cowpea with an output of over 2.15 million metric tonnes annually (FAOSTAT, 2017). However, varietal acceptance is often influenced by seed coat colour, texture and cooking duration. Cowpea production in West Africa has been abysmally low due to abiotic and biotic constraints such as pests, diseases, parasitic plants, and weeds. Yield losses are often more pronounced in areas with abundant rainfall and high relative humidity where incidence and severity abound.

Fungal diseases constitute major limitation to cowpea production globally, especially in sub-Saharan Africa where the crop is grown in commercial quantities. This is due to the devastating potential of diseases such as root and stem rot, charcoal rot, damping-off, and vascular wilts (Bastakoti et al., 2017).The prevalence of these diseases often results in the loss of cowpea plants at vegetative stage in the field and postharvest losses in storage. *Sclerotium rolfsii* Sacc., teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough is a soil-borne plant pathogenic fungus that is widely distributed in the warm temperate and tropical regions of the world (Mehri et al., 2013). Stem and root rot disease caused by *S. rolfsii* accounts for significant yield losses in common bean (*Phaseolus vulgaris* L.) (Gholami et al., 2020). The fungus survives during off-season as mycelium or sclerotia in infected plant tissue and

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soil. The hyphae and sclerotia germinate under high relative humidity and temperature to infect cowpea, which subsequently colonize and invade the root and stem tissue with typical silky white mycelium (Eslami et al., 2015) . Its initial symptoms of infection include brownish necrotic lesion and appearance of sclerotia on the stem and other parts of the plant including the soil surface. This is followed by a progressive drooping of leaves, decay of stem and roots leading to eventual death of infected plants.

The production of pectolytic enzymes enhances tissue penetration by the pathogen and this causes rapid deterioration of the host outer cell layer. These enzymes in conjunction with the production of oxalic acid, an exogenous non-specific metabolite, are important determinants of its pathogenicity status and carbohydrate metabolism (Cer & Morca, 2020). The soil-borne fungus thrives in soil organic matter which increases the multiplication rate and disease development (Paul et al., 2021). *Sclerotium rolfsii* is difficult to control by physical and cultural practices due to its wide host range of over 500 plant species (Le et al., 2012; Yaqub & Shahzad, 2005) and production of resting spores known as sclerotia (Sennoi et al., 2010).The decay starts on the stem at above ground level with clearly visible mass of mycelium projecting into the soil organic matter and infection courts occur on the roots. High temperature range of 25-35°C encourages rapid formation and development of mycelia and sclerotia. Entry to plant tissue is usually through wound, although direct penetration occurs. However, basal stem rot epidemics are dynamic and had been reported in warm moist climate (Thangavelu & Mustaffa, 2010; Nazerian & Piegham 2021).

The application of systemic fungicides like carbendazim and benomyl in the management of this soil-borne pathogen has proved effective in the short run in Nigerian farming systems (Dania et al., 2016). However, the undesirable effects of these chemical pesticides on the ecosystem have necessitated the use of reliable and cost effective alternative options in management of the disease. Biological control as a novel approach to plant disease management is deemed to be ecologically-friendly and safer than the use of synthetic pesticides (Sajeena et al., 2021). *Trichoderma* species are effective against many soil-borne fungi (Adandonon et al., 2006) where they have a comparative advantage of rapid colonization of the rhizosphere to the detriment of plant pathogens. However, limited information exists on the use of *Trichoderma* species for the management of *Sclerotium rolfsii* with a view to enhancing cowpea productivity. Therefore, this study sought to evaluate the efficacy of *Trichoderma* species against *Sclerotium rolfsii* under laboratory and screenhouse conditions as a baseline to field delivery of the antagonists.

MATERIALS AND METHODS

Experimental Location and Source of Materials

The research was conducted at the laboratory and screenhouse of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria in 2018. Three of the five *Trichoderma* species used in this study: *Trichoderma virens, T. atroviride,* and *T. koningii* were collected from the Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. *S. rolfsii* isolates were obtained from infected cowpea fields under natural conditions, while the two other *Trichoderma* isolates, *Trichoderma* CPT13 and *Trichoderma* CPT41, were obtained from mycological herbarium in the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria. A susceptible cowpea genotype, IT0K–815–5, was provided by the Genetic Resource Centre of IITA, while the synthetic funguforce fungicide used was purchased from an agricultural chemical store. Wheat seeds that were used as inoculum carrier were purchased from traders in the open market.

Pathogenicity of *Sclerotium rolfsii* Isolates

Pathogenicity study was first conducted to determine whether the isolates could cause stem and root rot disease on a susceptible cowpea genotype IT0K–815–5 to necessitate control trials. *S. rolfsii* isolates were cultured from infected cowpea plants showing symptoms of stem and root rot disease, especially at the stem base immediately above the soil that were collected from existing cowpea farms under natural conditions. Infected lesion was cut into small bits of about 0.5 mm × 0.5 mm using a pair of scissors that was previously

surface-sterilized in 5% sodium hypochlorite and rinsed in sterile distilled water. Inoculation of infected tissue pieces was done on potato dextrose agar (PDA) medium and incubated at 27-30°C for 3 days. Cultures were purified to obtain pure isolates of *S. rolfsii*. Identification was carried out following standard manual (Bissett, 1991; Barnett & Hunter, 2000) and confirmation at Germplasm Health Unit of IITA. *Trichoderma* species which had earlier been identified were purified on PDA.

Inoculation of healthy cowpea plants was done in the rhizosphere because the test pathogen is soil-borne and does not sporulate. Three hundred grammes of wheat seeds were packed into transparent 500 ml capacity cans, with the open end blocked with non-absorbent cotton wool, wrapped with aluminium foil and sterilised in an autoclave at 121°C for 45 minutes to eradicate inherent seed-borne inocula. After sterilization, water was drained off the seeds and allowed to cool at room temperature before inoculation with nine isolates of *S. rolfsii* separately. Inoculated cans were incubated at room temperature for 15 days to allow complete growth of the pathogen mycelia on the seeds. At full mycelial growth, healthy cowpea plants were inoculated with the inoculum at the rate of 7.5 g per pot in completely randomized design with four replications. Plants were examined for typical symptoms of stem and root rot disease such as stem rotting and appearance of whitish mycelia at two weeks after inoculation. The most virulent of the nine isolates, SR 06, was selected and used in the laboratory and screenhouse experiments.

Inhibitory Effect of *Trichoderma* Species on *Sclerotium rolfsii* Isolates SR 06 *In Vitro*

The *in vitro* antagonistic activity of five *Trichoderma* species: *Trichoderma virens, T. atroviride, T. koningii, Trichoderma* CPT13 and *Trichoderma* CPT 41 was evaluated using the dual culture method. A 3-mm mycelial disc was obtained from pure cultures of each of the *Trichoderma* isolates and the test pathogen. Isolate SR 06 was inoculated at the centre of the Petri dish while each *Trichoderma* species was inoculated at four equidistant points using three methods: Inoculation of the pathogen a day before *Trichoderma,* simultaneous inoculation of the pathogen and *Trichoderma,* and inoculation of the pathogen a day after *Trichoderma* (Dania et al., 2016). Control treatment consisted of PDA amended with sterile distilled water. The experiment was laid out in a completely randomized design with four replications. Inoculated Petri dishes were incubated at 27-30°C and daily mycelial growth was recorded for four days. Inhibitory effect of *Trichoderma* spp. on the pathogen was calculated by expressing radial growth of treatment as percentage of the control.

Evaluation of *Trichoderma* Species for the Management of Stem and Root Rot Disease of Cowpea *In Vivo*

The pot experiment was laid out in a completely randomized design and replicated four times. Loamy soil was sterilized using an Electrical Sterilizer Model SG 12-04-220, USA at 90°C for 2 h. The soil was allowed to cool and filled into 10 kg pots up to the collar region. Preparation of inoculum was done as previously described in the pathogenicity experiment. *Trichoderma* species were applied as soil drench at 4 ml per planting hole at a concentration adjusted to 2.5×10^8 using an hemocytometer (Dania et al., 2014) before sowing. Seven and half grammes of isolate SR 06-ramified wheat seeds were used to inoculate the soil around the base of healthy cowpea plants at two weeks after sowing while control plants were not inoculated with the pathogen. Four seeds were sown and these were later thinned to two per pot at one week after sowing. Plants treated with funguforce fungicide (63% mancozeb and carbendazim 12.5% WP) applied at 0.025/l served as positive check. The experiment consisted of seven treatments (Table 1).

Data were collected on a weekly basis on growth and yield parameters such as plant height, number of leaves, stem girth, number of pods per treatment, disease incidence, disease severity, number of seeds per treatment, 100 seed weight and yield. Plant height was measured using the metre-ruler while number of leaves was determined by hand counting each leaf. Stem diameter was measured using the Vernier caliper. Disease incidence was determined by counting the number of infected plants and expressed as percentage of the total number of plants assessed in each treatment.

Disease severity was assessed on a rating scale of 1-5: 1 = no symptoms, 2 = discolouration of the stem to dark brown, 3 = reduced stem size, appearance of mycelial or sclerotia on stem, 4 = decay of stem, drooping and wilting of the plant, 5 = death of the plant.

Data Analysis

All collected data were subjected to analysis of variance (ANOVA). Where there were significant differences (P<0.05), means were separated with Fisher's Least Significant Difference (LSD) using GENSTAT version 11 software.

RESULTS AND DISCUSSION

Pathogenicity of *Sclerotium rolfsii* Isolates

Results showed that all the *Sclerotium rolfsii* isolates (SR01-09) were pathogenic to the cowpea genotype IT0K–815–5, producing characteristic stem and root rot disease symptoms on inoculated cowpea plants (Table 2). The different isolates produced the same cultural features during re-isolation on PDA medium and re-inoculation of healthy plants also produced typical symptoms on the cowpea stems and roots in accordance with Koch's postulates. However, there was significant variation in pathogenicity potential among the isolates. The number of symptomatic cowpea ranged between 8 to 77 of 90 plants that were inoculated with *S. rolfsii* isolates per treatment. Isolate SR 06 had the highest infection rate of 85.56% which was significantly higher (p=0.031) than other treatments, while the lowest disease incidence of 8.89% was recorded in isolate SR 03. All plants in the control treatment were asymptomatic with no infection. Plants inoculated with isolate SR 09 and control plants had the longest and shortest shoot length of 14.76 and 36.20 cm, respectively. Shoot length differed significantly (p=0.024) and was highest in the control than other treatments. Healthy plants that were uninoculated but sprayed with sterile distilled water had significantly higher root length (25.77 cm) relative to other treatments. Isolates SR 02 and 07 produced plants with the shortest and longest root length of 7.01 and 18.32 cm, respectively. The period between inoculation of the cowpea plants and the appearance of visible symptoms (latent period) on the stem and roots did not differ significantly (p=0.073) among the treatments. However, lesion length differed significantly (p<0.05) among inoculated plants and was higher in control treatment. Isolate SR-06 was observed to be the most virulent with a lesion length of 13.83 cm, while isolate SR 01 with 5.60 cm lesion length was the least virulent. The lesion length of all the treatments was significantly higher than the control.

Several authors had implicated *S. rolfsii* in the incidence of stem and root rot disease of cowpea. Adandonon et al. (2006) reported *S. rolfsii* as the causal organism of damping-off and stem rot diseases of cowpea, which results in the death of seedlings and yield reduction. Farooq et al. (2011) and Paul et al. (2021) reported *S. rolfsii* as the pathogen causing root rot disease in sugar beet leading to rapid collapse of the vascular tissue and death of infected plants. Le et al. (2012) and Deepthi (2013) had established *S. rolfsii* as the inciting agent of stem rot disease in cultivated groundnut. All the isolates in this trial were observed to have varying degree of pathogenicity.

Table 1. Treatments used in the experiment

Treatment	Description
1	*Sclerotium rolfsii* isolate SR 06 + *Trichoderma virens*
2	*Sclerotium rolfsii* isolate SR 06 + *T. koningii*
3	*Sclerotium rolfsii* isolate SR 06 + *T. atoviride*
4	*Sclerotium rolfsii* isolate SR 06 + *Trichoderma* CPT13
5	*Sclerotium rolfsii* isolate SR 06 + *Trichoderma* CPT41
6	*Sclerotium rolfsii* isolate SR 06 + Funguforce
7	*Sclerotium rolfsii* isolate SR 06 (control)

Table 2. Pathogenicity of *Sclerotium rolfsii* isolates on cowpea seedlings

Isolate	No. of inoculated seedlings	No. of symptomatic seedlings	No. of asymptomatic seedlings	Percent infection	Root length (cm)	Shoot length (cm)	Latent Period (days)	Lesion length (cm)
SR 01	90	16cd	74b	17.8c	23.26d	10.07d	13.67a	5.60d
SR 02	90	10d	80ab	11.11d	16.71f	7.01e	10.71a	8.71c
SR 03	90	8.0d	82ab	8.89d	19.20e	11.26d	8.21a	7.23c
SR 04	90	43b	47bc	47.78b	27.23c	16.80b	11.2a	10.20b
SR 05	90	49ab	51bc	54. 4b	18.33e	14.37c	12.30a	11.37b
SR 06	90	77a	13c	85. 56a	24.81d	12.11c	13.86a	13.82a
SR 07	90	18d	72b	20. 0c	25.01d	18.32b	9.01a	8.01c
SR 08	90	38bc	52bc	42.22b	30.10b	13.88c	8.13a	7.16c
SR 09	90	22c	68bc	24. 44	14.76f	14.60c	10.79a	10.71b
Control	90	0de	90a	0e	36.30a	25.77a	0a	0.0e
Level of sig.		0.031	0.40	0.018	0.042	0.024	0.073	0.038
CV (%)		6.02	10.77	13.69	8.76	5.22	7.01	. 4.08

Remarks: SR = *Sclerotium rolfsii*; * CV = Coefficient of variation. Means followed by the same alphabet along the column are not significantly different (P<0.05) using Least Significant Difference (LSD).

Table 3. Inhibitory effect of biological control agents on mycelial growth of *Sclerotium rolfsii* isolate SR 06

Treatment	Inoculation of *S. rolfsii* before BCAs		Inoculation of *S. rolfsii* and BCAs in same day		Inoculation of *S. rolfsii* after BCAs	
	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)
Trichoderma. virens	58.9±1.53bc	34.5±0.24ab	44.1±1.80b	51.0±1.40b	3.8±0.05bc	96.0±3.75a
Trichoderma koningii	70.3±1.0ab	21.9±2.14bc	50.5±2.13ab	43.9±1.22c	10.4±0.01b	88.4±2.89a
Trichoderma atroviride	61.4±1.50b	31.8±1.08b	35.2±1.14bc	60.1±0.88ab	4.2±0.01bc	95.3±1.76a
Trichoderma CPT13	62.5±0.97b	30.6±1.05b	48.6±0.81ab	46.0±1.11bc	13.6±0.50ab	84.9±2.08a
Trichoderma CPT41	64.8±2.33b	28.0±0.01b	43.7±0.77b	51.4±1.60b	15.3±1.10ab	83±1.50a
Funguforce	50.7±1.05c	45.0±0.05a	22.7±1.50c	89.7±1.31a	2.8±0c	98.3±3.17a
Control	90.0±1.77a	0.0±0c	90.0±2.65a	0.0±0cd	90.0±2.33a	0.0±0b
Level of significance	0.043	0.036	0.015	0.032	0.029	0.065
CV (%)	19.77	15.20	17.63	15.08	14.33	21.01

Remarks: Data obtained at 7 days after inoculation. Each value represents mean +standard error. Means followed by the same alphabet along the column are not significantly different (P<0.05) using Least Significant Difference (LSD). * CV= Coefficient of variation.

These results are consistent with the findings of Sharf et al. (2021) that observed variability in virulence amongst *S. rolfsii* infecting chili pepper. It has been reported that there might be a positive correlation between sclerotial size and pathogenicity since larger sclerotia could secrete a high amount of oxalic acid and other cell wall degrading enzymes which are necessary for disease development (Chaudhary, Sing & Singh, 2021). In this study, however, this relationship was not observed since isolate SR 06 produced small-sized sclerotia but had the highest pathogenicity status among the isolates. It has been reported that the secretion of oxalic acid and endopolygalacturonase simultaneously with fast mycelial growth rate seems to be a major criterion for *S. rolfsii* infection (Manu et al., 2012).

Inhibitory Effect of *Trichoderma* Species on *Sclerotium rolfsii* Isolates SR 06 *In Vitro*

The inhibitory effect of the *Trichoderma* species on the mycelial growth of isolate SR 06 varied from 21.9-31.85% when the pathogen was inoculated before the *Trichoderma* spp. (Table 3). *Trichoderma atroviride* was most effective with 60% inhibition, while *T. koningii* had the lowest mycelial growth reduction of 43.9% when the biological control agents (BCAs) were inoculated same day with the test pathogen relative to the positive check treatment (89.7%) which comprised cowpea plants that were inoculated with isolate SR 06 but treated with synthetic funguforce fungicide. *T. virens* had the best mycelial reduction potential (96%) of isolate SR 06 when the pathogen was inoculated a day after the BCAs followed by *T. atroviride* with 95.3% reduction. Although the synthetic fungicide had the highest mycelial reduction of 98%, its performance was, however, not significantly higher (p=0.065) than *T. virens* and *T. atroviride*. The best results were recorded with the inoculation of the pathogen a day after the application of the *Trichoderma* spp. These results are consistent with the findings of Sangeetha et al. (2009) who reported the inhibitory effect of *Trichoderma* species against *Lasiodiplodia theobromae* causing crown gall disease of banana. Rapid growth and competitive ability of *Trichoderma* species, especially the colonisation of substrate had been reported by several authors (Dania et al., 2016; Harman, 2000; Pascale et al., 2017). A distinct inhibitory zone that was observed between *T. virens, T. atroviride* and *S. rolfsii* isolate and visible lysis of the pathogen mycelia *in vitro* may be due to the mechanism of antibiosis and production of secondary metabolites by the antagonists. This gives credence to the reports of Shoresh et al. (2010) and Martínez-Padrón et al. (2018) that the production of secondary metabolites enhances the ability of *Trichoderma* spp. to control plant pathogens. *Trichoderma* spp. has the inherent ability to inhibit the growth of fungi infecting plants through the production of effervescent metabolites or antibiotics which in varying concentrations interfere with substrate utilization and survival of invading fungi in plant tissue (Tomah et al., 2020). *Trichoderma koningii* had the least inhibitory effect on the test pathogen among the BCAs. The decline in vigour of *T. koningii* in the *in vitro* assay may be attributed to the effect of cold storage prior to its use in this experiment. This agrees with Bastakoti et al. (2017)) who submitted that the relative ability of *Trichoderma* species to exhibit biocontrol potential against a test pathogen is largely dependent on certain abiotic factors such as cold storage, pH, relative humidly and temperature. These may have reduced the competitive ability of *T. koningii* in this study. Protracted cold storage of isolates may also induce mutation in microorganisms which makes hitherto virulent pathogens to become avirulent and vice versa (Cai et al., 2013; Shang et al., 2020).

Cowpea plants that were inoculated with *Sclerotium* isolate SR 06 and treated with *T. atroviride* reached the highest height of 28.3 cm at 5 weeks after inoculation (WAI) (Fig. 1). However, there was no significant (p=0.081) difference in height among plants that were inoculated with the pathogen and treated with *Trichoderma* species and the control. The number of leaves increased progressively from the first to 5 WAI (Fig. 2). Plants that were inoculated with *S. rolfsii* and treated with *T. virens* had the highest number of leaves followed by those that were inoculated and treated with *T. atroviride*. Both treatments had a significantly (p=0.041) higher number of leaves than other treatments. Enzymes and antibiotics that are produced through plant-pathogen interaction have been reported to attack fungal pathogens, successfully degrade the fungal hyphae and permit penetration into the host cell thereby enhancing plant growth (Shoresh et al.,

2010). Several authors had reported the growth-enhancing ability of *Trichoderma* species in agricultural crop production. Pascale et al. (2017) established that *Trichoderma* and its secondary metabolites improved growth and quality of grapes. Similarly, Shang et al. (2020) showed the efficacy of *Trichoderma asperellum* TC01 against anthracnose and its ability to promote the growth of *Camellia sinensis* seedlings.

Evaluation of *Trichoderma* Species for the Management of Stem and Root Rot Disease of Cowpea *In Vivo*

The incidence of stem and root rot disease increased consistently up to 5 WAI (Fig. 3). Disease incidence was significantly (p=0.039) lower among cowpea plants with *Trichoderma* treatments than the control. Also, disease severity was significantly (p=0.028) lower in cowpea plants that were inoculated with *S. rolfsii* and treated with all *Trichoderma* species compared to the control with a rating of 5.3 (Fig. 4). Infected plants showed the development of whitish sclerotia and rotting of the stem base above soil surface (Fig. 5a) and drooping of leaves (Fig. 5b), while the control treatment comprised cowpea plants with healthy stems and asymptomatic leaves (Fig. 5c). Disease incidence and severity were significantly ($p<0.05$) higher in control treatments that were inoculated but without application of BCAs. Disease outbreak due to *S. rolfsii* may be favoured by increased temperature and relative humidity; while cycles of drought and wetness have been reported to encourage the germination of sclerotia (Kokub et al., 2007; Le et al., 2012). The occurrence of high amount of organic matter in cultivated farms provides substrate for mycelial growth and protection for pathogen sclerotia which enhances disease severity (Singh et al., 2003). *Sclerotium rolfsii* is rapidly spread by wind, water, poor cultural practices such as planting of infected seeds and cultivation on infected soil. The polyphagous nature of the pathogen, rapid mycelial growth and inherent ability to produce viable sclerotia contribute to the large economic losses associated with this fungus (Kokub et al., 2007). The reduction in disease incidence and severity in treatments with *Trichoderma* species may be attributed to the production of enzymes such as xylanases, cellulase and antibiotics that induce resistance in plants to pathogens (Kumari et al., 2015).

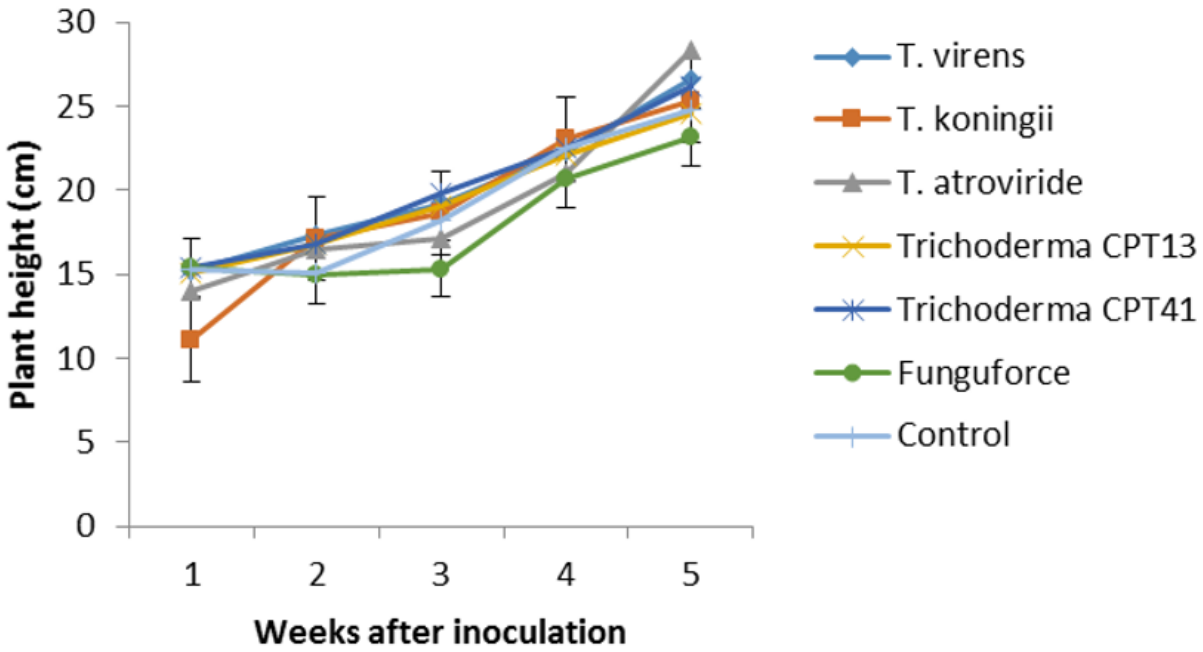

Fig. 1. Effect of treatments on the height of cowpea plants at 5 weeks after inoculation with *Sclerotium rolfsii*

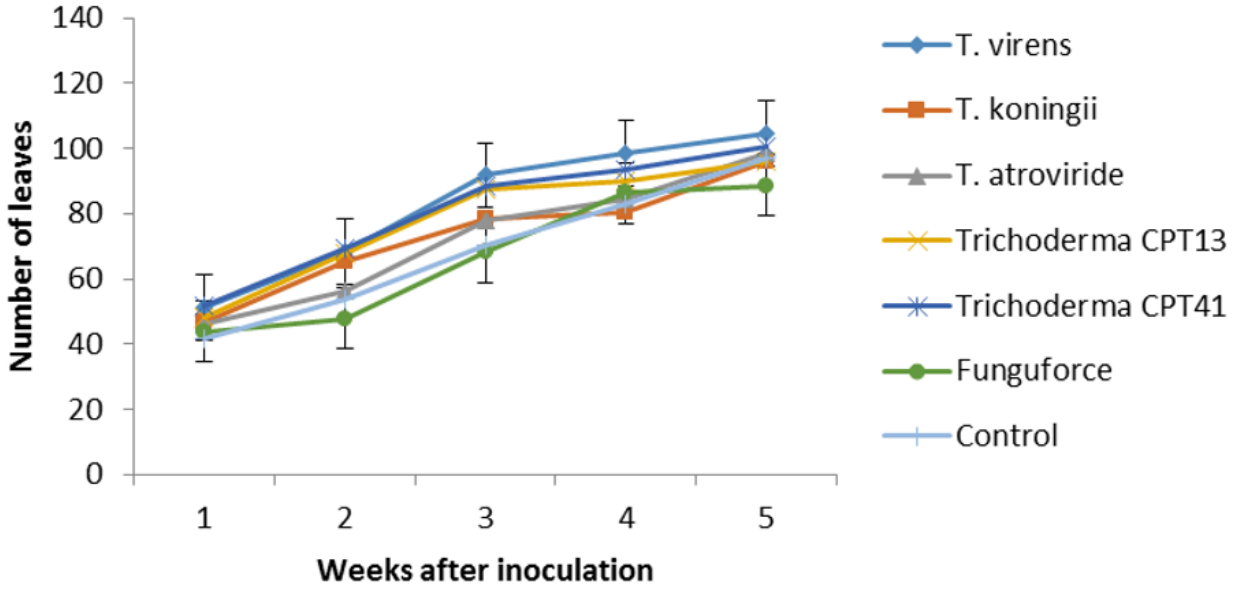

Fig. 2. Effect of treatments on number of leaves of cowpea plants at 5 weeks after inoculation with *Sclerotium rolfsii*

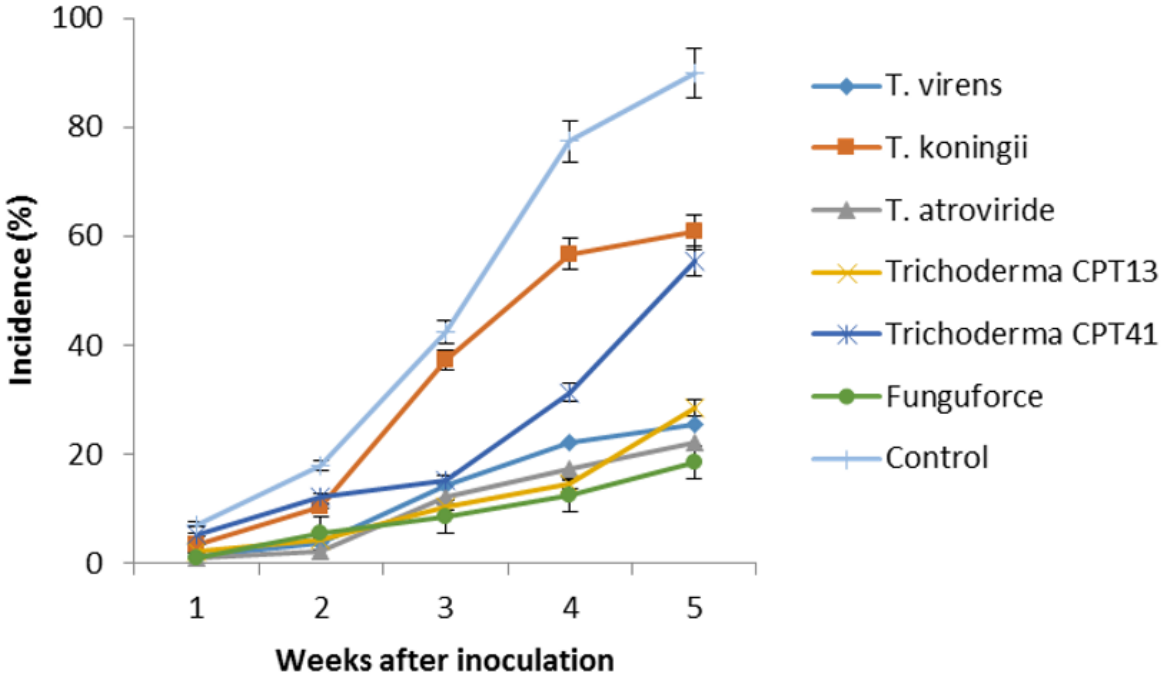

Fig. 3. Effect of treatments on incidence of stem and root rot disease of cowpea plants at 5 weeks after inoculation with *Sclerotium rolfsii*

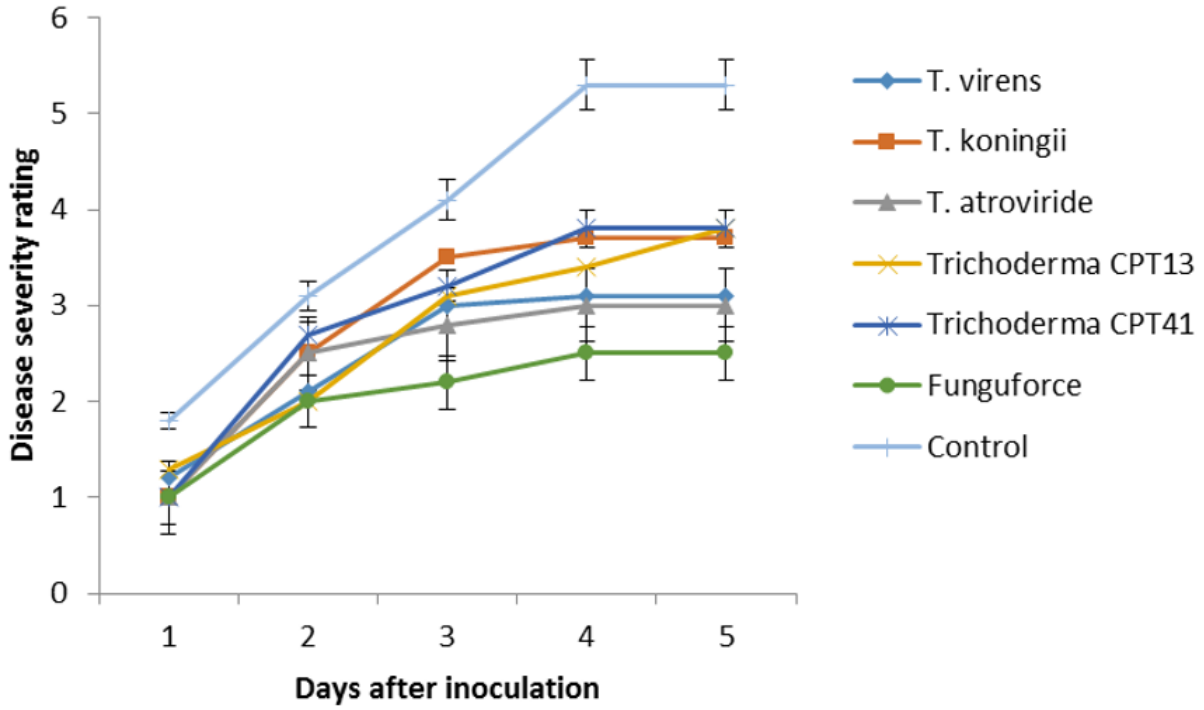

Fig. 4. Effect of treatments on severity of stem and root rot disease of cowpea plants at 5 weeks after inoculation with *Sclerotium rolfsii*

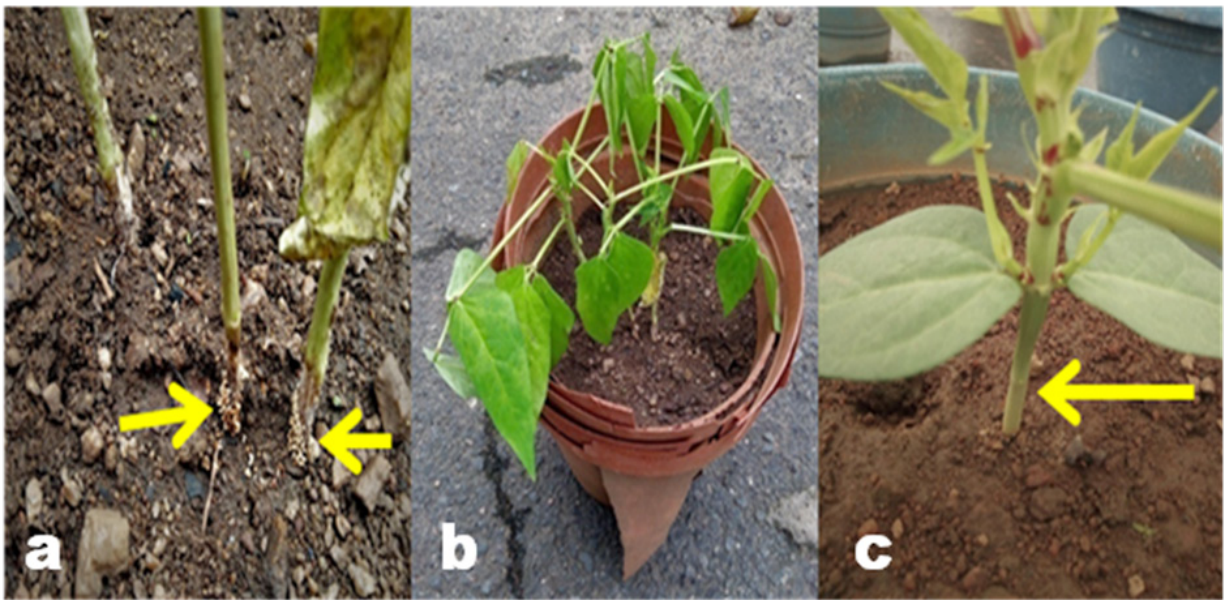

Fig. 5. Expression of symptoms in cowpea plants inoculated with *Sclerotium rolfsii*: a = Stem rot of cowpea plants showing presence of sclerotia, b = Drooping of leaves in infected plants, c = Healthy cowpea stem (control).

Table 4. Effect of biological control agents on yield parameters of cowpea

Treatments	No. of pods per plant	No. seeds per pod	Pod weight (g)	Pod length (cm)	100 seed weight (g)	Seed index (cm)	Harvest index (%)	Seed yield (kg/ha)
Trichoderma virens	4.1 ± 0.1a	20 ± 1.0a	2.1±0.02a	29.3±1.2a	17.59 ± 1.93a	0.5±0.01a	65.5±1.2c	384 .7± 3.99c
Trichoderma koningii	3.0 ± 0.05a	12 ± 1.5d	1.8±0.01a	25.8±1.5b	17.82 ± 0.95a	1.4±0.01a	63.3±2.7a	348.6 ± 5.95e
Trichoderma atroviride	3.5 ± 0.05a	15± 0.1c	2.5±0.05a	24.6±0.6b	19.11 ± 1.26a	0.7±0.02a	87.4±1.1b	435.5 ± 7.35a
Trichoderma CPT13	3.7 ± 0.10a	18 ± 1.3a	4.2±0.01a	27.7±2.1a	18.27 ± 0.39a	0.9±0.01a	78.1±2.2a	372,2 ± 6.19d
Trichoderma CPT41	2.5± 0.03a	15 ± 0.5c	2.9±0.01a	22.8±1.7c	15.42 ± 1.38b	1.1±0.05a	80.2±1.5a	333.8± 2.03f
Funguforce	2.0± 0.01a	17 ± 0.9b	1.7±0.02a	28.3±0.5a	14.93 ± 1.17b	0.8±0.02a	69.2±0.5b	422.6 ± 6.04b
Control	3.0± 0.03a	18 ± 1.2a	1.9±0.05a	19.4±0.8d	10.59 ± 0.31c	1.6±0.5a	85.5±1.8a	314.4 ± 2.71g
Level of significance	0.073	0.030	0.082	0.028	0.033	0.094	0.017	0.039
CV (%)	9.18	17.40	13.03	20.88	10.61	3.39	23.50	21.33

Remarks: Each value represents mean + standard error. Means followed by the same alphabet along the column are not significantly different (P<0.05) using Least Significant Difference (LSD).* CV = Coefficient of variation

Infected plants showed the development of whitish sclerotia in the screeenhouse experiment which are asexual reproductive propagules poduced by *S. rolfsii* and rotting of the stem base above soil line at 14 days after inoculation. Sclerotia are hard round mass of viable hyphae which serve as the source of primary inoculum in the development of *Sclerotium*-based plant disease epidemics (Fery & Dukes, 2011; Singh et al., 2003). Under favourable conditions, white mycelial growth and sclerotia appear at the soil base line of the stem on diseased cowpea causing necrosis of plant tissue at the point of contact with the pathogen mycelium (Flores-Moctezuma et al., 2006). Eslami et al. (2015) reported the production of oxalic acid by *S. rolfsii*, which is a toxic metabolite causing tissue death in infected plants. This metabolite acts in synergy with pectinolytic and cellulolytic enzymes in reducing the pH thereby enhancing the degradation of host plant tissue (Prasad et al., 2012).

The overall yield obtained in treatments with *Trichoderma* application varied between 333.8 and 435.5 kg/ha and was significantly (p=0.029) higher than that obtained in the negative control (Table 4). The highest seed yield of 435 kg/ha was obtained in plants that were inoculated with the pathogen and treated with *T. atroviride*, which was significantly higher than treatment with funguforce fungicide (positive control), while treatment with *Trichoderma* isolate CP 41 produced the lowest yield of 333.8 kg/ha. There was no significant difference (p=0.073) among treatments in the pod and seed weight. BCAs produce auxins and other metabolites that may encourage their establishment in cowpea rhizosphere with the tendency to stimulate lateral root development and ultimate increase in output (Hidangmayum & Dwivedi, 2018). Some *Trichoderma* species are resident in plants with a unique ability to migrate through the vascular tissues by the production of appressoria which facilitate the ability to penetrate plant tissue. The production of enzymes by these antagonistic fungi helps to stimulate photosynthetic processes, production of dry matter, development of plant defense systems through moderation of gene expression and resistance (Prasad et al., 2012). They have the capacity to improve plant yield under field conditions, particularly when environmental factors are optimal which enable BCAs to have a positive correlation with plant yield in addition to reducing the debilitating influence of plant pathogens and abiotic stress management in soil (Shoresh et al., 2010). Tomah et al. (2020) also reported the yield-promoting ability of *T. virens* against *Phytophthora capsici* on chili pepper.

CONCLUSION AND SUGGESTSION

This research showed the efficacy of *Trichoderma* species as viable substitute for synthetic fungicides in the management of stem and root rot disease of cowpea, particularly in terms of increased growth and yield of the crop. *Trichoderma atroviride* and *T. virens* effectively inhibited radial growth of the test pathogen in the *in vitro* assay, stimulated cowpea growth and significantly reduced the incidence and severity of stem and root rot disease. Results obtained in the application of these biological control agents compared favourably with those of the synthetic fungufoce treatment. Therefore, our research recommends the incorporation of harmless *Trichoderma* species as a vital component of an integrated management of the disease.

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