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Efficacy of some bio-control agent formulations on scab of cowpea and groundnut late leaf spot in the Nigerian savanna

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Abstract

Field experiment on fungicidal efficacy of *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus cereus* formulations against cowpea scab and groundnut late leaf spot was conducted. The trials were conducted in 2016 and 2017 rainy seasons on two farms of the Institute for Agricultural Research located at Samaru, Zaria (11^0 12′ N, 07^0 37′ E) in the Northern Guinea savanna and Minijbir, Kano (08^{0}31' E., I2^{0}03' N) in the Sudan savanna. The formulations were applied as seed treatment, foliar spray and also incorporated into the soil by broadcasting the powdered formulations on the ridges. The experiment was laid in Randomized Complete Block Design replicated three times with five treatments which consisted of the three formulations, Funguforce (Mancozeb + Carbendazim) and control. Data were taken on plant emergence, pod yield, disease incidence and severity. Irrespective of the treatments, incidence of scab at both Samaru and Minjibir were at par. However, Funguforce application gave lowest mean scab severities of 10% and 8.5% at Minjibir and Samaru respectively in 2017. In 2016 at Samaru, *P. fluorescens* had the lowest incidence (50.57 %) while *B. cereus* had the lowest severity (14.40 %) of groundnut late leaf spot. At Minjibir however, *T. harzianum* recorded the lowest incidence (48.75 %) and severity (18.22 %). The incidence and severity of groundnut late leaf spot at Samaru in 2017 did not significantly vary while at Minjibir lowest incidence (9.77 %) and severity (9.73 %) were recorded on Funguforce. The microbial formulations recorded an average pod yield increase of 10 – 58 % which was comparable with that of Funguforce. The use of formulations of *T. harzianum, P. fluorescens* and *B. cereus* in the management of fungal diseases of cowpea and groundnut in the Nigerian savanna is therefore recommended.

Keywords: Genetic-crosses, RILs, Polymorphisms, Genetic-Markers, EPN

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Introduction

The role of cowpea and groundnut in improving food security and farmers' income in the Nigerian savanna cannot be over emphasized. Cowpea and groundnut are the most economically important grain legume crops of the family Fabaceae that are of vital importance to the livelihood of several millions of people in the Nigerian savanna (Larry et al., 2008). The crops apart from improving soil fertility through nitrogen fixation also provide food, animal feed, alongside cash income to farmers in rural communities (Dugje et al., 2009). Though Nigeria is the largest producer of cowpea in the world and of groundnut in Africa, fungal diseases remain a serious challenge to producers of the crops (Alabi et al., 1993). Late leaf spot induced by *Phaeiosariopsis personata* (Berk & M. A. Curtis) Arx was reported as the most prevalent and economically important fungal disease of groundnut worldwide (Liu et al., 2013) that results to more than 50 % yield loss (Phat et al., 2019). Sphaceloma scab caused by *Sphaceloma* spp. is one of the most destructive diseases of cowpea in the Northern Guinea savanna zone of West and Central Africa (Adegbite and Amusa, 2008) and can cause up to 75 % yield loss (Emechebe and Shoyinka, 1985).One of the disease management strategies being advocated to farmers in Nigeria is the use of microbial antagonists which provide a safer, durable and sustainable alternative to the poisonous synthetic fungicides that are known to contaminate the environment (Akhtar et al, 2009).

Fungi and bacteria are the organisms most commonly used as antagonists in the control of plant diseases. Fungi of the genus *Trichoderma* have recorded success in controlling plant pathogens and also enhance plant growth through improvement of plant systemic resistance and increased root proliferation (Maureen, 2016). Species of *Bacillus* and *Pseudomonas* are among the bacteria that play an important role in plant growth promotion and activation of plant defense mechanism by triggering the induced systemic resistance through production of volatile compounds (Jamil et al., 2017). Despite this reported effectiveness of biological control of plant diseases which generally ranges from 30 to 50 %; success with bio-control agents is often unpredictable due to complicated mode of action and difficulty in obtaining a successful formulation (Dong-Dong, 2011). Also, bio-control agents exhibit specific and non-specific interactions and as such organisms of the same genus belonging to different species or even strains display variability in their interaction with pathogens (Rayees et al., 2014). It therefore becomes imperative to ascertain the identity of bio-control agents and test any biological formulation before recommending to farmers. The objective of this study was therefore to test the efficacy of powdered formulations of *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus cereus* in managing scab of cowpea and late leaf spot of groundnut under field conditions.

Materials and methods

Isolation of organism and product formulation

Cowpea and groundnut rhizosphere soils were collected from the research farm of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. Isolation of organisms and product formulation were conducted at the Microbiology Laboratory of Contec Global Agro Limited, Abuja. Soil samples were serially diluted and a dilution of 10^3 was used as stock for the isolation of the organisms. Using a micropipette, 0.1 ml of the sample was drawn and plated onto already prepared Corn Meal Agar (CMA) and Nutrient Agar (NA) and incubated at 27 ^{0}C in an incubator. Morphological characteristics of the colonies were used to differentiate between the target organisms. Colonies observed that resembled *Trichoderma* sp. based on color of mycelia and morphology were sub-cultured onto Potato Dextrose Agar (PDA) while those observed to resemble *Bacillus* or *Pseudomonas* spp. based on color and shape of colony were sub-cultured onto solidified NA. The cultures were sent to MACROGEN, Korea where they were subjected to molecular identification through DNA isolation, rRNA amplification with ITS-1(Forward) and ITS-4 (Reverse) primers. Further amplified products were subjected to partial sequencing and sequence data obtained was subjected to bio informatics analysis. BLAST search tool from the National Centre for Biotechnological Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify homologous sequences deposited at Genbank (http://www.ncbi.nlm.nih.gov/). A List of Prokaryotic Names with Standing in Nomenclature (LPSN) http://www.bacterio.net) was used to download 16S rDNA sequences of type strains deposited at Genbank.

The formulation for each of the organism was done using an established spore multiplication protocol by adopting solid state fermentation with maize as a solid substrate. Spores were collected through sterile distilled water and mixed into the desired quantity of talc powder for further formulation along with Carboxymethyl Cellulose (CMC) as stabilizer.

Field evaluation

The trial was laid out in a Randomized Complete Block Design (RCBD) with three replications. The test formulations were *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus cereus*. Funguforce (Mancozeb + Carbendazim) was used as chemical control. The treatments also included an untreated control making five treatments. Cowpea and groundnut were planted in 2016 and 2017 at Minjibir and Samaru located in the Sudan and Northern Guinea savanna respectively. The spacing was 0.75 m inter rows, 0.20 m intra row and two plants were sown per stand. Plots measuring 12 m^2 were separated by alley of 1.0 m wide. Nitrogen (N) was applied to all plots as urea (46% N) at 20 kg N ha^{-1} at 2 Weeks after Sowing (SAW); Phosphorus (P) was blanket-applied as SSP (18% P_2O_5) fertilizer at 20 kg P ha^{-1} while Potassium (K) was blanket-applied as Muriate of Potash (60% K_2O) at 20 kg K ha^{-1} at 5 cm away from base of cowpea and groundnut stand. Before planting, seeds were mixed with each of the three test formulations at the rate of 10 g /kg seed and 3 g of each of the formulations were mixed with 30 g of soil and broadcasted in each plot at time of planting. The suspension of the formulations were prepared by mixing 5 g in 1 Liter of water and sprayed at 15, 25, 45 and 60 days after sowing (DAS).The chemical control, Funguforce (Mancozeb + Carbendazim) was sprayed fortnightly and seeds in this treatment were dressed with Apronstar (Metalaxyl + Thiometoxam + Difenoconazole).

Data collection

The percentage of seedling emergence was determined two weeks after sowing as percentage of sowed seeds that emerged using the following formula.

$$\text{Seedling emergence ?\%?} = \frac{\text{Number of seedlings that emerged}}{\text{Number of seeds sown}} \times 100$$

Disease incidence of scab on cowpea and late leaf spot on groundnut was calculated using the formula below

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Individual plants were scored for disease severity using the scale in Tables 1 and 2 for groundnut leaf spot and cowpea scab respectively. Disease severity (%) was calculated using the following formula

$$\text{Disease severity (\%)} = \frac{\text{sum of all disease scores}}{\text{Total no. of plants assessed x Maximum disease score}} \text{x} 100$$

Pod yield was recorded after harvest and expressed per hectare.

Table 1: Scale used for assessing groundnut leaf spot severity

Description	Defolia (%)	Sco
1. No disease.	0	0
2. Lesion present largely on lower lea	1-5	1
3. Lesion largely on lower leaves; very Middle leaves; defoliation of some lower leaves	6-10	2
4. Lesions present on lower and midd on lower leaves; some defoliation of leaflets evident on lower leaves.	11-20	3
5. Lesions are present on all lower an lower leaves	21-30	4
6. Lesions severe on low[illegible] leaves but less severe; extensive d defoliation of some leaflets evident	31-40	5
7. Lesions present on all lower leaves defoliation of [illegible] leaves.	41-60	6
8. Defoliation of all lower and middle leaves and some defoliation of top	61-80	7
9. Defoliation of almost all leaves leav may be present but severe leaf spots	81-100	8

Source: Chiteka et al. (1988)

Table 2: Scale used for assessing cowpea scab severity

Description		% plant affected	Score
1.	No visible symptoms on either foliage or pods)	0	0
2.	scattered lesions on either foliage or pods	1-10	1
3.	extensive spotting of young stems and branches or on the pods	11-20	2
4.	stem lesions coalescing, covering half the plants or the pods	20-50	3
5.	foliage severely damaged or pod transformed into		

Source: Nakawuka and Adipala, (1997)

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9 and where the F-value was found to be significant, treatment means were separated using Least Significant Difference (LSD) at 5 % probability level.

Results

Identification of the micro organisms

Cultures on PDA were green with concentric rings which after microscopic examination were found to have flask-shaped conidia and were identified as Trichoderma sp. having matched the description by Shah et al. (2012). Two types of colony were observed on NA. One of the colonies was identified as *Pseudomonas fluorescens* as it was observed to be creamy white, mucous and gram negative which fluorescent under UV light on King's B medium. This corresponds to the description by Suman et al (2016). The other colony was dry, milky and gram positive with spores observed under light microscope. It was identified as *Bacillus* sp. having conformed to the description by Li et al. (2015). The sequence of the isolated organisms when subjected to BLAST search on NCBI database had homology with those of *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus cereus*. The materials submitted to NCBI had accession numbers designated as KY495199, KY495220 and KY495208 respectively. The organisms were thus identified as *Trichoderma harzianum* strain CGAJ2T-3; *Pseudomonas fluorescens* strain CGA FPBPF-034 and *Bacillus cereus* strain CGA FPFTS-012.

Field efficacy of the tested formulations

Plant emergence did not significantly differ ($P \leq 0.05$) across all the treatments for both crops and years (Tables 3 - 6). In 2016 disease incidence and severity on groundnut at Samaru were highest in untreated control (Table 3). Lowest disease incidence (50.57 %) was recorded on *P. fluorescens* while severity was lowest (14.40 %) on B. cereus at Samaru. A similar trend in disease incidence and severity was observed at Minjibir. The yield of bio-control agents and the chemicals plots at Samaru were at par and higher than the control. At Minjibir, no significant yield increase was recorded on treated plots compared with the control.

In 2017 cropping season, there was no significant difference ($P \leq 0.05$) in the incidence and severity of groundnut late leaf spot among the treatments at Samaru (Table 4). At Minjibir however, highest incidence (48.57 %) and severity (36.64 %) were recorded with control treatment while Funguforce had the lowest incidence (9.77 %) and severity (9.53 %). Highest pod yield of 1767 kg/ha at Samaru and 1641 kg/ha at Minjibir were obtained from *T. harzianum* and *P. fluorescens* respectively.

Table 3: Effect of microbial formulations on disease incidence and severity of late leaf spot, emergence and pod yield of groundnut at Samaru and Minjibir in 2016

Treatment	Emergence (%)		Disease incidence (%)		Disease severity (%)		Pod yield (kg/ha)	
	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir
T. harzianum	98.00	100.00	77.03^{ab}	48.75^{b}	19.65^{b}	18.22^{b}	2523.40^{a}	1351.50
P.fluorescens	96.50	89.60	50.57^{b}	56.06^{b}	16.13^{b}	22.28^{b}	2283.00^{a}	1433.90
B.cereus	94.60	92.00	51.40^{b}	73.95^{ab}	14.40^{b}	19.63^{b}	2319.20^{a}	1218.20
Funguforce	97.00	95.00	63.35^{b}	93.61^{a}	22.02^{ab}	33.17^{b}	2570.20^{a}	1195.30
Control	89.00	68.50	100^{a}	100^{a}	42.47^{a}	74.04^{a}	1066.70^{b}	666.90
LSD	29.78	39.25	25.84	29.52	21.43	39.48	559.80	783.26

Means with same superscript in a column were not significantly different at 5% probability level using Least Significant Difference (LSD)

Table 4: Effect of microbial formulations on disease incidence and severity of late leaf spot, emergence and pod yield of groundnut at Samaru and Minjibir in 2017

Treatment	Emergence (%)		Disease incidence (%)		Disease severity (%)		Pod yield (kg/ha)	
	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir
T. harzianum	89.60	78.00	8.33	16.73^{b}	4.17	15.77^{b}	1767.30^{a}	1329.80^{ab}
P.fluorescens	75.00	92.00	4.17	23.87^{ab}	4.17	20.87^{ab}	1665.40^{ab}	1640.50^{a}
B.cereus	85.00	90.00	8.33	23.73^{ab}	7.00	21.57^{ab}	1475.50^{ab}	1296.60^{ab}
Funguforce	90.00	89.00	3.33	9.77^{b}	3.33	9.53^{b}	1424.80^{ab}	1269.80^{ab}
Control	68.50	85.00	11.67	48.57^{a}	10.83	36.64^{a}	1210.90^{b}	908.80^{b}
LSD	36.67	43.65	21.54	27.78	9.83	20.63	548.81	585.06

Means with same superscript in a column were not significantly different at 5% probability level using Least Significant Difference (LSD)

There was no significant difference ($P> 0.05$) in disease incidence of cowpea scab at both Samaru and Minjibir in 2016 (Table 5). No significant difference in severity was also recorded at Samaru but at Minjibir, highest severity (21.74 %) was recorded in untreated plots while Funguforce treated plots had the lowest severity (10.55 %) which did not significantly differ from that of the bio-control agents. No significant difference in pod yield was observed at Samaru while at Minjibir, *P. fluorescens* had the highest yield (2044kg/ha) which was at par with B. cereus. The yields of *T. harzianum* and control were statistically similar.

In 2017 no significant difference ($P> 0.05$) was recorded in the number of cowpea plants infected with scab; however the severity of the disease significantly varied among the treatments (Table 6). Highest severity was recorded on control while the lowest was on Funguforce in both Samaru and Minjibir. *T. harzianum, P. fluorescens* and *B. cereus* had respectively resulted in disease reduction of 51.42, 39.32 and 37.16 % at Samaru; 26.15, 53.6 and 2.37 % % at Minjibir. Pod yield at Samaru significantly varied among the treatments with *T. harzianum* having the highest yield (1564kg/ha) and control had the lowest (1080kg/ha). Pod yields due to the bio-control agents and the chemical were statistically similar. There was no significant difference in the cowpea yield recorded at Minjibir though yields increase as high as 33.53 % was recorded on *T. harzianum*.

Table 5: Effect of microbial formulations on disease incidence and severity of scab, emergence and pod yield of cowpea at Samaru and Minjibir in 2016

Treatment	Emergence (%)		Disease incidence (%)		Disease severity (%)		pod yield (Kg/ha)	
	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir
T. harzianum	100.00	87.00	19.11	16.43	12.54	11.81[b]	1707.40	1284.4[b]
P.fluorescens	95.00	78.00	19.64	19.99	12.94	13.69[b]	1566.70	2043.60[a]
B.cereus	100.00	85.00	13.50	18.54	10.77	14.28[b]	1377.80	1794.80[a]
Funguforce	95.00	90.00	20.00	13.33	12.00	10.55[b]	1364.50	1944.80[a]
Control	75.00	80.00	37.78	39.50	17.22	21.74[a]	1230.10	884.00[b]
LSD	42.32	33.52	27.26	28.00	8.32	6.32	581.78	456.38

Means with same superscript in a column were not significantly different at 5% probability level using Least Significant Difference (LSD)

Table 6: Effect of microbial formulations on disease incidence and severity of scab, emergence and pod yield of cowpea at Samaru and Minjibir in 2017

Treatment	Emergence (%)		Disease incidence (%)		Disease severity (%)		pod yield (kg/ha)	
	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir	Samaru	Minjibir
T. harzianum	98.00	67.50	19.00	30.83	14.17[ab]	24.00[ab]	1564.20[a]	1445.30
P.fluorescens	93.00	84.00	20.00	19.17	17.70[ab]	15.07[b]	1413.40[a]	1147.40
B.cereus	100.00	73.50	22.33	36.67	18.33[ab]	31.73[a]	1387.90[ab]	1274.60
Funguforce	100.00	80.00	11.73	14.50	8.50[b]	10.17[b]	1489.70[a]	1369.10
Control	74.00	62.40	45.00	50.00	29.17[a]	32.50[a]	1080.00[b]	960.70
LSD	27.89	46.54	35.63	40.75	16.23	13.03	311.63	471.08

Means with same superscript in a column were not significantly different at 5% probability level using Least Significant Difference (LSD)

Discussion

This study shows that the application of the tested bio-control agents does not negatively affect crop emergence. This is in agreement with earlier report that Bacillus spp. used as seed treatment improved cowpea yield and germination (Maureen, 2016).Formulations of *Pseudomonas* spp. and *Bacillus subtilis* are also being used as cotton seed treatment in the USA (Brannen and Kenney, 1997). Dubey et al. (2011) have also reported an improvement in seed and crop performance in field of mungbean when treated with *Trichoderma* spp.

There was a disease reduction of up to 54 % by the application of the bio-control agents indicating the capability of the organisms in managing fungal diseases of legumes. In India, Nandi et al. (2016) reported that treatment of cowpea seeds with suspension of *P. fluorescens* gave a 37.31 % post emergence damping off reduction over untreated control and was even better than Carbendazim. Weekly spray of suspension of T. viride was shown to be as effective as Benomyl spray in reducing incidence of brown blotch on cowpea in the field (Bankole and Adebanjo, 1996). It was also reported from a field experiment in Brazil that combined inoculation of cowpea seeds with rhizobia and *Trichoderma* spp. showed effectiveness against *Rhizoctonia solani* and improvement in stand count (Aloisio et al., 2015).

The result of this study has also shown that the application of the tested formulations increased yield of cowpea and groundnut up to 50 %. A similar finding showed that yield increase of 55 – 150 % was obtained when cowpea plants were sprayed with suspension of *T. viride* (Bankole and Adebanjo, 1996). Another research reported promotion of cowpea growth and yield by *Trichoderma* spp. through synthesis of Indole acetic acid and phosphate solubilization (Changas et al., 2016). *P. fluorescens* was also found to increase tomato yield by 10 % compared with the non-inoculated control (Weller, 2007). Another research also reported reduced late leaf spot symptoms, increase in pod yield and plant growth by the application of *P. fluorescens* on groundnut (Meena and Marimuthu, 2012).The result of this study also agrees with the report of Hassan et al. (2014) that a commercially available Trichoderma based formulation, BAU in Bangladesh gave the highest germination percentage, lowest disease severity as well as highest vigor index and yield. The effectiveness of the tested formulations in this study indicates that use of talc as a carrier does not interfere with antagonistic activity of *T. harzianum, P. fluorescens* and *B. cereus*.

Conclusion

The study indicated that application of formulations containing spores of *T. harzianum; P. fluorescens* and *B. cereus* are effective in increasing yield and reducing scab and late leaf spot on cowpea and groundnut. It can be concluded that application of microbial formulations in the soils of Nigerian savanna is an effective way of managing fungal diseases of leguminous crops. It should therefore be incorporated into the Integrated Pest Management strategies of these crops in order to minimize the harmful effects of indiscriminate use of synthetic pesticides to farmers and the environment. Further studies on the effect of the tested microbial formulations on beneficial soil micro-organisms are thereby recommended.

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