BIOCONTROL ABILITY OF *Trichoderma harzianum* ON GROWTH, DISEASE INCIDENCE AND YIELD OF SELECTED COWPEA VARIETIES (*Vigna unguiculata* L.) INFESTED WITH *Colletotrichum lindemuthianum*

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ABSTRACT

Cowpea anthracnose caused by *Colletotrichum lindemuthianum* causes about 75 % yield reduction in Cowpea production in Nigeria. *Trichoderma harzianum* is a bioagent in controlling fungal diseases. Effect of *T. harzianum* was investigated on growth, disease incidence and yield of selected varieties of Cowpea infested with *C. lindemuthianum.* Four improved Cowpea varieties were selected IT07K-292-10, IT07K-318-33, IT98K-205-08, IT99K-573-11 and a local variety DANMISRA. Pure isolate of *Colletotrichum lindemuthianum* and *Trichoderma harzianum* were each cultured on PDA. Four concentrations of *T. harzianum* suspensions were prepared at $1x10^2$ conidia/ml, $1x10^4$ conidia/ml, $1x10^6$ conidia/ml, $1x10^8$ conidia/ml and control . Cowpea seedlings were artificially infested with $1x10^7$conidia/ml of *C. lindemuthianum* at 22 days after sowing. The experiment was arranged in a complete randomized design (CRD) with three replications. The result indicated that the infested variety IT99K-573-11 treated with *T. harzianum* at $1x10^8$ conidia/ml was insusceptible and had significantly ($p<0.05$) increased biomass, number of seeds and low disease incidence (19.1 %) while increase in chlorophyll content, seed number and decreased in the anthracnose incidence was observed at $1x10^8$ conidia/ml concentration. The cultivar IT99K-573-11 appeared tolerant hence is recommended to farmer for cultivation in *C. lindemuthianum* infested areas.

Keywords: Trichoderma, Colletotrichum, Cowpea, Anthracnose, Biocontrol

INTRODUCTION

Cowpea (*Vigna unguiculata L.*) is an annual, herbaceous and leguminous plant, with centre of origin and domestication reported to be in Africa (Carlos, 2004). It is a major source of Protein and a cheap source of quality protein for both rural and urban dwellers in Africa (Dube and Fanadzo, 2013). Cowpea diseases induced by species of pathogens belonging to various pathogenic groups (fungi, bacteria, viruses and nematodes) constitute one of the most important constraints to profitable Cowpea production, in all agro ecological zones where the crop is cultivated. Despite the increasing importance of Cowpea in the diet of man as a source of protein and agronomy, maximum yield potentials of the crop have not been revealed due to incidence and severity of anthracnose disease (Masangwa *et al.,* 2013). Cowpea Anthracnose caused by *C. lindemuthianum* is present in almost all areas where Cowpea is grown and is also an important fungal disease of field grown Cowpea capable of 75 % yield reduction in Nigeria (Enyiokwu *et al*., 2014).

The most frequently used control measure against pests and diseases is by synthetic pesticides usage whose intensive and indiscriminate use in agriculture has caused many problems to the environment. Such problems include water, soil, animals and food contamination; poisoning of farmers; elimination of non-target organisms; and selection of pest and weed tolerance to certain pesticides (Nidhi and Trivedi, 2002). Potential value of *T. harzianum* as bio-agents for the protection of several seedlings, potted outdoor and field diseases of crops (Howell, 2003). *Trichoderma* species also have the ability to interact with roots of diverse plant species leading to Induced Systemic Resistance (ISR) responses to a wide spectrum of pathogens and adverse environmental conditions (Shoresh *et al.,* 2010; Loritio *et al*., 2010). Hence there is the need to assess the effect of *T. harzianum* on Cowpea plant affected with *C. lindemuthianum*. The aim of the present study was to evaluate the effects of *Trichoderma harzianum* on growth, disease incidence and yield of selected varieties of Cowpea infested with *C. lindemuthianum*.

MATERIALS AND METHODS

Seed Collection and Identification of Fungi

Four improved varieties IT07K-292-10, IT07K-318-33, IT98K-205-08 and IT99K-573-11 and Local Variety (DANMISRA) were obtained from International Institute of Tropical Agriculture (IITA), Kano Station, Nigeria. The experiment was conducted in the Laboratory of Plant Pathology and Screen house of Plant Biology Department of Bayero University Kano.

Fungal Isolation; Rhizosphere soil of healthy Cowpea stand was collected from Gidantuku Village, Warawa, Kano State. Dilution plate method described by (Akhbar, 1966) was used for isolation of *Trichoderma* and identification using standard mycological key (Gams and Bissett, 1998). Cowpea leaves infested with *Colletotrichum lindemuthianum* were collected from Gidantuku Village, near Wudil river, Warawa Local Government of Kano State. The sterilized infected leaf was isolated using the method described by Pandey (2015). The pathogen was identified according to their morphological and cultural characters.

Preparation and Application of Inoculum

Colletotrichum lindemuthianum

The pure culture of the isolated *C. lindemuthianum* was harvested from 15-Days old culture grown on PDA, by adding 10 ml of distilled water to the plate and scrapping the culture using spatula. The spores were added to the beaker containing 100 ml of distilled water. The resulting suspensions were filtered through a double layer of cheese cloth. Cowpea Plants were evenly spread with 1 x 10^7 conidia/ml 21 days after sowing using a hand sprayer.

Trichoderma harzianum

Conidia of *Trichoderma* were harvested from 10 days old culture grown on PDA by adding 10 ml distilled water to the plate and scrapping the culture with spatula. The resulting suspensions were filtered through double layer of cheese cloth. Cowpea Plants were evenly sprayed with spore suspensions of *Trichoderma* concentrations i.e. (1x10^2 conidia/ml, 1x10^4 conidia/ml, 1x10^6 conidia/ml and 1x10^8 conidia/ml) at 22 days after sowing using a hand sprayer, 24 hours after inoculating 1x10^7 conidia/ml of *C. lindemuthianum*.

Plant Preparation

Three seeds were sown in a perforated pot containing 5 kg of sterilized sandy-loamy soil, amended with manure 1:5 ratio and analyzed for;

Number of Leaves**:** The entire number of plant leaves were countered at 3, 13 and 23 days after spray treatments.

Plant Height**:** The height of the plants was measured using a meter rule from the base of the plant to the terminal bud end at 3, 13 and 23days after spray treatment.

Shoot and root Biomass**:** The shoots /root of the harvested Cowpea plants were cut off, dried using oven at 40 °C and weighted using weighing balance (Scout™ pro, w=400 g).

***Chlorophyll Contents*:** Chlorophyll contents of tagged plant leaves were measured at 3, 13 and 23 days after spray treatment, using SPAD Chlorophyll Meter.

Disease Incidence (D.I)

The incidence of Anthracnose disease was calculated at 3 and 23 days after treatment, according to formula;

$$\text{D.I \%} = D.I = \frac{Number\ of\ infected\ leaves}{Total\ number\ of\ leaves} \times 100 \text{ (Ansari, 1995).}$$

***Number of pods and seeds*:** Total number of pods were counted at 15, 20 and 25 days after treatment while total number of each plant seeds were counted after harvesting.

Experimental Design and Data Analysis

Experiment was conducted in the screen house of Plant Biology Department. The concentrations of *T. harzianum* and *C. lindemuthianum* were measured using Haemocytometer (Goswani and Kistler, 2004). The experiment was laid out in a complete randomized design (CRD) with three replications. Data collected were analyzed using Analysis of Variance and means were separated using Duncan Multiple Range Test (DMRT) at 5 % probability level using SAS 9.0 version.

RESULTS AND DISCUSSION

Number of Leaves

The result on the effect of different concentrations of *T. harzianum* on number of leaves and interaction effect between different concentrations of *T. harzianum* and Cowpea Varieties are presented in Tables 1 and 2. There are significant differences ($p < 0.05$) in the treatments and varieties at 3, 13 and 23 days after treatment. The result showed that at 3, 13 and 23 DAT, the concentration of *T. harzianum* at 1×10^8 con/ml recorded the highest number of leaves while the control recorded the lowest number of leaves.

Cowpea varieties at 3, 13 and 23 DAT showed that, variety IT99K- 573-11 recorded the highest number of leaves (33.00, 44.00 and 49.00) respectively, while DANMISRA variety recorded lowest number of leaves (Tables 1). The highest percentage increase in number of leaves was recorded in variety IT98K-205-08 (58.6 % and 62.5 %) while Danmisra variety recorded the least (10 % and 11.8 %) respectively (Table 2).

Table 1: Main effect of *C. lindimuthianum* and *T. harzianum* infestations on number of leaves of Cowpea varieties.

Treatments (Conc./ml)	3 DAT	13 DAT	23 DAT
1×10^8	33.00 ± 1.09 ^{a}	41.00 ± 1.59 ^{a}	47.00 ± 1.77 ^{a}
1×10^6	31.00 ± 1.06 ^{b}	39.00 ± 1.87 ^{a}	42.00 ± 2.02 ^{a}
1×10^4	27.00 ± 1.02 ^{c}	34.00 ± 1.36 ^{b}	35.00 ± 1.50 ^{b}
1×10^2	26.00 ± 1.03 ^{c}	33.00 ± 1.44 ^{b}	34.00 ± 2.86 ^{b}
Control	23.00 ± 0.94 ^{d}	28.00 ± 2.60 ^{c}	31.00 ± 3.73 ^{b}
Varieties			
IT99K- 573-11	32.80 ± 1.30 ^{a}	44.00 ± 1.37 ^{a}	49.00 ± 1.11 ^{a}
IT98K-205-08	29.00 ± 1.54 ^{b}	36.00 ± 1.79 ^{b}	39.00 ± 2.20 ^{b}
IT07K-318-33	27.00 ± 1.07 ^{bc}	33.00 ± 1.54 ^{c}	33.00 ± 3.12 ^{c}
IT07K-292-10	27.00 ± 1.37 ^{bc}	32.00 ± 2.76 ^{c}	33.00 ± 3.79 ^{c}
DANMISRA	25.00 ± 0.88 ^{c}	31.00 ± 0.72 ^{c}	34.00 ± 0.77 ^{c}
V × T	NS	NS	NS

Means± standard error with the same letter in the column are not significant at 5 % using DMRT

Table 2: Treatment effect of *T. harzianum* on number of leaves of infested Cowpea varieties

TREATMENT					
13 DAT					
Varieties	**DANMISRA**	**IT07K-292-10**	**IT07K-318-33**	**IT98K-205-08**	**IT99K- 573-11**
Control	30 ± 0.3	30 ± 5.4	26 ± 0.9	29 ± 2.6	33 ± 2.0
1×10^{2}	30 ± 2.0	33 ± 1.0	28 ± 1.7	33 ± 3.2	41 ± 1.8
1×10^{4}	29 ± 1.2	33 ± 1.3	32 ± 1.2	34 ± 2.3	43 ± 1.0
1×10^{6}	31 ± 0.2	38 ± 3.4	36 ± 1.2	38 ± 1.2	49 ± 4.9
1×10^{8}	33 ± 2.6 (10.0)	37 ± 0.9 (23.3)	41 ± 1.2 (57.7)	46 ± 0.7	48 ± 0.7 (45.5)
IL (%)				(58.6)	
P-value $_{(0.05)}$	0.677475	0.0011	0.00004	0.00013	0.0006
23 DAT					
Control	34 ± 0.9	34 ± 1.8	29 ± 0.7	32 ± 2.1	37 ± 0.7
1×10^{2}	34 ± 1.7	34 ± 1.5	19 ± 3.2	36 ± 3.8	45 ± 1.5
1×10^{4}	32 ± 2.3	34 ± 2.0	33 ± 1.2	32 ± 1.5	45 ± 0.9
1×10^{6}	34 ±1.5	42 ± 3.7	38 ± 2.3	43 ± 2.1	54 ± 2.0
1×10^{8}	35 ± 2.4	46 ± 0.9 (26.5)	48 ± 2.6 (48.3)	52 ± 1.2 (62.5)	52 ± 0.6
(IL %)	(1.8)				(46.0)
P-value$_{(0.05)}$	0.708728	0.0371	0.0134	0.00008	0.001

Mean ± standard error of the three replicates, IL= Percentage increase in number of leaves

Plant Height

The result on the effect of different concentrations of *T. harzianum* on plant height and interaction effect between concentrations of *T. harzianum* and Cowpea varieties were presented in Tables 3 and 4. The result showed that there is significant different ($p < 0.05$) in the treatments and varieties at 3, 13 and 23 days after treatment. Similarly at 23 DAT, there is significant

Table 3: Effect of *C. lindimuthianum* and *T. harzianum infestations* on plant height of Cowpea varieties

Treatment (Con/ml)	**3 DAT**	**13 DAT**	**23 DAT**
1×10^{8}	76.19 ± 7.96 ^{ab}	98.03 ± 9.70 ^{a}	106.87 ± 10.44 ^{a}
1×10^{6}	73.25 ± 7.62 ^{ab}	90.08 ± 8.68 ^{a}	96.89 ± 9.15 ^{a}
1×10^{4}	66.26 ± 6.97 ^{bc}	76.45 ± 7.65 ^{b}	80.64 ± 7.91 ^{b}
1×10^{2}	63.23 ± 6.74 ^{cd}	69.33 ± 6.73 ^{b}	66.63 ± 8.39 ^{c}
Control	57.92 ± 7.33 ^{d}	60.23 ± 7.54 ^{c}	54.85 ± 9.27 ^{d}
Varieties			
IT99K- 573-11	113.84 ± 5.32 ^{a}	123.67 ± 6.3 ^{a}	129.14 ± 6.85 ^{a}
IT98K-205-08	64.84 ± 2.69 ^{b}	76.25 ± 4.28 ^{b}	82.38 ± 5.42 ^{b}
IT07K-318-33	63.34 ± 2.15 ^{b}	78.60 ± 3.46 ^{b}	79.10 ± 6.93 ^{b}
IT07K-292-10	60.74 ± 2.13 ^{b}	76.25 ± 7.10 ^{b}	77.41 ± 10.64 ^{b}
DANMISRA	34.08 ± 1.39 ^{c}	36.05 ± 1.39 ^{c}	37.74 ± 1.50 ^{c}
V×T	NS	NS	NS

Means ± standard error with the same letter within the column are not significant at 5 % using DMRT.

interaction between the treatment and varieties. Plant height increases in a concentration dependent pattern at 3, 13 and 23 DAT. During the 23 DAT he concentration of *T. harzianum* at 1×10^{8} con/ml, 1×10^{6} con/ml and control recorded plant height o of 106.87 cm 96.89 cm and 54.85 cm (Table 3).The tested Cowpea varieties at 3, 13 and 23

DAT showed that, variety IT99K- 573-11 recorded the highest plant height at 3, 13 and 23 DAT (113.84 cm, 123.67 cm and 129.14 cm) respectively, while variety DANMISRA recorded lowest plant height (34.08 cm, 36.05 cm and 37.74 cm). There were significant interactions between treatment and varieties ($p < 0.05$) at 23 DAT. The highest percentage increase in plant height was recorded in variety IT98K-205-08 (75.8.6 %), followed by variety IT99K- 573-11 (64.0 %) while the control is the lowest.

Table 4: Treatment effect of *C. lindimuthianum* and *T. harzianum* on plant height of infested Cowpea varieties at 23 DAT

Varieties	DANMISRA	IT07K-292-10	IT07K-318-33	IT98K-205-08	IT99K- 573-11
Control	30.6 ± 1.9	55.3 ± 9.1	62.6 ± 2.9	62.4 ± 4.3	97.3 ± 9.9
1×10^2	39.7 ± 4.8	65.2 ± 5.6	47.3 ± 3.9	68.3 ± 2.9	112.6 ± 7.2
1×10^4	37.2 ± 3.0	74.0 ± 2.9	86.5 ± 1.4	77.4 ± 2.3	128.1 ± 7.0
1×10^6	39.0 ± 1.0	108.2 ± 5.2	97.4 ± 3.1	99.8 ± 4.6	140.0 ± 7.2
1×10^8	42.1 ± 2.0	78.4 ± 8.7	101.7 ± 3.2	109.0 ± 6.7	159.2 ± 8.8
(% IPL)	(37.6)	(41.8)	(62.5)	(75.8)	(64.0)
P-value$_{(0.05)}$	0.134	0.0003	0.023	0.00005	0.102

Mean ± standard error, % IPL=Percentage increase in Plant Height.

Shoot Biomass

The result of the effect of different concentrations of *T. harzianum* on Shoot Biomass and interaction effect between different concentrations of *T. harzianum* and Cowpea varieties were presented in Tables 5 and 6. There is significant difference ($p < 0.05$) in the treatments, varieties and their interactions effect.

The concentration of *T. harzianum* at 1×10^8 con/ml recorded the highest shoot biomass (12.45 g) followed by 1×10^6 con/ml (11.13 g) while the control recorded the lowest shoot biomass (6.18 g). The result on the Cowpea varieties indicated that variety IT07K-318-33 recorded the highest shoot biomass (11.89 g) followed by IT99K- 573-11 (10.97 g) while lowest shoot biomass (6.18 g) was obtained with the DANMISRA variety (Tables 5). Interactions between treatment and varieties showed that, the highest shoot biomass was recorded in the 1×10^8 conidia/ml of *Trichoderma* while the highest percentage increase in shoot biomass was recorded in variety IT98K-205-08 (87.7 %), IT99K- 573-11 (85.70 %) while the lowest percentage increase in shoot biomass (50.0 %) was recorded in variety DANMISRA (Table 6).

Table 5: Effect of *C. lindimuthianum* and *T. harzianum* infestation on shoot biomass of Cowpea varieties

Treatments (Con/ml)	Weight (g)
1×10^8	12.45 ± 1.00 ^{a}
1×10^6	11.13 ± 1.07 ^{ab}
1×10^4	9.69 ± 0.76 ^{b}
1×10^2	7.42 ± 0.50 ^{c}
Control	6.18 ± 0.61 ^{c}
Varieties	
IT07K-318-33	11.89 ± 0.98 ^{a}
IT99K- 573-11	10.97 ± 1.04 ^{ab}
IT98K-205-08	9.59 ± 0.68 ^{bc}
IT07K-292-10	9.02 ± 0.90 ^{c}
DANMISRA	5.39 ± 0.40 ^{d}
V × T	NS

Means± standard error with the same letter within the column are not significant at 5 % using DMRT.

Table 6: Treatment effect of *C. lindimuthianum* and *T. harzianum* on shoot biomass of infested Cowpea varieties

Varieties	DANMISRA	IT07K-292-10	IT07K-318-33	IT98K-205-08	IT99K- 573-11
Control	3.6 ± 0.4	4.9 ± 1.0	8.9 ± 0.3	7.3 ± 1.1	6.2 ± 1.4
1×10^2	5.6 ± 1.1	7.5 ± 0.9	7.9 ± 1.9	7.4 ± 0.5	8.8 ± 0.2
1×10^4	5.2 ± 0.4	9.0 ± 1.0	12.3 ± 0.2	9.4 ± 0.3	12.6 ± 0.9
1×10^6	5.9 ± 0.7	9.7 ± 1.2	17.1 ± 0.2	10.2 ± 0.5	12.8 ± 1.1
1×10^8	6.7 ± 0.8	14.0 ± 1.2	13.2 ± 1.3	13.7 ± 0.8	14.7 ± 2.8
(% ISB)	(50.0)	(62.5)	(71.9)	(87.7)	(85.7)
P-value	0.1131	0.00177	0.00218	0.00034	0.034

Mean ± standard error, % ISB = Percentage increase in Shoot Biomass

Chlorophyll Contents

The result of the effect of different concentrations of *T. harzianum* on Chlorophyll contents and interaction effect between treatment of *T. harzianum* and Cowpea varieties were presented in Tables 7 and 8. The statistical analysis showed that there is significant difference ($p < 0.05$) between the treatment and varieties at 3, 13 and 23 DAT.

The concentration of *T. harzianum* at 1×10^8 con/ml recorded the highest chlorophyll contents at 3, 13 and 23 DAT (52.73, 56.58 and 60.84) respectively, followed by 1×10^6 con/ml (51.06, 53.97 and 57.67) while control treatment recorded the lowest value (46.19, 44.72 and 42.63) respectively. The Cowpea variety DANMISRA recorded the highest chlorophyll contents at 3, 13 and 23 DAT (55.06, 56.8 and 59.5) while the IT07K-318-33 variety is the lowest (47.4, 49.5, and 49.5) (Table 7). The highest percentage increase in chlorophyll contents were recorded in variety IT98K-205-08 at 3 and 13 DAT (21.4 % and 35.2 %) and variety IT99K-573-11 at 23 DAT (46.3 %). while the lowest percentage increase was recorded in variety DANMISRA at 3, 13 and 23 DAT (10.2 %, 8.4 % and 18.1 %) respectively (Table 7).

Table 7: Effect of *C. lindimuthianum* and *T. harzianum* infestation on chlorophyll contents of Cowpea varieties

Treatments/Varieties	3 DAT	13 DAT	23 DAT)
1×10^8	52.73 ± 0.76 [a]	56.58 ± 0.78 [a]	60.84 ± 1.09 [a]
1×10^6	51.21± 0.82 [ab]	53.97 ± 0.82 [b]	57.67± 0.80 [b]
1×10^4	50.84 ± 0.85 [b]	52.77 ± 1.07 [b]	54.26± 1.04 [c]
1×10^2	48.43 ± 0.94 [c]	49.31 ± 0.74 [c]	48.00 ± 1.53 [d]
Control	46.19 ± 1.44 [d]	44.72 ± 1.88 [d]	42.63 ± 2.10 [e]
Varieties			
DANMISRA	55.06 ± 0.87	56.80 ± 1.07	59.55 ± 1.35 [a]
IT98K-205-08	49.71 ± 0.86	51.85 ± 1.09	52.67 ± 1.66 [b]
IT07K-292-10	49.03 ± 1.28	49.82 ± 2.20	51.46 ± 2.8 [bc]
IT99K- 573-11	48.16 ± 0.50	49.36 ± 0.98	50.7 ± 1.91 [bc]
IT07K-318-33	47.45 ± 0.93	49.53 ± 1.26	49.05 ± 2.04 [c]
V × T	NS	NS	NS

Means ± standard error with the same letter within the column are not significant at 5 % by DMRT

Disease Incidence

The result on disease incidence and interaction effect of *T. harzianum* and infested Cowpea varieties were presented in Tables 8 and 9. There is significant different ($p < 0.05$) in the treatments and varieties at 3 and 23 DAT, the result also revealed significant interaction between the treatment and Cowpea Varieties at 3 and 23 DAT. The result at 13 and 23 DAT indicated a decreased in disease incidence by 14.39 % and 12.48 % at $1{\times}10^8$ con/ml while control treatment recorded about 45.95 % at 23 DAT. The Cowpea variety IT98K-205-08 recorded 15.54 % and 18.27 % declined in disease incidence at 3 and 23 DAT while the highest increase (28.75 % and 33.51 %) in disease incidence were recorded in variety IT07K-292-10 respectively (Table 8).

Table 8: Treatment effect of *C .lindimuthianum* and *T. harzianum* infestations on chlorophyll content of Cowpea varieties

Varieties	DANMISRA	IT07K-292-10	IT07K-318-33	IT98K-205-08	IT99K-573-11
3 DAT					
Control	55.9±1.3	41.5±0.7	42.2±0.6	40.3±1.1	46.9±0.1
$1{\times}10^2$	53.2±3.2	46.7±0.8	45.2±0.9	48.9±0.9	48.2±0.6
$1{\times}10^4$	55.8±2.2	49.8±1.4	49.4±0.4	50.4±0.4	48.7±1.3
$1{\times}10^6$	54.2±2.3	52.8±0.7	48.9±0.9	52.3±0.8	47.9±1.5
$1{\times}10^8$	56.1±1.2	53.5±0.6	51.5±0.5	52.6±0.4	49.0±1.8
(%IC)	(10.2)	(28.9)	(26.2)	(29.5)	(21.4)
P-value $_{(0.05)}$	0.823	0.00001	0.00002	0.00016	0.786
13 DAT					
Control	55.8±1.3	36.0±3.4	41.4±0.9	42.2±1.7	45.2±1.2
$1{\times}10^2$	51.9±1.8	47.5±1.1	48.9±1.0	51.1±1.1	46.9±1.7
$1{\times}10^4$	58.8±2.8	52.1±2.3	51.0±0.8	51.9±0.7	50.0±1.2
$1{\times}10^6$	56.9±2.1	55.9±0.8	51.2±1.3	54.6±0.8	51.2±1.3
$1{\times}10^8$	60.5±1.2	57.6±0.9	55.0±0.6	56.8±0.3	53.5±2.2
(%IC)	(8.4)	(32.5)	(32.8)	(35.2)	(21.7)
P-value $_{(0.05)}$	0.085	0.00011	0.00002	0.0002	0.0232
23 DAT					
Control	55.0 ±2.1	33.7±3.3	42.0±1.1	42.7±0.9	39.6±3.9
$1{\times}10^2$	54.7±1.6	47.8±2.2	43.2±5.2	50.1±1.7	44.1±0.5
$1{\times}10^4$	60.1±2.1	53.9±2.0	53.2±0.6	53.9±0.9	50.3±1.6
$1{\times}10^6$	61.3 ±1.5	58.9±0.5	57.3±0.9	56.5±1.6	54.2±1.5
$1{\times}10^8$ (%IC)	66.6±1.2	62.9 ±0.8	57.6±1.1	60.1±0.8	56.9±2.3
	(18.1)	(42.9)	(34.3)	(40.5)	(46.3)
P-value $_{(0.05)}$	0.0041	0.00001	0.0022	0.00002	0.0015

Mean ± Standard error, % IC= Percentage Increase in Chlorophyll Contents.

There is a noticeable interaction (at 5 %) between the varieties and treatments at 3 and 23 DAT. The highest percentage decrease (70.0 %) in chlorophyll contents was observed in variety IT07K-318-33 at 23 DAT while the DANMISRA variety recorded a minimum of 43.1 % (Table 9).

Table 8: Effect of *C. lindimuthianum* and *T. harzianum* infestations on disease incidence in Cowpea varieties at 3 and 23 DAT

Treatments	3 DAT%	23 DAT%
1×10^8	14.39 ± 1.12^{a}	12.48 ± 0.96^{a}
1×10^6	17.12 ± 1.32^{a}	16.39 ± 1.31^{b}
1×10^4	20.51 ± 1.28^{c}	21.17 ± 1.35^{c}
1×10^2	25.39 ± 1.49^{b}	31.51 ± 2.56^{d}
Control	35.39 ± 2.95^{d}	45.95 ± 4.80^{e}
Varieties		
IT07K-292-10	28.75 ± 3.02^{a}	33.51 ± 5.55^{a}
IT98K-205-08	26.03 ± 2.72^{b}	29.25 ± 4.15^{ab}
IT07K-318-33	23.47 ± 2.64^{b}	27.28 ± 4.23^{b}
IT99K- 573-11	19.00 ± 1.5^{c}	19.17 ± 2.10^{c}
DANMISRA	15.54 ± 1.08^{d}	18.27 ± 1.78^{c}
V×T	NS	NS

Means ± standard error with the same letter within the column are not significant at 5 % by DMRT

Yield component

The result on yield revealed a significant different ($p < 0.05$) in the treatments, varieties, and their interaction effect. The result showed that higher concentration of *T. harzianum* at 1×10^8 con/ml recorded the highest number of seeds (43.00) followed by Treatment 1×10^6 con/ml (39.00) while the control treatment recorded the lowest (20.00). The Cowpea variety IT99K- 573-11 recorded the highest number of seeds (38.00) followed by IT07K-318-33 (35.00) while DANMISRA is the lowest (26.00) (Tables 10).

Table 9: Treatment effect of *C. lindimuthianum* and *T. harzianum* infestations on disease incidence in Cowpea varieties at 3 and 23 DAT

Treatment					
3 DAT					
Varieties	**DANMISRA**	**IT07K-292-10**	**IT07K-318-33**	**IT98K-205-08**	**IT99K- 573-11**
Control	18.7 ± 1.2	48.9 ± 3.1	39.1 ± 1.1	41.6 ± 2.5	28.6 ± 1.0
1×10^2	18.7 ± 1.9	28.4 ± 1.6	27.7 ± 1.7	31.7 ± 2.2	20.5 ± 0.9
1×10^4	16.1 ± 2.2	22.5 ± 3.4	24.0 ± 1.0	23.0 ± 2.1	16.8 ± 1.1
1×10^6	12.6 ± 1.5	23.9 ± 1.8	14.1 ± 0.8	19.2 ± 2.2	15.8 ± 2.1
1×10^8	11.6±1.6	30.0±2.6	18.4±1.8	14.6±1.5	13.3±0.7
(% DI)	(38)	(38.7)	(52.9)	(64.9)	(53.5)
P-value$_{(0.05)}$	0.082	0.00034	0.000001	0.00008	0.00005
23 DAT					
Control	25.6±1.1	70.2±5.7	46.7±1.3	54.7±4.2	32.6±1.3
1×10^2	24.0±2.2	32.4±0.4	42.4±4.7	36.5±2.3	22.2±0.6
1×10^4	16.7±3.2	24.6±3.5	23.1+0.3	24.8±2.4	16.6±0.8
0^6	15.5±2.1	23.4±2.2	11.4±0.7	18.3±1.8	13.4±1.9
1×10^8	12.3±1.7	16.9±2.7	14.8±2.3	12.1±1.2	11.0±0.2 6
% DI	(43.1)	(62.8)	(70)	(36.4)	(2.4)
P-value $_{(0.05)}$	0.0023	0.00006	0.00002	0.000007	0.00001

Mean ± standard error of the Three Replication, % DI= Percentage decrease in Disease Incidence

The varieties and treatments interactions ($p < 0.05$) showed that, the highest seed number was recorded at Treatment 1×10^8 conidia/ml of *Trichoderma*. The highest percentage increase (82.6 %) in seed number was recorded in variety IT98K-205-08 while DANMISRA variety recorded low (40.9 %) (Table 11).

Table 10: Effect of *C. lindimuthianum* and *T. harzianum* infestations on yield of selected Cowpea varieties

Treatments(con/ml)	Seed
1×10^8	43.00 ± 1.81 ^{a}
1×10^6	39.00 ± 2.02 ^{ab}
1×10^4	35.00 ± 2.27 ^{b}
1×10^2	28.00 ± 2.68 ^{c}
Control	20.00 ± 3.46 ^{d}
Varieties	
IT99K- 573-11	38.00 ± 1.68 ^{a}
IT07K-318-33	34.87 ± 3.08 ^{a}
IT98K-205-08	34.60 ± 3.86 ^{a}
IT07K-292-10	32.00 ± 4.48 ^{a}
DANMISRA	26.00 ± 1.66 ^{b}
V×T	NS

Means ± standard error with the same letter within the column are not significant at 5 % by DMRT

DISCUSSION

Despite the increasing importance of Cowpea in the diet of man as a source of protein and agronomy, maximum yield potentials of the crop has not been revealed due to incidence and severity of anthracnose disease (Masangwa *et al*., 2013). The biological control activity of the *Trichoderma* against fungal pathogens has been reported (Joshi *et al*., 2010; Lone *et al*., 2012). In the present study, application of *T. harzianum* had significant effect in yield of Cowpea varieties. These results are in agreement with Lortio (2005) and Abd-EL-Hamed (2014) who reported *T. harzianum* application as spore suspension sprayed as foliar application to plant was the most promising way of increasing the plant growth parameters and reducing bean white rot and rust incidence. This is due to the main role of bio agents (such as *Trichoderma*) on decreasing the percentage of infectious diseases hence conserve and sustain the plant in a good health with very light attack.

Table 11: Interaction of *C. lindimuthianum* and *T. harzianum* infestations on yield of selected Cowpea varieties

Varieties	DANMISRA	IT07K-292-10	IT07K-318-33	IT98K-205-08	IT99K- 573-11
Control	22.0 ± 3.2	22.0 ± 0.9	26.0 ± 2.6	15.0 ± 3.7	35.0 ± 3.5
1×10^2	26.0 ± 2.9	28.0 ± 0.9	29.0 ± 1.4	24.0 ± 1.7	33.0 ± 1.9
1×10^4	20.0 ± 2.8	39.0 ± 3.2	36.0 ± 1.5	41.0 ± 4.1	37.3 ± 1.2
1×10^6	29.0 ± 2.9	44.0 ± 7.3	40.0 ± 0.3	43.0 ± 1.7	40.0 ± 2.9
1×10^8	31.0 ± 1.5	46.0 ± 0.6 (81.8)	42.6 ± 2.9	50.7 ± 1.2	44.0 ± 4.2
(% INS)	(40.9)		(65.4)	(82.6)	(53.3)
P-value$_{(0.05)}$	0.068	0.0048	0.398	0.0005	0.276

Mean ± standard error, % INS= Percentage increase in number of seed

The significant reduction of total chlorophyll contents in all the controls could be due to the effect of *C. lindimuthianum*. Damage to thylakoid membrane, reduction of ribulose 1-5 biphosphate regeneration and overall disturbance in plant photosynthesis network could be a cause of decline in chlorophyll contents of Cowpea leaves after pathogen infection (Petit *et al*., 2006). The application of *Trichorderma spp* enhanced the plant physiology that may direct the synthesis of chloroplast enzymes and enhance rubisco (Khodary, 2004), which resulted in increase of the total chlorophyll contents.

Anthracnose caused by *Colletotrichum* show irregularly shaped, dark brown on undersides of leaves and petioles and defoliation. However, a large body of evidence shows that pelleting seed and seed dressing with biocontrol fungal agents *Trichoderma harzianum* and *T. virens* are more effective than fungicide treatments against a wide range of root-infecting fungi and results in increased plant growth thus antifungal plant extracts can reduce dry root rot incidence and severity when optimal conditions for their use are provided (Dubey et al. 2011; Waheed et al., 2016; Ramzan et al. 2016). Antifungal plant extracts can reduce dry root rot incidence and severity when optimal conditions for their use are provided (Waheed et al., 2016).

The study showed that *T. harzianum* significantly reduced disease incidence of *C. lindimuthianum* in all the five Cowpea varieties. Idowu et al. (2016) reported significant effect of *T. harzianum* on disease incidence and severity of *P. aphanidermatum* on Okra. The results revealed that seed number were significantly increased with the foliar application of *T. harzianum*. The response of yield and its components may be attributes to availability and uptake of Nitrogen, Phosphorous and Potassium by plants in soil which positively has effect on vegetative growth. The increase in yield and its components may be attributed to the role of *T. harzianum* as bio agents on decreasing the percentage of infection and consequently promote yield. *Trichoderma spp* produce antibiotics and antifungal toxic metabolites which inhibit and disintegrate cell wall of pathogens by secreting enzymes like glucanase, cellulase, chitinase and protease (Lortio, 2005).

Trichoderma bio-agents can be effective for disease control and reducing mungbean yield loss (Maheshwari and Krishna, 2013; Batzer et al., 2022). *Trichoderma gamsii*, applied to maize kernels, has been proven effective in reducing pink ear rot caused by *Fusarium verticillioides* and root infections (Freimoser et al., 2019). Similarly, *Trichoderma harzianum*, *Pochonia clamydosporia*, *Paecilomyces lilacinus*, and *Pseudomonas fluorescens* strains have been found to inhibit the fungal pathogens of soybean roots (Herrera-Leon et al., 2007).

The versatile behavior of *Trichoderma* spp. can provide effective control of causal agent of *Fusarium graminearum* which caused Fusarium head blight of wheat through the colonization, and could be utilized to protect wheat spikes (Kinge et al., 2022). Similarly, the addition of *Trichoderma polysporum* spores to seeds in combination with liquid compost rich in organic matter supplied in fertigation during plant growth enhanced overall bioactivity towards melon wilt caused by *Fusarium oxysporum* f. sp. *melonis* under semiarid conditions (Marco S, 2022). This indicated that a combination of more biocontrol agents provides better control than the application of a single agent.

CONCLUSION

Foliar application of *T. harzianum* on Cowpea inhibited the growth of *C. lindimuthianum* under *In vivo* conditions. Higher concentrations of *T. harzianum* were found to enhance the number of leaves, plant height, shoot biomass and chlorophyll contents of the Cowpea varieties. IT99K-573-11 variety exhibited greater response to *T. harzianum* while DANMISRA appeared less sensitive. Minimum disease incidence occurred in IT98K-205-08 variety in contrast IT07K-292-10 revealed high susceptibility to *C. lindimthianum* disease incidence was recorded in variety. Farmers should be encouraged to grow variety IT99K-573-11 in the anthracnose affecting environments due to its tolerance to the disease. Foliar spray of *T. harzianium* isolate at 1×10^8 con/ml can be used to control diseases caused by *C. lindimthianum.* Research on pathogenicity and mechanism of *C. lindimthianum* mycotoxins infestation is worthy.

REFERENCES

Abd-EL-Hamed, W. F. M (2014). Integrated control for white rot caused Uromyces appendiculatus Unger of bean (Phaseolus vulgaris L). Ph.D. Thesis, Fac. of Agric., Fayoum Univ., pp: 145.

Akhtar, C. M (1966). The isolation of soil fungi-I, A simple method of isolating fungi from soil. The needle Method. Pakistan Journal of Agriculture Research, 4: 122-131.

Batzer, J.C. Singh, A. Rairdin, A. Chiteri, K. Mueller, D.S (2022) Mungbean: A Preview of Disease Management Challenges for an Alternative U.S. Cash Crop, Journal of Integrated Pest Management, 13 (1):4.

Carlos, G (2004). Cowpea: Post-harvest operation. International Centre for Tropical Agriculture. Danilo, M. (eds). Retrieved from www.fao.org/3/a-au994e.pdf on 6 June, 2013.pp 71.

Dube, E. Fanadzo, M (2013). Maximizing yield benefits from dual purpose Cowpea. Food Security, 5:769-779.

Dubey, S. C. Bhavani, R. Singh, B (2011). Integration of soil application and seed treatment formulations of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani,* Pest Management Science. 67: 1163–1168

Enyiukwu, D. N. Awurum, A. N. Ononuju, C.C. Nwaneri, J.A (2014). Biology and management strategies of Cowpea Anthracnose disease cause by Colletotichum species. Journal of Biochemistry and Biotechnology, 101(2) 52-65.

Freimoser, F. M. Rueda-Mejia, M. P. Tilocca, B. Migheli, Q (2019) Biocontrol yeasts: Mechanisms and applications. World Journal of Microbiology Biotechnology , *35*, 154

Gams, W. Bissett, J (1998). Morphology and Identification of Trichoderma. In: Kubicek CP, Harman GE, editors. Trichoderma and Gliocladium. Basic Biology, Taxonomy and Genetics. London: Taylor and Francis Ltd. pp. 3–34.

Herrera-León, S. Ramiro, R. Arroyo, M. Díez, R. Usera, M. A. Echeita, M. A (2007) Blind comparison of traditional serotyping with three multiplex PCRs for the identification of *Salmonella serotypes*. Research in Microbiology , *158*, 122–127.

Howell, C.R (2003). Mechanisms employed by Trichoderma species in the biological Control of Plant diseases: the history and evolution of current concepts, Plant Disease, 87(1): 4-10.

Idowu, O. O. Olawole, I. O. Idumu, O. O. Salami, O. A (2016). Bio-control Effect of Trichoderma asperellum (Samuels) Lieckf. and Glomusintraradices Schenk on Okra Seedlings Infected with Pythium aphanidermatum and Erwinia carotovora. American Journal of Experimental Agriculture, 10(4): 1-12.

Joshi, B. B. Bhatt, R. P. Bahukh, I. D (2010). Antagonistic and plant growth activity of Trichoderma isolates of Western Himalayas, Journal of Environmental Biology, 31,921,928.

Khodary, A. (2004). The effect of $mncl_2$ filler on physical properties of polystyrene films. physica B: Condense Matter, 344:297-307.

Kinge, T.R. Ghosh, S. Cason, E. D. Marieka, G (2022) Characterization of the Endophytic Mycobiome in Cowpea (*Vigna unguiculata*) from a Single Location Using Illumina Sequencing, Agriculture, *12*(3), 333.

Lone, M. A. Wani, M. R. Sheikh, S. A. Sahay, S. Dar, M. S (2012). Antagonistic potentiality of Trichoderma harzianum against Cladosporium spherospermum Aspergillus niger and Fusarium oxysporum, Journal of Biology Agriculture and Health care, 2:72-76.

Loritio, M. (2005). Molecular biology of the interactions between Trichoderma, Phytopathogenic, Fungi and Plants: Opportunities for developing novel disease control methods. Second Global Conference. “Plant Health Global Wealth”. Abst., pp: 162-163.

Lorito, M. Woo, S. L. Harman, G. E. Monte, E (2010). Translational research on Trichoderma: from omics to the field. Annual Review of Phytopathology 48: 395-417.

Maheshwari, S. K. Krishna, H (2013). Field efficacy of fungicides and bio-agents against *Alternaria* leaf spot of mungbean. Annal of Plant Protection Science. 21:364–367

Marco, S (2022) Sustainable Management of Diseases in Horticulture: Conventional and New Options, Horticulturae, *8*(6), 517.

Masangwa, J. I. Aveling, T. A. Kritziger, Q (2013). Screening of plant extracts for antifungal activities against Colletotrichum species of common bean (Phaseolus vulgaris) and Cowpea (Vigna unguiculata L.). Medical Microbiology, 23(1): 56-58.

Nidhi, S. Trivedi, P. C (2002). Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against Meloidogyne incognita and Fusarium oxysporum. Asian Journal of Experimental Science, 16: 21 – 28.

Pandey, S (2015) Efficacy of leaf extracts in controlling leaf blast and brown spot in rice (Oryza sativa L.). International Journal of Recent Scientific Research, 6, 7:5476-5479.

Petit, A. N. Vaillant, N. Boulay, M. Clement, C. Fontaine, F (2006). Alteration of photosynthesis in gripevine effected Esca. Phytopathology. 96:1060-1066

Ramzan, N. Noreen, N. Perveen, Z. Shahzad, S (2016). Effect of seed pelleting with biocontrol agents on growth and colonisation of roots of mungbean by root-infecting fungi, Journal of Science Food and Agriculture*,* 96: 3694–3700.

Shoresh, M. Harman, G. E. Mastouri, F (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopathology 48: 21-43.

Waheed, N. Jabeen, K. Iqba, S. Javaid, A (2016). Biopesticidal activity of *Calotropis procera* L. against *Macrophomina phaseolina*. African Journal of Traditional Complementary and Alternative Medicine. 13:163–167