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BIO-ACTIVITY OF *TRICHODERMA HARZIANUM* T_{22} AGAINST ROOT-KNOT NEMATODE OF SOME COWPEA VARIETIES.

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ABSTRACT

An experiment to test the bioactivity of *Trichoderma harzianum*, a bio-agent, against root-knot nematode of some cowpea varieties (Sampea 9, Sampea 10, Sampea 11, IT16K–91–1–1, IT07K – 187 – 55, IT06K – 123 – 1, 17845 – 2246 – 4^2, 1790K – 277 -2 and 1788D – 867 – 11) was conducted at the Teaching and Research Farm of the Faculty of Agriculture, University of Ilorin, Nigeria. The experimental design was a factorial type fitted into a completely randomized block design. Initial soil nematode population was assayed. Growth parameters, plant height and number of leaves were collected from two weeks to eight weeks after planting on a weekly basis. Number of pods and pod weights were also taken. At harvest, final nematode populations were counted. Data were subjected to analysis of variance and means separated using Duncan's Multiple Range Test (DMRT). Results showed that treated plants performed significantly ($P \leq 0.5$) higher in all the growth and yield parameters measured than their untreated counterparts. Conversely, soil nematode populations were significantly higher in the untreated plots than in the treated ones. Varietal differences played important role in the growth and yield response of plants to treatments. Though, all the cowpea varieties were susceptible at varied levels to root-knot nematode infection, *Trichoderma* treated plants were resistant while the control plants were susceptible and highly susceptible in the first and second year, respectively.

Keywords: *Trichoderma harzanium* T_{22}, *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne hapla*, Root-Knot.

INTRODUCTION

Cowpea, (*Vigna unguiculata* (L.) Walp.) is a major grain legume, fodder, green pod and leafy vegetable crop grown in drought-prone regions of Africa and other tropical and subtropical regions (Langyintuo *et al*., 2003). In most areas, cowpea is mainly grown by small-scale farmers who practice intercropping in their small land holdings (Singh *et al*., 2003). Growers with such small areas are always looking for maximization of their farm income through vertical expansion, achieved by either cultivating the land more than once per year and/or intercropping (Abou-hussein and Salman, 2005). The crop is the most important grain legume in West Africa, cheapest dietary and high quality vegetable protein of about 25-43%, providing a source of profitable revenue between 23 and 29% of selling price. It is also valued as accounting for up to 80% of total protein intake in Nigeria (Olowe, 2009).

Over the years the expected yield of cowpea has reduced due to certain factors like insect pest attack on pods, pathogen effect and nematode infestation (Umar and Simon, 2008). Root-knot nematodes cause root to appear knotted or galled with stunting. Affected plants often wilt because the root system is incapable of absorbing adequate amount of water and nutrients. Root-knot can also be harmful to cowpea because root injuries predispose the plant to secondary attack. The control of this pathogen becomes imperative in order to improve the quantity and quality of cowpea. Several methods of effectively controlling nematodes are available.

Plant extracts or residues used in control of nematode have advantage of cheapness and availability over the conventional methods (Izuogu *et al*., 2012; Oyedunmade *et al*., 2011). They also increase soil fertility as they acts as manure. Use of synthetic nematicides has proved very effective except for hazardous effects of chemicals, high cost, not being eco-friendly among others. Resistant varieties have been successfully used to control nematodes, but, hybrid resistance breaks down over time. The use of bio-agents is fast gaining grounds in controlling nematodes of agricultural crops in the tropics. This research therefore, aims at assessing the effectiveness of *Trichoderma harzianum* in the control of root-knot nematode, *Meloidogyne incognita* in nine varieties of cowpea (Sampea 9, Sampea 10, Sampea 11, IT16K – 91 – 1 – 1, IT07K – 187 – 55, IT06K – 123 – 1, 17845 – 2246 – 4, 1790K – 277 -2 and 1788D – 867 – 11) with respect to growth, damage reduction and yield improvement in the field.

MATERIALS AND METHODS

The two-year experimental trials were conducted between July and December 2011 and 2012 respectively at the University of Ilorin Teaching and Research farm, Ilorin, Nigeria. The piece of land used was well-drained-sandy-loam measuring 210m by 4m (840 m^2). The land was ploughed, harrowed and divided into two equal halves (plots) separated by 5m alley to avoid treatment interference. One plot was treated with *T. harzianum* and the other which received no treatment served as control. Soil samples from two plots were randomly collected for initial nematode population. Experimental lay-out was factorial fitted into a randomized block design having each treatment replicated five times.

Three varieties of cowpea (Sampea 9, Sampea 10, Sampea 11) were obtained from Institute for Agricultural research(IAR), Samaru Zaria, Nigeria while the remaining six varieties (1T16K-91-1-1, 1To7K-187-55, 1To6K-123-1, 17845-2246-4^2, 1790K-277-2 and 1788D-867-11) were obtained from International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. Soil nematode assay was carried out using Baerman's extraction method as described by Whitehead and Hemming (1965). The nematode genera present were identified with the use of stereo microscope. Nematode population density was counted with a tally counter. Pre-emergence herbicides paraquat was applied to the plots before planting. Two weeks after, heavily galled roots of *Celosia agentea* infected with *Meloidogyne incognita* (which had been previously identified) were incorporated into the two plots to increase the soil nematode population. Three seeds were planted per hole at a depth of 4-5 cm and 40 cm spacing. Two weeks after planting, seedlings were thinned down to one vigorous plant per stand. One month after incorporation of galled roots, soil nematode population was taken.

Cultured filtrate of *T. harzianum* was collected from Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo state, Nigeria. Thirty (30ml) of *T. harzianum* diluted in 15 litres of water was sprayed on one half using knapsack sprayer. The sprayed half served as the treated plot and the other which did not receive treatment served as control. Cultural practices which included regular hand rouging of weeds weekly and setting of traps and scare crow to control rodents and birds were maintained throughout the experiments.

Data on growth parameters, plant height and number of leaves were taken weekly. Similarly, data on number of pods as yield parameters was taken on the 7^{th} and 8^{th} weeks after planting. At final harvest on the 8^{th} WAP, plants were uprooted and rated for galling using the method describe by Taylor and Sasser (1978). Soil samples were also collected from treated and untreated cowpea rhizospheres to determine the final nematode population. All numerical data collected were subjected to Analysis of variance using GENSTAT. The means were separated using the DMRT at P=0.05. The rating scale used was adapted from Taylor and Sasser (1978) as indicated below:

Rating	**Number of galls**	**Host reaction**
0	0	Immune
1	1-2	Resistant
2	3-10	Moderately resistant
3	11-30	Susceptible
4	31 and above	Highly susceptible

Rating scale by Taylor and Sasser (1978).

RESULTS

Except for the first two weeks after planting (WAP) as shown in Tables 1 and 2, significant differences were observed in the height and number of leaves between *Trichoderma* treated and untreated control plants. Treated cowpea plants were significantly superior to their untreated counterparts in terms of growth, yield, soil nematode population and root galling indices measured.

Varietal differences played an important role in the growth and yield response of the plants to treatment. Varieties ITO7K-187-55 and 1784-2246-42 recorded significantly higher plant height than the other varieties. Though there were no significant differences in height amongst varieties, Sampea 10, Sampea 11 and 1784-2246-42, sampea 10 recorded the least mean plant height. Generally, IT16K-91-11-1, IT07K-187-55 and IT06-K-123-1 had significantly highest number of leaves while, Sampea 10, 1784-2246-4², 1790K-277-2 and 1788D-867-11 (in 2012 trial) had significantly least number of leaves throughout the period of trials.

Table 3 shows that there were significant differences between the yield of *Trichoderma* treated plants and the untreated controls. Numbers of cowpea pods were significantly higher in the treated than in the control plants. There were also significant differences among the varieties in terms of number of pods produced. Sampea 10, IT16K-91-11-1, IT07K-187-55, IT06K-123-1, 1788D-867-11 and Sampea 9 (only in 2012 trial) produced significantly higher number of pods than the other varieties.

Table 5 also shows that there were significant differences on the mean soil nematode population of *Trichoderma* treated and untreated cowpea plants in the two year experimental period. Nematode populations were significantly reduced in the treated soils. One month after treatment soil nematode population reduced from over 200 to as low as between 6-9 in 200ml soil and the untreated soil almost doubled the initial population. There were also significant differences among final soil nematode population of the different varieties of cowpea evaluated. Cultivars Sampea 10, IT16K-91-11-1, IT07K-187-55, IT067K-123-1 and 1788D-867-11 recorded significantly lower soil nematode population density among those that received treatment whereas among the untreated control plants, the same cultivars except Sampea 10 recorded the least soil nematode population in 2011. In 2012, IT16K-91-11-1 recorded the least number of soil nematode population among untreated control plants and was followed by IT07-187-55, IT06K-123-1 and 1788D-867-11.

Table 6 shows the effect of treatment on the root galling of infected cowpea varieties. Generally, all the *Trichoderma* treated plants were resistant to root-knot nematode through the period of study while the plants which did not receive any treatment were susceptible in the first year and highly susceptible in the second year.

Table 1. Effect of *Trichoderma harzianum* and varieties on mean plant height (cm) of root knot nematode infected cowpea

Treatments	**2011**				**2012**			
	2WAP	**4WAP**	**6WAP**	**8WAP**	**2WAP**	**4WAP**	**6WAP**	**8WAP**
Trichoderma	33.40a	52.67a	70.20a	73.07a	21.11	46.82	71.10	75.25
No Trichoderma	31.60ac	39.13b	42.33b	47.20b	20.17	27.02	40.12	47.73
SED	1.27	1.08	1.62	2.93	0.48	1.59	2.79	1.73
LSD	N.S	1.53	2.30	4.15	0.99	3.29	5.75	3.57
Sampea 9	25.60	44.61a	60.20a	65.30ab	22.50a	34.50a	53.60c	64.31c
Sampea 10	27.60	40.30a	53.12b	53.50c	24.31a	31.90b	47.80d	58.92d
Sampea 11	26.20	40.80a	55.50ab	61.60b	20.78b	34.10a	49.40d	60.24cd
IT6K-91-11-1	23.66	36.30ab	56.16ab	74.20a	20.70b	28.74b	58.25b	70.12b
IT07-K187-55	22.65	39.83a	54.50b	70.50a	21.90b	34.70a	65.51a	78.80b
IT06K-123-1	24.12	34.63b	59.00a	72.10a	25.67a	37.00a	63.42a	75.05a
1784-2246-42	22.70	34.00b	53.60b	64.30b	20.40b	26.40bc	55.40c	60.15cd
1790k-277-2	21.90	31.90b	47.80b	58.90c	19.90b	24.20c	54.50c	62.08c
1788D-867-11	23.10	34.70b	49.40b	60.20b	22.70a	29.60b	57.00b	62.25c
SED	1.55	1.32	1.99	3.60	1.13	1.98	1.89	3.21

Means within column followed by the same letter(s) are not significantly different P=0.05; WAP= Week after planting
SED= Standard Errors of Differences; N.S= Not Significant

Table 2. Effect of *Trichoderma harzianum* and varieties on mean number of leaves of Root Knot Nematode infected cowpea.

Treatment	**2011**				**2012**			
	2WAP	**4WAP**	**6WAP**	**8WAP**	**2WAP**	**4WAP**	**6WAP**	**8WAP**
Trichoderma	19.64	33.60a	71.73a	76.13a	20.10	29.87a	48.13a	71.93a
No Trichoderma	18.07	21.00b	34.73b	40.00b	19.00	22.05b	29.40b	37.53b
SED	0.87	1.01	1.72	1.16	0.98	1.00	1.92	1.25
LSD	N.S	2.08	3.55	2.41	N.S	1.97	3.97	2.49
Sampea 9	18.90b	35.30b	49.30a	52.00b	16.10c	27.50c	42.70b	50.80b
Sampea 10	18.90bc	34.90b	46.00b	51.30bc	18.00b	29.10c	39.00c	48.72c
Sampea 11	22.70a	34.20b	49.90a	55.60b	23.60a	34.60b	47.70ab	54.56c
IT6K-91-11-1	22.60a	37.30ab	54.10a	60.90a	17.00b	36.40b	49.50a	56.80a
IT07-K187-55	24.00a	41.30a	53.30a	64.00a	20.90ab	40.65a	51.30a	59.50a
IT06K-123-1	22.00a	37.70ab	52.00a	59.60a	24.10a	41.80a	51.96a	57.90a
1784-2246-42	17.10c	32.90bc	40.70c	49.30c	17.00b	29.01c	38.60c	46.10c
1790k-277-2	15.00c	29.00c	40.00c	47.00c	18.20b	28.74c	40.00c	47.98c
1788D-867-11	20.20b	33.70b	47.70b	54.30b	20.00	32.22bc	42.00b	48.60c
SED	1.19	1.77	2.10	2.35	1.46	2.35	1.70	1.96

Table 3. Effect of *Trichoderma harzianum* and varieties on mean number of pods of Root Knot Nematode infected cowpea.

Treatment	2011		2012	
	7WAP	8WAP	7WAP	8WAP
Trichoderma	25.00a	28.33a	22.33a	25.80b
No Trichoderma	10.60b	15.67b	10.13a	13.07b
SED	1.63	1.26	0.91	1.12
LSD	3.34	2.59	1.29	1.58
Sampea 9	19.40a	19.92b	16.50b	19.40a
Sampea 10	20.70a	20.60ab	19.00a	19.90a
Sampea 11	18.80b	19.00b	18.83a	17.00b
IT6K-91-11-1	18.30b	21.65a	18.60a	20.90a
IT07-K187-55	18.60b	23.50a	17.90ab	20.62a
IT06K-123-1	16.40c	20.90ab	19.50a	20.62a
1784-2246-42	17.60bc	18.20c	15.78c	16.35c
1790k-277-2	16.70c	17.00c	16.00c	18.20b
1788D-867-11	19.45a	24.20a	17.42ab	21.30a
SED	2.01	1.54	1.25	1.37

Table 4. **Impact of *T. harzianum* on growth and yield parameters of cowpea varieties at 8th weeks**

	2011	2012	2011	2012	2011	2012
Sampea 9	65.30ab	64.31c	52.00b	50.80b	19.40a	19.40a
Sampea 10	53.50c	58.92d	51.30bc	48.72c	20.70a	19.90a
Sampea 11	61.60b	60.24cd	55.60b	54.56c	18.80b	17.00b
IT6K-91-11-1	74.20a	70.12b	60.90a	56.80a	18.30b	20.90a
IT07-K187-55	70.50a	78.80b	64.00a	59.50a	18.60b	20.62a
IT06K-123-1	72.10a	75.05a	59.60a	57.90a	16.40c	20.62a
1784-2246-42	64.30b	60.15cd	49.30c	46.10c	17.60bc	16.35c
1790k-277-2	58.90c	62.08c	47.00c	47.98c	16.70c	18.20b
1788D-867-11	60.20b	62.25c	54.30b	48.60c	19.45a	21.30a
SED	3.6	3.21	2.35	1.96	2.01	1.37

Table 5.Effect of *Trichoderma harzianum* and varieties on mean nematode population (200 ml soil) one month after treatment.

2011			2012		
Initial nematode population	One month after treatment		Initial nematode population	One month after planting	
	Trichoderma	No Trichoderma		Trichoderma	No Trichoderma
232a	9b	401a	256	9b	507
260b	6a	453b	276	6a	480
SED 12.69	0.45	38.05	135.20	0.45	40.3

Final nematode population

Treatment	2011		2012	
	Trichoderma	No Trichoderma	Trichoderma	No Trichoderma
Sampea 9	9b	1244b	7a	1400c
Sampea 10	5a	1330c	7a	1522c
Sampea 11	9b	1520d	12c	1650cd
IT6K-91-11-1	7a	960a	8a	1115a
IT07-K187-55	6a	975a	7a	1300b
IT06K-123-1	7a	1005a	10b	1295b
1784-2246-42	11c	1510c	14c	1680b
1790k-277-2	9b	1372c	10b	1709d
1788D-867-11	7a	1002a	9a	1260b
SED	1.03	26.33	1.38	62.00

Table 6. Effect of treatment on root galling

Treatment	2011		2012	
	Rating	Host reaction	Rating	Host reaction
Trichoderma	1	Resistant	1	Resistant
No *Trichoderma*	3	Susceptible	4	Highly susceptible

DISCUSSION

The efficacy of *Trichoder maharzanum* T_{22} in the improvement of growth and yield of the cowpea varieties could be as a result of the bioactivities of this fungus against the soil nematodes; which may include feeding on the infective stages of parasitic nematodes especially the root-knot nematode, possible production of enzymes which would either control the nematode directly or inactivate their enzymes. According to Raja (2007) *Trichoderma* is well known for disease and nematode control on crop plants through production of several lytic enzymes and antibiotics controlling disease causing microbes, control of nematodes infestation by feeding on infective nematodes, inactivation of pathogen enzymes etc. Studies have shown that the fungus possess appropriate characteristics for biological control of nematodes for example fungal enzymes such as chitinases are capable of rupturing nematode egg shells contributing to parasitism of fungi on nematodes (Gortari and Hours, 2008). Sikora (2008) evaluated some non-pathogenic strains of *Fusariumoxysporum* and species of *Trichoderma* for their activity against plant parasitic nematodes and found them effective. In similar trials, the potential of *Trichoder maharzianum* to control root-knot nematode showed reduced galling and increased fresh shoot weight in nematode infected tomatoes (Sharon *et al*., 2001). Their result showed that the *Trichoderma* strains were able to control *M. javanica* separated eggs and the second stage juveniles in sterile in-vitro assays indicating that improved proteolytic activity of the antagonist may be important for the biological control of the nematodes. The variation in plant performance in terms of growth, yield, root galling and soil nematode population could be related to genetic variability among the treated plants. However, all the untreated varieties were susceptible to nematode infestation at varying degrees.

Varieties IT16K-91-11-1, IT07K-187-55, IT06K-123-1 and 1788D-867-11 which recorded significantly higher yield and reduced soil nematode population as well as reduced root galling proved to be more tolerant, resistant and superior to the other varieties. The implication of this is that yield reduction which is often caused by root-knot nematode infestation will be minimized if these varieties used are treated with a bioactive agent such as *Trichoderma harzianum* T_{22}. The bioagent invariably enhanced the resistance of some of the cowpea varieties better than others. Several sources of resistance to root-knot nematodes have been identified in some crops including cowpea. Ehlers *et al.* (2000) reported that virulence of rook knot nematode isolate within an area might change over time due to presence of individuals varying in fitness, most of which cannot reproduce on cowpea that contain the root knot resistant genes. Roberts *et al.* (1996) designated the gene locus for nematode resistant varieties of cowpea as RK. The gene confers resistance to many populations of *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica*.

Further studies have also identified more dominant resistant loci conferring resistance to root knot nematode in a number of crops and the best studied nematode resistance gene is *Mi*-1.2. This constitutively expressed gene (Martinez de llardiya and Kaloshian, 2001) confers resistance to *Meloidogyne incognita*, *M. javanica* and *M. arenaria*on tomatoes but not *M. hapla*, even though these four species are present sympatrically.

From our study, it could be deduced that *Trichoderma harzanum* T_{22} can be effectively used as good antagonist against root-knot nematodes *M. incognita* in cowpea fields. It reduced damage, improved growth, and resulted in high yield of cowpea. However, for optimum yield, combination of the treatment with varieties IT16K-91-1-1, IT07k-187-55, IT06K-123-1 and 1788D-867-11 is recommended in nematode infested fields in this zone.

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