Effect of *Pleurotusostreatus* on Root-knot Nematodes (*Meloidogyne* spp.) in Okra *(Abelmoschusesculentus* L (Moench))

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***Abstract*:- A greenhouse pot experiment was conducted to determine the effect of *Pleurotusostreatus* and its biocontrol efficacy against *Meloidogyne* spp., in okra cultivar; Kirikou F1.The Bearman's funnel extraction technique was used to extract nematodes while the Potato dextrose agar medium was used to culture the biocontrol agent. Seedlings of Okra cultivar; Kirikou F1 were nurtured in steam sterilized soil mixture of 1:1 loam and sand and were infected with 500; 1000; 1500 and 2000eggs/juveniles of *Meloidogyne* spp., The complete randomized block design (CRBD) with three replicates was adopted for the study. The experiment consisted of all possible combination of individual, concomitant and sequential inoculations of *P. ostreatus* and *Melodoigyne* spp. Nematode reproductive potential and other growth parameters; plant height (cm), root weight (g), stem girth (cm) and gall index were assessed in the 2nd, 4th, 6thand 8th weeks after inoculation (WAI). Results indicated that the introduction of 10g*P.ostreatus* to *Meloidogyne* spp., infested soil improved the fresh root weight of the seedlings, reduced the galling index and nematode population and had best effect on the growth parameters of okra plants as compared to the controls. It can be concluded that the fungus *P. ostreatus* cultivation can process the components of the plant material to some compounds with less harm to plant and more nematicidal effects.**

***Key words: Meloidogyne incognita, Pleurotusostreatus,* reproductive potential and biocontrol**

I. INTRODUCTION

Many species of phytonematodes have been found associated with rhizosphere of okra. Amongst these, root-knot nematode, *Meloidogyneincognita* is considered to be of great economic importance. It has a host range of 232 host genera (Krishnappa, 1985). The estimated losses in yield of okra due to plant parasitic nematodes are 20.40% on worldwide basis (Sasser, 1987).Soil borne plant pathogens such as bacteria, fungi and nematodes annually create major economic losses in many important crops. Some chemical compounds have been successfully used to control soil borne plant pathogens. Although in many cases, these pesticides appear to be the most economical and efficient means of controlling plant pathogens, toxicological, environmental and sociological concerns have led to drastic reduction in the availability of efficient commercial compounds. The use of fungicides may also lead to the appearance of new resistant strains of pathogens.

In the recent years, there has been a worldwide swing to the use of eco-friendly methods for protecting crops from pests and diseases (Rao*et al*., 1998). The use of biological technique in controlling plant diseases especially soil borne plant pathogens and nematodes have been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Barker and Panlitz, 1996; Eziashi*et al*., 2007).A type of compost that is known to secrete toxic compounds against nematodes but has not received enough attention is *Pleurotusostreatus*. *Pleurotusostreatus* is known to exude a toxin from the fungal hyphae, known as trans-2-decenedioic acid (Kwok, 1992). This toxin paralyzes the nematodes on contact, which allows the hyphae to move into position to colonize and digest the nematode. Studies on the effects of *P. ostreatus* on nematodes have been predominantly *in vitro*. (Palizi*et al*.,2009) demonstrated the use of *P.ostreatus* compost with sugar beets (*Beta vulgaris*) in the field by directly incorporating the mushroom compost into soil at 3% (w/w). The *P.ostreatus* compost suppressed more than 85% of sugar beet cyst nematode (*Heteroderaschachtii*) cysts. Although direct incorporation of the *P.ostreatus* substrate into the soil could ensure direct contact of the mushroom mycelia with the root system, the amendment rate needed for nematode suppression using this approach could be unfeasible in the field- or even at the garden-scale if ample supplies of the *P.ostreatus* compost are not available. The study therefore, was aimed at investigating the biocontrol effect of *Pleurotusostreatus* on root-knot nematodes (*Meloidogyne* spp.) in Okra (*Abelmoschusesculentus*).

II. MATERIALS AND METHODS

Sterilization of soil samples

Soil sample- a mixture of loam and sand was collected at Rumuchakara Community in Obio/Akpor Local Government Area of Rivers State. The soil was subjected to sterilization by heat for a period of 30 minutes at a temperature of 55^{o}c and bagged to a weight of 15kg. Afterwards, the soil sample was stored in a polythene bag and kept in a cool dry place for a

period of 2 weeks so as to ensure complete suffocation of any surviving soil parasitic nematodes.

Planting

Seedlings of *Abelmoschusesculentus* was sourced from Agrotropics Limited (LTD), Port Harcourt. The seedlings were raised in black polythene sowing bags containing steam-sterilized soil. The bagged plants were arranged in a Complete Randomized Block Design (CRBD) and in three replicates to facilitate analysis of the results. Each replicate contained four rows with five plants per row totaling twenty (20) plants in each replicate.

Isolation of Pleurotusostreatus

The fungus *Pleurotusostreatus* was isolated in the Faculty of Agriculture Demonstration farm, University of Port Harcourt under the following procedures: 200g of Irish potatoes was weighed, peeled, sliced and boiled for 15 minutes. The filtrate was passed through a mesh sieve into a container. 20g of agar powder was added and stirred vigorously. Glucose- D powder was added to it and stirred thoroughly. The mixture was sterilized for 15-20 minutes under pressure. When brought out, it was immediately poured into a Petri- dish where it solidifies and the internal tissue of a matured mushroom is inoculated into it. The Petri-dish is sealed tightly to avoid contamination by bacteria. The culture is carefully stored for 4 weeks before use. These fungal isolates were purified through subculturing from single spores and, then, identified to species level.

Extraction of nematodes was carried out from galled roots of waterleaf using the Bearman's Funnel Technique and identified using nematode identification key.

Inoculation of plants with nematodes

Three replicates each in four groups of okra were inoculated concurrently with 500il; 1000il; 1500il; and 2000ilat the base of the potted plant respectively after two weeks of sterilization. Five hundred (500) egg masses/juveniles (treatment 1), 1000egg masses/juveniles (treatment 2), 1500 egg masses/juveniles (treatment 3), 2000 egg masses/juvenile (treatment 4) respectively.

Analysis of data

Data collected were arranged in Tables and the relative prevalence of nematode in soil and root at each inocula level was calculated. Mean, Standard error of mean (SEM) and Analysis of variance (ANOVA) was also calculated using Microsoft Excel 2007. The confidence level was set at $P>0.05$.

III. RESULTS

Table 1 shows the mean response for various growth parameters for Okra cultivar, Kirikou F1 at 500, 1000, 1500, and 2000 inocula levels of *Meloidogyne* spp. treated with *P. ostreatus* 8 weeks after inoculation (WAI). Growth parameters of the okra cultivar such as height (cm), girth (cm),root weight (g) roots were also assessed within the period of the study. Result indicated that 10g of *P.ostreatus* (spawn) had inhibitory effect on the different inocula levels of *Meloidogyne* spp. Plant height showed apical competence from the 2nd week to the 6th week at 500il and 1000il. There was significant difference ($P<0.05$) in plant height at 500il and 1500il. The mean plant height was highest for 2000iland least for 1000il. The untreated plant showed no consistency in height throughout the period of study. Stem girth showed rapid increase for the first four weeks until the 6th week when girth size became constant while for the control girth size was not consistent. The mean response of the girth in Okra cultivar, Kirikou F1to the treatment was highest at the 500il and 1500il while for the control group the highest mean value was recorded at the 1500iland the least at the 2000il. There was significant difference ($P<0.05$) at the 500il for the treatment.

Root weight recorded for the treatment suggests that there was increase in fresh root weight at 500il but was highest at the 1000il for the control group although there was significant difference ($P<0.05$) at the 1500il for the treatment.

Fruition observed on the 8th week showed the occurrence of 5 fruits for the treated as compared to 3 fruits observed for the untreated at the 500il. Fruition was less at 1000il and 1500il at control while fruits recovered for the treatment at 1000il and 2000il was less. Results showed that more fruits were recovered from the treatment than for the control.

Table 2 shows the effect of *P. ostreatus* on the Reproductive potentials of Okra after inoculation. Gall formation on the rootlets was not observed at the 500il for the treatment but increased across the various levels. There was no consistency in the number of gall formed on the rootlets for the control. Gall index showed significant difference ($P<0.05$) at the 500il. Root bioassay to determine the abundance eggs/juvenile of *Meloidogyne* spp. in the roots suggests that less eggs/juveniles were recovered on the 2nd week for both treated and untreated group. Nematodes extraction from the root at the 8th week revealed higher recovery of eggs/juvenile of *Meloidogyne* spp. for the control. Nematode extraction from the soil, suggests that more eggs/juveniles of *Meloidogyne* spp. was recovered at the 1500il for both treatment and control at the 2nd and 4th week after inoculation.

Table 3 shows the Prevalence of *Melodiogyne* spp. recovered from soil and roots of Okra treated with *P. ostreatus*. Results revealed that more eggs/juveniles of *Meloidogyne* spp.were recovered from the soil (102) than the roots (94). At the 500il,the prevalence of*Meloidogyne* spp was 24 (23.52%) for soil and 17 (18.08%) for roots. At 2000il, there was a prevalence of 26 (25.49%) for soil and 26 (27.65%) for roots. Nematode recovery at 1500il had a prevalence of 28 (27.45%) for soil and 26 (27.65%) for roots while at 2000il, prevalence for soil was 24 (23.52%) and that of root was 25 (26.59%). The study revealed that more eggs/juvenile of *Meloidogyne* spp. was recovered at the 1500il for soil; 1000il and 1500il for root.

Table 4 shows prevalence of *Meloidogyne* spp. recovered from the soil and roots of Okra at control. Results revealed that there were more eggs/juveniles of *Meloidogyne* spp. recovered from the soil than the roots. Number of eggs/juvenile of *Meloidogyne* spp. recovered from the soil was 122 while that recovered from the roots were 120. The prevalence of *Meloidogyne* spp. at 500il for soil was 25 (20.49%) while the root had a prevalence of 27 (22.50%); 1000il had a prevalence of 29 (23.77%) for soil and 36 (30.00%) for root;1500il had 35 (28.68%) for soil and 27 (22.50%) for root and the 2000il had a prevalence of 33 (27.04%) for soil and 30 (25.00%) for roots. Result showed that the 1500il had the highest prevalence of eggs/juveniles of *Meloidogyne* spp. for the soil and the 1000ilhad the highest prevalence for the root. The study showed that more eggs/juveniles of *Meloidogyne* spp. was recovered from the untreated group when compared with the group treated with *P. ostreatus*.

Table 1: Effect of *P. ostreatus* on the growth parameters of Okra after inoculation

	Plant height (cm)		**Stem girth (cm)**		**Root weight (g)**		**No. of fruits**	
Inocula levels (il)	*Meloidogyne*spp. + *P. ostreatus*	Control (*Meloidogyne* spp.)	*Meloidogyne*spp. + *P. ostreatus*	Control (*Meloidogyne* spp.)	*Meloidogyne*spp. + *P. ostreatus*	Control (Meloidogyne spp.)	*Meloidogyne*s pp. + *P. ostreatus*	Control (Meloidogyne spp.)
500	33.85±2.73[a]	26.35±3.02	0.62±0.09[a]	0.52±0.09	0.55±0.12	0.67±0.15	5	3
1000	29.12±4.20	32.67±3.84	0.50±0.08	0.50±0.08	0.52±0.17	0.70±0.14	3	2
1500	30.20±3.01[a]	32.9±3.25	0.62±0.12	0.55±0.12	0.52±0.20[a]	0.55±0.05	4	2
2000	34.15±2.05	33.22±3.44	0.55±0.01	0.45±0.10	0.50±0.09	0.47±012	3	3

Each value is a mean of three replicates. Superscript 'a', indicate significant difference (at P<0.05)

Table 2: Effect of *P. ostreatus* on the Reproductive potentials of Okra after inoculation

	Gall index		**Nematodes in the roots**		**Nematodes in the soil**	
Inocula levels	*Meloidogyne*spp. + *P. ostreatus*	Control (*Meloidogyne* spp.)	*Meloidogyne*spp. + *P. ostreatus*	Control (Meloidogyne spp.)	*Meloidogyne*spp. + *P. ostreatus*	Control (*Meloidogyne* spp.)
500	0.00±0.00[a]	1.00±0.80	4.75±3.40	6.50±0.57	7.00±2.00	6.25±0.50
1000	0.50±0.57	0.25±0.50	7.00±1.63	8.50±1.00	6.00±1.29	7.75±2.06
1500	0.25±0.50	0.50±1.00	7.50±2.51	8.00±2.94	8.75±4.34	9.00±2.99
2000	1.00±0.81	1.00±1.41	6.75±2.06	9.25±3.30	7.50±3.00	8.50±1.91

Each value is a mean of three replicates. Superscript 'a', indicate significant difference (at P<0.05)

Table 3: Prevalence of *Melodiogyne* spp. recovered from soil roots of Okra treated with *P. ostreatus*

Inocula levels (il)	**500**	**1000**	**1500**	**2000**	**Total**
***Meloidogyne*spp. in the soil**	24 (23.52%)	26 (25.49%)	28 (27.45%)	24 (23.52%)	**102**
***Meloidogyne*spp. in the roots**	17 (18.08%)	26 (27.65%)	26 (27.65%)	25 (26.59%)	**94**

Table 4: Prevalence of *Melodiogyne* spp. Recovered from the soil and roots of Okra for control

Inocula levels (il)	**500**	**1000**	**1500**	**2000**	**Total**
***Meloidogyne*spp. in the soil**	25 (20.49%)	29 (23.77%)	35 (28.68%)	33 (27.04%)	**122**
***Meloidogyne*spp. in the roots**	27 (22.50%)	36 (30.00%)	27 (22.50%)	30(25.00%)	**120**

IV. DISCUSSION

Pleurotusostreatus is an easily cultivable mushroom that colonizes various crop residues as substrates *Pleurotus*spp. isable to degrade and convert lignocellulosic compounds into protein-rich biomass (Mamiro and Mamiro, 2011).In this study,the plant showed good apical competence for plant height at control across weeks after inoculation. This can be

explained by the findings of Ezigbo (1973), in his study on the effect of root- knot nematode on vegetables established that the first response to root knot nematode stimulation is the formation of galls.Galls are induced by surface feeding without actual entry of the larvae into the roots.The control plants attained the tallest heights from the 6th to the 8th week. The finding was in line with the findings of Ezigbo (1973), Singh *et al*. (1993) and Enopka *et al.* (1996). These authors in their various works on the effects of root-knot nematodes on vegetables observed some pathological changes in the inoculated plants. These pathological changes manifested in shoot heights, shoot weights, root weights, and most importantly in fruit development and maturation.On the 4th week *P. ostreatus* maintained consistent growth (34.7cm) while the control had a height of 32.0cm. The size of the girth was relatively small because of the nature of the cultivar but the treated plants had larger girth than the untreated. Root weight at 4th week, showed that the control recorded a progressive increase in weight than the treated plants. The gall infestation in the control group must have led to the sharp increase in weight (Frederick *et al.*, 2015). Galls are induced by surface feeding without actual entry of the larvae into the roots (Agwu and Ezigbo, 2005). On galls formation, Ezigbo (1973) reported the formation of lateral roots in the region of the galls. These additional lateral roots, enhances the uptake of water and mineral salts by the treated plants and this enhancement manifested as increased shoot height in the treated plants, until the damage of root cells by the entry of the second stage infective larva. Fruition started on the 8th week with 5 fruits recovered for the treated group while 3 fruits were recovered from the untreated at 500il.The findings of Khan *et al*. (1996) and Rao and Krishnappa (1994) were also supported by the findings in this study in which low inoculum levels of 4 and 8 egg masses gave the highest flower, fruit production and dry root weights of plants than the control. The total number of fruits recovered from the treated group was higher than that of control. The production of higher number of fruits could be due to a better translocation of water and nutrients to the shoots (Sikora and Fernandez, 2005) than the control plants whose roots were highly infested or galled, leading to a reduction in uptake and transportation of nutrients as reported by Hussey (1985) and Caveness and Ogunforowa (1985).

Nematodes recovered in the soil for the treatment revealed that there was a progressive decrease in the number of nematodes recovered atthe 6th and 8th week because juvenile penetration was greatly influenced by environmental factors. On the 4th week, there was no increase in nematode load in the roots. This trend was not sustained as the nematodes suddenly gained entry into the roots of okra by the 8th week as shown by their reduction in number in the soil. Nematodes recovered from the soil in the control were observed to reduce drastically by the 8th week as they have gained entrance into the plants. Gall infestation was minimal for the treated group on the 2nd and 4th week while on the 8th week, the number of galls increased both for treatment and control. In this study, there was high prevalence of eggs/juveniles of *Meloidogyne* spp. recovered from the soil and root of the control than those with treatment. More eggs/juveniles of *Meloidogyne* spp. recovered from the soil for both control and treatment than the roots. This shows that the soil was more compromised than the roots.

V. CONCLUSION

The results conclude that okra cultivar; Kirikou F1 was susceptible to *Meloidogyne* spp. infestation. This finding is consistent with studies by (Imafidor and Nzeako, 2008). The high prevalence of *Meloidogyne* spp.on okraat 1500il irrespective of treatments suggests that farmers cultivating crops in plots with high density of nematodes would require alternative measures because of inability of the treatment to inhibit nematodes activity at these levels. The introduction of *P. ostreatus* had a great inhibiting effect on *Meloidogyne* spp. root penetration due to their effective trap network 2WAI which limited motility of the juvenile and possibly the delayed egg hatch of nematodes.

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