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Management of *Meloidogyne incognita* with *Pleurotus ostreatus* and *P. tuberregium* in Soybean

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ABSTRACT

The effects of single or mixed inculcations of *Pleurotus ostreatus* and *P. tuberregium,* fungal species on the spread of plant parasitic nematode *Meloidogyne incognita* and growth of soybean (*Glycine max* L. Merrill) cultivars TGX 923-2E, TGX 1440-1E and TGX 1485-1D was studied under glasshouse conditions. Eight treatments including control were tested on the growth, nodulation, yield, galling incidence and development of nematode in soybean. Effects of mixed inoculation with *M. incognita* + *P. ostreatus*, *M. incognita* + *P. tuberregium*, *M. incognita* + *P. ostreatus* + *P. tuberregium* and including inoculation of only fungi in combination *P. ostreatus* + *P. tuberregium* and alone on plant growths and yields were significantly higher than that of only single inoculations of M. incognita. In other words growths on plants with inoculums of either single or mixed of *P. ostreatus, or P. tuberregium* were similar to control plants. Statistically *P. ostreatus* in the treatment where it appeared significantly ($P<0.05$) increased nodule weight, which stimulates plant growth characters when compared to *P. tuberregium* Progressively higher galling incidence and higher number of juvenile population of *M. incognita* were found in soybean with only single inoculation of *M. incognita*. Lower plant growth nodulation and yield per plant were also recorded in soybean with only nematode. But the harmful effects of nematodes were partially suppressed by *P. tuberregium,* while *P. ostreatus* showed complete control of nematode present in cultivar TGX 1440-1E. © 2010 Friends Science Publishers

Key Words: *Pleurotus* spp.; *M. incognita*; Growth; Nodulation; Yield; Galling; Soybean varieties

INTRODUCTION

Root-knot nematodes (*Meloidogyne* Spp.) are one of the most economically important pests causing severe damages to a wide variety of crops particularly to soybean (Adegbite, 2003) Among *Meloidogyne* species, *M. incognita* is the most prevalent, with approximate distribution in agricultural soils of Nigeria of 75% (Adegbite & Adesiyan, 2005). The use of of synthetic nematicides has been found to be the most effective method of controlling various species of root-knot nematodes (Onunuju & Fawole, 2000). However, the current use of nematicides is unsafe; the chemicals may result in poisoning of humans especially in the developing countries (Conway, 1995). Existing management procedure could be enhanced by the development of biocontrol strategies (Siddiqui *et al.*, 2003). Fungi can colonize and digest after coming in contact with nematodes (Kwock *et al.,* 1992; Thorn & Tsueneda, 1993; Heydari *et al.,* 2006). Therefore, the present study, was carried out to determine the individual effect as well as combined effect of *P. ostreatus*, *P. tuberregium* on *M. incognita* by measuring nodulation, gall incidence, growth and yield of three varieties of soybean.

MATERIALS AND METHODS

Experiments was carried out in Laboratories of Biological Sciences and Plant Health Management of Michael Okpara University of Agriculture, Umudike, following set-up pot plants carried out in a glasshouse of National Root Crops Research Institute Umudike, Abia State Nigeria during the period of July 2006 to January, 2007. Seed of soybean TGX 923-2E, TGX 1440-1E and TGX 1485-1D were obtained from National Cereals Research Institute Bedeggi Niger State, Nigeria. Inoculum for nematode was isolated from infected roots of grown *Celosia argentea.* Nematode eggs were then extracted from infected roots of *C. argentea cv* using NaOCL technique (Hussey & Barker, 1973). During the experimental harvest, nematodes juveniles from soybean planted were extracted using pie-pan method of (Whitehead & Hemming, 1965). The fungus, *P. ostreatus,* was procured from stock culture laboratory of Biological sciences and sub-cultures were prepared in a malt extract agar (MEA) and spawn was prepared using soaked millet grain and Gmelina sawdust for 24 h. This was inoculated with agar blocks containing 9 mm mycelial disc of *P. ostreatus* from pure culture plate and the

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spawn bottles were incubated at 29 ± 2°C for four days, thereafter, bulk substrate for spawn running was prepared under aseptic condition using vertiver grass, *Panicum maximum* and *Acacia* pods and pasteurization was done. However, 1 kg of each of the various materials was inoculated with 30 g of the spawn mushroom mycelia in each perforated buckets for eventual mushroom growth. After fructification, the spent substrate of *P. ostreatus* was collected as described by Okwujiako (unpublished). Then for *P. tuberregium*, no spawn was prepared rather the sclerotium was peeled free of the outer black surface and the white dry inner biomass was surface sterilized for inoculation.

Before planting of soybean, top soil was steam sterilized for 2 h at 80°C and allowed to cool to 27°C. Ninety-six perforated plastic pots were each filled with 10 kg of the sterilized soil. The pots were laid out in completely randomized design (CRD) and each variety consisting of thirty-two pots with a spacing of 120 cm apart. Two susceptible soybean seeds per variety TGX 923-2E, TGX 1440-1E and TGX 1485-1D were sown per pot and were later thinned to one healthy plant. Three weeks after planting, 5,000 eggs of *M. incognita* extracted from *C. argentea* were inoculated around the base of each seedling, using 20 mL of the nematode suspension. This was followed by inoculation of spent substrate of *P. ostreatus*, and *P. tuberregium* sclerotia using 50 g of each of the incoulum and the soil was properly closed. Eight treatments including control were used with four replications. The treatments included Nem+Po, Nem+Pt, Nem+Po+Pt, Nem alone, Po+Pt, Po alone, Pt alone, and control. (Where + = plus):

Nem+Po =*M. incognita* plus *P. ostreatus,*

Nem+Pt = *M. incognita* plus *P. tuberregium,*

Nem+Po+Pt = *M. incognita* plus *P. ostreatus* plus *P. tuberregium,*

Nem+Po+Pt = *M. incognita* plus *P. ostreatus* plus *P. tuberregium,*

Nem alone = *M. incognita*.

Po+Pt = *P. ostreatus* plus *P. tuberregium,*

Po alone = *P. ostreatus* alone,

Pt alone = *P. tuberregium alone,*

Control = no nematode and fungi.

After applying treatments, N.P.K. fertilizer was applied at the rate of 30 kg/ha at the fourth week after planting. This is equivalent to 10.45 mL of the fertilizer solution per pot and it was applied to all pots using a pipette. The pots were maintained at ambient temperature and plants were watered accordingly.

At the end of the experiment (90 DAP), the data were collected on plant height (CM), number of leaves, fresh and dry weight of shoot, root fresh weight, 50% flowering, number and dry weight of nodules, number and fresh weight of pods, grain yield per plant, number of galls, nematode populations in soil and root samples per pot. Data of this experiment were subjected to statistical analysis of variance (ANOVA) using the GLM procedure of SAS (2001). Treatment means were compared using Fishers' least significant difference at 5% probability level.

RESULTS

Results showed that growth of soybean *cv.* TGX 923-2E with treatment Nem+Po recorded the highest plant height (Table I). Highly significant and identical growth was also found in treatment Po alone, Po+Pt, Pt alone, including control and except in Nem+Po+Pt, Nem+Pt and Nem alone. However, all the treatments, including control differed significantly ($P<0.05$) from Nem alone. Number of leaves, fresh weight of shoots and dry weight of shoot performed greatly in Po alone treatment, highest weight and number of leaves observed in treatment Po alone (12.75) was not significantly ($P> 0.05$) different from other treatment except plants with only nematode (8.00) treatment. Nodule numbers and Nodule dry weight increased higher in plants with treatment Nem+Po (66.25,3.00 g) and this was similar to treatment Po alone, Po+Pt, Pt alone but differed from Nem+Pt (19.50), control (20.00) and Nem alone (2.50) and for dry nodule weight, Pt alone, Nem+Pt, control and Nem alone differed from the highest weight. Least number of nodule with corresponding dry weight were recorded in Nem alone, and for nodule weight, Pt alone, Nem+Pt, control and Nem alone differed from the highest. Early flowering was observed in treatment Po alone (32.50) and it was similar to control, Po+Pt, Nem+Po and Nem+Po+Pt. However, all differed significantly ($P< 0.05$) from treatment Nem alone (38.50), where flowering ability was delayed. In fresh weight of root, treatment Po+Pt recorded highest and is similar to other treatments and except Nem alone. Higher significant number of pods and fresh weight of pod were shown in treatment Po alone (61.23, 37.87 g), while Po+Pt recorded highest grain yield and they did not differ significantly ($P> 0.05$) with other treatment except that of Nem+Pt (50.75) and Nem alone (27.00) however, all the treatments including Nem+Pt differed significantly ($P> 0.05$) from plants with only nematode inocula. Highest plant height and number of leaves was indicated with treatment Po+Pt (35.75 cm, 14.50), respectively and these was not significantly different from control, Po alone, Pt alone and except on treatment Nem+Pt (29.50 cm), Nem+Po+Pt (29.75 cm) and Nem alone (20.75 cm). However, all these treatments differed significantly from that of Nem alone plant height, while in number of leaves Nem+Po (12.00), Nem+Pt (11.75), Nem+Po+Pt (11.75) and Nem alone (8.75) differed from significant number of leaves obtained in Po+Pt/Po alone (14.50), respectively (Table II). Following the influence of treatment on fresh and dry shoot weight inocula of Pt alone (60.55, 27.70) induced the highest fresh and dry weight of shoot and it was significantly ($P>0.05$) different from Po+Pt, Po alone and control except in plants with treatment Nem+Po (49.55), Nem+Pt (53.80) and Nem alone (27.10). In other words treatment Nem+Pt was not

Table I: Effect of Single or Mixed Inoculation with *P. ostreatus, P ostreatus* and Root-knot Nematode on Growth, Yield and Nodulation of *Glycine Max.* L. *Meril Cv* TGX 923-2E

Treatment	Plant height (cm)	Number of leaves	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	50% flowering	Nodule numbers	Nodule dry weight	Number of pods	Pods fresh weight (g)	Grain yield per plant (g)
Nem+Po	43.50	11.75	47.70	21.35	21.10	34.25	66.25	3.00	54.25	30.48	6.08
Nem+Pt	36.25	11.50	40.40	19.45	20.53	36.00	19.50	1.25	50.75	26.96	5.25
Nem+Po+Pt	36.50	12.50	43.96	22.75	21.15	35.25	41.00	1.80	56.00	30.15	5.95
Nem alone	24.75	8.00	19.23	12.10	12.65	38.50	2.50	0.15	27.00	14.28	2.10
Po+Pt	41.00	11.00	47.65	23.80	22.75	34.00	41.50	2.03	59.00	34.28	7.40
Po alone	41.50	12.75	50.13	24.28	22.55	32.50	46.00	1.75	61.27	37.87	6.80
Pt alone	41.00	12.00	46.03	18.78	21.38	34.50	34.00	1.65	56.00	28.85	6.13
Control	40.75	12.00	45.65	19.70	21.50	34.75	20.00	1.49	58.00	31.87	6.70
LSD (P<0.05)	6.39	1.94	12.94	5.89	2.36	2.36	33.78	1.58	14.68	10.78	2.16

Table II: Effect of single or mixed inoculation with *P. ostreatus, P. tuberregium* and *M. incognita* on growth, nodulation and yield of variety TGX 1440-1E

Treatment	Plant height cm	Number of leaves	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	50% flowering	Nodule numbers	Nodule dry weight	Number of pods	Pods fresh weight	Grain yield per plant
Nem+Po	30.75	12.00	49.55	22.43	36.98	39.50	47.25	2.28	82.00	38.81	6.00
Nem+Pt	29.50	11.25	38.80	18.48	34.60	42.25	21.25	1.25	72.00	30.72	5.55
Nem+Po+Pt	29.75	11.75	53.08	23.95	35.08	39.50	49.25	2.30	8.25	36.51	7.28
Nem alone	20.75	8.75	27.10	13.70	20.15	48.50	3.25	0.35	33.75	21.91	2.15
Po+Pt	35.75	14.50	55.10	23.90	40.68	34.00	31.75	1.80	82.75	40.19	7.33
Po alone	34.75	14.50	59.50	23.35	38.48	36.00	38.25	1.73	84.50	40.65	6.63
Pt alone	34.50	13.00	60.55	27.70	37.35	34.50	19.25	1.50	84.75	41.17	6.40
Control	32.25	13.00	53.60	24.10	37.35	38.50	20.25	1.20	83.75	39.49	6.48
LSD (P<0.05)	5.64	1.88	12.48	44.7	8.17	3.15	29.11	1.32	24.04	10.23	2.82

Table III: Effect of *P. ostreatus, P. tuberregium and M. incognita* either individual or combined inoculum on growth, nodulation, and yield of variety TGX 1485-1D

Treatment	Plant height (cm)	Number of leaves	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	50% flowering	Nodule numbers	Nodule dry weight	Number of pods	Pods fresh weight (g)	Grain yield per plant (g)
Nem+Po	42.00	13.00	62.70	23.40	33.33	34.25	48.50	3.48	58.00	31.88	6.50
Nem+Pt	40.75	12.25	4.35	21.55	27.88	35.00	19.50	1.38	52.00	26.96	5.95
Nem_Po+Pt	41.00	12.00	52.05	24.55	32.60	34.00	21.00	1.64	56.00	31.54	5.88
Nem alone	34.00	9.75	33.45	17.25	22.85	40.28	3.50	0.14	28.00	14.82	2.63
Po+Pt	43.25	11.75	64.70	28.38	36.85	36.25	22.75	1.80	60.00	14.80	6.83
Po alone	44.00	13.75	71.95	30.90	39.05	34.25	38.50	3.00	61.50	34.46	6.90
Pt alone	43.00	13.75	60.15	26.20	33.40	34.50	15.75	1.76	58.50	33.08	6.73
Control	42.25	13.25	64.23	25.20	36.25	34.75	14.25	1.70	59.00	33.68	6.58
LSD (P<0.05)	3.11	3.77	12.58	5.80	9.45	3.08	22.80	1.55	13.78	9.92	2.90

Table IV: Influence of treatments on number of root-galls, nematode population in roots and soil of each soybean varieties

Treatments	TGX 923-3E			TGX 1440-11E			TGAX 148-1D		
	Root Galls	Nem in Roots	Nem in Soils	Root Galls	Nem in Roots	Nem in Soils	Root Galls	Nem in Roots	Nem in Soils
Nem+Po	5.00	50.00	95.00	0.00	0.00	0.00	4.78	10.000	130.00
Nem+Pt	7.00	137.50	148.75	5.00	62.50	212.50	6.25	125.00	190.00
Nem+Po+Pt	5.25	70.00	152.50	2.00	5.00	87.50	6.25	125.00	177.50
Nem alone	19.00	325.00	700.00	17.00	300.00	692.00	20.50	422.50	817.00
Po+Pt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Po alone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt alone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD (P<0.05)	4.88	82.48	136.6	5.28	84.38	113.13	5.82	118.48	174.2

Each figure is an average of four replicate values obtained from each treatment

significantly (P> 0.05) different from plants with only nematode inocula. In fresh root, highest root weight was observed in treatment Po+Pt (40.68) and this was similar to other treatments except in plants with treatment Nem alone (20.15). Early flowering was indicated on treatment Po+Pt and it did not differ from Pt alone and Po alone but except in Nem + Po, Nem + Po + Pt and control. So far all plants flowered earlier than nematode alone. Again highest nodule number and nodule dry weight per plant were recorded in plants with treatment Nem+Po+Pt (47.28, 2.30 g) and was not significantly (P> 0.05) different from Po+Pt, Po alone, but except Pt alone, Nem+Pt, control and Nem alone. Inotherwords least number of nodule/nodule dry weight were recorded in Nem alone. With respect to number and fresh weight of pod, treatment Po alone with (84.75 & 41.17 g) recorded highest, while treatment Po+Pt gave the highest grain yield (7.33). Thus treatment Nem alone was found with lowest pod and grain yield (33.75, 21.91 g) and (2.15 g) and statistically it was different from the significant yield obtained from other treatments.

Treatment Pt alone produced tallest plant (44.00 cm) and highest number of leaves (13.75). In respect to plant height, significantly it was similar to control and treatment Nem+Po, Nem+Po+Pt, Po alone, but except on Nem+Pt and Nem alone. However, all the treatment and including control differed significantly (P<0.05) from nematode alone (Table III). Following the influence of treatments on fresh and dry weight of shoot and including weight of root, treatment Po alone recorded highest weight, thus significant fresh and dry weight of shoot obtained in Po alone (71.65 & 30.90) was comparable to control, Pt alone, Po+Pt, Nem+Po and except in Nem+Pt, Nem+Po+Pt and Nem alone, while for root weight of treatment Po alone (39.05) only Nem+Pt and Nem alone differed from significant weight obtained. So far, least shoot and root weight was observed only in treatment Nem alone. In terms of flowering, all treated plants and including control significantly (P< 0.05) flowered earlier than Nem alone. Higher significant effect on Nodule number and nodule dry weight was found with treatment Nem+Po having (48.50, 3.48 g) and this did not differ significantly (P> 0.05) from treatment Po+Pt, Po alone, but differed significantly from treatment Pt alone, Nem+Pt, Nem alone and control. Inotherwords, lowest Nodule number/nodule dry weight numbers was recorded in Nem alone. With respect to number of pods and its weight, treatment Po alone (61.50, 34.46 g) produced the highest, while on grain yield, treatment Pt alone (6.90 g) produced significant yield. Comparably, it did not differ significantly (P> 0.05) from control and other treatment except on plants treated with Nem alone (14.82) that has the lowest pod and yield production of (28.00, 14.80 & 2.63 g).

Plants inoculated with only nematode (Nem alone) had the highest number of root galls and nematode juvenile in both soil and roots of all the three soybean varieties (Table IV). On the number of galls, the two pathogen (fungi plus Nematode) significantly (P< 0.05) reduce the number of rootknot galls when compared with plants treated with nematode alone in all the varieties. No galling incidence was observed with plants with plants with treatment Nem+Po of variety TGX 1440-1E. Absence of root galls were also, observed in treatment (Po+Pt, Po alone, Pt alone & control) and they also appeared without any stage of nematodes in soil/root. Also treatment Nem+Po of variety TGX 1440-1E was found to achieve a complete control of nematode. For nematode population in roots, Nem+Po did not differ significantly (P> 0.05) with treatment Nem+Pt, Nem+Po+Pt of TGX 1440-1E and except Nem+Pt of variety TGX 923-2E and TGX1485-1D. Then for soil it was also observed that treatment Nem+Po was not significantly (P> 0.05) different from the larval mortality caused with Nem+Po+Pt but except with Nem+Pt, though all were significantly (P< 0.05) different from inoculum of nematode alone.

DISCUSSION

The study revealed that the combination of nematode with fungi or fungi-fungi or without any pathogenic inocula (control) in the three soybean varieties TGX 923-2E, TGX 1440-1E and TGX 1485-1D, recorded a significant response in respect to all the growth parameters measured when compared to the performance of plants treated with nematode alone in all the varieties. It was also noted that treatments with (Po+Pt, Po alone, Pt alone & control) proved most of the highest growth characteristics. This was probably as a result of absence of nematode. In case of their association with nematode, that is Nematode plus fungi (Nem+Po, Nem+Pt, Nem+Po+Pt). Similar trend of increasing growth and yields were also noted in most of the soybean varieties especially in treatment with Nem+Po (Nematode plus *P. ostreatus*). The effect of fungi on improving growth characteristics of plants infected with nematode has been shown by Sikora (1992), Hussey and Roncandori (1982). Considering the response of soybean shoot growth specifically, treatments with Nem+Pt, Nem+Po and Nem+Po+Pt differed significantly (P>0.05) from the highest shoot weight observed in Pt alone and Po alone (Table I & III). In other growth parameters there was found more or less increase. This is similar to the growth obtained by Waller and Bridge (1984), who revealed that combined effect of nematode and fungi, as a result of either of the pathogen acting antagonistically could cause changes in the host physiology of a test plant. Khan (1990) also suggested that lethal products secreted by fungus *Sclerotium rolfsii* disturbed the development of *M. javanica* in the fist month but ultimately, their association suppressed the growth of shoot of brinjal in the subsequent months. Results also confirmed reduction in plant growths, reduced number of nodules, pods and other growth parameters to soybean inoculated with only nematode. This was probably as a result of absence of nematode. This stunting growth observed by *M. incognita* has been reported by Anwar *et al.* (1996). Bhagawati and Phukan (1993) also reported a progressive decrease in all plant growth characters with high inoculum levels of *M. incognita* alone with the leguminous crop like pea. However, Ahmed (1993) observed reduction in the development of nodule in soybean with *M. incognita* alone. The single effect of *Meloidogyne* rather made the situation more vulnerable for suppressing nodulation and increasing root galls in all the soybean cultivars tested. The yield components were reduced significantly, corresponding with this; highest nematode population was observed in roots and soils of the infected soybean plants.

As for galling, Amarantha and Krishnappa (1989), Hussain and Bora (1998) reported higher galling incidence with only inoculum levels of *M. incognita* in sunflower and french bean, respectively. Higher inoculum level of only nematode like *M. javanica* inoculated with peanut and *M. incognita* with rice, bean and pigeon pea decreased the number of pods per plant as well as yield as reported by Tripathy and Pandhi (1992). Anver *et al*. (1997) and Hussain and Bora (1998) also reported that *M. incognita* population in French bean was found to be maximum with the maximum nematode inocula only. All these findings are in corroboration with the present study. Considering the performance of *Pleurotus* spp., the development of nodules were influenced by the species of *Pleurotus* in this study, the response of *P. ostreatus* inocula (Po), either in single or in combination with nematode or with *P. tuberreguim* nodulated heavier than treatment Nem+Pt, control and as well as pt alone. Ginterova (1979) and Singleton *et al.* (1992), similarly reported that the amount of nitrogen fixing depends on the presence of *P. ostreatus* and it could also related to the quantity and quality of Rhizobium it produces in soil. Progressively the combination effect of *P. ostreatus, or P. tuberreguim* with root-knot nematode resulted in reduction of root galls and as well reduced number of M. incognita in root and soil of three varieties of soybean. No galling incidence was found with plants treated with Nematode plus *P. ostreatus* (Nem+Po) in variety TGX 1440-1E and it was found to achieve complete control of nematode. Variation of toxin production among strains of fungi species has been observed by Hallman and Sikora (1996). Toxin droplets produced by *Pleurotus* species in nematode suppression has been (Fademi, 1990; Heydari *et al.,* 2006).

CONCLUSION

Both the species of *Pleurotus* are a source of nematicides, though influence of *P. ostreatus* either alone or in combination with nematode or in combination with nematode and *P. tuberregium* proved more effective in causing larval mortality, resulting in increased plant growth and nodulation than the combined effect of *P. tuberregium* plus nematode (Nem+Pt). However, further works will identify the actual active substrate of *P. tuberregium* that will make the fungus as effective as *P. ostreatus.* For now farmers should be encouraged to incorporate local edible mushrooms found within their farms as it is a toxin to nematode.

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