

Bio-activity of *Fusarium oxysporum* and *Phomopsis* sp. in the Management of Root-knot nematode (*Meloidogyne incognita*) infecting two Maize Varieties in an Open Field

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Abstract

This research was carried out at the Teaching and Research Farm of the University of Ilorin, to evaluate the use of some fungi as bio-agents in management of nematode pests of two varieties of maize. The experimental design was a Randomized Complete Block Design (RCBD), having two plots and four treatments with *Fusarium oxysporum*, *Phomopsis* sp., their combination and the control. Treatments were replicated three times. The varieties used were EVDT 99 and Oba Super II. The initial soil nematode population was increased by chopping galled roots of *Celosia argentea* into the soil. Two seeds per hole were planted out on the ploughed and harrowed ridges and were later thinned down to one seedling per stand. Data collections were done for a period of nine weeks and at harvest. The results showed that the plants on the treated plots performed significantly higher ($P=0.05$) than the control in terms of height, number of branches, yield and the reduction of soil nematode population and root galls. This study, established that at certain levels of application, *Fusarium oxysporum* and *Phomopsis* sp. exhibited fungal endophytes against parasitic nematodes of plant. Therefore, there is a need to identify the strains of the fungi which were used in this study in order to increase and incorporate them in sustainable agriculture

Keywords: Root knot nematode, *Fusarium oxysporum*, *Phomopsis* sp., Fungal Endophytes

Introduction

Maize or corn (*Zea mays* L.) is a plant belonging to the family Poaceae, genus *Zea*, species *mays*. Maize is one of the main cereal crops of West Africa, and is the third most important cereal crop after sorghum and millet in Nigeria (Ojo, 2000). It is a major important cereal being cultivated in the rainforest and the Savannah zones of Nigeria (Iken *et al.*, 2004). Special crops grown primarily for food, include; sweet corn and popcorn, although dent, starchy or floury and flint maize are also widely used as food. About 50 species exist and consist of different colours, textures, grain shapes and sizes. White and yellow varieties of *Zea mays* are preferred by most people depending on the region (Paliwal, 2000).

The grains are rich in vitamins A, C and E, carbohydrates, and essential minerals, and contain 9 % protein. It is also rich in dietary fiber and calories which are a good source of energy (Paliwal, 2000). In developing countries, maize is consumed directly and serves as staple diet for some 200 million people (Jéan du plessis, 2003). Maize is one of the most abundant food crops in Nigeria, about 80 % is consumed by man and animals while 20 % is utilized in variety of industrial processes for production of starch, corn sweetener, ethanol, cereal and alkali (Yahaya *et al.*, 2010). Maize can be hydrolyzed and enzymatically treated to produce syrups, while in some cases it is fermented and distilled to produce grain alcohol which is the traditional source of bourbon whisky (Ogunjinmi and Durojaiye, 2014).

Plant diseases are responsible for the loss of at least 10 % of global food production, representing a threat to food security (Strange and Scott, 2005). Maize production is faced with many constraints especially pests and diseases, among the agents causing diseases is the plant- parasitic nematode. Plant-parasitic nematodes caused serious crop losses worldwide and are among the most important agricultural pests (Koenning *et al.*, 1999). Roots damaged by nematodes are less able to supply plants with needed water and nutrients. This damage slows plant growth, lengthens the time to fruiting, reduces bunch weight and decreases the productive life of the plantation. Top-heavy plants may topple due to poor anchorage from damaged root system (Bancy *et al.*, 2014).

Although chemical nematicides are effective, easy to apply and show rapid effects, concerns about public health and environmental safety have been raised (Schneider *et al.*, 2003). Furthermore, nematicides are often too costly and difficult to access for smallholder farmers. The use of bio-agents is fast gaining grounds in controlling nematodes of agricultural crops in the tropics (Izuogu *et al.*, 2014). Therefore, efficient approaches to control nematodes that utilize a range of biological control options are needed (Sikora *et al.*, 2003).

This study investigated the efficacy of two fungi species as a bio-control agents on the nematode pests of two varieties of maize and compared the effectiveness of each bio-control agent, singly and in combination on the growth and yield of root-knot nematode infested varieties of maize.

Materials and Methods

Experimental Site: The open field experiments were conducted at the University of Ilorin Teaching and Research Farm, Ilorin Kwara State. The farm is situated at Latitude 8° 29 ' N and longitude 4° 35' E in the Southern Guinea Savannah of Nigeria at 310 m above sea level, the annual rainfall of Ilorin ranged from 1000 to 1200 mm.

Land Preparation: The farm land was ploughed, harrowed and ridged; a plot size of 44m by 26m was mapped out for the experiment. The plot was sprayed with pre-emergence herbicides (Paraquat and Atrazine).

Experimental layout: The experimental layout was a factorial type fitted into a randomized complete block design (RCBD) with three (3) replications.

Soil Sampling and Nematode Extraction: For initial soil nematode population, soil samples were randomly collected at 15cm depth from the plot with the aid of soil auger.

The extraction was conducted using the Baermann tray method as described by Whitehead and Hemming (1965).

Incorporation of Inoculum: Root-knot nematode galled roots of *Celosia argentea* were collected at Oyun area in Ilorin, Kwara State. The roots were chopped into small pieces and then incorporated into the soil (for both the control and treated plots). This was done a week prior to planting

and the objective was to increase the initial root-knot nematode population in the soil.

Varieties used and Planting Operation: Early Variety Drought Tolerant 99 (EVDT 99) (Variety 1) and Oba Super II (Variety 2) of different maize varieties. The improved varieties of maize were obtained from the Ministry of Agriculture, Ilorin Kwara State. Three seeds per hole were planted at 3cm depth and a spacing of 30cm in between, it was later tinned to one per stand. Planting was done only in July 2014.

Preparation of Inocula and Application: The treatment and preparations were done at the Bio-technology laboratory of the Faculty of Agriculture, University of Ilorin.

The identity of the organisms had been previously confirmed from the pathology laboratory of the International Institute of Tropical Agriculture (IITA).

Spores fragment were transferred aseptically unto fresh sterile Potato Dextrose Agar (PDA) with ethanol flamed inoculating needle in 9cm diameter Petri dishes. The plates were later incubated for 5 days at $25 \pm 2^{\circ}\text{C}$. Ten grams of the freshly harvested mycelia mat of each fungus was blended in 100ml of sterile water with Warring® blender for 10seconds to produce the inocula suspension. Two litres of each treatment was prepared and applied around the root zone at 10ml per plant stand. Treatment applications were done twice; a week after seedling emergence and a month after the first application.

Plate 1 and plate 2 were used to show the *Phomopsis* sp. and *Fusarium oxysporum* isolates respectively.

Data Collection: Data were collected on Plant height, Number of leaves, Root weight, Shoot weight, Fruit weight, Seed weight, Root gall rating and Soil analysis; Initial soil nematode population, nematode population at one month after planting and final soil nematode population, Physicochemical analysis. The data collection for number of leaves and plant height were performed weekly for a period of nine weeks while data on yield and final soil nematode populations were taken at harvest.

Root gall rating: The galled root rating was done using the Bridge and Page (1980) assessment method as described below;

0=No knots on roots

1=Few small knots, difficult to find

2=Small knots only but clearly visible. Main roots clean

3=Some larger knots visible. Main root clean

4=Larger knots predominate but main roots clean

5=50% of roots affected. Knotting on some main roots. Reduced root system.

6=Knotting on main roots knotted

7=Majority of main roots knotted

8=All main roots, including tap root, knotted. Few clean roots visible.

9= All roots severely knotted. Plant usually dying.

10= All roots severely knotted. No root system. Plant usually dead.

Statistical Analysis: The data collected were subjected to Analysis of Variance using IBM SPSS Statistics Version 21. The means were separated using the Duncan multiple range test at least significant difference $P = 0.05$.

Results and Discussions

Results

Table 1 shows significant differences between the treatments and control throughout from the third week of data collection. Treated plants recorded significantly higher height than the control but there were no significant differences between the treatments. There were also no significant differences between the used varieties of maize.

Table 2 shows significant differences between the treatments and control throughout the 9 weeks of data collection. Generally, treatment combination performed significantly higher than the single treatments in terms of number of leaves. There were no significant differences between the used varieties of maize.

Table 3 shows significant differences between the treatments and control on the mean root weight shoot weight, fruit weight and seed weight. Treated plants performed significantly higher than the control. It was observed that there were no significant differences between the treatments and likewise between the used varieties of maize.

Nematode Population Analyses

Table 4 shows the presence of *Meloidogyne*, *Pratylenchus*, *Scutellonema* *Hoplolaimus* and *Helicotylenchus* and their initial population from the collected soil samples.

Table 5 shows significant differences between the populations of nematode species obtained from the treated plot and the control plot after one month. Nematode densities were reduced in all the treated plots, not following a consistent trend for a particular treatment except for control where the nematode population recorded significant increase after one month. Table 6 shows significant differences between the population of nematode species obtained from the treated and the control plots at harvest. For *Meloidogyne*, *Pratylenchus* and *Scutellonema*, there were no significant differences between the treatments, but there were significant differences between the treatments in *Helicotylenchus* and *Hoplolaimus* populations.

Discussion

Results obtained from the different parameters measured, showed that *Fusarium oxysporum*, *Phomopsis* sp. and their combination had positive effect on the plant growth and yield as well as reduction in soil nematode population and root gall indices. The higher increase recorded by *M. incognita* in control plots one month after planting was as a result of the incorporation of galled roots at the beginning of the trial. The performance of *Fusarium oxysporum* and *Phomopsis* sp. Was not significantly ($P=0.05$) different from each other.

Recent studies provide strong evidence of the protective effects of fungal endophytes against plant-parasitic nematodes (Bancy *et al.*, 2014). Use of fungal endophytes to protect against plant-parasitic nematodes has previously been demonstrated in the open field. A number of studies have shown the beneficial effect of *F. oxysporum* endophytes on banana, primarily against *R. similis* (Niere, 2001) which is also supported in this study. A significant reduction in nematode densities and root galls was

observed in this study, following the inoculation with *F. oxysporum* and *Phomopsis* sp. translating to improved growth and yield of the crop, and reduction in nematode population which probably is as a result of fungal penetration and colonization of the root system. More factors might be responsible for the reduced nematode population; among these is inability of the female nematodes to produce eggs or large number of eggs as a result of the antagonistic effects of the bio-agents. The disruption of the environment by the antagonists could be an additional factor. Dababat *et al.*, 2008, in one of their studies confirmed that lower galling index was associated with a reduced egg masses production and female fecundity.

The results obtained from the study showed that, *F. oxysporum* and *Phomopsis* sp. treatments performed significantly higher at both singly and in combination. The effectiveness of the treatments might not be unconnected to the level used which was not pathogenic to the varieties of maize but antagonistic against the nematodes. Also, there could be a form of interactions (antagonism, parasitism, competition or mutualism) between the organisms when combined that may have increased their rate of controlling the nematode population. The level of colonization by *F. oxysporum* has been shown to be important where competition was suspected to be the mechanism leading to disease reduction (Niere, 2001; Vu, 2005). Studies showed that the negative or positive effect of bio-agents on plant growth could be due to a synergistic interaction between the organisms involved, in some cases the combined effect of the treatment could perform more positively or negatively than each organism alone in any experiment (Daami-Remadi *et al.*, 2009). On the basis of the results obtained, the varieties of maize were not significantly different ($P=0.05$) from each other and the treatments were able to control the nematode population on each variety. From the performance of the control plants, the two varieties of maize were susceptible to nematode pests. Generally, the treated plants performed significantly higher than the control trials.

Conclusion

The application of *Fusarium oxysporum* and *Phomopsis* sp. in nematode infected varieties of maize had a positive effect on the growth and yield of the crop. Although the galled roots present were not heavy and large in the untreated plants, their effect was evident with attendant lower yield, the

treated plants which had fewer, tinier galled roots recorded significantly higher yield.

Biological control is one of the control measures that have been found very effective and reliable by researchers. Further research is required to identify the strains of organisms that have been used in this study. This will elucidate a more robust knowledge about their characteristics and encourage their adoption in nematode management.

Table 1: Effect of *Fusarium oxysporum*, *Phomopsis* sp. and their combination on the mean height (cm) of *Zea mays*.

Treatments	WK 1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<i>Phomopsis</i> <i>spp</i>	18.33 ^a	35.67 ^a	52.77 ^b	80.93 ^b	100.28 ^b	144.52 ^a	178.00 ^a	181.57 ^a	181.70 ^a
<i>Fusarium</i> <i>oxysporum</i>	17.12 ^b	36.85 ^a	58.53 ^a	74.93 ^b	121.77 ^a	146.30 ^a	172.57 ^a	182.53 ^a	183.62 ^a
<i>Phomopsis</i> sp. and <i>Fusarium</i> <i>oxyporum</i>	17.05 ^b	34.63 ^a	52.17 ^b	96.47 ^a	119.58 ^a	145.00 ^a	173.27 ^a	181.75 ^a	182.00 ^a
Control	17.35 ^b	34.60 ^a	43.85 ^c	51.70 ^c	77.83 ^c	119.38 ^c	146.30 ^b	156.47 ^b	157. 22 ^b
S.E.M	0.214	1.167	1.415	3.746	2.488	2.197	3.177	2.323	2.439
Variety EVDT 99	17.167	34.30	48.53	76.88	104.50	136.73	167.72	176.13	176.40
Variety Oba Super II	16.758	32.08	50.13	75.13	105.23	140.88	167.35	175.03	175.87
S.E.M	0.152	0.825	1.001	2.649	1.759	1.553	2.247	1.642	1.725

Mean followed by the same letter are not significantly different P=0.05 using the new DUNCAN's Multiple Range Test

S.E.M= Standard Error of Mean

NS= Not significantly different

Table 2: Effect of *Fusarium oxysporum*, *Phomopsis* sp. and their combination on the mean number of leaves of *Zea mays*.

Treatments	WK 1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9
<i>Phomopsis spp</i>	5.00 ^{ab}	6.07 ^{ab}	7.10	7.67 ^{ab}	9.62 ^a	11.57 ^a	14.62 ^a	16.33 ^b	16.72 ^b
<i>Fusarium oxysporum</i>	5.55 ^a	6.75 ^a	6.95	7.6 ^{ab}	9.73 ^a	11.53 ^a	14.45 ^a	16.35 ^b	16.8 ^b
<i>Phomopsis</i> sp. and <i>Fusarium oxyporum</i>	5.38 ^a	6.47 ^a	6.85	7.73 ^b	9.77 ^a	11.62 ^a	14.4 ^b	16.35 ^a	17.28 ^a
Control	4.80 ^b	5.47 ^b	6.5	6.88 ^b	7.95 ^b	9.32 ^b	11.13	12.82 ^c	13.45 ^c
S.E.M	0.178	0.29	0.395	0.251	0.165	0.263	0.241	0.171	0.147
Variety EVDT 99	5.183	6.18	6.96	7.64	9.24	10.93	13.73	15.56	16.01
Variety Oba Super II	5.233	6.19	6.74	7.3	9.29	11.09	13.57	15.56	16.12
S.E.M	0.126	0.205	0.28	0.178	0.117	0.186	0.17	0.121	0.104

Mean followed by the same letter are not significantly different $P=0.05$ using the new DUNCAN's Multiple Range Test.

Table 3: Effect of *Fusarium oxysporum*, *Phomopsis* sp. and their combination on the mean root weight, shoot weight, fruit weight and seed weight of *Zea mays*.

Treatments	Root Weight	Shoot weight	Fruit weight	Seed weight
<i>Phomopsis sp.</i>	2.08 ^a	1.27 ^a	10.42 ^a	7.53 ^a
<i>Fusarium oxysporum</i>	2.10 ^a	1.27 ^a	10.25 ^a	7.00 ^a
<i>Phomopsis</i> sp. and <i>Fusarium Oxyporum</i>	1.85 ^a	1.23 ^a	9.92 ^a	7.05 ^a
Control	0.37 ^b	0.37 ^b	6.78 ^b	4.45 ^b
S.E.M	0.142	0.065	0.196	0.195
Variety EVDT 99	1.68	0.97	9.30	6.49
Variety Oba Super II	1.53	1.10	9.38	6.53
S.E.M	0.1	0.046	0.138	0.138

Mean followed by the same letter are not significantly different $P=0.05$ using the new DUNCAN's Multiple Range Test

Table 4: Nematode population at one week before planting (Initial Population)

Soil Samples	<i>Meloidogyne</i>	<i>Pratylenchus</i>	<i>Helicotylenchus</i>	<i>Scutellonema</i>	<i>Hoplolaimus</i>
1	60.00	32.50	16.25 ^b	7.50	6.25
2	36.25	24.5	47.25 ^a	26.25	17.5
3	66.00	27.25	23.75 ^b	21.75	3.75
S.E.M	6.567	7.944	5.252	5.080	3.005
Level of significance	NS	NS		NS	NS

Table 5: Nematode population at one month after planting

Treatments	<i>Meloidogyne</i>	<i>Pratylenchus</i>	<i>Helicotylenchus</i>	<i>Scutellonema</i>
<i>Phomopsis</i> sp	8.33 ^b	2.50 ^a	7.50 ^b	2.33 ^a
<i>Fusarium oxysporum</i>	1.67 ^a	6.67 ^b	1.50 ^a	1.05 ^a
<i>Phomopsis</i> sp. and <i>Fusarium oxyporum</i>	9.83 ^b	3.33 ^a	4.83 ^b	6.50 ^b
Control	169.17 ^c	76.17 ^c	43.33 ^c	56.67 ^c
S.E.M	23.099	12.415	15.086	12.911

Table 6: Nematode population at Harvest

Treatments	<i>Meloidogyne</i>	<i>Pratylenchus</i>	<i>Helicotylenchus</i>	<i>Scutellonema</i>	<i>Hoplolaimus</i>
<i>Phomopsis</i> sp.	4.33 ^a	3.17 ^a	2.33 ^a	0.50 ^a	0.33 ^a
<i>Fusarium oxysporum</i>	6.67 ^a	5.00 ^a	5.83 ^b	1.00 ^a	3.33 ^b
<i>Phomopsis</i> sp. and <i>Fusarium oxysporum</i>	5.33 ^a	4.50 ^a	2.50 ^a	2.50 ^a	2.67 ^b
Control	122.50 ^b	92.67 ^b	55.83 ^c	60.33 ^b	18.33 ^c
S.E.M	18.642	7.630	9.861	11.969	4.621

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