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Effects of Fungal Filtrates on Seed Germination and Seedling Growth of Maize (*Zea mays*, (L), in Rivers State Nigeria.

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Abstract

Investigation on effects of fungal filtrates of *Fusarium oxysporium, Macrophomina phaseolina* and *Culvularia lunata* on percentage germination of maize seed and growth of seeding using blotter and potted soil methods was carried out. *F. oxysporium, M. phaseolina* and *C. lunata* which were used as test fungi were isolated from maize seeds. Healthy maize seeds were soaked in culture filtrate of each test fungus for twenty-four (24) hours. The experiment was laid out in a complete randomized design in three replicates. Results of effects of fungal culture filtrates showed significant reduction in germination, shoot length and root length of maize seedlings (P=0.05) grown on both blotter and potted soil methods. *F. oxysporium* culture filtrate caused the highest reduction in germination of maize (50.0%; 39.7%), followed by *C. lunata* (38.6%; 32.3%), while *M. phaseolina* caused the least germination reduction (23.6, 22.6) for both blotter and potted soil methods respectively. A similar observation was made in shoot and root length reduction, with *F. oxysporium* taking the lead (51.0;55.8%) in shoot length reduction and (96.1; 54.3%) in root length reduction for blotter and potted soil methods respectively. However, *M. phaseolina* caused the least shoot length reduction (14.3;35.1%) while *C. lunata* caused the least root length reduction (20.5, 33.2%) for blotter and potted soil methods respectively. *F.oxysporium* also caused the highest reduction in vigour index, followed by *C.lunata*, while *M. phaseolina* did not show any significant reduction in vigour index. of maize seedling. This study concludes that fungal contamination have pathogenic effects on the growth of plants and can cause potential health hazard on human.

Key Words: *Zea mays*, germination, seedling, culture filtrate, fungi.

Introduction

Maize (*Zea mays*) is a cereal crop that is cultivated widely in a range of agro-ecological environment. It belongs to the poaceae (Graminae) family along with wheat, millet and rice. Maize grains are taken as substitute for other cereals and are prepared by boiling or roasted especially when fresh used for both animal and human food. Some varieties of maize are suitable for eating whole in form of sweet corn or often fried and eaten as popcorn.

It can be powdered into starch, the starch is largely used in making alcoholic beverages or taken as pap. Maize plant often attains 2.5m in height and may grow up to 12m in some natural strains. The endosperm forms most of the volume and weight of the corn seed, otherwise known as kernel. Maize is one of the most important cereal crops in the world.

Despite the numerous economic benefits, maize confers on the general populace, its production does not meet up with the demand.. Fungi accounted for 75% of seed-borne pathogens which have been found to cause infectious diseases such as rot, discoloration, necrosis and blight (Shethy, 1988). *Fusarium* spp. is reported to be the most important field

fungi worldwide and produce over a hundred secondary metabolites that are hazardous to the maize grains consumers'. (Owolade *et al.*, 2005). Maize grains and seedlings are susceptible to soil and seed-borne diseases as many seeds may decay before or after germination. Also, affected plants may suffer from stunted growth, reduced ear sized and even in severe condition, may die as a result of poor root system (Vincelli, 2008).

Fungi genera *Aspergillus, Fusarium, penicillium* and *Rhizoctonia* are known to produce mycotoxins and toxic metabolites. These mycotoxins had been reported to degrade seed quality and reduce their viability (Owolade *et al.*, 2005). Seed-borne pathogens play a significant role in seed rot. Seedling blight, Bipolaris leaf spot and culvularia leaf spot are etiologically caused by *Penicillium* spp, *Fusarium oxysporium*, *Aspergillus* spp, *Bipolaris maydis* and *Culvularia lunata* respectively (Debnath, 2012). Symptoms expression are affected by plant age, plant species and environmental factors. (Debnath, 2012)

The etiological agents of disease penetrate host plant by direct penetration using mechanical force or indirect penetration through enzyme action, wounds and natural openings such as lenticels, hydathodes and stomata (Agrios, 2005). As alternative to direct penetration, fungi attack foliage and develop infectious structures that may consider stomata as their penetration route. The dysfunction of stomata affects hosts on seedling physiological activities, which include transpiration and respiration. A lot of works have been done on myco deterioration of maize seeds. However, there is a particular gap in knowledge of how the seed borne fungi affect the seed germination and seedling vigour. This study seeks to ascertain effects of *Fusarium oxysporium, Macrophomina phaseotina* and *Culvularia lunata* filtrates on seed germination and seedling growth of maize (*Zea mays*).

Materials and Methods

Collection of Seeds

The grains of *Zea mays* were collected from National Agip Oil Company (NAOC) Green River Project, Rivers State Nigeria. These were kept in an air tight sterile polythene bag and moved to the plant Biology Laboratory of Rivers State University.

Viability Test of the Seeds

The test was carried out using floatation method as described by Anoliefo (2006). The grains with living embryo sank, were used for this research work, while unhealthy ones were used as substrates for isolation of seed-borne fungi of maize,

Isolation of Seed-Borne Fungi of Maize

The fungi, *Fusarium oxysporium, Macrophomina phaseolina* and *Culvalaria lunata* were isolated and identified from unhealthy maize grans obtained from NAOC, Green River Project, Port Harcourt, Rivers State using the standard blotter method, pure culture of each fungus was obtained by plating, using Potato Dextrose Agar (PDA). The fungi were identified as described by Barieth & Hunter 1991; Umechuruba & Elenwo, 1997).

Preparation of Fungal Filtrates

The medium Richard's solution was prepared by dissolving each of the chemical reagents separately with sterilized distilled water poured together in 1000ml measuring cylinder up to a litre. The dissolved Richard's solution was sterilized in an autoclave at $1.03 kglcm^3$ for 15 minutes. After sterilization, the solution was allowed to cool to attain room temperature before dispensing it into 150ml conical flasks. 10mm disc of 7 days old cultures of test fungi

grown on Potato Dextrose Agar (PDA) were inoculated into the conical flasks maintained in three replicates for 15 days at 25± 2^0C. Metabolites were mixed together, filtered and centrifuged at 5000mm for 10minutes.

Determination of Effects of Fungal Filtrates on Maize Grain Germination

One hundred and eighty (180) viable grains of maize were surface sterilized with 70% ethanol and rinsed three consecutive times with sterile distilled water. Sixty (60) grains were soaked in each fungal filtrate for 24 hours. After the period of soaking, the grains were rinsed separately with sterile distilled water. Twenty (20) out of the sixty (60) seeds were plated on Petri-dishes layered with three wet blotter papers as described by Patil *et al.* (2012). The treatment was done in triplicates.

Potted soil method was also investigated by sieving the soil to remove large particles, debris and stones. They were sterilized in autoclave at 121^0C for 1 hour. Four kilogram of sterilized soil was poured in each 7 x 7 pre-sterilized plastic pots. The treated grains were planted in the pot (3 grains per pot) replicated thrice. Maize grains treated and maintained with distilled water served as control. After seven (7) days of incubation, percentage of germination, shoot and root length of seedlings were measured daily for two weeks using transparent meter rule and the vigour index calculated using khokhar *et al.* (2013) formula. Vigour index (V.I) = Shoot length (cm) + Root length x percentage germination.

Data were subjected to statistical analysis, differences between treatments were tested by analysis of variance (ANOVA) and the mean comparison was done by Fishers least significant difference (FLSD).

Results

Effect of fungal filtrates of test fungi *F. oxysporiun, M. phaseolina* and *C.lunata* on maize seed germination, indicated that maize grains soaked in *F. oxysporium* filtrate caused the highest significant reduction in germination of maize grains (P = 0.05) using both blotter and potted soil methods (50.0%, 39.7%), while *M. phaseolina* caused the least reduction (23.6%; 22.6%) in germination as shown in Table 1.

Table 1: Effects of Fungal Filtrates on Germination of Maize Grains

Treatment	Blotter Method		Potted Soil Method	
	% Germination	% Germination Reduction	% Germination	% Germination Reduction
F. oxysporium	35.0[d]	50.0	46.7[d]	39.7
M. phaseolina	53.5[b]	23.6	60.0[b]	22.6
C. lunata	43.0[c]	38.6	52.5[c]	32.3
Control	70.0[a]	0	77.5[a]	0

Means followed by same letters are not significant at P = 0.05.

Effect of Fungal filtrates on seedling vigour of maize (Table 2 and 3) followed a similar trend, with *F. oxysporium* causing the highest reduction in vigour index (1.86; 15.7) in blotter and potted soil method respectively, followed by *C. lunata* (7.53; 22.4). While *M. phaseolina* (7.97; 28.8), did not show any significant reduction in vigour index of maize seedling.

Table 2: Effect of Fungal Filtrates on Seedling Vigour Index of Maize Using Blotter Method

Treatment	% Germination	Shoot Length (cm)	% Shoot Length Reduction	Root Length (cm)	% Root Length Reduction	Vigour Index (V.I)
F. oxysporium	35.0[d]	4.8[b]	51.0	0.5[d]	96.1	1.86
M. phaseolina	53.5[b]	8.4[a]	14.3	6.5[b]	48.8	7.97
C. lunata	43.0[c]	7.4[a]	24.5	10.1[a]	20.5	7.53
Control	70.0[a]	9.8[a]	0	12.7[a]	0	1.58

Means followed by same letters are not significant at P = 0.05.

Table 3: Effect of Fungal Filtrates on Seedling Vigour Index of Maize Using Potted Soil Method

Treatment	% Germination	Shoot Length (cm)	% Shoot Length Reduction	Root Length (cm)	% Root Length Reduction	Vigour Index (V.I)
F. oxysporium	46.7[d]	20.4[c]	55.8	13.2[c]	54.3	15.7
M. phaseolina	60.0[b]	30.0[b]	35.1	18.0[b]	37.7	28.8
C. lunata	52.5[c]	23.3[c]	49.6	19.3[b]	33.2	22.4
Control	77.0[a]	46.2[a]	0	28.9[a]	0	58.2

Means followed by same letters are not significant at P = 0.05.

Discussion

F. Oxysporium, *M. phaseolina* and *C. lunata* were found to be seed borne fungi of maize. Earlier workers reported *M. phaseolina* and *C. lunata* as pathogenic fungi of some arable crops. Chukunda *et al,* (2006) had reported *M. phaseolina* as fungi pathogen of okra and that pathogenic fungi are known to produce mycotoxins. These mycotoxins are located in different parts like the seed coat, endosperm or cotyledon (Patil e*t al.,* 2012). The fungal filtrate of *F. oxysporium* inhibited the seed germination of maize by 50.0%, hence causing the highest inhibition.

This confirms the assertion of Michelle and Thompson (2018) who reported *Fusarium verticilloides, Fusarium graminearum* and *Aspergillus flavus* as the most important fungal pathogens of maize. However, it negates the findings of Garuba *et al.* (2014) who reported *A. niger* as the most potent pathogenic fungus of maize, they noted that *A. niger* inhibited maize seed germination by 65.33%. The inhibition by *F. oxysporium* as observed in this study may be an indication that the test fungi produce toxic metabolites in the broth in which they were cultured. Eltariki *et al.,* (2018) reported that fungi of the genera *Aspergillus, Fusarium, Penicillium* and *Rhizoctonia* are known to produce toxic metabolites which are known as mycotoxins. These mycotoxins had been reported to degrade seed quality and reduce their viability. Magan & Aldred (2007); Eltariki *et al.* (2019) reported the predominant occurrence of *Fusarium* in wheat grain. This could be because, the fungus can tolerate adverse conditions such as high temperatures and drought for long periods in the form of chlamedospores (Agrios, 2012). *M. Phaseolina* and *C. lunata* showed significant mild inhibitory effect (with germination reduction of 23.6% and 38.6% respectively) on seed germination relative to *F.oxysporium.* The mild inhibition may indicate that the test fungi produced mild toxic metabolites in the broth in which they were cultivated. However, the percentage germination of maize seed in potted soil method was significantly higher relative to blotter method. Consequently, the percentage germination reduction was lower. That was not unexpected, given the fact that the soil has the ability to sustain plant growth by providing

essential plant nutrients and favourable chemical, physical and biological characteristic as a habitat for plant growth.

These nutrients in turn sustain the immunity of the plant to resist disease infection. It therefore suggests that, the potted soil medium may have provided the maize seedlings the required nutrients, it hence lower germination reduction relative to blotter method. This observation is in agreement with the findings of Emiri *et al.* (2012) who reported lower incidence and severity of Cocoyam Decline Disease (CDD) in nutrient enriched soils.

F. oxysporium had the highest percentage reduction, followed by *C. lunata* while the least germination reduction was recorded by *M. phaseolina* in potted soil method.

Conclusion

The culture filtrate of *F. oxysporium*, *C.lunata* and *M. phaseolina* inhibited seed germination in both blotter and potted soil method and seedling growth. However, the inhibitory effect of the fungal filtrate was mild in potted soil method.

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