



EFFECTS OF FUNGAL FILTRATES RELATIVE TO SOAKING PERIODS ON SEED GERMINATION OF MAIZE (*Zea mays* L. POACEAE)

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ABSTRACT

This work was carried out to investigate the effects of fungal filtrates on the seed germination and seedling growth of maize (*Zea mays* var. Obasuper). The filtrates of *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Fusarium oxysporium* were produced. The seeds were soaked in each filtrate for different periods. The results showed that 4-day-old filtrate of *Aspergillus niger* inhibited the germination of seeds (82.5%) at 2 hours pre-soaking period which was significantly different from the effects of other fungal filtrates. The plumule length of seeds soaked in 8-day-old culture of *P. chrysogenum* (1.00cm) was higher than the value obtained from seeds treated with *Fusarium oxysporium* filtrate (0.99cm) but showed no significant difference at significant level of 0.05.

Keywords: Fungal filtrate, Seed germination, *Zea mays*, Soaking period.

INTRODUCTION

Maize is grown throughout the world in a range of agro-ecological environments and a greater amount of the crop is cultivated more than any other grain (Chukunda et al., 2013). There are about 50 species characterized with different colors, textures and grain shapes and sizes. White, yellow and red are the most common types (IITA, 2004). Maize is a major important cereal being cultivated in the rainforest and derived savannah zones of Nigeria (Iken and Amusa, 2004). Maize is an important staple food for more than 1.2 billion people in Sub-Saharan Africa and Latin America (IITA, 2009). All parts of the crop are useful for food and animal feeds. The maize serves as feed for livestock and as raw materials for agro-based industries (Enuk et al., 2010). The fruit can be roasted or boiled on the cob. The dry grain can be processed into pop corn (guguru) and taken it with roasted groundnut. *Zea mays* is an annual grass of the Poaceae Family. Other grasses in this family include *Sorghum bicolor*, *Oryza sativa*, *Pennisetum americanum* and *Saccharum officinarum*. The plant is monoecious and the cob is being the female inflorescence while the tassel is being the male inflorescence (Olorode, 1984). The maize kernel is classified botanically as a caryopsis. It is covered with seed coat. The kernel is attached to the ear by the pedicel which remains attached to the base of the kernel. The endosperm forms the most volume and weight of the seed. The embryo is located in one base of the kernel.

The seeds and seedlings of *Zea mays* are susceptible to diseases caused by a number of soil borne fungi. The symptoms of affected seedlings may be stunted growth, yellowing and/or redding of the older leaves and marginal burning of leaves depending on the etiological fungi (Vincelli, 2008). Seed rot is

caused by *Pythium*, *Fusarium*, *Diplopodia*, *Rhizoctonia*, *Penicillium* and various seedborne bacteria. The pathogens will kill the embryo of the seed and their survival is favoured by prolonged wet and cold soil conditions. Soil temperature of 50 °F or lower favour seed rots development (Ruhl, 2012). Mycotoxins are secondary metabolites produce by pathogenic fungi in a favourable and susceptible hosts. The current work was therefore to elucidate the effects of fungal filtrates on the seed germination and seedling growth of *Zea mays* (maize).

MATERIALS AND METHODS

Isolation and identification of fungi

The infected maize seeds that were physically impaired were collected from Oja Oba market Ilorin. The seeds were surface-sterilized using 70% of ethanol and rinsed with sterile distilled water for three consecutive times. The isolation was carried out using the method of Chukunda et al., (2013) and a method adopted by Yusuf and Okunsanya (2008). The Potato Dextrose Agar was used as the medium. Identification of isolates was made using Barnett and Hunter (2010) and Campell and Stewart (1980).

Preparation of fungal filtrates

In the 7-day old pure culture of each isolate, a disc of 50mm in diameter of mycelia and spores was taken and introduced into 250ml conical flask. The mycelia and spores with 50ml Potato Dextrose Broth in the conical flask was incubated at room temperature (25±°C) for two different incubation period, that is 4 and 8-days. For each fungus, four conical flasks were used per incubation period. Filtration method was used in the separation technique. Sterile Whatman No 1 filter paper was used to obtain filtrate (crude mycotoxin) as was adopted by Haikail (2008).

Viability test for the maize seeds

All healthy maize seeds (OBASUPER variety) studied were examined. The test was carried out using Floatation Method as described by Anoliefo (2006). The seeds were sorted and cracked and broken ones were discarded. The intact seeds were put in a bowl containing water. The floated portion was discarded. The seeds that sank were considered viable due to presence of basic necessary component (embryo).

Sterilization of maize seeds

The seeds were swabbed with 70% ethanol for 2 minute and washed with several changes of sterile distilled water and blotted dry with sterile filter papers (Oladiran and Iwu, 1993).

Soaking of seeds in fungal filtrates

The seeds of *Zea mays* were soaked in 100% fungal filtrate of 4 and 8-day old for varying periods of time ranging from 2, 4, 6 to 8 hours. This soaking procedure was applied to all fungal filtrates under studies. The control seeds were soaked in distilled water. After soaking, treated seeds were placed on blotters moistened with sterile distilled water in petri dishes. The plates were incubated at room temperature for seven days. Percentage of germination was determined at the end of incubation period and both plumule and radicle lengths were measured using calibrated rulers. The seedling growth was studied using the moist-cotton method as adopted by Haikail (2008).

RESULTS AND DISCUSSION

Aspergillus niger, *Fusarium oxysporum*, *penicillium chrysogenum* and *Rhizopus stolonifer* were isolated from the infected seeds. The fungal filtrates (crude mycotoxins) of the isolates affected the seed germination relative to soaking periods. There was progressive reduction in germinations as the soaking period increased. The age of the culture filtrates played a significant and inhibitory role in the sprouting of the seeds. In 4-day old culture filtrates, 82.5% of the seeds treated with *A. niger* germinated from the soaking period and significantly different from the germinations of seeds soaked in all fungal filtrates including the control except *F. oxysporum* filtrate. At all the soaking periods, the effect of *P. chrysogenum* filtrate on germination showed no significant difference with other counterparts respectively except at 8hrs presoaking period where the results of the treatment was significantly different from ones obtained from the treatments of *A. niger*, *F. oxysporum* and *R. stolonifer* (Table 1). In 8-day old filtrates, the seeds treated with *R. stolonifer* filtrate recorded the highest plumule and radicle lengths across the soaking periods but significantly different from the values recorded for control experiment (Table 8). There was no significant difference in the wet weights of both treated and untreated (control) seeds as shown in Table 3.

Table 1: Effects of 4-day-old fungal filtrates on seed germination of maize at different soaking periods

Fungal filtrates	% Germination	Plumule length (cm)	Radicle length (cm)
2hrs pre-soaking period			
<i>Aspergillus niger</i>	82.5 ± 4.33 ^c	1.60 ± 0.22 ^a	5.66 ± 0.36 ^b
<i>Fusarium oxysporum</i>	85.0 ± 3.53 ^{bc}	1.65 ± 0.20 ^a	6.11 ± 0.49 ^{ab}
<i>Penicillium chrysogenum</i>	92.5 ± 4.33 ^{ab}	1.75 ± 0.27 ^a	6.31 ± 0.48 ^{ab}
<i>Rhizopus stolonifer</i>	93.7 ± 1.25 ^{ab}	1.88 ± 0.31 ^a	7.36 ± 0.50 ^a
Control	96.2 ± 2.39 ^a	2.00 ± 0.47 ^c	8.40 ± 0.46 ^a
4hrs pre-soaking period			
<i>Aspergillus niger</i>	78.75 ± 4.26 ^b	1.41 ± 0.21 ^b	5.56 ± 0.48 ^a
<i>Fusarium oxysporum</i>	81.25 ± 4.26 ^b	1.50 ± 0.28 ^{ab}	5.80 ± 0.56 ^a
<i>Penicillium chrysogenum</i>	85.00 ± 5.40 ^b	1.61 ± 0.20 ^a	6.27 ± 0.38 ^a
<i>Rhizopus stolonifer</i>	86.25 ± 5.54 ^b	1.74 ± 0.21 ^a	6.45 ± 0.53 ^a
Control	97.50 ± 1.44 ^a	2.55 ± 0.42 ^c	9.00 ± 0.70 ^a
6hrs pre-soaking period			
<i>Aspergillus niger</i>	67.50 ± 4.33 ^b	1.36 ± 0.15 ^a	5.07 ± 0.32 ^b
<i>Fusarium oxysporum</i>	75.00 ± 2.89 ^b	1.45 ± 0.18 ^{ab}	5.39 ± 0.45 ^{ab}
<i>Penicillium chrysogenum</i>	78.60 ± 8.50 ^b	1.54 ± 0.24 ^a	5.90 ± 0.33 ^{ab}
<i>Rhizopus stolonifer</i>	82.50 ± 1.44 ^{ab}	1.65 ± 0.21 ^a	6.25 ± 0.27 ^a
Control	97.50 ± 2.55 ^{ab}	3.06 ± 0.11 ^b	8.41 ± 0.42 ^a
8hrs pre-soaking period			
<i>Aspergillus niger</i>	53.75 ± 2.39 ^d	1.27 ± 0.22 ^c	4.76 ± 0.19 ^b
<i>Fusarium oxysporum</i>	68.75 ± 3.14 ^c	1.35 ± 0.17 ^{bc}	5.15 ± 0.32 ^b
<i>Penicillium chrysogenum</i>	76.25 ± 5.54 ^b	1.48 ± 0.19 ^{ab}	5.36 ± 0.25 ^{ab}
<i>Rhizopus stolonifer</i>	77.50 ± 3.22 ^b	1.59 ± 0.25 ^a	6.21 ± 0.25 ^a
Control	98.75 ± 1.25 ^a	3.26 ± 0.37 ^b	9.45 ± 0.68 ^a

Mean with the same letter(s) down the column are not significantly different at 0.05 α level



Table 2: Effects of 8-day-old fungal filtrates on seed germination of maize at different soaking periods

Fungal filtrates	% Germination	Plumule length (cm)	Radicle length (cm)
	2hrs pre-soaking period		
<i>Aspergillus niger</i>	61.25 ± 10.28 ^b	1.05 ± 0.12 ^b	5.10 ± 0.36 ^a
<i>Fusarium oxysporum</i>	76.25 ± 10.28 ^a	1.07 ± 0.15 ^{ab}	5.43 ± 0.46 ^a
<i>Penicillium chrysogenum</i>	71.25 ± 8.50 ^{ab}	1.30 ± 0.21 ^a	5.86 ± 0.61 ^a
<i>Rhizopus stolonifer</i>	77.50 ± 6.61 ^a	1.38 ± 0.10 ^a	5.98 ± 0.41 ^a
	4hrs pre-soaking period		
<i>Aspergillus niger</i>	57.50 ± 7.77 ^c	1.03 ± 0.81 ^c	4.96 ± 0.46 ^a
<i>Fusarium oxysporum</i>	67.50 ± 7.50 ^b	1.06 ± 0.15 ^b	5.19 ± 0.41 ^a
<i>Penicillium chrysogenum</i>	73.75 ± 6.25 ^a	1.23 ± 0.13 ^a	5.54 ± 0.46 ^a
<i>Rhizopus stolonifer</i>	75.00 ± 5.40 ^a	1.26 ± 0.08 ^a	5.72 ± 0.49 ^a
	6hrs pre-soaking period		
<i>Aspergillus niger</i>	53.75 ± 6.25 ^b	0.98 ± 0.15 ^c	4.74 ± 0.44 ^b
<i>Fusarium oxysporum</i>	66.25 ± 4.73 ^a	1.03 ± 0.17 ^b	4.88 ± 0.37 ^b
<i>Penicillium chrysogenum</i>	67.50 ± 4.33 ^a	1.16 ± 0.18 ^a	4.91 ± 0.61 ^b
<i>Rhizopus stolonifer</i>	72.50 ± 1.44 ^a	1.20 ± 0.16 ^a	5.56 ± 0.61 ^a
	8hrs pre-soaking period		
<i>Aspergillus niger</i>	45.00 ± 9.78 ^b	0.82 ± 0.11 ^c	4.30 ± 0.59 ^b
<i>Fusarium oxysporum</i>	58.75 ± 10.28 ^a	0.98 ± 0.15 ^{bc}	4.50 ± 0.19 ^b
<i>Penicillium chrysogenum</i>	60.00 ± 5.40 ^a	1.00 ± 0.94 ^{ab}	4.71 ± 0.44 ^{ab}
<i>Rhizopus stolonifer</i>	61.25 ± 3.75 ^a	1.15 ± 0.15 ^a	4.88 ± 0.49 ^a

Mean with the same letter(s) down the column are not significantly different at 0.05 α level

Table 3: Effects of fungal filtrates on seedlings of maize

Fungi	Root length (cm)	Shoot length (cm)	Wet weight (g)	Dry weight (g)
<i>Aspergillus niger</i>	8.20 ± 1.10 ^b	35.10 ± 3.70 ^b	15.89 ± 0.56 ^a	5.12 ± 0.13 ^c
<i>Fusarium oxysporum</i>	17.56 ± 1.20 ^a	43.40 ± 1.70 ^{ab}	19.40 ± 0.95 ^a	6.30 ± 0.20 ^{bc}
<i>Penicillium chrysogenum</i>	17.00 ± 0.91 ^a	46.30 ± 1.32 ^a	19.71 ± 0.55 ^a	8.90 ± 1.52 ^a
<i>Rhizopus stolonifer</i>	18.20 ± 1.46 ^a	47.20 ± 2.90 ^a	20.90 ± 3.30 ^a	9.51 ± 1.20 ^{ab}
Control	20.90 ± 1.50 ^a	52.30 ± 3.90 ^a	22.40 ± 1.52 ^a	13.20 ± 0.34 ^a

Mean with the same letter(s) down the column are not significantly different at 0.05 α level

The results had shown that the fungal filtrates of *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum* played inhibitory role in seed germination and seedling development. The inhibition may be considered as a result of toxic metabolites produced by the fungi in the media (Haikai, 2008). Fungal filtrates usually represent crude mycotoxins which are being produced by pathogenic fungi. Mycotoxins are harmful fungal metabolites that are released by relatively few but universally present fungi growing on grains, legumes and nuts (Agrios, 2005). These lethal metabolites are toxic to both plants and animals. Reduction in germination, root and shoot growth was recorded in the wheat treated with *Drechslera rostrata* and *Fusarium equiseti* filtrates (Anaso et al., 1981) and soybean treated with *Fusarium soloni*, *F. oxysporum*, *Aspergillus flavus*, *A.*

niger, *Alternaria tenuis* and *A. alternata* (Ibraheem et al., 1987). The metabolites produced by *Trichoderma* spp. influence the seed germination and seedling emergence (Clear and Valic, 2005). Agrios (2005) reported that different pathogenic fungi produce common or closely related mycotoxins: *Fusarium* produce fumonisins, *Aspergillus* produce aflatoxins, *Penicillium* produce citrinin and Luteoskyrin and both *Aspergillus* and *Penicillium* produce patulin. Mycotoxicoses, such as ergotism and fescue toxicosis, occur as a result of ingestion of infected seeds and other agricultural products.

Pathogenic seed-borne fungi produce mycotoxins that inhibit seed germination and growth. Researchers should intensify efforts on methods by which seeds can be treated so as to reduce the loss and promote seed survival.

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