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Effect of Filtrates of Pathogenic Fungi of Soybean on Seed Germination and Seedling Parameters

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Abstract: This work was carried out at the Girls College of Education, Jeddah, Saudi Arabia. Apparently healthy looking seeds of soybean (*Gylcin max*) pre-soaked in 25, 50, 75 and 100% concentration of 4, 8 and 12-day- old cell-free culture filtrates of seed-borne *Aspergillus niger*, *Fusarium culmorium, Penicillium* sp. and *Rhizoctonia solani* of soybean seeds for 2, 4, 6, 8 and 24 h were investigated for phytotoxicity. Results showed that all the fungal filtrates irrespective of filtrate concentrations, filtrate age and presoaking period significantly ($P \leq 0.05$ and $P \leq 0.01$) reduced percentages seed germination and seedling development when compared with the control. Percentage seed germination and seedling growth decreased with increase in filtrate concentration, filtrate age and presoaking time in all the fungal filtrates. Sterilized filtrate of *A. niger*, *F. culmorium*, *Penicillium* sp and *Rhizoctania solani* lost some of their phytotoxicity than non-sterilized filtrates.

Key words: seed-borne pathogenic fungi, soybean, seed germination, seedling parameters

INTRODUCTION

Fungal metabolites are substances discharged by fungi in their metabolic processes. The metabolites are products of some amino acids, cyclic peptides, aromatic, phenols, terpenoids and plant growth regulators[4,11,10]. These metabolites are many and diverse and they are known to cause diseases in plants, animals and humans who eat infected food[13,14]. Fungi of the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizoctonia* are commonly known to produce toxic substances, such as aflatoxin B1 and B2, Aspergellic acid, cyclopiczonic acid, kojic acid, naphthoquinones, fumonizin and fusaric acid[14] that threaten the health of our plants and animals.

The role of toxic metabolites of pathogenic fungi in plant disease development has been reported by several workers. Anaso *et al*.[1] found out that toxic metabolites of *Drechslera rostrata* and *Fusarium equiseti* retarded root growth of wheat. Reduction in percentage seed germination of soybean seeds was observed in seeds soaked in filtrates of *Phomopsis phaseoli*[6]. Soybean seeds soaked in cultures filtrates of *Fusarium solani*, *F. oxysporum*, *Aspergillus flavus*, *A. niger*, *Alternaria tenuis* and *A. alternata* for 24 hours showed reduction in percentage seed germination[7].

Filtrate from mycelial cultures of *Verticillum albo-atrum* was found to inhibit cell growth and reduced the viability of alfalfa (*Medicago sativo*) seeds[3]. Reddy *et al*. reported that the culture filtrate of seed-borne strains of *Alternaria alternate* (Fr.) Keissler inhibited the germination and vigour of sunflower seeds and maize kernels. Culture filtrates of *Fusaruim moniliforme*, *F. semitectum* and *F. oxysporum* gave very high percentage reduction in seed germination and also inhibited root and shoot growth of sorghum. Reduction in seed germination and yield of exotic potato cultures by filtrates of *Pythium* sp., *Chaetomium globosum* and *Rhizopus* sp. was reported by Dwivedi[2].

The present study was therefore, undertaken to evaluate the effect of filtrates of seed-borne fungi (*Aspergillus niger*, *Fusarium culmorium, Penicillium* sp. and *Rhizoctonia solani*) of soybean seeds on disease development as well as their phytotoxic effects.

MATERIALS AND METHODS

Pathogenic Fungi: Seed-borne fungi (*Aspergillus niger*, *Fusarium culmorium*, *Penicillium* sp. and *Rhizoctonia solani*) commonly associated with soybean seeds were used for this study. The fungi were isolated from soybean seeds and maintained on culture of potato dextrose agar (PDA) media.

Culture Filtrate Preparation: A disc (0.5 cm diameter) of mycelia and spores taken from the periphery of 6-day-old culture of each fungus grown on potato dextrose agar (PDA) medium was introduced into 250-ml conical flasks, each containing 50 ml of Sabouraud dextrose broth. The broth contains (g/l): peptone, 10 and glucose, 40. The flasks were incubated at 25 ± 1°C for 4, 8 and 12 days. Four flasks were

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used for each fungus per incubation period. The fungal filtrates were obtained by passing the culture through sterile Whatman No. 3 filter paper to obtain a cell free filtrate[15]. After that the filtrate was passed again through bacterial filter (0.45 µm).

Effect of Fungal Filtrated on Seed Germination: Seeds of soybean were surface sterilized with 1% sodium hypochlorite solution for 5 min and rinsed several times in sterile distilled water and subjected to the following treatment:

Presoaking in 4, 8 and 12-day-old fungal filtrates for varying periods of time (2, 4, 6 and 8 h) in undiluted filtrates (100%).

Presoaking seeds for 24 h in different concentrations 25, 50, 75 and 100% of 4, 8 and 12-day-old fungal filtrates.

Presoaking Seeds in Undiluted Fungal Filtrates for Varying Periods of Time: Sterile soybean seeds were presoaked in 4, 8 and 12-day-old undiluted fungal filtrates of each of the previously mentioned fungi for 2, 4, 6 and 8 h. The control seeds were presoaked in sterile distilled water for the same periods of time. At the end of each presoaking period, the seeds were removed from the filtrates and plated on 10-cm diameter Petri dishes each containing 3 layers of blotters soaked with sterile distilled water. About 12 seeds were sown per dish. The dishes containing the seeds were incubated for a week at 20 ± 2°C. Germination counts were made at the end of the incubation period and symptoms on seeds were recorded.

Presoaking Seeds in Different Concentrations of the Fungal Filtrates: In this experiment, seeds were surface sterilized as previously described and presoaked for 24 h in the different concentrations (25, 50, 75 and 100%) of 4, 8 and 12-day-old culture filtrates. Dilution was made with sterile distilled water, the control seeds were presoaked in sterile distilled water for 24 h. At the end of the presoaking period, seeds were plated on blotters moistened with sterile distilled water. The plates containing the seeds were incubated as described above and germination counts were recorded

Effect of Sterilization of Undiluted 12-day-old Fungal Filtrates on Seedling Growth: The effect of sterilized and non-sterilized fungal filtrates on seedling growth was studied using the moist-cotton method[15]. In this method, absorbent cotton wool was put in 100 ml measuring cylinder and after plugging the cylinder by absorbent cotton wool wrapped with aluminum foil. The sterile cotton wool in each sterile tube was moistening with 12-day-old sterilized or non-sterilized fungal filtrates. The cotton wool used for the control was soaked with sterile distilled water. Three seeds were placed in each cylinder, the cylinder contains the seeds were left at room temperature for 21 days. The end of the experiment, seedling heights was measured and the length of tap roots was also measured. The root system of each seedling was cut off, washed and dried to constant weight. At 80°C the shoot system of the seedlings was also determined after drying as above.

RESULTS AND DISCUSSIONS

Results: The effect of presoaking seeds in undiluted filtrates of seed-borne fungi for different periods of time on seed germination is shown in Table 1. Generally speaking, presoaking of seeds in fungal filtrates of the four seed-born fungi tested for the different period significantly ($P \leq 0.05$ and $P \leq 0.01$) reduced seed germination when compared with the control seeds.

The percentage of germination varied also with presoaking period, culture filtrates, as well as age of culture. Percentage seed germination decreased with increase in presoaking period while decrease in presoaking period enhanced percentage of seed germination (Table 1). 8 h presoaking in all the fungal filtrates gave the highest significant reduction in germination, while 2 h presoaking significantly gave the lowest reduction in seed germination. Fungal filtrates of *A. niger* and *F. culmorium* gave the lowest percentage of germination in all periods of presoaking, while filtrates of *Rhizoctonia solani* and *Penicillium* sp. gave the highest percentage of seed germination, but the percentage of seed germination was significantly lower than control.

The reduction in germination by all the filtrates increased with age of culture. The greatest reduction in germination was obtained in 12-day-old filtrates of all the seed-borne fungi tested.

Presoaking of seeds in various concentrations 25, 50, 75 and 100% of all the filtrates significantly reduced seed germination when compared with control (Table 2). Reduction in seed germination increased in concentrations of the fungal filtrates. Undiluted (100%) fungal filtrates of all the seed-borne fungi significantly reduced germination when compared with all the other concentrations tested. 25% fungal filtrate gave the lowest reduction in germination. However 25% fungal filtrate of all the fungi significantly decreased germination when compared with the control. The reduction in germination increased with increase in age of culture (Table 2). 12-day-old culture filtrate gave the highest reduction in seed germination, followed by 8 and 4-day-old fungal filtrate, respectively.

Table 1: Effect of Pre-soaking soybean seeds for varying Periods of time in 100% filtrate after incubation periods of 4, 8 and 12 days of seed-borne fungi of soybean seeds on percentage of seed germination

Fungal culture filtrate	Seed germination (%) after (days)			
	2	4	6	8
		4-day-old filtrate		
control	93.5±0.7	94.8±0.5	95.8±0.5	97.8±0.3
Aspergillus niger	62.1±0.4**	50.5±0.5**	31.7±0.4**	28.7±0.4*
Fusarium culmorium	63.9±0.5**	52.8±0.7**	43.3±0.3**	35.7±0.4**
Penicillium sp.	67.4±0.4**	57.1±0.5**	49.3±0.5*	40.6±0.2**
Rhizoctonia solani	72.1±0.7**	66.4±0.5**	49.6±0.5	42.7±0.7
		8-day-old filtrate		
Aspergillus niger	53.2±0.7**	38.3±0.3**	29.5±0.4**	21.1±1.1*
Fusarium culmorium	56.5±0.5**	43.3±0.3**	32.4±0.4*	26.6±1.0*
Penicillium sp.	50.1±0.7*	48.9±0.6**	36.7±0.8	29.5±0.4**
Rhizoctonia solani	58.3±0.4**	45.65±0.95*	40.3±0.6**	35.2±0.4**
		12-day-old filtrate		
Aspergillus niger	41.2±0.8**	34.9±0.5**	24.9±0.4**	21.3±0.8**
Fusarium culmorium	45.4±0.5**	37.6±0.3*	29.6±0.3**	24.1±0.7
Penicillium sp.	48.4±0.5**	39.9±0.5**	32.4±0.5	25.9±0.4*
Rhizoctonia solani	51.3±0.7**	45.5±0.6**	39.2±0.3*	19.6±0.5

*** Significant at 5%
** Significant at 1 %

Table 2: Effect of Pre-soaking soybean seeds for 24 hrs in various concentration of 4, 8 and 12-day-old culture filtrate of seed-borne fungi on the percentage of seed germination.

Fungal culture filtrate	Seed germination (%)				
	0.0	25	50	75	100
		4-day-old filtrate			
Aspergillus niger	97.2±0.3	62.0±0.4**	59.4±0.5**	46.2±0.3**	31.9±0.5**
Fusarium culmorium	97.2±0.3	63.7±0.3**	50.9±0.4**	54.9±0.4**	41.9±0.8**
Penicillium sp.	97.2±0.3	66.7±0.2**	64.0±0.4**	57.7±0.5**	47.7±0.6**
Rhizoctonia solani	97.2±0.3	62.0±0.7**	59.2±0.3**	46.0±0.4**	31.5±0.5**
		8- days old filtrate			
Aspergillus niger	97.2±0.3	53.7±0.5**	48.8±0.5**	40.9±0.5**	28.9±0.6**
Fusarium culmorium	97.2±0.3	57.1±0.4**	53.2±0.3**	31.8±0.5**	54.3±0.6**
Penicillium sp.	97.2±0.3	58.5±0.3**	54.2±0.3**	45.6±0.4**	33.9±0.3**
Rhizoctonia solani	97.2±0.3	52.5±0.3**	47.1±0.7**	38.6±0.9**	28.2±0.4**
		12- days old filtrate			
Aspergillus niger	97.2±0.3	50.6±0.4**	42.7±0.4**	31.6±0.3**	22.3±0.5**
Fusarium culmorium	97.2±0.3	54.3±0.6**	46.6±0.4**	14.3±0.6**	26.5±0.3**
Penicillium sp.	97.2±0.3	55.5±0.5**	47.2±0.3**	16.9±0.2**	31.0±0.5**
Rhizoctonia solani	97.2±0.3	49.7±0.7**	41.9±0.6**	51.5±0.5**	22.8±0.9

***Significant at 5%
** Significant at 1 %

Table 3: Effect of autoclaved and non-autoclaved 12 days- old culture filtrate of seed-borne fungi of soy bean seeds on seedling growth after 3-weeks of planting

Parameters	Control	Autoclaved filtrate			
		A niger	*F. culmorium*	*Penicillum* sp.	*R. solani*
Seedling length (cm)	38.7±0.5	35.43±0.8	31.3±0.5	33.4±0.3	31.1±0.4
Root length (cm)	7.9±0.3	5.9±0.1	4.6±0.1	5.7±0.1	4.8±0.2
Dry wt. of shoot (mg)	81.5±0.1	40.1±0.1	44.3±0.1	58.1±0.1	44.6±0.2
Dry wt. of root (mg)	36.0±0.2	22.8±0.2*	26.5±0.2	34.3±0.1	26.7±0.1**
		Non-Autoclaved filtrate			
Seedling length (cm)	38.7±0.5	30.8±0.5	26.3±0.4	30.3±0.3	27.3±0.6
Root length (cm)	7.9±0.3	5.2±0.2	3.9±0.1	4.5±0.1	3.2±0.1
Dry wt. of shoot (mg)	81.5±0.1	34.8±0.1	34.5±0.1	50.3±0.1	35.3±0.1
Dry wt. of root (mg)	36.0±0.2	26.8±0.1*	23.3±0.1*	28.5±0.1*	28.5±0.1**

* Significant at 5%
** Significant at 1 %

The results in Table (3) showed that non-sterilized fungal filtrates of all the test fungi significantly had deleterious effects on the seedling height, tap root length and dry weight of shoot and root system when compared with control. The inhibition was still evident after sterilizing the fungal filtrates of *A niger, F. culmorium, Penicillium* sp.and *R. solani.*

Discussion: The results of this study have shown that filtrates of *A. niger, F. culmorium, Penicillium* sp. and *R. solani* inhibited seed germination and seedling development of soybean Tables (1-3). This represents that the tested fungi produced toxic metabolites in the media in which they were grown, these metabolites can inhibit and reduce the percentage of seed germination and also retard seedling growth. Anaso *et al*.[1] reported that toxic metabolites produced by *Drechslera rostrata* and *Fusarium equiseti* retarded root growth of wheat. Hilty *et al*.[6] found that filtrate from *Phomopsis phaseoli* reduced seed germination of soybean seeds and also inhibited root growth and elongation at the concentration 0.1 and 0.01. This inhibition was attributed to the toxins secreted in the media. Fungal filtrates from *F. solani*, *F. oxysporum*, *A. niger*, *A. flavus*, *A. terreus* and *Alternaria alternata* were reported to reduce germination percentage of soybean seeds when seeds were soaked in the filtrate for 24 h[7].

The fungal filtrates of *A. niger*, *F. culmorium*, *Penicillium* sp. and *R. solani* still retained their toxicity after sterilization, these results agree with the results of Lukezic *et al*. and Paxton who reported that filtrates of some pathogens retain their toxicity after being autoclaved or heated. Anaso *et al*.[1] reported also that filtrates from *F. equiseti* are thermostable, while that from *D. rostrata* is thermolabile. Also Halfon-Meriri[5] reported that the autoclaved filtrates of *Aspergillus chevalieri* and *A. ocheraceus* have an inhibitory effect on popcorn seeds.

In this study the highest percentage reduction in seed germination and root and shoot elongation was obtained in 12-day-old culture filtrates of the tested four seed-borne fungi, Mahalinga *et al.* found that the maximum reduction in seed germination and root and shoot growth of sorghum was obtained with 30 and 45-day-old *Fusarium* filtrates, while the minimum reduction occurred with 15-day-old culture filtrate Diwivedi[2] found that 10-day-old culture filtrates of fungi associated with exotic true potato inhibited germination and tuber yield more than 4-day-old filtrates. Umechuruba and Nwachukwu[15] reported that *A. flavus, A. niger, F. moniliform*, *Penicillium* sp. produced various types of toxic metabolites and many of these metabolites are known to reduce germination and seedling development of African yam bean seeds, this results also agreed with the results reported by Nema[11], Vazquez *et. al*.[16] and Madhosingh[10].

Li *et al*.[9] found that there were fungal toxin was produced in soybean roots and translocated to foliage. Recently, Palmer *et al*.[12] reported that a phytotoxic protein was isolated from culture filtrates of *Verticillium dahliae* which reduce the germination of cotton seeds and inhibit the seedling growth.

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