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Original Article

In vitro Efficacy of Plant Extracts on Seed Germination and Fungal Infection of Six Varieties of Wheat (*Triticum aestivum* L.)

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ABSTRACT: The fungitoxicity of six plants extract viz., Allium sativum L., Azadirachta indica A. Juss., Citrus limon (L.) Burm. f., Datura metel L., Gynura procumbens Merr. and Thuja occidentalis L. were evaluated on seed germination and fungal infection of six wheat varieties (BARI Gom 25-30) at 5, 10 and 20% after 3, 5 and 7 days of incubation. After 7 days of incubation highest germination percentages of seed viz., 96, 94 and 92 were recorded in BARI Gom-25, BARI Gom-27 and BARI Gom-28 after being treated with A. sativum, respectively. After 7 days of incubation highest germination percentages of seed viz., 96, 94 and 96 were recorded in BARI Gom-26, BARI Gom-27 and BARI Gom-30 after being treated with T. occidentalis, respectively. After 7 days of incubation highest germination percentages of seed viz., 96, 96 and 94 were recorded in BARI Gom-25 BARI Gom-26 and BARI Gom-27 after being treated with G. procumbens, respectively. BARI Gom-25, BARI Gom-29 and BARI Gom-30 varieties showed lowest fungal infection viz., 8, 4 and 4% owing to A. sativum plant extract, respectively at 20% concentration after 7 days of incubation. BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28 and BARI Gom-30 varieties showed lowest fungal infection viz., 8, 6, 4, 4 and 4% owing to T. occidentalis plant extract, respectively at 20% concentration after 7 days of incubation. BARI Gom-26 and BARI Gom-30 varieties showed lowest fungal infection viz., 6 and 4% owing to A. indica plant extract, respectively at 20% concentration after 7 days of incubation. BARI Gom-25, BARI Gom-27 and BARI Gom-30 varieties showed lowest fungal infection viz., 8, 4 and 4% owing to C. limon plant extract, respectively at 20% concentration after 7 days of incubation. The present investigation suggests that A. sativum, A. indica, C. limon, G. procumbens and T. occidentalis plants can be used as best botanicals for increasing seed germination and inhibiting fungal infection of wheat varieties.

KEYWORDS: Efficacy, Plant extracts, Germination, Fungal infection, Six varieties, *Triticum aestivum*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered one of the most important cereal crops in the world and belongs to the family Poaceae¹. It provides nearly

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20% of world food requirement. About two third of the world population use wheat as stable food². It is the major grain used for human consumption in the temperate region. In Bangladesh, it is also an

important cereal crop. It is the second most important cereal crops next to rice in Bangladesh. By the time of independence (1971), Bangladesh had become highly dependent on wheat imports while dietary preferences were changing such that wheat was becoming a highly desirable food supplement to rice. Many important diseases of plants caused by fungi that spread through seeds³. Seed borne fungi are responsible for both pre and post-emergence death of grains, affect seedling and thus cause some reduction in vigor. and also variation germination in plant morphology⁴⁻⁵.Seed abnormality due to the influence of seed-borne fungi is very common and often accounts for a large percentage of crop losses⁶.

Each year about 20% of the wheat that otherwise would be available for food and feed is lost due to diseases⁷. Scientists and specialists after laboratory tests claimed that wheat blast is the first time in Bangladesh in which the seasonal crop got infected with this disease and wheat production would decrease by around 10-40 percent. Around 2,000 hectares of wheat fields had been damaged by wheat blast disease caused by Magnaporthe oryzae. The affected districts included Kushtia, Meherpur, Chuadanga, Jhenidah, Jessore, and Magura. Seed borne infection of fungal pathogens are not only for its association with seeds which cause germination failure and/or causing disease to the newly emerged seedlings or growing plants, but also contaminate the soil by its inocula permanently⁸. Soil is the main source of fungal infection⁹. The climate of Bangladesh is very much suitable for the growth of fungi.

Plant extracts can be successfully exploited in modern agriculture. Seed treatment by plant extracts may be an effective approach to reduce or eliminate seed-borne fungi and also increase grain germination¹⁰. Wheat Seed treatment with plant extracts is an ecofriendly measure for controlling seed-borne pathogens. Wheat seed treatment with aqueous extract of garlic and allamanda can be used to reduce fungal incidence¹¹. Seed treatment with plant extracts results in higher germination in different crops including wheat have been reported¹²⁻¹⁴. In view of the above facts, the research work was undertaken to find out the effects of plant extracts on seed germination and black point of wheat seeds caused by Bipolaris sorokiniana (Sacc.) Shoemaker and other associated fungi namely Alternaria spp., Curvularia spp. and Fusarium spp. with six varieties of T. aestivum.

MATERIALS AND METHODS

Six varieties of BARI Gom (25-30) of wheat seeds (*Triticum aestivum* L.) were collected from the Regional Wheat Research Centre, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur during the tenure of May 2015 to March 2016. Samples were collected after harvesting and placed in clean brown paper bags, labeled properly and preserved at room temperature for subsequent use.

The fungi associated with wheat seeds were isolated following "Tissue planting method"¹⁵. Identification of the isolates was determined based on morphological characteristics observed under a compound microscope following the standard literatures¹⁶⁻²¹. Pathogenecity test of isolated fungi were carried out following seed inoculation technique²².

The phytotoxic effect of plant extracts of six higher plants viz., Allium sativum L., Azadirachta indica A. Juss., Citrus limon (L.) Burm. f., Datura metel Gynura procumbens Merr. and L., Thuja occidentalis L. were evaluated on seed germination and fungal infection of wheat varieties. The desired parts of each plant were thoroughly washed in tap water; air dried and was prepared by crushing 100 gm of fresh materials with distilled water in ratio of 1:1 (w/v). The pulverized mass of a plant part was squeezed through four folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 minutes to remove particulate matter. The supernatants were filtered through Whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer flask. The requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10 and 20% concentration²³.Four hundred seeds of each wheat variety were surface sterilized with 10% chlorox solution. The sterilized seeds of each variety were soaked in 5, 10 and 20% concentrations of each plant separately for 24 hours. In control sets, seeds of each wheat variety were soaked in sterilized distilled water for 24 hours. Ten seeds were then placed on each moistened filter paper in sterilized Petri plate and incubated at $25\pm2^{\circ}$ C. Seed germination was recorded on the 3, 5 and 7 days interval of incubation.

RESULTS AND DISCUSSION

In this present study a total of ten fungal species i.e., Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Bipolaris sorokiniana, Curvularia lunata, Nigrospora sp., Penicillium sp., Rhizopus stolonifer and Trochoderma viride were recorded on wheat seeds. Among the isolated fungi Alternaria alternata, Bipolaris sorokiniana and



Curvularia lunata were found to be pathogenic to wheat seeds.

The results of effect of six plant parts extracts on seed germination at 5, 10 and 20% concentrations with control sets after 3, 5 and 7 days of incubation are presented in Tables 1-6. In contrast to the control sets, almost all the plant extracts showed comparatively better results on seed germination percentage of six wheat varieties (Tables 1-6). Table 1 showed that after 7 days of incubation highest germination percentage of seed (96) was recorded in BARI Gom-25 at 5% concentration after being treated with A. sativum and T. occidentalis whereas G. procumbens showed highest germination percentage of seed (96%) at 20% concentration. Table 2 showed that after 7 days of incubation highest germination percentage of seed (96) was recorded in BARI Gom-26 at 10% and 20% concentrations after being treated with Gynura procumbens and Thuja occidentalis. Table 3 showed that after 7 days of incubation highest germination percentage of seed (94) was recorded in BARI Gom-27 at 5% concentration after being treated with A. sativum, C. limon, and T. occidentalis whereas G. procumbens showed highest germination percentage of seed (96%) at 10% concentration.. Table 4 showed that after 7 days of incubation highest germination percentage of seed (92) was recorded in BARI Gom-28 at 20% concentration after being treated with A. sativum. Table 5 showed that after 7 days of incubation highest germination percentage of seed (96) was recorded in BARI Gom-29 at 20% concentration after being treated with D. metel whereas Τ. occidentalis showed highest germination percentage of seed (96%) at 5% concentration. Table 6 showed that after 7 days of incubation highest germination percentage of seed (96) was recorded in BARI Gom-30 at 20% concentration after being treated with Azadirachta indica whereas T. occidentalis showed highest germination percentage of seed (96%) at 5 and 10% concentrations.

In case of fungal infection, effects of the extracts of six fresh plant parts on fungal infection of six wheat varieties at 5, 10 and 20% concentrations with control sets after 3, 5 and 7 days of incubation are shown in Tables 1-6. In contrast to the control sets, almost all the plants extract treated wheat varieties showed lowest fungal infection (Tables 1-6). Table 1 revealed that BARI Gom-25 variety showed lowest fungal infection (8%) owing to *A. sativum, C. limon, G. procumbens* and *T. occidentalis* plant extract followed by *A. indica* (10%) and *D. metel* (12%) whereas control set

(without treatment) showed highest fungal infection (44%) at 20% concentration after 7 days of incubation. Table 2 revealed that BARI Gom-26 variety showed lowest fungal infection (6%) owing to A. indica and T. occidentalis plant extract followed by C. limon (8%), A. sativum (10%) and D. metel (10%) whereas control set (without treatment) showed highest fungal infection (38%) at 20% concentration after 7 days of incubation. Table 3 revealed that BARI Gom-27 variety showed lowest fungal infection (4%) owing to C. *limon* and *T. occidentalis* plant extract followed by A. sativum (8%), A. indica (10%), D. metel (10%) and G. procumbens (10%) whereas control set (without treatment) showed highest fungal infection (40%) at 20% concentration after 7 days of incubation. Table 4 revealed that BARI Gom-28 variety showed lowest fungal infection (4%) owing to T. occidentalis plant extract followed by A. sativum (6%), C. limon (6%), A. indica (8%), D. metel (8%) and G. procumbens whereas control set treatment) showed highest (without fungal infection (40%) at 20% concentration after 7 days of incubation. Table 5 revealed that BARI Gom-29 variety showed lowest fungal infection (4%) owing to A. sativum plant extract whereas control set (without treatment) showed highest fungal infection (36%) at 20% concentration after 7 days of incubation. Table 6 revealed that BARI Gom-30 variety showed lowest fungal infection (4%) owing to A. sativum, A. indica and T. occidentalis plant extract whereas control set (without treatment) showed highest fungal infection (40%) at 20% concentration after 7 days of incubation.

Laboratory evaluation of extracts of fresh plant parts revealed that all the extracts cause partial or complete effective on seed germination and fungal infection for all the six wheat varieties at 5, 10 and 20% concentrations after 3, 5 and 7 days of incubation.

In contrast to the present study Khan and Kumar $(1992)^{24}$ reported that the seed treatment with Garlic extract, Neem, Gagra, Vatpara, Bishkatali leaf extract reduced seed-borne prevalence of fungi and it also increased germination percentage of wheat seeds. Islam $(2001)^{25}$, Khaire *et al.* $(1992)^{26}$ and Hasan *et al.* $(2005)^8$ reported that seeds treated with plant materials did not adversely affect the seed germination. Rahman et al. (1999)²⁷ found that garlic extract was superior in terms of reducing seed-borne infections by Alternaria spp., sorokiniana, Curvularia **Bipolaris** lunata. Fusarium spp. of wheat to other extracts followed by ginger and neem. Ahmed (2002)²⁸ found that neem and garlic extracts were more effective



against *Bipolaris oryzae* at 1:1 dilution. Present investigation indicates that in case of seeds germination the differences between treatment and control plates are not remarkable. But effects of plant extracts on fungal infection of wheat seeds are satisfactory. So, efficiency gradients in the present study expressed that *Allium sativum* L., *Azadirachta indica*, *Citrus limon*, *Gynura procumbens* and *Thuja occidentalis* at 5, 10 and 20% concentrations were the best agent for increasing seed germination and inhibition of fungal infection of seeds of wheat varieties.

The present investigation suggests that A. sativum, A. indica, C. limon, G. procumbens and T. occidentalis plants can be used as best botanicals for increasing seed germination and inhibiting fungal infection of wheat varieties.

Table 1. Effect of plant extracts on seed germination and fungal infection of H	BARI Gom-25 variety at different concentrations.
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Sl. No.	Period infection							and fungal		
		(Days)	5% C	5% Conc.		10% Conc.		20% Conc.		
1.	. Allium sativum L	3	92	10		92	6	90	6	
		5	94	18		92	8	94	6	
		7	96	20		94	10	96	8	
2.	Azadirachta indica A.	3	90	12		90	8	92	6	
	Juss	5	90	18		92	10	92	6	
		7	92	24		92	14	94	10	
3.	3. <i>Citrus limon</i> (L.) Burm. F	3	92	8		92	8	92	6	
		5	94	16		92	8	92	8	
		7	94	16		94	10	96	8	
4.	Datura metel L.	3	88	12		92	10	90	8	
		5	90	30		92	14	92	10	
		7	92	22		94	14	94	12	
5.	Gynura procumbens	3	90	12		88	8	92	6	
	Merr.	5	92	18		90	12	94	8	
		7	94	18		94	16	96	8	
6.	Thuja occidentalis L.	3	92	10		92	8	92	4	
		5	94	16		94	8	94	4	
		7	96	16		96	10	94	8	
Control	(without treatment)	3	90	40		90	40	90	40	
		5	94	40		94	40	94	40	
		7	94	40		94	44	94	44	

Table 2. Effect of plant extracts on seed germination and fungal infection of BARI Gom-26 variety at different concentrations.

Sl.	Name of Plants	Germination	Per	Per cent germination of seeds and fungal infection							
No		Period (Days)	5% (5% Conc.		10% Conc.			20% Conc.		
1.	Allium sativum L.	3	88	10		90	6		92	6	
		5	92	20		92	12		92	8	
		7	92	20		94	14		94	10	
2	Azadirachta indica A.	3	88	14		88	8		88	4	
Jus	Juss	5	90	16		92	10		92	4	
		7	92	18		94	10		94	6	
3.	<i>Citrus limon</i> (L.) Burm. F	3	88	14		90	8		92	6	
		5	90	18		90	12		92	8	
		7	92	18		92	12		94	8	
4.	Datura metel L.	3	86	12		88	10		90	6	
		5	88	14		90	12		90	6	
		7	92	16		92	14		92	10	
5.	Gynura procumbens	3	92	14		94	8		92	6	
	Merr.	5	94	20		94	12		92	8	
		7	96	22		96	12		96	8	
6.	Thuja occidentalis L.	3	92	8		92	8		90	4	
	·	5	92	12		92	8		92	6	
		7	94	12		96	10		96	6	
Cont	trol (without treatment)	3	90	32		90	32		90	32	
		5	90	34		90	34		90	34	
		7	92	38		92	38		92	38	



SI. No.	Name of Plants	Germination Period (Days)	Per cent germination of seeds and fungal infection						
			5% C	conc.	10% Conc	2.	209	% Conc.	
1.	Allium sativum L.	3	88	10	92	8	90	0	
		5	92	16	92	8	92	4	
		7	94	18	94	10	94	8	
2.	Azadirachta indica A.	3	88	8	88	8	90	6	
	Juss	5	88	14	88	10	90	6	
		7	90	16	92	10	92	10	
3.	Citrus limon (L.)	3	90	8	90	4	88	0	
	Burm. F	5	94	16	90	4	90	4	
		7	94	16	92	10	92	4	
4.	Datura metel L.	3	88	12	90	8	88	8	
		5	90	18	92	10	90	10	
		7	90	20	92	10	92	10	
5.	Gynura procumbens	3	90	12	90	8	90	6	
	Merr.	5	90	18	92	12	90	6	
		7	92	20	94	12	92	10	
6.	Thuja occidentalis L.	3	92	10	90	6	90	0	
		5	92	16	92	8	92	4	
		7	94	16	92	8	94	4	
Control (without treatment)		3	90	34	90	34	90	34	
		5	90	38	90	38	90	38	
		7	92	40	92	40	92	40	

Table 3. Effect of plant extracts on seed germination and fungal infection of BARI Gom-27 variety at different concentrations.

Table 4. Effect of plant extracts on seed germination and fungal infection of BARI Gom-28 variety at different concentrations.

SI. No	Name of Plants	Germination Period (Days)	0 0								
•			5% Conc.		10%	Conc.	20% (20% Conc.			
1.	Allium sativum L.	3	82	8	84	4	88	4			
		5	86	14	90	6	90	4			
		7	90	18	90	6	92	6			
2.	Azadirachta indica	3	84	6	82	4	84	4			
	A. Juss	5	86	10	86	6	86	6			
		7	88	10	86	8	86	8			
3.	Citrus limon (L.)	3	84	8	86	4	84	2			
	Burm. F	5	84	12	88	8	88	4			
		7	86	16	88	8	88	6			
4.	Datura metel L.	3	86	12	80	4	86	6			
		5	90	14	84	8	88	8			
		7	90	16	84	8	90	8			
5.	Gynura procumbens	3	80	12	84	8	84	6			
	Merr.	5	84	16	86	8	84	6			
		7	84	18	86	10	86	10			
6.	Thuja occidentalis L.	3	84	6	88	4	84	0			
		5	86	6	90	6	88	4			
		7	90	8	92	6	90	4			
	trol (without	3	84	36	84	36	84	36			
trea	tment)	5	84	38	84	38	84	38			
		7	86	40	86	40	86	40			



Seed Germination and Fungal Infection

Table 5. Effect of plant extracts on seed germination and fungal infection of BARI Gom-29 variety at different concentrations.

Sl. No.	Name of Plants	Germination Period		Per cent germination of seeds and fungal infection						
		(Days)	5% C	onc.	10% (Conc.	20%	20% Conc.		
1.	Allium sativum L.	3	90	14	90	6	92	0		
		5	90	20	94	8	94	4		
		7	92	22	94	8	94	4		
2.	Azadirachta indica A.	3	90	10	90	6	88	4		
	Juss	5	94	16	94	8	92	8		
		7	94	18	94	10	94	10		
3.	Citrus limon (L.) Burm. f	3	90	8	90	8	92	6		
		5	92	16	92	8	92	8		
		7	92	16	94	10	94	8		
4.	Datura metel L.	3	92	14	90	6	92	6		
		5	92	18	92	10	94	6		
		7	94	22	94	12	96	8		
5.	Gynura procumbens	3	88	12	90	8	90	4		
	Merr.	5	92	16	92	8	92	6		
		7	92	16	92	10	92	6		
6.	Thuja occidentalis L.	3	92	12	90	4	92	4		
		5	92	16	90	6	92	6		
		7	96	16	94	8	94	6		
Control (without treatment)		3	88	30	88	30	88	30		
		5	90	34	90	34	90	34		
		7	90	36	90	36	90	36		

Table 6. Effect of plant extracts on seed germination and fungal infection of BARI Gom-30 variety at different concentrations.

Sl. No.	Name of Plants	Germination Period	Per cent germination of seeds and fung infection						
		(Days)	5% C	Conc.	10%	Conc.	20%		
							Conc		
1.	Allium sativum L.	3	90	16	90	6	88	2	
		5	92	20	92	8	92	4	
		7	92	22	92	8	92	4	
2.	Azadirachta indica A.	3	92	12	92	8	90	0	
	Juss	5	92	16	94	10	94	4	
		7	94	18	94	14	96	4	
3.	Citrus limon (L.) Burm. f	3	90	8	86	6	84	0	
		5	92	16	88	8	88	6	
		7	94	18	88	8	88	6	
4.	Datura metel L.	3	92	12	90	10	90	4	
		5	94	16	94	12	92	8	
		7	96	18	96	12	96	10	
5.	Gynura procumbens Merr.	3	90	14	92	8	92	6	
		5	94	20	94	10	94	8	
		7	94	20	94	10	94	8	
6.	Thuja occidentalis L.	3	90	10	90	6	92	0	
		5	90	12	94	6	94	2	
		7	94	12	96	8	96	4	
Con	trol (without treatment)	3	90	34	90	34	90	34	
		5	90	36	90	36	90	36	
		7	92	40	92	40	92	40	



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