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Incidence of seed-borne fungi of six cultivars of soybean, their pathogenicity test for inducement of damping-off disease and effect of gamma radiation on their incidence and seed germination

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Abstract: Soybean [*Glycine max* (L.) Merrill] is one of the most important oil seed crop. Seed-borne fungi are the major cause of deterioration during storage. Ten genera with 12 species of fungi were isolated from surface-sterilized seeds of six soybean cultivars (Giza 21, Giza 22, Giza 35, Giza 82, Giza 83 and Giza 111). *Aspergillus flavus, Fusarium semitectum* and *F. verticillioides* were isolated from soybean seeds of all tested cultivars with high incidence, while *Eurotium amstelodami* and *Syncephalastrum racemosum* were represented in Giza 22 only with low incidence. Other fungi were isolated with intermediate counts. Cultivars varied in their contents of seed-borne fungi and Giza 35 yielded the lowest content. From sixty-four major isolates tested for their pathogenicity, 61 isolates caused soybean pre- and post-emergence damping-off, while the 3 isolates of *Macrophomina phaseolina* did not significantly affect the percentage of damping-off incidence. Exposure of seeds of soybean to gamma radiation of 5 or 7 K Gray significantly reduced the incidence of all isolated fungi whereas exposure to 2 kg Gray was of no significant effect. All fungi disappeared when seeds were exposed to 7 K Gray except *F. semitectum* and *F. verticillioides* which gave low incidence percentage. Exposure soybean seeds to 5 or 7 K Gray incited a slight decrease in the percentage of seed germination.

Keywords: Soybean, seed-borne fungi, damping-off, gamma radiation, seed germination, *A. flavus*, *Fusarium* spp.

Introduction

Soybean, the "golden bean" is one of the foremost important oil seed crop known for its excellent protein (42-45%), oil (22%) and starch content (21%). It is a good source of vitamin-B complex, thiamine and riboflavin. Soybean protein is rich in valuable amino acids like lysine (5%) in which, most of the cereals are deficient. Its oil is the largest component of the world's oils. Inspite of phenomenal increase in area and soybean production, its productivity remains low because of lack of quality seeds (Venugopal Rao *et al*., 2015). The infected seeds failed to germinate or seedlings and plants developed in the field from infected seeds may escape the early infection but often may be infected at the later stages of the crop growth (Venugopal Rao *et al*., 2015).

Fungi are the major cause of deterioration during storage. Seeds in the field as well as in poor storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively, which is due to chemical breakdown or proteins, oil, and fatty acids by the seed-borne microbes (Welbaum, 2006).

In 2008 Shavan *et al*., isolated different isolates of *Colletotrichum dematium* and *Fusarium oxysporum* from soybean seeds and tested these isolates for their damping-off pathogenicity to soybean. These isolates appeared to be virulent from high to moderate to weak pathogenicity to soybean plants.

Safdar *et al*. (2013) studied the differences between eight soybean cultivars in its contents of seed-borne fungi. Common fungal genera isolated during this study included *Absidia, Aspergillus, Mucor, Curvularia, Drechslera, Fusarium, Phoma, Rhizopus* and *Penicillium. Aspergillus.* Neeti Saxena *et al*. (2015) studied the seed mycoflora of two varieties of soybean collected from fields of Adilabad district of Telangana, India and found that, thirty one fungal species belonging to thirteen different genera were isolated from PK-262 variety and thirty nine fungal species belonging to eighteen genera were isolated from PK-472 variety.

Maity *et al*. (2008) and (2009) reported that gamma radiation at doses of 3 K Gray reduced the level of the genera *Alternaria, Aspergillus, Trichoderma* and *Curvularia*, while Braghini *et al*. (2009) recorded the inhibitory effect of gamma radiation at doses from 5-7 K Gray on *Alternaria alternata*. Ahmed (2010) reported the inhibition effect of gamma at doses from 2.5 to 10 (K Gray) radiation on incidence % of *A. alternata, A. solani, Botrytis* sp., *Cladosporium* sp., *F. oxysporum, F. solani, M. phaseolina* and *R. solani* in seeds of watermelon, melon and squash.

The present work was constructed to cover isolation of seed-borne fungi of different soybean cultivars, test pathogenicity of major isolated fungi for soybean damping-off and determine an appropriate dose range of gamma radiation for soybean seeds to reduce the level of seed-borne fungi.

Materials and Methods

Isolation and identification of seed-borne fungi of surface sterilized seeds of six soybean cultivars:

Seeds of six cultivars of soybean (Giza 21, Giza 22, Giza 35, Giza 82, Giza 83 and Giza 111) were obtained from Crop Research Institute of Agriculture Research Center, Giza, Egypt, from the growing season 2012. Seeds were surface sterilized for 5 minutes in a 2% sodium hypochlorite solution followed by sterile distilled water rinses and dried between sterilized filter papers. Seeds were directly placed in sterilized Petri-dishes containing potato dextrose agar medium complemented with 0.06 mg/ml chloramphenicol (PDAC), ten seeds/plate. Four plates from each cultivar were incubated at room temperature (25±2°C) and observed daily from the fourth day up to 7 days. The fungi that appeared were examined, identified and counted. They were identified using appropriate keys for each genus and confirmed by Assiut University Mycological Center (AUMC). The following references were used for identification of fungal genera and species: Raper and Fennel (1965), Simmons (1967), Rifai (1969), Booth (1971), Sivanesan (1984), Pitt (1985), Moubasher (1993), Barnett and Hunter (1998), Leslie and Summerell (2006), Domsch *et al.* (2007) and Moubasher *et al*. (2011). Frequency percentage incidence of every fungus was calculated as follows:

$$\text{Percentage incidence of fungal species} = \frac{\text{Average counts of each fungal species per plate}}{\text{Average counts of total fungi per plate}} \times 100$$

Pathogenicity test for damping-off disease

Pathogenicity test was carried out under greenhouse conditions in the season of 2013. Pots (25 cm in diameter) were sterilized by immersing in 5% formalin solution for 15 minutes and left for two weeks to get rid of any toxicity. Sixty-four isolates, which were isolated from soybean seeds (Table 1) were inoculated on barley medium to prepare the inocula. Flasks (500 ml) contained 100 g clean barley grains and 100 ml water were mixed and autoclaved at 121°C for 20 minutes. Sterilized barley medium was inoculated with disk (5 mm diameter) taken from 7-days old pure culture from every isolate and incubated at 25±2°C for two weeks. Soil (50% sand and 50% clay w/w) was sterilized by autoclaving at 121°C for 1 hr. then left for 7 days before use. Sterilized pots were filled with sterilized soil at the rate of 4 kg soil/pot and mixed with the previously inoculated barley grains at a rate of 5 g/kg soil (w/w). Pots treated with uninoculated disks of agar media were used as a control.

Seeds of soybean cv. Giza 111 were surface sterilized by 2% sodium hypochlorite for 5 minutes, then washed several times with sterilized water and dried between sterilized filter papers. The sterilized seeds were sown in four replicate pots prepared as mentioned before at the rate of 10 seeds/pot.

Pots were regularly watered under greenhouse conditions. Percentages of pre- and post-emergence damping-off were recorded after 15 and 30 days from planting respectively.

Effect of gamma irradiation on soybean seed-borne fungi incidence and seed germination

Aliquots of one kg of fresh soybean seeds of (cv. Giza 111) with moisture ranged from 9-10% were put in jute bags for irradiation. The seeds were exposed to a ^{60}Co gamma source 2, 5 and 7 K Gray at dose rate of 1 K Gray/18 minutes at 25°C, using Gamma irradiation unit 5500 at Egyptian Nuclear and Radiological Regulatory Authority (ENRRA), Cairo, Egypt. Soybean naturally fresh seeds not exposed to radiation were kept as a control. The seed germination and incidence of seed-borne fungi were determined after radiation.

Results

Isolation of seed-borne fungi from different soybean cultivars

Data in Table (1) show that ten genera with 12 species of fungi were isolated from seeds of six soybean cultivars (Giza 21, Giza 22, Giza 35, Giza 82, Giza 83 and Giza 111).

Seeds of different cultivars varied in their contents of seed-borne fungi. *A. flavus*, *F. semitectum* and *F. verticillioides* were regularly isolated from all cultivars in relatively high percentages. *A. alternata*, *P. purpurogenum*, *T. harzianum* were encountered in 5 out of six cultivars, whereas *A. niger* in 3 cultivars, *Macrophomina phaseolina*, *Pochonia chlamydosporia*, and *C. globosum* in 2 cultivars only.

Pathogenicity test

Sixty-four fungal isolates isolated from soybean seeds (30 isolates of *F. semitectum*, 21 isolates of *F. verticillioides*, 4 isolates of *A. alternata*, 3 isolates of *P. cyhlamydosporia and* 3 isolates of *C. globosum* or *M. phaseolina*) were tested for their pathogenicity for inciting damping-off disease in seedlings of soybean Giza 111 cv.

Data in Table (2) indicate that all tested isolates caused pre-emergence damping-off except the three isolates of *M. phaseolina* and isolate No. 6 of *F. semitectum* which induced insignificant differences. Isolates of *F. semitectum* (No. 7 and 11), *F. verticillioides* (No. 36 and 37), *P. cyhlamydosporia* (No. 57), *A. alternata* (No. 53) and *C. globosum* (No. 60) induced the highest percentages of pre-emergence damping-off. In case of post-emergence damping-off, isolates of *F. semitectum* No. 13, 22 and 30 and isolate of *A. alternata* No. 55 significantly increased the percentages of this stage. The most virulent isolates, causing more than 90% death of seedlings, were the isolates of *F. semitectum* (No. 7, 8, 11, 12, 14, 16, 20 and 29), isolates of *F. verticillioides* (No. 36, 37 and 42), isolate of *A. alternata* No. 53 and isolate of *P. cyhlamydosporia* No. 57. Regularly the percentage incidences of infection in the pre-emergence stage were greater than those of the post-emergence.

Table 1: Percentage incidence of seed-borne fungal species calculated to the total fungi isolated from each of six soybean cultivars.

Culti-var	Isolate	Incidence (%)	Culti-var	Isolate	Incidence (%)
Giza 111	*Fusarium semitectum* Berk. & Rav.	19.00	Giza 82	*F. semitectum*	17.58
	Fusarium verticillioides (Saccardo) Nirenberg	11.57		*F. verticillioides*	12.65
	Pochonia chlamydosporia (Goddard) Zare & W. Gams	2.07		*P. chlamydosporia*	3.12
	Alternaria alternata (Fries) Keissler	3.85		*A. alternata*	14.07
	Chaetomium globosum Kunze	2.07		*C. globosum*	0.00
	Macrophomina phaseolina (Tassi) Goidanch	3.85		*M. phaseolina*	12.58
	Aspergillus flavus Link	24.67		*A. flavus*	16.05
	Aspergillus niger van Tieghem	0.00		*A. niger*	7.89
	Penicillium purpurogenum Stoll	17.20		*P. purpurogenum*	0.00
	Trichoderma harzianum Rifai	15.40		*T. harzianum*	15.95
	Eurotium amstelodami Mangin	0.00		*E. amstelodami*	0.00
	Syncephalastrum racemosum Cohn ex Schroter	0.00		*S. racemosum*	0.00
Giza 21	*F. semitectum*	19.50	Giza 83	*F. semitectum*	15.15
	F. verticillioides	11.85		*F. verticillioides*	15.87
	P. chlamydosporia	3.85		*P. chlamydosporia*	0.00
	A. alternata	18.20		*A. alternata*	8.82
	C. globosum	0.00		*C. globosum*	0.00
	M. phaseolina	0.00		*M. phaseolina*	6.45
	A. flavus	11.15		*A. flavus*	11.81
	A. niger	6.62		*A. niger*	11.15
	P. purpurogenum	19.50		*P. purpurogenum*	19.13
	T. harzianum	9.00		*T. harzianum*	8.55
	E. amstelodami	0.00		*E. amstelodami*	0.00
	S. racemosum	0.00		*S. racemosum*	0.00
Giza 22	*F. semitectum*	18.68	Giza 35	*F. semitectum*	11.99
	F. verticillioides	5.22		*F. verticillioides*	17.85
	P. chlamydosporia	0.00		*P. chlamydosporia*	0.00
	A. alternata	0.00		*A. alternata*	7.96
	C. globosum	0.00		*C. globosum*	9.89
	M. phaseolina	0.00		*M. phaseolina*	11.05
	A. flavus	25.03		*A. flavus*	28.95
	A. niger	15.10		*A. niger*	0.00
	P. purpurogenum	1.77		*P. purpurogenum*	12.11
	T. harzianum	27.01		*T. harzianum*	0.00
	E. amstelodami	2.50		*E. amstelodami*	0.00
	S. racemosum	4.55		*S. racemosum*	0.00

L.S.D. 5% = 8.17%

Table 2: Pathogenicity test of 64 fungal isolates from soybean seeds from six cultivars for the percentage incidence of damping-off of Giza 111 cultivar.

Isolate No.	Isolates	Cultivar Giza	Damping-of (%)		
			Pre	Post	Total
1	*F. semitectum*	111	65.0	10.0	75.0
2	*F. semitectum*		75.0	0.0	75.0
3	*F. semitectum*		52.5	10.0	62.5
4	*F. semitectum*		75.0	10.0	85.0
5	*F. semitectum*		70.0	10.0	80.0
6	*F. semitectum*		20.0	30.0	50.0
7	*F. semitectum*		90.0	2.5	92.5
8	*F. semitectum*		95.0	0.0	95.0
9	*F. semitectum*	82	85.0	2.5	87.5

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Isolate No.	Isolates	Cultivar Giza	Damping-of (%)		
			Pre	Post	Total
10	*F. semitectum*		80.0	2.5	82.15
11	*F. semitectum*		92.5	2.5	95.0
12	*F. semitectum*		82.5	10.0	92.5
13	*F. semitectum*		37.5	17.5	55.0
14	*F. semitectum*		55.0	7.5	62.5
15	*F. semitectum*	83	60.0	12.5	72.5
16	*F. semitectum*		90.0	2.5	92.5
17	*F. semitectum*		80.0	5.0	85.0
18	*F. semitectum*	35	70.0	10.0	80.0
19	*F. semitectum*		75.0	5.0	80.0
20	*F. semitectum*		80.0	15.0	95.0
21	*F. semitectum*		75.0	7.5	82.5
22	*F. semitectum*	22	57.5	22.5	80.0
23	*F. semitectum*		65.0	10.0	75.0
24	*F. semitectum*		62.5	10.0	72.5
25	*F. semitectum*		47.5	12.5	60.0
26	*F. semitectum*		65.0	7.5	72.5
27	*F. semitectum*	21	77.5	7.5	85.0
28	*F. semitectum*		55.0	15.0	70.0
29	*F. semitectum*		85.0	7.5	92.5
30	*F. semitectum*		67.5	22.5	90.0
31	*F. verticillioides*	111	62.5	2.5	65.0
32	*F. verticillioides*		80.0	0.0	80.0
33	*F. verticillioides*		70.0	0.0	70.0
34	*F. verticillioides*		77.5	2.5	80.0
35	*F. verticillioides*	82	77.5	2.5	80.0
36	*F. verticillioides*		90.0	0.0	90.0
37	*F. verticillioides*	83	92.5	0.0	92.5
38	*F. verticillioides*		85.0	2.5	87.5
39	*F. verticillioides*	35	82.5	5.0	87.5
40	*F. verticillioides*		80.0	5.0	85.0
41	*F. verticillioides*		65.0	7.5	72.5
42	*F. verticillioides*		87.5	7.5	95.0
43	*F. verticillioides*		65.0	2.5	67.5
44	*F. verticillioides*		55.0	15.0	70.0
45	*F. verticillioides*		80.0	2.5	82.5
46	*F. verticillioides*		77.5	5.0	82.5
47	*F. verticillioides*	22	77.5	5.0	82.5
48	*F. verticillioides*		52.5	5.0	57.5
49	*F. verticillioides*	21	57.5	15.0	72.5
50	*F. verticillioides*		72.5	0.0	72.5
51	*F. verticillioides*		65.0	12.5	77.5
52	*A alternata*	111	70.0	5.0	75.0
53	*A alternata*	82	92.5	5.0	97.5
54	*A alternata*		77.5	7.5	85.0
55	*A alternata*	21	47.5	27.5	75.0
56	*P. chlamydosporia*	111	82.5	5.0	87.5
57	*P. chlamydosporia*	82	100.0	0.0	100.0
58	*P. chlamydosporia*	21	77.5	5.0	82.5
59	*C. globosum*	111	80.0	0.0	80.0
60	*C. globosum*	35	92.5	5.0	97.5
61	*C. globosum*		85.0	2.5	87.5
62	*M. phaseolina*		27.5	5.0	32.5
63	*M. phaseolina*	83	25.0	7.5	32.5
64	*M. phaseolina*	111	22.5	5.0	27.5
65	Control		25.0	0.0	25.0
L.S.D. 5%			27.58	13.97	

Effect of gamma radiation on incidence of soybean seed-borne fungi and seed germination in Giza 111 cultivar

Data in Table (3) show that the doses of 5 and 7 K Gray reduced significantly the percentage incidence of all tested fungi (*F. semitectum, F. verticillioides, A. alternata, M. phaseolina, A. niger, A. flavus, P. purpurogenum* and *Cladosporium sp.).* Exposure of seeds to 2 K Gray did not significantly affect incidence of *F. semitectum, F. verticillioides* and *M. phaseolina* while it significantly decreased the incidence of *A. alternata, A. niger, A. flavus* and *P. purpurogenum*. All tested fungi disappeared when seeds were exposed to 7 K Gray except *F. semitectum* and *F. verticillioides* which showed significantly low percentage incidence.

Data in Table (4) indicate that exposure soybean seeds to 2 K Gray did not significantly affect the percentage of seed germination while exposure to 5 or 7 K Gray incited a slight significant decrease in percentage of seed germination.

Table 3: Effect of gamma radiation on percentage incidence of soybean seed-borne fungi calculated to 40 seeds in each treatment.

Isolates	**Seed-borne fungi incidence %**			
	Control	**Gamma dose / K Gray**		
		2	**5**	**7**
F. semitectum	40	35	15	5
F. verticillioides	35	25	10	5
A. alternata	30	15	5	0
M. phaseolina	15	5	0	0
A. niger	45	10	5	0
A. flavus	45	15	10	0
P. purpurogenum	25	10	5	0
Cladosporium sp.	10	0	0	0

L.S.D 5% = 15.10%

Table 4: Effect of gamma radiation on soybean seed germination percentage.

Gamma dose/k Gray	Seed germination (%)
2	90
5	85
7	82
0.0	95

L.S.D. 5% = 7.2

Discussion

Seed-borne fungi cause a world problem for soybean seed quality and seed germination. Venugopal Rao *et al.* (2015) reported that in spite of phenomenal increase in area and soybean production, its productivity remains low because of lack of quality seeds. They added that the infected seeds failed to germinate or seedlings of plants developed in the field may escape the early infection but often may be infected at the later stages of the crop growth.

A. flavus, F. semitectum and *F. verticillioides* were regularly isolated from soybean seeds of all tested cultivars with high incidences while other species were encountered in 5, 3 or 2 cultivars. Cultivars varied in their contents of seed-borne fungi and Giza 35 gave the lowest incidence. Alemu (2014), Dwivedi and Gopal (2014), Patharkar and Hedawoo (2014) and Venugopal Rao *et al.* (2015) revealed the presence of *A. flavus, A. niger* and *Fusarium* sp., *Alternaria* sp. and *Macrophomina phaseolina* in soybean seeds. Differences between soybean cultivars in their contents of seed-borne fungi were also reported by Safdar *et al.* (2013) who tested eight cultivars and Neeti Saxena *et al.* (2015) who tested two cultivars.

Sixty-four of major fungal isolates isolated from soybean seeds were tested for their pathogenicity for inciting damping-off disease on seeds of soybean Giza 111 cv. All tested isolates caused pre-emergence damping-off except the three isolates of *M. phaseolina* and isolate of *F. semitectum* No. 6. The pathogenic isolates varied for their virulence from high, moderate to weak rates of soybean damping-off. Percentages of pre-emergence damping-off stage were consistently greater than those of post-emergence damping-off stage. Shovan *et al.* (2008) tested 33 isolates of *F. oxysporum* from soybean seeds, among them, 3 isolates appeared to be highly virulent, 7 isolates were virulent, 13 isolates moderate virulent and 10 isolates weakly virulent. This confirms the important role of soybean seed-borne fungi in causing pre- and post-emergence damping-off. Pathowska and Marzana (2013) found that soybean was mainly infected by *F. culmorum* and *F. oxysporum*.

Exposure of soybean seeds to gamma radiation of 2 K Gray reduced, but mostly insignificantly, the incidence of all isolated fungi (*F. semitectum, F. verticillioides, A. alternata, M. phaseolina, A. niger, A. flavus, P. purpurogenum* and *Cladosporium* sp.). Exposure to 5 K Gray or 7 K Gray reduced significantly the incidence of all isolated fungi. All fungi disappeared when seeds were exposed to 7 K Gray except *F. semitectum* and *F. verticillioides* which exhibited significantly low incidence percentage. On the other hand, soybean seed germination was not significantly affected by exposing seeds to 2 K Gray while 5 or 7 K Gray dose induced slight decrease in percentage of seed germination.

These data confirm the findings of Maity *et al.* (2004), Ferreira *et al.* (2007), Maity *et al.* (2008), Maity *et al.* (2009) and Ahmed (2010) who reported that exposing seeds of different crops to Gamma radiation decreased percentage of seed-borne fungi incidence. Gamma radiation at proper dose could be a useful process for the preservation of seed against the deterioration by seed-borne fungi in storage and protection against diseases they incite.

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