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STUDIES ON SEED-BORNE FUNGI IN SOYBEAN AND THEIR CONTROL
1- DISCOLORATION OF SOYBEAN SEED BY *FUSARIUM* SPP., QUALITY LOSSES AND PRODUCTION OF TOXINS.

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Abstract

Species of *Fusarium* and other fungi were isolated from soybean seeds, causing complete discoloration of seed coat; a reddish discoloration (including both dark and light red). There was no evidence that the infections by *Phomopsis* sp. or *Fusarium* spp. were preceded by *Peronospora manshurica. Fusarium oxysporum, F.accuminatum, F.graminearum* and *Phomopsis* spp. These fungi were the most predominant pathogens isolated from both dark or lightly discolored seeds, in surface disinfested or non disinfested seeds.

Both deoxynivalenol (DON) and HT-2 toxins were present in discolored seeds. The extensively discolored red seeds contained the largest amount of the toxin, with the highest concentration localized in the seed coats.

Germination of the reddish discolored seeds and iodine number were decreased. There was an increase in the free fatty acids contents, and a little change in total oil and protein content, while only meal color was deleteriously affected.

INTRODUCTION

Discoloration of soybean seed (*Glycine max* (L.) merrill), is a fungal disease caused by several species of fungi (Anderson, 1985 and Russin *et al.*, 1988). These fungi include *Cercospora soja, C.Kikuckii, Peronospora manshurica, Phomopsis* spp. and *Fusarium* spp. produce reddish discolorations of mature soybean seeds (El-Gantiry, 1985; Wicklow *et al.*, 1987 and Clear *et al.* 1989). These fungi also produce the mycotoxins deoxynivalenol (DON) and Zearalenone. External discolorations

reduce the aesthetic appeal of the beans and thereby their marketability (Clear *et al.*, 1989).

Soybean seed discoloration by fungal infections can lower the quality of oil and flour (Hepperly and Sinclair, 1987) and this invasion may be accompanied or followed by a decrease in germination and an increase in fat acidity value (Dorworth and Christensen, 1968).

The purpose of this paper is to describe the relationship between discoloration of soybean seeds and pathogenic fungal populations, seed germination, toxin production and seed quality.

MATERIALS AND METHODS

Sample collection :

Soybean seed samples were collected from different regions of Assiut and Sohag Governorates during 1996 growing season. The seed samples were grouped according to their color into three categories; a) dark red discoloraion b) light red discoloration c) not discolored (normal) Fig. (1).

Determinations of seed-borne pathogens:

Half of the examined seeds were surface sterilized with 2.0% sodium hypochlorite for 2 min., rinsed in steriled distilled water then dried between two filter papers. The second half of the seeds were left without sterilization. Non surface disinfcted seeds were then placed in sterilized plates containing potato-dextrase agar (PDA) medium acidified with lactic acid (pH. 4.7). Five seeds were placed in each plate (9cm in diameter). The plates were incubated at 19-22^{o}C under fluorescent light (16 hrs. light and 8 hrs. dark). According to ISTA (1976), then 400 seeds from each treatment (categories) were examined. The developed fungal colonies were purified and then identified according to their cultural characteristics (Nelson *et al.*, 1983; Russin *et al.*, 1988 and Clear *et al.*, 1989).

Germination tests:

Germination percentage was determined by placing 100 seeds from each treatment into Pertri dish containing moist paper towels. The plates were incubated at 25^{o}C for 6-7 days. Any seed that produced a hypocotyl was counted as germinated.

Table 1. Fungi isolated from surface and nonsurface disinfested Clark soybean seed.

Fungi	Seed color/fregency (%) of the isolated fungi		
	Normal	Light red	Dark red
Surface-disinfested seeds			
Fusarium accuminatum	2	20	48
F.equiseti	-	2	6
F.graminearun	1	10	22
F.moniliforme	-	18	14
F.oxysporum	1	30	84
F.solani	2	4	8
F.semitectum	-	2	4
Phomopsis sp.	1	6	20
Rhizoctonia solani	-	8	4
Alternaria alternata	-	2	6
Curvularia spp.	-	-	2
Non-surface-disinfeted seeds			
Mucor hemalis	1	4	4
Aspergillus flavus	-	2	6
A.niger	2	8	8
Nigrospora spherica	-	1	2
Penicillium spp.	4	2	10
Phomopsis sp.	2	8	18
Rhizopus spp.	4	1	4
Verticillium spp.	-	2	1
Alternaria tenuis	1	6	12
Fusarium oxysporum	4	26	80
F.equiseti	-	10	4
F.accuminatum	6	24	54
F.solani	2	4	6
F.graminearum	1	9	18
F.moniliforme	-	6	2

Mycotoxin analysis:

Seed sample (normal, dark and light red) were screened for the presence of toxin at levels equal to or above 0.05 Ug/ml. Extraction procedures reported by Ware *et al.*, (1986) and toxin estimation described by Nowicki *et al.*, (1988) were adopted.

Quality tests:

Protein content in the examined seed samples was determined using the method described by Davidson (1980), oil content was estimated according to Walker (1987), fatty acid value was calculated according to Daun *et al.*, (1983) and meal color differences were detected as reportad by Daun (1978).

RESULTS

Nineteen fungal species, frequently associated with discolored soybean seed samples, were screened and presented in (Table 1). *Fusarium oxysporum* and *F.accuminatum* were the most frequently isolated fungi followed by *F.graminearum* and *Phomopsis* sp. Nevertheless, *Curvularia* spp., *Nigrospora spherica* and *Verticillium* spp. were the least isolated fungi from surface and nonsurface sterilized discolord seeds. In two samples, 80% or more of the dark red discolored seeds were associated with *F.oxysporum*, and between 48% and 54% by *F.accuminatum*. In the light reddish discolored seeds, between 26% and 30% were infected by F.oxysporum and 20% to 24% by *F.accuminatum*. Percentage of fungal isolation were greater in the dark red seeds. Saprophytic fungi were found only in the nonsurface sterilized seeds. Oospores of *Peronospora manshurica* were present on less than 1.6% of the examined seeds.

Results of germination of the discolored soybean seeds are presented in (Table 2). Percent seed germination of both types of discolored seeds obviously decreased in comparison with the normal seeds (control), and this reduction was most pronounced in the dark red seeds. In all samples, decreased germinability was preceded by invasion with *Fusarium oxysporum* and certain other fungi.

Abnormal seedlings were generally covered with fungal growth. Seedlings which escaped from seed decay frequently developed necrotic lesion on the cotyledons and occasionally the terminal bud or root become infected, resulting in eventual death of the seedlings.

Table 2. Germination of colored soybean seeds when incubatedat 25oC for 6-7 days.

Discolored seed	Germination[a)]%	Reisolation[b]			
		A	B	C	D
Light red seeds	50	-	+	+	-
Dark red seeds	32	+	+	+	-
Non reddish seeds (normal)	96	-	-	-	-
L.S.D. at 5%	12.7				

a) Average of 100 seeds.

b) Presence (+) or absence (-) of the fungus (*F.oxysporum* and *Fusarium* spp.) in surface disinfected host tissue pieces excised from : A, tap root apex; B, hypocotyl; c, lower stem; and D, shoot apex.

Toxin analysis of soybean seeds is presented in Table (3). Unlike normal seeds, discolored soybean seeds contained different levels of DON and HT-2 toxins. However, lower concentration of HT-2 or DON toxins was observed in the light red seeds, where traces of HT-2 were found only in the light reddish seeds.

Table 3. Levels (ug/ml.) of the mycotoxins in discolored soybean seed.

Discolored seed	Toxins detected ug/ml	
	DON[a]	HT-2
Light red seeds	0.18	trace
Dark red seeds	1.09	0.23
normal (Control)	ND[b]	ND

a) Deoxynivalenol (DON).

b) ND = not detected at a level > 0.05 ug/ml.

The greatest concentration of DON and HT-2 toxins was located in the seed coats of the reddish soybean seeds, with the greatest weight of toxin remaining in the meal (Table 4).

Table 4. Levels (ug/ml.) of the mycotoxins deoxynivalenol (DON) and HT-2 and their distribution in soybean seeds with reddish discolored seed coats[a)]

Toxin	Seed coat only	Remaining portion of seed
DON	6.12	3.01
HT-2	0.70	0.16

a) only seeds which had more than 50% of the seed coat discolored red were used. Total weight of seed coat fraction 4.28g; total weight of remaining portion of seed 13.64 g.

Studies on biochemical aspects of soybean seeds discoloration data are presented in Table (5). Data clearly show high reduction in the iodine number and appreciable increase in free fatty acids of the oil. No differences were observed in protein content, total oil, color between the discolored sample groups, but there was a reduction when compared with normal seeds (control). Most changes were more obvious in case of dark red color.

Meal color differed among samples, as indicated by dominant wavelength and purity, whether or not the seed coats had been removed. The dominant wavelength varied from 550 nM for the dark red discolored seeds to 562 nM for the light red discolored and 594 nM for nondiscolored ones. Percent of purity (Daun, 1978) ranged from 0.9% for dark red to 3.4% for light red color seeds and 8.8% for the normal seeds.

Table 5. Effect of soybean seed discoloration on protein, oil content and other chemical properies of the oil produced.

Discolored seed	Protein as percent initial dry weight	Total oil as % of initial dry weight	Free fatty acids as % oleic acid	Iodine number
Light red seeds	37.4[a)]	17.6a)	47.64[a)]	126.3
Dark red seeds	36.6[a)]	16.6	54.70	105.3
Normal seeds (control)	40.2	20.0	30.54	137.5
L.S.D. at 5%	2.9	1.4	5.4	10.1

a) Each value is the average of 3 replicates.

DISCUSSION

Seed infections by seed-borne pathogens, reduce the quality and value of seed and have been shown to reduce germination and seedling emergence. The ability of infected seed to germinate may be related to threshold level of the associated fungi (internally and / or externally) as reported by Kilpatrick (1957) and Anderson (1985).

Seeds infected with specific pathogen(s) which produce toxin may affect the health of human and/or animal consumers (Marquardt, 1983). Infected seeds can be classified according to their appearance, shape, size, shrivelling, deformation of the testa, etc. The larger size of the more discolored seeds is difficult to explain, but may be due to physiological factors, location and cultivar differences or to mixing of large-seeded soybean samples, which were greatly discolored, with smaller-seeded samples, which were relatively clean (Spaeth and Sinclair, 1984 and Tu *et al.*, 1988).

In the present investigation, the count of the fungi recovered from soybean seeds reached nineteen different species. This considerable number of the isolated fungi was probably due to the high moisture content and nutritional aspects of soybean seeds which are more liable to be colonized by fungi (Tu, 1982).

Under harvest conditions, it is common to find elevated levels of *Fusarium* and *Phomopsis* on soybean seeds (Wilcox *et al.*, 1974 and Nedrow and Harman, 1980). Excessive fungal growth in the field can result in red discolorations of the soybean seeds. *F.graminearum* was reported for the first time in 1986 on the USA crop (Wicklow *et al.*, 1987), and in the same year it was seen in Canadian harvest. Clear *et al.* (1989) found that discolored soybean seeds (light and deep red color) tend to harbour most of the isolated fungi. *Fusarium* spp., and *F.graminearum* were the most frequently isolated fungi. This is possibly due to that both fungi attack soybean in the field and during storage, while shrivelling and discoloring of the seeds is caused by *Phomopsis* spp. (Hepperly and Sinclair, 1978 and Martens *et al.*, 1984). Although *F.graminearum* was described by Wilcox *et al.*, (1974) as a secondary coloniser of seeds already infected by *Peronsopora monshurica*, the virtual absence of oospore in encrusted seeds in our samples indicates that invasion and discoloration of mature soybean seeds by *Fusarium* spp. does not require primary colonization by *Peronsopora manshurica*.

Beside the reddish discoloration, heavy invasion by *Fusarium oxysporum* and/or other fungi caused a decrease in germination of soybean seeds (Nedrow and Harman, 1980). As The percentage of seeds infected with seed-borne fungi increased the germination decreased and the percentage of abnormal seedlings and ungerminated seeds decayed and colonized by fungi increased. This appeared to contribute to the reduction in germination of soybean seeds (Christensen, 1972 and Kabeere and Taligoola 1983).

In the red dscolored seeds, *Fusarium* spp. and *F.graminearum* most likely produced DON toxin, as this species is a well known producer of DON in the field. It was reported to contaminate some of the fields as reported from the USA in 1986 by Wicklow *et al.*, (1987).

Growth of the associated fungi on soybean seeds caused marked changes in color of the seed testa. These changes depended upon the fungal species; however, the deep dark or light red were the most detected colors of infected soybean seeds. These results confirmed those obtained by Clear *et al.*, (1989), who found that HT-2 and DON were detected in reddish discolored seeds of soybean. DON, another toxin produced by *F.graminearum*, was also reported from the USA and Egypt beans (Wicklow *et al.*, 1987., Tu, 1982 and Arafa *et al.*, 1996). The HT-2 toxin was produced by *Fusarium* spp and *F.sporotrichioides* (Joffe, 1986 and Marasas *et al.*, 1984). However, the highest concentrations of HT-2 were found in the red seeds, whereas *Fusarium oxysporum, F.accuminatum, F.graminearum* and other fungi were often observed in the red discolored seed fractions.

Seed-borne fungi associated with soybean seeds caused changes in color of the seed coat and quality of oil and flour. These changes depend upon the fungal species, moisture contents of seeds, high temperatures and humidity during seed maturation (Wilcox *et al.*, 1985 and Clear *et al.*, 1989). Early work by Milner (1950) reported that high levels of fungi on soybean seeds caused drop in iodine number, increase in fatty acids and little changes in total oil. Others have found that high levels of *Phomopsis* spp., *Fusarium* spp and *F.graminearum* infections can lower the quality of oil and flour (Hepperly and Sinclair, 1978).

Finally, deformation of soybean seed testa may be considered as an indicator for the presence of plant pathogens. Discolored seed were poor in germination, seedling emergence and may contain some toxic substances. Therefore, deformed seeds should be discarded from any seed lots to be sown or use for human and animal diet.

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دراسات على الفطريات المصاحبة لبذرة فول الصويا وطرق مقاومتها
١ - تلون بذور فول الصويا بأنواع فطر الفيوزاريوم وتأثيرها على الجودة، وإنتاج السموم

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عزلت من بذور فول الصويا أنواع من فطر الفيوزاريوم وأنواع لفطريات أخرى مصاحبة لتلون غلاف البذره. منتجة لونا أحمر وهو اما خفيف أو قاتم اللون.

الاصابة بأنواع من فطرى الفيوزاريوم وفومبسس غير واضح فى كونها مسبوقة بالإصابة بالفطر بيرونوسبورا منشوريكا.

الفطريات الأكثر تكرارا وسياده فى العزل من البذور الملونة (أحمر خفيف أو قاتم) سواء كانت هذه البذور معقمه سطحيا أو غير معقمة هى *F.accuminatum, F.oxysporum Phomopsis, F.graminearum*.

أحتوت البذور الملونة على السموم DON, HT-2 وكان تركيزها أعلى فى البذور ذات اللون الأحمر القاتم، أما أغلفة البذور الملونة فإنها أحتوت على أعلى تركيز من هذه السموم.

تأثرت جودة بذور فول الصويا بتلونها حيث قلت نسبة الإنبات، الرقم اليودى بينما زاد محتواها من الأحماض الدهنية الحرة، كما حدث نقص فى كمية المحتوى الزيتى والمحتوى البروتينى، وصفات الدقيق.