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Legume Seed Deterioration Caused by Some Mould Fungi Affecting Seed Quality

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ABSTRACT

Fabaceae, is one of the most important source of plant protein for animal, poultry and human consumption in all over the world. Many fungi are serious parasites of legume in maturing and stored seeds and their invasion have resulted various damage**.** *In vitro*, seed health testing resulted that**,** a total of 11 fungal species belonging to 8 genera namely *Alternaria alternata, Aspergillus flavus, A. niger, A. parasiticus, Aspergillus terreus, Epicocum* sp., *Fusarium* spp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. were isolated and identified from tested beans (*Phaseolus vulgaris* L.), Lupine (*Lupinus termis* L.), pea (*Pisum sativum* L.) and soybean (*Glycine max* L.) seed samples. Toxic effect of filtrates of these fungi has different degrees of deterioration including decreases viability of seeds (germinability), protein, lipids, carbohydrates and energy value than control. Also, the obtained results revealed that legume seed contaminated samples by *Aspergillus flavus, A. parasiticus* and *A. terreus* had lower protein, lipid, total carbohydrates, available carbohydrates and energy value than un-contaminated samples. Infected legume meals were darker in color. Data of Hunter instrument indicated that contaminated legume seed meal samples had lower "L" value and increased the rate of total color differences (**Δ**E) compared with control (Healthy). The highest color differences were recorded for bean contaminated with *A. terreus*. On the other hand, the results presented that crude oil extracted from contaminated soybean had higher iodine value, peroxide value, moisture content, acidity and saponification value compared with Healthy seeds.

Key words: Legume seed, deterioration, fungi, germination, mycotoxin, quality

Introduction

Over 800 million people having no access to adequate food and about two billion faced with hunger and malnutrition (Oladipo *et al.,* 2015). A legume (Fabaceae) is one of the most important sources of plant protein for human feeding, animal, and poultry in all over the world. Legumes are generally reliable sources of slow release carbohydrates and are rich in proteins.

Many fungi are serious parasites of legume which might cause severe infection for legume seeds under both of field and storing conditions leading to various damages including, seed yield reduce quantitatively and qualitatively, discolorations, decrease seed germination, mycotoxin production and total decay. Species of *Aspergillus, Penicillium* and *Fusarium* are responsible for most spoilage and seed damage during storage. They cause reduction in cooking or baking quality, and nutritive values, produce undesirable odors and color, and change appearance of stored food grade grains (Castillo *et al.,* 2004; Quenton *et al.,* 2003).

There are many saprophytic and pathogenic fungi commonly isolated from seeds. These include the mainly saprophytic genera *Mucor, Rhizopus, Trichoderma, Cladosporium, Penicillium, Chaetomium* and *Aspergillus* as well as the mainly pathogenic genera *Pythium* and *Alternaria. Fusarium, Acremonium* and *Phoma* (Schafer & Kotanen, 2004). Five fungal genera *i.e. Alternaria, Aspergillus, Epicoccum, Fusarium* and *Trichoderma* were isolated from some legume seeds as beans, cowpea, and lupine (Embaby & Mona, 2006).

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Compositional changes and nutritive values of some legume seeds due to infection with fungi were studied by several investigators (Embaby & Abdel-Galil, 2006; Morkinas *et al.,* 2005; Aziz & Mahrous, 2004). On the other hand, Embaby *et al.,* (2013) reported that *A. parasiticus* and *Fusarium moniliform* were found to be decrease the biochemical components (protein, fat, carbohydrates and ash contents) of the tested legume seeds *i.e.* bean, pea and soybean. Furthermore, moisture was found to be a causative factor in fungal infection compared to the healthy seeds. Mubashir *et al.,* (2012) mentioned that there was reduction in oil quantity, color and odor in case of infested three varieties of mustard i. e. Basanti, Kalasona and Kaveri AK-47 cvs., as compared to healthy seeds, in case of variety Basanti the color in controlled conditions was light brown while in infested oil by *Aspergillus flavus* was light yellow, in Kalasona variety it was yellow in controlled condition while it was slightly yellow color in infested oil however in Kaveri AK-47 the color changed from brown to red.

This study was conducted to: 1- isolation and identification of some legume seed-borne fungi *i.e.* beans, Lupine, pea and soybean seeds, 2- investigate the changes in some chemical and physical properties of tested seeds caused by some mould fungi, and 3- investigate the changes in physicochemical properties of crude oil extracted from soybean seeds that infected with mould fungi were studies.

Materials and Methods

Collection of seed samples:

The method described by Neergaard (1997) has been adopted for the collection of seed samples during 2014/2015 season. Accordingly, four random samples of seeds (each of half Kg) were collected *i.e.* beans (*Phaseolus vulguris* L.), lupine (*Lupinus termis* L.), pea (*Pisum sativum* L.) and soybean (*Glycine max* L.) from local super market in Egypt. The seed samples were brought to Plant Pathology Laboratory. The samples were enclosed in paper bags with proper labeling and kept in the refrigerator at 5 ± 1°C until used for subsequent studies (Ahmed *et al.,* 2013).

Detection of Seed Microflora:

Seed microflora were isolated by using different methods such as standard blotter paper method and agar plate method as recommended by Mathur & Olga, (2003); Neergaard, (1997) and ISTA, (1993). Seed samples were divided into two groups; the first group was disinfected with sodium hypochlorite solution (1%) for 2 min, while the second group was untreated (non-disinfected). All seed samples were washed several times by sterilized water (SW), then dried between two sterilized filter papers and plated on potato dextrose agar (PDA test medium) as well as on sterilized filter papers with enough moisture (blotter test) in sterilized Petri dishes. Five seeds/dish and three dishes were used as replicates for each treatment as described by Kumlachew, (2014); Mathur & Olga, (2003); El Nagerabi & El-Shafie, (2000); Neergaard, (1997) and Agarwal & Sinclair, (1993). All dishes were incubated for 5-7 days at 25 ±2°C. All fungal growths were transferred and purified using hyphal tip and/or single spore techniques onto PDA medium in the presence of antibiotic (traces of Streptomycin).

Identification of fungi:

Developing fungi were cultured on PDA slants (5-7 days old) then identified in Plant Pathology Department, National Research Centre (NRC), El-Dokki, Egypt based on growth morphology of colony and microscopic characters and the available of literature according to Maren & Johan, (1988) and Raper & Funel, (1965) for *Aspergillus* spp.; Nelson *et al.*, (1983) and Booth, (1977) for *Fusarium* spp.; Barent & Hunter (1977) for the genera of imperfect fungi; *Aspergillus* spp. and *Penicillium* spp. according to Pitt & Hocking(1997).

Data Collected:

Frequency percent of the isolated fungi were calculated according to: the number of isolates of a genus or species/total number of fungal isolates x 100 according to Castillo *et al.* (2004) and Kumlachew, (2014). Type and frequency of occurrence of identified fungal species was recorded according to Kumlachew, (2014). Frequency of occurrence of a pathogen was computed by dividing occurrence of the individual pathogen to the total population. Percent of seed infection is determined as the proportion of legume seed showing any symptom of infection and calculated as:
Infection (%) = Number of infected seeds/total of tested seeds x 100.

Germination capacity test:

Germination percentage was calculated using the following formula by Kumlachew, (2014) and Monira *et al.,* (2012).
Germination % = Number of germinated seeds/total of tested seeds x 100

Measuring the inhibitory effect on seed germination:

Preparation of culture filtrates and performance of germination assays

Two discs of mycelial agar plugs (5 mm diameter) from the margin of 7 days old culture of each isolate were propagated as pure culture into 100 mL autoclaved SMKY broth (Sucrose 200 g, $MgSO_4$ $7H_2O$ 0.5 g, KNO_3 3 g, yeast extract 7 g/l) in 250 mL conical flasks and incubated at 25±2°C for 14 days. After this period the mycelia mat was removed by filtering through a Whatman filter paper No.1 under clean conditions (Deepavali, and Nilima 2012& 2015; and Celar and Valic, 2012).

Toxic effect of culture filtrates on germination seeds

The filtered culture filtrate was used as crude aflatoxin. Each filtrate was collected and a concentration of 50 and 100% of *A. flavus* and *A. parasiticus* filtrate was used. A sterile blotting paper in a Petri dish was then appropriately moistened with the culture filtrate. Seeds of tested legumes were soaked in crude aflatoxin extracted from culture filtrate of *A. flavus* and *A. parasiticus* for 5 min. In the control treatment sterile distilled water was used.

Seed germination test:

The treated and non-treated seeds were sown (5 seeds/plate) into Petri plates containing filter paper with thin layer of sterile cotton soaked with sterile distilled water then sealed with parafilm and incubated in a growth chamber at 25±2°C with 80% relative humidity. The experiments were carried out in three replications. After standardized incubation periods, toxic effect of culture filtrates on germination seeds were measured by calculated the number of germinated seeds (= Number of germinated seeds/total of tested seeds x 100) (Deepavali, and Nilima 2012 & 2015 and Celar, and Valic, 2012).

Preparation of legume meals:

Contaminated legume seeds were dried in an air-drying oven at 60^{o}C for 48 hr. Both of contaminated and non- contaminated legume seeds were ground to get homogenous fine powder using laboratory mill. All samples were stored in air tight container at 5±2°C until used.

Chemical composition of legume meals:

Legume meal samples were analyzed for Protein, fat, ash, crude fiber and moisture contents according to AACC, (2000) methods.

Crude Protein (%):

Buchi AutoKjeldahl-370 (B 811, Switzerland) was used for nitrogen determination according to the instructions given in the manual (AACC, 2000). One-gram sample was digested with 20 mL concentrated sulphuric acid in the presence of catalysts (selenium and potassium sulphate), converting nitrogen of sample into ammonium sulfate, which was then distilled into 4 % boric acid solution by boiling with 32 percent sodium hydroxide, converting it into ammonium hydroxide. The titrant (0.25 Normal sulphuric acid) was used against the amount of the ammonium hydroxide present in the distillate. Results were recorded as percent nitrogen or percent protein(nitrogen x protein factor).

Fat (%):

Crude fat from samples was extracted by adopting AACC, (2000) method No. 30-20 using soxhlet extractor. Five grams flour was placed in cellulose thimbles, fitted into the extractor. The crude fat was extracted with hexane. The extracted fat in cups was weighed and calculated as percent fat.

$$\text{Crude fat Percent} = \frac{\text{Weight of extracted fat}}{\text{Weight of sample}} \times 100$$

Ash (%):

Ash content was determined by AACC, (2000). Method No. 08-01.In a dried and pre- weighed crucible 3g sample was ignited in muffle furnace (Carbolite 1100, USA) at 550°C overnight to complete burning of all organic matter. Remaining 36 matter was cooled, weighed and calculated as percent ash or minerals.

$$\text{Ash Percent} = \frac{\text{Weight of residue}}{\text{Weight of Sample}} \times 100$$

Crude Fiber (%):

Crude fiber was determined according to AACC, (2000) method No.926.09. One g sample was digested with 100 mL of 1.25 percent sulphuric acid in a beaker under reflux for 30 minutes and then filtered through sintered glass crucible under vacuum. The residue was then washed with hot distilled water till neutralized. The washed material was again transferred to beaker and refluxed for 30 minutes with 100 mL of 1.25 percent Sodium hydroxide. Digested material was again filtered and washed with hot water until neutralized. The washed material was dried at 130ºC for1 hour, cooled in a desiccators and weighed. The dried residue was ignited for 6 hours and reweighed the crucible with burnt material (ash). Crude fiber was calculated by using the following formula :

$$\text{Crude Fiber Percent} = \frac{A - B}{\text{Weight of Sample}} \times 100$$

Where, A = weight of crucible and residue; B = weight of crucible and ash

Total carbohydrates (%):

Total carbohydrates (%) were calculated from the equation described by Samati and Rajagopal (1996) as follow :

$$\text{Total Carbohydrates (\%)} = 100 - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\text{crude fiber})$$

Energy value:

Caloric value was calculated according to the following equation (FAO/WHO, 1974) **:**

$$\text{Energy value} = 4\ (\%\ \text{protein} + \text{carbohydrates}\ \%) + 9\ \text{fat}\ (\%)$$

Moisture (%):

Moisture was determined by AACC, (2000) method No. 44-19. Two grams flour was placed in preheated and weighed metallic dish and dried in a hot air oven at 130°C for 2 hours or till constant weight. The loss in weight was calculated as percentage of moisture content (percent MC) of flour.

$$\text{MC Percent} = \frac{W1 - W2}{\text{Weight of Sample}} \times 100$$

Where, MC= Moisture content; W_1= Weight of sample and metal dish before heating; W_2= Weight of sample and metal dish after heating**.**

Changes in physical properties of legume meals:

Color quality:

Color quality of Non-contaminated (control) and contaminated samples were measured using a spectrocolorimeter with the CIE color scale (Hunter, Lab scan XE - Reston VA, USA). This instrument was standardized against the white tile of Hunter Lab Color standard (Lx No, 16379): X= 77.26, Y= 81.94 and Z= 88.14.The L, a and b values according to Hunter, (1975). Total color difference (ΔE) was calculated as :

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$

Where, ΔE= Total color difference; L= Lightness; a=Redness; b=Yellowness

Physical properties of soybean oil

Oil extraction:

Contaminated and non- contaminated soybean meal samples were fed into a soxhlet extractor with petroleum ether (60 - 80°C) as a solvent. The solvent was then distilled off under vacuum at 45 °C in a rotary evaporator (Attah and Ibenesi, 1990).

Changes in physicochemical properties of soybean oil:

The refractive indexes (RI), Iodine value (g/kg), saponification value (mg/kg oil), peroxide value (mg/kg oil), acidity (%) and moisture content (%) of soybean oil extracted from contaminated and non- contaminated soybean seed samples were determined according to AOAC., (2005).

Result and Discussion

Microorganisms associated with legume seed samples:

Isolation from legume seeds yielded 624 isolates, out of them 543 fungal isolates and 81 bacterial isolates. Fungal isolates gave higher frequency of contaminated seed samples (87.02%) than bacterial isolates (12.98%). The percentages of associated fungi were recorded in Table 1.

Fungal frequency:

Isolation of mycoflora associated legume seed samples indicated that, eight fungal genera were isolated and identified as, *Alternaria* 8.65%*, Aspergillus* (*A. niger* 5.60%*, A. flavus* 15.22%*, A. parasiticus* 34.93%*,* and *A. terreus* 4.96%)*, Epicocum* sp. 0.16%, *Fusarium* sp. 8.49%*, Mucor* sp. 0.32%*, Penicillium* spp. 8.01%, *Rhizopus stolonifer* 0.16% and *Trichoderma* sp. 0.48% . Also, data presented that, Soybean seed gave higher frequency occurrences 269 equal 43.10% followed by beans 29.96%**,** pea 16.50%, but lupine was less fungal frequency which recorded 10.41%. El-Nagerabi and El-Shafie, (2000) stated that, the genus *Aspergillus* (10 species, 8 varieties) was the most common,

followed by *Rhizopus* (1 species), *Fusarium* (6 species) and *Alternaria* (5 species) while the remaining genera (*Chaetomium, Cladosporium, Curvularia, Drechslera, Penicillium, Phoma, Emericella, Mucor, Sclerotium, Ulocladium*) displayed lower levels of contamination of Lupine (*Lupinus termis*) seeds by using PDA and blotter methods**.** Domijan *et al.* (2005) reported that, the most common seed borne fungi on dry beans (*P. vulgaries*) were *Alternaria* spp. *Aspergillus* spp., *Fusarium* spp., *Botrytis* spp., *Chaetomium* spp., *Penicillium* spp., *Rhizopus* spp., *Cladosporium* spp. and *Trichothecium* spp. These fungi are transmitted by seeds and can be preserved as conidia in the coat or as mycelia at the seeds surface**.**

Table1: Frequency of some legume seed-borne microorganisms

Microorganism		Tested legume seed samples				Total
		Beans	Lupine	Pea	Soybean	
Alternaria alternata	T.C*	20.00	1.00	12.00	21.00	54.00
	%	3.20	0.16	1.92	3.36	8.65
Aspergillus niger	T.C	13.00	3.00	3.00	16.00	35.00
	%	2.08	0.48	0.48	2.56	5.60
Aspergillus flavus	T.C	24.00	8.00	28.00	35.00	95.00
	%	3.84	1.28	4.48	5.60	15.22
Aspergillus parasiticus	T.C	62.00	41.00	35.00	80.00	218
	%	9.93	6.57	5.60	12.82	34.93
Aspergillus terrus	T.C	30.00	0.00	0.00	1.00	31.00
	%	4.80	0.00	0.00	0.16	4.96
Epicocum sp.	T.C	1.00	0.00	0.00	0.00	1.00
	%	0.16	0.00	0.00	0.00	0.16
Fusarium sp.	T.C	15.00	3.00	18.00	17.00	53.00
	%	2.40	0.48	2.88	2.72	8.49
Mucor sp.	T.C	1.00	1.00	0.00	0.00	2.00
	%	0.16	0.16	0.00	0.00	0.32
Penicillium sp.	T.C	17.00	8.00	7.00	18.00	50.00
	%	2.72	1.28	1.12	2.88	8.01
Rhizopus sp.	T.C	0.00	0.00	0.00	1.00	1.00
	%	0.00	0.00	0.00	0.16	0.16
Trichoderma sp.	T.C	3.00	0.00	0.00	0.00	3.00
	%	0.48	0.00	0.00	0.00	0.48
Bacterial isolates	T.C	1.00	0.00	0.00	80.00	81.00
	%	0.16	0.00	0.00	12.82	12.98
Total		187.00	65.00	103.00	269.00	624.00
		29.96	10.41	16.50	43.10	

**T.C = Total colony count*

Legume seed deterioration caused by fungi

Toxic effect of aflatoxin on seed germination (toxicity):

Effect of fungal filtrate on percentage of germination of tested legume seed samples were recorded in Table 2. Data show that toxic effect has different degrees including delay of seed germination and yellowish seedlings were found in all fungal filtrates with the two concentrations 50 and 100%. All tested of fungal filtrates were found to be reduce the percentage of germination tested legume seed samples. Also, data illustrate that, increase reduction percent with increasing the concentration of fungal filtrates from 50 to 100% respectively. On the other hand, higher reduction percent was recorded with *A. flavus* filtrate at 100% concentration which gave zero percent of lupine germination seed (completely inhibition of germination seeds) and hundred percent of reduction compared with un-treated (control 100% of germination), as well as *A. parasiticus* filtrate on Soybean seeds at 50 &100% concentrates respectively. In bean seeds, *A. flavus* filtrate at 100% was found to be decreased germination percent from 100% to 80% with 20% of reduction (decreasing percent) while gave 86.6% of germination seeds when treated with 50% of fungal filtrate with 13.3% of reduction (decreasing percent). *A. parasiticus* was found to be decrease germination percent of bean seeds from 100% to 13.3% with 86.6% of reduction percent (decreasing percent) while gave 20% of germination seeds with 13.3% of reduction (decreasing percent) with 80% of reduction (decreasing

percent) when treated with 100% and 50% of fungal filtrate respectively. *A. parasiticus* filtrate was found to be reduce germination percent of pea seeds from 100% to 73.3% with 26.6% of reduction percent (decreasing percent) while gave 20% of germination seeds with 13.3% of reduction (decreasing percent) when treated with 100% of fungal filtrate but 50% conc. of fungal filtrate not affected. Abbasi, (2013) suggested that depletion of available oxidizable materials in meristematic cells might cause deterioration. In indoor seeds the effect of *Aspergillus flavus* was remarkable. Initially the germination percentage was 100% in healthy and 70 % in infested seeds which increased to 90 % on final observation where as healthy remained the same. The decrease in germination ability by fungal infection may be purpose due to damaging of the embryo or by depletion of nutrient reserves. The decrease in germination percentage may be due to production of toxic metabolites. The infestation on culture filtrate of *A. flavus* was unable to check germination as quality of seed would have been very viable but with time it could be observed that the health of emerging seedling root length was affected and thus there was a noticeable difference in vigour index of healthy and infested seeds of all the three genotypes of *Arachis hypogea*. The importance of production of toxic metabolites is obvious, when the pathogen is seed borne. The seed borne fungi are known to affect adversely seed germination and seedling vigor possibly due to production of toxic metabolites. Deepavali and Nilima (2012) reported that, the germinability of Maize seeds was highly reduced when treated with crude aflatoxin filtrate extracted from *Aspergillus flavus*. Aflatoxins are biologically active secondary metabolites produced by *A. flavus*. Aflatoxin B_1 alters the physiology of seeds and seedlings. Aflatoxin B_1 also restricts plant growth by inhibiting seed germination, seedling growth and other physiological processes of plants. Aflatoxins affect certain plants by inhibition of seed germination. Deepavali and Nilima (2015) found that, Germination percent and seedling growth in terms of root - shoot length and chlorophyll content in leaf was found to be reduced in treated maize grains with crude mycotoxin from culture filtrate of *A. niger*.

Table 2: Effect of fungal filtrate on germination percent of tested legume seeds

Legume seeds type	Un-treated		Treated by Fungal filtrate				D**	%D***
	NG	%	Fungal filtrate type	% Conc.	NG*	%		
Beans	15	100	*A. flavus*	100	12	80	3	20
				50	13	86.6	2	13.3
	15	100	*A. parasiticus*	100	2	13.3	13	86.6
				50	3	20	12	80
Lupine	15	100	*A. flavus*	100	0	0	15	100
				50	10	66.6	5	33.3
Pea	15	100	*A. parasiticus*	100	11	73.3	4	26.6
				50	15	100	0	0.0
Soybean	1	6.67	*A. parasiticus*	100	0	0	1	6.67
				50	0	0	1	6.67

NG = Number of germinated seeds, **D= Decreased, * %D=Percentage*

Reduction in weight loss (g)

Reduction in weight loss (g) (of 200 legume seeds) after 15 days from inoculation caused by toxigenic fungi were studied. Data in table 3 present that, all toxigenic fungi were found to reduce dry weight (g) of all tested legume seeds after 15 days from inoculation. Higher reduction percent was recorded with *A. flavus* which reduce dry weight of tested Soybean seeds from 492.50 to 111.40g and loosed 381.10 equal 77.38 reduction percent and Lupine seeds from 475.86 to114.25g and loosed 361.61g equal 76.00% reduction followed by *A. parasiticus* which reduce Soybean seeds from 478.76 to 124.13g and loosed 354.63g equal reduction percent and Beans seeds from 499.33 to 158.78 g and loosed 340.55g equal 68.20% reduction.

Moderate affected was recorded with *A. terreus* which reduce dry weight seeds from 463.42 to 156.10g and loosed 307.32g equal 66.32 reduction percent. Less affecting of tested fungi was recorded with *A. parasiticus* isolated from Pea seeds which reduced dry weight from 437.12 to 161.57g and loosed 275.55g equal 63.04% reduction.

Table 3: Legume seed deterioration of (200 seeds) caused by fungi

Tested legume seeds	Tested fungi	Weight (g)		L (g)	% R
		F	D		
Beans	*A. parasiticus*	499.33	158.78	340.55	68.20
	A. terreus	463.42	156.10	307.32	66.32
Lupine	*A. flavus*	475.86	114.25	361.61	76.00
Pea	*A. parasiticus*	437.12	161.57	275.55	63.04
Soybeans	*A. parasiticus*	478.76	124.13	354.63	74.07
	A. flavus	492.50	111.40	381.10	77.38

F = Fresh weight (g), D = Dry weight (g), L = Loss (g) = F-D, R = % reduction = F-D/F

Changes in some chemical composition

Changes in some chemical composition of tested legume seed deterioration caused by toxigenic fungal isolates were recorded in Table 4. Moisture content of control sample (Untreated) was in a narrow range of 8.87 – 10.70%. The highest moisture was found in bean seeds which recorded 10.70% while the lowest value for soybean seeds 8.87%. The contaminated (inoculated) samples showed higher values of moisture content compared to control sample (Untreated). For instance, while the moisture content of soybean seeds contaminated by *A. parasiticus* was 12.13%, that of the control was only 8.87%. The increment in moisture was 36.75%. Similar findings were noticed for other contaminated samples. The results are in agreement with Embaby and Abdel-Galile (2006). Protein content of control legume samples showed very wide range being 26.51% for pea seeds, and reached to 46.20% for soybean seeds. Other control samples displayed intermediated values being 36.23% for bean seeds, and 37.45% for lupine seeds. While in case of the contaminated samples results in the same table revealed that was slight reduction in the protein content. The reduction percent reached 4.38, 2.38, 2.87 and 4.5% for lupine seeds contaminated with *A. flavus*, pea seeds, bean and soybean seeds contaminated with *A. parasiticus* respectively. The total lipid, total carbohydrates, available carbohydrate contents and energy value of contaminated samples under test showed the same trend as that of protein. These results demonstrated that the fungus utilized the basic compound of these seeds for its growth. Aziz and Mahrous (2004) reported that, *A. flavus* utilizes carbohydrates for its growth and aflatoxin production. The difference in crop composition was mainly due to the influence of the pathway to use the major energy source of each seed. These results agreed with those reported by Morkunas *et al.* (2005). A remarkable high crude fiber content was noticed for all control samples. The highest crude fiber percent was found in the lupine seeds (9.77%) followed by pea seeds (6.34%). Such values were increased markedly by contamination with fungi. For instance, the highest increment percent was 31.63% for bean seeds contaminated with *A. parasiticus* followed by the same seeds contaminated with *A. terreus* (21.63%). Ash content showed a parallel trend as that of crude fiber. Lupine sample contaminated with *A. flavus* ranked first (40.31%) followed by bean seeds contaminated by *A. parasiticus* (20.13%).

Table 4: Changes in some chemical composition

Tested seeds	Type of treatment	Chemical composition changes							
		P	L	A	C F	CHO*	CHO**	E	M
Beans	Control	36.23	6.96	4.72	4.30	47.79	43.49	381.52	10.70
	A. parasiticus	35.19	6.84	5.67	5.66	46.64	40.98	366.24	10.86
	A. terreus	40.50	6.70	5.31	5.23	42.26	37.03	391.34	11.02
Lupine	Untreated	37.45	6.68	3.87	9.77	42.23	32.46	378.84	9.47
	A. flavus	35.81	6.60	5.43	10.00	42.16	32.16	371.28	9.76
Pea	Control	26.51	7.07	5.73	6.34	54.35	48.01	361.71	9.92
	A. parasiticus	25.88	7.00	5.97	7.50	53.65	46.15	351.12	10.07
Soybean	Control	46.20	26.25	6.90	5.42	15.23	9.81	477.21	8.87
	A. parasiticus	44.12	26.13	7.78	5.97	16.00	10.03	475.65	12.13

*P= Protein, L =Lipid, A = Ash, CF =Crude Fiber, E = Energy, M = Moisture, *CHo =Total carbohydrate, **CHO = Available carbohydrate*

Changes in color quality

Color attributes of contaminated and non-contaminated legume meal samples were recorded in Table 5. Data show that, contaminated samples were darker in color compared with control. The lightness (L) values of contaminated samples were in a narrow range 30.95 – 70.64 for control samples. This lightness reduction was more pronounced in soybean sample contaminated with *A. parasiticus*. Comparison among (a) values (degree of redness) of samples indicated that bean sample contaminated with *A. terreus* was clearly redder as compared to the control or other contaminated samples. The higher (a) values of contaminated samples may be attributed to the presence of the red to brown pigments naturally produced by fungus. Values of (b) (degree of yellowness) of samples ranged from 15.35 to 30.45. Again, sample tended to have lower (b) values indicating lower degree of yellowness than those of control sample. Total color differences (ΔE) ranged between 8.74 to 44.25. The highest color differences were recorded for bean meal contaminated with *A. terreus* followed by soybean meal contaminated by *A. parasiticus*.

Table 5: Changes in color properties

Tested Seeds	Type of treatment	Color properties			
		(L)	(a)	(b)	ΔE
Beans	Untreated	81.71	1.88	15.35	0.00
	A. parasiticus	64.00	7.18	22.90	19.97
	A. terries	39.14	11.15	23.07	44.25
Lupine	Untreated	77.73	5.15	27.57	0.00
	A. flavus	70.64	6.31	23.01	8.47
Pea	Untreated	68.53	-1.82	19.83	0.00
	A. parasiticus	55.90	6.96	22.60	15.63
Soybean	Untreated	71.61	2.99	30.45	0.00
	A. parasiticus	30.95	8.85	18.54	42.77

(L) = Lightness, a= Redness, b= yellowness, ΔE = Total color differences

Changes in some physico-chemical properties of soybean oil

Effect of *A. parasiticus* infection on some physico-chemical properties of extracted soybean oil was recorded in Table 6. Results show that, control sample (Healthy) had 101.06g/kg oil iodine value, indicating a high degree of un-saturation. While in case of the contaminated sample revealed that there was slight increase in the iodine value to 114.92 g/kg oil equal 12.6% reduction. The increment % reached 13.71%. Dhingra *et al*., (1998), demonstrated the quality of the oil obtained from soybean grains stored at 25°c and infected with *Aspergillus niger* and reported that no significant variation in the iodine value for the crude oil compared to healthy (control). Peroxide value indicated a value of 9.98mg/kg oil for the healthy (control) sample and this value increased to 15.94 mg/kg oil for contaminated sample which recorded 37.39% reduction. The increment was 59.72%. It has been shown that oil become rancid when the peroxide value ranges from 20.0 to 40.0 mg/kg oil (Ajayi *et al*., 2006). Same finding was noticed when moisture content and acidity of oil were considered. For example, oil acidity percent of contaminated sample was 62.80%, that of the control was only 1.95%.

Table 6: Changes in some physico-chemical properties of soybean seed oil deterioration caused by *A. parasiticus*

Tested of soybean seed sample				
Physico-chemical properties	H	C	H - C	%R
Iodine value (g/kg oil)	101.06	114.92	13.86	12.6
Peroxide value (mg/kg oil)	9.98	15.94	5.96	37.39
Refractive index	1.4645	1.4605	-0.005	-0.31
Acidity (%)	1.95	62.80	60.85	96.90
Saponification No. (mg/kg oil)	197.30	199.70	2.40	1.20
Moisture (%)	0.05	0.08	0.03	37.5

H = Healthy (control), C =Contaminated, %R = %Reduction

The refractive index is considered one of the most important physical characteristics of oil, as it is useful for estimating degree of their saturation as well as for identification processing purposes, establishing their purity and observing the progress reaction such as catalytic hydrogenation, oxidation and isomerization. Since the oil protect them from oxidation (Amro *et al.,* 2002 and De Leonardis *et al*., 2007). Data in the same table reveal that, the refractive index was 1.4645 for healthy (control) sample, while it was 1.4605 for contaminated sample. Saponification number (SN) is an indicator of the average molecular weight and, hence chain length. The Saponification number (SN) of un-contaminated sample (control) was 197.30 mg/kg oil, and this value increased because of contamination of soybean grain with *A. parasiticus* to 199.70 mg/kg oil equal 1.20% reduction. The increment was 1.22%.

Conclusion

From the present study, it may be concluded that the seed-borne pathogenic fungal diseases are responsible for the economic loss . It was observed that *Aspergillus flavus* and *A. parasiticus* were most frequent fungi. Seed-borne fungal species are responsible to mycotoxin formation and reduce or delay the seed germination in legume seeds as well as the physico-chemical properties of legume seed quality and soybean oil .

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