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# Seed borne fungal pathogens associated with common Egyptian seeds and their efficiency to produce saponin hydrolase enzyme

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**Abstract :** Seed borne fungi are a serious problem worldwide causing diseases and poor quality on many imported crops. Several fungal isolates were isolated from seed samples collected from commercial markets in Egypt. Seeds of different crop cultivars collected or stored were contaminated with fungi ranging from 37.50 to 100% with an average of 72.224% on PDA medium. The fungal infection showed high infection in cotton and peanut seeds, where detected 100% infection followed by broad bean seeds (93.75%) and lentil (92.85) without significant difference. Also, regarding the total fungal count as cfu, it is clear that the fungal count per 100 seeds was ranged from 129.1 to 831.3 cfu/100 seeds on PDA with an average of 327.707cfu/100 seeds. Moreover, mungbean seeds showed low fungal densities than the corresponding figures of different seed cultivars. The following 20 fungal species belonging to eleven genera were observed and identified as *Aternaria alternata*, *Alternaria tenuis, Aspergillus amstelodam, Aspergillus flavus, Aspergillus ochraceous, Aspergillu sniger, Aspergillus paraziticus, Aspergillus regulosus, Aspergillus ruber, Aspergillus sydowii, Aspergillus terreus, Chaetomium globosum, Fusariumgra minearum, Fusarium oxysporum, Fusariums olani, Helminthosporium sativum, Mucor spp., Macrophomina phasolina, Nigrospora sphaerica, Penicillium spp.,Rhizoctonia solani and Rhizopusnigricans.* Saponin hydrolase screening results showed that 20 fungal isolates (66.67%) had the ability to produce saponin hydrolase enzyme, furthermore, only 2(10%) isolates had a high ability to produce saponin hydrolase enzyme in the medium, including 2 isolates of *Aspergillus flavus* which produce 64.26 U/ml and 52.23 U/ml.
**Keywords**: Seed borne fungi.Saponin hydrolase, Soyasapogenol B.

## Introduction

Seeds have the most potential in crop production. Seeds free from fungal infection are essential for good plant production. Previous investigators reported that fungal pathogens may be associated with seeds externally or internally[1]. Generally, common Egyptian seeds are important in nutrition sources and occupy great figures in local consumption and export. Different fungal species are known to infect seeds during storage in which, *Aspergillus, Penicillium and Fusarium* species are the main cause of spoilage and germ tube damage of seeds[2,3].

Various plants have been identified for their potential to produce saponins showed antifungal activities[4], like soyasaponins (SS) of soybean[5], avenacin A-1 of oats[6] andα-tomatine of tomato[7], which could effectively prevent infection by phytopathogenic fungi. Saponins are plant glycosides, which commonly consist

of an aglycone and various carbohydrate moieties[8]. Fungi which could overcome these antimicrobial plant defenses produce saponin-detoxifying enzymes, which hydrolyze the carbohydrate moieties of saponins[5]. Saponin-detoxifying enzymes such as, soybean saponin hydrolase, avenacinase and tomatinase, which are produced by the soybean pathogen *Neocosmospora vasinfecta var. vasinfecta*, the oat leaf pathogen *Septortia avenae* and the tomato pathogen *Septoria lycopersici*, respectively, suggesting that the phytopathogenic fungi have common saponin detoxification mechanisms[5]. The potential of phytopathogenic fungi to infect host plants is facilitated by a reduction ofthe antifungal activities of saponins. Consequently, these enzymes may determine the range of plants susceptible to phytopathogenic fungi[9].

Among these saponins, SS are oleananetriterpenoid glycosides found in soy and other legumes, such as mung beans, green peas, cowpeas, lentils, chick peas, lupine seeds and alfalfa[10].These saponins have been divided into A, Band E groups according to their aglycone (soyasapogenol) structures[11]. Soyasapogenols A and B are generally showed higher biological activities compared to their glycosides[12]. Giving this fact, it is not surprising that soyasapogenol B (SB) have several biological activities such as, anticancer, antivirus, antimutagenic, hepatoprotective, and anti-inflammatory[5,13,14]. SB could be produced by an enzyme reaction using microorganisms having SS hydrolyzing activity. Examples of fungi, that can hydrolyze SS and produce SB,*Eupenicilliumbrefeldianum*PF122[15], *Aspergillusoryzae*PF1224[15], and *Aspergillus parasiticus*[16].

Therefore, the present study aim was to isolate and identify the seed borne fungi associated with some Egyptian seeds and also investigate their ability to produce saponin hydrolase enzyme as a template of saponin-detoxifying enzymes.

## Materials and Methods

### Sampling

The study was conducted in National Research Centre of Egypt. A Total number of 100 seeds of each of moringa, pope corn, broad bean, lupine, mung bean, cow pea, chick pea, lentil, wheat, cotton, pea nut, soybean, green bean and maize were collected from different places and put in a polyethylene bag, sealed and put in another bag which was also sealed. Storage of seeds in a double-bag container minimizes the loss of water content and yet furnishes sufficient aeration during storage at 4˚C.

### Isolation of seed borne fungi

Laboratory analysis was done to detect the seed borne fungi associated with different seed according to Foor *et al.*[17]. Seeds were surface disinfected by soaking in 2% sodium hypochlorite for 3 min, followed by 70% ethanol for 2 min and then thoroughly washed in sterile water. Drain excess water, dried between two layers of sterilized filter papers. The seeds were platted on potato dextrose agar (PDA) medium at rate of 4 seeds/dish. The plates were incubated at 27±2˚C for 7 days. The cultures were single-spore following the standard protocol of Burgess *et al.*[18]. After 7 day of incubation, the cultures were transferred onto PDA for species identification according to colony morphology and microscopic examination[19,20,21,22].

### Screening for fungi producing saponin hydrolase

Saponin hydrolase production was performed in a production medium consisting of (g/l) 10 SS, 40 malt extract, 20 yeast extract, 2 $KH_2PO_4$, 2 $(NH_4)_2SO_4$, 0.3 $MgSO_4 \cdot 7H_2O$ and 0.3 $CaCl_2 \cdot 2H_2O$ with a pH adjusted to 7.0[5]. Each isolate was cultured in Erlenmeyer flasks (250 ml) containing 50 ml of production medium and incubated for 72 h at 30°C on a rotary shaker at 150 rpm. Then, the enzyme activity of saponin hydrolasewas detected in the cultural filtrate for each isolate.

### Measurement of saponin hydrolase activity

Unless otherwise stated, saponin hydrolase activity was measured in cultural filtrate as follows. To 1ml of 2% soybean saponin suspended in 0.2M acetate (pH 5), 1ml of enzyme solution was added, and the mixture was allowed to react at 40°C for 1h. A blank for each cultural filtrate was carried out without the addition of substrate (soybean saponin). Reaction products were extracted with 2ml of ethyl acetate. The quantity of SB in the sample was analyzed by high-pressure liquid chromatography (HPLC). One unit of enzyme activity is defined as the amount of enzyme that produces 1 µmole of aglycone (SB) per hour from the substrate.

### SB analytical methods

Thin layer chromatography(TLC)was carried out on pre-coated silica gel plate (Merck, silica gel 60F-254). The plate was chromatographed for SB with a solvent system of benzene: ethyl acetate: acetic acid (12:4:0.5, v/v/v). SB having Rf value of 0.35 was detected on TLC plates by acid charring (10% $H_2SO_4$, 120◦C, 10 min). HPLC was performed with Waters Alliance HPLC System (Model NO.E2695 XE Separations Module, Austria) under the following conditions: column, Sun Fire Prep C18 (5µm,10x150mm); column temperature, 40°C; mobile phase, acetonitrile-methanol-water (50: 15: 35); flow rate, 1 ml/min; and UV detector operating 200 nm. 100 µl of ethyl acetate containing reaction products was diluted with 900 µl of the mobile phase. 10 µl of this dilution was analyzed by HPLC, and the quantity of SB in the sample was determined by comparison with authentic SB[5].

### Statistical analysis

The collected data were statistically computed using the software MSTATE-C for Windows. Least significant differences values at $P<0.05$ were used to separate treatment means when ANOVA indicated a significant F value.

## Results and Discussion

### Prevalence of seed borne fungi

Data presented in Table 1 and Fig. 1 show that stored seeds of different crop cultivars collected were contaminated with fungi ranging from 37.50 to 100% with an average of 72.224% on PDA. The fungal infection results showed high infection in seeds of cotton (Fig. 1d) and peanut (Fig. 1e) detecting 100% infection followed by broad bean (Fig. 1g) seeds (93.75%) and lentil (92.85) (Fig. 1c) without significant difference. On the other hand, seeds of mungbean (Fig. 1f) and moringa (Fig. 1l) showed the lowest densities of fungal contamination detecting 37.50 and 43.75%, respectively. Whereas, a high percentage of fungal infection was found in seeds of chickpea (Fig. 1k) recorded 80.0%and wheat (Fig. 1j, 85.0%). On the other hand, a moderate fungal infection percentage ranged from 55.0 to 72.5% was detected in lupine (Fig. 1i), maize (Fig. 1h), green bean (Fig. 1a), popcorn (Fig. 1b), cowpea (67.79 %) and soybean (72.50%).

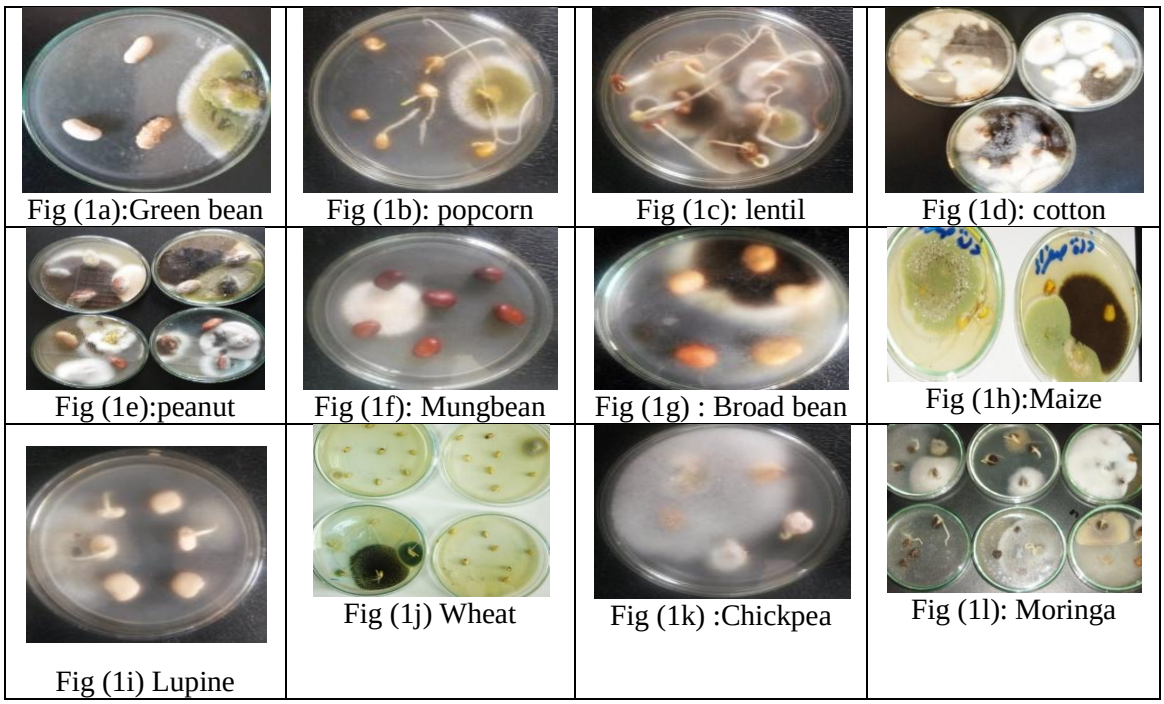

Fig (1a):Green bean | Fig (1b): popcorn | Fig (1c): lentil | Fig (1d): cotton

Fig (1e):peanut | Fig (1f): Mungbean | Fig (1g) : Broad bean | Fig (1h):Maize

Fig (1i) Lupine | Fig (1j) Wheat | Fig (1k) :Chickpea | Fig (1l): Moringa

**Fig.1: Seed borne fungi infected common Egyptian seeds**

In different places of the world, many researches have been carried out on seed borne fungi infected cotton seeds[23,24]and peanut seeds[25,26]. The fungal isolates infected lentil seeds has been extensively studied by several investigators[27,28]. In a study of the seed borne fungi, Elwakil *et al*.[29]isolated fungi infected broad bean and mungbean seeds. Moreover, numerous investigations have been carried out on seed borne fungi on soybean seeds[30], chickpea seeds[31], maize[32], popcornseeds[33], wheat[34],bean[35], cowpea[36] and lupine[36]. Also, association of moring aseed borne fungi causing sever damaged in seeds was reported by El-Mohamady *et al*.[37] and Sahab and Nawar[38].

**Table 1: Percentage of fungal infection and number of different fungal counts (cfu/100 seeds) associated with different seed cultivars collected from local markets on PDA medium**

| Seed name | Scientific name | % of infection | cfu/100 seeds |
|---|---|---|---|
| **Broad bean** | *Vicia faba* | 93.75 *AB* | 287.5 CD |
| **Lupine** | *Lupinus termis* | 55.00 *DEF* | 177.5 E |
| **Mungbean** | *Vigna radiata* | 37.50 *F* | 129.1 E |
| **Cowpea** | *Vigna sinensis* | 67.79 *BCDE* | 175.0 E |
| **Chickpea** | *Cicerarientinium* | 80.00 *ABCD* | 225.0 DE |
| **Lentil** | *Lens esculenta* | 92.85 *AB* | 225.0 DE |
| **Peanut** | *Arachi shispida* | 100.0 *A* | 831.3 A |
| **Soybean** | *Glicine hispida* | 72.50 *ABCDE* | 440.0 B |
| **Green bean** | *Phaseolus vulgaris* | 56.25*CDEF* | 225.0 DE |
| **Wheat** | *Triticum vulgare* | 85.00 *ABC* | 335.0 C |
| **Popcorn** | *Zea mays var. everta* | 68.75 *BCDE* | 175.0 E |
| **Maize** | *Zea mays* | 55.00*DEF* | 180.0 E |
| **Moringa** | *Moringa oleifera* | 43.75 *EF* | 737.5 A |
| **Cotton** | *Gassypium barbadense* | 100.0 *A* | 445.0 B |
| **Average** | | **72.224** | **327.707** |

Three replicates were used for each treatment.
Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.
Means followed by the same letters are not significantly differed

Concerning the total fungal count as cfu, it is clear that the number of fungi per 100 seeds was ranged from 129.1 to 831.3 cfu/100 seeds on PDA with an average of 327.707cfu/100.Mungbean seeds showed low fungal densities than the corresponding figures of different seed cultivars, as the total fungal count (cfu/100seeds)was significantly lowered from 129.1 in mung bean to 831.3cfu/seeds (6.44 folds increase) in peanut. Results also showed that the seeds of legume plants contained fungi ranged from 175.0 to 831.3 cfu/100 seeds and can be arranged in descending order as follows: peanut, soybean, broad bean, green bean, chickpea, cowpea, lentil lupine and mungbean. The obtained results almost agree with Embaby *et al.*[3],Embaby and Abdel-Galil[36] and Shovan *et al*.[39]. On the other hand, the cfu/100 seeds were 175 and 180 for pope corn and maize respectively, while moringa seeds were found associated with high number of fungi reached 737.5cfu/seeds. Similar observation was reported by other investigators[37,38].

**Frequency occurrence of fungi infected seeds**

Data presented in Table 2 indicate that the internal infection was recorded within seeds of each sample. Great variation in types and in number of propagations among samples was noticed and many fungal isolates which found in seeds of legumes were absent in seeds of cereal or other seeds.

**Table2: Numbers (cfu/g) and frequency occurrence percentages of fungi associated with various seed cultivars on PDA medium for 7 days at 27±2°C.**

| Isolated fungi | Cultivars | | | | | | | | | | | | | | % ave. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Moringa | Popcorn | Broad bean | | Mung bean | Cowpea | Chiickpea | Lentil | Wheat | Cotton | Peanut | Soybean | Beans | Mays | |
| *Alternaria alternata* | 6.9 (2) | - | - | - | -- | | 11.1 (1) | - | 13.3 (2) | - | - | 11.8 (2) | - | - | 3.28 (7) |
| *Alternaria tenuis* | - | 11.1 (1) | - | 14.3 (1) | - | 14.3 (1) | - | 11.1 (1) | - | 15.8 (3) | 6.06 (2) | - | 3.4 (1) | 7.7 (1) | 5.14 (11) |
| *Aspergillus amstelodami* | - | - | - | - | - | - | - | - | - | - | 6.06 (2) | - | - | - | 0.93 (2) |
| *Aspergillus flavus* | 13.9 (4) | 11.1 (1) | 18.0 (2) | 28.6 (2) | 14.3 (1) | 14.3 (1) | - | - | 13.3 (2) | 10.5 (2) | 12.1 (4) | 24.5 (4) | 10.3 (3) | 30.8 (4) | 14.9 (30) |
| *Aspergillusochraceous* | - | - | - | - | - | - | - | - | - | 10.5 (2) | 12.1 (4) | 5.88 (1) | 13.8 (5) | - | 5.61 (12) |
| *Aspergillusniger* | 6.89 (2) | 11.1 (1) | 9.09 (1) | 14.3 (1) | 14.3 (1) | - | 11.1 (1) | 11.1 (1) | 6.7 (1) | 10.5 (2) | 12.1 (4) | 11.8 (2) | 3.4 (1) | 7.7 (1) | 8.88 (19) |
| *Aspergillus paraziticus* | - | - | - | | - | - | - | - | - | - | - | - | 3.4 (1) | - | 0.97 (1) |
| *Aspergillus regulosus* | - | - | - | - | 28.6 (2) | - | - | - | - | - | 6.06 (2) | - | - | - | 1.87 (4) |
| *Aspergillus ruber* | - | - | - | | | - | - | - | - | - | - | - | 6.9 (2) | - | 0.93 (2) |
| *Aspergillus sydowii* | 6.89 (2) | - | - | - | - | - | - | - | 6.7 (1) | 5.26 (1) | 6.06 (2) | - | - | - | 2.83 (6) |
| *Aspergillus terreus* | 10.3 (3) | - | 18.0 (2) | 14.3 (1) | 14.3 (1) | 14.3 (1) | 11.1 (1) | 11.1 (1) | 13.3 (2) | 5.26 (1) | - | 11.8 (2) | 17.2 (5) | 7.7 (1) | 9.81 (21) |
| *Chaetomium globosum* | 6.89 (2) | - | - | - | - | - | - | - | - | - | 6.06 (2) | - | - | - | 1.87 (4) |
| *Fusariumgraminearum* | - | 22.2 (2) | - | - | - | - | - | - | 13.3 (2) | - | - | - | - | 15.4 (2) | 2.83 (6) |
| *Fusariumoxysporum* | 6.89 (2) | - | 18.0 (2) | 14.3 (1) | - | - | 11.1 (1) | - | - | - | 6.06 (2) | - | 6.9 (2) | - | 4.67 (10) |
| *Fusarium solani* | 3.45 (1) | 22.2 (2) | - | 14.3 (1) | - | 28.6 (2) | - | 22.2 (2) | 13.3 (2) | 10.5 (2) | 6.06 (2) | 11.8 (2) | - | 15.4 (2) | 8.41 (18) |
| *Helminthosporium sativum* | - | - | - | - | - | - | - | - | 13.3 (2) | - | - | - | - | - | 0.93 (2) |
| *Mucor spp.* | 3.45 (1) | 11.1 (1) | 9.09 (1) | - | 28.6 (2) | - | 11.1 (1) | - | 6.7 (1) | 5.26 (1) | 3.03 (1) | - | - | 7.7 (1) | 4.67 (10) |
| *Macrophomina phasolina* | 10.3 (3) | - | - | - | - | 28.6 (2) | 11.1 (1) | 22.2 (2) | - | - | 6.06 (2) | 11.8 (2) | 3.4 (1) | - | 6.07 (13) |
| *Nigrospora sphaerica* | 10.3 (3) | - | - | - | -- | - | - | - | - | - | - | - | - | - | 1.41 (3) |
| *Penicillium spp.* | - | - | - | - | - | - | 11.1 (1) | 11.1 (1) | - | 10.5 (2) | 6.06 (2) | - | 13.8 (4) | - | 4.67 (10) |
| *Rhizoctonia solani* | 6.89 (2) | - | 18.0 (2) | - | - | - | 22.2 (2) | 11.1 (1) | - | 10.5 (2) | 6.06 (2) | 5.88 (1) | - | 7.7 (1) | 6.07 (13) |
| *Rhizopus nigricans* | 6.89 (2) | 11.1 (1) | 9.09 (1) | - | - | - | - | - | - | 5.26 (1) | - | 5.88 (1) | 13.8 (4) | - | 4.67 (10) |
| **Total** | 29 | 9 | 11 | 7 | 7 | 7 | 9 | 9 | 15 | 19 | 33 | 17 | 29 | 13 | |

Twenty two species belonging to eleven genera were detected and they were classified as, *Aternaria alternata, Alternaria tenuis, Aspergillus amstelodam, A. flavus, A. ochraceous, A. niger, A. paraziticus, A. regulosus, A. ruber, A. sydowii, A. terreus, Chaetomium globosum, Fusarium graminearum, Fusarium oxysporum, F.solani, Helminthosporium sativum, Mucor spp.,Macrophomina phasolina, Nigrospora sphaerica Penicillium spp.,Rhizoctonia solani, Rhizopus nigricans.*

According to their occurrence frequency the genera and species were grouped as major and minor component include: *Aspergillu flavus*(30%), *spergillus terreus*(21%), *Aspergillus niger* (19%), and *Fusarium solani* (18%) were the most frequently isolated fungi. On the other hand, the seed borne fungi which causing, seed stunt, wilt and damping off diseases),i.e., *Macrophomina phasolina* and *Rhizoctonia solani* were also found in high frequency (13%). These important plant pathogenic fungi are always detected associated with of different seeds and have the ability to cause severe damage[40,41]. Additionally, analysis of seeds from different sources detected fungi that can produce mycotoxins which is considered as another major problem associated with seeds. The present data revealed that the highest percentage of infection with *Aspergillus flavus* was obtained inmaize(30.8%), in lupine(28.6%) and in soybean(24.5%).

**Table 3: Efficiency of isolated fungi to produce saponin hydrolase enzyme**

| **Fungal species** | **No. of tested isolate** | **No. of (+) isolate** | **Saponin hydrolase activity (U/ml)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ***Alternaria tenuis*** | 1 | 0 | - | - | - | - | - | - | - |
| ***Aspergillus flavus*** | 7 | 7 (100%) | 18.75 | 17.85 | 37.60 | 35.73 | 43.54 | 52.23 | 64.26 |
| ***Aspergillus ochraceous*** | 4 | 2 (50%) | 0.67 | 0.88 | - | - | - | - | - |
| ***Aspergillus niger*** | 3 | 1 (33%) | - | - | 23.01 | - | - | - | - |
| ***Aspergillus paraziticus*** | 1 | 1 | 50.63 | - | - | - | - | - | - |
| ***Aspergillus ruber*** | 2 | 1 (50%) | - | 1.11 - | - | - | - | - | - |
| ***Aspergillus sydowii*** | 2 | 1 (50%) | - | 6.22 - | - | - | - | - | - |
| ***Aspergillus terreus*** | 4 | 4 (100%) | 15.34 | 19.18 | 27.30 | 10.55 | - | - | - |
| ***Fusarium graminearum*** | 1 | 1 | 1.13 | - | - | - | - | - | - |
| ***Fusarium oxysporum*** | 1 | 0 | - | - | - | - | - | - | - |
| ***Helminthosporium sativum*** | 1 | 1 | 0.96 | - | - | - | - | - | - |
| ***Pacilomyces* sp.** | 1 | 1 | 0.77 | - | - | - | - | - | - |
| ***Pencillium chrisogenum*** | 1 | 0 | - | - | - | - | - | - | - |
| ***Curvulania lunata*** | 1 | 0 | - | - | - | - | - | - | - |
| **Total** | 30 | 20 | | | | | | | |

**Screening of fungal isolates for saponin hydrolase enzyme production**

The tested isolates were propagated on saponin hydrolase production medium and the saponin hydrolase activity was measured in cultural filtrate. Data in Table (3) show that of the 30 fungal isolates screened for the production of saponin hydrolase enzyme only 20 isolates (66.67%) had the ability to produce saponin hydrolase enzyme. Moreover, only 2out of20 (10%) fungal isolates had a high ability to produce saponin hydrolase enzyme in the medium, including 2 isolates of *Aspergillus flavus* produced 64.26 and 52.23 U/ml. While, the other 5 isolates of *A. flavus* produced moderate amount of saponin hydrolase enzyme in amount ranged from 18.75 to 43.53 U/ml. The isolate of *A. paraziticu s*also produced the enzyme in high amount reached 50.63 U/ml and this is in accordance of previous results reported by[16]. While, the 4 isolates of *A. terreus* produce enzyme between10.55 and 27.30 U/ml and the isolate of *A. niger* detect enzyme production reached 23.01 U/ml. In this context, Kudou *et al.*[42]reported that 126 *Aspergillus* sp. have the ability to produce saponin hydrolase enzyme. Other 8 isolates belonging to, *Aspergillus ochraceous, Aspergillus ruber,*

*Aspergillus sydowii, Fusarium graminearum, Helminthosporium sativum, Pacilomyces* sp., gave lower values of enzyme production (between 0.67 to 6.22 U/ml). On the other hand, the tested isolates of *Alternaria tenuis, Curvularia lunata, Fusarium oxysporum,* and *Pencillium chrisogenum* showed no enzyme activity.

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## References

1. Singh D, Mathur SB. Location of fungal hyphae in seeds. In: Singh D, Mathur SB, eds. Histopathology of seedborne infections. Boca Raton, FL, USA: CRC Press, 2004, 101–168.
2. Castillo MD, Gonzalez HHL, Martinez EJ, PacinAM, Resnik SL. Mycoflora and potential for mycotoxin production of freshly harvested black bean from the Argentinean main production area. Mycopathologia, 2004, 158: 107-112
3. Embaby EM, Reda M, Abdel-Wahhab MA,Omara H,Mokabel AM. Occurrence of toxigenic fungi and mycotoxins in some legume seeds. J Agri Technol., 2013, 9: 151-164.
4. Morrissey JP, Osbourn AE. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. MicrobiolMolBiol Rev. 1999, 63: 708-724.
5. Watanabe M, Sumida N,Yanai K, Murakami T. A Novel Saponin Hydrolase from *Neocosmospora vasinfecta* var. *vasinfecta*. J Appl Environ Microbiol. 2004, 70: 865-872.
6. Morrissey JP, Wubben JP, Osbourn AE. *Stagonospora avenaesecretes* multiple enzymes that hydrolyze oat leaf saponins. MolPlantMicrobe Interact,2000, 13: 1041-1052.
7. Roldan-Arjona T, Perez-EspinosaA, Ruiz-RubioM. Tomatinase from *Fusarium oxysporum* f. sp. Lycopersicidefines a new class of saponinases.Mol Plant Microbe Interact, 1999, 12:852-861.
8. Lin J, Wang C. Soybean saponins: Chemistry, analysis and potential health effects. In: Soybeans as Functional Foods and Ingredients, Liu KS, ed.AOCS Press, Champaign, 2004,73-100.
9. Bowyer P, ClarkeBR, LunnessP, DanielsMJ, OsbournAE. Host range of a plant pathogenic fungus determined by a saponin-detoxifying enzyme. Science, 1995, 267:371-374.
10. Ruiz RG, Price KR, Arthur AE, Rose ME, Rhodes MJC, Fenwick RG. Effect of soaking and cooking on the saponin content and composition of chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). J AgricFood Chem.,1996, 44: 1526-1530.
11. Berhow MA, Cantrell CL, Duval SM, Dobbins TA, Maynes J, Vaughn SF. Analysis and quantitative determination of group B saponins in processed soybean product. Phytochem Anal., 2002, 13: 343-348.
12. GurfinkelLDM, Rao AV. Soyasaponins: The relationship between chemical structure and colon anticarcinogenic activity. Nutrition and cancer, 2003, 47: 24-33.
13. Kuzuhara H, Nishiyama S, Minowa N, Sasaki K. Effects of triterpene compounds on cytotoxicity, apoptosis, and immune response in cultured cells. J Nat Med., 2006, 60: 113-120.
14. Zhang W, Popovich DG. Effect of soyasapogenol A and soyasapogenol B concentrated extracts on hep-G2 cell proliferation and apoptosis. J Agric Food Chem., 2008, 56: 2603-2608.
15. Watanabe M, Sumida N, Yanai K, Murakami T. Cloning and characterization of saponin hydrolases from *Aspergillus oryzae* and *Eupenicillium brefeldianum*. Biosci Biotechnol Biochem., 2005, 69: 2178-2185.
16. Amin HA, Hassan YM, Yehia SM. Biotransformation of soybean saponin to soyasapogenol B by *Aspergillus parasiticus*. Egyptian Pharmaceutical Journal, 2013, 12: 40-45.
17. Foor SR, Tenne FD, Sinclair B.Occurrence of seed borne microorganisms and germination in culture for determining seed health in soybean.Plant Disease Rept., 1976, 60: 970-973.
18. Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D. Laboratory Manual for Fusarium Research.University of Sydney. Sydney,1994.
19. Pitt JI, Hocking AD. Fungi and food spoilage. 2nd ed. Blackie Academic and Professional, London and New York, 1997,593.
20. Barnett HL, Hunter BB. Illustrated Genera of Imperfecti Fungi. Minneapolis: Burgess publishing Co., 2000, 241.

21. Domsch KH, Gams W, Anderson TH. Compendium of soil fungi.2nd ed. IHW-Verlag, Eching, 2007, 1-672.
22. Samson R, Houbraken J, Thrane U, Frisvad J, Andersen B. Food and indoor fungi.CBS-KNAW,2010.
23. Abd El-Rahim MA, Omar MR, El-Nagar MA, Yassin MA, Amer OE. Pathological assessment of seed borne fungi involved in cotton seedling Damping-off. J Plant Sci., 2012, 7: 85-95.
24. El-Samawaty AMA, Omar MR, El-Naggar MA, YassinMA, Amer OE. Pathological Assessment of Seed Borne Fungi Involved in Cotton Seedlings Damping-off. J Plant Sci, 2012, 7: 85-95.
25. Adiver SS,Anahosur KH. Pod rot of groundnut Caused by Fusarium. Indian phytopathol., 2002, 55:315-318.
26. Embaby EM, Abdel-Galil M. Detection of fungi and aflatoxins contaminated peanut samples (*Arachishypogaea*L.). J Agri Technol., 2014, 10: 4213-437.
27. Abdel-Hafez SII. Mycoflora of bean, broad bean, lentil, lupine and pea seeds in Saudi Arabia. Mycopathologia,1984, 88: 45-49.
28. Kaiser WJ. Fungi associated with the seeds of commercial lentils from the US Pacific Northwest. Plant Disease, 1992, 76: 605-610.
29. Elwakil MA, El-Refai IM, Awadallah OA, Mohammed MS. Seed borne pathogens of faba bean in Egypt: Detection and pathogenicty. J Plant Pathology,2009, 8: 90-97.
30. Morsy AA, Sahab AF, Diab MM, Nofal MA. Determining seed health of soybean (*Glycinemax*) by the effect of seed borne fungi on germination, invasion and occurrence in culture. Egypt J Phytopathology,1982, 14: 75-82.
31. Dawar SH, Sayed F, Ghaffar A. Seed borne fungi associated with chickpea in Pakstan. Pak J Bot., 2007, 39: 637-643.
32. Somda I, Sanou J, Sanon P. Seed borne infection of farmer saved maize by pathogenic fungi and their transmission to seedlings.J Plant Pathology, 2008, 7: 98-103.
33. Doohan FM, Brennan J, Cooke BM. Influence of climatic factors on Fusarium species pathogenic to cereals. Eur J Plant Pathol.,2003, 109: 755–768.
34. Embaby EM, AyaatNM, Abd El-Hamid NH, Abdel-GalilMM, Yaseen AA, YounosMA. Detection of Fungi and mycotoxin affected wheat quality. J ApplSciRes., 2012, 8(7): 3382-3392.
35. Sabry YM, Hosseny MH, El-Shakh KAA, Obiadalla AH, Mohamed YA. Seed borne fungal pathogens associated with common bean (*Phaseolus vulgaris* L.) seeds and their impact germination. J Environ Studies,2013, 11: 19-26.
36. Embaby EM, Abdel-Galil MM. Seed Borne Fungi and Mycotoxins Associated with Some Legume Seeds in Egypt. J ApplSciRes., 2006, 2: 1064-1071.
37. El-Mohamedy RSR, Abdalla AM, Adam SM. Preliminary studies on response of Moringa oleifera plants to infection with some soil borne plant pathogenic fungi. Int J Curr. Microbiol App Sci., 2014, 3:389-397.
38. Sahab AF, Nawar LS.Chemical and Phytochemical composition of Moringa seed powder and Antifungal activity of seed extracts against seed borne fungi. International Journal of ChemTech Research, 2015, 8: 686-695.
39. Shovan LR, Bhuiyan MKA, Sultana N, Begum JA, Pervez Z. Prevalence of Fungi Associated with Soybean Seeds and Pathogenicity Tests of the Major Seed-Borne Pathogens. Int J Sustain Crop Prod., 2008, 3: 24-33.
40. Tanaka MA. Survival of *Fusarium moniliforme* in corn seeds kept in two storage conditions. J Phytopathology,2001, 26: 58-62.
41. Neves WS, Parreira DF, Ferreira PA, Lopes EA. Phytosanitary status of *Jatropha curcas* seeds from Jequitinhonho and Mucuri Valleys. RevistaTropica CienciaseBiologicas, 2009, 3: 17-23.
42. Kudou S, Tsuizaki I, Shimoyamada M, Uchida T and Okubo K. Screening for microorganisms producing soybean saponinhydrolase. J AgricBiolChem., 1990, 54:3035-3037.


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