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Evaluation of some plant extracts for controlling mycoflora causing spoilage of stored cereals and legumes

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Abstract

This study aimed to isolate and identify seed-borne fungi associated with some cereals and legumes and the possibility of their control by some plant extracts. The studied cereals and legumes were *Sorghum bicolor*, *Triticum aestivum*, *Oryza sativa*, *Lens esculentus*, *Vigna sinensis*, *Arachis hypogea* and *Vicia faba*. Thirteen fungal species were isolated from these cereals and legumes. Five fresh plants called *Allium sativum*, *Aloe vera*, *Mentha basilicum*, *Musa acuminate* and *Eucalptus rostrata* and eleven dried plants called *Datura stramonium*, *Zingiber officinale*, *Azadirachta indica*, *Jatropha curcas*, *Euphorbia peplis*, *Ocimum basilicum*, *Carum carvi*, *Rosmarinus officinalis*, *Nigella sativa*, *Cuminum cyminum* and *Citrullus colocynthis* as medicinal plants were screened for their antifungal activities. Aqueous extracts of all mentioned plants were tested against *Aspergillus niger*, *A. flavus* and *Penicillium chrysogenum in vitro* because they represented the highest percentage of occurrence on seeds and grains. *Allium sativum* and *Zingiber officinale* exhibited maximum antifungal activity, whereas *Datura stramonium* and *Citrullus colocynthis* showed moderate activity. In addition, these plant extracts were tested against the germination of seeds and grains by using blotter plate method. Treated seeds and grains by plant extracts showed an increase of their germination (%) and reduction of seed-borne fungal infection.

Keywords: Antifungal activity, Cereals, Legumes, Medicinal plants, Seed-borne mycoflora

Introduction

Legumes (Fabaceae) are one of the most important plant families all over the world. It is used for human feeding, animals, poultry and other consumption. Legumes are the second after grasses (cereals) in providing food crops for world agriculture [1]. In comparison to cereal grains, the seeds of legumes are rich in high quality protein, providing man with a highly nutritious food resource [2].

Cereal grains have been the principal component of human diet for thousands of years and have played a major role in shaping human civilization. Around the world, rice, wheat, and maize, and to a lesser extent, are important

staples critical to daily survival of billions of people. More than 50% of world daily caloric intake is derived directly from cereal grain consumption [3].

Seed-borne fungi have been found to affect the growth and productivity of crop plants including legumes and cereals [4, 5]. Presence or absence of seed-borne fungi on seed surface is one of the important aspects that determine the quality of seeds and grains. Attempts have been made to reduce seed-borne fungi by chemical treatment of the seeds and some successes have been reported. Seed dressings are used to eliminate most surface infestation of seeds but have relatively little effect on internally borne organisms [6]. However, the application of these fungicides may not always be desirable, owing to their toxic effects on non-target organisms [7].

An urgent need for alternatives to fungicides for the control of seed-borne fungi are important In recent years, much attention has been given to nonchemical systems for seed treatment to protect them against seed-borne pathogens. Plant extracts have played a significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds. Many authors reported the effective and safe use of plant extracts for controlling seed-borne fungi [8-11]. However, in this study, the effects of water extracts of some plants on seed-borne fungi of some legumes and cereals; and on seed germination were investigated.

Materials and methods

Collection of legumes and cereals

Some stored cereals and legumes of economically important crops were collected from some markets in Damietta Governorate, Egypt such as sorghum (*Sorghum bicolor*), wheat (*Triticum* aestivum), rice (*Oryza sativa*), lentil (*Lens esculentus*), cowpea (*Vigna sinensis*), peanut (*Arachis hypogea*) and faba bean (*Vicia faba*).

Isolation and identification of Fungi

Fungal isolation from collected samples was carried out by using direct plating method. First, grains and seeds were surface sterilized using 2.5% sodium hypochlorite for 1 min and rinsed with distilled water and dried between sterile Whatman No. 1 filter papers. Then, five grains or seeds spaced out in the Petri dishes containing PDA medium (200 g potato + 20 g glucose + 20 g agar in liter distilled water). An antibacterial agent chloramphenicol (50 ppm) was used to inhibit the growth of bacteria. The Petri dishes were incubated at 27±2 ºC for 5-7 days and examined daily for fungal growth [12]. The observed fungal colonies were sub-cultured to get pure culture. All isolates were identified according to their microscopic and macroscopic characteristics according to some authors [13-19].

Preparation of plant extracts

For dried samples, the plant materials were air-dried under the shade at 25-29ºC until they became dry and crispy. Dried parts of the plants (Table 1) were ground using a blender and sieved to remove coarse particles. Ten grams from each fine powdered plant material was placed in a 250 ml beaker and extracted with 25 ml of distilled water for 48 hr. Mixture was vigorously stirred for 5 min. three times per day to prevent decay of water extracts. For fresh samples, water extract of fresh samples were prepared as follow: ten grams of each plant material were cut into small pieces using a sharp knife. The cutting plant material was mixed with 25 ml of distilled water by using electrical blender. The homogenate was placed in a closed container and was left for 48 hr. [20].

Assay of antifungal activity

The antifungal activity assay was carried out by the standard method according to Gurgel *et al*. [21]. Potato dextrose agar media were sterilized by autoclaving at 121°C for 15 min and cooled to 45°C. Then, about 20 ml of PDA medium was poured into Petri dishes and allowed to solidify. Plant extracts were tested against *Penicillium chrysogenum, Aspergillus niger* and *A. flavus* because they exhibited the highest percentage of occurrence on seeds and grains. The fungus was spread over the media by using sterile cotton swab. Five-mm diameter of sterile filter paper (Whatman No.3) discs previously soaked in aqueous plant extract (400 and 500 mg l^{-1}) were placed on the surface of the plates. The plates were incubated at 28°C for 48-72 hr. The experiment was performed in triplicates to minimize the error ratio. At the end of incubation period, the inhibition zones around each disc

were measured to an accuracy of 0.1 mm and the effect was calculated as a mean of triplicate tests to evaluate the antifungal activity.

Effect of plant extracts on incidence of fungi and seed germination

Three replicates of 25 seeds per Petri-dish for each of the treated seeds including the controls were plated using the Blotter method as recommended by Mathur and Kongsdal [22]. These gave a total of 75 seeds for each treatment. They were then incubated for 7–10 days and then examined for seed-borne fungi. Records on incidence of seed-borne fungi and germination of treated seeds were then taken.

Statistical analysis

Analysis of variance (ANOVA) was performed on all transformed data (i.e. using the arcs in percentage transformation) collected in respect of parameters studied on effects of plant extracts and separation of treatment means was done using the LSD at 5% level of significance.

Results

Fungi isolated from grains and seeds

Results obtained in Table 1 showed that thirteen fungal species were isolated from different seeds and grains. *Aspergillus niger*, *A. flavus* and *P. chrysogenum* showed the highest percentage of occurrence.

Antifungal activity of plant extracts against fungal species

Warm water extracts of five fresh plant samples namely Garlic, Aloe, Peppermint, Banana and Camphor were tested against some fungal isolates (*Aspergillus niger*, *A. flavus* and *P. chrysogenum*), only Garlic bulb had the highest antifungal activity. It is worth to mention that the antifungal activity of Garlic extract varied from one fungal species to another. The diameter of inhibition zone for *Aspergillus niger, A. flavus* and *P. chrysogenum* were 22.83, 12.50 and 10.83 mm at 400 mg ml^{-1}, while at 500 mg ml^{-1} they were 24.33, 18.0 and 13.17 mm, respectively (Fig. 1).

Table 1. List of the isolated fungal species from grains and seeds.

Isolated fungal species	Grains and Seeds							
	Sorghum bicolor	*Triticum aestivum*	*Oryza sativa*	*Lens esculentus*	*Vigna sinensis*	*Arachis hypogaea*	*Vicia faba*	% of occurrence
Acremonium gramineum	-	5.4	-	-	-	-	-	5.4
Alternaria alternata	5.4	-	-	-	-	-	-	5.4
Aspergillus flavus	-	-	-	-	-	8.1	-	8.1
Aspergillus niger	2.7	-	-	2.7	-	2.7	2.7	10.8
Cladosporium herbarium	-	2.7	-	-	-	-	-	2.7
Curvularia lunata	2.7	-	-	-	-	-	-	2.7
Epicoccumn nigrum	-	-	2.7	-	-	-	2.7	5.4
Fusarium moniliforme	2.7	-	-	-	2.7	-	-	5.4
Mycelia sterilia	-	2.7	-	-	-	-	-	2.7
Penicillium chrysogenum	5.4	8.1	2.7	8.1	2.7	5.4	8.1	40.5
Rhizopus oryzae	-	-	-	2.7	-	-	2.7	5.4
Rhizopus stolonifer	-	2.7	-	-	-	-	-	2.7
Trichothecium roseum	-	-	2.7	-	-	-	-	2.7

- =absence

The antifungal activities of eleven dried plant water extracts (Datura, Ginger, Bitter apple, Jatropha, Basil, Neem, Caraway, Rosemary, Euphorbia, Black seeds and Cumin) were tested against the fungal species. Datura leaves extract exhibited the highest antifungal activity against all tested fungi except *Aspergillus niger*. The highest sensitive species to water extract of Datura leaves were *Aspergillus flavus* and *Penicillium chrysogenum* with inhibition zone of 12.50 and 12.33 mm at 400 mg ml^{-1}, and 14.0 and 20.83 at 500 mg ml^{-1}, respectively (Fig. 2).

Water extract of dried powdered Ginger rhizome exhibited antifungal activity against all tested fungi with inhibition zone of 8.83, 8.167 and 8.0 mm at 400 mg ml^{-1}, and 9.83, 11.67and16.83 at 500 mg ml^{-1}, respectively (Fig. 3). Water extract of dried bitter apple fruit showed antifungal activity against *Penicillium chrysogenum* only with inhibition zone of 22.83 mm at 500 mg ml^{-1} (Fig. 4).

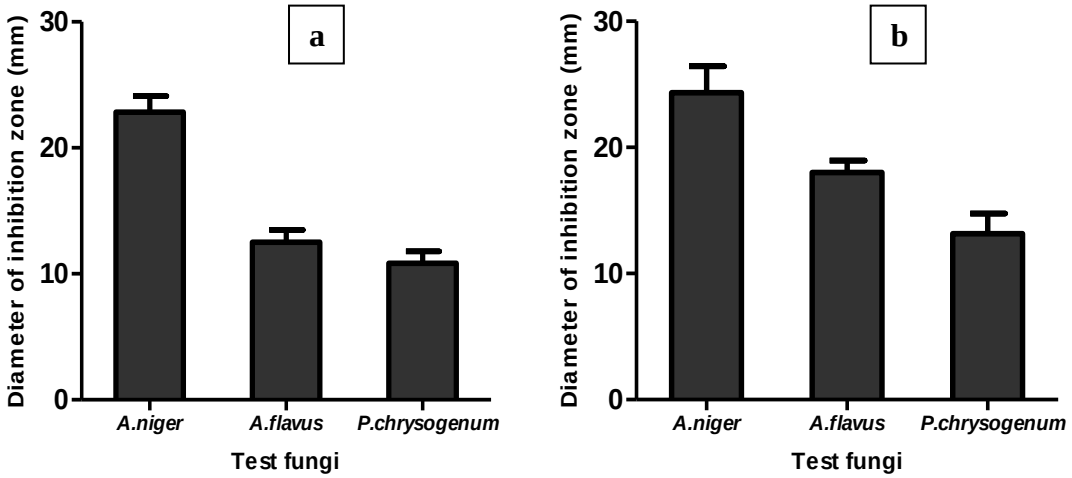

Fig. 1 Antifungal activity of fresh *Allium sativum* bulb water extract at 400 mg ml^{-1} (a) and 500 mg ml^{-1} (b) against fungal species.

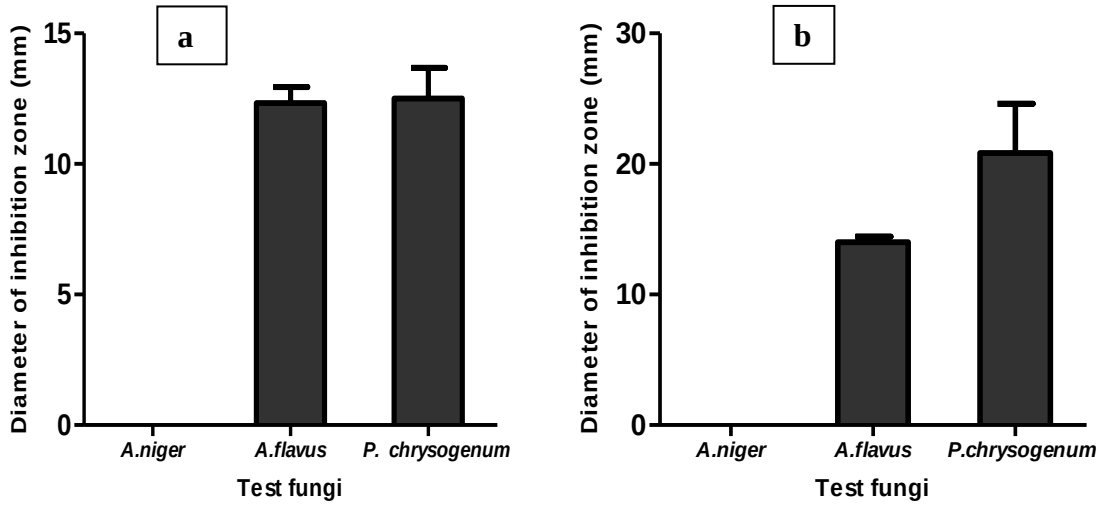

Fig. 2 Antifungal activity of dried *Datura stramonium* leaves water extracts at 400 mg ml^{-1} (a) and at 500 mg ml^{-1} (b) against fungal species.

Effect of plant extracts on seeds and grains germination and fungal growth.

Results obtained in Table 2 showed the effect of different plant extracts on seeds and grains germination and fungal growth. In this experiment, four different plant extracts were used to controlling seed-borne fungi on seeds and grains which were Bitter apple, Ginger, Datura and Garlic. Sorghum grains treated with these extracts showed high seed germination with 93.3, 96.0, 97.3 and 94.7 %, respectively, while the untreated (control) grains showed 90.7 %. The fungal growth appeared only on grains treated with Bitter apple extract with 72.0 %, while the control was 96.0 %. Wheat grains treated with these extracts showed high grain germination with 88.0, 94.7, 92.0 and 93.3 %, respectively, while the control was 65.3%. The fungal growth appeared only on the grains treated with Bitter apple extract with 34.7 %, while the control was 90.7 %. Rice grains treated with these extracts

showed no germination. The fungal growth appeared only on grains treated with Bitter apple extract with 33.3 %, while the control seeds were 53.3%.

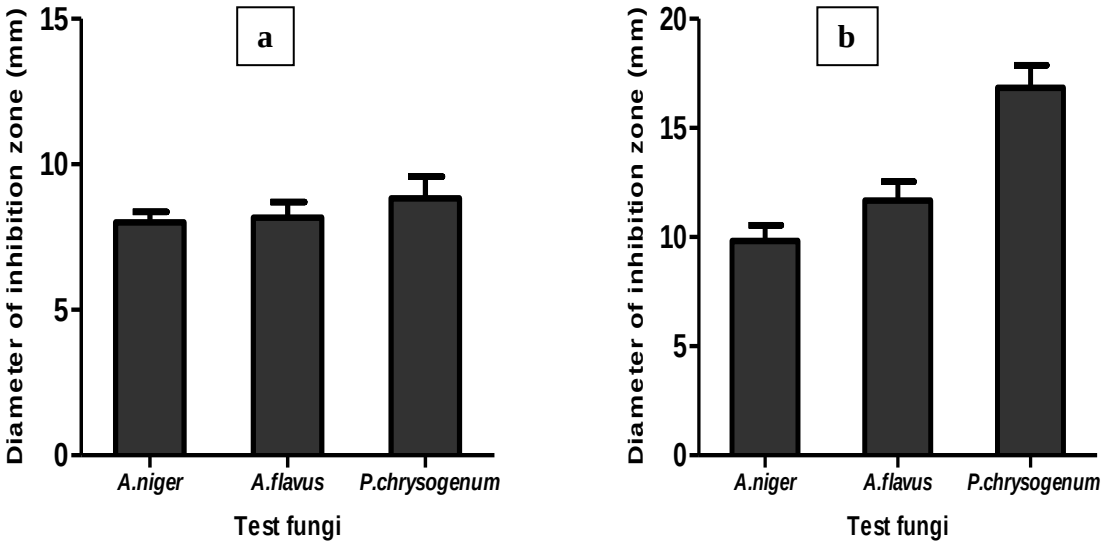

Fig. 3 Antifungal activity of dried *Zingiber officinale* powder extracts at 400 mg ml^{-1} (a) and at 500mg ml^{-1} (b) against fungal species.

Lentil seeds treated with these extracts showed high seed germination with 80.0, 98.7, 96.0 and 98.7%, respectively, while the control seeds were 73.3 %. The fungal growth appeared only on the seeds treated with bitter apple and Datura extracts with 26.7 and 53.0 %, respectively, while the control was 88.0 %. Cowpea seeds treated with these extracts showed high seed germination with 77.3, 93.3, 90.7 and 97.3 %, respectively, while the control was 60.0 %. The fungal growth appeared only on the seeds treated with Bitter apple and Datura extracts with 17.3 and 8.0 %, respectively, while the control was 61.3%. Faba been seeds treated with these extracts showed high seed germination with 78.7, 93.3, 90.7 and 98.7, respectively, while the control seeds was 77.3 %. The fungal growth appeared only on the seeds treated with Bitter apple extract with 48.1 %, while the control was 93.3%.

Discussion

Seeds and grains play a vital role in the production of healthy crops. Healthy seeds and grains is the foundation of healthy plant; a necessary condition for good yields [23]. Many pathogenic fungi are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the productivity. Therefore, control of seed-borne fungi is extremely important and the damaging effects can be relieved through integrated approaches [24].

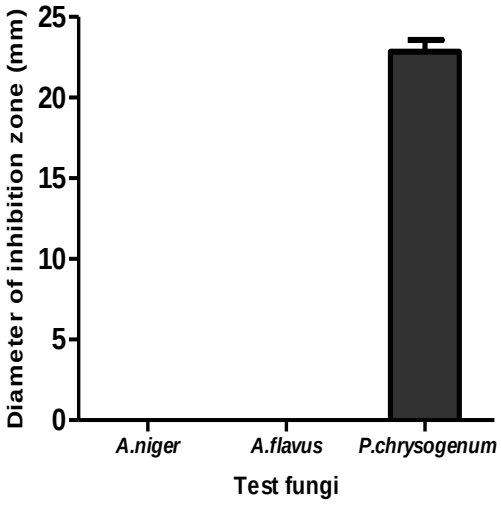

Fig. 4 Antifungal activity of water extracts of dried *Citrullus colocynthis* fruit at 500 mg ml^{-1} against fungal species.

Deteriorations by fungi are due to unhygienic conditions of storage and this in turn is associated with initial high moisture content of the stored products or absorption of moisture during storage due to defects in the storage system [25]. In the present study, the most

common fungi isolated from seeds and grains were *Aspergillus niger*, *A. flavus* and *Penicillium chrysogenum*. These fungal species are very important for different reasons; they cause deteriorations in stored commodities [26-29], bring about reduction in the quality and quantity of agricultural products in storage and transit and also create health hazards in animals and human beings by producing toxic metabolites in the form of mycotoxins in the stored commodities [30,31]. *Penicillium chrysogenum* is a common fungus that can inhabit a wide variety of habitats [32].

From all samples extracted from fresh plants, only Garlic bulb exhibited the highest antifungal activity against isolated fungi. Lawson [33] reported that the most abundant sulfur compound in Garlic is allicin (S-allylcysteine sulfoxide), which is present at 10 mg g^{-1} fresh Garlic or 30 mg g^{-1} dry weight. Various chemical constituents in garlic products, including non-sulfure compounds such as saponins, may contribute to the essential biological activities of garlic [34].

Datura leaves aquous extract possessed antifungal activity against all tested fungi except *Aspergillus niger*. Datura plants contain tropane alkaloid such as hyoscyamine, scopolamine and atropine but the seeds, leaves and the flowers contain the highest level of alkaloids. Besides its hallucinogen activity, Datura reported to have antimicrobial activity [35, 36]. Hussain *et al.* [37] reported that the leaf extract of *Datura stramonium* reduced the development of rust pustules on the leaves of wheat. The inhibitory effect of the plant extracts might be attributed to the presence of antifungal compounds. Water extract of ginger rhizome exhibited antifungal activity against all tested fungi. Ficker *et al.* [38] reported that gingerols and gingerdiol are the main antifungal principles presented in ginger rhizomes. Water extract of bitter apple fruit showed antifungal activity against *Penicillium chrysogenum* only. The antifungal effect of bitter apple attributed to the presence of the active compounds colocynthidin and colocynthin alkaloids which may be disrupt cytoplasmic membrane of the microorganisms through their action on lipids and protein [39].The present results indicated that the treated seeds and grains with plant extracts gave very good effects on their germination and also reducing seed-borne infections.

Table 2. Screening of plant species for antifungal activities

Scientific name	Common name	Family name	Part used	Antifungal activity (mm)
Allium sativum L.	Garlic	Liliaceae	Bulb (Fresh)	10.83-24.33
Aloe vera L.	Aloe	Liliaceae	Leaves (Fresh)	-
Azadirachta indica A. Juss	Neem	Meliaceae	Seeds (Dry)	-
Carum carvi L.	Caraway	Apiaceae	Seeds (Dry)	-
Eucalyptus rostrata L.	Camphor	Myrtaceae	Leaves (Fresh)	-
Citrullus colocynthis L.	Bitter apple	Cucurbitaceae	Fruits (Dry)	22.83
Cuminum cyminum L.	Cumin	Apiaceae	Seeds (Dry)	-
Datura stramonium L.	Datura	Solanaceae	Leaves (Dry)	12.33-20.83
Euphorbia peplis L.	Euphorbia	Euphorbiaceae	Leaves (Dry)	-
Jatropha curcas L.	Jatropha	Euphorbiaceae	Leaves and seeds (Dry)	-
Mentha piperita L.	Peppermint	Lamiaceae	Leaves (Fresh)	-
Musa acuminate L.	Banana	Musaceae	Leaves (Fresh)	-
Nigella sativa L.	Black seeds	Ranunculaceae	Seeds (Dry)	-
Ocimum basilicum L.	Basil	Lamiaceae	Leaves and seeds (Dry)	-
Rosmarinus officinalis L.	Rosmary	Lamiaceae	Leaves (Dry)	-
Zingiber officinale Rosc.	Ginger	Zingiberaceae	Rhizome (Dry)	8.0-16.83

- = no antifungal activity

These results are in agreement with the findings of [40] who found that garlic extract was superior in terms of reducing seed-borne infections by *A1ternaria* spp., *Bipolaris sorokiniana*, *Curvularia lunata* and *Fusarium* spp. infecting wheat grains. Mondall *et al*. [41]

reported that seed treated with the Garlic extract, reduced seed-borne prevalence and increased germination percentage of wheat seeds. In addition, aqueous extracts of Garlic bulb and ginger rhizomes were significantly exhibited antifungal activity against all tested fungi. These aqueous extracts were strongly inhibited seed infection.

The treated seeds and grains with the extracts of Datura showed very low infection by fungi rather than bitter apple. The effect of extracts depends on their concentrations. These results are in agreement with the findings of Reddy *et al.* [42]. They reported that the plant extracts completely inhibited *A. flavus.* Some important seed-borne pathogens like *Fusarium oxysporum, Aspergillus niger, A. flavus, Penicillium* spp. and *P. vexans* can be managed by using some plant extracts [43]. Exploitation of naturally available chemicals from plant protection would be a prominent role in development of future commercial pesticides for crop protection strategies, with special reference to manage plant diseases [44].

Conclusion

The study confirms that the natural plant extracts may be safer for human health than chemical fungicides to combat the fungi that cause deterioration of stored grains and seeds. The using of these extracts can also reduce wastage during storage process, as well as increasing seed germination during agriculture. Datura, bitter apple, garlic and ginger extracts are recommended as antifungal agents for preserving grains and seeds.

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الملخص العربى

تقييم بعض المستخلصات النباتية لمقاومة الفلورا الفطرية المسببة لفساد الحبوب والبقوليات المخزنة

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تهدف الدراسة إلى عزل وتعريف بعض الفطريات المسببة لفساد بعض الحبوب والبقوليات واستخدام بعض المستخلصات النباتية لتثبيط نمو هذه الفطريات ومعرفه تأثير هذه المستخلصات على نمو الحبوب والبذور وقدرتها على اختزال النمو الفطري. وقد تم دراسة حبوب وبذور نباتات الذرة العويجة والقمح والأرز والعدس واللوبيا والفول البلدى والفول السودانى. وقد تم عزل ثلاثة عشر فطرا من على هذه الحبوب والبذور وكانت أكثرها حدوثا هي اسبرجليس نيجر واسبرجليس فلافس وبنيسيليوم كريسوجينم. وقد تم اختبار مستخلصات خمسة نباتات طازجة وهى فصوص الثوم والصبار والنعناع الفلفلى والموز والكافور وأحدا عشر نباتا جافاً وهى الزنجبيل والنيم والجاتروفا واللبينة والريحان والكراوية واكليل الجبل والحبه السوداء والكمون والحنظل لمعرفة أيهم أكثر فعالية على هذه الفطريات. أظهرت الدراسة أن المستخلصات النباتية المائية لكل من فصوص الثوم وأوراق الداتورا وريزومات الزنجبيل وثمرة الحنظل لها قدره عالية في تثبيط نمو الفطريات الأكثر حدوثا. كما أظهرت الدراسة أن المستخلصات المائية لكل من فصوص الثوم وريزومات الزنجبيل كان لهما قدره عالية في تثبيط الفطريات الثلاثة بينما ثبط المستخلص المائى لأوراق الداتورا فطرين فقط هما اسبرجليس فلافس وبنيسيليوم كريسوجينم بينما ثبط المستخلص المائى لثمار الحنظل فطراً واحداً فقط وهو بنيسيليوم كريسوجينم. وعند معالجه الحبوب بالمستخلصات النباتية بعد إنباتها لمده عشرة أيام وجد ان هذه المستخلصات تنشط إنبات هذه الحبوب والبذور كما انها تختزل نمو الفطريات عليها عند مقارنتها بالحبوب غير المعالجة.