Mycobiota Associated with Imported Seeds of Vegetable Crops in Sudan

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Abstract: Seedborne fungi present on or inside the seed or as contaminant with the seed debris adversely affect seed viability, germination, emergence, plant growth vigour and eventually production and productivity. In the present study, seedborne fungi of 7 imported vegetables were examined. 29 species of fungi belonging to 23 genera were recovered from the seeds. Of these fungi, 6 species and one genus are new records to the mycoflora of Sudan, whereas different species are new to the micobiota of these vegetables. These include 15 species for Beta vulgaris, Petroselinum crispum (14 species), Solanum melongena (12 species), Portulaca oleracea (8 species and 1 genus), Eruca sativa (5 species and 3 genera), Corchorus olitorius (2 species), and Cucumis sativa (1 species). The seeds were highly contaminated with saprophytic and pathogenic fungi (25-100%), which apparently inhibited seed germination (15-79%) and seedling emergence (12-75%) of these vegetables. The genus Aspergillus and Drechslera (4 species each) were the most prevalent genera followed by Alternaria, Curvularia, Fusarium, Pithomyces (2 species) and 1 species for the remaining genera (Aureobasidium, Beltrania, Chaetomium, Cladosporium, Mennoniella, Microascus, Rhizopus, Sordaria, Stachybotrys, Stemphylium, Trichothecium, Ulocladium, and Wolleria). Therefore, there is urgent need for development of proper standard laboratory seed testing methods, fungal eradication measures, and adopting strong legislations and quarantine regulations. The use of certified and high grade seeds is a priority.

Keywords: Beta vulgaris, Corchorus olitorius, Cucumis sativa, Eruca sativa, Petroselinum crispum, Portulaca oleracea, Seedborne fungi, Solanum melongena, Sudan.

INTRODUCTION

Vegetables are the fresh edible parts of herbaceous plants. They are important food of significant nutritive value for their high carbohydrates, vitamins, fibers, mineral contents, which associated with maintenance of health and disease prevention. They may be edible roots, stems, leaves, fruits or seeds [1-3]. Many studies have reported their uses and consumption methods [4-6].

In Sudan, various vegetables are grown in both irrigated and rain-fed plots, in a total area of about 273000 hectares which represents 3% of the total cultivated area with average production of about 3.4 million tons of vegetables [7]. Generally, the horticultural crops represent 12% of the national agricultural income compared to 17% cotton and 29.6% cereals and oil seeds. Introduction of exotic species and varieties of vegetables started early, mainly from Egypt, USA, Denmark, Holland, France and other countries [8]. Beta vulgaris L. (Beet, Selg) of the family Amaranthaceae is known as beet root and the leaves and roots eaten as salads or pickled, and as folk medicine in many parts of the World. Corchorus olitorius L. (Malvaceae), jute, Jew mallow “Molukhiyah” in Arabic, “Khudra” means “green” in Sudanese Arabic, is cooked as green leaf vegetable in most countries of the Middle East, North Africa, and some African countries. The seeds and leaves extracts of C. olitorius possess antibacterial activity against wide range of bacterial species [9, 10]. Cucumis sativa L. (Cucumber) of the family Cucurbitaceae is a common cultivated vegetable with many different varieties and grown in green houses and outdoors. Eruca sativa Mill (Brassicaceae) is known as garden rocket or “Gargeer” in Arabic and is cultivated in many places throughout the World as vegetable salad. Petroselinum crispum Mill (Parsley) of the family Apiaceae is an herb that is widely cultivated in temperate, subtropical and tropical areas as spice and vegetable salad. Portulaca oleracea L. (Portulaceae) which is known as purslane “Rigla” or “Begla” in Arabic is edible and used fresh as salad or cooked as a vegetable similar to Spinach. It is also used in traditional Chinese folk medicine and in many regions it is considered as invasive weed [11]. It has been reported to have antifungal and antibacterial activities [12-14]. Solanum melongena L. (Eggplant, Aubergine), Bazjan” in Arabic belongs to Solanaceae with raw fruits cooked or roasted in oil until charred and used as food for many countries in Asia, Middle East, North and South Africa.

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Fungi are the principal organisms associated with seeds causing devastating effects on seed germination, seedlings emergence, plant growth vigour and eventually quality and quantity of the production. In the Sudan, seeds for cultivation of vegetables are imported from different countries of various climatic conditions. Therefore, various studies were conducted on vegetables diseases aiming at improving their productivity and to enhance their uses and market value. However, the seedborne fungi of *Beta vulgaris* seedborne and seed transmitted causing many diseases to On the other hand, numerous fungi were reported as phylloplane and seedborne mycoflora of *C. olitorius* were reported [25, 26]. Many fungi were either isolated from the seeds of *E. sativa* or as foliar and root diseases [32-36]. In Sudan, there is increasing demand for production of high quality vegetable crops for both local consumption and export. This depends mainly on the use of healthy seeds which are free from seedborne pathogenic fungi. Therefore, seed health testing for fungi is an important practice and step towards management of plant diseases [37]. In Sudan, vegetable seeds are imported by local seed companies under low level of quarantine regulations and seed testing facilities. Besides, no research has been conducted on the seedborne mycoflora of these vegetables and little to negligible information available on their diseases. Therefore, the present study was undertaken to improve recognition and to assess the quality and the incidence of seedborne mycobiota associated with seven imported vegetable crops namely *Beta vulgaris*, *Corchorus olitorius*, *Cucumis sativa*, *Eruca sativa*, *Petroselinum crispum*, *Portulaca oleracea*, and *Solanum melongena* and to evaluate their effect on seed germination and seedlings emergence levels. This will enrich our knowledge about the nature of these fungi and contribute to effective control measures through adopting strong quarantine regulations and efficient seed testing methods.

**METHODS**

**Collection of the Seed Samples**

The seed samples of 7 vegetable crops, namely *Beta vulgaris* (Beet, Selg), *Corchorus olitorius* (Jew mallow), *Cucumis sativa* (Cucumber), *Eruca sativa* (Rocket salad), *Petroselinum crispum* (Parsley), and *Portulaca oleracea* (Purslane, Rigla), which were imported from Egypt and *Solanum melongena* (Eggplant) from Holland (Netherlands), were purchased from seed companies in Khartoum State, Sudan. The working samples were drawn and examined according to rules of the International Seed Testing Association [38].

**Seed Germination**

In this study, the blotter method was used according to the rules of the International Seed Testing Association [38]. For this, 400 seeds from each sample were inoculated on sterilized moistened filter paper in Petri dishes (Blotter). The seeds were spaced according to their size at equal distance. The inoculated plates were incubated in Gallenkamp illuminated incubator at 26°C under alternating cycle of 12 hours near ultraviolet light and darkness to stimulate fungal sporulation of many of the seedborne fungi [38]. The incubated seeds were kept moistened by adding sterile distilled water throughout the incubation period (7-15 days) and the percentage of seed germination was recorded.

**Emergence of Seeds in Soil**

For testing the emergence levels of the seeds from the soil, 200 seeds from each type of the selected vegetables were sown in pots filled with uniform mixture of sand and silt (2:1). The seeds were covered with soil layer of 1-3 cm deep depending on the seed size. The seeds were sown at the rate of 20 seeds per pot and were kept in the Botanical garden of the Department of Botany, University of Khartoum, which is of partial shade and average temperature of between 27°C and 29°C. The average percentage of seed emergence was recorded for each vegetable crop.

**Isolation and Estimation of Fungi**

For isolation of the seedborne fungi, routine agar plate method which was suggested by many authors was adopted [39-41]. In this method, 400 seeds from each sample were surface disinfected in 1% sodium hypochlorite for 5 min and washed with several changes of sterile distilled water. The treated seeds were then inoculated aseptically on Potato Dextrose Agar (PDA) and incubated at 28°C±2°C for 1-2 weeks. Then, the colonies of fungi which were developed around the seeds were examined, identified microscopically and the average levels of contamination and fungi incidence were recorded. The identification of the isolated fungi was confirmed using many taxonomic books, monographs and taxonomic papers [42-49]. For non-sporulating fungi, mycelial fragments were inoculated on Malt Extract Agar (MEA) and incubated at 28°C ± 2°C to stimulate sporulation of these fungi and were then identified to species level. Some of these fungi were illustrated (Appendices: Fig. 1-22).

**RESULTS AND DISCUSSION**

Twenty nine species of fungi belonging to 23 genera were isolated from seeds of the 7 imported vegetables viz. *Beta vulgaris* (Beet, Selg), *Corchorus olitorius* (Jew mallow), *Cucumis sativa* (Cucumber), *Eruca sativa* (Rocket salad), *Petroselinum crispum* (Parsley), *Portulaca oleracea* (Purslane, Rigla), and *Solanum melongena* (Eggplant) (Table 1). Of these isolates, 6 species and one genus are new records for the mycoflora of Sudan, whereas different species are considered new for the seeds of each vegetable (Table 1). These seeds were highly contaminated with saprophytic and potentially pathogenic fungi (25-100%) and exhibited very low levels of seed germination (15-79%) and seedling emergence (12-75%) (Table 2). The genus *Aspergillus* and *Drechslera* (4 species each) were the most dominant genera followed by *Alternaria*, *Curvularia*, *Fusarium*, *Pithomyces* (2 species) and one species for the...
Table 1. Incidence percentage of fungi in different vegetables seeds.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Pathogenic and saprophytic nature of the fungi</th>
<th>Incidence%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Record Type</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Alternaria alternata (Fig. 1)</td>
<td>P³</td>
<td>1</td>
</tr>
<tr>
<td>Alternaria citri (Fig. 2)</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>PS⁸</td>
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</tr>
<tr>
<td>Aspergillus flavus</td>
<td>P</td>
<td>8.5</td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td>P</td>
<td>10.25</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>P</td>
<td>3.75</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>S⁴</td>
<td>1</td>
</tr>
<tr>
<td>Aureobasidium pullulans (Fig. 3)</td>
<td>S</td>
<td>2.75*</td>
</tr>
<tr>
<td>Beltrania santapai (Fig. 4)</td>
<td>S</td>
<td>NS¹</td>
</tr>
<tr>
<td>Chaetomium sp.</td>
<td>PS</td>
<td>-</td>
</tr>
<tr>
<td>Chaetomium globosum (Fig. 5)</td>
<td>P</td>
<td>2.25*</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>PS</td>
<td>2.5</td>
</tr>
<tr>
<td>Cladosporium cladosporiodes (Fig. 6)</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Curvularia lunata (Fig. 7)</td>
<td>P</td>
<td>2.25*</td>
</tr>
<tr>
<td>Curvularia pallescens (Fig. 8)</td>
<td>P</td>
<td>1.0*</td>
</tr>
<tr>
<td>Drechslera hawaiensis (Fig. 9)</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>Drechslera papendorfii (Fig. 10)</td>
<td>P</td>
<td>2.0*</td>
</tr>
<tr>
<td>Drechslera spicifera (Fig. 11)</td>
<td>P</td>
<td>1.0*</td>
</tr>
<tr>
<td>Drechslera rostrata (Fig. 12)</td>
<td>P</td>
<td>2.0*</td>
</tr>
<tr>
<td>Eurotium sp.</td>
<td>PS</td>
<td>1.0</td>
</tr>
<tr>
<td>Fusarium semitectum (Fig. 13)</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium solani (Fig. 14)</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>Lophotrichas sp.</td>
<td>PS</td>
<td>NS</td>
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<td>Memnoniella echinata (Fig. 15)</td>
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</tr>
<tr>
<td>Microascus trignosporus (Fig. 16)</td>
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<tr>
<td>Penicillium spp.</td>
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<td>1.25</td>
</tr>
<tr>
<td>Phoma sp.</td>
<td>PS</td>
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<tr>
<td>Pithomyces chartarum (Fig. 17)</td>
<td>P</td>
<td>NS</td>
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Table 1. contd…

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Pathogenic and saprophytic nature of the fungi</th>
<th>Incidence%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Record Type</td>
<td>Beta vulgaris (Beet, Sek)</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Rhizopus stolonifer</td>
<td>S</td>
<td>2.0</td>
</tr>
<tr>
<td>Sordaria fimicola</td>
<td>S</td>
<td>NS</td>
</tr>
<tr>
<td>Stachybotrys aurantes (Fig. 19)</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Stemphylium botryosum (Fig. 20)</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>Trichothecium roseum (Fig. 21)</td>
<td>S</td>
<td>1.25*</td>
</tr>
<tr>
<td>Ulocladium botrytis (Fig. 22)</td>
<td>S</td>
<td>5.75*</td>
</tr>
<tr>
<td>Wellernia sebi</td>
<td>S</td>
<td>NS</td>
</tr>
<tr>
<td>Mycelia sterilia</td>
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<td>3.0</td>
</tr>
</tbody>
</table>

1*: Not detected.
2*: New record for the vegetable crop.
3*: New record for the mycoflora of Sudan.

Table 2. Average contamination, germination and emergence of different vegetables seeds.

<table>
<thead>
<tr>
<th>Seed Types</th>
<th>Contamination %</th>
<th>Germination %</th>
<th>Emergence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta vulgaris</td>
<td>55</td>
<td>65</td>
<td>20</td>
</tr>
<tr>
<td>Corchorus olitorius</td>
<td>25</td>
<td>72</td>
<td>70</td>
</tr>
<tr>
<td>Cucumis sativa</td>
<td>100</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Eruca sativa</td>
<td>35</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td>Petroselinum crispum</td>
<td>40</td>
<td>64</td>
<td>60</td>
</tr>
<tr>
<td>Portulaca oleracea</td>
<td>30</td>
<td>79</td>
<td>75</td>
</tr>
<tr>
<td>Solanum melongena</td>
<td>27</td>
<td>77</td>
<td>60</td>
</tr>
</tbody>
</table>

remaining genera (Aureobasidium, Beltrania, Chaetomium, Cladosporium, Memnoniella, Microascus, Rhizopus, Sordaria, Stachybotrys, Stemphylium, Trichothecium, Ulocladium, and Wellernia).

Many researchers are studying the seedborne and plant pathogenic fungi of different crops including vegetables. Of the negligible studies of B. vulgaris (Beet), Pérez Vicente et al. (2011) [24] stated that Verticillium dahliae and V. albo-atrum are seedborne and seed transmitted of this vegetable. A number of fungi were reported causing different diseases to this plant which include; Phoma betae (Leaf spot) [22]; Fusarium oxysporum f.sp. betae (Fusarium yellow) [23]. Cercospora beticola (Leaf spot) [50]. In the present study, 15 species and 12 genera Aureobasidium: pullulans, Beltrania santapui, Chaetomium globosum, Curvularia lunata, C. pallescens, Drechslera papendorffii, D. spicifera, D. rostrata, Lophothrix sp., Memnoniella echinata, Microascus trignosporus, Pithomyces chartarum, Sordaria fimicola, Trichothecium roseum, and Ulocladium botrytis were isolated for the first time as seedborne fungi of beet (Table 1). In the study of the phylloplane mycoflora of Corchorus olitorius [25] reported a number of fungi including Alternaria, Aspergillus, Cladosporium, Fusarium and Penicillium. In similar study, many fungi were reported as seedborne of C. olitorius such as Aspergillus spp. Penicillium spp., Macrophomina phaseolina, Fusarium oxysporum, F. moniliforme (G. fujikuroi) and Sclerotium rolfsii (Corticium rolfsii) [19]. In this investigation, 8 species of fungi (Alternaria alternata, Aspergillus spp., Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aureobasidium
pullulans, Chaetomium globosum, Memnoniella echinata) were isolated from the seeds of C. olitorius and of these fungi Chaetomium globosum and Memnoniella echinata are new records to Jew mallow (Table 1). Some of the previously reported as seedborne and pathogenic fungi were recovered from the current seeds [9, 25] (Table 1). For cucumber (Cucumis sativus), 7 seedborne fungi were isolated from this vegetable in Bangladesh [28]). These include Aspergillus flavus, A. niger, Fusarium oxysporum, F. moniliforme, Penicillium spp., Doratomyces sp. and Rhizopus stolonifer which ranged from 1 to 17%. Also A. flavus, A. versicolor, Chaetomium globosum, Cunninghamamella sp., Curvularia lunata, Fusarium oxysporum, F. solani, F. verticillioidei, Myrothecium roridum, Phoma spp., and Trichurus sp., were reported as seedborne of cucumber [27]. Moreover, Fusarium solani, Fusarium spp., A. niger, Colletotrichum spp., Macrophomina phaseolina, Rhizoctonia spp. were reported as seedborne fungi on cucumber seeds [29, 51, 52]. In the present study, Drechslera rostrata was newly isolated from cucumber seeds and some of the previously recorded seedborne fungi such as were recovered [27-29, 51, 52] (Table 1). In similar studies of seedborne fungi of Eruca sativa, A. flavus, A. niger, Penicillium chrysogenum, P. funiculosum, and Rhizopus stolonifer were reported as seedborne species [30]. Gibberella fujikuroi was also reported as seedborne fungus [31], whereas other fungi were reported as foliar diseases such as Alternaria brassicae (Alternaria blight) [32], Peronospora parasitica (Downy mildews) and Albugo candida (white rust) [33], Plasmidio-phora brassicae (clubroot) [34], Sclerotinia sclerotiorum, Rhizoctonia solani, and Golovinomyces orontii [35], Fusarium equiseti (leaf spot) [36]. In this study, many of the common saprophytic seedborne fungi [41] were reisolated from the seeds of E. sativa including Alternaria alternata, A. flavus, Cladosporium spp., Phoma sp., Penicillium spp., Stachybotrys botryosum and Ulocladium botryoides (Table 1). No seedborne fungi were reported in/on the seeds of Petrosoinum crispum. However, a number of fungi were associated with different diseases of this plant. Septoria blight [15], Phytophthora root rot [16], powdery mildews [17], Sclerotinia sclerotiorum [18], and downy mildews [19]. In this study, 14 species and 11 genera Alternaria alternata, Aspergillus flavus, A. nidulans, Chaetomium globosum, Cladosporium cladosporioides, Curvularia lunata, C. pallescens, Drechslera spicifera, D. rostrata, Fusarium semitectum, Memnoniella echinata, Rhizopus stolonifer, Stemphylium botryosum, and Ulocladium botryoides were isolated as new records for parsley seeds (Table 1). Fungi on or inside the seeds of P. oleracea has never been reported. Nonetheless, many plant pathogenic fungi were known to infect this plant; Verticillium dahlia (Verticillium wilt) [11], the obligate parasite Wilsoniana portulacaee (white blister disease), Drechslera portulacaee [20], and the highly pathogenic Dichotomphthora portulacaee [21]. In the present report, 8 species and one genus are considered new seedborne fungi for this vegetable. These include Alternaria alternata, Aspergillus flavus, A. terreus, Aureobasidium pullulans, Cladosporium cladosporioides, Curvularia lunata, Drechslera spicifera, Memnoniella echinata, and Phoma sp. (Table 1). The seedborne fungi of Solanum melongena (Eggplant) were not studied. Therefore, in this investigation 12 species of fungi including Aspergillus flavus, A. nidulans, A. niger, A. terreus, Chaetomium globosum, Cladosporium cladosporioides, Curvularia lunata, Fusarium solani, Memnoniella echinata, Rhizopus stolonifer, Stemphylium botryosum, and Wollertia sebia are considered new for the mycoflora of eggplant (Table 1).

Many fungal species of saprophytic genera cause destructive diseases to various plants. Although, common genera like Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Drechslera, Fusarium, Macor, Penicillium, Rhizopus, and Ulocladium are saprophytic in nature, some species of these genera can cause serious plant diseases [39-41, 51]. In our investigations, many species of these genera were recovered from the seeds of the imported vegetables (Table 1) and displayed high levels of seed contamination (25-100%) which adversely affect the seed viability and associated with low levels of germination (15-79%), and seedling emergence (12-75%) as concluded by many authors [51, 53]. In similar studies on different plants, these fungi were reported to cause various plant diseases; leaf lesion of Poo paratensis (Curvularia pallescens), seed rot of Sorghum bicolor (Drechslera spicifera), leaf spot on wide host range (D. rostrata), seedling blight (Curvularia lunata), and Fusarium solani wilt [54].

CONCLUSION

The nature of fungi associated with the seeds of 7 imported vegetable crops and their effects on seed germination, seedling emergence was were studied. The seeds were highly contaminated with saprophytic and pathogenic fungi (25-100%) which evidently reduced seed germination (15-79%), and seedling emergence (12-75%). Some of these fungi are new records to the seed mycoflora of these vegetables and to the fungal flora of Sudan. Since these fungi can be naturally present in the seeds, their hazardous effects on plant growth vigour and productivity warrant further investigations on their control. Therefore, it is apparently important to develop and implement eco-friendly and effective eradication measures for these seedborne contaminants. The development of proper seed testing methods, and strong quarantine regulations for import and export is a priority.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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APPENDICES

Fig. (1). *Alternaria alternata* (A) Conidia (B) Conidiophores.

Fig. (2). *Alternaria citri* (A) Conidia (B) Conidiophores.
Fig. (3). *Aureobasidium pullulans* (A) Conidia (B) Conidiophores (C) Hyphae (D) Chlamydospore-like cells.

Fig. (4). *Belterania santapui* (A) Conidia (B) Conidiophores.
Fig. (5). *Chaetomium globosum* (A) Peritheciun (B) Terminal hairs (C) Lateral hairs (D) Asci & ascospores.

Fig. (6). *Cladosporium cladosporioides* (A) Ramoconidia (B) Conidia (C) Conidiophores.
Fig. (7). *Curvularia lunata* (A) Conidia (B) Conidiophores.

Fig. (8). *Curvularia pallescens* (A) Conidia (B) Conidiophores.
**Fig. 9:** *Drechslera hawaiensis* (A) Conidia (B) Conidiophores.

**Fig. 10:** *Drechslera papendorfii* (A) Conidia (B) Conidiophores
Fig. 11. *Drechslera spicifera* (A) Conidia (B) Conidiophores.

Fig. (12). *Drechslera rostorata* (A) Conidia (B) Conidiophores.
Fig. (13). *Fusarium semitectum* (A) Conidia (B) Chlamydospores.

Fig. (14). *Fusarium solani* (A) Conidia (B) Conidiophores (C) Chlamydospores.
Fig. (15). *Memnoniella echinata* (A) Conidia (B) Conidiophores (C) Phialides.

Fig. (16). *Microascus trignosporus* (A) Perithesium (B) Ascus and ascospores.
Fig. (17). *Pithomyces chartarum* (A) Conidia (B) Conidiophores.

Fig. (18). *Pithomyces sacchari* (A) Conidia (B) Conidiophores.
Fig. (19). *Stachybotrys aurantes* (A) Conidia (B) Conidiophores.

Fig. (20). *Stemphylium botryosum* (A) Conidia (B) Conidiophores.
Fig. (21). *Trichothecium roseum* (A) Conidia (B) Conidiophores.

Fig. (22). *Ulocladium botrytis* (A) Conidia (B) Conidiophores.
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