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Leguminicolous fungi associated with some seeds of Sudanese legumes

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Abstract. *Abdulwehab SA, El-Nagerabi SAF, Elshafie AE. 2015. Leguminicolous fungi associated with some seeds of Sudanese legumes. Biodiversitas 16: 269-280.* The mycoflora associated with seeds evidently deteriorate seed viability, germination, emergence and plant growth performance leading to apparent losses in production and productivity. In the present investigation, seedborne fungi of six legumes were screened. Twenty six species of fungi from 14 genera were isolated from this seeds. Of these isolates, 6 species are new reports to the mycoflora of Sudan, whereas some species are new records to the mycoflora of these legumes. These include 6 species for *Cajanus cajan*, *Cicer arietinum* (10 species), *Dolichos lablab* (7 species), *Medicago sativa* (8 species), *Phaseolus vulgaris* (10 species), and *Vigna unguiculata* (11 species). The seeds are obviously contaminated with saprophytic and pathogenic fungi (17-64%) which evidently inhibited seed germination (41-86%), and seedling emergence (29-81%). The *Alternaria*, *Aspergillus* and *Fusarium* (4 species each) were the most prevalent fungi followed by *Curvularia*, *Drechslera* (3 species), *Fusariella*, *Ulocladium* (2 species) and one species for the remaining genera (*Aureobasidium*, *Acremonium*, *Memnoniella*, and *Rhizopus*). Hence, there is a high need for establishment of standard seed testing methods with strong legislations in order to meet the international quarantine regulations. The use of certified seeds by the farmers is recommended.

Key words: *Cajanus cajan, Cicer arietinum, Dolichos lablab, Medicago sativa, Phaseolus vulgaris, Vigna unguiculata*, seedborne, Sudan

INTRODUCTION

Legumes of the family Fabaceae are one of the most important plants cultivated as pulse or forage crops in many tropical and temperate regions. They are rich sources of plant protein and oil as human food and animals feed (Embaby and Abdel-Galil 2006; Swami and Alane 2013; Saleem and Ebrahim 2014). In Sudan, several leguminous crops such as *Cajanus cajan*, *Cicer arietinum*, *Dolichos lablab*, *Medicago sativa*, *Phaseolus vulgaris*, and *Vigna unguiculata* are cultivated as green manure for improving the soil fertility and reduced the amount of the expensive nitrogen fertilizer. *Cajanus cajan* (L.) Millsp. belong to the family Fabaceae and is placed in Papilionaceae of semi-arid tropics is grown as grain crops with high levels of important amino acids and proteins. It is cultivated for consumption of its dry seeds and the unripe green seeds serve as a cooked vegetable. The forage of this plant fed to livestock, and the stems are used for firewood and as hedgerow for windbreak (Pal et al. 2011; Singh and Kaur 2012). *Cicer arietinum* L. (Chickpea, Gram) also known as "Humus, Kabkabeik" in Arabic, is important pulse legume throughout the globe (Duke 1981).

The green immature pods and dry seeds are used as green vegetable or dry pulse boiled or fried, where the green and dried stems and leaves are used for feeding livestock. *Dolichos lablab* (Hyacinth lablab, Bonavista, and Egyptian bean) and "Lubia Afin" in Arabic is grown for food where young immature pods are cooked and eaten like green bean. Young leaves are used in salads and older leaves are cooked like Spinach. Starchy root tubers, immature and dried seeds can be boiled and eaten (Sarwatt et al. 1991). *Medicago sativa* L. (Medic, Alfalfa, Lucerne, Queen of Forage) which is also known as "Barsim hegazi" in Arabic was originated near Iran and endemic to Mediterranean region. It is extensively grown in warm temperate and cool subtropical regions as the most widely adapted agronomic crop. It is highly valued as forage legume having highest feeding values (Duke 1981). The seeds are used in many folk medicines and as lactigenic. *Phaseolus vulgaris* L. is known as common bean, kidney bean or "Fasulia" in Arabic is one of the five cultivated species of this genus as major grain legume crop, third in importance after soybean and peanuts, but first in direct human consumption (Broughton et al. 2003). It is grown for its green leaves, green pods, and immature and dry seeds. The dry bean are eaten in cooked dishes, bean flour, whereas the dry leaves, threshed pods and stalks are fed to animals and used as fuel for cooking in Africa and Asia. *Vigna unguiculata* (L.) Walp. which is known as cowpea, black-eyed pea or "Lubia helo" in Arabci is an annual legume that was domesticated in West Africa.

It is important grain legume and leaf vegetable in much of Africa and part of Asia. It is used as human diet, forage, cover, and green manure crop in many part of the world. Tender shoot tips, leaves, immature pods and seeds can be consumed as well as dry seeds for making flour. Nonetheless, the seeds of these legumes are susceptible to

fungal contamination, resulting in seeds deterioration (Rathod et al. 2012; Swami and Alane 2013). These fungi are of saprophytic or pathogenic nature which affects seed germination, emergence from soil, plant growth vigor and storability (Saleem and Ebahim 2014). Worldwide, many studies have reported about the biology of the seedborne and plant diseases associated with these leguminous crops (Nath et al. 1970; Deo and Gupta 1980; Nakkeeran and Devi 1997; Rathod et al. 2012).

The *Alternaria*, *Aspergillus*, *Curvularia*, *Drechslera*, *Eurotium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Sclerotium*, and *Gliocladium* were the most common fungal genera isolated from different legume seeds: broad bean (*Vicia faba*), kidney bean (*Phaseolus vulgaris*), lupine (*Lupinus termis*), cowpea (*Vigna sinensis*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*) under different environmental conditions throughout the World (Tseng et al. 1995; Ruiz et al. 1996; El-Nagerabi and Elshafie 2000; Kritzinger et al. 2003; Castillo et al. 2004; Kumar et al. 2004; Domijan et al. 2005; Embaby and Abdel-Galil 2006; Attitalla et al. 2010). *Fusarium semitectum*, *F. graminearum*, *F. chlamydosporum*, *F. equiseti*, *F. proliferatum* and *F. subglutinans* were the most dominant fungal species in the seeds of *Phaseolus vulgaris* and *Vigna sinensis* from Argentina and South Africa (Castillo et al. 2004; Kritzinger et al. 2003). *Alternaria alternata, Sclerotinia sclerotiorum, Rhizoctonia solani*, *F. semitectum*, and *Acremonium strictum* were the dominant species in black bean whereas *A. alternata*, *Lasiodiplodia theobromae*, *Drechslera spicifera* and *F. moniliforme* were isolated from cowpea (Castillo et al. 2004). The most common seedborne fungi isolated from *Phaseolus vulgaris* were from *Alternaria*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, and *Trichothecium* genus (Domijan et al. 2005).

In Sudan, many leguminous crops are cultivated under different climatic conditions. These seeds are locally produced or imported and stored by the farmers under poor quarantine regulations and legislations. Therefore, seed contamination can occur by seedborne fungi which adversely affect the production and productivity of these crops. There is a high possibility for isolation of many saprophytic and pathogenic fungi from various substrates including seeds (Elshafie 1985, 1986). A few studies were conducted on some of the seedborne fungi associated with locally produced leguminous crops such as peas, soybean (El-Nagerabi et al. 2000a, 2000b), guar, lupine (El-Nagerabi and Elshafie 2000, 2001a, 2001b), faba bean (El-Nagerabi et al. 2001), and fenugreek (El-Nagerabi 2000). Therefore, the present study was designed to investigate the quality and the incidence level of the seedborne fungi from six leguminous crops namely *Cajanus cajan*, *Cicer arietinum*, *Dolichos lablab*, *Medicago sativa*, *Phaseolus vulgaris*, *Vigna unguiculata* and to assess their effect on seed germination and seedling emergence. These information may improve knowledge about invading fungi and control measures and good management practices to prevent some fungi in legumes.

MATERIALS AND METHODS

Collection of the seed samples

Ninety seed samples from six leguminous crop namely *Cajanus cajan*, *Cicer arietinum*, *Dolichos lablab*, *Medicago sativa*, *Phaseolus vulgaris*, and *Vigna unguiculata* were purchased from seed companies in Khartoum State, Sudan. The samples were collected and tested as recommended by the rules of the International Seed Testing Association (ISTA 1966).

Seed germination

In this study, the blotter method was used according to the procedure adopted by the International Seed Testing Association (ISTA 1966). For this, 400 seeds from each sample were inoculated on sterilized moistened filter paper in Petri plates (Blotter). The seeds were aseptically spaced according to their size at equal distance. The inoculated plates were incubated in Gallenkamp illuminated incubator at 28ºC under alternating cycle of 12 hours near ultraviolet light and darkness to enhance sporulation of many of the seedborne fungi. The seeds were kept moistened by adding sterile distilled water throughout the incubation period of two weeks and the percentage of seed germination was recorded.

Emergence of seeds in soil

The emergence levels of the seeds from the soil was tested by sowing 200 seeds from each type of the selected legumes 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 27ºC and 29ºC. The average percentage of seed emergence was recorded for each vegetable crop.

Isolation and estimation of fungi

The seedborne fungi were isolated using agar plate method (ISTA 1966). Four hundred seeds from each sample were surface disinfected with 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 28ºC ±2ºC for two weeks. The fungal colonies developed around the seeds were examined, and identified microscopically. The average levels of contamination and incidence were reported.

Identification of isolated fungi

The isolated fungi were identified using macroscopic features based upon colony morphology and microscopic observations of mycelia and asexual/sexual spores (Barnett 1955; Raper and Fennell 1965; Pitt 1979; Ellis 1971, 1976; Sutton 1980; Webster 1980; Nelson et al. 1983; Samson et al. 1995; Barnett and Hunter 1998, 2003; Barac et al. 2004). For non-sporulating fungi, mycelial fragments were inoculated on Malt Extract Agar (MEA) and incubated at

28ºC ± 2ºC to stimulate their sporulation and were then identified to species level. Some of these fungi were illustrated (Figures 1-18).

RESULTS AND DISCUSSION

Twenty six species of fungi which belong to 14 genera were recovered from 90 seed samples of six leguminous crops namely *Cajanus cajan* (pigeon pea), *Cicer arietinum* (chickpea), *Dolichos lablab* (hyacinth), *Medicago sativa* (alfalfa), *Phaseolus vulgaris* (kidney bean), and *Vigna unguiculata* (cowpea). From these isolates, 6 species are new records to the mycoflora of Sudan, whereas different fungal species were reported for the first time in the seeds of the tested legumes (Table 1). The seeds were evidently contaminated with both saprophytic and pathogenic fungi (17-64%) and displayed variable levels of seed germination (41-86%), and seedling emergence from the soil (29-81%) (Table 2). The genera of *Alternaria*, *Aspergillus* and *Fusarium* (4 species each) were the most dominant species followed by *Curvularia*, *Drechslera* (3 species), *Fusariella*, *Ulocladium* (2 species) and one species for the remaining genera (*Aureobasidium*, *Acremonium*, *Memnoniella*, and *Rhizopus*).

Worldwide, many researchers investigating the biology of the seedborne mycoflora from numerous crops such as fruits, vegetables, cereals, and legumes (Nath et al. 1970; Deo and Gupta 1980; Nakkeeran and Devi 1997; Rathod et al. 2012). The seeds of legumes were found to be heavily infested with numerous mycoflora (Swami and Alane 2013). However, published results on seedborne fungi of leguminous cops are very few to negligible. Many fungi are serious parasites of the seed primordial, maturing and stored seeds and grains. Their invasion is associated with various damages such as seedling growth and yield loss. Twelve genera of fungi (*Alternaria*, *Aspergillus*, *Ascochyta*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Penicillium*, *Pythium*, *Rhizoctonia*, *Rhizopus*, and *Verticillium*) were isolated from different legume seeds in Iraq (Sarhan 2009). Twenty four seedborne fungi belonging to different genera were detected from 145 seed samples of major legume cops in Pakistan (Rauf 2000). Of these fungi, *Alternaria alternata*, *Ascochyta* spp., *Colletotrichum* spp., *Fusarium* spp., and *Macrophomina phaseolina* were the most frequent and common pathogens of these crops. *Alternaria*, *Aspergillus*, *Curvularia*, *Drechslera*, *Eurotium*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Mucor*, *Rhizopus*, *Sclerotium*, and *Gliocladium* were the most common fungal genera isolated from various legume seeds (Tseng et al. 1995; Ruiz et al. 1996; El-Nagerabi and Elshafie 2000; Kritzinger et al. 2003; Castillo et al. 2004; Kumar et al. 2004; Domijan et al. 2005; Embaby and Abdel-Galil 2006; Attitalla et al. 2010). *Fusarium*, *Penicillium*, *Rhizopus*, *Cladosporium* and *Alternaria* were the main seedborne pathogens on the surface of five major seeds of leguminous plants from Ningxia, China (Liu et al. 2002).

In Saudi Arabia, 46 fungal species belonging to 26 genera were isolated from the seeds of 5 legumes (broad beans, chickpeas, cowpeas, kidney beans, and peas) (Saleem and Ebrahim 2014). The most prevalent genera were *Aspergillus*, *Emericella*, *Mucor*, *Mycosphaerella*, *Penicillium*, and *Rhizopus*, whereas the most common species were *A. flavus*, *A. fumig*atus, *A. niger*, *A. ochraceus*, *A. terreus*, *Emericella nidulans*, *Mucor racemosus*, *Mycosphaerella tassiana*, P*enicillium chrysogenum*, and *Rhizopus stolonifer*. In the present study, the seeds of the six legume crops were highly infested with different types of fungi (17-64%) which evidently affected the seed germination (41-86%), and seedling emergence (29-81%) (Table 2). Of the few studies on the seedborne mycoflora of *Cajanus cajan* (pigeon peas), *Alternaria tenuissima* (seed or seedling rot, pod spot or rot), *Botryosphaeria xanthocephalus*, *Cercospora cajani*, *C. instabilis* (spotting), *Colletotrichum cajani* (seed or seedling rot, spotting, blight, pod spot or rot), *Creonectria grammicospora*, *Fusarium semitectum*, and *Megalonctria pseudotrichia* (seedling rot) were reported (Kaiser 1981). The diseases of economic importance at the present are *Fusarium* wilt (*F. udum*), and *Phytophthora* blight (*P. drechsleri* f. sp. *cajan*i) (Marley and Hillocks 1996; Kumar et al. 2010). *Cladosporium* species has been reported by Tarr (1963) as the main cause of sooty mould of pigeon peas in Sudan. In the present study, *Alternaria alternata*, *A. tenuis*, *Aureobasidium pullulans*, *Curvularia brachyspora*, *C. pallescens*, *Drechslera rostrata*, and *Fusarium solani* were isolated for the first time as seedborne fungi of pigeon peas (Table 1). *Cladosporium* spp. (18%) and *Alternaria alternata* (4.5%) which were associated with sooty mould of this crop are encountered in high levels of incidence comparable to other fungal isolates. The remaining isolates were recorded before in similar studies carried on the seedborne fungi of pigeon pea (e.g. Malone and Muskett 1964; Singh and Khapre 1978). Chickpea (*Cicer arietinum*) has been found attacked by 172 pathogens including 67 species of fungi (Nene et al. 1996). The plants are infected with a large number of fungal diseases viz. blight (*Ascochyta rabiei*, *A. alternata*, *Colletotrichum dematium*, *Stemphylium sarciniforme*), wilt (*Fusarium oxysporum*), powdery mildew (*Leveillula taurica*), dry root rot (*Rhizoctonia bataticola*), stem rot (*Sclerotinia sclerotiorum*), wet root rot (*R. so*lani), and foot rot (*Operculella padwickii*) (Kaur 1995; Singh and Sharma 2005; Dubey et al. 2007).

Twenty six fungal species belonging to 15 genera were isolated from 14 seed samples of chickpea collected from different areas of Pakistan (Ahmed et al. 1993; Dawar et al. 2007). In India, *Alternaria alternata*, *Chaetomium* spp., *Penicillium citrinum*, *A. niger*, *A. flavus*, *Rhizopus stolonifer*, and *Fusarium oxysporum* were isolated from the seeds of this crop (Agarwal 2011). In the present investigations, 18 species of fungi belonging to 12 genera were recovered from the seeds of highly contaminated chickpea (40%). Of these isolates, *A. tenuis*, *A. tenuissima*, *A. nidulans*, *A. terreus*, *Acremonium strictum*, *Curvularia lunata*, *Drechslera papendorfii*, *D. spicifera*, *Fusariella aegyptiaca*, and *Ulocladium atrum* are considered new to the seeds of this crop (Table 1). Some of the previously reported as seedborne fungi and pathogenic to this plant

Table 2. Incidence percentage (I%), number of cases isolation (NCI, out of 90 samples), and occurrence remarks (OR) of seedborne fungi of leguminous plants

Fungal isolates	Incidence percentage (I%)						
	Cajanus cajan	*Cicer arietinum*	*Dolichos lablab*	*Medicago sativa*	*Phaseolus vulgaris*	*Vigna unguiculata*	*NCI/OR*
Alternaria alternata (Figure 1)	4.5 N[1]	8.25	1.5	1.0 N	6.25	4.0 N	87H
A. dianthi (Figure 2)	-[3]	-	2.5 N	-	0.25 N	-	36M
A. tenuis (Figure 3)	1.75 N	2.25 N	1.5 N	-	3.75 N	2.0	49H
A. tenuissima (Figure 4)	1.25	3.0 N	-	-	4.0 N	-	41H
Aspergillus flavus	2.5	4.5	3.75	0.75 N	2.0	3.0	88H
A. nidulans	1.0	0.25 N	0.5	-	-	2.75	32M
A. niger	13.5	22.5	32.5	0.5 N	7.5	2.5	92H
A. terreus	1.5	2.5 N	-	1.25 N	1.0	1.5	34M
Aureobasidium pullulans (Figure 5)	1.25 N	-	0.5 N	-	0.1	-	27L
Acremonium strictum	-	1.25 N	-	-	2.3 N	2.0 N	18L
Cladosporium spp.	18.0	7.3	2.0	3.5	5.0	3.5	72H
Curvularia brachyspora (Figure 6)	2.75 N	-	-	-	1.5 N	-	30M
C. lunata (Figure 7)	-	1.0 N	-	0.5 N	-	-	27L
C. pallescens (Figure 8)	1.25 N	-	2.5 N	-	-	2.0 N	39M
Drechslera papendorfii (Figure 9)	-	3.0 N	-	-	1.5	-	25L
D. rostrata (Figure 10) [2]NS	1.5 N	-	-	2.75 N	-	2.25 N	33M
D. spicifera (Figure 11)	2.0	3.5 N	1.75 N	0.25 N	3.3	1.75	76H
Fusariella aegyptiaca (Figure 12) NS	-	0.25 N	-	-	0.25 N	-	12R
Fusariella intermedia (Figure 13) NS	-	-	-	-	2.0 N	-	9R
Fusarium equiseti (Figure 14)	-	1.5	3.25N	-	-	-	13R
F. moniliforme	-	10.25	-	-	-	-	10R
F. semitectum (Figure 15)	-	2.5	2.25 N	-	3.0	2.0 N	31M
F. solani (Figure 16)	2.0 N	-	-	-	0.75	-	15L
Memnoniella echinata NS	1.0	1.75	-	-	-	-	11R
Myrothecium roridum	-	-	-	-	0.25 N	-	5R
Penicillium spp.	3.0	4.25	5.0	-	-	-	38M
Rhizopus stolonifer	8.25	6.5	4.5	0.5	19.5	30.0	89H
Ulocladium atrum (Figure 17) NS	-	1.75 N	-	-	0.25 N	-	26L
Ulocladium botrytis (Figure 18) NS	-	-	-	2.25 N	-	2.75 N	21L

Note: [1]N: New record to the crop, [2]NS: New record to the mycoflora of Sudan, [3]-: Not detected, OR: Occurrence remarks, out of 90 samples, H: High, more than 45 samples, M: Moderate, between 30-45 samples, L: Low, between 15-29 samples, R: Rare, less than 15 samples.

Table 2. The percentage of seed germination and contamination of different leguminous **crops**

Legume species	Contamination %	Germination %	Emergence%
Cajanus cajan (pigeon pea)	31	68	54
Cicer arietinum	40	86	73
Dolichos lablab	64	41	29
Medicago sativa	17	86	81
Phaseolus vulgaris	44	66	52
Vigna unguiculata	47	57	45

were recovered from the present seeds (Ahmed et al. 1993; Kaur 1995; Nene et al. 1996; Singh and Sharma 2005; Dawar et al. 2007; Dubey et al. 2007; Agarwal 2011). Of the few studies on the seedborne mycoflora of *Dolichos lablab* (*Lablab purpureus*), the genera of *Aspergillus*, *Fusarium*, and *Penicillium* were the most important fungi (Chalaut and Perris 1994). In India, *Trichothecium roseum*, *Alternaria* sp., and *Fusariu*m sp. were linked to severe multiple infections of lablab resulting in discolored pods, and shrunken disfigured seeds (Siddaramaiah et al. 1980). Seed germination was suppressed by *A. niger* while *A. chevalieri*, *A. flavus*, *A. candidus*, *A. niveus*, and *A. alternata* caused staining and necrosis of 23-37% of cotyledons and twisting in 19-27% (Prasad and Prasad 1987). According to Tarr (1963), many diseases of *Dolichos lablab* were caused by fungal species such as leaf spot (*A. alternata*, *Cladosporium* sp.), wilt (*Macrophomina phaseolina*, *Phyllosticta* spp.). In the present study, 12 species of fungi were isolated from the seeds of this crop and of these isolates *Alternaria dianthi*, *A. tenuis*, *Aureobasidium pullulans*, *Curvularia pallescens*,

Drechslera spicifera, Fusarium equiseti, and *F. semitectum* are considered new to the mycoflora of this crop (Table 1).

Some of the previously reported as seedborne and/or pathogenic fungi were recovered from the current seed samples of *D. lablab* (Tarr 1963; Siddaramaiah et al. 1980; Prasad and Prasad 1987; Chalaut and Perris 1994). For Lucerne (*Medicago sativa*), *Mycosphaerella pinodes, Botrytis cinerea, Phoma herbarum* (*Phoma exigua* var. *exigua*) var. *medicagi*nis, *Fusarium roseum* and *Stemphylium botryosum* (*Pleospora tarda, Pleospora herba*rum) were the most frequently encountered seedborne fungi (Leach 1960). *Fusarium avenaceum* was detected for the first time on the seed of Lucerne by Kellock et al. (1978). *Fusarium acuminatum, F. avenaceum, F. equiseti, F. fusarioides, F. oxysporum, F. poae, Diaporthe phaseolorum* and *Phoma sorghina* were proven pathogenic to a range of pasture legumes including Lucerne (Trenteva 1974; Nik and Parbery 1977). Many fungal species isolated from the seeds of this plant by many authors in similar mycological investigations. These include *Cercospora dematium* f.sp. *truncata, C. zebrina* (*C. medicaginis*), *Colletotrichum trifolii* (anthracnose), *F. sporotrichoides, F. oxysporum, F. sambucinum, F. avenaceum, Ascochyta infectoria, Stemphylium* spp. (leaf spot), *Pleospora herbarum, Stemphylium loti, S. sarciniforme, Sclerotinia sclerotiorum,* and *S. trifoliorum* (crown rot), *Verticillium albo-atrum (*wilt) (Leach 1960; Maloy 1968; Trenteva 1974). In the present research, *Alternaria alternata, Aspergillus flavus, A. niger, A. terreus, Curvularia lunata, Drechslera rostrata, D. spicifera,* and *Ulocladium botrytis* were newly isolated from the seeds samples of Lucerne (Table 1). In similar studies on seedborne fungi of common bean (*Phaseolus vulgaris*) collected from Riyadh region, Saudi Arabia, various fungi from 11 genera were isolated such as *A. alternata, A. flavus, A. flavus* var. *columnaris, A. niger, A. ochraceus, A. ustus, Botrytis* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium* spp., *Penicillium chrysogenum, Phoma* sp., *Rhizopus stolonifer, Stemphylium* sp., and *Ulocladium* sp. (El-Samawaty et al. 2014).

The most common seedborne fungal species isolated from common bean crops grown in 13 counties of the Republic of Croatia belong to genera of *Cladosporium* (98%), *Alternaria* (75%), *Aspergillus* (75%), *Rhizopus* (72%), *Penicillium* (69%), *Fusarium* (38%), *Botrytis* (27%), *Trichothecium* (24%), and *Chaetomium* (18%) (Domijan et al. 2005). In Egypt, *A. niger* (43.2%), *A. ochraceus* (2.4%), *A. parasiti*cus (0.8%), *A. flavus* (0.8%), *Aspergillus* spp. (4.8%), *Epicoccum* sp. (0.8%), *Fusarium oxysp*orum (2.4%), *Fusarium* spp. (5.6%), and *Trichoderma* spp. (11.2%) were isolated from common bean seeds (Embaby and Abdel-Galil 2006). Various seedborne fungi were isolated from the seed of this crop such as *Alternaria brassicicola, Ascochyta phaseolina, A. boltshauseri* (leaf spot), *Aspergillus glaucus, Botrytis cinerea* (chocolate spot), *Rhizoctonia solani (*damping off), *Fusarium solani* f.sp. *phaseoli* (stem rot), *F. solani, Phyllosticta phaseolina, Aspergillus flavus* (rot), *F. oxysporum* (wilt), *A. glaucus, Colletotrichum dematium, Drechslera tetramera, F. equiseti, F. oxysporum, F. moniliforme, F. semitectum, Phoma solani, Phoma* sp., *Phomopsis sojae, Colletotrichum lindemuthianum, Macrophomina phaseolina, Sclerotinia sclerotiorum, Rhizoctonia* sp., *Botrytis cinerea, Uromyces appendiculatus, Trichothecium roseum, Phytophthora* sp., *Diaporthe* sp., *Elsinoe phaseoli, Rhizoctonia solani,* and *Alternaria alternata,* (e.g. Winter et al. 1974; Gomes and Dhingra 1983; Sesan and Dumitras 1979). In the present results, 18 species were recovered from the seeds of this crop. Of these isolates, *Alternaria dianthi, A. tenuissima, A. tenuis, Acremonium strictum, Curvularia brachyspora, Drechslera papendorfii, Fusariella aegyptiaca, F. intermedia, Myrothecium roridum,* and *Ulocladium atrum* were recovered for the first time from the seed of common bean (Table 1). In similar mycological investigations on the seedborne fungi of cowpea (*Vigna unguiculata*), *A. niger* (62.2%), *A. parasiticus* (6.7%), *Aspergillus* spp. (15.6%), and *Fusarium* spp. (4.4%) were the most frequently isolated from this crop (Embaby and Abdel-Galil 2006).

Cowpea seeds collected from Riyadh region, Saudi Arabia were found contaminated with *Alternaria alternata, A. flavus, A. flavus var. columnaris, A. niger, A. parasiticus, A. ustus, Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp., *Nigrospora* sp., *Penicillium* spp., *Rhizopus stolonifer,* and *Ulocladium* sp. (El-Samawaty et al. 2014). In Benin, West Africa, *A. flavus*, a fungus that produces aflatoxins, was the most frequently encountered (Houssou et al. 2009). Many fungi were isolated from the seeds of cowpea by several authors such as *Myrothecium leucotrichum, Rhizoctonia solani, Ascochyta* sp., *Colletotrichum lindemuthianum, C. truncatum, Fusarium oxysporum, Corticium rolfsii, Colletotrichum capsici, Cercospora canescens, Aspergillus* spp., *A. flavus, A. niger, Botrytis cinerea, Cacumisporum* sp., *Cephalosporium* sp., *Chaetomium* sp., *Curvularia verruculosa, Diaporthe phaseolorum, Drechslera hawaiiensis, D. spicifera, Fusarium equiseti, F. fusarioides, Memniella* sp., *Nigrospora* sp., *Penicillium* spp., *Phoma* sp., *Pithomyces* sp., *Alternaria infectoria, Stachybotrys* sp., *Cencephalastrum racemosus, Pestalotiopsis nagniferae, A. terreus, C. lunata, Pleospora infectoria, Rhizoctonia bataticola,* and *Rhizopus stolonifer* (e.g. Singh and Khapre 1978; Enechebe and McDonald 1979; Sesan and Dumitras 1979).

Different pathogenic fungi were associated with many devastating diseases of cowpea plant including *Ascochyta phaseolorum* (leaf and pod spot), *Cladosporium vignae* (Leaf spot), *Cercospora canescens, C. cruenta, C. lunata, Phyllosticta phaseolorum, Sporidesmium bakeri, Uromyces vignae, Capnodium* spp., and *Cladosporium herbarum* (sooty mould), *Fusarium oxysporum f. sp. tracheiphilum* (wilt), *Rhizoctonia solani, Myrothecium leucotrichum, Aspergillus terreus* (brown lesions), *Curvularia lunata, Rhizoctonia bataticola* and *F. conc*olor (post-emergence death and stem lesions), *Oidium* sp., and *Sphaerotheca fuliginea* (powdery mildew), and *Macrophomina phaseoli* (wilt) (Singh and Khapre 1978; Enechebe and McDonald 1979; Siddaramaiah et al. 1980). In the present research, *Alternaria alternata, A. terreus, A. flavus, A. nidulans, A. niger, Acremonium strictum, Cladosporium* spp., *Curvularia*

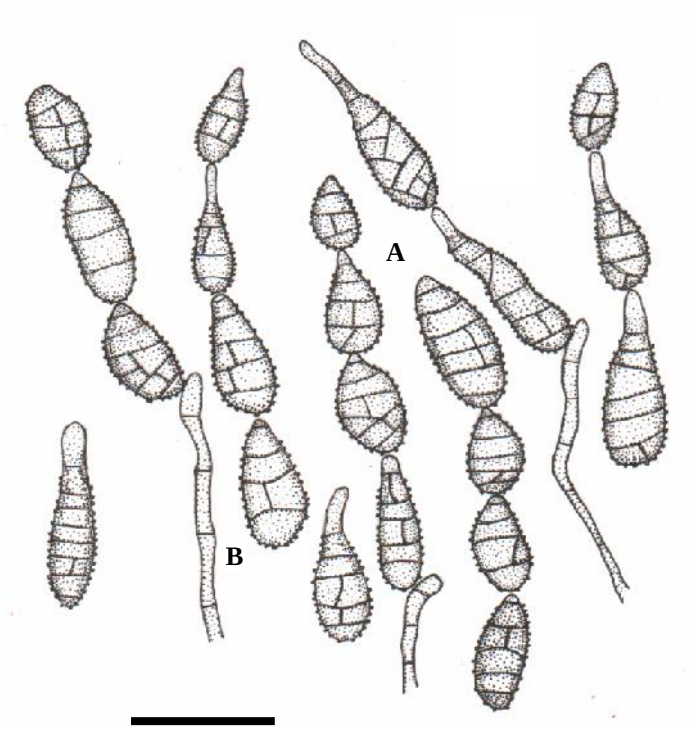

Figure 1. *Alternaria alternata* (A) Conidia, (B) Conidiophores. Bar = 50 µm

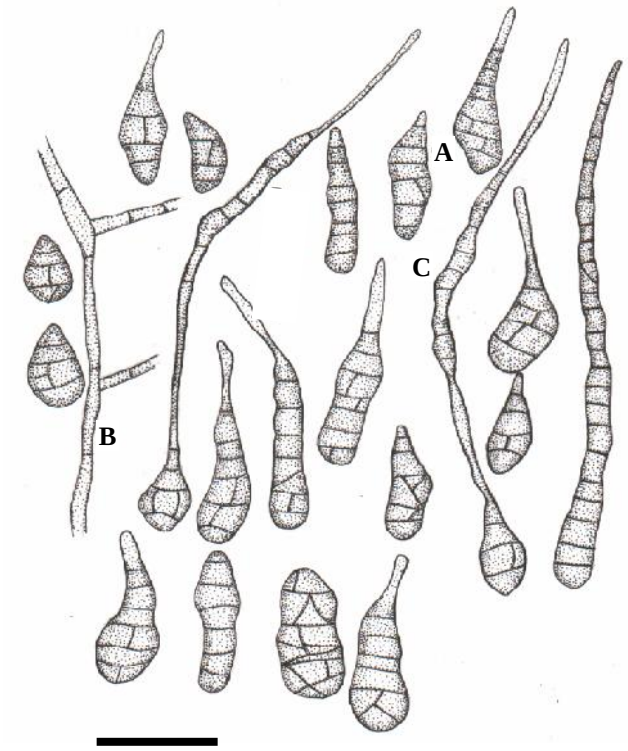

Figure 2. *Alternaria dianthi* (A) Conidia, (B) Conidiophores, (C) Chlamydospores. Bar = 50 µm

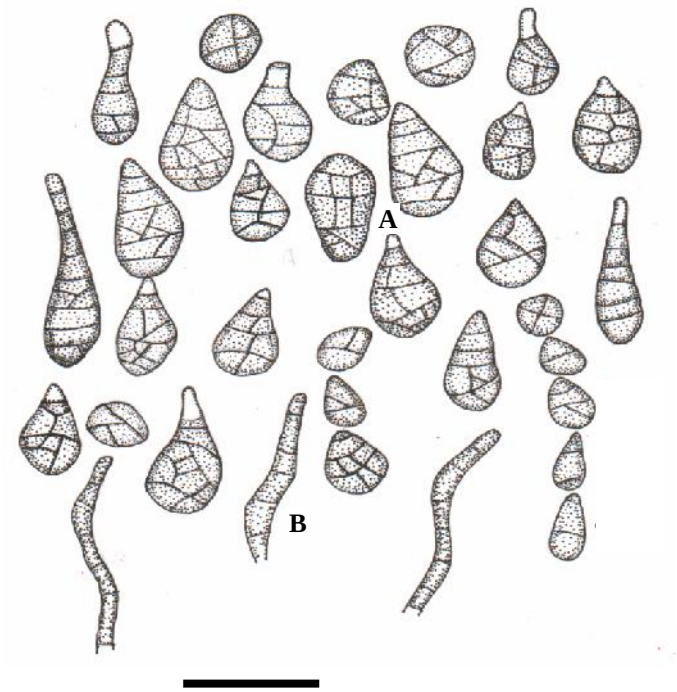

Figure 3. *Alternaria tenuis* (A) Conidia, (B) Conidiophores. Bar = 50 µm

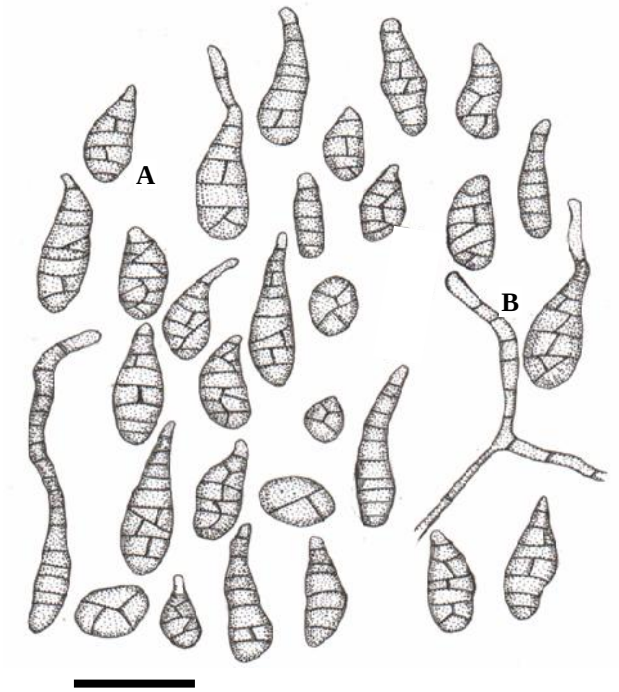

Figure 4. *Alternaria tenuissima* (A) Conidia, (B) Conidiophores. Bar = 50 µm

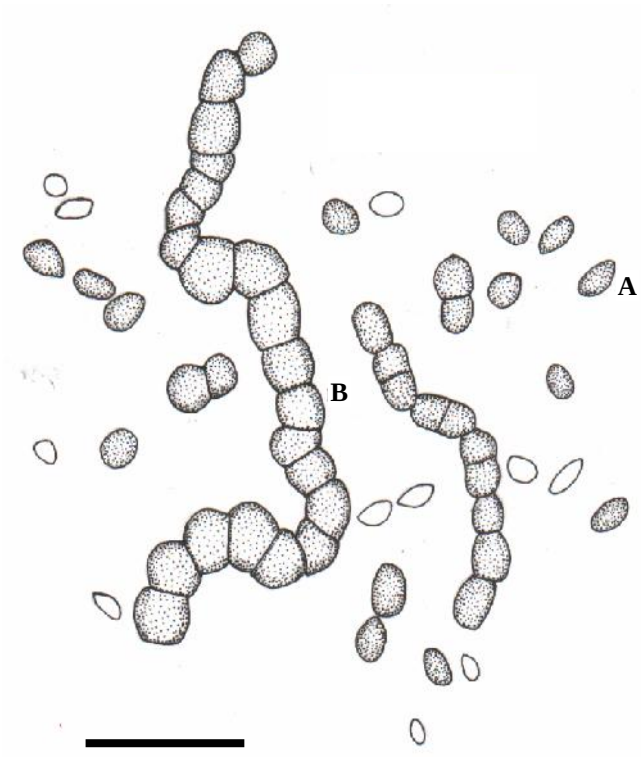

Figure 5. *Aureobasidium pullulans* (A) Conidia, (B) Conidiophores. Bar = 50 µm

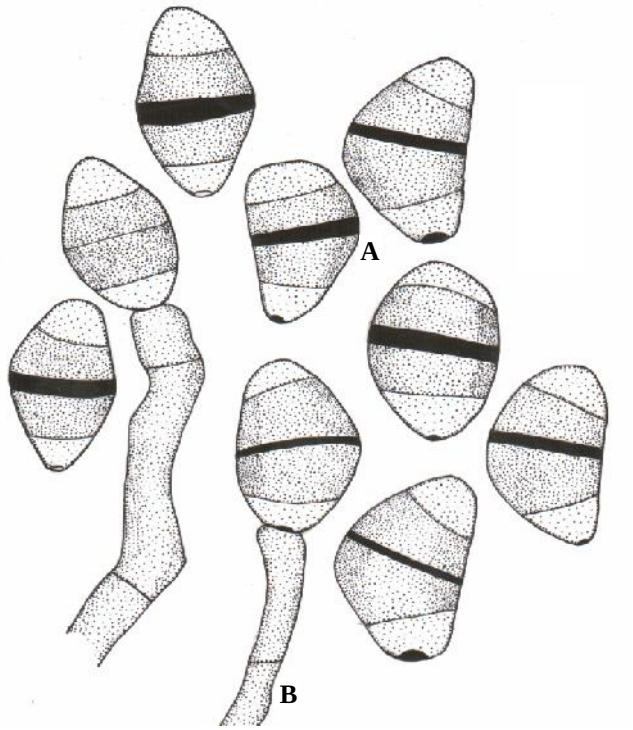

Figure 6. *Curvularia brachyspora* (A) Conidia, (B) Conidiophores. Bar = 25 µm

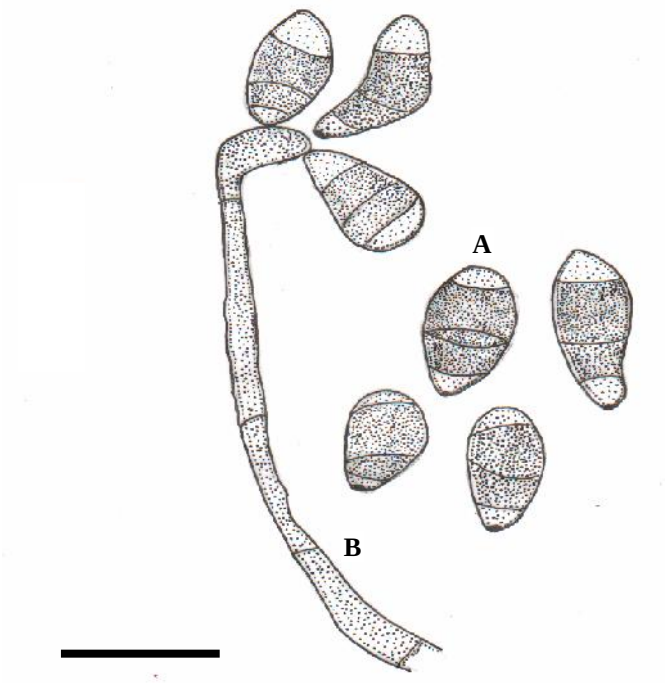

Figure 7. *Curvularia lunata* (A) Conidia, (B) Conidiophores. Bar = 25 µm

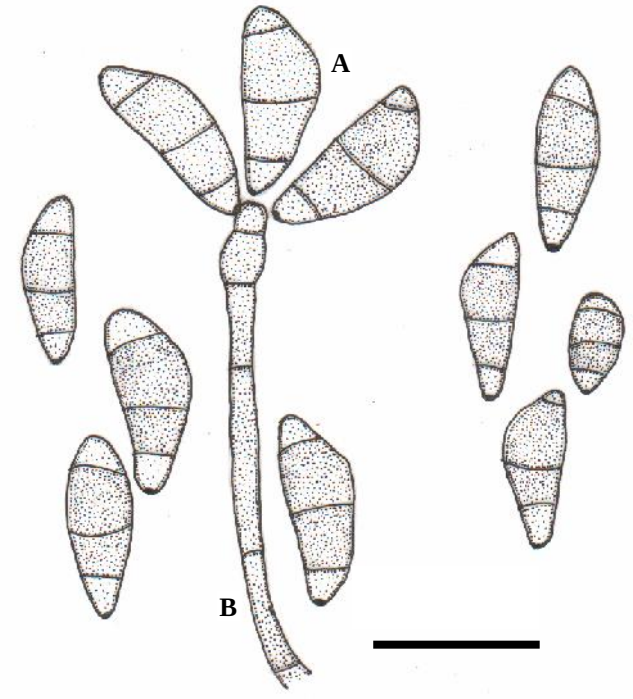

Figure 8. *Curvularia pallescens* (A) Conidia, (B) Conidiophores. Bar = 25 µm

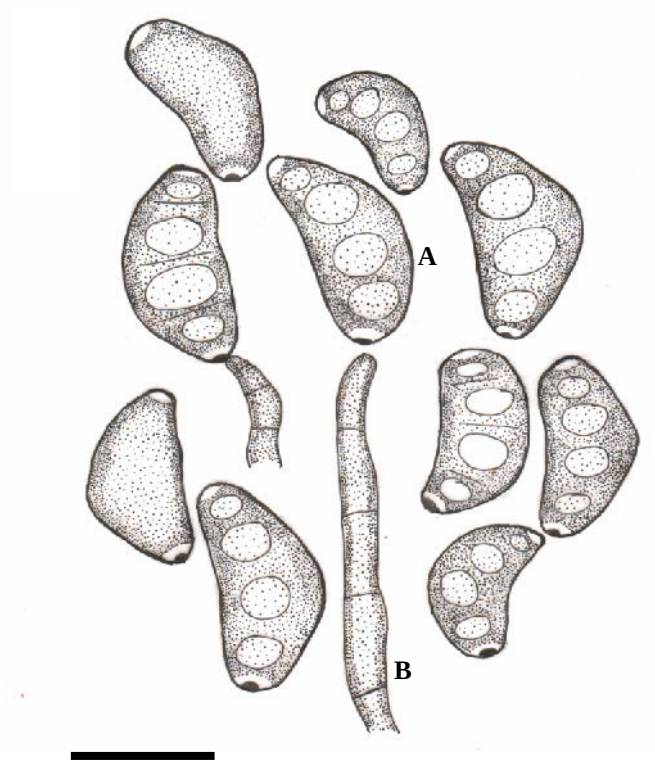

Figure 9. *Drechslera papendorfii* (A) Conidia, (B) Conidiophores. Bar = 25 µm

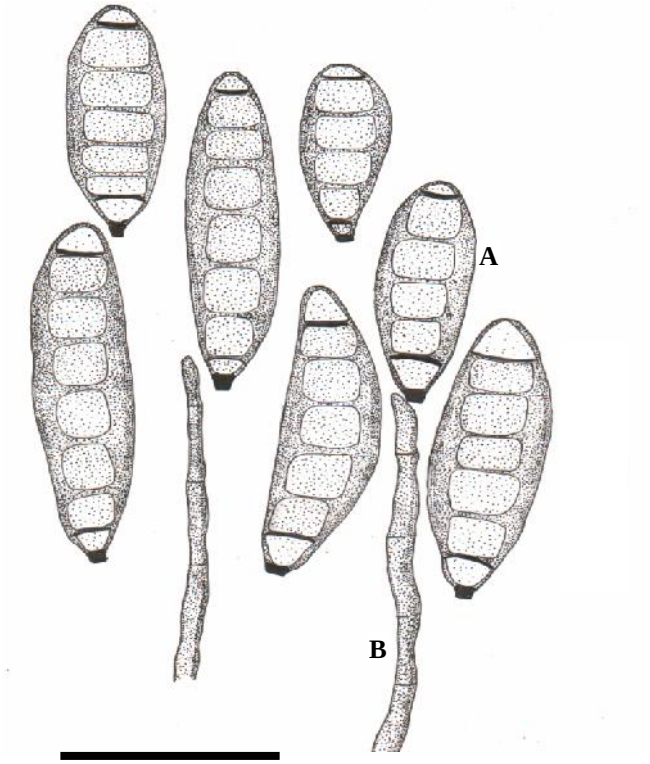

Figure 10. *Drechslera rostrata* (A) Conidia, (B) Conidiophores. Bar = 50 µm

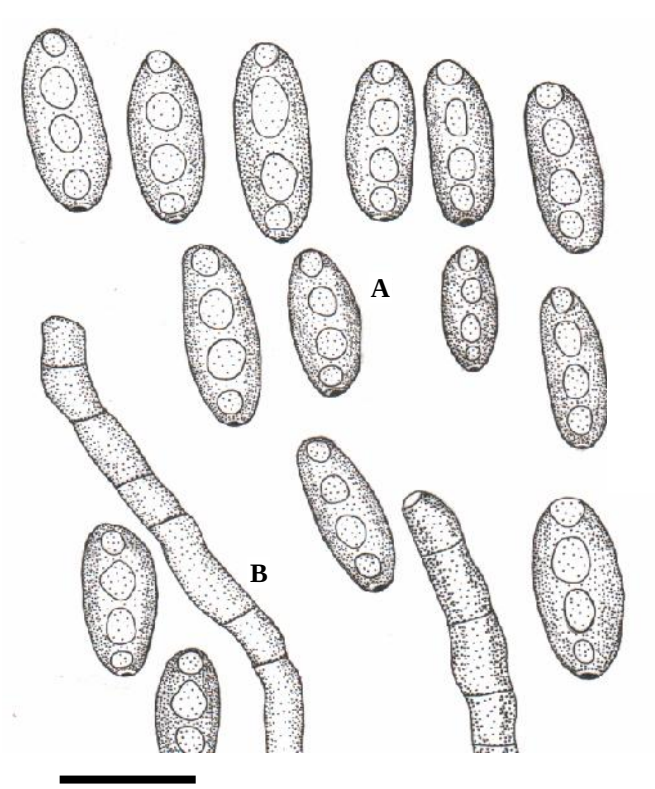

Figure 11. *Drechslera spicifera* (A) Conidia, (B) Conidiophores. Bar = 50 µm

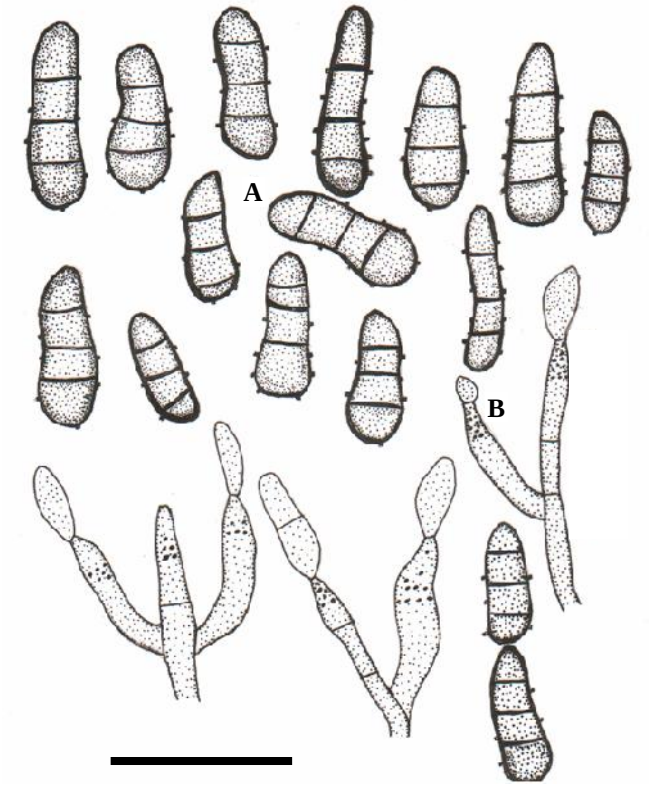

Figure 12. *Fusariella aegyptiaca* (A) Conidia, (B) Phialides. Bar = 25 µm

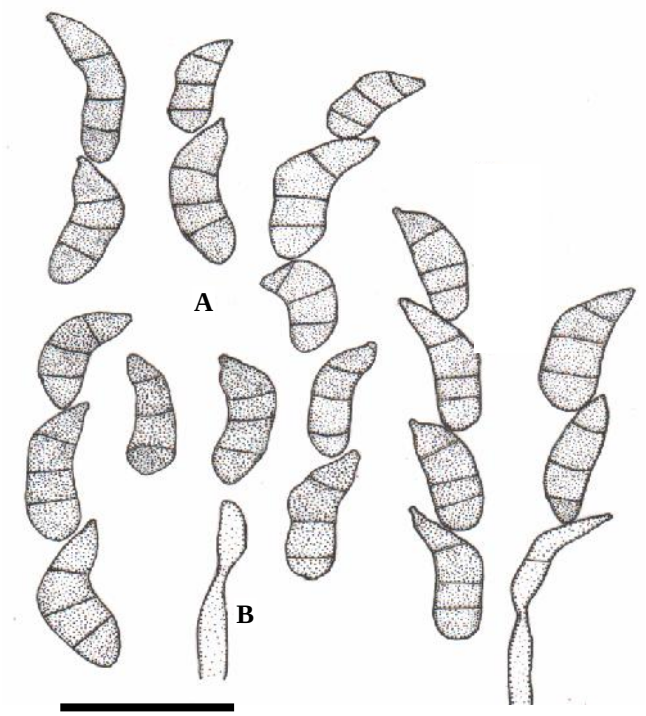

Figure 13. *Fusariella intermedia* (A) Conidia, (B) Phialides. Bar = 25 µm

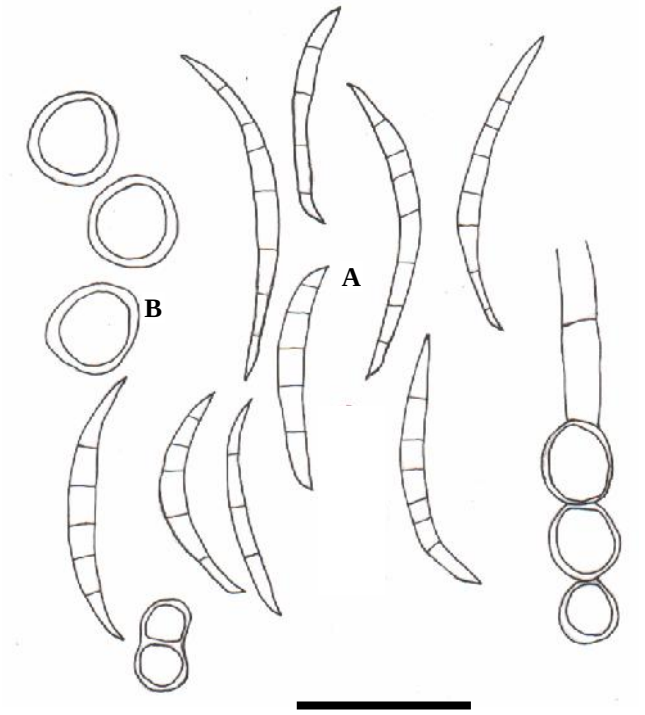

Figure 14. *Fusarium equiseti* (A) Macroconidia, (B) Chlamydospores. Bar = 25 µm

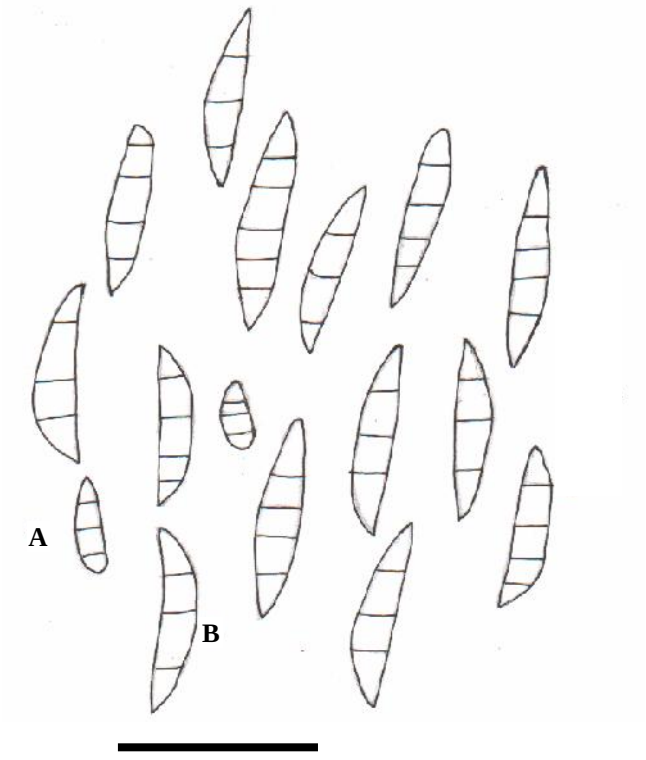

Figure 15. *Fusarium semitectum* (A) Primary microconidia, (B) Secondary macroconidia. Bar = 25 µm

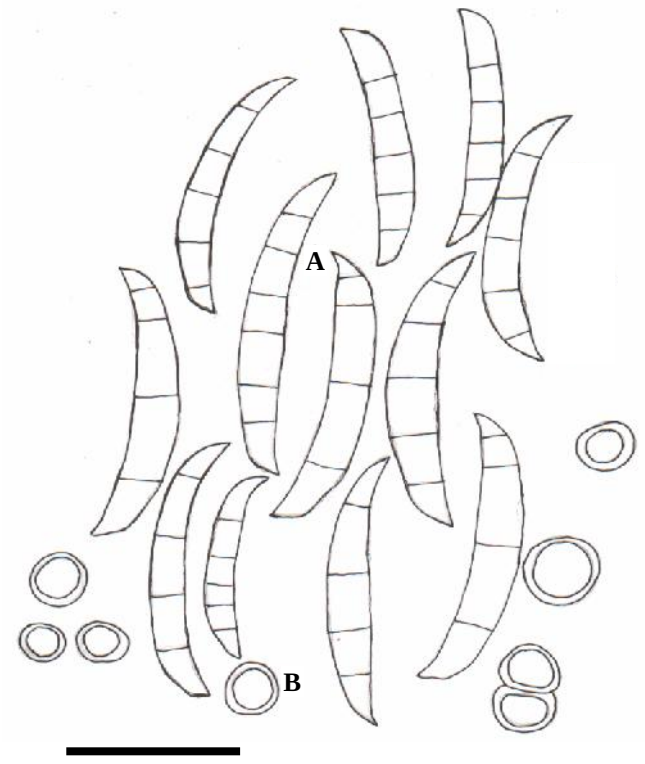

Figure 16. *Fusarium solani* (A) Macroconidia, (B) Chlamydospores. Bar = 25 µm

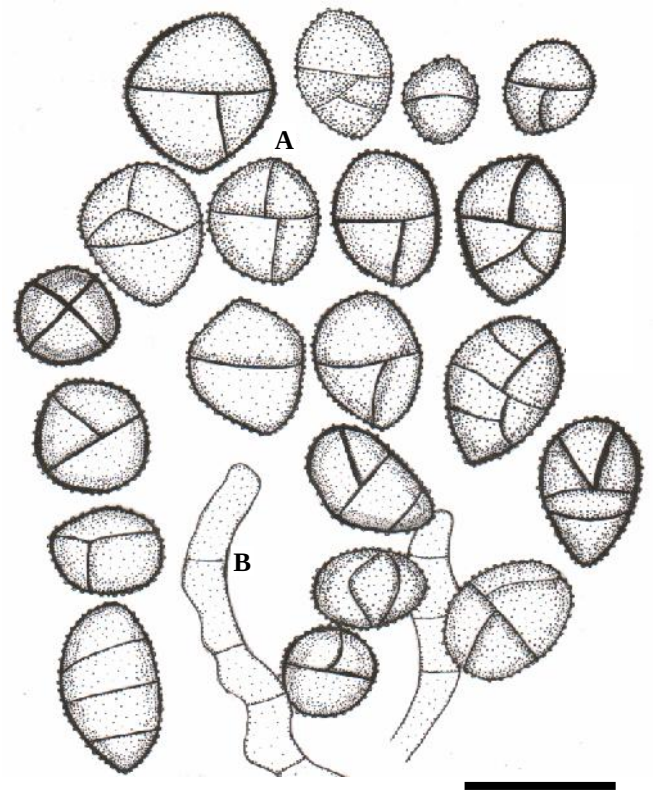

Figure 17. *Ulocladium atrum* (A) Conidia, (B) Conidiophores. Bar = 25 µm

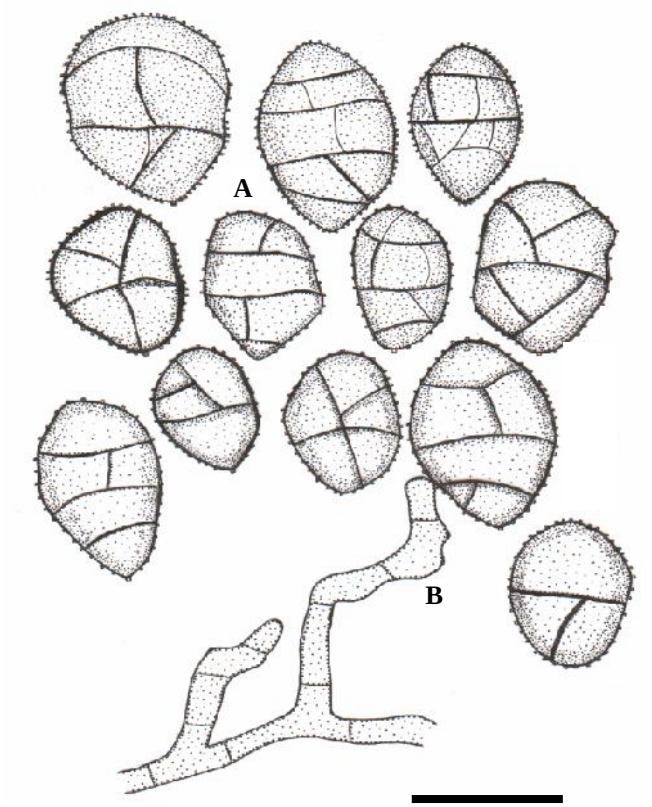

Figure 18. *Ulocladium botrytis* (A) Conidia, (B) Conidiophores. Bar = 25 µm

pallescens, Drechslera rostrata, D. spicifera, F. semitectum, Rhizopus stolonifer, and *Ulocladium botrytis* were recovered from the seeds of cowpea (Table 1). Of these isolates, 6 species are considered new to this crop including *A. alternata, Acremonium strictum, Curvularia pallescens, D. rostrata, F. semitectum, Ulocladium botrytis* (Table 1).

Numerous saprophytic fungal genera were linked with hazardous plant diseases to many plants. The main genera of *Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Drechslera, Fusarium, Penicillium, Rhizopus, Uloc*ladium, are commonly known as saprophytes, however, some species of these genera can cause destructive plant diseases (Kamble et al. 1999; El-Nagerabi and Elshafie 2002). In our results, many species of these genera were isolated from the seed samples of the tested legumes (Table 1) and showed high levels of contamination (17-64%), which apparently affected seed germination (41-86%) and seedling emergence (29-81%) as concluded by many authors (Kamble et al. 1999; El-Nagerabi and Ahmed 2001; Abdelwehab et al. 2014). In similar pathogenicity studies on different plants, some of these fungi were considered pathogenic and caused various plant diseases; leaf lesion of *Poa pratensis* (*Curvularia pallescens*), seed rot of *Sorghum bicolor* (*Drechslera spicifera*), leaf spot (*D. rostrata*), seedling blight (*C. lunata*), and *Fusarium solani* wilt (Richardson 1997; Abdelwehab et al. 2014).

The fungal flora invaded the seeds of six leguminous crops and their evident effect on seed germination and seedling emergence were investigated. The seeds were evidently contaminated with both saprophytic and pathogenic mycoflora (17-64%) which apparently reduced seed germination (41-86%), and seedling emergence (29-81%). These fungal isolates are natural contaminant on different seeds whereas some of them are new reports to the mycoflora of these legumes and to the fungal flora of Sudan. Therefore, their devastating effects on seed germinations, plant growth and vigor and eventually losses in production and productivity together with suitable control measures need further intensive investigations and evaluation. Setting proper seed testing method, and proper quarantine regulations and legislations is urgently required.

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