



## Occurrence and isolation of some *Fusarium* species associated with crown and root rot disease in some wheat fields of Duhok, IKR, Iraq

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### Abstract

The current work was conducted on crown and root rot disease of wheat incited by *Fusarium* species. A survey was carried out in several wheat fields at Sumel, Duhok province including: (Camone, Hajsne, Namrik, Sharia and Sumel center) during booting and heading stages of wheat in April to May and Ripening in May to Jun during the growing season 2014/15. The symptoms were obvious in all the locations as yellowing, stunting, and browning discoloration of lower stems and roots. The highest disease incidence in both stages was found in Hajsne (67.2% and 74.4%, respectively), while the lowest disease incidence was recorded in Camone (21.6%) at the first survey and increased to (41.6%) in the second survey in Namrike. *Fusarium graminearum*, *F. oxysporum*, *F. solani*, *F. avenaceum*, and *F. nivale* were isolated from root and crown of wheat. *F. graminearum* was isolated at high frequency (56%) followed by *F. oxysporum* (23%). The prevalence of *Fusarium* species was varied with concern to locations. The highest prevalence of *F. graminearum* was reported in Camone (70%) followed by Namrike (64%) and the lowest prevalence found in Sumel center (29%), while *F. oxysporum* and *F. solani* were the superior species in Sumel center (36%) and Hajsne (22%) respectively. *Fusarium* spp. were isolated from all wheat fields in both stages, at (Booting to Heading) it was higher accumulated in roots with (42%), increased to (58%) in the (Ripening) which mostly isolated from crown. Pathogenicity test of isolated *Fusarium* species was performed in greenhouse. *F. graminearum*, *F. oxysporum* and *F. solani* resulted more disease incidence and severity than remaining tested species with obvious symptoms of crown and root discoloration, reducing of wheat growth and wilting.

### Introduction

Wheat (*Triticum aestivum* L.) affected by various diseases, root and crown rot are diseases, which cause losses in most countries around the world that produce wheat [1]. This disease has been found in many regions of the world, and particularly under dry condition and caused yield losses of more than 50% [2]. *Fusarium* species are a varied group of fungi that have an effect on cereals through rotting of seeds, root, crown, seedling, basal stem, and spike [3]. The disease is characterized by several symptoms such as yellowing, seedling death, stunting of seedling and mature plant, dark brown lesions on root, crown, sub crown internodes and stem. Afterword other symptoms appear like tiller death prematurely which contains little grains or its empty [4].

Wheat infections with *Fusarium* species are economically important in cereal growing countries. In Iran the disease causes severe problems in main wheat growing regions [5]. In South Dakota the disease is the most destructive and familiar in wheat field. It can cause 3-4% losses of crop in a year. The Loss may be huge in a year because of the climatic conditions favored by the disease [6]. In the pacific Northwest of America Paulitz *et al.* (2002) [3] determined that 76% of the plants in winter wheat fields can be infected with *Fusarium* crown rot with estimated losses of 18% in heavily infected fields with a negative impact of US\$76/ha. Yield losses have been reported 25-58% in Australia, amounting to US\$80 million, due to reduced grain yield and quality [7]. In Iraq, there are some studies on fusarium crown and root rot (FCRR) disease on wheat plants [8]; [9] and [10]

Various *Fusarium* species causes crown and root rot disease including *F. culmorum*, *F. graminearum*, *F. equiseti*, *F. pseudograminearum*, *F. acuminatum*, *F. nivale*, *F. avenaceum*, While *F. graminearum*, *F. culmorum* and *F. pseudograminearum*, are epidemiologically the highly important species involved in this disease [11]. Khalifah (2013) [9] showed that *F. pseudograminearum* and *F. graminearum* are the causal agents of wheat crown rot in several wheat fields of Iraq. While Taha (2014) [10] showed the presence of the disease in Erbil and Ninevah and isolated *F. graminearum*, *F. culmorum* and *F. poae*, from the infected wheat roots.

The aim of the study is to determine the occurrence and distribution of *Fusarium* Crown and Root Rot (FCRR) at Sumel district which represents one of the major wheat producing area of Duhok Governorate.

## Materials and Methods

### A. Field survey and sampling

A survey was performed in five wheat growing areas of Sumel district including (Camone, Hajsne, Namrik, Sharia and Sumel center) in Duhok province. Durum and soft wheat samples were collected in two periods depending on the plant growth stage according to Zadoks scale [12], the first period was (Booting to Heading, Scale 40 - 59) in April to May the second period was ( Ripening, Scale 90 -99 ) in May to Jun during 2014/15 growing season.

Five fields were selected; field size of each was approximately 6-8 hectares. At each field five sampling points were made in (W) pattern [13]. At each point 25 plants were collected, the total number of plants in each field was 125 plants, depending on the symptoms appear obviously as leaves yellowing and crown rot with browning roots and discolored subcrown internode. Samples were removed with as much of the root system as possible and kept in cold and dry place after washing of the roots in the lab, disease incidence and severity in each field was calculated based on root and crown discoloration. Diseases incidence in each region was calculated according to this formula:

$$\% \text{ Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total No. of examined plants}} \times 100$$

Severity ratings depending on the discoloration from the roots to the crown above the soil line were assigned in the lab based on a scale scoring from 0 to 4 (Figure: 1) where: 0 = Healthy plant, 1 = (1-10 %) Brown discoloration, 2 = (11-25 %) Brown discoloration, 3 = (26-50 %) Brown discoloration, 4 = (51-100 %) Brown discoloration. Disease severity (DS) was estimated according to McKinney (1923) [14] as follows:

$$DS = \sum d / d_{max} \times n$$

Where DS is the disease severity, d is (the disease rating on each plant), d<sub>max</sub> is (the maximum disease rating possible) and n is (the total number of plants examined in each replicate).

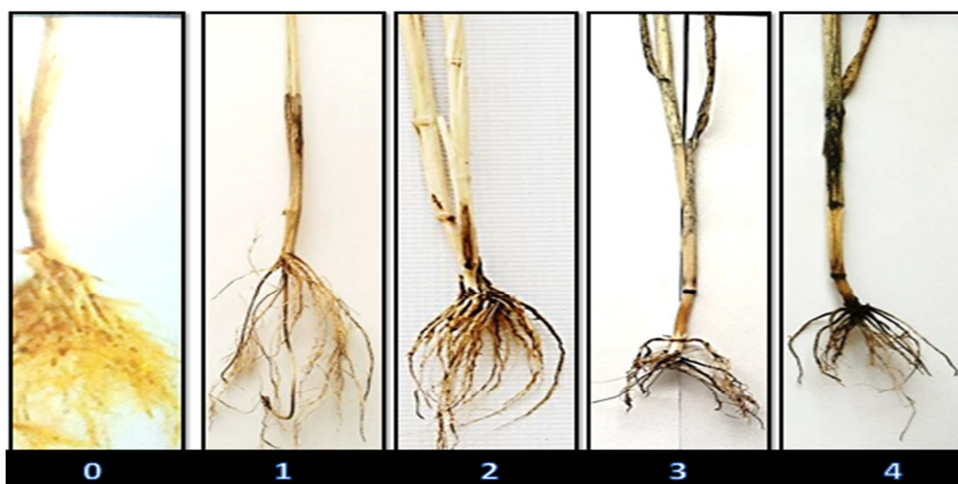


Figure -1: Estimation severity of wheat crown and root rot on a 0 to 4 scale. 0 = Healthy plant, 1 = (1-10 %) Brown discoloration, 2 = (11-25 %) Brown discoloration, 3 = (26-50 %) Brown discoloration, 4 = (51-100 %) Brown discoloration..

**B. Isolation and identification of the fungi associated with crown and root of wheat plant**

Fungi were isolated from root and crown using the following method; sixty plants from each field (12plants/point) washed under running tap water for half hour. Crown, root, nodes and spike sections (1cm) were taken from each selected wheat plant and surface disinfected with 1.5% sodium hypochlorite (NaOCL) for three minutes, rinsed in distilled water twice, and dried on clean filter papers. Then plant parts were cultured on sterilized potato dextrose agar (PDA) medium (Himedia laboratories Pvt. Ltd.-India) with adding 250mg/L. of chloramphenicol with 5 pieces /plate; there were two replications for each plant section in each point making the total plate of 70-80 plates per field. The plates were incubated at 25±1°C for 5-7 days under 12/12 hrs. photoperiod. Suspected fungi were purified in PDA plate and slants for further identification. The frequency of each fusarium species was recorded according to the following formula:

$$\% \text{ Frequency} = \frac{\text{Colony No. of isolated fungus}}{\text{Total number of colonies}} \times 100$$

The prevalence determination of *Fusarium* spp. was recoded according to locations and infected plant parts by those formula:

$$\% \text{ Prevalence} = \frac{\text{Colony No. of each Fusarium sp. in each location or plant parts}}{\text{Total colony No. of Fusarium spp. in each location or in all plant parts}} \times 100$$

The cultures resembling *Fusarium* species were sub-cultured on carnation leaf agar (CLA) which is a natural substrate media prepared by putting 10-12 leaf pieces of sterile carnation into a petri dish and then adding 2% sterile water agar (20g of agar in 1L of distilled water with 250mg/L. of chloramphenicol). The plates were incubated for 6-10 days for more sporulation and then identification confirmed based on their morphological characters including: colony appearance, morphology of microconidia, macroconidia, chlamydospores, and conidiophores according to [15]; [16]; [17] and [18].

**C- Pathogenicity trial of potted wheat plant in greenhouse**

Pathogenicity experiment was conducted on Simeto cultivar in the greenhouse at College of Agriculture/ University of Duhok. five *Fusarium* species (*F. avenaceum*, *F. graminearum*, *F. nivale*, *F. oxysporum*, and *F. solani*) isolated during field survey were tested. Before sowing seeds in plastic pots (12cm height × 11cm width), wheat seeds were surface disinfected by washing for 5min in running tap water, immersed in 75%

ethanol and in 0.5% NaOCl for 1min, rinsed twice in distilled water, finally seeds dried under a laminar flow hood. Seeds were sown at a depth of 2cm in each pot that were filled with sterilized soil (sand and peat 2:1) by autoclave (121°C and pressure 1.5 kg/cm<sup>2</sup> for 20 minutes twice). Each replicate consisted of a pot, and two seeds were planted in each. Fourteen days after planting, seedlings were inoculated at the base of the shoot using spore suspension technique [19]. A volume of 10ml of a spore suspension ( $1 \times 10^6$  spores/ml) was added at the base of each plant stem. Inoculation technique was applied after 14 days of planting. Disease severity and incidence of crown and root rot of each plant were assessed 35 days after inoculation. The experiment was done in a Complete Randomized Design (CRD) in three replications. Means of the treatments were compared by Duncan Multiple Range Test at 5% level.

## Results and Discussion

### A. Field survey

Crown and Root Rot disease symptoms were obvious in all five locations that showed yellowing, stunting, and browning discoloration of lower stems and roots. In the booting to heading plant growth stage, the highest disease incidence and severity were recorded in Hajsne (67.2%, 0.35) and the lowest one found in Camone (21.6%, 0.07, respectively). The fields located in Hajsne (36° 56' 42.5" N; 42° 50' 31.09" E), Elevation (661m) and Camone (36°50'25.15" N; 42° 46' 01.81" E), Elevation (394m). In the ripening stage, the maximum disease incidence was in Hajsne (74.4%) and minimum disease incidence occurred in Namrike (41.6%), the highest disease severity was also recorded in Hajsne (0.49) and the lowest one was reported in Namrike (0.11) located in (36°44' 28.68" N; 42°53' 23.46" E), Elevation (418) (Table 1).

Table- 1: Disease severity and incidence of crown and root rot disease at surveyed wheat locations of Sumel district.

Location	Booting to Heading stage		Ripening stage	
	Disease severity	%Disease incidence	Disease severity	%Disease incidence
Camone	0.07	21.6	0.15	47.2
Hajsne	0.35	67.2	0.49	74.4
Namrike	0.13	45.6	0.11	41.6
Sharia	0.12	40	0.15	43.2
Sumel center	0.25	56.8	0.21	45.6

### B. Isolation and identification of the suspected fungi from wheat crown and root

The predominant fungus in this study was *Fusarium* spp. when isolated at ratio 62% from wheat tissues. *Fusarium* spp. has been isolated as the most common fungal pathogen from roots and crown of wheat in various regions of the world, as in Iran [20], North Dakota [21] and Southern Chile [22]. In addition to *Fusarium* spp., several fungal species were isolated from wheat root and crown during investigation including *Alternaria* spp. 17%, *Rhizoctonia* spp. 12%, *Macrophomina* spp. and *Aspergillus* spp. 3% each, *Bipolaris* spp. 2% and *Penicillium* spp. at ratio 1%. The occurrence of these species was in agreement with Tunali *et al.* (2008) [23] who isolated different fungi with *Fusarium* spp. from root and crown of wheat through a survey of a two-year period in Turkey.

Results showed isolation of five *Fusarium* species from roots and crown tissues of wheat namely (*F. graminearum* 56%, *F. oxysporum* 23%, *F. solani* 14%, *F. nivale* and *F. avenaceum* 3% for each. Records of this study were consistent with results from Australia, New York, Southern France [24] and Canada [25] who demonstrated that *F. graminearum* was predominant fungus in these regions. *F. graminearum* was also

reported as superior fungus as the cause of wheat root and crown rot in most wheat producing areas of Iran [5].

The predominant species that cause the disease in a region based on the amount of inoculums and climatic conditions; it may differ within regions and between regions [26]. Thus, *F. graminearum* occurs in arid and semiarid conditions as mentioned by Cook (2010) [11], since high temperature increase development of *F. graminearum*, while colder climate contribute to the proliferation of *F. avenaceum* [27], other species particularly *F. nivale* and *F. avenaceum* prefer lower temperature; therefore they exhibit at low frequency, and their infection levels are very dependent of weather conditions [28]. *F. avenaceum* and *F. solani* had been isolated from wheat by [29]. Demirci and Dane (2003) [30] isolated *F. oxysporum* from stem base of winter wheat in eastern Anatolia.

#### **Prevalence of *Fusarium* species in the locations of wheat grown.**

Prevalence of *Fusarium* species associated with crown and roots of wheat were varied with respect to locations (Table 2). The highest prevalence of *F. graminearum* was reported in Camone (70%) followed by Namrike (64%) and the lowest prevalence found in Sumel center (29%). *F. oxysporum* was most isolated from Sumel center (36%) and the least isolation was recorded in Sharia (10%), *F. solani* was the superior species in Hajsne (22%) followed by Sharia (17%). *F. nivale* was found only in Sharia (25%), while *F. avenaceum* was reported only in Sumel center (18% and 6%) consistently.

Table -2: Prevalence of *Fusarium* spp. in the survey locations of Sumel district:

<i>Location</i>	<i>(%) Prevalence of Fusarium species</i>				
	<i>F. graminearum</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>F. avenaceum</i>	<i>F. nivale</i>
<i>Camone</i>	70	19	11	0	0
<i>Hajsne</i>	54	24	22	0	0
<i>Namrike</i>	64	24	12	0	0
<i>Sharia</i>	48	10	17	0	25
<i>Sumel center</i>	29	36	11	18	0

#### **Prevalence of *Fusarium* spp. in the wheat plant parts**

Figure (2) demonstrated the colonization of fusarium crown and root rot (FCRR) fungi in entire plant parts in both stages of plant growth. Substantially, *Fusarium* spp. were isolated from all wheat parts particularly on root, crown and 1<sup>st</sup> node but in ripening stage prevalence of *Fusarium* spp. became higher than booting to heading stage except in roots which became lower. Wang (2015) [31] proved that after wheat root inoculation, *F. graminearum* can reach the spikes of wheat. At flowering stage fungi biomass was dropped in roots but highly accumulated in stem base. Also Boughalleb *et al.* (2008) [32] isolated *F. graminearum*, *F. solani* and *F. oxysporum* from root, crown and head of wheat.

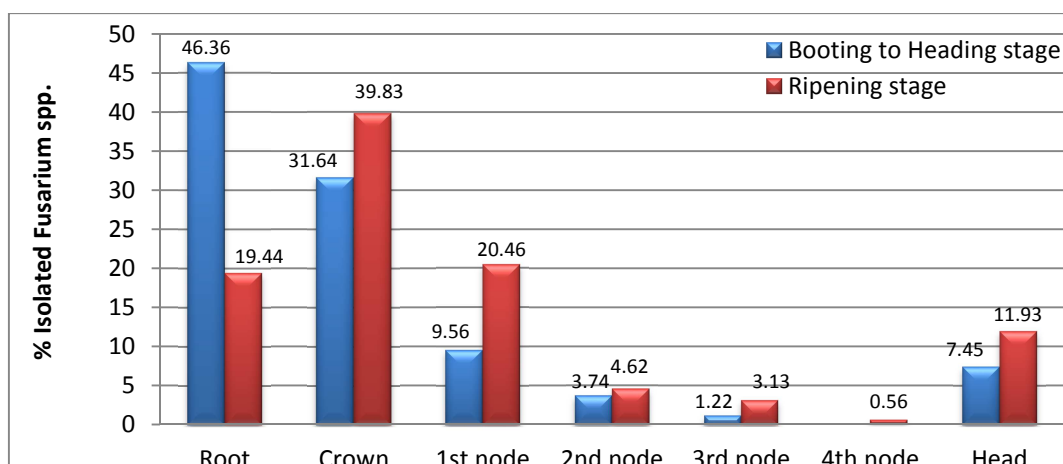


Figure -2: Percentage of *Fusarium* spp. isolated from wheat plant parts in both stages of growth.

Results of field survey indicated the high level of presence and distribution of disease in Sumel district; this might be due to the semiarid conditions of Sumel, that help the occurrence of the disease causing reduction in the amount of root density and crown rot [33] and [34]. Other factors associated with the prevalence of specific *Fusarium* species on wheat such as cropping history, climatic conditions especially soil moisture and temperature, host susceptibility, and fungal pathogenicity [35] and. Also plant debris and infected seeds are essential for fungi survival and represent as sources of inoculum for next cropping [36]. Our findings are in general agreed with studies performed in other wheat growing regions of Iraq and Kurdistan region by (Khalifah, 2013 [9] and Taha, 2014 [10]).

#### **Morphological identification**

***Fusarium graminearum*** Schwabe, Flora Anhaltina 2: 285 (1839).Figure (3) A-E.

Colony characters on PDA is white to pale yellow, rose, becoming brown at aged colony with dense mycelium on the surface and creamy color on the bottom. Macroconidia: slender to falcate, apical cell tapered and basal cell foot shaped, dorsal side smooth and curved, ventral side straight, Produced from lateral phialides which may become grouped on branched conidiophores; three septate, 30.6-32.5 x 3.7 $\mu$ m, five to seven septate, 35- 40 x 3.7-5 $\mu$ m. Microconidia: absent. Chlamydo spores: slow to form, usually formed in macroconidia but also formed in mycelium (10-12.5 $\mu$ m). The same or most of these characters were also shown on CLA. These characters were consistent with descriptions of (Booth, 1977 [15]; and Leslie and Summerell, 2006 [18]).

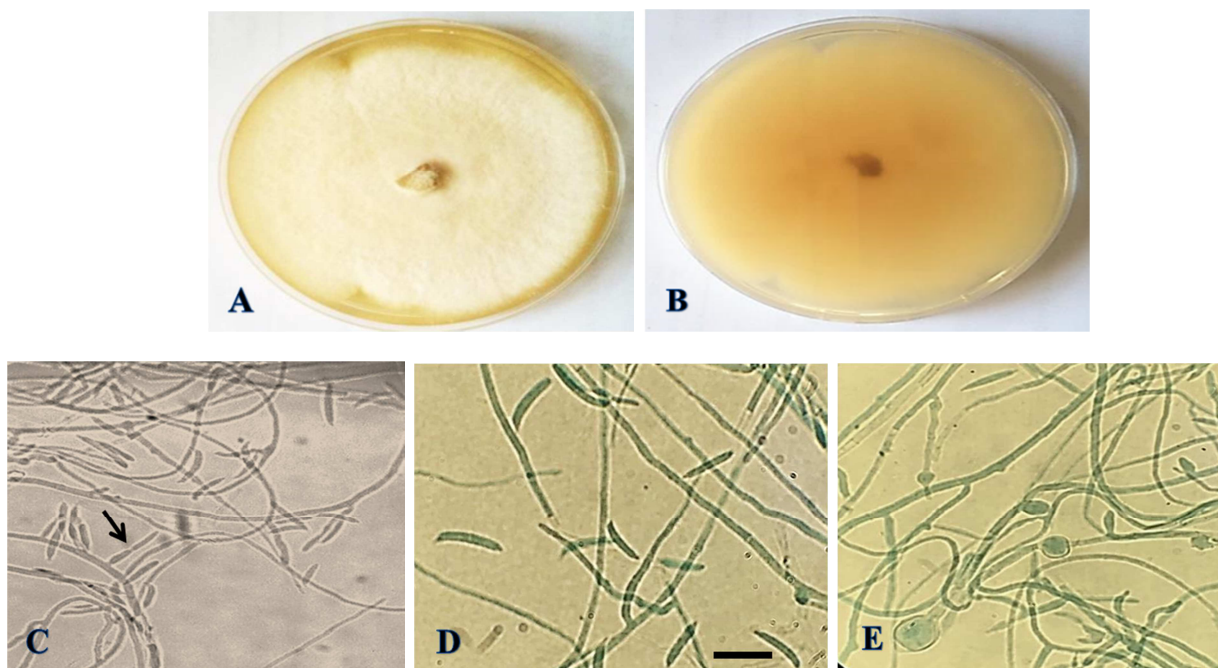


Figure- 3: *Fusarium graminearum*, (A) Colony surface on PDA; (B) Colony reverse; (C) Simple lateral phialides with macroconidia; (D) Macroconidia; (E) Chlamydospores. Scale bar: E=35µm (400X).

*Fusarium avenaceum* Fries Saccardo, Sylloge Fungorum 4:713(1886). Figure (4) A-D.

Colony characters on PDA: dense aerial mycelium, with white to light yellow color on the surface with rapid growth, light yellow from below. Macroconidia: two types of macroconidia produced primary and secondary macroconidia. Primary macroconidia: 1-3 septate, 15-25 x 3.75-5µm produced from conidiogenous cell. Secondary macroconidi: 4-7 septate, narrow, slender, long, straight to curved 40-47.5 x 3.75µm. Apical cell: elongated, and basal cell: foot shaped. Chlamydospores: rarely formed in conidia but absent in mycelium, also no chlamydospores were produced on CLA. Measurements of our isolates were consistent with other investigations (Booth, 1977 [15]; Nelson *et al.*, 1994 [16]; Summerell *et al.*, 2003 [17]).

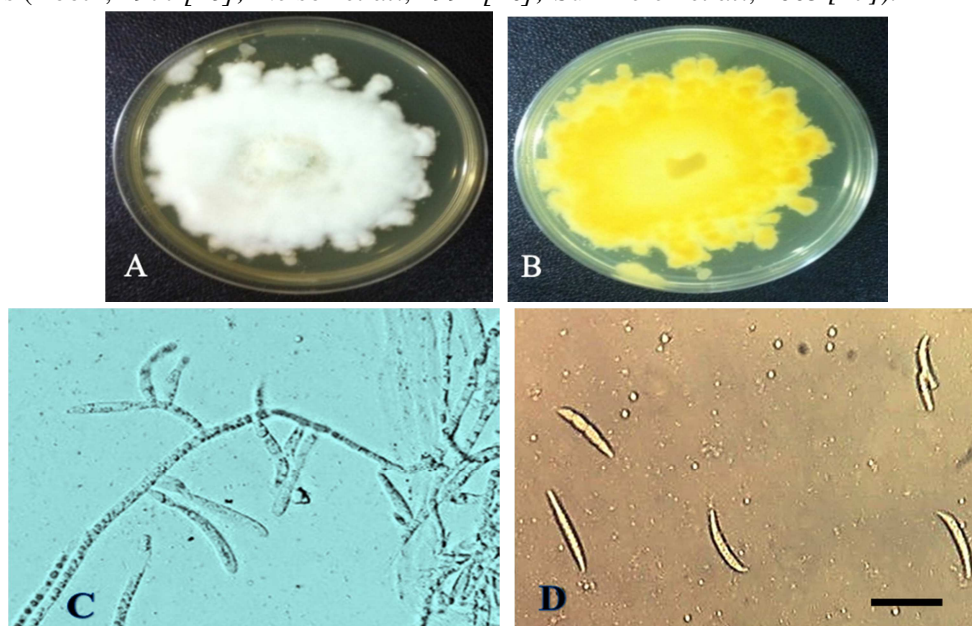


Figure- 4: *Fusarium avenaceum*, (A) Colony surface on PDA; (B) Colony reverse; (C) Formation of primary conidia; (D) Secondary conidia Scale bar: D=42.5µm (400X).

***Fusarium nivale*** (Fr.) Ces., Rabenh. Klotzch, Herb. Mycol. Ed. I. No. 1439, 1850. Figure (5) A-D.

Cultural pigmentation on PDA: white to pale peach to apricot color on the surface. Conidia: straight to slightly curved shape with pointed apex, 1-3 septate, 10-25 x 2.5-5µm, with the presence of abundant proliferating phialides. Chlamydo spores: absent. The result was in agreement with (Barnett and Hunter, 1972 [37]; Booth, 1977 [15]).

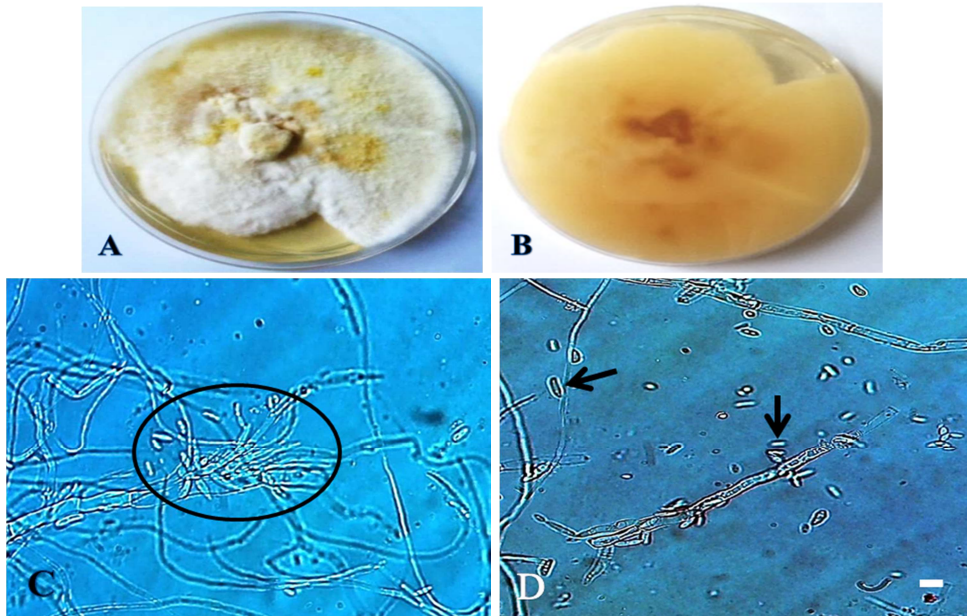


Figure- 5: *Fusarium nivale*, (A) Colony surface on PDA; (B) Colony reverse; (C) Proliferating phialides; (D) Macro- and Micro Conidia Scale bar: E= 20µm (400X).

***Fusarium oxysporum*** Schlecht., *Flora Berol.* 2: 139, 1824. Figure (6) A-E.

Colony characters on PDA: white to violet color on the surface and dark purple on the bottom side. Microconidia: oval to kidney shape, produced from simple lateral short phialides, with no septate, 5-7.5 x 2.5-3.75 µm. Macroconidia: straight to slightly curved, 3-5 mostly 3septate, 25- 47.5 x 3.75-5 µm. Pointed to curve at ends. Chlamydo spores: globose, intercalary or terminal, hyaline with thick wall, singly or in pairs. On CLA similar conidial characters were produced. This result was consistent with (Booth, 1977 [15]; Nelson *et al.*, 1994 [16] and Leslie and Summerell, 2006 [18]).

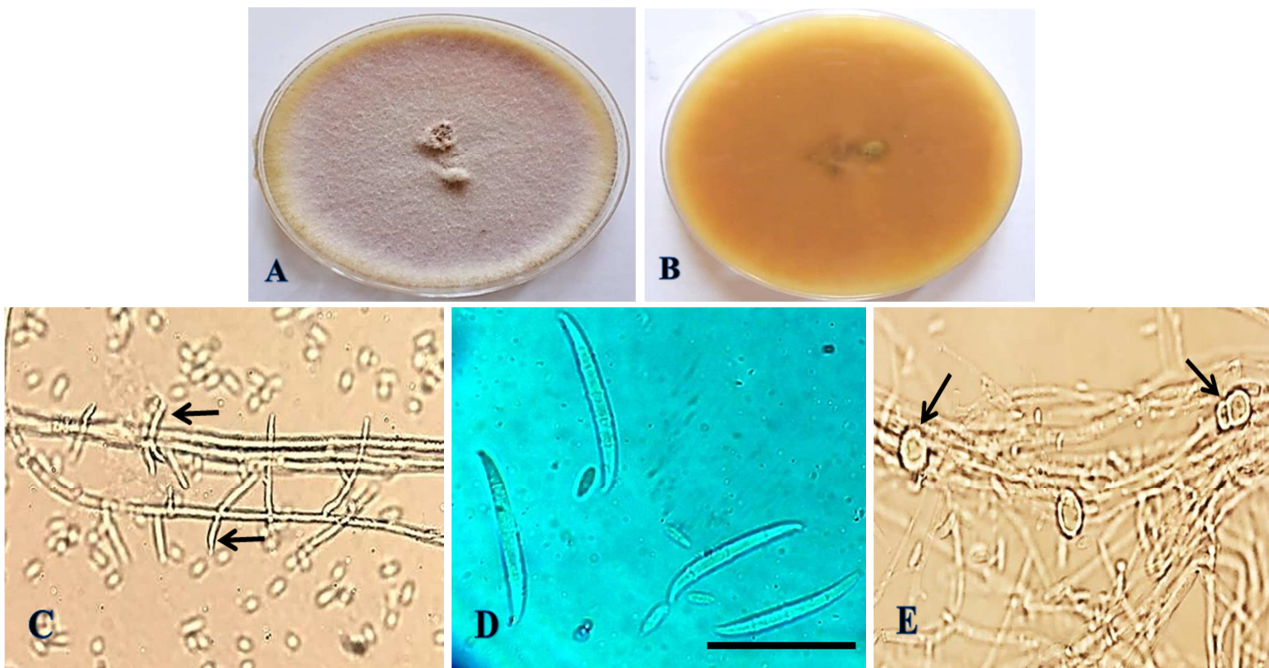


Figure- 6: *Fusarium oxysporum*, (A) Colony surface on PDA; (B) Colony reverse; (C) Short lateral phialides with microconidia; (D) Micro- and Macroconidia; (E) Chlamydospores. Scale bar: D = 30µm (400X).

*Fusarium solani* (Mart.) Saccardo, Michelia 2(7) :296 (1881). Figure (7) A - D.

Colony Characters on PDA: white to creamy on the surface and yellow to light orange on the bottom with sparse mycelium. Microconidia: oval to fusiform, 1 septate, 7.5-10 x 2.5-3.75 µm formed from long lateral phialides. Macroconidia: wide, strait to fusiform, 1-5 septate, 35-37.5 x 3.75-5 µm. Chlamydospores: globose, rough to smooth wall, single or in pair born on short lateral branch, these Characters also were obvious on CLA. This result was coordinate with description of (Booth, 1977 [15]; Nelson *et al.*, 1994 [16]; and Summerell *et al.*, 2003 [17]).

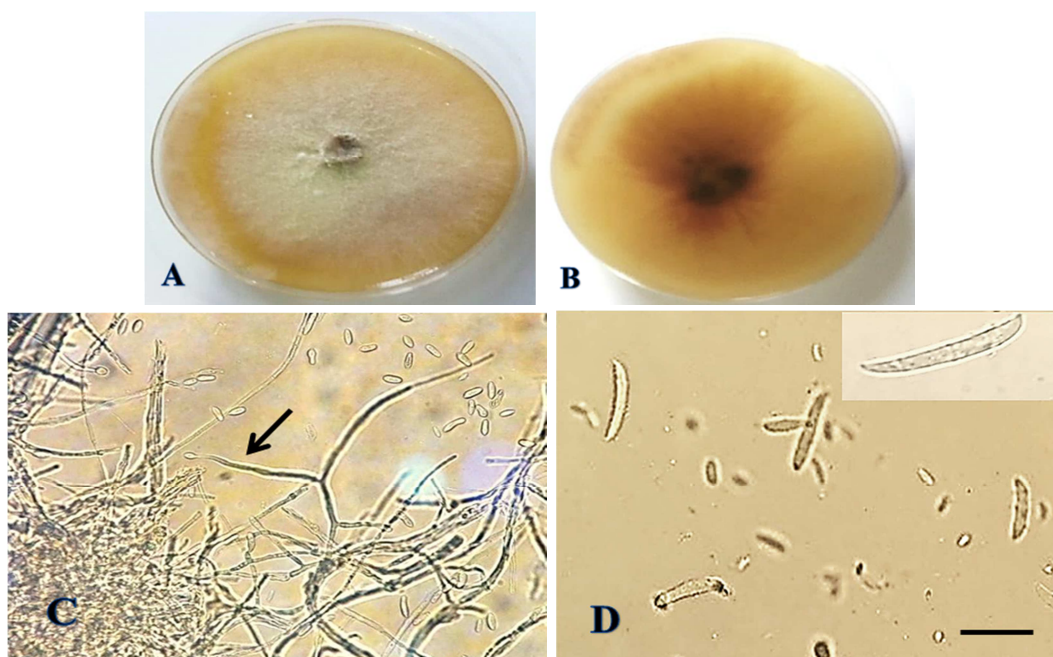


Figure -7: *Fusarium solani*, (A) Colony surface on PDA; (B) Colony reverse; (C) Long monopialide and microconidia; (D) Micro- and macroconidia. Scale bar: D = 35 µm (400X).

### C. Pathogenicity trail of potted wheat plant in greenhouse

Results of pathogenicity test in greenhouse revealed that all tested fungi caused worthwhile infection of wheat seedlings with symptoms of stunting and blight seedlings, rotting of roots and crown. The most virulent were *F. oxysporum* (0.78), *F. solani* (0.70) and *F. graminearum* (0.66). The low disease severity was (0.45) with *F. nivale* (Table 3). Root length of wheat seedling was also reduced and the most affected one was *F. oxysporum* (0.83cm) that was significantly varied from all other fungi. Seedling height was significantly different with control treatment.

Table- 3: Pathogenicity test of Fusarium species and their effect on disease severity, seedling height and root length.

<i>Fungi</i>	<i>Disease severity</i>	<i>Seedling height(cm)</i>	<i>Root length(cm)</i>
<i>F. graminearum</i>	0.66 ab*	20.5 b	4.83 ab
<i>F. oxysporum</i>	0.78 a	20.66 b	0.83 c
<i>F. solani</i>	0.70 ab	24 b	3.66 b
<i>F. avenaceum</i>	0.54 bc	21.16 b	3.91 b
<i>F. nivale</i>	0.45 c	24.83 b	4.91 ab
<i>Control</i>	0 d	31.66 a	6.5 a

\*Means of different letters in each column differ significantly depending on Duncan's Multiple Range test ( $P \leq 0.05$ )

Results were in line with Kane *et al.* (1987)[38] who recorded that *F. graminearum* was more aggressive in causing pre-emergence and postemergence seedling mortality than *F. avenaceum*, while both fungi reduced number of tillers and grain yield. Also Fernandez and Chen (2005) [39] showed that *Fusarium graminearum* was more pathogenic as crown rot to *Fusarium avenaceum* in greenhouse conditions. Gargouri-Kammoun *et al.* (2009) [40] reported that *F. graminearum* produced highest disease severity, while *F. avenaceum* and *F. nivale* were weak pathogenic species under controlled conditions. Through a pathogenicity test on wheat roots, Hajieghrari (2009) [5] revealed that *F. graminearum* and *F. solani* are involved in destruction of wheat root tissues. Gordon *et al.* (1989) [41] demonstrated that *F. oxysporum* and *F. solani* are wheat root colonizers during a pathogenicity test performed in the greenhouse.

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