





# Pathogenicity of *Fusarium* species associated with crown and root rot of wheat (*Triticum aestivum*) under different condition

Patogenicidad de especies de *Fusarium* asociados a daño del ápice y la raíz del trigo (*Triticum aestivum*) bajo diferentes condiciones

Author:

 Bareen Sidqi Shareef Al-Tovi<sup>1</sup>  
 Raed Abduljabbar Haleem<sup>2</sup>

## SCIENTIFIC RESEARCH

### How to cite this paper:

Al-Tovi B.S.S., and Haleem R. A., Pathogenicity of *Fusarium* species associated with crown and root rot of wheat (*Triticum aestivum*) under different condition. *Innovaciencia*. 2019; 7 (1): 1-9.

<http://dx.doi.org/10.15649/2346075X.659>

### Reception date:

Received: 15 January 2019

Accepted: 5 May 2019

Published: 25 October 2019

### Keywords:

*Fusarium* species; Wheat; Pathogenicity; In-vivo; In-vitro; Crown and root rot.

## ABSTRACT

This study was conducted to test the pathogenicity of *Fusarium* species, the causes of crown and root rot disease of wheat crop, under three different conditions (Laboratory, Greenhouse and Field) and to show the best method for pathogenicity among different conditions. Pathogenicity test of six isolates of *Fusarium* species (*F. graminearum*, *F. oxysporum*, *F. avenaceum*, *F. nivale*, *F. solani* and *F. udum*) was tested on durum (Simeto) cultivar of wheat by test tube method in the laboratory, the tested fungi had substantial effect on seed germination. *F. oxysporum* showed the highest germination failure (44.44%) which significantly differed with other species. In the greenhouse, seedlings were inoculated by spore suspension at the base of each plant stem. The most virulent fungus after 35 days of inoculation was *F. oxysporum* (0.78) followed by *F. solani* (0.70) and *F. graminearum* (0.66), while the lowest disease severity was recorded by *F. udum* (0.16). Also in the field pathogenicity experiments of three *Fusarium* species (*F. graminearum*, *F. oxysporum* and *F. solani*) were performed on a durum (Simeto) and soft (Cham6) cultivars. Spore suspension was applied at the 2- to 3-leaf Zadoks's growth stage. Disease severity was calculated at two stages of wheat growth (Booting and Ripening). The most virulent fungus was *F. graminearum* (0.42) that was significantly different from other fungi. This work indicated that *F. graminearum*, *F. oxysporum* and *F. solani* showed higher infection than remaining tested species under three conditions. Pathogenicity test in laboratory by test tube method (*In-vitro*) appeared more effective than greenhouse and field experiments

<sup>1</sup> Plant Protection Department, College of Agriculture, University of Duhok, Duhok Province, Kurdistan Region –Iraq. E-Mail: barin.tovi89@yahoo.com.

<sup>2</sup> Plant Protection Department, College of Agriculture, University of Duhok, Duhok Province, Kurdistan Region –Iraq. E-Mail: raed.haleem@uod.ac.

## INTRODUCTION

*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 <sup>(1)</sup>. Crown rot, foot rot and root rot are economically essential diseases of cereal in various grains producing regions around the world and appear to be especially developed by dry climatic conditions <sup>(2)</sup>. Various pathogen species in the genera *Fusarium* cause crown and root rot disease which includes *Fusarium culmorum*, *F. graminearum*, *F. equisetum*, *pseudograminearum*, *F. acuminatum*, *F. nivale*, *F. avenaceum*, but *F. graminearum*, *F. culmorum* and *F. pseudograminearum*, are epidemiologically the highly important species involved in this disease <sup>(3)</sup>. This disease has been found in many regions of the world, and particularly under dry condition and caused yield losses of more than 50% <sup>(4,5)</sup>. Wheat fungal infections caused by *Fusarium* species are important economically in cereal growing countries including Bulgaria <sup>(6)</sup>. In the pacific Northwest of America Paulitz *et al.* <sup>(1)</sup> 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. Yield losses have been reported 25-58% in Australia, amounting to 80 million US dollars, due to reduced grain yield and quality <sup>(7)</sup>. According to surveys conducted across Duhok province-Iraq, *Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium avenaceum*, *Fusarium nivale* and *Fusarium udum* were isolated from root and crown of wheat <sup>(8)</sup>. There are other studies in Iraq identified *Fusarium* as a causal agent of crown and root rot (FCRR) on wheat plants <sup>(9,10,11)</sup>. Pathogenicity of *Fusarium* species and its aggressiveness on wheat crop evaluate by different methods <sup>(12)</sup> such as: seeds inoculation by putting them on inoculated filter paper <sup>(13)</sup>, seedling inoculation by adding spore suspension to the stem <sup>(14)</sup> or by agar plug mycelium placed next to the lower stem of seedling <sup>(15)</sup>.

This study was undertaken to investigate the virulence of *Fusarium* species isolated from the same location (Sumel district) causes *Fusarium* crown and root rot of wheat (FCRR) under different conditions (*In-vitro* Laboratory and greenhouse and *In-vivo* Field conditions).

## MATERIALS AND METHODS

### Fungal Isolates

Fungal isolates were obtained from crown and root of wheat randomly selected from fields surveyed at Sumel district- Duhok province during 2014-2015. Collected plants were washed thoroughly under running tap water for half hour. Crown and root was taken from each stem. These sections were surface disinfected with 1% sodium hypochlorite (NaOCl) for 2 minutes, rinsed twice in sterilized water, and dried on sterile filter papers. Sections was plated onto full strength potato dextrose agar (PDA) medium that made by (Himedia laboratories Pvt. Ltd.-India) 39.5g.of medium per liter of distal water mended with 250mg of chloramphenicol to inhibit bacterial growth sterilized by autoclave at 121°C for 20mins and 1.5 kg/cm<sup>2</sup>pressure. Plant parts were cultured on sterilized PDA with 5 pieces /plate. The plates were incubated at 25±1°C for 7 days under a 12/12 h photoperiod. Suspected isolates were purified, identified and transfer to fresh media. *Fusarium* species were identified based on their morphological characters including: colony appearance, morphology of microconidia, macroconidia, chlamydospores, and conidiophores according to Booth <sup>(16)</sup>; Nelson *et al.*, <sup>(17)</sup>; Summerell *et al.*, <sup>(18)</sup>; Leslie and Summerell <sup>(19)</sup>.

### Pathogenicity Test

#### Test tube method

The pathogenic nature of six species of *Fusarium* (*Fusarium graminearum*, *F. oxysporum*, *F. avenaceum*, *F. nivale*, *F. solani* and *F. udum*) was tested on durum wheat cultivar (Simeto) by test tube method in the laboratory of College of Agriculture/ University of Duhok. The test tubes (16cm x 2cm) were prepared by filling the tubes bottom with 5cm of cotton. Five milliliter of distilled water was added to each tube and lids were covered with aluminum foil then autoclaved in 121°C and pressure 1.5 kg/cm<sup>2</sup> for 1h. Wheat seeds which surface disinfected by 1% of sodium hypochlorite (NaOCl) for two minutes and washed twice in sterilized distilled water were placed on the moist cotton swab in each test tube (3seeds/ tube). Adjacent to seeds one disk of 5mm of fungal isolate was placed. After inoculation again, the tubes

were sealed with aluminum foil and were putted in incubator at  $25 \pm 1^\circ\text{C}$  for incubation. This test was done according to (20).

The tubes were arranged in complete randomized design (CRD). After two weeks, percentage of seeds germination, percentage of infection, disease severity, and the length of root and green growth were recorded using the following formula:

$$\% \text{ Germination} = \frac{\text{No. of germinated grains}}{\text{Total No. of grains}} \times 100$$

$$\% \text{ Incidence} = \frac{\text{No. of infected seedlings}}{\text{Total No. of examined seedling}} \times 100$$

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 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) (21) as follows:  $DS = \sum d/d_{\text{max}} \times n$

Where DS is the disease severity, d is (the disease rating on each plant),  $d_{\text{max}}$  is (the maximum disease rating possible) and n is (the total number of plants examined in each replicate).

### Pathogenicity trial of potted wheat plant under greenhouse conditions

Pathogenicity experiment was conducted under greenhouse condition at College of Agriculture/ University of Duhok on durum (Simeto) wheat cultivar. Six *Fusarium* isolates (*F. avenaceum*, *F. graminearum*, *F. nivale*, *F. oxysporum*, *F. solani* and *F. udum*) were tested. Before sowing seeds in plastic pots (12cm height  $\times$  11cm width), wheat seeds were surface disinfected by the following method: seeds were washed for 5min by running tap water, immersed in 70 % ethanol and in 1% NaOCl for 1min, rinsed twice in dis-

tilled 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 ( $121^\circ\text{C}$  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 (22). A volume of 10ml of a spore suspension ( $1 \times 10^6$  spores/ml) was added at the base of each plant stem. All plants were watered daily. Disease severity and incidence of crown and root rot of each plant were assessed 35 days after inoculation (22). The experiment was done in a Complete Randomized Design (CRD) which consisted of three replications.

### Pathogenicity trial of potted wheat plant under field conditions

Pathogenicity experiments were conducted in the field of college of Agriculture/ University of Duhok on a durum (Simeto) and soft (Cham6) cultivars of wheat. Three *Fusarium* species (*F. graminearum*, *F. oxysporum* and *F. solani*) isolated during field survey were tested. Before seeds sowing in plastic pots (25x 25 cm), seeds were surface disinfected and air dried. Seeds were sown at a depth of 2cm by 20seeds/pot that contains field clay soil serialized by 1% Formaldehyde, accordingly the soil mixed with peat moss at (2:1). Spore suspension was prepared and applied as mentioned previously at the 2- to 3-leaf Zadoks's growth stage (23). Inoculation was applied to four pots and plants were watered as needed.

At both stages of wheat growth (Booting and Ripening), roots and crown from 10 plants/ pot were washed and severity of crown and root rot was assessed and calculated according to McKinney (1923) (21). The trail was done as factorial in a Randomized Complete block Design (RCBD) with four replications.

### Data analysis

Data were analyzed using Statistical Analysis System (version 8.2; SAS, 2003). Data were subjected to analysis of variance (ANOVA) and pooled together after testing the homogeneity of variance ( $P \leq 0.05$ ). Means of the treatments were compared by Duncan Multiple Range Test at 5% level (24).

## RESULTS

### Test tube method

In-vitro results of pathogenicity test of *Fusarium graminearum*, *F. oxysporum*, *F. solani*, *F. avenaceum*, *F. nivale* and *F. udum* on Simeto cultivar showed the distinctive symptoms of brown to dark brown even black decaying and rotting of seeds, roots, crown area and lower stem to above; root growth and seed-

ling vigor were reduced (Figure 1). Statistical analysis of pathogenicity test showed that the highest and considerable disease incidence and severity were recorded by *F. oxysporum* (0.56) and with no significant difference with *F. solani* and *F. graminearum* (0.53 and 0.50 respectively); with entirely incidence for each that significantly different with fungi (*F. avenaceum* and *F. nivale*). The lowest pathogenicity was shown by *F. nivale* (0.20 and 44.44%) (Table 1).



**Figure 1.** Pathogenicity shows of FCRR disease on wheat seedlings by test tube method.

The tested fungi had substantial effect on seed germination, *F. oxysporum* showed the highest germination failure (44.44%) which significantly differed with other species. The germination rate with *F. graminearum* and *F. solani* were (77.77%) for each. In contrast, wheat seeds with inoculum of *F. nivale*, *F. udum* and *F. avenaceum* germinated entirely. The most affected fungus on seedling height was *F. oxysporum* which was reduced its growth remarkably to 7.6cm. No significant difference appeared between *F. gram-*

*inearum*, *F. solani* and *F. udum* (12.5cm, 11.97cm and 12.52cm respectively), *F. avenaceum* and *F. nivale* were in low effect on the wheat seedlings. The virulence of *F. oxysporum* progressed to root length which led to produce seedling with no or very short roots (1.66cm) with significant difference with tested fungi followed by *F. solani* and *F. graminearum*, the lowest effect was caused by *F. nivale* (7.16cm) compared to control treatment (18.67cm).

**Table 1.** Pathogenicity test of *Fusarium* species and its effect on seed germination rate, seedling height and root length of wheat.

Fungi	Disease severity	%Disease incidence	% Germination	Seedling height(cm)	Root length(cm)
<i>F. graminearum</i>	0.50 a	100 a	77.77 a	12.5 b	5.66 bc
<i>F. oxysporum</i>	0.56 a	100 a	44.44 b	7.66 c	1.66 d
<i>F. solani</i>	0.53 a	100 a	77.77 a	11.97 b	3.55 cd
<i>F. avenaceum</i>	0.26 b	66.66 b	100 a	15.99 a	5.66 bc
<i>F. nivale</i>	0.20 b	44.44 c	100 a	16.66 a	7.16 b
<i>F. udum</i>	0.21 b	88.88 a	88.88 a	12.52 b	6.44 b
Un inoculated (control)	0 c	0 d	100 a	16.83 a	18.67a

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

Results of pathogenicity test in greenhouse revealed that all tested species of *Fusarium* caused worthwhile infection of wheat seedlings with diagnostic symptoms of stunting seedlings, seedling blight, rotting of roots and crown. The most virulent was *F. oxysporum* (0.78) with no significant effect of *F. solani* and

*F. graminearum*. The low disease severity was (0.16) caused by *F. udum* (Table 2). Root length of wheat seedling was reduced and the most affected fungus was *F. oxysporum* (0.83cm) that was significantly varied from all other tested species. Seedling height was also reduced with significant difference from control treatment except *F. udum*.

**Table 2.** Pathogenicity test of isolated fungi and there effect on seedling height and root length under greenhouse conditions.

Fungi	Disease severity	Seedling height(cm)	Root length(cm)
<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>F. udum</i>	0.16 d	25.91 ab	5 ab
<b>Control</b>	0 d	31.66 a	6.5 a

### Pathogenicity trial of potted wheat plant in the field

Pathogenicity trial under field condition showed the diagnostic symptoms of Fusarium Crown and Root

Rot include dark brown to black lesion on root, sub crown internode and stem bases forming large area of dead tissue in crown (Figure 1).



**Figure 2.** Symptoms of FCRR disease on wheat resulted from pathogenicity test in the field showing extend in browning and rotting of root, crown and stem (1: Healthy plant, 2: Infected plant).



Field experiment demonstrated that disease severity in ripening stages was more obvious than in booting stage on both cultivars (Simeto and Sham 6) (Table 3). The most virulent fungus was *F. graminearum* (0.42) that was significantly different from others.

There was no significant difference between *F. oxysporum* and *F. solani*. Whereas the effect of fungi on tested cultivar showed that in both stages of wheat growth durum wheat (Simeto) cultivar was more affected by fungi than soft (Sham6) cultivar.

**Table 3.** Pathogenicity trial of potted wheat plants in the field.

Cultivars	Fungi	Booting stage	Ripening stage	Effect of cultivars
Simeto	<i>F. graminearum</i>	0.28 cd	0.49 a	0.38 a
	<i>F. oxysporum</i>	0.23 de	0.41 ab	0.32 b
	<i>F. solani</i>	0.18 e	0.37 b	0.27 b-d
	Healthy plant	0 f	0 f	0 e
Cham6	<i>F. graminearum</i>	0.20 de	0.36 bc	0.28 bc
	<i>F. oxysporum</i>	0.16 e	0.28 cd	0.22 cd
	<i>F. solani</i>	0.15 e	0.28 cd	0.22 cd
	Healthy plant	0 f	0 f	0 e
Effect of stages	<i>F. graminearum</i>	0.24 c	0.42 a	
	<i>F. oxysporum</i>	0.19 cd	0.34 b	*****
	<i>F. solani</i>	0.16 d	0.32 b	
	Healthy plant	0 e	0 e	

## DISCUSSION

Results of test tube method revealed that wheat seed germination was consistent with the results of other workers who demonstrated that infected wheat seeds with *Fusarium* spp. is often associated with reduced germination, seedling vigor (25, 26) and emergence (27). The highest disease incidence reflected the lowest germination rate, and the least disease incidence gave the highest germination rate (28). When seeds germinate and root tips elongate, nutrients exudate, stimulating the fungal spores to germinate and growth of infection hyphae to penetrate plant tissues. Before or soon after emergence affected seedlings may die even if they are weakly affected (29, 30). Results of pathogenicity test was in line with Brennan *et al.* (2003) (31) who proved that generally *F. graminearum* was more pathogenic than *F. avenaceum* and *F. nivale*, and with result of pathogenicity test of *F. solani* done by Matnyet *et al.* (32). Also similar results reported by Wang (33) who showed that successful invasion of wheat roots by *F. graminearum* produced adverse effect on the root and seedling growth, after 14 days of inoculation half of tested wheat plants were wilted.

Under greenhouse condition, the results were in line with Kane *et al.* (34) who recorded that *F. graminearum* was more aggressive in causing pre and post emergence seedling mortality than *F. avenaceum*, while both fungi reduced number of tillers and grain yield. Also Fernandez and Chen (2005) (35) showed that *F. graminearum* was more pathogenic as crown rot to *F. avenaceum* in greenhouse conditions. Gargouri-Kammounet *et al.* (12) reported that *F. graminearum* produced highest disease severity, while *F. avenaceum* and *F. nivale* were weak pathogenic species under controlled conditions.

In contrast to our results, Jenkinson and Parry (36) and Arseniuket *et al.* (37) recorded that *F. avenaceum* was as, or more, pathogenic than *F. culmorum* and *F. graminearum* to wheat seedlings, while Specht and Rush (38) reported that *F. avenaceum* produced no mortality to winter wheat.

Through a pathogenicity test on wheat roots, Hajjegrhari (39) revealed that *F. graminearum*, *F. udumand* and *F. solani* are involved in destruction of wheat root tissues. Gordon *et al.* (40) demonstrated that *F. oxysporum* and *F. solani* are wheat root colonizers during a

pathogenicity test performed in the greenhouse.

Under Field conditions, result was in agreement with the result of Chekali *et al.* (41) who revealed that soft wheat and durum wheat cultivars grown in Tunis seem to be susceptible to disease but soft wheat cultivars had a partial resistance. Our result was corresponding to the result of other workers who showed that damage of disease severity of crown rot is highly affected by plant stress as reach to the maturity stage, because maturation of wheat occurs in midsummer when temperature is high and water available in the soil is limited (42, 43). Also Covarelli *et al.* (44) recorded that after inoculation of *Fusarium* spp. to the base of wheat seedlings, symptoms of disease appeared at the stem base and reached to upper nodes when plant reached to maturity. Paulitz *et al.* (4) revealed that after dry climate during anthesis and ripening severity of crown rot is increased. Infection the crown and root might then be a source of inoculum for infection of heads the following seasons or might constitute a significant fungal survival mechanism under dry conditions.

## CONCLUSION

We conclude that *Fusarium graminearum*, *F. oxysporum* and *F. solani*, the causes of crown and root rot of wheat, were highly virulent and more pathogenic than remaining tested species under three conditions (Laboratory, Greenhouse and Field condition). Additionally, our results indicated that Test tube method in laboratory is simple, rapid and reliable method for pathogenicity studies of *Fusarium* species.

## REFERENCES

1. Paulitz T. C., Smiley R. W. and Cook R. J. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. *Can. J. of Plant Pathol.*, 2002; 24(4): 416-428.  
<https://doi.org/10.1080/07060660209507029>
2. Burgess L.W., Backhouse D., Summerell B. A. and Swan L. J., Chapter 20 in *Fusarium*- Paul E. Nelson Memorial Symposium. Edited by Summerell B.A., Leslie J.F., Backhouse D. Bryden W.L and Burgess L.W. APS Press, The American Phytopathological Society, St. Paul, Minnesota, United States of America. 2001; ISBN 0-89054-268-6. pp. 271-294.
3. Cook, R. J., *Fusarium* root, crown, and foot rots and associated seedling diseases. Compendium of wheat diseases and pests. 3<sup>rd</sup> ed. The Pennsylvania State University Press, University Park, Pennsylvania, USA, 2010; 37-39.
4. Mergoum, M., Lyamani, A. and Nsarellah, N., Root rot of wheat. *Al Awamia Magazine National Government publication Morocco*, 1995; 89:1-24.
5. Nicol, J. M., Bagci, A., Hekimhan, H., Bolat, N., Braun, H. J. and Trethowan, R., Strategy for the identification and breeding of resistance to dry land root rot complex for international spring and winter wheat breeding programs. Page 283 in: *Proc. 4th Int. Crop Sci. Congr.*, Brisbane, Australia, 2004.
6. Beev, G., Denev, S. and Pavlov, D., Occurrence and distribution of *Fusarium* species in wheat grain. *Agricul. Scie. and Technol.* 2011; 3(2):165-168.

7. Chakraborty, S., Obanor, F., Westecott, R. and Abeywickrama, K., Wheat crown rot pathogens *Fusarium graminearum* and *F. pseudograminearum* lack specialization. *Phytopathology*, (2010); 100:1057-1065.  
<https://doi.org/10.1094/PHTO-01-10-0007>
8. Al-Tovi, B.S., Prevalence and Molecular identification of *Fusarium* Crown and Root Rot of Wheat in Sumel-Duhok Province. M.Sc. Thesis, College of Agriculture, University of Duhok, Kurdistan region-Iraq. 2017.
9. Hameed, M. A., Rana, R. M. and Ali, Z., Identification and characterization of a novel Iraqi isolate of *Fusarium pseudograminearum* causing crown rot in wheat. *Genet. Mol. Res*, 2012; 11(2): 1341-1348. <https://doi.org/10.4238/2012.May.15.4>
10. Khalifah, M.H., Molecular identification of wheat crown rot pathogen and evaluation of some protective methods. M.Sc. thesis, College of Agriculture, University of Baghdad, Baghdad, Iraq. 2013; (In Arabic).
11. Taha, Z.A., Wheat root rot in Ninevah government and its biological control. M.Sc. thesis, College of Agriculture and Forestry, University of Mosul, Iraq. 2014; (In Arabic).
12. Gargouri-Kammoun, L., Gargouri, S., Rezgui, S., Trifi, M., Bahri, N. and Hajlaoui, M. R., Pathogenicity and aggressiveness of *Fusarium* and *Microdochium* on wheat seedlings under controlled conditions. *Tunisian J. of Plant Prot.*, 2009; 4(2):135-144.
13. Brennan, J.M., Fagan, B., van Maanen, A., Cooke, B.M. and Doohan, F.M., Studies on *in-vitro* growth and pathogenicity of European *Fusarium* fungi. *Eur. Journal of Plant Pathology*.(2003); 109: 577-587. <https://doi.org/10.1023/A:1024712415326>
14. Carter, J. P., Rezanoor, H. N., Holden, D., Desjardins, A. E., Plattner, R. D. and Nicholson, P., Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. *Eur. J. of Plant Pathol.*, 2002; 108(6): 573-583.  
<https://doi.org/10.1023/A:1019921203161>
15. Tunalı, B., Nicol, J., YeldaErol, F. and Altıparmak, G., Pathogenicity of Turkish crown and head scab isolates on stem bases on winter wheat under greenhouse conditions. *Plant Pathol. J.* 2006; 5: 143-149.  
<https://doi.org/10.3923/ppj.2006.143.149>
16. Booth, C., *Fusarium*, Laboratory guide to the identification of the major species. Commonwealth Mycological Institute. Academic press. UK, 1977; ISBN 0 85198 3839.
17. Nelson, P.E., Dignani, M.C. and Anaissie, E.J., Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clinical Microbiology Reviews*, 1994;7(4): 479-504.  
<https://doi.org/10.1128/CMR.7.4.479>
18. Summerell, B.A., Salleh, B. and Leslie, J.F., A utilitarian approach to *Fusarium* identification. *Plant Disease*, 2003; 87(2): 117-128.  
<https://doi.org/10.1094/PDIS.2003.87.2.117>
19. Leslie, J.F. and Summerell, B.A., Techniques and methods. *Fusarium laboratory manual*. Blackwell Publishing Ltd., Oxford, UK; 2006.  
<https://doi.org/10.1002/9780470278376>
20. Asad, S.I., Shamim. A. and Iftikhar, A., Characterization of *Bipolaris sorokiniana* isolated from different agro-ecological zones of wheat production in Pakistan. *Pakistan. Journal Botany*, 2009; 41(1): 301-308.
21. McKinney, H. H., Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J. of Agricult. Res.*, 1923; 26(5), 195-218.
22. Mitter, V., Zhang, M. C., Liu, C. J., Ghosh, R., Ghosh, M. and Chakraborty, S., A high-throughput glasshouse bioassay to detect crown rot resistance in wheat germplasm. *Plant Pathology*, 2006; 55(3): 433-441.  
<https://doi.org/10.1111/j.1365-3059.2006.01384.x>
23. Zadoks, J. C., Chang, T. T. and Konzak, C. F., A decimal code for the growth stages of cereals. *Weed Res.*, 1974;14(6): 415-421.  
<https://doi.org/10.1111/j.1365-3180.1974.tb01084.x>
24. SAS., SAS/STAT User's Guide, Version 8.2, 1<sup>st</sup> printing, Vol. 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina, USA, 2003.
25. Bechtel, D. B., Kaleikau, L. A., Gaines, R. L. and Seitz, L. M., The effects of *Fusarium graminearum* infection on wheat kernels. *Cereal Chemistry*, 1985; 62(3):191-197.
26. Akınanmi, O. A., Mitter, V., Simpfendorfer, S., Backhouse, D. and Chakraborty, S., Identity and pathogenicity of *Fusarium* spp. isolated from wheat fields in Queensland and northern New South Wales. *Aust. J. Agric. Res.* 2004; 55: 97-107.  
<https://doi.org/10.1071/AR03090>



27. Humphreys, J., Cooke, B. M. and Storey, T., Effect of seed borne *Microdochium nivale* on establishment and grain yield. *Plant Varieties and Seeds* 1995; 8:107–117.
28. Debnath, M., Sultana, A. and Rashid, A. Q. M. B., Effect of Seed-borne Fungi on the Germinating Seeds and their Bio-control in Maize. *Journal of Environ. Sci. and Natural Res.*, 2012; 5(1):117-120. <https://doi.org/10.3329/jesnr.v5i1.11564>
29. Rodriguez-Galvez, E. and Mendgen, K., The infection process of *Fusarium oxysporum* in cotton root tips. *Protoplasma*, 1995; 189(1-2): 61-72. <https://doi.org/10.1007/BF01280291>
30. Deacon J. W. and Donaldson S.P., Molecular recognition in the homing responses of zoosporic fungi, with special reference to *Phytium* and *Phytophthora*. *Mycol Res* 1993; 97:1153-1171. [https://doi.org/10.1016/S0953-7562\(09\)81278-1](https://doi.org/10.1016/S0953-7562(09)81278-1)
31. Brennan, J.M., Fagan, B., van Maanen, A., Cooke, B.M. and Doohan, F.M., Studies on *in-vitro* growth and pathogenicity of European *Fusarium* fungi. *Eur. J. of Plant Pathol.* 2003; 109: 577-587. <https://doi.org/10.1023/A:1024712415326>
32. Matny, O. N., Chakraborty, S., Obanar, F. and Al-Ani, R. A., Molecular identification of *Fusarium* spp causing crown rot and head blight on winter wheat in Iraq. *J. of Agricult. Technol.*, 2012; 8(5): 1677-1690.
33. Wang, Q., The pathosystem wheat (*Triticum aestivum*) root-*Fusarium graminearum*: complex plant responses and fungal strategies, Ph.D. dissertation, Justus Liebig University Giessen, Germany. 2015.
34. Kane, R. T., Smiley, R. W. and Sorrells, M. E., Relative pathogenicity of selected *Fusarium* species and *Microdochium bolleyi* to winter wheat in New York. *Plant Dis.* 1987; 71:177-181. <https://doi.org/10.1094/PD-71-0177>
35. Fernandez, M. R. and Chen, Y., Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. *Plant Dis.*, 2005; 89(2): 164-169. <https://doi.org/10.1094/PD-89-0164>
36. Jenkinson, P. and Parry, D. W., Isolates of *Fusarium* species from common broadleaved weeds and their pathogenicity to winter wheat. *Mycol. Res.* 1994; 98:776-780. [https://doi.org/10.1016/S0953-7562\(09\)81054-X](https://doi.org/10.1016/S0953-7562(09)81054-X)
37. Arseniuk, E., Goral, T. and Czembor, H. J., Reaction of triticale, wheat and rye accessions to graminaceous *Fusarium* spp. infection at the seedling and adult plant growth stages. *Euphytica* 1993; 70:175-183. <https://doi.org/10.1007/BF00023757>
38. Specht, L. P. and Rush, C. M., Fungi associated with root and foot rot of winter wheat and populations of *Cochliobolus sativus* in the Texas Panhandle. *Plant Dis.* 1988; 72:959-963. <https://doi.org/10.1094/PD-72-0959>
39. Hajieghrari, B., Wheat crown and root rotting fungi in Moghan area, Northwest of Iran. *Afri. J. of Biotechnol.*, 2009; 8(22):6214-6219. <https://doi.org/10.5897/AJB09.1087>
40. Gordon, T. R., Okamoto, D. and Jacobson, D. J. colonization of muskmelon and non susceptible crops by *Fusarium oxysporum* f. sp. melonis and other species of *Fusarium*. *Phytopathology*, 1989; 79(10):1095-1100. <https://doi.org/10.1094/Phyto-79-1095>
41. Chekali, S., Gargouri, S., Berraies, S., Gharbi, M.S., Nicol, M.J. and Nasraoui, B., Impact of *Fusarium* foot and root rot on yield of cereals in Tunisia. *Tunisian J. of Plant Protec.t* 2013; 8: 75-86.
42. Papendick, R. I. and Cook, R. J., Plant water stress and development of *Fusarium* foot rot in wheat subjected to different cultural practices. *Phytopathology* 1974; 64:358-363. <https://doi.org/10.1094/Phyto-64-358>
43. Smiley, R. W., Gourlie, J. A., Easley, S. A. and Patterson, L. M., Pathogenicity of fungi associated with the wheat crown rot complex in Oregon and Washington. *Plant Dis.*, 2005; 89(9): 949-957. <https://doi.org/10.1094/PD-89-0949>
44. Covarelli, L., Beccari, G., Steed, A. and Nicholson, P., Colonization of soft wheat following infection of the stem base by *Fusarium culmorum* and translocation of deoxynivalenol to the head. *Plant Pathol.*, 2012; 61(6), 1121-1129. <https://doi.org/10.1111/j.1365-3059.2012.02600.x>