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RESEARCH ARTICLE

Comparison of Aggressiveness of Four *fusarium* spp. That cause Head blight on Bread wheat, Durum wheat and Barley

Nachaat Sakr

Department of Agriculture, Atomic Energy Commission of Syria (AECS), Damascus, P.O. Box 6091, Syria.

Corresponding Author:
Nachaat Sakr, Email: ascientific33@aec.org.sy

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A B S T R A C T

Fusarium head blight (FHB) is a significant disease affecting wheat and barley and is caused by several *Fusarium* species. However, contradictory data is available about their comparative pathogenicity on small grain cereals. Also, little research have compared the pathogenicity of individual *Fusarium* isolates on different plant parts (heads vs. detached leaves, detached heads, and coleoptiles) on cereals. This study aimed to assess the comparative pathogenicity of four *Fusarium* species at the earliest and latest growth stages under artificial infection with eight bread wheat, durum wheat and barley cultivars with diverse quantitative resistance levels. Four *Fusarium* species (*F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiesti*) were identified using an aggressive index involving nine pathogenic components generated under *in vitro*, growth chamber and field conditions to explore the relationships among *Fusarium* fungi and cereal plants. All the 16 tested FHB isolates and species were found to be pathogenic and induced typical FHB symptoms on small plant parts and adult heads. High level of variation in aggressiveness was observed among isolates within the species tested. Nevertheless, there was a similar comparative pathogenicity among the four tested *Fusarium* species when testing on the head and small plant parts tests. The origin of FHB pathogens may play a crucial role in this pathogenic similarity. This study suggests that screening for resistance to FHB requires the use of aggressive isolates or a mixture of several isolates. In addition, it indicates a lack of adaptation of these four tested *Fusarium* species. Our data clarifies for the first time the nature of comparative pathogenicity of diverse FHB species on durum wheat. It will provide critical information for cereal breeders to develop and improve FHB-resistant cultivars.

Keywords: Fusarium head blight, comparative pathogenicity, *Hordeum vulgare*, *Fusarium* pathogens, *Triticum* spp.

INTRODUCTION

Cereals are cultivated across more acreage than any other agricultural crops globally, with staple foods such as wheat, including bread (*Triticum aestivum*) and durum (*T. durum*), barley (*Hordeum vulgare*) and many other plants of Poaceae family being the most largely sown (FAO, 2022; Ahmad *et al.,* 2025). The genus *Fusarium* is one of the most relevant fungi invading cereal crops in several parts worldwide (Nazir *et al.,* 2012; Stepien, 2020). Several *Fusarium* species are occurring commonly and widespread as pathogens of *Triticum* and *Hordeum*, resulting in diverse fungal diseases: root and foot rot, crown rot, and Fusarium head blight (FHB) (Agrios, 2004). Nevertheless, FHB is one of the most destructive diseases on bread wheat, durum wheat and barley (Moonjely *et al.,* 2023) economic damages occur due to decreases in kernel yield and quality, particularly from the degradation of gluten proteins by the fungi leading to lower bakery characters (Buerstmayr *et al.,* 2020; Atiq *et al.,* 2024; Usman *et al.,* 2025). Also, kernels could be contaminated with mycotoxins that, in some cases, are regulated at the maximum permitted values, making wheat/barley trading more difficult (Ma *et al.,* 2019). In addition, these mycotoxins are noxious for human and animal health. In plants, they can result in chlorosis, dwarfism, and inhibition of shoot and root elongation and act as a pathogenicity element in FHB (Sakr, 2023). Deoxynivalenol (DON) is the principal trichothecene present in cereals (Moonjely *et al.,* 2023) and cereal-based products (Fernando *et al.,* 2021).

Aggressiveness is an attribute of a pathogen originally identified by Van Der Plank (1968) as the quantity of disease produced by a pathogenic strain on a susceptible host. The aggressiveness of FHB isolates accounts on their DON-producing ability (Mesterhazy, 2002). DON-non-producing strains of *Fusarium* provoked a low score of disease intensity in *Triticum* and *Hordeum* plants (Eudes *et al.,* 2001). Bai *et al.,* (2001) observed that the DON-non-producing strains still could infect wheat heads but could not spread beyond the initial invasion site, proposing that DON is an aggressiveness component rather than a pathogenicity component. Different works found diversity in aggressiveness among FHB strains isolated from diverse locations of the world within a country and even within *Fusarium* populations from individual fields (Xue *et al.,* 2019; Toth *et al.,* 2020). Aggressiveness of *Fusarium* pathogens is not geographically structured since fungal strains with high, medium, and low estimates of aggressiveness make up the FHB population in a single part (Miedaner *et al.,* 2021). Most works highlighted a high level of genetic variation in FHB species within individual field populations or *Fusarium* populations isolates over a definite geographical scale (Buerstmayr *et al.,* 2020). Knowledge of strain features in terms of aggressiveness in any part is requested for predicting the pathogenic capacity of FHB species and for the deployment of resistant wheat/barley cultivars in a given location (Fernando *et al.,* 2021).

Examination of bibliography reveals that pathogenicity of *Fusarium* species on barley may not be the same as on bread wheat, highlighting contradictory data about the comparative pathogenicity of several *Fusarium* pathogens implicated in this destructive disease on bread wheat and barley. Fernandez and Chen (2005) reported that *F. graminearum* and *F. culmorum* were the most pathogenic species on bread wheat, although the latter was more pathogenic than the former. *F. poae* and *F. equiseti* were the least pathogenic species, whereas *F. avenaceum* had intermediate pathogenicity in the seed and head analyses, but low pathogenicity in the seedling experiment (Fernandez and Chen, 2005). However, Xue *et al.,* (2006) tested the pathogenicity of eight *Fusarium* pathogens causing head blight in barley, i.e., *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. crookwellense*, *F. acuminatum*, *F. sporotrichioides*, *F. equiseti* and *F. poae*. All pathogens analyzed caused FHB symptoms on *H. vulgare* cultivars, but only *F. graminearum*, *F. culmorum* and *F. crookwellense* resulted in severe head blight development (> 65% proportion of infected florets) and were found to be highly pathogenic. *F. avenaceum* had 48% proportion of infected florets, which was significantly lower than those of the three highly pathogenic pathogens and was moderately pathogenic. The remaining species had < 15% proportion of infected florets and were weakly pathogenic (Xue *et al.,* 2006). Till now, no reports are available on comparative pathogenicity on durum wheat. In addition, little research have compared the pathogenicity of individual *Fusarium* isolates on different plant parts (heads vs. detached leaves, detached heads, and coleoptiles) on cereals.

Regarding aggressiveness of different FHB species on bread wheat, pathogenicity analyses of *Fusarium* species on cereal roots vs. heads or crowns vs. heads have been previously conducted (Kane *et al.,* 1987; Mihuta-Grimm *et al.,* 1989; Arseniuk *et al.,* 1993; Wong *et al.,* 1995; Golinski *et al.,* 2002). *Fusarium graminearum*, *F. culmorum*, *F. acuminatum*, *F. poae*, *F. avenaceum*, *F. sporotrichioides* and *F. equiseti* are sampled frequently from FHB infected grains (Clear *et al.,* 1996; McCallum *et al.,* 2000). Of these species, *F. graminearum* and *F. culmorum* are considered the most important causal agents of FHB disease complex globally (Clear *et al.,* 1996; Tekauz *et al.,* 2000). Research on the pathogenicity of these *Fusarium* pathogens on *Triticum aestivum* showed that only *F. graminearum* and *F. culmorum* were highly pathogenic, *F. sporotrichioides* was intermediate, and the other *Fusarium* species were weakly pathogenic (Stack and McMullen, 1985; Wong *et al.,* 1995; Stack *et al.,* 1997). *F. avenaceum* was also found to be as more pathogenic or equally than *F. graminearum* and *F. culmorum* (Buerstmayr *et al.,* 2009). In Australia, Liddell (1985) observed that *F. crookwellense* is also highly pathogenic on *T. aestivum*, resulting in more severe root and crown rot than *F. graminearum* and *F. culmorum*. In Japan, *Fusarium crookwellense* was also sampled at 1991 from scabby *T. aestivum* grains and confirmed to cause typical head blight symptoms on *Triticum* and *Hordeum*

(Sugiura *et al.,* 1994). Under controlled conditions, Xue *et al.* (2004) showed that *F. crookwellense* is highly pathogenic on *Triticum aestivum*, causing severe disease symptoms. High level of diversity in aggressiveness was also found among the pathogens analyzed: *F. graminearum sensu stricto* strains were the most aggressive, followed by those of *F. boothii*, and lastly by *F. crookwellense* (Malihipour *et al.,* 2021). In some of these works, strains from several sources of each of the pathogen were analyzed. However, little research have compared the pathogenicity of individual *Fusarium* strains on underground- and above tissue of *Triticum aestivum*. Arseniuk *et al.,* (1993) observed a negative link between seedling blight and head blight of winter bread wheat caused by a spore mixture of different *Fusarium* species.

On barely, all *Fusarium* species have been proved to cause head blight symptoms, except *F. equiseti* and *F. acuminatum* (Perkowski *et al.,* 1995; Salas *et al.,* 1999; Sugiura *et al.,* 1994), but little data is available on the comparative pathogenicity of these pathogens. McCallum and Tekauz (2002) found that *H. vulgare* differs from *T. aestivum* with regard to the profile of the pathogenic species causing head blight disease in nature and the plant growth stage most susceptible to *Fusarium* infection. When cereal plants were artificially infected with a wild-type and DON production gene disrupted *F. graminearum*, Jansen *et al.,* (2005) reported a new pathway of infection in *Hordeum vulgare*, but not in *Triticum aestivum*.

A better understanding of the comparative pathogenicity of *Fusarium* pathogens on small-grain cereals, i.e., wheat and barley, is important in developing head blight-resistant cultivars globally. In this work, we compared the pathogenicity of 16 fungal isolates for four *Fusarium* pathogens causing FHB, i.e., *F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiesti*, on eight bread wheat, durum wheat and barley with different levels of quantitative resistance. We also compared the relative pathogenicity of *Fusarium* isolates on heads vs. small plant parts, i.e., detached leaves, detached heads, and coleoptiles.

MATERIALS AND METHODS

Plant materials, fungal isolates, and inoculum preparation: Throughout this study, a set of widely grown high-yielding eight Syrian cereal cultivars with favorable agronomic and quality traits (high harvest index, earliness, shorter plants and improved biomass partitioning to the grain) and resistance to different fungal diseases including FHB disease. According to earlier head blight resistance experiments by infection of seedlings, detached leaves and heads, heads and florets on wheat and barley cultivars, i.e., area under disease progress curve (AUDPC) of Petri-dish infection, latent period (LP) detached leaf infection, and coleoptile length reduction CD of a coleoptile inoculation determined *in vitro*, disease severity (DS) and disease incidence (DI) determined utilizing a detached head test in a growth chamber, and DI determined utilizing a spike artificial infection and DS determined utilizing a spikelet artificial infection in the growth chamber, DI and DS determined utilizing a spike artificial infection in the filed over three consecutive growing seasons (Sakr, 2023), moderately resistant cultivars, i.e., Arabi Aswad (barely, AS) and Bohoth10 (bread), moderately susceptible cultivars, i.e., Arabi Abiad (barley, AB), Cham4, and Douma4 (bread), susceptible to moderately susceptible cultivars, i.e., Cham7 and Cham9, a susceptible cultivar, i.e., Acsad65 (durum) were included to cover a wide genetic and resistant variability.

Sixteen single-spored cultures belonging to four *Fusarium* pathogens causing head blight, i.e. (*F. equiseti* (one isolate), *F. verticillioides* (synonym *F. moniliforme*) (4 isolates), *F. culmorum* (5 isolates), and *F. solani* (6 isolates)), representative of the level in pathogenicity ability were individually and artificially inoculated on the above-cited wheat and barley cultivars to better understand the comparative aggressiveness of fungi on plant materials. To ensure adequate pathogenicity on the tested wheat and barley plants, pathogenic reactions were analyzed with the main *Fusarium* species present in Syrian wheat fields because FHB pathogens were not recovered from Syrian barley fields till now. Although *F. graminearum* is considered the major causative of FHB complex worldwide (Moonjely *et al.,* 2023), this species was not found in the surveyed region in our study Ghab Plain, one of the principal Syrian wheat production areas, as observed in other reports investigating the composition of FHB complex species in our surveyed area during spring of three seasons (2008-2010) (Al-Chaabi *et al.* 2018). In fact, the selection of FHB species used in our study (*F. culmorum* (31.3%), *F. solani* (37.5%), *F. verticillioides* (25.0%) and *F. equiseti* (6.3%)) was reflective of other populations recovered from Ghab Plain (Al-Chaabi *et al.* 2018); *F. culmorum* was the most frequent causing agent in Syria. These fungal cultures were utilized in earlier studies and proved their aggressiveness capacity (Sakr, 2023). Through the 2015 flowering season, the sampling of these FHB isolates, referred herein as non-moisture treated isolates, was achieved from naturally invaded wheat heads in Ghab Plain with a FHB history, one of the chief wheat production areas in Syria, through nine infected FHB locations. By freezing at -16°C or cold storage in sterile distilled water (SDW) at 4°C, *Fusarium* isolates were preserved until used for inoculation (Sakr, 2020).

To produce conidia for artificial inoculations, isolates were cultured in Petri dishes on PDA at 22ºC under continuous darkness for 10 days to permit *Fusarium* sporulation and development. The macroconidia were harvested from 7-day-old cultures following covering of fungal cultures with 10 ml of SDW. In order to remove the pieces of agar and mycelia, filtrating through 2 layers of sterile cheesecloth was carried out as described by Sakr (2023). Inoculum was directly adjusted to requested values as FHB inoculum sources (1×10^6 conidia/ml for latent period (LP, days) and area under disease progress curve (AUDPC, ranging from 0 (not aggressive) to 1 (very aggressive)) criteria, 2×10^5conidia/ml for coleoptile length reduction (CLR, %) criterion, and 5×10^4spores/ml for aggressiveness criteria on adult heads) under an optical microscope with a Neubauer chamber.

Measuring comparative aggressiveness of FHB species: At the seedling and adult plant stages, nine pathogenic responses of three bread cultivars, three durum cultivars and two barley cultivars were used to measure comparative aggressiveness of *Fusarium* species under *in vitro*, climatic growth chamber and field conditions. The evaluation of pathogenic reactions of all cultivars infected with *Fusarium* fungi were previously conducted according to methods described by Sakr (2023): area under disease progress curve (AUDPC) of Petri-dish infection, latent period (LP) detached leaf infection, and coleoptile length reduction (CLR) of a coleoptile inoculation determined *in vitro*, disease severity (DS, %) and disease incidence (DI, %) determined utilizing a detached head test in a growth chamber, and DI determined utilizing a spike artificial infection and DS determined utilizing a spikelet artificial infection in the growth chamber, DI and DS determined utilizing a spike artificial infection in the filed over three consecutive growing seasons. There were no significant effects for interaction year × fungus/cultivar (environmental findings for the station were somewhat similar over the three growing seasons (Sakr 2023)), field observations were exhibited as the means of the three growing seasons. Aggressiveness index (A index) was employed to elucidate the links among *Fusarium* fungi and cereal plants (Sakr, 2024). This index encompassed nine components related to the aggressiveness of FHB isolates.

$$A\ index = (DS^{FC} + DI^{FC} + DS^{CC} + DI^{CC} + DS^{DHT} + DI^{DHT} + CD + AUDPC)/ LP$$

Where: A FHB fungus with lower value of LP and higher values of DS^{FC}, DI^{FC}, DS^{CC}, DI^{CC}, DS^{DHT}, DI^{DHT}, CD, and AUDPC was considered as more pathogenic than a FHB fungus with higher values of LP and lower value of DS^{FC}, DI^{FC}, DS^{CC}, DI^{CC}, DS^{DHT}, DI^{DHT}, CD, and AUDPC.

The usefulness of three distinguished *in vitro* bio-experiments, i.e., latent period (LP) of detached leaf infection, area under disease progress curve (AUDPC) of Petri-dish infection and coleoptile length reduction (CLR) of a coleoptile inoculation was investigated herein to analyze the comparative pathogenicity of different *Fusarium* species. The experiments were laid out in a completely randomized design with three replications. The experiment was repeated three times. For LP analysis, wheat/barley seeds were then grown in 15 cm plastic pots in sterilized soil in a growth chamber with 16 hours of light per day at a daytime/nighttime temperature of 20 °C. For AUDPC analyses, inoculated and control treatments were incubated in an incubator in the dark at 22ºC. For CLR analyses, treatments were incubated with a 16 h photoperiod under 15ºC.

Regarding adult head analyses under controlled conditions, the 16 FHB isolates were individually inoculated on eight wheat and barley cultivars in a growth chamber at 20°C day/night temperature, and 16/8 h light/dark cycle to DI and DS on discrete heads of the same cultivar as indicators of the pathogenicity and quantitative resistance. Wheat/barley seeds were surface sterilized with 5% sodium hypochlorite solution for 8 min and then washed six times in sterile distilled water. They were sown into plastic 15-cm pots containing sterilized clay soil. The potting soil consisted of 57% clay, 39% loam and 2% sand. The experimental design was a completely randomized design, comprising three replicates for each isolate. Three pots per replicate were left non-inoculated as control treatment. Following emergence, plants were thinned and nitrogen fertilizer was applied twice at two dates: emergence and tillering. Both types of inoculations, head and floret, were made on the discrete heads of the same cultivar in two separate experiments. For field experiments, wheat and barley plants were grown under natural climatic conditions with the same experimental design.

All experiments were arranged in a factorial design (e.g., two-way ANOVA with *Fusarium* isolates × cultivar interaction).

It is widely observed that resistant wheat/barley cultivars exhibit durable resistance to nearly all FHB isolates worldwide (Fernando *et al.,* 2021). In our report, combined analysis of bio-experiments showed that isolate × cultivar interactions were significant as revealed by Sakr (2023). These findings are in harmony with earlier reports that showed a significant interaction between *Triticum* and *Hordeum* and isolates of *Fusarium* pathogens (Xue *et al.,* 2019).

STATISTICAL ANALYSES

Data were evaluated using analyses of variance using DSAASTAT add-in version 2011. The percentages were transformed before statistical analysis using the angular transformation to stabilize variances. To compare the values of aggressiveness of fungal isolates and species, ANOVA incorporating the Fisher's LSD test at P>0.05was used.

RESULTS AND DISCUSSION

In any environment, knowledge of quantitative components in terms of pathogenicity is important for predicting the aggressiveness capacity of *Fusarium* pathogens (Asif *et al.,* 2023; Moonjely *et al.,* 2023). This requires that their evaluations should be carried out under several experimental conditions (Sakr, 2023) since environmental/climatic conditions infect *Fusarium*/cereal responses because of cultivar/ isolate by environment interaction (Buerstmayr *et al.,* 2020; Fernando *et al.,* 2021). Intensity/damage of FHB isolates/species generally varies from location to location and from growing season leading to change in aggressiveness shifts and disease outbreaks of FHB population that result in potential erosion in wheat/barley resistance (Sakr, 2022).

DON as a key aggressiveness factor (Ma *et al.,* 2019). While the four tested *Fusarium* species, i.e., *F. solani*, *F. culmorum*, *F. verticillioides*and *F. equiseti*, are considered of FHB pathogens (Moonjely *et al.,* 2023), and great pathogenic variability was observed in the tested FHB isolates (Sakr, 2023); it can be concluded that these tested pathogens produce toxins in the cereal grains since the toxins may act as aggressiveness factors and enhance the pathogenicity of *Fusarium* fungi in cereal crops and lead to FHB disease (Fernando *et al.,* 2021). We hypothesized that these pathogens are toxin-producing species in spite of the toxin analyses were not conducted on these fungi.

Our data highlighted that there was a similar comparative aggressiveness, the quantitative component of pathogenicity that is expressed horizontally, irrespective of plant cultivars or species (Van der Plank, 1968), in the head and small plant parts tests among the four tested *Fusarium* species, i.e., *F. culmorum*, *F. equiseti*, *F. solani*, and *F. verticillioides*, indicating a lack of adaptation of these *Fusarium* pathogens. The origin of *Fusarium* pathogens may play a significant role in this pathogenic similarity. The differences in aggressiveness among the sixteen FHB isolates within each of the four analyzed *Fusarium* species were significant in our investigation. The presence of diverse scores of aggressiveness among *Fusarium* isolates has practical implications that must be taken into account when screening *Triticum* and *Hordeum* for FHB resistance (Parry *et al.,* 1995; McMullen *et al.,* 2012). It is significant that FHB isolates with a known level of aggressiveness be employed in cultivar assessment experiments, so that valid comparisons can be conducted between cultivars (Ma *et al.,* 2019; Buerstmayr *et al.,* 2020). However, significant interactions were found between the eight wheat and barley cultivars across the sixteen *Fusarium* isolates. This further demonstrates that analyses governing isolate aggressiveness of FHB populations were more complicated than *Triticum*/*Hordeum* host reaction to environmental conditions and DON production (Miedaner *et al.,* 2021).

Head blight aggressiveness is probably the outcome of timely expression of different genes, including production of specific metabolites, hormones, cell-wall-degrading enzymes, and mycotoxins that change the *Triticum*/*Hordeum*host's resistance reaction (Fernando *et al.,* 2021). In the present work, comparing to the SDW control treatment, *Triticum*/*Hordeum* plants growing in the existence of 16 FHB pathogens under diverse experimental conditions, i.e., *in vitro*, climatic growth chamber and field variables, showed notable *Fusarium* symptoms (Figure 1), suggesting an important effect of *Fusarium* fungi on the growth of *Triticum*/*Hordeum* plants over the earliest and latest development stages. Scores of "Aggressiveness index" among the analyzed *Fusarium* pathogens were presented in Table 2. All isolates and species were proven to be pathogenic and produced typical FHB symptoms on small wheat/barley organs and adult spikes (Table 1). ANOVA showed pronounced differences among *Fusarium* isolates for values of "Aggressiveness index" that varied from 5.9 to 7.5 among the analyzed FHB pathogens. All comparisons were significant under LSD at P>0.001. FHB score ranged from 0 (not aggressive) to 10 (very aggressive isolate. Findings presented in Table 1 exhibited that the isolates F29 of *F. solani* and F16 of *F. verticillioides* were the most pathogenic with a value of "Aggressiveness index" of 7.4; followed by other analyzed *Fusarium* isolates, and isolate F35 of *F. solani* was the less pathogenic one with a value of "Aggressiveness index" of 5.9. "Aggressiveness index" did differentiate *Fusarium* pathogens within and among species. High level of diversity in aggressiveness was found among isolates within the species tested; suggesting that screening for resistance to *Fusarium* necessities the use of aggressive isolates or a mixture of several isolates. Employment of highly aggressive FHB isolates as an effective tool for achieving adequate distinguishing among *Triticum*/*Hordeum* cultivars and selecting wheat and barley cultivars with high levels of resistance to head blight is recommended in *Fusarium* nurseries and wheat/barley breeding programs (Fernando *et al.,* 2021). Also, utilization of a mixture of FHB isolates of the pathogen should be preferred for artificial head/floret inoculations reflecting the parameters that occur in natural epidemics (Moonjely *et al.,* 2023).

Table 1. Scores of aggressiveness index encompassing nine aggressiveness components of a set of 16 fungal isolates of four Fusarium head blight species infecting eight wheat and barley cultivars of Syrian origin

Fungal isolates (identification)	Nine aggressive components									Aggressiveness index (value × 10)
	LP (days × 10)	AUDPC (value × 100)	CD (%)	DI^{DHT} (%)	DS^{DHT} (%)	DI^{CC} (%)	DS^{CC} (%)	DI^{FC} (%)	DS^{FC} (%)	
F1 (*F. culmorum*)	59	40	59	47	47	48	49	45	34	6.3
F2 (*F. culmorum*)	55	40	61	50	46	50	47	46	34	6.8
F3 (*F. culmorum*)	55	49	51	52	52	53	52	48	36	7.2
F28 (*F. culmorum*)	53	44	59	42	39	43	39	45	33	6.5
F30 (*F. culmorum*)	57	44	60	43	42	43	43	45	34	6.2
F7 (*F. solani*)	61	50	51	46	45	46	46	48	36	6.1
F20 (*F. solani*)	58	46	52	63	44	62	39	54	43	7.0
F26 (*F. solani*)	52	44	62	52	38	53	35	48	37	7.1
F29 (*F. solani*)	52	41	59	52	44	52	47	53	40	7.4
F31 (*F. solani*)	54	41	59	43	39	44	44	41	31	6.3
F35 (*F. solani*)	64	48	52	51	47	49	47	47	36	5.9
F15 (*F. verticillioides*)	46	34	67	35	29	36	28	36	28	6.4
F16 (*F. verticillioides*)	45	40	65	43	37	45	38	41	32	7.5
F21 (*F. verticillioides*)	57	40	63	46	37	46	36	46	37	6.2
F27 (*F. verticillioides*)	49	35	67	39	31	39	29	36	28	6.2
F43 (*F. equiseti*)	54	43	59	44	44	43	48	44	34	6.7
	P=0.001	P=0.001	P=0.001	P=0.001	P=0.001	P=0.001	P=0.001	P=0.001	P=0.001	P=0.001

Figure 1. Fusarium head blight symptoms on small plant parts (a), and heads (b) of Syrian barley cultivar Arabi Abiad and wheat cultivar Cham7 inoculated with *Fusarium* isolates, respectively, compared with negative water control.

Latent period (LP) of detached leaf inoculation, area under disease progress curve (AUDPC) of Petri-dish inoculation and coleoptile dwarfing (CD) of a coleoptile infection detected *in vitro*, disease incidence (DI) and disease severity (DS) detected using a detached head test (DHT) under controlled conditions, and disease incidence (DI^{CC}) detected using a head artificial inoculation and disease severity (DS^{CC}) detected using a floret artificial inoculation under controlled conditions in a growth chamber, disease incidence (DI and disease severity (DS) detected using a head artificial inoculation under field conditions. A index = (AUDPC + CD + DI^{DHT} + DS^{DHT} + DI^{CC} + DS^{CC} + DI^{FC} + DS^{FC})/LP (Sakr, 2024). FHB score ranged from 0 (not aggressive) to 10 (very aggressive isolate. Pathogenic responses of all isolates on wheat and barley cultivars were presented by Sakr (2023).

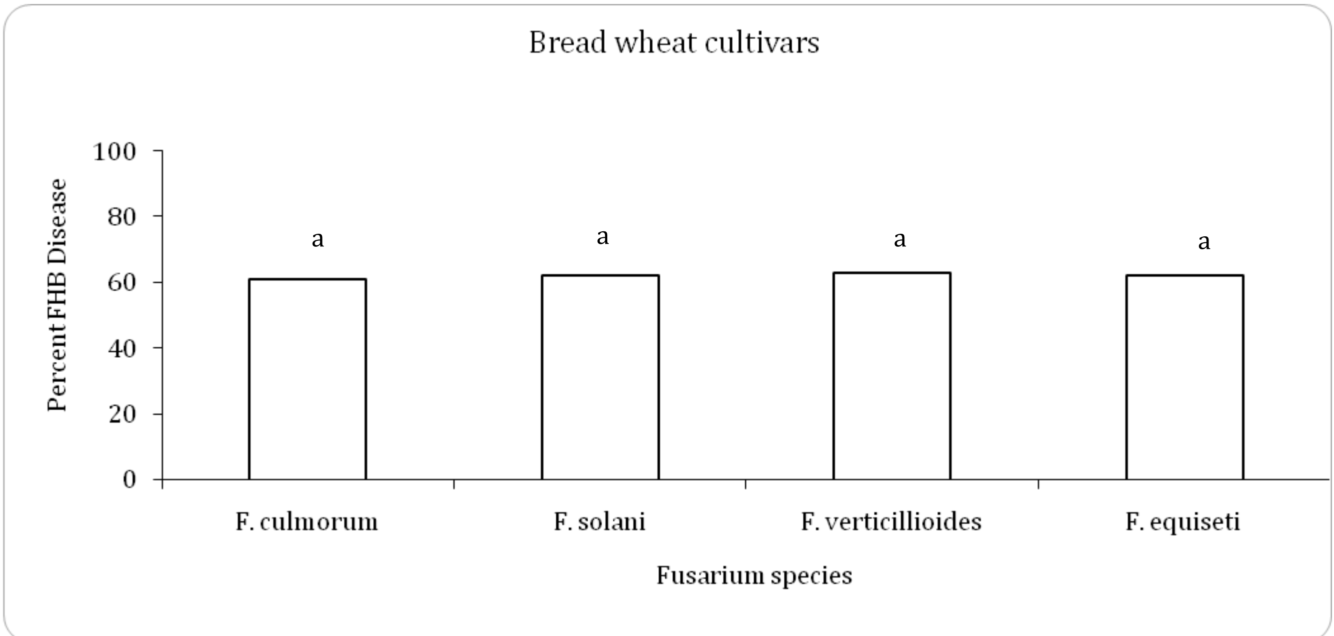
Our pathogenic diversity was not structured according to the geographic location, and several levels of aggressiveness were indicated in the same geographic part (McDonald and Linde, 2002). The importance of differences in our “Aggressiveness index” was indications of aggressiveness of individual pathogens (Xue *et al.,* 2019; Mesterhazy, 2020). All analyzed *Triticum*/*Hordeum* plants exhibited a qualitative pattern in which *Fusarium* isolates expressed either high aggressiveness or low aggressiveness (Sakr, 2023). The board range of diversity of pathogenicity among head blight isolates in our research has been supported by other works analyzing aggressiveness of diverse *Fusarium* pathogens (Xue *et al.,* 2019). DON production, however, is a crucial factor in the aggressiveness of *Fusarium* pathogens in *Triticum* and *Hordeum* (Fernando *et al.,* 2021) and other cereals (Moonjely *et al.,* 2023) as it exacerbates spread of FHB disease; in spite of it does not contribute to the initiation of *Fusarium* infection (Toth *et al.,* 2020).

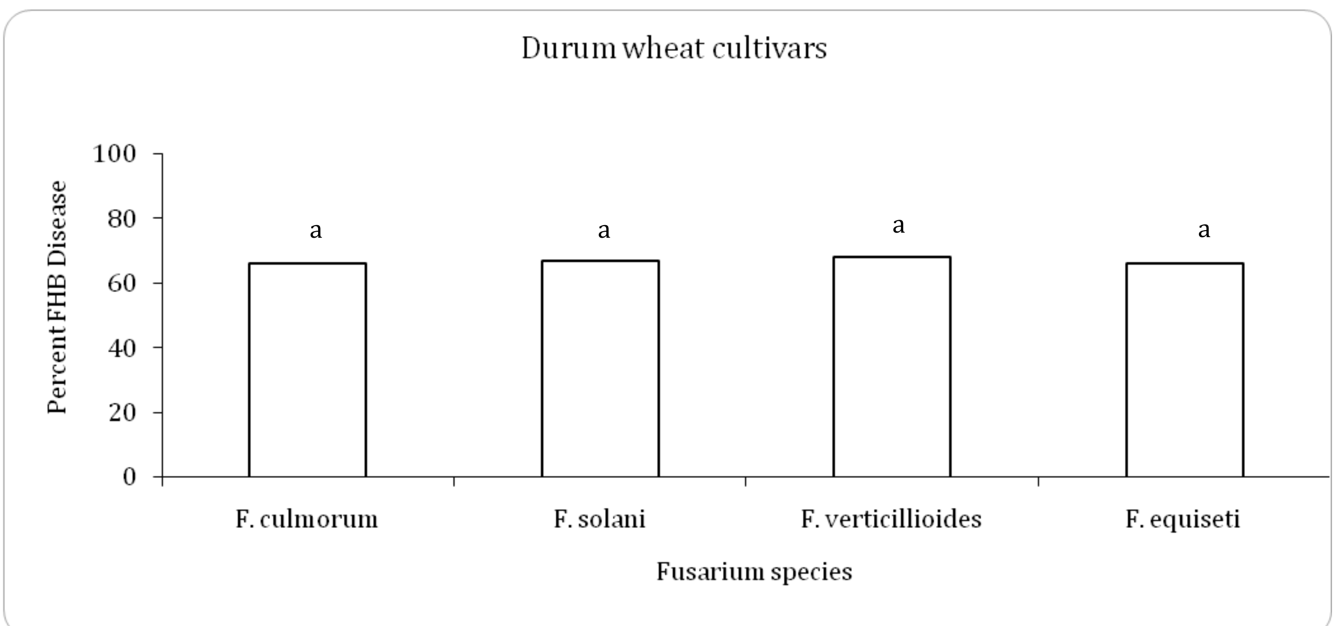
In our report, it was not possible to cluster the FHB isolates based on their species origins in the head and small plant parts tests on bread wheat, durum wheat and barley cultivars (Figures 2 and 3), suggesting an apparent lack of a difference in pathogenicity betweenthe four tested *Fusarium* species, i.e.,*F. equiseti*, *F. culmorum*, *F. verticillioides* and *F. solani*. In accordance with our observations, Fernandez and Chen (2005) reported an apparent lack of a difference in aggressiveness between *F. graminearum* and *F. culmorum* on *T. aestivum*. Our findings are not comparable with other works exhibiting that the four *Fusarium* species involved in the present work were classified as highly, moderately and weakly pathogenic on *T. aestivum* plants. In previous works, *F. verticillioides* and *F. solani* was distinguished by low pathogenicity, *F. equiseti* was moderate, and *F. culmorum* was highly pathogenic (Xue *et al.,* 2004, 2006). The apparent link of a difference in pathogenicity between the four tested species found in our study does not agree with Balmas *et al.,* (1995), Mihuta-Grimm and Forster (1989), or Wong *et al.,* (1995), who observed that a given *Fusarium* pathogen was more pathogenic on heads than another *Fusarium* pathogen. Arseniuk *et al.,* (1993) and Wong *et al.,* (1995) also reported that *F. equiseti*, *F. poae,* and/or *F. avenaceum* were the pathogens least pathogenic to *T. aestivum* heads. In inoculation analyses on *T. aestivum*, Stack and McMullen (1985) and Wong *et al.,* (1995) found that only *F. graminearum* and *F. culmorum* were identified to be highly pathogenic, among nine and seven *Fusarium* species analyzed, respectively. Xue *et al.,* (2004) further showed that *F. crookwellense* was among the most pathogenic pathogen, identical to *F. graminearum* and *F. culmorum* in causing FHB on *T. aestivum*. *F. avenaceum* is weakly pathogenic on *T. aestivum* (Stack and McMullen, 1985; Wong *et al.,* 1995; Xue *et al.,* 2004). Kammoun *et al.,* (2009) highlighted that *F. pseudograminearum* and *F. culmorum* had the highest aggressiveness, whereas the lowest is that of *M. nivale* var. *nivale* and *F. avenaceum*. The variations in these data may be attributable to the contrasting strains and *T. aestivum* host cultivars employed in this work and previous studies. The origin of FHB cultures may also play a crucial role in this pathogenic similarity. The analyzed isolates of *F. equiseti*, *F. culmorum*, *F. verticillioides* and *F. solani* species sampled from Ghab plain, a limited geographic area, may show low degrees of genotype/gene flow which leads to population size levels and low mutation, and consequently these genetic and physiological traits may enhance the level of pathogenic similarity between species as observed for other pathogens (McDonald and Linde, 2002).

Generally, our findings from the detached leaf, detached head, and coleoptile pathogenicity analyses also agree with data on fungal pathogenicity on head in *Triticum* and *Hordeum* plants (Figures 2 and 3). In comparable to our data, findings from the seedling and seed pathogenicity analyses also agree with other works on fungal pathogenicity on crowns and/or roots or lower culms, in spite of relative variations among the *Fusarium* species have in some cases differed (Fernandez and Chen, 2005). In addition, *F. culmorum* was found to be as more pathogenic to *Triticum aestivum* seedlings than *F. avenaceum* (Akinsanmi*et al.,* 2004), more pathogenic to *Triticum aestivum* and *Hordeum vulgare* seeds than *F. avenaceum*, *F. poae,* or *F. graminearum* (Kammoun *et al.,*, 2009), and it caused foot and crown rot of *T. durum* seedlings to a greater extent than *F. graminearum* (Brennan *et al.,* 2003). *F. avenaceum, F. graminearum,* and *F. culmorum* were also observed to be more pathogenic to winter *Triticum aestivum* seedlings than *F. poae* and *F. equiseti* (Akinsanmi*et al.,* 2004). Nevertheless, in contrast to our findings, Arseniuk *et al.,* (2004) and Jenkinson and Parry (1994) observed that *F. avenaceum* was as, or more, pathogenic to winter *Triticum aestivum* seedlings than *F. graminearum* and *F. culmorum*, whereas Kane and Smiley (1987) and Specht and Rush (1988) found that there was no mortality of winter *Triticum aestivum* caused by *F. avenaceum*.

This work has also shown an overall identical relative susceptibility of heads and small plant parts of *T. aestivum*/*T. durum* and *Hordeum vulgare* to the *Fusarium* pathogens analyzed (Figures 2 and 3). Nevertheless, whereas *F. equiseti*, *F. culmorum*, *F. verticillioides* and *F. solani* were equally pathogenic to heads and caused similar discoloration of detached leaves, detached heads, and coleoptiles in the seed test, *F. culmorum* influenced cereal host emergence and growth to a greater extent than other *Fusarium* species. The greater relative pathogenicity of *Fusarium* pathogens on heads than on small plant parts might be contributed to stimulatory compounds exist in anthers (Fernando *et al.,* 2021). Identical notifications had been reported by Fernandez and Chen (2005) who observed that *F. graminearum* and *F. culmorum* were more aggressive as crown rot pathogens than *F. avenaceum* under controlled greenhouse conditions. *F. avenaceum* was less pathogenic to seedlings than to heads or seeds, in spite of in the seed analysis this fungus also caused as little discoloration of crowns/lower culms and sub-crown internodes as *F. poae* and *F. equiseti*. The observation that

F. avenaceum was less pathogenic to seedlings than to seeds might be explained, at least partly, by variations in the maturity of cereal plant tissue between germinating seeds and seedling stems (Fernandez and Chen, 2005). On the other hand, Smiley *et al.,* (2005) reported that *F. pseudograminearum* and *F. culmorum* exhibited the highest levels of FHB disease intensity in the greenhouse. Further, Tunali *et al.,* (2006) proved that *F. pseudograminearum* and *F. culmorum* were the most pathogenic species in seedling analysis. Brennan *et al.,* (2003) also found that *F. graminearum* and *F. culmorum* were generally more pathogenic than *M. nivale* and *F. avenaceum* when analyzing the rate of coleoptile's elongation. Besides, Tunali *et al.,* (2003) observed that FHB isolates of the same *Fusarium* species from crown and head tissue did not change greatly in their aggressiveness on the crown. Nevertheless, Akinsanmi *et al.,* (2006) observed that isolates sampled from stubble and crown were more aggressive toward crown rot, whereas FHB isolates sampled from flag leaf node were more aggressive for head blight disease, proposing a potential adaptation of isolates toward either disease.





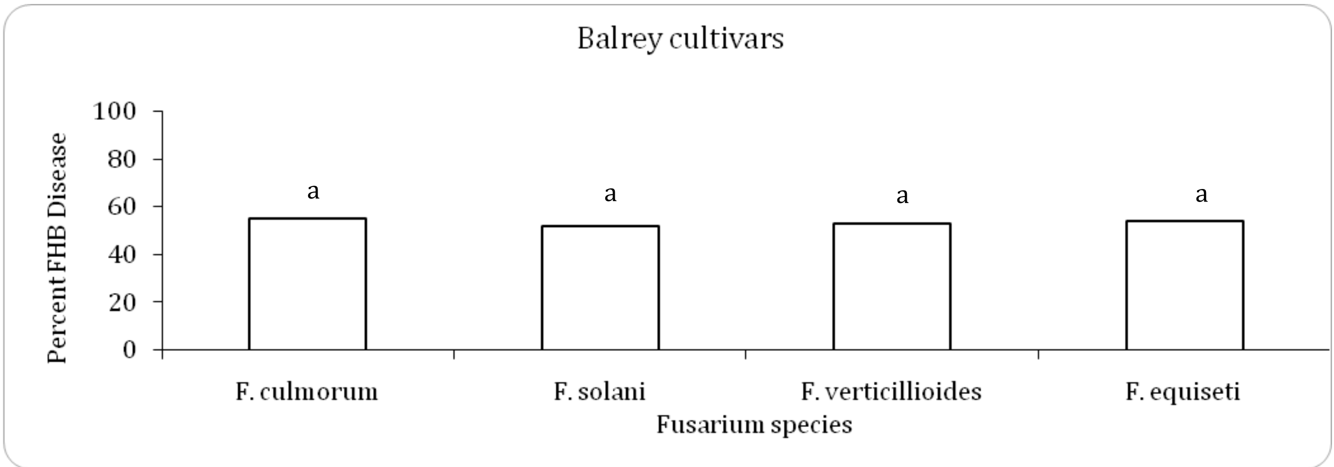
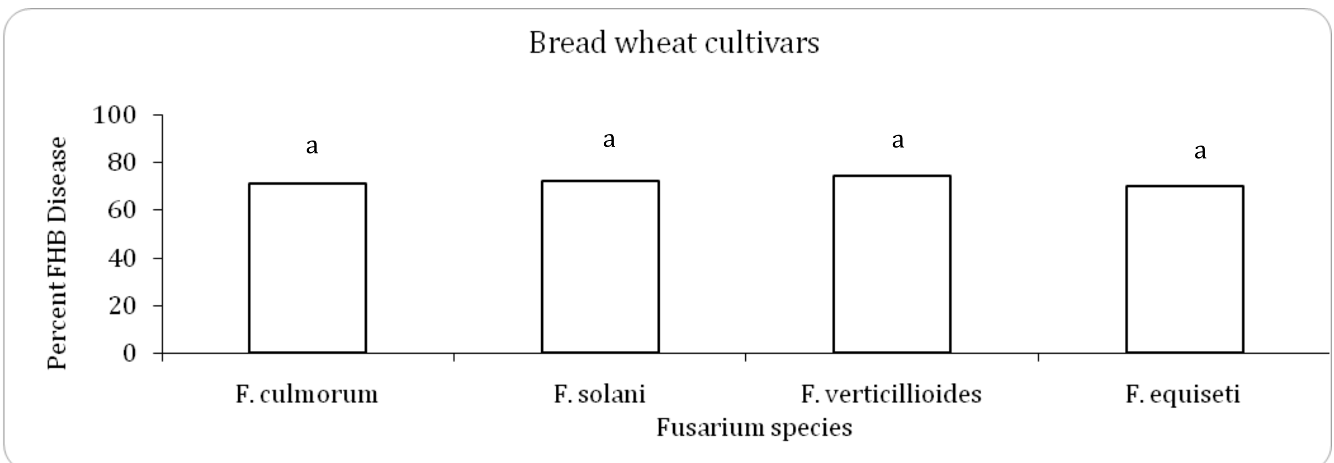
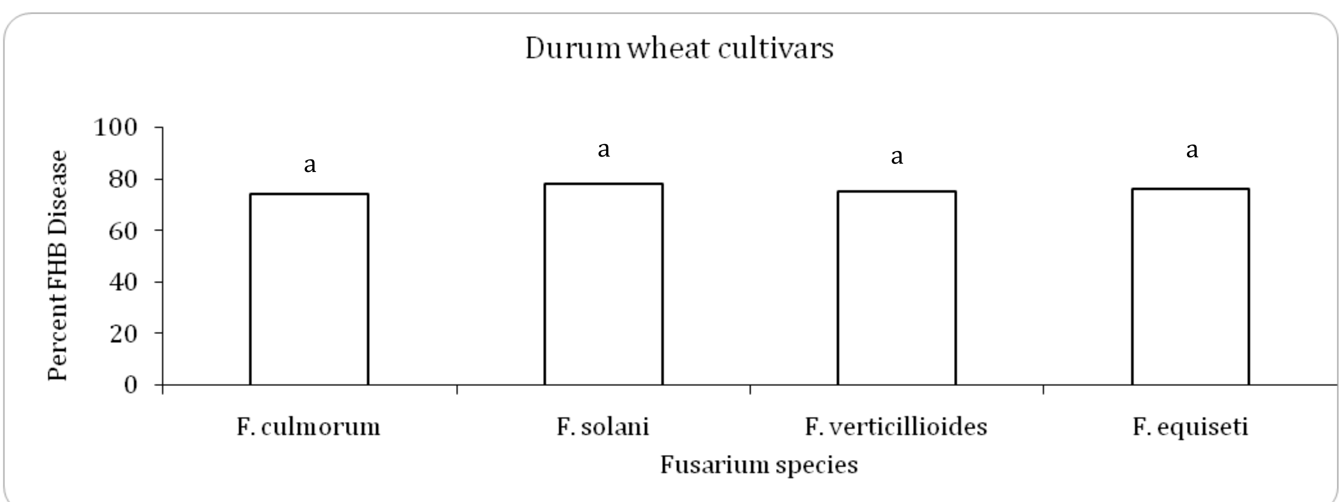


Figure 2. Mean of aggressiveness index involving nine aggressiveness components of four Fusarium head blight species infecting eight wheat and barley cultivars of Syrian origin in the small plant parts tests, detached leaves, detached heads, and coleoptiles, under in vitro conditions. Pathogenic reactions of all cultivars infected with *Fusarium* fungi were previously evaluated according to methods described by Sakr (2023). According to the Fisher's LSD test, means followed by the same letter within a linage are not significantly different at P>0.05.





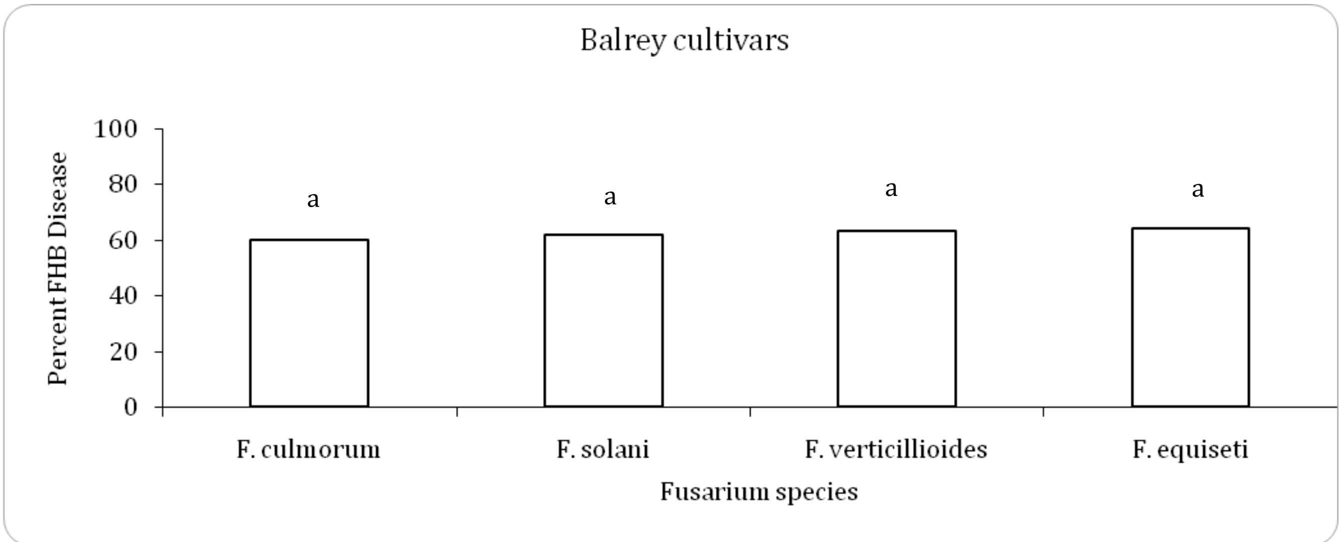


Figure 3. Mean of aggressiveness index involving nine aggressiveness components of four Fusarium head blight species infecting eight wheat and barley cultivars of Syrian origin in the head tests under growth chamber and field conditions. Pathogenic reactions of all cultivars infected with Fusarium fungi were previously evaluated according to methods described by Sakr (2023). According to the Fisher's LSD test, means followed by the same letter within a linage are not significantly different at P>0.05.

Whether or not there is adaptation, it will have crucial implications for *Fusarium* epidemiology and management. All these data show that infected plant debris and seeds might play a crucial role in the survival of the FHB pathogens and contribute as source of inoculum for head blight and crown rot. Thus, Akinsamni *et al.,* (2004) concluded that all sources of inoculum should be taken into account in the epidemiology of both fungal diseases. Infected plant organs at or below soil level might then be a source of inoculum for infection of *Triticum* and *Hordeum* spikes the following season(s), or might constitute a significant fungal survival mechanism under dry conditions.

CONCLUSION

The aggressiveness of isolates is the most important trait for breeding purposes. Wheat/barley breeders can use aggressive isolates for resistance tests in wheat and barley plants. All the 16 tested FHB isolates and species were found to be pathogenic and induced typical FHB symptoms on small plant parts and adult heads. High level of variation in aggressiveness was observed among isolates within the species tested. The origin of FHB pathogens may play a crucial role in this pathogenic similarity. The tested isolates of *F. culmorum*, *F. verticillioides*, *F. solani*, and *F. equiesti* species collected from Ghab plain, a limited geographic area, may exhibit low degrees of gene/genotype flow which result in low mutation and population size levels, and consequently these physiological and genetic characteristics may increase the level of pathogenic similarity between species. This study suggests that screening for resistance to FHB requires the use of aggressive isolates or a mixture of several isolates. In addition, it indicates a lack of adaptation of these four tested *Fusarium* species referring to the complex polygenic background of aggressiveness in the interaction in FHP-wheat/barley pathosystem. Our data clarify for the first time the nature of comparative pathogenicity of diverse FHB species on durum wheat. Further research is required to assess the difference in pathogenicity between *Fusarium* single isolates and species on wheat and barley. It will provide critical information for cereal breeders to develop and improve FHB-resistant cultivars.

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Contribution of Authors:		
Nachaat Sakr	:	Design experiments, conduct research and writing manuscript