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Effect of epidemiological factors on the growth and development of *Fusarium graminearum* and head blight of wheat

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Abstract

Fusarium head blight (FHB), is a serious disease causing significant reduction in yield and quality of wheat grains throughout the world. An in vitro experiment was conducted to find out the suitable temperature and pH for the growth and sporulation of *Fusarium graminearum*, the main causal agent of FHB. The results showed that the maximum growth of the fungus (90.00 mm), highest sporulation of 5.5×10^6 conidia per ml and perithecia formation occurred at 25°C. A pH of 5 resulted in the maximum growth (86.00 mm) of the fungus, highest sporulation (5.7×10^6 conidia/ ml) and perithecia formation. The studies on the effect of weather variables viz., temperature, relative humidity and rainfall on the development of FHB in wheat crop sown at three different dates i.e. 31st October, 15th November and 1st December during *Rabi* season of 2019-20 and 2020-21 revealed that all the weather parameters contributed positively in the development of the disease and the disease severity was highest on late sown crop during both the cropping years.

Key words: Wheat, *Fusarium graminearum*, temperature, pH, weather

Wheat (*Triticum aestivum* L.) is an extensively grown cereal crop across the world. India is the world's second-largest producer of wheat. It is attacked by a large number of fungal diseases resulting in severe economic losses. Fusarium head blight (FHB) caused by a complex of *Fusarium* species is though a minor disease in the country, but with the changing climatic conditions due to global warming it is appearing in severe form at some locations. *Fusarium* spp. are widely distributed across all the geographic regions and can cause diseases either individually or as a complex (Doohan *et al*. 1998). In India, *Fusarium graminearum, F. poae, F. avenaceum, F. compactum, F. verticillioides, F. oxysporum, F. pallidoroseum (F. semitectum)* and *Microdocium nivale* are reported to be associated with FHB (Roy 1974; Brahma and Singh 1985; Singh and Aujla 1994; Kaur *et al*. 1999; Mann and Nanda 1999; Saharan *et al*. 2003; Gupta 2019). Among these, Fusarium graminearum is considered to be the major causal agent of FHB (Panwar *et al*. 2016).

FHB has consequences beyond the yield and kernel quality losses, since mycotoxins produced by the fungus contaminate raw grain and processed wheat products, putting human and cattle health at risk (Edwards *et al*. 2009). Climatic factors such as temperature, rainfall, humidity and soil pH influence the growth, survival and spread of the pathogen which ultimately affects the disease development and crop damage.

Wheat crop is vulnerable to FHB infection from the anthesis (flowering) to the soft dough stage of kernel development. Prolonged wet weather and high humidity during these growth stages favour FHB infection. McMullen *et al*. (1997) observed that the disease resulted in reduction in yield and quality of wheat in nations where the warm and humid climate prevailed. Epidemics of FHB are sporadic and influenced by a number of factors including the local and regional environment, physiological condition and genetic make-up of the host and adaptability and virulence of the pathogen (Fernando *et al*. 2000).

Each fungus in the FHB disease complex has its own set of biological and environmental requirements, which help to explain the reason of varying frequency of these species from one location

to other. These factors are especially important in the mycotoxicosis epidemiology, because the mycotoxin production by different *Fusarium* species is differentially affected by environmental factors (Di Menna *et al*. 1991; Jimeneza *et al*. 1996). The ability of *Fusarium* species to survive in extreme conditions (broad temperatures and pH levels) make them a potent pathogen. The present study depicts the effect of different temperature and pH levels on the survival of pathogen, which will ultimately be quite useful in designing the management strategy for FHB in the field.

Materials and Methods

Isolation of the pathogen

The pathogen was isolated from the infected earheads of wheat by Agar plate method using Potato Dextrose Agar (PDA) as basal medium. The infected grains were disinfected with 1 per cent sodium hypochlorite solution for one minute and then washed thrice with sterile distilled water. The grains were dried under the fold of sterilized blotting paper and aseptically transferred in to sterilized Petri plates containing sterilized and solidified PDA medium with the help of a sterilized forcep. The plates were incubated at 25±1ºC in the BOD incubator till the fungal mycelium fully covered the surface of the medium. The fungus was identified by mounting in lactophenol and following the standard keys (Booth 1971). The pure culture of the fungus was obtained by single hyphal tip method (Dhingra and Sinclair 1985) and preserved in the refrigerator at 4°C for further studies. The pathogenic behavior of the isolated pathogen was ascertained by spray inoculation of spore suspension (5×10^5 conidia/ ml) on healthy spikes of wheat variety HPW 89.

Effect of temperature

The effect of different temperature levels *viz*., 10, 15, 20, 25, 30 and 35ºC on the mycelial growth, sporulation and perithecia formation of the pathogen was studied under in *vitro* conditions. The mycelial discs of 5 mm diameter were cut from margins of actively growing colony of *F. graminearum* and inoculated into 90 mm Petri plates containing 20 ml of solidified PDA medium and then incubated at different temperature regimes *viz*., 10, 15, 20, 25, 30 and 35ºC. Each treatment was replicated thrice in a Completely Randomized Design (CRD). After 7 days of incubation, the colony diameter was measured with a scale and sporulation (conidia/ml) was also recorded with the help of a haemocytometer.

Perithecia formation in culture was studied using the method of Klittich and Leslie (1988) with some modifications. The cultures were grown on PDA in 9 cm diameter Petri dishes at different temperatures mentioned above with 24 h lighting of a 15 W fluorescent bulb. After 7 days of incubation, 1 ml of 2.5% Tween 60 (Sigma) was poured over the culture in each plate and spread across the surface with a sterile glass rod and then cultures were again incubated at respective temperatures. The cultures were regularly observed for the formation of perithecia. Perithecia production was recorded after12 days post treatment.

Effect of pH

The effect of different pH levels *viz*., 4, 5, 6, 7 and 8 was studied on the mycelial growth, sporulation and perithecia formation of the pathogen under in *vitro* conditions. Different pH levels of PDA medium were maintained by the method described by Sharma *et al*. (2005). The pH of the medium was adjusted using HCl (0.1N) and NaOH (0.1N) before autoclaving. After autoclaving the medium was poured (20 ml) in each Petriplate (90 mm) and allowed to solidify. The Petriplates were then inoculated centrally with 5 mm diameter mycelial discs cut from the actively growing culture of *F. graminearum*. Each treatment was replicated thrice in a Completely Randomized Design (CRD). Inoculated Petriplates were incubated at a temperature of 25±1°C in the BOD incubator. After 7 days of incubation, the colony diameter was measured with a scale and sporulation (conidia/ml) was also recorded with the help of a haemocytometer.

Perithecia formation in culture was studied following the method of Klittich and Leslie (1988) with some modifications. The cultures maintained at different pH levels as mentioned above (4, 5, 6, 7 and 8) were incubated at room temperature (23-25°C) under a 24 h lighting of 15 W fluorescent bulb. After 7 days of incubation, 1 ml of 2.5% Tween 60 (Sigma) was poured over the culture and spread across the surface with a sterile glass rod and then cultures were incubated at room temperature. The cultures were regularly observed for the formation of perithecia. Perithecia formation was recorded 12 days post

treatment.

Effect of weather parameters on disease development

To study the role of environmental factors (temperature, relative humidity and rainfall) on the progression of FHB of wheat at three different sowing dates *i.e.* early (31st Oct), timely (15th Nov) and late (1st Dec), a field experiment was conducted at Rice and Wheat Research Center (RWRC), Malan by sowing artificially inoculated seed of susceptible wheat variety HPW 89 during *Rabi* season of 2019-20 and 2020-21. Inoculated seeds were sown in plots of 1.0 ×1.0 m^2 size with row to row spacing of 20 cm. The temporal progress of the disease in terms of disease severity (%) was recorded after every 10th day starting from the first appearance of the disease on ten randomly selected spikes. The data on agrometeorological factors *viz*., temperature, relative humidity and rainfall was taken from the RWRC, Malan. Disease severity (%) on spikes of crop of each sowing date was correlated with the meteorological factors (mean temperature, mean RH, total rainfall) to obtain simple, partial and multiple correlations, regression equations and coefficients of determination (R^2).

Results and Discussion

Identification and pathogenicity of pathogen

The fungus produced white, yellow to pale pink coloured aerial floccose mycelium on PDA, often becoming vinaceous with brown tinge in the later stages and beared only macroconidia. Microscopically, the hyphae were hyaline, filamentous, septate and branched. The macroconidia were canoe shaped with tapered apical cell, basal cell foot shaped, 5-6 septate and measured 45.0-68.0 × 4.1-4.3 µm in size. The fungus was identified as *Fusarium graminearum* as its morphocultural characteristics resembled with the descriptions of Booth (1971). In pathogenicity test, wheat spikes inoculated individually with freshly sporulating cultures of *F. graminearum* produced typical symptoms of the disease thus, confirmimg the pathogenic behavior of the test pathogen. The inoculated spikes initially showed water soaked lesions at the base or middle of the glumes followed by salmon pink fungal growth along the edges of glumes. The inoculated spikes gave bleached appearance. The infected grains were shriveled with floury discoloured interior. The initial symptoms on earheads appeared on glumes after 7 days of inoculation in potted plants. However, the formation of conidia was observed after 9 days of inoculation on spikes.

Effect of temperature

Perusal of the data in Table 1 revealed that *F. graminearum* grew at a broad range of temperature from 10 to 35°C with 25°C being the optimum temperature for mycelial growth of the pathogen. The maximum mycelial growth (90 mm) of the pathogen was recorded at 25°C followed by 30°C (87.60 mm). However, the pathogen showed 48.00, 45.67 and 35.67 mm mycelial growth at 35, 20 and 15°C, respectively. The minimum mycelial growth (31.67 mm) of the pathogen was recorded at 10°C. A decrease in mycelial growth was observed with the increase in temperature from 25 to 35°C. The mycelial growth also decreased with the decrease in temperature from 25 to 10°C.

The data also indicated that temperature had a great influence on sporulation (conidia/ ml) and perithecia formation. The highest sporulation (5.5×10^6 conidia/ ml) and high perithecia formation was

Table 1. Effect of temperature on growth and sporulation of *Fusarium graminearum*

Temperature (°C)	Mycelial growth* (mm)	Sporulation (Conidia/ ml)	Perithecia abundance
10	31.67(34.23)	1.1×10^6	Very less
15	35.67(36.65)	1.8×10^6	Less
20	45.67(42.50)	2.4×10^6	Moderate
25	90.00(71.53)	5.5×10^6	High
30	87.60(69.41)	5.2×10^6	Less
35	48.00(43.84)	2.3×10^6	Nil
CD (P=0.05)	(1.05)		

* Figures within parenthesis are arc sign transformed values

observed at 25°C, followed by 30°C with sporulation of 5.2×10^6 conidia per ml and less perithecia formation. However, sporulation of 2.4×10^6, 2.3×10^6 & 1.8×10^6 conidia per ml with moderate, nil and less perithecia formation were observed at 20, 35 and 15°C, respectively. Minimum sporulation (1.1×10^6 conidia/ ml) with very less perithecia formation were observed at 10°C.

Effect of pH

It is evident from the data in Table 2 that *F. graminearum* can grow at a wide range of pH ranging from 4 to 8, however, pH 5 was observed to be optimum for mycelial growth of the pathogen. The maximum mycelial growth (86.00 mm) of the pathogen was observed at pH 5 followed by pH 6 (81.33 mm). However, the pathogen showed only 74.00 and 46.67 mm mycelial growth at pH 7 and 4, respectively. The minimum mycelial growth (30.33 mm) of the pathogen was observed at pH 8.

It is also shown by the data that pH had a great influence on the sporulation (conidia/ ml) and perithecia formation. The highest sporulation (5.7×10^6 conidia/ ml) and high perithecia formation were recorded at pH 5 and followed by pH 6 with sporulation of 5.3×10^6 conidia per ml and high perithecia formation. However, lesser sporulation of 3.6×10^6 & 2.4×10^6 conidia per ml were observed with moderate and less perithecia formation at pH 7 and 8, respectively. The minimum sporulation (1.9×10^6 conidia/ ml) and very less perithecia formation were observed at pH 4.

Effect of weather variables on disease development

The data on maximum and minimum temperature (°C), maximum and minimum RH (%), rainfall (mm) and disease severity (%) were recorded after every 10th day starting from the first appearance of the disease.

It is evident from Figure 1 that an increase in mean temperature, mean relative humidity and rainfall

Table 2. Effect of pH on growth and sporulation of *Fusarium graminearum*

pH level	Mycelial growth* (mm)	Sporulation (Conidia/ ml)	Perithecia abundance
4	46.67(43.07)	1.9×10^6	Very less
5	86.00(68.00)	5.7×10^6	High
6	81.33(64.38)	5.3×10^6	High
7	74.00(59.32)	3.6×10^6	Moderate
8	30.33(33.40)	2.4×10^6	Less
CD (P=0.05)	(1.65)		

* Figures within parenthesis are arc sign transformed values

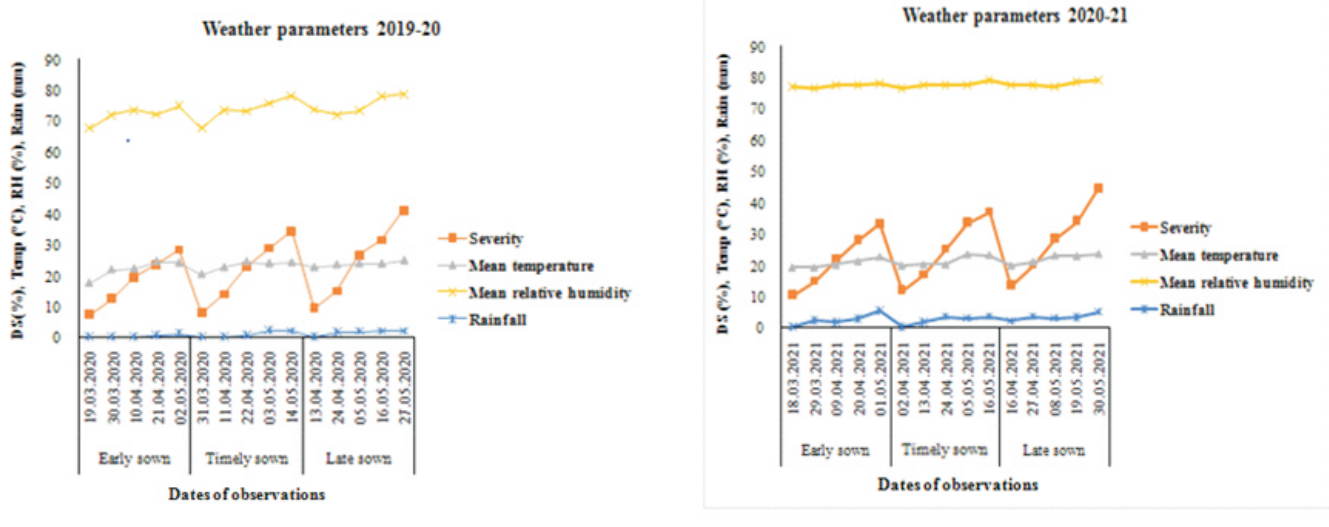

Figure 1. Effect of weather parameters on disease development on the crop sown at different sowing dates

resulted in gradual increase in the disease severity on all the three dates of sowing (DOS), during 2019-20 and 2020-21. The disease severity was more in 2020-21 as compared to 2019-20 probably due to more favourable environmental conditions.

The data presented in Table 3 revealed that terminal disease severity was higher on late (1st December) sown crop in comparison to early (31st October) and timely (15th November) sown crop in both the cropping years i.e. 2019-20 and 2020-21. The terminal disease severity was 41.23, 34.33 and 28.43 per cent on 1st December, 15th November and 31st October sown crop, respectively in the cropping year 2019-20. Whereas, in 2020-21, it was 44.56, 36.90 and 33.23 per cent on 1st December, 15th November and 31st October sown crop, respectively. The disease severity of three dates of sowing was correlated with weather variables *viz.*, mean temperature, mean relative humidity and rainfall and correlation coefficients (simple, partial and multiple) along with regression equations were derived.

The simple correlation coefficients indicated that the disease severity on wheat crop of three dates of sowing (31st October, 15th November and 1st December) had positive correlation with mean temperature (0.900, 0.859 & 0.936), mean relative humidity (0.863, 0.919 & 0.826) and rainfall (0.855, 0.926 & 0.773) in the cropping year 2019-20. However, it showed significant positive correlation with mean temperature in case of 1st and 3rd date of sowing, mean relative humidity and rainfall (2nd DOS). In 2020-21 also the simple correlation coefficients showed positive correlation of disease severity with mean temperature (0.955, 0.905 & 0.927), mean relative humidity (0.854, 0.848 & 0.734) and rainfall (0.890, 0.863 & 0.862) for three dates of sowing *i.e.* 31st October, 15th November and 1st December, respectively. However, it showed significant positive correlation with mean temperature in case of 1st, 2nd and 3rd date of sowing and rainfall in 1st date of sowing.

Partial correlation coefficients also showed positive correlation of disease severity with mean temperature (0.512, 0.619 & 0.809; 0.193, 0.969 & 0.984), mean relative humidity (0.601, 0.605 & 0.884; 0.645, 0.364 & 0.933) and rainfall (0.832, 0.869 & 0.509; 0.657, 0.952 & 0.782) for three dates of sowing (31st October, 15th November and 1st December) during both the cropping years (2019-20 and 2020-21), respectively.

The multiple correlation coefficients were highest for 1st Dec sown crop (0.986 & 0.996) followed by 15th Nov (0.984 & 0.995) sown crop and least (0.975 & 0.977) for 31st Oct sown crop during 2019-20 and 2020-21, respectively.

Similarly, coefficients of determination (R^2) suggest that the highest effect of weather variables on disease severity occurred on late sown crop (97.30 & 99.30 %) followed by timely (96.80 & 99.00 %) and least (95.00 & 95.50 %) in early sown crop during two consecutive years, respectively.

The regression equations for three dates of sowing during 2019-20 indicated that a unit change in mean temperature, mean relative humidity and rainfall changed the disease severity by 0.923, 1.041 & 6.335; 1.688, 0.812 & 5.166 and 8.296, 1.826 & 2.763 per cent while, in 2020-21 by 1.013, 6.218 & 2.280; 3.313, 0.800 & 3.404 and 4.906, 4.346 & 2.039 per cent, respectively.

From the above results it can be concluded that the optimum temperature and pH for the maximum growth, sporulation and perithecia formation of the Fusarium graminearum were 25°C and pH 5, respectively. All the three weather variables (temperature, relative humidity and rainfall) individually as well as collectively influenced FHB development in wheat.

The present findings are corroborated by the observations of Brennan *et al*. (2003) and Hudec and Machova (2010) who also reported 25°C to be the optimum temperature for the growth of *F. graminearum*. Rossi *et al*. (2002) observed 32ºC as the optimum temperature for macroconidia production of *F. graminearium.* Tschanz *et al*. (1976) and Dufault *et al*. (2006) reported that the optimum temperature for perithecia formation ranged from 15 to 28.5°C and 12 to 28°C, respectively. Manstretta and Rossi (2016) observed that perithecia were produced at a temperature range of 5-30°C (optimum being 21.7°C), whereas, no perithecia were formed at 35 and 40°C, and just a handful produced at 5°C.

Several *Fusarium* species have been observed to grow and sporulate at a pH range of 5.0 to 6.0 (Cochrane 1958) but Agarwal and Sarbhoy (1978) found that acidic pH favoured growth of *Fusarium*

Table 3. Effect of weather parameters on severity of Fusarium head blight under field conditions

Year of experiment	Date of sowing	Disease severity (%)	Correlation coefficient						Multiple correlation coefficient (R)	Coefficient of determination (R2)	Regression equation
			Simple			Partial					
			DS x MT	DS x MRH	DS x R	DS x MT	DS x MRH	DS x R			
2019-20	31st Oct	28.43	0.900*	0.863	0.855	0.512	0.601	0.832	0.975	0.950	$Y=-79.633+0.923X_1+1.041X_2+6.335X_3$
	15th Nov	34.33	0.859	0.919*	0.926*	0.619	0.605	0.869	0.984	0.968	$Y=-82.555+1.688X_1+0.812X_2+5.166X_3$
	1st Dec	41.23	0.936*	0.826	0.773	0.809	0.884	0.509	0.986	0.973	$Y=-313.398+8.296X_1+1.826X_2+2.763X_3$
2020-21	31st Oct	33.23	0.955*	0.854	0.890*	0.193	0.645	0.657	0.977	0.955	$Y=-484.909+1.013X_1+6.218X_2+2.280X_3$
	15th Nov	36.90	0.905*	0.848	0.863	0.969	0.364	0.952	0.995	0.990	$Y=-116.018+3.313X_1+0.800X_2+3.404X_3$
	1st Dec	44.56	0.927*	0.734	0.862	0.984	0.933	0.782	0.996	0.993	$Y=-425.794+4.906X_1+4.346X_2+2.039X_3$

*Significant at 5 per cent level; DS= Per cent disease severity (%); MT= Mean temperature (°C); MRH= Mean relative humidity (%); R= Rainfall (mm)

species. Sharma *et al*. (2005) found that pH 5.5 showed the highest mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *lini*. Similarly, Pal *et al*. (2019) reported the maximum mycelial growth (86.33 mm) and the highest sporulation of 8.2 x 10^6 per ml of *Fusarium oxysporum* f.sp. *lini* at pH 5.5.

The effect of environmental (temperature, relative humidity & rainfall) factors on the disease development has been earlier studied by various workers whose observations are in agreement with the present findings. Shaner (2003) observed that FHB development was favoured by warm temperatures, high rainfall and relative humidity. Saharan *et al*. (2004) observed that FHB was more prevalent in Northern India because of warm and humid weather during the months of March and April. Weather factors such as moderately high temperature (15 to 30°C), frequent precipitation, prolonged periods (48–72 hrs) of high moisture and occurrence of air currents favoured FHB infection in wheat (Lenc *et al*. 2015).

Conflict of interest: The authors declare that there is no conflict of interest in this research paper.

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