Indian Journal of Ecology (2022) 49(5): 1782-1785
DOI: https://doi.org/10.55362/IJE/2022/3735

Manuscript Number: 3735
NAAS Rating: 5.79

Physiological Studies on *Fusarium equiseti* and *Fusarium chlamydosporum*, the Cause of Pod Rot Disease in Mungbean [*Vigna radiata* (L.) Wilczek] in India

Harwinder Singh Buttar, Amarjit Singh and Asmita Sirari[1]

Department of Plant Pathology, [1]Department of Plant Breeding and Genetics
Punjab Agricultural University, Ludhiana-141 004, India
E-mail: harwinderbuttar59@gmail.com

***Abstract*:** Mungbean is an important pulse crop grown in different seasons, *viz*., *Kharif, rabi* and summer in India. Pod rot disease, caused by *Fusarium equiseti* and *Fusarium chlamydosporum*, is emerging as a destructive disease affecting the quantity and quality of mungbean worldwide. Climatic factors like temperature and pH influence the growth, survival and infestation of these pathogens. In the present study, response of *F. equiseti* and *F. chlamydosporum* to different temperature and pH was assessed by analyzing there *in vitro* growth rate (mm/day) on Potato Dextrose Agar (PDA) medium. The results from the study revealed that the temperature range 25-35^{0}C and pH range 5.5-7.5 was found conducive for growth *F. equiseti* and *F. chlamydosporum* and pod rot development. This attributes make *F. equiseti* and *F. chlamydosporum* a widely distributed and potent pathogen of mungbean.

Keywords: Fusarium, Mungbean, pH, Pod rot, Temperature

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most important short duration pulse crop that is cultivated in different seasons, *viz*., *kharif* and *rabi*/summer in India. It is also used as green manure crop having the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixation, which helps in decreasing soil nutrient depletion (Nadeem et al 2004, Pataczek et al 2018). However, this crop is susceptible to several abiotic and biotic stressors, which significantly reduce productivity. Fungal infections are a significant barrier to the development of mungbeans among biotic stressors. The major diseases of mungbean caused up to 40–60 percent yield reduction in mungbean (Kaur et al 2011). Among the diseases, yield loss of 10-44 percent by charcoal rot/dry root rot (*Macrophomina phaseolina*), 33-44 percent by Rhizoctonia root rot (*Rhizoctonia solani*), 30-70 percent by anthracnose (*Colletotrichum lindemuthianum* or *C. gloeosporioides*), up to 97 percent yield losses by Cercospora leaf spot (*Cercospora cruenta* or *C. canescens*) disease and up to 40 per cent losses by powdery mildew (*Erysiphe polygoni* or *Podosphaera fusca*) in mungbean have been reported (Bashir and Malik 1988, Khajudparn et al 2007, Singh et al 2013a, Bhat et al 2014, Shukla et al 2014). Different stages of mungbean such as seedling, vegetative and reproductive can be attacked by fungal pathogens and cause severe yield loss or complete production failure. Pod rot disease of mungbean, caused by *Fusarium equiseti* and *Fusarium chlamydosporum,* has emerged in India in recent years particularly in the crop grown in *Kharif* season (Buttar et al 2021).

Fusarium spp. are found in all geographic regions, including soils, plants and air. However, the presence of Fusarium in the soil and the infestation it causes in plants are influenced by a number of different factors. For the occurrence and the pattern of infestation by different Fusarium species, geographical conditions, particularly climate, are significant. Temperature, soil pH, and humidity are examples of climatic variables that have an impact on the development, survival and spread of Fusarium species, as well as the crop damage by these pathogens. The ability of Fusarium species to produce the diseases either singly or in complexes makes it difficult to determine how these factors affect disease (Doohan et al 1998). Climate may have both direct (Such as affecting reproduction) and indirect (e.g. an effect of soil and vegetation type) effects on the occurrence of *Fusarium* species. Both of the fungi that cause pod rot disease have slightly distinct biological and environmental requirements, which may help to partially explain why various locations have varied frequencies of these species. There are several findings on how different Fusarium species respond differently to environmental changes, primarily those related to temperature, source, and moisture (Conrath et al 2002). Environmental variables including temperature, moisture, and pH have a substantial impact on the production of toxins by various fungus species, and these elements are also crucial for mycotoxicosis

epidemiology (Jimeneza et al 1996). *Fusarium* spp. are powerful pathogens that can endure a broad range of temperature and pH conditions and may survive in soil for a very long period. In light of this, investigated in the current study the effect of temperature and pH on the development and sporulation of *F. equiseti* and *F. chlamydosporum* inducing pod rot disease of mungbean in India.

MATERIAL AND METHODS

Isolation of pathogens from the symptomatic plants: Infected pods of mungbean with symptoms such as discolored, soft, distorted, shriveled mature pods; white powdery growth of mycelium at any part of pods; rotting of the pods; white mouldy growth on seeds; rotting and shriveling of seeds were collected from farmer fields, Krishi Vigyan Kendras and research farms of Punjab Agricultural University, Ludhiana during 2018-2021. Samples were transported to the laboratory in paper bags for additional examination and isolation. Pods were cleaned under running tap water and chopped into little pieces in the lab. Pods were divided into little pieces and cleaned in running water in the lab and placed onto water agar medium after sterilization (Burgess et al 1994) and incubated under a typical growth condition (Salleh and Sulaiman 1984). These cultures were transferred to Potato Dextrose Agar (PDA) and subsequently purified using the single spore isolation technique (Hansen 1926). The purified cultures incubated at 25 ± 1°C in the incubator. All the cultures were maintained on PDA and SNA (Spezieller Nährstoffarmer Agar) (Nirenberg 1976) medium for further studies. The same method was applied to isolate the pathogens from infected seeds.

Effect of temperature on pathogens growth: In order to study the growth of the *F. equiseti* and *F. chlamydosporum* under different temperature levels, the 100 ml of Potato Dextrose Broth (PDB) medium was poured in each glass jars (250ml) and sterilized at 15 lbs/inch^2 in an autoclave for 20 min and the active cultures of *F. equiseti* and *F. chlamydosporum* were independently inoculated into each jar using 5 mm discs. The inoculated jars were then incubated at different levels of temperature *viz*., 0, 5, 10, 15, 20, 25, 28, 30, 35 and 40°C in five replications. At each temperature level, the dry mycelial weight and sporulation was calculated after 9 days. The sporulations of both fungi were calculated by using Haemocytometer slide (0.01 cm) under the compound microscope as described by Tyagi and Pudel (2014).

Effect of pH on pathogens growth: Effect of different pH levels *viz*., 2.0, 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 on the growth of pod rot pathogens was studied. 100 ml autoclaved Potato Dextrose Broth (PDB) medium was poured into sterilized glass jars under aseptic conditions. The pH levels of medium were measured by the 'Elico' digital (LI 127 model) pH meter. The fifferent levels of pH were prepared by using HCl (0.1N) and NaOH (0.1N) for each jar. For replication purpose, five jars per each pH level were inoculated centrally with 5 mm culture disc of the actively growing culture of *F. equiseti* and *F. chlamydosporum* separately under aseptically conditions. Inoculated jars were incubated at 25 ± 1°C. Observations on the dry mycelial weight were taken after 9 days at each pH level. Sporulation was recorded by counting the number of macro-conidia and micro-conidia with the help of haemocytometer as described by Tyagi and Pudel (2014).

Statistical analysis: The statistical design was completely randomised in a factorial configuration of 2x10 (2 pathogens x 10 temperature/pH levels) over 20 treatments with 4 replicates each. For statistical analysis, the data were subjected to Duncan's Multiple Range Test (DMRT) at P= 0.05 by using R Statistical Software (v4.1.2; R Core Team 2021).

RESULTS AND DISCUSSION

Effect of different temperature levels on the growth of *F. equiseti* and *F. chlamydosporum*: In the present study, the effect of temperature and pH on mycelial growth and sporulation was studied. Among the external factors that influence fungi' growth, temperature plays an important role. Each fungus has its optimal temperature range for growth and sporulation. In the present study, maximum dry mycelial weight of *F. equiseti* and *F. chlamydosporum i.e*., 5.404 mg and 5.190 mg, respectively were obtained at 28°C. However, temperatures below 10°C and above 35°C were found to affect the fungal growth (Table 1). Maximum sporulation of *F. equiseti* (1.65 x 10^6 conidia ml^{-1}) and *F. chlamydosporum* (1.50 x 10^6 conidia ml^{-1}) was observed at 30°C, followed by *F. equiseti* (1.60 x 10^6 conidia ml^{-1}) and *F. chlamydosporum* (1.45 x 10^6 conidia ml^{-1}) at 28°C. Minimum sporulation of *F. equiseti* (0.25x 10^6 conidia ml^{-1}) was observed at 10°C. However, *F. chlamydosporum* showed the minimum sporulation of 0.15 x 10^6 conidia ml^{-1} at 10°C, at par with 15°C (Table 1).

Pathogens were incubated at different temperatures to study the optimum temperature for growth. It was concluded that the significant growth of the pathogens was observed at 10-35°C of temperature. The ideal temperature for best development of both fungi was 28°C, which was near to room temperature; nevertheless, moderate growth and sporulation was recorded at 30°C because it demonstrated maximum colony growth next to a temperature of 28°C and greatest in conidia generation. Gupta et al. (2010) discovered that when

the temperature was 28°C, radial development of *F. oxysporum* f. sp. *psidii* and *F. solani* was at its peak. *Gibberella fujikuroi* showed great growth and sporulation at 30°C, followed by 25°, 20°, and 35°C, according to Ahamad et al. (2002). Desai et al. (2016) reported that *F. udum* showed maximum growth and sporulation at 28°C on PDA. Daami-Remadi et al. (2006) discovered that the temperature optimum for maximal mycelial development of *F. oxysporum* f. sp. *tuberose* was between 25 and 30°C, whereas it was 30°C for *F. solani*. The results of previous researchers (Boughalleb 2001, Daami-Remadi et al 2004) and current analysis also reflect the conclusion that temperatures between 25 and 35 °C were favourable for the development and sporulation of Fusarium.

Effect of different pH levels on the growth of *F. equiseti* and *F. chlamydosporum*: The pH of the medium directly affects the growth rate, amount of mycelium, and other life processes (Lilly and Barnett, 1951). At the favorable reaction of the solution, fungus utilizes the substrate effectively and yields maximum mycelium. The results of the present investigation indicated that maximum growth of fungus *F. equiseti i.e*., 4.399 mg and *F. chlamydosporum* (4.637 mg), was observed at pH 6.5. The least growth of fungus *F. equiseti i.e*., 1.292 mg and *F. chlamydosporum i.e.,* 1.286 mg was observed at pH 4.0. However, both fungi were unable to grow on the media with a pH level below 4.0 (Table 2). Similarly, maximum sporulation of *F. equiseti i.e.* 1.65 x 10^6 conidia ml^{-1} and *F. chlamydosporum i.e.* 1.45 x 10^6 conidia ml^{-1} was observed at pH 6.5 followed by *F. equiseti* (1.55 x 10^6 conidia ml^{-1}) and *F. chlamydosporum* (1.40 x 10^6 conidia ml^{-1}) at pH 6.0. Minimum sporulation of *F. equiseti* (0.20 x 10^6 conidia ml^{-1}) was observed at pH 8.0 and *F. chlamydosporum* (0.10 x 10^6 conidia ml^{-1}) was observed at pH 4.0 (Table 2).

Fusarium spp. may survive in a wide pH range, from 4.0 to 8.0. However, the findings showed that for *F. equiseti* and *F. chlamydosporum*, a suitable pH for maximum mycelial mass and spore production was 6 to 6.5. Thus, it is abundantly obvious that the tested Fusarium spp. preferred an pH less than 7 for the development and sporulation of its spores. The current results are consistent with the preceding studies. At

Table 1. Effect of different temperature regime on the growth and sporulation of *F. equiseti* and *F. chlamydosporum*

Temperature (^{0}C)	*F. equiseti*		*F. chlamydosporum*	
	Mean dry mycelial weight (g)	Sporulation (conidia ml^{-1})	Mean dry mycelial weight (g)	Sporulation (conidia ml^{-1})
0	0.000^{G}	-	0.000^{F}	-
5	0.000^{G}	-	0.000^{F}	-
10	1.476^{F}	0.25 x 10^6	1.131^{E}	0.15 x 10^6
15	2.409^{E}	0.30 x 10^6	2.399^{D}	0.15 x 10^6
20	3.613^{C}	1.00 x 10^6	3.297^{C}	0.70 x 10^6
25	4.731^{B}	1.45x 10^6	4.212^{B}	1.25 x 10^6
28	5.404^{A}	1.60 x 10^6	5.190^{A}	1.45 x 10^6
30	4.740^{B}	1.65 x 10^6	5.142^{A}	1.50 x 10^6
35	3.036^{D}	0.80 x 10^6	3.146^{C}	1.10 x 10^6
40	0.035^{G}	-	0.000^{F}	-

The values following the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P= 0.05

Table 2. Effect of different pH levels on the growth and sporulation of *F. equiseti* and *F. chlamydosporum*

pH	*F. equiseti*		*F. chlamydosporum*	
	Mean dry mycelial weight (g)	Sporulation (conidia ml^{-1})	Mean dry mycelial weight (g)	Sporulation (conidia ml^{-1})
2.0	0.000^{G}	-	0.000^{G}	-
3.0	0.000^{G}	-	0.000^{G}	-
4.0	1.292^{F}	0.50 x 10^6	1.286^{F}	0.10 x 10^6
5.0	2.513^{E}	0.85 x 10^6	2.874^{D}	0.35 x 10^6
5.5	2.816^{D}	1.10 x 10^6	3.892^{BC}	1.20 x 10^6
6.0	3.442^{C}	1.55 x 10^6	4.526^{A}	1.40 x 10^6
6.5	4.399^{A}	1.65 x 10^6	4.637^{A}	1.45 x 10^6
7.0	4.104^{B}	1.50 x 10^6	4.101^{B}	1.35 x 10^6
7.5	3.457^{C}	1.10 x 10^6	3.626^{C}	0.95 x 10^6
8.0	1.302^{F}	0.20 x 10^6	2.005^{E}	0.25 x 10^6

The values following the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P= 0.05

pH 6.0, *Fusarium oxysporum* f. sp. *ciceri* saw its fastest growth (Desai et al. 1992). *G. fujikuroi* was grown on broth medium at four different pH levels by Ahamad et al (2002) and at pH 5 saw great growth and sporulation. Sharma et al (2005) investigated how pH affected *F. oxysporum* f. sp. *lini* growth and sporulation and found that the tested *Fusarium* spp. could sporulate and thrive at 5.5 pH. The current findings support prior researchers' accounts of their physiological research on *Fusarium* spp. (Kulkarni 2006, Chittem and Kulkarni 2008). Conclusively, the findings of this study will help to improve understanding of the epidemiology of *F. equiseti* and *F. chlamydosporum*, as well as forecast the risk of pod rot disease in mungbean. With this knowledge, breeders may be better able to recognise the resistant mungbean cultivars, taking into consideration the fact that temperature has a significant impact on the development of pod rot and the aggressiveness of pathogens. Quantifying the impact of environmental factors may aid in the creation of more effective management approaches by identifying environmental conditions to restrict disease development through soil pH modification, planting dates and the usage of cultivars that may exhibit a temperature-dependent responses.

REFERENCES

Ahamad S, Agrawal DK, Udit N and Chauhan SS 2002. Effect of temperature, pH, light and inoculation period on growth, sporulation, biomass and gibberellic acid production. *Annals of Plant Protection Science* **10**(2): 343-348.

Bashir M and Malik BA 1988. Diseases of major pulse crops in Pakistan-a review. *Tropical Pest Management* **34**: 309-314.

Bhat FA, Mohiddin FA and Bhat HA 2014. Reaction of green gram (*Vigna radiata*) to *Cercospora canascens* (ELL.) and Mart. *Indian Journal of Agriculture Research* **48**: 140-144.

Boughalleb N 2001. Decay of watermelon in Tunisia: Identification of the causal organism and biology of *Fusarium* spp. Mémoire de Diplôme d'Etudes Approfondies en Protection des Planteset Environnement. *Ecole Supérieure d'Horticulture et d'Elevage de Chott Mariam* p. **76**.

Burgess LW, Summerell BA, Bullock P and Backhouse D 1994. *Laboratory Manual for Fusarium Research*, 3rd edn. Department of crop science, University of Sydney, Sydney, Australia, p 133.

Buttar HS, Singh A, Sirari A and Sharma S 2021. First report of identification and characterization of pathogen causing pod rot in mungbean. In: Abstract of *National Symposium on Strategic plant disease management for food security*. Indian Society of Plant Pathologists, 6 December to 7 December, 2021. ICAR-Central Potato Research Institute Shimla, Himachal Pradesh. Pp. 26.

Chittem K and Kulkarni S 2008. Effect of Media on the Growth of *Fusarium oxysporum* f. sp. g*erberae* and *Fusarium oxysporum* f. sp. *dianthi*. *Karnataka Journal of Agriculture Science* **21**(2): 303-304.

Conrath U, Pieterse CMJ and Mauch-Mani B 2002. Priming in plant-pathogen interactions. *Trends in Plant Science* **7**: 210-216.

Daami-Remadi M and El Mahjoub M 2004. Appearance in Tunisia of *Fusarium oxysporum* f. sp. *tuberosi* causing vascular wilting and tuber dry rot of potato. *EPPO Bulletin* **34**: 407-411.

Received 12 June, 2022; Accepted 25 September, 2022

Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F and El Mah-joub M 2006. Effect of temperature on aggerressivity of Tunisian *Fusarium* sp. causing potato (*Solanum tuberosum* L) tuber dry rot. *Journal of Agronomy* **5**: 350-355.

Desai S, Hegde KK and Desai S 1992. Identification of suitable method and time for artificial inoculation of maize with stalk rotting fungi. *Indian Phytopathology* **45**(3): 381-82.

Desai UA, Andoji YS and Shivaji SS 2016. Influence of temperature and different culture media on growth of *Fusarium udum* (Butler), causal organism of wilt of pigeon pea. *International Journal of Biology Research* **4** (1): 42-45.

Di Menna ME, Lauren DR and Smith WA 1991. Effect of incubation temperature on zearalenone production by strains of *Fusarium crookwellense*. *Mycopathologia* **116**: 81-86.

Doohan FM, Parry DW, Jenkinson P and Nicholson P 1998. The use of species-specific PCR-based assays to analyse Fusarium ear blight of wheat. *Plant Pathology* **47**: 197-205.

Gupta VJ, Misra AK and Gaur RK 2010. Growth characteristics of *Fusarium* spp. causing wilt disease in *Psidium guajava* L. in India. *Journal of Plant Protection Research* **50**(4): 452-62.

Hansen HN 1926. A simple method of obtaining single-spore cultures. *Science* **64**: 384.

Jimeneza M, Manez M and Hernandez E 1996. Influence of water activity and temperature on the production of zearalenone in corn by three Fusarium species. *International Journal of Food Microbiology* **29**: 417-421.

Kaur L, Singh P and Sirari A 2011. Biplot analysis for locating multiple disease resistant diversity in mungbean germplasm. *Plant Disease Research* **26**: 55-60.

Khajudparn P, Wongkaew S and Thipyapong P 2007. Mungbean powdery resistant identification of genes for resistant to powdery mildew in mungbean. *African Crop Science Conference Proceedings* **8**: 743-45.

Kulkarni SP 2006. *Studies on Fusarium oxysporum Schlecht Fr f. sp. gladioli (Massey) Snyd. & Hans. causing wilt of Gladiolus.* M.Sc. (Agric.) thesis, Univ. Agric. Sci., Dharwad, India, p. 245.

Lilly VG and Barnett HL 1951. *Physiology of Fungi*. McGraw Hill Book Company Inc., New York, p. 464.

Nadeem MA, Ahmad R and Ahmad MS 2004. Effect of seed inoculation and different fertilizer levels on the growth and yield of mungbean (*Vigna radiata* L.) *Journal of Agronomy* **3**: 40-42.

Nirenberg HL 1976. Untersuchungen uber die morphologische und biologische differenzierung in der Fusarium section Liseola. *Mitteilungen aus der biologischen bundesanstalt fur land-und forstwirtschaft (Berlin-Dahlem)* **169**: 1-117.

Pataczsek L, Zahir ZA, Ahmad M, Rani S, Nair R, Schafleitner R, Cadisch G and Hilger T 2018. Beans with benefits: The role of mungbean (*Vigna radiata*) in a changing environment. *American Journal of Plant Science* **9**: 1577-1600.

Salleh B and Sulaiman B 1984. Fusaria associated with naturally diseases plants in Penang. *Journal of Plant Protection* **1**: 47-53.

Sharma RL, Singh BP, Thakur MP and Thapa SK 2005. Effect of media, temperature, pH and light on the growth and sporulation of *Fusarium oxysporum* f. sp. *lini* (Bolley) Snyder and Hensan. *Annals of Plant Protection Sciences* **13**(1):172-174.

Shukla V, Baghel S, Maravi K and Singh SK 2014. Yield loss assessment in mungbean [*Vigna radiata* (L.) Wilczek] caused by anthracnose [*Colletotrichum truncatum* (Schw.) Andrus and moore]. *Bioscan* **9**: 1233-1235.

Singh J, Mishra KK and Singh AK 2013a. Current status of web blight of mungbean. *Asian Journal of Soil Science* **8:** 495-504.

Tyagi S and Paudel P 2014. Effect of different pH on the growth and sporulation of *Fusarium oxysporum*: The causal organism of wilt disease of tomato. *International Journal of Basic and Applied Biology* **2**(1): 103-106.