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Effect of abiotic factors on growth of *Macrophomina phaseolina* causing *Macrophomina* stem blight and dry root rot diseases of pigeonpea

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Abstract
In North Eastern Karnataka pigeonpea growing regions, *Macrophomina phaseolina* is one of the devastating plant pathogens that causes dry root rot and stem blight diseases. Studies on the effects of pH, temperature, and relative humidity on the mycelial development of *M. phaseolina* cultures causing stem blight and dry root rot diseases were carried out under *in vitro* conditions. The findings showed that the pH range of 6 to 8 is ideal for growth, perpetuation and establishment of *M. phaseolina*. The pathogen grew best at temperatures between 30 and 35 °C, whereas between 40 and 45 °C, no growth was seen. At 80–100% relative humidity, the largest colony diameter was observed for both the pathogens. The results reveal that the during the weather parameters during the pigeonpea cultivation are favourable for the growth and spread of the disease and strategies should be devised to control the disease through different approaches.

Keywords: abiotic, *Macrophomina phaseolina*, *Macrophomina*, pigeonpea, *Cajanus cajan* L.

Introduction
Pulses are the main source of protein for vegetarians and pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most sought pulse crops across Asia and many other countries. It belongs to the family Fabaceae, has deep-root system, C3 in growth habit and is a short-day plant widely adapted to a range of soil types, temperatures, and rainfall (Nsiah, 2012 and Musokwa and Mafongoya, 2021) [16, 15]. Pigeonpea can tolerate temperatures as high as 35 °C. However, it can be killed by heavy frost. An average annual rainfall between 600 and 1,000 mm is most suitable. It is known for its drought tolerance (Hemavathy *et al*., 2023) [9].
Despite India, being the largest producer of pigeonpea accounting for about 90 percent of world area and production, it is still importing pigeonpea from neighbouring Myanmar and many African countries engaged in pigeonpea production. There have been constraints in the productivity over the past five years (Sarkar *et al*., 2018; Ganguly and Gulati 2022) [20, 8] and for the current fiscal, 0.89 million tonnes were imported in April 2023 and it is likely to cross 1.2 million tonnes by March 2024 (https://www.nasdaq.com).
Amongst biotic factors, damage due to pests and diseases have always been predominant in pigeonpea and is affected by more than 20 diseases. The changing climate is another factor favouring many minor and lesser-known diseases emerging in to major epidemics of pigeonpea. *Macrophomina* stem blight and dry root rot are known and emerging diseases, drastically affecting pigeonpea production across major pigeonpea-growing regions of South and Central India (Mallikarjun *et al*., 2023) [12]. The dry and warm climate favourable to the disease development is frequently witnessed in the past three years. Given this and the fact that the climate is changing, study on the relationship between epidemiological factors and pathogen growth was deemed crucial. Therefore, the current investigation was undertaken with the objectives of knowing the effects of three abiotic variables like temperature, pH, and relative humidity on the pathogen development, its perpetuation and spread.

Materials and Methods
Isolation of pathogen cultures: Pigeonpea plant samples infested with *Macrophomina* stem blight and dry root rot were collected from ZARS, Kalaburagi research fields and farmers' fields.

Plants showing conspicuous blight symptoms of grey coloured spindle shaped lesions with black and brown margins and wilted with dry root rot symptoms were collected separately and brought to laboratory. The upper epidermal layer of symptomatic and diseased stem portion was peeled off, discoloured inner tissues of 2 to 3 mm were collected in sterile petri pate using scalpel blade. These pieces were surface sterilized with 0.1 percent mercuric chloride solution for 1 minute and subsequently washed thrice with sterile distilled water aseptically and transferred to PDA plates. The plates were stored in BOD incubator at 30 ±1 °C and observed for growth of fungal pathogen.

The primary morphological characteristics of suspected mycelial cultures such as colour of mycelia, growth habit, fluffiness, and formation of sclerotial bodies were assessed for considered for its identification. Further confirmation of their identity was done by observing hyphal characters, its branching and formation of conidia or sclerotial bodies.

Response of pathogens to different pH levels

The pathogen cultures were grown on PDA plates prepared with different pH levels *viz*., 6, 6.5, 7, 7.5, 8, 8.5 and 9 respectively. Different pH level media was prepared by adjusting the final pH of the media before autoclaving using either 0.1N NaOH or 0.1N HCl. The autoclaved media was transferred to 90mm Petri plates aseptically and allowed for solidification. The 7-days old pathogen cultures were used for the study, 5mm mycelial discs were cut aseptically using corkborer. The discs were placed at the centre of the plates. For each pH level and for each pathogen, three replications were maintained. The plates were incubated at 30 ^{0}C in BOD incubator maintaining 12-hours dark and 12 hours photoperiod conditions and observed for the growth of the culture regularly at 24, 48 and 72 hours after inoculation. The colony diameter was measured in all the three Petri plates of each pH level and average diameter was calculated. Based on the response of both the pathogens against all the pH levels conclusions were arrived.

Response of pathogens to different temperatures

The stem blight and dry root rot causing pathogens were assessed for their growth responses at different temperature levels *viz*., 25, 30, 35, 40, and 45°C. A 5mm-diameter mycelial discs were excised using cork borer from a 7-day-old pure cultures of the pathogens separately and placed at the centre of the 90mm Petri dish containing PDA media. They were then incubated at respective temperatures in BOD incubator maintaining 12-hours dark and 12 hours photoperiod conditions. Three plates were maintained for each temperature regime. Culture growth was observed at 48, 72, and 96 hours after incubation. Fungal colony's diameter was measured in each plate and average diameter was calculated. Based on the response of both the pathogens against all the temperature levels conclusions were arrived.

Response of pathogens to different relative humidity (RH)

The pathogen cultures were subjected for growing at six different RH levels *i.e.*, 50 percent, 60 percent, 70 percent, 80 percent, 90 percent, and 100 percent. Mycelial discs of 5mm from a 7-days old culture were aseptically transferred to PDA in Petri plates. By dissolving various quantities of sulphuric acid in distilled water, varied relative humidity levels were maintained in the desiccator. Without adding sulphuric acid to the distilled water in the desiccator, 100% relative humidity was achieved. A total of three replications were kept. At 24, 48and 72 hours following incubation, the average growth of the fungal colony was noted.

Results and Discussion

The pathogen culture isolated from stem blight symptomatic tissue initially produced white hyaline mycelia that eventually turned black or grey indicating the development of sclerotia. The shape of sclerotia varied from round to irregular. The average size of sclerotia varied from 97.27-137.02µm in diameter (40X). The pathogen took 72 h to reach the edge of the Petri plate when grown on PDA media shown in Fig.1. Mycelia were septate, produced right angle and acute angle branching from mother hyphae (Marquez *et al*., 2021) [13]. The aerial mycelium production was also noticed occasionally (Mishra and Kumari, 2021) [14]. Similar variation in size of sclerotia (84-148µm) was observed by Chikkana (2014) [4] from the *Macrophhomina* culture isolated from stem canker.

The mycelium of culture isolated from the root was pale white in colour with septa in the initial growth stages, later it turned to dark brown to black in colour as sclerotia formation started. Sclerotia were round, oval and hitherto irregular in shape and size ranged from 66.42-76.19 µm in diameter (40X). Mycelium was septate and produced branching structures that extended outward from mother hyphae at right and acute angles (Marquez *et al*., 2021) [13] and the pathogen took 48h to reach the periphery of the Petri plate on PDA media. (Fig.2)

Response of pathogens at different pH levels

The hydrogen ion concentration measured as pH level in soil, water and other host cells plays crucial role in growth, establishment and reproduction of every microbe including plant pathogens. It determines supply of nutrients and acts as vital buffering agent for every kind of metabolic activities across the microbial world.

The results obtained from both the pathogens showed that both stem blight and dry root rot cultures had similar growth patterns when subjected to the growth on Potato Dextrose Agar media at different of pH levels. The maximum mean radial colony growth of *Macrophomina* causing dry root rot culture was seen at pH 6.0 (19.1mm) and also of stem bight culture (18.9mm at pH6.0), followed by pH 6.5 (18.2 mm of dry root culture and 16 mm of stem blight culture) at 24 hours after inoculation. While the least growth was noticed at pH 9.0 for both the pathogens *i.e*., 8.4 mm for dry root culture and 8.1 for stem blight culture (Fig.3, 4 and Table.1).

Maximum colony growth was seen for both the cultures at 72 h after inoculation at a pH 6.0 *viz*., 87.7 mm for dry root culture and 85.1mm for stem culture. Interestingly no much difference in the growth was observed at pH 6.5, 7.0 and 7.5 for both the cultures. There was substantial and significant difference in growth was observed across other pH concentrations. PDA media with pH 9 had the least growth of stem blight (20.5 mm) and root culture (37 mm). The current findings are in line with those of Surinder *et al*. (2013) [24] who observed higher relative growth and higher mean dry mycelial weight of *Macrophomina phaseolina* inciting stem canker disease in pigeonpea at pH 6 and 7. Kumar and chaudhary (2020) [11] also reported alike results in charcoal rot of soybean caused by *M. phaseolina*.

In the present study, least mycelial growth of both the pathogens was observed at pH 9 and 8.5, earlier also

Chowdary (2007) [5] reported least mycelial growth at pH 8 (59mm). Similarly, Csöndes *et al*. (2007) [6] also noticed decreased growth of *Macrophomina* at pH more than8.0. Bhupathi and Theradimani (2018) also reported pH 6-7 are the preferential range for growth of *M. phaseolina* and more than 8 is not preferred by the pathogen.

Response of pathogens at different temperatures

Significant influence of temperature on growth of the both the pathogen was observed. Maximum colony growth of *Macrophomina* culture causing stem blight was observed at 35 ºC (88.6 mm) after 96hours of incubation which was significantly superior over other temperatures evaluated. The next best temperature supported the growth of the pathogen culture was 30 °C (83.3 mm). Moderate growth was observed at 25 °C (76.0 mm). At 40 °C and 45°C, no mycelial growth was observed even at 96 hours after inoculation (Fig.3, 4 and Table2). Similar findings were observed by Srinivas *et al*. (2017) [21]. They reported maximum mycelial growth of *M. phaseolina* causing dry root rot in chickpea at 35 ºC followed by 30 and 25 ºC. Similarly, also recorded highest mycelial growth of *M. phaseolina* between 25 and 35 ºC. It is evident from the present investigation that 30-35 ºC temperature is very ideal for growth and establishment of the pathogen, it could not produce any mycelium at 40 ºC and above temperatures indicating this temperature is detrimental to the pathogen.

The *Macrophomina* culture isolated from the root samples infected with Dry Root Rot (DRR) disease grew faster at 30 ºC and attained the maximum growth with mean colony diameter of 88.6mmafter 72 hours of inoculation. At 30 and 25 ºC temperature levels, mean colony diameter growth was 83.3 mm after 72 hours of incubation. No mycelial growth was seen at 40 and 45 °C temperature levels up to 72 hours (Fig. 3, 4 and Table 3). It is quite natural that both stem blight and dry root rot causing pathogens are same, they were isolated from different parts of the same host but behaving similarly towards different temperature levels. The observations of present study are in line with Kumar and chaudhary (2020) [11] who recorded highest growth of *M. phaseolina* around 30 °C and growth was slower at temperatures below 25 and above 35 °C. Earlier, Csöndes *et al*. (2007) [6] also stated 25 to 35 ºC was the most favourable temperature for growth and development of *M. phaseolina*. The mycelial growth slowed at 10, 15 and 40 ºC. Similarly, Akhtar *et al*. (2011) [1] also opined 30-35 ºC as the optimum temperature for fungal growth and microsclerotia production of *M. phaseolina* infecting sesame. These previous findings are providing substantial evidence to conclude the present findings and support the conclusion.

Macrophomina phaseolina has been reported to grow at varying temperature levels. From the present study it was concluded that, the ideal temperature for growth and development of both the pathogens is 30-35 °C. Viana and Souza (2002) [25] also reported 35 ºC as the most suitable temperature for growth and multiplication of *M. phaseolina*. Khamari *et al*. (2018) [10] studied growth of stem and root rot fungus of sesame and found that 30-35 ºC was optimal for growth of *M. phaseolina*. In another study by Sunkad *et al*. (2023) [23] also maximum mycelial growth of *M. phaseolina* was observed at 35 °C and no growth were observed at 45 and 50 °C.

In our study both the *Macrophomina phaseolina* cultures failed to grow at 40 and 45 ºC.Interestingly both the pathogens began to grow after the plates were removed from 40 and 45 ºC temperature levels and kept at room temperature (32-35 ºC), indicating that sclerotia formed during the growth and development of the pathogen can survive under elevated temperatures, The ability of sustaining higher temperatures making this pathogen more widely distributed across different agroecology's of varying temperature levels. Similar results were also obtained by Sukanya *et al*. (2016) [22] who reported no growth of sorghum charcoal rot fungus at 45 ºC and Oviya *et al*. (2019) [17] while studying on *M. phaseolina* infecting blackgram also reported zero growth of the pathogen at 40 ºC. Salunkhe *et al*. (2009) [18] also reported no growth of *M. phaseolina* at 40 ºC.

Response pathogens at different relative humidity levels

Among the weather parameters next to temperature, humidity is the vital epidemiological component in determining the growth and establishment of plant pathogens. It significantly influences physiology of fungal growth as well as the disease outbreak.

The findings of the present study showed that colony diameter of *Macrophomina phaseolina* cultures causing stem blight and dry root rot were significantly influenced by different RH levels. Significantly highest mean mycelial colony growth was observed at 80 percent RH for both stem blight (88.7mm) and root rot (90.0mm) cultures of *M. Phaseolina* (Fig. 3, 4 and Table 4), followed by 90 percent RH which showed 85.1mm growth for stem blight and 86.1mm for dry root rot culture and 100 percent (80.6 mm for stem and 82.3 mm for root culture). A laboratory investigation by Ali *et al*. (1998) [2] revealed that *Rhizoctonia* spp. causing crown rot of carrot grew efficiently at RH level of 80 to100 percent and it declined at the lower humidity levels, the present findings agree with these reports.

The least mycelial growth was observed at 50 percent RH level with lowest mycelial colony growth of 38.1 mm diameter for stem blight and 40.6 mm for dry root rot cultures respectively, followed by 60 percent RH level which showed mycelia colony growth of 45.5 mm for stem blight and 51.1mm for root culture cultures. Similarly, least growth of *Macrophomina phaseolina* causing root rot in mungbean was observed at 50-60% relative humidity (Gahlot *et al*., 2022) [7].

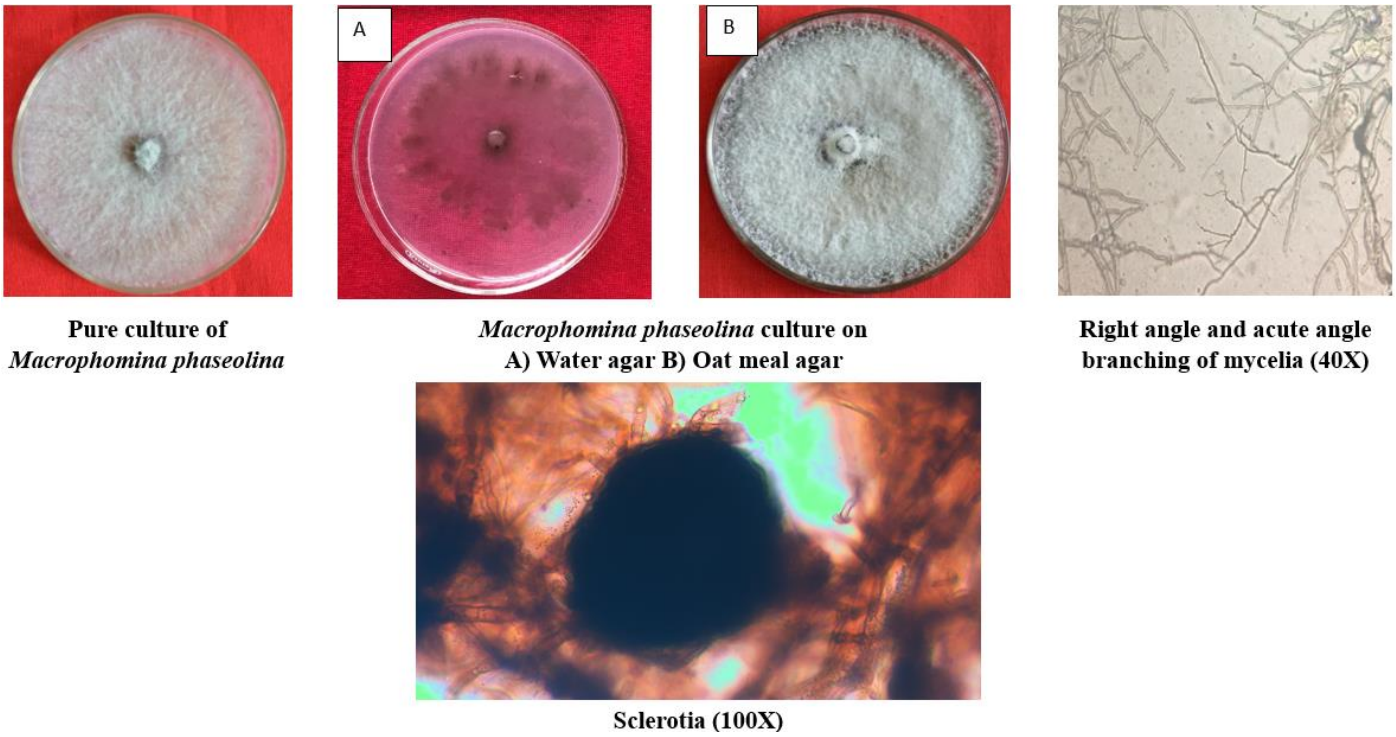

Fig 1: Morphological and cultural characters of *Macrophomina phaseolina* (stem blight culture) on artificial media

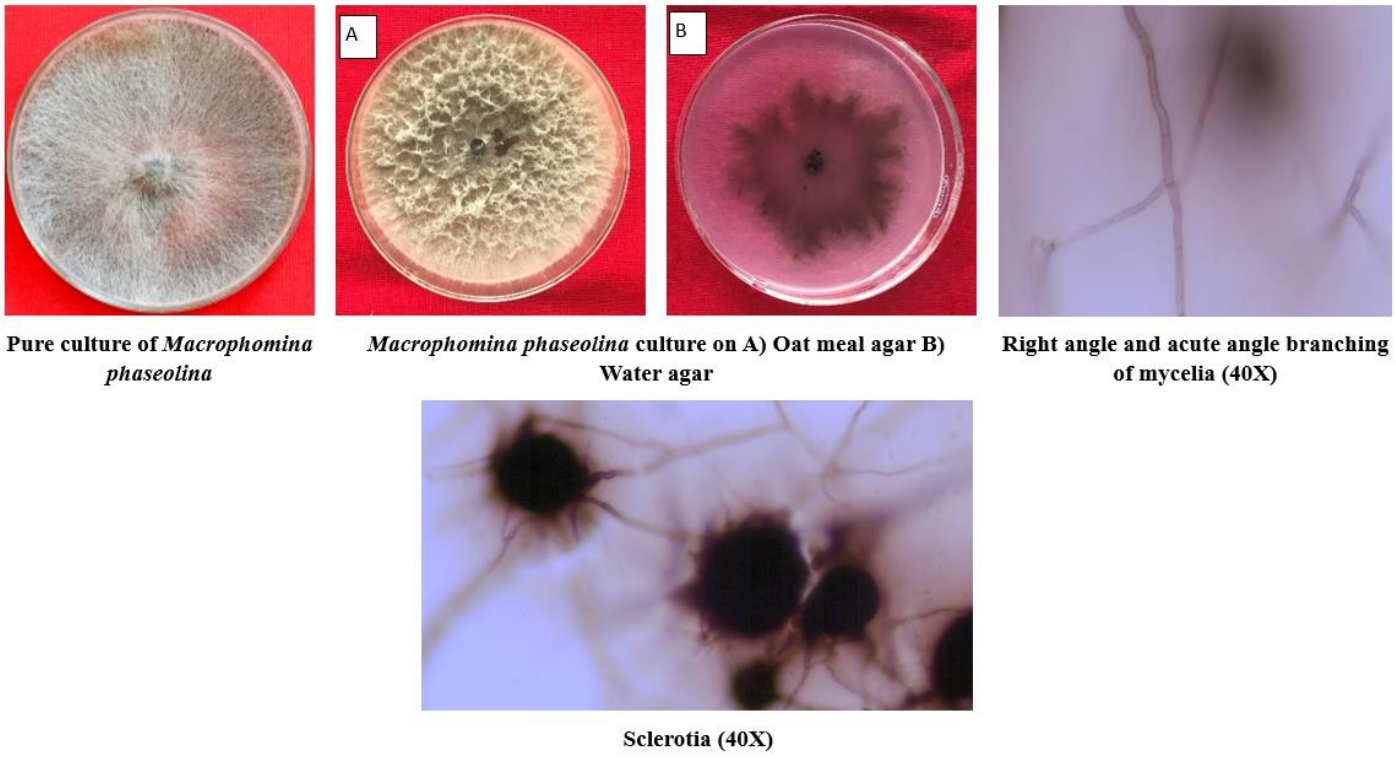

Fig 2: Morphological and cultural characteristics of *Macrophomina phaseolina* (dry root rot culture) on artificial media

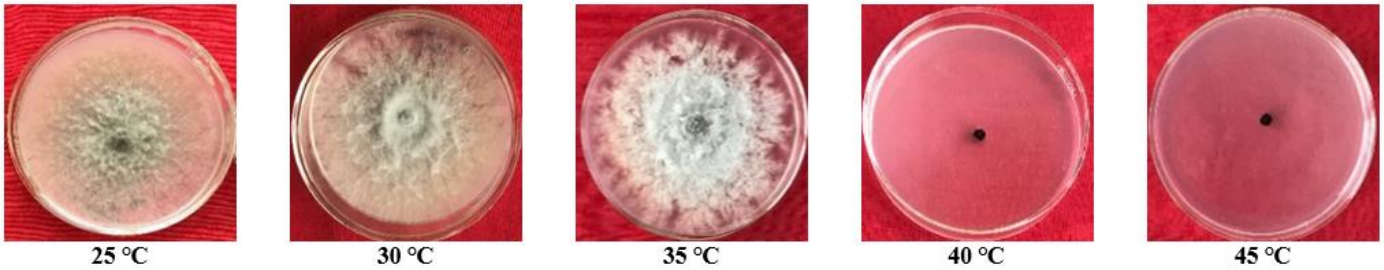

Fig 3: Effect of pH, temperature and relative humidity on growth of *Macrophomina phaseolina* (stem blight culture)

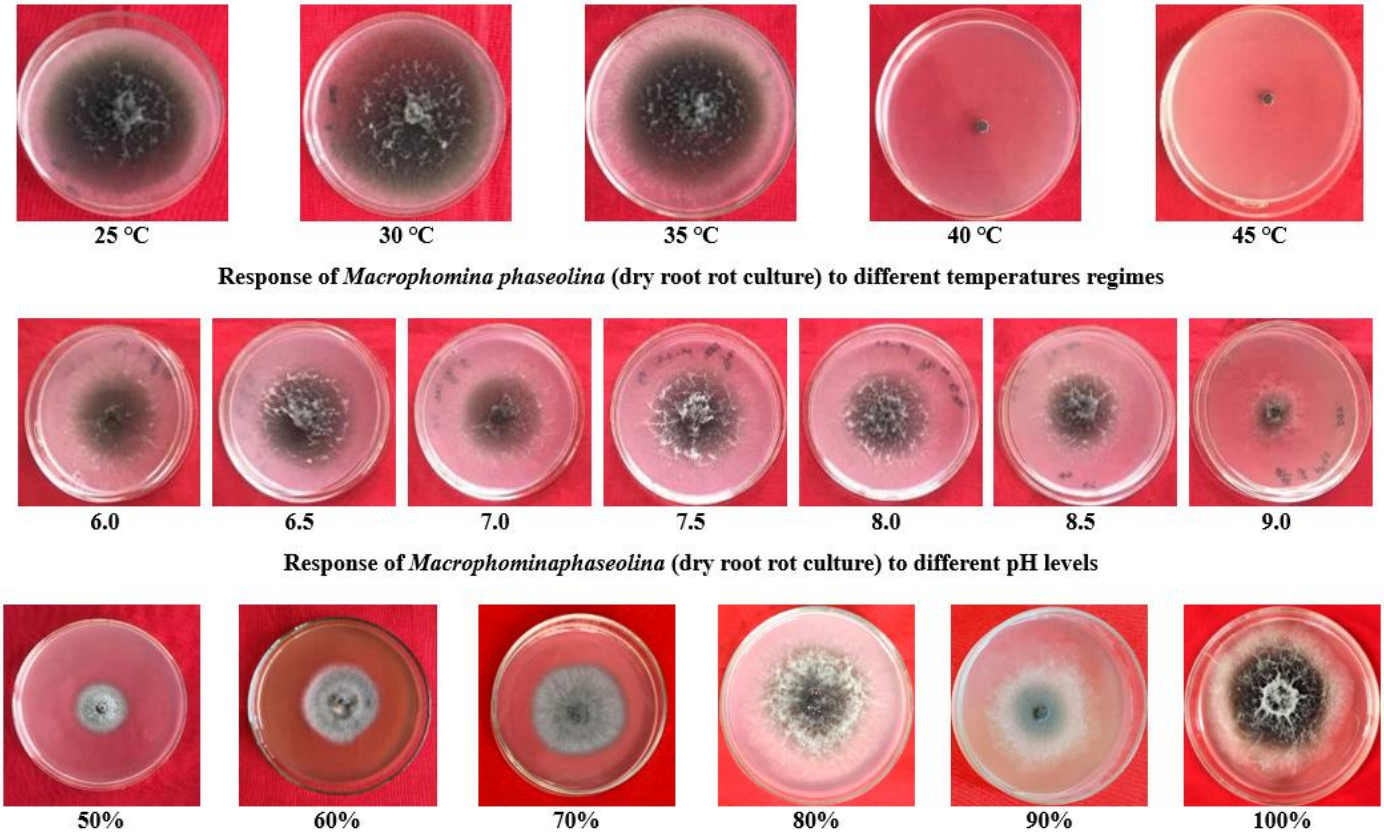

Fig 4: Effect of pH, temperature and relative humidity on growth of *Macrophomina phaseolina* (dry root rot culture)

Table 1: Effect of different pH levels on growth of *Macrophomina phaseolina* cultures causing stem blight and dry root rot diseases of pigeonpea.

Sl. No.	pH levels	Dry root rot culture mycelial growth (mm)		Stem blight culture mycelial growth (mm)	
		24 HAI	72HAI	24 HAI	72 HAI
1	6.0	19.1	87.7	18.9	85.1
2	6.5	18.2	80.1	16	80.4
3	7.0	16.2	75.9	14.8	74.3
4	7.5	14.6	72.2	14	70.4
5	8.0	14.1	68.2	12.9	62.2
6	8.5	11.9	64.1	10.7	58.6
7	9	8.4	37	8.1	20.5
	S. Em±	0.17	0.58	0.29	0.18
	CD 1%	0.69	2.35	1.17	0.75

Table 2: Effect of different temperature levels on growth of *Macrophomina phaseolina* culture causing stem blight of pigeonpea at different time intervals

Sl. No	Temperature (℃)	Mycelial growth (mm)			
		24HAI	48HAI	72HAI	96HAI
1	25	12.6	33.3	63.3	76.0
2	30	8	40	73.1	83.3
3	35	14.6	40	74.6	88.6
4	40	0	0	0	0
5	45	0	0	0	0
	S. Em±	0.09	0.12	0.11	0.05
	CD @1%	0.37	0.49	0.47	0.20

Table 3: Effect of different temperature levels on growth of *Macrophomina phaseolina* causing dry root rot disease of pigeonpea at different time intervals.

Sl. No,	Temperature (℃)	Mycelial growth (mm)		
		24HAI	48HAI	72HAI
1	25	25.2	58	83.4
2	30	21.2	73	88.6
3	35	26	75.1	83.4
4	40	0	0	0
5	45	0	0	0
	S. Em±	0.27	0.24	0.17
	CD @1%	1.08	0.98	0.70

Table 4: Effect of different relative humidity levels on growth of *Macrophomina phaseolina* causing stem blight and dry root rot diseases of pigeonpea at 4 days of incubation

SI. No.	Relative humidity (%)	*Macrophomina* stem blight culture mycelial growth (mm)	Dry root rot culture mycelial growth (mm)
1	50	38.1	40.6
2	60	45.5	51.1
3	70	78.5	73.4
4	80	88.7	90.0
5	90	85.1	86.1
6	100	80.6	82.3
	S. Em±	0.19	0.84
	C.D @ 1%	0.77	3.39

Conclusion

It is evident from the current findings that maximum growth of *M. phaseolina* was at a pH range of 6-8, temperature of 30-35 ^{0}C and relative humidity of 70 to 100 percent. These factors are usually found starting from the sowing of the pigeonpea till the physiological maturity across the study region. The prevailing weather factors during the entire season are highly favourable for the pathogen and it is necessary to take the preventive and precautionary measures for effective control of both Stem blight and Dry Root Rot diseases caused by *M. phaseolina* using different strategies.

References

1. Akhtar KP, Sarwar G, Arshad HMI. Temperature response, pathogenicity, seed infection and mutant evaluation against Macrophomina phaseolina causing charcoal rot disease of sesame. Arch Phyto Pathol Plant Prot. 2011;44(4):320-330.
2. Ali A, Hall AM, Gladders P. The biology and pathology of Rhizoctonia solani and Rhizoctonia oryzae isolated from crown rot of carrots in the UK. In: Brighton Crop Protection Conference: Pests & Diseases-1998. Brighton, UK; November 16-19; 1998. p. 875-880.
3. Bhupathi P, Theradimani M. *In vitro* studies of carbon, nitrogen sources and pH on mycelia growth of blackgram root rot caused by Macrophomina phaseolina. Pharm Innov J. 2018;7(6):33-35.
4. Chikkanaswamy. Biology and management of Macrophomina stem canker of pigeonpea caused by Macrophomina phaseolina (Tassi) Gold. M. Sc. (Agri) Thesis. Univ. Agric. Sci., Raichur; c2014.
5. Chowdary NB. Influence of different abiotic conditions on the growth and sclerotial production of Macrophomina phaseolina. Indian J Seric. 2007;46(2):186-188.
6. Csondes I, Kadlicsko S, Gaborjanyi R. Effect of different temperature and culture media on the growth of Macrophomina phaseolina. Commun. Agric. Appl. Biol. Sci. 2007;72(4):839-848.
7. Gahlot N, Ahir RR, Abhinav SK, Choudhary S. Effect of temperature, pH and relative humidity on growth of Macrophomina phaseolina and evaluation of cultivars of mungbean against root rot pathogen. J Pharm Innov. 2022;11(4):709-712.
8. Ganguly K, Gulati A. Pulses Value Chain-Pigeon Pea and Gram. In: Agricultural Value Chains in India: Ensuring Competitiveness, Inclusiveness, Sustainability, Scalability, and Improved Finance. 2022. p. 253-286.
9. Hemavathy TA, Anantharaju P, Kavitha S, Dhanushkodi. Investigation of pigeonpea (*Cajanus cajan* (L.) Millspaugh) germplasms for drought tolerance dynamics. J Pharm Innov. 2023;12(6):1064-1067.
10. Khamari B, Satapathy SN, Roy S, Patra C. Adaptability of Macrophomina phaseolina, the incident of stem and root rot disease of sesame to different regimes of temperature, pH and light period. Int. J Curr Microbiol App Sci. 2018;7(12):761-766.
11. Kumar S, Chaudhary B. Effect of different media, temperature, and pH on growth and microsclerotia formation of Macrophomina phaseolina causing charcoal rot of soybean. World. 2020;2:3-8.
12. Mallikarjun K, Gururaj S, Yenjerappa ST, Sunil K, Rachappa VH, Muniswamy S. Emerging disease dynamics in pigeonpea induced by climate change. Paper presented at: National Symposium on Plant Health Management: Current Trends and Novel Mitigation Strategies. UAS, Raichur; September 11-12; 2023. p. 131.
13. Marquez N, Giachero ML, Declerck S, Ducasse DA. Macrophomina phaseolina: General characteristics of pathogenicity and methods of control. Front Plant Sci. 2021;12:1-16.

14. Mishra PK, Kumari M. Morpho-cultural and pathogenic variability in Macrophomina phaseolina isolates from soybean. Pharm Innov J. 2021;10(3):777-785.
15. Musokwa M, Mafongoya P. Pigeonpea yield and water use efficiency: A savior under climate change-induced water stress. Agronomy. 2021;11(5):1-14.
16. Nsiah AS. Role of pigeonpea cultivation on soil fertility and farming system sustainability in Ghana. Int. J Agronomy. 2012;12(2):1-9.
17. Oviya R, Akila R, Sabarinathan KG, Mehetre ST, Rammamoorthy V. Studies on various factors influencing growth and pathogenicity of Macrophomina phaseolina in blackgram. J Pharmacogn Phytochem. 2019;8(3):3545-3549.
18. Salunkhe V, Armarkar S, Ingle RW. Effect of nutritional and physiological factors on growth and sclerotial formation of Rhizoctonia bataticola (Tan B.) Butler isolates. J Plant Dis Sci. 2009;4(1):44-48.
19. Sameer Kumar CV, Satheesh Naik SJ, Mohan N, Saxena RK, Varshney RK. Botanical description of pigeonpea [*Cajanus cajan* (L.) Millsp.]. In: The Pigeonpea Genome. 2017. p. 17-29.
20. Sarkar S, Panda S, Yadav KK, Kandasamy P. Pigeon pea (*Cajanus cajan*) an important food legume in the Indian scenario- A review. Legume Res. 2018;43(5):601-610.
21. Srinivas P, Ramesh Babu S, Sharma M, Narayan Reddy P, Pushpavathi B. Effect of temperature on Rhizoctonia bataticola and dry root rot in chickpea. Int. J Curr. 2017;6(6):3349-3355.
22. Sukanya R, Jayalakshmi SK, Girish G. Effect of temperature and pH levels on the growth of Macrophomina phaseolina (Tassi) Goid. Infecting sorghum. Int. J Agric Sci. 2016;8(37):176-182.
23. Sunkad G, Dore D, Patil M, Joshi R, Kumar M. Impact of temperature, moisture, and CO2 on the growth of the pathogen and severity of emerging dry root rot disease of chickpea in Karnataka. J Agric. Meteorol. 2023;25(2):312-319.
24. Surinder K, Bahadur CV, Kaur BS, Singh DG. Life Science. Int J Life Sci. 2013;1(2):81-88.
25. Viana FMP, Souza NLD. Effect of temperature-water tension interaction on germination of Macrophomina phaseolina microsclerotia. Fitopatol. Bras. 2002;27:268–272.