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IN VITRO PHYSIOLOGICAL AND CONIDIAL CHARACTERIZATION OF *ALTERNARIA TENUISSIMA* INDUCING LEAF BLIGHT IN KODO MILLET (*PASPALUM SCROBICULATUM* L.)

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ABSTRACT

Kodo Millet (*Paspalum scrobiculatum* L.) is an ancient crop with diverse and numerous health benefits. Earlier, it was known to have countable number of diseases but now this crop is facing many health issues. One of the major diseases involved in limiting the production and causing substantial losses is leaf blight caused by *Alternaria tenuissima*. The present study was carried out in Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India. The experiment was set in Completely Randomized Design (CRD) and was replicated thrice *in vitro*. Investigations involved the study of physiological and conidial characterization studies. Five temperature range *viz.*, 15°C, 20°C, 25°C, 30°C and 35°C were studied. On other hand, seven pH ranges *viz.*, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were evaluated. Results regarding these experiments were that the mycelial growth and sporulation of the pathogen was quite good in temperature of 25°C and pH 5.5 respectively. As environment plays a very critical role in growth and sporulation of the pathogens, hence these *in vitro* studies could effectively be used under *in vivo* conditions to break the pathogen's life cycle in order to get more enhanced returns by the farmers. In addition, these studies could be used to give proper guidance to farmers for proper and effective management of the pathogen.

Key words : Kodo millet, Leaf blight, *Alternaria tenuissima*, Temperature, pH, Mycelial growth, Conidial characterization.

Introduction

Kodo millet (*Paspalum scrobiculatum* L.) is an ancient, small seeded and hardy crop which thrives very well in limited number of resources available (Jain and Sharma, 2010). The crop is believed to be originated in Africa but was later domesticated in India approximately 3000 years ago. The crop is a principal member of “Sri Anna” and is cultivated by the farmers of low-income group usually within the traditional systems followed by them (de Wet *et al.*, 1983). Chromosome number of kodo millet is $2n=4x=40$ and approximately 400 species come under the genus “*Paspalum*” and is often an annual crop.

Kodo millet is monocot and its seeds are roughly 2.00 mm long and 1.5 mm wide which appears to be light brown to dark grey in its colour texture. Plant are slender grows up to 90 cm tall, leaf blades are linear, glabrous or pubescent, up to 40 cm long with the basal leaf sheath glabrous or pilose. Inflorescence is composed off more than five racemes that are alternatively arranged on a short to elongated primary axis (Clayton, 1975).

Different vernacular names used in different parts of the country are Kodon in Hindi, Harka in Kannada, Varagu in Tamil and Malayalam, Arika/Arikelu in Telugu, Kodra in Punjabi, Marathi and Gujarati and Kodua in

Oriya. Globally, the crop is cultivated in tropical and subtropical areas. Primarily the crop is grown in India. Besides India, kodo millet is also grown in Indonesia, Philippines, Thailand, Vietnam, Bangladesh, Myanmar and western part of Africa. In India, the crop is largely grown in the states of Madhya Pradesh, Chattisgarh, Tamil Nadu, Telangana, Maharashtra, Gujarat, Karnataka, Uttar Pradesh and Jharkhand (Hariprasanna, 2023).

In recent times, kodo has gained lot of importance due to high nutritive values, agronomic characters and number of health benefits. Kodo's nutritive values (per 100 gram) are as follows; 65.9g of CHO, 8.3g of protein, 1.4g of fat, 9.0g of fibre, 2.6g of minerals, 0.5mg of Fe, 188mg of P, 27mg of Ca, 0.09mg of riboflavin, 0.33mg of thiamine and 0.2mg of niacin (Deshpande *et al.*, 2015). Lecithin is present in high amount in the grains, which is good for strengthening of nervous system. Gluten is absent in kodo millet and is good for people who are gluten intolerant. Kodo millet increases insulin sensitivity for people suffering from diabetes. Low glycaemic index, rich in antioxidants like polyphenols are unique properties of the grains. Consumption of kodo millet reduces the serious problems of bloating, stomach cramping, flatulence, constipation and lower the problems of cardiovascular diseases (NAAS, 2013; Rajalakshmi *et al.*, 2014).

Several plant pathogens which include fungi, viruses, bacteria, nematodes and phanerogamic partial root parasites are reported to cause diseases at various crop growth stages that limits the sustainable yields of kodo millet (Nagaraja *et al.*, 2007). Among fungal pathogens, *Rhizoctonia solani* (Banded leaf blight), *Sporisorium paspali thunbergii* (Head smut), *Ephelis oryzae* (Udbatta), *Alternaria alternata* (leaf blight), *Puccinia substriata* (Rust) and several species of *Helminthosporium* were reported to infect the crop and can cause significant economic loss in grain yield under favourable climatic conditions (Jain and Sharma, 2010).

Leaf blight caused by *Alternaria* spp. and *Helminthosporium* spp. were reported long back in kodo millet as a minor disease but now leaf blight is becoming a major and an emerging problem in kodo millet (Nagaraja *et al.*, 2016). Few studies were done on characterization of pathogen, hence, needs more studies on different aspects of pathogen as well as disease.

Materials and Methods

Present investigation was carried out in the Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India (latitude: 23.21°N, longitude:



Image 1 : Leaf blight of Kodo Millet (*Paspalum scrobiculatum* L.).



Image 2 : Burnt appearance in Kodo Millet.

79.96°E). Diseased samples of Kodo millet were collected from RARS, Dindori, Madhya Pradesh, India (latitude: 22.9418° North and longitude: 81.0768° East) (Images 1 and 2). Samples were then taken to the Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India for further studies. Major symptom recognized during the collection of the diseased samples were the blighting/burning of the leaf's apical (tip) region which gradually progressed downwards towards the older part of the leaf (Gupta *et al.*, 1994).

After bringing the fresh infected samples from the field to the laboratory, small pieces of infected part of about 2-3 mm in size were cut with the help of sterilized scalpel, washed with sterile water 2-3 times and then in 0.1% of NaOCl (Sodium Hypochlorite Solution) in an alternative manner for 20 – 30 seconds each. This procedure is repeated three to four times.

Potato Dextrose Agar (PDA) medium was used to isolate the targeted pathogen. PDA powder was used to prepare PDA medium and then it was autoclaved at 121°C at 15psi for 20 minutes. After autoclaving the PDA

medium was added with streptomycin sulphate powder (100 ppm) in order to avoid the bacterial contamination. 10 ml of PDA medium was poured into 90 mm of sterilized Petri plates and left for 10 -12 minutes to solidify under UV light in laminar air flow chamber. 4 pieces of small surface sterilized samples were kept into the plates in an evenly distributed manner. Above mentioned work was done in totally aseptic condition to avoid aseptic condition to avoid any type of contamination. For 5 – 6 days, at an appropriate temperature of $27 \pm 2^\circ\text{C}$ Petri plates were incubated in biological oxygen demand (BOD) incubator and the growth and sporulation of the pathogen was also monitored. To locate the colonies and identification of the pathogen, microscope analysis was done. Pure culture of the pathogen was maintained in the slants and were stored for long term use at 4°C .

Temporary mounts were prepared from the pure culture of the pathogen. Measurements of conidia were taken using binocular microscope (Leica company) and software (Leica Application Suite). Initially, light microscope was used for locating the presence of conidia. Morphological characters *viz.*, growth, septation of the conidia, size (length and width) and shape of the conidia were observed. The measurement of spores from 9 different spots on temporary mounts were observed under the 100X magnification microscope. The mean values of these measurements were calculated.

This particular experiment was set up to identify the best suitable temperature for the growth of mycelia and sporulation of the pathogen. Different temperatures were used for the experiment were; 15, 20, 25, 30 and 35 degrees, respectively. 100 ml medium of was poured in the conical flask of capacity 150 ml tightly packed with non-absorbent cotton plug and aluminium foil. These flasks were then sterilized in an autoclave for 20 minutes at 121°C and 15 psi. The sterilized Petri plates of 90 mm were poured with the potato dextrose agar @ 10 ml per Petri plate. The test pathogen from 7-day old culture was taken and disc was cut of 5mm and transferred to these plates, sealed with parafilm and kept in an inverted position inside the BOD incubator at different temperatures mentioned above. The replication taken was four. The experiment ended on the 7th day and spore count by making temporary mount was done. Micrometry was done to ensure proper details regarding length and width of the pathogen in the treatments which showed spore count microscope (Leica company) and software (Leica Application Suite) at 100X.

Various pH range *viz.*, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were taken. The pH concentration of the media was

maintained carefully by adding of 0.1N NaOH and 0.1N HCl in the PDA medium. Each trial was conducted in triplicates. 100 ml medium was poured in the conical flask of 150 ml capacity and pH was adjusted as mentioned above using pipette. The flasks containing PDA were then sterilized in an autoclave for 20 minutes at 121°C and 15 psi. The medium was poured in sterilized Petri plates of 90 mm @ 10 ml. The 7 days old culture disc of 5mm was taken and then was inoculated into the Petri plates. Plates were kept in an inverted position in BOD incubator at $27 \pm 2^\circ\text{C}$. When plate got full, the spore count was done by making suspension of pathogen by cutting 5mm disc from each treatment with sterilized cork borer and suspending it in the test tube then shaking it properly. Temporary mount was prepared and spores were count from nine different microscopic fields. Micrometry was done to ensure proper details regarding length and width of the pathogen in the treatments which showed spore count microscope (Leica company) and software (Leica Application Suite) at 100X.

The data have been evaluated using OPSTAT online software in completely randomized design (CRD).

Results

Effect of temperature levels on growth and sporulation of *Alternaria tenuissima*

Effect on temperature levels on radial growth of mycelium, sporulation and conidial character of *A. tenuissima* were studied and results are presented in Table 1, Fig. 1 and Image 3. Significant variations in radial growth of fungus were observed at 48 hours to 192 hours after incubation. Radial growth recorded 48.3 mm to 74.4 mm in different temperature levels. Maximum mycelial growth was recorded at temperature of 25°C (74.4 mm) followed by 67.7 mm at 20°C , 58.6 mm at 30°C and 55.4 mm at 35°C . Minimum growth of the fungus was noted at 15°C temperature (48.3 mm). Excellent sporulation of *A. tenuissima* was recorded at 25°C , where it was fair at 20°C . In rest of the temperature levels, sporulation was not observed.

Average conidial length of $27.10 \mu\text{m}$ and $30.87 \mu\text{m}$

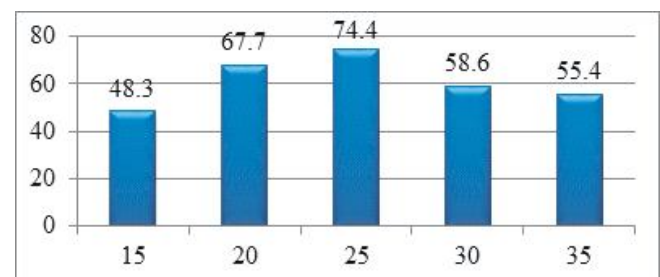


Fig. 1 : Effect of different temperature levels on mycelial growth of *Alternaria tenuissima*.

Table 1 : Effect of different temperature on mycelial growth of *Alternaria tenuissima*.

S. no.	Temperature	Radial growth (mm) after (hrs)							Sporulation Index
		48	72	96	120	144	168	192	
1	15	9.2	15.0	20.3	24.9	32.4	39.3	48.3	-
2	20	12.7	20.7	30.3	40.7	51.2	57.0	67.7	++
3	25	22.0	30.6	40.2	46.3	53.4	62.3	74.4	++++
4	30	10.4	16.3	24.6	38.5	45.6	52.7	58.6	-
5	35	10.1	17.0	24.6	30.5	37.7	46.9	55.4	-
	SEm±	0.42	0.79	0.94	0.84	0.90	0.93	0.86	
	CD(5%)	1.28	2.39	2.86	2.56	2.73	2.83	2.61	

[Scale of sporulation – (++++)= Excellent (>60 conidia per microscopic view); (+++) = Good (41-60 conidia per microscopic view); (++) = Fair (21-40 conidia per microscopic view); (+) = Poor (Less than 20 conidia per microscopic view); (-) = No sporulation.]

Table 2 : Conidial characteristics of *Alternaria tenuissima* as influenced by different temperatures.

S. no.	Temperature	Length of conidia		Width of conidia		Range of longitudinal septa	Range of transverse septa
		Range	Mean	Range	Mean		
1	15	-	-	-	-	-	-
2	20	24.38-31.87	27.10	9.88-12.38	11.33	1-3	-
3	25	20.51-43.16	30.87	8.65-15.52	12.53	2-4	0-2
4	30	-	-	-	-	-	-
5	35	-	-	-	-	-	-

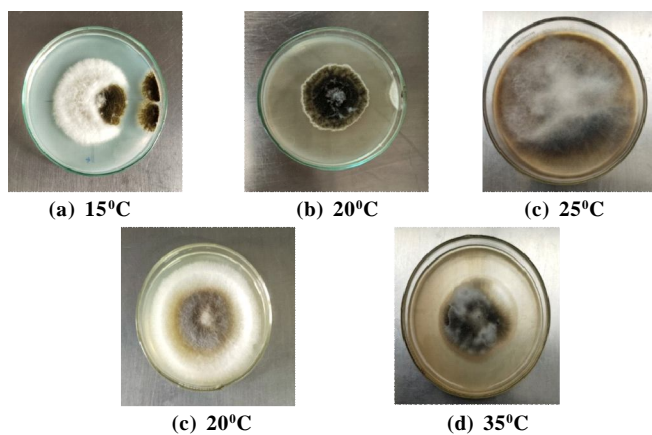


Image 3 : Effect of different temperatures on mycelial growth of *Alternaria tenuissima*.

was recorded at 20°C and 25°C temperature levels, respectively. Whereas average conidial width was 11.33 µm at 20°C and 12.53 µm at 25°C. Number of longitudinal septa was 2 to 4 and transverse septa were 0 to 2 at 25°C temperature level. Only longitudinal septa (1 to 3) were observed at 20°C (Table 2 and Image 4).

Effect of pH on growth, sporulation and conidial character of *A. tenuissima*

Effect of hydrogen ion concentration (pH) of the medium on fungal growth and sporulation of *A. tenuissima* was studied in seven pH, i.e., 4.5, 5.0, 5.5,

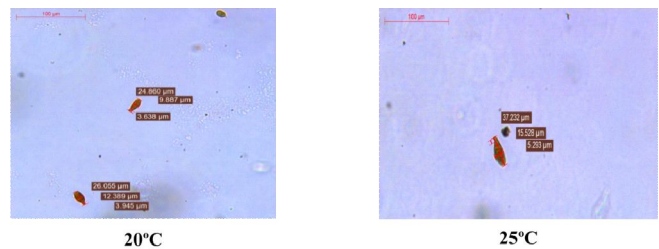


Image 4 : Conidial Characterization of *A. tenuissima* as influenced by different temperatures (10 x10 X).

6.0, 6.5, 7.0 and 7.5. Results are presented in table 3 and depicted in Fig. 2 and image 5. Radial mycelial growth varied from 73.3 mm to 89.6 mm was recorded at different pH after 264 hrs often incubation maximum mycelial growth was recorded at pH 5.5 (89.6 mm), which was closely followed by pH 6.0 (87.4 mm). Next best radial growth was noted at pH 6.5 (85.3 mm) followed by pH 7.0 (81.5 mm) which was significantly at par at pH 5.0 (80.5 mm) and 7.5 (80.2 mm). Least growth was recorded at pH 4.5 (73.3 mm). Poor to excellent sporulation was observed at different pH levels. Excellent sporulation was recorded at 5.5, whereas good sporulation was found at pH 6.5. Sporulation was fair at pH 5.0, 6.0, 7.0 and 7.5. Poor sporulation was observed at pH 4.5.

Conidial characteristics as influenced by pH levels are presented in Table 4 and Image 6, which reveals the variation in length, width and septation of conidia at

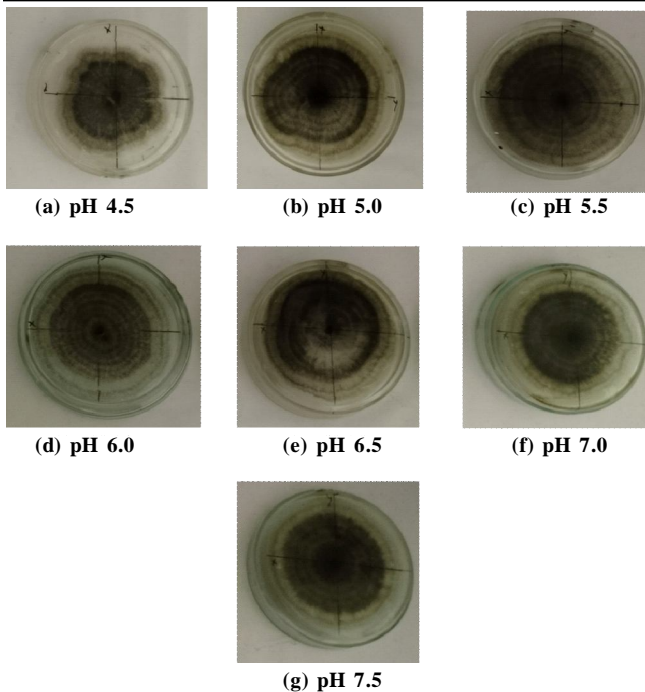
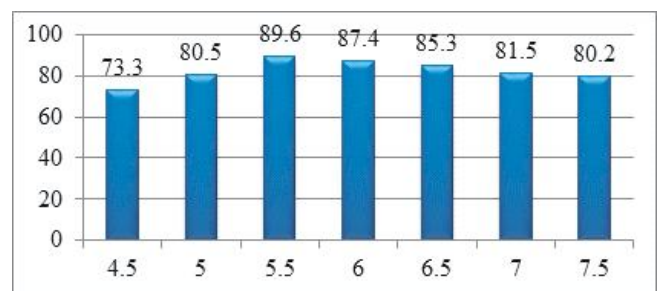
Table 3 : Effect of different pH on mycelial growth of *Alternaria tenuissima*.

S. no.	pH	Radial growth (mm) after (hrs)										Sporulation Index
		48	72	96	120	144	168	192	216	240	264	
1	4.5	15.1	20.7	26.4	32.3	38.9	43.4	53.3	57.7	65.3	73.3	+
2	5.0	11.4	21.2	30.3	38.3	46.3	54.5	62.4	68.7	74.3	80.5	++
3	5.5	16.1	28.9	38.0	46.0	54.2	62.8	71.8	81.0	87.5	89.6	++++
4	6.0	14.0	25.1	35.6	41.5	50.2	58.3	67.7	77.7	83.1	87.4	++
5	6.5	14.3	24.0	34.6	40.3	49.4	57.4	66.1	74.7	79.0	85.3	+++
6	7.0	14.8	23.1	33.3	39.2	46.6	53.6	60.8	69.3	75.5	81.5	++
7	7.5	13.6	22.8	29.9	37.1	45.1	52.9	59.5	68.6	74.9	80.2	++
	SEm±	0.65	1.02	0.70	0.81	0.88	0.86	0.91	0.98	1.11	0.68	
	CD (5%)	1.98	3.13	2.14	2.50	2.69	2.64	2.79	2.99	3.39	2.06	

[Scale of sporulation – (++++) = Excellent (>60 conidia per microscopic view); (+++) = Good (41-60 conidia per microscopic view); (++) = Fair (21-40 conidia per microscopic view); (+) = Poor (Less than 20 conidia per microscopic view); (-) = No sporulation.]

Table 4 : Conidial Characteristics of *Alternaria tenuissima* as influenced by different pH.

S. no.	pH	Length of conidia		Width of conidia		Range of longitudinal septa	Range of transverse septa
		Range	Mean	Range	Mean		
1	4.5	25.75-45.72	31.29	7.79-10.19	8.74	0-3	0-2
2	5.0	20.17-43.12	29.42	6.79-11.54	9.25	0-3	0-2
3	5.5	13.88-30.32	23.49	7.06-10.67	8.79	2-4	0-1
4	6.0	19.27-47.12	29.81	7.85-12.56	9.02	2-4	0-2
5	6.5	21.02-31.68	24.66	7.63-11.46	9.35	1-3	-
6	7.0	21.42-49.32	33.86	7.01-11.14	8.82	1-5	0-1
7	7.5	24.78-46.04	32.18	8.23-11.39	10.15	3-5	0-2

**Image 5 :** Effect of different pH on mycelial growth of *Alternaria tenuissima*.**Fig. 2 :** Effect of different pH ranges on mycelial growth of *Alternaria tenuissima*.

different pH. Average conidial length varied from 23.49 μm to 33.86 μm was maximum at pH 7.0, whereas minimum conidial length was noticed at pH 5.5. Conidial width varied from 8.74 μm to 10.15 μm was maximum at pH 7.5 and minimum at pH 4.5. Variation in number of longitudinal septa (3 to 5) were observed at pH 7.5, whereas minimum number of longitudinal septa were found at pH 4.5 and 5.0 (0 to 3). In rest of the pH levels, longitudinal septa vary from 0-3 to 2-4. Number of transverse septa was more or less similar 0 to 2 at different pH level concentrations examined.

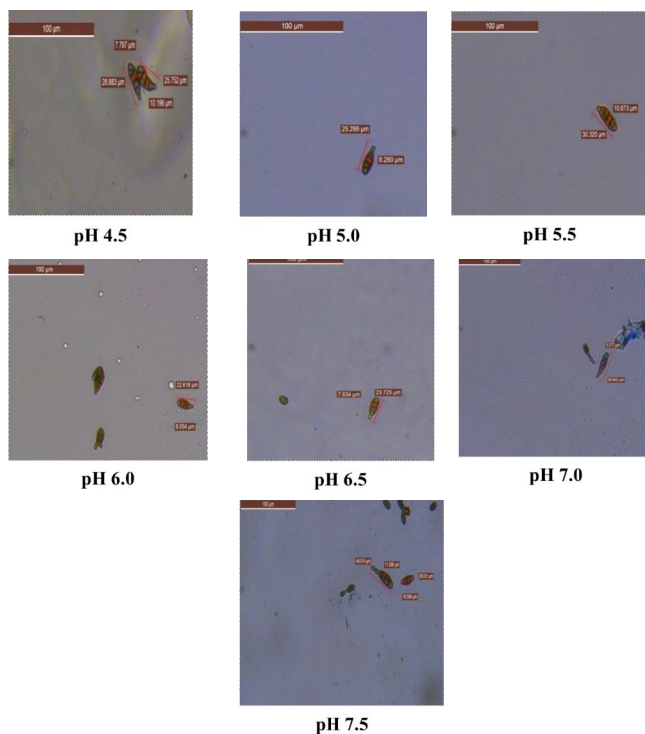


Image 6 : Conidial characterization of *A. tenuissima* as influenced by different pH ranges (10 x10 X).

Discussion

During the investigation, five ranges of temperature were used *viz.*, 15°C, 20°C, 25°C, 30°C and 35°C. Among these five temperatures ranges 25°C (74.4 mm) after 192 hours of incubation gained the maximum growth and sporulation. Hasija (1970) also suggested that 15-35°C range was good for the growth of the pathogen. The findings coincided in line with that of Verma (1970), Kumar and Arya (1978), Singh *et al.* (2001a), Singh *et al.* (2001 b), Kantwa *et al.* (2015) and Hariprasad *et al.* (2018). Maheshwari *et al.* (2000) suggested that best growth of *A. alternata* was observed at 28°C, which is close to 25°C. Hubballi *et al.* (2010) reported the same findings where 25°C was proved to be the best optimum temperature after 30°C. Sporulation was excellent in temperature of 25°C, whereas it was fair in 20°C. Verma (1970), Maheshwari *et al.* (2000), Singh *et al.* (2001 a), Singh *et al.* (2001 b), TianShu *et al.* (2009), Kantwa *et al.* (2015) and Hariprasad *et al.* (2018) found the same that 25°C was the best and optimum temperature for the sporulation of different *Alternaria* spp.

Among 7 different pH range *viz.*, pH 4.5, pH 5.0, pH 5.5, pH 6.0, pH 6.5, pH 7.0 and pH 7.5. In our findings, best growth was seen in pH 5.5 (89.6 mm) and least was observed in pH 4.5 (73.3 mm). The findings of Singh *et al.* (2001 a) suggests that *A. alternata* grew best at pH 5.5. Hasija (1970) proved and showed that the best growth

of *A. tenuis* (*A. alternata*) was between the pH range of 5.4 - 7.4. Regarding sporulation, the best and excellent sporulation was seen in pH 5.5 and it was good in pH 6.5. Findings of Verma (1970) explains that *A. alternata* sporulated best in pH 6.6 which was closely related to our finding for good sporulation in pH 6.5. Maheshwari *et al.* (2000) recorded highest sporulation at pH range of 5.5 – 6.5.

Conclusion

In conclusion, the optimum temperature recorded for the fast and high mycelial growth and excellent sporulation of *Alternaria tenuissima* was 25°C temperature with pH 5.5 could be responsible for the devastating disease studied here in the experiments, *i.e.*, leaf blight caused by *Alternaria tenuissima*.

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Conflict of interest

Authors have no conflict of interest to disclose.

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