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EFFECT OF TEMPERATURE & RELATIVE HUMIDITY ON SPORULATION AND SPORE GERMINATION ON *ALTERNARIA SOLANI* INCIDENCE OF EARLY BLIGHT OF TOMATO

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ABSTRACT

Tomato output is severely hampered by Alternaria leaf blight, which is brought on by *Alternaria solani*. At JNKVV Jabalpur, an experiment was conducted in a laboratory experiment (2019) to determine the impact of temperature and relative humidity on the sporulation of *Alternaria solani*, it was discovered that, after 72 hours of inoculation, a temperature of 25°C and a relative humidity of 90% were ideal for sporulation. Additionally examined were the effects of temperature and relative humidity on sporulation of *Alternaria solani* on a host in an *in vitro* condition. *Alternaria solani* sporulation was detected on the leaves that were kept in a moist environment. The maximum sporulation on leaves was seen when the temperature reached 25°C and the relative humidity reached 90%. Additionally, a lab experiment was run to determine the impact of temperature and relative humidity on spore germination. The maximum spore germination occurred after 72 hours of incubation at a temperature of 25°C and a relative humidity of 90%. It was followed by 30°C temperature and 100 per cent relative humidity.

***Key words*:** Tomato, Alternaria blight, sporulation, Spore germination.

Introduction

Tomato (*Lycopersicon esculentum* Mill) ranks as one of the most crucial vegetable crops globally, second only to potatoes in significance. Widely cultivated worldwide, it serves as a primary source of essential vitamins A, B and C. India holds the second position in both tomato cultivation area and production volume. In India, tomatoes are cultivated across approximately 8.85 lakh hectares, yielding a production of 19,696.0 metric tons with a productivity rate of 24.4 metric tons per hectare during the 2016-17 period. The prominent tomato-growing states in India include Orissa, Bihar, Karnataka, Andhra Pradesh, Maharashtra, Madhya Pradesh and Tamil Nadu. In Madhya Pradesh specifically, tomato cultivation covers an area of 100.00 thousand hectares, producing 3,102.00 metric tons with a productivity rate of 31.02 metric tons per hectare (Anon, 2017).

Tomato plants are susceptible to various diseases from seedling emergence to harvest. Among these, early blight caused by *Alternaria solani* stands out as the most critical and limiting factor in tomato production (Datar and Mayee, 1981). This disease significantly impacts yield, causing considerable losses (Munde *et al*., 2013). The incidence of early blight varies with location, reaching up to 70 percent in some instances (Munde *et al.*, 2013 and Pachori and Sharma, 2016). The influence of weather conditions on disease development has been observed by researchers worldwide at different times (Sangeetha and Siddaramaih, 2007; Devi and Chanu, 2012). As morphological characters and phylogenetic analysis, *Alternaria solani* bear large, long- beaked and non-catenated spores (Simmons, 2000). The mycelium

consists of septa, branched, light brown hyphae which turned darker with age. The conidiophores are short, 50-90 µm long and dark in colour. Conidia are 120-296 ×12-20 µm in size, beaked, muriform, dark colour and born singly. Conidia contained 5-10 transverse 3 septa and 1-5 longitudinal septa (Singh, 1987). In view of the above facts the present study was undertaken and results are embodied here in.

Material and Methods

Effect of temperature and relative humidity on sporulation of *Alternaria solani* on Culture and Host

To find the ideal temperature and relative humidity for sporulation of *Alternaria solani* on the culture and host, an experiment was carried out *in vitro*.

Twenty ml of media were dispensed into each sterilized Petri dish. Using a sterilized cork borer, five-millimeter discs of pure *Alternaria solani* culture was cultivated on PDA for seven days were excised from the culture's edge. Each Petri dish was then inoculated with one culture disc at the center and inverted. To investigate sporulation, these inoculated Petri plates were subjected to various temperatures and relative humidity levels ranging from 15, 20, 25, 30 and 35°C and 60, 70, 80, 90 and 100% over different incubation periods. Conidia enumeration was conducted using a hemocytometer.

The leaves were properly cleansed in sterile water after being carefully collected from a field with specific symptoms. The Petri plates with moist blotter, glass slides and glass rods that came with them were used to create a moist chamber where the leaves were stored. The leaves were incubated at various relative humidity levels and temperatures including 60, 70, 80, 90 and 100 percent and 15, 20, 25, 30 and 35^{0}C, respectively. Following the procedure outlined by Buxton and Mellanby (1934), these relative humidity levels were maintained using concentrated H_2So_4 and distilled water in Table 1. To record sporulation, one ml of distilled water was used to wash the leaves. Using a hemocytometer, sporulation was detected at various intervals. For each treatment, three replications were kept. Petri plates were checked for sporulation at 24, 48 and 72 hours. The measurement of sporulation was done with the aid of a hemocytometer.

Table 1: Quantity of H_2So_4 and distilled water for different humidity level.

Humidity level (%)	H_2So_4(ml)	Distilled Water (ml)
60	37.5	62.5
70	32.5	67.5
80	26	74
90	10	90
100	0	100

Table 2: Effect of temperature on sporulation of *Alternaria solani* on culture and host (*In vitro*).

Temp. (°C)	Incubation hours					
	Culture (Spores per cm^2)			Host (Spores per cm^2)		
	24	48	72	24	48	72
15	96.96	106.7	138.88	295.92	304.48	326.7
20	141.18	154.29	185.29	325.92	352.77	362.03
25	190.55	218.89	256.55	365.73	367.59	412.03
30	163.18	180.7	232.59	326.84	349.07	370.37
35	147.59	162.33	197.03	306.47	310.44	319.44
Factors	SEm±	CD at 5%		SEm±	CD at 5%	
Temp. (A)	8.32	24.14		12.23	N/A	
Hrs. (B)	6.44	N/A		9.48	N/A	
Interaction (A×B)	14.41	41.82		21.19	61.52	

*Average of Three replications.

Effect of temperature and relative humidity on spore germination of *Alternaria solani* on culture

The goal of the experiment was to determine the ideal temperature and relative humidity for spore germination of *Alternaria solani*. For this, the cavity slide approach was used. Conidial suspension was created in culture tubes using sterile water. Each slide had two chambers, each of which received one ml of conidial suspension. For each treatment, three replications were retained. In a wet chamber created with Petri plates filled with a moist blotter and glass rods, cavity slides were stored. At 24, 48 and 72 hours, spore germination was observed.

In order to determine the impact of temperature and relative humidity on spore germination, cavity slides containing spore suspension were kept in Petri dishes at various temperatures and five relative humidity levels, including 15, 20, 25, 30 and 35^{0}C and 60, 70, 80, 90 and 100% at various time intervals. These humidity levels were maintained by utilizing distilled water and H_2So_4 in various concentrations.

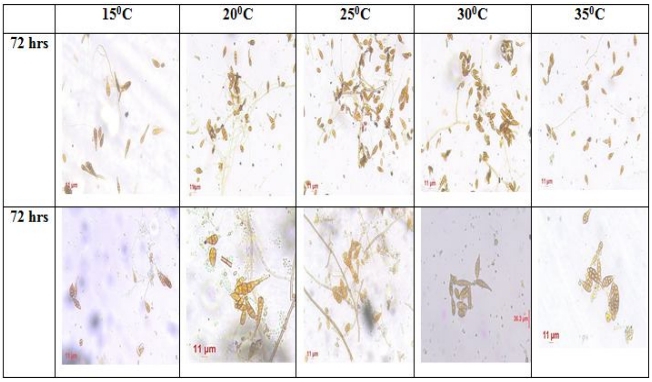

Fig. 1: Effect on temperature on sporulation of *Alternaria solani* on culture and host after 72 hrs of incubation.

Results and Discussion

Effect of temperature on sporulation of *Alternaria solani* on culture and host

The results shown in Table 2 and Fig. 1 in culture showed that temperature and incubation time had a substantial impact on *Alternaria solani* sporulation. After 72 hours of incubation, the highest level of sporulation was noted. Temperatures of 25, 30, 35, 20 and 15°C each had 232.59, 197.03, 185.29 and 138.88 spores per cm^2 as their maximum sporulation, respectively. Temperature and incubation time interacted significantly to affect sporulation. The maximum sporulation was recorded at 25°C temperature. Sharma and Ahir (2018), Somappa *et al.*, (2013) and Aruna kumara (2006) have reported the similar observation that the sporulation of *Alternaria solani* at 25°C temperature followed by 30°C and 35°C temperature sporulation was recorded at 10°C temperature.The variations in sporulation of *Alternaria solani* in present studies, support the views of earlier workers (Ansari *et al.*, 1989) who have reported that growth and sporulation was influenced by temperature.

In the host, the data shown in Table 2 and Fig. 1 showed that after 72 hours of incubation, the greatest sporulation of 412.03 per cm^2 was seen at a temperature of 25°C followed by 370.37 and 362.03 at 30 and 20°C respectively. At a temperature of 35°C *Alternaria solani* showed the least amount of sporulation. Sporulation was significantly impacted by the incubation time. The 25 and 30°C temperature also support the sporulation of *Alternaria solani*. The present results are coincided with the findings of Khare (1979) and also same result was found by Kaul and Saxena (1988).

Effect of relative humidity on sporulation of *Alternaria solani* on culture and host

The data in Table 3 and Fig. 2 showed that, in culture, sporulation varied with varying humidity and incubation

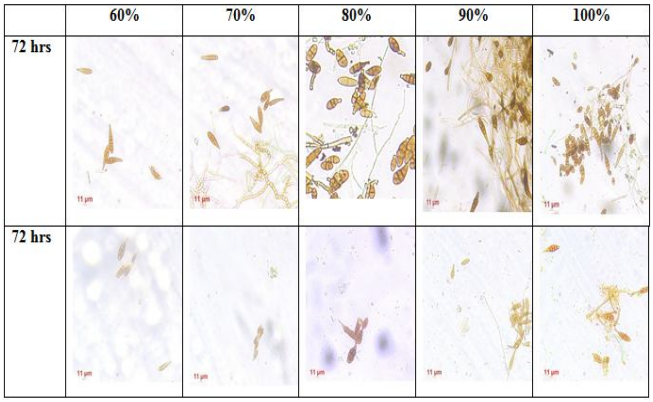

Fig. 2: Effect on relative humidity on sporulation of *Alternaria solani* on culture and host after 72 hrs of incubation.

Table 3: Effect of relative humidity on sporulation of *Alternaria solani* on culture and host (*In vitro*).

Relative Humidity (%)	Incubation hours					
	Culture (Spores per cm^2)			Host (Spores per cm^2)		
	24	48	72	24	48	72
15	141.18	166.85	210.63	298.96	306.7	315.77
20	163.55	201.18	244.66	321.84	331.59	340.85
25	198.51	229.51	300.74	330.81	340.29	363.4
30	243.7	292.92	379.63	373.59	398.26	426.99
35	208.7	254.73	332.48	336.10	371.03	400.44

Factors	SEm±	CD at 5%	SEm±	CD at 5%
Temp. (A)	8.903	25.839	10.435	30.283
Hrs. (B)	6.897	N/A	8.083	N/A
Interaction (A×B)	15.421	44.755	18.073	52.452

*Average of Three replications.

times. Both humidity and the length of the incubation time had a big impact on sporulation. At 90% relative humidity, the highest sporulation of 379.63 per cm^2 was reported. This was followed by 332.48, 300.74, 244.66 and 210.63 per cm^2 at 100%, 80%, 70% and 60% relative humidity respectively. The incubation period also had a big impact on sporulation. 72 hours of incubation with 90% relative humidity resulted in the highest sporulation, which was measured at 379.63 per cm^2. The maximum sporulation was recorded at 90 per cent relative humidity. Sharma and Ahir (2018), Somappa *et al.*, (2013) and Aruna kumara (2006) have reported the similar observation that the maximum sporulation of *Alternaria solani* 90 per cent relative humidity followed by 100 per cent relative humidity and the minimum sporulation was recorded at 10°C temperature.

The data in Table 3 and Fig.2 make it clear that the influence of relative humidity and incubation period on sporulation in the host was determined to be statistically significant. Maximum sporulation occurred at a relative humidity of 90% reaching 426.99 spores per cm^2 with

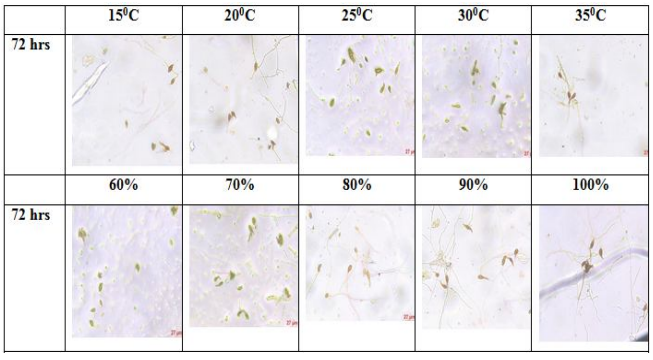

Fig. 3: Effect on temperature and relative humidity on spore germination of *Alternaria solani* after 72 hrs of incubation.

Table 4: Effect of temperature on spore germination of *Alternaria solani*.

Temp. (⁰C)	Incubation hours					
	24	%	48	24	%	48
15	9.667	34.52	11.333	40.46	13.333	47.60
20	13.000	46.42	16.667	59.53	18.000	64.28
25	17.333	61.89	22.667	80.92	26.667	95.21
30	14.333	51.17	18.667	66.64	19.333	69.03
35	13.000	46.42	15.333	54.75	17.667	63.07
Factors	**SEm ±**			**CD at 5%**		
Temp. (A)	0.725			2.105		
Hrs. (B)	0.562			1.556		
Interaction (A×B)	1.256			3.645		
*Average of Three replications.						

Table 5: Effect of relative humidity on spore germination of *Alternaria solani*.

Relative Humidity(%)	Incubation hours					
	24	%	48	24	%	48
60	12.000	42.85	13.333	47.60	15.667	55.92
70	12.667	45.21	14.667	52.35	17.333	61.89
80	14.667	52.35	17.667	63.07	21.000	75.00
90	18.667	66.64	22.333	79.75	27.667	98.81
100	17.000	60.71	20.333	72.60	23.667	84.52
Factors	**SEm ±**			**CD at 5%**		
Temp. (A)	0.650			1.886		
Hrs. (B)	0.503			1.395		
Interaction (A×B)	1.125			3.266		
*Average of Three replications.						

100, 80, 70 and 60 percent having 400.44, 363.4, 340.85 and 315.77 spores per cm^2 respectively. Statistically significant differences between incubation periods and sporulation were discovered. The relative humidity played vital role with references to sporulation. The relative humidity ranged from 60 to 100 per cent respectively. Maximum sporulation observed at 90 per cent relative humidity. The 90 and 100 per cent relative humidity also support the sporulation of *Alternaria solani*. The present results are coincided with the findings of Khare (1979).

Effect of temperature and relative humidity on spore germination of *Alternaria solani*

The information in Table 4 and Fig. 3 showed that temperature and incubation time had an impact on the spore germination. Temperature and incubation time had a substantial impact on the percentage of spore germination. After 72 hours of incubation, the temperature at which the maximum spore germination (95.21%) was observed was 25°C followed by temperatures of 69.03, 64.28, 63.07 and 47.60⁰C and temperatures of 20, 30 and 35⁰C respectively. Comparisons between various incubation periods were discovered to be statistically significant. The data shown in Table 5 and Fig. 3 showed that after 72 hours of incubation, the maximum spore germination (98.81%) was obtained at 90% relative humidity, followed by 84.52, 75.00, 61.89 and 55.92% at 100%, 80.7% and 60% relative humidity, respectively. Incubation time also had a substantial impact on the percentage of spores that germinated, and variations between them were also found to be statistically significant.

Five temperature (15, 20, 25, 30 and 35⁰C) and relative humidity (60, 70, 80, 90 and 100%) were used to observe their effect on spore germination of *A. solani*. Spore germination of *A. solani* varied with different temperature and relative humidity levels. Maximum spore germination was recorded in 25⁰C temperature and 90 per cent relative humidity against minimum spore germination at 15⁰C temperature and 60 per cent relative humidity. However, temperature 30⁰C and 100 per cent relative humidity had significant effect on spore germination of *A. solani*. Similar results were also reported by Stevenson and Pennypacker (1988).

Conclusion

It was concluded the conditions that favored the highest sporulation of *Alternaria solani* on culture were observed at 25°C with a relative humidity of 90% followed by 30°C with 100% humidity. Additionally, peak sporulation on the host leaf was observed at 25°C with a relative humidity of 90%. Conversely, the lowest sporulation was recorded at 15°C with a relative humidity of 60%. Optimal spore germination occurred at 25°C with a relative humidity of 90%, with secondary favorable conditions noted at temperatures of 30°C, 20°C, 35°C and 15°C along with relative humidity levels of 100%, 80%, 70% and 60%, respectively.

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Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest exists.

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