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Influence of Temperature and Relative Humidity on Spore Germination and Early Blight Disease Development of Tomato Caused by *Alternaria solani*

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Abstract

Early blight of tomato caused by *Alternaria solani* is one of the most important and devastating disease of tomato which causing extensive yield losses to the crop. Environmental factors play an important role in the development of disease. To investigate the congenial conditions to develop the disease and germination of fungal spores an experiment was conducted in the year 2019 and 2020 at vegetable pathology laboratory of Dr Yashwant Singh Parmar University of Horticulture and Forestry Nauni Solan, Himachal Pradesh, India. Under investigations effect of temperature and relative humidity (RH) on conidial germination and early blight diseases development of tomato caused by *Alternaria solani* was studied under *in vitro* and *in vivo* conditions. Under *in vitro* conditions, maximum germination of conidia (75.54%) was recorded at 30°C after 24 hours and minimum (23.24%) was recorded at 15°C. length of germ tube (129.55 µm) was maximum at 25°C, whereas, minimum (67.09 µm) was observed at 15°C. In case of relative humidity maximum conidial germination (95.18%) and germ tube length of 238.95 µm was observed at 100% level. Under *in vivo* conditions disease severity was maximum (81.48%) at 30°C followed by 25°C (78.21%), whereas, minimum (18.88%) was recorded at 40°C. Similarly, the maximum (88.09%) disease severity was observed at 100% RH level while, it was minimum at 38% level of humidity. Overall, the relative humidity of more than 90% and temperature range of 25–30°C was found most congenial for the development of disease.

Keywords: *Alternaria*, Early blight, relative humidity, temperature

1. Introduction

Tomato *(Solanum lycopersicum* L.), (2n=24) belongs to the family solanaceae is one of the most popular vegetables and a most important horticultural crop grown all over the world. Tomato is commonly consumed in our daily life and it is a good source of antioxidants (Sgherri et al., 2008). With high nutritional value, it provides a balance source of Vitamin A, C and E needed to maintain good human health (Olaniyi et al., 2010). Although, many factors contributed in destruction of successful cultivation as well as marketing of quality tomato, of which diseases play an important role. More than two hundred diseases have been reported to infect tomato in the worldwide which destroy the quality and reduce the yield of crop (Atherton and Rudich, 1986). The reduction in potential yield of tomato is mainly due to susceptibility of crop to various insect-pests and diseases. Among the fungal diseases, alternaria leaf blight of tomato is the worst damaging one (Abdel-Sayed, 2006; Abada et al., 2008). This disease is caused by various species of *Alternaria* but *Alternaria solani* is dominant and economically important throughout the world and cause heavy losses (Tymon et al., 2016; Foolad et al., 2002). In a global study of soilborne fungi, *Alternaria* was the most abundant plant pathogen (Delgado-Baquerizo et al., 2020). The disease caused by it an economically most important disease of tomatoes in the India, USA, Australia, The UK and Israel where significant yield reduction has been reported. In United States, 20 to 30% (Christ and Maczuga, 1989), in Israel 5 to 40% (Olanya et al., 2009) and in India 35 to 78% yield losses have been observed (Datar and Mayee, 1972, Basu, 1974, Jones et al., 1993, Mathur and Shekhawat, 1986). The pathogen causing this disease is a word wide spread pathogen and various investigations carried out all over the world, find that the investigated *Alternaria* genotypes are spread across the world (United States: Adhikari et al., 2021; Ding et al., 2019; Morris et al., 2000; Petrunak and Christ, 1992; Weir et al., 1998; South Africa: Van der Waals et al., 2004; India: Prakash et al., 2022; Upadhyay et al., 2019; Varma et al., 2007; Brazil: Lourenco et al., 2011; China: Meng et al., 2015; Russia: Kokaeva et al., 2022). Symptoms of this disease include small to irregular brown spots that give bull eye appearance on older plant leaves. These spots enlarge in diameter lead to concentric rings surrounded by yellow halo which resulted in destruction of foliage and the fruits are damaged directly by the pathogen (Ding et al., 2019; Zhao et al., 2023; Rotem, 1994; Jones and Perez, 2023). As the disease progress the plant become weakens which increases its susceptibility to infection by reducing the photosynthetic leaf area and increasing the imbalance between nutrient demand in the fruits and nutrient supply from the leaves (Rowell, 1953). Seedling, stem, blossom blight and fruit drop symptoms are also produced by this pathogen (Agrios, 2005). Moist conditions and temperature are key environmental factors which regulate the germination and growth of this fungus (Cooke and Whipps, 1993). In general, species of *Alternaria* causing many diseases of crop plants are highly resistant to unfavourable weather conditions and can develop under a wide range of temperature and relative humidity levels (Rotem, 1994). Therefore, to investigate the requirement of these factors detailed studies were undertaken to determine the effects of relative humidity and temperature on conidial germination, germ tube length and early blight disease development of tomato caused by *Alternaria solani.*

2. Materials and Methods

The experiment was conducted at vegetable pathology laboratory, Dr. Yashwant Singh Parmar University of Horticulture and Forestry Solan (Nauni), Himachal Pradesh 30.8645° N, 77.1695° E India, by slide germination technique in Completely Randomized Design (CRD) during the year 2019 for two months. Seven different temperatures viz. 10, 15, 20, 25, 30, 35 and 40°C were analyzed. One drop of the conidial suspension prepared from freshly sporulating colonies, containing approximately $5\times l0^4$ conidia ml^{-1} was put on the cavity slides. Two such cavity slides were then kept on glass rod triangles placed in the 9 cm diameter petriplates lined with the wet sterilized blotting papers to provide relative humidity of 95% or more. The petriplates were incubated at different temperatures viz. 10, 15, 20, 25, 30, 35 and 40°C in BOD incubators. Similarly, to study the effect of different relative humidity levels on conidial germination, seven different levels of relative humidity viz., 100, 89.9, 80.5, 70.4, 60.7, 49 and 38% were maintained as per the method of Stevens (1916). To maintain the relative humidity levels of 100, 89.9, 80.5, 70.4, 60.7, 49 and 38%, solutions of 0, 19.61, 27.32, 33.43, 38.03, 44.00 and 50% sulphuric acid, respectively were prepared. Equal volume of required solutions was poured in Petri plates (90 mm) which were used as humidity chambers. One drop of the conidial suspension prepared from freshly sporulating colonies, containing approximately $5\times l0^4$ conidia ml^{-1} was placed on sterilized, clean dry cavity slide. Two such slides were kept in each Petri plate. The sides of each Petri plate were sealed with parafilm and subsequently transferred to an incubator maintained at 28±2°C. All the treatments were replicated three times. After 24 hours of incubation, slides were microscopically examined to record conidial germination and germ tube length. About 100 conidia selected randomly from different microscopic fields were examined to calculate % germination and germ tube length. Per cent conidial germination was calculated by the formula given below.

Percent conidial germination=(No. of conidia germinated/Total no. of conidia observed)×100

Further, effect of different temperature regimes and relative humidity levels was studied under pot culture conditions in Completely Randomized Design (CRD). Seedlings of susceptible tomato cv. 'Heem Sohna' raised in 10 cm diameter size plastic pots. Plants were inoculated with conidial suspension of 3×10^4 spore's ml^{-1} through spray inoculation method. The inoculated plants were then maintained in polythene cover under high humidity to provide leaf wetness for 24 hrs and then transferred to growth chambers maintaining different temperature levels viz. 15, 20, 25, 30, 35 and 40°C.

Similarly, in order to determine the effect of different relative humidity levels on the severity of disease, 30–35 days old tomato seedlings of cv. 'Heem Sohna' were transplanted in disposable plastic cups. After seven days of transplanting when seedlings got well established, inoculation was done with suspension of conidia 3×10^4 spore's ml^{-1} by spray inoculation method. Subsequently after inoculation, plastic cups containing plants were transferred to desiccators kept at ambient temperature for 48 hrs. Different relative humidity (RH) levels viz., 100, 89.9, 80.5, 70.4, 60.7, 49 and 38% were maintained as per the method of Stevens (1916). Observations on severity of disease were recorded after 15 days of inoculation.

3. Results and Discussion

3.1. Effect of temperature on conidial germination and germ tube length

In present investigations we observed that conidia of *A. solani* could germinate at a wide range of temperature from 15–40°C. Germination percentage of condia showed significant variation among various temperature regimes tested and it was ranged from 14.11–64.44% and 23.24–75.54% after 18 and 24 hours, respectively (Table 1). Whereas, maximum germination (75.54%) was recorded at 30°C after 24 hours and minimum (23.24%) was recorded at 15°C (Figure 1). Germination of conidia increased initially with increase in temperature but declined after 30°C. Similarly, germ tube length of *A. solani* increased initially with increase in temperature. Maximum germ tube length (129.55 µm) was recorded at 25°C followed by 113.59 µm at 30°C, whereas, minimum 67.09 µm was observed at 15°C. Data indicated that with increase in temperature, length of germ tube increased up to 25°C and started to decline thereafter.

Table 1: Effect of different temperature regimes on conidial germination and germ tube length of *A. solani* causing early blight of tomato under *in vitro* conditions

Temperature (°C)	Conidial germination (%)		Germ tube length (µm)	
	18 hours	24 hours	18 hours	24 hours
15	14.11 (22.04)	23.24 (28.80)	43.46	67.09
20	31.97 (34.40)	42.54 (40.69)	66.98	93.45
25	60.55 (51.07)	67.36 (55.14)	104.82	129.55
30	64.44 (53.38)	75.54 (60.34)	94.52	113.59
35	51.94 (46.09)	71.70 (57.85)	80.20	95.81
40	22.04 (27.96)	27.56 (31.65)	39.27	53.63
SEm±	0.91	0.68	0.92	0.84
CD (*p*=0.05)	2.83	2.14	2.89	2.62

Figures in the parenthesis are arc sine transformed values

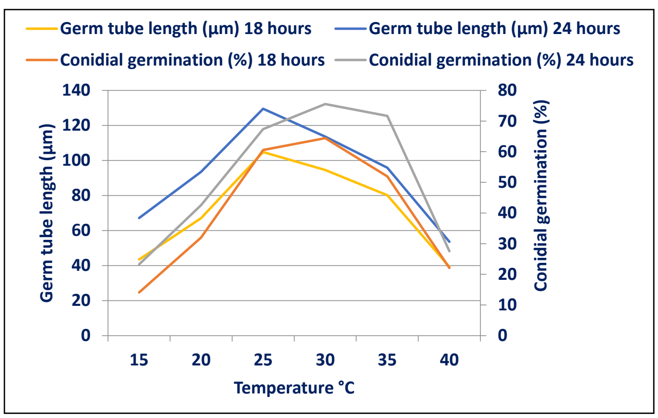

Figure 1: Effect of different temperature regimes on conidial germination of *A. solani* causing early blight of tomato

The observations of present investigations are in conformity with the findings of Degenhardt et al. (1982) who observed that conidial germination of *A. solani* can occur at wide range of temperature, however, germination occur most quickly when the temperature was between 21–28°C and as the temperature decreases, the time period it takes for germination increases. The results of conidial germ tube length at various temperatures are also in consonance with the findings of Vloutoglou (1996) who reported that germ tube of *Alternaria* was elongated maximum at 25°C and with dry interruptions at any time stopped the germination and germ tube of conidia.

In another study optimal conditions for germination of conidia are reported at 25°C with moistened host tissue and 100% humidity (Thomidis et al., 2023).

3.2. Effect of relative humidity on conidial germination and germ tube length

Perusal of the data presented in table (Table 2) revealed that high relative humidity favours the germination and elongation of germ tube of conidia. It is clearly evident from the data that germination of *A. solani* conidia can occurred at wide range of relative humidity levels from 38–100%. The maximum germination (95.18%) of conidia was observed at 100% level of humidity followed by 89.9% which resulted in 84.21% germination of conidia whereas minimum germination (6.8%) of conidia was observed at 38% level of humidity (Figure 2).

Table 2: Effect of different relative humidity levels on conidial germination and germ tube length of *A. solani* causing early blight of tomato under *in vitro* conditions

Relative humidity (%)	Conidial germination (%)	Germ tube length (µm)
100	95.18 (77.38)	238.95
89.9	84.21 (66.57)	177.89
80.5	71.05 (57.43)	81.60
70.4	59.13 (50.22)	43.85
60.7	48.14 (43.91)	29.67
49	33.13 (35.12)	14.61
38	6.8 (15.05)	4.24
SEm±	0.72	1.35
CD (*p*=0.05)	2.23	4.13

Figures in the parenthesis are arc sine transformed values

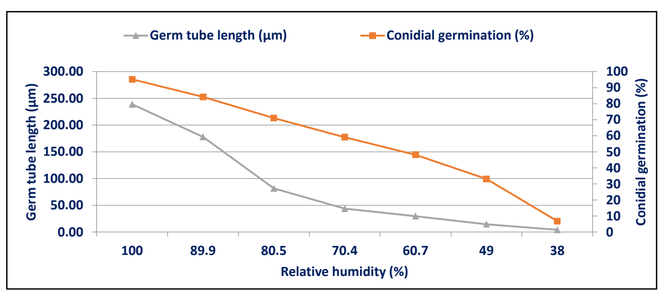

Figure 2: Effect of different relative humidity levels on conidial germination of *A. solani* causing early blight of tomato

Similar pattern was observed in case of germ tube elongation and maximum germ tube length (238.95 µm) was observed at 100% level of humidity followed by 89.9 (177.89 µm), 80.5 (81.60 µm) and 70.4% (43.25 µm), respectively. With decrease in relative humidity levels, conidial germination percentage and germ tube length (µm) of conidia decreased. Conidial germination and germ tube length decreased with decrease in relative humidity levels in descending order. The results of present investigations are in support with the findings of Degenhardt et al. (1982), Borkar and Patil (1995) and Green and Bailey (2000), who reported that *Alternaria* spp. required relative humidity above 95% for conidial germination and maximum conidial germination and germ tube length was recorded at 100% level of relative humidity.

3.3. Effect of different temperature regimes on disease development

Temperature plays a significant role in development of early blight disease of tomato. Experiments conducted in present investigations reveals maximum disease severity (81.48%) at 30°C followed by 25°C (78.21%) whereas, it was minimum (18.88%) at 40°C (Table 3). Slightly increase in disease severity level was observed initially with raise in temperature upto 30°C and later on it started to decline. Our findings are in support with the findings of Borkar and Patil (1995), who studied the effect of various parameters in relation to *Alternaria* leaf blight disease development and recorded temperature range of 25.9°C to 33.7°C congenial for disease development (Figure 3).

Table 3: Effect of different temperature regimes on early blight disease development of tomato

Temperature (°C)	Disease severity (%)
15	26.67 (31.07)
20	46.16 (42.77)
25	78.21 (62.16)
30	81.48 (64.50)
35	65.72 (54.14)
40	18.88 (19.48)
SEm±	0.73
CD (p=0.05)	2.29

Figures in the parentheses are arc sine transformed values

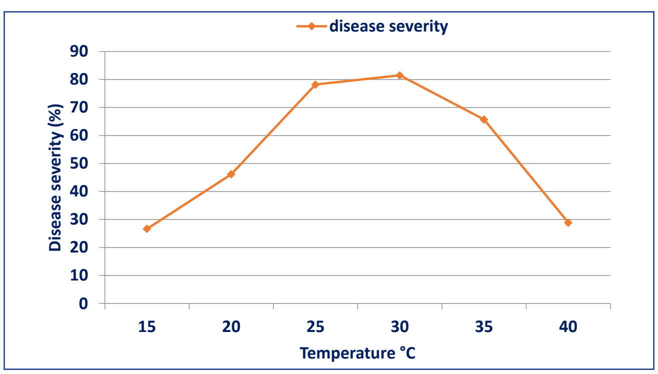

Figure 3: Effect of different temperature regimes on early blight disease development of tomato under pot culture conditions

3.4. Effect of different relative humidity levels on disease development

Relative humidity had significant influence on the disease severity when seedlings transplanted in disposable cups were exposed to various RH levels. Data (Table 4) revealed that disease severity varied according to different relative humidity levels and maximum (88.09%) disease severity was recorded at 100% RH level followed by 89.9 (82.53%) and 80.5% (75.39%) level of relative humidity.

Table 4: Effect of different relative humidity levels on early blight disease development of tomato under pot culture conditions

Relative humidity (%)	Disease severity (%)
100.0	88.09 (69.80)
89.9	82.53 (65.27)
80.5	75.39 (60.24)
70.4	64.28 (53.27)
60.7	43.65 (41.33)
49.0	24.6 (29.71)
38.0	8.73 (17.14)
SEm±	0.57
CD (*p*=0.05)	1.77

Figures in the parentheses are arc sine transformed values

Minimum disease severity (8.73%) was recorded at 38% level of humidity. Severity of the disease was increased with increase in RH levels and at maximum humidity level disease severity reached at maximum level (Figure 4). These observations are in conformity with the findings of Degenhardt et al. (1982), who observed that relative humidity level above 95% were congenial for conidial germination and development of early blight disease. Chaerani and Voorrips, 2006 also reported complete defoliation of tomato crop under heavy rainfall, high humidity and high temperature conditions. Pandey, 2011 observed that maximum relative humidity of 54–93% and minimum relative humidity 20–68% was congenial for development of early blight in tomato.

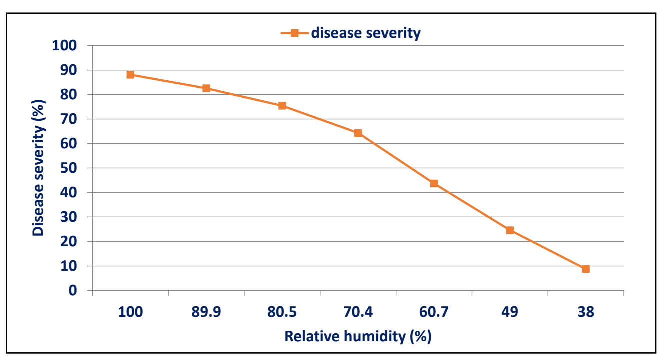

Figure 4: Effect of different relative humidity levels on early blight disease development of tomato under pot culture conditions

4. Conclusion

Environmental factors such as temperature and relative humidity play key role in development of any disease. Optimum temperature and relative humidity are essential prerequisite to develop early blight disease of tomato caused by *Alternaria solani*. For conidial germination temperature range of 25–30°C with 100% relative humidity is most favourable for germination of conidia. The development of disease on tomato host was highest at 30°C under maximum humid conditions.

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