

IMPACT OF DIFFERENT RANGE OF pH AND TEMPERATURE ON GROWTH AND SPORULATION OF *Alternaria solani* ISOLATED FROM INFECTED TOMATO PLANTS

ANUPAMA SINHA^{1a} AND MD. ANZER ALAM^b

^aResearch Scholar, Department of Botany, J.P. University, Chapra, Bihar, India

^bDepartment of Botany, Ganga Singh College, J.P. University, Chapra, Bihar, India

ABSTRACT

Tomato is considered to be the most widely used vegetables in the world in general and India in particular. However, the crop is damaged by different pathogens in which *Alternaria solani* is most important. From the infected fruits the fungus was isolated, and pure culture was maintained on Potato Dextrose Agar medium. From these cultures inoculum was taken and cultured in liquid culture medium adjusted with ten different pH (3.0-8.0) and incubated at ten different temperatures (10^o-44^oC) to observe the effect on growth and sporulation in *Alternaria solani*. Mycelial growth was measured on the solid medium as radial growth, while the dry mycelial weight was considered as the growth rate in the liquid medium. Maximum radial growth in the solid medium was noted in the culture adjusted at pH 7.0 which was 86.28 mm and at 7.5 it was 85.24 mm. Similarly at 28^oC the radial growth was 82.76 mm followed by 32^oC which was 81.18 mm. The dry mycelial weight at 7.0 pH was 1620 mg/ l of culture, while at 7.5 it was 1540 mg respectively. The dry mycelial weight at 28^oC was 1686 mg/l of culture, while it was 1570 mg at 32^oC. Neither the lowest pH nor the lowest temperature, similarly neither the highest pH nor the highest temperature could promote better mycelial growth. Sporulation was better in the conditions where the mycelial growth was noted better.

Keywords: Mycelial growth, Sporulation, Fungal pathogen, Culture conditions, Dry mycelial weight, Tomato.

Tomato, (*Lycopersicon esculentum*) of Solanaceae is being cultivated in the different part of India. The yield as well as the quality of the fruits is degraded by the most common fungal pathogen known as *Alternaria solani* that causes severe early blight disease of the crop. Pandey *et al*; (2003) have reported that in the experimental plots the loss is up to 80%. Ellis (1971) reported the structure of conidia in culture conditions. Rath and Padhi (1973) reported that the sporulation rate in culture conditions can be enhanced by exposing the three days old culture in direct sunlight, for 10 minutes. Similarly incubation of culture at 25^oC enhances the sporulation rate. Impact of different range of temperature and pH on growth and sporulation of *Alternaria solani* in solid and liquid medium have been reported by Stevenson and Penny Palker (1988); Rodriguez and Santa (1991); Prasad *et al*; (1973); Rath and Padhi (1973); Soddauskienet *et al*; (2003) etc. Impact of different culture media, pH and temperature on growth and sporulation of *Alternaria solani* has been observed by, Hubballiet *et al*; (2010); Albert *et al*; (2012); Tawareet *et al*; (2014); Kumar *et al*; (2015); Kumawatet *et al*; (2016) and Kumar *et al*; (2017). In the present research work experiments were done to observe the impact of different culture conditions (ecological) on growth and sporulation of *Alternaria solani*.

MATERIALS AND METHODS

Alternaria solani which was isolated from the infected tomato plant was maintained as pure culture on PDA. 10 days old above culture was used for inoculums. For the study of impact of pH on mycelial growth and sporulation of the above fungus the pH, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were adjusted by adding few drops of N/HCl or NaOH. Similarly, 10 temperatures 10, 12, 16, 20, 24, 28, 32, 36, 40 and 44^oC were maintained separately in BOD incubator. For radial growth under different pH and temperature PDA medium was used. While for mycelial dry mass the liquid culture was used.

5 mm culture disc from 10 days old cultures was inoculated in the above medium. While for pH the temperature was kept constant, for temperature the pH was maintained constant. Radial growth was measured on 10th day of incubation, for both the parameters. Similarly, for dry mycelial weight 10 days old culture was filtered through the pre-sterilized and weighted Whatman filter paper No.-1. The mycelial mat was washed with hot water to remove any traces of culture medium. First the fresh weight was taken and then the filter paper along with the mycelial mat was placed in BOD incubator at 60^oC and on alternate day the weight was taken. This was repeated till the constant weight was obtained. This was considered as the dry mycelial weights. All the

experiments were done in triplicate and in each experiment 15th cultures were used. The mean of the above have been presented in the tables 1 – 4.

RESULTS AND DISCUSSION

Effect of different pH and temperature on growth and sporulation of *Alternaria solani* have been studied. For the growth both solid and liquid medium were used. On the solid medium the growth rate was analysed through radial growth of the fungus while in the liquid medium the dry mycelial weight was considered as the parameters for the growth. Perusal of the table 1, clearly indicates that the maximum radial growth of the fungus was at pH, 7.0 which was 86.28 mm followed by the radial growth at pH 7.5 which was 85.24. Likewise the radial growth was the highest at 28^oC that was 82.76 as is indicated by the data presented in table 2. This was followed by the radial growth at 32^oC that was 81.18 from table 1 and 3, it may be further noted that radial growth of the fungus was poor at lowest pH and temperature. Again after a certain pH and temperature the growth was affected rather not promoted. Growth rate of the fungus at different pH ranges was also observed. Here the analysis was done for dry mycelial weight which indicated the growth rate. Here again the highest dry mycelial weight was found at 7.0 pH which was 1620 mg followed by 7.5 pH that was 1540 mg respectively.

Similarly, the highest dry mycelial weight was obtained at 28^oC (1686 mg) followed by 1570 mg at 32^oC. From the table it may be noted that up to 7.0 pH from 3.0 and up to 28^o C from 10^oC there was an increasing trends and beyond this again decreasing trend was found. Both hydrogen ion concentrations (pH) of the medium in which the fungus is inoculated and the temperature at which the culture is incubated has marked impact on growth and sporulation of the fungus. Sinpfendorfer *et al*; (2001); Abubaker *et al*; (2013) have reported that hydrogen ion concentration of the medium in which the fungus is cultured, significantly influence the growth of the fungus either directly or indirectly. Findings of the present work corroborate with the above findings because here also the best growth was found in the neutral pH as well slightly acidic or alkaline conditions.

Temperature at which the fungus is incubated also influences the growth & sporulation of the fungus. That all the fungi have lowest, optimum, and highest temperature for growth and sporulation. (Verma, 1989). Effect of temperature on growth and sporulation of fungi has been

reported by Singh (1979); Rodriguez and Santa (1991); Maheshwari *et al*; (2000); Sinpfendorfer *et al*; (2001); Sodlauskienė and Survilienė 2003(); Yadhavand Khan (2008); Sharma *et al*; (2011); Mishra and Mishra (2012); Singh and Chauhan (2013); Taware *et al*; (2014) and Pradnya Raniand Kulkarni(2015). Findings of the present work are in agreement with the findings of the above workers. In case of temperature it may be noted that there was an increasing trends in both the radial growth as well as dry mycelial weight up to 28^oC, beyond which there was again declination.

Temperature affects the physiology of the organism and thus depending on this the growth rate and the sporulation, both were affected at the lower and highest temperature. It may further be concluded that sporulation rate was directly related with the growth of the fungus. So excellent rate of sporulation was noted at the pH and temperatures where the mycelial weight was the highest.

Here in the present study best mycelial growth both radial and dry mycelial weight was noted at 7 pH, while the best temperature for the above at 28^oC. Yang and Lian (1998) reported the effects of environmental conditions on the mycelial growth of *Gonodermaluclidum* in shake flask cultures and observed the optimal temperature 30-35^oC. Here also the maximum mycelial growth was at 28^oC. So the findings are supported by the above findings. Onilude *et al*; (2012) however, reported that in case of *Trichoderma* the maximum growth was between 30 to 45^oC. However, such exceptional cases are not common and mostly 28 to 30^oC temperatures are mostly found to be optimal for general fungi.

CONCLUSION

It may be concluded that for better growth and sporulation there is optimal pH and temperatures, below or above it, both the growth and sporulation are affected. The findings of the present work will help in the research being carried out by different workers in the field of plant pathology.

ACKNOWLEDGEMENTS

Authors are thankful to the Principal of Ganga Singh College, Chapra for providing necessary facilities for the above research.

Table 1:

Impact of different pH ranges on radial growth and sporulation of *Alternaria solani*

pH	Mean of the radial growth	Sporulation
3.0	22.64	+
4.0	32.52	+
4.5	38.46	++
5.0	49.36	++
5.5	56.28	+++
6.0	73.72	+++
6.5	78.22	++++
7.0	86.28	++++
7.5	85.24	++++
8.0	70.12	++

Table 2:

Mycelial growth and sporulation of *Alternaria solani* at different pH, as indicated by dry mycelial weight

pH	Dry mycelial weight / mg	Sporulation
3.0	362	+
4.0	580	+
4.5	678	++
5.0	890	++
5.5	936	+++
6.0	1180	+++
6.5	1360	++++
7.0	1620	++++
7.5	1540	++++
8.0	1175	+++

+ Poor, ++ Average, +++ Better, ++++ Excellent

Table 3:

Effect of different temperatures on radial growth and sporulation of *Alternaria solani*

Temperature (In °C)	Radial growth in mm	Sporulation
10	18.64	+
12	32.28	+
16	42.72	++
20	61.86	++
24	76.58	+++
28	82.76	++++
32	81.18	++++
36	70.12	+++
40	66.42	+++
44	30.26	+

Table 4:

Effect of different temperature on mycelial growth (dry mycelial weight) & sporulation of *Alternaria solani*

Temperature (In °C)	Dry mycelial weight in mg	Sporulation
10	366	+
12	585	+
16	682	++
20	896	++
24	1005	+++
28	1686	+++
32	1570	++++
36	1538	++++
40	1276	++++

44	1070	+++
+ Average,	+ + Good,	+ + + Better,
		+ + + + Best,
		+ + + + + Excellent
REFERENCES		
Abubaker A, Suberu H.A., Bello IM.,Abdulkadir R, Daudu Q.A., Lateef A.A. 2013. Effect of pH on mycelial growth and sporulation of <i>Aspergillus parasiticus</i> .Journal of Plant Sciences. 1: 64-67.		mycelial and spores yield by <i>Trichoderma viride</i> in batch and fed batch cultures. Annals of Microbiology. 63: 547-553.
Albert S., Pandya B., and Padhiar A. ,2012. Evaluation of colony characteristics and enzymatic activity of some fungi for potential use in co-culture in bio pulping.Asian Journal of Biological and Life Sciences 1: 83-88.		Pandeya K.K., Pandeya P.K., Kallou G. and Banerjee M.K. 2003.Resistant to early blight of tomato, with respect to various parameters of disease epidemics. J. of Gen. Plant Pathol. 69: 364-371.
Ellis, M.B. (1971): Dermataceoushyphomycetes – CMI Kew, BoCo, Survey England.		Pradnyarani N. and M.S. Kulkarni ,2015.Effect of different pH, temperature and solid media on growth of pure blotch of onion caused by <i>Alternaria porri</i> ELLIS. The Bioscan 10(4): 1839-1843.
Hubbali M, Nakkeeran S, Raghuchander T, Anand T and Samiyappan R ,2010. Effect of environmental conditions on growth of <i>Alternaria alternata</i> causing leaf blight of noni.World Journal of Agricultural Science. 6: 171-177.		Prasad A., Chaudhary R.S. and Chaudhary S.K. ,1992. Effect of different pH levels on growth and sporulation of <i>Fusarium moniliforme</i> V.s Wr, causal organism of wilt of maize. Bio. J., 4(1 &2): 75-78.
Kumar Narendra, SushmaNeema, Vibha and RatalLal Sharma ,2017. Effect of media and pH on mycelial growth and sporulation of <i>A. sesame</i> .International Journal of Chemical Studies. 5(4): 294-296.		Prasad B., Dutt B.L. and Nagaich B.B.,1973. Inducing sporulation in <i>Alternaria solani</i> – I, Effect of water treatment. Mycopathologia et Mycologia Applicata. 49: 141-146.
Kumar Virendra, K.K. Pandey and K.K. Mishra ,2015.Study of variability and sporulation by isolates of <i>Alternaria solani</i> of <i>Lycopersicon esculentum</i> (MILL.)Asian Journal of Science and Technology, 6(4): 1264-1270.		Rath G.C. and Padhi N.N. ,1973. Sporulation of <i>Alternaria solani</i> in pure culture. India Phytopathology, 26:495-501.
Kumawat, Tarun Kumar, Anima Sharma, SeemaBhadauria ,2016.Influence of liquid culture media, temperature and hydrogen ion concentration on the growth of mycelium and sporulation of <i>Arthroderma multifida</i> . Int. J. Pharm. Sci. Rev. Res. ,41(2): 136-141.		Rodriguez, A.C.M. and Santana R. (1991): Effect of new culture media and temperature on the growth <i>in vitro</i> of <i>Alternaria solani</i> . Centro.Agricol., 18: 86-88.
Maheshwari S.K., Singh D.V., Singh S.B. ,2000. Effect of temperature and pH on growth and sporulation of <i>Alternaria alternata</i> causing alternaria leaf spot of dolichos bean.ICAR Report.		Sharma M and Sharma M.,2009. Influence of environmental factors on the growth and sporulation of Geophilic keratinophiles from soil samples of Public Park. Asian J. Exp. Sci., 23: 307-312.
Mishra, P.T. and Mishra V. ,2012.Effect of media, temperature and pH on growth of <i>Alternaria alternata</i> causing leaf spot of cotton.Ann. Pl. Protec. Sci. 20(1): 246-247.		Sharma S.K., Sharma P., Agrawal R.P. (2011): Effect of temperature & pH combination on growth pattern of dermatophytes, isolated from HIV (+)ve patient. Asian Journal of Biochem&Pharma- Res. 3: 307-312.
Onilude A.A., Adbayo-TayoBC.,Odeniyi A.O., Banjo D., Garuba E.O. ,2012.Comparative		Simpfendorfer S, Harden T.J., Murray G.C. ,2001. Effect of temperature and pH on the growth and sporulation of <i>Phytophthora</i> Spp. Australian Plant Pathology, 30: 1-5.
		Singh A., Shahid M., Srivastva M., Pandey S, Sharma A. and Kumar V.,2014.Optimal physical parameters for growth of <i>Trichoderma</i> species at varying pH, temperature and agitation.Virology and Mycology, 3: 127-131.

- Singh P. and Chauhan M. ,2013. Influence of environmental factors on growth of bindings deteriorating fungi, *Aspergillus flavus* and *Penicillium chrysogenum*. International Journal of Pharmaceutical Sciences and Research, 4: 425-429.
- Sodlauskienė A., and Surviliene E. ,2003. Influence of environmental conditions upon the development of *Alternaria* genus fungi *in vitro*. *Sodininkystė ir daržininkystė* 22: 160-166.
- Stevenson R.E., and Pennypacker S.P.,1988. Effect of radiation, temperature and moisture on conidial germination of *Alternaria solani*. *Phytopathology*, 78: 926-930.
- Taware M.R., V.M. Gholve, S.S. Wagh, D.P. Kuldhar, D.V. Pawar, and A.A. Chavan ,2014. Effect of different culture media, temperature, pH, carbon and nitrogen sources on mycelial growth and sporulation of *Alternaria carthami* causing *Alternaria* blight of Safflower. *International Journal of Plant Protection*.7(2):349-353.