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Research article

THE EFFECT OF MEDIA AND LIGHT ON *IN VITRO* SPORULATION OF *ALTERNARIA SOLANI*

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ABSTRACT: Early blight of tomato incited by *Alternaria solani* is an economically significant disease especially in commercial tomato cultivation under greenhouse and field conditions. Since, *A. solani* is a shy sporulator, the present investigation was taken to assess the optimum *in vitro* conditons for growth and sporulation of early blight pathogen. Ten *A. solani* isolates obtained from diseased leaf samples collected different crop growing areas of India were used in the present study. The effect of different incubation periods, fluorescent light, cold-water treatment and media were evaluated. Our results revealed maximum sporulation of *A. solani* on tomato fruit extract agar medium (TFEA) under continuous light for 7 days at 25^{0}C, followed by cold-water treatment and further incubated in darkness at 20^{0}C for 48 h. The sporulation of test pathogen was however sparse on V-8 juice agar. Further, the *A. solani* isolates on V-8 juice agar did not respond to the treatments imposed. Among the isolates, JAS (Jhajjar) isolate, that is more versatile in its ability to produce spores recorded irregular margin with abundant aerial mycelium.

Keywords: *Alternaria solani,* early blight of tomato, sporulation, fluorescent light, tomato fruit extract agar

INTRODUCTION

Early blight of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is an economically important and widely distributed disease throughout the world and occurs in mild to severe form in different parts of India. In India, *A. solani* causes destructive leaf spots, stem cankers and fruit rot (Datar, 1979). *A. solani* usually doesn't sporulate readily in pure culture. Several methods have been employed for conidial production by mutilation of mycelium, exposing cultures to different light sources, dehydration of the mycelium and chemical treatment of the culture (Rands, 1917; Charlton, 1953; Lukens, 1960, Kozlovski and Kvasnyuk, 1984).

Douglas (1972) studied the effect of fluorescent illumination and temperature on the sporulation of *A. solani* and advocated that maximum sporulation could be obtained under continuous light at 25^{0}C. Prasad *et al*. (1973) reported that sporulation was induced when fully grown cultures were given dip or spray treatment with distilled water (cold or hot) and thereafter kept partially covered at different temperatures. Shahin and Shepherd (1979) obtained profuse sporulation of *A. solani* in pure culture by when agar blocks from cultures on primary media were transferred to the surface of a sporulation medium of water agar plus $CaCO_3$ followed by incubation in the dark at 18^{o}C. Vakalounakis (1983) observed large masses of conidia, when mycelial discs of *A. solani* were inoculated onto sterile solanaceous leaf discs placed on water agar plates at 20^{o}C.

Zhu *et a*l. (1985) observed that *A. solani* sporulated profusely on the corn meal agar when illuminated with fluorescent lamp for 8 h at 18^{o}C. Between the two media with or without $CaCO_3$, highest sporulation of *A. solani* was obtained in PDA plus $CaCO_3$ (Moretto and Barreto, 1995). Benlioghlu and Delen (1996) demonstrated that T-media containing tomato juice was found to be the most suitable with 6 days dark at 25^{o}C, 12 h light alternated with 12 h dark for sporulation of different isolates of *A. solani*. While Liuchienhui *et al*. (1997) got maximum sporulation when mycelia were homogenized and transferred to a second V-8 juice agar medium after 10 days at 28^{o}C. Sporulation increased in V-8 medium with mycelial wounding or exposure to lower temperature at 18^{o}C. Demirci (1997) obtained abundant sporulation of *A.solani* isolates when grown on the potato leaf extract agar under 6 days continuous fluorescent light at 25^{o}C and then incubated at 20^{o}C in the dark.

Sporulation *in vitro* was not satisfactory with some of the Indian isolates with the above-mentioned methods. The present investigation was undertaken to evaluate the combined effect of fluorescent light, cold-water treatment and media on the sporulation of *A. solani* isolates.

MATERIAL AND METHODS

Early blight infected tomato leaf samples were collected from Northern states, i.e., Haryana (HAS-I, Hisar; HAS-II, Hansi, Hisar; JAS, Jhajjar; RAS, Rohtak; YAS, Yamunanagar), Himachal Pradesh (HAAS, Hamirpur), New Delhi (DLAS, Delhi), Punjab (LAS, Ludhiana), and Southern states, *i.e*., Andhra Pradesh (SAS, Srikakulam) and Karnataka (DAS, Dharwad) of India. The samples obtained were surface sterilized with 0.1% mercuric chloride and then washed twice in sterile, distilled water. Leaf bits of approximately 5mm were excised from the lesion edges and plated on potato dextrose agar (PDA) medium and incubated in dark at 25^{0}C. *A. solani* cultures were identified based on morphological characteristics and spore dimensions. In this study, the combined effect of fluorescent light, culture media and cold-water treatment were evaluated for inducing sporulation in *A. solani* isolates. The photoperiods of (i) continuous light, (ii) 16h of light plus 8h of darkness, (iii) 8h of light plus 16h of darkness and (iv) constant darkness; three culture media [Potato Dextrose Agar, V-8 Juice agar and tomato fruit extract agar (Instant mashed tomatoes 100g; dextrose 20g; agar-agar 20g; distilled water adjusted up to 1000ml)] were tested for induction of sporulation in ten *A. solani* isolates.

A. solani isolates were grown in 90 mm diameter Petri plates containing 20ml of above mentioned media. After incubation for 7 days at 25 $\pm$ 1^{o}C at different photoperiods, the cultures were given spray treatment with sterile distilled cold-water (4^{0}C) by means of a hand atomizer under aseptic conditions (Prasad *et al*., 1973). Water treated culture plates were kept in an incubator at 20 $\pm$ 1^{o}C in such a manner that they remained partially covered. The treatments were replicated thrice. The following treatments were imposed.

The following are the list of treatments imposed (*in vitro*) for inducing sporulation in early blight pathogen of tomato induced by *Alternaria solani*

Treatment	Photoperiod conditions		
	First 7 days incubation at 25^{o}C	Cold-water treatment	Incubation at 20 ^{o}C for 48 h
CD+D	Continuous dark	+ & -	Dark
CL+L	Continuous light	+ & -	Light
CD+L	Continuous dark	+ & -	Light
CL+D	Continuous light	+ & -	Dark
16 h L plus 8 h D + 16 h L plus 8 h D	16 h light plus 8 h dark	+ & -	16 h light plus 8 h dark
16 h D plus 8 h L + 16 h D plus 8 h L	16 h dark plus 8 h light	+ & -	16 h dark plus 8 h light
16 h L plus 8 h L + D	16 h light plus 8 h light	+ & -	Dark
16 h D plus 8 h L + L	16 h dark plus 8 h light	+ & -	Light
16 h L plus 8 h D + L	16 h light plus 8 h dark	+ & -	Light
16h D plus 8h L + D	16 h dark plus 8 h light	+ & -	Dark

**A. solani* cultures in control were cultured and incubated at 25^0 C for 9 days without any cold-water treatment
**Treatments were imposed on three media (PDA, V-8 juice agar, tomato fruit extract agar) were used
Three replications per treatment were maintained

The *A. solani* cultures in different media without any cold water treatment and incubated in dark at 25^{0}C for 9 days served as control. Altogether, there were 23 treatments including control. For control, the culture plates were incubated at 25^0 C in dark for nine days. Three replications were maintained for each of the treatments. Observations on sporulation were recorded at the end of 9th day. A five class rating system (Douglas, 1972) was used in evaluating sporulation (class 0= no sporulation; class 1= one to very few; class 2= sparse but easy to observe; class 3= abundant and present over most of the culture; class 4= profuse and covering the entire culture surface. The data were analyzed using GENSTAT statistical package and the treatment means are separated.

RESULTS AND DISCUSSION

The sporulation of *A. solani* isolates was significantly affected by fluorescent light plus cold-water treatment and culture media. Among the treatments evaluated, maximum mean sporulation of *A. solani* isolates was obtained when the cultures were grown on tomato fruit extract agar medium (TFEA) under continuous light for 7 days at 25^{0}C followed by cold-water treatment and then incubated in darkness at 20^{0}C. The effect of media and fluorescent light were found to be very distinct compared to cold-water treatment.

The significant effects of interaction of fluorescent light and culture media are in accordance with the results reported by Demirci (1997), who stated that abundant sporulation of *A. solani* could be obtained when grown on potato leaf extract agar under 6 days continuous fluorescent light at 25^0C and then incubated at 20^0C in the dark. Tomato fruit extract medium was found to be superior over potato dextrose agar and V-8 juice agar in inducing sporulation of *A. solani* isolates (Fig.1). The aerial growth of the isolates was profuse on TFEA and the colony color was dark grayish. PDA was also efficient in inducing sporulation of *A. solani* isolates with moderate to abundant aerial mycelium and grayish growth of the colonies. However, sporulation of *A. solani* isolates was sparse on V-8 juice agar with whitish growth of the colony. The isolates grown on V-8 juice agar didn't respond to the treatments imposed. Growth varied among different isolates, with those having the ability to sporulate abundantly generally having the most profuse growth. The isolate JAS, that is more versatile in its ability to produce spores recorded irregular margin with abundant aerial mycelium. The results are in accordance with that of Douglas (1972) who reported that the isolates having the ability to sporulate tend to grow profusely on culture media.

The light effects were very distinct and differed significantly from one another. The treatment of continuous light followed by darkness was more effective than all other treatments. The treatments of continuous light and 16 h light plus 8 h darkness were at par with each other and found superior to 16 h dark and 8 h light. Further, the treatment of initial growth of the isolates for 7 days under 16 h light plus 8 h dark followed by incubation under continuous darkness was also found to induce good amount of spores. These results are in contrary to that of Lukens (1960) who reported that conidiophores of *A. solani* require a 12 h dark period to produce conidia.

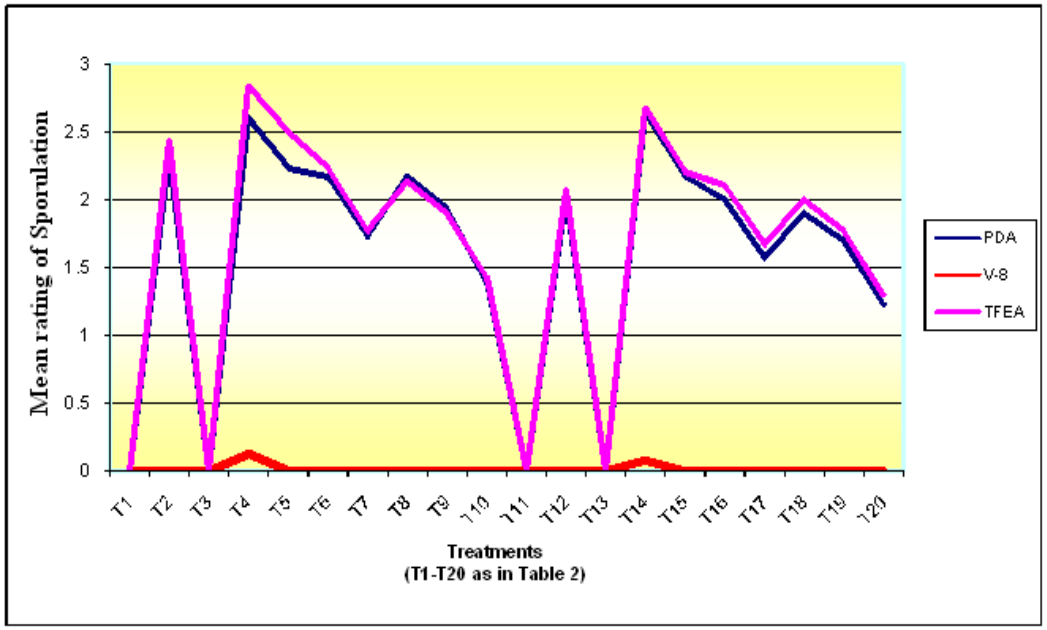

Fig-1: The effect of fluorescent light plus cold water treatment and media on the sporulation of *Alternaria solani*

T_1= CD+D; T_2= CL+L; T_3= CD+L; T_4= CL+D; T_5=16h L and 8h D + 16h L and 8h D; T_6=16h L and 8h D + CD; T_7=16h L and 8hD + CL; T_8=16hD and 8h L + 16hD and 8h L; T_9=16h D and 8h L + CD; T_{10}=16h D and 8h L+ CL; T_{11}= CD-D; T_{12}= CL-L; T_{13}= CD-L; T_{14}= CL-D; T_{15}=16h L and 8h D - 16h L and 8h D; T_{16}=16h L and 8h D – CD; T_{17}=16h L and 8h D – CL; T_{18}=16h D and 8h L - 16h D and 8h L; T_{19}=16h D and 8h L – CD; T_{20}=16h D and 8h L- CL

Where **CD=** Continuous dark: **CL=** Continuous light; **D=** Dark; **L=** Light; **+,** Cold-water treatment; **–,** without cold-water treatment

The growth of isolates for 7 days under continuous light, 16 h light plus 8 h dark or 16h dark plus 8 h light at 25^0C were found to induce conidiophores and there by conidia. However, none of the isolates produced spores under continuous darkness. This is contrary to the report by Benlioghlu and Delen (1996), who demonstrated that T-media containing tomato juice was most suitable for sporulation of *A. solani* isolates under six days darkness at 25^0C.

In the present study, it was observed that initial growth under continuous fluorescent light for 7 days at 25^0C induced conidiophores in *A.solani* isolates and further incubation under darkness at 20^0C produced conidia. These results tend to agree with that of Leach (1967) who stated that *Alternaria dauci* is a diurnal sporulator and has two distinct phases of photosporogenesis, an inductive phase leading to formation of conidiophores and a terminal phase resulting in the formation of conidia.

Cold-water treatment after 7 days of incubation under different light conditions enhanced the sporulation in most of the treatments. Prasad *et al*., (1973) suggested that cold-water treatment of fully-grown cultures induce sporulation in *A. solani*. However, the light effects were more prominent than the cold-water treatment in inducing sporulation in *A. solani* isolates.

The mean rating of sporulation of *A. solani* isolates on three culture media at different light conditions was presented in

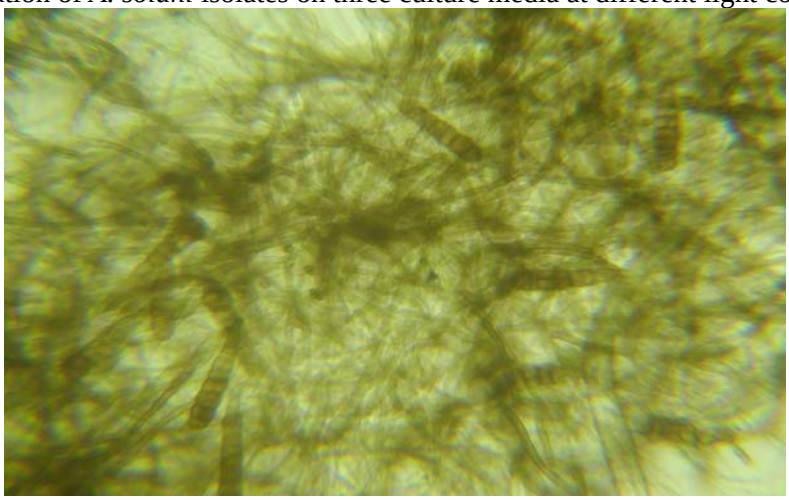

Plate 1: Sporulation in JAS isolate of *Alternaria solani* (400x)

Table 1: The effect of fluorescent light plus cold-water treatment and media on the sporulation of *Alternaria solani*

Treatment	Mean rating of sporulation			Mean
	PDA	V-8 juice agar	TFEA	
CD+D*	0.00**	0.00	0.00	0.00
CL+L	2.33	0.00	2.43	1.59
CD+L	0.00	0.00	0.00	0.00
CL+D	2.60	0.13	2.83	1.86
16h L & 8h D + 16 h L & 8h D	2.23	0.00	2.50	1.58
16h L & 8h D + CD	2.17	0.00	2.23	1.47
16h L & 8h D + CL	1.73	0.00	1.77	1.17
16h D & 8h L + 16h D & 8h L	2.17	0.00	2.13	1.43
16h D & 8h L + CD	1.93	0.00	1.90	1.28
16hD & 8hL + CL	1.40	0.00	1.43	0.94
CD-D	0.00	0.00	0.00	0.00
CL-L	2.03	0.00	2.07	1.37
CD-L	0.00	0.00	0.00	0.00
CL-D	2.63	0.07	2.67	1.79
16h L & 8h D - 16 h L & 8h D	2.17	0.00	2.20	1.46
16h L & 8h D - CD	2.00	0.00	2.10	1.37
16h L & 8h D - CL	1.57	0.00	1.67	1.08
16h D & 8h L - 16h D & 8h L	1.90	0.00	2.00	1.30
16h D & 8h L - CD	1.70	0.00	1.77	1.16
16h D & 8h L - CL	1.23	0.00	1.30	0.84
Mean	1.59	0.01	1.65	--
Source	CD (1%)			
Treatment (T)	0.09			
Media (M)	0.04			
T x M	0.17			

** Mean rating of sporulation with light plus cold-water x media interaction

***CD,** Continuous dark: **CL,** Continuous light; **D,** Dark; **L,** Light; **+,** Cold-water treatment; **–,** without cold-water treatment

Table 2. Most of the isolates responded to the treatment, continuous light for 7 days followed by incubation at continuous dark. Isolate JAS proved to be the most versatile in its ability to produce spores (Plate 1). It sporulated at every treatment except under continuous darkness. Good sporulating ability was also observed with the isolates LAS, HAAS, YAS and RAS. However, the sporulating ability was less in the isolates DLAS, DAS, HAS-II, HAS-I and SAS. No correlation was observed between the ability of an isolate to sporulate and its pathogenicity. All the isolates under tests were pathogenic on tomato plants. Investigation on isolate virulence and abundance of sporulation *in vitro* didn't reveal any correlation. The findings are in agreement with that of Douglas (1972) who advocated that there is no correlation between isolate virulence and abundance of sporulation.

The selection of appropriate media and light conditions are important for abundant sporulation of *A. solani*. In addition, the cultures need to be incubated at temperatures around 25^0C for 6 to 7 days and further incubation should be at lower temperatures. The present study shows that abundant sporulation could be obtained on tomato fruit extract agar under continuous light for 7 days at 25^0C followed by cold-water treatment and further incubation at 20^0C in darkness.

Table 2: The effect of cold-water treatment and fluorescent light on the sporulation* of *Alternaria solani* isolates

Trt No.	Treatment**	Isolate										
		HAS-I	HAS-II	RAS	JAS	YAS	LAS	DLAS	HAAS	DAS	SAS	Mean
T_1.	CD+D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
T_2.	CL+L	1.22	1.11	1.67	2.33	1.55	2.11	1.44	1.89	1.00	0.78	**1.59**
T_3.	CD+L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
T_4.	CL+D	1.44	1.33	2.11	3.00	1.89	2.44	1.67	2.11	1.33	1.22	**1.85**
T_5.	16h L and 8h D + 16h L and 8h D	1.11	1.22	1.78	2.22	1.89	2.11	1.44	1.78	1.22	1.00	**1.58**
T_6.	16h L and 8h D + CD	0.89	1.00	1.67	2.00	1.78	2.11	1.55	1.67	1.11	0.89	**1.47**
T_7.	16h L and 8hD + CL	0.56	0.67	1.44	1.44	1.67	1.55	1.33	1.67	0.78	0.56	**1.17**
T_8.	16hD and 8h L + 16hD and 8h L	0.89	0.78	1.44	2.00	1.55	1.78	1.44	2.00	1.11	1.33	**1.43**
T_9.	16h D and 8h L + CD	0.67	0.78	1.33	1.89	1.78	1.89	1.33	1.44	1.00	0.67	**1.28**
T_{10}.	16h D and 8h L+ CL	0.45	0.33	1.00	1.33	1.44	1.33	1.11	1.44	0.78	0.22	**0.61**
T_{11}.	CD-D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
T_{12}.	CL-L	1.00	1.22	1.33	2.11	1.55	1.78	1.44	1.67	0.89	0.67	**1.37**
T_{13}.	CD-L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
T_{14}.	CL-D	1.55	1.22	2.00	2.89	1.89	2.22	1.78	2.00	1.22	1.11	**1.79**
T_{15}.	16h L and 8h D - 16h L and 8h D	1.00	1.33	1.67	2.22	1.78	2.11	1.33	1.44	0.89	0.78	**1.46**
T_{16}.	16h L and 8h D - CD	0.78	0.89	1.44	1.89	1.55	1.89	1.44	1.78	1.00	1.00	**1.37**
T_{17}.	16h L and 8h D - CL	0.78	0.56	1.22	1.44	1.78	1.44	1.22	1.44	0.89	0.33	**1.08**
T_{18}.	16h D and 8h L - 16h D and 8h L	0.78	1.00	1.33	1.78	1.44	1.44	1.33	1.78	1.00	1.11	**1.30**
T_{19}.	16h D and 8h L – CD	0.45	0.67	1.22	1.55	1.67	2.00	1.22	1.33	0.89	0.56	**1.16**
T_{20}.	16h D and 8h L- CL	0.33	0.11	0.89	1.22	1.22	1.11	1.00	1.44	0.78	0.33	**0.84**
	Mean	0.69	0.71	1.17	1.57	1.32	1.47	1.11	1.34	0.78	0.67	
CD (P=0.01)		Treatment (T) 0.09		Isolate (I) 0.07		T x I 0.31						

*** Mean rating of sporulation on three culture media based on five class rating system: class 0, no sporulation; class 1, one to very few; class 2, sparse but easy to observe; class 3, abundant and present over most of the culture; class 4-profuse and covering the entire culture surface. Ten isolates, three cultures in triplicate on three culture media.**

****CD- Continuous dark, CL - Continuous light, D – Dark, L – Light, (+) - Cold-water treatment, (–) - Without cold-water treatment**

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