The Bioscan 8(1): 101-104, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

STUDIES ON GROWTH CONDITIONS OF THE TOMATO ALTERNARIA LEAF SPOT CAUSING *ALTERNARIA SOLANI* L

JAGGAL SOMAPPA*, K. SRIVASTAVA, B. K. SARMA, CHHATTAR PAL[1] AND RAVINDRA KUMAR
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi – 221 005
[1]Department of Mycology and Plant Pathology, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi – 221 005
e-mail: somappa35552@gmail.com

KEYWORDS
Tomato
Growth and sporulation
Alternaria leaf spot
Alternaria solani

Received on :
11.09.2012

Accepted on :
04.01.2013

***Corresponding author**

ABSTRACT
Alternaria leaf spot is the second most important disease of tomato after leaf curl virus causing severe loss every year. Studies of liquid media revealed that *Alternaria solani* growth was best on Potato Dextrose Broth (34.1mg) followed by Czapeck's medium (58mm) and sporulation was maximum on Potato Dextrose Agar (13.2×10^6 spores/mL). The pathogen also sporulated maximum at temperature 25ºC and RH 100%.

INTRODUCTION

Tomato [*Lycopersicon esculentum* Mill (2n = 2x = 24)] is the world's largest vegetable crop and known as protective food both because of its special nutritive value as well as also for its wide spread production. Tomato is being extensively grown as an annual plant all over the world. India is the second largest tomato producer in the world after China, accounting for about 11% of the world tomato production (Indian Horticulture Database, 2011). During 2010-11, the area and production of tomato in India was about 0.865 million hectare and 16.82 million tonnes, average productivity 19.6 tonnes per hecatre (Indian Horticulture Database, 2011). The leading tomato growing states are Uttar Pradesh, Karnataka, Maharashtra, Haryana, Punjab and Bihar. A wide gap exists between the potential yield and the yield realized at the farmer's field, which is largely because of number of biotic and abiotic stresses to which the tomato crop is exposed. Among the biotic stresses, Alternaria leaf spot disease caused by *Alternaria solani* (Ellis and Martin), is one of the most common and destructive disease of tomato crop especially in Northern plains and peninsular plateau regions of India. Leaf spot, stem lesion and fruit spots are most damaging symptoms of the disease and yield losses up to 79% damage by *A. solani* were reported from Canada, India, USA and Nigeria (Basu, 1974; Datar and Mayee, 1981; Sherf and MacNab, 1986; Mathur and Shekawat, 1986; Gwary and Nahunnaro, 1998).

Considering the prevalence and significance of the disease and important of the crop, present investigation was under taken to determine the best medium and optimal growth conditions of the pathogen *in vitro*.

MATERIALS AND METHODS

Different synthetic and semi-synthetic media were prepared by weighing the different constituents of each medium and then adding the distilled water to make up the volume 1000mL and autoclaved at 1.045 kg/cm^2 for 20 minutes. In solid media agar-agar powder was added where as, in liquid media no agar-agar was added. In solid media 20mL of agar medium was poured in each petridish whereas, in liquid media 20mL of the medium was dispensed in each of 100mL conical flask. Inoculation was done with 5mm disc of mycelia mat taken from 7 days old fungal culture and incubated at 25 $\pm$1ºC for 7 days.

Growth and sporulation on solid media

Growth on solid media such as Potato Dextrose Agar (extract of peeled potato 200g + dextrose 20g + agar-agar 20g + distilled water to made total volume 1000mL), Czapeck's agar (Sucrose 30 g + Sodium nitrate 2g + Dipotassium phosphate 1g + Magnesium sulphate 0.500 g + Potassium chloride 0.500g + Ferrous sulphate 0.010 + Agar-agar 15g + distilled water to made total volume 1000mL), Martin's (Glucose 10g + Peptone5g + KH_2PO_4 0.5g + $MgSO_4$ - $7H_2O$ 0.5g + Agar 12g + Yeast extract 0.5g + Streptomycin sulfate 30mg + distilled water to make total volume 1000mL) and Plain agar (agar-agar 20g + Distilled water 1000mL) medium was determined by measuring the colony diameter along the two

diagonals passing through the centre of the colony and sporulation was recorded by using haemo-cytometer. The measurements were recorded at an interval of 7 days. The various solid media such as synthetic media and semi-synthetic media used in the present study.

Growth and sporulation on liquid media

For measuring growth in liquid media the mycelia was harvested by filtration through Whatman's filter paper no.42, washed with distilled water and dried at 60ºC till it had constant weight after cooling in desiccators.

Net weight of mycelium was calculated as follows:

Net weight of mycelium = (Wt. of mycelium + filter paper)-(Wt. of filter paper).

Both synthetic media and semi-synthetic broth media were used in the present study. Observation on mycelial weight and sporulation were recorded after 7 days of incubation. Spoulation was recorded by using haemo-cytometer.

Effect of temperature on growth and sporulation

Effect of temperature on growth and sporulation of *Alternaria solani* was studied *in vitro*. The solid and liquid media mentioned above were used for the study. The pathogen was incubated at 5 different temperature viz., 15, 20, 25, 30 and 35ºC. Observations on radial growth and sporulation were recorded after 7 days incubation.

Effect of relative humidity on growth and sporulation

To study the effect of relative humidity on mycelia growth of *Alternaria solani*, five different humidity levels *i.e.* 60, 70, 80, 90 and 100 per cent were maintained by using the concentrated sulphuric acid and sterilized distilled water in different proportions suggested by (Buxton and Mellanby, 1934). Observation on radial growth and sporulation were recorded after 7 days of incubation.

RESULTS AND DISCUSSION

Growth and sporulation of *Alternaria solani* on different solid media

Among the four different solid media under study, maximum radial growth was recorded on Czapeck's agar medium (58.00mm) followed by Potato Dextrose Agar (37.00mm), Martin's medium (7.33mm) and plain agar (5.0mm). Potato Dextrose Agar was found best with sporulation ($13.20x10^6$ spores/mL) (Fig.1). It was followed by Czapeck's ($11.10x10^6$spores/mL), where as minimum sporulation of this fungus was observed on Martin's medium ($6.70 x10^6$spores/mL) and Plain Agar ($5.10 x10^6$ spores/mL) (Table 1).

Table 1: Growth and sporulation of *Alternaria solani* on different solid media (After 7 days of incubation at 25 ± 1ºC)

Medium	Average colony diameter (mm)*	Number of spores/mL ($\times 10^6$)*
Czapeck's medium	58.0	11.1
Martin's medium	7.33	6.7
Plain agar	5.0	5.1
Potato dextrose agar	37.0	13.2
SEm±	0.52	0.25
CD	2.50	1.23

CD at 1% level of significance. *Average of three replications

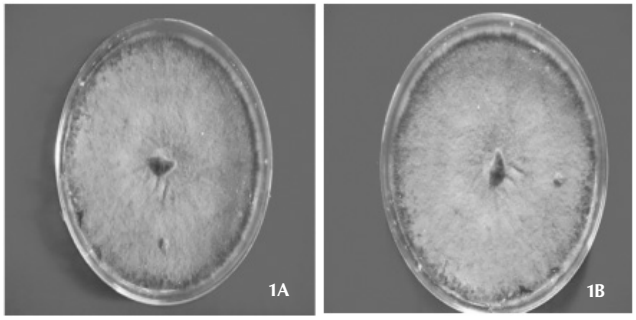

Figure 1: Growth and sporulation of *Alternaria solani* on PDA medium

Growth and sporulation of *Alternaria solani* on different liquid media

To find out a suitable liquid medium for the mycelial growth and sporulation of *Alternaria solani*, three different lequid media were tested *in vitro*. Inoculated flasks were incubated at 25 ± 1ºC for 7 days and mycelial mats were harvested, dried and weighed. From the data presented in Table 2, it is evident that all media, under study, supported the growth and sporulation of *Alternaria solani*. Maximum growth was recorded on Czapeck's broth medium (76.77mg) followed by Potato Dextrose Broth medium (34.1mg) and Martin's broth medium (14.4mg). Potato Dextrose Broth medium was best for sporulation (16.90×10^6 spores/mL) (Fig. 2) followed by Czapeck's broth medium (14.83×10^6 spores/mL) and Martin's broth medium (4.47×10^6 spores/mL).

It can be concluded that PDB was the best supporter of growth and sporulation. The PDB was selective medium for further studies.

Growth and sporulation of the fungus differed in solid and liquid media

Maximum growth and sporulation was recorded on Potato

Table 2: Growth and sporulation of *Alternaria solani* on different liquid media (After 7 days of incubation at 25 ± 1ºC)

Medium	Average mycelia weight (mg)*	Number of spores/mL ($\times 10^6$)*
Potato dextrose broth	34.10	16.90
Czapeck's medium	76.77	14.83
Martin's medium	14.40	4.47
SEm±	0.41	0.17
CD	2.17	0.91

CD at 1% level of significance *Average of three replications.

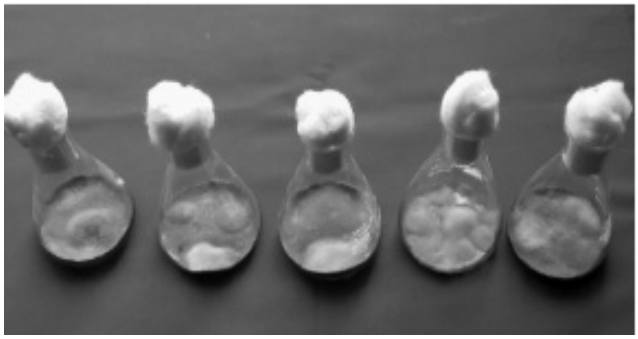

Figure 2: Growth and sporulation of *Alternaria solani* on PDB medium

Table 3: Effect of temperature and relative humidity on the growth and sporulation of *A. solani in vitro*

S.No.	Temperature (ºC)	Average mycelial growth (mm)*	Spores/mL ($\times 10^6$)*	Relative humidity(%)	Average mycelial growth (mm)*	Spores/mL ($\times 10^6$)*
1	15	10.33	3.84	60	33.20	6.13
2	20	21.30	10.70	70	38.33	9.27
3	25	37.47	13.80	80	46.30	13.57
4	30	34.00	12.17	90	53.33	16.17
5	35	17.67	5.20	100	54.30	17.00
	SEm ±	0.33	0.19		0.47	0.22
	CD	1.48	0.85		2.11	1.03

CD at 1% level of significance. *Average of three replications.

Dextrose Broth (PDB) followed by Czapeck's medium and Martin's medium. The moderate growth and sporulation was recorded on Czapeck's medium, where as Martin's medium was proved least supporter of growth and sporulation. Khatun *et al*. (1996) observed that maximum growth of *Alternaria brassicae* was found on Potato Dextrose Agar. The same observation was also reported by Gemawat and Ghosh (1979), Rotem (1994) and Kumar *et al*. (2008) working with *Alternaria solani*. The growth pattern on PDB and PDA medium was the best in *in vitro* conditions.

Growth and sporulation of *Alternaria solani* on different temperature

The temperature range for the growth varies for all micro-organisms as well as for host pathogen interaction. It is evident from the data presented in Table 3 that the fungus grows at all temperature (15 to 35ºC). Maximum mycelial growth (37.47mm) and sporulation (13.80 x 10^6spores/mL) was observed at 25ºC. A sudden fall in mycelial growth and sporulation was observed at 30ºC and 35ºC. However, 20ºC and 30ºC also favoured good growth and sporulation of *Alternaria solani* but differ significantly from growth at 25ºC. It can be concluded that 25ºC is the optimum temperature for mycelial growth and sporulation of *Alternaria solani* (Table 3). In the present study, result showed that maximum growth and sporulation of fungus were observed at 25ºC followed by 30, 20, 35 and 15ºC. Degenhardt *et al*. (1982) have reported that an isolate of *Alternaria brassicicola* from cabbage required more than 15ºC temperature and more than 95% realative humidity for best conidial germination. Taber *et al*. (1968) reported that the conidia of *Alternaria brassicae* grew best at 20-25ºC. Neergaard (1945) observed a good growth and sporulation of various species of *Alternaria* at different temperatures varies from 23-28ºC.

Effect of relative humidity on growth and sporulation of *Alternaria solani*

To evaluate the effect of atmospheric moisture, the fungus was exposed directly to different level of relative humidity. It was observed (Table 3) that all the five humidity levels (60 to 100 per cent) induced the growth and sporulation of *Alternaria solani*. Significantly best mycelial growth (54.30mm) and sporulation (17.00 x10^6spores/mL) was recorded at 100 per cent relative humidity followed by growth and sporulation at 90 per cent (53.33mm and 16.17 x10^6spores/ml) humidity level. A significant decrease in mycelial growth and sporulation was observed at 80 per cent, 70 per cent and 60 per cent humidity level. The sporulation was supported by all the humidity levels except at 60 per cent. Maximum sporulation was observed at 100 per cent relative humidity. Sinha *et al*. (1992) reported that severe leaf spot disease of rapeseed and mustard was associated with 21-25ºC temperature and 90% relative humidity. Similar observations were also recorded by Singh *et al*. (2001).

REFERENCES

Basu, P. K. 1974. Measuring early blight, its progress and influence on fruit losses in nine tomato cultivars. *Can. Plant Dis. Surv*. **54:** 45-51.

Buxton, P. A. and Mellanby, K. 1934. Measurement and control of humidity. *Bulletin of Entomology Research*. **25:** 171-175.

Datar, V. V. and Mayee, C. D. 1981. Assessment of losses in tomato yield due to early blight. *Indian Phytopath*. **34:** 191-195.

Degenhardt, K. J., Petrie, G. A. and Morrall, A. A. 1982. Effect of temperature on spore germination and infection of rapeseed by *Alternaria brassicae*, *Alternaria brassicicola* and *A.raphani*. *Candian J. Pl. Patho*. **4(2):** 115-118.

Gemawat, P. D. and Ghosh, K. M. 1979. Studies on the physiology of growth of *Alternaria solani*. *Indian J. Mycol. Pl. Pathol*. **9:** 138-139.

Gwary, D. M. and Nahunnaro, H. 1998 Epiphytotics of early blight of tomatoes in Northeastern Nigeria. *Crop Prot*. **17**: 619-624.

Indian Horticulture Database. 2011. National Horticulture Board, Department of Agriculture and cooperation, Government of India. www. nhb.gov.in

Khatun, F., Hossain, M. A., Hossain, M. M. and Dey, D. K. 1996. Effect of culture media, temperature, pH and nitrogen sources on growth and sporulation of *Alternaria brassicicola*. *Bangladesh J. Plant Pathology*. **12(1/2):** 29-32.

Kumar, V., Haldar, S., Pandey, K. K., Singh, R. P., Singh, A. K. and Singh, P. C. 2008. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. *World J. Microbiol. Biotechnol*. **24:** 1003–1009.

Mathur, N. and Shekawat, R. 1986. Chemical control of early blight in kharif sown tomato. *Indian J. Mycol. Plant Pathol*. **16:** 235–240.

Neergaard, P. 1945. Danish species of *Alternaria* and *Stemphylium*. E. Munsgarrd Copenhaugue. p. 560.

Rotem, J. 1994. The genus *Alternaria* biology, epidemiology, and pathogenicity, 1st Ed. The American Phtyopathological Society, St. *Paul, Minnesota*. **48:** 203.

Sherf, A. F. and Macnab, A. A. 1986. Vegetable diseases and their control. John Wiley and Sons, New York. pp. 634-640.

Singh, V., Kumar, P. and Sinha, A. P. 2001. Influence of different

media, pH and temperature on growth and sporulation of *Alternaria alternate* (Fr.) Keissler causing *Alternaria* blight of chickpea. *Legume Res.* **24(4):** 238-242.

Sinha, R. K. P., Rai, B. and Sinha, B. B. P. 1992. Epidemiology of leaf spot of rapeseed mustard caused by *Alternaria brassicae*. *J. Appli. Bio.* **2(1-2):** 70-75.

Taber, R. A., Vanterpod, T. C. and Taber, W. A. 1968. A comparative nutritional study of *Alternaria raphani*, *Alternaria brassicae* and *Alternaria brassicicola* with special reference to *Alternaria raphani*. *Phytopathology*. **58:** 609-616.