INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 8 | ISSUE 2 | OCTOBER, 2015 | 359-363

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/8.2/359-363

Studies on physiological parameters of *Alternaria alternata* (Fr) keissler-The incitant of leaf blight of groundnut

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ARITCLE INFO

Received : 24.02.2015
Revised : 05.09.2015
Accepted : 20.09.2015

KEY WORDS :
Alternaria alternata, Physiological parameters, Leaf blight, Groundnut

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ABSTRACT

Leaf blight caused by *Alternaria alternata* (Fr) Keissler was observed on leaves of groundnut. Out of six different solid and liquid media and different levels of temperature, relative humidity and pH tested for mycelial growth and sporulation of fungus, the maximum mycelial growth and sporulation were observed on potato dextrose agar at 25°C temperature, 100 per cent relative humidity and pH 6.5.

How to view point the article : Kantwa, S.L., Shekhawat, K.S. and Tetarwal, J.P. (2015). Studies on physiological parameters of *Alternaria alternata* (Fr) keissler-The incitant of leaf blight of groundnut. *Internat. J. Plant Protec.*, **8**(2) : 359-363.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is mainly grown as an oilseed *Kharif* crop in India. Groundnut is also known as peanut, earthnut, monkey-nut, goober nut, pinda and manillanut. De Candolle (1886) stated its origin to be in Brazil (South America). The groundnut is presently grown on a commercial scale in about 82 different countries in the world. In India, the total coverage under this crop is about 6.7 million hectares with an annual seed production of 7.0 million tonnes. Leaf blight of groundnut caused by *Alternaria alternata* was reported by Balasubramanian (1979). It is an important disease in the major groundnut growing areas of the state. The disease occurs widely in the crop grown in sandy soil in the Rajasthan, where climatic conditions are dry and temperature remains high. Under favourable conditions, spread of disease is rapid; involving all the plants in a field and such blighted plants can be recognized.

MATERIAL AND METHODS

Inoculation was done with 2 mm disc of mycelial bit taken from 7 days old fungal culture and incubated at 25 ± 2^0C for 7 days. Each treatment was replicated four times with CRD design. Observations on radial mycelial growth and sporulation were recorded after 7 days of incubation and sporulation was recorded using Haemocytometer.

Growth and sporulation on solid media :

Growth on solid media was determined by measuring the colony diameter along with the two diagonals passing through the centre of colony by

excluding initial diameter (2 mm) of bit. The solid media used in the present study were Asthana media, Corn meal agar, Czapeck's dox agar, Oat meal agar, Potato dextrose agar and Richard's medium.

Growth and sporulation on liquid media :

In case of liquid media, the mycelial growth was harvested by filtration through Whatman's filter paper No. 42, washed repeatedly, dried at 60^0 C till it had constant weight after cooling in desiccators. Observation on dry mycelial weight and sporulation were recorded after 7 days of incubation. The liquid media used in the present study were Asthana broth media, Corn meal broth media, Czapeck's dox broth media, Oat meal broth media, Potato dextrose broth media, and Richard's broth media.

Effect of temperature on mycelial growth and sporulation :

Effect of temperature on growth and sporulation of *Alternaria alternata* was studied *in vitro*. Twenty ml of sterilized potato dextrose agar medium was poured in each sterilized Petri-plate. Inoculation was made with 2 mm disc taken from 7 days old fungal culture and incubated at 7 different temperatures *viz.*, 20, 25, 30, 35, 40, 45 and 50^0C.

Effect of relative humidity on mycelial growth and sporulation :

To study the effect of relative humidity on mycelial growth and sporulation of *Alternaria alternata*, six different levels of relative humidity *i.e.* 50, 60, 70, 80, 90 and 100 per cent were maintained by using concentrated sulphuric acid and sterilized distilled water in different proportions. The different relative humidity levels were maintained by the method suggested by Buxton and Mellan (1934). Petriplates containing PDA medium were inoculated with 2 mm disc of 7 days old culture of test fungus. Inoculated Petriplates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportions and incubated at 25 ± 2^0C for 7 days.

Effect of hydrogen ion concentrations on mycelial growth and sporulation :

The effect of hydrogen ion concentration on the growth and sporulation of *Alternaria alternata* was determined by adjusting the pH of broth medium at 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 by using citrate phosphate buffer (Singh *et al.*, 2005) before sterilization with the help of pH meter. Aliquate of 20 ml medium were dispensed in 100 ml conical flask and autoclaved at 1.045 kg/cm^2 for 20 minutes. Inoculations were made with 2 mm disc of mycelial mat obtained from 7 days old culture of *Alternaria alternata*. Observations on dry mycelial weight and sporulation were recorded after 7 days of incubation at 25 ± 2^0C for 7 days.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented in Table 1 to 5.

Growth and sporulation on different solid and liquid media :

Out of Six different solid and liquid media under study, the potato dextrose agar was significantly superior in supporting maximum radial growth (88 mm) and sporulation (35.00 x 106/ml) than other media, followed by Richard's and Oatmeal media on which the fungus showed reasonable growth (74.25 mm and 60.25 mm) and sporulation (30.25 and 26.00 x 106 /ml), respectively. Minimum growth and sporulation of the fungus was observed on Asthana medium (14.25 mm and 20.25 x 106 /ml). It can be concluded that potato dextrose agar medium was best supporter of growth and sporulation of the fungus. Maximum growth and sporulation were recorded on potato dextrose broth medium (54.75 mg and 13.75 x 106 /ml) followed by Richard's and Oatmeal broth media. Cornmeal and Asthana media were found poor supporter for mycelial growth and sporulation. It can be concluded that PDA and potato dextrose broth medium were best supporters for growth and sporulation of the fungus. Singh *et al.* (2001) observed that potato dextrose agar medium supported better mycelial growth and sporulation of *Alternaria alternata* followed by Richard's and Czapeck's media. Maheshwari *et al*. (2001) found maximum growth and sporulation of *Alternaria alternata* on potato dextrose agar as well as potato broth media followed by oatmeal agar media.

Effect of temperature on mycelial growth and sporulation :

The temperature range for the growth varies for all micro-organisms as well as for host-pathogen interactions. Maximum mycelial growth (88.00 mm) and

sporulation (35.50 x 106 /ml) was observed at 25^{0}C. A gradual decrease in mycelial growth and sporulation was observed at 30^{0}C, 35^{0}C and 40^{0}C. However the temperatures 20^{0}C and 30^{0}C favoured good growth and sporulation of *Alternaria alternata* but differed significantly from the growth at 25^{0}C. No mycelial growth and sporulation were observed at 45^{0}C and 50^{0}C temperatures. It can be concluded that 25^{0}C is the optimum temperature for mycelial growth and sporulation of *Alternaria alternate.* Singh *et al*. (2001) reported that optimum temperature and pH for mycelial growth and sporulation of *A. alternata* were 25^{0}C and 6.5, respectively Maheshwari *et al*. (2000) reported that optimum temperature for mycelial growth and sporulation of *A. alternata* was 28^{0}C (Thaware *et al.*, 2010 and Ginoya and Gohel, 2015).

Effect of relative humidity on mycelial growth and sporulation :

All the six humidity levels induced the growth and

Table 1 : Radial growth and sporulation of *Alternaria alternata* on different solid media

Sr. No.	Medium	Mycelial growth (mm)*	No. of spore/ml (x 10^6)*
1.	Asthana media	44.25 (41.69)	20.25 (26.74)
2.	Corn meal media	50.00 (45.00)	22.50 (28.31)
3.	Czapeck's media	57.50 (49.31)	25.25 (30.16)
4.	Oat meal media	60.25 (50.91)	26.00 (30.65)
5.	Potato dextrose agar	88.00 (69.73)	35.00 (36.27)
6.	Richard's medium	74.75 (59.83)	30.25 (33.36)
	S.E.±	0.73	0.26
	C.D. (P=0.05)	2.24	0.79

* Average of four replications
Figures given in parenthesis are angular transformed values

Table 2 : Dry weight of mycelia and sporulation of *Alternaria alternata* on different liquid media

Sr. No.	Medium	Dry mycelial weight (mg)*	No. of spore/ml (x 10^6)*
1.	Asthana media	20.25 (26.74)	5.75 (13.87)
2.	Corn meal media	26.75 (31.14)	7.25 (15.62)
3.	Czapeck's media	42.00 (40.39)	9.75 (18.19)
4.	Oat meal media	45.75 (42.56)	10.75 (19.13)
5.	Potato dextrose	54.75 (46.57)	13.75 (21.76)
6.	Richard's medium	49.25 (44.57)	12.50 (20.70)
	S.E.±	0.49	0.18
	C.D. (P=0.05)	1.50	0.54

* Average of four replications
Figures given in parenthesis are angular transformed values

Table 3 : Effect of temperature on the mycelial growth and sporulation of *Alternaria alternata in vitro*

Sr. No.	Temperature (^{0}C)	Mycelial growth (mm)*	No. of spore/ml (x 10^6)*
1.	20	41.50 (40.10)	15.75 (23.38)
2.	25	88.00 (69.73)	35.50 (36.57)
3.	30	66.00 (54.33)	23.75 (29.16)
4.	35	49.75 (44.85)	18.25 (25.29)
5.	40	10.25 (18.67)	3.25 (10.38)
6.	45	0.00 (0.00)	0.00 (0.00)
7.	50	0.00 (0.00)	0.00 (0.00)
	S.E.±	0.30	0.15
	C.D. (P=0.05)	0.92	0.47

* Average of four replications
Figures given in parenthesis are angular transformed values

Table 4 : Effect of relative humidity on the mycelial growth and sporulation of *Alternaria alternata*

Sr. No.	Relative humidity (%)	Mycelial growth (mm)*	No. of spore/ml (x 10^6)*
1.	50	42.00 (42.39)	11.25 (19.59)
2.	60	49.25 (44.57)	14.75 (22.58)
3.	70	67.50 (55.24)	25.25 (30.16)
4.	80	80.50 (63.79)	32.50 (34.75)
5.	90	86.00 (68.02)	35.00 (36.27)
6.	100	88.25 (69.95)	37.25 (37.90)
	S.E.±	0.70	0.37
	C.D. (P=0.05)	2.14	1.12

* Average of four replications
Figures given in parenthesis are angular transformed values

Table 5 : Mycelial growth and sporulation of *Alternaria alternata* at different hydrogen ion concentrations

Sr. No.	pH level	Dry mycelial weight (mg)*	No. of spore/ml (x 10^6)*
1.	5.5	67.50 (55.24)	21.00 (27.27)
2.	6.0	72.00 (58.05)	23.00 (28.65)
3.	6.5	95.00 (77.07)	30.50 (33.52)
4.	7.0	91.00 (72.54)	28.75 (32.42)
5.	7.5	80.50 (63.79)	26.00 (30.65)
6.	8.0	76.50 (61.00)	25.25 (30.16)
7.	8.5	62.00 (51.94)	19.75 (26.38)
	S.E.±	0.66	0.29
	C.D. (P=0.05)	2.03	0.88

* Average of four replications
Figures given in parenthesis are angular transformed values

sporulation of *Alternaria alternata*. Maximum mycelial growth (88.25 mm) and sporulation (37.25 x 10^6/ml) were recorded at 100 per cent relative humidity, closely followed by 90 per cent (86.00 mm and 35.00 x 10^6 /ml) RH level. A significant decrease in mycelial growth and sporulation was observed at 80, 70 and 60 per cent humidity. Minimum mycelial growth (42.00 mm) and sporulation (11.25 x 10^6 /ml) was observed at 50 per cent relative humidity. Chen *et al.* (2000) reported that the conidial germination of *Alternaria alternata* was maximum at relative humidity of 90 to 100 per cent. Singh *et al.* (2001) observed that 100 per cent relative humidity was best for maximum growth and number of conidia of *Alternaria tenuissima*.

Effect of hydrogen ion concentrations on mycelial growth and sporulation :

Seven different pH levels ranging from 5.5 to 8.5 with a regular inter val of 0.5 unit were studied on potato dextrose broth medium. The fungus grew on wide range of pH from 5.5 to 8.5. Maximum dry mycelium weight and sporulation were recorded at pH 6.5 (95.00 mg and 30.50 x 10^6/ml). It was closely followed by pH 7.0 and 7.5. The mycelium weight and sporulation harvested from the broth media with pH 6.5 and 7.0 were significantly more than any other pH range tested. Least mycelial growth and sporulation (62.00 mg and 19.75 x 10^6 /ml) was recorded at pH 8.5. Hasija (1970) reported 6.6 as the optimum pH for growth and sporulation of *Alternaria alternata*. Similar observations were also reported by Verma (1970) where pH was 6.6 for maximum growth and sporulation of *Alternaria tenuis* (*Alternaria alternata*). Maheshwari *et al.* (2000) reported that optimum pH for mycelial growth and sporulation of *A. alternata* was 6.5. Dabbas *et al.* (2009) studied on effect of seed dressing chemical on seed germination and seedling infection of mungbean against *Alternaria alternata* and Neeraj and Verma (2010) on biochemical alterations caused by *Alternaria alternata* in *Raphanus sativus* L. var. (Mino Early).

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