The Bioscan 10(4): 1839-1843, 2015 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF DIFFERENT pH, TEMPERATURE AND SOLID MEDIA ON GROWTH OF PURPLE BLOTCH OF ONION CAUSED BY *ALTERNARIA PORRI* ELLIS

N. PRADNYARANI* AND M. S. KULKARNI
Department of Plant Pathology, Kittur Rani Channamma College of Horticulture,
Arabhavi, University of Horticultural Sciences, Bagalkot - 587 104, Karnataka
e-mail: pradnyarani.nidagundi@gmail.com

KEYWORDS
Onion
Alternaria porri
Media
Temperature
pH

Received on :
16.09.2015

Accepted on :
22.12.2015

***Corresponding author**

ABSTRACT
Purple blotch of onion caused by *Alternaria porri* is an important and destructive disease in onion growing areas of Karnataka. *In vitro* studies were conducted to know best solid media, temperature and pH on mycelial growth of *Alternaria porri*. Among the different solid media tested potato dextrose agar showed maximum mycelial growth (83.00 mm) with good sporulation. The maximum growth phase of the fungus was on 12th day of incubation (207.00 mg). The optimum mycelial growth and sporulation was observed at temperature of 28^0 C and no growth and sporulation was observed at 15^0 C. The most suitable pH level for growth was ranged from 5.0 to 7.0.

INTRODUCTION

The Karnataka state has a diverse range of agro-climatic conditions which are highly suitable for growing of onion throughout the year. In the Karnataka, 13.6 thousand hectares area comes under onion cultivation with productivity of 15.1 metric tonnes per hectare. Onion is an extremely important vegetable crop not only for internal consumption but also as highest foreign exchange earner among the fruits and vegetables.

Crop failure with low productivity is due to the threat posed by many pest and diseases. Among the diseases, purple blotch caused by *Alternaria porri* is the major constraint to onion bulb and seed production. The disease is found in almost all the major onion growing areas of Karnataka. The incidence of purple blotch is more serious in *kharif* season (disease incidence of 62%) than in *Rabi* season (disease incidence 38%) (Quadri *et al*., 1982).

In order to culture *Alternaria porri* in the laboratory, it is necessary to furnish essential elements and compounds in the medium for their growth and other life processes. Not all media are not good for growth of *Alternaria porri*, nor there is a universal substrate or artificial media upon which the fungi can grow. So the media which provided good growth and sporulation was evaluated. Temperature has profound effect on the growth and sporulation of *Alternaria porri*. Optimum temperature favours the growth and development of *Alternaria porri*. Hydrogen ion concentration of the medium can affect the rate and the amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951).

Hence the present investigations were taken under *in vitro* conditions to know the growth and morphological characteristics of the pathogen using different synthetic and non synthetic solid media and pH.

MATERIALS AND METHODS

An *in vitro* experiment was conducted during 2013 at KRC College of Arabhavi to find out the suitable different solid media with growth phase, temperature and pH of *Alternaria porri*. Experiment was designed in complete randomized design (CRD).

Growth on solid media

The growth of the fungus on solid media was studied on ten different media *viz.,* Asthana and Hawker's agar, Corn meal agar, Czapek's Dox agar, Malt extract agar, Oat meal agar, Potato dextrose agar, Richards's agar, Sabouraud's agar, Yeast dextrose agar and yeast malt agar. Fifteen ml of each of the medium was poured to each petriplates separately. Such petriplates were aseptically inoculated with 5 mm disc cut-outs from periphery of the seven day old culture and incubated at 28^0 C for a period of seven days at the end of which observations were recorded. Each treatment was replicated thrice following completely randomized design. The colony diameter was recorded. The data on radial growth was analyzed statistically (Ainsworth, 1971 and Tuite, 1969).

All the above mentioned media were dissolved in 400 mL distilled water and agar-agar is dissolved in 400 ml distilled water separately. Both the solutions were mixed thoroughly and the volume was made up to 1000 ml with distilled water and sterilized at 15 lbs for 15 minutes.

Growth phase

Twenty ml of potato dextrose broth was pipette into each of the 100 ml conical flasks. After sterilization these flasks were inoculated with 5 mm disc of eight day pure culture and incubated at room temperature 28° C ± 1° C. A set of three flasks were harvested every 48 hr starting from two days of inoculation. Cultures were filtered through Whatman No. 42 filter paper which was previously dried to a constant weight in hot air oven at 60° C. The mycelial mat on the filter paper was thoroughly washed with distilled water to leach out any salts associated with the mycelium. Subsequently, the filter papers along with mycelial mat were dried to constant weight cooled in a desiccator and weighed on electronic balance. The data were analyzed statistically (Lilly and Barnett, 1951).

Temperature studies

Different temperature ranges were tried for growth and sporulation of the pathogen at *viz*., 15, 20, 22, 25, 28, 30 and 35°C. Twenty ml of sterilized PDA media was dispensed in 90 mm diameter petridishes and incubated aseptically with 5 mm disc of the pathogen from a seven days old culture. Petridishes were incubated at different temperatures and each treatment was replicated three times following completely randomized design. At the end of eight days of incubation, observations on colony diameter and sporulation were recorded (Ramjegathesh and Ebenezar, 2012).

Hydrogen ion concentration (pH) studies

Potato dextrose broth was used as a basal medium. pH of the liquid medium was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). The pH of the medium used was 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The culture was inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated at 28°C for ten days. Four replications were maintained in each treatment. Dry mycelial weight of the fungus was recorded. Results were analyzed statistically (Ramjegathesh and Ebenezar, 2012).

RESULTS

The result of the present study on temperature, pH and different solid media with growth phase has been presented under following sub heads

Growth on solid media

The growth performance of *Alternaria porri* was studied on ten different solid media as described in "Materials and Methods". The aim of the study was to know the best medium for the growth of the fungus. The radial growth of the fungus was measured. The results are presented in the Table 1.

There was significant difference with respect to growth of *Alternaria porri* between solid media used for cultural studies. The growth of fungus covered entire area of petriplates after twelve days of incubation in potato dextrose agar. On seventh day of incubation the maximum radial growth was observed on yeast dextrose agar (70.00 mm) followed by Asthana and Hawker's media (65.33 mm), but on twelfth day of incubation the maximum radial growth was observed on potato dextrose agar (83.00 mm) followed by Asthana and Hawker's media (82.33 mm) and yeast dextrose agar (77.67 mm). The growth of the fungus on seventh and twelve days were least in Richard's agar (42.33 mm & 61.00 mm) and it was on par with growth on yeast malt agar on seventh day of incubation (43.67 mm). The results indicated that the fungus grew well on both synthetic and non-synthetic media. Growth on potato dextrose agar was initially white and later turned to brownish black in colour. It was smooth and with distinct ring. Rings were distinct in all the media except Richard's agar and colony was white in colour. Margin was smooth in most of the media *viz*., yeast dextrose agar, corn meal agar, potato dextrose agar, malt extract agar, yeast malt agar, oat meal agar, Sabouraud's agar and Czapeck's agar. The colour on the growth of the fungus appeared as white at initial days of incubation later became light greyish in colour. The mycelial growth was fluffy in malt extract agar, yeast malt agar and Richard's agar and it was flat in yeast dextrose agar, Ashtana and Hawker's media, Sabouraud's agar and Czapeck's agar.

However, two methods of growth measurements namely, radial growth of the fungus on solid media and dry mycelial weight in liquid media were employed in present studies. Among the solid media, maximum radial growth of the fungus was observed on potato dextrose agar (83.00 mm) followed by Asthana and Hawker's media (82.33 mm) and yeast dextrose agar (77.67 mm). This could be because of the fact that these media may be having some organic substances, which other media may be lacking. The least growth was observed in Richard's agar (61.00 mm).

Maximum sporulation was observed on potato dextrose agar and Asthana and Hawker's media and the sporulation was moderate on rest of the media except Richard's agar and Czapeck's agar. The cultural characters of *Alternaria porri* on different solid media after 12 days of incubation and are presented in Table 2.

Growth phase

This experiment was conducted as explained in "Materials and Methods" to know the period when the fungus attains

Table 1: Studies on growth of *Alternaria porri* on ten different solid media

Media	Colony diameter (mm) Growth on 7th day	Growth on 12th day
Yeast Dextrose Agar	70.00	77.67
Asthana and Hawker's media	65.33	82.33
Corn Meal Agar	61.33	76.33
Potato Dextrose Agar	64.33	83.00
Malt Extract Agar	53.67	66.00
Richard's Agar	42.33	61.00
Oat Meal Agar	47.67	67.00
Czapeck's Agar	60.67	74.67
Yeast Malt Agar	43.67	68.00
Sabouraud's Agar	52.00	67.33
S.Em ±	0.72	1.03
CD at 1%	2.16	3.03
CV	2.23	2.46

Table 2: Studies on cultural characteristics of *Alternaria porri* on different solid media after 12 days of incubation

Medium	Growth character	Sporulation
Yeast Dextrose Agar	Moderate growth, smooth margin, rings distinct and light greyish colony	+ +
Asthana and Hawker's media	Slow growth, irregular margin, whitish to light grey colony and rings indistinct	+ + +
Corn Meal Agar	Circular, smooth, ring's distinct and light greyish colony	+ +
Potato Dextrose Agar	Good growth, smooth margin, rings distinct and light greyish colony	+ + + +
Malt Extract Agar	Slow growth, smooth, circular and ring's distinct	+ +
Yeast Malt Agar	Slow growth, smooth, ring's lightly distinct and white to light greyish colour	+ +
Oat Meal Agar	Moderate growth, smooth margin and light greyish colony	+ +
Richard's Agar	Slow growth, ring's indistinct, irregular and white mycelial growth	+
Sabouraud's Agar	Slow growth, circular, ring's lightly distinct and light greyish to white	+ +
Czapeck's Agar	Moderate growth, circular, ring's distinct and light greyish colour	+

+ + + + = >75 conidia per microscopic field; + + + = 50-75 conidia per microscopic field; + + = 25-50 conidia per microscopic field; + = 1-25 conidia per microscopic field.

Table 3: Studies on dry mycelial weight of *Alternaria porri* in potato dextrose broth

Sl. No.	Days after inoculation	Mean dry mycelial weight (mg)
1	2	85.63
2	4	103.02
3	6	148.54
4	8	179.56
5	10	198.20
6	12	207.00
7	14	197.14
8	16	179.15
9	18	154.37
10	20	131.21
Mean		158.40
S. Em ±		1.60
C. D at 1 %		4.72
CV		1.75

Table 4: Effect of temperature on the growth and sporulation of *Alternaria porri*

Temperature (°C)	Colony diameter (mm) 8th day	15th day	Sporulation at 15th day
15	0.00	0.00	-
20	26.7	62.7	+ +
22	27.7	74.3	+ +
25	29.3	74.3	+ + +
28	41.7	89.0	+ + + +
30	30.0	74.3	+ + +
35	28.3	59.0	+ +
S.Em ±	0.13	0.16	
CD at 1%	0.40	0.50	
CV	8.72	4.58	

maximum growth. The fungus was grown on potato dextrose broth and dry mycelial weights were recorded at two days interval from the second day to twentieth day after inoculation. The results are presented in Table 3.

There was significant difference among the different treatments. The growth of the fungus was maximum (207.00 mg) on 12th day of incubation, followed by weight on 10th day (198.20 mg). The mycelial growth of the fungus increased with increase in number of days of incubation and reached its peak on 12th day and thereafter decreased drastically. Therefore, 12th day incubation was taken as the maximum growth period.

The experiment in the growth phase was conducted to find out the number of days required to obtain maximum growth of the fungus. The results indicated that, the growth increased with number of days of incubation and reached its peak on 12th day (207.00 mg) and thereafter it decreased. The decline in growth after 12 days of incubation might be due to autolysis of the fungus.

Temperature studies

In the present investigation the effect of different temperature levels on the growth of *Alternaria porri* was studied as explained in "Materials and Methods". Data presented in Table 4.

Effect the growth of the fungus differed significantly with temperature levels. The maximum radial growth on eight days and fifteen days (4.17 cm and 8.90 cm, respectively) was noticed at 28°C, followed by 25° C and 30° C (7.43 cm and 7.43 cm) after fifteen days of incubation. The next best temperature level was 20° C (6.27 cm) after fifteen days of incubation. The least growth was recorded at 15° C (0.00 cm).

Even though there was good sporulation at the temperature of 28° C (+ + + + - >75 conidia per microscopic field) followed by 25° C and 30° C (+ + + - 50-75 conidia per microscopic field). The growth of fungus was on par in these treatments. No sporulation was observed at 15° C. As the temperature increased the sporulation decreased initially up to 28° C and even with the decrease in temperature the sporulation was also decreased. Hence optimum temperature is required for good growth and sporulation of the fungus lies between 25° C to 30° C.

Temperature affects almost every function of the fungi and each fungus has its temperature range for growth and sporulation. In the present investigation, maximum mycelial growth (8.90cm) of the fungus was recorded at 28° C and this was followed by 25° C (7.43 cm) and 30° C (7.43 cm), indicating optimum temperature range to be 25° C to 30° C and least growth was obtained at 35° C (5.90 cm) and 15° C (0.00 cm).

pH studies

In the present experiment, effect of different levels of pH on the growth of *Alternaria porri* was studied at different pH levels as explained in "Materials and Methods". Data presented in Table 5.

Maximum growth of the fungus with highest mean dry weight of mycelium was observed at the pH range of 6 and 7 (1.37

Table 5: Effect of pH on the growth of *Alternaria porri* in potato dextrose broth

pH	Mean dry weight of mycelium (mg)	Sporulation
4	1.33	+
5	1.34	+ + +
6	1.37	+ +
7	1.37	+ +
8	1.33	+
9	1.32	+
S.Em ±	0.01	
CD at 1%	0.03	
CV	1.22	

mg and 1.37 mg, respectively) followed by pH range of 5 (1.34 mg). The growth of the fungus decreased considerably at pH range beyond 7.0. Good sporulation was observed at the pH range of 5.0 and decreased with increase in the pH range.

Hydrogen ion concentration of the medium has a profound effect upon the rate and the amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951). The fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This shows importance of hydrogen ion concentration for the better fungal growth. Maximum fungal growth was observed in broth adjusted to pH 6 and 7, where in it has shown equal growth in both the ranges. The growth was poor at pH 9.0 (+). The fungus did not grow at highly acidic levels of pH 4.0 (+) and below. Growth and sporulation of *Alternaria porri* was found to be maximum at P^H 5.0 (+ + +)

DISCUSSION

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture fungus in the laboratory, it is necessary to furnish essential elements and compounds in the medium for their growth and other life processes. Not all media are not good for all fungi, nor there is a universal substrate or artificial media upon which all fungi can grow. So, different media including both synthetic and non-synthetic were tried for *Alternaria porri* in the present investigation. Growth is determined by the methods of measurement selected. Some methods are useful only for particular organisms or for special problems. No method is general, that could be recommended for all (Cochrane, 1958). However, two methods of growth measurements namely, radial growth of the fungus on solid media and dry mycelial weight in liquid media were employed in present studies. Among the solid media, maximum radial growth of the fungus was observed on potato dextrose agar (83.00 mm). The results obtained are in agreement with Shivakumar (1999) and Chethana (2000), followed by Asthana and Hawker's media (82.33 mm) and yeast dextrose agar (77.67 mm). This could be because of the fact that these media may be having some organic substances, which other media may be lacking. The results obtained are in agreement with earlier studies by Nolla (1927) and Sitarama and Mehta (1982). The least growth was observed in Richard's agar (61.00 mm).

The experiment in the growth phase was conducted to find out the number of days required to obtain maximum growth of the fungus. The results indicated that, the growth increased with number of days of incubation and reached its peak on 12th day (207.00 mg) and thereafter it decreased. These results are in agreement with Shivakumar (1999). The decline in growth after 12 days of incubation might be due to autolysis of the fungus. In general non-synthetic media supported the better growth than synthetic media, which is in agreement with Hanumanthaiah (1976).

Temperature affects almost every function of the fungi and each fungus has its temperature range for growth and sporulation. In the present investigation, maximum mycelial growth (8.90cm) of the fungus was recorded at 28ºC which is in conformity with the results obtained by Ramjegathesh and Ebenezar (2012) and this was followed by 25ºC (7.43 cm) and 30ºC (7.43 cm), indicating optimum temperature range to be 25ºC to 30ºC and least growth was obtained at 35ºC (5.90 cm) and 15ºC (0.00 cm). Cochrane (1958) stated that, most fungi made atleast some growth between 25ºC and 30ºC. These results are in agreement with findings of Taber *et al*. (1968) for *Alternaria raphani*, Verma (1970) for *Alternaria solani*. Similarly, Hanumanthaiah (1976) in *Alternaria tenuissima*. Maximum conidial germination was observed at 25ºC (Datar, 1994). Ramteke and Kamble (2011) reported that growth of *Fusarium solani* was good at 20ºC and 30ºC and it was relatively low at 10ºC and 35ºC.

Hydrogen ion concentration of the medium has a profound effect upon the rate and the amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951). The fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This shows importance of hydrogen ion concentration for the better fungal growth. Maximum fungal growth was observed in broth adjusted to pH 6 and 7, where in it has shown equal growth in both the ranges. The growth was poor at pH 9.0 (+). The fungus did not grow at highly acidic levels of pH 4.0 (+) and below. Growth and sporulation of *Alternaria porri* was found to be maximum at pH 5.0 (+ + +) which is in conformity with the report of Ramamohan and Vijaylakshmi (2000), Madhavi *et al*. (2012) and Ramjegathesh and Ebenezar (2012). Jaruhar and Prasad (2011) reported that pH level 6.0 was optimum for growth as well as the sporulation of the *Fusarium oxysporum* sclecht. f. sp. *lentis*. sporulation of chlamydospores was however found best in the pH level 4.0.

REFERENCES

Ainsworth, G. C. 1971. Dictionary of fungi by Ainsworth and Bisby's. Common Wealth *Mycological Institute, Ferrylane, Kew Surrey, UK*, p. 663.

Ajrekar, S. L. 1922. Report of the work done under the plant pathologist to the government of Bombay, Poona, for the year 1920-21, Bombay, Department of Agriculture *Annual Report* 1920-21, pp. 121-122.

Chethana, B. S. 2000. Studies on *Alternaria* leaf blight of onion (*Allium cepa*. L.). *Karnataka. J. Agril. Sci.* p. 242.

Cochrane, V. W. 1958. Physiology of fungi. *J. Wiley and Sons Inc., New York*, p. 524.

Cooke, M. C. and Ellis, J. B. 1879. New jersy fungi, *Grevillea*, **8**: 11-16.

Datar, V. V. 1994. Investigation on purple blotch of onion in India. *Acta Horticulture*. **358:** 259-263.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. *CMI, Kew*, Boco, Surrey, *England*.

Hanumanthaiah, B. 1976. Studies on leaf spot disease of redgram (*Cajanus cajan* (L.) Mill Sp.) caused by *Alternaria tenuissima* (Fr.) Wiltshire, Karnataka. M. Sc. (Agri.) Thesis. *University of Agricultural Sciences, Banglore*. p. 68.

Jaruhar, H. B. and Prasad, A. 2011. Effect of different pH levels on the growth and sporulation of *Fusarium oxysporum* schlecht. F. sp. *lentis* (Vasudeva and srinivasan) the causal organism of wilt disease of lentil. *The Bioscan*. **6(1):** 289-219.

Lilly, V. G. and Barnett, H. L. 1951. Physiology of Fungi. *Mc Graw Hill Book Co. Inc., London, UK.,* pp. 30-80.

Madhavi, M., Kavitha, A. and Vijayalakshmi, M. 2012. Studies on *Alternaria porri* (Ellis) Ciferri pathogenic to onion (*Allium cepa* L.). *Arch. Appl. Sci. Res*. **4(1):** 1-9.

Nolla, J. A. B. 1927. A new *Alternaria* disease of onions (*Allium cepa* L.). *Phytopathology*. **17:** 115-132.

Ponnappa, K. M. 1970. Leaf blight of onions (*Allium cepa* L.) caused by *Alternaria cepulae* Ponnappa and Deshpande sp. nov.I. Comparative morphology and physiology. *Nova Hewigia*. **47:** 547-564.

Ramamohan, N. and Vijayalakshmi, M. 2000. Studies on *Alternaria porri* pathogenic to onion. *Microbiol. Res*. **155:** 129-131.

Ramjegathesh, R. and Ebenezar, E. G. 2012. Morphological and physiological characters of *Alternaria alternata* causing leaf blight disease of onion. *Int. J. Pl. Pathol*. **3:** 34-44.

Ramteke, P. K and Kamble, S. S. 2011. Physiological studies in *Fusarium solani* causing rhizome rot of ginger (*Zinger officinale* Rosc.). *The Bioscan.* **6(2):** 195- 197.

Shivakumar. 1999. Studies on leaf blight of onion (*Allium cepa* var. *cepa* Linn.) caused by *Alternaria porri* (Ellis) Cif. *Karnataka. J. Agril. Sci*. **2:** 304.

Sitarama, R. and Mehta, B. K. 1982. Certain nutritional aspects of *Alternaria porri* on onion. *Indian. J. Mycol. Pl. Pathol*. **12:** 96-98.

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

Tuite, J. 1969. Plant pathological methods; fungi and bacteria. *Burgess Publishing Co., Minneapolis, USA*, pp. 1-238.

Verma, V. 1970. Effect of temperature and hydrogen-ion concentration on three pathogenic fungi. *Sydowia*. **23:** 164-168.