Original Research Article

Nutritional and Physiological Requirement of *Alternaria porri* (Ellis) Cif Causing Purple blotch of Onion

ABSTRACT

An experiment was carried out to study the nutritional requirement and response of different physiological parameters viz., temperature and pH on mycelial growth of different isolates of *Alternaria porri* (Ellis) Cif causing purple blotch of onion. The five different pH levels, temperature, carbon sources and nitrogen sources were tested for their effect on mycelial growth and sporulation of *A. porri*. The results showed that the maximum mycelial growth and sporulation of *A. porri* was observed at pH 7.0 and minimum at pH 9.0. In temperature range the maximum mycelial growth and sporulation was recorded at 30 ^{o}C and minimum at 10 ^{o}. The mycelial growth of *A. porri* was different on Czapek's dox agar basal medium with different carbon sources. Maximum radial growth was observed on maltose followed by sucrose and minimum on dextrose maltose. The mycelial growth of *A. porri* grown on varied Czapek's dox agar basal medium with different nitrogen sources. Maximum mycelial growth and sporulation was observed on potassium nitrate followed by sodium nitrate and minimum on urea, respectively.

***Keywords*:** Onion, Purple blotch, *Alternaria porri*, Temperature, pH, Media

Introduction

Onion (*Allium cepa* L., 2n=16), a bulbous, biennial herb is the most important and one of the five most important fresh market vegetables worldwide (Cramer, 2000). Among vegetables, onion often called as "queen of kitchen" is one of the oldest known and an important crop grown in India. It contains the lachrymatory principle, a strong antibiotic, having fungicidal, bacterial and nematidicial properties (Yadav *et al.,* 2017). It also contains chemical compounds with potential anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties, such as

quercetin (Slimestad *et al.,* 2007). It is also of high medicinal value in controlling human and plant diseases (Wanggikar *et al.*, 2014).

Several factors have been identified for the low productivity of onion in India. The most important factors responsible are the diseases like purple blotch, downy mildew, Stemphylium blight, basal rot and storage rots and non-availability of varieties resistant to biotic and abiotic stresses (Ravichandran *et al.,* 2017). Among the foliar diseases, purple blotch is one of the most destructive diseases, commonly prevailing in almost all onion growing pockets of the world, which causes heavy loss in onions under field conditions. For the first time the purple blotch of onion caused by *Alternaria cepulae* was observed by Ponnappa (1970) in Karnataka. This was not recognized as a major foliar and inflorescence disease until recently, however now a day it is one of the important diseases. The name "Purple blotch" for this disease was proposed by Nolla (1927). He named the causal organism as *Alternaria alli* which was later amended as *Alternaria porri*.

The present study was undertaken to understand the physiological conditions required for the growth and sporulation of the pathogens associated with purple blotch complex. The identification of suitable culture medium, pH levels and host substrate for the growth and sporulation of the pathogens would aid in preparation of inoculum required for creation of artificial epiphytotic conditions and thus, would be instrumental in disease resistance breeding as well as evaluation of fungicides. The study would be useful in devising promising strategy for the integrated management of *A. porri* singly as well as in complex.

Materials and Methods

The experiment was conducted at Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner (Rajasthan). Naturally infected onion leaves showing typical well-developed purple blotch symptoms were collected from Agricultural Research Station, Bikaner and brought to laboratory. The infected leaves were thoroughly washed with tap water and then immediately examined under a compound microscope for preliminary identification of the pathogen. Isolation of the fungus was made by tissue isolation technique. Typical diseased spots on leaves were selected and cut into small bits with the help of a sterilized blade. These bits of diseased tissues were washed with sterilized distilled water and disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 to 60 seconds. These disinfected bits were immediately washed thrice with sterilized distilled water to remove excess mercuric chloride. These bits were placed on the

surface of Petri plates containing potato dextrose agar (PDA) and incubated at 27±1℃ for 10 days. The resulting fungus culture was purified by hyphal tip technique in PDA slant. The pure culture was maintained on PDA by storing it under refrigeration at 5℃ and making periodical transfers at every forthnight

Effect of hydrogen ion concentration (pH)

The study of different pH levels was undertaken with a view to ascertain the effect of different hydrogen ion concentration of the medium on growth of the fungus. The initial pH of the basal medium before autoclaving was adjusted from 5.0 to 9.0 with a difference of 0.5 using N/10 NaOH or N/10 HCl. After autoclaving, the pH was again tested. The inoculated Petri plates were incubated at 28±2^{0}C for 7 days.

Effect of temperature

It is a well-known phenomenon that the temperature yields considerable influence on the biochemical activity of pathogens. 20 ml of PDA was poured in each of sterilized Petri dish. Each Petri dish was inoculated aseptically by placing in the center a 5 mm disc from actively growing 7 days old culture on PDA. The inoculated Petri dishes were incubated at 10, 15, 20, 25, 30, 35 and 40℃ temperature for 7 days.

Effect of carbon sources

To find out the effect of various carbon sources on growth of *Alternaria porri*, the sucrose content of basal medium Czapek's dox agar was substituted by adding different sources of carbon on equivalent basis (12.63 g in 30 g of sucrose). Inoculated Petri dishes containing basal medium supplemented with different carbon sources were incubated at 28±2°C for 7 days and the mycelial growth was recorded. Carbon sources used were: glucose ($C_6H_{12}O_6$), dextrose ($C_6H_{12}O_6$), fructose ($C_5H_{12}O_6$), maltose ($C_{12}H_{22}O_{11}$) and sucrose ($C_{12}H_{22}O_{11}$) as control.

Effect of nitrogen sources

To find out the effect of various nitrogen sources on growth of *Alternaria porri*, sodium nitrate of basal medium Czapek's dox agar medium was substituted by adding different sources

of nitrogen on equivalent basis (329 mg in 2 g of sodium nitrate). The inoculated Petri dishes containing basal medium supplemented with different nitrogen sources were incubated at 28±2°C for 7 days and observation for mycelial growth of each isolate was recorded. Nitrogen sources were studied: urea, ammonium chloride, potassium nitrate, ammonium nitrate and sodium nitrate as control.

Result and discussion

Effect of hydrogen ion concentration (pH)

In present studies maximum mycelial growth was observed at pH 7.0 and minimum mycelial growth was observed at pH 9.0 (Table 1 and Fig 1). The results are in accordance with the results found by Hubbali (2010). Khare *et al.* (2012) concluded that the best growth and sporulation occurred at pH 6 and pH 7. Poor growth with less spores was observed at pH 5 and very little growth and no sporulation at pH 9.0.

Effect of temperature

Temperature yields considerable effect on growth of fungal organisms (Table 2 and Fig. 2). In the present studies increased mycelial growth of *A. porri* have been observed from 10-40^{0}C. Maximum growth was observed at 10^{0}C and minimum mycelial growth was observed at 20℃. These results show the maximum growth at 30℃, which are in total conformity with the results obtained by Singh & Prasada (1972), they reported that 30^{0}C as the optimum temperature for mycelial growth of *A. porri* followed by 20 and 25℃.

Effect of carbon sources

The mycelial growth of *Alternaria porri* was found different, when grown on Czapek's dox aga basal medium with different carbon sources (Table 3 and Fig. 3). Maximum mycelial growth of *A.porri* was observed on maltose followed by sucrose, fructose and starch compared to dextroses as control which are very similar and conformity with the result concluded by Ramjegathesh and Ebenezar 2012), who recorded that maximum mycelial growth was found in maltose, followed by sucrose and fructose, while the dextrose had the minimum mycelial growth as compared to control.

Effect of nitrogen sources

Similarly, mycelial growth of *Alternaria porri* varied while grown on Czapek's dox agar basal medium provided with different nitrogen bases (Table 4 and Fig. 4). *A. porri* had significantly higher growth on nitrogen sources *viz.*, potassium nitrate, followed by ammonium nitrate and ammonium chloride. Among all the tested nitrogen sources urea was found as least supportive to the mycelial growth of *A. porri.* The present findings are in line with the studies of Ramjegathesh and Ebenezar (2011), who concluded that potassium nitrate produced maximum mean mycelial growth (8.07 cm) followed by sodium nitrate (7.38 cm), while urea had minimum mean mycelial growth in *A.porri.*

Conclusion

The mycelial growth of *A. porri* grown on varied Czapek's dox agar basal medium with different nitrogen sources. Maximum mycelial growth and sporulation was observed on potassium nitrate followed by sodium nitrate and minimum on urea, respectively.

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Table 1 Effect of different pH levels on mycelial growth and sporulation of *Alternaria porri*

pH	Mycelial growth (mm)*	Sporulation
5.0	51.31	++
6.0	72.14	+++
7.0	85.74	++++
8 .0	70.68	++
9.0	47.64	+
S Em (±)	0.82	
CD (P = 0.05)	2.47	
CV (%)	2.50	

***** Mean of 4 replications

+:poor, ++:fair, +++:good, ++++:excellent

Table 2 Effect of different temperature on mycelial growth and sporulation of *Alternaria porri*

Temperature (℃)	Mycelial growth (mm)	Sporulation
10	21.76*	+
15	46.03	++
20	66.50	++
25	81.03	+++
30	87.40	++++
35	59.70	+++
40	25.23	++
S.Em (±)	0.60	
CD (P=0.05)	1.81	
CV (%)	2.17	

***** Mean of four replications

+:Poor, ++:Fair,+++: Good,++++: Excellent

Table 3. Effect of different carbon sources on mycelial growth and sporulation of *Alternaria porri*

Carbon Source	Mycelial growth (mm)	Sporulation
Dextrose	73.85*	+++
Maltose	88.26	++++
Sucrose	82.15	++++
Starch	77.33	+++
Fructose	77.98	+++
S.Em (±)	0.47	
CD (P=0.05)	1.43	
CV (%)	1.19	

*Mean of four replications
+:Poor, ++:Fair, +++:Good, ++++:Excellent

Table 4. Effect of different nitrogen sources on mycelial growth and sporulation of *Alternaria porri*

Nitrogen source	Mycelial growth (mm)	Sporulation
Peptone	62.83*	++
Urea	31.90	+
Ammonium chloride	48.38	++
Potassium nitrate	80.75	++++
Ammonium nitrate	37.33	+
Sodium nitrate	73.88	+++
S.Em(±)	0.65	
CD (P=0.05)	1.96	
CV (%)	2.26	

*Mean of four replications
+: Poor ++: Fair +++: Good ++++: Excellent

Fig. 1: Effect of different pH levels on mycelial growth and sporulation of *Alternaria porri*

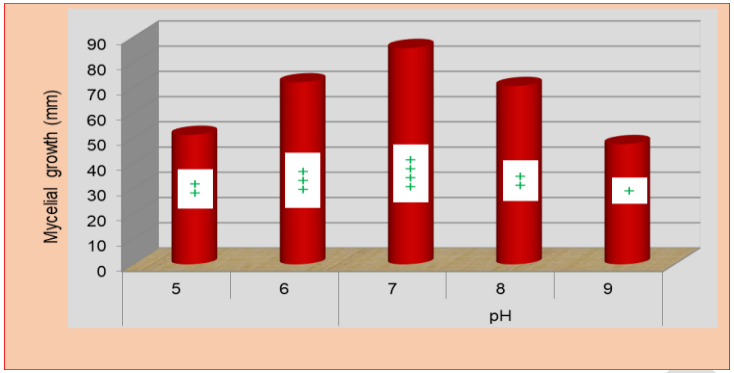
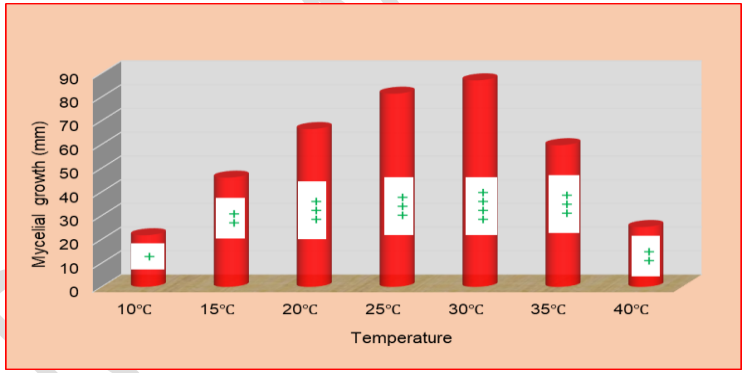


Fig. 2: Effect of different temperature on mycelial growth and sporulation of *Alternaria porri*



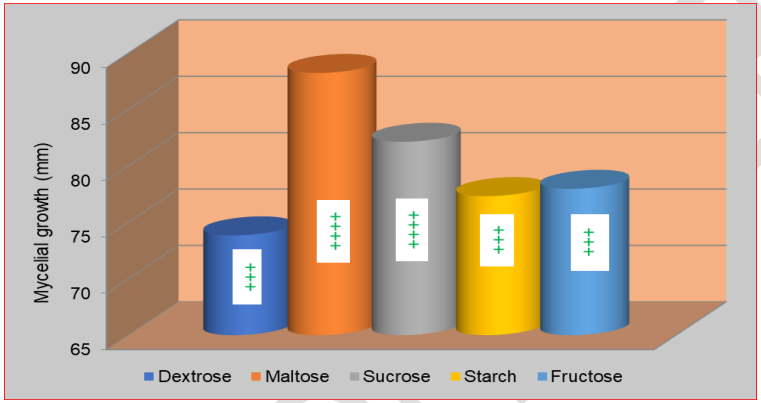


Fig. 3: Effect of different carbon sources on mycelial growth and sporulation of *Alternaria porri*

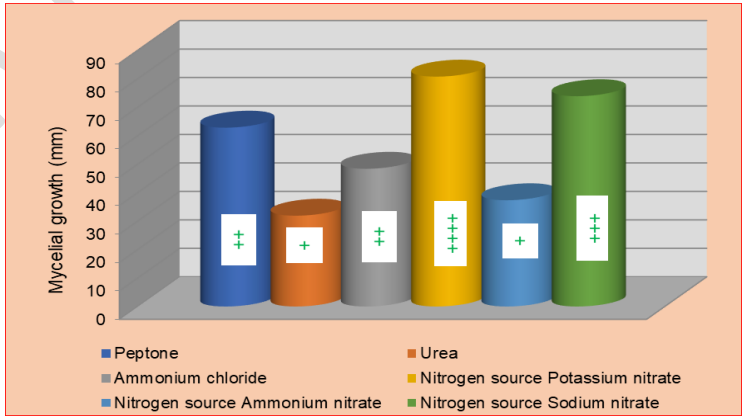


Fig. 4: Effect of different nitrogen sources on mycelial growth and sporulation of *Alternaria porri*