Physiological studies on *Alternaria porri* caused purple blotch of onion under *In-vitro* conditions

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Summary

The effect of nutrient media, temperature, pH level, carbon and nitrogen ion concentration were studied on mycelial growth and sporulation of *Alternaria porri* caused purple blotch of onion. The investigations revealed that Potato dextrose agar was the best culture medium for *A. porri*. The maximum mycelial growth of *A. porri* was recorded on 30°C temperature (85.74 mm) and pH 7.0 (83.40 mm). *A. porri* grew significantly better response to the source of carbon nutrient media on mycelial growth and observed the maximum mycelial growth on maltose (88.26 mm) based medium with highest sporulation and potassium nitrate-based media source of nitrogen gave maximum mycelial growth (80.75 mm) and sporulation of *A. porri.* The present findings are useful for the preparation of inoculums required for resistance breeding and fungicidal evaluation against pathogen *A. porri.*

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Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crops grown throughout the world. Among the diseases, purple blotch caused by *Alternaria porri* (Ellis) Cif. is the major disease of onion worldwide affecting the foliage severely resulting in crop loss ranging from 30 to 100 per cent both in seed and bulb crop from year to year and are more prevalent in a warm and humid environment (Gupta & Pathak 1988; Upmanyu & Sharma 2007). The disease was recorded as more severe on seed crop as compared to bulb crop (Qadri et al. 1982; Gupta & Pathak 1988) causing sometimes 100 per cent loss of the seed production (Singh et al. 1992; Haldhar et al. 2017). Studies were conducted on the cultural and physiological variability of *A. porri* to understand the nature of the pathogen and found the most significant growth of mycelial in Czapeck's agar (Priya et al. 2018). The effect of different culture media, pH levels and natural substrates on mycelial growth and sporulation of *A. porri* was studied by Yadav et al. 2017. The effect of nutrient media, temperature, hydrogen ion concentration and photoperiod were studied under in vitro conditions to record the maximum colony growth and spore count (Tahira et al. 2019; Maheshwari et al. 2022). The present study was undertaken to understand the physiological conditions required for the growth and sporulation of the pathogens associated with purple blotch. The identification of suitable temperature, pH levels and host substrate for the growth and sporulation of the pathogens would aid in the preparation of inoculum required for the 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 a promising strategy for the preparation of inoculums required for resistance breeding and fungicidal evaluation against pathogen *A. porri*.

Material and Methods

Isolation and purification of *A. porri*

Isolations of the pathogens were made from the diseased leaf tissue of onion collected from the research area. Typical diseased spots on the leaves were selected and cut into bits of about 1 to 1.5 mm with the help of a sterilized scalpel, washed with sterilized distilled water and disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) solution (30 to 60 seconds). These disinfected bits were immediately rinsed in double sterilised distilled water repeatedly to remove the traces of mercuric chloride and toweled on sterilised filter paper, before their being aseptically transferred to Petri plates containing 20 ml of autoclaved potato dextrose agar (PDA) in a laminar flow and incubated at 28±2°C in BOD incubator for 7 d. The resulting fungal culture was purified by hyphal tip technique in PDA slant for *A. porri.*

Effect of different media on growth and sporulation of *A. porri*

Six different semi-solid media viz., Potato dextrose agar, Onion leaf extract agar, Carrot onion leaf extract agar, V8 juice agar, Carrot dextrose agar and Czapek's dox agar were used to study the growth and sporulation of *A. porri*. Twenty ml of each sterilized medium was aseptically poured into the sterilized Petri plates in a laminar flow. The actively growing 5 mm mycelial disc was cut with the help of a sterilized cork borer from 7 d old culture of *A. porri* raised on PDA and the Petri plate was seeded with an actively growing 5 mm mycelial disc of *A. porri* with the help of a sterilized inoculating needle. Four replications were kept in each treatment under a completely randomized design. The Petri plates were incubated at 28±2°C maintained in a BOD incubator.

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Effect of different pH levels on growth and sporulation of *A. porri*

To determine the optimum level of pH for growth of *A. porri*, 100 ml of potato dextrose agar was dispensed in Erlenmeyer flasks of 250 ml capacity, and the different pH levels of 5.0, 6.0, 7.0, 8.0 and 9.0 were adjusted with the help of digital pH meter by adding 0.1 N HCl (hydrochloric acid) or NaOH (sodium hydroxide) solutions. The flasks were plugged with non-absorbent cotton wrapped in muslin cloth and sterilized in an autoclave at 121°C temperature and 15 psi pressure for 20 min. After autoclaving, when the medium was lukewarm, 20 ml of medium was poured into the Petri plates under aseptic conditions in the laminar airflow. The Petri plates were inoculated with 5 mm mycelial discs of each fungus cut with the help of sterilized cork borer from 7 d old culture raised on PDA. Four replications were kept for each treatment under a completely randomized design. The inoculated Petri dishes were incubated at 28±2°C maintained in a BOD incubator.

Effect of different temperatures on growth and sporulation of *A. porri*

To determine the optimum level of temperature for the growth of *A. porri*, 20 ml of PDA was poured in each sterilized Petri dish. Each Petri dish was inoculated aseptically by placing in the centre a 5 mm disc from actively growing 7 days culture on PDA. The inoculated Petri dishes were incubated at 10°C to 40°C with a difference of 5°C for 7 days maintained in a BOD incubator.

Effect of carbon and nitrogen source for the mass multiplication of *A. porri*

To find out the effect of various carbon sources on the growth of *A. porri*, the sucrose content of basal medium Potato dextrose agar was substituted by adding different sources of carbon on an 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 ($C_6H_{12}O_6$), maltose ($C_{12}H_{22}O_{11}$) and sucrose ($C_{12}H_2 2O_{11}$). To find out the effect of various nitrogen sources on the growth of *A. porri*, sodium nitrate of basal medium Potato dextrose agar medium was substituted by adding different sources of nitrogen on an 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.

Observation of the mycelial growth and sporulation of *A. porri*

The observations for colony growth of the pathogen were recorded after every 24 h of incubation for 7 consecutive days and the actively growing mycelium was categorized based on visual observations. Sporulation of pathogen was observed from 7 days culture of each treatment by making the spore suspension. A single block of 5 mm diameter was cut out from the colony near the margin by sterilized corkborer and was transferred to 5 ml sterile distilled water in a test tube, where it was mixed thoroughly to make a uniform spore suspension. One small drop of spore suspension was taken on haemocytometer and spore count under low power (10X) objective of the microscope. Sporulation was categorized by Bains et al. 2019 as below:

No. of spore	Designation
0	- (nil)
1 - 10	+ (poor)
11-20	++ (fair)
21-30	+++ (good)
31 and above	++++ (excellent)

Results and Discussion

Effect of different media on growth and sporulation of *A. porri*

The colony characteristics of the fungus varied significantly among the different media. The data presented in Table 1 revealed that all the tested media significantly varied in terms of mean growth in diameter of *A. porri*. The fungus showed considerable growth on all the tested media. The mean growth varied from 30.20 mm to 84.50 mm on all the tested media. The maximum radial growth on 7th day of incubation was recorded on potato dextrose agar (84.50 mm) followed by Czapek's Dox agar (82.30 mm) while the least growth was recorded on Onion leaf extract agar (30.20 mm) and V8 juice agar (42.80 mm). While Yadav et al. 2017 recorded the maximum mycelial growth on oatmeal agar (87.50 mm) followed by potato dextrose agar (87.43 mm) and Czapek's Dox agar (84.65 mm) while the least radial growth (34.15 mm) was recorded on V8 juice agar followed by Richard's agar (54.25 mm). However, Raju & Mehta 1982; Chethana et al. 2010 recorded that Potato dextrose agar was the best medium for the colony of *A. porri*.

Table 1. Effect of different media on growth and sporulation of *A. porri*

Different media	Mycelial growth (mm)	Sporulation
Potato dextrose agar	84.50	**++++**
Onion leaf extract agar	30.20	**+**
Carrot onion leaf extract agar	63.85	**++**
V8 juice agar	42.80	**+**
Carrot dextrose agar	68.90	**+++**
Czapek's dox agar	82.30	**++++**
CD (P ≥ 0.05)	**5.25**	

+ : poor; ++ : fair; +++ : good; ++++ : excellent;

Effect of different pH levels on mycelial growth and sporulation of *A. porri*

The data presented in Table 2 revealed significant differences among pH levels of PDA medium in terms of mean diameter growth of the *A. porri*. The mean growth varied from 26.64 to 85.74 mm on all the tested pH levels. The maximum radial growth (85.74 mm) on 7th

day of incubation was recorded at pH 7.0 followed by 78.14 mm at pH 6.0 while the minimum growth (26.64 mm) was recorded at pH 9.0. The maximum sporulation of *A. porri* was recorded at pH 7.0. In present studies, mycelial growth was observed maximum at pH 7.0 and minimum at pH 9.0. Tahira et al. 2019 observed the maximum fresh biomass at pH 6.0 and the least was at pH 8.0. Whereas, 5.0, 5.5, 6.0, 6.5 and 7.0 pH exhibited intermediate level of biomass production were recorded. The highest spore production was verified at pH 6.0 however least production of spore was at pH 8.0. They observed that the declining growth and spore production were noticed with the increase in pH (Meena & Yadav 2022).

Table 2. Effect of different pH levels on growth and sporulation of *A. porri*

pH level	Mycelial growth (mm)	Sporulation
5.0	51.31	**++**
6.0	78.14	**+++**
7.0	85.74	**++++**
8.0	40.68	**++**
9.0	26.64	**+**
CD (P ≥ 0.05)	**7.47**	

+ : poor; ++ : fair; +++ : good; ++++ : excellent;

Effect of different temperature on mycelial growth and sporulation of A. porri

The mycelial growth of *A. porri* was studied by incubating Petri dishes at different temperature ranging from 10°C to 40°C. The maximum mycelial growth and sporulation were observed on the 25°C to 30°C temperature range. However, the highest mycelial growth (83.40 mm) and sporulation were observed at 30°C followed by 25°C (81.03 mm), while minimum mycelial growth (18.25 mm) was observed at 40°C (Table 3). Tahira et al. 2019 conducted studies to optimise of temperature on potato dextrose agar medium and observed the temperature of 28°C maximum colony growth was 86.1 mm and sporulation was while at 34°C least colony growth 56.7 mm and lowest spore production.

Table 3. Effect of different temperature on growth and sporulation of *Alternaria porri*

Temperature (°C)	Mycelial growth (mm)	Sporulation
10	21.76	**+**
15	46.03	**++**
20	66.50	**++**
25	81.03	**++++**
30	83.40	**++++**
35	43.70	**+++**
40	18.25	**++**
CD (P ≥ 0.05)	**8.81**	

+ : poor; ++ : fair; +++ : good; ++++ : excellent;

Effect of carbon and nitrogen source for the mass multiplication of *A. porri*

The mycelial growth and sporulation of *A. porri* were studied on Potato dextrose agar basal medium with different carbon sources. The maximum growth and sporulation were observed on maltose (88.26 mm) followed by sucrose (82.15 mm), fructose (77.98) and starch (77.33 mm) while minimum mycelial growth (73.85 mm) was observed on dextrose (Table 4). The effect the source of nitrogen on mycelial growth and sporulation of *A. porri* was studied on Potato dextrose agar basal medium (Table 5). The maximum mycelial growth was observed on potassium nitrate (80.75 mm) followed by sodium nitrate (73.88mm), peptone (62.83 mm), ammonium chloride (48.38 mm), ammonium nitrate (37.44 mm) and urea (31.90 mm).

Table 4. Effect the source of carbon on growth and sporulation of *A. porri*

Carbon Source	Mycelial growth (mm)	Sporulation
Dextrose	73.85	**+++**
Maltose	88.26	**++++**
Sucrose	82.15	**++++**
Starch	77.33	**+++**
Fructose	77.98	**+++**
CD (P ≥ 0.05)	**4.43**	

+ : poor; ++ : fair; +++ : good; ++++ : excellent;

Table 5. Effect the source of nitrogen on growth and sporulation of *A. 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	**+++**
CD (P ≥ 0.05)	**5.96**	

+ : poor; ++ : fair; +++ : good; ++++ : excellent;

Conclusion

The present investigations revealed that Potato dextrose agar was the best culture medium for *A. porri* and grew best at 30°C temperature on pH 7.0. Maltose was recorded the best carbon source and potassium nitrate was observed the best nitrogen source on the mycelial growth and sporulation of *A. porri*. The present study was undertaken to identify the best suitable aspects on basis of their cultural and sporulation characteristics of *A. porri*. The most suitable practice is useful for the preparation of inoculums for testing of fungicidal evaluation and screening of breeding materials etc.

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Declaration of interests

The authors have no conflict of interest to declare.

Data sharing

All relevant data are within the manuscript.

References

1. Bais RK, Ratan V, Kumar S & Somesh. 2019. Influence of temperature, pH and light on growth and sporulation of *Alternaria solani* (Ellis and Martin) jones and grout causing early blight of tomato (*Solanum lycopersicum* L.) under In vitro condition. *International Journal of Current Microbiology and Applied Scien*ces, 8(12): 36-46.
2. Chethana BS, Kachapur MR, Manjunath B & Kumawat GL. 2010. Effect of culture media and various carbon and nitrogen sources on growth of *Alternaria porri* (Ellis) Cif. causing purple blotch of onion. *Environmental Ecology,* 28: 2393-2395.
3. Gupta RBL & Pathak VN 1988. Yield losses in onions due to purple blotch disease caused by *Alternaria porri*. *Phytophylactica,* 20: 21-23.
4. Haldhar SM, Jat GC, Deshwal H, Gora JS & Singh D. 2017. Insect Pest and Disease Management in Organic Farming. Book 'Towards Organic Agriculture' edited by B. Gangwar and N. K. Jat. Today & Tomorrow's Publishers, New Delhi, Page: 359-390, ISBN: 81-7019.
5. Maheshwari SK, Choudhary BR, Haldhar SM and Berwal MK. 2022. Effectiveness of botanicals, inorganic salts and fungicide against Fusarium wilt of muskmelon under hot arid region of Rajasthan Journal of Agriculture and Ecology, 14: 21-25.
6. Meena PK & Yadav DL. 2022. Evaluation of potato genotypes against early blight (*Alternaria alternata*) of potato (*Solanum tuberosum* L.). *Journal of Agriculture and Ecology*, 14: 99–103. https://doi.org/10.58628/JAE-2214-214.
7. Priya RU, Arun R, Sataraddi & Kavitha TR. 2018. Studies on cultural and physiological variability of *Alternaria porri* (Ellis) Cif. – A causative of purple blotch of onion (*Allium cepa* L.). *International Journal of Current Microbiology and Applied Sci*ence, 7(08): 3284-3291.
8. Qadri SMH, Srivastava KS, Bhope SR, Pandey UB & Bhagchan Dani PM. Fungicidal bioassay against some important pathogens of onion. *Pesticides*, 16: 11-16.
9. Raju KS & Mehta BK. 1982. Certain nutritional aspects of *Alternaria porri* on onion. *Journal of Mycology and Plant Pathology,* 12: 96-98.
10. Singh D, Dhiman JS, Sidhu AS & Singh H. 1992. Current status of onions in India: Strategies for disease resistance breeding for sustained production. *Onion Newsletter Tropics*, 4: 43-44.
11. Tahira JJ, Khan SN & Siddique M. 2019. In-vitro growth of *Alternaria porri* the causal agent of purple blotch of onion. *International Journal of Biology and Biotechnology*, 16(2): 401-405.
12. Upmanyu S & Sharma RC. 2007. Seed yield losses in onion by purple blotch (*Alternaria porri*) and its management. *Indian Phytopathology*, 60(3): 370-372.
13. Yadav RK, Singh A, Jain S, pandit M & Dhakal R. 2017. Physiological studies on *Alternaria porri* and *Stemphylium vesicarium* causing purple blotch complex in onion. *Horticultural Biotechnology Research*, 3: 13-21.

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