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REGULAR ARTICLE

PHYSIOLOGICAL STUDIES ON *ALTERNARIA PORRI* AND *STEMPHYLIUM VESICARIUM* CAUSING PURPLE BLOTCH COMPLEX IN ONION

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ABSTRACT

Effect of different culture media, pH levels and natural substrates on mycelial growth and sporulation of *Alternaria porri* and *Stemphylium vesicarium,* incitants of purple blotch complex of onion was investigated. Maximum colony growth of *A. porri* and *S. vesicarium* was recorded on oat meal agar and Richard's agar while, oat meal agar and V8 juice agar recorded the maximum sporulation, respectively. Similarly, pH 5.0 and 6.0 on potato dextrose agar (PDA) recorded the maximum colony growth of *A. porri* and *S. vesicarium*, respectively. None of the pH levels on PDA supported the sporulation of *A. porri* while maximum sporulation of *S. vesicarium* was recorded on pH 5.0. Onion seed stalks and garlic leaves were found to be the most suitable natural substrates for mass multiplication of *A. porri* and *S. vesicarium*, respectively. The present findings are useful for preparation of inoculums required for resistance breeding and fungicidal evaluation against purple blotch complex.

Keywords: *Alternaria porri,* Mycelial growth, Purple blotch complex, Sporulation, *Stemphylium vesicarium*

INTRODUCTION

Onion (*Allium cepa* L.) is one among the most important vegetable crops grown throughout the world. Among the diseases, purple leaf blotch (PLB) caused by *Alternaria porri* (Ellis) Cif. and *Stemphylium* leaf blight (SLB) caused by *Stemphylium vesicarium* (Wallr.) Simmons, are the major diseases of onion world-wide 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 [1, 2, 3, 4, 5, 6, 7, 8] and are more prevalent in warm and humid environment [9, 10]. Both diseases are, however, more severe on seed crop as compared to bulb crop [11, 12, 13] causing sometimes 100 per cent loss of the seed production [14]. The PLB as well as SLB occur and progress synchronously on the same umbel bearing stalk. The weather preferences for both being similar, the loss is additive. Since, the typical symptoms are found either colonised by *A. porri*, *S. vesicarium* or mixture of both, the symptoms are generally indistinguishable and considered to be a disease complex [9, 10, 15]. Uddin *et al.* [16] also reported that the SLB pathogen (*S. vesicarium)* is first to initiate infection, which is followed by subsequent infection by the pathogen of PLB (*A. porri)* and hence, the disease is designated as purple blotch complex (PBC). The difficulty in sporulation of *A. porri* in culture media as well as on host under normal conditions has been reported by Skiles [17], Fahim [18], Rotem and Bashi [19] and Gupta and Pathak [20].

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*, *S. vesicarium* singly as well as in complex.

MATERIALS AND METHODS

Isolation, purification and confirmation of identity of pathogens

Isolations of the pathogens were made from the diseased leaf tissue collected from different locations of Punjab. Typical diseased spot on the leaves were selected and cut into bits of about 1 to 1.5 mm with the help of sterilized

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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 sterilized distilled water repeatedly to remove the traces of mercuric chloride and toweled on sterilized filter paper, prior to their being aseptically transferred to Petri plates containing 20 ml of autoclaved potato dextrose agar (PDA) in a laminar flow and incubated at 25±1°C in BOD incubator for 10 d. The resulting fungal culture was purified by hyphal tip technique in PDA slant both for *A. porri* and *S. vesicarium*.

Identification of each fungus under study was made after examining 100 conidia under compound microscope (40x) from the 10-day old pure culture of each pathogen obtained from infected leaves of onion. Stage and ocular micrometer were used to measure the length, breadth, beak length and number of septa of the fungus after the calibration of the microscope. The average length and breadth of the conidial body, beak and number of septa were recorded. These observations were compared with those of the standard measurements given by Ellis [21] and Simmons [22] to identify the pathogens. After the confirmation of identity of the pathogens, the culture of the fungus was purified by single spore isolation technique. As single spore isolates were identical, they were multiplied for further use.

Effect of different media on growth and sporulation of *Alternaria porri* and *Stemphylium vesicarium*

Six different semi-solid media *viz.*, potato dextrose agar (PDA), oat meal agar, Czapek's Dox agar, Richard's agar, V8 juice agar and rye agar B were used to study growth and sporulation of *A. porri* and *S. vesicarium*. 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 sterilized cork borer from 10 d old culture of *A. porri* and *S. vesicarium* raised on PDA and the Petri plate was seeded with actively growing 5 mm mycelial disc of the respective fungus with the help of sterilized inoculating needle. Four replications were kept in each treatment under completely randomized design. The Petri plates were incubated at 25±1°C and observations on colony diameter of the respective fungus were taken from four directions at 45° angle. The linear growth of the respective fungus was measured in millimeter at 24 h interval for 10 consecutive days. Rate of growth of each fungus on various test media was determined by slope of the regression line. For quantifying the sporulation, five mycelial discs of 5 mm was cut from the 10-days old culture and eluted in 5 ml of distilled water in a glass vial for each replicate. The vials were shaken thoroughly for 2-3 min and the eluted spores were counted with the help of haemocytometer.

Effect of different pH levels on growth and sporulation of *Alternaria porri* and *Stemphylium vesicarium*

To determine the optimum level of pH for growth of *A. porri* and *S. vesicarium*, 100 ml of potato dextrose agar was dispensed in Erlenmeyer flasks of 250 ml capacity, and the different pH levels of 4.0, 5.0, 6.0, 7.0 and 10.0 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 air flow. The Petri plates were inoculated with 5 mm mycelial discs of each fungus cut with the help of sterilized cork borer from 10 d old culture raised on PDA. Four replications were kept for each treatment under completely randomized design. The inoculated Petri dishes were incubated at 25±1°C maintained in a BOD incubator. The observations for colony growth of each fungus were recorded after every 24 h of incubation for 10 consecutive days. Rate of growth of each fungus on various test pH levels was determined by slope of the regression line. The data on sporulation of respective fungus was taken on 10-days old culture with the help of haemocytometer.

Effect of different natural substrates for the mass multiplication of *Alternaria porri* and *Stemphylium vesicarium*

Eight natural substrates *viz.*, onion leaves, onion seed stalks, onion bulbs, garlic cloves, garlic leaves, onion seeds, rye grains and maize grains were tested with a view to find out the most suitable host substrate which favours the best sporulation of each fungus. Hundred grams of each substrate supplemented with 2 g sucrose was soaked overnight in 250 ml Erlenmeyer flasks. Then excess of water was drained-off and the flasks were plugged with non-absorbent cotton wrapped in muslin cloth before sterilization. The flasks were sterilized in an autoclave at 121°C temperature and 15 psi pressure for 20 min for three consecutive days. Four replications were kept in each treatment under completely randomized design. The substrate in flasks was inoculated with actively growing 5 mm mycelial disc of each pathogen separately under aseptic conditions in the laminar flow. The inoculated flasks were incubated at 25±1°C in BOD incubator and the actively growing mycelium was categorized on the basis of visual observations. The sporulation was measured with haemocytometer after 15 d of incubation. For taking data on sporulation, one gram of each substrate bearing growth of the pathogen was eluted in 5 ml of distilled water in a glass vial for each replicate. The vials were shaken thoroughly for 2-3 min and the eluted spores were counted with the help of haemocytometer.

Statistical analysis

The data were subjected to analysis of variance as per completely randomized design (CRD) using statistical analysis software SPSS 22.0 (SPSS Inc., USA) and the significance of differences between the treatment means were compared using Least Significant Difference (LSD) and Tukey's honest significant difference (HSD) test at 5 per cent level for proper interpretation of results.

RESULTS AND DISCUSSION

Effect of different media on growth and sporulation of *Alternaria porri*

The colony characteristics of the fungus varied significantly among the different media (table 1). The data presented in table 2 revealed that all the tested media significantly varied in terms of mean radial growth of *A. porri*. The fungus showed considerable growth on all the tested media. The mean radial growth varied from 27.62 mm to 49.15 mm on all the tested media**.** The maximum radial growth on 10thday of incubation was recorded on oat meal 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, the radial growth of 87.50, 87.43, 84.65, and 82.10 mm recorded on oat meal agar, potato dextrose agar, Czapek's Dox agar and rye agar B were statistically at par with each other as per by Tukey's HSD test ($P \leq 0.05$). Highest linear growth rate of *A. porri* was recorded on oat meal agar (9.58 mm/day) followed by potato dextrose agar (9.49 mm/day) and Czapek's Dox agar (8.95 mm/day) while the least linear growth rate was recorded on V8 juice agar (3.15 mm/day) followed by Richard's agar (5.32 mm/day). Among all the media tested, only oat meal agar and rye agar B supported the production of conidia. None of other tested media favoured sporulation of *A. porri*. Oat meal agar supported significantly higher sporulation (2.62 x 10^5conidia/ml) than Richard's agar (1.30 x 10^4 conidia/ml) as per by Tukey's HSD test ($P \leq 0.05$).

The present findings are in close agreement with Agale *et al.* [23] who recorded maximum mycelial growth and fair sporulation of *A. porri* on oat meal agar. Potato dextrose agar was the best medium for the colony growth of *A. porri* [24, 25, 26]. *A. porri* has been reported to grow well on carbohydrate rich media like oat meal agar, corn meal agar, Czapek's Dox agar and Cook's II agar, potato dextrose agar and onion agar [27, 28, 18, 24, 25].

Effect of different media on growth and sporulation of *Stemphylium vesicarium*

The colony characteristics of the fungus varied significantly among the different media (table 3). The data presented in table 4 revealed that all the tested media significantly varied in terms of mean radial growth of *S. vesicarium*. The fungus showed considerable growth on all the tested media. The mean radial growth varied from 31.14 to 33.46 mm on all the tested media. The maximum radial growth (65.30 mm) on 10^{th}day of incubation was recorded on Richard's agar followed by potato dextrose agar (60.95 mm) and Czapek's Dox agar (56.90 mm) while the least radial growth (53.35 mm) was recorded on V8 juice agar followed by rye agar B (55.58 mm). Richard's agar and potato dextrose agar were found to be statistically at par with the respect to radial growth of the fungus as per Tukey's HSD test ($P \leq 0.05$). Highest linear growth rate of *S. vesicarium* was recorded on Richard's agar (6.67 mm/day) followed by potato dextrose agar (6.43 mm/day) and rye agar B (5.62 mm/day) while least growth rate was recorded on V8 juice agar (5.11 mm/day) followed by Czapek's Dox agar (5.20 mm/day) and oat meal agar (5.40 mm/day). All the tested media fairly supported the production of conidia of *S. vesicarium*. V8 juice agar supported the highest sporulation (9.21 x 10^6conidia/ml) followed by rye agar B (9.80 x 10^5 conidia/ml) and oat meal agar (8.60 x 10^5 conidia/ml) while the least sporulation was recorded on Czapek's Dox agar (6.00 x 10^4conidia/ml) followed by potato dextrose agar (2.20 x 10^5 conidia/ml). However, all the other media except V8 juice agar were statistically at par with each other with respect to sporulation as per Tukey's HSD test ($P \leq 0.05$).

Chowdhury [26] reported V-7 juice agar and V-7 juice mixed with potato dextrose agar the most suitable culture media for mycelial growth and sporulation of *S. vesicarium*. Kim *et al.* [27] reported higher sporulation of *S. solani* and *S. lycopersici* on V8 juice agar medium followed by potato carrot agar and potato dextrose agar, respectively. Kumar [29] reported the highest sporulation of *S. botryosum* on V8 juice potato dextrose agar medium (V8P) followed by V8 juice agar, V8P+2 per cent tamarind juice medium and V8P+4 per cent tamarind juice medium, respectively.

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

The data presented in table 5 revealed significant differences among pH levels of PDA medium in terms of mean radial growth of the *A. porri*. The fungus grew well at all tested pH levels except pH 10.0 at which the growth stopped after 3 d of incubation. The mean radial growth varied from 9.06 to 36.97 mm on all the tested pH levels. The maximum radial growth (68.53 mm) on 10^{th} day of incubation was recorded at pH 5.0 followed pH 4.0 (51.40 mm) while the minimum radial growth (9.50 mm) was recorded at pH 10.0 followed by pH 7.0 (37.68 mm). The highest linear growth rate of (7.28 mm/day) was recorded at pH 5.0 followed by pH 4.0 (4.70 mm/day) while the least growth rate (0.22 mm/day) was recorded at pH 10.0 followed by pH 7.0 (3.72 mm/day). None of the tested pH levels of potato dextrose agar supported the production of conidia of *A. porri*.

The present findings revealed that the fungus was favoured by slightly acidic medium and pH range of 4.0-6.0 was ideal for its growth. The present findings are consistent with those of Saeed *et al.* [30], Jash *et al.* [31], Agale *et al.* [23], Vijayalakshmi *et al.* [32] and Ramjegathesh and Ebenezar [33] who reported the maximum growth of *A. porri* at pH 5.0 and found pH range of 4.0-6.0 ideal for the growth of the fungus. Angell [28] reported that the pathogen could grow over a wide range of pH from 3.8-9.0.

Effect of different pH levels on growth and sporulation of *Stemphylium vesicarium*

The data presented in table 6 revealed significant differences among pH levels of the PDA medium in terms of mean radial growth of *S. vesicarium*. The mean radial growth varied from nil to 34.03 mm on all the tested pH levels. The fungus grew well at all tested pH levels except pH 10.0, at which no growth was recorded at all. The maximum radial growth (65.00 mm) was recorded at pH 6.0 followed by pH 5.0 (44.33 mm), pH 7.0 (35.48 mm) and pH 4.0 (27.98 mm) after 10 d of incubation while pH 10.0 supported no growth of the fungus at all. The highest linear growth rate (7.11 mm/day) was recorded at pH 6.0 followed by pH 5.0 (3.90 mm/day) while the least growth rate (2.33 mm/day) was recorded at pH 4.0. Maximum sporulation of the fungus (2.31 x 10^6conidia/ml) was recorded at pH 5.0 followed by pH 4.0 (8.30 x 10^5conidia/ml), pH 6.0 (2.70 x 10^5conidia/ml) and pH 7.0 (1.70 x10^5 conidia/ml).

The present finding, that pH range of 5.0-6.0 is ideal for the growth of the fungus, is similar to those of Padhi and Synder [34], Rajani *et al.* [35], Huq [36], Rahman *et al.* [37] and Hosen [38] who have also reported pH range of 5.0-6.0 as ideal for the growth of *Stemphylium* species. Hosen [38] reported the maximum growth of *S. botryosum* at pH 5.5 followed by pH 6.0 while Rahman *et al.* [37] recorded the maximum colony growth of *S. botryosum* at pH 6.0. Rajani *et al.* [35] found pH 5.5 as optimum for colony growth of *S. lycopersici*. Padhi and Synder [34] reported pH 5.5 as the optimum level, which recorded the luxuriant mycelial dry weight of *S. botryosum*

while Huq (2003) found maximum growth of *S. botryosum* at 6.0 followed by pH 7.0.

Effect of different natural substrates on mass multiplication of *Alternaria porri*

The data presented in table 7 revealed significant variation in mycelial growth, colour and sporulation of *A. porri* on different natural host substrates. The colour of mycelium was pinkish white on onion leaves, creamy white on onion seeds stalks, orange white on onion bulbs, yellowish white on garlic cloves, dirty white on garlic leaves, dirty white on rye grains and greyish white on maize grains.

Rye grains supported excellent mycelial growth of *A. porri* while mycelial growth was good on onion leaves, onion seed stalks, onion bulbs and garlic leaves. Poor mycelial growth was observed on onion seeds and maize grains. Among eight natural substrates, only two substrates *viz.*, onion leaves and onion seed stalks were found to support the sporulation of *A. porri*. None of the other natural substrates was found to support the sporulation of the fungus. Onion seed stalks supported the significantly higher sporulation (8.26 x 10^5 conidia/ml) than onion leaves (2.80 x 10^5 conidia/ml) as per Tukey's HSD test ($P \leq 0.05$).

The pathogen *A. porri* has been reported to be a sparsely sporulating fungus in culture media as well as on host under normal conditions by many workers [17, 18, 19, 20, 39]. Gupta and Pathak [20] reported that none of the five isolates of *A. porri* from onion could sporulate on the conventional media. Yadav *et al.* [40] reported that sorghum and wheat grains supported luxuriant colony growth and sporulation of *A. solani* while barley, pearl millet and maize recorded no colony growth and sporulation of the fungus. As the literature is silent regarding the effect of natural substrates on mass multiplication of *A. porri*, the present findings remain uncompared and new.

Table 1: Effect of different media on colony characteristics of *Alternaria porri*

Medium	Colony characteristics of the fungus
Czapek's Dox agar	Growth fast, slightly fluffy, uniformly spreading, creamy white with few off-white concentric rings
Oat meal agar	Growth fast, slightly fluffy, uniformly spreading, faint olive green to dark olive green, depressed at the centre with distinct zonation.
Potato Dextrose agar	Growth fast, slightly fluffy, uniformly spreading, slightly depressed at the centre with distinct concentric zonations of light to dark brown, off-white to olive green colour.
Richard's agar	Growth slow, fluffy, irregularly spreading, creamy white, depressed at the centre and slightly raised at the periphery
Rye agar B	Growth fast, smooth, uniformly spreading, faint olive green with concentric rings at the periphery
V8 juice agar	Growth very slow, fluffy, irregularly spreading, variably raised at periphery, creamy white and slightly depressed at the centre

Table 2: Effect of different media on growth and sporulation of *Alternaria porri*

Media	Radial growth (mm) after incubation (days)										Mean	Growth Rate (mm/day)	Sporulation (mean±S. E x 10^5conidia/ml)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10			
Czapek's Dox agar	7.85^{a}	13.35^{abc}	20.95^{a}	29.35^{b}	37.85^{b}	50.95^{c}	58.65^{b}	68.45^{bc}	77.25^{ab}	84.65^{a}	44.93^{b}	8.95	0^{b}
Oat meal agar	8.13^{a}	12.40^{bc}	21.88^{a}	33.40^{ab}	44.38^{a}	59.45^{a}	68.88^{a}	73.45^{ab}	82.08^{a}	87.50^{a}	49.15^{a}	9.58	$(2.62\pm0.40)^{a}$
Potato dextrose agar	9.15^{a}	16.38^{ab}	20.23^{a}	32.53^{ab}	38.05^{b}	57.83^{ab}	69.15^{a}	75.30^{a}	81.55^{a}	87.43^{a}	48.76^{a}	9.49	0^{b}
Richard agar	6.90^{a}	$10.90b^{c}$	12.38^{b}	18.00^{c}	22.75^{c}	28.50^{d}	34.00^{c}	41.50^{d}	47.00^{c}	54.25^{b}	27.62^{c}	5.32	$(0.13\pm0.14)^{b}$
Rye agar B	9.10^{a}	18.40^{a}	24.40^{a}	35.50^{a}	42.10^{ab}	52.10^{bc}	59.10^{b}	66.50^{c}	74.40^{b}	82.10^{a}	46.37^{b}	8.12	0^{b}
V8 juice agar	6.51^{a}	9.30^{c}	11.43^{b}	14.62^{c}	18.46^{c}	21.20^{e}	24.60^{d}	27.81^{e}	31.62^{d}	34.15^{c}	19.97^{d}	3.15	0^{b}
Mean	7.94	13.45	18.54	27.23	33.93	45.00	52.40	58.84	65.65	71.68			

Values in columns with different superscripts are significantly different ($P \leq 0.05$) according to Tukey's HSD test

	LSD ($P \leq 0.05$)	S. Em±
Media =	1.26	0.64
Interval (day) =	1.64	0.83
Media x Interval (day) =	4.01	2.03
Sporulation =	0.48 x 10^5	0.23 x 10^5

Table 3: Effect of different media on colony characteristics of *Stemphylium vesicarium*

Media	Colony characteristics of the fungus
Czapek's Dox agar	Growth fast, fluffy, irregularly spreading, unevenly raised, creamy white
Oat meal agar	Growth uniformly spreading, creamy white fluffy raised around centre and thin at the periphery
Potato Dextrose agar	Growth fast, fluffy, uniformly spreading, umbonate, greenish grey to greyish brown, raised and creamy white at the centre
Richard's agar	Growth fast, velvety, submerged, uniformly spreading brownish grey
Rye agar B	Growth irregularly spreading, raised around the centre, dirty white
V8 juice agar	Growth fast, fluffy, uniformly spreading, greenish grey to dirty white

Table 4: Effect of different media on growth and sporulation of *Stemphylium vesicarium*

Media	Radial growth (mm) after incubation (days)										Mean	Growth Rate (mm/d)	Sporulation (mean±SE x 10^6conidia/ml)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10			
Czapek's Dox agar	8.30^{a}	14.28^{a}	20.03^{a}	26.53^{a}	30.13^{a}	35.28^{a}	40.75^{a}	44.40^{b}	50.05^{bc}	56.90^{bc}	32.66^{ab}	5.20	0.06±0.003^{b}
Oat meal agar	7.40^{a}	11.00^{a}	18.33^{a}	25.98^{a}	32.88^{a}	37.88^{a}	41.15^{a}	45.45^{ab}	49.53^{bc}	55.78^{bc}	32.54^{ab}	5.40	0.86±0.02^{b}
Potato dextrose agar	6.98^{a}	9.50^{a}	13.28^{a}	21.35^{a}	28.90^{a}	33.90^{a}	41.33^{a}	50.33^{ab}	56.05^{ab}	60.95^{ab}	32.26^{ab}	6.43	0.22±0.03^{b}
Richard's agar	7.63^{a}	11.15^{a}	15.10^{a}	20.83^{a}	28.33^{a}	34.00^{a}	42.23^{a}	52.48^{a}	57.55^{a}	65.30^{a}	33.46^{a}	6.67	0.24±0.03^{b}
Rye agar B	6.58^{a}	9.85^{a}	16.08^{a}	23.48^{a}	29.15^{a}	34.98^{a}	39.75^{a}	45.08^{ab}	50.85^{ab}	55.58^{bc}	31.14^{b}	5.62	0.98±0.06^{b}
V8 juice agar	6.95^{a}	12.6^{a}	19.15^{a}	24.40^{a}	29.45^{a}	33.63^{a}	39.15^{a}	44.23^{b}	48.58^{c}	53.35^{c}	31.15^{b}	5.11	9.21±0.33^{a}
Mean	7.30	11.40	16.99	23.76	29.80	34.94	40.73	46.99	52.10	57.98			

Values in columns with different superscripts are significantly different (P≤0.05) according to Tukey's HSD test

	LSD (P≤0.05)	S. Em±
Media =	1.60	0.81
Interval (day) =	2.07	1.05
Media x Interval (day) =	5.07	2.57
Sporulation =	3.98 x 10^6	1.89 x 10^6

Table 5: Effect of different pH levels on growth of *Alternaria porri*

pH levels	Radial growth of mycelium(mm) on PDA after incubation (days)										Mean	Growth Rate (mm/day)	Sporulation (conidia/ml)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10			
4	7.13^{ab}	14.38^{a}	18.38^{b}	22.70^{a}	28.38^{a}	32.33^{b}	36.03^{b}	41.33^{b}	45.48^{b}	51.40^{b}	29.75^{b}	4.70	0
5	6.53^{bc}	12.53^{b}	19.83^{a}	22.63^{a}	29.23^{a}	36.53^{a}	52.53^{a}	58.53^{a}	62.83^{a}	68.53^{a}	36.97^{a}	7.28	0
6	7.53^{a}	9.63^{c}	14.63^{c}	19.93^{b}	24.63^{a}	28.63^{c}	31.33^{c}	34.63^{c}	38.63^{c}	41.53^{c}	25.11^{c}	3.92	0
7	6.08^{c}	7.68^{d}	12.68^{d}	16.68^{c}	20.38^{c}	24.68^{d}	28.68^{d}	31.68^{d}	35.38^{d}	37.68^{d}	22.16^{d}	3.72	0
10	6.80^{abc}	7.80^{d}	9.50^{e}	9.50^{d}	9.50^{d}	9.50^{e}	9.50^{e}	9.50^{e}	9.50^{e}	9.50^{e}	9.06^{e}	0.22	0
Mean	6.81	10.40	15.00	18.29	22.42	26.33	31.61	35.13	38.36	41.73			

Values in columns with different superscripts are significantly different (P≤0.05) according to Tukey's HSD test

	LSD (P≤0.05)	S. Em±
pH =	0.28	0.14
Interval (day) =	0.41	0.21
pH x Interval (day) =	0.91	0.46

Table 6: Effect of different pH levels on growth of *Stemphylium vesicarium*

pH levels	Radial growth of mycelium(mm) on PDA after incubation (days)										Mean	Growth rate (mm/day)	Sporulation (mean±S. E x 10^6conidia/ml)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10			
4	7.08^{a}	8.73^{bc}	11.65^{b}	14.80^{b}	16.48^{d}	18.48^{d}	20.40^{d}	22.73^{d}	25.55^{d}	27.98^{d}	17.39^{d}	2.30	0.83±0.04^{b}
5	8.08^{a}	13.58^{a}	15.75^{a}	20.30^{a}	23.90^{b}	28.00^{b}	30.75^{b}	34.73^{b}	40.23^{b}	44.33^{b}	25.96^{b}	3.90	2.31±0.12^{a}
6	7.30^{a}	10.33^{b}	13.45^{b}	19.93^{a}	27.58^{a}	34.73^{a}	43.90^{a}	56.40^{a}	61.73^{a}	65.00^{a}	34.03^{a}	7.11	0.27±0.01^{c}
7	6.65^{a}	8.08^{c}	11.43^{b}	14.80^{b}	20.08^{c}	23.15^{c}	26.73^{c}	29.55^{c}	31.98^{c}	35.48^{c}	20.79^{c}	3.37	0.17±0.009^{cd}
10	0.00^{b}	0.00^{d}	0.00^{c}	0.00^{c}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0	0^{d}
Mean	5.82	8.14	10.46	13.97	17.61	20.87	24.36	28.68	31.90	34.56			

Values in columns with different superscripts are significantly different (P≤0.05) according to Tukey's HSD test

	LSD (P≤0.05)	S. Em±
pH level =	0.71	0.36
Interval (day) =	0.99	0.50
pH x Interval (day) =	2.21	1.12
Sporulation =	0.18 x 10^6	0.08 x 10^6

Table 7: Effect of different natural substrates on mass multiplication of *Alternaria porri*

Natural substrate	Mycelial growth	Mycelial colour	Sporulation (mean±SE x 10^5conidia/ml)
Garlic cloves	+	Yellowish white	0^c
Garlic leaves	++	Dirty white	0^c
Maize grains	+	Greyish white	0^c
Onion bulbs	++	Orange white	0^c
Onion leaves	++	Pinkish white	$(2.80\pm0.11)^b$
Onion seed stalks	++	Creamy white	$(8.26\pm0.05)^a$
Onion seeds	+	Creamy white	0^c
Rye grains	+++	Dirty white	0^c
LSD ($P\leq0.05$)			0.08

+++(Excellent);++(Good);+(Poor);-(No growth), Values in columns with different superscripts are significantly different ($P\leq0.05$) according to Tukey's HSD test

Table 8: Effect of different natural substrates on mass multiplication of *Stemphylium vesicarium*

Natural substrate	Mycelial growth	Mycelial colour	Sporulation (mean±S. E x 10^6conidia/ml)
Garlic cloves	-	-	0c
Garlic leaves	+++	Greyish white	1.16±0.03a
Maize grains	-	-	0c
Onion bulbs	++	Creamy white	0c
Onion leaves	+++	Creamy white	0.72±0.01b
Onion seed stalks	++	Creamy white	1.11±0.08a
Onion seeds	-	-	0c
Rye grain	+++	Pinkish white	1.14±0.05a
LSD ($P\leq0.05$)			0.07

+++(Excellent);++(Good);+(Poor);-(No growth), Values in columns with different superscripts are significantly different ($P\leq0.05$) according to Tukey's HSD test

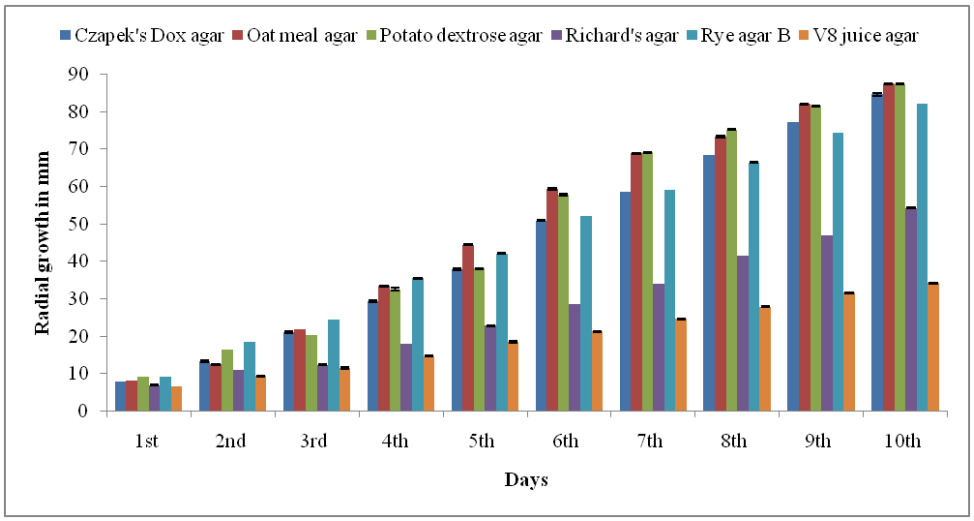

Fig. 1: Effect of different media on radial growth of *Alternaria porri*

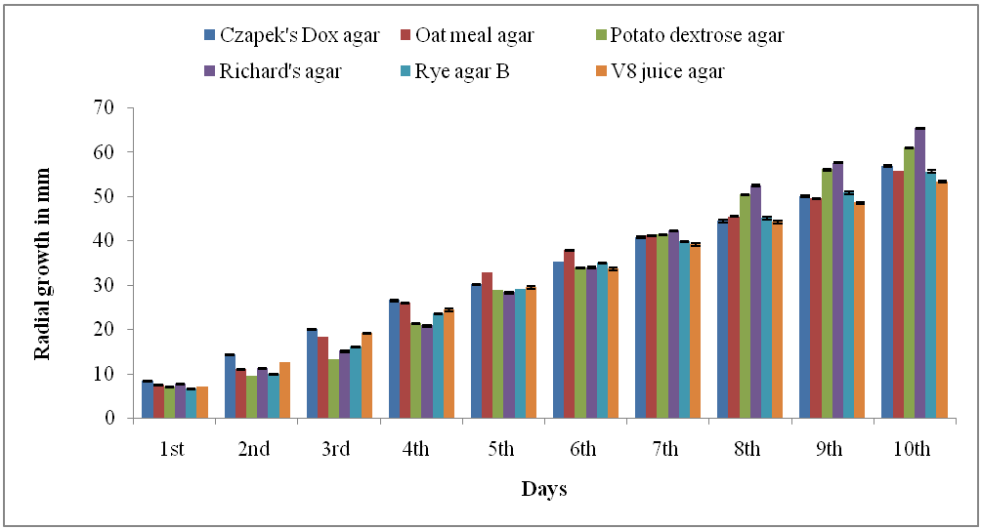

Fig. 2: Effect of different media on radial growth of *Stemphylium vesicarium*

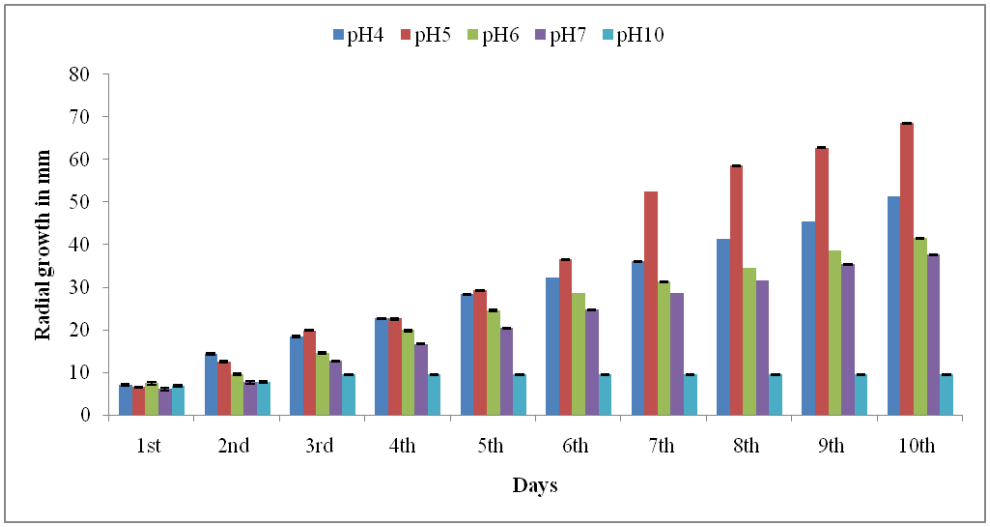

Fig. 3: Effect of different pH levels on radial growth of *Alternaria porri*

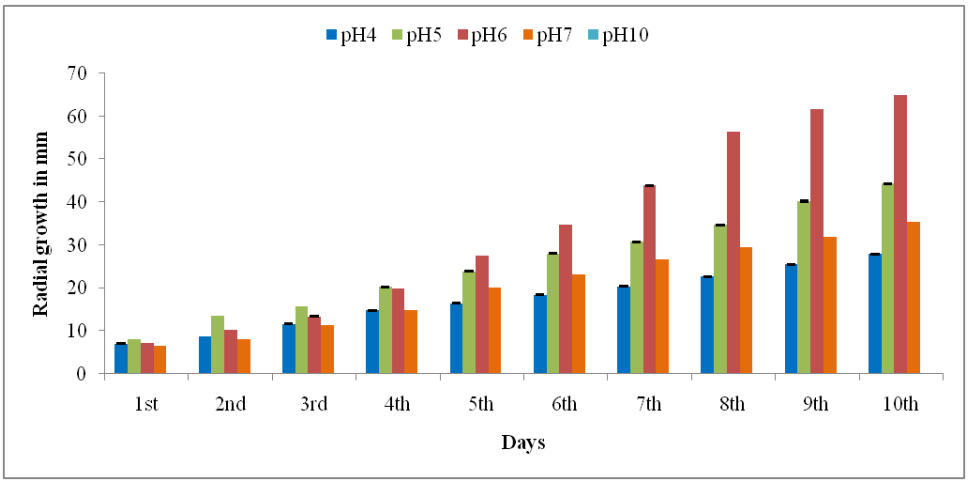

Fig. 3: Effect of different pH levels on radial growth of *Stemphylium vesicarium*

Effect of different natural substrates on mass multiplication of *Stemphylium vesicarium*

The data presented in table 8 revealed significant variation in mycelial growth, colour and sporulation of *S. vesicarium* on different natural host substrates. The colour of mycelium was creamy white on onion leaves, onion seed stalks and onion bulbs, pinkish white on rye grain and greyish white on garlic leaves. No mycelial growth was recorded on maize grains, garlic cloves and onion seeds. Excellent mycelial growth was recorded on onion leaves, garlic leaves and rye grains while good mycelial growth was recorded on onion seed stalks and onion bulbs. Garlic leaves recorded numerically highest sporulation (1.16 x 10^6 conidia/ml) followed by rye grain (1.14 x 10^6 conidia/ml), onion seed stalks (1.11 x 10^6conidia/ml) and onion leaves (7.20 x 10^5 conidia/ml), respectively. However, garlic leaves, rye grain and onion seed stalks were statistically at par with respect to sporulation by Tukey's HSD test ($P \leq 0.05$). As the literature is silent regarding the effect of natural substrates on sporulation and growth of *S. vesicarium*, the present findings remain uncompared and new.

CONCLUSION

The present investigations revealed that oat meal agar was the best culture medium for *A. porri* while Richard's agar and V8 juice agar for *S. vesicarium*. *A. porri* and *S. vesicarium* grew best at pH 5.0 and pH 6.0, respectively while the natural substrates, onion seed stalks and garlic leaves, were the most suitable. The findings are useful in preparation of inoculums required for resistance breeding and fungicidal evaluation against purple blotch complex of onion.

AUTHORS CONTRIBUTIONS

Authors contributed equally to the overall study and manuscript preparation and approved the final version of the manuscript for publication.

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