



Epidemiological factors affecting purple blotch (*Alternaria porri*) progression in garlic

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Abstract

Purple blotch caused by *Alternaria porri* Ellis (Cif.), poses a serious threat to garlic cultivation. This study investigated key epidemiological factors influencing disease development, specifically inoculum concentration, age of host and pathogen culture, and its ability to infect other plant species on disease development. A spore concentration of 1×10^5 conidia/ml resulted in highest disease severity (36.38%) within a shortest incubation period (6 days), establishing as optimal inoculum load. Among varying culture ages, 14-day-old culture exhibited peak virulence with disease severity (33.77%) and infection rate (0.04 r/day). Host susceptibility increased with plant age with maximum disease progression in 60-day-old plants. Host range assessments demonstrated that it could infect onion, tomato, and chilli with incubation period ranging from 7-12 days, while potato and cabbage remain unaffected, highlighting moderate host specificity. These findings enhance understanding of *A. porri* pathogenesis and provide framework for developing effective screening and disease management strategies in garlic.

Keywords: Artificial inoculation, garlic, *Alternaria porri*, pathogenicity, host range

Garlic (*Allium sativum* L.) is a herbaceous annual bulbous spice and medicinal crop grown all over the world. It is the second most widely cultivated bulb crop after onion, belongs to family *Amaryllidaceae*. Globally, China is the major producer of garlic contributing 14 per cent of the total world area and about 22.2 million tons production followed by India with total production of 3.1 million metric tonne over an area of 180 thousand hectare (Anonymous 2024). Long day garlic is exclusively grown in temperate areas, such as Himachal Pradesh, Jammu & Kashmir and Uttarakhand. In Himachal Pradesh, garlic is grown over an area of 3940 Ha with a total production of 2.58 thousand tonnes and contributes to about 0.77 per cent in India's share (Indiastat 2024). Garlic is mainly cultivated in Kangra, Kullu, Mandi and Sirmaur districts of Himachal Pradesh with Sirmaur being the leading district in garlic cultivation.

Garlic is prone to several pathogens like fungi, bacteria and viruses. Purple Blotch caused by

Alternaria porri (Ellis) Cif. is the most serious and devastating diseases of garlic resulting in yield reduction (quantity and quality) and losses up to 97 per cent have been reported upon leaves and bulb infection (Kareem *et al.* 2012). In India, yield loss ranging from 2.5 to 97 per cent was reported due to purple blotch (Nanda *et al.* 2016).

Accurate and consistent screening for disease resistance in plants requires artificial inoculation at a susceptible growth stage using an appropriate and standardized inoculum concentration. Artificial inoculation not only ensures uniform disease development but also plays a critical role in various aspects of plant pathology, including studies on epidemiology, etiology, host-pathogen interactions, disease resistance, and management strategies (Thakur & Banyal 2022). The genus is significantly important for causing wide spread and economically significant diseases across a range of crops such as cereals, oil crops, spices, vegetables and ornamentals. These

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pathogens may exhibit host specificity or infect a broader host spectrum, with warm and humid conditions favoring disease development (Sharma and Ratnoo 2019). Host range of any pathogen is one criteria which shows its virulence and host preference. Understanding these physiological factors that influence disease development, such as age of fungal culture and host plant age which can significantly alter the severity and progression of disease. Therefore, this investigation was carried out in order to assess the effect of age of culture, inoculum load, host-age and host range on the development of purple blotch of garlic. Thereby, standardizing these factors of *A. porri* on garlic for pathogenesis studies and to enhance disease management strategies.

Materials and Methods

Alternaria porri (Ellis) Cif. culture was isolated from diseased garlic leaf samples using standard leaf bit methodology (Dhingra and Sinclair 1985). Fungal culture was purified by single spore method (Choi *et al.* 1999) and the isolate was maintained on potato dextrose agar (PDA). The fungus was identified on the basis of morpho-cultural characteristics (Ellis 1971). The spores were harvested by flooding a ten days old well-sporulated fungal culture plates on PDA medium with 10 ml of sterilized distilled water and gently scraping the surface with a sterilized spatula to form spore suspension. The resulting spore suspension was then filtered through a double layer of sterilized muslin cloth to remove mycelial bits and remains of media. The filtered spore suspension was re-suspended in deionized water. *A. porri* conidia being long beaked, tend to clog together among themselves and with bits of media and mycelium. This prevented accurate calculation of the spore concentration. Therefore, a clear spore suspension was preferred. After the final wash, supernatant was discarded and spores were resuspended in water containing 0.05 per cent Tween-20.

Inoculum load

Different inoculum concentrations (spores/ml) were evaluated to standardize the optimum inoculum concentration required for successful infection and development of purple blotch symptoms on garlic plants. Two month old seedlings of susceptible garlic variety Agrifound White were spray inoculated with

different conidial concentrations viz., 4×10^3 , 1×10^4 , 5×10^4 , 1×10^5 and 5×10^5 conidia/ml. Simultaneously, control treatment by spraying sterilized water was also maintained. The inoculated plants were placed in net house by maintaining RH > 90 per cent for 48 hours at temperature 25-30°C and thereafter the pots were watered daily until the development of disease symptoms. Five plants were taken for each replication and the treatments were replicated thrice in CRD. Observation on incubation period was recorded for each treatment. Per cent Disease Index (PDI) was recorded at weekly interval using a 0-5 rating scale based on the percentage of leaf area affected by the disease. A rating of 0 was assigned to plants showing no visible symptoms, categorized as Immune (I). A score of 1, corresponding to 0-10% of leaf area covered, indicated a Resistant (R) reaction. A rating of 2, with 11-20% leaf area affected, denoted Moderately Resistant (MR) plants. Plants with 21-50% of the leaf area diseased were rated as 3, classified as Moderately Susceptible (MS). A rating of 4, indicating 51-75% infection, was considered Susceptible (S). The highest severity, with more than 75% of leaf area covered, received a score of 5, signifying a Highly Susceptible (HS) reaction (Sharma 1986).

The PDI values were expressed using the formula given by McKinney (1923). Disease progression was measured by calculating AUDPC and apparent rate of infection (r) as per logistic equation given by Vander Plank (1963).

$$PDI = \sum \frac{\text{Severity grade} \times \text{Number of leave}}{\text{Maximum grade} \times \text{Total number of leaves scored}} \times 100$$

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1} + 1}{2} \times (t_{i+1} - t_i)$$

Where, y_i = Disease severity at the i^{th} observation

t_i = time (in days) at the i^{th} observation

n = total number of observations.

$$r = \frac{2.303}{t_2 - t_1} \log_{10} \frac{x_2(1 - x_1)}{x_1(1 - x_2)}$$

Where, r = apparent infection rate per day

$t_2 - t_1$ = time interval between first and last observation

x_1 & x_2 = proportion of leaf area covered by lesion at t_1 and t_2 time intervals, respectively

$(1 - x_1)$ & $(1 - x_2)$ = proportion of healthy leaf area at t_1 and t_2 time intervals, respectively

Age of pathogen culture

Alternaria porri culture isolated from the infected garlic leaves on PDA was maintained in the laboratory at 25°C for different durations viz., 7 days, up to 35 days old culture. The pathogenicity tests were carried out in polyhouse conditions by spraying spore suspension (1×10^5 conidia/ml) from 7, 14, 21, 28 and 35 days old culture on two month old seedlings of susceptible garlic variety Agrifound White grown in pots. The inoculated plants were placed in net house by maintaining RH > 90 per cent for 48 hours at temperature 25-30°C and thereafter, the pots were watered daily until the development of disease symptoms. Five plants were taken for each replication and the treatments were replicated thrice in CRD. The incubation period was recorded for each treatment and per cent disease index (PDI) was recorded using rating scale given by Sharma (1986). PDI was recorded at weekly interval using the formula given by McKinney (1923). The AUDPC and infection rate were calculated as described earlier.

Host age

Garlic cloves of susceptible variety Agrifound White sown (five cloves/pot) in pots were spray inoculated at 20, 30, 40 and 50 days after sowing. For this staggered sowing was done at 10 days interval up to 60 days. Plants were grown in controlled conditions. The pathogenicity tests were carried out in polyhouse conditions by spraying spore suspension (1×10^5 conidia/ml). Five plants were taken for each replication and each treatment was replicated thrice in pots along with an uninoculated control. The inoculated plants were placed in net house by maintaining RH > 90 per cent for 48 hours at temperature 25-30°C and thereafter the pots were watered daily until the development of disease symptoms. Incubation period and per cent disease index (PDI) were recorded and AUDPC and infection rate were calculated as described earlier.

Host range of *Alternaria porri*

To assess the host range of *A. porri* the trial was performed on onion (*Allium cepa*), chilli (*Capsicum annum*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*) and cabbage (*Brassica oleracea*), commonly reported to be infected by *Alternaria* sp., or taxonomically related to garlic. Under polyhouse conditions, seeds of these hosts were

sown in pots (20 cm diameter) filled with sterilized potting mixtures with equal proportions (v/v) of soil, sand and clay. These plants at the age of 30 days were spray inoculated with *A. porri* isolate of garlic. Seedlings were inoculated until run-off with conidia suspension using a hand-held atomizer. Control plants were sprayed with distilled water. The inoculated set and uninoculated set of test plants were maintained in a polyhouse having 24-28°C temperature and 85-95 per cent RH. Plant-pathogen interactions were evaluated using incubation period and disease severity measured as Leaf Area Damage. The inoculated plants were kept under observation up to 30 days for any symptoms development. Disease severity was visually assessed daily for 15 days following inoculation.

Statistical analysis

The data obtained from experiments were subjected to statistical analysis. The differences exhibited by treatments in various experiments were tested for their significance by employing standard statistical procedures (CRD) as described by Gomez and Gomez (1984). The critical difference (CD) was calculated in each experiment to establish the least significant difference amongst the treatments. All the data were analysed by using OPSTAT and Microsoft excel 2007.

Results and Discussions

Inoculum load

The effect of different inoculum levels on disease development was studied and the data have been presented in Table 1. Significant variation in incubation period, disease severity, AUDPC and rate of infection was recorded at different inoculum loads. The results revealed that all the plants inoculated with inoculum at different concentrations showed varied infection. Inoculum load of 1×10^5 spores/ml gave maximum disease severity (36.38%) with shortest incubation period of 6 days followed by 5×10^5 (31.75%) with 7 days of incubation period. The disease progress was also significantly high at 1×10^5 spores/ml inoculum load which was evident by highest AUDPC (506.89) and apparent infection rate (0.04 per day). This was followed by 5×10^5 conidial concentrations with 447.17 and 0.04 of AUDPC and rate of infection, respectively. However, minimum disease severity (16.91%) was observed at inoculum level 4×10^3 with

Table 1. Effect of inoculum load of *Alternaria porri* on the development of purple blotch of garlic

Inoculum load (spores/ml)	Incubation period (days)	Disease severity (%) after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
4x10 ³	10	11.8	17.6	21.33	16.91	237.94	0.04
1x10 ⁴	9	14.2	21.2	27.31	20.90	293.85	0.05
5x10 ⁴	8	20.5	28.32	36.84	28.55	400.37	0.04
1x10 ⁵	6	26.7	38.45	44.00	36.38	506.89	0.04
5x10 ⁵	7	22.4	34.44	38.41	31.75	447.17	0.04
LSD (0.05)		1.0	2.1	1.4	-	19.36	0.02

incubation period of 10 days. It also gave minimum AUDPC and apparent infection rate i.e. 237.94 and 0.04, respectively. From the data in Table 1, it is clear that increase of inoculum load from 4x10³ to 1x10⁵ spores/ml, a significant reduction was observed in the incubation period i.e. 10 days to 6 days with a significant increase in disease severity.

The findings of the present study are corroborated with previous studies highlighting the role of inoculum concentration in disease concentration. Gupta and Pathak (1986) reported maximum disease incidence (100%) and severity (68.8%) at an inoculum concentration of 3.28 x 10⁵ mycelial propagules/cm³, with a corresponding reduction in incubation period. Late appearance and poor development of purple blotch were observed as the density of inoculum decreased. Conversely, lower inoculum densities led to delayed symptom onset and reduced disease development. A positive correlation between inoculum concentration and symptom development has also been demonstrated for other *Alternaria* species (Vloutoglou 1994). In garlic, Bhardwaj (2018) identified 6x10⁴ conidial/ml as the ideal inoculum concentration for rapid onset of *A. porri* infection, while inoculum concentration below 2x10⁴ noticeably

delayed the infection process. Adequate amount of inoculum is a pre-requisite for initiation of symptoms and disease progress as this is likely to be proportional to the amount of pathogenicity factors required for initial host invasion (Sinha *et al.* 2024).

Age of pathogen culture

The present study elucidates the significant impact of the age of *Alternaria porri* culture on the epidemiology of purple blotch in garlic. The results demonstrate that the pathogen's virulence, as measured by incubation period, disease severity, AUDPC, and apparent infection rate, varies considerably with the age of the inoculum. The data presented in Table 2 revealed that 14 days old culture was most virulent with highest disease severity (33.77%) and shortest incubation period (5.7 days) along with maximum AUDPC and infection rate of 474.53 and 0.04 r/day, respectively. Minimum disease severity (12.70%), AUDPC (179.23) as well as infection rate (0.08) was recorded with 35 days old pathogen culture. The disease severity obtained with 7 (24.22%) and 21 (22.00%) days old culture was statistically at par with each other. Pathogen culture older than 14 days indicated successively reduced virulence as evident from delayed disease appearance and slow disease

Table 2. Effect of age of culture of *Alternaria porri* on the development of purple blotch of garlic

Age of culture (days)	Incubation period (days)	Disease severity (%) after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
7	6.3	18.60	23.00	31.06	24.22	334.81	0.04
14	5.7	24.20	34.27	42.84	33.77	474.53	0.04
21	7.2	17.40	21.80	26.80	22.00	307.3	0.03
28	7.6	9.10	15.33	25.01	16.48	226.69	0.07
35	8.0	5.90	13.10	19.11	12.70	179.23	0.08
LSD (0.05)		1.1	1.67	0.99	-	14.29	0.01

progress. The pathogen virulence was also observed less with 7 days old culture, indicating that about 2 weeks old pathogen culture is most suitable for development of disease and sporulation. The rate of sporulation and growth rate significantly decreased after 14 days pertaining to the depleting nutrients in the culture media.

The findings are in conformity with those of Suheri and Prince (2001), who reported the older cultures of *A. porri* (28 and 35 days) with longer incubation periods (5.5 and 6.3 days), exhibited reduced mean disease severity (16.48% and 12.70%), and lower AUDPC values (226.70 and 179.24) in development of purple blotch of onion, respectively. This decline in virulence with culture age may be attributed to physiological senescence, reduced conidial capacity and depletion of essential nutrients in the aging mycelium. Similar observations were made by Kumar *et al.* (2013), who observed that young, actively growing cultures of *Alternaria* spp. exhibit enhanced virulence, due to increased metabolic activity and spore viability. These findings align with previous studies on *Alternaria* spp., where pathogen virulence and host susceptibility are influenced by both the physiological state of the pathogen and the host tissue (Bhushan *et al.* 2025). Moreover, Singh *et al.* (2021) observed that 14 day-old cultures of *A. porri* induced shortest incubation period and highest disease severity (27.47%), followed closely by 7 and 21 day-old cultures (23.27% and 23.53%, respectively). Sinha *et al.* (2024) suggested that 14 days old culture of *A. solani* as compared to 7, 21, 28 and 35 days old was most virulent with highest mean disease severity and shortest incubation period.

Effect of host age

A pot experiment was laid out in CRD with five replications. Five cloves per pot of susceptible

Agrifound White were grown on plastic pots and 20, 30, 40 and 60-days old plants were spray inoculated with 1×10^5 conidia/ml of *A. porri*. Inoculated plants were maintained for high humidity throughout the disease development period by regular spraying of water. Observations for incubation period and disease severity on 0-5 scale were recorded. All the inoculated plants at different ages showed infection and significant differences (Table 3) in the disease index were found among the plants inoculated at different ages. Highest per cent disease index (29.43%) was recorded on plants inoculated at 60 days with AUDPC (402.12) as well as infection rate (0.05) followed by plants inoculated at 50 days with 27.26 per cent disease severity, 382.10 value of AUDPC with infection rate of 0.06 r/day. Plants inoculated at 40 days (24.67%, 350.74, 0.07) and 30 days (19.93%, 271.53, 0.05) showed less per cent disease index, AUDPC and rate of infection relative to 60 days and 50 days old plants, respectively. Plants inoculated at 20 days showed the least per cent disease index (13.13%), AUDPC and infection rate of 173.60 and 0.07 r/day. From the above data it is evident that with the increase in the host age, there was a significant increase in disease development (Table 3). All the treatment dates are statistically significant to each other. The susceptibility of garlic crop to purple blotch directly correlated with crop age.

According to Horsfall and Dimond (1957), the increased severity of symptoms on older leaves was attributed to their lower sugar content. The findings of the present study corroborated by Gupta and Pathak (1986) who reported lowest incubation period and highest disease incidence and severity in 60 days old onion plants being the most susceptible compared to 30 and 40 days old plants with poor development of the disease. Khare and Nema (1984) reported that onion plants of 25 and 33 days old did not develop any

Table 3. Effect of host age of *Alternaria porri* on the development of purple blotch of garlic

Host age (DAS)	Incubation period (days)	Disease severity (%) after disease appearance				AUDPC	Apparent rate of infection (r/day)
		7	14	21	Mean		
20	9.0	7.80	10.20	21.40	13.13	173.60	0.07
30	7.8	14.20	17.80	27.78	19.93	271.53	0.05
40	7.4	13.40	26.20	34.41	24.67	350.74	0.07
50	7.0	16.60	27.40	37.77	27.26	382.10	0.06
60	6.2	20.60	26.60	41.09	29.43	402.12	0.05
LSD _(0.05)		1.4	1.2	1.7	-	12.1	0.02

DAS - Days after sowing

symptom. Only white specks were observed on 41 and 49 days old plants, which became completely dark on 58 days old plants. Disease symptoms first appeared on the older leaves. Suheri and Prince (2001), Kareem *et al.* (2012) and Kumar *et al.* (2013) also reported the work on the effect of host age on susceptibility to purple blotch of onion, *Alternaria* blight of garlic and chilli. Sharma and Ratnoo (2019) reported that 15-day-old plants exhibited the lowest disease severity (10.74%) with the longest latent period (45 hours), while 40-day-old plants showed the highest disease severity (38.31%) and the shortest latent period (25 hours). These observations reinforce that older garlic plants are more susceptible to *A. porri*, likely due to age-related physiological changes that facilitate pathogen invasion and disease development.

Host range of *Alternaria porri*

The pathogen was artificially inoculated on different plant species for their sensitivity to *A. porri*. Onion, tomato and chilli plants showed prominent symptoms of *A. porri* infection whereas, potato and cabbage showed no symptom of the disease (Table 4). The result revealed that in onion and tomato typical disease symptom were expressed 7 and 9 days, respectively, as concentric leaf spots developed with greyish black centre with disease severity 49.80 and 34.13 per cent, respectively. However, it took 12 days in chilli with initial symptoms as small, light brown lesions on leaves and gave 22.48 per cent disease

severity. No symptoms were observed on potato and cabbage. The pathogen was re-isolated from tested plant leaves and the morphological characters of the reisolated pathogen were compared with the original culture and these were similar in all respects. Hence, purple blotch of garlic (*A. porri*) has wide host range.

Our results are supported by Rotem (1994) and Mangala *et al.* (2006), as they also reported the pathogenicity of *A. alternata* in many other species from the Solanaceae, Brassicaceae and Cucurbitaceae families. Sharma and Ratnoo (2019) evaluated the host susceptibility of *A. porri* against several plant species belonging to same or different botanical families through artificial inoculation and found the highest per cent disease index in onion (43.33%) followed by garlic (36.51%), tomato (36.37%) and brinjal (36.14%). These results collectively underscore the wide host adaptability of *A. porri* and related species, suggesting their potential to cause significant cross-infection among economically important crops belonging to different botanical families.

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Table 4. Host range of *Alternaria porri* under inoculated condition in pots

Sr. No.	Host	Scientific Name	Reaction	Incubation period (Days)	Disease severity (%)
1.	Onion	<i>Allium cepa</i>	+	7	49.80
2.	Tomato	<i>Lycopersicon esculentum</i>	+	9	34.13
3.	Chilli	<i>Capsicum annum</i>	+	12	22.48
4.	Potato	<i>Solanum tuberosum</i>	-	-	-
5.	Cabbage	<i>Brassica oleracea</i>	-	-	-

‘+’ = compatible reaction

‘-’ = incompatible reaction

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