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Effect of weather variables on the biology of *Alternaria porri*. of onion under controlled environmental condition

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Abstract
The effect of temperature and Relative humidity on spore germination of *Alternaria porri* isolates were studied under controlled environmental condition (growth chamber). The growth rate experiment was conducted according to the virulence of the isolate through pathogenicity. Among the 15 isolates, three isolates (AP_1, AP_8 and AP_{15}) were selected according to their aggressiveness towards disease production. The effect of temperatures on *A. porri* was investigated to study their influence on the conidial germination, length of germ tube (µm) and number of germ tube /conidia. The result showed that the germination % and length of germ tube (µm) were greatly influenced by different temperatures. Maximum conidial germination (71.60%) was observed at 20^0C at 6 hr after incubation period followed by 25^0C (65.55%). Moderate conidial germination (57.57%) was observed at 30^0C and minimum conidial germination was recorded at 35^0C (48.64%) and their difference was statistically significant. Maximum growth rate was observed at 25^0C for all these 3 isolates (10.00, 11.02 and 9.72 mm/day) followed by 30^0C (9.70, 9.96 and 8.91 mm/day respectively). According to the maximum growth rate on 25^0C one isolate (AP_8) was selected for study on spore germination, length of germ tube and no. of germ tube/conidium on 4 different Relative Humidity i.e. 65, 75, 85 and 95% and it was observed that maximum conidial germination was observed at 95% RH (97.42%) followed by 85 %RH (93.77%) and minimum conidial germination was observed at 75% RH (9.50%).

Keywords: onion, *Alternaria*, temperatures, Relative Humidity, spore germination, length of germ tube and number of germ tube per conidia

Introduction
Onion (*Allium cepa* L.) a member of family Liliaceae is a vegetable crop of global importance and is known as protective food because of its special nutritive and medicinal value. It owns potent medicinal value in ayurvedic and homoeopathic therapy. It is also regarded as an anticancer source of food. Allyl-propyl-di-sulphide is the main ingredient responsible for pungency in bulbs, which help to prevent cancer. The production of onion was 18928.32 million tonne with an area of 1173.360 million ha. During 2014-15 in India (Anonymous 2014-2015) [1[]]. Due to their enormous commercial and medicinal value, onion is cultivated in almost all countries of the world and consumed across the globe. Although, India is the largest producer of vegetables in the world, the productivity is very low at12.5 tonnes/ha as against to 15.8 tonnes/ha in China and 44.21 tonnes/ha in Japan. Though onion is considered as an important fresh market vegetable and medicinal crop, it shows susceptibility to numerous foliar, bulb and root pathogens that ultimately reduce yield and its quality. Purple blotch of onion caused by *Alternaria porri* (Ellis) ciff. is an important destructive disease worldwide and needs timely attention for its managements. The influence of environment on incidence of disease was studied by some workers from different part of countries and reported that high rainfall and high humidity favoured the disease development. *Alternaria porri* on onion occurred following a long period of relative humidity (> 90 per cent) or dew deposition and temperature ranges between 20-250 C (Gupta and Pathak, 1986; Evert and Lacy, 1996) [4, 3]

Materials and Methods
Collection and isolation of pathogen
Leaves of onion infected by pathogen showing typical dark brown, circular to irregular spots were collected from following locations and *A. porri* was isolated from these infected leaves by standard tissue isolation technique in the laboratory. The infected leaf bits will be surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 30 seconds and repeatedly washed separately in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilized Petri plates (1-2 leaf bits per Petri dish) containing potato dextrose agar

(PDA). The Petri plates will be incubated at room temperature (27±1°C) and observed periodically for the growth. Bit of fungal growth developed from the infected tissue was transferred to PDA slants. Then the mycelial tip or single spore isolation will be done for purification of the pathogen. Then such slants with pure culture will be used for further studies

Proving the pathogenicity

Onion seedlings will be raised in earthen pots filled with sterilized soil. Plants will be thoroughly cleaned with sterilized distilled water using moist cotton. Later, the plants will be sprayed with distilled water. They will be covered with polythene bags for 24 hr. The inoculum suspension from ten day old culture will be prepared in sterile distilled water and sprayed on to be the plants by using atomizer. Similarly control plants will be sprayed with sterile distilled water for comparison. The seedlings will be covered with polyethylene bags and incubated for 120 hr. to ensure successful penetration of the pathogen into the tissue. The polythene bags removed after five days and seedlings kept under greenhouse conditions. Observations will be made regularly for the appearance and development of symptoms. After appearance of disease symptoms, re-isolation will be made from the diseased tissues of artificially infected plants. The isolate obtained will be compared with the original culture for confirmation of fungus under study.

The effect of temperature and Relative humidity on spore germination of *Alternaria* isolates were studied under controlled environmental condition (growth chamber).

Effect of temperatures, Relative Humidity for growth, spore germination, length of germ tube and number of germ tube per conidia of *A. porri*

An *A. porri* isolate isolated from onion plants were used for this study. Seven days old culture of *Alternaria* grown on PDA plates was flooded with distilled water and the spores were released by agitation with sterile brush. Spore concentration of 10^4spores/ml was prepared by diluting the concentrated spore suspension with sterilized distilled water with the help of haemocytometer. 100µL of spore suspension after vortexed with a spin mix tube shaker was loaded onto slides containing 2 % water agar block and incubated in Petri dishes on distilled water-saturated filter paper at different temperatures *viz.*, 20^0C, 25^0C, 30^0C and 35^0C respectively and different RH 65-100% at the difference of 5%.

The conidia were considered germinated if the germ tubes were protruded from them. The experiment was conducted in a controlled environmental where a relative humidity of 95 % was maintained throughout the course of experiment. The slides containing *Alternaria* conidia were fixed at different time interval as mentioned earlier with the help of lactophenol. Observations on percent germination of spore, number of germ tube /conidia, length of germ tube/conidia at different time interval were recorded with the help of microscope at 20x and 40x. Germination percentage was then expressed as number of germinated conidia/total number of conidia assessed x 100, using a light microscope.

Result and Discussion

The infected disease specimens particularly the leaves of onion were collected from different districts of West Bengal during survey and the pathogens were isolated from the infected leaves showing typical symptom. Pathogenicity test was performed by inoculating different isolates collected to a set of seedlings of its original hosts under growth chamber condition. Plants were inoculated with a conidial suspension of (1×10^4 conidia/ml) until leaf runoff. The presence/absence of leaf spot symptoms was evaluated 12 days after inoculation and it was confirmed that all the 15 isolates were able to produce the symptom similar to that was observed in the field. Variation in disease severity was recorded on host genotypes with various test isolates. The isolate AP_1 from Nadia district produced highest PDI 22.31% followed by AP_2 (21.46%), AP_3, AP_6, AP_7, AP_4 and AP_5 (20.28%, 19.70%, 19.56%, 18.71% and 18.15% respectively). In case of Hooghly district, AP_8 isolate was found to be highly virulent with 24.47% followed by AP_{10}, AP_9, AP_{13}, AP_{11} and AP_{12} (20.16%, 20.15%, 20.11%, 20.05% and 19.76% respectively). In North 24 Parganas district, the maximum PDI was noticed in AP_{15} with 21.23% followed by AP_{14} 20.26%. Among the fifteen isolates it was found that, the isolate AP_8 produced maximum PDI in comparison to others (Table 1). According to disease severity the isolates grouped in following descending order AP_1> AP_2>AP_{15}> AP_3> AP_{14}> AP_{10}> AP_9>AP_{13}> AP_{11}> AP_{12}>AP_6> AP_7> AP_4>AP_5.

Table 1: Disease severity (PDI) of different isolates under artificial growing condition

Districts	Isolates	Percent Disease Index (%)
Nadia	AP_1	22.31 (28.53)
	AP_2	21.46(27.94)
	AP_3	20.28(27.12)
	AP_4	18.71(25.99)
	AP_5	18.15(25.59)
	AP_6	19.70(26.71)
	AP_7	19.56(26.61)
Hooghly	AP_8	24.47(29.98)
	AP_9	20.15(27.03)
	AP_{10}	20.16(27.03)
	AP_{11}	20.05(26.96)
	AP_{12}	19.76(26.75)
	AP_{13}	20.11(27.00)
N-24-Parganas	AP_{14}	20.26(27.11)
	AP_{15}	21.23(27.78)
	Sem±	0.28
	CD @ 5%	0.83

Figures in parenthesis are angular transformed values

Reisolated and purified cultures from these artificially infected leaves were similar to that of original culture. Similar experimental result for the pathogenic variability was also reported by Ramjegathesh and Ebenezar (2012) [6]. The plants which were not inoculated with the fungal spore suspension did not show any symptom of the disease. Considering the disease severity of all the isolates, the maximum PDI producing (most virulent) isolate are AP_8 (Puratanbaga, Balagarh) of Hooghly district; $AP_1$and AP_2 (In-check farm, Kalyani) of Nadia district and AP_{15} (Amdanga) of North 24 Parganas district.

Similar technique was adopted by Koike and Henderson (1998) and Docampo and Conci (1996) [2] to test the level of pathogenicity on leek and garlic respectively.

Effect of temperatures and relative humidity on *Alternaria* spp.

Effect of temperatures on growth rate

The growth rate experiment was conducted according to the virulence of the isolate through pathogenicity. Among the 15 isolates, three isolates (AP_1, AP_8 and AP_{15}) were selected according to their aggressiveness towards disease production. The growth rate of these three *Alternaria* isolates grown

under different temperature regimes (20^0C, 25^0C, 30^0C and 35^0C) and their differences were statistically significant. The three isolates (AP_1, AP_8 and AP_{15}) showed different growth rate at different temperatures and their difference was statistically significant. Maximum growth rate was observed at 25^0C for all these 3 isolates (10.00, 11.02 and 9.72 mm/day) followed by 30^0C (9.70, 9.96 and 8.91 mm/day respectively). At 20^0C, all the three isolates produced medium growth rate (7.87, 8.98 and 7.19 mm/day) though their differences were statistically significant. Minimum growth rate was obtained at 35^0C (5.38, 6.07 and 5.06 mm/day) and their differences were statistically significant except the latter two isolates (Table 2).

Table 2: Effect of temperatures on growth rate (mm/day) of *Alternaria* isolates

Isolates	Growth rate on different temperature (mm /day)			
	20^0C	25^0C	30^0C	35^0C
AP_1	7.87	10.00	9.70	5.38
AP_8	8.98	11.02	9.96	6.07
AP_{15}	7.19	9.72	8.91	5.06
Sem±	0.23	0.26	0.11	0.34
CD at 5%	0.55	0.64	0.27	0.84

Effect of temperatures on spore germination, length of germ tube and number of germ tube/conidia on *Alternaria* isolates.

The effect of temperatures on *A. porri* was investigated to study their influence on the conidial germination, length of germ tube (µm) and number of germ tube /conidia (Plate 1). The result (Table 3) showed that the germination % and length of germ tube (µm) were greatly influenced by different temperatures. Maximum conidial germination (71.60%) was observed at 20^0C at 6 hr after incubation period followed by 25^0C (65.55%). Moderate conidial germination (57.57%) was observed at 30^0C and minimum conidial germination was recorded at 35^0C (48.64%) and their difference was statistically significant.

Temperature had a significant influence on the length of germ tube and the length of germ tube showed significant differences at 12 hr after incubation period. There was a significant increase in the length of germ tube at 20^0C and beyond which on the length of germ tube abruptly decreased. Maximum length of germ tube (441.76µm) was recorded at 20^0C followed by 409.56 µm at 25^0C and their difference was statistically significant. Medium length of germ tube (353.27 µm) was noted at 30^0C and Minimum length of germ tube was measured at 35^0C (103.30 µm). Different temperature had a significant influence on the number of germ tube at 12 hr after incubation period and their differences were statistically significant. Maximum number of germ tube/conidia was recorded at 25^0C (2.53) statistically at par with 20^0C (2.35).Minimum number of germ tube/conidia was recorded at 35^0C (1.27) followed by 30^0C (1.49) and their differences were not statistically significant.

Table 3: Effect of temperatures on spore germination, length of germ tube and number of germ tube/conidia on *Alternaria* isolates

Temperature (0C)	Germination (%) @ 6 hr	Length of germ tube (µm) @ 12 hr	No. of germtube/conidium @ 12 hr
20º C	71.60 (58.12)	441.76	2.35
25 º C	65.55 (54.36)	409.56	2.53
30º C	57.57 (49.64)	353.27	1.49
35 º C	48.64 (44.51)	103.30	1.27
Sem±	0.42	2.06	0.16
CD at 5%	0.94	4.67	0.36

Figures in parenthesis are angular transformed values

Effect of RH on spore germination, length of germ tube and no. of germ tube/conidium on *Alternaria* isolate.

According to the maximum growth rate on 25^0C one isolate (AP_8) was selected for study on spore germination, length of germ tube and no. of germ tube/conidium on different relative humidity. Relative humidity is a major component of mycelial growth and sporulation, spore germination, spore production of *Alternaria* isolates. To find out the optimum relative humidity (RH %) for maximum germination, length of germ tube and number of germ tube, 4 different RH i.e. 65, 75, 85 and 95% were maintained and it was observed that with increasing humidity, there was a significant increase in the conidial germination of *A. porri* and their difference was statistically significant (Plate 2). Maximum conidial germination was observed at 95% RH (97.42%) followed by 85 %RH (93.77%) and they are statistically at par with each other. Minimum conidial germination was observed at 75% RH (9.50%) and no germination has been observed at 65% RH (Table 4).

The length of germ tube 12 hr after incubation period at different RH showed that with increase in RH, there was a significant increase in the length of germ tube. Maximum length of germ tube (µm) was noted on 95% RH (404.75 µm) followed by 85% RH (380.25µm) and their differences were statistically significant. Minimum length of germ tube was recorded at 75% RH (46.06 µm) whereas, no germ tube was observed at 65 % RH. Different number of germ tubes/conidia after 12 hr of incubation at different RHs showed differential numbers and their differences were statistically significant. Here also, with increase in RH, there was a significant increase in the number of germ tube/ conidia. Maximum number of germ tube was obtained at 95% RH (2.58/ conidia) statistically at par with 85% RH (2.43/ conidia). At 60% RH, no germination was observed whereas minimum number of germ tube was observed at 75% RH (1.01/conidia).

Table 4: Effect of RH on spore germination, length of germ tube and no. of germ tube/conidium on *Alternaria* isolate

Relative humidity (%)	Conidial germination after 6 hr (%)	Length of germ tube (µm) after 12 hr	No. of germ tube/conidia after 12 hr
65	0	0	0
75	9.50 (18.43)	46.06	1.01
85	93.77 (76.15)	380.25	2.43
95	97.42 (81.71)	404.75	2.58
S.Em (±)	1.43	4.55	0.12
CD at 5%	4.21	13.90	0.38

Figures in parenthesis are angular transformed values

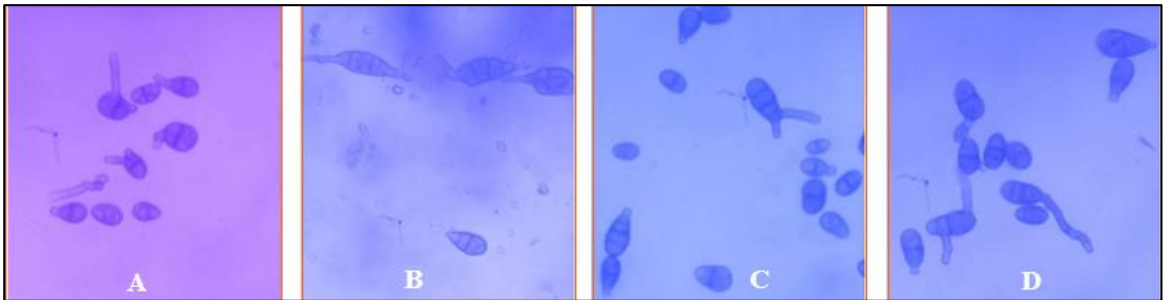

Plate 1.1: Effect of temperature on biology of *Alternaria isolates*. (A), (B), (C) and (D) Conidial germination after 6 hr of incubation at 20^0C, 25^0C, 30^0C and 36^0C

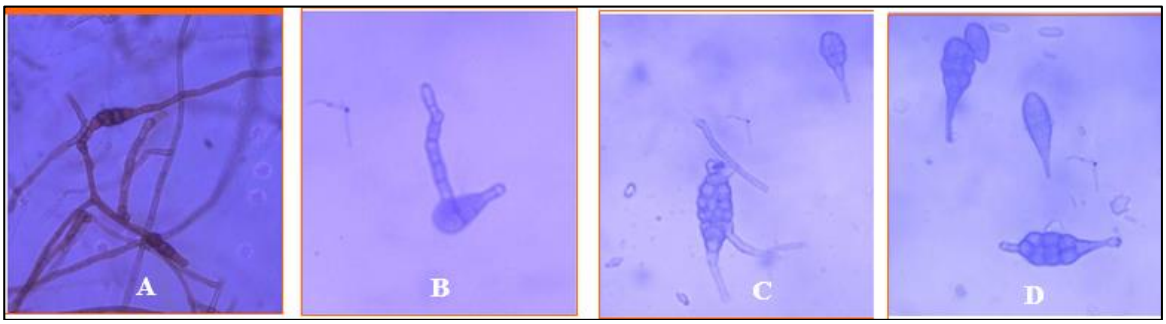

Plate 1.2: (A), (B), (C) and (D) Length of germ tube after 12 hr of incubation at 20^0C, 25^0C, 30^0C and 36^0C

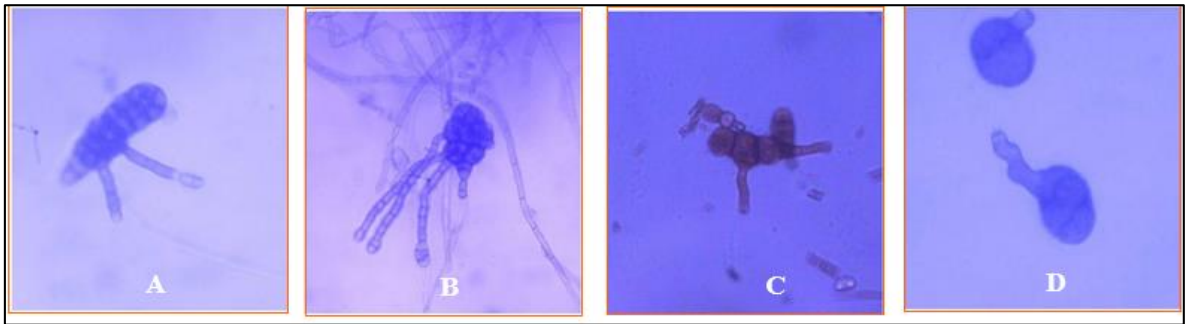

Plate 1.3: (A), (B), (C) and (D) No. of germ tube/conidia after 12 hr of incubation at 20^0C, 25^0C, 30^0C and 36^0C

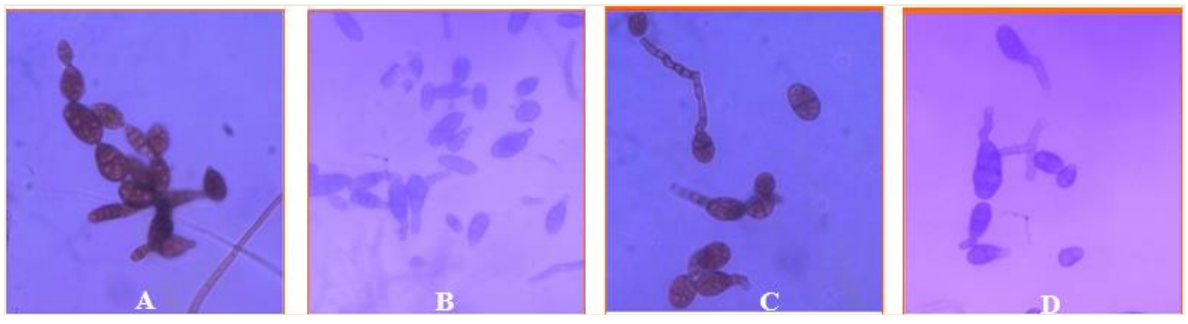

Plate 2.1: Effect of relative humidity on biology of *Alternaria isolates*. (A), (B), (C) and (D) Conidial germination after 6 hr of incubation at 65%, 75%, 85% and 95%

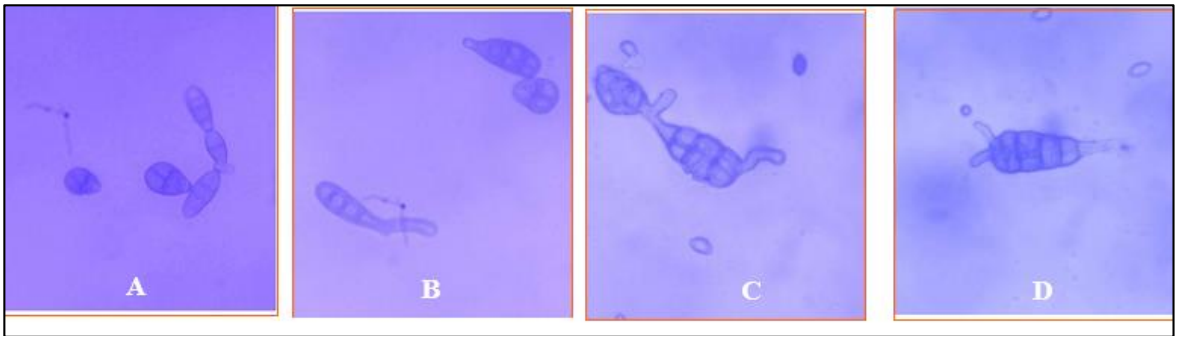

Plate 2.2: (A), (B), (C) and (D) Length of germ tube after 12 hr of incubation at 65%, 75%, 85% and 95%

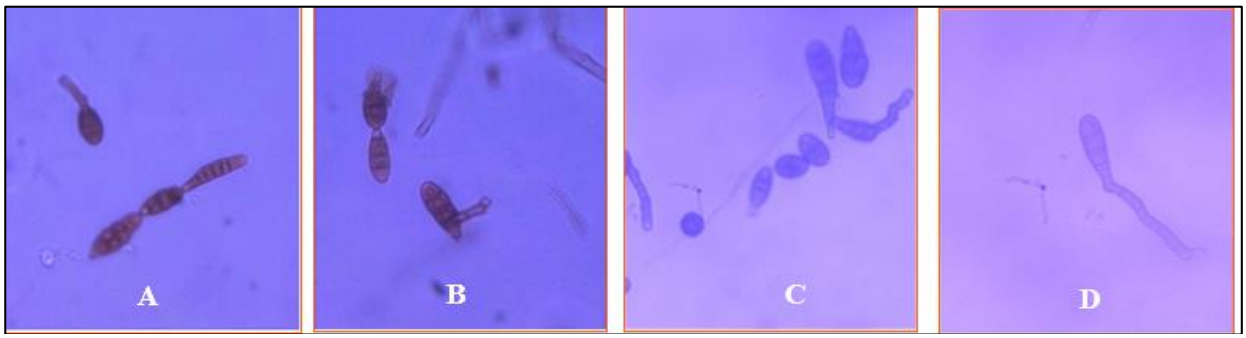

Plate 2.3: (A), (B), (C) and (D) No. of germ tube/conidia after 12 hr of incubation at 65%, 75%, 85% and 95%

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