

## STUDIES ON SOME PHYSIOLOGICAL ASPECTS OF *Phomopsis vexans* CAUSING PHOMOPSIS BLIGHT AND FRUIT ROT OF EGGPLANT

M. R. Islam<sup>1</sup>, M. B. Meah<sup>2</sup>, M. R. Islam<sup>3</sup>, M. M. Islam<sup>4</sup> and A. N. Faruq<sup>5</sup>

### ABSTRACT

The effect of temperature, light, culture media and pH on the mycelial growth and sporulation behaviours of *Phomopsis vexans*, causal organism of *Phomopsis* blight and fruit rot of eggplant were studied in the laboratory. The pathogen grew best at 25°C, under 12/12h cycle light and pH 5.5 levels of culture media. Number of spores per millilitre was also maximum at 25°C temperature, 12/12h cycle light and at pH 5.5. Eggplant Fruit Extract Agar media gave the maximum growth and sporulation of *P. vexans*. Two types of conidia,  $\alpha$  (alpha) and  $\beta$  (beta) were detected in *P. vexans*. Five groups of isolates showed variation in growth and sporulation under different levels of temperature, light, culture media and pH. Alpha and beta conidia varied in size for five isolates groups. Existence of variation in isolates of *P. vexans* revealed the existence of the variability of *P. vexans* occurs in Bangladesh.

**Key words :** *Phomopsis vexans*, fruit rot, eggplant, phomopsis blight

### INTRODUCTION

Eggplant (*Solanum melongena* L.) also known as brinjal is one of the most popular and important vegetable crops worldwide and also in Bangladesh. The eggplant is extensively grown in Bangladesh round the year. Due to its taste and year round availability, it is one of the widely consumed vegetable in the country. Eggplant is a nutritious vegetable and has got multifarious use as a food item (Bose and Som 1986, Rashid 1993). The crop is highly susceptible to different diseases in Bangladesh. Among these 12 diseases, *Phomopsis* fruit rot caused by *P. vexans* (Sacc & Syd.) is the major constraint in successful cultivation of eggplant in the country (Das, 1998). The pathogen is externally and internally seed-borne. The spores may easily be dispersed by rain splashes. They are also disseminated by rotten parts and insects (Singh, 1992). About 20-30% fruit rots due to this disease have been estimated in Bangladesh (Das, 1998; Khan, 1999). Recent investigations have revealed that *Phomopsis* blight and fruit rot causes about 21% fruit rot and 7% seed rot in eggplant (Anon, 2002).

Physiological studies of the microorganism are the basis of applied research. The growth of the fungus is governed by a number of factors. Of them, temperature, light, culture media, hydrogen ion concentration are important (Cochrane, 1958). Without any knowledge of physiology of the fungus, the applied research will be meaningless and will never reach the door of the goal. The physiological studies provide information about the most suitable temperature/light/culture media/pH for growth of the fungus as well as sporulation. Abundant growth and sporulations are the most important criteria for infection of crop varieties/variety screening and to adopt control measures against the pathogen. Different aspects of physiology of *P. vexans* have been studied (Divinagracia, 1972; Harada *et al.*, 1973; Chowdhury and Hasija, 1980; Islam *et al.*, 1990). In view of the above facts, the present research work was designed to study the effect of temperature, light, culture media and pH on the growth and sporulation of *P. vexans* and also to study the variability of *P. vexans* existing in Bangladesh.

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<sup>1</sup>Scientific officer, SRDI, <sup>2</sup> Professor, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh, <sup>3</sup> Professor and <sup>5</sup>Assistant Professor, Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, <sup>4</sup> SSO, Bangla Agricultural Research Institute, Gazipur, Bangladesh.

## MATERIALS AND METHODS

### **Isolation of *Phomopsis vexans***

The present study was carried out during the period from March 2002 to April 2003. Diseased fruit samples of eggplant were collected from 17 eggplant growing areas of Bangladesh. The samples were plucked and placed in polythene bag in airtight condition, later kept in the refrigerator overnight for further study. Diseased fruit samples with typical symptoms were selected and washed separately under running tap water to remove dust particles and then air dried. The infected fruits were surface sterilized with ethanol followed by flaming in spirit lamp and cleaning with cotton swab soaked in alcohol. Small pieces (2-3mm) of fruit tissues at the junction of diseased and healthy portion were cut with the help of sterilized blade and were placed on potato dextrose agar (PDA) plates. The inocula were incubated at  $25\pm 2^{\circ}\text{C}$  for seven days to allow the pathogen to grow. After incubation, white mycelia of *P. vexans* grew from the inocula. Similar growth was observed on almost all inocula. The organism was aseptically transferred to several fresh PDA plates. Pure culture of the pathogen was prepared and preserved in PDA plates.

### **Grouping of Isolates of *Phomopsis vexans***

Thirty two isolates of *P. vexans* were collected from 17 eggplant growing districts of Bangladesh (Table 1). The isolates were isolated from the samples based on location of diseased sample collection. They were maintained on PDA medium at  $25 \pm 1^{\circ}\text{C}$  for studying cultural and morphological characteristics of the isolates. Based on the cultural properties, the isolates were grouped. A representative isolate from each group was selected for further investigation.

### **Studies on physiology of the fungi**

The effect of temperature, light, culture media, and pH on the growth and sporulation of *P. vexans* was studied. PDA was used as basal medium. PDA plates acidified with one drop of 5% lactic acid were inoculated with 5 mm mycelial disc of 10 days old fungal culture on PDA and incubated at  $15 \pm 1^{\circ}\text{C}$ ,  $20 \pm 1^{\circ}\text{C}$ ,  $25 \pm 1^{\circ}\text{C}$ ,  $30 \pm 1^{\circ}\text{C}$ ,  $35 \pm 1^{\circ}\text{C}$  for 7 days. For the effect of light mycelial disc of 10 days old culture of *P. vexans* were incubated at room temperature ( $25 \pm 1^{\circ}\text{C}$ ) under four different conditions of light, viz. 24h light, 24h dark, 12/12h cycle light and darkness under normal fluorescent light and UV light for 7 days. Observations were made on the radial colony growth, colour, and consistency of the colony and spore production. Sporulation was determined at 30 days age of the culture. For determining the effect of pH of culture media seven pH levels were tested. The media in the flasks (after autoclaving) were adjusted to pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. The media were inoculated with 5 mm mycelial disc of the fungus in the center and incubated at  $25^{\circ}\text{C} \pm 2$  for 7 days. The pH was adjusted with 0.1N HCl and 1 N NaOH. All treatments were replicated four times. Observations were made on the linear growth, colour and consistency of the colony and spore production. Spores produced in a treatment after incubation of 30 days, were counted by a Haemocytometer.

### **Counting spores of *P. vexans***

After incubation, the degree of sporulation of the fungus was determined by adding 10 ml sterile water in each petridishes. Then surface of the fungal culture was scrapped gently with a wire loop to disperse the spores. The suspension was decanted into a conical flask and sieved to separate the hyphal fragments from the spore suspension. Finally, from the suspension 1 ml was poured into the Haemocytometer cells, under microscope, the spores in five unit cells were counted at 10x magnification. Putting the average spore number per unit cell in the formula the number per millilitre was determined.

$$\text{No. of spores per cubic mm suspension} = \frac{\text{No. of spores counted} \times \text{dilution}}{\text{No. of smallest square counted}} \times 4000$$

All laboratory experiments were set in completely randomized design (CRD). Minimum of four replications were maintained. Experimental data were analyzed following appropriate statistical methods. Treatment means were compared following Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

### Effect of temperature

The mycelial growth and degree of sporulation of 5 groups of isolates at different temperature regimes are shown in Table 1. In the present investigation, the isolates of the fungus were able to grow at temperature range from 15 to 30°C. Comparatively higher growth and sporulation was obtained at 25°C for all groups of isolates except group-2. Group-2 showed highest growth at 20°C temperature. The mycelial growth gradually reduced with the increase or reduction of temperature from 25°C temperature. In all cases, the growths were completely inhibited at 35°C. At 25°C the highest growth was obtained for group-1 followed by group-3. Group-4 had the highest growth at 30°C while Group-2 had the highest growth at 20°C.

Growth pattern and consistency of the colonies were dissimilar in different groups. At 25°C the growth pattern and colony consistency were fluffy with concentric ring and compact and thick in group-1; embedded, concentric ring not distinct/absent and compact and thin in group-2; slightly fluffy with concentric ring and loose and thin in group-3; fluffy with distinct concentric ring and compact and thick in group-4; and embedded with slightly fluffy, distinct concentric ring, and compact and thin in group-5. This colony behaviour were also seen at 30°C (Table 1), except Group-2 and 4. Sporulation was the highest at 25°C. Group-2 isolate had the highest sporulation at 20°C while group 4 had the highest at 30°C. On an average, isolate group 2 had the highest sporulation followed by group 1 and 3 while group 5 had the lowest. Temperature 20°C and 25°C supported the highest sporulation followed by temperature 30°C. Among all the groups, the maximum sporulation was observed in group-2 at 20°C which was followed by group-1 at 25°C. Among the 5 groups the minimum sporulation was detected in group-2 at 15°C. At 35°C no spores were produced in any group. Statistical analysis revealed that the growth and sporulation of the isolates at different temperatures differed significantly.

### Effect of light

Light affected the mycelial growth and sporulation appreciably in PDA media at 25±1°C (Table 2). Twelve hours light supported the highest growth in all groups and the highest value was 8.35 cm in group-3. Twelve hours. UV light showed the second highest performance in all groups. There were not much variations in different light treatments but alternate light and darkness produced maximum radial mycelial growth. The minimum growth was observed in continuous darkness in all isolate groups of *P. vexans*. Colony consistency and growth pattern did not vary with different types of light but they varied among the groups.

Trend in sporulation was similar to that of growth in all groups. Sporulation capacity of *P. vexans* was enhanced in alternate light and darkness. Maximum spores were recorded at 12/12 h cycle in all groups and the highest value was (1565×10<sup>4</sup> spores/ml) in Group-1. The number of spores dropped sharply at 12h. UV light and very poor spores were produced at 24h darkness. Group-4 isolates produced the lowest number of spores at all light conditions.

### Effect of pH of culture media

The pH of PDA greatly affected the mycelial growth and sporulation when the fungus was grown at 25±1°C (Table 3). The highest mycelial growth was observed at pH 5.5 in every group. The second

highest growth was observed at pH 5 in all groups except group 5. The growth at pH 7 was near to the growth of pH 5. The lowest growth was observed at pH 4 in most of the groups.

**Table 1. Effect of different temperature on the growth and sporulation of five groups of isolates of *Phomopsis vexans***

Temperature (OC)	Linear growth in cm after 7 days*	Growth rate/24 hrs	Growth pattern	Colony consistency	Days to sporulation	Numbers of spores/ml (-000)**	
<b>Group- 1</b>	15	4.48	0.64	Fluffy with concentric rings	Compact and thick	11	325
	20	7.8	1.11	Fluffy with concentric rings	Compact and thick	13	1175
	25	7.93	1.13	Fluffy with concentric rings	Compact and thick	10	10050
	30	7.08	1.01	Fluffy with concentric rings	Compact and thick	21	650
	35	0.00	0.00	0.00	0.00	0.0	0.0
<b>Group-2</b>	15	3.93	0.56	Embedded, concentric rings not distinct/absent	Compact and thin	12	150
	20	6.8	0.97	Embedded, concentric rings not distinct/absent	Compact and thin	14	15200
	25	7.53	1.08	Embedded, concentric rings not distinct/absent	Compact and thin	12	2200
	30	7.98	1.18	Embedded, concentric rings not distinct/absent	Compact and thin	14	500
	35	0.0	0.00	0.00	0.00	0.0	0.0
<b>Group-3</b>	15	4.4	0.63	Slightly fluffy with concentric ring	Loose and thin	13	475
	20	6.98	0.99	Slightly fluffy with concentric ring	Loose and thin	21	350
	25	7.48	1.07	Slightly fluffy with concentric ring	Loose and thin	10	5200
	30	6.23	0.89	Slightly fluffy with concentric ring	Loose and thin	15	550
	35	0.0	0.0	0.00	0.00	0.0	0.0
<b>Group-4</b>	15	5.4	0.77	Fluffy with distinct concentric ring	Compact and thick	12	350
	20	6.38	0.91	Fluff]' with distinct concentric ring	Compact and thick	11	550
	25	7,53	1.08	Fluffy with distinct concentric ring	Compact and thick	9	650
	30	6.85	0.98	Fluffy with distinct concentric ring	Compact and thick	12	6625
	35	0.0	0.00	0.00	0.00	0.0	0.0
<b>Group-5</b>	15	5.6	0.80	Embedded with slightly concentric rings are distinct	Compact and thin	9	350
	20	7.53	1.08	Embedded with slightly concentric rings are distinct	Compact and thin	11	450
	25	7.63	1.09	Embedded with slightly concentric rings are distinct	Compact and thin	12	1325
	30	6.8	0.97	Embedded with slightly concentric rings are distinct	Compact and thin	12	600
	35	0.0	0.00	0.00	0.00	0.0	0.0
S_ (P<0.05)i	0.08246					459.8	
LSD (P<0.05)	0.2323					1334	

Total (Av.) radial growth after 7 days of incubation; \*\* Counting was done after 30 days of incubation

**Table 2. Effect of different types of light on the growth and sporulation of five groups of isolates of *Phottopsis vexans***

Light	Linear growth in cm after 7 days*	Growth rate/24 hrs	Growth pattern	Colony consistency	Days to sporulation	Numbers of spores/ml ('000)**
<b>Group-1</b> All time light	7.10	1.01	Fluffy with concentric rings	Compact and thin	9	11700
All time dark	6.88	0.98	Fluffy with concentric rings	Compact and thin	10	8800
f 2 h. light	7.58	1.08	Fluffy with concentric rings	Compact and thin	6	15650
12h.UV light	7.35	1.05	Fluffy with concentric rings	Compact and thin	6	13500
<b>Group-2</b> All time light	7.38	1.05	Embedded, concentric ring absent	Loose and thin	7	880
All time dark	7.03	f.00	Embedded, concentric ring absent	Loose and thin	7	160
1 2 h. light	7.88	1.13	Embedded, concentric ring absent	Loose and thin	11	8550
12h,UV light	7.58	1.08	Embedded, concentric ring absent	Loose and thin	8	6500
<b>Group-3</b> All time light	7.95	1.13	Slightly fluffy with concentric ring	Compact and thin	6	6300
All time dark	7.13	1.01	Slightly fluffy with concentric ring	Compact and thin	7	1500
1 2 h. light	8.35	1.19	Slightly fluffy with concentric ring	Compact and thin	7	7500
12h.UV light	8.03	1.15	Slightly fluffy with concentric ring	Compact and thin	8	6500
<b>Group-4</b> All time light	6.70	0.95	Fluffy with distinct concentric ring	Compact and thick	7	500
All time dark	6.45	0.92	Fluffy with distinct concentric ring	Compact and thick	8	150
1 2 h. light	7.63	1.09	Fluffy with distinct concentric ring	Compact and thick	6	750
12h.UV light	6.93	0.99	Fluffy with distinct concentric ring	Compact and thick	6	650
<b>Group-5</b> All time light	7.25	1.04	Embedded with slightly concentric rings are distinct	Slightly compact and thin	9	1500
All time dark	6.80	0.97	Embedded with slightly concentric rings are distinct	Slightly compact and thin	7	1100
12h. light	7.53	1.08	Embedded with slightly concentric rings are distinct	Slightly compact and thin	6	5500
12h.UV light	7.38	1.05	Embedded with slightly concentric rings are distinct	Slightly compact and thin	7	4670
S <sub>x</sub> (P<0.05)	0.1883					353.7
LSD (P<0.05)	0.5326					1011

\*Total (Av.) radial growth after 7 days of incubation

\*\* Counting was done after 30 days of incubation.

**Table 3. Effect of different pH levels of culture medium PDA on the growth and sporulation of five groups of isolates of *Phonopsis vexans***

	PH	Linear growth in cm after 7 days*	Growth rate/24 hrs	Growth pattern	Colony consistency	Days to speculation	Numbers of spores/(ml rooo)**
<b>Group-1</b>	4	7.30	1.04	Fluffy with concentric rings	Compact and thick	14	1500
	4.5	7.23	1.03	Fluffy with concentric rings	Compact and thick	12	1666
	5	8.68	1.24s	Fluffy with concentric rings	Compact and thick	9	1916
	5.5	8.75	1.25	Fluffy with concentric rings	Compact and thick	8	5200
	6	8.13	1.16	Fluffy with concentric rings	Compact and thick	8	2250
	6.5	7.13	1.02	Fluffy with concentric rings	Compact and thick	9	650
	7	8.25	1.18	Fluffy with concentric rings	Compact and thick	14	550
<b>Group-2</b>	4	6.38	0.91	Embedded, concentric rings not distinct/absent	Compact and thin	9	125
	4.5	7.45	1.06	Embedded, concentric rings not distinct/absent	Compact and thin	13	150
	5	8.10	1.16	Embedded, concentric rings not distinct/absent	Compact and thin	12	1225
	5.5	8.83	1.26	Embedded, concentric rings not distinct/absent	Compact and thin	11	6437
	6	7.20	1.03	Embedded, concentric rings not distinct/absent	Compact and thin	15	4000
	6.5	6.98	0.99	Embedded, concentric rings not distinct/absent	Compact and thin	17	1750
	7	7.10	1.01	Embedded, concentric rings not distinct/absent	Compact and thin	16	7000
<b>Group-3</b>	4	7.05	1.00	Slightly fluffy with concentric ring	Loose and thin	10	3500
	4.5	7.73	1.10	Slightly fluffy with concentric ring	Loose and thin	10	2500
	5	8.35	1.19	Slightly fluffy with concentric ring	Loose and thin	12	1225
	5.5	8.73	1.25	Slightly fluffy with concentric ring	Loose and thin	11	8800
	6	7.58	1.07	Slightly fluffy with concentric ring	Loose and thin	13	725
	6.5	6.80	0.97	Slightly fluffy with concentric ring	Loose and thin	16	450
	7	8.33	1.19	Slightly fluffy with concentric ring	Loose and thin	20	250
<b>Group-4</b>	4	7.08	1.01	Fluffy with distinct concentric ring	Compact and thin	16	125
	4.5	7.93	1.13	Fluffy with distinct concentric ring	Compact and thin	12	175
	5	7.98	1.14	Fluffy with distinct concentric ring	Compact and thin	12	350
	5.5	8.53	1.22	Fluffy with distinct concentric ring	Compact and thin	10	450
	6.0	6.83	0.98	Fluffy with distinct concentric ring	Compact and thin	17	250
	6.5	6.58	0.94	Fluffy with distinct concentric ring	Compact and thin	8	175
	7.0	7.55	1.08	Fluffy with distinct concentric ring	Compact and thin	17	150
<b>Group-5</b>	4.0	6.60	0.94	Embedded slight concentric rings	Compact and thin	16	250
	4.5	7.53	1.08	Embedded slight concentric rings	Compact and thin	13	220
	5.0	6.23	0.89	Embedded slight concentric rings	Compact and thin	13	5500
	5.5	8.75	1.25	Embedded slight concentric rings	Compact and thin	7	7750
	6.0	6.83	0.98	Embedded slight concentric rings	Compact and thin	14	1430
	6.5	6.88	0.98	Embedded slight concentric rings	Compact and thin	13	800
	7.0	7.78	1.11	Embedded slight concentric rings	Compact and thin	15	520
S <sub>i</sub> (P<0.05)		0.1138					119.5
[TSD7P^05)		0.3151					

\* Total (Av.) radial growth after 7 days of incubation, \*\* Counting was done after 30 days of incubation 46

At pH 5.5, the isolates differed in colony consistency and radial growth with increase in pH over 5.5, mycelial growth decreased sharply which continued till pH 6.5 followed by a slight increase at pH 7.0 except for Group-2 which continued to decrease.

Sporulation was the highest at pH 5.5 in all groups. The pH range which supported next higher growth was 5.0 to 6.0. Poor growth was recorded at pH 4.0 for all groups except group-3. Group-4 exhibited the poorest sporulation at all pH except at 4.5. On an average isolate group-1 had the highest sporulation followed by group-2 at all pH while isolate group-5 produced the lowest. Average sporulation was the highest at pH 5.5, the second highest was at pH 5.0 and the lowest at pH 6.5. Changes in pH level influenced sporulation (Plate 5b) of all isolates except group-4 which maintained a constant low amount of sporulation. At pH 4.0 to 5.0, sporulation was lower with wide variation among the groups which suddenly increased to the highest level at pH 5.5 which again sharply decreased with increase in pH level with exception of group-5.

Experiments on the effect of different temperatures on mycelial growth of *P. vexans* reveal that the pathogen can grow over a temperature range between 15 to 30°C and best growth was found at 25°C in all groups. The growth at 20°C and 30°C was numerically different but statistically similar. These was in line with the reports of Divinagracia (1972), Harada *et al.* (1973), Chinenye (1974), Chowdhury and Hasija (1980), Ahmad (1987), Islam *et al.* (1990), Hossain *et al.* (1992), and Sugha *et al.* (2002) who found 25°C as the best temperature to obtain maximum mycelial growth of *P. vexans*. The mycelial growth was inhibited at 35°C and this is supported by the findings of Divinagracia (1972).

The findings of the present study do not agree with Pawar and Patel (1957) who reported temperature range of 7-11°C as minimum, 28°C as optimum and 35-40°C as maximum for the growth of *P. vexans* and also with Singh (1992) who stated 29°C as the optimum temperature for growth of *P. vexans*. Maximum sporulation occurred at 25°C as it was found during the sporulation of *P. vexans* which declined with rise and fall of the incubating temperature. This finding agrees with the report of Harada *et al.* (1973) who obtained similar results while working with *P. molt.* However, Punithalingam and Holliday (1972) found 28-30°C as optimum temperature for the growth of *P. vexans*. Akter (1999) also obtained maximum sporulation at 25°C. However, the present findings do not agree with the report of Chinenye (1974) who obtained enormous spores at 28°C.

Incubating *P. vexans* under light and darkness revealed that an alternate cycle of 12/12 h enhanced growth and sporulation. But the growth of the fungus under continuous light, continuous dark and 12/12 h UV light was lower. At 12 h light gave the highest sporulation and 12h UV light gave the second highest sporulation of *P. vexans*. Continuous dark showed the lowest sporulation. The present findings do agree with the report of Divinagracia (1969) that light was needed for abundant sporulation of *P. vexans* and also with that of Harada *et al.* (1973) who found that formation of Pycnidia and spores were dependent on light intensity and temperature while working with *P. mail.*

Under the present investigation it appears that *P. vexans* can grow over a range of pH levels ranging from 4.0 to 7.0 with best growth and sporulation at pH 5.5 and lowest at pH 4.0. The findings do agree with Akter (1999) and also with Hasija and Chowdhury (1980) found maximum spores produced at pH 5.5.

Variation in isolate groups in respect of growth and sporulation in different temperature, light, pH and nutrition indicates the existence of variability among the isolates of *P. vexans*.

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