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CULTURAL, MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF *PHOMOPSIS VEXANS* ISOLATES FROM DIFFERENT BRINJAL (*SOLANUM MELONGENA* L.) GROWING REGIONS OF KARNATAKA, INDIA

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ABSTRACT

In the present study, an attempt was made to record the cultural, morphological and physiological characteristics of 23 isolates of *Phomopsis vexans* from different brinjal (*Solanum melongena* L.) growing regions of Karnataka, India. It was observed that all the isolates were varied considerably in the cultural characteristics such as mycelial type, colony colour, colony diameter, margin type and zonation when they were grown on five different media. All the media supported the pycnidia formation, but the isolate Pv7 was not found to produce pycnidia on all the tested media. The physiological tests indicated that all isolates showed maximum growth in the plates containing PDA medium at pH 5.6 and pH 6. Similarly, 27° C was found suitable for *P. vexans* growth and pycnidia formation. By analyzing the results, these isolates were broadly categorized into five groups which are not in accordance with their agro-climatic conditions.

KEYWORDS: ***Phomopsis vexans*, *Solanum melongena* L. Pycnidia, α and β – conidia.**

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INTRODUCTION

Phomopsis vexans (Sacc. & Syd.) Harter [Telomorph: *Diaporthe vexans* (Gratz)] is a fungal pathogen causing leaf blight and fruit rot disease in brinjal (*Solanum melongena* L.). The disease has been considered as the major constraint for cultivation of brinjal across world[1,7,13]. It causes over 50 per cent loss in production and productivity in various regions of world[3] and to the extent of 10–20 per cent in India[13]. The seeds were prime infection source and serve as a substrate for pathogen survival[12]. Severely infected seedlings die in the nursery stage, in some case the infection is latent and expresses after transplantation. The disease can spread quickly when mature pycnidiospores (α and β conidia) are released from pycnidia and dispersed by splashing rain water, insects and contaminated equipment. The pycnidiospores germinate rapidly when free moisture is present on the leaves, stems or fruits. The pathogen survives in brinjal plants, crop debris, seeds and soil[14]. Even though extensive studies were carried out previously on this pathogen/disease, there are very few reports available on the cultural, morphological and physiological variability observed in the isolates of *P. vexans* from different parts of world[2,4,5,6,8,9,10,11]. This microbe is highly versatile to changing environmental conditions and shows high variability across different agro-climatic regions. Hence, the present study was aimed to observe and record the cultural, morphological and physiological characteristics of *P. vexans* isolated from naturally infected brinjal plant materials collected from different agro-climatic zones of Karnataka, India.

MATERIALS AND METHODS

Collection of Phomopsis vexans

A total of 23 isolates of *P. vexans* were collected from the culture collection of the Department of Biotechnology, University of Mysore, Mysore, Karnataka (India) and designated as Pv1 to Pv23. These fungal cultures were previously isolated from naturally infected plant materials collected from major brinjal growing regions of Karnataka (India) and used for the further experiments.

Cultural and morphological characterization of Phomopsis vexans

Five different growth media such as Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Czapek Dox Agar (CDA), Malt Extract Agar (MEA) and Glucose Peptone Yeast Extract Agar (GPY) were used to observe the cultural characteristics of *P. vexans* isolates. A mycelial plug (5 mm diameter) of 7-days-old culture of each isolate of *P. vexans* was inoculated at the center of Petri plates (90 mm diameter) containing each medium. The plates were incubated for 7 days at 28 ± 2° C under dark condition. After an incubation period, the cultural characters of *P. vexans* isolates were observed and recorded. The cultural characters included were growth characteristics, mycelial type, colour of the colony, colony diameter, shape, margin type and zonation. A minimum of three plates were maintained for each isolate on each medium and the whole experiment was repeated three times. At the end of the incubation period, the formation of pycnidia and sporulation were observed under microscope and recorded. α and β conidia were observed under a compound microscope.

Physiological characterization of Phomopsis vexans

Physiological characterization of *P. vexans* isolates was carried out by growing them on PDA medium adjusted to different pH and incubated at different temperatures. The pH of the medium was adjusted to 4, 6, 8, 10, 12 and control pH (5.6) using 1 M HCl and 1 M NaOH. A mycelial plug (5 mm diameter) of 7-day-old culture of each isolate of *P. vexans* was inoculated at the center of Petri plates containing PDA medium with different pH. The plates were incubated for 7 days at 28 ± 2° C under dark condition. After incubation period, the plates were observed for the growth of *P. vexans* isolates. Further, the growth response of these isolates to different temperatures was studied by inoculating them at the center of Petri plates containing PDA medium and incubated at different temperatures (4, 15, 27 and 40° C). In each experiment, three plates were maintained for each isolate and the experiment was repeated three times.

Statistical analysis

The data were statistically analyzed and subjected to arcsine transformation and analysis of variance (ANOVA) using SPSS, ver. 17 (SPSS Inc., Chicago, IL).

RESULTS

In the present study, all the isolates of *P. vexans* showed considerably varied cultural characteristics on PDA, OMA, CDA, MEA and GPY as shown in the Table 1. The cultural characteristics of *P. vexans* isolate Pv1 on five different growth media was represented in the Fig. 1 (a – e). Among the five media tested, all isolates were grown and sporulated well on PDA medium. The average mycelial growth of *P. vexans* on PDA, OMA, CDA, MEA and GPY was found to 86, 70, 81, 52 and 75 mm, respectively. The PDA medium was found to be most suitable growth medium and also supports pycnidia formation and sporulation. Among the 23 tested isolates, all isolates were found to produce pycnidia except the isolate Pv7 which recorded only mycelial growth on all the growth media used. The colour and size of the pycnidia were varied from isolate to isolate with respect to growth media. In majority of the isolates, the colour of the pycnidia was black, but some are showing the brown, green or gray (Table 1). Production of α and β – conidia were also observed for each isolate under microscope (Fig. 2) and it was found that α – conidia was frequently produced in these isolates in comparison with β – conidia on all tested media. The Fig. 3 represented the mature pycnidiospores ooze out of pycnidia of *P. vexans* isolate Pv1 through the ostioles. The physiological tests indicated that all the isolates of *P. vexans* recorded maximum growth in the plates containing PDA medium with pH 5.6 and pH 6, as the pH of the medium becomes more basic or acidic the growth of *P. vexans* was found to decreased (Table 2; Fig. 1 f – k), at or

below pH 3 and above pH 12 the fungal growth was completely absent. The maximum growth of *P. vexans* isolates was also recorded on PDA medium, incubated at 27° C but their growth was completely absent at 4 and 40° C (Table 3; Fig. 1 l – o).

DISCUSSION

In the present study, the cultural characterization indicated that all 23 isolates of *P. vexans* were recorded considerably varied results for the cultural characteristics on five different growth media used. The isolates were found to grow faster and produced pycnidia well on PDA medium. The findings showed that the PDA medium is the best growth medium for the formation of pycnidia as well as sporulation in comparison with other growth media tested. The formation of pycnidia was absent in most of the isolates of *P. vexans* on GPY medium. There was also variation present among different isolates in their pycnidia colour on different growth media used. Such variations have been previously reported by Islam and Pan[8], Akhtar and Chaube[2] and Islam et al.,[10]. These differences indicated that there was an existence of variability among the isolates. Islam and Pan[8] also observed that the variation in morphological characters, growth rate, sporulation, colony diameter, dry weight, colour, texture, zonation, arrangement of pycnidia and its relative abundance in the Pv colonies isolated from diseased aubergines from various locations in India. Akhtar and Chaube[2] studied the variability in the isolates of *P. vexans* and revealed that there were significant and substantial differences in radial growth and morphological characters of the isolates on PDA medium. They also showed that the germinability of pycnidiospore significantly differed from different isolates and the colour of the pycnidia developed was black in some isolates while brown in some. Recently, Islam et al.,[10] characterized and categorized forty four isolates of *P. vexans* isolated from different eggplant cultivars into five groups based on their cultural and morphological properties like mycelial growth, colony colour, colony consistency, pycnidial distribution in the growing media, pycnidial size, spore (α and β) size and sporulation rate, and it has been observed that five groups of isolates distinctly differed on all the parameters evaluated with few exceptions. Our studies also revealed that the morphological characterization under microscope indicated that all the isolates also showed variable results for sporulation and formation of pycnidia, and production of α and β – conidia on all the media tested. In the present study, the physiological characterization indicated that all the isolates showed the maximum growth in the plates containing PDA medium at pH 5.6 and pH 6. It also showed that the optimum incubation temperature for their maximum growth was at 27° C on PDA medium. The existence of physiological differences among the isolates of *P. vexans* was reported earlier by Islam et al.,[6]. Islam et al.,[9] also revealed that *P. vexans* can grow over a wide range of pH of medium ranging from 4.0 to 7.0 and temperature range between 15 to 30° C with best growth and sporulation at pH 5.5 and 25° C which required for their cellular growth and several metabolic activities. Similar findings of Akter[4] and also with Hasija and Chowdhury[5] were found to show the maximum growth and spores of *P. vexans* produced at optimum temperature and pH of 25° C and 5.5 respectively. Our results showed that there was no growth of *P. vexans* isolates found to record at pH 3 and at low (4° C) and high (40° C) temperatures. Our results are in corroboration with Islam et al.[9] where they recorded that the mycelial growth of *P. vexans* was lowest at pH 4.0 and inhibited at 35° C. MaZuo[11] also revealed that *P. vexans* couldn't grow when the pH of the medium was less than 3 and couldn't survive both at low (10° C) and high (35° C) temperatures. By analyzing the results obtained, these isolates were broadly divided into five groups based on their cultural and physiological characteristics. These groups didn't relate to the agro-climatic regions of origin of fungus. In conclusion, the present study was observed that the significant differences in cultural, morphological and physiological characteristics among 23 isolates of *P. vexans* and the observations are substantiated to others similar work done earlier.

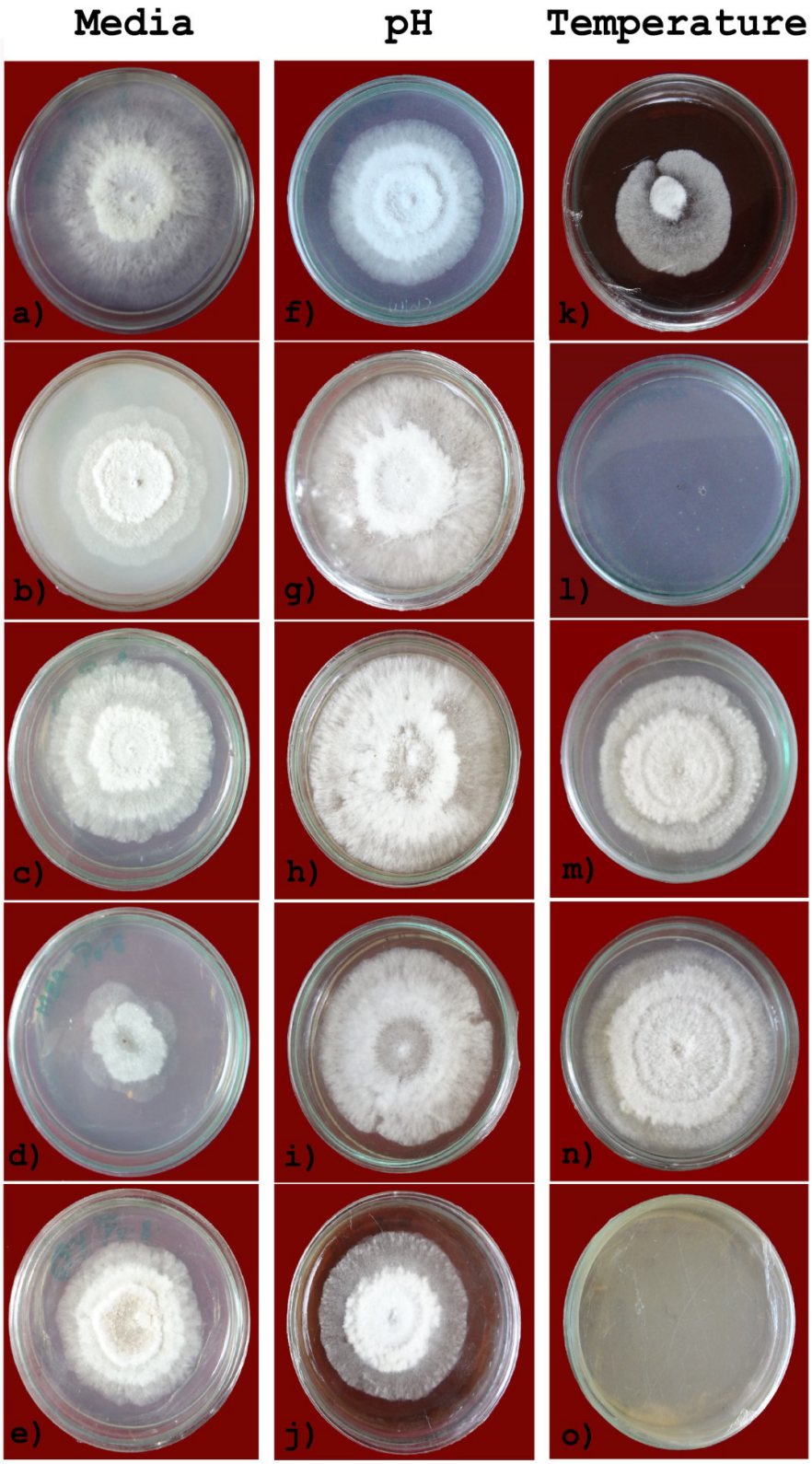

Figure 1

Cultural, morphological and physiological characteristics of P. vexans isolate Pv1. a) – e) The growth characteristics of P. vexans on five different growth media: a) Potato Dextrose Agar (PDA); b) Oat Meal Agar (OMA); c) CzapekDox Agar (CDA); d) Malt Extract Agar (MEA); e) Glucose Peptone Yeast Extract Agar (GPY). f) – k) The growth characteristics of P. vexans on PDA medium at different pH: f) pH 4; g) Control pH (5.6); h) pH 6; i) pH 8; j) pH 10; k) pH 12. l) – o) The growth characteristics of P. vexans on PDA medium at different temperatures: l) 4° C; m) 15° C; n) 27° C; o) 40° C.

Figure 2
Alpha (α) and beta (β) – conidia of P. vexans isolate Pv1

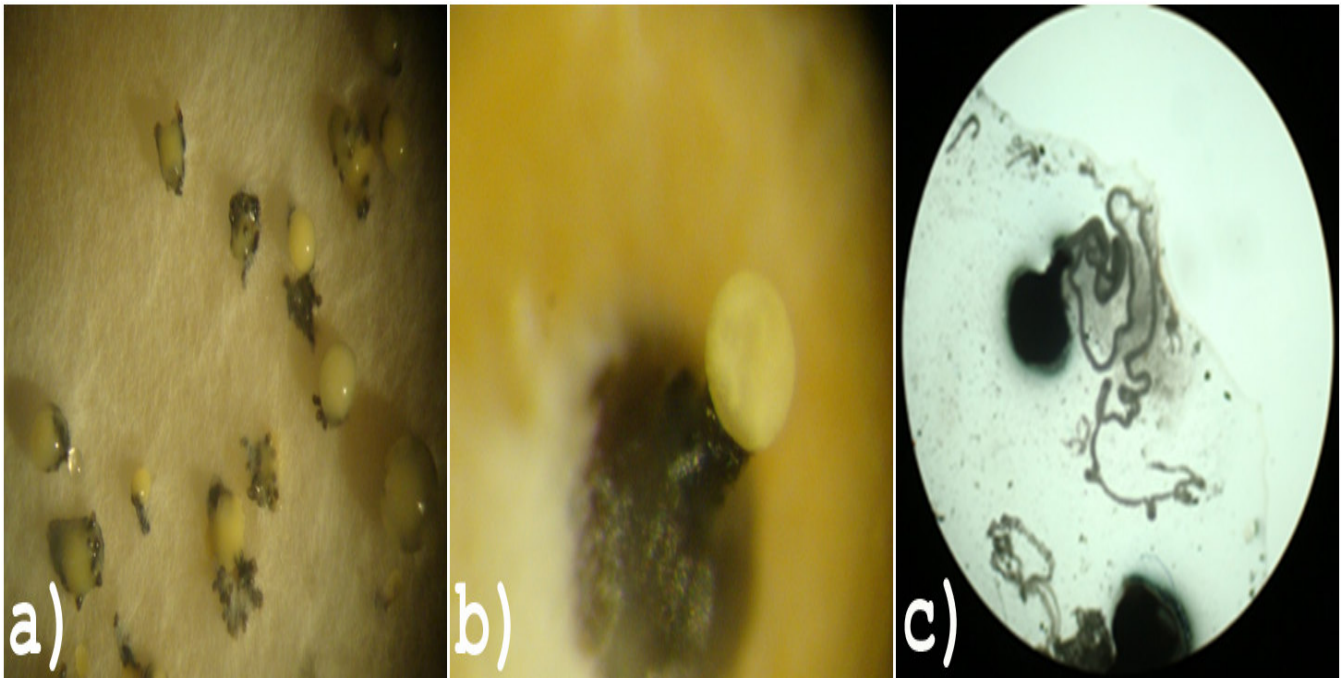

Figure 3
The mature pycnidiospores ooze out of pycnidia of P. vexans isolate Pv1 onto the PDA medium via the ostioles. a) Bunch of pycnidia with pycnidiospores ooze out on PDA medium; b) A pycnidium with pycnidiospores ooze out on PDA medium; c) The pycnidiospores ooze out of a pycnidium under microscope.

Table 1

Cultural and morphological characteristics of Phomopsis vexans isolates on different growth medium

Isolate Code	Colony characteristics					Colony diameter (mm)					Pycnidia					Conidia (α/β)				
	PDA	OMA	CDA	MEA	GPY	PDA	OMA	CDA	MEA	GPY	PDA	OMA	CDA	MEA	GPY	PDA	OMA	CDA	MEA	GPY
Pv1	A	A	A	E	B	85±1.73	78±4.04	88±2.64	49±3.46	75±2.88	Bl, Sm	Bl	Bl, La	Ab	Ab	+/+	+/+	+/+	+/+	+/+
Pv2	B	E	A	E	E	81±3.05	77±3.21	84±2.08	52±2.30	73±2.00	Bl, Sm	Bl	Bl, La	Bl, Sm	Ab	+/–	+/–	+/–	+/–	+/–
Pv3	A	A	A	D	A	84±1.52	81±3.46	90±2.88	51±2.08	77±2.64	Bl, La	Bl	Ab	Ab	Ab	+/+	+/+	+/+	+/+	+/+
Pv4	A	A	A	D	A	83±0.57	80±2.88	83±1.73	46±2.64	82±1.15	Bl, La	Bl	Bl, La	Ab	Ab	+/+	+/+	+/+	+/+	+/+
Pv5	C	A	A	E	E	74±2.30	73±2.30	83±2.88	44±3.78	60±1.73	Bl	Bl	Bl, La	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv6	A	A	A	E	B	90±2.88	79±3.78	90±3.46	53±3.46	83±3.46	Bl	Bl	Bl, La	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv7	A	A	A	D	C	85±2.64	65±2.64	68±3.00	58±1.73	84±3.78	Ab	Ab	Ab	Ab	Ab	–/–	–/–	–/–	–/–	–/–
Pv8	A	A	A	A	D	90±3.21	65±0.57	78±2.30	40±1.15	70±2.64	Bl, La	Bl	Bl, La	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv9	A	A	A	E	E	81±2.30	52±2.30	54±2.64	48±0.57	76±2.88	Bl, La	Bl	Bl, La	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv10	A	A	A	A	E	90±1.15	75±2.08	88±3.21	51±3.21	85±2.30	Bl, La	Bl	Ab	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv11	A	D	D	A	E	85±2.88	74±2.64	80±3.21	58±4.04	74±2.51	Gre	Bl	Gr	Bl	Ab	+/+	+/+	+/+	+/+	+/+
Pv12	D	D	C	A	D	88±2.00	70±2.08	45±2.64	55±3.21	84±2.30	Bl, Sm	Bl	Ab	Bl	Gre	+/+	+/+	+/+	+/+	+/+
Pv13	E	B	B	D	C	84±2.64	75±2.30	80±4.35	57±3.21	79±2.88	Bl	Bl	Ab	Bl	Ab	+/+	+/+	+/+	+/+	+/+
Pv14	A	A	A	B	E	85±3.21	46±3.21	73±2.64	53±4.58	70±3.21	Bl	Bl	Gr	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv15	D	C	D	B	D	88±1.73	47±2.64	83±1.73	59±3.78	71±3.46	Bl	Bl	Gre	Bl	Bl	+/+	+/+	+/+	+/+	+/+
Pv16	A	C	D	D	D	90±3.21	75±3.00	85±2.64	56±1.73	78±2.88	Bl	Bl	Gr	Bl, Sm	Gre	+/+	+/+	+/+	+/+	+/+
Pv17	D	A	B	B	C	85±3.46	74±2.64	88±4.35	55±2.30	76±4.16	Bl	Bl	Bl, La	Bl	Ab	+/+	+/+	+/+	+/+	+/+
Pv18	D	A	B	A	E	88±2.88	75±3.46	82±3.05	56±2.88	73±3.21	Bl	Bl	Bl, La	Bl	Ab	+/+	+/+	+/+	+/+	+/+
Pv19	A	A	D	B	E	84±3.78	68±2.64	87±2.64	58±2.00	75±3.05	Br, La	Br	Gr	Bl	Ab	+/+	+/+	+/+	+/+	+/+
Pv20	A	D	B	B	E	88±4.04	70±3.78	88±2.30	52±2.51	65±2.64	Bl	Bl	Ab	Bl	Ab	+/–	+/–	+/–	+/–	+/–
Pv21	A	A	A	A	C	90±4.61	72±2.88	84±3.46	50±2.88	70±2.64	Bl	Bl	Ab	Bl	Ab	+/–	+/–	+/–	+/–	+/–
Pv22	B	A	A	D	E	88±3.60	74±2.30	88±2.64	52±2.30	74±3.21	Bl	Bl	Ab	Bl, Sm	Ab	+/+	+/+	+/+	+/+	+/+
Pv23	C	B	A	B	D	90±2.08	75±2.08	90±3.78	49±3.21	72±2.88	Bl, Sm	Bl	Ab	Bl	Gre	+/+	+/+	+/+	+/+	+/+

PDA – Potato Dextrose Agar; OMA – Oat Meal Agar; CDA – CzapekDox Agar; MEA – Malt Extract Agar; GPY – Glucose Peptone Yeast Extract Agar.

Bl – Black ; Br – Brown; Gre – Green; Gr – Grey; Sm – Small; La – Large; Ab – Absent. '+' indicates positive; '–' indicates negative.

A – Growth fluffy, colony compact and thick, creamish with distinct concentric ring.

B – Growth embedded, colony compact and thick, creamish with distinct concentric ring.

C – Growth fluffy, colony compact and thick, whitish with no distinct concentric ring.

D – Growth fluffy, colony compact and thick, brownish/greenish/grayish with distinct concentric ring.

E – Growth embedded, colony compact and thin, yellowish with distinct concentric ring.

Fungal growth: fast (> 60 mm), medium (40–60 mm) and slow (< 40 mm).

Values are means ± standard error from triplicates of three separate experiments.

Table 2
Growth of Phomopsis vexans isolates on Potato Dextrose Agar medium at different pH

Sl. No.	Isolate Code	Colony diameter (mm)					
		pH					
		4	Control (5.6)	6	8	10	12
1.	Pv1	60±4.35	85±2.30	82±2.51	79±3.46	71±2.08	43±3.00
2.	Pv2	41±1.15	81±2.08	80±1.15	77±1.15	72±2.30	51±4.04
3.	Pv3	33±2.88	84±2.64	78±1.73	77±2.88	70±2.88	44±3.05
4.	Pv4	25±2.00	83±3.21	80±2.88	79±4.35	72±2.64	41±2.30
5.	Pv5	15±3.21	73±3.46	70±2.30	62±3.21	61±2.30	36±4.04
6.	Pv6	34±2.64	90±2.88	79±3.46	77±2.30	75±2.30	62±3.21
7.	Pv7	19±2.30	85±2.64	81±3.05	77±1.73	79±5.03	51±4.35
8.	Pv8	24±2.08	90±1.15	79±4.35	76±3.21	75±3.21	42±2.88
9.	Pv9	29±3.05	81±4.35	80±3.21	79±3.78	76±3.46	50±2.30
10.	Pv10	35±1.73	90±3.46	81±2.30	77±3.46	73±1.73	46±3.78
11.	Pv11	40±2.51	85±3.21	82±2.30	80±4.35	77±3.46	53±2.51
12.	Pv12	20±2.88	88±4.58	80±2.30	78±3.21	73±2.51	50±2.88
13.	Pv13	38±3.78	84±1.73	79±3.78	76±2.64	72±2.30	47±3.46
14.	Pv14	22±1.15	85±3.21	71±3.05	72±2.30	68±3.46	52±2.30
15.	Pv15	45±2.08	88±2.00	80±2.08	80±2.30	78±1.73	63±2.64
16.	Pv16	26±2.30	90±3.21	81±2.30	78±1.73	72±3.21	48±3.21
17.	Pv17	35±3.21	85±3.46	80±3.21	70±2.08	74±3.05	45±5.68
18.	Pv18	41±3.46	88±2.88	80±4.61	79±5.03	71±4.04	50±3.46
19.	Pv19	24±2.08	84±2.88	80±4.93	78±3.78	72±3.05	47±3.46
20.	Pv20	37±3.60	88±4.04	82±3.78	77±4.04	70±2.30	52±2.30
21.	Pv21	42±2.88	90±2.08	89±2.88	80±1.15	73±3.00	51±2.88
22.	Pv22	29±3.21	80±3.05	80±4.16	76±3.46	68±3.21	45±2.64
23.	Pv23	30±2.30	87±3.60	79±4.58	75±3.21	67±3.46	46±3.46

Values are means ± standard error from triplicates of three separate experiments.

Table 3
Growth of Phomopsis vexans isolates on Potato Dextrose Agar medium at different temperatures

Sl. No.	Isolate Code	Colony diameter (mm)			
		Temperature (° C)			
		4	15	27	40
1.	Pv1	NG	75±1.73 (+)	88±3.78 (+)	NG
2.	Pv2	NG	65±1.15 (–)	85±1.73 (+)	NG
3.	Pv3	NG	78±2.30 (–)	82±2.08 (+)	NG
4.	Pv4	NG	80±2.88 (+)	90±2.88 (+)	NG
5.	Pv5	NG	78±2.51 (–)	84±1.52 (+)	NG
6.	Pv6	NG	80±3.78 (+)	88±4.04 (+)	NG
7.	Pv7	NG	77±3.60 (–)	85±2.64 (–)	NG
8.	Pv8	NG	75±3.21 (–)	80±3.21 (+)	NG
9.	Pv9	NG	82±2.64 (+)	88±1.73 (+)	NG
10.	Pv10	NG	75±2.30 (–)	85±2.88 (+)	NG
11.	Pv11	NG	82±3.78 (+)	88±3.60 (+)	NG
12.	Pv12	NG	75±3.21 (–)	82±2.51 (+)	NG
13.	Pv13	NG	80±4.04 (+)	80±3.21 (+)	NG
14.	Pv14	NG	70±5.68 (–)	78±4.16 (–)	NG
15.	Pv15	NG	75±3.78 (–)	82±3.05 (–)	NG
16.	Pv16	NG	80±3.21 (–)	83±3.78 (+)	NG
17.	Pv17	NG	65±2.64 (+)	85±3.21 (–)	NG
18.	Pv18	NG	70±5.19 (–)	84±2.64 (+)	NG
19.	Pv19	NG	68±3.21 (–)	80±2.88 (+)	NG
20.	Pv20	NG	70±4.61 (–)	80±3.21 (+)	NG
21.	Pv21	NG	69±2.88 (–)	79±2.30 (–)	NG
22.	Pv22	NG	72±3.05 (–)	72±2.08 (–)	NG
23.	Pv23	NG	62±0.57 (–)	88±1.15 (+)	NG

NG – No growth; (+) indicates the sporulation of P. vexans after 14 days; (–) indicates no sporulation of P. vexans after 14 days.
Values are means ± standard error from triplicates of three separate experiments.

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Conflict of Interest
No conflict of interest declared.

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