Indian Phytopathology (2022) 75:691–701
https://doi.org/10.1007/s42360-022-00501-z

RESEARCH ARTICLE

Cultural analysis and growth kinetics of *Pestalotiopsis psidii* (Pat.) Mordue causing scabby fruit canker in guava (*Psidium guajava* L.)

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Received: 2 March 2021 / Revised: 30 December 2021 / Accepted: 8 April 2022 / Published online: 7 May 2022

Abstract

Different nutrient media, temperature regimes and pH levels were evaluated for the growth of *Pestalotiopsis psidii* causing scabby fruit canker in guava. Among seven different nutrient media evaluated, potato dextrose agar gave maximum mycelial growth (90.00 mm) and growth rate (0.50 mm/h) with creamish white, fluffy mycelium exhibiting beautiful ring pattern after seven days of incubation whereas, maximum number of acervuli was produced on Richard's agar medium (1552.00) upto 40 days. Out of seven different temperature regimes, 28 °C was observed to be the best temperature for the myceliual growth (90.00 mm) with maximum growth rate (0.50 mm/h) after 7 days whereas, maximum number of acervuli was produced on 15 °C (457.00) up to 40 days. Among seven pH levels, pH 7.0 was observed to be the best pH for supporting the maximum mycelial growth (90.00 mm) and growth rate (0.50 mm/h) after 7 days whereas, maximum number of acervuli was produced at pH 5.0 (1476.67) up to 40 days. The growth rate of the fungus was highest between 48 and 120 h of incubation in different experiments.

Keywords *Pestalotiopsis psidii* · Cultural characters · Scabby fruit canker · Guava

Introduction

Guava (*Psidium guajava* L.) the "apple of the tropics" or "poor man's apple" belonging to Myrtaceae family is one of the most popular fruit crops of tropical and subtropical regions in the world (Radha and Mathew 2007). It is grown in soils of various textures, drainage and pH (ranging from 4.5 to 9.4). Guava grows naturally in areas of high annual rainfall and can also withstand drought conditions (Mortan 1987). It is also an excellent source of vitamin C (50–300 mg/100 g fruit), niacin, riboflavin and vitamin A (Soares et al. 2007). It ranks fourth among most important fruit crops after mango, banana and citrus (Annonymous 2017).

As the guava cultivation is increasing and gaining popularity, it has led to an increase in guava diseases (Lin et al. 2003). Scabby fruit canker caused by *Pestalotiopsis psidii* (Pat.) Mordue, is one of the most common and devastating fruit diseases in guava. The disease affects all developmental stages of guava fruit. The disease was first recorded from Bombay and the pathogen was identified as *Pestalotia psidii* Pat. (Chibber 1911). Later, the disease was reported from Mysore (Narsimhan 1938; Venkatakrishniah 1952), Thane, Dharwar, Poona (Patel et al. 1950), Ponta Valley, Himachal Pradesh (Verma and Sharma 1976) and Lucknow (Mishra and Prakash 1986).

Pestalotiopsis is a genus characterized by spores/conidia having mostly four-euseptate and pigmented median cells with two to four apical appendages which arise as tubular extensions from the apical cell and a centric basal appendage is also present (Jeewon et al. 2002). It is a complex genus and is difficult to classify to the species level because of variation within the species in terms of characters such as growth rate, conidial morphology and fruiting structure characteristics (Karakaya 2001).

Cultural studies of any organism help to define the nutritional, chemical, and environmental requirements for its growth and metabolism. These studies not only enhance understanding of any natural ecosystem within infected hosts or in the environment, but also help in the determination of molecular architecture and screening for novel compounds.

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Physiological studies are the foundation of fundamental and applied research on any fungal organism (Jong and Birmingham 2001). Although, few such studies have been conducted on *P. psidii* (Rehman et al. 2003; Younis et al. 2004; Keith et al. 2006), but much research has not been conducted on physiological studies of this pathogen. Keeping in view the importance of disease and pathogen, present investigations were planned with an objective to find out cultural requirements of the fungus.

Materials and methods

The study was conducted in the Research Laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India.

Collection, isolation and identification of the pathogen

The diseased samples of fruits showing typical symptoms of canker were collected from the experimental farm, Department of Fruit Science, COHF, Neri, Hamirpur (H.P.) and thoroughly washed repeatedly in tap water.

Thereafter, small pieces of the diseased tissues were taken from the young lesions on infected fruits along with some healthy tissue with the help of sterilized blade and surface sterilized with 0.1 per cent mercuric chloride for 10–15 s. After rinsing with sterilized distilled water thrice, these tissues were placed on the sterilized blotting sheets to pat dry and were further placed on the potato dextrose agar (PDA) medium poured in sterilized Petri plates and slants with sterilized inoculating needle. The inoculated plates as well as slants were incubated at 28 ± 1 °C in BOD incubator. After the initiation of the fungal growth in the inoculated plates/ slants, an agar bit from the periphery of actively growing mycelium was taken and placed on sterilized PDA slants to purify the culture. These purified cultures were then maintained in the refrigerator at 4–5 °C for use in further experiments.

The pathogen was preliminarily identified on the basis of cultural and microscopic characters. For the confirmation of identification, the isolated fungal culture was sent to Indian Type Culture Collection (ITCC), New Delhi.

Effect of different nutrient media on the growth of *P. psidii*

Seven different nutrient media viz., potato dextrose agar (PDA), Czapek's dox agar (CDA), Richard's agar (RA), malt extract agar (MEA), oat meal agar (OMA), corn meal agar (CMA) and guava decoction agar (GDA) were evaluated for the growth of the *P. psidii*. A 5 mm bit of test fungus was taken with the help of sterilized cork borer from the pure culture plate and inoculated in the centre of the Petri plate poured with respective medium and then incubated in BOD incubator at 28 ± 1 °C. Each treatment was replicated thrice and data were recorded regularly at 24 h intervals up to seven days (168 h) in terms of average diametric growth (mm) and cultural characteristics like colour of the mycelium, type of growth, growth pattern etc. Based on the diametric growth of the fungus in different media at particular point of time, growth curves were plotted by taking mycelial growth verses time on Y and X axis, respectively. Growth rate (mm/h) of the fungus on each medium was further calculated as per the formula given below:

$$\text{Growth rate } r_g(\text{mm/h}) = dgt_2 - dgt_1/t_2 - t_1$$

where: dgt_1 is the diametric mycelial growth (mm) at time t_1 and dgt_2 is the diametric mycelial growth (mm) at time t_2.

To visualize small changes in the growth rates during the growth curve and to determine the time point after which the growth rate changed significantly, a calculation model based on the area under the kinetic curve (AUKC) was developed. With this model the relative AUKC (rAUKC) at each interval was estimated by using following formula:

$$\text{rAUKC} = \left(dgt_1 + dgt_2/2\right) \times t_2 - t_1.$$

The changes in rAUKC i.e. ΔrAUKC, were calculated for each time point by subtracting the rAUKC for each time point from the corresponding rAUKC of the previous time point. The ΔrAUKC is an estimate of changes in the slope of the growth curve and thus an estimate of the growth rate of fungus. When the ΔrAUKC value increases linearly over time, the mycelial growth increases with a constant rate, and when the ΔrAUKC value decreases or goes to zero over time, the growth rate decreases or goes to zero, respectively. Thus, increasing ΔrAUKC values corresponded to high growth rates. The ΔrAUKC values were used in order to distinguish different phases in the growth of filamentous fungi and the time employed for each phase. ΔrAUKC values in each nutrient media were plotted against various time intervals to see the trend of growth rate in different media with time.

In addition, time taken for the formation of acervuli and number of acervuli produced up to 40 days of incubation was also recorded. Based on these studies, best nutrient medium was selected for further experiments.

Effect of different temperature regimes on the growth of the *P. psidii*

To study the effect of different temperature regimes on the mycelial growth of the pathogen, Petri plates containing the best nutrient medium selected were inoculated with culture

bit (5 mm dia.) of the test fungus and subjected to different temperature regimes viz., 15, 20, 25, 28, 30, 32 and 35 °C in different BOD incubators up to 168 h. Each treatment was replicated thrice and data were recorded regularly at 24 h intervals in terms of average diametric growth (mm) and cultural characteristics as mentioned above. Data in terms of days to acervuli production and total number of acervuli produced were also recorded up to 40 days of incubation. Growth rate and rAUKC values of the fungus at each temperature was further calculated as mentioned earlier. Based on these studies, best temperature was selected for further experiments.

Effect of different pH levels on the growth of the *P. psidii*

To see the effect of different pH levels on the mycelial growth of *P. psidii,* the best nutrient medium was adjusted to different pH levels viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 with the help of 1 N HCl and 1 N NaOH and poured in Petri plates further inoculated with a culture bit of 5 mm diameter. These Petri plates were then incubated at best temperature up to 168 h to record the data. Each treatment was replicated thrice and data were recorded regularly at 24 h intervals in terms of average diametric growth (mm) and cultural characteristics up to 7 days (168 h), as well as acervuli production up to 40 days as mentioned earlier. Growth rate and rAUKC values of the fungus at each pH level was further calculated.

Statistical analysis

Data recorded in three experiments were further subjected to statistical analysis for completely randomized design by using online software OPSTAT.

Results and discussion

Collection, isolation and identification of the pathogen

The diseased samples of fruits were collected from the experimental farm, Department of Fruit Science, COHF, Neri, Hamirpur (H.P.). The symptoms on fruits were recorded as minute, brown or rust coloured, unbroken, circular, necrotic areas, which in later stages of infection tear opened the epidermis in a circinate manner. The lesion was elevated at the margin and sunken inside. This crater like appearance was more prominent on fruits than on leaves (Fig. 1).

The pathogen was isolated and purified on potato dextrose agar (PDA) medium and maintained in PDA slants at 4–5 °C in the refrigerator. The cultural and microscopic

Fig. 1 Symptoms of fruit canker of guava under field conditions

characteristics of the pathogen were studied in detail. The mycelium was creamish white in colour having cottony and fluffy growth on PDA and a ring pattern of growth was observed in the Petri plate. Acervuli of the fungus could be observed in the culture plate after three weeks of incubation. Microscopically, the mycelium was hyaline and conidia of the fungus were observed to be fusiform, septate, 5 celled with three central cells dark brown and apical cells hyaline, one of the apical cells bearing 3–4 transparent long appendages and other terminal cell tapering into single appendage (Fig. 2). The conidia measured between 19.8 and 52.8 µm in length and 3.3–16.5 µm in width. Based on preliminary examination, the pathogen was identified as *Pestalotiopsis psidii*. The identity of the pathogen was finally confirmed as *P. psidii* from Indian Type Culture Collection, New Delhi under the Identification No. ITCC 11, 301.20.

The results are in conformity with the findings of Venkatkrishniah (1952) who also reported that the mycelium of *P. psidii* makes a thick, pure white, cottony growth and acervuli develop as black, shining, moist crusts in cultures. The conidia are spindle shaped, dark coloured, in mass and 5-celled. The upper end conical cell bears usually 3 long, slender, colourless, simple appendages. The appendages measure 7–17 µm long while, the conidia measure 17–24 × 5–7 µm on the host. These results were further supported by the findings of Keith et al. (2006) who also identified the causal agent of scabby fruit canker as *Pestalotiopsis psidii* based on the structure of the conidia.

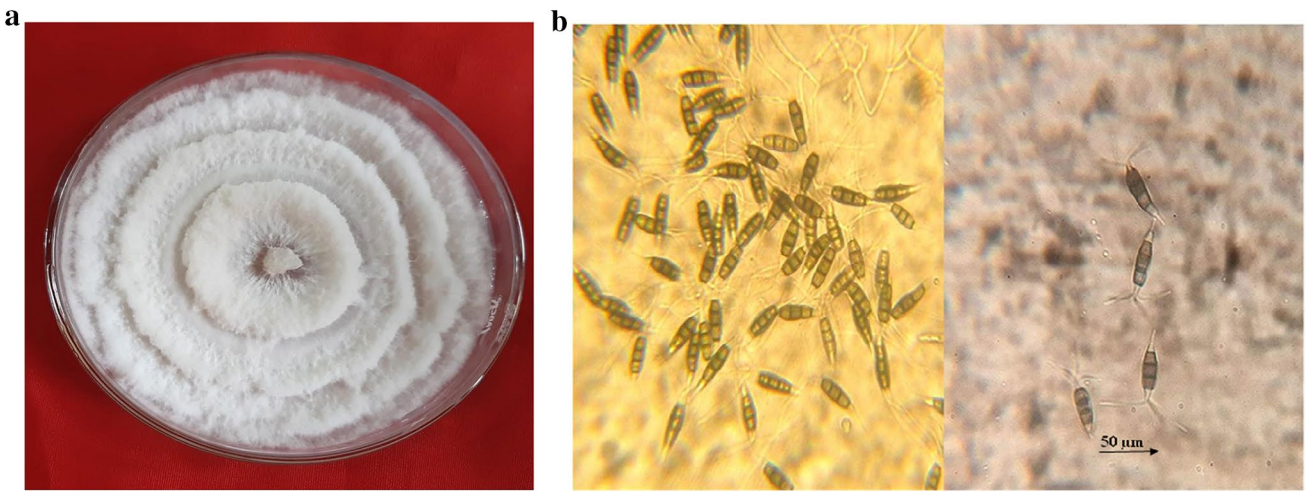

Fig. 2 **a** Pure culture of *Pestalotiopsis psidii*. **b** A micrograph of *Pestalotiopsis psidii* exhibiting mycelium and conidia

Effect of different nutrient media

Data recorded in terms of average diametric growth (mm) after 7 days (168 h) of incubation and growth rate of the test fungus, further calculated on the basis of mycelial growth of the fungus at 24 h interval upto 168 h (7 days) on different test nutrient media have been presented in Table 1. It is clear from the table that the fungus grew well on all the nutrient media under study. Significantly maximum diametric growth (90 mm) was recorded in PDA followed by the growth in CDA (87.00 mm), GDA (86.40 mm), OMA (84.87 mm) and RA (82.23 mm). However, mean minimum diametric growth (55.67 mm) was recorded in CMA followed significantly by that in MEA (66.97 mm) after 168 h of incubation. The growth curves obtained reflected linear to sigmoid growth in different phases of time (Fig. 3). As far as growth rate of the fungus was concerned, irrespective of the time interval, mean growth rate of the test pathogen was significantly maximum in PDA (0.50 mm/h) followed by CDA, RA and GDA on which the fungus exhibited equal mean growth rate (0.48 mm/h) which was statistically at par with the growth rate in OMA (0.47 mm/h). However, minimum mean growth rate of the test fungus was recorded in CMA (0.29 mm/h) significantly followed by that in MEA (0.37 mm/h). Irrespective of the nutrient medium used average growth rate was significantly maximum (0.60 mm/h) between 48 and 72 h of incubation while, the minimum average growth rate (0.29 mm/h) was recorded between 144 and 168 h of inoculation. Body of the table reveals that average growth rate of the test fungus was maximum (0.82 mm/h) on RA between

Table 1 Effect of different nutrient media on mycelial growth and growth rate of *Pestalotiopsis psidii*

Nutrient medium	Average diametric growth (mm) after 168 h	Growth rate (mm) after time (h)							Overall mean
		0–24	24–48	48–72	72–96	96–120	120–144	144–168	
Potato dextrose agar	90.00	0.40	0.59	0.79	0.54	0.39	0.57	0.24	0.50
Czapek's dox agar	87.00	0.29	0.54	0.48	0.59	0.54	0.51	0.44	0.48
Richard's agar	82.23	0.38	0.52	0.82	0.63	0.32	0.41	0.30	0.48
Malt extract agar	66.97	0.30	0.59	0.57	0.48	0.20	0.31	0.12	0.37
Oat meal agar	84.87	0.32	0.53	0.56	0.72	0.28	0.60	0.29	0.47
Corn meal agar	55.67	0.28	0.37	0.27	0.27	0.20	0.32	0.34	0.29
Guava decoction agar	86.40	0.36	0.56	0.73	0.47	0.44	0.44	0.36	0.48
Overall mean		0.33	0.53	0.60	0.53	0.34	0.45	0.29	

			$CD_{p\geq0.05}$	$SE_{(d)}$
$CD_{p\geq0.05}$	0.98	Nutrient medium	0.03	0.01
$SE_{(d)}$	0.45	Time interval	0.03	0.01
		Interaction	0.07	0.03

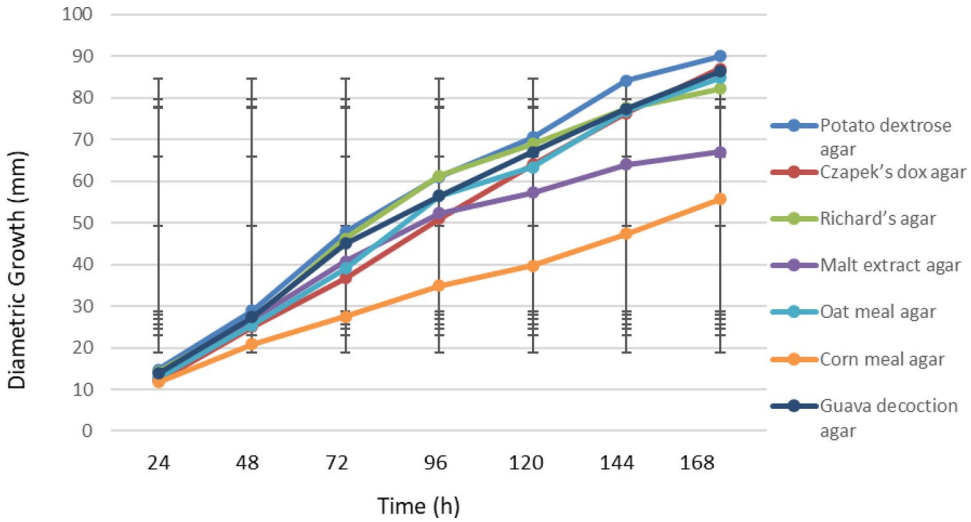

Fig. 3 Growth of *Pestalotiopsis psidii* as affected by different nutrient media

48 and 72 h of incubation while minimum (0.12 mm/h) growth rate was recorded on MEA between 144 and 168 h of inoculation. The ΔrAUKC values plotted against time indicated a peak in growth rate between 72 and 96 h of incubation in almost all the test media except CMA (Fig. 4).

Production of acervuli as affected by different solid media was also studied. The data presented in Table 2 depict that significantly mean maximum time for production of acervuli (37.00 days) was recorded on MEA followed by PDA (25.00 days) and CMA (16.00 days). However, mean minimum time for production of acervuli was recorded on OMA (8.00 days) which was statistically at par with CDA and GDA which took equal time (9.00 days) for the production of acervuli. As far as total number of acervuli produced in each medium was concerned, maximum acervuli were produced (1552.00) on RA which was statistically at par with CDA (1455.33). However, significantly mean minimum number of acervuli (1.00) was recorded on MEA followed by GDA (10.00), OMA (16.00), CMA (30.00) and PDA (269.67). Cultural characteristics of *P. psidii* recorded in each test nutrient medium revealed that colour of the mycelium varied from light cream on CMA to cream on CDA and MEA and creamish white on PDA, RA and GDA to pure white on OMA. As far as the growth pattern was concerned, ring pattern was recorded to be predominant pattern in four (PDA, CDA, OMA and CMA) out of seven media tested. On RA, the fungus grew in a ray pattern, while, on MEA the growth pattern was recorded to be wavy in appearance. Interestingly, on GDA a beautiful capitulum floral pattern of growth was observed. However, the type of growth in most of the media tested was cottony except for CMA, where sparse growth was recorded. But, only on PDA the growth was recorded to be moderately fluffy and on rest of the media, it was suppressed. However, on OMA, it was neither fluffy nor suppressed but a flaky growth of the mycelium was observed.

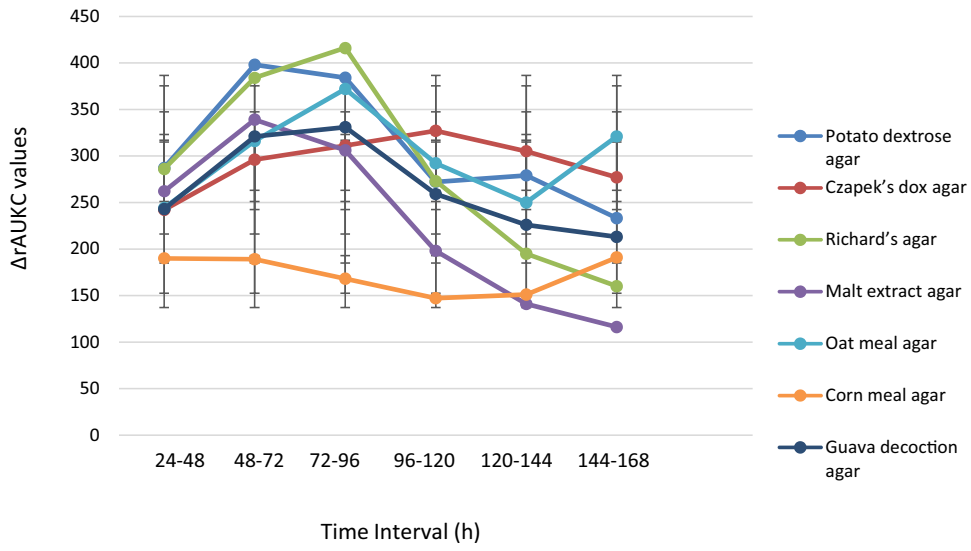

Fig. 4 Growth rate of *Pestalotiopsis psidii* as affected by different nutrient media

Table 2 Effect of different nutrient media on the acervuli production and cultural characters of *Pestalotiopsis psidii*

Nutrient medium	Days to acervuli production	No. of acervuli	Colour of mycelium	Growth pattern	Type of growth
Potato dextrose agar	25.00	269.67 (2.43)	Creamish White	Ring	Cottony and moderately fluffy
Czapek's dox agar	9.00	1455.33 (3.16)	Cream	Ring	Cottony but suppressed
Richard's agar	10.00	1552.00 (3.19)	Creamish white	Ray	Cottony but suppressed
Malt extract agar	37.00	01.00 (0.00)	Cream	Wavy	Completely suppressed
Oat meal agar	8.00	16.00 (1.19)	Pure white	Ring	Cottony but flaky
Corn meal agar	16.00	30.00 (1.47)	Light cream	Ring	Sparse and completely suppressed
Guava decoction agar	9.00	10.00 (1.00)	Creamish white	Capitulum like	Cottony but suppressed
$CD_{P\geq0.05}$	1.77	0.07			
$SE_{(d)}$	0.82	0.03			

Figures in parentheses indicate log transformed value

Nutrients present in any nutrient medium directly or indirectly influence the growth of any microorganism, due to their varied nutritional requirements for mycelial growth and sporulation. During present investigations, maximum mycelial growth was supported by PDA followed by RA medium. Growth rate was also highest in PDA followed by RA and CDA. The peak of growth rate was recorded between 72 and 96 h of incubation indicating that the fungus grows fastest in this duration and after that the nutrients start exhausting leading to a gradual decline in the growth rate. The colour of mycelium varied from pure white to cream. The growth pattern was generally ring pattern. However, the number of acervuli produced was maximum in RA followed by CDA and PDA and time taken for acervuli production was 25 and 10 days in PDA and RA, respectively. The present findings were in accordance with Younis et al. (2004) who reported that potato dextrose agar produced the maximum mycelial growth and acervuli followed by Richard's agar. Our findings are also confirmed by Keith et al. (2006) who reported that *P. psidii* produced greyish to white colonies which later developed black acervuli. The acervuli production of *P. psidii* varied from abundant to moderate to sparse and acervuli did not develop within 12 days of growth on PDA. Results are further supported by Tandan (1950) who reported that sporulation in *P. psidii* is abundant on PDA and Richard's medium. In our findings, the acervuli production was delayed on PDA while, on RA it was quite quick. This may be attributed to the fact that PDA is too rich in nutrients that it encourages the mycelial growth with loss of sporulation until the nutrients start exhausting (El-Gali 2017). The ring pattern observed in the mycelial growth of fungus on different media is supported by the findings of El-Gali (2017) who also reported a ring growth pattern of different *Pestalotiopsis* species. However, there are no reports in the literature regarding growth rate of this fungus on different nutrient media, so these results cannot be compared with any available data. However, fastest growth rate of the test fungus on PDA can also be attributed to the availability of more nutrients in this medium ultimately leading to faster metabolism and growth. Based on these studies PDA was selected as the best medium and used in subsequent experiments.

Effect of different temperature regimes on the mycelial growth of *Pestalotiopsis psidii*

Data presented in Table 3 clearly depict that significantly maximum average diametric growth (90 mm) was recorded at 25 and 28 °C after 168 h of incubation followed significantly by that at 30 °C (85.63 mm). However, minimum average diametric growth (30.97 mm) was recorded at 15 °C after same duration followed significantly by that at 35 (36.83 mm), 20 (41.88 mm) and 32 °C (49.03 mm). The fungus grew exponentially up to 72 h of incubation at most of the temperatures after which, it attained somewhat stationery phase except at 28 and 30 °C where the growth became stationery after 144 h of incubation (Fig. 5). Growth rate of the test fungus at different temperatures under study was recorded to be maximum (0.50 mm/h) at 28 and 25 °C at which the fungus exhibited equal mean growth rate followed significantly by that at 30 °C (0.48 mm/h). However, minimum mean growth rate (0.15 mm/h) of the fungus was recorded at 15 °C significantly followed by that at 35 °C (0.20 mm/h) which was statistically at par with growth rate at 20 °C (0.21 mm/h) irrespective of the incubation intervals. However, keeping aside the temperature regimes, average growth rate was equal and maximum (0.52 mm/h) between 24–48 h and 48–72 h of incubation while, the minimum average growth rate (0.13 mm/h) was recorded between 144 and 168 h of incubation. The body of the table reveals that growth rate of the test fungus was significantly maximum at 28 °C between 24 and 48 h (0.88 mm/h) of incubation while, minimum growth rate of the fungus was recorded at 35 °C (0.04 mm/h) between 144 and 168 h of inoculation and between 120 and 144 h at 15 °C which was statistically

Table 3 Effect of different temperature regimes on mycelial growth and growth rate of *Pestalotiopsis psidii*

Temperature (°C)	Average diametric growth (mm) after 168 h	Growth rate (mm/h) between interval (h)							Overall mean
		0–24	24–48	48–72	72–96	96–120	120–144	144–168	
15	30.97	0.08	0.26	0.32	0.13	0.12	0.04	0.07	0.15
20	41.88	0.14	0.38	0.25	0.14	0.19	0.31	0.08	0.21
25	90.00	0.40	0.61	0.64	0.59	0.59	0.39	0.27	0.50
28	90.00	0.40	0.88	0.86	0.46	0.39	0.33	0.18	0.50
30	85.63	0.42	0.55	0.66	0.57	0.61	0.40	0.12	0.48
32	49.03	0.15	0.42	0.56	0.23	0.13	0.12	0.16	0.25
35	36.83	0.20	0.53	0.31	0.14	0.11	0.05	0.04	0.20
Overall mean		0.26	0.52	0.52	0.33	0.31	0.23	0.13	

			$CD_{p\geq0.05}$	$SE_{(d)}$
$CD_{p\geq0.05}$	1.24	Temperature	0.02	0.01
$SE_{(d)}$	0.57	Time interval	0.02	0.01
		Interaction	0.04	0.02

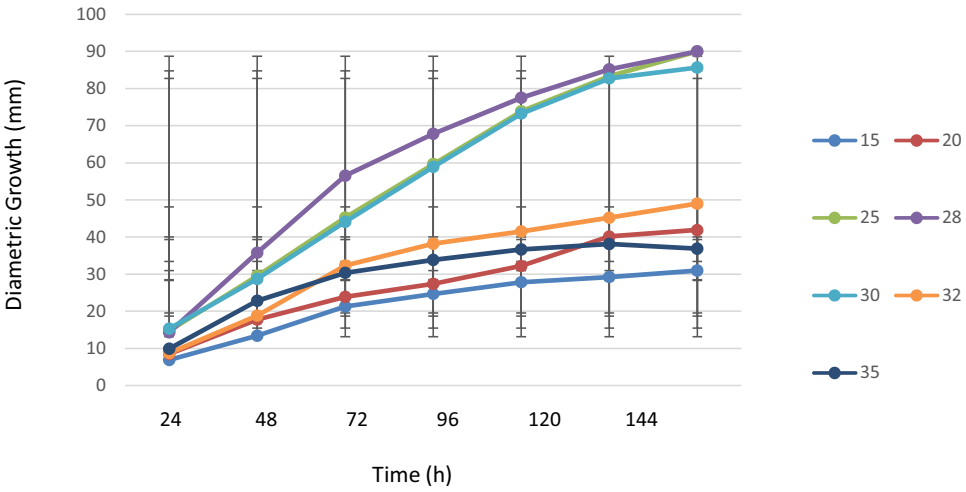

Fig. 5 Growth of *Pestalotiopsis psidii* as affected by different temperature regimes

at par with growth rate at 35 °C between 120 and 144 h (0.05 mm/h). An intermediate range of growth rate was recorded after different duration of incubation at rest of the temperatures studied. The ΔrAUKC values plotted against time indicated a peak in growth rate between 48 and 72 h of incubation at almost all the test temperatures (Fig. 6).

Data recorded in terms of time taken for acervuli production at a particular temperature and total number of acervuli produced at each temperature upto 40 days of incubation (Table 4) depict that significantly mean maximum time for production of acervuli (27.67 days) was recorded at 28 °C followed by 30 °C (25.67 days) which was statistically at par with 25 °C (25 days). However, the minimum time for the production of acervuli was recorded at 15 °C (20.33 days) significantly followed by 35 °C (22 days) which was statistically at par with 20 °C (22.33 days) which further did not differ significantly from 32 °C. As far as total number of acervuli produced at each temperature was concerned, maximum mean number of acervuli were produced (457.00) at 15 °C followed significantly by 20 °C (418.33) and 30 °C (407.33) which did not differ significantly from 35 °C (405.33) as well as 25 °C (404.67). Significantly minimum number of acervuli (384.67) was produced at 32 °C followed by that at 28 °C (396.00). Cultural characteristics of *P. psidii* were recorded at each temperature reveal that colour of the mycelium varied from creamish white and cream at 25, 28 and 30 °C to white at 20, 32 and 35 °C and transparent at 15 °C. As far as the growth pattern was concerned, a ring pattern was recorded at three (25, 28 and 30 °C) out of seven temperature tested. At 15, 20, 32 and 35 °C the fungus grew in a plain pattern. However, the type of growth was recorded to be suppressed at 15 °C and it was cottony and suppressed at 20 °C. At 25 and 30 °C growth was cottony and moderately fluffy initially and then started suppressing. At 32 and

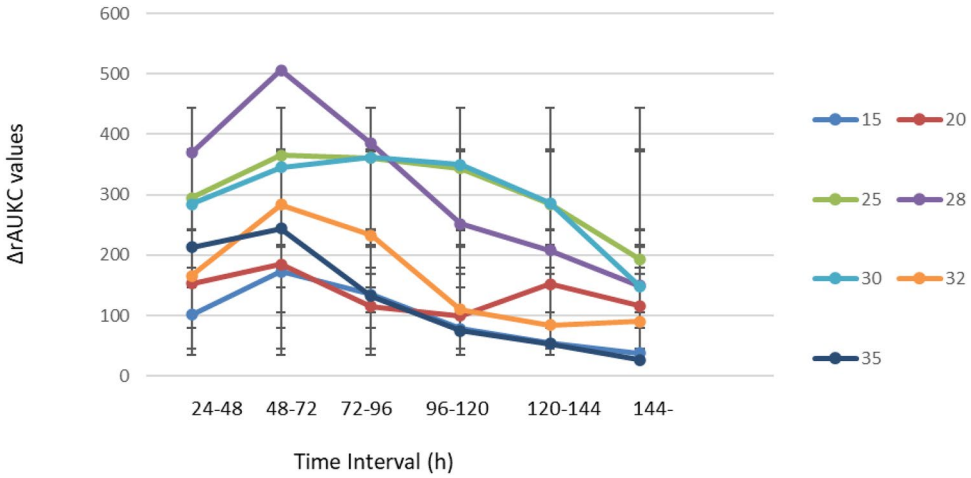

Fig. 6 Growth rate of *Pestalotiopsis psidii* as affected by different temperature regimes

Table 4 Effect of different temperature regimes on acervuli production and cultural characters of *Pestalotiopsis psidii*

Temperature (°C)	Days to production of acervuli	No. of acervuli	Colour of mycelium	Growth pattern	Type of growth
15	20.33	457.00 (2.65)	Transparent	Plain	Suppressed
20	22.33	418.33 (2.62)	White	Plain	Suppressed and cottony
25	25.00	404.67 (2.60)	Creamish white	Ring	Moderately fluffy and cottony for 2 days suppressed thereafter
28	27.67	396.00 (2.59)	Creamish White	Ring	Cottony and moderately fluffy
30	25.67	407.33 (2.60)	Creamish white	Ring	Moderately fluffy and cottony upto 3 days and then starts suppressing
32	23.33	384.67 (2.58)	White	Plain	Moderately fluffy, cottony and compact.
35	22.00	405.33 (2.60)	White	Plain	Half fluffy cottony and half suppressed
$CD_{p\geq0.05}$	1.73	0.01			
$SE_{(d)}$	0.80	0.01			

Figures in parentheses indicate log transformed value

35 °C it was recorded to be compact but fluffy and cottony. The growth of the fungus was recorded to be properly cottony and moderately fluffy at 28 °C which was also recorded to be the best temperature in terms of mycelial growth and growth rate.

Temperature of incubation also affected the mycelial growth, rate of growth, acervuli production and growth pattern of *P. psidii*. The test fungus grew well at a temperature range of 25–30 °C while, 28 °C being the optimum temperature. The growth rate at 25 and 28 °C was however same. These results are supported by the findings of Keith et al. (2006) who reported that fungus grew well at the temperature range of 10–35 °C but optimum range recorded was 22–28 °C. The findings of Younis et al. (2004) further supported our results, who concluded that optimum temperature for the maximum growth and acervuli production was recorded to be 30 °C followed by 35, 25 and 20 °C. These results were further in close conformity with Tandan (1950) and Rahman et al. (2003) who reported optimum temperature for the growth of the fungus in the culture to be 30 °C. Ramaswamy et al. (1984) also confirmed these findings who reported that spores of *P. psidii* germinate maximum at 30 °C while, below 15 °C or above 40 °C, it does not germinate. During present studies, acervuli production started faster at 15, 20 and 35 °C. It could be attributed to the fact that at these temperatures, the fungus could not grow well and thus entered into reproductive phase from vegetative phase. The temperature also affected the growth pattern of the fungus which was ringed and cottony at 25–30 °C but, plain at rest of the temperatures studied. This was also due to fast growth of the fungus at 25–30 °C as at these temperatures, the fungus might have exhausted all the nutrients available at a particular zone of the nutrient medium in the plate and after that, it moved to next zone ultimately resulting in a ring pattern and cottony growth. Based on these studies 28 °C was selected as the best temperature and used in subsequent experiments.

Effect of different pH levels on mycelial growth of the *Pestalotiopsis psidii*

It is clear from the Table 5 that the maximum mean diametric growth (90.00 mm) was recorded at pH 7.0 which was statistically at par with the growth at pH 6.5 (88.63 mm) which further did not differ significantly from growth at pH 6.0 (87.42 mm) and pH 5.5 (87.17 mm). However, significantly minimum mean diametric growth (51.30 mm) was recorded at pH 5.0 followed by pH 7.5 (81.67 mm) and pH 8.0 (83.78 mm). The growth curves plotted indicated that the fungus grew exponentially at all the test pH levels indicating that all these pH levels could support the fungal growth (Fig. 7) although, the level of growth varied according to the pH level being minimum at pH 5.0.

It is clear from the table that irrespective of the time interval, mean growth rate of the test pathogen was significantly maximum at pH 7.0 (0.50 mm/h) which was statistically at par with pH 6.5 and pH 5.5 at which the fungus exhibited equal mean growth rate i.e. 0.49 mm per hour which was further statistically at par with growth rate at pH 6.0. However, minimum mean growth rate of the fungus was recorded at pH 5.0 (0.27 mm/h) significantly followed by growth rate at pH 7.5 (0.45 mm/h) which did not differ significantly from growth rate recorded at pH 8.0 (0.46 mm/h) and pH 6.0 (0.48 mm/h). Irrespective of the pH levels, average growth rate was significantly maximum (0.68 mm/h) between 24 and 48 h of incubation while, the minimum average growth rate (0.17 mm/h) was recorded between 144 and 168 h of incubation. The body of the table reveals that growth rate of the test fungus was maximum at pH 7.0 (0.95 mm/h) between 24 and 48 h of incubation which was statistically at par with the growth rate at pH 6.5 between 24 and 48 h (0.93 mm/h). However, minimum growth rate was recorded on at pH 6.0 (0.09 mm/h) between 144 and –168 h of inoculation which was statistically at par with the growth rate (0.12 mm/h) at pH 7.5 between 144 and 168 h of incubation. An intermediate range of growth rate of the fungus was observed at rest

Table 5 Effect of different pH levels on mycelial growth and growth rate of *Pestalotiopsis psidii*

pH	Average diametric growth (mm) after 168 h	Growth rate (mm/h) between interval (h)							Overall mean
		0–24	24–48	48–72	72–96	96–120	120–144	144–168	
5.0	51.30	0.13	0.31	0.41	0.38	0.22	0.28	0.16	0.27
5.5	87.17	0.53	0.87	0.28	0.64	0.39	0.57	0.16	0.49
6.0	87.42	0.56	0.54	0.55	0.67	0.60	0.40	0.09	0.48
6.5	88.63	0.48	0.93	0.28	0.55	0.61	0.42	0.19	0.49
7.0	90.00	0.46	0.95	0.26	0.60	0.58	0.40	0.27	0.50
7.5	81.67	0.36	0.65	0.54	0.70	0.63	0.18	0.12	0.45
8.0	83.78	0.49	0.51	0.65	0.47	0.63	0.34	0.16	0.46
Overall mean		0.43	0.68	0.42	0.57	0.52	0.37	0.17	

			$CD_{p\geq0.05}$	$SE_{(d)}$
$CD_{p\geq0.05}$	1.65	pH	0.03	0.01
$SE_{(d)}$	0.76	Time interval	0.03	0.01
		Interaction	0.07	0.03

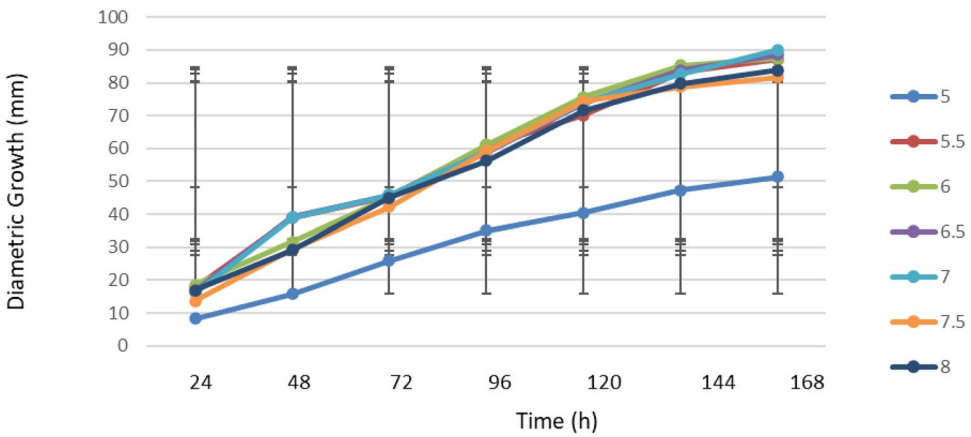

Fig. 7 Growth of *Pestalotiopsis psidii* as affected by different pH levels

of the pH levels between different intervals of incubation. The ΔrAUKC values plotted against time indicated a peak in growth rate between 96 and 120 h of incubation at almost all the test pH values (Fig. 8).

Effect of different pH levels on the production of acervuli of the test fungus and total number of acervuli produced at each pH level upto 40 days of incubation were recorded and have been presented in Table 6.

A perusal of the data presented in Table 6 clearly depicts that significantly maximum time (28.67 days) for production of acervuli was recorded at pH 8.0 (Fig. 4.6) which was statistically at par with that at pH 7.5 (28.33 days) followed significantly by pH 7.0 (26.33 days). However, the minimum time (19.33 days) for production of acervuli was recorded at pH 5.0 which was statistically at par with that at pH 5.5 (21.00 days) which further did not differ significantly from time taken to acervuli production at pH 6.0 (22.33 days). The latter was again statistically at par with time taken for acervuli production at pH 6.5 (23.00 days). As far as total number of acervuli production each pH was concerned, significantly maximum acervuli production was recorded at pH 5.0 (1476.67) followed by pH 5.5 (1168.00), pH 6.0 (981.00), pH 6.5 (871.00) and pH 7.5 (767.67). However, minimum acervuli production was recorded at pH 7.0 (393.67) followed significantly by pH 8.0 (656.33).

The colour of the mycelium varied between white at pH 5.0 to creamish white at pH 5.5 to cream at pH 6.0 to 8.0. As far as the growth pattern was concerned, ring pattern was recorded to be the predominant pattern at six (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) out of seven pH levels tested. At pH 5.0 the fungus grew in an unspecific pattern while, at pH 7.5 and 8.0 the growth pattern was recorded to be ring pattern with clear distinction of rings. However, the type of growth in most of the pH levels tested was cottony and fluffy. But at pH 5.0 the growth was cottony but little suppressed and at pH 8.0, the growth was cottony and little fluffy in the beginning but suppressed at the end.

Studies on effect of pH levels on growth and cultural characters of *P. psidii* revealed that pH 7.0 supported the best growth of the fungus with highest rate of growth followed by pH 6.5 and 6.0. These findings are somewhat in accordance with Younis et al. (2004) who reported that maximum

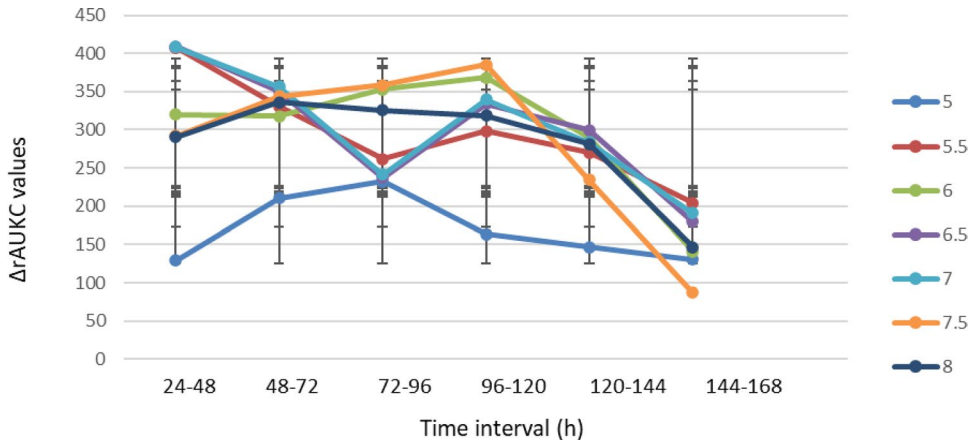

Fig. 8 Growth rate of *Pestalotiopsis psidii* as affected by different pH levels

Table 6 Effect of different pH levels on the acervuli production and cultural characters of *Pestalotiopsis psidii*

pH	Days to production of acervuli	No. of acervuli	Colour of mycelium	Pattern	Type of growth
5.0	19.33	1476.67 (3.16)	White	Unspecific	Cottony but little suppressed
5.5	21.00	1168.00 (3.06)	Creamish white	Ring	Cottony but little suppressed
6.0	22.33	981.00 (2.98)	Cream	Ring	Cottony and fluffy
6.5	23.00	871.00 (2.93)	Cream	Ring	Cottony and fluffy
7.0	26.33	393.67 (2.59)	Whitish cream	Ring	Cottony and fluffy
7.5	28.33	767.67 (2.88)	Cream	Ring with clear distinction of rings	Cottony and fluffy
8.0	28.67	656.33 (2.81)	Cream	Ring with clear distinction of rings	Cottony, little fluffy in the beginning but suppressed at the end
$CD_{P\geq0.05}$	1.85	0.02			
$SE_{(d)}$	0.85	0.01			

Figures in parentheses indicate log transformed value

growth of fungus was supported at pH 6.5 followed by 6, 5, 7 and 8.The results are further supported by the findings of Upadhyay and Dwivedi (1980) who reported that pH level 6 is optimum for the growth and sporulation of the *P. psidii.* These results were also in conformity with Hopkins (1996) who reported that growth and conidial germination occurred at a range of pH 2.6–7.6. During present studies, the acervuli production was delayed at pH 7.0 and number of acervuli produced was also minimum at this pH which could be attributed to the fact that the fungus will try to remain in vegetative phase at best supportive pH and will enter the reproductive phase only when the nutrients are about to finish or otherwise, the conditions become unfavouarble. Based on these studies pH 7.0 was selected as the best pH level and used in subsequent experiments.

Growth rate peaks obtained in all the three experiments varied between 48 to 96 h of incubation indicating this period to be the optimum time for exponential growth of the fungus. The same was also clear from the growth curves obtained in all the three experiments.

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