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Full Length Research Paper

Effect of carbon sources, substrates, leachates, and water grades on germinability of *Phomopsis vexans*

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The five isolates of the pathogen *Phomopsis vexans* formed both types of conidia that is α and β conidia. Both the conidia were exposed to plant parts, plant leachates, and different grades of water as well as to different carbon sources. Among the substrates, leaf promoted maximum germination of α conidia. Among leachates, leaf leachate was found best and glucose among the carbon sources was most effective in enhancing germination of the pathogen isolates.

Key words: Phomopsis blight, *Phomopsis vexans*, brinjal.

INTRODUCTION

The Phomopsis leaf blight and fruit rot caused by *Phomopsis vexans* (Sacc. & Syd.) Harter, (Tel: *Diaporthe vexans* Gratz) is a very destructive disease and consi-dered to be the major constraint for limited production and productivity of brinjal. This pathogen causes over 50% losses in production and productivity in various parts of the world (Nolla, 1929). In India, Panwar et al. (1970) reported that the losses due to Phomopsis fruit-rot ranged to the extent of 10 – 20%. Although there are very few reports on conidial physiology of pathogen (Kumar, 1997). Hence, keeping in view, the present investigations were carried-out to study the sources of nutrition on conidial physiology of naturally occurring populations of *P. vexans*.

MATERIALS AND METHODS

To study the effect of nutritional factors for the germinability of five isolates of *P. vexans* (Pv 10, Pv 11, Pv 16, Pv 25 and Pv 32), different carbon sources such as glucose, sucrose and dextrose were used, different types of substrates viz., leaf, fruit and stem,l eachates of host plant viz., leaf, fruit and stem of the susceptible cultivar “Pant Rituraj” and water types (sterilized water, distilled sterilized water, deionized water and tap water).

Three carbon sources were taken at 100 and 1000 ppm concen-trations in sterilized water. To make the conidial suspension, mea-sured amount of sugar solution was mixed in conidial mass of the isolates separately. Conidial suspension of each isolate was placed at 20 µl in cavity glass slide in triplicate manner. All the plates were incubated at 27 ± 1°C. The germination was microscopically observ-ed at an interval of 12 h.

Spore suspensions of five isolates were prepared by suspending the spores in 2% gelatin treated distilled water and desired concen-tration was maintained. Then individual plant parts as substrates were put in the plates where 3 layers of moistened filter papers were placed. Now the conidial suspension of each isolate was dropped at 10 l separately.

Spore suspensions of five isolates were prepared by suspending the spores in plant leachates prepared by emerging plant parts in water in 1:1 ratio for 24 h. Spore suspensions of five isolates were prepared by mixing the spores in different types of water separately. The cavity glass slides were put in the petri plates where 3 layers of moistened filter papers were placed. Now conidial suspension of each isolate was placed at 20 µl on cavity glass slide in triplicate manner.

RESULTS AND DISCUSSION

Leachates of host plant organs such as leaf, stem, and fruits and different grades of waters may affect the extent and magnitude of spore germination. It was exactly in this context, that they were evaluated for their effects on germination of both α and β conidia of 5 isolates of *P. vexans* and the results so obtained are given in following Tables. Neither α nor β conidia of the isolates germinated during the first 24 h except for the α conidia of isolate Pv36 in which germination was initiated by the end of 24th

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h. In the remaining isolates the initiation of germination of α conidia was recorded by the end of 36th h except for the isolate Pv 25 in which germination initiated at the end of 48th h. In isolate Pv 10 only leaf and fruit leachates stimulated the germination after 36 h. While in stem leachate, sterilized water and distilled sterilized water, germination started after 48 h of incubation.

For complete germination of α conidia, in leaf and fruit leachates above 60 h of incubation period were observed. In stem leachate and sterilized water for complete germination approximately 72 h were required. De-ionized water and tap water were least effective and the germination of α conidia started after 60 h.

It is evident from the Table 1 that the effect of leachates and types of water on conidial germination was different than what was observed in isolate Pv 10. Minimum (36 h) time was required to start germination of α conidia in leaf leachate. Whereas, fruit, stem leachates as well as sterilized water took 48 h to initiate germination of α conidia. There was no difference in the effectiveness of distilled sterilized water, de-ionized water and tap water to start conidial germination and germination started after 60 h. Fruit leachate was similar to that of stem leachate and sterilized water with respect to their effectiveness and germination was completed after 72 h. No germination/elongation of β conidia of all five isolates were observed even after incubation period of 72 h.

Data presented in the Table 1 revealed that fruit leachate, stem leachate, sterilized water, distilled sterilized water as well as tap water were similar in their effectiveness with respect to stimulation of germination of α conidia of Pv 16. The germination commenced after 48 hs. But germination was completed only in fruit leachate and sterilized water after 72 h of incubation period. Though, the germ tube was only half fold elongated in stem leachate, distilled sterilized water, de-ionized water as well as tap water after 72 h of incubation period.

Results revealed that none of the leachates of plant parts could initiate germination/elongation of α conidia of Pv 25 up to 36 h. Germination of α conidia started after 48 h in leaf leachate, while in fruit and stem leachates germination started after 48 h. Among the water types, sterilized water took minimum (48 h) time to stimulate germination of α conidia. Whereas, in rest of the water types, germination of α conidia started after 60 h. None of the leachates/water types supported the advance stimulation of germination/elongation of β conidia.

Results recorded in the Table 1 and Figure. 1 showed that leaf leachate took minimum (24 h) time to stimulate germination of α conidia of Pv 36, whereas, in rest of the leachates/water type, germination was initiated after 36 h. Germination of α conidia was completed after 60 h in all the treatments except leaf leachate, where germination was completed within 48 h.

After all, evaluation of leachates and water types on conidial germination revealed that leaf leachate was most effective in initiating germination of α conidia over other treatments. Among the water types, sterilized water was relatively more effective that others but less effective than different plant leachates. It is evident from the observations that distilled sterilized water and de-ionized water were least effective to promote germination of α conidia. The observations further revealed that none of the leachates/water types could stimulate germination of β conidia.

Plants, during their growth, development and cellular differentiation, exude/secrete different kinds of compounds, principally the energy rich organic compounds. It is well known that these secretions affect micro-organisms. In the present experiment different plant organs (leaf, fruit and stem) along with microbiologically important grades of water were evaluated for their effects on germinability of conidia of isolates of *P. vexans.* The results are given in the Table 2**.**

The α conidia of isolates Pv 10 showed highest germination on leaf (47.85%) followed by fruit (41.29%). It was minimum in de-ionized water (33.69%). The differences in germination between the seven treatments tried were statistically significant from each other in isolates Pv 11, Pv 16, Pv 25 and Pv 26. The patterns and differences in germination remained almost the same, both at isolate level and treatment level.

The study of the Table 2 and the data analysed horizontally revealed differences. On leaf, there was higher germination of α conidia of isolate Pv 36. In the remaining isolates, it was around 50%. However, differences between them were statistically significant. On fruit, germination of α conidia of isolate Pv 36 was maximum. In the rest of isolates approximately just above 45% α conidia germinated. Almost similar results were recorded with respect to germinability of α conidia in different grades of water used for testing germination.

The results of the experiments conducted *in-vitro* on the effects of two concentrations of three carbon sources on germinability of α conidia of 5 isolates of *P. vexans* are given in Table 3 and Figure 2. This study was conducted with twin purposes i.e. to find out the best carbon source that stimulates germination of alpha coni-dia of *P. vexans* and the reaction of the isolates to concentrations and sources to find variability among them.

It is evident from the results recorded that increase in percent germination with energy sources was significant when compared to check. Among the carbon sources, glucose as carbon source was most suited and stimulated maximum germination in all the five isolates. Dextrose was found least conducive and effective as far as spore germination of the isolates of *P. vexans* is concerned. Among the isolates, Pv 10 was most sensitive. The germination in the isolate was increased by 15.19 and 34.18% at 100 and 1000 ppm of glucose, respectively. The isolate Pv16 was observed to be least sensitive with minimum change in germination per cent that is 4.39 and 17.96% at 100 and 1000 ppm of glucose, respectively. It

Table 1. Effect of different leachates and water types on spore germination of *P. vexans*

Leachate/ water	Conidial germination after different incubation period																		
	36 h					48 h					60 h					72 h			
	Pv10	Pv11	Pv16	Pv25	Pv36	Pv10	Pv11	Pv16	Pv25	Pv36	Pv10	Pv11	Pv16	Pv25	Pv36	Pv10	Pv11	Pv16	Pv25
Leaf leachate	+	+	+	-	++	++	++	++	+	+++	+++	+++	+++	+++					
Fruit leachate	+	-	-	-	+	++	+	+	-	++	+++	++	++	++	+++		+++	+++	+++
Stem leachate	-	-	-	-	+	+	+	+	-	++	++	++	+	++	+++	+++	+++	++	+++
Sterilized Water	-	-	-	-	+	+	+	+	+	++	+	++	++	++	+++	+++	+++	+++	+++
D. S. Water	-	-	-	-	+	+	-	+	-	++	++	+	++	+	+++	++	++	++	++
Deionized Water	-	-	-	-	+	-	-	-	-	++	+	+	+	+	+++	+	++	++	++
Tap Water	-	-	-	-	+	-	-	+	-	++	+	+	++	+	+++	+	++	++	++

- = No change; + = Germination started; ++ = Half fold germ tube elongated; +++ = Germ tube elongated; D. S = Distilled sterilized

Table 2. Effect of carbon sources on germination of conidia of *P. vexans* isolates

Isolate	Conidial germination (%)					
	Glucose (ppm)		Sucrose (ppm)		Dextrose (ppm)	
	100	1000	100	1000	100	1000
Pv 10	56.33 (48.63)	70.67 (57.22)	60.67 (51.16)	67.67 (55.34)	55.67 (48.25)	63.00 (52.53)
Pv 11	64.67 (53.55)	76.00 (60.66)	66.33 (54.55)	70.67 (57.21)	83.33 (52.74)	69.33 (56.37)
Pv 16	69.67 (56.58)	81.00 (64.15)	71.78 (57.84)	80.33 (63.69)	49.35 (44.61)	75.00 (60.07)
Pv 25	61.24 (51.50)	73.00 (58.69)	63.67 (52.96)	69.00 (56.16)	59.33 (50.38)	66.00 (54.36)
Pv 36	78.57 (62.43)	93.67 (75.70)	81.34 (64.46)	92.33 (74.00)	76.00 (60.66)	81.33 (64.42)

C. D. at 5% for a = 4.24; C. D. at 5% for b = 3.67; C. D. at 5% for a x = 5.95
Values in parenthesis are angular transformation

Table 3. Effect of different substrates and water types on germination of α conidia of different isolates of *P. vexans.*

Substrate/Water grade	Conidia Germination (%) of different isolates				
	Pv 10	Pv 11	Pv 16	Pv 25	Pv 36
Leaf	55.41 (47.85)	65.35 (54.52)	59.22 (52.14)	61.50 (50.47)	88.83 (73.19)
Fruit	43.64 (41.29)	48.38 (43.98)	46.03 (43.38)	43.95 (42.59)	88.16 (69.37)
Stem	40.12 (38.45)	43.45 (41.18)	42.50 (41.83)	32.78 (36.17)	78.33 (61.91)
Sterilized Water	35.68 (32.51)	39.65 (39.72)	40.77 (39.66)	32.76 (21.33)	75.00 (58.49)
Distilled Sterilized Water	35.29 (33.84)	37.79 (36.75)	39.13 (38.87)	33.08 (28.96)	64.83 (55.02)
De-ionized Water	33.69 (30.35)	34.79 (29.42)	36.55 (34.46)	29.95 (27.65)	61.66 (53.58)
Tap Water	36.04 (30.45)	40.00 (38.76)	38.12 (40.45)	33.14 (30.84)	70.00 (57.81)

C. D. at 5% for a = 1.04; C. D. at 5% for b = 1.23; C. D. at 5% for a x b = 2.76
Values in parenthesis are angular transformation.

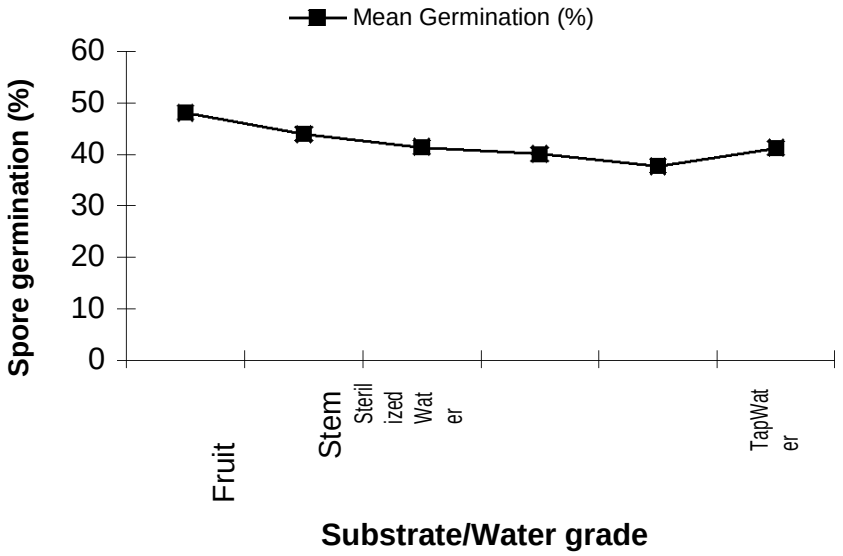

Figure 1. Effect of substrates and water grades on germination of *P. vexans* isolates.

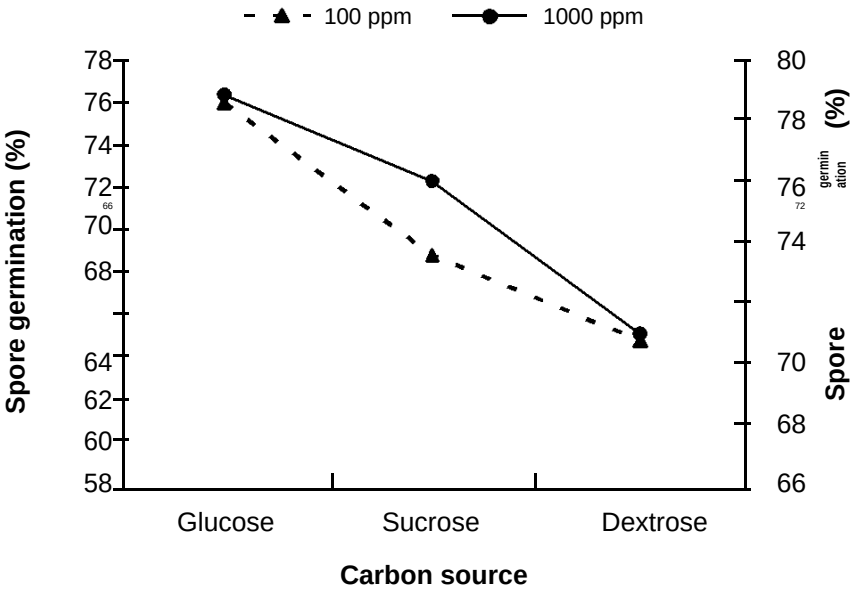

Figure 2. Cumulative effect of sugar solution on germination of conidia of *P. vexans* isolates.

is clear from the data that isolate Pv 16 was least sensitive to all the carbon sources.

Alpha and beta conidia were exposed to substrates, leachates, exudates and different moisture contents with differences in their constituents. It was interesting to observe the differences in incubation period required for germination of different isolates even in a particular plant part, leachate as well as in different grades of water. Leaf and leaf leachate promoted germination of α-conidia. Among water grades sterilized water promoted germination significantly. Glucose was the best source of carbon.

Results revealed that none of the plant parts, leachates, grades of water and carbon sources could initiate germination/elongation of β conidia of all the isolates even after 72 h of incubation period. Pv 25 up to 36 h. Our findings further establish the fact that it is the only conidia which germinates. Beta conidia of *P. vexans* did never germinate and is in accordance with previous findings (Vishunavat, 1992; Singh and Chand, 1986) whereas; it is contrary to the findings of Kumar and Sugha (1999).

In every source of carbon, the difference in conidial ( )

germination was different in pathogen isolates. It is obviously so, may have different requirements. It was tried as source of energy for annulment of fungistasis. Sources may have differences in their physico-chemical and biological components.

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