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Influence of Culture Media on Growth and Sporulation of *Phytophthora capsici* the Cause of Quick Wilt of Black Pepper

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Abstract

The influence of eight culture media *viz.*, Carrot agar (CA), Corn meal agar (CMA), Host leaf extract agar (HLEA), Malt extract agar (MEA), Oat meal agar (OMA), Potato carrot agar (PCA), Potato dextrose agar (PDA) and Rye-B agar were evaluated for supporting growth and sporangial production of *Phytophthora capsici*. Among the different solid culture media tested, both CA and Rye-B agar supported significantly the maximum colony growth (90.00 mm) each followed by CMA (88.33 mm) and OMA (87.50 mm). All eight media were used to test their ability to induce sporangial production, using different solutions *viz.*, unsterilized soil extract, sterilized soil extract, distilled water, tap water and sterilized distilled water. The Rye-B agar medium was found to be significantly superior to other media, recording the highest number of sporangia (136.5/microscopic field) under 40 x followed by CA (88.3/microscopic field). Some morphological characters of *P. capsici* observed on different culture media. *P. capsici* produced brown coloured lemon shaped sporangia which are umbellate type in branching with hyphal swellings on all tested media. No chlamydospores were observed on any of the media even in the aged culture.

Keywords : Phytophthora capsici, colony growth, sporangia

The King of Spices - Black pepper (*Piper nigrum* L.) is one of the economically important spice crops in India since ancient times and it belongs to the family Piperaceae. Foot rot disease of black pepper caused by *Phytophthora capsici* is a major production constraint in India (Saju George *et al.*, 2015). *P. capsici* is an oomycete plant destroyer that causes blight and foot rot of black pepper and fruit rot on other important commercial crops. It was first reported on chilli peppers by Leonian at a New Mexico Agricultural Research Station in Las Cruces in 1922. Phytophthora foot rot, which is considered to be the most devastating disease of black pepper creating huge losses of around 25-30 per cent (Thomas and Naik, 2017). About the description of the fungus, very limited information is available on its biology, including suitable artificial media required for growth and sporulation (Dianevys *et al.*, 2014; Kavitha *et al.*, 2013). The present study insights in to further mycological and pathological research on the fungus and disease, including timely scheduling of management strategies to combat against the pathogen. Therefore studies were conducted to know the influence of different solid media and solutions for the mycelial growth and sporulation of *P. capsici.*

Material and Methods

The pathogen was isolated from black pepper vines showing typical symptoms of foot rot disease by standard tissue isolation technique. Small bits measuring about 3 mm size were cut off from the leaves showing lesions in such a way that it contained both infected and healthy portions and these bits were surface sterilized in 0.1 per cent mercuric chloride for 30 seconds followed by three washings in sterile distilled water. The bits were further transferred to sterile discs of blotting paper. The dried bits were subsequently transferred to CMA incorporated with antibiotics *viz.*, natamycin, ampicillin, rifampicin and hymaxazole andincubated at 20-25 °C for five days.

P. capsici from soil underneath infected black pepper vines was obtained by following apple baiting technique (Lee and Varghese, 1974). Green or yellow apples were surface sterilized and moistened soil was inserted into a hole made on a fleshy fruit, sealed in plastic bags and incubation for 72 hours at 25±1°C for infection. After 4-5 days, brown discolouration was observed around the plugs. A firm rot indicates the presence of Phytophthora, a soft rot the presence of saprophytic organisms. Small amount of healthy

tissues from around the discoloured patch were taken transferred to CMA (incorporated with antibiotics) medium under aseptic conditions. A loop full of fungal culture developed on CMA in the Petri-plates was taken on a glass slide and observed under the microscope for the presence of sporangia.

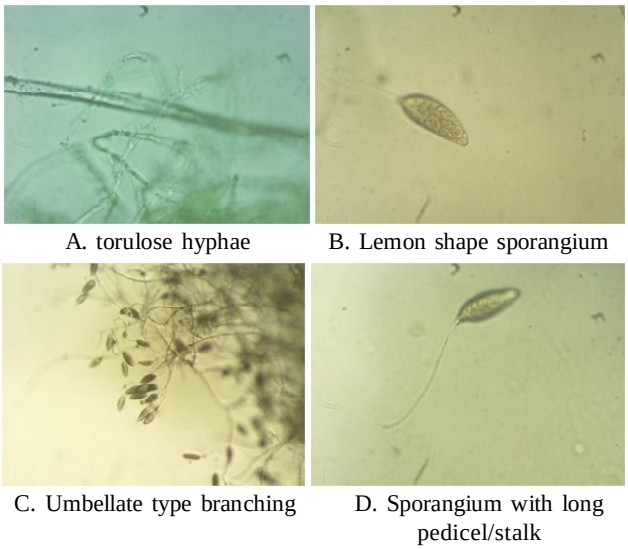
The pathogen was identified as *P. capsici* based on morphological characteristics observation such as mycelial structure, shape and branching type of sporangia.

The influence of eight culture media *viz.*, Carrot agar (CA), Corn meal agar (CMA), Host leaf extract agar (HLEA), Malt extract agar (MEA), Oat meal agar (OMA), Potato carrot agar (PCA), Potato dextrose agar (PDA) and Rye-B agar on growth and sporulation of *P. capsici* were studied. The composition of the above media was obtained from "Ainsworth and Bisby's Dictionary of the Fungi" by Ainsworth (1971) and the media prepared by following standard procedures (Tuite, 1969). The radial measurements of the colony were taken when the maximum growth was attained in any one of the media tested. Colony growth was measured along two diameters at right angles and averaged. Mycelial density was determined by following visual rating 1-4 scale: 1-Mycelium submerged, no aerial growth; 2- Scanty aerial mycelial growth, spreading as a thin layer on the surface of the medium; 3-Aerial mycelial growth moderate, covering half the height of inner Petri dish; 4- Profuse aerial mycelial growth, totally covering the inner Petri dish.

All eight media were used to test their ability to induce sporangial production, using different solutions *viz.*, unsterilized soil extract (USSE), sterilized soil extract (SSE), distilled water (DW), tap water (TW) and sterilized water (SW). Mycelial discs of *P. capsici* grown on different culture media as described earlier was separately transferred to petri dishes containing the liquid substrate and exposed to continuous light for twelve hours in laminar air flow and sporangial production was evaluated by counting number of sporangia per microscopic field at 40 x of compound microscope. In each treatment five observations were made and the data were averaged and subjected to statistical analysis. Certain morphological characters of *P. capsici* grown on different culture media were observed with reference to the mycelial characters, sporangial characters like shape, colour, papilla, branching type and presence or absence of chlamydospores.

Results and Discussion

The pure culture of *Phytophthora capsici* was successfully isolated on CMA and identified based on the morphological characteristics of mycelia and sporangia. The fungus was white to grayish white on CMA and produced aseptate mycelium with torulose hyphae and lemon shape, umbellately branching sporangia with long pedicels (Fig. 1) as described by (Diaz-Najera *et al.*, 2015 and Farhana *et al.*, 2013).



A. torulose hyphae B. Lemon shape sporangium

C. Umbellate type branching D. Sporangium with long pedicel/stalk

Fig. 1: Morphological characteristics of *P. capsici*

The results of the cultural studies on solid media indicated that the both CA and Rye-B agar supported significantly the maximum colony growth (90.0 mm) followed by CMA (88.33 mm) and OMA (87.50 mm) (Fig. 2) which were statistically on parwith CA and

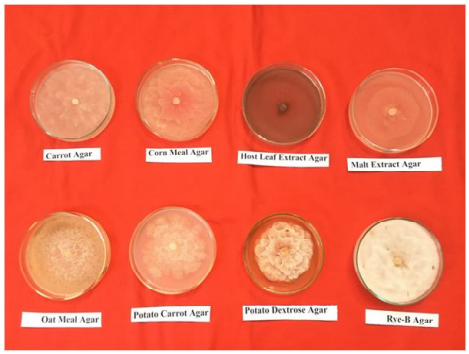


Fig. 2 : Effect of different solid media on growth of *P. capsici*

Rye-B agar (Table I). The presence of plant sterols in Rye-B agar and OMA supported maximum growth of *P. capsici* (Kavitha *et al.,* 2013). The least radial growth was recorded on HLEA (59.0 mm).

Based on aerial mycelial growth on different solid culture media, the growth on Rye-B agar recorded the density rating 4, on CA it was 3, the density ratings were 2 on OMA, PCA, and PDA, while the least density rating was 1 observed on HLEA and MEA (Table I).

Mycelial growth patterns of *Phytophthora capsici* varied on different solid media. Mycelium was creamy whitish on Rye-B agar with indefinite growth pattern, on CA, CMA, PCA, OMA and PDA the fungus produced almost whitish to slightly greyish mycelium with pettaloid in growth pattern except rosaceous on PDA, whereas on MEA and HLEA instead of pettaloid / chrysanthemum structure, radiate mycelial growth pattern was observed (Fig. 2). Similar findings were observed from processing Pumpkin isolates of *P. capsici* exhibited four growth patterns in culture: cottony, rosaceous, petaloid and stellate (Islam *et al.,* 2005). Similar findings were observed by Shashidhara (2009).

All eight media were used to test their ability to induce sporangial production, using different solutions *viz.*, unsterilized soil extract, sterilized soil extract, distilled water, tap water and sterilized distilled water. The Rye-B agar medium was found to be significantly superior to other media, recording the highest number of sporangia (136.5/microscopic field) under 40x followed by CA (88.3 / microscopic field). Significantly lower sporangial production was found on HLEA (15.1/microscopic field) (Table II). Similar results were obtained by several workers. Muzzamil

TABLE I

Effect of different solid media on cultural characters of P. capsici

Media	Growth of the fungus (mm)	Growth characters		
		Mycelial density rating	Colour of the colony	Type of growth
Carrot agar	90.00 (71.56)	3	Whitish	Petaloid
Corn meal agar	88.33 (70.02)	2	Whitish to grey	Petaloid
Host leaf extract agar	59.00 (50.18)	1	Light greyish	Slightly radiate
Malt extract agar	62.03 (51.95)	1	Whitish to grey	Slightly radiate
Oat meal agar	87.50 (69.29)	2	Whitish to grey	Slightly pataloid
Potato carrot agar	69.33 (56.36)	2	Whitish to grey	Petaloid
Potato dextrose agar	62.60 (52.29)	2	Whitish	Rosaceous
Rye-B agar	90.00 (71.56)	4	Creamy white	Indefinite
SEm ±	0.76			
CD at 1%	3.12			
CV (%)	1.72			

* Figures in parentheses indicate angular transformed values

Mycelial density

1-Mycelium submerged, no aerial growth

2- Aerial mycelial growth scanty, spreading as a thin layer on the surface of the medium

3- Aerial mycelial growth moderate, covering half the height of inner Petri dish

4- Aerial mycelial growth profuse, fully covering the inner Petri dish

TABLE II

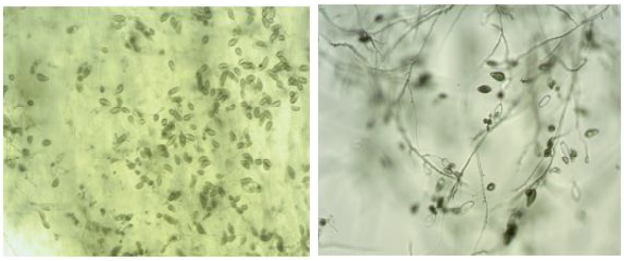
Effect of different solutions on sporangial production of P. capsici *grown on different culture media*

Different media	No. of sporangia / microscopic field					
	Sterilized soil extract	Unsterilized soil extract	Distilled water	Tap water	Sterilized water	Mean
Carrot agar (CA)	95.00	171.7	28.3	33.3	113.3	88.3
Corn meal agar (CMA)	56.7	125.0	20.3	22.7	88.3	62.6
Host leaf extract agar (HLEA)	12.3	23.3	8.3	13.7	18.0	15.1
Malt extract agar (MEA)	85.0	122.7	35.0	48.3	85.0	75.20
Oat meal agar (OMA)	85.0	119.3	25.3	39.0	94.7	72.66
Potato carrot agar (PCA)	52.3	63.3	26.0	41.6	73.3	51.30
Potato dextrose agar (PDA)	21.7	35.00	42.0	55.3	61.7	43.14
Rye-B agar	117.3	192.8	104.33	126.3	141.7	136.5
Mean	65.7	106.6	36.2	47.5	84.5	
		Media (M)	Solutions(S)	M x S		
SEm ±		0.87	0.68	1.94		
CD at 1%		3.24	2.56	7.26		
CV (%)		4.95				

and Touseef (2016) reported that Rye has a lower glucose level, which is conducive to sporangial production. They evaluated different culture media on mycelium growth and sporangium production of *Phytophthora infestans* and found that optimum growth and sporangial production occurred on Rye Agar medium followed by Oatmeal agar. Kavitha *et al.* (2013) reported that CA medium was good for sporangial production of *P. capsici.*

Among different solutions tested, highest number of sporangia (106.6 / microscopic field) was observed in unsterilized soil extract, which was statistically significantly superior over other solutions (Fig. 3a). Second best solution was sterilized water (84.5 / microscopic field) followed by sterilized soil extract (65.7 / microscopic field). Lowest number of sporangia (36.2 / microscopic field) was recorded in distilled water (Table II) (Fig. 3b). Kavitha *et al.* (2013) reported that presence of some organic substances in soil water might have stimulating ability to induce sporangial production.

The interaction studies indicated that Rye B agar medium submerged with USSE solution recorded the maximum number of sporangia (192.8/microscopic field) which was significantly superior over others (Fig. 3). Next best sporangial induction was recorded on CA submerged with USSE (171.7/microscopic field). The poor sporangial induction was observed on HLEA when submerged with distilled water (8.3/ microscopic field). The interaction studies revealed that the ability of the culture medium to induce



A. Sporangial production in USSE B. Sporangial production in DW

Fig. 3: Effect of different solutions on sporangial production of *P. capsici*

TABLE III

Morphological characters of P. capsici on *different solid culture media*

Character	Culture media							
	CA	CMA	HLEA	MEA	OMA	PCA	PDA	Rye-B
Hyphal swellings	+	+	+	+	+	+	+	+
Chlamydospore production	-	-	-	-	-	-	-	-
Sporangiophore branching	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate
Sporangial shape	Leminiform	Leminiform	Leminiform	Leminiform	Leminiform	Leminiform	Leminiform	Leminiform
Papillate	Semipapillate	Semipapillate	Semipapillate	Semipapillate	Semipapillate	Semipapillate	Semipapillate	Semipapillate
Sporangial colour	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Sporangial production on agar medium	-	-	-	-	-	-	-	-

sporangial production was highly influenced by the type of solution.

Morphological characters of *P. capsici* were studied on eight different solid media (Table III). Hyphal swellings were recorded on all the tested media and no chlamydospores were observed on any of the media even in aged cultures. There was no variation in shape and colour of sporangia observed on all tested media. The fungus produced brown, lemon shaped, semipapillate sporangia which are umbellate type in branching (more than one sporangia raise from same point of sporangiophore). No sporangial production noticed on agar media. Similarly, Diaz-Najera *et al*. (2015) reported that torulose hyphae and lemon shape of sporangia with long pedicels are characteristics of *P. capsici.*

From the present study it is surprise to observe that most of media which supported the maximum growth did not encourage the highest number of sporangia showing no correlation between vegetative growth and sporangial production. Type of solution used influenced to greater extent for sporangial production. This study also revealed that sporangial production can be assumed to be the result of combined effect of culture media and solution used.

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