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AD Saykar
Senior Research Fellow, Directorate of Plant Protection, Quarantine and Storage, Faridabad (Central Integrated Pest Management Centre, Ranchi, Jharkhand, India

PG Borkar
Assistant Professor, of Plant Pathology, Dr. KKV., Dapoli, Maharashtra, India

HT Valvi
Senior Research Fellow, IPM Division, Directorate of Plant Protection, Quarantine and Storage, Faridabad, Haryana, India

Correspondence
AD Saykar
Senior Research Fellow, Directorate of Plant Protection, Quarantine and Storage, Faridabad (Central Integrated Pest Management Centre, Ranchi, Jharkhand, India

Evaluation different culture media for growth of *Phytophthora colocasiae* Racib. Causal agent of leaf blight disease of *Colocasia* under *in vivo* conditions

AD Saykar, PG Borkar and HT Valvi

Abstract
Phytophthora colocasiae Racib. was recently the most important pathogen of *Colocasia* (*Colocasia esculenta*) in India and no more study is yet done on its culture growth on common medium. Eight artificial media, Oat meal agar medium (OMA), Potato dextrose agar medium (PDA), Host leaf extract agar medium (HLEA), Carrot agar medium (CAM), Tomato agar medium (TAM), Soybean agar medium (SAM), Water agar medium (WAM) & V8 agar medium (V8) were assessed *in vitro* to determine the growth characteristics of the pathogen. After evaluation for growth of the pathogen, on oat meal agar medium (OMA) mycelial disks from pure culture were placed on each culture medium and incubated for 7 days at room temperature (27 ±1 °C). OMA was the most effective medium as it recorded maximum (90mm) of mycelial growth of the pathogen, after 7 days of inoculation. It was followed by HLEA (87.67mm), CAM (87.33mm), PDA (84.67mm), TAM (54.67mm), WAM (41mm), SAM (38mm) and V8 (28.33mm) was the least effective medium. The mycelium was hyaline, coenocytic, sporangia elongated lemon to pear shaped with a conspicuous lid like structure at the distal end.

Keywords: *Phytophthora colocasiae*, culture media, *Colocasia esculenta*

Introduction
Colocasia is a tuber crop belonging to Araceae family. The world production of *Colocasia* is estimated at 11.8 million tons per annum (Vishnu *et al.*, 2012) [15] produced from about 2 million hectares with average yield of 6 t/ha (Singh *et al.*, 2012) [12]. Most of the global production comes from developing countries characterized by small holder production systems relying on minimum external resource input (Singh *et al.*, 2012) [12].

It is grown throughout India due to its wide adaptability, large scale acceptability and high return unit area-1 (Gurung, 2001) [6]. In India, it is grown in Andhra Pradesh, Uttar Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra (Konkan region), Tamil Nadu and West Bengal. It grows well in lowland and upland areas. The corms, cormels and leaves of *Colocasia* are eaten as fried and cooked vegetable. Various delicious dishes are prepared by using different plant parts.

Leaf blight of *Colocasia* was reported for the first time in India, by Butler and Kulkarni, (1913) [1]. It was a major limiting factor causing 25-30 per cent yield losses in taro plantation in Ponape, Hawai and India (Trujillo and Aragaki 1964; Gollifer and Brown 1974; Jakson *et al*. 1980; Misra 1997) [13, 4, 8]. The disease was earlier reported from *Konkan* region by Gadre and Joshi (2003) [3].

There is tremendous scope for popularizing this crop as an economically profitable crop among the farmers in the *Konkan* region. Area under this crop is increasing due to the efforts taken by AICRP on Tuber crops functioning at Central Experiment Station, Wakawali, of Dr. BSKKV., Dapoli. In this situation, it was necessary to understand the time of occurrence of the disease, to find out different medium suitable for the late blight of *Colocasia* at *in vivo* condition.

Materials and Methods
Isolation
Fresh samples of diseased leaves, showing leaf blight symptoms were brought to the laboratory in paper bags.

These samples were washed with running tap water to remove extraneous material. Small bits of desired size were cut by taking care that each bit contained half infected and half healthy portion. Such bits were then disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) for 1 minute followed by three washings in distilled sterile water to remove the traces of mercuric chloride. These bits were then placed on sterilized blotters for drying. Properly dried bits were transferred aseptically in sterilized Petri plates containing sterilized, solidified Oat Meal Agar medium (OMA). The plates were incubated in BOD incubator at 26 ± 1º C for 7 days. The growth of the isolate was transferred to OMA slants and maintained as stock culture for further studies.

For the identification of the causal organism

The re-isolated, pure fungal culture was identified by comparing its morphological and colony characters with the information available in the reviewed literature as well as on the standard websites for fungal identification.

Evaluation of different culture media for growth of the pathogen

In all, 8 culture media having different composition were prepared and the pure culture of the pathogen was inoculated in Petri plates poured with media to study the growth and colony characters of the pathogen. The media used in this experiment were as follows. The composition of each medium is mentioned in Table no.1.

Some earlier workers had used different media to study the radial growth of the pathogen (Guo and Ko, 1993; Padmaja *et al*., 2015) [5, 9]. The composition of the media used in present study was as per the suggestions of earlier workers. Three replications were maintained per treatment. The observations on colony diameter were recorded when Petri plates containing OMA were fully covered with mycelial growth. The experiment was carried out completely randomized design (CRD).

Each prepared medium was transferred to a flask, and plugged with cotton and sterilized in an autoclave at 121 °C for 20 min. prior to pouring the agar media into sterilized Petri dishes.

Table 1: Details of different media composition for the growth of *P. colocasiae.*

S. No.	Name of Medium	Composition	Quantity
1	Potato Dextrose Agar (PDA) medium	Peeled potato	200.00 g
		Dextrose	20.00 g
		Agar-agar	20.00 g
		Distilled water	1000 ml
2	Host Leaf Extract Agar (HLEA) medium	Host Leaf Extract	200 g
		Distilled water	200 ml
		Agar-agar	20 g
		Final volume	1000ml
3	Vegetable 8 (V8) Agar medium	V8-agar	44.3 g
		Distilled water	1000ml
4	Oat Meal Agar medium (OMA)	Oat meal agar	72.5 g
		Distilled water	1000ml
5	Water Agar Medium (WAM)		20 g
		Distilled water	1000 ml
6	Tomato Agar Medium (TAM)	Tomato	312.5 g
		Agar- Agar	20 g
		Distilled water (Final volume)	1000ml
7	Soya bean Agar Medium (SAM)	Soaked soya bean	57.5 g
		Distilled water	325 ml
		Agar- Agar	20 g
		Final volume	1000ml
8	Carrot Agar Medium (CAM)	Carrot	200 gm
		Agar- Agar	20g
		Distilled water (Final volume)	1000ml

Evaluation of the different media

Mycelial disks of 2 mm diameter were cut with a sterilized cork borer from 10 days old pure culture and placed at the center of 9 cm diameter dishes containing each freshly prepared medium. The dishes were incubated at room temperature (27 ±2 °C) for 7 days. Data on mycelial growth diameter and cultural characteristics were recorded daily. Three plates were used per medium and the experiment was repeated thrice.

Statistical analysis

The data obtained in all the experiments were statistically analyzed using methods suggested by Gomez and Gomez (1986). The standard error (S. Em.) and critical difference (C.D.) at level P = 0.01 were worked out in lab experiment. Results obtained in all the experiments were compared statistically.

Results and Discussion

Different culture media were prepared as described earlier and the effect of these media on growth of the pathogen were recorded. The results are presented in Table no. 2. It is revealed from the data presented in Table no 2 that all the treatments were statistically significant. The colony of the fungal pathogen grew profusely and reached to periphery of the Petri plates containing oat meal agar medium within 7 days. The mean colony diameter on all the media under study ranged between 28.33-90 mm. OMA was the most effective medium as it recorded maximum (90mm) of mycelial growth of the pathogen. It was followed by HLEA (87.67mm), CAM (87.33mm), PDA (84.67mm), TAM (54.67mm), WAM (41mm), SAM (38mm) and V8 (28.33mm) was the least effective medium. Though the mean colony diameter on PDA was less than that on HLEA, the growth of the pathogen was sparse on HLEA while it was robust on PDA. On HLEA the

colony exhibited distinct zones indicating termination of mycelium and further resurgence. The results of present findings are comparative with those of Padmaja *et al*., (2015) [9] who recorded the maximum radial growth of the pathogen (86 mm) on carrot agar and 72 mm on HLEA. But their results in terms OMA (52.0 mm) are contradictory to present findings where OMA has recorded maximum growth. So also they recorded minimum growth of the pathogen on PDA medium (22 mm) but this is also contradictory to the results of this study.

On carrot agar medium the growth appeared to be slightly sparse one side while it was layered on the opposite side. In T5 (TAM) the colony growth was indeterminate while in T6 it was determinate with entire margin. On water agar, the growth was scanty and mycelial strands were clearly visible only at the periphery. Many workers have reported that V8 medium is suitable for *Phytophthora* but in the present study it proved to be the least effective medium.

Many workers (Fortes and Pecknold, 1981; Tsopmbeng *et al.*, 2012; Mbong *et al.*, 2015) [2, 14] reported that V8 is the best medium for mycelial growth and sporulation of *P. colocasiae*. But the results of present study are not in agreement with those of earlier workers as the least mean mycelial growth was recorded on V8 medium. This may be due to the occurrence of different races of the pathogen in different agro climatic regions. Sahu *et al.* (2000) [11] reported better growth on PDA while Guo and Ko (1993) [5] reported that soybean agar medium and tomato agar medium were superior to V8 juice agar medium. These results are in concurrence with findings of present study.

Moreover, the edge of the colony was charred and surrounded by a zone of diffused metabolites. The fungus possessed an ability to utilize a wide spectrum of nutrients as a source of energy. Many workers have reported that V8 medium is suitable for *Phytophthora* but in the present study it proved to be the least effective medium.

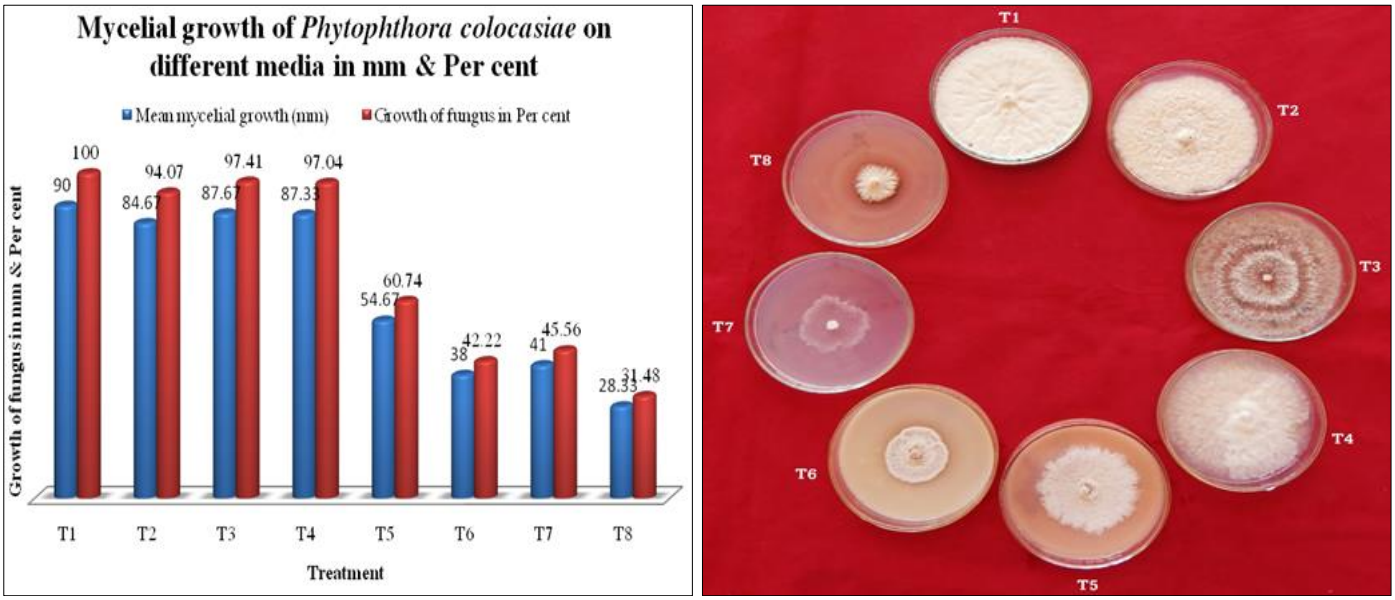

Plate 1: Evaluation of culture media for growth of the *Phytophthora colocasiae* Racib

Table 2: Mycelial growth of *Phytophthora colocasiae* on different media in mm

Treat.no	Name Of media	Mean mycelial growth (mm)	Growth (%)
T1	OMA	90.00	100.00
T2	PDA	84.67	94.07
T3	HLEA	87.67	97.41
T4	CAM	87.33	97.04
T5	TAM	54.67	60.74
T6	SAM	38.00	42.22
T7	WAM	41.00	45.56
T8	V8	28.33	31.48
SEM ±		0.333	
CD at 1%		1.377	

(*****Figures in parentheses are arc sine values)

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