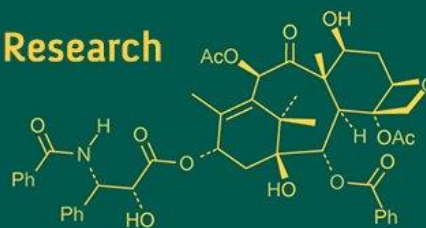


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Evaluation of different culture media for the growth of *Phytophthora palmivora*, the causal agent of koleroga disease of arecanut, under *in vitro* conditions

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Abstract

Koleroga (fruit rot) caused by *Phytophthora palmivora* is an important disease of arecanut (*Areca catechu* L.) that causes considerable yield losses, particularly during the monsoon season. The present study was conducted to evaluate different culture media for the *in vitro* growth and cultural characteristics of *Phytophthora palmivora*. Eight media, viz., Oat Meal Agar, Sabouraud's Dextrose Agar, Carrot Agar, V8 Juice Agar, Richard's Agar, Martin's Agar, Nutrient Agar and Potato Dextrose Agar, were evaluated in a Completely Randomized Design with three replications. Five-millimeter mycelial discs from a 10-day-old culture were inoculated onto the respective media and incubated at 27 ± 2 °C. Significant differences in mycelial growth were observed among the media tested. Potato Dextrose Agar recorded the maximum colony diameter (90.00 mm), followed by Carrot Agar (89.33 mm), V8 Juice Agar (80.33 mm) and Oat Meal Agar (75.50 mm). The minimum growth was recorded on Martin's Agar (53.17 mm). Colony colour, texture and margin also varied with the culture medium. PDA supported profuse, dense and cottony white mycelial growth and was identified as the most suitable medium for the *in vitro* growth and maintenance of *Phytophthora palmivora*. The findings provide a suitable basis for further laboratory studies on the biology and management of koleroga disease of arecanut.

Keywords: Arecanut, *Areca catechu*, koleroga, *Phytophthora palmivora*, culture media, mycelial growth

1. Introduction

The arecanut palm (*Areca catechu* L.), a plantation crop in tropical and subtropical regions, is a member of the Arecaceae family. It is typically thought to be indigenous to the Philippines or Malaysia, where it is cultivated as a mixed crop or monoculture. It is extensively grown in Bangladesh, Thailand, Malaysia, Philippines, Sri Lanka and India. Karnataka, Kerala, Assam, Meghalaya, Tamil Nadu and West Bengal are the main locations for arecanut growing in India. Karnataka alone is responsible for more than 69% of the country's production. Karnataka, Kerala and Assam together account for over 90% of the total area under arecanut cultivation as well as production.

According to the Ministry of Agriculture & Farmers Welfare, Govt. of India, the total area under arecanut cultivation in India for 2024-2025 was 1040.27 thousand hectares, with Karnataka accounting for the largest share of 741.18 thousand hectares, followed by Kerala (96.05 thousand hectares), Assam (69.38 thousand hectares) and Meghalaya (55.93 thousand hectares). Similarly, production levels varied, with Karnataka producing 1097.75 thousand tonnes followed by Meghalaya (87.26 thousand tonnes), Kerala (85.50 thousand tonnes), Assam (55.48 thousand tonnes), totaling 1491.23 thousand tonnes nationwide.

Arecanut, in both raw and processed forms, has long been used for sociocultural, religious and medicinal purposes. Its widespread use in betel leaf preparations and availability in fresh, dried, boiled, roasted, baked and cured forms have increased its market demand. Traditionally valued as a masticatory aid, arecanut possesses several pharmacological properties, including anti-inflammatory, analgesic, antidiabetic, hypolipidemic, antibacterial, antifungal, antiviral, hepatoprotective, wound-healing and antitumor activities, many of which have been scientifically validated (Rashid *et al.*, 2015) [6].

In China, arecanut is used as an ingredient in more than 30 medicinal formulations (Peng *et al.*, 2015) [5].

In India, koleroga was first recorded from the erstwhile Mysore state (Butler, 1906) [2] and later from the prevailing Dakshina Kannada and Uttara Kannada districts of Karnataka and some places of Malabar and Cochin in Kerala (Coleman, 1910) [3]. Further, Coleman (1910) [3] opined that, the occurrence of the disease was increased year after year due to the raise in inoculum level within the endemic regions of South India resulting extreme economic loss. This illness typically first manifests itself right after the monsoon season begins and lasts until September.

The fungus that causes koleroga disease attacks fruits while they are still green, typically manifesting two to three weeks after the start of the monsoon. The spores begin their life on the moist surface of green nut and spread through the husk and fruit stalk tissues, weakening the fruit's attachment and causing premature nut fall. The white mycelial material that swiftly covers the entire fruit is present on fallen fruits. Alternating warm and moist spells promote the growth of the spores and the disease spread. The disease spreads into the core shoot and eventually kills the tree in two to three years if the fungus activity is left unchecked (Balanagouda *et al.*, 2021) [1].

The growth and cultural characteristics of *Phytophthora palmivora* are influenced by the composition of the culture medium, making the selection of a suitable medium essential for its isolation, maintenance and further biological studies. Therefore, the present study was undertaken to

evaluate different culture media for the *in vitro* growth of *Phytophthora palmivora*, the causal agent of koleroga disease of arecanut.

2. Materials and Methods

2.1 Evaluation of different culture media for growth of the pathogen

A total of eight culture media with different compositions were prepared to evaluate the growth and colony characteristics of *Phytophthora palmivora*. The pathogen was inoculated onto Petri plates containing each medium under aseptic conditions and the composition of individual culture media is presented in Table 1. The media were prepared according to the standard compositions described by earlier workers. The experiment was conducted in a Completely Randomized Design (CRD) with three replications per treatment.

Each prepared medium was dispensed into flasks, plugged with non-absorbent cotton and sterilized in an autoclave at 121 °C (15 psi) for 15 minutes. After sterilization, the molten media were aseptically poured into sterile Petri plates and allowed to solidify before inoculation. Mycelial discs of 5 mm diameter were cut from a 10-day-old pure culture using a sterilized cork borer and placed at the center of 9 cm diameter Petri plates containing the respective media. The inoculated plates were incubated at room temperature (27 ± 2 °C) and colony diameter was recorded when maximum mycelial growth was attained in any of the tested media.

Table 1: Details of different media composition for the growth of *Phytophthora palmivora*

Sr. No.	Name of Medium	Composition	Quantity
1	Oat Meal Agar	Oat meal	60 g
		Agar-agar	20 g
		Distilled water	1000 ml
2	Sabouraud's Dextrose Agar	Sucrose	40 g
		Peptic digest of animal tissue	10 g
		Agar-agar	20 g
		Distilled water	1000 ml
3	V8 Juice Agar	V-8 juice	8.3 g
		L-asparagine	10 g
		Yeast extract	2 g
		Calcium carbonate	2 g
		Glucose	2 g
		Agar-agar	20 g
		Distilled water	1000 ml
		Peeled carrot	200 g
4	Carrot Agar	Dextrose	20 g
		Agar-agar	20 g
		Distilled water	1000 ml
		Potassium nitrate	10 g
5	Richard's Agar	Monopotassium dihydrogen phosphate	5 g
		Magnesium sulphate	2.5 g
		Ferric chloride	0.02 g
		Sucrose	50 g
		Agar-agar	20 g
		Distilled water	1000 ml
		Peptic digest of animal tissue	23 g
6	Martin's Agar	Starch	1 g
		Sodium chloride	5 g
		Dextrose	2.5 g
		Agar-agar	20 g
		Distilled water	1000 ml
		Peeled potato	200 g
7	Potato Dextrose Agar	Dextrose	20 g
		Agar-agar	20 g
		Distilled water	1000 ml
		Beef extract	3.0 g
8	Nutrient Agar	Peptone	5.0 g
		Agar-agar	20 g
		Sodium chloride (NaCl)	0.5 g
		Distilled water	1000 ml
		Distilled water	1000 ml

2.2 Statistical analysis

The data obtained in all the experiments were statistically analyzed. 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.

3. 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 Potato Dextrose Agar medium within 10 days. The mean colony diameter on all the media under study ranged between 53.17 to 90 mm. Potato Dextrose Agar was the most effective medium as it recorded maximum (90mm) of mycelial growth of the pathogen. It was followed by Carrot Agar (89.33 mm), V8 Juice Agar (80.33 mm), Oat Meal Agar (75.50 mm), Sabouraud's Dextrose Agar (61.00 mm), Nutrient Agar (56.83 mm), Richard's Agar (56.50 mm) and Martin's Agar (53.17 mm) was the least effective medium. The results of present findings are comparative with those of Sushma (2022) [7].

Table 2: Cultural characteristics of *Phytophthora palmivora* on different solid media

Tr. No.	Medium used	Mean Colony Dia. (mm)*	Colony Colour		Margin
			Front	Rear	
T ₁	Oat Meal Agar	75.50	Sparse white	Pale yellow	Regular
T ₂	Sabouraud's Dextrose Agar	61.00	Dull white	Whitish	Irregular
T ₃	Carrot Agar	89.33	Dense cottony white	Whitish	Regular
T ₄	V8 Juice Agar	80.33	Sparse white	Light brown	Regular
T ₅	Richard's Agar	56.50	Dull white	Whitish	Regular
T ₆	Martin's Agar	53.17	Dense cottony white	Pale yellow	Irregular
T ₇	Nutrient Agar	56.83	Sparse white	Whitish	Irregular
T ₈	Potato Dextrose Agar	90.00	Dense cottony white	Whitish	Irregular
SE(m) $\pm$		0.57			
C.D at 1%		2.36			

(*) = Average of three replication

Although the mean colony diameter of *Phytophthora palmivora* was higher on PDA compared to some other media, the growth was more vigorous and dense on PDA. In contrast, the pathogen exhibited sparse mycelial growth on certain media, with distinct zonation patterns indicating cessation and subsequent resumption of mycelial development.

The colony margin of *Phytophthora palmivora* varied among the different culture media tested. A regular margin was observed on Oat Meal Agar, Carrot Agar, V8 Juice Agar and Richard's Agar, whereas an irregular margin was recorded on Sabouraud's Dextrose Agar, Martin's Agar, Nutrient Agar and Potato Dextrose Agar. The findings are comparable to those reported by Nath *et al.* (2015) and Sushma (2022) [4, 7].

Phytophthora palmivora exhibited dense, cottony white mycelial growth on Potato Dextrose Agar, Martin's Agar

and Carrot Agar. Dull white mycelial growth was observed on Sabouraud's Dextrose Agar and Richard's Agar, while sparse white growth was recorded on Oat Meal Agar, V8 Juice Agar and Nutrient Agar. On the rear, whitish coloration was observed on Sabouraud's Dextrose Agar, Carrot Agar, Richard's Agar, Nutrient Agar and Potato Dextrose Agar. Pale yellow pigmentation was observed on Oat Meal Agar and Martin's Agar, while the colonies exhibited a light brown coloration on V8 Juice Agar. These results are comparative with those of Nath *et al.* (2015) and Sushma (2022) [4, 7].

Moreover, the colony margin was irregular and surrounded by diffused metabolites, indicating the fungus could utilize a wide range of nutrients. Although V8 Juice Agar has been reported as a suitable medium for *Phytophthora spp.*, Potato Dextrose Agar proved to be the most effective medium for the growth of *Phytophthora palmivora*.



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

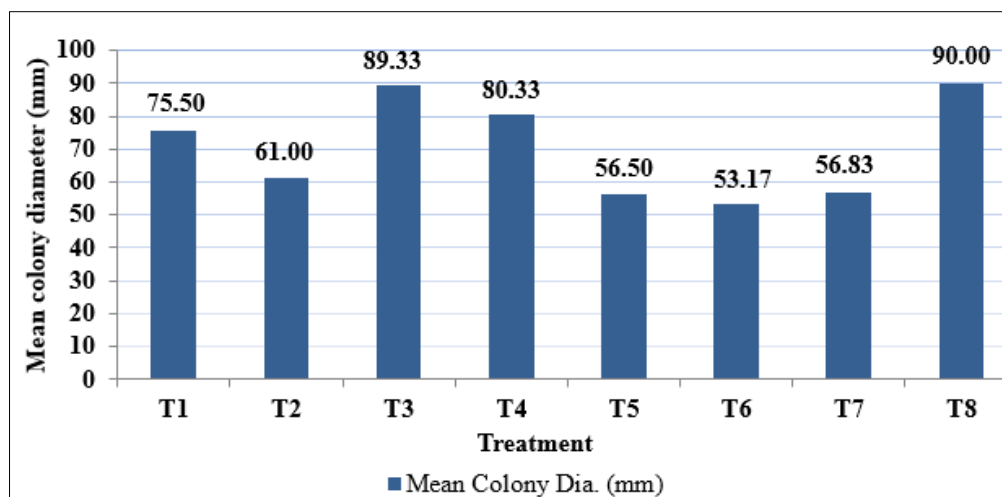


Fig 1: Evaluation of culture media for growth of the *Phytophthora palmivora*

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