

EFFECTS OF GRAIN AND NUT SOURCES ON *PHYTOPHTHORA NICOTIANAE* VAR. *PARASITICA* GROWTH AND SPORULATION

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Abstract: Influence of various grain and nut sourced culture media [viz. Oatmeal agar (OMA), Whet meal agar(WMA), Black wheat agar (BWA), Green gram agar(GGA), soybean meal agar(SMA), Pistachio agar (PIA)] were tested to evaluate the influence of mycelial growth and sporangial production of *Phytophthora nicotianae* var. *parasitica*. Among the culture media tested, both PIA and SMA agar exhibited maximum colony growth (90.00 mm) followed by GGA (89.00 mm), WMA (88.00 mm), OMA(86.66) and BWA(84.66). The mycelial growth pattern was similar in all the cultures with a slightly radiate pattern. But the SMA exhibited the highest sporangia production in water.

Keywords: Cultivation, Growth, Nut, Nutrition, *Piper betle*

INTRODUCTION

Betelvine (*Piper betle* L.) is an important medicinal crop and is prone to many diseases among them Betelvine leaf rot caused by *P. nicotianae* var. *parasitica* is a serious disease for betelvine cultivation. The disease is most frequently seen during monsoons. Typical symptoms are observed mainly on leaves with distinct grey-brown zonations or expanding, circular, dark brown, necrotic spots, without any zonation. Phytophthora leaf rot may cause yield loss of 30-100% leaf (Maiti and Sen 1997). Phytophthora leaf and foot-rot disease occurrence was first time reported by Dastur (1926).

P. nicotianae var. *parasitica* mycelia thrives well at 19-30°C while 22 ± 2°C is optimum for growth and development under in vitro conditions. Sporangia will form mostly when mycelial discs are suspended in water, which contains several motile spores called zoospores. Water with soil solution improves the progenitive ability to produce zoosporangia. Sporangia of *P. nicotianae* var. *parasitica* are virtually spherical to pyriform (pear-shape), hyaline, papillate (pointed at the tip), caducous (sporangia fall from the pedicel) with short stalks (Water house 1974) and have a long pedicel (stalk) attached to the base of the spore. (Erwin and Riberiro, 1996).

P. nicotianae var. *parasitica* is heterothallic which means it has two mating types and both mating types are needed for the production of sexual spores called oospores. Oogonium is red or orange (a mother cell that develops oospores) in some types of media and has thick walls. The presence of the mating type of another type causes some Phytophthora species to produce oospores. The presence of the mating type of another type causes some Phytophthora species to produce oospores. Zoospores are produced and discharged when moisture is present

Splashing irrigation or rainwater can disperse sporangia under natural conditions. . Before

encysting, these zoospores swim for a short while up to an hour. The amount of time that zoospores swim depends on environmental parameters such as water temperature, nutrition, pH, and other elements.

Phytophthora spp. is most commonly grown on Corn meal agar and V8 agar medium under in vitro conditions. It grows slowly when compared to other fungi due to less saprophytic ability (Prasad *et al.*, 2017) and nutrition wasn't standardised as per the requirements of the development of Phytophthora. Hence, different media were explored to select the suitable medium for the growth of the test organism under in vitro conditions. The present study insights to understand the influence of Six grain and nut-sourced culture media for the mycelial growth and sporulation of *P. nicotianae* var. *parasitica*

MATERIALS AND METHODS

Phytophthora leaf rot-causing organism was isolated from betel vine leaves with typical symptoms of leaf rot disease by standard tissue isolation technique. Small bits of leaf specimen with both infected and healthy portions measuring about 4-5 mm in size were cut off from the lesions and surface sterilized in 1 per cent sodium hypochlorite for 60 seconds followed by rinsing in sterile distilled water in three plates and placed on sterile blotting paper to get surface dried. Subsequently, the sample bits were transferred to water agar media incorporated with antibiotics (Cefotaxime) and incubated at 20-25 °C for five days. Later the actively growing mycelium was cut and placed on the CMA agar, incubated in BOD for 5 days at 22 ± 2°C. Later a loop full of mycelia from the CMA plate was taken on a glass slide to observe the presence of sporangia and mycelial characters under the microscope. A mycelial disc was placed in a petri-dish containing sterile soil solution and incubated for 48 hrs at room temperature and observed for sporangia and its morphology

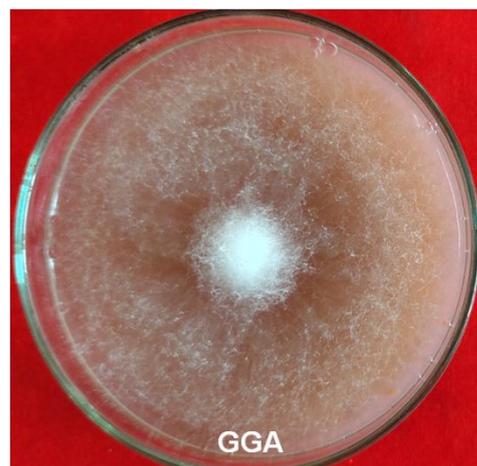
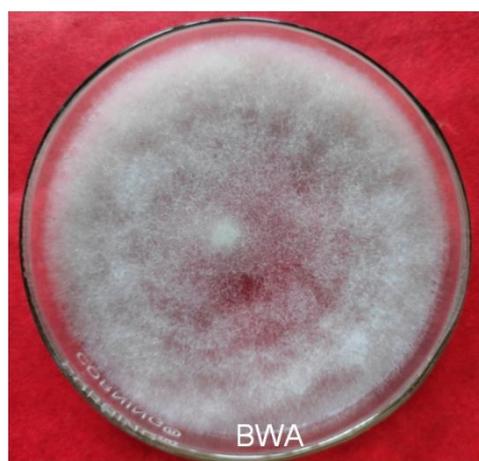
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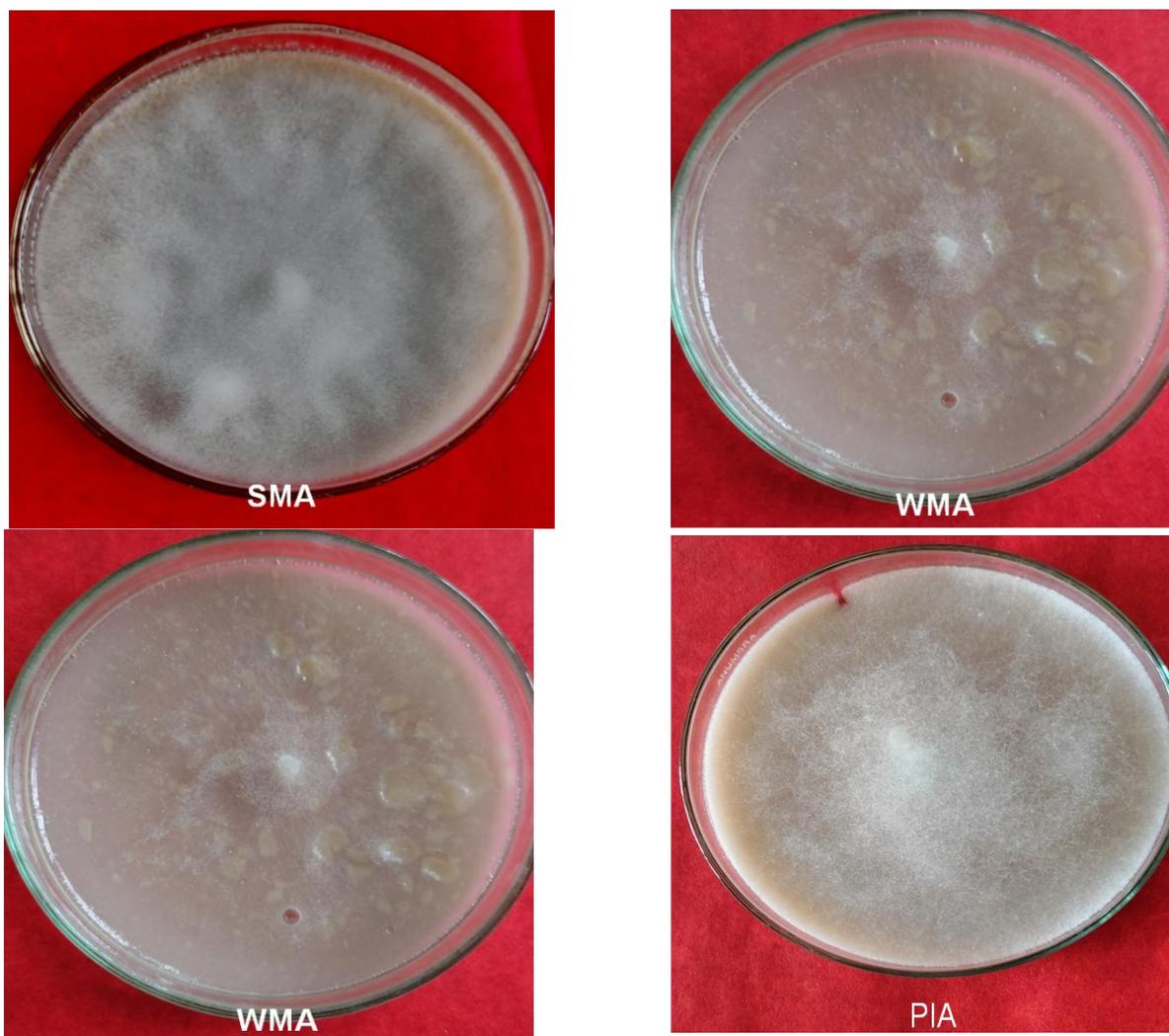
based on the morphological characteristics such as mycelial structure, shape and branching type of sporangia the organism was identified as *P. nicotianae* var. *parasitica* and later confirmed by molecular characterisation.

The influence of Oatmeal agar (OMA), Wheat meal agar (WMA), Black wheat agar (BWA), Green gram agar (GGA), soybean meal agar (SMA), Pistachio agar (PIA) on growth and sporulation of *P. nicotianae* var. *parasitica* were studied. The composition of the above natural media was 10 per cent of extract of nutrient source and 2 percent base material (Agar), i.e., nuts and grains at 10 grams soaked overnight in 100 ml distilled water, later grounded and the solution was filtered using a muslin cloth. Thereafter 2g of agar was added to the solution and heated until the agar dissolves. It was autoclaved and cooled down to 40°C temperature, and then poured into 90 mm sterile petriplates at three replications for each treatment. Actively growing Mycelia from Phytophthora culture plate were excised at 5mm size discs and were inoculated in each media. The radial measurements of the colony were recorded once the maximum growth of mycelia was observed in any one of the media. The Mycelial

density of the Phytophthora was determined by a visual rating scale of 1-4: (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).

As mentioned earlier, mycelial discs of different culture media were transferred separately to Petri dishes containing sterile tap water, incubated at standard room temperature, and exposed to daylight and night darkness for 48 hours. Later sporangial production was evaluated by counting the number of sporangia per microscopic field at **10X** of a compound microscope. In each treatment, five observations were made and the data were averaged and subjected to statistical analysis the numerical were round-off to the nearest value. Certain morphological characters of ***P. nicotianae* var. *parasitica*** 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

All culture media exhibited satisfactory mycelia growth among them SMA and PIA influenced 100 per cent of mycelial radial growth. Whereas Padmaja, et al.,(2015) obtained the best growth of *P.colocasiae* on carrot agar (86 mm), followed by papaya sucrose agar (80.6mm) and less growth of the pathogen was recorded on PDA (22 mm). The growth OMA was 86.66 mm radial growth which was on par with the results of Prasad et al., (2017) 81.80 mm at seven days of incubation. The growth of mycelia was represented in Plate 1 and illustrated graphically in fig.1

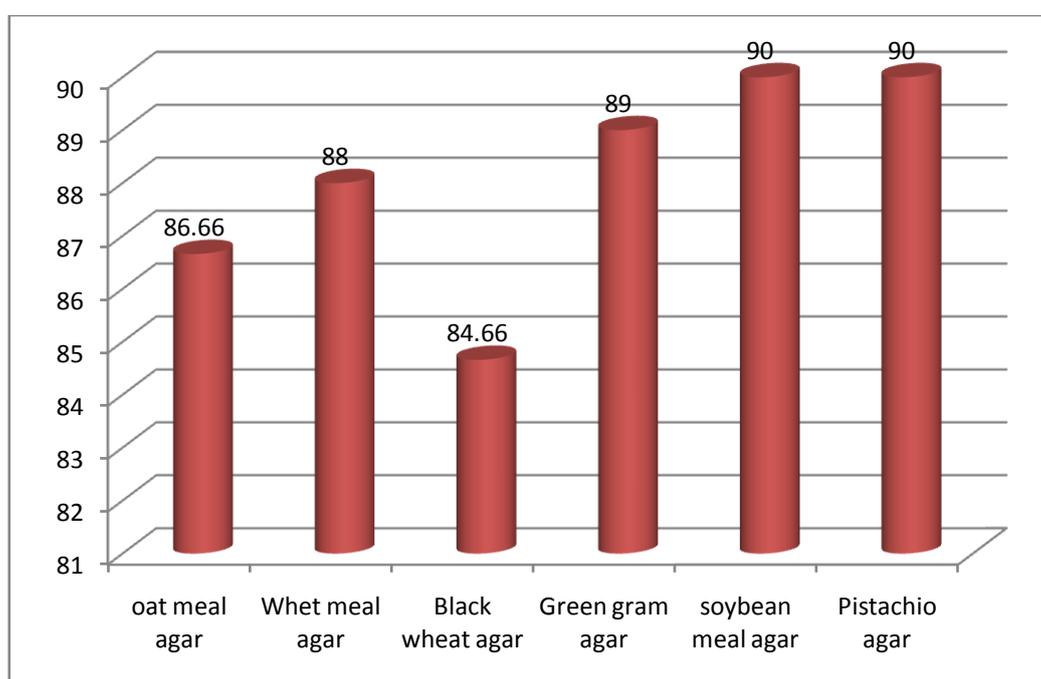
In the context of sporulation soybean meal agar medium was found to be significantly superior to other media, recording the highest number of sporangia (134/microscopic field) under 10 x followed by OMA (125/microscopic field) followed by PIA(123 /microscopic field), GGA

(120/microscopic field), WMA(117/microscopic field), BWA (113/microscopic field).

The mycelial pattern was similar in all the media with a slightly radiate pattern. While mycelial density was superior in OMA and SMA. WMA exhibited the least mycelial growth density with a rating of 2. Maximum mycelial growth was attained in the culture media by eight days of incubation. Some morphological characters of *P. nicotianae var. parasitica* were observed on different culture media. Sporangia are brown-coloured lemon-shaped with hyphal swellings on all tested media. No chlamyospores were observed on any of the media even. Still, the development of Oogonium was observed in Green gram agar media which is spherical in shape and ranged between 22 -28 μ m size diameter as mentioned by **Cacciola and Magnano** (1988). But antheridium wasn't found in the culture as it is heterothallic.

Table 1. Effect of different media on growth and development of *P. nicotianae var. parasitica*

Nutrient source	Mycelial growth (mm)	Sporangia on media	Sporangia in water	Mycelium density rating	Type of growth
Oat meal agar (OMA)	86.66	4	125	4	Slightly radiate
Whet meal agar(WMA)	88	0	117	2	Slightly radiate
Black wheat agar(BWA)	84.66	0	113	3	Slightly radiate
Green gram agar (GGA)	89	3	120	3	Slightly radiate
Soybean meal agar (SMA)	90	0	134	4	Slightly radiate
Pistachio agar (PIA)	90	4	123	3	Slightly radiate
C.D.	1.748				
SE(m)	0.561				

**Fig 1.** Mycelial growth of *P. nicotianae var. parasitica* on different media**REFERENCE**

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