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PERFORMANCE OF DIFFERENT GROWTH MEDIA FOR THE GROWTH AND SPORULATION OF *PHOMOPSIS* SP. OF CASHEW

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Abstract

The experiment was conducted at Regional Fruit Research Station, Vengurle, Sindhudurg (M.S.), India on the growth of *Phomopsis anacardii* causing leaf blight disease on cashew. The present work investigated the performance of different growth media for the growth and sporulation of *Phomopsis* sp. of cashew. The mycelial growth, colony character and sporulation pattern of fungus grown on five different culture media namely, malt extract agar (MEA), tryptone dextrose agar (TDA), potato carrot agar (PCA), potato dextrose agar (PDA) and host leaf agar were observed after 10 days of incubation at 23±1°C. The colour of colony, culture characteristics and sporulation of the fungus were greatly influenced by the type of growth medium used. The best mycelial growth was recorded in 12 h alternating light/dark followed by total light and total dark conditions, respectively. Among the five media, host leaf agar media found to be the best for growth of fungi, *Phomopsis* sp. followed by PDA, but least growth of fungus observed on TDA medium. The maximum growth of *Phomopsis* sp. was observed in temperature range of 23 to 30°C. The suitable pH level for growth of fungus was ranged between 5.8 to 7.0.

Key words : Performance, growth media, growth, sporulation, *Phomopsis* sp., cashew.

Introduction

Cashew is an important cash crop that traded worldwide. The Indian Cashew Industry is export oriented and hence called as dollar earning crop of the country. India is largest producer, processor, consumer and exporter in the world contributing for 26.40 per cent and 46.09 per cent of the world production and export respectively, during 2006-07. Commercial cultivation of cashew is taken up in eight states of our country mainly in west and eastern coast *viz*., Andhra Pradesh, Goa, Karnataka, Kerala, Maharashtra, Orissa, Tamilnadu and West Bengal. Cashew is also grown in few pockets of Assam, Chhattisgarh, Gujarat, Meghalaya, Nagaland and Tripura. India has an area of 9.53 lakh ha (2010-11) under cashew with an estimated annual production of about 6.74 lakh tonnes of raw cashew nut. India is the third largest producer and exporter of cashew in the world next only to Vietnam and Nigeria. In Maharashtra State, it is grown mainly in Ratnagiri and Sindhudurg districts. Cashew trees are genuinely tropical and very frost sensitive. The trees grow in a wide spectrum of climatic regions between the 25° N and S latitudes. Although, the cashew can withstand high temperatures, a monthly mean of 25°C is regarded as optimal. Yearly, rainfall of 1000 mm is sufficient for production, but 1500 to 2000 mm can be regarded as optimal. Diseases constitute limiting factors in production of cashew in Konkan region of Maharashtra, India. In the tropics, the fight against maladies of phyto-pathological nature would be of preventive measures, aimed at allowing the plant to develop in suitable conditions like proper drainage of the ground for seedlings, right spacing between plants, ensuring normal circulation of air and giving adequate sunlight above ground for the grown up trees. Further, selection of varieties showing greater natural resistance to certain disease, combined with their vegetative propagation, are some measures to protect health of the cashew trees. However, when more areas are covered with improper phytosanitation, the loss due to disease is likely to increase. Though, it is hardy crop but due to increase in area and change in climatic condition cashew crop also affected by different diseases. Hence, study was undertaken to know the performance of *Phomopsis anacardii* on different growth media for the growth and sporulation of *Phomopsis* sp. of cashew.

Materials and Methods

The experiment was carried out at Regional Fruit Research Station, Vengurle, distt. Sindhudurg (M.S.),

India. The diseased samples were collected from field and brought into the laboratory and isolation of diseased sample was done. Isolated pathogen was sent for identification at Indian Agricultural Research Institute, New Delhi, Indian type culture collection. *Phomopsis* sp. was found in all of the lesions and was identified. The typical cashew leaf blight diseased leaf samples were collected from farmers' fields from 6 talukas namely Vengurla, Kudal, Sawantwadi, Kankawali, Devgad and Malvan. These samples were brought to the laboratory for isolation of disease causing fungi. The methodology was followed according to Menge *et al*. (2013).

The pathogen was isolated on potato dextrose agar (PDA) medium. Cashew leaves showing leaf blight symptoms were cut into small pieces of 1.2 cm, surface sterilized by sodium hypochloride for 1 minute and washed in sterilized distilled water three times. The leaf bits were placed in petri plates containing moist filter paper and incubated for four days at 23±1°C. Sporulated leaf bits were shaken onto new PDA medium to release spores thereafter the plates were incubated for four days at 23±1°C.

Maintenance of the culture

The pure cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in laboratory at 23±1°C for 10 days. Such mother culture slants were preserved at 5°C in refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies.

Effect of culture media on mycelial growth

Five different culture media were used to find out best media for the mycelial growth of the fungus. Cultural characters of six isolates of *Phomopsis* sp. from six talukas were studied on five different media. The growth characters of *Phomopsis* sp. were studied on five different culture media namely, malt extract agar (MEA), tryptone dextrose agar (TDA), potato carrot agar (PCA), potato dextrose agar (PDA) and host leaf agar. To carry out the study, 20 ml look warmed medium was poured in 90 mm petri plates, such petri plates were inoculated with 8 mm disc cut from periphery of actively growing culture and incubated at 23±1°C for 10 days. Observations were recorded when the fungus had completely covered the Petri plate in any one of the media. The colony diameter was recorded. The fungus colony colour and sporulation were also recorded.

i. Potato Dextrose Agar (PDA) – Potato infusion 200, dextrose 20.0, agar 15 and distilled water 1000 mL.

ii. Tryptone Dextrose Agar (TDA)- Tryptone 10.0, Glucose 5.0, Bromocresol purple 0.04, agar 12.0 and distilled water 1000 mL.

iii. Malt Extract Agar (MEA)- malt extract 30.0, peptone from soymeal 3.0, agar 15.0 and distilled water 1000 mL.

iv. Potato Carrot Agar (PCA); grated potato 20g, grated carrot 20g, agar 20g and distilled water 1000 mL prepared according to Tuite, 1969.

v. Host Leaf Agar -young cashew leaves 200 g, agar 20 g and distilled water 1000 mL.

Morphological studies of the pathogen

Spores of diseased sample were taken from infected host tissue and mounted on a clean glass slide. To characterize isolates by colony morphology, single germinating conidia were transferred to Petri dishes containing PDA. Dishes were incubated at 23±1°C in 12 h alternate light and darkness for six days. After incubation, cultures were examined for colony color, colony texture, and the development of pigments or crystals in the agar medium. To characterize isolates by sporulation habit, single germinating conidia were transferred to petri dishes containing PDA were incubated for one week and observations were recorded.

Results and Discussion

The experiment was conducted at Regional Fruit Research Station, Vengurle on the growth of *Phomopsis* sp. causing leaf blight disease on cashew. To check the best growth of fungi, *Phomopsis* sp., five different media namely PDA, TDA, MEA, PCA and host leaf extract agar were selected and incubated for 10 days. The results showed that the growth behavior of six isolates on five different media showed significant difference in colour, morphology with sporulation in PDA (table 1).

The maximum growth was observed on host leaf extract medium and PDA supported maximum growth of fungal colony followed by malt extract agar, tryptone dextrose agar and potato carrot agar (table 2). PDA was one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth (Xu *et al*., 1984; Maheshwari *et al*., 1999; Saha *et al*., 2008). There were variations among the colony characters of the isolates collected from six talukas. Most of the isolates (A2, A3, A5, A6) had white colonies, whereas A1 and A4 produced dull white coloured colonies. The media became brownish in all isolates with time.

Sharma and Pandey (2010) studied the mycelial

Table 1 : Cultural characters of *Phomopsis* spp. isolates in PDA medium.

S. no.	Isolate	Colony colour	Sporulation
1.	A1	White	+++
2.	A2	Dull white	++
3.	A3	White	++
4.	A4	White	+++
5.	A5	Dull white	++
6.	A6	White	+++

++ = Poor sporulation, +++ = Good sporulation.

Table 2 : Growth of *Phomopsis* sp. isolates in different media.

S. no.	Isolate	PDA	TDA	MEA	PCA	Host Leaf Agar
1.	A1	White	Dull white	Dull white	Dull white	Dull white
2.	A2	Dull white	White	Dull white	White	White
3.	A3	White	Dull white	White	Dull white	White
4.	A4	White	Dull white	Dull white	White	Dull white
5.	A5	Dull white	White	White	White	White
6.	A6	White	White	Dull white	White	Dull white
Sporulation		**Better**	**Medium**	**Good**	**Medium**	**Better**

PDA = Potato Dextrose Agar, TDA = Tryptone Dextrose Agar, MEA = Malt Extract Agar, PCA = Potato Carrot Agar.

growth rate, colony character and sporulation pattern of ten fungal isolates, grown on three different culture media *viz*., Potato Dextrose Agar (PDA), Czapek's Dox + Yeast Extract Agar (CYA) and Lignocellulose Agar (LCA) were observed after seven days of incubation at 25±1°C. The colony diameter, culture characteristics (texture, surface and reverse colouration, zonation) and sporulation of selected test fungi were greatly influenced by the type of growth medium used. LCA exhibited comparatively higher mycelial growth in six test fungi, whereas all the ten isolates revealed heavy sporulation on this culture medium. *Penicillium* sp. and *Acremonium kiliense* exhibited maximum colony growth on PDA, while *Chaetomium funicola* and *Fusarium oxysporum* showed highest growth on CYA medium. Okunowo *et al*. (2010) observed least sporulation and minimum mycelia growth of *Myrothecium roridum* on Czapek's Dox agar, which may be due to the presence of chloride ion in the test medium. Zhae and Simon (2006) reported that the type of culture media and their chemical compositions significantly affected the mycelia growth rate and conidial production of *Phoma exigua*.

It is necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996). Osono and Takeda (1999) stated that Lignocellulose Agar (LCA) because of its low glucose content suppresses the overgrowth of fast growing species and induces sporulation, hence this medium is useful for fungal identification.

Conclusion

Our findings revealed that culture media differentially influenced the growth, colony character and sporulation of the test fungi. Out of five test media employed in the present study. Host leaf agar, PDA and MEA were found to be the most suitable for good sporulation while PDA reproduced most visible colony morphology. It was concluded that instead of using any single culture medium, a combination of two or more media will be more appropriate for routine cultural and morphological characterization of fungi to observe different colony features.

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