Response of Different Media on Growth and Sporulation of Alternaria Alternata Causing Fruit Rot of Pomegranate

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ABSTRACT

Alternaria alternata (Fr.) Keissl is one of the most serious post-harvest diseases throughout the World. It causes severe post-harvest losses of perishable due to A. Alternata ranging up to 10 to 50 percentages. In this view, a total of fifteen isolates of A. alternata were isolated from infested pomegranates in the various locality of Maharashtra. Infected pomegranates were rinsed with sterile distilled water up to 5 to 6 times then cut into smaller pieces and placed aseptically at equidistant onto sterile culture media augmented with streptomycin (20µg/ml) in petriplate. Potato dextrose agar, Czapek Dox Agar,Corn Meal Agar and Martin Rose Bengal agar was undertaken to ascertain the requirement of different culture media on growth and sporulation of A. alternata. The culture plate was incubated at 22 ± 2ºC for seven days. Growing mycelium was transferred to slants and pure culture of the pathogen was obtained. Potato dextrose agar medium (90.00 mm) and Corn meal agar (75.33 mm) show maximum growth and sporulation of A. alternata whereas Martins Rose Bengal agar (48.17 mm) and Czapek Dox Agar (51.67 mm) shows minimum growth and sporulation.

Keywords: Pomegranates, A. alternata, culture media.

INTRODUCTION

Fruit rot of pomegranate caused by Alternaria alternata (Fr.) Keissl is a filamentous heterothallic septate mycelium, an ascomycetes comprising great variability in the mycelia growth and sporulation. Conidial germination of A. alternata is induced by different physical and chemical signals including the presence of quality nutrients (Apet and et al., 2014). Conidia of A. alternata are typically nutrient-dependent, they do not readily germinate in sterile water and they usually require an exogenous input of nutrients for germination. In addition, it has been proposed that nutrient dependent of phytopathogenic fungi may use germination stimulating compounds from a host plant as an alternative chemical when nutrient concentrations are too low for conidial germination and diverse carbon sources are effective at low concentrations (10 µg/ml) to induce sporulation in A.alternata (Filonow, 2002, Dahiwale et al., 2009 and 2012). Rich media such as malt extract induced rapid germination and early germ tube branching. The mechanism of nutrient sensing by A. alternata is unknown (Forsberg and Ljungdahl, 2001). The current study has illustrated the effect of such several culture media on the growth and sporulation of A. alternate.

MATERIALS AND METHODS

The infected pomegranate samples were brought from the field to the laboratory and used for the isolation study. The infected samples were surface sterilized with 0.1% $HgCl_2$solutions for two minute with gentle agitation. Samples were rinsed with sterile distilled water up to 5 to 6 times then cut into smaller pieces and placed aseptically at equidistant onto sterile PDA augmented with streptomycin (20µg/ml) in petriplate. Plate was incubated at 22 ± 2ºC for seven days, examined daily for the growth of the organism. Pure culture of the pathogen was obtained and the pathogen was purified using a PDA medium. Growing mycelium was transferred to slants. The identification was carried out by macroscopic and microscopic observation which confirmed the A. alternata (Samson et al., 1984). A. alternata was obtained and maintained at 4OC in the PDA medium tilted in sterile tubes and used for further study wherever necessary. The response of different media on growth and sporulation of A. alternata was used for detailed studies. Potato Dextrose Agar Medium (Hawksworth et al.,1983), Czapek's Dox Agar (Onions et al., 1981), Corn meal Agar (Baron and Finegold, 1990), Martin Rose Bengal Agar (Dahiwale and

Suryawanshi, 2012) were used for growing A. alternata. Potato dextrose agar medium (PDA) showing maximum growth and sporulation of A. alternata and this medium is used for further study.

RESULTS AND DISCUSSION

A. alternata were grown on four different media viz. PDA, CDA, CMA and MRBA in order to find the best medium suitable for the growth of pathogens. Total four media were taken for isolation and purification of A. alternaria. Media were prepared using standard techniques. In case of pure culture of the A. alternaria was obtained onto sterile PDA, CDA, CMA and MRBA medium. Corn meal agar (CMA) (75.33 mm) and Potato dextrose agar medium (PDA) (90.00 mm) shows maximum growth and sporulation in seven days where as Martin Rose Bengal agar (MRBA) (48.17 mm) and czapek dox agar (CDA) (51.67 mm) shows minimum growth and sporulaton. (Fig 1 and Table 1). The A. alternaria was purified using PDA medium. Plate was incubated at 22 ± 2ºC for seven days. Growing mycelium was transferred to slants. The identification was carried out by macroscopic and microscopic observation which confirmed the A. alternaria (Samson et al., 1984). Similar result reported by Reddy and Gupta, (1981) the growth and sporulation of Alternaria helianthi while Mohanthy et al., (1981) reported Richards B medium shows maximum growth of A.alternata. Morphological characteristics of the cultures on potato carrot agar and V8 agar media after seven days exhibited typical characteristics of Alternaria mali (Simmons, 1999). PDA, Oatmeal agar, Czapek Dox, Richards, Walkman's agar medium, water agar and Martins Rose Bengal Agar media were also studied by Hubballi (2010), maximum growth was observed on PDA.

Table 1: Effect of different media on germination and sporulation of Alternaria alternate

Sr.No.	Medium	Colony diam (mm)	Sporulation
1	Potato dextrose agar	90.00	++++
2	Czapek Dox Agar	51.67	++
3	Corn Meal Agar	75.33	+++
4	Martins Rose Bengal Agar	48.17	+

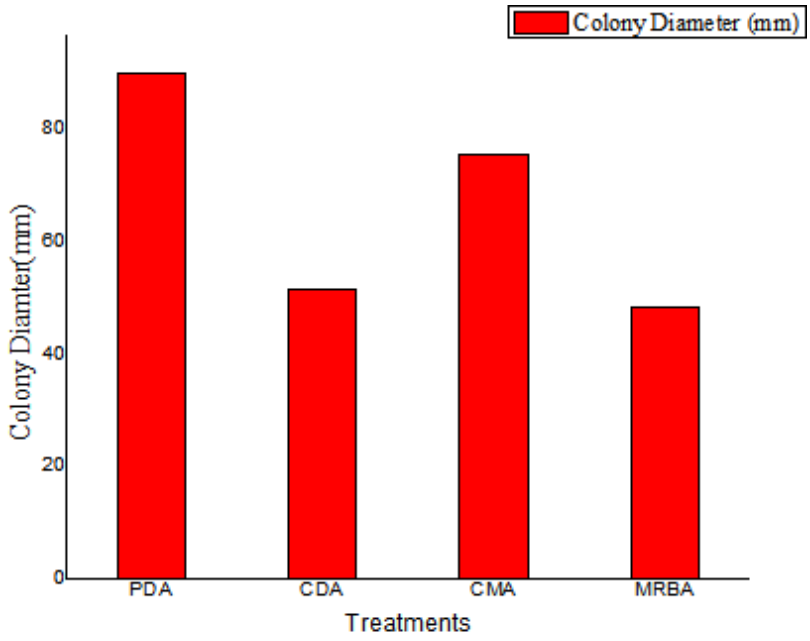

Fig 1: Effect of various media on radial growth of *A. alternata* infecting Pomegranate (graph)

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