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In vitro Studies of carbon, Nitrogen sources and pH on mycelia growth Blackgram Root rot caused by *Macrophomina phaseolina*

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Abstract
Blackgram, also known as urdbean, mash, black maple etc. an important short-duration pulse crop grown in many parts of India. Blackgram is subjected to different biotic constraints, root rot being the serious one. The root rot pathogen *Macrophomina phaseolina* was isolated from the diseased stems and roots of blackgram collected from seven different places of Tamil Nadu. Among the seven isolates screened, K_2m_7 collected from Kallipatti in Namakkal district was identified as a virulent culture. The virulent culture K_2m_7 was used in all experiment in the subsequent study. The pH 7.0 was found to favour maximum mycelial growth of pathogen irrespective of the isolates. Among different carbon sources tested in both solid media and liquid broth, glucose was identified as best carbon source for maximum mycelia growth and mycelia dry weight (7.50 cm and 7.20 g) respectively. Among different nitrogen sources tested both in solid media and liquid broth, peptone was identified as best nitrogen source for maximum mycelia growth and mycelia dry weight (7.47 cm and 7.77 g).

Keywords: Blackgram, *Macrophomina phaseolina*, K_2m_7, pH, Carbon and Nitrogen sources.

Introduction
In Tamil Nadu, blackgram is grown in area of 2.15 lakh ha with a production of 0.7 lakh tonnes during 2005-2006 (Ravindran and Anita, 2008) [12]. During 2010-2011, the productivity of blackgram is 528 kg/ha (Anonymous, 2011) [3]. The pathogen survives in the form of free sclerotia in the soil or as sclerotia embedded in diseased plant tissues (Sheik and Ghaffar, 1979) [15].

Dark lesions appear on the epicotyls and hypocotyls followed by seedling death due to obstruction of xylem vessels. In plants, the pathogen causes red to brown lesions on roots and stems with production of dark mycelia and black microsclerotia and ultimately the plant becomes defoliated and wilted (Abawi and Pastor-Corrales, 1990) [1].

Macrophomina phaseolina is soil and seed- borne pathogenic fungus produces cushion shaped black sclerotia (Wheeler, 1975) [16]. Its prevalence can be enhanced by different physiological and ecological factors such as low moisture content, high temperature and heat (Dhingra and Sinclair 1978) [6]. The disease recently observed in severe proportions in the blackgram growing areas of Tamil Nadu leading to severe loss in the yield. The disease may cause up to 100 per cent yield losses (Bashir and Malik, 1988) [4]. The loss in grain weight due to the disease in *Rabi* cultivars varies from 18.53 to 63.22 per cent (Anonymous, 1999) [2].

Moniz and Bhide (1963) [9] working with the Gujarat strain of *Macrophomina phaseolina* observed that, dextrose, sucrose, raffinose, levulose and maltose supported good growth and sclerotial formation of the fungus. Patil and Kulkarni (1965) [11] reported that, arabinose, dextrin, glucose, lactose and sucrose supported good growth of *Macrophomina phaseolina* isolated from cotton, sesame, groundnut and castor. Shanmugam and Govindswamy (1973) [14] obtained significantly higher growth of *Macrophomina phaseolina* in asparagine followed by glutamine, peptone and potassium nitrate. *R. bataticola* metabolized a number of nitrogen compounds for the growth and that the amount of growth varied with the type of nitrogen source.

Chowdary and Govindaiah (2007) [5] observed that the growth of *M. phaseolina* (causal agent of root rot of mulberry) was maximum (90.0 mm) at pH 7.0, whereas it recorded 59.0mm at pH 8.0. At different levels of pH (6.0, 6.5 and 7.5), growth of pathogen varied 62.3, 84.3 and 87.6 mm respectively. Similarly, maximum number (120) of sclerotia 9 mm mycelia disc was recorded at pH 7.0 and the same was found minimum (16.3) at pH 6.0. The growth rate of

pathogen and number of sclerotia varied at different levels of pH. Bainade *et al.* (2006) studied various pH levels for the growth of *M. phaseolina* causal agent of leaf spot of mung bean. They observed that the pH levels of 7.0 (neutral) recorded 89.66 mm growth followed by pH 8.0 (61.33 mm growth).

Materials and Methods

Utilization of different carbon and nitrogen sources of solid media

The Czapek dox medium was substituted with different carbon sources *viz*. glucose, maltose, sucrose, fructose, dextrose, lactose and different nitrogen sources *viz*., ammonium nitrate, potassium nitrate, urea, sodium nitrate, ammonium sulphate and peptone. The medium without nitrogen and carbon sources served as control. The sterilized warm medium was poured in the sterilized Petri dish and allowed to solidify and inoculated with seven days old nine mm culture disc of the pathogen. The plates were incubated at room temperature (28±2 ºC) for 7 days. The diameter of mycelia growth was recorded. Three replications were maintained.

Utilization of different carbon and nitrogen sources in liquid broth

The Czapek dox broth was substituted with different carbon sources *viz*, glucose, maltose, sucrose, fructose, dextrose, lactose and different nitrogen sources *viz.*, ammonium nitrate, potassium nitrate, urea, sodium nitrate, ammonium sulphate and peptone. The broth without nitrogen and carbon sources served as control. The sterilized warm broth was inoculated with seven days old nine mm culture disc of the pathogen. The flasks were incubated at the room temperature (28±2 ºC) for 7 days. The mycelia dry weight was recorded. Three replications were maintained.

Effect of different pH levels on growth of *M. phaseolina* under *in vitro*

One hundred ml of potato dextrose agar broth was prepared and transferred to
250 ml conical flask and the pH of the broth was adjusted to different pH levels *viz*., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 with 0.1 N HCl or 0.1 N NaOH by using digital pH meter (Medox) and sterilized in an autoclave at 15 lb for 20 min. A nine mm PDA culture disc of actively growing *M. phaseolina* was placed at the centre of each conical flask containing the broth under aseptic conditions. The flasks were incubated at room temperature (28±2 ºC) for seven days. Three replications were maintained for each pH level. The mycelia dry weight of the pathogen was measured at 7 days after incubation.

Result and Discussion

A survey was conducted to assess the incidence of blackgram root rot disease in different districts of Tamil Nadu. The incidence of root rot ranged between 18.3 to 70 per cent. The pathogen was isolated and purified. These isolates were maintained in PDA slants and sand maize media for further studies.

The growth of the pathogen in different carbon sources both solid and liquid exhibited significant differences among the treatments. Glucose as carbon source promoted significant mycelia growth and mycelia dry weight of 7.50 cm and 7.20 g respectively followed by maltose which recorded the mycelia growth of 6.60 cm and mycelia dry weight of 6.93 g and the least mycelia growth (4.30 cm) and mycelia dry weight (5.51 g) was observed in lactose. Among the isolates, the maximum mycelia growth (6.60 cm) and mycelia dry weight (6.55 g) was observed in K_2m_7. The least mycelia growth (5.30 cm) and mycelia dry weight (5.87g) were recorded in Km_4 isolate (Table1&2). The monosaccharide glucose followed by maltose appeared to be the most favourable carbon sources, even in their combination in the disaccharide sucrose (Saleh and Garni, 2001) [13]. Pallavi Pal and Purshotam Kaushik (2012) [10] reported that effect of carbon and nitrogen sources on the growth of *Rhizoctonia solani* the fungus showed the maximum growth at yeast extract followed peptone and sodium nitrate.

Among the six different nitrogen sources of both solid and liquid tested, peptone promoted significant mycelia growth (7.47 cm) and mycelia dry weight (7.77 g) followed by ammonium nitrate which recorded the mycelia growth of 7.04 cm and mycelia dry weight of 7.24 g. The minimum mycelia growth (5.71 cm) and mycelia dry weight (5.11 g) was observed in ammonium sulphate supplemented media. The K_2m_7 isolate recorded the maximum mycelia growth (6.84 cm) and mycelia dry weight (6.65 g). The lowest mycelia growth and mycelia dry weight (5.60 cm and 5.35 g) was observed in Km4 (Table 3 and 4). All the carbon sources were found to be more or less equally good while peptone was the best among the nitrogen sources (Khanzada *et al*., 2003) [7].

The experimental results showed that the maximum mycelia growth was observed at pH 7.0 (7.02 cm) followed by pH 6.0 (6.37 cm). The minimum mycelia growth of 3.6 cm was recorded at pH 8.0. Less growth was observed in below pH 5.0 and above pH 7.5 (Table 5). Kulkarni (2000) [8] reported that variation due to change in pH level was evident in *Macrophomina phaseolina* isolates. Highest growth was observed at pH 7.0 closely followed by pH 6.0 indicating preferential range to be between pH 6.0 and 7.0.

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