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Physiological studies on *Ascochyta rabiei* (Pass.) Lab.

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Abstract

The effect of culture media, carbon and nitrogen sources, pH levels and temperature were studied on mycelial growth of *Ascochyta rabiei*. Maximum growth of the fungus was found on chickpea extract agar medium. Glucose was found to be the best source of carbon while potassium nitrate and sodium nitrate were better sources of nitrogen. The most suitable pH level for growth of the fungus was 7.0 and 7.5. Growth of *A. rabiei* was maximum at 25^0 C after 15 days of inoculation while it is not significantly different from that of 20^0C after 21 days of inoculation. The growth was reduced drastically below 10^0C and above 30^0C.

Key words: *Ascochyta rabiei*, culture media, pH, carbon, nitrogen, mycelial growth.

Introduction

Blight caused by *Ascochyta rabiei* (Pass.) Lab. is the most destructive disease of chickpea (*Cicer arietinum* L.) in the parts of Indian sub-continent and the Mediterranean region (Reddy and Singh, 1990). In Pakistan, Blight is the major constraint to chickpea production and it caused about 48% yield reduction during 1978-79 and 1979-80 seasons (Malik and Tufail, 1984). Colonies of the fungus on artificial media are flat, with submerged sparse mycelium, white at first, becoming dark and smoky on oat-meal agar (OMA), while on potato dextrose agar (PDA) at 20-25^{0}C they are creamy to pinkish at first, darkening with time (Nene, 1982; Nene, 1984). Pycnidia are formed within 4-5 days and appear in concentric rings on artificial media such as oatmeal agar (Reddy *et al*., 1992), chickpea seed meal agar (Jan and Wiese, 1991), potato dextrose agar (Porta-Puglia *et al*., 1996) and seed meal dextrose agar (Reddy and Kababeh, 1985). Bedi and Aujla (1970) reported that on OMA medium pycnidia developed best at pH 7.6 to 8.6 at 20^0C. Besides oatmeal agar medium, chickpea seed meal agar medium has been found to be a good medium for the growth and pycnidial production (Kaiser, 1973; Tripathi, 1985). Kaiser (1973) reported that maximum spore production occurred on 8% chickpea seed meal agar (CSMA), while mycelial growth was greatest on CSMA or OMA at 15-20^0C. Khalil and Khan (1986) developed a new medium, which supports better growth and pycnidial production. A number of studies have demonstrated that the optimum temperature for growth, pycnidial production and spore germination is around 20^0C (Bedi and Aujla, 1970; Chauhan and Sinha, 1973; Kaiser, 1973; Maden *et al*., 1975; Zachos *et. al*., 1963). Temperatures below 10^0C and above 35^0C are unfavourable to the fungus (Chauhan and Sinha, 1973; Kaiser, 1973). Maden *et al*. (1975) reported that pycnidia did not form at 4^0C nor at 28^0C and above, and that the colonies were pinkish-brown with zonation and maximum pycnidial formation in near UV light but light pink, fluffy, without zones and pycnidia in darkness. The optimum temperature for growth, pycnidial production and spore germination is 20-22^{0}C, whereas continuous light increases sporulation (Haware, 1998). Preliminary studies on some physiological aspects of this fungus were initiated to understand the requirement of fungus for its mass production.

Material and Methods

Studies of the following physiological aspects of *A. rabiei* were conducted *in vitro*.

1. Effect of culture media

Five culture media viz; Chickpea seed meal extract agar (CSMA) (chickpea seed meal extract 20 g, dextrose 20 g and agar 20 g), Potato dextrose agar (potato starch 20 g, dextrose 20 g and agar 20 g), Cornmeal agar (cornmeal 20 g, dextrose 20 g and agar 20 g), Czapek (Dox) agar (sodium nitrate 2g, potassium nitrate 1g, magnesium sulphate 0.5g, potassium chloride 0.5 g, ferrous sulphate 3 g, sucrose 30 g and agar 20g) and Sabouraud's agar (dextrose 40g, peptone 10g and agar 20) were used to find out the most suitable one for the mycelial growth of the fungus. Each culture medium was prepared in one litre of water and autoclaved at

120^0C at 15 psi for 20 minutes. These were cooled to 40-45^0C and then poured in 90 mm petri dishes for solidification.

2. Effect of different Carbon and Nitrogen Sources

CSMA medium (in one liter of water) was used as the medium for studying the effect of carbon and nitrogen sources.

a) Carbon sources

Three carbon compounds viz; glucose 13.5 g, sucrose 12.5 g and starch 12.5 g were tried individually as supplement of carbon source in CSMA medium.

b) Nitrogen sources

Three nitrogen compounds viz; Potassium nitrates 10 g, Sodium nitrate 8.5 g and Peptone 2.5 g were tried.

3. Effect of different pH levels

The test fungus was inoculated on CSMA medium whose pH was adjusted to 6.0, 6.5, 7.0, 7.5 and 8.0.

4. Effect of Temperature

The fungus *A. rabiei* was inoculated in CSMA medium using five petri dishes for each temperature. Temperatures applied for this experiment were 5, 10, 15, 20, 25, 30 and 35 ^{0}C, respectively.

All these experiments were conducted in five replicates. Plates were inoculated by placing an equal amount of inoculum (4mm plugs cut with the help of cork-borer) in the centre of the petri dishes. Plates were incubated at 22 ± 2^0C (except for the study of temperatures) when observations on radial growth were recorded after 7, 14 and 21 days of inoculation.

Results and Discussion

1. Effect of culture media

Maximum growth (6.5 cm) of the fungus was found on chickpea seed meal agar medium after 21 days of incubation at 22 ± 2^0C. It was followed by Sabouraud's agar (5.75 cm) and Cornmeal agar 5.68 cm (Fig-1). Kaiser (1973) and Tripathi (1985) had also reported that CSMA medium has been found to be a good medium for the growth and pycnidial production of *A. rabiei*. Kaiser (1973) further confirmed that maximum spore production occurred on 8% chickpea seed meal agar (CSMA), while mycelial growth was greatest on CSMA at 15-20^0C.

2. Effect of different carbon and nitrogen sources

The results of this experiment indicated that glucose (5.53 cm) was the best source of carbon (Fig-2) while sucrose and starch did not give better results. The fungus may utilize certain simple forms of C-compounds. It converted the complex carbon compounds into simple form which may be readily metabolized (Bais *et al*., 1970). Chauhan and Sinha (1973) reported that spore germination is improved in the presence of N/50 and N/25 malic acid and carbon food.

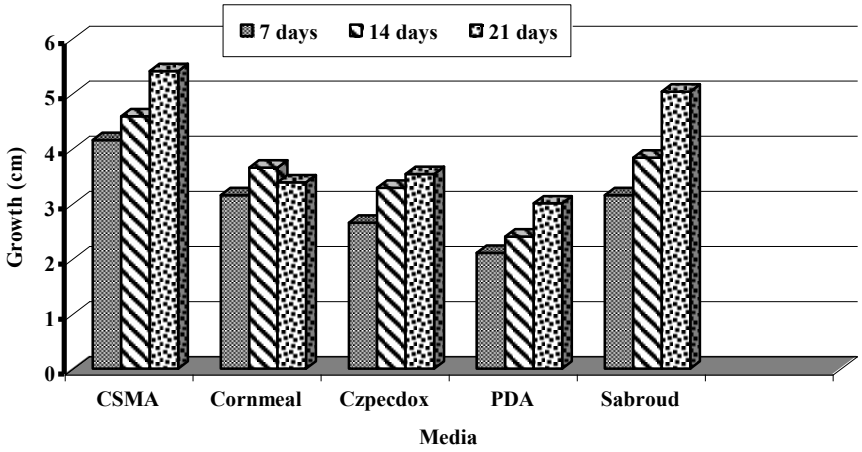

Fig.1: Effect of different culture media on the radial growth of *A. rabiei.*

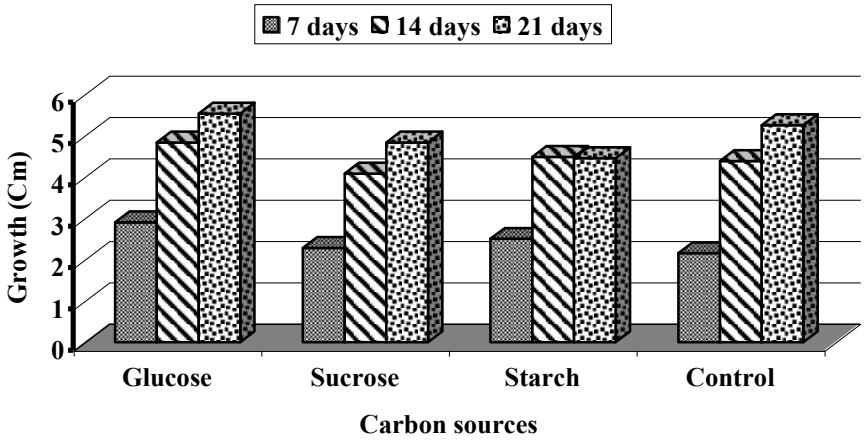

Fig. 2: Effect of carbon sources on the radial growth of *A. rabiei.*

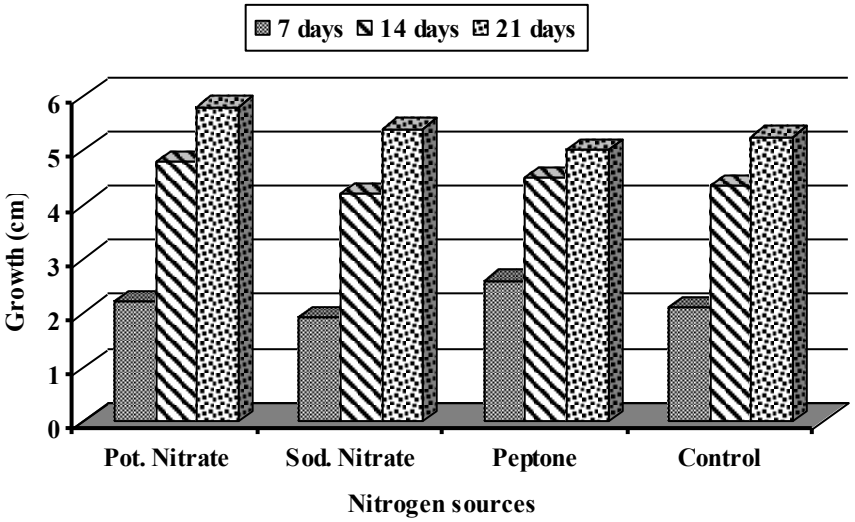

Fig. 3: Effect of nitrogen sources on the radial growth of *A. rabiei*

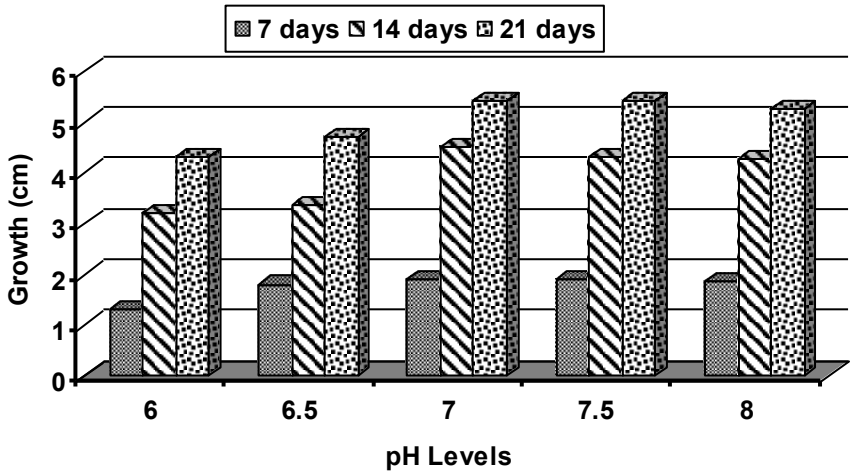

Fig. 4: Effect of different pH levels on the radial growth of *A. rabiei*

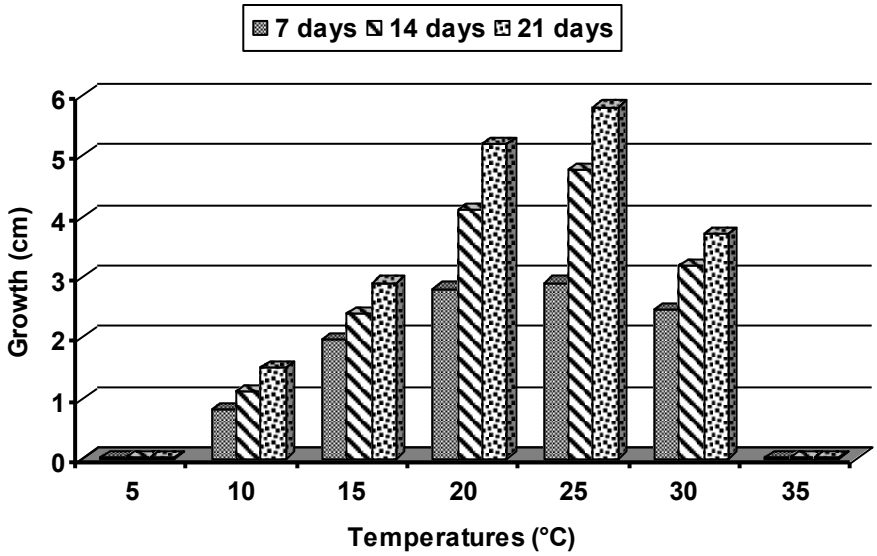

Fig. 5: Effect of temperature on the radial growth of *A. rabiei*

It is evident (Fig-3) that potassium nitrate and Sodium nitrate were found to be better sources of nitrogen for *A. rabiei*. On potassium nitrate (KNO_3), the growth of fungus was 5.8 cm after 21days of inoculation. Similar observations were made by Brock (1951) for the mycelial study of *Morchella esculenta.*

3. Effect of different pH levels

The best pH level for the mycelial growth was 7.0 (Fig-4) where the maximum average growth of 5.35 cm was recorded after 21 days of inoculation followed by 7.5 and 8.0 where the maximum average growth was 5.33 and 5.20 cm, respectively. The results of the present study are in agreement with those achieved by Bedi and Aujla (1970) who reported that best pH range is 7.6 to 8.6 at 20^0C for this fungus.

4. Effect of Temperature

As evident from Fig-5, the fungus was grown at most of the temperatures tried for the radial growth. However, the growth of fungus was drastically reduced below 10^0C and above 30^0C, as these temperatures did not favour the growth of the fungus. It was observed that at 25^0C, the fungus had maximum diameter (5.69 cm) while at 20^0 C, it was 5.13 cm after 21 days of inoculation. There was no significant difference on the growth at the temperatures of 20^0C and 25^0C. On the contrary, minimum growth of the fungus was 1.43 cm at 10^0C and 3.65 cm at 30^0C. There was no growth of the fungus at 0^0C and at 35^0C. Similar observations had been reported that temperatures below 10^0C and above 35^0C are unfavourable to the fungus (Chauhan and Sinha, 1973; Kaiser, 1973). Maden *et al*. (1975) reported that pycnidia did not form at 4^0C nor at 28^0C and above.

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