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Full Length Research Paper

Physiological and nutrition requirements for the determination of *Alternaria helianthi* in sunflower

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Ten isolates of *Alternaria helianthi* were collected from different sunflower growing areas of Tamil Nadu. Most isolates preferred pH levels of 6.96-7.15, temperature 29.22-33.93°C and incubation time of 24 h. The nutritional studies were taken up to know the best source of carbon and nitrogen required for the growth and sporulation of the fungus. Six different carbon sources tried, Glucose was found to be the best source of carbon for the growth and the seven different nitrogen sources tested, ammonium nitrate supported good growth and sporulation.

Key words: Sunflower, *Alternaria helianthi*, temperature, pH, incubation period, carbon and nitrogen sources.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops in the world and it ranks third in area after soybean and groundnut. Cultivated sunflower, a member of the family *Asteraceae* (Compositae) is believed to have been first domesticated in the central part of the United States. Presently, in India sunflower is cultivated over an area of 21.62 lakh ha with a production of 12.24 lakh tonnes (Anonymous, 2006). The major sunflower growing states in the country are Tamil Nadu, Karnataka, Maharashtra, and Andhra Pradesh. Among these, Karnataka occupies first position accounting 53% of total area and 35% of total production of India (Shankergoud et al*.,* 2006).

Among the several biotic stresses for successful sunflower production, susceptibility to the diseases is one of the major constraints. Gulya and Masirevic (1991) listed 80 pathogens occurring on sunflower. In Tamil nadu, the major diseases of sunflower are *viz.,* necrosis virus disease, Alternaria blight, rust, collar rot and downy mildew. Among these *Alternaria* blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara have been considered as a potentially destructive disease in many parts of the sunflower growing countries (Allen et al*.,* 1983a, b, c; Morris et al., 1983; Lipps and Herr, 1986 and Anon, 2004).

The metabolic and catabolic activity of an organism varies depending upon the H ion concentration existing in the surrounding environment. Hence, pH plays vital role in deciding the nature and activities of microorganisms. *A. helianthi* exhibited the maximum mycelial growth at pH 6-7. Temperature affects the physiological function of the fungi, which in turn affect the phenotypic expression. For each fungus, there is a particular temperature below which it will not grow. Likewise, there is a particular temperature above which the growth ceases. A temperature of 25°C was reported to be the optimum for

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A. helianthi is 29.22-33.93°C. Chaturvedi, (1966) reported that *Alternaria alternata* utilized fructose, lactose, maltose and arabinose effectively. Rane and Patel (1956) found ammonium nitrate, potassium nitrate, sodium nitrate and peptone as the best nitrogen sources for the growth of *A. macrospora*.

MATERIALS AND METHODS

Physiological characters

Growth of A. helianthi isolates on different pH

The effect of pH on the growth of the pathogen was studied. The method followed by (Kiryu, 1939) using PDA. The pH of the medium was adjusted to get different pH levels *viz* 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 using N/10 hydrochloric acid or N/10 sodium hydroxide and the medium is transferred to sterile Petri dishes and these dishes were inoculated with 10mm cultural disc of the respective isolates. The plates were incubated at the room temperature (28 ± 2°C) for 10 days. The mycelial growth of the fungal colonies were determined 10 days after inoculation, replications were maintained for each treatment.

Effect of different temperature on A. helianthi

Conidial germination of *A. helianthi* was studied at 7, 14, 20, 21, 35 and 42°C. A drop of conidial suspension was placed in cavity slide with water and thoroughly mixed. The cavity slides were then placed in Petri dishes lined with moist blotter paper. All the Petri dishes were incubated at the specified temperature in the incubator (Borkar, 1995). Three replication was maintained for each treatment. The spore germination was calculated after 24 h.

Effect of different incubation period on A. helianthi

Conidial germination of *A. helianthi* was studied at different hours *viz.,* 2, 4, 6, 8, 16, 24 h. A drop of conidial suspension is thoroughly mixed. The cavity slides were then placed in Petri dishes lined with moist blotter paper. All the Petri dishes were incubated at the specified hours in the incubator. Three replication were maintained for each treatment.

Effect of carbon sources on the growth of A. helianthi

The Richard's agar medium was substituted with different carbon sources *viz*., Carboxy methyl cellulose, Glucose, Fructose, Manitol, Sucrose, and Starch. The sterilized warm medium was poured in sterilized Petri dishes and allowed to solidify and inoculated with five-days-old 10 mm culture disc of the pathogen. Then these plates were incubated at room temperature (28 ± 2°C) for 10 days. The treatments were replicated three times and the diameter of mycelial growth was recorded in each treatment (Bias et al*.,* 1970).

To study the effect of carbon source in the liquid medium Richards medium without agar and was prepared the sucrose substituted with the various carbon sources. The fungal disc (10 mm diameter) transferred from 15 days old PDA culture at the rate of one into each 250 ml in sterilized liquid medium in the conical flask. Suitable controls were maintained without carbon source and incubated at room temperature (28 $\pm$ 2°C). After 15 days of incubation, mycelial mats were collected and dried in hot air oven at 60°C for 24 h and dry weight of mycelia mats was taken.

Effect of nitrogen sources on the growth of A. helianthi

The Richard's agar medium was substituted with different nitrogen sources *viz.,* Ammonium nitrate, Ammonium molybdate, Ammonium oxalate, Ammonium sulphate, Urea, Thiourea, sodium nitrate, and Potassium nitrate, all the nitrogen sources served as control. The sterilized warm medium was poured in sterilized Pertri dishes and allowed to solidify and to this five-days-old 10 mm culture disc of the pathogen was inoculated. Then these plates were incubated at room temperature (28 $\pm$ 2°C) for 10 days. The diameter of mycelial growth was recorded. Three replications were maintained in each treatment (Bias et al*.,* 1970).

To study the effect of nitrogen source in the liquid medium, Richard's medium without agar was substituted with the various nitrogen sources used. The fungal disc (10 mm diameter) transferred from 15 day old PDA culture at the rate of one into each 250 ml in sterilized liquid medium in the conical flask suitable controls were maintained without carbon source and incubated at room temperature (28 ± 2°C). After 15 days of incubation, mycelial mats were collected and dried in hot air oven at 60°C for 24 h and dry weight of mycelia mats was taken.

RESULTS AND DISCUSSION

Growth of *A. helianthi* isolates on different incubation period

Among the six incubation periods tested, the present study revealed that conidial germination of *A. helianthi* and it was maximum (38.62%) in 24 h incubation period

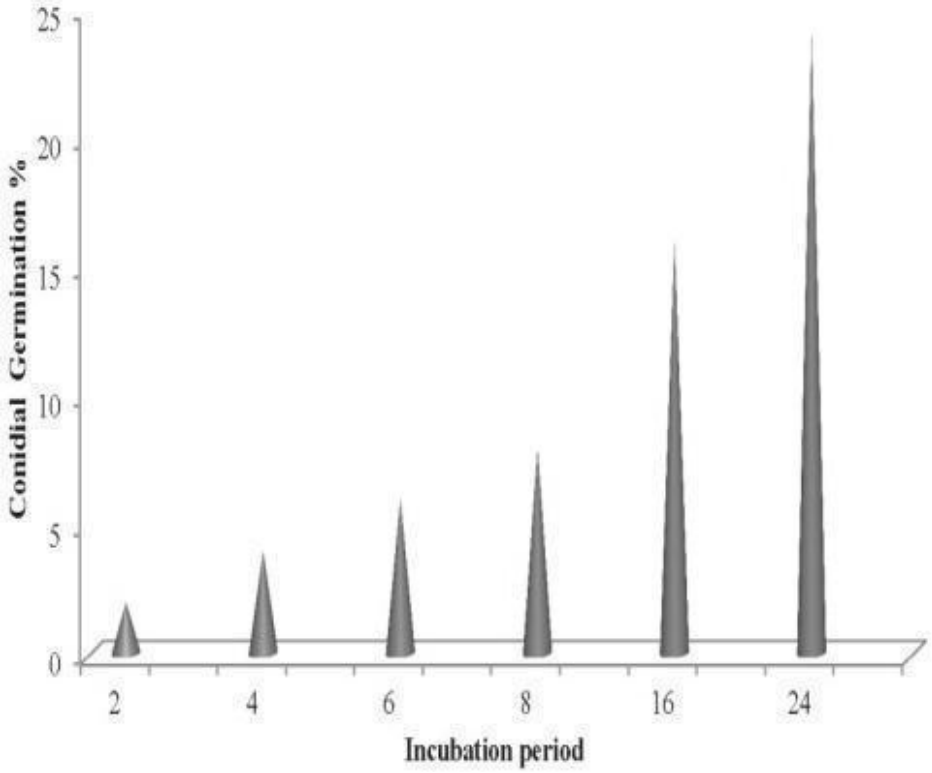

Figure 1. Growth of *A. helianthi* isolates on different period.

followed by 16 h (29.21%) (Figure 1). Eyal et al. (1977) found that the area of necrosis caused by *Septoria* state of *Leptosphaeria nodorum* Muller on Wheat was markedly affected by length of the post inoculation. Allen et al., (1983) found that 12 hrs period of leaf wetness was required to give maximum infection and repeated periods of dew and high relative humidity promoted the expansion of lesions. The conidial germination was observed and recorded after 24 h germination was calculated (Table 6).

Growth of *A. helianthi* isolates on different temperature

Temperature is most important physical environmental factor for regulating growth and reproduction of fungi (Reference). The present study found that optimum temperature for conidial germination of *A. helianthi* appears to be 20°C with 33.93% germination (Figure 2). Similar observation also made by (Alien et al., 1983).The fungus grew well from 18 to 30°C but growth was more rapid at 28 and 30°C. Infectivity increases as conidia production temperature increased. Infectivity was greatest when conidia have thick cell walls, high percent germination, and high number of germ tubes (Reddy and Gupta, 1981).

Growth of *A. helianthi* isolates on different carbon sources

The aggressive isolate I3 (*A. helianthi*) recorded the maximum mycelial growth in all the carbon and nitrogen sources indicating the wide adaptability (Figure 3).

Among the carbon sources isolate I3 registered the maximum mycelial growth and mycelial dry weight in glucose amended medium (6.56 cm, 2.12 g) followed by fructose (5.40 cm, 1.50 g) *A. helianthi* being the foliar pathogen preferred the glucose for growth similar observation were made earlier by Bias et al*.,* (1970). The fungus may utilize certain complex form of carbon compound in to simple form as reported by Bias et al., (1970). Hence increase or decrease in sugar content in plant tissues may affect the disease development.

Growth of *A. helianthi* isolates on nitrogen sources

In case of nitrogen source result revealed that Potassium nitrate favoured maximum mycelial growth and mycelial dry weight of *A. helianthi* (7.51 cm and 3.47 g) for the isolate I3 (Figure 4). Ammonia is known to leave the cells by passive diffusion as undissociated ammonia molecules. Rapid fall in pH due to assimilation of

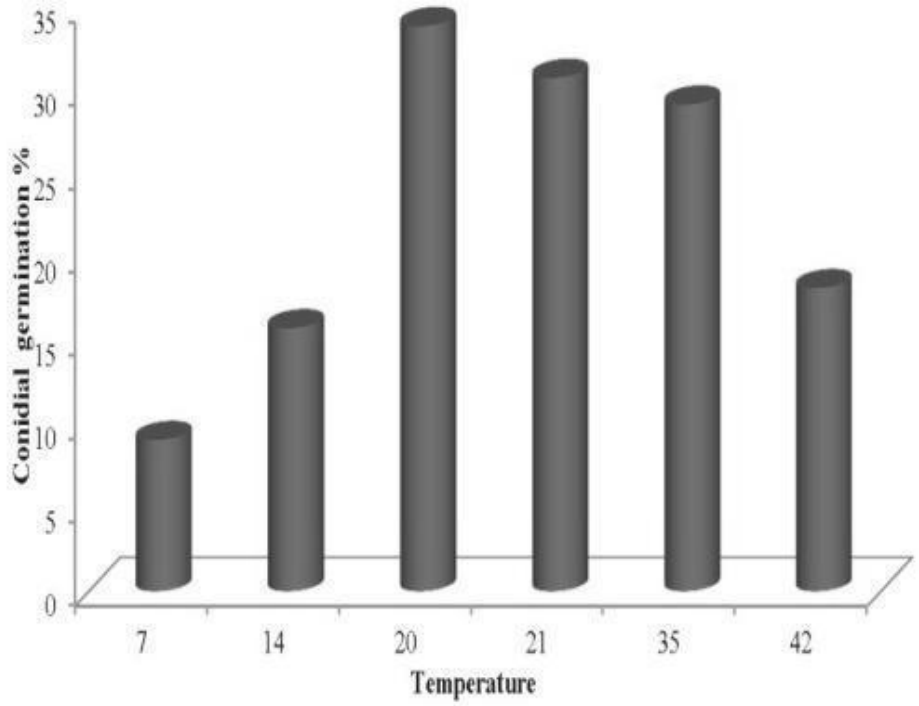

Figure 2. Growth of *A. helianthi* isolates on different temperature.

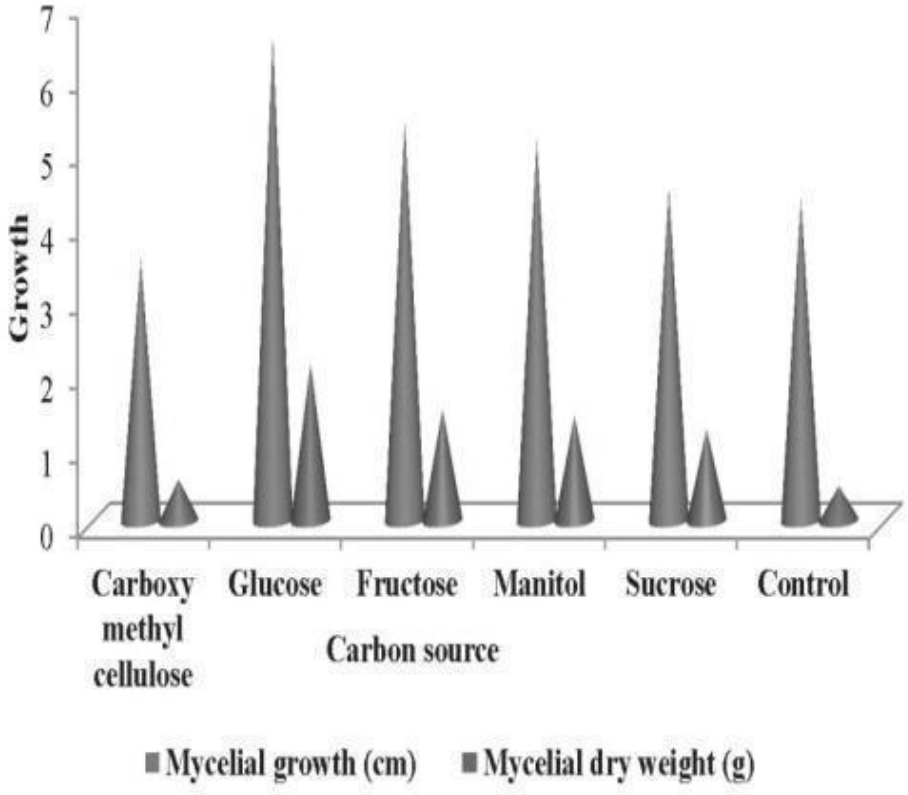

Figure 3. Effect of different carbon sources on growth of *A. helianthi in vitro*.

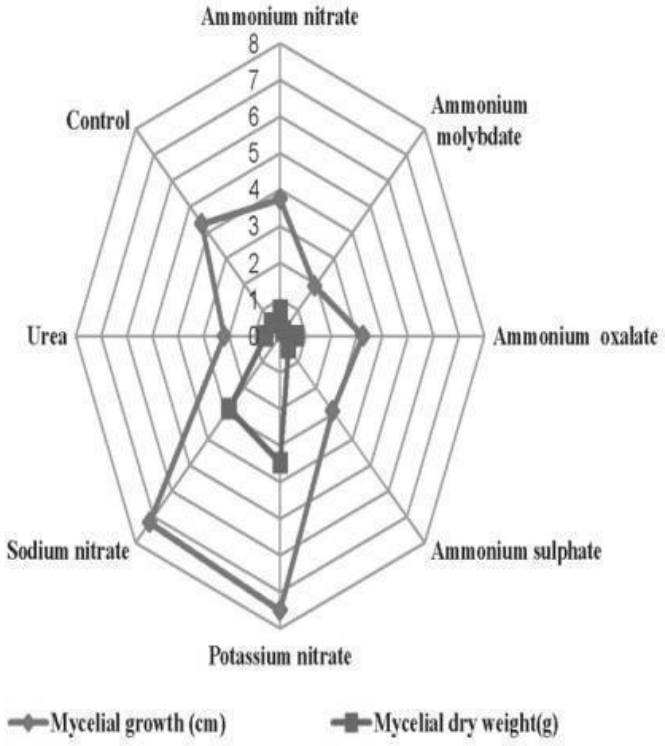

Figure 4. Effect of different nitrogen sources on growth of *A. helianthi* under *in vitro*.

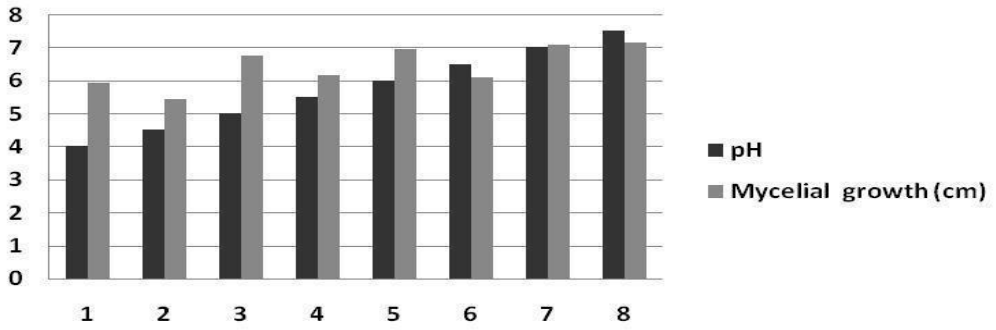

Figure 5. Growth of *A. helianthi* on different pH.

ammoniacal nitrogen has been observed in several fungi this result coincides with Cochrane (1958). The maximum mycelial growth was recorded in Potassium nitrate amended medium followed by Sodium nitrate, Ammonium nitrate and Thiourea.

Growth of *A. helianthi* isolates on different pH

The metabolic and catabolic activity of an organism varies depending upon the H ion concentration existing in the surrounding environment. Hence, pH plays vital role in deciding the nature and activities of microorganisms**.** All the isolates were recorded maximum mycelial growth at the pH 6.0 followed by at pH 7.5 (Figure 5). The aggressive isolate I3 of *A. helianthi* recorded the maximum mean mycelial growth of 7.15 cm at pH 7.5 indicating its adaptability to wide range of pH levels. Scientific work done by Prathebha et al. (2008) pointed out that the fungus grew well over a wide range of pH 6

and 7. *A. helianthi* could grow and sporulate over a wide range of pH from 4.5 to 10.0 with maximum at neutral pH and steep fall after wards reported by Narasimha and Rajagopalan (1978).

Conclusion

Isolates of *A. helianthi* were studied under varied temperature, incubation periods and pH levels along with the utilization of different carbon and nitrogen sources. In this study, it has been revealed that Potato dextrose agar media supported the fungal growth at higher range when compared to other media used. Most of the isolates preferred temperature range of 18 to 30°C and pH 5.0 for the growth and sporulation of *A. helianthi*. The conidial germination was maximum of 38.62% in 24 h incubation period. Considering the nutritional parameters, Glucose was found to be the best source of carbon for the growth and sporulation of this pathogen. In case of nitrogen sources, ammonium nitrate supported the maximum growth of the pathogen.

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