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IN VITRO GROWTH OF *ALTERNARIA PORRI*, THE CAUSAL AGENT OF PURPLE BLOTCH OF ONION

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ABSTRACT

Effect of nutrient media, temperature, hydrogen ion concentration and photoperiod were studied under *in vitro* conditions to record the maximum colony growth and spore count. Maximum growth was recorded on 28°C on PDA media having pH 6.5 with 16/8 light and dark period. The maximum growth on PDA was 87 mm and spore count was 4.36×10^6 spores/mL while the lowest growth was observed in MEA media which was 47mm and spore was counted as 1.48×10^6 spores/mL. The present studies were found useful to prepare mass inoculum and also by maintain physiological factors attack of *Alternaria porri* can be decreased in controlled environment.

Key words: *Alternaria porri*, Temperature, pH, PDA, MEA

Introduction

Role of weather shift or climate change in determining production, distribution and pathogenicity has been described by many workers. These workers studied the impact of environmental gradients temperature and humidity individually and in wide range of combinations with carbon sources. Strong evidence for dependence of microbes with drought and site specific ecology plays major role in determining fungal physiology expressions on the production and distribution diversity of fungal communities (Everts and Lacy 1990; Simons 2007; Primicia *et al*,. 2016). However, lack of understanding prevails on impact of climate change and variability on fungal phenology, Variability in modifications for trait selection, including spore size and dispersal characteristics. Compositional and trait modifications of fungal communities would have important consequences for fungal biogeography, interactions with plant communities, and ecosystem functions (Andrew *et al.*, 2016).

There had been a long debate on taxonomic description of *A. porri* and it has a wide range of plant host for its survival. Most of them belonged to Allium family i.e. Leek (*Allium*) (purple blotch, Koike and Henderson, 1998), Velvet Bean (*Mucuna pruriens*) (Ye *et al.*, 2013).It is saprophytic in nature and shows sensitivity towards incubation conditions, climatic and nutrient factor for its growth and sporulation. The present studies are designed to understand best suited conditions for growth and multiplication of *Alternaria porri* in vitro conditions.

MATERIALS AND METHODS

Nutrient media

To evaluate the nutrients supplies to the fungal pathogen, different culture media i.e. OLEA (Onion leaf extract agar), PDA (Potato dextrose agar), MEA (Malt extract agar), V8 (v8 juice agar), fresh CDA (carrot dextrose agar) and fresh COLEA (Carrot onion leaf extract agar medium)were prepared by household juicer blender, poured and inoculated with *A. porri* under aseptic conditions. Petri plates with test pathogen were incubated at 26 $\pm$ 2°C for7 days. Fungal mycelial growth was measured after 48 hours till the achievement of maximum growth. Colony growth in terms of mycelial growth and number of milliliter was calculated (Stępalska and Wołek, 2009)

Temperature

Temperature of *A. porri* was optimized by plating 5mm fungal plug on PDA (selected nutrient medium) in aseptic environment. Plates were incubated at 16°C, 19°C, 22°C, 25°C, 28°C, 31°C and 34°C for 7 days. Fungus response was calculated by calculating the colonial radial growth of the pathogen and number of spore /mL count.

pH (Hydrogen ion concentration)

pH Optimization for the maximum fungal growth of *A. porri* was studied on most suitable medium (PDA) and incubated at suitable temperature (26 $\pm$ 2° C).Optimization of pH level of nutrient medium was maintained at 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 by adding few drops of concentrated HCl. Petri plates were replicated thrice. Data was recorded as maximum fungal growth and number of spores.

Photoperiod

Fungal mycelial growth of *A. porri* was checked under different light regimes. Four light regimes 24h dark, 24h fluorescent light, 12 h light, 12 h dark and 16 h dark8h light incubation was used at suitable medium and incubated at suitable temperature. Data regarding maximum fungal growth and spore/mL was recorded.

All experimental data were statistically analyzed by using Statistix 8.1 software. One-way analysis of variance was performed by using completely randomized block design (CRD) and means of treatments were compared through least significance difference LSD at p = 0.05 level.

RESULTS

Synthetic growth medium

Colony growth of *Alternaria porri* measured on 90 mm diameter Perti dishes revealed that highest growth of 87 mm was observed on PDA medium, whereas Onion leaf extract agar medium, Carrot onion leaf extract agar medium, V8 juice agar medium, Carrot dextrose agar medium and Czepek mediums had 70.56, 76.16, 63.84, 78.86 and 55.97 mm colonial diameter respectively. Minimum radial growth (47.3mm) was recorded in malt extract agar medium. It was interested to note that spore count was not in line with radial growth development. Maximum spore count4.36×10^6 spores/mL recorded on PDA medium while least number of spore (1.48×10^6 spores/mL) was observed on MEA growth medium (Fig. 1).

Optimization of pH

Seven different pH levels viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were used on PDA medium and incubated at 28°C for suitable mycelial growth of *A. porri*. Declining radial growth and spore production was noticed with the increase of pH. Maximum fresh biomass was 502 mg at pH 6.0 and least was 152 mg at pH8.0. whereas5.0, 5.5, 6.0, 6.5 and 7.0 pH exhibited intermediate level of biomass production with4.96, 3.98, 4.39, 3.02, 3.56 and 2.96 mg biomass was recorded, respectively. Highest spore production (4.96×10^6 spore/mL) was verified at pH 6.0 however least (1.06×10^6 spores/mL) production of spore was at pH 8.0 (Fig. 2).

Temperature optimization

Optimization of temperature studies were conducted on potato dextrose agar medium which showed best result among all tested media. At 28^{o}C maximum colony growth was86.1mm and sporulation was (5.15×10^6 spores/mL) on PDA medium. Colony growth was 0,59.3, 63.3, 74.5, 56.1, 46.1 and 1.81mm at 16,19, 22, 25, 31and 34°C, respectivelywhile at 34°C least colony growth 56.7 mm and lowest spore production (1.81×10^6spores/mL) was noted(Fig 3).

Photoperiod optimization

Three different Photoperiod (12h light, 12h dark and 16h alternate light and dark) were maintained at 28^0C. Maximum fungal growth and spore production was 52 mm and (3.9×10^6 spores/mL) at 16/8 alternate light and dark period respectively while least mycelial growth and spore production was 40mm and 2.12×10^6 spores/mL was noted at 12h dark period (Fig.4).

DISCUSSION

Monowara *et al.* (2017) claimed that variability studied in factors influencing sporulation and colony growth may lead to underst and host-pathogen interaction. For the onset of disease severity temperature, pH, mycelial growth, conidial formation and colonial development pattern plays an important role (Brzonkalik*et al.,* 2012;Chohan *et al.,* 2015). Considering the abovementioned facts, studies were designed to investigate various physiological factors which supports morphology, pathogenicity and other biological features of *A porri*.

Nutrient availability and temperature had major effect on the growth and conidial size of *A. porri*. Among the growth media, PDA proved to be the best followed by V8 medium. The difference in fungal colony diameter was significant at $P<0.05$. Maximum colonial diameter of tested fungus was 79.6mm on PDA. Similar results were obtained by Kumar *et al*. (2012) and (Yadav *et al.*, 2017) also agreed to this statement. In current studies it was noted that MEA showed less support towards colony growth as the lowest colony development with least number of conidia (1.52×10^6 spores/mL) were recorded in this medium. Nolla (1927) have opined this medium may contain some organic compounds which other media may be lacking and influences the colonial growth and sporulation of *A. porri.*

Temperature is one of the important physical factors that triggers the growth and sporulation of *A. porri*. Maximum colonial growth 86mm was recorded at 28°C. These results are verified with the studies of Ramjegathesh and Ebenezar (2012). In 2000 Chethana stated that, most of the fungi can grow between 25°C to 30°C. Minimum colonial growth 57 mm and 1.81×10^8 spores/mL was recorded at 34°C. Pradnyarani (2015) also studied the similar temperature range and concluded least colonial growth of *A. porri* induced at 35°C temperature.

Hydrogen ion concentration (pH) of the growth medium had a very significant effect on fungal growth and many other life processes (Abubakar *et al.,* 2013). Lilly and Barnett, 1951 suggested that maximum fungal growth can be observed in broth adjusted to pH 6 and 7 and further described importance of hydrogen ion concentration for the better fungal growth. In the present studies pH scale (5.5, 6, 6.5, 7, 7.5 and 8) were investigated in optimization process. However high pH did not support the colony growth and decreasing trend in radial growth and spore production was observed with Increase in pH level. At very low pH (acidic) fungus did not grow well. The highest fresh weight of biomass 9.0 mg was observed at pH 6.5 and least (5mg) at pH8.0.

While pH 6.0 supported highest spore production (4.98×10^6 spores/mL), however minimum spore production (1.06×10^6 spores/mL) was recorded at pH 8.0. pH level 6.0 for optimum fungal growth and sporulation was supported by Madhavi *et al.*(2012). Light is also one of the important physical factors which affect fungal development including the formation of reproductive structures (Fuller *et al.,* 2013; Fanelli *et al*., 2012). Optimization studies of Photoperiod conducted at 12h light, 12h dark and 16/8h alternate light and dark. Tested isolates respond differently towards applied light regimes and among studied light regime highest mycelial growth (56 mm) and spore production (4.05×10^6 spores/mL) was observed at 16/8 h alternate light and dark period whereas least was observed at 12h light.

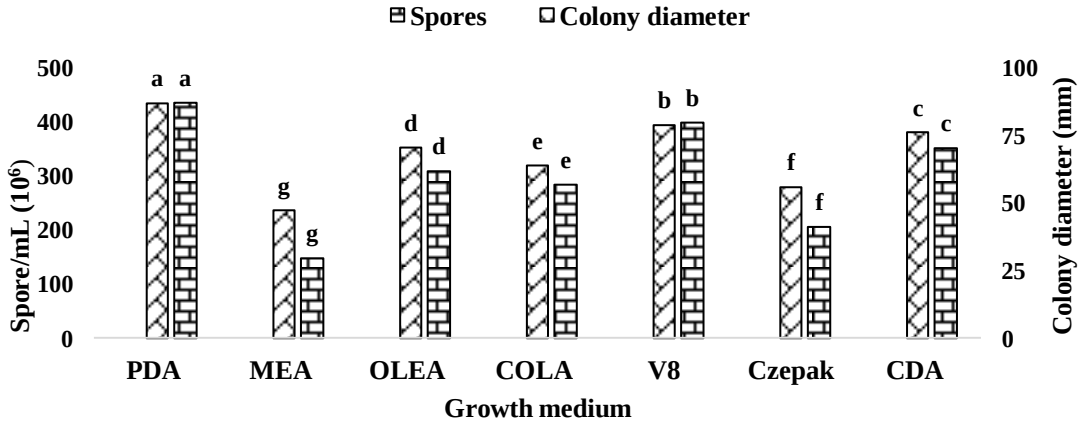

Fig1. Effect of nutrient medium on colonial growth and spore count of *Alternaria porri.*
PDA= Potato dextrose agar; MEA=Malt extract agar; OLEA= Onion leaf extract agar; COLA= Carrot onion leaf extract agar; V8= V8 juice agar; CDA= Carrot dextrose agar

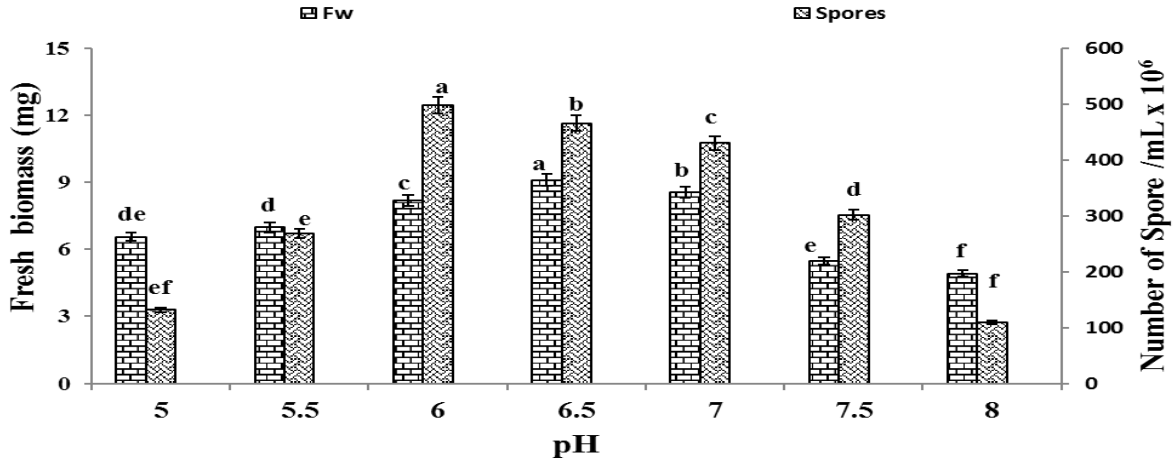

Fig. 2. Effect of pH on fresh biomass and spore count of *Alternaria porri.*

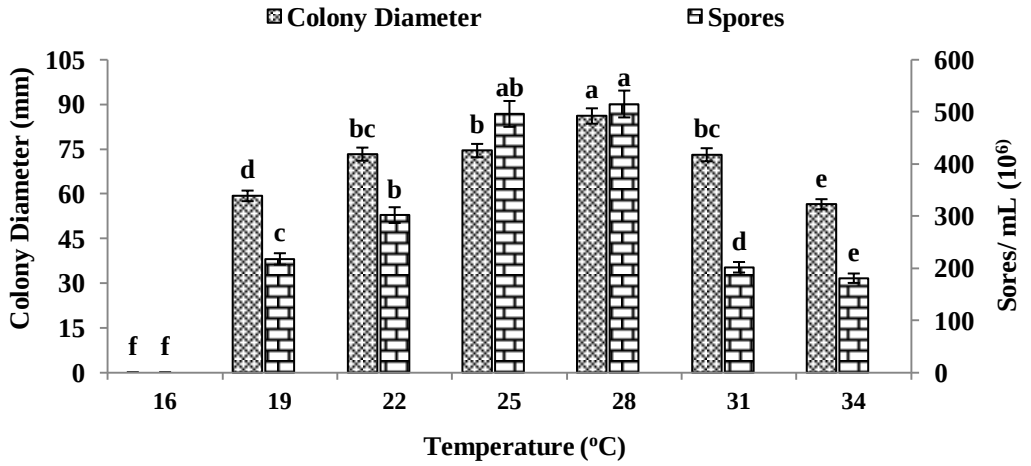

Fig.3. Effect of various temperatures on colonial growth and spore count of *Alternaria porri.*

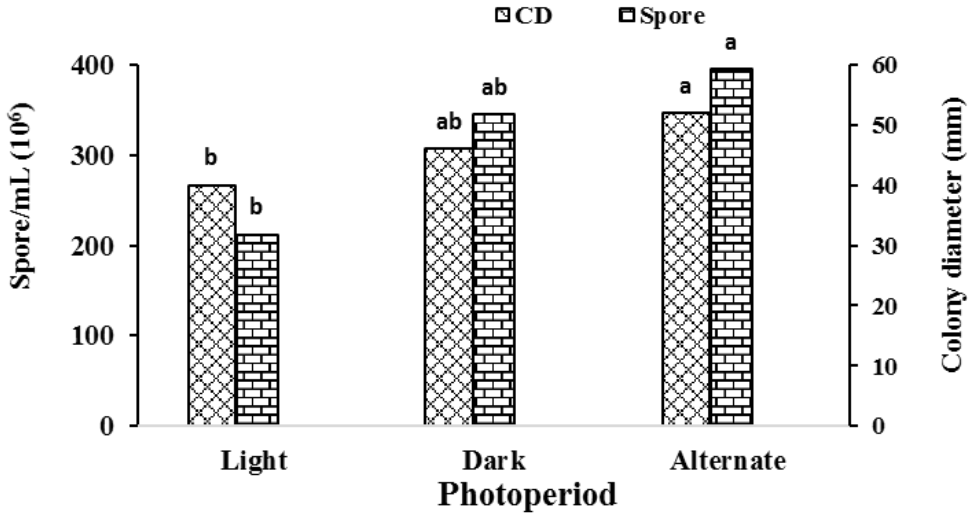

Fig. 4. Effect of photoperiod on colony diameter and spore count of *Alternaria porri.*

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