Plant Archives Vol. 19, Supplement 2, 2019 pp. 817-819 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF NUTRITIONAL FACTORS ON THE MYCELIAL GROWTH AND BIOMASS PRODUCTION OF *PYTHIUM APHANIDERMATUM* (EDSON) FITZP. CAUSING TOMATO DAMPING OFF

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Abstract

The disease is both soil borne and seed borne and is world wide in distribution. Exploratory studies were made on culture media and various nutrient sources on the mycelial growth of *Pythium aphanidermatum***.** Among the different media tested, Potato dextrose agar medium supported maximum mycelia growth and biomass production of *Pythium* spp. (87.82 mm and 508.95 mg). Among these, isolates I_4 significantly recorded maximum mycelial growth in all medium which recorded mean mycelial growth and mycelia dry weight of *P. aphanidrermatum* (72.33 mm and 358.06 mg). The growth of the pathogen in different carbon sources amended solid and liquid media, exhibited significant differences between the treatments. Of these, glucose as carbon source promoted maximum mycelial growth of 89.80 mm and mycelia dry weight of 620.57 mg in liquid media. With regard to the different nitrogen sources tested both in solid and liquid media, Sodium nitrate promoted maximum mycelial growth (90.00 mm) and mycelia dry weight (610.54 mg) followed by ammonium nitrate which recorded the mycelia growth of 87.25 mm and mycelia dry weight of 607.00 mg. Hence, each and every pathogen may require different nutritional sources for their growth and development.

***Keywords*:** Tomato, *Pythium*, culture media, Carbon sources, Nitrogen sources

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops in the world. It is one of the important food and cash crops for many low-income farmers in the tropical countries. (Prior *et al.*, 1994).Tomato is a solanaceae family and belongs to the genus *Lycopersicon*, grown for its edible fruits (Jones, 1999). The ripened fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food (Dias, 2012). In India, the tomato-growing in an area of approximately 894 ha of the total vegetable-growing area with total production accounting for approximately 19167 of total vegetable production (Anonymous, 2017).

Among the pathogens that affect the tomato crop, soil-borne fungal pathogens, including genus belonged to *Pythium, Sclerotium*, *Fusarium*, *Rhizoctonia,* and *Verticillium* genera causing the root rot or damping- off and wilt which affect the quality with yield reduction (Mandal *et al.,* 2017). Stanghellini (1974) found that worldwide distribution of *Pythium* spp. cause damping-off of many plants. Martin and Loper (1999) reported that *Pythium* species are ubiquitous soil-borne oomycetes that rank from opportunistic up to highly virulent pathogens on many plant species. Elshahawy *et al.* (2018) Symptoms observed on affected tomato plants developed symptoms of wilting, dead plant, root rot with crown and stem rot on above ground plant parts.

They mainly infect young plant tissues and cause pre- and post-emergence damping off or reduce the vigor and growth of surviving seedlings. Besides, they infect mature plant roots, resulting in severe necrosis and stunting Numerous media have been developed for selective isolation *Pythium* spp. from soil Rao *et al.* (1978). Khalil (2002) observed that faster utilisation of sole carbon sources by the resident root microflora has also been indicated using Biolog GN2 panels after exposure of tomato roots to the fungal root pathogen *P. ultimum.* This research was undertaken to determine the activity could be incorporated in to a selective media, carbon and nitrogen sources for isolated species of *P. aphanidermatum* in tomato.

Material and Methods

Isolation, Purification and Identification of the Pathogen

Naturally infected tomato seedlings showing typical symptoms of damping off were collected from tomato growers of around Tamil Nadu for isolation and further investigations. The infected areas of seedlings were thoroughly washed in running water to remove the adhering soil. The small pieces of infected seedlings were surface sterilized by dipping in 0.1% $HgCl_2$ for 1-2 minutes followed by two washings in sterilized water and finally were transferred aseptically on Potato Dextrose Agar (PDA) medium in Petri plates. These plates were then incubated at 28±2°C in an incubator for 3 days. The emerging fungal growth was transferred on fresh medium of potato dextrose agar in Petri plates. Identification of the pathogen was done by comparing the morphological characters (Elshahawy *et al.,* 2018).

Growth characters of *P. aphanidermatum* (I_4) isolates on different solid media

In order to compare the growth of *P.aphanidermatum* on different solid media and liquid broth viz., Potato dextrose, Czapek dox, Carrot dextrose, Beet root agar, Peas, Beans and host extract. The prepared solid medium, 15 ml of sterilized medium was poured in sterile Petri dish (10 cm) and allowed to solidify. The pathogen was inoculated at the centre of the plate by placing a seven days old nine mm culture disc of the pathogen. The plates were incubated at room temperature (28 ± 2°C) and three replications were maintained. The radial growths of the mycelia were measured seven days after inoculation. The prepared broth, 100 ml was transferred to 250 ml Erlenmeyer flask and

autoclaved at 20 lb for 15 min, and cooled. The flasks were separately inoculated with seven days old nine mm culture disc of the pathogen. Seven days after inoculation, the mycelia mat was filtered through a pre weighed whatman No. 1 filter paper, dried in hot air oven at 100°C until constant weight was obtained. The mycelia dry weight was obtained by subtracting the weight of the filter paper.

Utilization of different carbon and nitrogen sources on the growth of *P. aphanidermatum* (I_4) in solid media

The Czepek's Dox agar medium was substituted with different carbon sources *viz*. glucose, maltose, sucrose, fructose, dextrose, lactose, manitol and different nitrogen sources *viz*., ammonium nitrate, ammonium oxalate, ammonium sulphate, urea, sodium nitrate, sodium nitrite and peptone separately. 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. Three replications were maintained. The plates were incubated at room temperature (28 ± 2°C) for 4 days. The diameter of mycelial growth was recorded.

Utilization of different carbon and nitrogen sources on the growth of *P. aphanidermatum* in liquid broth

The Richard's broth was substituted with different carbon sources *viz*, glucose, maltose, sucrose, fructose, dextrose, lactose, manitol and different nitrogen sources *viz.,* ammonium nitrate, ammonium oxalate, ammonium sulphate, urea, sodium nitrate, sodium nitrite and peptone separately. 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. Three replications were maintained. The flasks were incubated at the room temperature (28±2°C) for 4 days. The mycelial dry weight was recorded as in previous case.

Statistical Analysis

The data were statistically analyzed using the Wasp version 2.0 developed by the Indian Council of Agricultural Research, Goa (Gomez and Gomez, 1984) Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$) and means were compared by Duncan's Multiple Range Test (DMRT). Laboratory experiments were laid out in Randomized Block Design (RBD).

Results and Discussion

Growth of *P. aphanidermatum* on different solid and liquid media

The experiment on growth of *P. aphanidermatum* on different solid and liquid media revealed that, the isolates displayed variations of growth in different solid media. Among the seven solid media, Potato dextrose agar medium was significantly on par and recorded the maximum mycelial growth and biomass production of 87.82 mm and 508.95 mg respectively followed by Carrot dextrose agar (86.65 mm and 426.48 mg) and Czepek' Dox agar (83.58 mm and 380.78 mg) respectively was statistically on par while host extract agar recorded minimum growth of 38.08 mm and 196.73 mg (Table 1). Among these, isolates I_4 significantly recorded maximum mycelial growth in all medium which recorded mean mycelial growth and mycelia dry weight 72.33 mm and 358.06 mg followed by I_7 70.04 mm and 346.78 mg respectively. The minimum mycelial growth was observed in I_6 (65.24 mm and 318.66 mg) (Table 2). Sharma and Pandey (2010) reported that ten dormant oomycetes fungi isolated from decaying vegetables wastes observed from the influence of three different culture media on the mycelial growth, colony characters and sporulation patterns. Jukte *et al.* (2016) similar to *P. aphanidermatum* was re-isolated on PDA from artificially diseased seedlings of Parbhani kranti cultivar of okra. The cultural and morphological characteristics observed were found exactly identical to those of the original fungus isolated from naturally diseased okra plants. Ibrahimet and Riad (2018) observed that in tomato causing *P. aphanidermatum* grown in potato dextrose agar medium. Alhussaen (2019) similar finding the morphology characterization of the selective four isolates of *P. aphanidermatum* indicated that growth on the PDA produced thick white cottony mycelia growth with fluffy topography.

Growth of P. *aphanidermatum* in different carbon sources

The growth of the pathogen in different carbon sources amended solid and liquid media, exhibited significant differences between the treatments. Sucrose as carbon source promoted significant mycelial growth of 89.80[a] mm and mycelia dry weight of 620.57 mg in liquid media followed by Dextrose which recorded mycelial growth of 88.78 mm and mycelia dry weight of 617.85 mg. The least mycelial growth of 64.67mm and mycelia dry weight of 385.67 mg was observed in Manitol (Table 3). Khalil and Alsanius (2009) findings that carbohydrates mentioned as growth stimulators of *P. aphanidermatum.* Accordingly, Van Buyten and Höfte (2013) similar finding these *in vitro* experiments the carbon sources may be used as outer and inner root colonization processes of each isolates viz., *P. arrhenomanes*, *P. graminicola* and/or *P. inflatum*. Muthukumar *et al.* (2019) similar finding that tested nine different carbon sources utilization among them maximum mycelia growth and biomass production observed in sucrose followed by dextrose and glucose production in soil born pathogen.

Growth of *P. aphanidermatum* in different nitrogen sources

Among the six different nitrogen sources tested both in solid and liquid media, Sodium nitrate promoted significant mycelial growth (90.00 mm) and mycelia dry weight (610.54 mg) followed by ammonium nitrate which recorded the mycelia growth of 87.25 mm and mycelia dry weight of 607.00 mg. The minimum mycelia growth (78.66 mm) and mycelia dry weight (447.78 mg) was observed in Ammonium oxalate supplemented as a nitrogen sources (Table 4). Desai (1974) observed that asparagine supported the better linear growth of *P. debaryanum* (vegetables) than other nitrogen sources used. Sodium nitrate was found to be excellent source for the growth of many fungi. Tamilvanan *et al.* (2005) who explained that ammonium nitrate was the best among the nitrogen sources tested. Muthukumar (2009) found that the nitrogen sources calcium nitrate, ammonium chloride, ammonium nitrate, and potassium nitrate recorded the maximum mycelial growth and dry weight of pathogen in *P. aphanidermatum*. Muthukumar *et al.* (2019) reported that use of various nitrogen sources peptone followed by potassium nitrate recorded maximum mycelia growth and mycelia dry weight production in soil born pathogen.

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