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EEFECT OF DIFFERENT MEDIA ON GROWTH AND CULTURAL CHARACTERISTICS OF *ALTERNARIA JASMINI* CAUSING JASMINE LEAF BLIGHT

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Abstract

Laboratory studies were conducted on the effect of culture media on the growth and bio-mass production of *A. jasmini* causing leaf blight of jasmine. In the present study, potato dextrose agar medium significantly supported the maximum (72.10 mm) growth followed by host extract medium (68.59 mm), beetroot dextrose agar (65.51 mm) and peas dextrose agar (63.60mm). Among the isolates tested, I_1 from B. mutlur had the highest mean mycelial growth of (75.94 mm) followed by I_5 from Melakadu (74.45 mm) and I_2 from Theethampalayam (72.01 mm). Among the liquid media tested, potato dextrose broth recorded the highest mean mycelial dry weight (619.31 mg /100 ml) followed by host extract broth (592.48 mg/100ml). and isolate I_1 recorded the highest mean mycelial dry weight (665.47mg/100ml), where as the least mean mycelial dry weight (500.30 mg/100ml) was found in I_6 in carrot dextrose broth. In general, all the seven isolates grow well on PDA and produced sporulation. Of these, isolate I_1 recorded maximum mycelial growth (89.00 mm) and quick sporulation (9 days). The colony colour and shape of conidia in all the isolates were similar (Brown colour) and conidial colour varied from brown, golden brown and deep brown. The colony colour of all the isolates were similar (Brown) with slight variation in the margin either roundish or irregular with white margin. The number of cells in each conidia may varied from 2-9. Zonation is present in isolate-I_1, where as absent in remaining isolates. Hence, each and every pathogen require various culture media for their growth and development.

Key words : Mycelial growth, mycelia dry weight, Cultural characters.

Introduction

Jasmine (*Jasminum sambac*) is a highly valuable ornamental plant for home gardens and commercial cultivation. Flowers and buds are used for making garlands, bouquets and for religious offering, while vein is used as hair adornment. The flowers also used for the production of perfumed hair oils and attars. Jasmine essential oil has a sweet and floral aroma. It is regarded as unique, as it blends well with other floral extracts and which is highly valued throughout the world. For its high grade perfumes, which is used in soap and cosmetic industries and in flavoring mouth wash liquids? The flowers should preferable be picked at night for extraction of essential oils. In India, Jasmines are cultivated throughout the country. Tamil Nadu is the leading producer of jasmine in the country with an annual production of 130070 t from the cultivated area of 12590 ha (Anonymous, 2016). In Tamil Nadu, jasmine is produced mainly in Madurai District, with an area of 1503 ha while the district produces nearly 15150 t per year (Anonymous, 2016) and is transported to Mumbai / Bombay in trade, as well as being exported to other countries.

The jasmine plants suffer due to several diseases caused by the fungal, bacterial and viral pathogens and are of major constraints causing economic yield loss. Among the fungal diseases, leaf spot of jasmine is caused by *Alternaria jasmini* is becoming common disease on jasmine cause serious losses to jasmine plant. The pathogen infects the crop mainly under dry and warm conditions and it was air borne in nature. Peak incidence occurs during rainy season. *A. jasmini* affected leaves are evidenced by formation of brown, necrotics spots with concentric rings on the leaf tip of the leaves, spreading rapidly in the rainy season. The infected leaves curl and start drying from margins. In severe cases, the

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young shoots also dry up. The flower production is very much reduced in infected plants and may cause yield loss up to 50 % (Conn and Tewari, 1990). In the present study was aimed on the use of various culture media on the mycelia growth and cultural characteristics of *A. jasmini*.

Materials and Methods

Isolation of pathogen

Jasmine plants showing typical symptoms of leaf blight were collected from different places *viz.,* B. mutlur, Theethampalayam, Vallampadugai, Melur, Melakadu, Morepalayam and Salur. Isolation of leaf blight pathogen *i.e., Alternaria jasmini* was made by tissue segment method (Rangaswami, 1958). Fresh leaves showing typical symptoms were collected and edge of the lesions were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in changes of sterile distilled water thrice and then placed on Potato Dextrose Agar (PDA) medium in Petri dish. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified based on their cultural and morphological characters.

Cultural characters

Growth characters of *A. jasmini* isolates on different solid media

In order to compare the growth of *A. jasmini* on different solid media *viz.,* Bean dextrose agar, Beet root agar, Carrot dextrose agar, Corn dextrose agar, Czapek dox agar, Host extract agar, Peas dextrose agar and Potato dextrose agar medium. Sterilized warm medium was poured into sterilized Petri dishes (9 cm) at the rate of 15 ml and allowed to solidify. The isolates of the pathogen was inoculated at the centre of the plate by placing a nine day old nine mm culture disc. The plates were incubated at room temperature (28 ± 2°C) for nine days and three replications were maintained and radial growth of the mycelium was measured.

Growth characters of *A. jasmini* isolates on different liquid media

Bean dextrose broth, Beet root dextrose broth, Carrot dextrose broth, Corn dextrose broth, Czapek's dox broth, Host dextrose broth, Peas dextrose broth and Potato dextrose broth were prepared without adding agar. From the prepared broth, 100 ml was distributed in 250 ml Erlenmeyer flasks and autoclaved at 1.05 kg cm^{-2} for 15 min and cooled. The flasks were separately inoculated with a nine day old culture of nine mm disc of the pathogen. Nine days after incubation, the mycelia mat was filtered through a pre weighed what man No.1 filter paper, dried in hot air oven at 100°C until constant weight was obtained. The mycelial dry weight was obtained by subtracting the weight of the filter paper.

Cultural and Conidial characters of *A. jasmini*

From the nine day old culture plates, nine mm culture disc of the pathogen was cut by using a sterilized cork borer and placed at the center of the each sterile Petri dish containing 20 ml of previously sterilized and solidified PDA medium. The plates were incubated at room temperature (28 ± 2°C) for nine days. The growth and morphological characters of the isolates *viz.,* conidia shape, colour, septation, number of cells per conidium, were observed under microscope (magnification 45x) by using ocular and stage micrometer. In addition to this, sporulation, colony growth, colony colour, margin and zonation was also observed.

Statistical analysis

The data on the effect of the treatments on the growth of pathogen and disease incidence were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT). The data on disease incidence was arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1eveloped by the Biometrics Unit of the International Rice Research Institute, The Philippines.

Results and Discussion

Effect of different solid media on growth of *A. jasmini* isolates

In order to culture fungus in the laboratory, it is necessary to furnish essential elements and compounds in the medium for their growth and other life processes. All media are not equally good for all fungi, nor there an universal substrate or artificial media upon which all fungi can grow. So, different media including both synthetic and non synthetic media were tried for *A. jasmini* in the present investigation.

All the eight media tested were supported the growth of *A. jasmini* in different manner. Among the solid media tested, potato dextrose agar medium significantly supported the maximum (72.10 mm) growth followed by host extract medium (68.59 mm), beetroot dextrose agar (65.51 mm) and peas dextrose agar (63.60mm), while carrot dextrose agar medium supported the least (49.93 mm) growth. Among the isolates tested, I_1 from B. mutlur

had the highest mean mycelial growth of (75.94 mm) followed by I_5 from Melakadu (74.45 mm) and I_2 from Theethampalayam (72.01 mm). The minimum mean mycelial growth (63.85 mm) was recorded in I_6 from Salur. Among the liquid media tested, potato dextrose broth recorded the highest mean mycelial dry weight (619.31 mg/100 ml) followed by host extract broth (592.48 mg/100ml), while carrot dextrose broth had least mean mycelial dry weight (403.11 mg/100ml). Among the isolates I_1 recorded the highest mean mycelial dry weight (665.47mg/100ml), where as the least mean mycelial dry weight (500.30 mg/100ml) was found in I_6 in carrot dextrose broth. Fungi secure food and energy from the substrate upon which they live in nature. Similarly, Jeeva Priscila (2014); Narender Kumar *et al.,* (2017) and Sharma *et al.,* (2018) reported that PDA medium as well as PDA broth favoured the fungal growth and promoted maximum mean mycelial growth and mean mycelial dry weight of *A. jasmini* on jasmine*, A. alternata* on leaf spot of calotropis and *A. cucumerina* var. *cyamopsidis* causing *Alternaria* leaf blight of cluster bean. These results are in agreement with that of Mohan (1996) and Karthikeyan (1999) reported that PDA was also found to be the suitable medium for culturing of *A. palandui* inciting leaf blight of onion. Our results also corroborated with the findings of Suman Lata *et al.,* (2013) and Ginoya and Gohel (2015) reported that best media for growth and sporulation of *A. lini* and *A. alternata* was potato dextrose agar followed by oat meal agar media.

Cultural and Conidial characteristics of *A. jasmini* isolates

In general, all the seven isolates grow well on PDA and produced sporulation. Of these, isolate I_1 recorded maximum mycelial growth (89.00 mm) and quick sporulation (9 days). This was followed by isolate I_5 (87.60 mm; 12 days). The colony colour and shape of conidia in all the isolates were similar (Brown colour)

Table 1: Growth of *A. jasmini* isolates on different solid media *in vitro.*

S.No.	Isolates	Diameter of Mycelial growth (mm)*								
		Beans dextrose agar	Beetroot dextrose agar	Carrot dextrose agar	Corn dextrose agar	Czepeck's dox agar	Host dextrose agar	Peas dextrose agar	Potato dextrose agar	Mean
1.	I_1	70.48^{a}	81.26^{a}	61.23^{a}	67.50^{a}	78.05^{a}	84.53^{a}	75.82^{a}	88.67^{a}	75.94
2.	I_2	56.72^{d}	67.89^{c}	52.47^{d}	59.79^{d}	52.92^{d}	71.10^{d}	70.55^{d}	79.38^{bc}	72.01
3.	I_3	65.91^{b}	75.20^{b}	57.86b	62.17^{cd}	62.21^{c}	79.86^{b}	72.21b^{cd}	81.41^{b}	66.93
4.	I_4	60.51^{c}	71.41^{c}	54.49^{c}	61.44^{d}	61.62^{c}	76.00^{c}	71.17^{cd}	78.83^{bc}	65.45
5.	I_5	69.11^{a}	80.00^{a}	60.92^{a}	65.65^{ab}	75.46^{a}	82.41^{ab}	74.46^{ab}	87.59^{a}	74.45
6.	I_6	57.92^{d}	69.11^{c}	53.84^{cd}	60.31^{d}	60.21^{c}	74.68^{cd}	71.12^{cd}	76.43^{cd}	63.85
7.	I_7	68.23^{ab}	79.26^{a}	58.63^{b}	64.23^{bc}	67.38^{b}	80.17^{b}	73.44^{abc}	84.51^{d}	69.60
Mean		56.11	65.51	49.93	55.13	57.23	68.59	63.60	72.10	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs C.D (P = 0.05%).

Isolates - 1.002, Media - 1.035, Isolates × Media - 2.929

Table 2: Growth of *A. jasmini* isolates on different liquid media *in vitro.*

S.No.	Isolates	Mycelial dry weight (mg)*								
		Beans dextrose agar	Beetroot dextrose agar	Carrot dextrose agar	Corn dextrose agar	Czepeck's dox agar	Host dextrose agar	Peas dextrose agar	Potato dextrose agar	Mean
1.	I_1	603.92^{a}	749.38^{a}	498.52^{a}	532.05^{a}	624.73^{a}	760.51^{a}	669.31^{a}	885.33^{a}	665.47
2.	I_2	532.57^{b}	640.23^{c}	475.31^{b}	521.89^{a}	550.85^{c}	702.99^{b}	639.11^{ab}	723.81^{c}	598.22
3.	I_3	498.72^{c}	619.82^{c}	451.07^{cd}	470.39^{b}	523.27^{d}	681.02^{b}	612.71^{b}	675.23^{d}	566.53
4.	I_4	480.31^{c}	529.36^{d}	433.52^{de}	467.05^{b}	507.21d^{e}	603.21^{c}	514.88^{c}	612.89^{e}	518.55
5.	I_5	552.57^{b}	698.23^{b}	482.55^{ab}	526.22^{a}	571.63^{b}	747.11^{a}	641.66^{ab}	760.31^{b}	622.54
6.	I_6	479.11^{c}	515.00^{d}	417.60^{e}	460.32^{b}	481.20^{f}	554.60^{d}	501.30^{c}	593.25^{e}	500.30
7.	I_7	501.81^{c}	639.33^{c}	466.30^{bc}	480.66^{b}	502.77^{e}	690.41^{b}	620.81^{b}	703.66^{c}	575.72
Mean		456.13	548.92	403.11	432.32	470.21	592.48	524.85	619.31	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs C.D (P = 0.05%).

Isolates - 8.738, Media - 8.220, Isolates × Media - 24.71

Table 3: Cultural characteristics of *A. jasmini* isolates on PDA.

S.No.	Isolates	Mean colony diameter (mm)	Dry taken for spore production	Colour of the colony	Margin of the colony	No. of cells	Shape of conidia	Colour of conidia	Zonation
1.	I_1	89.00[a]	9	Brown	Roundish	3-9	Obclavate	Golden brown	Present
2.	I_2	80.66[c]	15	Brown	Roundish	3-8	Obclavate	Brown	No
3.	I_3	81.00[c]	17	Brown	Irregular with white margin	3-5	Obclavate	Brown	No
4.	I_4	79.33[cd]	18	Brown	Irregular with white margin	2-5	Obclavate	Golden brown	No
5.	I_5	87.60[ab]	12	Brown	Roundish	3-9	Obclavate	Deep brown	No
6.	I_6	77.00[d]	20	Brown	Roundish	2-4	Obclavate	Brown	No
7.	I_7	84.66[b]	15	Brown	Roundish	3-5	Obclavate	Deep brown	No
CD (P=0.05%)					3.472				

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs

and conidial colour varied from brown, golden brown and deep brown. The colony colour of all the isolates were similar (Brown) with slight variation in the margin either roundish or irregular with white margin. The number of cells in each conidia may varied from 2-9. Zonation is present in isolate-I_1, where as absent in remaining isolates.

Rajender *et al.,* (2013) studied cultural, morphological and pathogenic characteristics of *A. helianthi* isolates, causing sunflower blight. They reported that all the isolates showed considerable variation in respect to colony margin, colony growth, pigmentation, colour of aerial mycelium and sporulation. Six isolates showed circular growth pattern and rest showed irregular growth with wavy margin. Devappa and Thejakumar (2016) reported that the conidiophores of *A. alternata* on chilli leaf spots were short to long, simple or branched arising singly. Conidiophores were golden to brown coloured with 2-9 transverse and 0-2 longitudinal septa. Conidia were borne in long chains (6-11) on conidiophores, they were thick walled, beaked and brown in colour. Brian *et al.,* (2017) reported that the isolates of *Alternaria* spp. from sesame were cultured to homogeneity, all isolates developed loose, cottony and greyish-green to olive brown colonies on PDA after incuba-tion at 25ºC for 7 days in the dark. Anil *et al.,* (2017) reported that the septation of twelve isolates of *Alternaria* spp. causing leaf blight of cotton shows conidia ranged from 1-7 vertical and 3-9 horizontal septations. Chethana *et al.,* (2018) reported that out of fifty six *Alternaria* isolates seventeen were selected as a descriptive isolates exhibited ash, ashy black, ashy white, ashy green and blackish green colour mycelium. These earlier reports support the present observations.

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