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EFFECT OF MEDIA, NITROGEN SOURCES AND TEMPERATURE ON THE GROWTH AND SPORULATION OF *CURVULARIA LUNATA* CAUSING CURVULARIA LEAF SPOT OF BLACKGRAM

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ABSTRACT
Curvularia leaf spot of Blackgram is an important disease in the class of fungal diseases of black gram. The effects of different media, nitrogen (N) sources and temperatures on *Curvularia lunata* causing curvularia leaf spot of Blackgram, were examined to determine optimal conditions for mycelial growth and sporulation. *In vitro* studies were carried out to select the suitable media for proper growth and sporulation using both solid and liquid media. Amongst solid media, Potato dextrose agar (7.60 cm) and Host extract agar (6.73 cm) were the best for fungus growth as well as for sporulation followed by Conn's agar and Czapek (Dox) agar. Whereas in liquid media, Richard's (834.33mg), Czapek (Dox) (830.00mg), supported best growth of the fungus and same composition of media were also suitable for the sporulation. Different sources of nitrogen were evaluated for exploring suitable sources of nitrogen for growth and sporulation. It was observed that potassium nitrate (435.00mg) was the best source of nitrogen for the growth of the fungus followed by urea (371.33mg) and calcium nitrate (360.00mg). Different temperatures (8, 15,20,25,28 30 35 and 37°C) were tested for suitable fungal growth and it was observed that fungus can grow up to temperature range of 15°-37°C. Whereas 28°C (830.00 mg) were the optimum for the growth of the fungus followed by 30°C (734.66 mg). Identified suitable media could be used for growth and sporulation study and temperature ranged could be used for epidemiological study.

INTRODUCTION

Urdbean or Blackgram (*Vigna mungo* L.) Hepper is the most important ancient pulse crop of the *'Vigna'* group and its grain contains about 24 per cent protein and is the richest one in source of phosphoric acid (Aykroyd, 1941). Though this crop is worldwide distribution, it is mainly grown in India at Maharashtra, Tamil Nadu, West Bengal, Uttar Pradesh, Madhya Pradesh, Orissa, Andhra Pradesh and Rajasthan. Various factors are responsible for low productivity of black gram in India, among them diseases are major concerns. Curvularia leaf spot of black caused by *Curvularia lunata* (Wakker) (Boedijn, 1933) is the most serious fungal disease of this crop. This disease was first reported by Singh and Singh (1973) from CSA University of agriculture and Technology Kanpur. Mainly disease was observed in the month of August and September to caused the severe infection most of the varieties/cultures grown at farmer fields. The disease occurs during all growing regions of black gram. Various culture media showed differential effects on the growth and cultural characteristics of different fungal pathogen on various host plants (Singh and Kaiser, 1994). Temperature and nitrogen sources affect the growth of *Fusarium solani* (Ramteke and Kamble, 2011). The ever the increasing severity of the Curvularia leaf spot of black gram in black gram growing area, prompted to investigate the effect of various factors on the mycelial growth and sporulation of *C. lunata*. Therefore, experiments were planned to observe the effects of culture media, nitrogen source, and temperatures on mycelial growth and spore production of *C. lunata*.

MATERIALS AND METHODS

Solid and liquid media

The cultural studies of the pathogen were carried out on solid and liquid media. Eight solid media i.e Potato dextrose agar, Host extract agar, Czapek dox agar, Richard's agar, Brown's starch agar, Asthana and Hawker's agar, Coon's agar and Das Gupta's agar medium and 6 liquid media (Richard's, Czapek (dox), brown's starch, Asthana and Hawker's, Coon's and Das Gupta's) were used. Each sterilized media was poured in Petridishes. Three replications were kept for each medium. Sterilized Petridishes containing media were inoculated using corkborer (5 mm diameter of fungus) with fresh culture of *C. lunata* and incubated in B.O.D. at 28°C and after 7 days fungal growth was recorded by measuring the diameter of the growing fungus. Similarly, liquid media was prepared without agar in 50ml conical flasks were used for each medium and three replications were maintained for each medium. Sterilized media containing flask were inoculated with fresh culture of *C. lunata* and incubated in B.O.D. at 28°C for further

growth. After 10 days of fungal growth, the mycelium mats were filtered using Whatman filter paper and the filter paper with the mycelial mats was dried in the hot air oven at 60°C for 24 hrs. Then after, weight of filter paper was deducted from the total weight to find out the actual weight of the fungus (Senthamizhlselvan *et al.*, 2010).

Effect of different sources of nitrogen on the growth and sporulation of *C. lunata*

Seven different organic and inorganic nitrogen compounds viz., Ammonium sulphate, Ammonium chloride, Potassium nitrate, Magnesium nitrate, Calcium nitrate, Sodium nitrate, and Urea were used as sources of nitrogen compounds. These nitrogen compounds were amended in the Richard's medium. Medium without any nitrogen compounds was kept as the control. Three replications were made for each treatment and pH in each treatment was adjusted to 6.6 before autoclaving. Fresh culture of *C. lunata* was inoculated in containing sterilized media, the flasks were incubated at 28°C for 10 days and after that filtered though Whatman filter paper. Remaining procedures were followed as mentioned in case of liquid media study.

Effect of different temperatures on growth and sporutation of *C. lunata*

The effect of eight different temperatures viz., 8°C, 15°C, 20°C, 25°C, 28°C, 30°C, 35°C and 37°C were studied using synthetic medium (Richard's medium) in different incubators. Fresh culture of *C. lunata* was inoculated in containing sterilized media, the flasks were incubated at different for 10 days and after filtered though Whatman filter paper. Remaining procedures were followed as mentioned above. The intensity of sporulation of *C. lunata* was determined using standard methods as recommended by Wilson and Knight (1952) and Tuite (1969).

RESULTS AND DISCUSSION

Effect of solid and liquid media on the growth and sporulation of *C. lunata*

Fungi can be grown on a variety of media comprising of known and unknown constituents but *in vitro* they require some specific media for their best vegetative and reproductive growth. Keeping view of this in mind, eight different solid and six liquid media were used for the growth of the fungus and sporulation in present study. It is evident from Table No. 1 that potato dextrose agar medium (7.60 cm) supported the best growth of the fungus followed by host extract agar (6.73cm) , Coon's agar, Czapek Dox agar, Brown's starch agar, whereas Das Gupta's agar (3.50 cm) medium supported lowest growth of the fungus. Regarding intensity of sporulation, excellent sporulation was obtained on PDA medium whereas poor spouralation was recorded on Das Gupta's agar medium. Similar line of finding also reported by Rudha *et al.* (2008) that potato dextrose agar medium supported best medium for the mycelium growth and sporulaion for *C. lunata*. Tandel *et al.* (2012) also reported that PDA medium was best supported for growth of *Macrophomina phaseolina*. The best medium for the vegetative and reproductive growth of this fungus in case of liquid medium was the Richard's medium (834.33 mg) followed by Czapek's (Dox) medium and Coon's medium (Table 2). Poor growth was observed on Brown starch medium (69.66 mg). Intensity of sporulation was excellent on Richard's and Czapek's (Dox) medium and poor on Brown's starch medium. All the media differed significantly with each other for the growth of the fungus. Begum *et al.* (2009) also reported

Table 1: Growth diameter of C. lunata on different solid media at 28 ± 1°C

Media	*Average diameter of the Fungus (cm)	Sporulation
Potato dextrose agar	7.60	Excellent
Host extract agar	6.73	Excellent
Czapek dox agar	5.70	Good
Richard's agar	4.53	Good
Brown's starch agar	4.80	Good
Asthana and Hawker's agar	3.60	Fair
Coon's agar	5.83	Good
Das Gupta's agar	3.50	Poor
SEm ±	0.007	
CD (p = 0.05)	0.015	

*Means of three replications

Table 2: Dry Mycelial Weight of *C. lunata* on different liquid media

Media	*Av. mycelial dry weight (mg)	Sporulation
Richard's	834.33	Excellent
Czapek (Dox)	830.00	Excellent
Brown's starch	69.66	Poor
Asthana and Hawker's	137.33	Good
Coon's	501.66	Good
Das Gupta's	329.00	Fair
SEm ±	0.81	
CD (p = 0.05)	61.78	

*Means of three replications

Table 3: Average dry mycelial weight of fungus on different nitrogen sources

Nitrogen Sources	*Av. Dry Weight of mycelium (mg.)	Sporulation
Ammonium sulphate	314.66	Fair
Ammonium chloride	216.66	Fair
Potassium nitrate	435.00	Excellent
Magnesium nitrate	350.00	Good
Calcium nitrate	360.00	Good
Sodium nitrate	175.00	Poor
Urea	371.33	Excellent
Control	134.33	Poor
SEm ±	7.02	
CD (p = 0.05)	14.8	

*Means of three replications

Table 4: Average dry weight (mg) of mycelium at different temperatures

Temperatures (°C)	*Av. dry weight (mg.)	Sporulation
8	00	-
15	144.66	Poor
20	347.33	Poor
25	530.66	Fair
28	830.00	Excellent
30	734.66	Good
35	369.33	Fair
37	230.00	Poor
SEm	3.48	
CD (p = 0.05)	7.37	

*Means of three replications

that Richard's and Czapek's (Dox) medium was supported for the vegetative and reproductive growth of *C. lunata*.

Effect of different sources of nitrogen on the growth and sporulation of the pathogen

Result revealed in Table 3 that Potassium nitrate (435.00 mg) was the best source of nitrogen for the growth and sporulation of the fungus followed by urea, calcium nitrate and magnesium nitrate. Sodium nitrate (175.00 mg) was the poor source of nitrogen for the growth and sporulation of the fungus. Vibha and Sinha (2005) also reported that the potassium nitrate as nitrogen source is most appropriate for growth and sporulation of the different cellulolytic fungi, *C. lunata, Trichoderma harzianum,Penicillium citrinum, ,Aspergillus flavus* etc.

Effect of different temperatures on the growth and sporulation of *C. lunata*:

Result revealed that the optimum temperature for the growth of the fungus was 28°C (833.00 mg) followed by 30°C (734.66 mg). The fungus was able to grow at wide temperature range 15°C - 37°C, but was quite unable to grow at 8°C (Table 4). It is also clear that excellent sporulation was obtained at 28°C, good at 30°C, and poor at 37°C and 20°C. However, the fungus failed to sporulate at 15°C. It was observed that 28°C was the optimum temperature for the growth and sporulation of *C. lunata*, the minimum and maximum temperature for the growth and sporulation 15°C and 37°C, respectively. Ramteke and Kamble (2011) also reported that *Fusarium solani* grow at wide range of temperature (10°C -35°C) Almaguer *et al.* (2012) also observed the optimum temperature range 10°C - 40°C for the growth and germination of conidia of the fungi i.g. *C. clavata, C. pallescens, C. trifolii, C. aeria* and *C. spp* etc.

Present study finding concludes that the *C. lunata* can grow various sources of media, different sources of nitrogen and wide range of temperature. Studied range of temperature could be utilized for epidemiological study and accordingly management strategies could be developed for managing this disease.

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