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EFFECT OF DIFFERENT SOLID AND LIQUID MEDIA ON GROWTH OF *COLLETOTRICHUM LINDEMUTHIANUM* IN *IN VITRO* CONDITION

Nakrani B.R., Mit Patel and V.M. Jitaliya*
Department of Plant Pathology, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar- 385 506, Gujarat, India
*Corresponding author E-mail: vmjitaliya@gmail.com
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ABSTRACT

The study was conducted to find out the effect of different solid and liquid media on growth and sporulation of *Colletotrichum lindemuthianum* in *in vitro* condition, causing anthracnose disease in black gram [*Vigna mungo* (L.) Hepper] commonly known as urdbean. Experiment was carried out at the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during the year 2021-2023. Potato dextrose, Czapek's-Dox, Richard's, Oat meal, and Corn meal solid as well as liquid media evaluated in terms of colony colour, colony diameter, colony pattern, sporulation, conidial length and breath, and mycelial weight. The result indicated that in solid media, Potato dextrose agar was found best followed by Richard's agar for the growth and excellent sporulation whereas in liquid media, maximum dry mycelial weight and excellent sporulation was observed in Richard's broth followed by Czapek's broth.
Keywords **:** *Vigna mungo,* black gram, anthracnose, *Colletotrichum lindemuthianum*

Introduction

Pulses in India have long been considered as the poor man's only source of dietary protein. Pulses play an important role in improving livelihood, nutritional security of farmers and populations in under developed countries. Apart from being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in ecological agriculture (Kannaiyan, 1999). The black gram [*Vigna mungo* (L.) Hepper] commonly known as urdbean, is an annual semi erect to spreading herb belonging to the family *Leguminosae*. Black gram is locally known as Urad dal (Hindi), Minumulu (Telgu), Ulundu Paruppu (Tamil), Uddina bele (Kannada), Masakalai dala (Bengali), Biri dali (Oriya), Adad dal (Gujrat), Kali dal, Udid (Marathi) in India. It has been growing in India, Pakistan, Bangladesh, Sri Lanka, Thailand, Vietnam, Indonesia, South China and Malaysia since ancient times. India is said to be the origin of black gram (Piper and Morse, 1914). India as the primary Urd bean origin centre with Central Asia as a secondary location (Vavilov, 1926).

Black gram has high nutritional value containing, fat (1.4%), protein (24%), carbohydrate (59.6%), calcium (154 mg), phosphorus (385 mg), iron (9.1 mg), thiamine (0.4 mg), riboflavin (0.37 mg), niacin (2 mg) and beta carotene (38 mg) per 100 g seeds (Gopalan *et al.,* 1971). It is a nutritive fodder for animals, especially milch animals. The leaves and stems are the most common sources of fodder, but seeds, pods and pod husks are also used. Black gram crop is itself a mini-fertilizer factory, as it has unique characteristics of maintaining and restoring soil fertility through fixing atmospheric nitrogen through symbiotic association with *Rhizobium* bacteria, which are present in the root nodules. Black gram can fix atmospheric nitrogen to the tune of 30 kg nitrogen per hectare per year. Black gram can be used as green manure and a cover crop. The crop is suitable for intercropping with different crops such as sorghum, cotton, pearl millet, green gram, maize, groundnut and soybean for increasing production and maintaining soil fertility (Parashar, 2006). India is the world's leading producer of black gram, accounting for more than 70 per cent of global output, followed by Myanmar and Pakistan (Anon., 2020). In India, the black gram area increased by 386 per cent in *Kharif* 2020-21, from 1.88 lakh ha in 2019-20 to 8.77 lakh ha in 2020-21. Madhya Pradesh (4.45 lakh ha), Maharashtra (1.79 lakh ha),

Rajasthan (0.71 lakh ha), Karnataka (0.58 lakh ha), Telangana (0.11 lakh ha) and Andhra Pradesh (0.04 lakh ha) are the major *kharif* growing states (Anon., 2020). In Gujarat, black gram is primarily grown in the *kharif* season in the Kutch, Banaskantha, Saurashtra, Mahesana and Panchmahal districts, with adequate but erratic rainfall. During the summer, however, it is grown extensively in the districts of Kheda, Vadodara and Panchmahal (Anon., 2020).

Biotic and Abiotic stresses cause significant yield reduction in black gram. According to estimate made in India, nearly (10-15 %) of food legumes production is lost due to diseases alone. The micro-organisms *viz.,* fungi, bacteria, viruses and nematodes infect the black gram, causing a variety of diseases and among these, fungal diseases are particularly important. The major diseases infecting black gram are anthracnose (*Colletotrichum* spp.), powdery mildew (*Erysiphe polygoni*), cercospora leaf spot (*Cercospora canescens*), root rot (*Rhizoctonia solani*), stem canker (*Macrophomina phaseolina*), bacterial leaf blight (*Xanthomonas phaseoli*), yellow mosaic and leaf crinkle causing significant yield losses (Agarwal, 1991).

Among the various fungal diseases, the occurrence of anthracnose disease in black gram is commonly observed in most of the cultivated areas. Anthracnose continues to be one of the major constraints in black gram cultivation caused by *Colletotrichum* spp. is world's most important seed and soil-borne disease (Agarwal, 1991). At least four species of *Colletotrichum* have been found associated with green gram and black gram causing anthracnose in different parts of the world (Saxena and Sinha, 1977). It has been reported to possess high pathogenic variability and more than 100 races of *C. lindemuthianum* have been identified worldwide (Sharma *et al.,* 2007). Anthracnose pathogen (*Colletotrichum* spp.) attacks all aerial parts of plants at all stages of development. Symptoms are black, circular, sunken spots with a dark centre and bright red-orange margins appear on leaves and pods. The cotyledons of seedlings show dark brown to black sunken spots, which may bear pink spore masses of the fungus in wet weather and become blighted due to infection shortly after seed germination. In the event of a severe infection, the affected parts, particularly the leaves, wither. The pathogen perennates on infected seeds and in the soil on diseased plant debris. The secondary infection takes place through airborne conidia. The disease is most common in areas with cool and wet weather and it can result in a yield loss of up to cent per cent. Various researchers have estimated yield losses due to anthracnose between 24 to 67 per cent (Deeksha and Tripathi, 2002), 18.2 to 86.6 per cent (Laxman, 2006) and 21.36 to 60.07 per cent (Kulkarni, 2009).

Materials and Methods

Effect of different solid media

The study was carried out at the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during the year 2021-2023 to find out the effect of different solid media on growth and sporulation of *C. lindemuthianum*. For this twenty ml of each below listed medium (Table 1) were poured in 90 mm diameter Petri plates and inoculate aseptically with 5 mm mycelial disc of seven days old culture in the center, after 8 days of incubation at 27±2 ^{0}C. Variations were measured with respect to colony colour, colony diameter, colony pattern, sporulation (Table 2), length and breadth of conidia.

Table 1 : Composition of various solid media

Potato Dextrose Agar		**Corn meal agar**	
Peeled potato	200 g	Corn meal	**50 g**
Dextrose	20 g	Agar	20 g
Agar	20 g	Distilled water	1000 g
Distilled water	1000 ml	**Richard's agar**	
Czapeck's Dox agar		Potassium nitrate	**10 g**
Sucrose	30 g	Potassium dihydrogen phosphate	5 g
Sodium nitrate	2 g	Magnesium sulphate	2.50 g
Dipotassium phosphate	1 g	Ferric chloride	0.02 g
Magnesium sulphate	0.5 g	Sucrose	50 g
Ferrous sulphate	0.01 g	Agar	20 g
Potassium chloride	0.50 g	Distilled water	1000 ml
Agar	20 g	**Oat meal agar**	
Distilled water	1000 ml	Oat meal	30 g
		Agar	20 g
		Distilled water	1000 ml

Table 2 : Sporulation grade

Sr. No.	Score	Grade	Description [Conidia per microscopic field (100 X)]
1.	++++	Excellent	>151
2.	+++	Good	101 – 150
3.	++	Fair	51 – 100
4.	+	Poor	1 – 50
5.	-	No sporulation	-

Effect of different liquid media

The study was conducted to find out the effect of different liquid media listed in the Table 1 without adding the agar-agar powder on growth and sporulation of *C. lindemuthianum.* For this 20 ml of each medium were poured in 150 ml flask and inoculated aseptically with 5 mm mycelial disc of seven days old culture. After 8 days of incubation at 27 ± 2 ^{0}C, dry mycelium weight (mg) and sporulation was recorded.

The cultures were filtered through previously weighed Whatman No. 42 filter paper of 12.5 cm diameter. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to get rid of the salts likely to be associated with the mycelial mass. The filter paper along with the mycelial mat were dried to a constant weight at 60 °C for 48 h in electric oven, cooled in a desiccator and weighed immediately on an analytical balance. The difference between final and initial weight of filter discs were taken as the weight of the mycelia.

Results and Discussion

Effect of different solid media

The diversity in cultural and morphological characters of *C. lindemuthianum* was studied on five solid media at temperature (27 ± 2 ^{0}C). The colony diameter, colony colour, colony pattern, sporulation and size of the conidia were recorded after eight days from incubation. The data presented in Table 3 were suggested that the fungus recorded maximum growth on potato dextrose agar (85.75 mm) and was found superior over other media, next to Richard's agar (56.25 mm) and corn meal agar (55.50 mm). The minimum growth was observed in oat meal agar (46.25 mm).

Table 3 : Effect of different solid media

Solid media	Colony diameter (mm)	Colony colour	Colony pattern	Sporulation	Size of conidia (µm)	
					Length	Breadth
Potato Dextrose agar	85.75	White	Cottony smooth and circular	++++	12.00-14.83	3.20-4.23
Czapek's Dox agar	48.50	Creamy white	Flat irregular and fluffy	+++	10.40-16.59	2.80-4.90
Oat meal agar	46.25	Yellowish White	Raised circular	+++	12.20-17.27	3.00-5.10
Richard's agar	56.25	White	Regular smooth and circular	++++	13.02-15.77	3.20-4.70
Corn meal agar	55.50	White	Raised Cottony and fluffy	++	07.80-17.40	3.40-3.69

The maximum sporulation was found in potato dextrose agar and oat meal agar while fair in corn meal agar. With respect to the colony colour, it varied from white to creamy white. The growth pattern varied from flat to fluffy with smooth to irregular margins. The fungus showed white coloured with cottony smooth and circular margin mycelia on potato dextrose agar with excellent sporulation, on Czapek's dox agar the colony were creamy white in colour and irregular margin having good sporulation, while on oat meal agar showed yellowish white colour and margin was raised circular with excellent sporulation, while Richard's agar and corn meal agar media produced white colour mycelia with slight smooth to fluffy growth having poor to fair sporulation. With respect to the conidia size, 12.00-14.83 × 3.20-4.23 µm was observed on potato dextrose agar, 10.40-16.59 × 2.80-4.90 µm on Czapek's dox agar, 12.20-17.27 × 3.00-5.10 µm on oat meal agar, 13.02-15.77 × 3.20-4.70 µm on Richard's agar, 07.80-17.40 × 3.40-3.69 µm on corn meal agar (Plate 1).

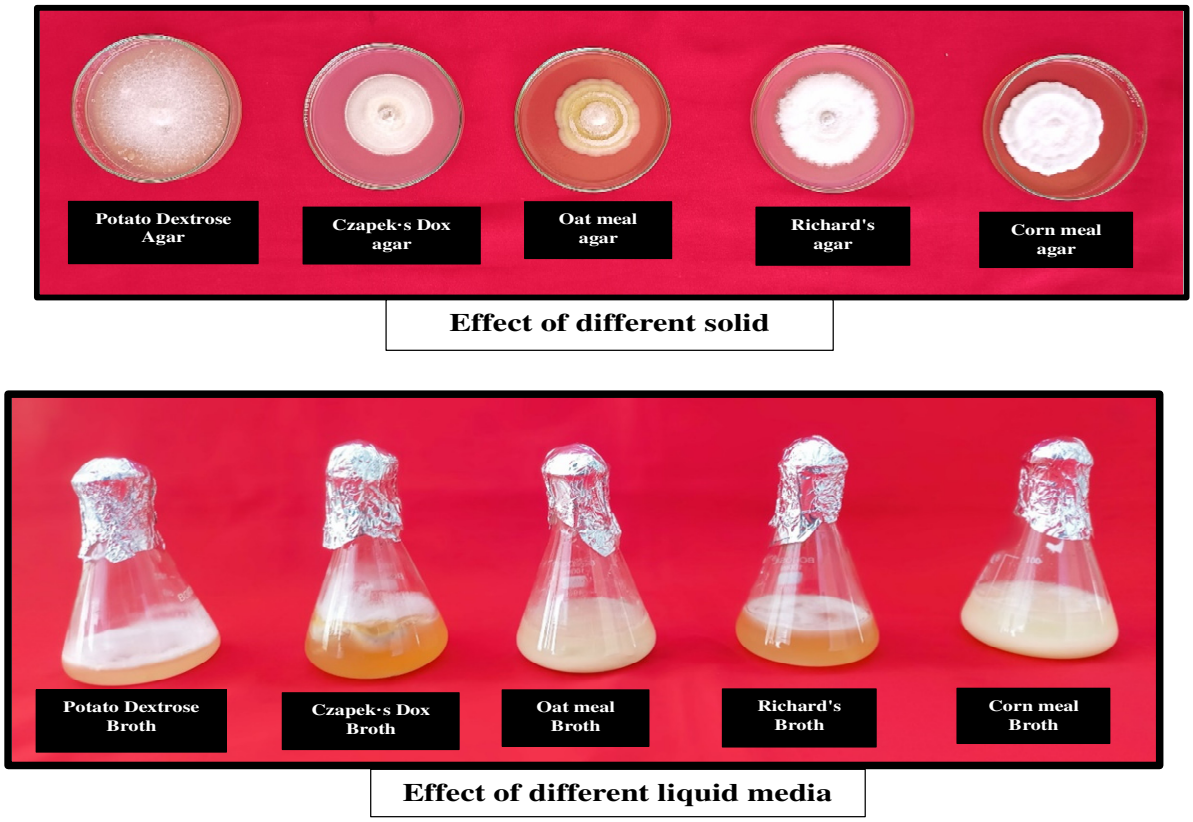

Plate 1: Effect of solid and liquid media on *C. lindemuthianum in vitro*

These results are conformity with the result obtained by Akhtar (2000) reported fresh potato extract is the best source for the growth of *Colletotrichum gloeosporioides*. Kulkarni and Raja (2019) investigated cultural studies of *Colletotrichum truncatum* of green gram showed that the potato dextrose agar was best followed by oat meal agar for the growth and excellent sporulation.

Marak *et al.* (2019) used different culture media for the growth of *Colletotrichum truncatum* infecting green gram and they found that colony colour varied from white or white with light brown centres which later changed to black or dark to light brown with increase in the age of the fungal cultures and fluffy or cottony mycelial growth with slight variations and regular to irregular white margin. PDA and RA produced maximum mycelial growth (90 mm) at 10 days after inoculation.

Effect of different liquid media

Average dry mycelial weight and sporulation of *C. lindemuthianum* grown on five different liquid media after eight days of incubation was recorded. Data from the Table 4 indicated that, there was difference between the liquid media. Richard's Broth supported the maximum dry mycelial weight (178.22 mg) and was superior over other media. Czapek's dox broth (170.23 mg), Potato dextrose broth (168.30 mg), Oat meal Broth (162.30 mg) and Corn meal Broth (157.03 mg) were found best but differed significantly with each other. Excellent sporulation was observed in potato dextrose medium and Richard's broth, while fair sporulation was noticed in Czapeck's dox Broth, oat meal Broth and corn meal medium (Plate 1).

These findings were close enough with the results of Hiremath *et al.* (1993) and Ekbote *et al.* (1997) reported that, maximum dry mycelial growth of *C. gloeosporioides* was recorded in Richard's broth (339.00 mg). Kulkarni and Raja (2019) investigated cultural studies of *C. truncatum* of green gram, maximum dry mycelial weight and excellent sporulation were observed in Richard's medium followed by Czapeck's medium.

Table 4 : Effect of different liquid media

Liquid media	Dry mycelium weight (mg)	Sporulation
Potato Dextrose Broth	168.30	++++
Czapek's Dox Broth	170.23	++
Oat meal Broth	162.30	++
Richard's Broth	178.22	++++
Corn meal Broth	157.03	++

Conclusion

Based on present investigation it can be concluded that black gram is one of the important pulse crops, suffering seriously from anthracnose diseases. Isolation made from infected plant and based on cultural and morphological characterization; it was identified as a *Colletotrichum lindemuthianum*. The pathogen is airborne in nature spread rapidly under congenial conditions. The maximum growth was observed in potato dextrose agar in solid media and in liquid media Richard's broth having maximum dry mycelial weight.

Conflict of interest

The authors, affiliated with declare no conflicts of interest.

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