INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 9 | ISSUE 1 | APRIL, 2016 | 47-51

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/9.1/47-51

# Effect of culture media and temperature on growth and sporulation of *Colletotrichum lindemuthianum* of urdbean *in vitro*

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ARITCLE INFO

**Received** : 05.10.2015
**Revised** : 07.02.2016
**Accepted** : 21.02.2016

KEY WORDS :
Blackgram (*Vigna mungo* L. Hepper), Culture media, Temperature

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ABSTRACT

Anthracnose (*Colletotrichum lindemuthianum*) fungus is an important in the urdbean legumes crop. In the present investigation, the rate of growth of Anthracnose (*C. lindemuthianum*) has been compared in various temperature, culture media solid and liquid while in case solid media types *viz.*, simple PDA (Potato Dextrose Agar), Potato carrot sucrose agar, Carrot juice agar, Blackgram leaf extract agar, Richard's agar, Czapeck's (Dox) agar, Asthana and Hawker's agar had tested. Among all the solid media tested, maximum mycelial growth was obtained in Potato dextrose agar medium (80.04 mm), which was statistically at par with Richard's agar medium (76.52 mm).Similarly in the liquid media, maximum dry mycelial weight was recorded in Richard's broth medium (444.34 mg) after 10 days of inoculation which was significantly superior over rest of the broth medias and followed potato dextrose agar broth (429.03 mg). Temperature plays an important role in infection and disease development. Maximum mean colony diameter of fungus was recorded at temperatures of 30 $^0$C (80.92 mm) and 25 $^0$C (78.62 mm) which was significantly superior over all other temperatures. Lowest mean colony diameter was obtained at temperatures of 10 $^0$C (24.01 mm) and 40 $^0$C (21.37 mm). This is important for further study of disease management.

**How to view point the article :** Sardhara, M.J., Davara, D.K., Moradia, A.M. and Kapadiya, H.J. (2016). Effect of culture media and temperature on growth and sporulation of *Colletotrichum lindemuthianum* of urdbean *in vitro*. *Internat. J. Plant Protec.*, **9**(1) : 47-51.

## INTRODUCTION

Blackgram (*Vigna mungo* L. Hepper) is commonly known as urd, udad, udid, mash and urad in India. Blackgram is originated from India (De Candolle, 1986). Blackgram is an important pulse crop of Indian subcontinent, cultivated almost throughout the country in all the three seasons such as in *Kharif*, *Rabi*, and Summer. India is the largest producer of urdbean in the world. In India the major urdbean growing states are Andhra Pradesh, Maharashtra, Gujarat, Uttar Pradesh,

Madhya Pradesh, Tamil Nadu, Rajasthan, West Bengal, Karnataka, Assam, Bihar, Punjab, Jammu and Kashmir, etc. India is the world producer as well as consumer of blackgram. It produce about 1.5 to 1.8 million tons of urdbean annually for about 3.3 million hactares of area with an average productivity of 520 kg/ha. About 10 per cent of india total pulse production (Anonymous, 2013). In Gujarat, it covers an area of about 101400 hectares with production of 613 mt and productivity of 605 kg/ha (Anonymous, 2013). With its high protein content (20.8 – 30.5 %) and richest source of phosphoric acid among pulses. The dried seeds may be eaten whole or split, coocked or fermented, or parched, milled and ground into flour, whole or split, they are used to make dhal, soups, and curries and are added to various spiced or fried dishes. Plant residues are used either as animal fodder or chopped into the soil as a green manure.

Anthracnose of black gram belongs to fungi kingdom, sorariomycetes class, incertae sedis subclass, phyllachorales order, phyllachoraceae family, *Colletotrichum* genus and *lindemuthianum* species. It has been known for some time that the fungus can be grown in a culture. The culture is generally made of a nutrient rich agar, as the fungus generally has a hard time germinating in water due to the water solubility of the appressorium. For optimal growth, the culture should be kept at $22^0$C and at a pH of 8, although it can be grown in temperatures between 0-$34^0$ C and pH levels between 3-11.

Several species of *Colletotrichum* cause serious anthracnose diseases of important annual crop and ornamental plants. Some of them produced their teleomorph, *Glomrella singulata* with some frequency and are some times referred to as Glomrella diseases. Such species also causes cankers and dieback of woody plant. Only few of pathogen produce serious anthracnose *viz.*, bean, cotton cucurbits, onion, pepper, tomato and strawberry. *Colletotrichum lindemuthianum* is often present in or on the seed produced in infected pods. Infected seed may show yellowish to brown sunken lesions.

Crops have been used as forage or as a green manure (Lawn and Ahn, 1983). A number of widespread and serious diseases were caused by fungal, bacterial, and viral pathogens. Disease may be one of the major constraints to yield throughout much of Asia. Most serious of the fungal diseases are anthracnose (*Colletotrichum lindemuthianum*), cercospora leaf spot (*Cercospora canescens*), root rot (*Macrophomina phaseolina*), rust (*Uromyces appendiculatus*), bacterial diseases *viz.*, bacterial leaf spot or blight (*Xanthomonas phaseoli*), and halo blight (*Pseudomonas phaseolicola*) are important ones. In case of viral diseases, yellow mosaic virus, diseases (Agrawal, 1991). The anthracnose of blackgram is caused by *C. lindemuthianum* (Sacc. and Magn.) was first reported in Assam in devastating form and caused heavy loss in yield under favourable climatic conditions. This disease is now widespread in India and occurs every year on all *Colletotrichum* spp. but in severe form only in *Vigna mungo*. The initial symptoms appear as leaf spots and brown blotches. Small, circular, brown, spots appear mainly on leaves. Later the spots enlarge and develop concentric rings giving the target board effect. The concentric rings are dark brown while the in between area is grey or dirty grey. The spots may be sickle shaped, circular to irregular in shape with grey centre and measure 3-5 mm in diameter. The spotted portion often becomes papery and falls off the leaves producing shot holes, the circular spots appear on pods of blackgram (Agrawal, 1991).

The anthracnose disease was observed in severe form on the Pulse Research Station of the Junagadh Agricultural University, Junagadh, in the year 2014 on the *Vigna mungo* and *C. lindemuthianum* (Sacc. and Magn.) was observed to be constantly associated upon isolation with the disease. Basic studies like isolation, purification, morphological studies of fungus symptomatology and pathocenicity was carried out.

## MATERIAL AND METHODS

Naturally infected urdbean leaves showing typical well-developed anthracnose symptoms were collected from Pulse Research Station, Junagadh Agricultural University, Junagadh 2014-15. Isolation of the fungus was made by tissue isolation technique. The typical spots on leaves were selected and cut into small bits with the help of a sterilized blade. The care was taken to select both initial as well as developed spots. Bits of diseased tissues were washed with distilled sterilized water and disinfected with 0.1 per cent mercuric chloride solution for 30 to 60 seconds. The bits thus disinfected were immediately washed thrice with distilled sterilized water in sterilized Petri plates. The bits than transfer with sterilized forceps on surface sterile the blotting paper to

removed excess water. The surface sterilize bits of diseased tissues were then transferred directly on the surface of potato dextrose agar in Petri plates under aseptic condition. Inoculated Petri plates were then incubated at 26 ± 1$^0$ C. The emanating fungal culture was purified after eight days of incubation by hyphal tip isolation and then by single spore isolation technique. The pure culture was maintained on PDA by storing it under refrigeration at 5$^0$ C and making periodical transfers every fortnight.

Growth and sporulation of *C. lindemuthianum* was measured by transferring 20 ml of agar agar based sterile medium to sterile Petri plates. After solidification, 5 mm diameter culture block of 9 days old pure culture of *C. lindemuthianum* was cut with the help of sterilized cork borer and placed in the centre of the Petriplates. The Petriplates were incubated at room temperature (26 ± 1 $^o$C) for 8 days and observations were also recorded after 10 days of incubation. The radial fungal growth was measured in mm from each fungal colony in two directions at right angles. Visual observations on mycelial growth and substratum colour of solid media were also recorded. Sporulation of the fungus was examined under the microscope (0.1 ml/fungal bit) using following signs.

The composition of liquid media was same as in solid media except omission of agar agar. 50 ml of liquid media were dispensed in 150 ml conical flask and plugged with non-absorbent cotton. Then it was subjected for sterilization in the autoclave at 1.036 kg/cm$^2$ (15 lbs psi) for 20 minutes. Pure culture block of 5 mm diameter from 9 days old culture of *C. lindemuthianum*, was cut with the help of sterilized cork borer was placed in the medium with the help of sterile forceps under aseptic conditions replicating four times. Out of these, one replication was kept for counting sporulation of fungus. The rest of the replications were kept for recording dry mycelium weight of the fungus, which were incubated at room temperature for a period of 10 days. After incubation period, the mycelium mats were filtered through a previously weighed filter paper and was thoroughly rinsed with warm sterile distilled water. The filter papers with mycelium mats were dried in an oven at 60$^0$ C for 24 hrs and dry weight of the mycelium was recorded. Spore count was recorded from fourth replication. The whole mycelial substrate was homogenized in 50 ml sterilized distilled water with the help of homogenizer. The homogenate was filtered through muslin cloth. A drop of the filtrate was examined and the numbers of conidia were recorded and graded as above.

The growth of *C. lindemuthianum* was tested at 10, 15, 20, 25, 30, 35 and 40$^0$C. Potato dextrose agar was poured into 90 mm diameter Petriplates. After solidification, 4 mm disc from actively growing cultures were cut and inoculated to solidified petriplates and incubated for 10 days in the incubators adjusted to required temperature levels. Each treatment was replicated thrice. After incubation period, radial growth and sporulation from solid media were recorded as described earlier.

## RESULTS AND DISCUSSION

Seven solid media including synthetic and semi-synthetic were tested for their suitability for the growth and sporulation of the fungus *C. lindemuthianum* at room temperature. Among all the solid media tested, maximum mycelial growth and excellent sporulation were obtained in Potato dextrose agar medium (80.04 mm), which was

**Table 1 : Growth and sporulation of *C. lindemuthianum* on different solid media *in vitro***

| Medium | Solid media colony diameter after 10 days (mm) | Sporulation |
|---|---|---|
| Potato dextrose agar | 80.04 | ++++ |
| Potato carrot sucrose agar | 75.17 | +++ |
| Carrot juice agar | 73.07 | +++ |
| Blackgram leaf extract agar | 60.22 | ++ |
| Richard's agar | 76.52 | ++++ |
| Czapeck's (Dox) agar | 67.96 | +++ |
| Asthana and Hawker's agar | 70.76 | +++ |
| S.E. ± | 1.19 | - |
| C.D. (P=0.05) | 3.60 | - |
| C.V. % | 2.86 | - |

Sporulation: ++++ = abundant; +++ = good; ++ = moderate; + = scanty; - = no sporulation formation

**Table 2 : Growth and sporulation of *C. lindemuthianum* on different liquid media *in vitro***

| Medium | Dry mycelial weight (mg) | Sporulation |
|---|---|---|
| Potato dextrose agar | 429.03 | ++++ |
| Potato carrot sucrose agar | 394.10 | +++ |
| Carrot juice agar | 398.33 | +++ |
| Blackgram leaf extract agar | 401.82 | ++ |
| Richard's agar | 444.34 | ++++ |
| Czapeck's (Dox) agar | 310.47 | ++ |
| Asthana and Hawker's agar | 364.94 | +++ |
| S.E. ± | 4.85 | - |
| C.D. (P=0.05) | 14.71 | - |
| C.V. % | 2.14 | - |

Sporulation: ++++ = abundant; +++ = good; ++ = moderate; + = scanty; - = no sporulation formation

**Table 3 : Growth and sporulation of *C. lindemuthianum* on temperature *in vitro***

| Temperature (ºC) | Mean colony diameter (mm) | Sporulation |
|---|---|---|
| 10 | 24.01 | - |
| 15 | 35.25 | + |
| 20 | 49.20 | ++ |
| 25 | 78.62 | ++++ |
| 30 | 80.92 | ++++ |
| 35 | 60.29 | +++ |
| 40 | 21.37 | - |
| S.E. ± | 0.76 | - |
| C.D. (P=0.05) | 2.31 | - |
| C.V. % | 2.65 | - |

Sporulation: ++++ = abundant; +++ = good; ++ = moderate; + = scanty; - = no sporulation formation

statistically at par with Richard's agar medium (76.52 mm) (Table 1). Potato carrot sucrose agar medium (75.17 mm) found next best in mycelia growth and remain at par with Carrot juice agar (73.07 mm). These solid media were found better for the growth and sporulation of *C. lindemuthianum* and they were reported earlier better by Kumar and Yadav (2004); Vinod and Benagi (2009) and Prema *et al*. (2011). Similarly in liquid media maximum dry mycelial weight was recorded in Richard's broth medium (444.34 mg) after 10 days which was significantly superior over rest of the broth medias (Table 2). In order of merit, the next best media was potato dextrose agar broth (429.03 mg). Excellent sporulation was observed in Potato dextrose agar and Richard's agar in both solid and liquid medium. Similarly result obtain by Vinod and Benagi (2009) juice agar and Richard's agar were good for growth and sporulation of *C. lindemuthianum* under *in vitro* and Ashutosh *et al*. (2012) and Prema *et al*. (2011) also confirmed these findings. In case of temperature, the fungus *C. lindemuthianum* was grown on potato dextrose agar medium at different temperatures *viz.*, 10, 15, 20, 25, 30, 35 and 40 $^0$C to know the suitable temperature requirement for maximum fungal growth and sporulation was recorded at 30 $^0$C followed by 25 $^0$C and it was least at 40$^0$C (Table 3). Hence, the temperature range of 25 to 30 $^0$C. The present results are in agreement with the results obtained by Singh and Shukla (1986); Wasantha and Rawal (2008); Vinod and Benagi (2009) and Prema *et al*. (2011).

## Conclusion :

Among seven media studied, potato dextrose agar was the best media for the growth of the fungus in liquid media, rechard's medium was best for the growth and sporulation of *C. lindemuthianum*. The optimum range of temperature for this fungus was 25$^0$C to 30$^0$C. However, maximum growth and sporulation of *C.*

*lindemuthianum* was recorded at $30^0$C temperature. This research is useful for further study of morphology *C. lindemuthianum* fungi.

## REFERENCES

**Agrawal, S.C. (1991).** Diseases of greengram and blackgram, R.A.K. College of Agriculture, Sehore, M.P. pp.52-61.

Anonymous (2013). Directorate of Agriculture, Gujarat State, Gandhinagar.

**Ashutosh, P., Lallan, P.Y., Muthukumar, M., Ugam, K.C. and Brajesh, K.P. (2012).** Effectiveness of cultural parameters on the growth and sporulation of *colletotrichum gloeosporioides* causing anthracnose disease of mango. *J. Bio. Sci.*, **12** : 123-133.

**De Candolle, A.P. (1986).** *Origin of cultivated plants*. Second ed., Hafner Publ. Co., New York, USA, p.468.

**Haytowitz, D.B. and Mathews, R.H. (1986).** Composition of Foods: Legumes and Legume Products. Agricultural Handbook, USDA, USA., pp.257-262.

**Kumar, S. and Yadav, B.P. (2004).** Influence of different media on radial growth, biomass production and sporulation of *Colletotrichum gloeosporioides* and *Colletotrichum capsici*. Causing anthracnose disease of betelvine. *RAU J. Res.*, **15** : 74-76.

**Lawn, R.J. and Ahn, C.S. (1983).** 'Grain legumes crops' published by Collins Professional and Techanical Books, pp. 584-618.

**Prema, R.T., Prabakar, K., Mohammed, F.P., Kathikeyan, G. and Thiruvengadam, R. (2011).** Morphological and Physiological Characterization of *Colletotrichum musae* the Causal Organism of Banana Anthracnose. *World J. Agric. Sci.*, **7** : 743-754.

**Singh, R.R. and Shukla, P. (1986).** Cultural Studies on *Colletotrichum truncatum* causing anthracnose of blackgram. *Indian J. Mycol. Pl. Path.*, **16** : 172-174.

**Vinod, T. and Benagi, V.I. (2009).** Studies on the cultural and nutritional characteristics of *Colletotrichum gloeosporioides*, the causal organism of papaya anthracnose. *Karnataka J. Agric. Sci.*, **22** : 787-789.

**Wasantha, K.L. and Rawal, R.D. (2008).** Influence of carbon, nitrogen, temperature and ph on the growth and sporulation of some indian isolates of *colletotrichum gloeosporioides* causing anthracnose disease of papaya. *Tropical Agri. Res. & Exten.*, **11** : 7-12.