International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 3058-3062

Received: 13-01-2018
Accepted: 14-02-2018

BB Thombre
Department of Plant Pathology, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

OD Kohire
Department of Plant Pathology, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

Correspondence
BB Thombre
Department of Plant Pathology, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India

Nutritional and physiological studies of *Macrophomina phaseolina* (Tassi) Goid causing mungbean blight

BB Thombre and OD Kohire

Abstract
Sucrose (90.00 mm), Fructose and Dextrose (83.50 mm) among carbon and Potassium nitrate (82.14 mm) as well as Sodium nitrate (78.33 mm) among nitrogen sources yielded maximum growth and sporulation of the test pathogen. The optimum temperature for growth and sporulation was 30 to 35 ^{0}C. The pathogen could grow and sporulate in the pH range of 5 to 8. Among the light intensities alternate cycle of dark and light (each 12 hrs.) (90.00 mm) supported the best growth and sporulation.

Keywords: Blight, Mungbean, *Macrophomina,* nutritional, physiological

Introduction
The Mungbean (*Vigna radiata* (L.) Wilczek, *Phaseolus radiata* L. is a highly nutritious source of proteins and calories in the Asian diet (Engel, 1978) [6]. It is easily digestible and in the absence of milk it is considered as an excellent food for infants. It is one of the most important sources of proteins (24%) in vegetarian diet in addition to fats (1.3%), minerals (3.5%) and carbohydrates (56.7%). Besides, it provides vitamin A, Riboflavin (0.3 g/mg) and essential amino acids like Arginine (0.50%), Histidin (0.35%), Isoleucine (0.35%) and Valine (0.32%). The Mungbean is one of the thirteen food legumes grown in India and third most important pulse crop of India, after chickpea and pigeonpea.
Being a crop of short duration with wide adaptability, Mungbean is grown all around the year as a sole crop, in double and multiple cropping systems and as an intercrop under most adverse and semi-arid conditions. As a leguminous crop, mungbean is cultivated in countries like Burma, Ceylon, South and North America, China, Africa and India. India alone accounts for about 2/3rd of total global production of Mungbean. Major states in the country growing mungbean are Maharashtra, Orissa, Andhra Pradesh, Madhya Pradesh, Gujarat, Rajastan, Bihar, Uttar Pradesh and Karnataka. *Rhizoctonia bataticola* (*Macrophomina phaseolina*) has been reported to infect the Mungbean plant parts *viz*., leaf (leaf blight), stem (stem blight), stalk (stalk rot), root (root rot), collar region (collar rot), blossom and fruit rot (Saksena, 1979) [16]. *Macrophomina Phaseolina* (Tassi) Goid has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species (Khan 2007) [7]. Keeping in view the present investigation was carried out to find a which carbon, Nitrogen source, pH levels, light and Temperature regimes will suit the growth and sporulation of *Macrophomina phaseolina.*

Materials and Methods
Nutritional and Physiological studies
All the nutritional and physiological studies were undertaken using representative MpJ isolate (Jalna) of *M. phaseolina*

Effect of carbon sources
To study the effect of different carbon sources on growth and sporulation of *M. phaseolina*, carbon sources *viz*., Mannitol, Xylose, Maltose, Dextrose, Lactose, D- Galactose, Glucose, Fructose, Starch, Sucrose, and C- control were used. For the assessment of suitable carbon source, Richards medium without carbon (50 g/lit) was used as basal stock medium. Sucrose when used @ 5.25 g provides 21.052 g of carbon 100/ml. On this basis for each test carbon source, 200 ml Richards agar medium was prepared in 250 ml capacity glass conical flasks and

sterilized in autoclave at 15 lbs/inch^2 pressure for 20 min. Autoclaved and cooled Richards agar medium amended separately with various carbon sources was poured (@ 20 ml/plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the medium, plates (three plates / carbon source / replication) were inoculated by placing in the centre 5 mm mycelial disc of actively growing 7 days old pure culture of *M. phaseolina* (MpJ isolate). Each carbon source was replicated thrice. Carbon control (Richard's medium without any carbon source) was also maintained. Plates were incubated at room temperature (27 ± 2 ^{0}C). Observations on colony diameter and sporulation were recorded after at a week and two weeks of incubations, respectively.

Effect of nitrogen sources

To study the effect of different nitrogen sources on growth and sporulation of *M. phaseolina*, nitrogen sources *viz.*, Ammonium nitrate, Peptone, Sodium nitrate, Potassium nitrate, Urea, Lueceine and N-control were used. For the assessment of suitable nitrogen source, Richards medium without nitrogen (10 g / lit) was used as basal stock medium. As the nitrogen provides 0.872 of nitrogen through 1.386 g/100 ml.

For each test nitrogen source, 200 ml of Richards agar medium was prepared and poured in glass Conical flasks (250 ml cap.) and sterilized in autoclave at 15 lbs/inch^2 pressure for 20 min. Autoclaved and cooled medium was poured (@ 20 ml/plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the medium, plates (three plates/nitrogen source/replication) were inoculated by placing in the centre 5 mm mycelial disc of actively growing 7 days old pure culture of *M. phaseolina* (MpJ isolate). Each nitrogen source was replicated thrice. Nitrogen control (with at any source of nitrogen) was also maintained. Plates were incubated at room temperature (27 ± 2 ^{0}C). Observations on colony diameter and sporulation were recorded after ten days of incubation.

Effect of temperature

The growth of *M. phaseolina* was tested at 15, 20, 25, 30, 35, 40 and 45 ^{0}C. Potato dextrose agar (20 ml/plate) was poured into 90 mm diameter sterile glass Petriplates (90 mm dia.). After solidification, 5 mm disc from actively growing 7 days old culture of *M. phaseolina* were cut and inoculated to solidified Petriplates and incubated for 15 days in the BOD incubator (Make: MAC, Delhi) adjusted to required temperature levels. Each treatment was replicated thrice. After incubation period, colony diameter and sporulation from solid media were recorded.

Effect of different pH

The effect of various pH levels (hydrogen ion concentration) was studied using PDA as basal medium. For the purpose, 200 ml PDA medium was poured in glass beakers (250 ml cap.) and its pH levels (4.0 to 8.5) were separately adjusted using pH meter (Make: MAC, Delhi) by adding 0.1 N HCL / NaoH. Then the PDA medium adjusted with various pH level was sterilized at 15 Lbs / inch^2 in an autoclave for 20 min. Autoclaved and cooled PDA medium with various pH levels was poured (20 ml / plate) separately in sterile glass petri plates (90 mm dia). Three plates / pH level / replication were maintained. Plates were inoculated by placing in the centre 5mm mycelial disc of actively growing 7 days old pure culture of *M. phaseolina* and incubated at temperature of 27 ± 2 ^{0}C. Observations on colony diameter, colony colour and sporulation were recorded after ten days of incubation.

Effect of light.

The effect of light on the growth and sporulation of *M. phaseolina* was studied by exposing the culture.

1. Continuous natural light
2. Continuous fluorescent light
3. Continuous darkness.
4. Alternate darkness and light (each for 12 hrs.)

Autoclaved and cooled PDA medium was poured is sterile glass Petri plates (three plates/treatments /replication). These PDA plates were inoculated aseptically by placing in the centre a 5 mm mycelial disc of actively growing a week old pure culture of *M. phaseolina* (MpJ isolate). These inoculated plates were exposed to various treatments of light *viz*., continuous natural light, continuous fluorescent light, continuous darkness and alternate darkness and light (each for 12 hrs.). All the treatment plates were incubated at 27 + 2 ^{0}C for the period of two weeks. Observations on colony diameter and sporulation were recorded at 14 days of incubation.

The number of sclerotia and pycniospores were observed microscopically and graded as below. (Tandel and Sabalpara, 2011) [22]

Sr. No.	Score	Grade	Number (Sclerotia or pycnidiospores/ microscopic filed) at 100 X
1	++++	Excellent	> 50
2	+++	Good	30-50
3	++	Fair	21-30
4	+	Poor	10-20
5	-	No sporulation	-

Result and Discussion

Nutritional and physiological studies

Effect of carbon sources

The capacity of the fungus to utilize various carbon sources is governed by its ability to produce enzymes. These enzymes convert complex carbon compounds into simpler forms, which are subsequently utilized for growth and reproduction. All of the ten carbon sources (Table 1) tested encouraged better growth of *M. phaseolina* except carbon control. However, significantly highest mean mycelial growth (90.00 mm) was recorded with Sucrose. The second and third best carbon sources found were Fructose (89.33 mm) and Dextrose (83.50 mm). Rest of carbon sources recorded radial mycelial growth in the range of 16.83 (Starch) to 63.50 mm. (each Glucose and Maltose). All the carbon sources tested exhibited a wide range of sporulation from poor (+) to excellent (++++). However, carbon sources *viz.*, Sucrose, Fructose and Dextrose recorded excellent (++++) sporulation. Rest of carbon sources exhibited good (+++) to Poor sporulation (+). Mukhopadhyay and Nandi (1975) [13] reported that sucrose, fructose and glucose were best carbon sources for mycelial growth and sclerotial production. Das (1998) [4] reported that glucose favoured maximum growth of *M. phaseolina* followed by maltose, sucrose and fructose, while sucrose was excellent carbon source for sclerotial production. The result in the present study is in agreement with the findings of Mukhopadhyay and Nandi (1975) [13] and Das (1988) [4].

The sources of carbon *viz*., Sucrose, Fructose, Dextrose, Maltose, Glucose, and D – Galactose were reported to support maximum growth and sporulation in *M. phaseolina* earlier as reported by several workers (Shanmugam and Govindaswamy, 1973; Diazfranco, 1984; Mehta and Gupta, 1992; Vishnupriya, 2006; Kumar and Gaur, 2010) [19, 5, 12, 23, 10]

Table 1: Effect of carbon sources on growth and sclerotia / pycniospores production of *M. phaseolina* (isolate MpJ)

Carbon sources	Colony dia. (mm)*	Colony colour	Sclerotia / Pycniospores per microscopic field
Manitol	35.60	Whitish flat	++
Xylose	26.78	Black	++
Maltose	63.50	Whitish Black	++
Dextrose	83.50	Whitish	++++
Lactose	40.17	Whitish	++
D-Galactose	60.17	Whitish Black	+++
Glucose	63.50	Black	+++
Fructose	89.33	Whitish Black cottony	++++
Starch	16.83	Black	+
Sucrose	90.00	Whitish Black	++++
C- Control	14.00	Black	-
S.E. ±	3.69	--	--
C.D. (P=0.05)	10.82	--	--

* Mean of three replications

++++: Excellent; +++: Good; ++: Fair; +: Poor; -: No

Effect of nitrogen sources

In the present study to determine the utilization of different nitrogen compounds (Table 2) by *M. phaseolina*, it was observed that potassium nitrate was found most suitable and encouraged maximum radial mycelial growth (82.14 mm). The second best nitrogen source was found Sodium nitrate (78.33 mm), followed by Peptone (66.73 mm), Urea (55.00 mm), Ammonium nitrate (48.53 mm). All the nitrogen sources tested, exhibited poor (+) to excellent (++++) sporulation. Mukhopadhyay and Nandi (1975) [13] reported that among nitrates, Potassium gave good results. Khune *et al.* (1993) [9] also reported that Potassium nitrate at the higher concentration supported luxuriant growth as well as high sclerotial formation of *M. phaseolina.* The result in the present study is in agreement with the finding of Mukhopadhay and Nandi (1975) [13] and Khune *et al.* (1993) [9]. The sources of nitrogen *viz.*, potassium nitrate and sodium nitrate, ammonium nitrate and peptone were reported to support maximum growth and sporulation in *M. phaseolina* earlier as reported by several workers (Shanmugam and Govindaswamy; 1973, Khanzada *et al.*, 2003; Kumar and Gaur, 2010; Tandel and Sabalpara, 2011) [19, 8, 10, 22].

Table 2: Effect of nitrogen sources on growth and sclerotia / pycniospore production of *M. phaseolina* (isolate MpJ)

Nitrogen source	Colony dia. (mm)*	Colony colour	Sclerotia / Pycniospores per microscopic field
Ammonium nitrate	48.53	Whitish cottony	++
Potassium nitrate	82.14	Whitish	++++
Sodium nitrate	78.33	Whitish	++++
Urea	55.00	Whitish	+++
Peptone	66.73	Whitish grey	++++
Lueceine	44.72	Whitish black	++
N-Control	13.07	Whitish	_
S.E. ±	2.77	--	--
C.D. (P=0.05)	8.39	--	--

* Mean of three replications,

++++: Excellent; +++: Good; ++: Fair; +: Poor; -: No

Effect of temperature regimes

The present study on temperature requirement (Table 3) showed that the isolate, MpJ could grow in the temperature range of 15-40ºC. The optimum temperature was 25-35 ^{0}C which allowed significantly highest growth over rest of the temperature levels. The best temperatures for mycelial growth found were 35 ^{0}C (90.00 mm), 30 ^{0}C (78.84 mm) and 25 ^{0}C (69.12 mm). This was followed the temperatures 20 ^{0}C (45.39 mm) and 15 ^{0}C (17.93 mm). All the temperature regimes tested exhibited a wide range of sporulation from none (-)to excellent (++++). However, excellent (++++) sporulation was recorded at temperature 35 ^{0}C. Good (+++) sporulation were recorded at temperature 30 ^{0}C at 25 ^{0}C and 40 ^{0}C and at the temperature 45 ^{0}C there was no any sporulation of the test pathogen. Temperature higher than optimum (35 ^{0}C) showed pronounced adverse effect on growth as compared to the temperatures lowers than optimum. Patel and Patel (1990) [14] found that the optimum temperature for growth and sclerotial formation by *M. phaseolina* was 35^0 C, both declined at temperature below 15 ^{0}C and above 40 ^{0}C. Sharma *et al.* (2004) [20] also found a higher temperature range of 25-35 ^{0}C for the growth of four isolates of *M. phaseolina* on different crops. The present results are in agreement with the results obtained by Patel and Patel (1990), Sharma *et al.* (2004), Bainade (2005), Salunkhe *et al.* (2009) Kumar and Gaur (2010) Akhtar *et al.* (2011) Csondes *et al.* (2012) [14, 20, 2, 17, 10, 1, 3].

In the present study, the excellent fungal growth and sporulation was observed at 35 ^{0}C followed by 30 ^{0}C. Hence, the temperature range of 25 to 35 ^{0}C can be suitable to obtain excellent fungal growth and sporulation of *M. phaseolina*.

Table 3: Effect of temperatures regimes on growth and Sclerotia / pycniospores production of *M. phaseolina* (isolate MpJ).

Temp. levels (^{0}C)	Colony dia.* (mm)	Sclerotia / Pycniospores per microscopic field
15	17.93	-
20	45.39	+
25	69.12	+
30	78.84	+++
35	90.00	++++
40	51.28	++
45	0.00	+
S.E. ±	0.64	--
C.D.	2.69	--

* Mean of three replications

++++: Excellent; +++: Good; ++: Fair; +: Poor; -: No

Effect of pH levels

Any living organism requires a particular medium for the growth and development. The pH of the media should be optimum for growth. A wide range of pH (Table 4) supported the growth of *M. phaseolina*. The best pH level for mycelial growth was found to be 7.0 (90 mm). This was followed by

the pH levels *viz.,* 6.5 (87.42 mm), 7.5 (84.30 mm) and 6.0 (83.93), 8.0 (80.00 mm) and 5.5 (77.52 mm). The sporulation was also influenced by pH and is known to play a crucial role. In the present investigation, excellent (++++) sporulation was recorded at the pH of 6.5 and 7.0. Good (+++) sporulation was recorded at the pH of 5, 5.5, 7.5 and 8.0.

The present results are in line with the Singh *et al*. (1974) [18]. They reported that pH 7.0 was optimum for growth and abundant sclerotial production of *Rhizoctonia bataticola*. Ratnoo and Bhatnagar (1991) [15] reported best growth and sclerotial formation of *Macrophomina phaseolina* at pH 7.0. Sharma *et al.* (2004) [20] observed that pH between 6.5 to 7.0 favoured the growth of all four isolates of *Macrophomina phaseolina* from different crops. Lokesha and Benagi, (2004) [11] they reported that Good growth was observed at pH values ranging from 6 to 8. Salunkhe *et al.* (2009) [17] reported that there was no significant difference in radial growth in isolates at pH range 5 to 9 but at pH 7.0 growth of all isolate was comparatively superior.

Table 4: Effect of pH on growth and sclerotia /pycniospores production of *M. phaseolina* (isolate MpJ).

pH levels	Colony dia.* (mm)	Sclerotia / Pycniospores per microscopic field
5.0	74.31	++
5.5	77.52	+++
6.0	83.93	+++
6.5	87.42	+++
7.0	90.00	++++
7.5	84.30	++++
8.0	80.00	+++
8.5	69.10	++
S.E. ±	0.68	--
C.D. (P=0.05)	2.80	--

* Mean of three replications

++++: Excellent; +++: Good; ++: Fair; +: Poor; -: No

Effect of light

In the present study, the results (Table 5) revealed that best mycelial growth was found at alternate dark and light (each for 12 hrs. duration) with 90.00 mm growth. This was followed by continuous electric light (83.00 mm), continuous dark (67.33 mm) and continuous sunlight (64.67 mm). However, excellent (++++) sporulation was recorded at alternate dark and light (each 12 hrs. duration). Good (+++) sporulation was recorded at continuous electric light. The present findings are in agreement with the study conducted by Sobrinho *et al*. (2004) [21] these authors verified that the highest rates were obtained under a daily 12-hour-light/dark-exposition period during the whole incubation period. It was also verified that a slight increase in the dark period was enough to inhibit the sporulation process.

Based on the findings, it can be recommended that for obtaining maximum fungal growth and sporulation, *M. phaseolina* culture should be exposed to alternate dark and light (each for 12 hrs. duration).

Table 5: Effect of light on growth and sclerotia / pycniospores production of *M. phaseolina.* (isolate MpJ)

Light	Colony dia.* (mm)	Sclerotia / Pycniospores per microscopic field
Continuous sunlight	64.67	++
Continuous electric light	83.00	+++
Continuous dark	67.33	++
Alternate dark and light	90.00	++++
S.E. ±	1.67	--
C.D. (P=0.05)	5.46	--

* Mean of four replications

++++: Excellent; +++: Good; ++: Fair; +: Poor; -: No

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