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Effect of temperature, pH and relative humidity on growth of *Macrophomina phaseolina* and Evaluation of cultivars of mungbean against root rot pathogen

Nitisha Gahlot, RR Ahir, Abhinav, SK Munnysha and Sushila Choudhary

Abstract
Mungbean is sensitive to a variety of fungal, bacterial and viral diseases. Root rot of caused by *Macrophomina phaseolina* is considered as the most serious disease, particularly in all the mungbean cultivating areas of Rajasthan and India. Due to favorable weather circumstances, the disease spread widely across the state and resulting in significant yield losses. In the present study influence of various temperature, pH and humidity levels were studied on the growth of the pathogen. Maximum mycelial growth (90mm) was observed at 30 ºC temperature and at 80% relative humidity and maximum dry mycelial weight (615 mg) was observed at 6.5 pH. Thirteen cultivars of mungbean were tested through artificial inoculation in pot condition, against root rot of mungbean. None of variety was found immune or resistant to root rot pathogen. Cultivars, RMG-1140, RMG-1144, RMG-1086, RMG-1079 were observed susceptible and RMG-1132 and Check were found highly susceptible for the disease.

Keywords: Mungbean, *Macrophomina phaseolina*, temperature, pH, relative humidity, cultivars

Introduction
Mungbean [*Vigna radiata* (L.) Wilczek] is popular pulse crop in India. Green gram, golden gram, moong and celera bean are some of its other names. It belongs to *Fabaceae* family. Due to the fact that it is leguminous plant, it has the ability to fix the atmospheric nitrogen by symbiotic nitrogen fixation and also utilized as green manure crop. It is self pollinated crop that is grown in arid semi arid regions throughout the *kharif* and summer seasons. It is draught tolerant and grows best in well drained loamy to sandy soil. Mungbean is a high protein source (24.5%) with high quality lysine (460mg/g N) and tryptophan (60mg/g N) and 51% carbohydrate content. It comprises substantial quantity of ascorbic acid (Vitamin-C) and consists riboflavin (0.21mg/100g) and minerals (3.84g/100g) when sprouted (Gopalan *et al.*, 1995) [7].

Root rot incited by *Macrophomina phaseolina* (Tassi) Goid has been rated as most devasting disease of mungbean. The pathogen attacks on all parts of plant *i.e.* root, stem, branches, petiols, leaves, pods and seeds. Root infected by *M. Phaseolina* show necrotic lesions. (Bouhot, 1967) [3]. Infection of pathogen occurs primarily during the flowering and pod formation stage (Singh *et al.*, 1990) [12] or during seed development stage (Trapero-Casas and Jimenez – Diaz, 1985) [15]. Yellowing of the leaves was the common indication of root rot disease and these leaves may drop off in two to three days. The plant may wilt within a week. At ground level, dark lesions on the stem can be noticed. When the plants are pulled out from the soil and examined, root rot symptoms can be seen on the basal stem and main roots. In advance stage on the affected tissues, scattered sclerotial bodies can be visible in the early stages (Singh and Srivastava, 1988) [13].

Macrophomina phaseolina survives in/on seed and stays in the soil as black sclerotia which are formed in great numbers on infected host tissues and then distributed in the soil during tillage operations (Sheikh and Ghaffar, 1978) [11]. Sharma and Singh (2001) observed that mungbean seed infection by *Rhizoctonia bataticola* causes 10.85% grain yield losses and 12.3% protein content losses (Kaushik *et al.*, 1987) [8]. Management strategies of this disease include a large range of options but farmers largely depend on fungicides due to its higher control efficiency over other methods. However, wider use of fungicidal can cause hazards to human health and known to increase environmental pollution. Objective of this research were to determine factors like temperature, pH and RH required for the growth of pathogen under lab conditions and to evaluate mungbean cultivars against root rot of mungbean disease.

Material and Method

Physiological studies was done by testing effect of various temperature, humidity and pH levels on mycelial growth of *Macrophomina phaseolina* causing root rot of mungbean.

1. Effect of temperature

It is a well-known phenomenon that the temperature exerts considerable influence on the biochemical activity of pathogens. Twenty ml of PDA was poured in each of sterilized Petri dish. Each Petri dish was inoculated aseptically by placing in the centre a 5 mm disc from actively growing 7 days old culture on PDA. The inoculated Petri dishes were incubated at 25, 30, 35 and 40^{0}C temperature for 7 days with five replications. Observations on mycelial growth were recorded after 7 days of incubation.

2. Effect of hydrogen ion concentration (pH)

The study of different pH levels was undertaken with a view to ascertain the effect of different hydrogen ion concentrations of the medium on growth of the fungus *Macrophomina phaseolina*. The initial pH of the basal medium before autoclaving was adjusted from 6.0 to 8.0 with a difference of 0.5 using N/10 NaOH or N/10 HCl. After autoclaving the pH was again tested. The inoculated Petri plates were incubated at 30±1 ^{0}C for 7 days with four replications. Observations on mycelial growth were recorded after 7 days of incubation.

3. Effect of relative humidity

To study the effect of relative humidity on mycelial growth of *Macrophomina phaseolina*, six different levels of relative humidity i.e. 50, 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportion in glass desiccators according to the method suggested by Buxton and Mellan by (1934). The composition of the acid solution used was as follows. Petriplates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *Macrophomina phaseolina*, with the help of sterilized cork borer. Inoculated petriplates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 30±1ºC for 7 days with four replications. Observations on mycelial growth were recorded after 7 day of incubation.

Evalution of cultivars of mungbean against *Macrophomina phaseolina*

Thirteen cultivars of mungbean received from RARI, Durgapura, Jaipur (Rajasthan), were evaluated against dry root rot under pot condition. Inoculum multiplied on sorghum medium was applied in pot (20gm/pot) to increase the disease pressure. Inoculum was added ten days before sowing. Seeds were washed thoroughly with sterilized water. Five seeds of each mungbean cultivars were sown in each pot. Three replications of each treatment were maintained under pot condition, in which three un-inoculated susceptible mungbean cultivar sown pots served as check. Observations were recorded after 40 days of sowing and continued up to 60 days. On the basis of disease incidence cultivars were categorized as per criterion followed by Nagamma *et al*. (2015) [10].

Category	Per cent disease incidence % (PDI)
Resistance	0-20
Moderately susceptible	21-50
Susceptible	51-80
Highly susceptible	81-100

The following cultivars were screened against root rot of mungbean, RMG- 492, RMG- 1092, RMG-1028, RMG-975, RMG-1098, RMG-1143, RMG-1141, RMG-1140, RMG-1144, RMG-1086, RMG-1079, RMG-1132 and surface sterilized, un-inoculated seed sown in pot served as check. The disease incidence of root rot was recorded for each cultivar lines.

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

Results and Discussion

Physiological studies

1. Effect of temperature

The entire microorganisms grow under certain range of temperature within which a minimum, optimum and maximum temperature could be located. It is evident from the data (Table 1) that the fungus grows at all the temperature levels ranged from 25 to 40 ˚C. Maximum mycelial growth (90 mm) of fungus was observed at 30 ˚C at 7th day of incubation followed by 35 ˚C (76.52 mm). A gradual decrease in mycelial growth was observed at 25 °C (55.24 mm) and minimum mycelial growth (42.92 mm) at 40 °C.

Table 1: Effect of temperature on mycelial growth of *Macrophomina phaseolina* after 7 days of incubation

S. no.	Temperature (°C)	Mycelial growth (mm)*
1.	25	55.24
2.	30	90.00
3.	35	76.52
4.	40	42.92
	SEm+	1.29
	CD (p=0.05)	3.97

*Average of five replications

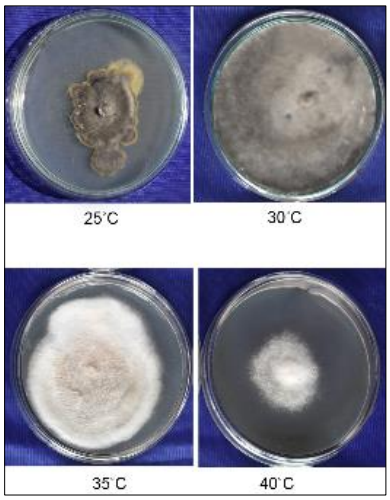

Fig 1: Effect of temperature on mycelial growth of *Macrophomina phaseolina* after 7 days of incubation

2. Effect of relative humidity

It was observed that all the six humidity levels include the growth of *Macrophomina phaseolina.* Perusal of data (Table 2) showed that maximum mycelial growth (90 mm) of *Macrophomina phaseolina* was observed at 80 per cent relative humidity followed by 90 per cent mycelial growth (85.72). A significant decrease in mycelial growth (80.34) was observed at 100 per cent relative humidity and minimum mycelial growth (36.72 mm) was observed at 50 per cent relative humidity.

Table 2: Effect of relative humidity on mycelial growth of *Macrophomina phaseolina* after 7 days of incubation at 30 + 1 °C

S. no.	Relative humidity (%)	Mycelial growth (mm*)
1.	50	36.72
2.	60	39.16
3.	70	65.32
4.	80	90.00
5.	90	85.72
6.	100	80.34
	S.Em+	1.37
	CD (p=0.05)	4.27

*Average of five replications

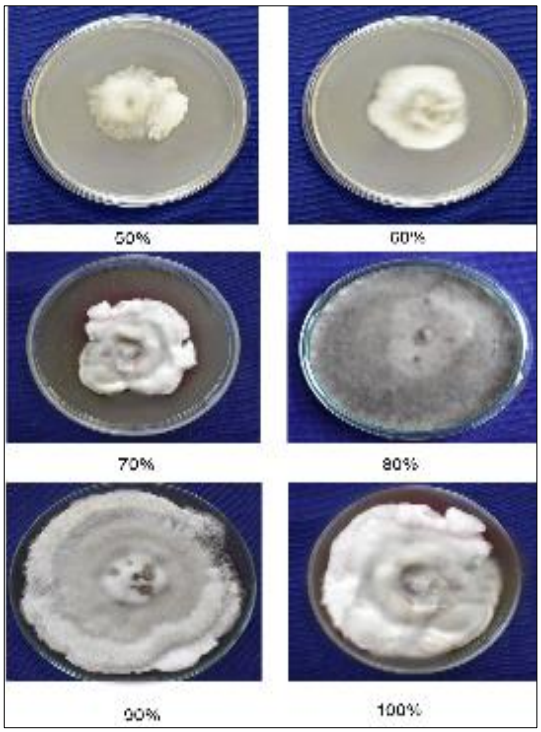

Fig 2: Effect of relative humidity on mycelia growth of *Macrophomina phaseolina* after 7 days of incubation 30±1 ^{0}C

3. Effect of pH

It was observed that all the five pH levels include the growth of *Macrophomina phaseolina* Perusal of data (Table 3) showed that maximum dry mycelial weight (615 mg) of *Macrophomina phaseolina* was observed at 6.5 pH level. A significantly decrease in dry mycelial weight was observed at pH 7.0 (532mg), at 6.0 (420 mg) and at 7.5 (365 mg). Minimum dry mycelial weight (305 mg) was observed at 8.0 of pH level.

The occurrence and development of disease and most of the organisms grow between 0 to 42ºC (Wolf and Wolf, 1947). The present findings, follow the result of Kaur *et al.* (2013) [5] and Khan *et al.* (2012) [6] who mentioned 30 ºC temp as higher growth of mycelium temp followed by 35 ºC, also the pathogen *Macrophomina phaseolina* grew efficiently at 80 to 100 per cent relative humidity, whereas, decline was observed at lower humidity levels. Maximum mycelial growth (90 mm) of *Macrophomina phaseolina* has observed at 80 per cent relative humidity and these observations persue with the result of earlier workers (Ali *et al.*, 1998 and Barcelo and Vega, 1988) [1, 2]. To evaluate the effect of pH on mycelium growth of the fungus, it was exposed directly to different levels of pH. Observations of pH, persue the results of Kaur *et al.* (2013) [5] and Sukanya *et al.* (2016) [14].

Table 3: Effect of pH on mycelial growth of *Macrophomina phaseolina* after 7 days of incubation at 30+ 1 ^{0}C

S. no.	pH level	Dry weight of mycelial growth (mg)*
1.	6.0	420.00
2.	6.5	615.00
3.	7.0	532.00
4.	7.5	365.00
5.	8.0	305.00
	S.Em+	7.95
	CD (p=0.05)	24.49

*Average of five replications

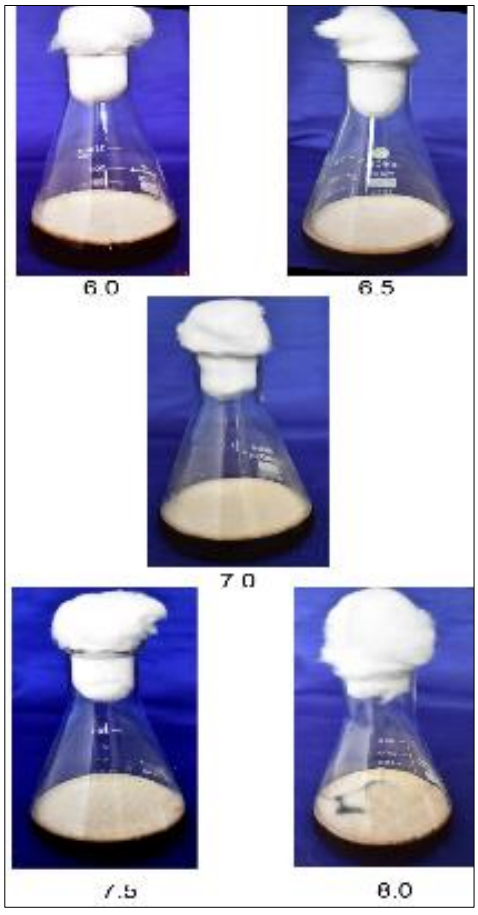

Fig 3: Effect of pH on mycelial growth of *Macrophomina phaseolina* after 7 days of incubation at 30 + 1 ^{0}C

Evalution of cultivars of mungbean against *Macrophomina phaseolina*

Thirteen cultivars were screened against *Macrophomina phaseolina* under artificial conditions (Table 4). None of variety was found immune or resistant to dry root rot

pathogen. Cultivars RMG- 492, RMG- 1092, RMG-1028, RMG-975, RMG-1098, RMG-1143 and RMG-1141 were found moderately susceptible, RMG-1140, RMG-1144, RMG-1086, RMG-1079 were observed susceptible RMG-1132 and Check were found highly susceptible. Lokesha and Benagi (2006) [9] tested twenty four pigeon pea genotypes against dry root rot. Two genotypes, PT-221 and ICPL-90097 had high resistant reaction and V-50, TAT-9621, ICPL-89049 and GS-1 recorded moderately resistant reaction. Choudhary *et al.* (2011) [4] screened twenty five germplasms of mungbean against dry root rot of mungbean caused by *Macrophomina phaseolina* under field conditions. Three genotypes namely MSJ-118, KM-4-44 and KM-4-59 were found resistant to dry root rot. Nagamma *et al.* (2015) [10] were screened chickpea varieties against dry root rot disease in sick plot. They observed that only thirteen varieties *viz*. GNG-1958 (AVT-2), GNG-1999, CSJ-303, NG-3004, CSJ-753, RSG-888, PhuleG-04305, IPCK-07-62, RVSSG-12, HK-08-212, PhuleG-09305, AKG-2002-1K and ICCV-08317 showed resistant reaction under field condition.

Table 4: Evaluation of cultivars of mungbean against *Macrophomina phaseolina*

S. No.	Name of cultivars	Disease incidence (%)	Reaction of cultivars
1	RMG-492	33.33 (35.26)	MS
2	RMG-1140	58.33 (49.80)	S
3	RMG-1144	58.00 (49.60)	S
4	RMG-1092	51.67 (45.96)	MS
5	RMG-1028	50.00 (45.00)	MS
6	RMG-1086	49.15 (44.51)	MS
7	RMG-975	66.66 (54.73)	S
8	RMG-1079	58.33 (49.80)	S
9	RMG-1098	51.12 (45.64)	S
10	RMG-1143	41.66 (40.20)	MS
11	RMG-1141	49.56 (44.75)	MS
12	RMG-1132	91.87 (73.43)	HS
13	Check	63.34 (52.74)	S
	S.Em+	2.87	
	CD (p=0.05)	8.84	

* Mean average of three replications
Figure in parentheses are angular transformed values
Where, Resistance (0-20), MS – Moderately susceptible (21-50), S-Susceptible (51-80), HS- Highly susceptible (81-100)

Conclusion

Physiological studies showed that, mungbean root rot pathogen *Macrophomina phaseolina* prefers high temperature *i.e.* 30ºC, pH 6.5, and 80% relative humidity to grow significantly as well as for disease development. Also, the present study showed the absence of complete resistance to *Macrophomina phaseolina* in the evaluated cultivars of mungbean.

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