Original Research Article

Effect of different temperature, lights, carbon & nitrogen sources and pH on the sporulation and mycelial growth of *Macrophomina phaseolina* isolated from fenugreek *in vitro*.

ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) also known as *Methi* belongs to family *leguminoceae*. The study of physiological parameters for the mycelial growth and sporulation of the *M. phaseolina* was undertaken by culturing the pathogen in different temperature, lights**,** carbon & nitrogen sources and pH conditions. Among the temperature, maximum mycelial growth and sporulation was observed at 35°C.In all the tested different lights maximum mycelial growth and sporulation of *M. phaseolina* was observed in black light. Among the carbon and nitrogen sources maximum mycelial growth and sporulation were observed in sucrose and L-alanine. Respectively, in all the tested different pH levels maximum mycelial growth and sporulation was recorded at 7.0 pH.

INTRODUCTION

Fenugreek believed to be originated from South East Europe and West Asia is cultivated throughout India and other parts of world for leafy vegetables, condiment, medicinal purposes and fodder also. It is rich source of minerals, protein, vitamin A and C. Fenugreek seed is also used as dye making and for extraction of alkaloids or steroids. The dried leaves and flowers are used for flavour. The leaves (per 100 gram of edible portions) contain moisture (per 100 gram of edible portions) 86.1 g, thiamine 0.05 mg, fat 0.9 g, calcium 360 mg, protein 4.4 g, oxalic acid 13 mg, fiber 1.1 g, iron 17.2 mg, potassium 51 mg, mineral 1.5 g, sulphur 167 mg, carbohydrates 6.00 g, vitamin A 6450 IU, magnesium 67 mg, nicotinic acid 0.7 mg, sodium 76.1 mg, vitamin C 54 mg, phosphorus 51 mg and chlorine 165 mg (Bose and Som, 1986).

Fenugreek has medicinal value as it cure cardiovascular diseases, inflammatory diseases, cancer and chronic diseases. It contain anti-inflammatory, antimutagenic, antioxidant (Srinivas, 2014). Now-a-days, it is used as food stabilizer, adhesive and emulsifying agent due to its high fibre, protein and gum content. The protein of fenugreek is found to be more soluble at alkaline pH (Meghwal and Goswami, 2012).

Fenugreek is utilized in many parts of the world in different forms and has been regarded as a treatment for many ailments known to man (Laila *et al*., 2013).

Fenugreek is infected by several fungal, bacterial and viral diseases. Major fungal diseases of fenugreek are Cercospora leaf spot (*Cercospora traversian*), Charcoal rot (*Macrophomina phaseolina* (Tassi.) Goid.), Wilt (*Fusarium oxysporum*

Schlecht.), Downy mildew (*Peronospora trigonellae* Gaum*),* Rhizoctonia root rot (*Thanatephorus cucumeris* Kuhn), Powdery mildew (*Leveillula taurica* (Lev.) Arm), Root rot (*Sclerotinia trifoliorum* Sacc.) and Rust (*Uromyce strigonellae* Pass.) etc.

Macrophomina phaseolina (Tassi.) Goid. infects more than 500 plant species worldwide (Sinclair, 1982) and causes charcoal rot disease in several agronomical important crop including soyabean, maize, sorghum, cotton and fenugreek.

METHOD AND MATERIAL

Collection and Isolation of *Macrophomina phaseolina*

Collection of root rot infected samples and isolation of *Macrophomina phaseolina* from infested fenugreek plants were carried out from field. The infested root samples were gently washed in tap water for removing the soil particles adhering on root surface. The washed root parts were cut into small pieces and surface sterilized in 0.1% sodium hypochloride solution in Petri plates for 1-2 minutes followed by repeated washing in sterilized distilled water. The surface sterilized pieces were transferred aseptically on Potato Dextrose Agar (PDA) medium (Appendix-I) in Petri plates and kept in BOD incubator for 7 days at 28±2°C for growth of the pathogen.

Effect of temperature

It is a well known phenomenon that the temperature effect the considerable influence on the biochemical activity of pathogens. 20 ml of Potato Dextrose Agar was poured in each of sterilized Petri plate. Each Petri plate was inoculated aseptically by placing a 5 mm disc in the centre from actively growing 7 days old culture of pathogen. The inoculated Petri plates were incubated at 20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C. Temperature respectively for 7 days and observation of growth and sporulation was recorded.

Effect of light

Effect of different light intensity on the mycelial growth of *M. phaseolina*was tested by keeping the inoculated Petri plates wrapped in coloured sheets. Present study has been taken red, black, yellow, green and normal light conditions. The inoculated petri plates were inubated at 28±2°C for 7 days with three replications. Mycelial growth was recorded after 7 days of incubation.

Effect of carbon and nitrogen sources

To find out the effect of various carbon sources, like glucose, sucrose, maltose, fructose and lactose on growth of *Macrophomina phaseolina*. The sucrose content of basal medium Czapek's dox agar was substituted by adding different sources of carbon on equivalent basis (12.63 g in 30 g of sucrose). Inoculated Petri plates containing basal medium supplemented by different carbon sources were incubated at 28±2°C for 7 days and the mycelial growth and sporulation were recorded.

To find out the effect of various nitrogen sources on growth of *Macrophomina phaseolina*, sodium nitrate of basal medium Czapek's dox agar medium was substituted by adding different sources of nitrogen on equivalent basis (329 mg in 2 g of sodium nitrate) to study the effect of different nitrogen sources on the growth of *Macrophomina phaseolina*. The inoculated Petri dishes containing basal medium supplemented with different nitrogen sources were incubated at 28±2°C for 7 days and observation for mycelial growth of each isolate and sporulation was recorded. Nitrogen sources studied were Ammonium chloride, L-alanine, L-agrinine and Glutamic acid.

Effect of hydrogen ion concentration (pH)

The study of different pH levels *i.e.* 6.0, 6.5, 7.0, 7.5, 8.0 were undertaken with a view to ascertain the effect of different hydrogen ion concentration of the medium on growth of the fungus. The initial pH of the basal medium before autoclaving was adjusted 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 28±2°C for 7 days and observation of growth and sporulation was recorded. The number of sclerotia were observed microscopically and graded as below. (Tandel and Sabalpara, 2011)

Chart 1 : Gradation and score

Score	Grade	Number of sclerotia /microscopic field) at 100X	Score
++++	Excellent	>50	++++
+++	Good	30-50	+++
++	Fair	21-30	++
+	Poor	10-20	+
-	No sporulation	-	-

RESULT AND DISCUSSION

Effect of temperature

Five different temperature *viz*., 20°C, 25°C, 30°C, 35°C and 40°C were tested on mycelium growth and sporulation of *M. phaseolina*. Data indicated in table 1 revealed

that among the tested temperature, highest mycelial growth was recorded at 35°C (90.00 mm) followed by 30°C (80.62 mm), 25°C (68.72 mm), 40°C (49.41 mm) and found least at 20°C (46.61 mm). All the temperature regimes tested showed a wide range of sporulation from none (-) to excellent (++++). However, excellent (++++) sporulation was observed at temperature 35°C, good (+++) sporulation were recorded 30°C and at the temperature 20°C and 40°C there was poor (+) sporulation of the test fungus. In the present study, the excellent mycelial growth and maximum sporulation was recorded at 35°C followed by 30°C. Hence, the temperature range of 30°C to 35°C can be favorable to obtain maximum mycelial growth and sporulation of *M. phaseolina*.

Our findings are similar to the results reported by Thombre and Kohire (2018) who tested seven different temperatures and observed the best temperatures for mycelium growth found were 35°C (90.00 mm), 30°C (78.84 mm) and 25°C (69.12 mm). This was followed by the temperatures 20°C (45.39 mm) and 15°C (17.93 mm) and excellent (++++) sporulation was recorded at temperature 35°C. Good (+++) sporulation were observed at temperature 30°C at 25°C and 40°C and at the temperature 45°C there was not any sporulation of the fungus. Temperature higher than optimum (35°C) showed pronounced adverse effect on growth as compared to the temperatures lowers than optimum. Similar results were also obtained by Patel and Patel (1990).

Table 1 Effect of different temperature on the mycelial growth and sporulation of *M. phaseolina in vitro*

Temperature(°C**)**	**Mycelial growth (mm)**	**Sclerotia per microscopic field**
20	46.61 (43.03)*	+
25	68.72 (55.97)	++
30	80.62 (63.86)	+++
35	90.00 (71.53)	++++
40	49.01 (44.41)	+
S.Em±	0.70	
CD (P = 0.05)	2.24	

*Figure in parentheses are angular transformed value ++++; Excellent, +++; Good, ++; Fair, +; Poor, -; Absent

Effect of light

To study the effect of different lights on the mycelial growth and sporulation of *M. phaseolina* five different lights conditions were used *viz*., normal, red, black, yellow and

green light conditions. Data presented in table 2 showed that maximum mycelial growth was observed in black light (90.00 mm) followed by normal light (86.77 mm), yellow (84.08 mm), red (78.23 mm) and green (77.26 mm). Excellent (++++) sporulation was observed at black light, good (+++) sporulation was observed at normal light and at the red and green lights there was poor (+) sporulation of the test pathogen. In the present study, the excellent fungal growth and maximum sporulation was observed at black light followed by normal light. Based on the findings, it can be recommended that for obtaining maximum mycelial growth and sporulation, *M. phaseolina* culture should be exposed in black to normal light. Similarly, findings were recorded by Khamari *et al.* (2018) and Sobrinho *et al*. (2004) that maximum mycelial growth was observed in black light.

Effect of carbon and nitrogen sources

Carbon sources *viz*., glucose, sucrose, maltose, fructose, and lactose were used to observe the effect of carbon sources on mycelial growth and sporulation of *M. phaseolina*. Data showed in table 3 revealed that highest mycelial growth (90.00 mm) in sucrose followed by fructose (87.61 mm), maltose (64.58 mm), glucose (64.41 mm) and lactose (41.13 mm). Similarly other physiological parameters all the carbon sources showed a wide range of sporulation from none (-) to excellent (++++). However, carbon source sucrose was recorded excellent (++++) sporulation. Rest of carbon sources also good (+++) to poor (+) sporulation were recorded. On the basis of results, it can be recommended that for obtaining maximum mycelial growth and sporulation *M. phaseolina* culture should be exposed in sucrose to fructose carbon sources.

Table 2 Effect of different lights on the mycelial growth and sporulation of *M. phaseolina in vitro*

Lights	Mycelial growth (mm)	Sclerotia per microscopic field
Normal	86.77 (68.65)*	+++
Red	78.23 (62.17)	++
Black	90.00 (71.53)	++++
Yellow	84.08 (66.47)	+++
Green	77.26 (61.49)	++
S.Em± CD(P=0.05)	0.56 1.80	

*Figure in parentheses are angular transformed value ++++; Excellent, +++; Good, ++; Fair, +; Poor, -; Absent

Table 3 Effect of different carbon sources on the mycelial growth and sporulation of *M. phaseolina in vitro*

Carbon sources	Mycelial growth (mm)	Sclerotia per microscopic field
Glucose	64.41 (53.35)*	++
Sucrose	90.00 (71.53)	++++
Maltose	64.58 (53.45)	++
Fructose	87.61 (69.39)	+++
Lactose	41.13 (39.62)	+
S.Em± CD (P = 0.05)	0.56 1.81	

*Figure in parentheses are angular transformed values ++++; Excellent, +++; Good, ++; Fair, +; Poor, -; Absent

Nitrogen sources *viz*., ammonium chloride, glutamic acid, L-alanine and L-agrinine were tested the effect of nitrogen sources on mycelial growth and sporulation of *M. phaseolina*. Data showed in table 4 revealed that highest mycelial growth (84.78 mm) in L-alanine followed by glutamic acid (83.49 mm) and L- arginine (62.75 mm). However, ammonium chloride (48.49 mm) yielded the lowest mycelial growth. All the tested nitrogen sources showed a wide range of sporulation from none (-) to excellent (++++). However, nitrogen sources L-alanine and glutamic acid was observed excellent (++++) sporulation. Remaining nitrogen sources showed good (+++) to poor (+) sporulation respectively, L-agrinine and ammonium chloride were recorded. Based on the findings, it can be recommended that for obtaining maximum mycelial growth and sporulation *M. phaseolina* culture should be exposed in L-alanine to glutamic acid nitrogen sources.

Similar, findings were recorded by Das (1998), Mukhopadhyay and Nandi (1975), Kumar and Choudhary (2020) and Thombre and Kohire (2018) observed that maximum mycelial growth and sporulation was observed with carbon sources sucrose and nitrogen sources L-alanine.

Table 4 Effect of different nitrogen sources on the mycelial growth and sporulation of *M. phaseolina in vitro*

Nitrogen sources	Mycelial growth (mm)	Sclerotia per microscopic field
Ammonium chloride	48.49 (44.12)*	+
Glutamic acid	83.97 (66.42)	++++

L-alanine	84.78 (67.03)	++++
L-arginine	62.75 (52.37)	++
S.Em± CD (P = 0.05)	0.82 2.72	

*Figure in parentheses are angular transformed values++++; Excellent, +++; Good, ++; Fair, +; Poor, -; Absent

Effect of different pH

Five different pH levels were used to study the effect of pH on mycelium growth and sporulation of *M. phaseolina.* Data showed in table 5 revealed that among the tested pH, highest mycelial growth and sporulation was observed at pH 7.0 (90.00 mm) followed by pH 6.5 (87.92 mm), pH 7.5 (84.74 mm), pH 6.0 (83.08 mm) and found minimum at 8.0 pH (79.53 mm). Excellent (++++) sporulation were observed at pH 7.0 and pH 6.5, good (+++) sporulation were recorded at pH 7.5 and pH 6.0, Fair (++) sporulation were recorded at pH 8.0 of the test pathogen. Hence, the pH range of 6.5 to 7.0 can be suitable to obtain excellent mycelial growth and sporulation.

Similar results were obtained by Salunkhe *et al.* (2009) and Thombre and Kohire (2018) they reported that maximum mycelial growth and sporulation was found to be 7.0 pH (90.00 mm). Followed by pH levels *viz*., 6.5 (87.42 mm), 7.5 (84.30 mm) and 6.0 (83.93 mm), 8.0 (80.00 mm) and 5.5 pH (77.52 mm). The maximum sporulation also influenced by 7.0 pH followed by 6.5 pH.

Table 5 Effect of different pH on the mycelial growth and sporulation of *M. phaseolina in vitro*

pH levels	Mycelial growth (mm)	Sclerotia per microscopic field
6.0	83.08 (65.70)*	+++

6.5	87.92 (69.66)	++++
7.0	90.00 (71.53)	++++
7.5	84.74 (67.03)	+++
8.0	79.53 (63.13)	++
S.Em± CD (P = 0.05)	1.01 3.23	

*Figure in parentheses are angular transformed values ++++; Excellent, +++; Good, ++; Fair, +; Poor, -; Absent

CONCLUSION

It can be concluded unequivocally considering the results In physiological studies, favourable temperature for mycelial growth and sporulation was 35°C. Maximum mycelial growth and sporulation favoured by black light condition. From the carbon and nitrogen sources sucrose and L-alanine were favour the maximum mycelial growth and sporulation. Best pH for the mycelial growth and sporulation of *Macrophomina phaseolina* was 7.0 pH.

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