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Influence of Carbon, Nitrogen, Temperature and pH on the Growth of *Cercospora nicotianae* Causes Frog Eye Leaf Spot Disease of Tobacco

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The paper gives an account of the variations in nutritional and physiological characteristics found in different isolate of *Cercospora nicotianae* causing frog eye leaf spot disease of tobacco, in India. The pathogen under study varied in their ability to grow in different temperature and pH levels and to utilize different carbon and nitrogen sources. Fructose was found to be the best source of carbon for the growth and sporulation of this pathogen. Among the nitrogen sources tested, Ammonium nitrate supported the maximum growth of the fungus followed by potassium nitrate.The optimum temperature range is between 20 to 30ºC levels are identified as ideal for growth and sporulation. The least dry mycelial weight was found at below 20ºC and above 40º C. The pathogen grew well at pH of 5 to pH 6 favoured maximum growth and development of the fungus. Whereas, least growth was observed at pH 3.

Keywords: Tobacco, frog eye leaf spot, *Cercospora nicotianae*, Nutritional, physiological studies.

India is one of the principal tobacco producing countries of the world and has attained its commercial importance in India. It is mainly grown in states of Maharashtra, Karnataka uttar Pradesh, Gujarath, West Bengal, Andhra Pradesh and Tamil Nadu. The successful cultivation in recent years has met with different traumas such as pest and diseases and climate for the farmers of Karnataka, Maharashtra and Andhra Pradesh in sorthern Karnataka**.**The tobacco leaves is prone to many diseases, among which frog eye leaf spot is caused by *C nicotianae* Ell. and Eve. is an important destructive disease. In India, frog eye leaf spot disease was first reported by Vasudeva (1963). The fungus derive food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon which all the fungi can grow and reproduce. Therefore studies were conducted in different suitable media to identify surface medium for the growth and sporulation of *C. nicotianae.*

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MATERIALS AND METHODS

Studies of the following nutritional and physiological aspects of *C. nicotianae* were conducted *in vitro.*

Tobacco leaves infected with frog eye leaf spot were collected from infected field and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by following standard tissue isolation technique. Identification of the fungus was carried out based on the morphological characters of the isolated fungus.

Nutritional studies

The utilization of carbon and nitrogen nutrition was studied by replacing the sucrose and potassium nitrate in the basal medium with various nitrogen and carbon compounds on the molecular weight basis. Czapek's (dox) broth was used as a basal medium for studying carbon and nitrogen. Fourty milliliters of the medium dispensed in 150 ml conical flasks were sterilized and used for inoculation with the fungus. All the flasks were inoculated with 5 mm diameter mycelial disk obtained from 7 day old single spore cultures of *C. nicotianae* isolates and incubated at 28±10C for ten days. The cultures were filtered through Whatman No. 42 filter paper and the dry mycelial weight was measured by subtracting the initial weight of the filter paper from the weight of the filter paper along with the mycelial mat.

Carbon Utilization

Carbon compounds tested in the study were dextrose, fructose, sucrose, lactose, glucose and maltose. The carbon requirement of the fungus was studied in Czapek's (dox) broth, the quantity of carbon compounds added was determined based on their molecular weights. So as to provide equivalent amount of carbon as sucrose present in basal medium. Each flask containing different carbon sources was inoculated with a 5mm mycelial disk of 12 day's old fungal cultures and incubated for 19 days.

Nitrogen Utilization

Six different nitrogen sources used in this study were urea, potassium nitrate, ammonium nitrate, ammonium choloride and sodium nitrate.Different nitrogen sources were added into Czapek's (dox) broth on the basis of their molecular weights, so as to provide an equivalent amount of nitrogen as sodium nitrate in the basal medium.All the nitrogen sources were dissolved properly and then sterilized at 1.1 kg cm-2 pressure for 20 min. Each treatment was replicated thrice. The flasks were inoculated as described earlier and incubated at 25^0C for 19 days. The mycelial growth was harvested and recorded the dry mycelial weight. The results were analyzed statistically.

Physiological studies

Temperature Requirement

The pathogen was subjected to different temperature conditions to study the best-suited temperature level for the growth and sporulation of the fungus. potato dextrose broth was used in one experiment to study the growth characters. Fourty milliliters of potato dextrose broth was poured into each 100 ml conical flask and inoculated with 5 mm mycelial disc of each isolate. Inoculated petriplates and conical flasks containing Richard's medium were incubated at 15^0 C, 20^0 C, 25^0 C, 30^0 C, 35^0 C, 40° C and room temperature. The experiment was replicated thrice.. The dry mycelial weight at each temperature level was recorded after incubating for 18 days and the data were analyzed statistically.

Hydrogen-lon concentration (pH)

The pH is the most crucial physical factor regulating vegetative growth as well as reproductive activity of the fungus. Effect of pH on the growth of *Cercospora nicotianae* was also tested in the laboratory using liquid cultures containing different pH levels. potato dextrose broth medium was used to study the effect of pH of medium on the growth characters. Fourty milliliters of potato dextrose broth medium was poured into a 100ml conical flask under aseptic conditions. The Reaction of the medium was adjusted to the desired pH by adding 0.1N NaOH or 0.1N HCl (Naik *et al* 1988). The medium was buffered with Disodium hydrogen phosphate citric acid buffer according to the schedule of Vogel (Vogel 1951). Flasks were sterilized at 121 0C at 15 *psi* for 20 minutes. Each flask was inoculated with each isolate using 5 mm diameter mycelial disc in sterile conditions. Inoculated flasks were incubated at 25^0C for 18 days and the dry mycelial weight and extent of sporulation were obtained**.** . Each treatment was replicated thrice. The dry mycelial weight was obtained after incubating for 18 days as described earlier and the results were analyzed statistically.

RESULTS AND DISCUSSION

Effect Carbon utilization

The effect of different carbon sources on the mycelia growth was significant. It has been shown that different fungi respond differently with a particular compound and the fungus exhibited marked variation in the utilization of different carbon sources. All the carbon sources favoured the growth of *C. nicotianae*. However, among the tested carbon sources Fructose (34.07 mg) supported the Maximum dry mycelia weight of the fungus followed by dextrose (27.00 mg), lactose (25.47 mg), and sucrose (23.07 mg). However, the least dry mycelial weight was recorded on Glucose (11.57 mg). The results are in confirmation with that of by wokers. [1, 3 and 4]

Nitrogen utilization

Nitrogen is a very important element for the growth and development of pathogen because it induces protein synthesis but all the sources of nitrogen are not equally good for the growth of the fungi. Among the different tested nitrogen sources, Ammonium nitrate, was efficiently utilized by *C.nicotianae* showing highest dry mycelial weight of 24.53 mg which was significantly superior to rest of the sources tested, followed by ammonium chloride (22.01 mg) and potassium nitrate(18.73 mg) respectively. The least dry mycelial weight of 13.47 mg and 11.87 mg was recorded in sodium nitrate and urea. Similar results were also reported by earlier worker [1].Utilization of nitrogen sources differ significantly among different isolates as presented in the Table 2.

Physiological studies

Temperature

Among the external abiotic factors which influence the growth of fungi, temperature plays an important role. Temperature affects almost every function of fungi, including growth, spore germination and reproduction. The fungus under study grew best at temperature of 25^{o}C (59.40 mg

Table 1. Effect of different Carbon source on growth of *C.nicotianae*

S. No	Carbon source	Dry mycelial weight (mg)
1	Sucrose	23.70
2	Dextrose	27.00
3	Fructose	34.07
4	Lactose	25.47
5	Glucose	11.57
6	Maltose	19.03
S.Em±	0.30	
CD (1%)	1.33	

Table 2. Effect of different nitrogen source on growth of *C.nicotianae.*

S. No.	Nitrogen source	Dry mycelial weight (mg)
1	Sodium nitrate	13.47
2	Potassium nitrate	18.73
3	Ammonium nitrate	24.53
4	Urea	11.87
5	Ammonium chloride	22.01
S.Em±	0.24	
CD (1%)	1.10	

Table 3. Effect of different temperature level on the growth of *C. Nicotianae*

S. No.	Temperature (^{0} C)	Dry mycelial weight (mg)
1	15	34.67
2	20	46.80
3	25	59.40
4	30	55.70
5	35	25.93
6	40	17.77
7	control	51.87
S.Em±	0.70	
CD (1%)	2.97	

Table 4. Effect of different pH levels on the growth of *C. nicotianae*

S. No.	pH level	Dry mycelial weight (mg)
1	3	23.20
2	4	34.39
3	5	54.78
4	6	49.71
5	7	38.38
S.Em±	0.41	
CD (1%)	1.87	

dry mycelia weight), which was significantly superior to all other temperature levels followed by, 30°C (55.70 mg), at room temperature 27°C (51.87 mg) and 20° C (46.80 mg).The least dry mycelia growth was recorded at 35 and 40°C temperature (25.93 and 17.77 mg). whereas optimum temperature range is between 20 to 30°C levels are identified as ideal for growth of *C. nicotianae*.. The sporulation was excellent at 30° C, good at 25° C and moderate at 35° C. Poor sporulation was recorded at 20° C and no sporulation at 15° C. Light brown mycelial colour was observed at 20° C and 35°C. Similarly, findings were reported by worker[3] reported, 20-30°C as an optimum temperature range for a *C. Nicotianae* and also Similar result was also obtained on *C. canescens* by earlier worker [5].

Hydrogen ion concentration (pH)

The pH is the most crucial physical factor regulating vegetative growth as well as reproductive activity of the fungus. Growth of the fungus was obtained at all the pH levels tested but it was maximum at pH 5 where it was 54.78 mg after 19 days of inoculation . pH 6 (49.71 mg) and pH 7 (38.38 mg) were also favourable. Whereas the least dry mycelia weight of 23.20 mg was recorded at pH 3. Growth of the fungus decreased by increasing or decreasing the pH level from the neutral level. The results of the present study are in agreement with those achieved by earlier researcher [3] .This fungus can tolerate a wide range of pH 4-8 was reported by worker [2].

CONCLUSION

The paper gives an account of the variations in nutritional and physiological characteristics found in *Cercospora nicotianae* causing frog eye leaf spot disease of tobacco, in India. The pathogen under study varied in their ability to grow in different temperature and pH levels and to utilize different carbon and nitrogen sources. Fructose was found to be the best source of carbon for the growth and sporulation of this pathogen. Among the nitrogen sources tested, Ammonium nitrate supported the maximum growth of the fungus followed by potassium nitrate.Physiological studies revealed that temperature has lot of influence on pathogen growth by influencing maximum dry mycelial weight was recorded at the temperature ranging from 20° C to 30° C. The least dry mycelial weight was found at below 20°C and above 40° C. The effect of pH on the growth of pathogen revealed that pH 5.0 to 6.0 favoured maximum growth and development of the fungus. Whereas, least growth

REFERENCES

1. Khatib., Studies on leaf spot of greengram caused by *Cercospora canescens Ellis and Martin. M.Sc. (Agri) Thesis, Univ. Agric. Sci.,* 2012; Bangalore (India).
2. Lim, Y. S. and Kim, B. S., Sporulation of *Cercospora capsici* causing *Cercospora* leaf spot of pepper. *Res. Pl. Dis.,* 2003; **9**(3)**:**162-165.
3. Mallappa., Studies on frog eye leaf spot of bidi tobacco caused by *C. nicotianae* Ell. & Eve. *M. Sc. (Agri) Thesis, Univ. Agric. Sci.,* 2007; Dharwad (India).
4. Prasanth S.P., Investigations on purple seed stain of soybean caused by *Cercospora kikuchii. M.Sc. (Agri.) Thesis,* Univ. Agric. Sci., 2004; Dharwad (India).
5. Poornima., Studies on *leaf spot of palak Cercospora beticola Sacc. M. Sc. (Agri) Thesis,* Univ. Agric. Sci., 2010; Dharwad (India).