Research Paper :

Effect of different carbon and nitrogen sources on the growth and sporulation of *Alternaria alternata* (Fr.) Keissler causing leaf blight of cowpea

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SUMMARY

The pathogenic fungus was isolated on PDA medium. The pathogen was taxonomically identified as *Alternaria alternata* (Fr.) Keissler. The colony of *Alternaria alternata* was circular, grayish black with whitish growth on the upper surface on PDA with profuse growth and sporulation. The good growth and sporulation of the test fungus was obtained on Maltose as a source of carbon while Ammonium nitrate was found to be good nitrogen source for growth of the test fungus.

Key words : *Alternaria alternata*, Carbon and nitrogen source, Cowpea

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India grows a variety of pulse crops on about 223.91 lakh hectares with annual production of 133.81 lakh tonnes. In Maharashtra, pulses are cultivated on 34.32 lakh hectares area with a productivity of about 584 kg $^{-ha}$ (Anonymous, 2007). Among the pulses, cowpea [*Vigna unguiculata* (L.) Walper] is nutritionally the most important legume crop containing 63.6 per cent carbohydrates, 24.8 per cent proteins, 1.9 per cent fats, 6.3 per cent fiber, 3-3.8 per cent ash and 9-11 per cent moisture. It is a rich source of Calcium and Iron.

Area under cowpea in India is to the tune of 1.5 million hectares with annual production of 0.5 million tonnes (Reddy, 2004). In Maharashtra, it is cultivated on 11800 hectares area with a productivity of about 390 kg $^{-ha}$ (Apte and Jadhav, 2002). In the Konkan region of Maharashtra cowpea is grown as a sole crop, mostly during late *Kharif* or *Rabi* or *summer* season after rice on 1200 hectares area with a productivity of 400 kg^{-ha} (Apte and Jadhav, 2002).

Among the various diseases of cowpea, the leaf blight caused by *Alternaria alternata* was noticed in severe form on cowpea crop at the farms of Agril. Botany and Agronomy, College of Agriculture, Dapoli during summer sown crop in the year 2008. The disease incidence was observed to be more than 40 per cent. Prevalence of such newly introduced leaf blight disease on cowpea in Konkan region was found damaging. Since no research was undertaken, on this disease in Konkan, therefore, it was felt necessary to carry out the investigation on the physiological aspects of the causal organism.

MATERIALS AND METHODS

To study the effect of various carbon sources on the growth and sporulation of the test fungus, the amount of carbon present in 50 g of sucrose in the basal medium (Richard's medium) was calculated and replaced by an equivalent amount of carbon compounds calculated on the basis of their molecular weight (Table 1).

Richard's medium broth of 100 ml quantity was prepared without sucrose for each carbon source and 25 ml of medium was dispensed in each 100 ml conical flasks. Four replications per treatment were maintained. One additional treatment was kept as control without adding any carbon source. After sterilization, the flasks were inoculated with 7 days old culture of the test fungus and incubated 27 ± 1^{0}C for 10 days. After incubation period, the mycelial mat was filtered through Whatman's No. 42 filter paper. Before use, the filter papers were previously oven dried at 70^{0}C for 3 consecutive days until constant dry weight was achieved and weighed (W_1) after keeping them in dessicator. The mycelial mat over the filter paper disc was washed three times with sterilized water in order to remove the traces of salts adhering to mycelial mat and then, filter papers along with

mycelial mats were dried upto constant weight in an oven at 70^{0}C for 48 hours. After drying, the filter papers were cooled in moisture free dessicator and weighed (W_2) immediately. The weight of dry mycelium (W_3) was calculated as $W_3=W_2–W_1$. Out of four replications, three were used for recording dry mycelial weight and fourth replicate was used to count the number of spores per microscopic field at low power and accordingly, spore formation grades were assigned.

To study the effect of nitrogen sources, the amount of nitrogen present in 10 g of potassium nitrate in the basal medium was calculated and replaced with an equivalent amount of nitrogen present in inorganic and organic compounds (Table 2). The rest of the procedure was the same as described earlier in case of carbon studies.

Table 1 : Effect of different carbon sources on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Carbon sources	Quantity g/lit	Average dry mycelial wt. (mg)*	Sporulation
1.	Glucose	52.62	358.33	++
2.	Maltose	49.9	445.00	++++
3.	Sucrose	50.0	293.83	+
4.	Starch	47.36	397.17	+++
5.	Mannitol	53.20	292.00	+
6.	Sorbitol	53.20	245.00	+
7.	Citric acid	61.39	0	-
8.	Control	-	91.50	-
S.E. ± :			3.95	
C.D. (P=0.01)			16.63	

* Mean of three replications.
Sporulation: + + + + Excellent, + + + Good, + + Moderate, + Poor, - Nil.

RESULTS AND DISCUSSION

Seven different carbon sources *viz*., glucose, maltose, sucrose, starch, mannitol, sorbitol and citric acid were tried to determine their effect on growth and sporulation of *Alternaria alternata.* The observations recorded on dry mycelial weight of the test fungus on different carbon sources are depicted in Table 1. Basal medium (Richard's medium) without carbon source served as control.

Data of Table 1 reveal that the maximum mycelial growth of the test fungus was recorded on maltose followed by starch, glucose, sucrose, mannitol and sorbitol. No growth was observed in citric acid. Excellent sporulation was recorded in the medium containing maltose as carbon source. Good sporulation was observed in starch. Glucose supported moderate sporulation. Poor sporulation was recorded in sucrose, mannitol and sorbitol, where as no sporulation was observed in citric acid.

These findings are similar to those of Goyal (1977) who reported that maltose was the best source of carbon for growth and sporulation of *Alternaria alternata* followed by sucrose, starch and lactose. Shinde (1991) also observed that the maltose was the best carbon source for growth and sporulation of *A. alternata*. No growth and sporulation was observed in citric acid. Nair (1997) had similar views regarding citric acid in which no growth and sporulation of *A. alternata* was observed by him.

Data presented in the Table 2 reveal that the maximum growth of the test fungus was observed in Ammonium nitrate followed by Ammonium sulphate, Sodium nitrate, Urea, Potassium nitrate and Ammonium chloride. These results are in conformity with Nair (1997) who observed that Ammonium nitrate supported

Table 2 : Effect of different nitrogen sources on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Media	Quantity g/lit	Average dry mycelial wt. (mg)*	Sporulation
Inorganic compounds				
1.	Ammonium nitrate	3.94	442.00	++++
2.	Ammonium sulphate	6.50	419.00	++++
3.	Potassium nitrate	10.0	358.00	++
4.	Sodium nitrate	8.37	408.33	++++
5.	Ammonium chloride	11.63	239.33	+
Organic compounds				
6.	Urea	2.95	364.00	++
7.	Control		192.67	+
S.E. ± :		2.99		
C.D. (P=0.01)		12.95		

* Mean of three replications.
Sporulation: + + + + Excellent, + + + Good, + + Moderate, + Poor, - Nil.

maximum growth followed by Sodium nitrate and Potassium nitrate. Shinde (1991) reported maximum growth of marigold isolate of *Alternaria alternata* on Ammonium nitrate followed by Sodium nitrate and Potassium nitrate. Similarly, Goyal (1977) recorded maximum growth of *A. alternata* on Sodium nitrate, Ammonium nitrate and Ammonium sulphate.

The excellent sporulation was observed in Ammonium nitrate, Ammonium sulphate and Sodium nitrate, where as Potassium nitrate and Urea supported moderate sporulation. Poor sporulation was observed in Ammonium chloride and control (without nitrogen source). These finding are in accordance with those of Ingawale (1996) who reported the excellent sporulation of *A. alternata* in Ammonium nitrate and good sporulation in Sodium nitrate and ammonium sulphate. However, Goyal (1977) and Nair (1997) recorded excellent sporulation of *A. alternata* in Potassium nitrate. These reports seem to be contrary to the present findings, where moderate sporulation was observed in potassium nitrate and urea.

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