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Effect of carbon and nitrogen sources on growth and sporulation of *Fusarium moniliforme*

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In the present investigation, the potent producer of GA_3 *Fusarium moniliforme* Isolate III (ITCC No. 4916) has been tested for nutritional requirements. The growth and sporulation of this isolate was studied on different carbon and nitrogen sources. Six carbon sources (glucose, fructose, lactose, starch,galactose and xylose) were tested. Out of which, glucose was found to be best carbon source. The supply of different forms of nitrogen (sodium nitrate, ammonium sulphate, ammonium chloride, ammonium nitrate, glycine and aspartic acid)showed glycine was the best source for the maximum growth and sporulation. Thirty six differents combinations and concentrations of C : N ratio tested for Isolate III. Among these ratio's 30 :1.0 was found to be the best combination for maximum growth and sporulation

Key words: *Fusarium moniliforme*, carbon, nitrogen, C : N ratio .

INTRODUCTION

Fusarium moniliforme (Gibberella fujikuroi) is the most potent fungus leading to the discovery of the growth promoting subtances and production of gibberellins. Sanchez-Marroquin (1963) tested 43 strains of *Fusarium* species and reported the gibberellic acid production level in *F. moniliforme*. In India, Thakur and Vyas (1983) screened several isolates of *Fusarium* and tested the growth promoting activity by qualitative and quantitative analysis. There are several factors like superior isolate, optimum temperature, pH, incubation period, basal medium and nutritional compounds (carbon, nitrogen) and their ratio's which are affecting the growth and sporulation of fungus. Generally, the fungi exhibit heterotrophy and obtain their carbon from various organic carbohydrate sources. Bechet and Iordeche (1983) studied the growth of *F. solani* on different culture media with various carbon and nitrogen sources and later on studied the development of *F. oxysporum var. solani* on media with different sources of carbon and nitrogen. Bechet and Angelescu (1985) studied *F. avenaceum* in relation to the effect of carbon and their development *in vitro*. Sanchez-Marroquin (1963) reported that a variety of organic and inorganic nitrogen sources, including those from plant and animal, were evaluated by various workers in order to study their effect on the production of GA_3. El-Masry (1991) grew *F. moniliforme* on media containing ammonium nitrate at 500 mg N lit^{-1} of nitrogen source. Mandal and Chaudhuri (1960) studied different carbon and nitrogen sources and had varied effect on induction of chlamydospore in *F. moniliforme*. El-Masry (1991) used 2% destran as carbon source, ammonium nitrate 500 mg N lit^{-1} as nitrogen source, for the synthesis of dextrose by *F. moniliforme*. There is a direct correlation between growth and sporulation of fungus and the production of secondary metabolites. For this it is essential to test the physiological parameters for the particular isolate which is to be consider as important. Now-a-days mycologists and microbiologist are busy in using gibberellin in various crop production especially in rice and grapes in India thereby paying exorbitant price in the market because it is being imported from China and U.S.A.

MATERIALS AND METHODS

Utilization of carbon sources

Effect of various carbon sources on the growth and sporulation was studied adding different carbon compounds singly in the place of sucrose in the basal medium. Six carbon sources viz., glucose, fructose, lactose, starch, galactose and xylose were tested for their utilization by *F.moniliforme* Isolate III grown on

Richard's medium. Three replicates of each treatment were maintained. Various sources of carbon were added so as to supplement an equal amount of carbon present in 50 g of sucroce, a constituent of Richard's medium. Starch was added equal in weight of sucrose due to it's unknown composition. These experiments were conducted in 50 ml of broth poured into 250 ml of conical flask. The pH of the medium was adjusted at 5.0 for obtaining good growth of *F.moniliforme* isolate. After sterilization at 121°C for 20 minutes and removing the lag effect, these flasks were inoculated in triplicate for each treatment with 5.5 mm. disc of actively growing mycelium of the selected fungi. Inoculated flasks were incubated at 30±1°C for 15 days. Mycelial mats were collected on Whatman's No. 42 filter paper and dried in hot air oven at 70°C for three consecutive days, the dried mats were weighed on Sartorius pan balance and the values tabulated after deducting the weight of original filter paper. Proper controls were also maintained.

Utilization of nitrogen source

Effect of six nitrogen compounds viz., sodium nitrate, ammonium sulphate, ammonium chloride, ammonium nitrate, glycine and aspartic acid was studied on *F.moniliforme* Isolate III.Three replications of each treatment were maintained besides control. Various sources of nitrogen present in 10 g of potassium nitrate as constituent of Richard's medium were replaced. The remaining procedure was the same as outlined in carbon utilization studies.

Utilization of interaction of carbon and nitrogen (C: N) compounds on growth and sporulation

Since, glucose as carbon and glycine as nitrogen source supported maximum growth of these fungi amongst other combination tested, it was therefore thought desirable to study the C: N ratio at various concentrations ranging from 5:1, 10:2.5, 15:5.0, 20:7.5, 25:10 and 30:15 (C: N ratio expressed in g^{-1}) and see their effect on growth and sporulation of *F. moniliforme* Isolate III. The remaining procedure of experimental details are same as in the carbon and nitrogen utilization studies.

RESULTS AND DISCUSSION

Isolate III was grown on six different carbon compounds and their effect on growth and sporulation was recorded (Fig. 1). It is was not significant in the absence of carbon. Glucose supported best growth and sporulation in the present study. In case of fructose, growth of fungus was slightly less than glucose followed by galactose, Starch, xylose and lactose. However, excellent sporulation was observed in case of galactose while good sporulation was observed in xylose and lactose. Starch supported moderate sporulation. Based on these experimental results, glucose was selected as a good carbon source for the growth and sporulation. Mitra (1979) undertook the carbon requirement study of three isolates of *F. moniliforme var. subglutinans* and glucose was selected as the best carbon source for maximum growth and sporulation. Jackson and Freer (1991) reported that glucose also provide excess carbohydrate in media and significantly increased fusarin C synthesis. Galactose was found to be poor source of carbon for *F. udum* by Natarajan (1958). Oritsejafor (1986) found xylose to be comparatively poor sources of carbon for growth and sporulation of *F. oxysporum sp. elaeidis.* Effect of six nitrogen sources clearly evident (Fig. 2). that maximum growth of the fungus was recorded on glycine followed by ammonium sulphate, sodium nitrate, ammonium nitrate whereas ammonium chloride and aspartic acid supported poor growth.Excellent sporulation was observed on glycine .Good sporulation was observed on ammonium sulphate and sodium nitrate. Moderate to poor sporulation was observed on ammonium nitrate.No sporulation was observed on ammonium chloride and aspartic acid .No growth was observed in the control flasks .On the basis of experimental findings,glycine was selected as good source of nitrogen for the growth and sporulation .Mitra (1979) obtained the maximum growth in L-glycine for four isolates of *F. moniliforme var. subglutinans.* The author also found that the aspartic acid showed poor growth. Among inorganic salts ammonium sulphate showed the best results followed by sodium nitrate. Minimum growth and sporulation were recorded when ammonium nitrate and ammonium chloride were used as nitrogen sources. Oritsejafor (1986) observed that the growth of *F.oxysporum f.sp. elaeidis* was moderate on ammonium sulphate and poor sporulation was observed on DL-aspartic acid. Jackson and Freer (1991) reported that the highest level of fusarin C producion on ammonium sulpthate as the nitrogen source. Maximum growth and sporulation was rccorded on glycinc. Poor growht was observed on

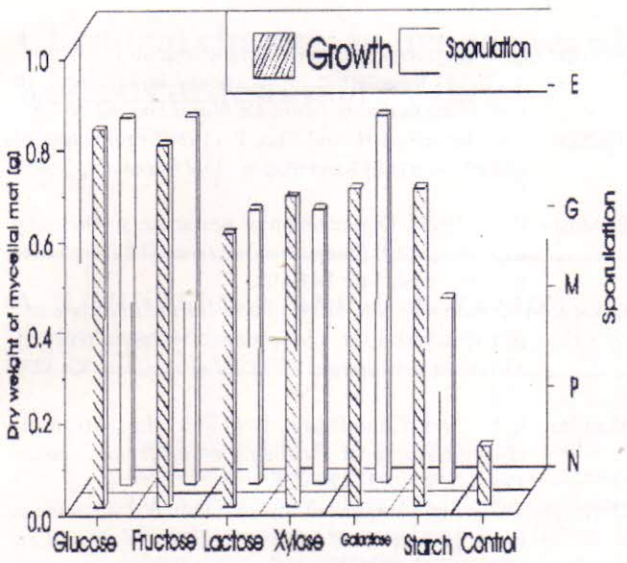

Fig. 1. Effect of carbon sources on growth and sporulation of F, *moniliforme* on Richard's medium after 15 days of incubation at 30°C

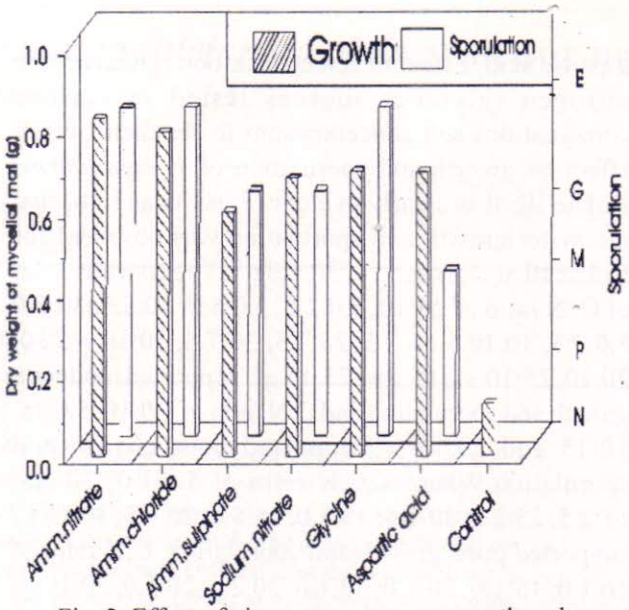

Fig. 2. Effect of nitrogen sources on growth and spourulation of *F. moniliforme* on Richard's medium after 15 days of incubation at 30°C

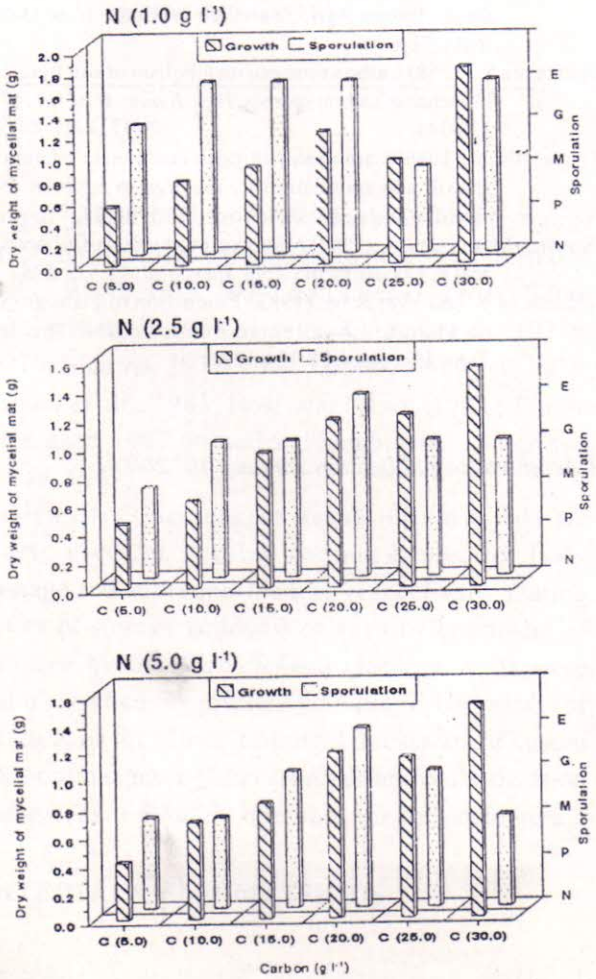

Fig. 3a. Effect of C:N ratio on growth and sporulation of F, *moniliforme* on Richard's medium after 15 days of incubation at 30°C

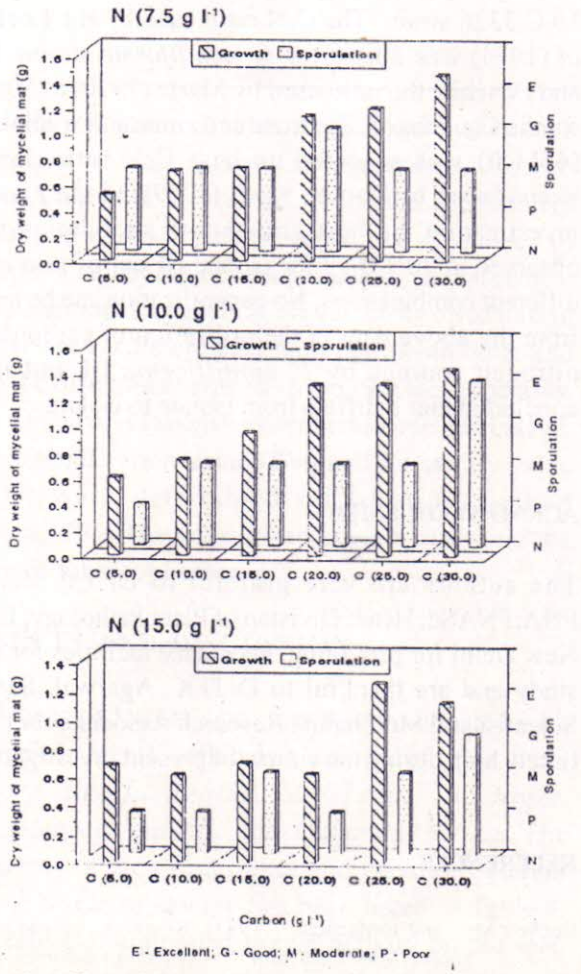

Fig. 3b. Effect of C:N ratio on growth and sporulation of F, *moniliforme* on Richard's medium after 15 days of incubation at 30°C

aspartic acid. Effect of selected carbon (glucose) and nitrogen (glycine) sources tested in various combinations and concentrations to see their overall effect on growth and sporulation of *F. moniliforme* Isolate III. It is clearly evident (Figs. 3a and 3b) that the better growth and sporulation was observed for Isolate III at C : N ratio of 30:1.0 g^{-1}. The concentration of C: N ratio of 25:10, 5.0:2.5, 5.0:5.0, 10.5.0, 30:5.0 5.0:7.5, 10.10.5,15:7.5, 25:7.5, 30:7.5, 10:10, 15:10, 20:10, 25:10,15:15, and 25.15 g^{-1}. supported moderate growth and sporulation and C:N ratio of 5.0:10, 5.0:15, 10:15 and 20:15 g^{-1} supported poor growth and sporulation.Whereas C:N ratio of 5.0:1.0; 10:2.5; 15:2.5; 25:2.5; 30:2.5; 15:5.0; 25:5.0; 20:7.5; 30:15 g^{-1} supported good growth and sporulation. C:N ratio of 10:1.0; 15:1.0; 20:1.0; 30:1.0; 20:2.5, 20:5.0, 30:10 g^{-1} supported excellent growth and sporulation.Sanchez-Marroquin (1963) selected glucose and ammonium sulphate (30:1.0) as the best ratio for the *F. moniliforme* 10 C 3326 strain. The C:N ratio used by Bu 'Lock *et al* (1974) was 30:1.0 for *F. moniliforme* strains I,II, and IV while the ratio used by Mertz (1970) as 30:6.0 for the *G.fujikuroi.* Dextrose and ammonium sulphate (65:1.0) was selected as best C:N ratio for *F. moniliforme* by Darken *et al.* (1959). In the present investigation the best growth and sporulation was observed at 30:1.0 g^{-1} for isolate III out of thirty six different combinations. No generalization can be made from the above data in the utilization of carbon and nitrogen sources by *F. moniliforme* III and it is concluded that it differs from isolate to isolate.

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