

Studies on improving growth and sporulation of *Pyricularia grisea* causing leaf blight of pearl millet

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Pyricularia grisea (Cke.) Sacc. from pearl millet (*Pennisetum americanum* (L.) was grown on ten different liquid media. Ramakrishnan's medium was the best for growth but due to poor sporulation Glucose Asparagine Vitamin (GAV) medium was selected. The maximum growth and excellent sporulation was recorded at 25°C and pH 5.5. The growth was excellent on starch followed by sucrose amongst the fifteen different carbon sources used. Eleven inorganic and eight organic nitrogen sources were tried for the growth and sporulation of the fungus. Maximum growth and sporulation were recorded on nitrate nitrogen while serine and glycine were found to be excellent sources for organic nitrogen. There was no correlation observed between growth and sporulation. Almost every grade of sporulation was noticed with significantly good or poor growth.

Key words : *Pyricularia grisea*, pearl millet, leaf blight, growth, sporulation

INTRODUCTION

Mehta *et al.* (1952) reported *Pyricularia* sp. on pearl millet (*Pennisetum americanum* (L.) Leeke) while on the same host Prasada and Goyal (1970) reported *P. penniseti* from Rajasthan. Mathur and Mathur (1972) recorded for the first time a leaf spot disease of hybrid bajra and proposed *Pyricularia grisea* f. sp. *penniseti* f. nov. as a host specific pathogen to *Pennisetum americanum* (L.) Leeke. Since no work has been made on the nutritional studies on the organism, the present investigation was designed to study the physiology of the pathogen in detail with special reference to the environmental factors viz. temperature and pH along with some nutritional requirement on the growth and sporulation.

MATERIALS AND METHODS

Monoconidial isolate of a virulent culture of *P. grisea* isolated from NHB₃ hybrid bajra was used. The isolate has been deposited to ITCC, New Delhi

The organism was grown on seven synthetic media viz.. Asthana and Hawker's, Brown's, Czapek's, Glucose asparagine vitamin's, Glucose peptone, Ramakrishnan's, and Richard's

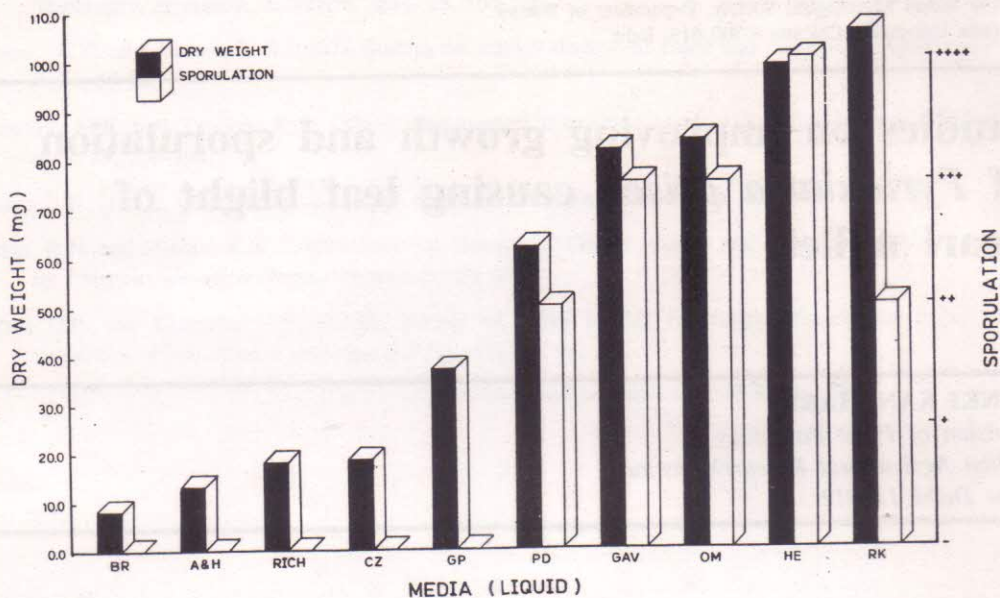


Fig 1- Growth and sporulation of *P. grisea* on different liquid media : BR-Brown, A&H-Asthana and Hawker, RICH-Richards, CZ-Czapek, GP-Glucose Peptone, PD-Potato Dextrose, GAV-Glucose Asparagine Vitamin, OM-Oat Meal, HE-Host Extract, RK-Ramakrishnan

alongwith three natural media viz., Potato dextrose agar, Oat meal and Host extract. Natural media were taken only for comparative studies. Twenty ml of medium was used in 100 ml Erlenmeyer conical flasks. The experiments were run in quadruplicates. Five mm of mycelial disc of *P. grisea* from 15 days old cultures were used for inoculation of each

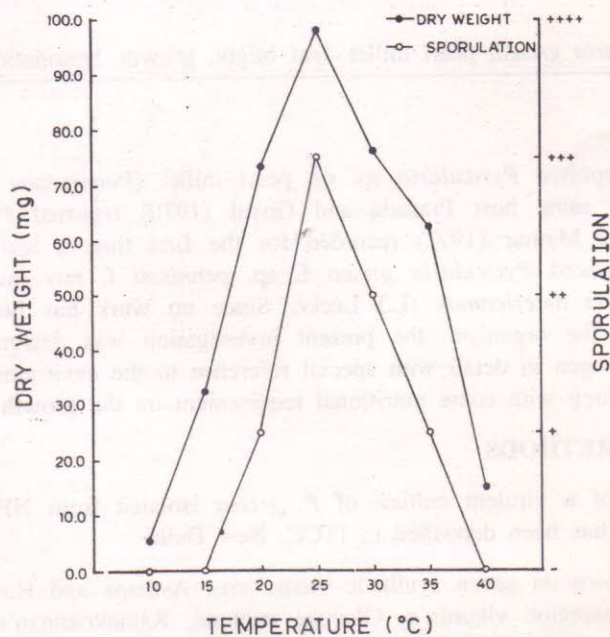


Fig 2. Effect of temperature on growth and sporulation of *P. grisea*.

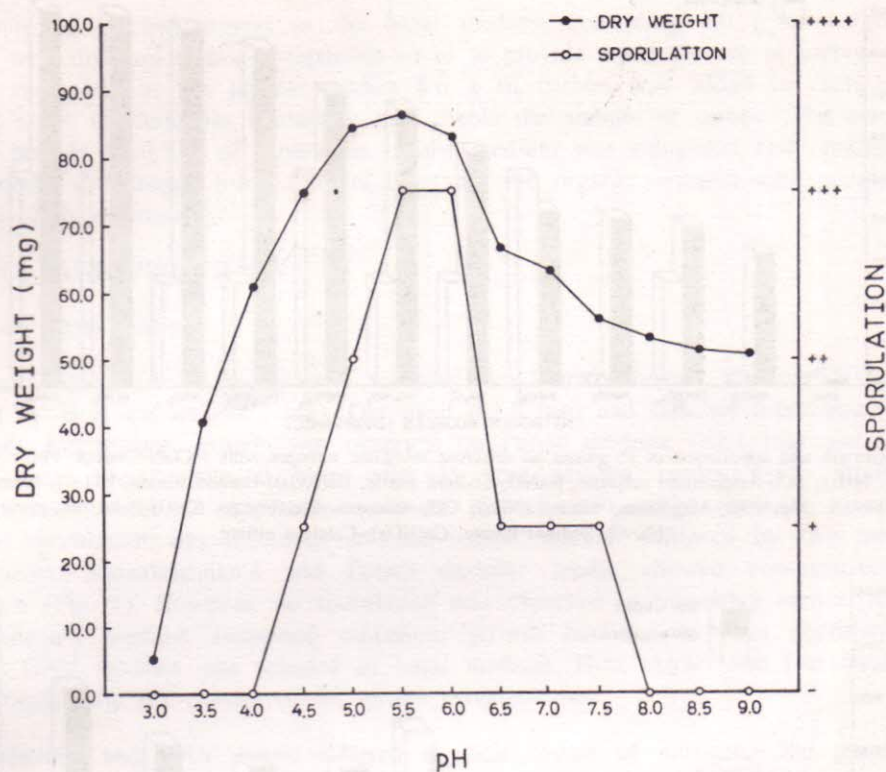


Fig 3. Effect of pH levels on growth and sporulation of *P. grisea*.

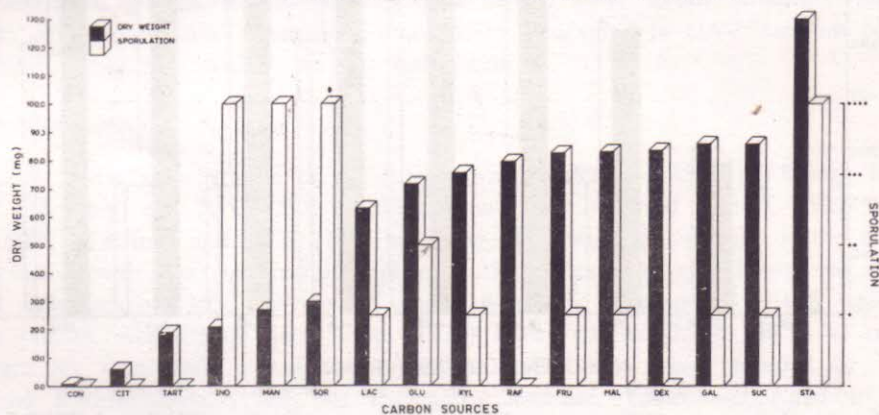


Fig 4. Growth and sporulation of *P. grisea* on different carbon sources : CON-Control, CIT-Citric Acid, TART-Tartaric acid, INO-Inositol, MAN-Mannitol, SOR-Sorbitol, LAC-Lactose, GLU-Glucose, XYL-Xylose, RAF-Raffinose, FRU-Fructose, MAL-Maltose, DEX-Dextrin, GAL-Galactose, SUC-Sucrose, STA-Starch.

flask which was then incubated for 15 days at 25°C. After the incubation period the mycelial mats were washed with distilled water and dried to a constant weight at 60°C. The data were analysed statistically and the results have been discussed at 5 per cent level of probability. Sporulation was recorded on the basis of visual observation. Sporulation was rated as Poor, Fair, Good and Excellent.

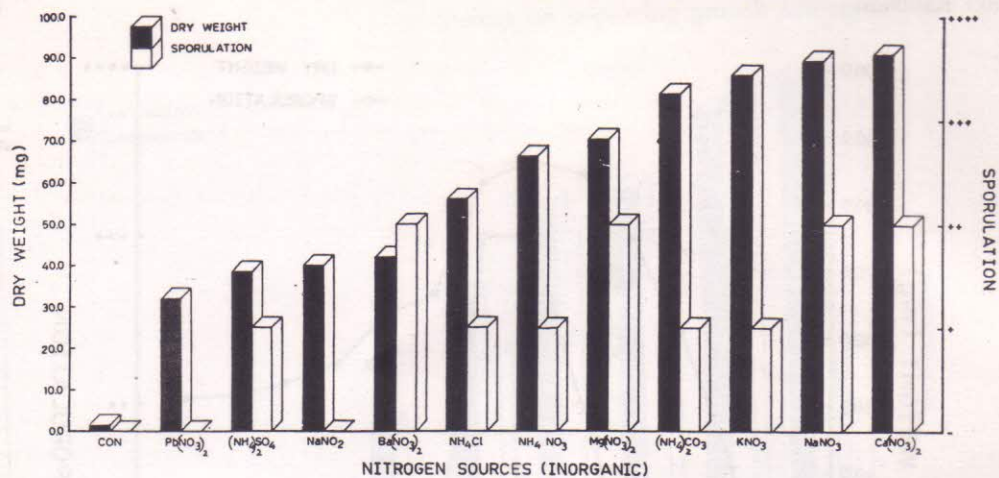


Fig 5. Growth and sporulation of *P. grisea* on different inorganic nitrogen salts : CON-Control, Pb(NO₃)₂-Lead nitrate, (NH₄)₂ SO₄-Ammonium sulphate, NaNO₂-Sodium nitrite, Ba(NO₃)₂-Barium nitrate, NH₄CL-Ammonium chloride, Mg(NO₃)₂-Magnesium nitrate, (NH₄)₂ CO₃-Ammonium carbonate, KNO₃-Potassium nitrate, NaNO₃-Sodium nitrate, Ca(NO₃)₂-Calcium nitrate.

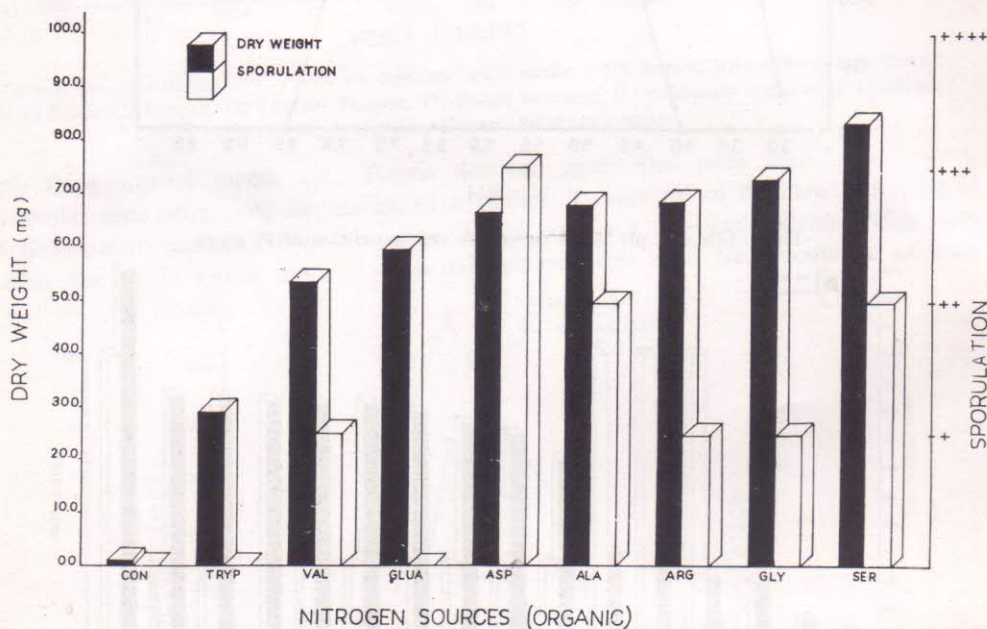


Fig 6. Growth and sporulation of *P. grisea* on different organic nitrogen salts : CON-Control, TRYP-Tryptophan, VAL-Valine, GLUA-Glutamic Acid, ASP-Asparagine, ALA-Alanine, ARG-Arginine, GLY-Glycine, SER-Serine

To determine the temperature which favours the maximum growth and sporulation of the fungus, an experiment was conducted with incubation temperature of 10, 15, 20, 25, 30, 35 and 40°C. To study the effect of pH, the fungus was grown in sets of thirteen different pH adjusted medium ranging from 3.0 to 9.0. The pH was adjusted by 0.1N HCL and 0.1N NaOH with the help of Beckman's pH meter.

Ten grams of glucose present in the basal medium containing 4.0 g of carbon was replaced with different carbon compounds so as to provide equal amount of carbon (4.0 g) in each case. But in the present studies 8.0 g of carbon was added in each case as modified GAV medium was containing just double the amount of carbon. The amount of nitrogen present in 0.5 g of asparagine of the medium was calculated and replaced with equal amount of nitrogen from different inorganic and organic nitrogen salts calculated on the basis of their formulae.

RESULTS AND DISCUSSION

Growth on liquid media

Maximum growth of *P. grisea* was recorded on Ramakrishnan's medium (105.6 mg) followed by Host extract (98.7 mg). Oat meal (83.6 mg) and Glucose asparagine vitamin (81.7 mg). Intermediate growth was observed on Potato dextrose (62.1 mg) and Glucose Peptone (36.8 mg) while it was poor in rest of the media tried. Statistically, Ramakrishnan's medium supported maximum growth with only moderate sporulation. Excellent sporulation was recorded on host extract medium followed by Oat meal and GAV media. Ramakrishnan's and Potato dextrose media showed comparatively less sporulation (Fig. 1). However, no sporulation was observed in remaining media. Although Ramakrishnan's medium supported maximum growth but due to poor sporulation the synthetic GAV medium was selected as basal medium. Host extract and Oat meal being natural media were not considered for further investigations.

Ramakrishnan's and GAV media differed in their source of nitrogen. The presence of peptone as a source of nitrogen in Ramakrishnan's medium could possibly be responsible for the maximum growth. It also contains most of the water soluble vitamins (Stokes *et al.*, 1944) which could also stimulate growth while asparagine in GAV medium could be responsible to make this medium best for sporulation.

Environmental factors

Temperature : Maximum growth of the fungus was obtained at 25°C (97.8 mg) followed by 30°C (76.1 mg) and 20°C (73.8 mg). Fair growth was observed at 35°C (62.6 mg) and poor at 10°C (5.6 mg) and 40°C (15.0 mg) (Fig. 2). Statistical analysis of the data also revealed that growth was significantly higher at 25°C. Maximum sporulation was observed at 25°C followed by 30°C. While no sporulation was observed at 10.15 and 40°C. Nisikado (1927), Abe (1933) and Yoshi (1936) have reported 25 to 30°C as optimum temperature for *Pyricularia oryzae* while similar observations were recorded by Thomas (1940) and Ramakrishnan (1948) for *Pyricularia setariae*. Yadav and Agnihotri (1982) observed temperature range allowing sporulation was much narrower than the range of growth in *Pyricularia penniseti*. The optimum for different strains varied from 25 to 28°C.

Hydrogen-ion concentration : *Pyricularia grisea* tolerated a wide range of pH. There was a gradual increase in the growth and sporulation of the fungus upto pH 5.5 and beyond that it declined. Maximum growth was observed at pH 5.5 (86.3 mg) followed by at pH 5.0 (84.5 mg). pH 6.0 (82.8 mg) and pH 4.5 (74.7 mg). Poor growth of 5.3 mg was observed at pH 3.0 (Fig.3). Statistical data revealed that growth of the fungus was significantly higher at pH 5.5 and there was no difference between growth of the fungus at pH 8.5 and

9.0. Yadav and Agnihotri (1982) found that their strains of *P. penniseti* though different in pH optima, grew best in the range of pH 5.5 to 6.4. Apparently, *P. grisea* and *P. penniseti* grew best in acidic media. In the present study excellent sporulation was recorded at pH 5.5 and 6.0 followed by pH 5.0. Poor sporulation was obtained at pH 4.5, 6.5, 7.0 and 7.5. No sporulation was observed at pH 3.0, 3.5, 4.0, 8.0, 8.5 and 9.0. Yadav and Agnihotri (1982) did not observe any sporulation of *P. penniseti* at pH 4.5 while present study have shown that the fungus *P. grisea* poorly sporulated at this pH. Moreover, the fungus changed pH of the culture filtrate towards acidity, after its growth when grown of pH > 7.0

Carbon

The fungus could grow in all the fifteen carbon sources tested in the present studies. Maximum growth was recorded in the medium containing starch as the carbon source followed by sucrose and glucose. Almost no growth was recorded in the medium devoid of carbon (Fig. 4). Statistical analysis of data revealed that the growth of the fungus was significantly higher in the medium containing starch as the carbon source whereas no statistical differences was observed between the medium containing sucrose and galactose.

In the present investigation, sorbitol, mannitol, inositol and starch supported excellent sporulation of *P. grisea*. No sporulation was noticed in the media containing citric acid, tartaric acid, raffinose and dextrin.

Nitrogen

Among inorganic nitrogen compounds, maximum growth was observed on calcium nitrate followed by sodium nitrate, potassium nitrate and ammonium carbonate. Negligible growth was observed in control (Fig. 5). Statistically also calcium nitrate was found significantly superior over all the inorganic salts. Maximum sporulation was recorded in the media containing nitrate of sodium, magnesium and barium. However, no sporulation was observed on media containing lead nitrate, sodium nitrite and in the absence of nitrogen source. Among organic nitrogen sources maximum growth was observed in serine closely followed by glycine, arginine, alanine, and asparagine while maximum sporulation was obtained from asparagine (Fig. 6). Yadav (1976) observed maximum growth for *P. penniseti* on potassium nitrate followed by sodium nitrate but no growth was observed with sodium nitrite. Among organic nitrogen sources asparagine proved best for growth and sporulation of the fungus followed by alanine, leucine and proline. The poor utilization of nitrite nitrogen by *P. grisea* may be due to toxicity particularly in acidic pH levels of the medium. In general, urea, sodium nitrate, potassium nitrate, ammonium oxalate and ammonium tartarate were found to be better sources than others. Robbins (1937) mentioned that there was no instance recorded in the literature of an organism being able to utilize nitrate nitrogen and unable to utilize ammonical nitrogen. It is clear from the above findings that there is differential utilization of nitrite and nitrate by the fungus.

There was no correlation between growth and sporulation throughout the present study. Almost every grade of sporulation was noticed with significantly good or poor growth. All the sources of carbon and nitrogen tried, supported varying vegetative growth and induced variable sporulation in *Pyricularia grisea* strain used in this study.

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