International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 503-505

Received: 22-05-2017
Accepted: 24-06-2017

Rajendra Nasnwa
Department of Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India

SL Godara
Professor & Head, Department of Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India

GL Kakraliya
Department of Pathology, SKNAU, Jobner, Rajasthan, India

Sajjan Choudhary
Department of Pathology, SKNAU, Jobner, Rajasthan, India

OP Bana
Department of Pathology, SKNAU, Jobner, Rajasthan, India

Correspondence
Rajendra Nasnwa
Department of Pathology, College of Agriculture, SKRAU, Bikaner, Rajasthan, India

Physiological Variation among Isolate of *Drechslera setariae* of Inciting Leaf Spot of Pearl Millet

Rajendra Nasnwa, SL Godara, GL Kakraliya, Sajjan Choudhary and OP Bana

Abstract
Maximum mycelial growth of *Drechslera setariae* was observed on sucrose as compared to glucose, maltose and fructose. The pathogen *D. setariae* having significantly better growth on three nitrogen sources *viz.*, ammonium chloride, L-arginine and L-alalnine, compare to glutamic acid. Among all the tested nitrogen sources glutamic acid was found at least supportive to the mycelial growth of the pathogen. The effect of temperature studies revealed that maximum mycelial growth of pathogen was observed at 25 ^{0}C. Minimum mycelial growth was observed at 35 ^{0}C temperature. Similarly optimum pH maximum mycelial growth of pathogen was found at optimum pH 6.5. The findings showed good growth over a range of pH 6.0 to 8.0.

Keywords: Nitrogen, Carbon, Temperature, pH, *Drechslera setariae*

Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] 2n = 14 locally known as bajra, bari, sajja, combo, ganti or kambam, is an allogamous crop having protogynous nature. It belongs to the family in *Poaceae*. It originated in West Africa and from there introduced to India. It is an important food and forage crop in Africa and Asia and important forage in American. It has great potential because of its suitability to the extreme limits of agriculture. A total of 21,392 germplasm accessions including 750 accessions of wild species of genera *Pennisetum* and *Cenchrus*, assembled from 50 countries are conserved at International Crops Research Institute for the Semi-Arid Tropics **(**ICRISAT**)** gene bank. The pearl millet grains are very nutritious and form the staple diet of approximately 10 per cent of the population in India. It has high protein with slightly superior amino acid profile. It is a good source of protein (11.5%), fat (4.1-6.4%), carbohydrate (59.8-78.2%) and also rich good amount of minerals particularly phosphorus and iron (2.8%). India is the largest producer of pearl millet with an annual production of 9.25 million tonnes from an area of 7.0 million hectares with productivity being 1250 kg/ha (Anonymous, 2014-15a) [1]. Pearl millet is mainly grown in Rajasthan, Uttar Pradesh, Gujarat, Maharashtra, Haryana, Karnataka, Tamil Nadu, Madhya Pradesh, and Andhra Pradesh states of the country. Rajasthan occupies first position in area and production of pearl millet in India. In Rajasthan, it is cultivated on 40.76 lac hectares area with the production of 44.56 lac tonnes and productivity of 1093 kg/ha (Anonymous, 2014-15b) [2]. Major pearl millet producing districts of Rajasthan are Alwar, Bharatpur, Karoli, Dholpur, Swai Madhopur, Jaipur, Jhunjhunu, Churu, Bikaner, Jaisalmer and Barmer.
Drechslera setariae was isolated from the rotted seeds and infected parts of seedlings. The seed used was obtained from Mysore, India, and was found to be infected with *D. setariae*, which has been reported from the United States to be seed-borne in pearl millet, causing seed rot, blight and leaf spotting (Wells & Winstead, 1965 and Wells & Burton, 1967) [12, 11]. The disease has also been noticed in India by Bhowmik (1972) [4] and Balasubramanian (1980) [3].

Materials and Methods
Effect of temperatures: Temperature has considerable influence on the biochemical activity of pathogens. Twenty ml of basal medium Czapek's dox agar was poured in each of sterilized Petri plate. Each Petri plate was inoculated aseptically by placing in the centre a 5 mm mycelial Petri plate from actively grown seven days old culture of isolate of *D. setariae* on

basal m edium. The inoculated Petri plates were incubated at 15, 20, 25, 30 and 35ºC temperature for seven days.

Effect of hydrogen ion concentrations (pH): The study of different pH level was 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 from 6.0, 6.5, 7.0, 7.5 and 8.0 with a difference of 0.5 using N/10 NaOH or N/10 HCl. After autoclaving the pH was again tested. The inoculated Petri plate were incubated at 28 ±1 ºC for seven days.

Effect of carbon sources: To find out the effect of various carbon sources on growth of *D. setariae* the sucrose content of basal medium Czapek's dox agar was substituted by adding different source of carbon on equivalent basis (12.63g in 30g of sucrose). Inoculated Petri dishes containing basal medium supplemented with different carbon sources were incubated at 28± 1ºc for 7 days and the mycelial growth was recorded. Carbon sources used were glucose (C_6H_{12} O_6), sucrose (C_6H_{22} O_{11}), maltose ($C_{12}H_{22}$ O_{11}) and fructose ($C_{12}H_{22}$ O_{11}).

Effect of nitrogen sources: The ammonium chloride of basal medium Czapek's dox agar medium was substituted by adding different sources of nitrogen on equivalent basis (329mg in 2 g of Ammonium chloride) to study the effect of different nitrogen sources on the mycelial growth of *D. setariae*. Nitrogen sources studied were Ammonium chloride, L-alanine, L-arginine, Glutamic acid and control being without nitrogen source.

Results and Discussion

Effect of different temperatures: The effect of temperature on mycelial growth of *Drechslera setariae* was studied by incubating Petri plate at different temperatures ranging from 15 to 35 ^{0}C. The mycelial growth of *D. setariae* at 15 ^{0}C (35.00 mm), 20 ^{0}C (52.33 mm), 25 ^{0}C (76.66 mm), 30 ^{0}C (68.00 mm) and 35 ^{0}C (27.67 mm) was observed. The maximum mycelial growth of the pathogen was observed at 25 ^{0}C as compared to 30 ^{0}C. The minimum mycelial growth was observed at 35 ^{0}C temperature. It becomes evident from the study that the maximum mycelial growth of *D. setariae* was observed at 25 ^{0}C and it was found significantly superior over treatments, (Table-1).

Table 1: Effect on mycelial growth of *Drechslera setariae* at different temperatures

Temperature (^{0}C)	Mycelial growth (mm)
15	35.00
20	52.33
25	76.66
30	68.00
35	27.67
SEm±	0.35
CD at 5%	1.09
CV (%)	2.60

Effect of pH: Hydrogen ion concentration also affected the mycelial growth of *Drechslera setariae* tested over a wide range of pH 6.0 to 8.0. The maximum mycelial growth of *D. setariae* was recorded at pH 6.5 (78.50 mm) followed by pH 6.0 (69.33 mm). The minimum mycelial growth was observed at pH 8.0 (47.00 mm). The data presented in Table-2. Revealed that there was significantly increased mycelial growth at pH 6.5 followed by pH 6.0 and pH 7.0.

Table 2: Effect on mycelial growth of *Drechslera setariae* at different pH levels

pH levels	Mycelial growth (mm)
6.0	69.33
6.5	78.50
7.0	57.33
7.5	54.67
8.0	47.00
SEm±	0.47
CD at 5%	1.49
CV (%)	2.99

Effect of carbon sources: The effect on mycelial growth of *Drechslera setariae* grown on Czapek's dox agar basal medium with different sources *viz*., glucose, sucrose, maltose and fructose were studies in this assay. The maximum mycelial growth on sucrose (61.67 mm) followed by glucose (59.00 mm) carbon sources were observed. The mycelial growths of *D. setariae* were found comparatively less on glucose, maltose and fructose. All the carbon sources supported better mycelial growth of *D. setariae* over control. (Table-3)

Table 3: Effect of different carbon sources on mycelial growth of *Drechslera setariae*

Carbon sources	Mycelial growth (mm)
Glucose	59.00
Maltose	53.33
Sucrose	61.67
Fructose	52.33
Control	43.40
SEm±	0.35
CD at 5%	1.11
CV (%)	2.54

Effect of nitrogen sources: Mycelial growths of *Drechslera setariae* grown on Czapek's dox agar basal medium with different nitrogen sources were evaluated. Significantly better mycelial growth were recorded on all the four nitrogen sources *viz*. Ammonium chloride, L-alanine, L-arginine and Glutamic acid as compared to control. The maximum mycelial growth was observed on Ammonium chloride nitrogen source followed by L-arginine. The results presented in Table-4, that revealed mycelial growth on Ammonium chloride was found significantly higher as compared to other treatments.

Table 4: Effect of different nitrogen sources on mycelial growth of *Drechslera setariae*

Nitrogen sources	Mycelial growth (mm)
Ammonium chloride	67.33
L-alanine	61.33
L-arginine	61.67
Glutamic acid	42.33
Control	40.00
SEm±	0.37
CD at 5%	1.15
CV (%)	2.60

The mycelial growths of *Drechslera setariae* were different when grown on Czapek's dox agar basal medium with different carbon sources. Maximum mycelial growth of *D. setariae* was observed on sucrose as compared to glucose, maltose and fructose, which are very much similar and conformity with the results concluded by Harding *et al.*

(1975) [7] reported the sucrose as the best carbon source for the *Drechslera spp.*

Similarly, mycelial growth of *Drechslera setariae* varied while grown on Czapek's dox agar basal medium with different nitrogen sources. *Drechslera setariae* having significantly higher growth on nitrogen sources *viz*., ammonium chloride L-alalnine, L-arginine and glutamic acid. Among all the tested nitrogen sources, the ammonium chloride supported as maximum mycelial growth of *D. setariae*, which are very much similar and conformity with the results concluded by Patil *et al*. (2015) [10] investigated on the mycelial growth of *Drechslera sorokiniana* [*Cochliobolus sativus*] on different media, C and sources, and at different temperature and pH. Soluble starch and ammonium chloride were the best carbon and nitrogen sources respectively, for the growth of the fungus. Kansara *et al.* (2012) [12] evaluated that nine nitrogenous sources tried sodium nitrate, ammonium phosphate, asparagines, ammonium sulphate and ammonium chloride proved to be the best for the growth and sporulation of the pathogen.

Evaluated the growth of *Drechslera sorokiniana* causing foot rot disease in wheat, as indicated by mat weight, differed with different sources and level of nitrogen. In general, higher mat weights were obtained in media containing organic sources of nitrogen than in media containing inorganic sources. Almost the order of mat production with different sources were asparagine > urea > potassium nitrate > ammonium nitrate > ammonium sulphate.

Temperature wields considerable effect on growth of fugal organisms. In present studies increasing trend of mycelial growth of *D. setariae* have been observed from 20 ^{0}C to 30 ^{0}C. Maximum of mycelial growth was observed at 25 ^{0}C. Decreasing trend of growth was found between 30 ^{0}C to 35 ^{0}C and minimum of mycelial growth was observed at 35 ^{0}C. These results show the maximum growth at 25 ^{0}C, which are very much similar and conformity with the results concluded by Nagaraja *et al.* (1992) [9] the pathogen made maximum of mycelial growth on the 10th day of seeding on Richard's medium maintained at 25 ^{0}C at a pH of 5.8. Studies on various diseases of barley, the leaf blight caused by *Helminthosporium sativum* was attaining importance in Karnataka. Physiological studies revealed that temperature of 25 ^{0}C and pH of 7.0 was found to be optimum for maximum germination of conidia. Barley leaf extract media supported maximum germination of conidia of *H. sativum*, which was at par with two per cent sucrose solution. Penetration studies revealed that the mode of entry of this pathogen is direct by producing aspersorium on cuticle and indirect by penetration of hyphae through stomata.

Maximum mycelial growth of *D. setariae* was observed at pH 6.5, which are very much similar and conformity with the results concluded by Didvania *et al.* (2012) [6] reported that bell pepper blight caused by *Drechslera bicolor* was observed on leaves and fruits, the pathogenicity were confirmed on bell pepper plants and fruits of bell pepper, chilli, tomato and brinjal. Initial symptoms on bell pepper plants appeared on 7th days and inoculations in diseased fruits incubation period varied in between 6-8 days, at the 25±2 ^{0}C temperature, 100 % RH, brown light and pH 6.5.

References

1. Anonymous. Ministry of Agriculture, Govt. of India, 2014-2015a.
2. Anonymous. Department of Agriculture, Govt. of Rajasthan, 2014-2015b.
3. Balasubramanian KA. Association of *Drechslera setariae* with downy mildew affected pearl millet. Curr. Sci. 1980; 49(6):233-234.
4. Bhowmik TP. *Bipolaris setariae* on two new host in india. Indian Phytopathol. 1972; 25:590-591.
5. Chandrshekara ME, Kalappanavar IK, Kulkarni S. Physiology and host parasitic relationship of leaf blight of barley. Karnataka. J. Agril. Sci. 2005; 18(3):694-697.
6. Didvania S, Shah R, Jadon KS. A new disease of bell pepper (*capsicum annum var.grossum*) caused by *Drechslera bicolor* its pathophysiology efficacy of fungicides and botanicals. Plant pathol. J. 2012; 11(2):68-72.
7. Harding H. Effect of pH and sucrose concentration on conidium size and septaion in four Bipolaris Species. Canadian J. Bot. 1975; 53(15):1457-1464.
8. Kansara SS, Joshi DM, Dhingani JC. Physiological studies of *Drechslera state* of *Trichometasphaeria holmii* causing leaf spot/blight of Heliconia. J. App. Microbiol. 2012; 6(4):1945-1950.
9. Nagaraja SV, Kulkarni S, Hegde RK. Physiological studies *Drechslera sorokiniana* (Sacc.) Subram. and Jain, a causal agent of leaf spot of Dioscorea sp. Mysore J. Agric. Sci. 1992; 26(3):261-264.
10. Patil JS, Kulkarni S, Hegde RK. Studies on leaf blight of barley caused by *Drechslera sorokiniana* (Sacc.) Subram and Jain. Physiological studies on *Drechslera sorokiniana*. *Curr. Res*. 2015; 13(4-6):43-47.
11. Wells HD, Burton GW. *Helminthosporium setariae* on pearl millet, *Pennisetum typhoides*, as affected by age of host differences. Crop Sci. 1967; 7:621-622.
12. Wells HD, Winstead EE. Seed borne fungi in Georgia grown and western grown pearl millet seed on safe in Georgia during 1960. Pl. Dis. Rep. 1965; 49:487-489.