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Khatal MP
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

Thakare CS
Assistant Professor, Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

HN Markad
Ph.D. Scholar, Department of Plant Pathology, Post Graduate Institute, Rahuri, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

SS Hurule
Assistant Professor, Department of Plant Pathology, College of Agriculture, Sonai, Maharashtra, India

Correspondence
Khatal MP
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

Cultural and physiological study of *Curvularia hawaiiensis* (Bugnic. ex M. B. Ellis) causing leaf spot of pearl millet

Khatal MP, Thakare CS, HN Markad and SS Hurule

Abstract
Among the all pearl millet diseases, leaf spot caused by *Curvularia hawaiiensis* has been gaining importance in recent years by causing considerable losses in high yielding varieties and hybrids, than native cultivars. Fungi have been isolated from the leaf spot of Pearl millet *Curvularia hawaiiensis* was found dominant pathogen. The cultural studies result revealed that, the maximum growth was recorded on yeast dextrose agar and potato dextrose agar medium with colony diameter 8.96 cm and 8.86 cm, respectively as circular colony thick growth of mycelium with dark brownish in colour, followed by host leaf extract agar medium 8.50 cm produced circular colony with dark greenish colour of the mycelium and oat meal agar 8.33 cm produced smooth circular colony with dark greenish raise fluffy mycelial growth. Temperature studies revealed that, significantly maximum growth of the pathogen was recorded at temperature 25º and 30 ºC. Results on pH studies stated that, maximum mycelial growth was obtained at pH 4.0 and the test fungus could tolerate a wide range of pH 4.0 to 6.0 significantly.

Keywords: Cultural and physiological study of *Curvularia hawaiiensis*, pearl millet

Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] commonly known as bajra, sajja, combo, bari, ganti or kambam. Its belongs to family *Poaceae*. It is an important food and forage crop in Asia, Africa and important forage in America. It has great potential because of its suitability to the extreme limits of agriculture. The pearl millet grains are very good nutritious and form the staple food or diet of approximately ten per cent of population in India. It has high quality protein with 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 in good amount of minerals particularly phosphorus and iron (2.8%). Due to its rich composition of proteins and minerals, pearl millet has many health benefits.

The area under cultivation of pearl millet was 7.128 million ha with grain production of 10.08 metric tons and productivity of 1132 kg ha^{-1} in India (Anon., 2017) [2]. India is a major Pearl millet producing country with 43.3% of the world area and 42% of world production. In Maharashtra state area under pearl millet cultivation is about 15.3 % share in all India, the productivity of pearl millet is about 673 kg ha^{-1} (Agropedia). The crop is cultivated in almost all the districts of Maharashtra state, except konkan area, however, the major pearl millet growing districts are Nashik, Dhule, Ahmednagar, Pune, Satara, Sangali, Aurangabad and Solapur.

Pearl millet (*Pennisetum glaucum* L. R. Br.) is being affected by several fungal diseases. The pearl millet crop grown extensively with a limiting factor that invariably attacked by fungal diseases like Downey Mildew (*Sclerospora graminicola*), Smut (*Moesziomyces penicillariae),* Ergot (*Claviceps fusiformis),* Blast (*Pyricularia grisea),* Rust (*Puccinia substriata),* Leaf spot (*Bipolaris, Cercospora, Curvularia, Drechslera, Exeserohilum, Pyricularia) etc.* Among these, leaf spot is gaining importance in recent years by causing considerable losses in high yielding varieties and hybrids, than native cultivars. *Curvularia* is a hyphomycete fungus which is a facultative pathogen of many plant species and of the soil.

Materials and Methods
Present investigation on studies on leaf spot of Pearl millet (*Pennisetum glaucum* L.) was carried out during 2018-19 at field condition of Bajra Research Scheme, Department of Plant Pathology, College of Agriculture, Dhule.

Cultural character on solid media

The agar based sterilized media were poured aseptically in 90 mm diameter previously sterilized petri plates. The petri plates were inoculated aseptically after solidification by placing 5 mm dia. mycelia disc of 10 days old culture of *Curvularia hawaiiensis* maintained on PDA medium at the centre. Three replications were kept for each medium recording growth and sporulation. The Petri plates were incubated at 27 ± 2 ^{0}C temperatures in BOD incubator. The radial growth was daily measured till the plates were covered with the pathogen mycelium and colour of the growth of pathogen on different media recorded by using standard colour chart. The data thus, obtained was statistically analyzed.

Physiological study

The growth fungi was tested at different temperature *viz*., 20^0, 25^0, 30^0, 35^0, 40^0 and 45 ^{0}C. Potato dextrose agar was poured into 90 mm diameter petri plates. After solidification, 5 mm actively growing culture were cut and inoculated to the media containing Petri plates and incubated for 7 days in the incubator adjusted to required temperature levels. Each treatment replicated at thrice.

The pH of the medium was adjusted before autoclaving with the help of HCL (0.1 N) and NaOH (0.1 N) using pH meter. Stock solution of Richard's liquid medium was prepared and distributed in 100 ml quantities in 250 ml Erlenmeyer conical flasks adjusted to different pH values by means 'Beckmens' pH meter with glass electrode and by adding to its approximate quantities of N/10 HCL and N/10 NAOH solution. The flasks were then inoculated with a bit of the fungus and incubated at room temperature (27$\pm$1 ^{0}C) for 10 days. The mycelium mat was filtered and dried to content weight at 40 ^{0}C. Dry weight of the mycelia mat was recorded.

Results and Discussion

Cultural study

Cultural character of the test pathogen was studied on seven different synthetic and semi-synthetic media. After seven days of inoculation the growth and colony character of pathogen on different media were observed. It was observed that the treatment differences in respect of colony diameter and growth characteristics. A colour change of different media was observed by using standard colour chart. Sporulation and spore count was recorded after 120 hrs (Table 1; Fig. 1).

The result (Table 1) revealed that, the maximum growth was recorded on yeast dextrose agar and potato dextrose agar medium with colony diameter 8.96 cm and 8.86 cm, respectively as circular colony thick growth of mycelium with dark brownish in colour followed by host leaf extract agar medium 8.50 cm produced circular colony with dark greenish colour of the mycelium and Oat meal agar 8.33 cm produced smooth circular colony with dark greenish raise fluffy mycelial growth.

The next best treatment was Richard's agar media has produced circular colony of 8.13 cm growth. The mycelium was light brown colour and raised fluffy growth followed by Peptone rose bengal agar produced 7.13 cm circular with dark brownish colour colony with raised fluffy growth, Saboround's agar 6.33 cm Light brownish circular colony with raised fluffy mycelium growth.

The cultural character of the pathogen on different semi synthetic media under study indicated yeast dextrose agar, potato dextrose agar, oat meal agar and richard's agar are the excellent media for growth and sporulation of *Curvularia hawaiiensis* from Pearl millet crop. These results are matching with results of Kore (1973) [7] and Mahilal *et al*. (2014). These observation were also confirmed with the Singh (1971) [11], Gadage (1972) [4] and Kore and Bhide (1981) [8].

Table 1: Effect of different culture media on colony diameter, growth character and sporulation of *C. hawaiiensis*, causing leaf spot of Pearl millet.

Tr. No. No.	Culture medium	Average Colony diameter (cm)* 7 DAI	Colony character	Sporulation
T_1	Potato dextrose agar	8.86	Colony circular, Dark brown in colour, center raised, excellent growth.	++++
T_2	Oat meal agar	8.33	Colony smooth circular, Dark greenish in colour, raised fluffy mycelim growth.	+++
T_3	Host leaf extract agar	8.50	Colony circular with smooth, Dark greenish colour, submerged growth of mycelium.	+
T_4	Saboround's agar	6.33	Colony smooth with circular, light brownish in colour, raised fluffy growth of mycelium.	+++
T_5	Richard's agar	8.13	Colony circular with smooth, light brownish and white in colour, raised fluffy growth.	++++
T_6	Peptone rose bengal agar	7.13	Colony circular, Dark brownish in colour, raised fluffy growth.	++
T_7	Yeast dextrose agar	8.96	Colony circular, light brownish in colour, center raised, excellent growth.	++++
	S.E. $\pm$	0.11		
	C.D. at 5%	0.33		

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

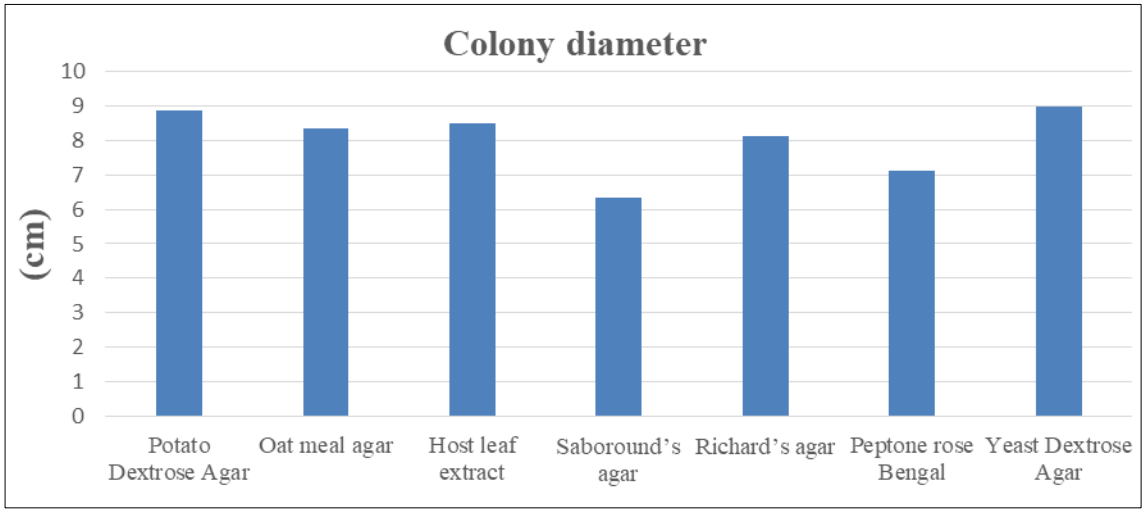

Fig 1: Effect of different cultural media on colony diameter and sporulation of *Curvularia hawaiiensis*

Physiological study

Temperature

Fungi are very sensitive to temperature. Their growth is greatly influenced by temperature of incubation. It was observed from the results (Table 2; Fig. 2) that, the significantly maximum growth of the pathogen (9.0 cm) was recorded at temperature 25º and 30 ºC and it was followed by at 20 ºC (8.93 cm) and 35º C (8.8 cm) and they were at par to each other. The next best treatment was 40 ºC (7.9 cm). The test pathogen recorded the least growth at temperature 45 ºC (1.76 cm). These results are conformity with reports of Jackson (1959) [6] and Mathur *et.al.* (1960) [10].

Table 2: Effect of different temperature on growth and sporulation of *Curvularia hawaiiensis*

Tr. No.	Temp. ºC	Ave. Colony Dia. (cm)* 7 DAI	Colony character	Sporulation
T_1	20	8.93	Colony circular, mycelium whitish at center and brownish at periphery.	++++
T_2	25	9.0	Colony circular, mycelium blackish colour, excellent growth.	++++
T_3	30	9.0	Colony circular, mycelium blackish colour, excellent growth.	++++
T_4	35	8.8	Colony circular, mycelium blackish colour, excellent growth.	++++
T_5	40	7.9	Colony circular, slow growth of mycelium, whitish at center and greenish at periphery.	++
T_6	45	1.76	No mycelial growth.	-
	S.E.±	0.19		
	C.D. at 5%	0.60		

* Mean of three replication

Sporulation:- - Nil,+ Poor,++ moderate, +++ Good, ++++ Excellent.

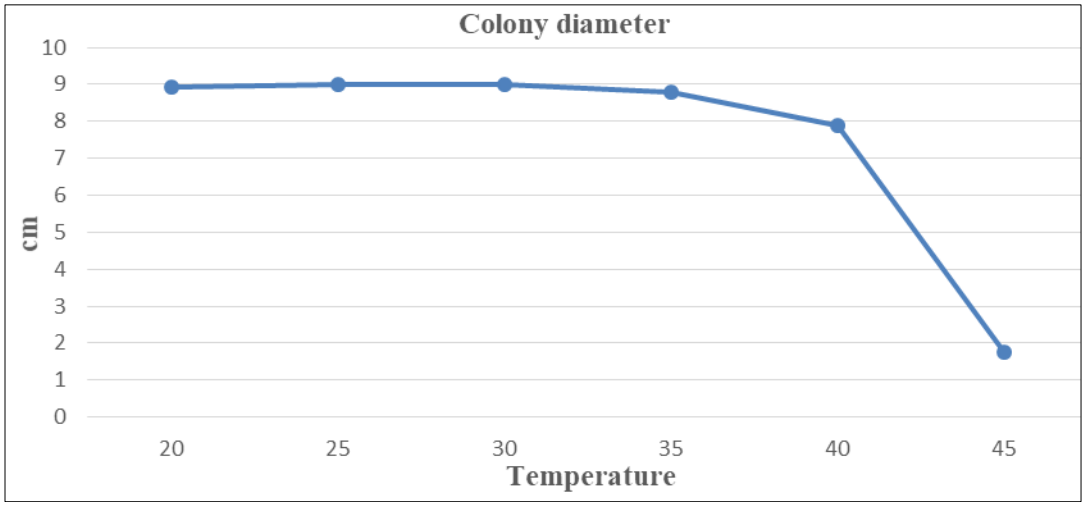

Fig 2: Effect of different temperature on growth of *Curvularia hawaiiensis*

Effect of H^+ ion concentration on growth of the fungus

The growth of the pathogen in culture as influenced by hydrogen ion concentration of the medium was studied in Richard's broth adjusted to different pH values. Observations on dry mycelial weight were recorded after 20 days of inoculation.

From the results (Table 3; Fig. 3) it was revealed that the fungus could tolerate a wide range of pH 4.0 to 6.0 significantly. The maximum mycelial growth was obtained at pH 4.0 (9.66 mg) and it was followed by pH 5.0 (5.76 mg), then the mycelial growth reduced from pH 6.0 and above.

The optimum pH was at 4.0. The results are confirmity with Hasija (1971) who reported that organism grew better at pH 5.4. and Aulakh (1970) reported that these organism grew better at pH 5.5-6.0.

Table 3: Effect of H^+ ion concentration on growth of *Curvularia hawaiiensis*.

Tr. No.	pH level	Average mycelial dry weight * (mg) 10 days after inoculation.
T_1	4	9.66
T_2	5	5.76
T_3	6	3.47
T_4	7	2.85
T_5	8	1.30
T_6	9	1.19
	S.E. ±	0.11
	C.D. at 5%	0.34

*Mean of three replication

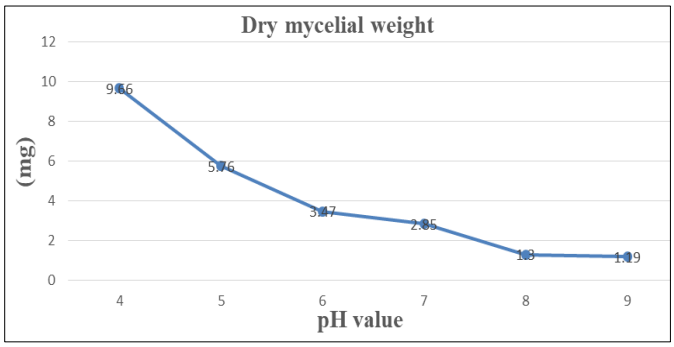

Fig 3: Effect of H^+ ion concentration on growth of *Curvularia hawaiiensis.*

Conclusion

Hence, from ongoing results and discussion, it is concluded that, the excellent growth and sporulation of *C. hawaiiensis* was observed on yeast dextrose agar and potato dextrose agar medium. The colony of the *C. hawaiiensis* was circular and thick growth on the upper surface of PDA, which turned into dark brown. Temperature studies indicated that test fungus was significantly excellent growth and sporulation at temperature 20^0 to 35 ^{0}C and it was reduced at 40 ^{0}C and above. p^H studies revealed that the test pathogen grew best at pH 4.0, moderate growth was found at p^H 5.0-6.0, while low on pH 7.0, 8.0 and 9.0.

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