EFFECT OF DIFFERENT MEDIA, TEMPERATURE, PH AND NITROGEN SOURCES ON GROWTH AND DEVELOPMENT OF *HELMINTHOSPORIUM ORYZAE* CAUSING BROWN LEAF SPOT OF RICE

Abstract

The pathogen *Helminthosporium oryzae* was subjected to different cultural conditions viz., media, temperature, pH and nitrogen sources under *in vitro* conditions. The maximum radial growth of Czapeck (DOX) Agar,Oat Meal Agar&Potato Dextrose Agar was recorded highest with average radial growth of 90.00 mm followed by Richard's agar (88 mm), Water agar.. On this medium, the colony appeared greyish-white to dark brown, thick, leathery slightly raised and profuse mycelia with brown-coloured conidia. The average mycelial growth of fungus recorded when exposed to continuous light was 58.60 mm and 51.41 mmat alternate cycles of light and at complete dark highest mycelial growth was recorded 72.80 mm. Growth of the pathogen when evaluated at different pH levels, a maximum radial growth of the fungus was recorded at pH 7.0 (90.00) andleast radial growth of the fungus was recorded at pH 4.5 (44.89 mm) and pH 5.0 (60.33 mm).Among the different nitrogen sources maximum growth was recorded in potassium nitrate and least was recorded in Ammonium chloride.

Keywords: radial growth, media, temperature, nitrogen, pH

1. Introduction

Rice (*Oryza sativa* L.) is a most important staple food grain for more than 3billion people around the world. Ricebelongs to family Poaceae (old Gramineae) and consist of 24 recognized species of genus *Oryza*. Out of theseonly two major rice species *Oryzasativa* and *Oryzaglaberrima* are cultivated worldwide.Rice is a kharif season crop in India, 25ºC temperature and rainfall of more than 100 cm is appropriate for rice cultivation. An ideal food to include in a healthy diet is rice. It has a decent amount of fiber, protein (7.2%), carbohydrate (78%), vitamins and minerals such as thiamine (2.8 mg/g), iron (38 ppm), and riboflavin (0.5 mg/g) (Duraisamy*et al*.,2019). On some special occasions, rice flour is prepared to make a variety of traditional recipes like rice phirni, rice chakli, modak, etc.Rice is the second most important cereal crop after wheat, which feed about 45% of world

population and provides 15% calories need (Anonymous 2018). It isthe staple food crop of southern and eastern parts of India (Anonymous,2022).

Like other crops, rice is also suffered from various biotic and abiotic stresses. Various biotic causes include the disease caused by fungi, bacteria, viruses, viroid's, nematodes, phytoplasma and also insects and weeds.Abiotic stressincludes high and low temperatures, salinity, drought, flooding and nutritional deficiency like Khaira disease and White leaf disease. Various pathogenic microorganism includes fungi bacteria viruses causing disease which reduce crop yield. Approx 12–20% of crop losses are caused by fungi (Rajan, 1987). Major diseases are Blast (*Magnoporthe grisea*boriosis and cavara), Brown spot (*Helminthosporiumoryzae* Brenda de haan), False smut (*Ustiloginoidea virens*), Bunt (*Neovossia horrible*padwick and khan), Sheath rot (*Sarocladiumoryzae*sawada), and Stem rot (*Sclerotium oryzae*cattahea), Sheath blight (*Rhizoctonia solani)* Seedling blight (*Corticiumrolfsii*curzi) Foot rot or the bakanae disease (*Fusarium moniliforme*sheld) and Bacterial leaf blight(*Xanthomonas oryzaepv. oryzae*)**.**

Among these, brown leaf spot caused by *Bipolaris oryzae* is one of the major diseases of rice. Brown leaf spot of rice caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker (= *Helminthosporium oryzae* teleomorph = *Cochliobolusmiyabeanus*) is known to occur in Japan since 1900. It is also called as 'nai-yake' i.e. seedling blight, sesame leaf spot and Helminthosporiopsis. The disease has been reported to occur in all the rice growing countries including Japan, China, Myanmar, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America and Philippines (Khalili *et al.*, 2012). In India, it is known to occur in most rice growing states. It was first reported from Madras by Sundraraman in 1919. The disease is more severe in dry or direct seeded rice in the states of Bihar, Chhattisgarh and Madhya Pradesh. The disease has great importance in several countries and has been reported to cause enormous losses ingrain yield (up to 90%) particularly when leaf spotting

phase assumes epiphytotic proportions observed during great Bengal famine in 1942 (Ghose *et al*., 1960).

2. Material and Methods

In vitro experiments were conducted in Biocontrol laboratory, Department of Plant Pathology, College of Agriculture, S.V.P.U.A.T, Meerut, U.P.

2.1.Collection of diseased specimen and isolation of pathogen

The infected leaves showing typical brown leaf spot symptoms were collected from naturally infected paddy plants from the field CRC, S.V.P.U.A.T, Meerut, U.P. The brown spot pathogen was isolated and purified on Potato Dextrose Agar Medium.

2.2.Morphological and physiological studies

The morphological characters of the fungus were studied on 10 solid media. To study the physiological characteristics like temperature and pH,Six different temperature levels viz., 15, 20, 25, 30, 35& 40°Cand eight levels of pH viz., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 & 8.0 were studied. Three replications were maintained at each treatment for all the experiments. The radial growth, colony characters like colony colour, topography, margin and sporulation were recorded. The dry mycelial weight of the fungus was harvested by draining the medium through Whatman No.1 filter paper. The filter paper with fungal mycelial mat was dried in a hot air oven at 60°C for 48 hours. After 48 hours the dry mycelial weight of the pathogen was recorded.

2.3.Nitrogen sources

The following nitrogen sources viz., ammonium chloride, peptone, calcium nitrate, ammonium nitrate, potassium nitrate& prolineat different concentrations (1.0 1.5 and 2%) on Potato Dextrose broth. Three replications were maintained at each source for both the

experiment. The dry mycelial weight of the pathogen was recorded using above mentioned procedure. Analysis of the experimental data was done by using Completely Randomised Design (CRD) for the laboratory studies as suggested by Panse and Sukathme (1985).

3. Result and Discussion

3.1.Studies on the morphological and physiological characters of the pathogen

Different morphological characters like shape, colony color, texture, edge and radial growth were studied on ten different media are shown in Table 1. The shape of conidia was observed as slightly curved and wide in the middle with 5-9septations. Fully matured conidia are brownish or fuliginous with septate mycelia. Morphological characters of the mycelium and conidia confirm with the reports of Kumari *et al.*, (2015), wherein they observed that, the spore size varied from (5.34-7.48 µm x 4.10- 5.51 µm) under 10X of compound microscope, where in different isolates grown in PDA medium. From among the 10 different solid media tested, most supporting medium for the growth of the fungus was Czapeck (DOX) Agar,Oat Meal Agar&Potato Dextrose Agar which recorded a highest average radial growth of 90.00 mm followed by Richard's agar (88 mm), Water agar. However, the lowest average radial growth of 25 mm was recorded on Rose Bengal agar after an incubation period of 9 days as indicated in Table 2 and Figure 3. The results are in accordance with Arshad *et al*., (2013) wherein they recorded maximum growth of the pathogen on potato dextrose agar with 57.80 mm. Physiological characters like different light regimes, temperature, pH, results indicated that, the exposure of the fungus to complete darkness for 8 days recorded the maximum average mycelial growth of 72.80 mm over other two treatments tested (Table 3). The average mycelial growth of fungus recorded when exposed to continuous light was 58.60 mm and 51.41 mmat alternate cycles of light and at complete dark highest mycelial growth was recorded 72.80 mm. Similarly, Hau and Rush, (1980) observed that short-cycle of 12 hrs of complete darkness found to be good light regime for sporulation.

Among the 8 temperature levels, 30°C proved to be the best temperature with maximum radial growth of 80.12 mm followed by 25°C (65.18 mm) as shown in Table 4. Minimum radial growth of 35.56 mm was recorded at 40°C. These results are in line with the Ram Dayal and Joshi, (1968), Ou, (1985), Ahmed *et al*., (2011) and Arshad *et al*., (2013), wherein Arshad *et al*., (2013) reported that, growth of the fungus was best at temperature levels ranged from 25°C to 30°C with 38-57 mm radial growth on PDA medium. Thus, from the present investigation, temperature levels ranging from25°C to 30°C proved to be the best for the growth of the pathogen. Growth of the pathogen when evaluated at different pH levels, a maximum radial growth of the fungus was recorded at pH 7.0 (90.00) followed by 7.5 (89.34), 6.5 (88.70). Lowest radial growthof the fungus was recorded at pH 4.5 (44.89 mm) and pH 5.0 (60.33 mm) (Table 5). The results recorded in the present investigation are similar to the results obtained by Naresh *et al*., (2009). They reported that, growth and sporulation of *Bipolarissorokiniana* occurred at pH 6.0-6.5 with radial growth of 58.5-89.0 mm on PDA.

3.2.Studies on the effect of different nitrogen sources on the growth of the pathogen

The effect of sixnitrogen sources on growth of *H. oryzae* was studied in Potato Dextrose broth at three concentrations (1.0, 1.5 and 2.0%)revealed that, potassium nitrate proved to be significantly superior over the other nitrogen sources tested, which recorded maximum average dry mycelial weight of 103.39 mg followed by peptone 93.78 mg, Proline (89.17). Whereas least average dry mycelial weight was recorded in Ammonium chloride (Table 6). The results obtained from the present study are in accordance with Naza *et al*., (2012). They reported that, from among the four nitrogen sources tested on radial growth of *Cochliobolusheterostrophus*potassium nitrate recorded maximum average radial growth of 90 mm.

Table. 1. Cultural and morphology variability of *Helminthosporium oryzae*on different solid media.

Sr.No.	Media	Colony	Texture/Edge	Growth
1	Malt Extract Agar	Greyish brown	Leathery thick & raised colony	Profuse mycelia with conidia
2	Yeast Extract Agar	Whitish Grey	Flat colony	Thin mycelium with no conidia
3	Oat Meal Agar	Greyish Brown	Waxy, thick with raised colony	Profuse mycelia with conidia
4	Corn Meal Agar	Greyish to dark brown	Flat colony	Thin mycelium with no conidia
5	Water Agar	Light brown	Flat thread like colony	Thick mycelia no conidia
6	Czapeck (DOX) Agar	Greyish	Waxy, thick with raised colony	Thick mycelia no conidia
7	Sabouraud's dextrose Agar	Greyish at center & white at periphery	Leathery thick & raised colony	Scanty mycelia with conidia
8	Rose bengal Agar	Greyish to brown	Waxy, thick with slightlyraised colony	Thick mycelia no conidia
9	Richards Agar	Greyish at center & white at periphery	Waxy, thick with slightlyraised colony	Profuse mycelia with conidia
10	Potato Dextrose Agar	Greyish at center & white at periphery	Waxy, thick with slightlyraised colony	Profuse mycelia with conidia

Table. 2. Growth on different media of *Helminthosporium oryzae.*

Sr.No.	Media	Type of Media	Radial growth(mm)
1	Malt Extract Agar	Non-synthetic	63
2	Yeast Extract Agar	Semi-synthetic	62
3	Oat Meal Agar	Synthetic	90
4	Corn Meal Agar	Synthetic	67
5	Water Agar	Semi-synthetic	70
6	Czapeck (DOX) Agar	Synthetic	90
7	Sabouraud's dextrose Agar	Synthetic	45
8	Rose bengal Agar	Non-synthetic	25
9	Richards Agar	Synthetic	88
10	Potato Dextrose Agar	Synthetic	90
		Sem (±)	**0.59**
		CD @ 1%	**1.77**
		CV (%)	**1.56**

Table. 3. Effect of different light regimes on growth of *H. oryzae* and its colonycharacters

Sr. no.	Light regimes	Mean colony radial growth (mm)	Colony Character

1	Alternate cycles of (12 hours of light & 12 hours of dark)	51.41	Light brown colour with moderate mycelium growth
2	Complete light (24hours)	58.60	Light brown colour with good mycelium growth
3	Complete dark (24hours)	72.80	Dark brown with good mycelial growth
	Sem (±)	**0.50**	
	CD @ 1%	**1.79**	
	CV (%)	**1.44**	

Table.4. Effect of different temperature on growth of *H.oryzae*

Sr. No.	Treatments	Mean Radial growth(mm)
1	15	43.70
2	20	50.60
3	25	65.18
4	30	80.12
5	35	43.22
6	40	35.56
	Sem (±)	**0.71**
	CD @ 1%	**2.20**
	CV (%)	**2.32**

Table. 5. Effect of different pH levels on growth of *H. oryzae*

Sr.No.	pH	Mean radial growth (mm)
1	4.50	44.89
2	5.00	60.33
3	5.50	74.49
4	6.00	87.78
5	6.50	90.67
6	7.00	91.21
7	7.50	89.34
8	8.00	83.45
	Sem (±)	**0.81**
	CD @ 1%	**2.46**
	CV (%)	**1.81**

Table.6. Growth of *H. oryzae* on different nitrogen sources

Sr.No.	Nitrogen sources	Average dry mycelial weight (mg)			Mean
		1.00	1.50	2.00	
1	Calcium nitrate	76.51	82.92	79.71	79.71
2	Potassium nitrate	99.41	107.37	103.39	103.39
3	Ammonium sulphate	82.65	89.38	86.02	86.02
4	Ammonium chloride	55.20	63.15	59.18	59.18
5	Peptone	87.45	100.11	93.78	93.78
6	Proline	85.94	92.39	89.17	89.17
	Sem (±)	**0.43**	**0.36**	**0.64**	
	CD @ 1%	**1.37**	**1.15**	**2.03**	
	CV (%)	**0.92**	**0.72**	**1.14**	

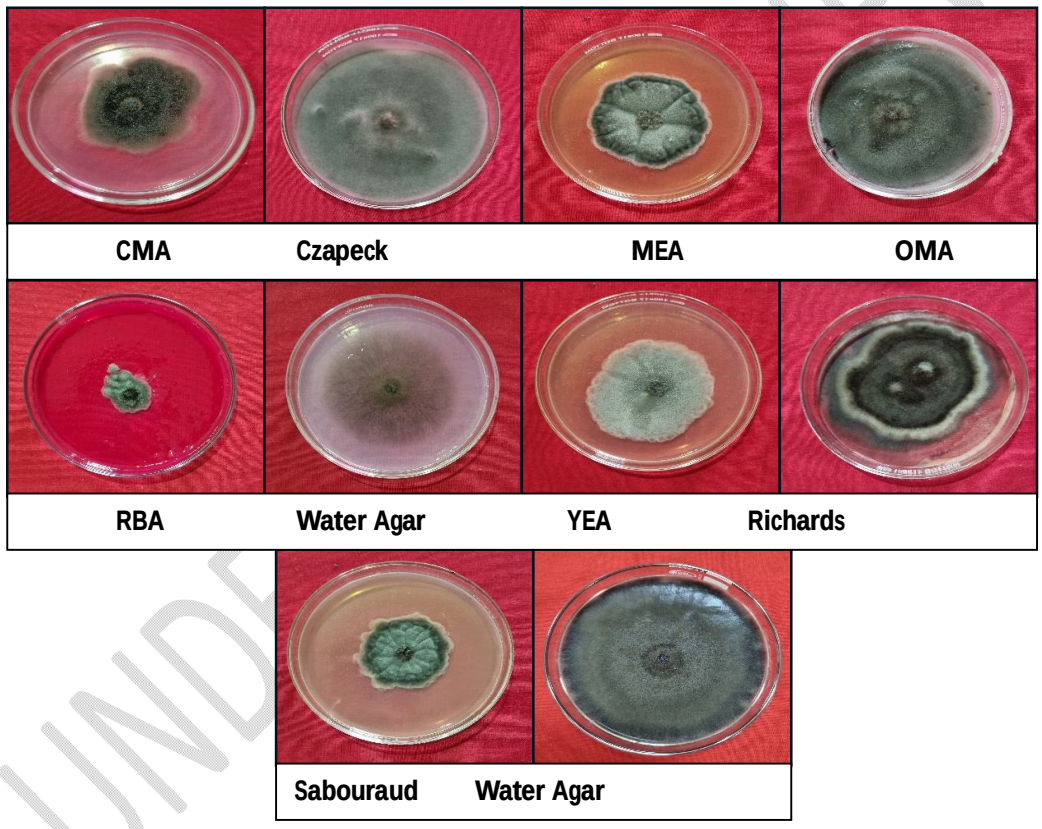

Fig.1. Radial growth of different media of *H. oryzae*

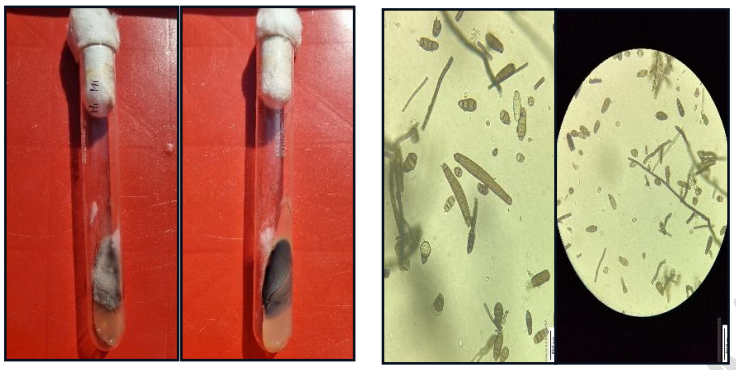

Fig.2. Morphology & cultural characters of *H. oryzae*

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