Int.J.Curr.Microbiol.App.Sci (2018) *7*(7): 1713-1722

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 07 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.707.203

Effect of Media, Temperature, Light, pH and Nutrient Source on Growth and Development of *Bipolaris oryzae* Causing Brown Leaf Spot of Paddy

C. Channakeshava* and N.S. Pankaja

[1]*Department of Plant Pathology, College of Agriculture, V.C. Farm, Mandya, India*

**Corresponding author*

Keywords

Media, Temperature, pH, Light and nutrition, Radial growth and dry mycelial weight

Article Info

Accepted:
15 June 2018
Available Online:
10 July 2018

A B S T R A C T

The pathogen *Bipolaris oryzae* was subjected to different cultural conditions viz., media, temperature, pH, light and nutrient source under *in vitro* conditions. The maximum radial growth of 84.83 mm was recorded on paddy leaf extract agar followed by potato dextrose agar (61.33 mm). On this medium, the colony appeared greyish-white to dark brown, thick, leathery slightly raised and profuse mycelia with brown colored conidia. Similarly on liquid media, maximum dry mycelial weight of 113.06 mg was recorded on paddy leaf extract broth followed by potato dextrose broth (98.28 mg).Maximum radial growth of 70.67 mm,62.83 mmand68.00 mm was recorded at 30°C,25°C and complete dark regime, respectively. Maximum dry mycelial weight of 113.0 mg was recorded at pH 7.0 followed by 103.0 mg at pH 7.5 and 97.32 mg at pH 6.5.Among the different carbon and nitrogen sources maximum growth was recorded in glucose (89.57, 102.86, 112.73 and 128.88 mg)and ammonium peptone (79.10, 97.43, 103.61 and 115.24 mg) at 0.5, 1.0, 1.5 and 2.0% concentration respectively.

Introduction

Rice is one of the major staple foods in the world and a pillar for food security in many developing countries. Rice has occupied the central position in Indian agriculture with 24 % of gross cropped area. It contributes 42 % of total food grain production and 45 % of total cereal production of the country. Karnataka is one of the major rice growing states in India where it occupies an area of 13.43 lakh ha with a production of 39.53 lakh tonnes and productivity of 3.098 t/ha (2013-14). There are about 40 diseases reported on rice to be caused by fungi and bacteria. Among these diseases, brown leaf spot caused by *Bipolaris oryzae* has been reported to occur in all rice growing regions of India (Gangopadhyaya 1983 and Ou 1985). The disease is of great importance in several countries and has been reported to cause enormous loss in grain yield (upto90%) particularly when leaf spotting phase assumes epiphytotic proportions as observed in Great Bengal Famine during 1942 (Ghose*et al*., 1960). The disease especially occurs in environment where water supply is scarce combined with nutritional imbalance particularly lack of nitrogen (Baranwal *et al*., 2013).

The growth of fungi is controlled by many factors. Culture media, temperature and light are some of the important factors influencing the growth of fungi. Every living organism requires food for its growth and reproduction and fungi are not an exception. Culturing of fungi under laboratory conditions implies that the medium should contain all the essential elements and compounds required for growth and other life processes. However, no medium is equally suitable for all fungi. Therefore, the present investigation was undertaken to measure the growth rate on different culture media, to determine optimum temperature, light, pH, and nutrient requirement of the pathogen

Materials and Methods

In vitro experiments were conducted in Plant Pathology laboratory, at the Department of Plant Pathology, College of Agriculture, V.C. Farm, Mandya, University of Agricultural Sciences, Bangalore during 2015-16.

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 in and around College of Agriculture, V.C. farm, Mandya, Karnataka. The pathogen was isolated and purified on potato dextrose agar medium.

Morphological and physiological studies

The morphological characters of the fungus were studied on 10 solid and 10 liquid media. Whereas, paddy leaf extract agar and broth was used to study the physiological characteristics like temperature, light and pH, respectively. Three different light regimes v*iz.*, continuous light (fluorescent light of 40 watts), alternate cycle of 12 hour light and 12 hour dark and continuous darkness, eight different temperature levels *viz*., 5, 10, 15, 20, 25, 30 and 35°Cand eleven levels of pH *viz*., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 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.

Carbon and nitrogen sources to estimate the mycelial weight of the pathogen

The following carbohydrates were used as carbon source *viz.*, glucose, dextrose, lactose, maltose, mannitol, sucrose, starch, cellulose and fructose and nitrogen source *viz.*, ammonium chloride, peptone, calcium nitrate, ammonium nitrate, potassium nitrate, aspergine and proleine at 4 different concentrations (0.5, 1.0 1.5 and 2%) on Richard's 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).

Results and Discussion

Studies on the morphological and physiological characters of the pathogen

Different morphological characters like size, shape, colony color, texture, edge, radial growth and dry mycelial weight were studied on 10 different media are shown in Table 1. The shape of conidia was observed as slightly curved and wide in the middle with 5-9

septations. Fully matured conidia are brownish or fuliginous with septate mycelia. The size of the conidia recorded was 11-14 x 2-3.5 µm (Fig. 1, 2 and 3). 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 paddy leaf extract agar which recorded a highest average radial growth of 90.00 mm followed by potato dextrose agar (61.33 mm).However, the lowest average radial growth of 29.33 mm was recorded on Sabouraud's agar after an incubation period of 8 days as indicated in Table 2 and Figure 4. 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. The highest average dry mycelial weight of 116.06 mg was recorded on paddy leaf extract broth followed by potato dextrose broth (98.28 mg). However lowest dry mycelial weight of 37.60 mg was recorded on Sabouraud's broth, followed by Nutrient broth (41.38 mg) (Table 2). Similar results were recorded by Ahmed *et al.,* (2011). They reported that highest dry mycelial weight of 75.80 mg on potato dextrose broth compared with other media tested.

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 68.00 mm over other two treatments tested (Table 3). The average mycelial growth of fungus recorded when exposed to continuous light was 55.00 mm and 48.50 mmat alternate cycles of light and dark. 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 70.67 mm followed by 25°C (62.83 mm) as shown in Table 4. Minimum radial growth of 36.17 mm was recorded at 5°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. Maximum dry mycelial weight of 103.14 mg was obtained at 30^0C followed by 25^0C (81.53 mg).Thus, from the present investigation, temperature levels ranging from25^0C to 30^0C proved to be the best for the growth of the pathogen. The results are confirmatory with Ahmed *et al.,* (2011). Wherein he reported a maximum dry mycelial weight of 75.80 mg, 181.80 mg at 30°C and 35°C temperatures respectively.

Growth of the pathogen when evaluated at different pH levels, a maximum dry mycelial weight of the fungus was recorded at pH ranged from 6.5-7.5 with dry mycelial weight 97.32-113.0 mg. Lowest dry mycelial weight of the fungus was recorded at pH 4.0 (49.91 mg) and pH 4.5 (62.98 mg) (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 *Bipolaris sorokiniana* occurred at pH 6.0-6.5 with radial growth of 58.5-89.0 mm on PDA.

Studies on the effect of different carbon and nitrogen sources on the growth of the pathogen

The effect of nine carbon sources on growth of *B. oryzae* was studied in Richard's broth at four concentrations (0.5, 1.0, 1.5 and 2.0%).

Table.1 Colony morphology of *B. oryzae* on different solid media

Sl. No.	Media	Color	Luster	Texture/Edge	Growth
1	Potato dextrose agar	Greyish at center, white at periphery	Dull white	Waxy, thick with raised colony	Profuse mycelia with conidia
2	Nutrient agar	Whitish olivine	Shinning	Waxy with slightly raised colony	Scanty mycelia
3	Oat meal agar	Greyish brown	Dull brown	Waxy, thick with raised colony	Profuse mycelia with conidia
4	Richard's agar	Greyish at center, white at periphery	Dull white	Leathery, thick and raised colony	Profuse mycelia with conidia
5	Malt extract agar	Greyish olivine	Dull brown	Thick, waxy with slightly raised colony	Profuse mycelia with conidia
6	Kirchoff's agar	Greyish	Dull white	Thick, waxy withslightly raised colony	Scanty mycelia with conidia
7	Sabouraud's dextrose agar	Greyish at center, white at periphery	Dull white	Leathery, thick and raised colony	Scanty mycelia with conidia
8	Czapeck'sDox agar	Greyish	Shining	Flat, thick brown border with stripes	Thick mycelia with conidia
9	Paddy leaf extract agar	Greyish-white to Dark brown	Dull brown	Thick, leathery with slightly raised colony	Profuse mycelia with conidia
10	Paddy seed extract agar	Dark brown	Dull brown	Leathery with slightly raised colony	Scanty mycelia with conidia

Table.3 Effect of different light regimes on growth of *B. oryzae* and its colony characters

Sl. No.	Light regimes	Mean colony radial growth (mm)	Colony characters
1	Alternate cycles of (12hrs light and 12hrs dark)	48.50	Light brown color with Moderate mycelia growth
2	Complete light (24hrs)	55.00	Light brown with good mycelial growth
3	Complete dark (24hrs)	68.00	Dark brown colony with good growth
	Sem (±)	**0.43**	
	CD@1%	**1.45**	
	CV (%)	**1.32**	

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

Sl. No.	Media	Type of media	Mean radial growth (mm)	Mean dry mycelial weight (mg)
1	Potato dextrose agar/broth	Semi-synthetic	61.33	98.28
2	Nutrient agar/broth	Synthetic	55.83	41.38
3	Oat meal agar/broth	Semi- synthetic	39.17	75.92
4	Richard's agar/broth	Synthetic	60.50	71.03
5	Malt extract agar/broth	Non-Synthetic	59.83	80.84
6	Kirchoff"s agar/broth	Synthetic	51.67	54.43
7	Sabouraud's dextrose agar/broth	Synthetic	29.33	37.60
8	Czapeks (Dox) agar/broth	Synthetic	48.17	56.79
9	Paddy leaf extract agar/broth	Semi-synthetic	90.83	113.06
10	Paddy grain extract agar/broth	Semi-synthetic	38.67	70.52
	Sem (±)		**0.54**	**0.41**
	CD@1%		**1.61**	**1.22**
	CV (%)		**1.78**	**1.03**

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

Sl. No.	Treatments (°C)	Mean radial growth (mm)	Mean dry mycelial weight (mg)
1	5	36.17	37.72
2	10	36.83	49.80
3	15	39.57	73.55
4	20	48.50	77.33
5	25	62.83	81.53
6	30	70.67	103.14
7	35	48.00	65.37
8	40	37.17	52.45
	Sem (±)	**0.76**	**0.49**
	CD@1%	**2.29**	**1.46**
	CV (%)	**2.81**	**1.25**

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

Sl. No.	pH	Mean dry mycelial weight (mg)
1	4.0	49.91
2	4.5	62.98
3	5.0	67.41
4	5.5	83.11
5	6.0	89.24
6	6.5	103.25
7	7.0	113.00
8	7.5	97.32
9	8.0	83.60
10	8.5	83.82
11	9.0	78.14
	Sem (±)	**0.51**
	CD@1%	**1.49**
	CV (%)	**1.16**

Table.6 Growth of *B. oryzae* on different carbon sources

Sl. No.	Carbon sources	Average dry mycelial weight (mg)				Mean
		Concentration (%)				
		0.5	**1.0**	**1.5**	**2.0**	
1	Glucose	89.26	102.39	112.73	128.88	108.31
2	Maltose	72.37	82.14	82.16	85.49	80.54
3	Dextrose	35.77	45.96	80.16	89.11	62.5
4	Sucrose	87.52	98.20	107.14	121.76	103.65
5	Mannitol	61.89	67.01	91.03	91.23	77.79
6	Starch	51.00	81.43	92.28	92.84	79.38
7	Fructose	49.35	78.46	80.59	90.16	74.64
8	Lactose	39.29	43.06	77.87	80.96	60.29
9	Cellulose	67.27	69.12	81.77	82.40	75.14
		Carbon sources (T)		**Concentrations (C)**		**T X C**
	Sem (±)	**0.30**		**0.20**		**0.59**
	CD@1%	**1.11**		**0.74**		**2.11**
	CV (%)	**1.28**				

Table.7 Growth of *B. oryzae* on different nitrogen sources

Sl. No.	Nitrogen sources	Average dry mycelial weight (mg)				Mean
		Concentration (%)				
		0.5	**1.0**	**1.5**	**2.0**	
1	Ammonium chloride	37.88	52.35	60.66	67.94	54.70
2	Peptone	79.29	85.92	98.00	108.16	96.09
3	Calcium nitrate	58.04	73.21	77.39	93.27	75.47
4	Ammonium sulphate	78.17	80.91	86.09	89.00	83.54
5	Potassium nitrate	81.73	97.43	103.38	115.24	99.44
6	Aspergine	77.67	83.32	85.62	86.20	83.20
7	Proline	57.49	82.46	89.32	89.37	79.66
		Nitrogen sources (N)		**Concentrations (C)**	**N X C**	
	Sem (±)	**0.21**		**0.16**	**0.42**	
	CD@1%	**0.79**		**0.60**	**1.59**	
	CV (%)	**0.90**				

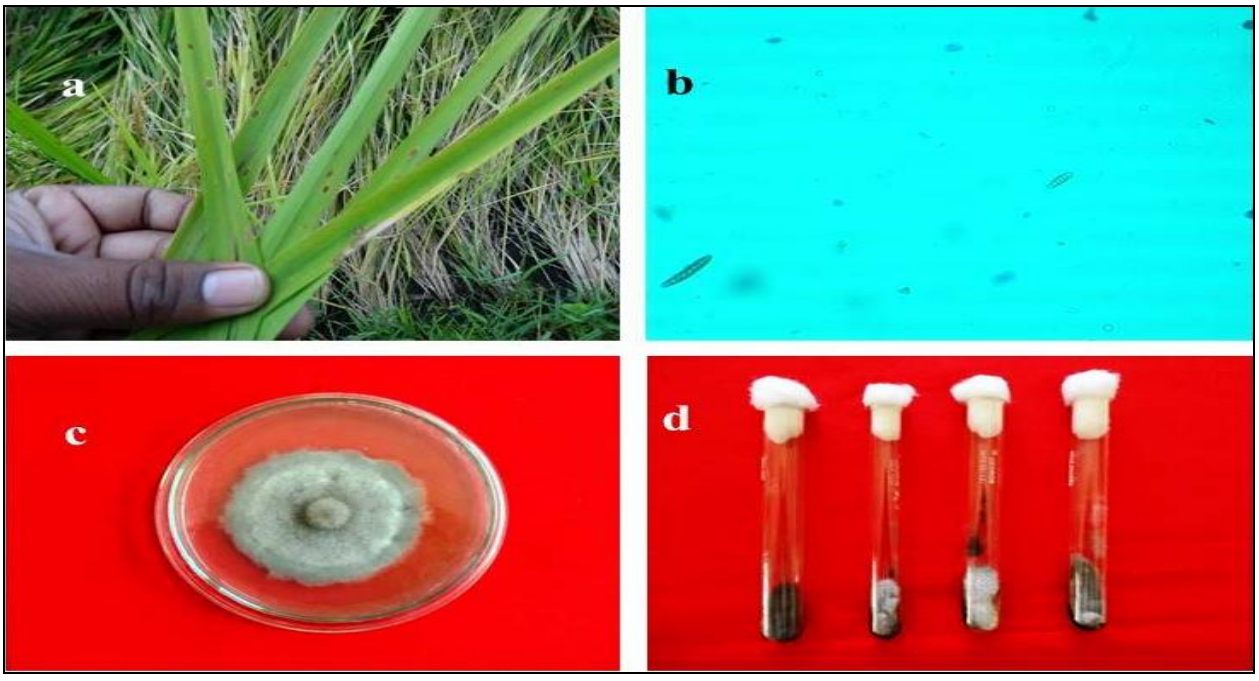

Fig.1 a): Paddy leaves showing brown spot disease symptoms b) Conidia of *B. oryzae* under 10X c) Colony of *B. oryzae* on PDA d) Pure culture of *B. oryzae* PDA Slants

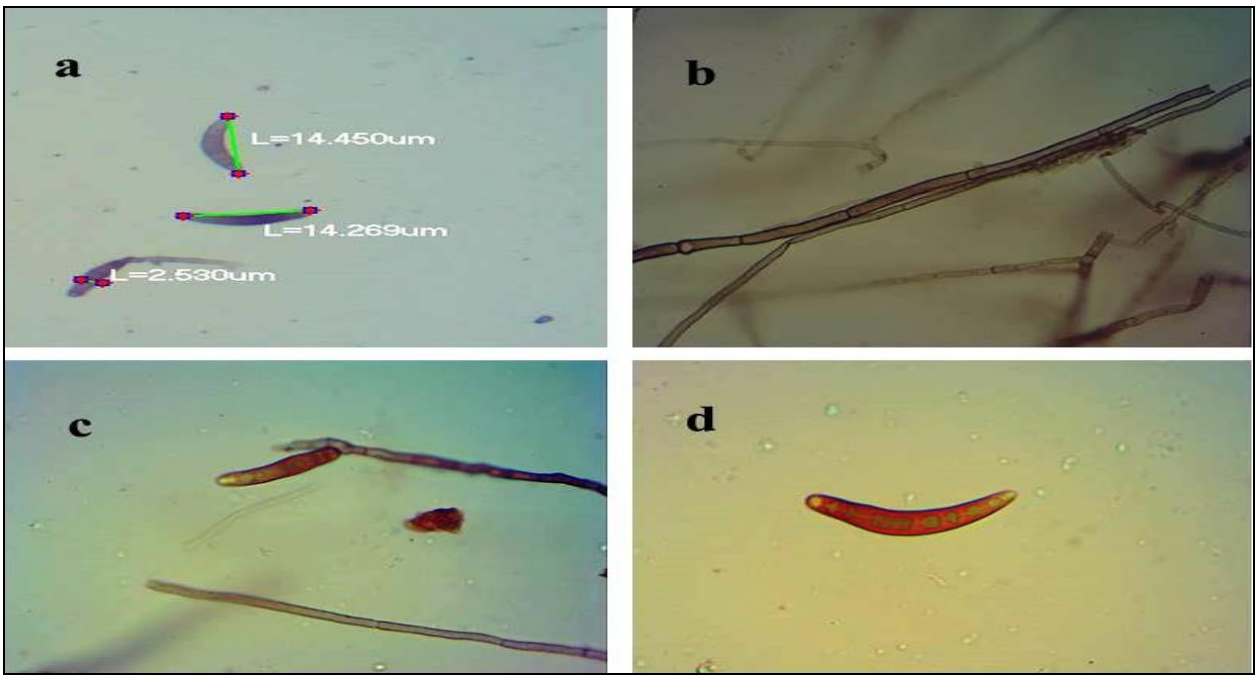

Fig.2 Microscopic view of conidia of *B. oryzae*
(a) Conidia under 10x (11-14 x 2-3.5 µm) b) Septate mycelia c) Conidia under 40x
d) Conidia under 100x

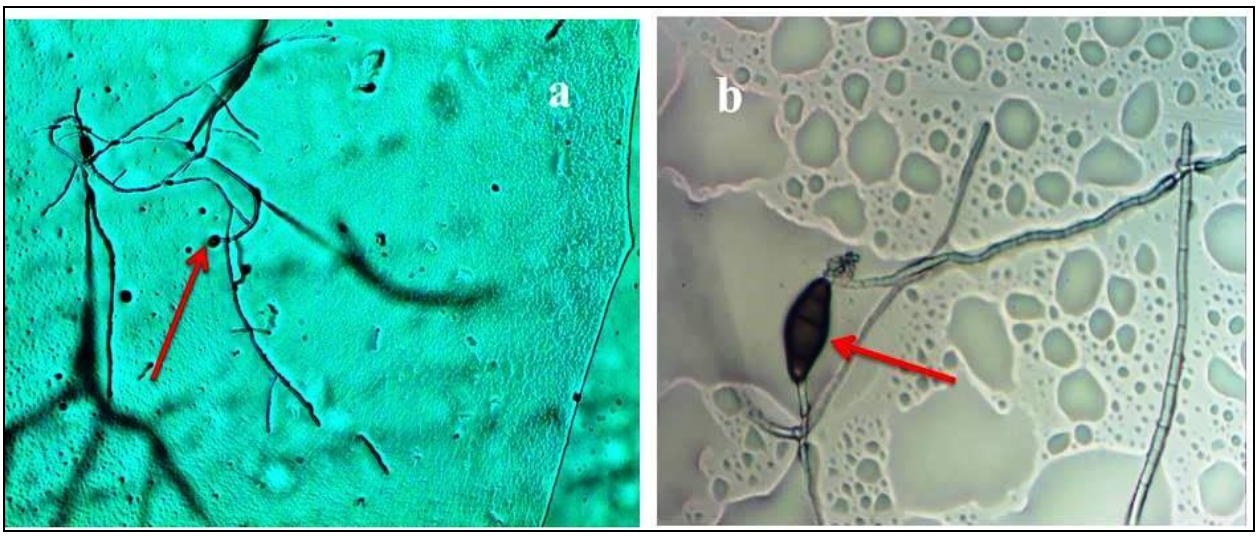

Fig.3 Germinating spores of *B. oryzae* on sterilized water; a) Spore germination 24 hrs after incubation on distilled water; b) Spore germination on both the sides of the conidia

Fig.4 Radial growth of *B. oryzae* on different solid media
T_1: Potato dextrose agar; **T_2:** Nutrient agar; **T_3:** Oat meal agar; **T_4:** Richard's agar; **T_5:** Malt extract agar; **T_6:** Kirchoff's agar; **T_7:** Sabouraud's dextrose agar; **T_8:** Czapek's (Dox) agar; **T_9:** Paddy grain extract agar; **T_{10}:** Paddy leaf extract agar

The results indicated that, the dry mycelial weight varied with carbon sources. However, glucose recorded the maximum average dry mycelial weight of 108.31 mg which is significantly superior over the other carbon sources, followed by sucrose with 103.65 mg from all the concentrations tested (0.5, 1.0, 1.5 and 2.0%). Least minimum average dry mycelial weight of 60.29 mg was recorded in lactose and is indicated in Table 6. Riaz *et al.*, (1974) also found similar effect of glucose on dry mycelial weight of *Helminthosporium oryzae* where in they reported maximum average dry mycelial weight of 79.48 mg at 5%. Further they also reported that, out of monosaccharides and oligosaccharides tested, glucose and sucrose found to be the best carbon sources at 5% with 100% spore germination.

The effect of seven different nitrogen sources at 4 different concentrations (0.5, 1.0, 1.5 and 2.0%) on Richard's broth revealed that, potassium nitrate proved to be significantly superior over the other nitrogen sources tested, which recorded maximum average dry mycelial weight of 99.44 mg followed by peptone 96.09 mg. Whereas, Aspergine was on par with ammonium sulphate which recorded 83.54 and 83.20 mg average dry mycelial weight respectively. Least average dry mycelial weight of 75.47 mg was recorded in calcium nitrate (Table 7). 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 *Cochliobolus heterostrophus* potassium nitrate recorded maximum average radial growth of 90 mm.

References

Ahmed, S. G., Garg, V. K., Pandith, A. K., Anwar, A. and Aijaz, S., 2011, Disease incidence of paddy seedlings in relation to environmental factors under temperature agronomic conditions of Kashmir valley. *J. Res. Dev.,* 11:29-38.

Arshad, H. M. I., Hussain, N., Khan, J. A., Sallem, K. and Baber, M. M., 2013, Behavior of *Bipolaris oryzae* at different temperatures, culture media, fungicides and rice germplasm for resistance. *Pakistan J. Phytopath.*, 25(1): 84-90.

Baranwal, M. K., Kotasthane, A., Magculia, N., Mukherjee, P. K., Savary, S., Sharma, A. K., Singh, H. B., Singh, U. S., Sparks, A. H., Variar, M. andZaidi, N.,2013, A review oncrop losses, epidemiology and disease management ofrice brown spot to identify research priorities and knowledge gaps. *European J. Pl. Pathol*., 136: 443-457.

Gangopadhyay, S., 1983, Current concepts on fungal diseases of rice. *Today and tomorrow's* Printers and Publishers, *New Delhi,* 349 pp.

Ghose, R. L. M., Ghatge, M. B. and Subramanian, V., 1960, *Rice in India* (revised edn.)ICAR, *New Delhi*, 474 pp.

Hau, F. C. and Rush, M. C., 1980, A system for inducing sporulation of *Bipolaris oryzae. Pl. Dis.*, 64:788-789.

Kumari, S., Kumar, A. and Rani, S., 2015, Morphological characterization of *Bipolaris oryzae* causing brown spot of paddy in Bihar. *Int. Educ. Res. J.*, 1(5): 85-87.

Naresh, P., Biswas, S. K., Kumar, U. and Rajik, M., 2009, Effect of media, pH, Temperature, Host range and fungicides on *Bipolariss orokiniana. Ann. Pl. Protec. Sci.*,17(2): 394-397.

Naza, I., Sehar, S., Perveen, I., Rehman, A., Hameed, A., Ahmad, Y. and Ahmed, S., 2012, Optimization of cultural conditions for *Cochliobolus heterotrophus* isolates from infected maize plants from different Agricultural zones of Pakistan. *British Microbiol. Res. J.*, 2(4): 233-242.

Ou, S. H., 1985, *Rice disease* 2nd edn. Kew, *Commonwealth Mycological Institute*. 380 pp.

Panse, V. G. and Sukathme, P. V., 1985, *Statistical method for Agricultural workers.* ICAR Publications, pp. 145-155. New Delhi.

Riaz, M., Ahmed, A. N., Mirza, J. H. andNasir, M. A., 1974, Effect of different carbon sources on spore germination of *Helminthosporium oryzae. Pakistan J. Bot.,* 6(1): 65-68.

Sundraraman, S., 1919, *Helminthosporium* disease of rice. *Bull. Res. Inst. Pusa*128: 1-7.

How to cite this article:

Channakeshava, C. and Pankaja, N.S. 2018. Effect of Media, Temperature, Light, pH and Nutrient Source on Growth and Development of *Bipolarisoryzae* Causing Brown Leaf Spot of Paddy. *Int.J.Curr.Microbiol.App.Sci.* 7(07): 1713-1722.
doi: https://doi.org/10.20546/ijcmas.2018.707.203