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Effect of different growth media on radial growth, dry mycelial weight and sporulation of *Bipolaris sorokiniana*

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Abstract

Bipolaris sorokiniana is a hemibiotrophic, phytopathogenic fungus causing spot blotch of wheat. It is prevalent in warmer and humid wheat growing regions of the world and has special significance in the Eastern Gangetic Plains (EGP) of South Asia, which includes India, Nepal and Bangladesh. Spot blotch pathogen has a worldwide distribution and is particularly aggressive under high relative humidity and temperature associated with imbalanced soil fertility. An experiment was conducted to find out a suitable culture media for growth and sporulation of *Bipolaris sorokiniana*. Findings of present investigations revealed that out of nine growth media (potato dextrose, Asthana Hawker's, Czapek's, Richards', malt extract, oat meal, standard nutrients, corn meal and wheat leaf decoction media) tested, Oat meal medium was best suited media for radial growth and biomass production of *Bipolaris sorokiniana* as highest colony diameter as well as dry mycelial weight was recorded on this media. For sporulation of *Bipolaris sorokiniana*, potato dextrose agar media was best as highest numbers of spores/ml were recorded in this media.

Keywords: *Bipolaris sorokiniana*, spot Bloch, wheat, growth media, radial growth, dry mycelial weight, sporulation.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the oldest and most important cereal crop. It is the second important staple cereal food in India after rice and has played vital role in stabilizing the food grain production in the country. Wheat contributes nearly one third of the total food grain production in India and one tenth of the global production is contributed from India. India has emerged as the second largest producer of wheat in the world by harvesting a record production after China. Wheat serves as a staple food for more than one billion people in the world and contributes about 20 per cent of total food calories for human being.

Wheat crop is affected by many fungal diseases. Among these spot blotch has emerged as the number one problem in hot and humid wheat cultivating regions [1]. Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) SHOem. a hemibiotrophic, phytopathogenic fungus is prevalent in warmer and humid wheat growing regions of the world. This has special significance in the Eastern Gangetic Plains (EGP) of South Asia, which includes India, Nepal and Bangladesh [2]. Spot blotch pathogen has a worldwide distribution and is particularly aggressive under high relative humidity and temperature associated with imbalanced soil fertility [9]. An experiment was conducted in the department of Plant Pathology, RPCAU, Pusa to find out a suitable culture media for growth and sporulation of *Bipolaris sorokiniana*.

2. Materials and Methods

Effect of nine growth media, namely Potato dextrose, Asthana Hawker's, Czapek's, Richards' medium, Malt extract's, Oat meal, Standard nutrients, Corn meal and Wheat leaf decoction's media, were tested. All the growth media were prepared according to the standard formulae given by [8], [10], [1], [3]. All the solid media were sterilized in an autoclave at a pressure of 15 lbs p.s.i. for 15 minutes. Liquid media were sterilized at 10 lbs p.s.i. for 10 minutes and the process was repeated after 24 hours. For inoculating different solid media in Petri-plates, 7 days old culture grown on PDA medium was used. The size of the inoculum was standardized by cork borer of 5 mm diameter. The inoculum (5mm diameter) was placed at the centre of the plate in an inverted position. For inoculating different liquid media in 100 ml Erlenmeyer flasks containing 25 ml broth medium, one disc of 5 mm diameter of fungal mycelium was

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allowed to float on the medium. All inoculations were carried out under aseptic conditions. The inoculated Petri-plates and flasks were incubated at $28 \pm 1^\circ\text{C}$ in B.O.D. incubator for 7 days and 21 days, respectively.

Radial growth of the regular colonies was measured in two directions at right angle with the help of linear scale. In case of irregular colonies measurements were recorded at the broadest and narrowest diameter and an average of two different directions were taken as growth. The mycelial mats were harvested with the help of Whatman filter paper no. 42 and washed thoroughly with hot water to remove the traces of suspended sugars. Mycelial mats along with filter papers were dried at 60°C for 24 hrs and then cooled in desiccators. The mycelial mats were weighed and again dried in oven until the constant weights were obtained. Weight of mycelial mat was calculated with the help of the formula: $DW = (W_2 - W_1)$, where, DW = Dry weight of mycelial mat, W_2 = Weight of fungus along with filter paper and W_1 = Weight of filter paper. For estimation of sporulation, at the end of the incubation period a five mm disc of the culture was cut from the near center portion of the plate and suspended in sterilized water (10 ml) and shaken well, so that the spores were dislodged. One drop of this spore suspension was placed on a haemocytometer and the numbers of spores in 4 squares at random, were counted. The number of spores per ml was calculated with a haemocytometer, using the formula: No. of spores/ml (N) = $n \times 16 \times 10^3$, where, N = Total No. of spores counted/ml, n = Average number of spores in each smallest square of haemocytometer

3. Results and Discussion

Data presented in Table 1 and illustrated in fig. 1 reveals that after seven days of incubation maximum colony diameter, i.e. 84.25 mm was recorded on oat meal agar medium which was statistically superior to colony diameter observed on rest of the media tried except potato dextrose agar. Oat meal agar medium was statistically at par with potato dextrose agar in which 83.25 mm growth was observed. This indicates that maximum growth of *Bipolaris sorokiniana* was supported by oat meal agar medium followed by potato dextrose agar medium which recorded 83.25 mm colony diameter. Potato dextrose agar medium was also statistically superior to other media tried in supporting radial growth. Next to potato dextrose agar medium, maximum growth was observed on Richard medium, i.e. 66.50 mm which is statistically inferior to oat meal agar and potato dextrose agar medium. Richards's medium was statistically superior to remaining all media tried except wheat leaf decoction medium which supported 63.25 mm growth. In standard nutrients medium 55.25 mm growth of colony diameter recorded which was statistically superior to corn meal agar, Asthana Hawker's medium and Czapek's agar medium but it was statistically at par with malt extracts medium in which 52.75 mm growth was observed. Asthana Hawker medium recorded only 30.00 mm of colony diameter, which was statistically superior to only Czapek's agar medium which supported 18.75 mm growth and was at par

with corn meal agar in which 29.50 mm growth of colony diameter observed. Least growth (18.75 mm) of colony diameter observed on Czapek's agar medium was statistically inferior to all other media tried. Data presented in Table 1 clearly indicates that oat meal agar medium is best suited medium for radial growth of *Bipolaris sorokiniana* followed by potato dextrose agar medium whereas least growth of pathogen was supported by Czapek's agar medium.

The fungus sporulated more or less in all the media used but highest sporulation was observed on potato dextrose agar medium. Maximum sporulation (5.18×10^4 spores/ml) was recorded on potato dextrose agar. After potato dextrose agar, second best medium for supporting sporulation was oat meal agar in which sporulation was 4.87×10^4 spores/ml. Richard medium was also good for supporting sporulation in which 4.06×10^4 spores/ml was observed. On malt extract, Czapek's agar, standard nutrients and Asthana Hawker medium sporulation was 3.31×10^4 , 2.75×10^4 , 2.25×10^4 and 2.06×10^4 spores/ml, respectively. Lowest sporulation (1.12×10^4 spores/ml) was found in corn meal agar medium. It can be concluded that potato dextrose agar is best suited for sporulation of *Bipolaris sorokiniana* followed by oat meal agar medium whereas corn meal agar medium is worst.

Data presented in Table 1 also revealed that maximum dry mycelial weight of 387.00 mg of *Bipolaris sorokiniana* was recorded on oat meal medium which was statistically superior to dry mycelia weight recorded on rest of the media. Next best medium for supporting the growth of *Bipolaris sorokiniana* was Richards' medium which yielded 346.50 mg dry mycelial weight; however, it was statistically inferior to oat meal medium. Richards's medium was statistically superior to rest of the medium in supporting biomass production. In other media biomass production ranged between 190.25 to 326.00 mg, the minimum in Czapek's agar medium. All the media used in the present investigation were significantly different from each other in supporting biomass production but maximum biomass production was supported by oat meal medium followed by Richards' medium. Minimum biomass production was on Czapek's medium.

Similar findings were observed by many other workers. Misra and Munankami (1970) [4] reported that, *H. tetramera* Mckinney made maximum growth on potato dextrose agar and found almost equally good growth on Richards's, oat meal and corn agar. Patil (1982) [6] observed the maximum growth of *D. sorokiniana* of barley on potato dextrose agar and maximum dry mycelial weight of *D. sorokiniana* in Richards's medium followed by potato dextrose broth. Raguchander *et al.* (1988) [7] have also reported good growth of *B. sorokiniana* on oat meal and potato dextrose agar and the maximum growth of *B. sorokiniana*. However, according to Narayan (2004) [5] potato dextrose agar medium and Richards's medium were best suited for radial growth, sporulation and biomass production of *Helminthosporium sativum*.

Table 1: Effect of growth media on radial growth, dry mycelial weight and sporulation of *Bipolaris sorokiniana*

Media	Colony diameter (mm)		Sporulation (No. of spores $\times 10^4$ /ml)	Dry mycelial weight (mg)*
	5 days*	7days*		
Asthana Hawker	15.75	30.00	2.06	219.00
Czapek's	14.25	18.75	2.75	190.25
Richards'	46.25	66.50	4.06	346.50
Malt extract	45.00	52.75	3.31	259.75

Oat meal	71.75	84.25	4.87	387.00
Corn meal	29.00	29.50	1.12	208.75
Standard nutrients	39.25	55.25	2.25	288.75
Wheat leaf decoction	49.00	63.25	3.00	305.75
Potato dextrose	72.75	83.25	5.18	326.00
CD 5%	6.57	5.87		7.94
SE(m)	2.25	2.01		2.72

*Average of 4 replications

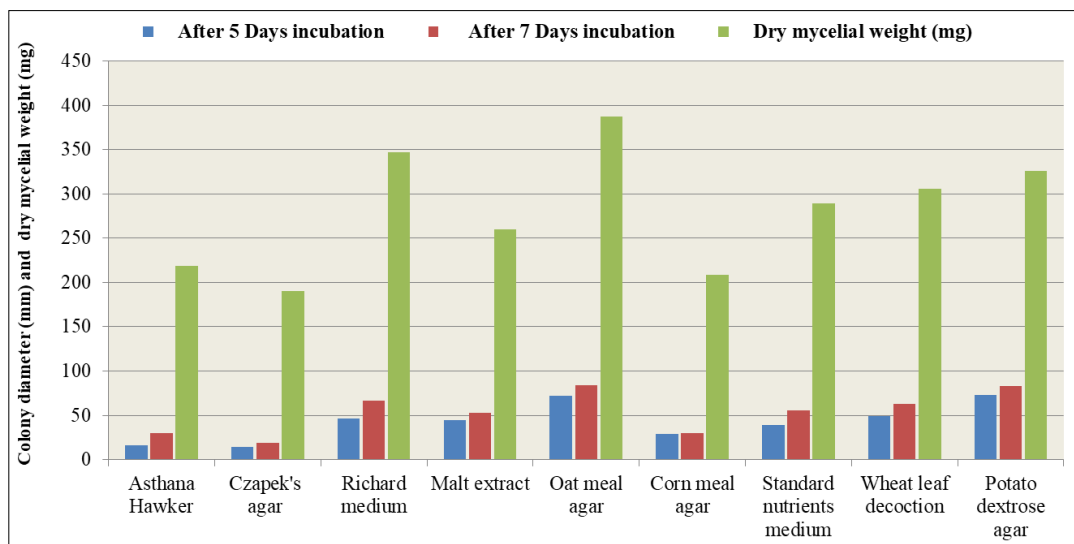


Fig 1. Effect of growth media on radial growth and dry mycelial weight of *Bipolaris sorokiniana*

4. Conclusion

Oat meal agar medium and oat meal medium are best suited medium for radial growth and biomass production of *Bipolaris sorokiniana*, respectively. However, potato dextrose agar is best suited for sporulation of *Bipolaris sorokiniana*.

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