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Assessment of Some Culture Media for Optimum Growth and Sporulation of *Bipolaris sorokiniana* Causing Spot Blotch of Wheat

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Abstract

An experiment was conducted during January–June, 2022 at Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya for the evaluation of the different culture media on the growth of the fungus *Bipolaris sorokiniana* causing spot blotch disease in wheat. Five different growth media, *viz.*, Potato Dextrose Agar (PDA), wheat seed extract+PDA (WSPDA), wheat leaf decoction+PDA (WLPDA), carrot juice extract+PDA (CPDA), and oatmeal agar (OMA), were used in the experiment. PDA supplemented with different plant extracts exhibited differential growth and sporulation patterns on *B. sorokiniana*. The maximum colony diameter of 8.09 mm at 9 days after inoculation demonstrated that the OMA provided the best growth conditions. Moreover, based on the Tukey HSD test, the AUGPC of OMA was found to be the highest (34.68±1.3 cm^2) and significantly different from all the other media used in the study. The OMA was followed by the WLPDA, which had an AUGPC of 22.6±1.79 cm^2. The fungus sporulated in all the media considered for the present study, with OMA recording the highest sporulation with 44×10^4 spores ml^{-1}, followed by WLPDA with 41×10^4 spores ml^{-1}. The lowest level of sporulation was seen in CPDA, with a concentration of 15×10^3 spores ml^{-1}. Based on the aforementioned results, it can be inferred that oatmeal agar was the most suitable medium for the development and sporulation of *B. sorokiniana*, followed by a mixture of wheat leaf decoction and PDA. On the other hand, PDA alone and carrot juice PDA were shown to be the least suitable media.

Keywords: *Bipolaris sorokiniana*, OMA media, sporulation, spot blotch

1. Introduction

Wheat plays a pivotal role in sustaining over a billion people worldwide, serving as a fundamental crop that contributes around 20% of the total calories in our diet (Zewdu et al., 2024). In 2020, the global wheat production reached approximately 776.5 million mt. It is projected to increase to 778.3 million mt in 2021 and 770.3 million mt by 2022 (Anonymous, 2022). Having said that, the crop is exposed to a number of biotic and abiotic stresses. Among the biotic constraints, spot blotch of wheat emerges as a major threat to wheat production in warmer and humid regions such as Eastern Gangetic Plains of India, Nepal, Bangladesh, China, and Africa (Patsa et al., 2018b; Joshi et al., 2007; Laila et al., 2010). It is widespread throughout the wheat growing regions, mainly in the North Western and North Eastern Plains Zone of India (Patsa et al., 2018a; Jesumaharaja et al., 2023). In India, the average yield loss due to spot blotch was reported to be between 18–22%, but could potentially reached up to 87% under severe conditions on susceptible varieties (Gupta et al., 2018; Singh et al., 2023).

Microbes are extremely diverse in nature. They survive in various habitats and have varying requirements to achieve their optimum growth and development. Nutritional availability, pH, osmotic pressure, and temperature are of vital importance for growth (Ganeshamurthy et al., 2021). Different microbes require different media for their effective growth and sporulation. Therefore, the preparation of a suitable culture media is considered to be a pre-requisite study. Almost 99% of all microorganisms are still unculturable as the ideal media composition identical to its environmental conditions could not be replicated in the laboratory (Ferrari et al., 2005; Vartoukian et al., 2010). The development of microorganisms in artificial media is influenced by several physical and chemical variables. To support the growth of microorganisms in a controlled laboratory environment, culture medium, also known as growth media containing required nutrients, energy

sources, growth-promoting agents, minerals, etc. is required (Su et al., 2012).

Culture media are the food material in which the microbes grow. Bacteria are commonly cultured in an agar-less media, mostly in nutrient broth (Aneja, 2005), whereas, fungal growth media are usually complex, rich with carbohydrate and nitrogen source, at pH range between 5 and 6, and with an ideal temperature range from 15 to 37˚C. Fungal culture media can be natural or synthetic (Senanayake et al., 2020). The particular pathogen, *B. sorokiniana* exhibits a range of morphological characteristics, including variations in colony color, colony diameter, conidial shape and size, as well as sporulation. These variations occur across diverse growth media, such as potato dextrose agar, carrot agar, corn meal, and wheat leaf decoction media (Nasreen et al., 2017). According to Ismail et al. (2023) Potato dextrose agar, corn meal agar and V8 agar media shows variation in mycelial growth and sporulation of B*. sorokiniana* and it has the capability for reproduction using PDA culture media. In their study, Hu et al. (2024) found that *P. indica* exhibited the highest growth rate on Potato Dextrose Agar, followed by Oatmeal Agar, and Luria-Bertani Agar. Additionally, they determined that Oatmeal Agar was the most optimal medium for spore generation. Therefore, it is necessary to investigate the growth patterns and the sporulation nature of the *B. sorokiniana* isolate collected to analyze the best growth media to ensure successful *in-vitro* culture maintenance and enhanced sporulation for further studies in the screening of disease resistance. In the present study, five different growth media were evaluated to determine the best one for the optimum growth and sporulation of the *B. sorokiniana* isolate causing severe spot blotch in wheat.

2. Materials and Methods

The study was conducted during January–June, 2022 at Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Coochbehar, West Bengal, India.

2.1. Preparation of various growth media

In the present investigation, a total of five different media viz., potato dextrose agar, PDA incorporated with wheat seed extract (WSPDA), PDA incorporated with wheat leaf extract (WLPDA), PDA incorporated with carrot juice extract (CPDA), and oatmeal agar (OMA) were prepared and tested for its effectiveness in the growth and sporulation of *B. sorokiniana* fungus during January–June, 2022.

PDA was prepared by first boiling 200 g of cleanly sliced potato tuber in 500 ml of water. The water was strained out and in it 20 g dextrose and 20 g agar were added. A pinch (0.2 g) of chloramphenicol was then added. The final volume was then made up to 1 l with distilled water (Table 1). In a similar manner, WLPDA, WSPDA, and CPDA were prepared following the above procedure, with the only difference being the inclusion of 100 g of wheat leaf, wheat seed, and carrot juice extract, respectively, along with 100 g of sliced potato. The OMA was prepared by mixing 35 g of oats, 3–5 g of sucrose, and 0.2 g of chloramphenicol in 1 l of distilled water (Table 1). After preparation, all the media were autoclaved at 121°C at 15 psi for 15 min. Then the media were poured in Petri plates and slants for further use in culturing the fungus, *B. sorokiniana.*

Table 1: Composition of different culture media for growth and reproduction of *B. sorokiniana*

Media	Volume (L)	Ingredients
PDA	1	Sliced potato (200 g), dextrose (20 g), agar (20 g), Chloramphenicol (0.2 g)
WSPDA	1	Sliced potato (100 g), Wheat seed (100g), Dextrose (20 g), Agar (20 g), chloramphenicol (0.200 g)
WLPDA	1	Sliced potato (100 g), Wheat leaf (100g), Dextrose (20 g), Agar (20 g), chloramphenicol (0.200 g)
CPDA	1	Sliced potato (100 g), carrot juice (100g) Dextrose (20 g), Agar (20 g) chloramphenicol (0.200 g)
OMA	1	Oats (35 g), Sucrose (3-5 g), Agar (20g), Chloramphenicol (0.200 g)

2.2. *Collection, isolation, and identification of B. sorokiniana*

From the experimental field of the Department of Plant Pathology, UBKV, Cooch Behar, West Bengal (736 165), India, suspected diseased leaf samples with distinct spot blotch symptoms were taken for the isolation of the pathogen, *B. sorokiniana*. A thorough rinsing with running water removed debris from leaf samples. Infected plant tissues were sliced into 5×5 mm^2 pieces with a sterile scalpel. Infected tissues and green, healthy, actively growing leaf tissue segments were selectively excised from lesions, focusing on the advanced margins. After soaking the sliced pieces in 2% sodium hypochlorite solution for 30 seconds, they were washed with sterile distilled water three (3) times and placed on blotting paper to dry out extra moisture. Each leaf piece was placed on PDA–containing petri plates and slants after drying. The samples are incubated for 7 days at 25±2°C in a BOD incubator. In order to make a pure culture of *B. sorokiniana*, a tiny portion of the established hypha from the outermost region of the actively growing hyphal tips was transferred to PDA plates and slants under laminar airflow and kept in an incubator (Noman et al., 2018). The morphological features and fungal growth were observed and recorded.

2.3. *Inoculation of B. sorokiniana onto different growth media*

From the previously prepared ten days old pure culture of *B. sorokiniana,* fungal discs were cut off using a sterilized cork borer and inoculating needle and transferred into different petri plates and slants (under a laminar airflow) containing various growth media that were prepared previously. The culture was incubated at 25±2°C under a 12 hour light-dark

cycle for two weeks to ensure optimal growth followed by regular sub culturing.

2.4. Morphological characters, growth of B. sorokiniana and its sporulation

The color of the fungal colonies, colony texture, and colony margins in each of the different culture media were recorded regularly. Microscopic characteristics of the fungus were examined using a seven to ten days old culture of *B. sorokiniana* grown in each of the five different culture media under a compound microscope. The fungal growth was also recorded by measuring the colony diameters on the same axis using a transparent scale (in cm) at 2 days intervals. The area under growth progress curve (AUGPC) was then calculated using the formula, $AUGPC=\Sigma_{i=1} [\{(y_i+y_{i+1})/2\} (t_{i+1}- t_i)]$ where, y_i is the growth at i^{th} day, $t_{i+1}- t_i$ is the duration between two observations, and n is the number of observations (Campbell and Madden, 1990). Further, the sporulation of the fungus was measured using a hemocytometer. After the incubation for 7 days, the fungal spores were collected from each of the culture plates supplemented with varied media using sterile distilled water, and a spore suspension was prepared. The suspension was observed under a hemocytometer to determine the spore count of the fungus. All the measurements were taken in triplicates.

2.5. Statistical analysis

One-way ANOVA (analysis of variance) was conducted to test the statistically significant differences between the mean values of AUGPC under varied culture media at $p<0.01$. Further, a post hoc test (Tukey's HSD test) was performed to check the pairwise mean differences between each culture media. All the statistical analysis was performed in SPSS 27 software.

3. Results and Discussion

3.1. Morphological study

Based on the visual observation of the fungal plates, *B. sorokiniana* exhibited a dark, blackish colony with whitish growth in PDA and WLPDA while OMA showed a slightly dark or greyish-white to a light-brown colored colony with a dark center (Figure 1, Table 2). On the other hand, WSPDA and CPDA exhibited a light greyish colony with whitish growth. OMA and WLPDA showed regular colony margins while

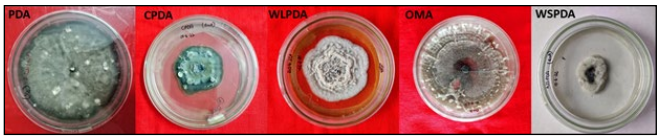

Figure 1: Growth pattern of *B. sorokiniana* in response to various culture media used

WSPDA, CPDA, and PDA showed irregular wavy colony margins (Figure 1, Table 2). Under the microscopic observations, the fungal spores were perceived to be olive-brown to dark brown in color, slightly curved, oblong to ellipsoidal, and widest at the near middle portion tapering towards both ends. The conidia were formed singly at the tip of the conidiophores. They are smooth-walled and have abruptly rounded basal ends. The spores have an average of 2–10 transverse septa.

Various microorganisms possess distinct nutrient needs for their growth and reproduction. Consequently, it is imperative to have a suitable growth medium as a fundamental prerequisite when cultivating these microorganisms in a controlled environment (*in vitro*) to establish a pure culture. This pure culture is essential for conducting comprehensive studies aimed at developing effective disease management strategies against the pathogen. The fungal pathogen *B. sorokiniana*, responsible for wheat spot blotch, displays diverse morphological traits when grown in different growth media and exhibits variations across different geographical locations. Therefore, in the current investigation, we assessed the impact of five distinct growth media viz., PDA, WLPDA, WSPDA, CPDA, and OMA on the morphology and growth of *B. sorokiniana*. This evaluation aimed to identify the most suitable medium for establishing a pure culture and sustaining its growth and sporulation. The pure culture will be further employed in future studies for artificial inoculation during resistance screening in the field.

3.2. Effect of different growth media on radial growth and sporulation of B. sorokiniana

The radial growth of the fungus was recorded regularly and it was observed that OMA media gave the best growth conditions which was evident by the maximum colony diameter of 8.09 mm at 9 days after inoculation followed by WLPDA (4.96 mm), CPDA (3.04 mm), while the least growth of the fungus was observed in WSPDA and PDA with a colony diameter of 2.6 mm (Table 3). Further, the AUGPC was estimated for all

Table 2: Morphological characteristics of *B. sorokiniana* colony observed under different growth media

Media	Media color	Colony color	Colony margin	Colony texture
PDA	White	Slight fluffy, blackish colony with whitish growth	Irregular or wavy	Compact and thick
WSPDA	Light brown	No fluffy growth with greyish black colony	Irregular	Loose and fluffy
WLPDA	Yellowish/ light orange	Blackish colony with whitish growth	Regular	Compact and less fluffy
CPDA	Yellowish	Whitish grey with whitish growth	Irregular	Light and less fluffy
OMA	White	Greyish white to light brown	Regular	Light and thin

Table 3: Effect of different growth media on radial growth and sporulation of *B. sorokiniana*

Media	Growth (in cm)				AUG-PC** (cm^2)	Sporulation (spores ml^{-1})
	2 DAI	5 DAI	7 DAI	9 DAI		
PDA	1.60	2.04	2.34	2.67	13.03± 0.69[a]	7×10^4
WSPDA	1.87	2.08	2.23	2.60	13.09± 0.38[a]	6×10^4
WLPDA	1.96	3.40	4.44	4.96	22.6± 1.79[b]	7×10^4
CPDA	1.96	2.40	2.77	3.04	15.61± 0.34[a]	15×10^3
OMA	3.07	4.93	6.83	8.09	34.68± 1.3[c]	44×10^4

five media by taking the colony diameters on different days after inoculation. It was observed that the OMA gave the highest AUGPC value of 34.68±1.3 cm^2 which was found to be statistically significantly different from all the other media used in the study (Table 3 Figure. 2). The OMA was followed by WLPDA media with an AUGPC of 22.6±1.79 cm^2 while the remaining three other media, WSPDA, CPDA, and PDA were found to give the least growth to the fungus (Table 3 Figure. 2). The fungus, B. sorokiniana was found to be successfully sporulated in all the media used in the study, although it was evident that the highest sporulation was observed on the OMA medium (44×10^4 spores ml^{-1}), followed by WLPDA and PDA (7×10^4 spores ml^{-1}). WSPDA was found to give a sporulation of 6×10^4 spores ml^{-1} and CPDA gave the least sporulation of 15×10^3 spores ml^{-1}, among all the five media (Table 3). Based on the present study, *B. sorokiniana* gave a blackish-to-whitish colony growth in various culture media used, and the colony margins were found to be irregular or wavy except for the fungus grown in OMA and WLPDA (Figure 1, Table 2). The colony color of *B. sorokiniana* was previously reported to be highly affected by different plant extracts supplemented into the PDA media. The PDA media supplemented with plant

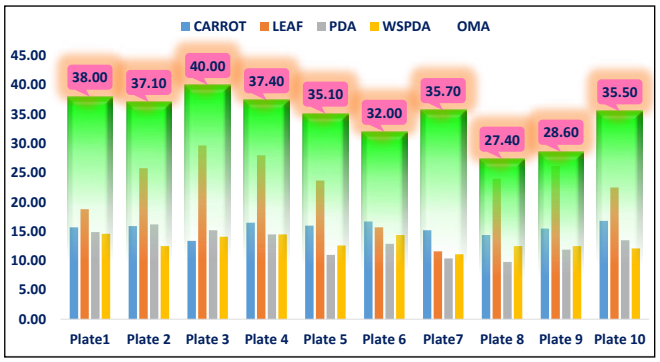

Figure 2: Comparison of the different media used for culturing *B. sorokiniana* with respect to oatmeal agar

extracts enhanced vegetative growth and sporulation of *B. sorokiniana* (Nasreen et al., 2017). However, the colony margin was reported to be irregular when grown in PDA as well as those PDA media which were supplemented with different plant extracts (Nasreen et al., 2017). Based on the study of 18 different isolates of *B. sorokiniana*, the colony colour of the fungus was reported to range from dark grey to creamy to olivaceous black colour with a wavy to smooth margin, with or without fluffy velvety growth (Deepti and Kumar, 2018). The fungus spores appeared to have an olive-brown to dark-brown colour when observed under a microscope and had a range of 2–10 transverse septations. Safari and Kaviani (2008) reported that the mycelium of *Bipolaris spp.* has a cottony grey olivaceous growth with a brownish tinge colour and the conidia were found to bear 5–12 septations.

Among the five media used, OMA was found to be most suited for the growth and sporulation of the fungus, *B. sorokiniana* with an AUGPC value of 34.68±1.3 cm^2 which is statistically significantly different from the rest of the media used (Table 3, Figure 2). This finding is aligned with the previous study conducted (Chowdhury et al., 2016; Raguchander et al., 1988). However, according to Ismail et al. (2023) Potato dextrose agar, corn meal agar and V8 agar media shows variation in mycelial growth and sporulation of B. *sorokiniana* and it has the potential for reproduction using PDA culture media. In another study, a similar fungus, *Helminthosporium tetramera* was observed to have good growth on various media viz., PDA, Richards's media, oatmeal agar, and corn agar although the maximum growth was found in PDA (Misra and Munankami, 1970).

4. Conclusion

Maximum mycelial growth of *B. sorokiniana* was observed in OMA followed by WLPDA. Also, the maximum sporulation was also found in OMA. Based on the study, it can be concluded that the OMA is the best-suited medium for the growth and sporulation of the fungus, *B. sorokiniana,* followed by WLPDA, whereas, CPDA and PDA are the least favorable media for culturing the fungus.

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