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Efficacy of Plant Extracts on Morphology and Cultural Characteristics of *Bipolaris sorokiniana*, Causing Black Point Disease of Wheat in Bangladesh

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Abstract

Black point of wheat caused by *Bipolaris sorokiniana* (Bs) is very destructive disease of wheat in Bangladesh and all over the world. The symptoms of diseases caused by *Bipolaris sorokiniana* can vary depending on the host plant and environmental conditions. The conidia of Bs germinate from two poles, which differ physiologically as indicated by the rate, growth character, of length, breadth and number of septa on different media. The aim of this study was to evaluate the efficacy of selected plant extracts on the morphology and cultural characteristics of the fungus *Bipolaris sorokiniana* causing black point disease in wheat crops in Bangladesh. The efficacy of fifteen plant extracts on the growth and characteristics of Bs was conducted *in vitro* in 2019-2020. The treatments were: Untreated control, T_1—Black berry Leaves, T_2—Guava Leaves, T_3—Lantena camera leaves, T_4—Eucalyptus Leaves, T_5—Turmeric Leaves, T_6—Khoksha Leaves, T_7—Papaya leaves, T_8-Gurlic Bulb, T_9—Chili dust, T_{10}—Nigella seeds, T_{11}—Turmeric dust, T_{12}—Cloves, T_{13}—Bohera fruits, T_{14}—Black pepper and T_{15}—Neem leaves. After 15 days of inoculation of *Bipolaris sorokiniana*, colony color and shapes were Gerrish blackish, greenish blackish, greyish blackish and shapes were round or irregular. In our study, conidia color was dark brown to light brown, conidiophore color was brown, dark brown, grayish brown, dark olivaceous, light brown and conidia shapes were elliptical, oblong or slightly curved. The septation of conidia was 2 - 5 and highest septation was seen in T_4 and T_{14} treatments. The highest conidial length was 17.79 µm recorded in T_2 treatment and lowest was 6.62 µm T_9 treatment where conidial breath was 8.27 µm in control and lowest was 3.79 µm in T_8 treatment. Mycelial growth rate of *Bipolaris sorokiniana* was different in different treatments in different days af-

ter inoculation (DAI) where at 7 DAI, % reduction of mycelial growth over control was highest in Bohera and Neem Leaves treatments respectively.

Keywords

Bipolaris sorokiniana, Black Point, Botanicals, Wheat

1. Introduction

Bipolaris sorokiniana, also known as (teleomorph, *Cochliobolus sativus*), is a fungal pathogen that can cause a variety of diseases in plants, including cereal crops such as wheat and barley, and is a wheat pathogen that affects all parts of the wheat plant, including seeds, roots, shoots, and leaves. Its impact on wheat production is significant, causing diseases such as black point, head blight, Leaf Spots, Leaf Blight of wheat, root rot, crown rot, and spot blotch wheat and barley. As one of the top ten most widely grown crops in the world, wheat is vulnerable to several diseases, and *Bipolaris sorokiniana* is among the pathogens that cause losses in wheat production globally. It is important to note that the symptoms of diseases caused by *Bipolaris sorokiniana* can vary depending on the host plant and environmental conditions. Four biological specializations of *Helminthosporium sativum* differ physiologically as indicated by the rate, growth character, of length, breadth and number of septa on same and different media and they produce different degrees of infection on the same cereal and grasses [1]. Redial mycelial growth rate, color of the colony, surface texture of the colony, conidia production ability and shape and color of the conidia are differed in different isolates of *B. sorokiniana* [2]. About 37 races of *Helminthosporium sativum* are based on morphological and pathological characters [3]. Different measurements of conidia are in different isolates of *B. sorokiniana* [4]. The conidia of *Bipolaris sorokiniana* is fusoid, straight or curved with bipolar germination and characterized by thick walled, elliptical conidia (60 - 120 μm × 12 - 20 μm) with 4 - 8 septa. *Helminthosporium* differs from *Bipolaris*, *Drechslera* and *Exserohilum* by forming parallel-walled, erect conidiophores and Bipolaris presents bipolar germination of conidia from two poles [5]. The septation of conidia ranged from 2 - 10 septa [6]. In Brazil, spot blotch in wheat is caused by *Bipolaris sorokiniana* (Sacc) Schoem. Other fungi have been isolated from wheat seeds as *Bipolaris bicolor* (Mitra) Shoem that causes as well spot blotch but with minor lesions and can be the most deleterious disease for the producers [7] [8] [9]. Morphological characteristics such as conidia (size, shape, and septation) are used as the primary characters for the practical and working identification of Bipolaris genus [10]. Morphological Variability of *B. sorokiniana* has been reported [11] and more or less of same type of conidia of *Bipolaris sorokiniana* having straight to curved, 3 - 12 septa with olive brown color [12]. *Bipolaris sorokiniana* (Sacc.) Shoemaker [5] [13] teleomorph *Cochliobolus sativus*) is the causal agent of common root rot, leaf spot, seedling blight, head blight of wheat

and barley and black point of grains. Several synonyms of the anamorph have been used like *Helminthosporium sorokiniana*, *Drechslera sorokiniana* and *Helminthosporium sativum* [14]. Seed sample of grade-0 seeds (free from infection) sowed good result of germination, seedling vigor, leaf blight severity, grain formation and yield and yield contributing characters in different severity grades (0 - 5) seeds [15].

Among 20 isolates of *Helminthosporium sativum* when grown in PDA, wheat extract agar and V-8 juice agar, found a distinct difference in colony morphology due to cultural variation [16]. Geographical racial differentiation of *Bipolaris sorokiniana* has the tendency for isolates from warm and dry regions to be less virulent and most virulent isolates came from southern and central Africa [17]. 83 isolates of *Helminthosporium sativum* are from seven locations of Bangladesh and they found variable mycelia growth and conidia production ability of the isolates on PDA in respect of location [18]. Physiological and morphologic al variation such as colonies were ash brown, olive green, light green or dark green in color with regular or wavy margins, fluffy, spread or velvety texture and number of cells per conidium varied from 3 - 10 and length and width of conidium varied from 35 - 270 μm and 15 - 65 μm depending on isolates of *Bipolaris sorokiniana* in Bangladesh [19]. Among 27 isolates of *Bipolaris sorokiniana* belonged to cluster I, II, III and IV. And [20] identified seven morphological and physiological divergences. The disease is characterized by brown to black discoloration usually restricted to the embryonic end of the grain, but in case of severe infection, the whole grain may be discolored and shriveled [21]. The conidia of *Bipolaris sorokiniana* form quickly at room temperature usually 25˚C ± 1˚C whereas the optimum temperature for mycelia growth and germination of conidia of *B. sorokiniana* were 10˚C - 40˚C [22] [23]. The conidiophores are 6 - 10 × 110 - 220 μm, brown, erect, unbranched, single or clustered, septate [24] and also reported that conidia are 15 - 28 × 40 - 120 μm, slightly curved, oblong, fusiform to broadly ellipsoid, olive brown to dark brown, tapered towards the end and have a prominent scar, smooth walled and having 3 - 10 thick-walled transverse septa [25] [26]. The conidiophores are long, septate, simple, dark brown to olivaceous, at the base and somewhat paler at the growing tip, successive conidia are seen the regular intervals on the conidiophores [24]. Eight years experiment was conducted and reviewed the literature on causal organisms which *Alternaria* and *Helminthosporium* were the main responsible fungi of *Bipolaris* [27] and associate pathogen with Black point in Manitoba were *A. tenuis*, *A. peglioni*, *H. sativum*, and *H. teres.* The black point is a major cause of down-grading wheat [28] [29] and microscopic morphology *B. sorokiniana* conidia were longer than 75 μ and less than 20 μ wide. 5 to 9 Pseudosepta were present at any age with 6 and *B. bicolor* conidia size ranged from 40 to 78 μ long and from 12.1 to 17.3 μ wide, were straight, ellipsoid, with round edges, occasionally similar to an inverted club, rarely curve, from medium brown to dark brown in color, in more advanced developmental stages, cells from the edges tend to become more hya-

line and pseudosepta in these cells seemed to be thicker, whereas the central part became almost black and made pseudosepta impossible to count [30]. Results of the observations on *B. bicolor* are in accordance with the description by the same characteristics of the fungus causing leaf spot in peach palm and to determine the efficacy of plant extracts on radial mycelial growth, cultural characteristics and spore morphology of *Bipolaris sorokiniana* causing black point of wheat [31].

2. Materials and Methods

2.1. Collection of Seed Samples

Wheat seed samples were collected from Bangladesh Agricultural Development Corporation (BADC), Rajshahi, Bangladesh, Katakhali Bazar and Bagha Bazar of Rajshahi District. The seeds were collected in cotton bags and were sun dried for three days. Then the seeds were put in polyethylene bag in air tight condition and preserved in the refrigerator at 5˚C temperature for isolation of *B. sorokiniana*.

2.1.1. Detection of Seed-Borne Fungi

Health status of all the seed samples was studied for detection of fungi following blotter incubation method [32].

2.1.2. Seed Health Study (Blotter Method)

In this method, three pieces of filter paper (whatman No. 1) were soaked in sterilized water and placed at the bottom of 9cm diameter glass petridish. Firstly, these seeds were surface sterilized with 0.1% solution of $HgCl_2$ (Mercuric chloride) for 30 - 45 seconds to remove contamination followed by 4 - 5 washing in sterilized water and were later dried using sterilized blotters. Two hundred seeds from each sample were taken randomly and then placed on the moist filter paper. In each petridish 25 seeds were plated. The petridishes with seeds were then incubated at 22˚C ± 2˚C under 12/12 hours alternating cycles of NUV and darkness in the incubation room of the Botanical Pesticides Lab the Institute of Environmental Science, University of Rajshahi for seven days. After incubation the seeds were examined under stereo microscope (Figure 1).

(a) (b)

Figure 1. Diseased seeds (a) and healthy seeds (b).

2.2. Isolation of the Pathogen

Bipolaris sorokiniana was isolated from seeds following the method was used [18] and the isolates were grown PDA plates at 22˚C - 24˚C for 7 - 10 days. At the end of incubation period, the mycelium grown from the planted tissues were examined under stereobinocular microscope and single conidium isolation was done. The isolates were grown on PDA plates for 7 - 10 days at 25˚C ± 1˚C for sporulation. The collected seed samples were surface sterilized by mercuric chloride (1:1000) for 30 seconds, rinsed thrice in sterilized water and were placed on three layered moist filter paper in petridish and incubated following ISTA rules [33]. After 7 days of incubation the seeds were observed under stereobinocular microscope and the single conidium of *B. sorokiniana* from the infected seed was transferred on to PDA and the plates were incubated at 25˚C for luxuriant growth. The cultures from the plates were transferred to PDA slants using hyphal tip culture method and preserved in refrigerator at 5˚C for further study. The collected isolates were designated based on its location and source. The composition of PDA was as: Peeled potato—200 g Dextrose sugar—20 g Agar-agar—20 g Water, added to make total volume upto 1000 ml (**Figure 2**).

Identification of Fungi

After incubation period the seed were examined and identified under microscope based on the growth characters of the fungi. Slides were prepared out of the associated fungi on seeds to identify under compound microscope for better identification if necessary. The fungi were identified following the keys as Physiological and morphologic al variation such as variable mycelia growth and conidia production ability, different colonies color such as ash brown, olive green, light green or dark green in color with regular or wavy margins, fluffy, spread or velvety texture and number of cells per conidium varied from 3 - 10 and length and width of conidium varied from 35 - 270 µm and 15 - 65 µm depending on isolates of *Bipolaris sorokiniana* in Bangladesh [18] [19] [34] [35] [36] (**Figure 3**).

2.3. Experiment and Treatments

This experiment was conducted at Botanical Pesticides Laboratory, the Institute of Environmental Science, University of Rajshahi, Bangladesh during 2019-2020.

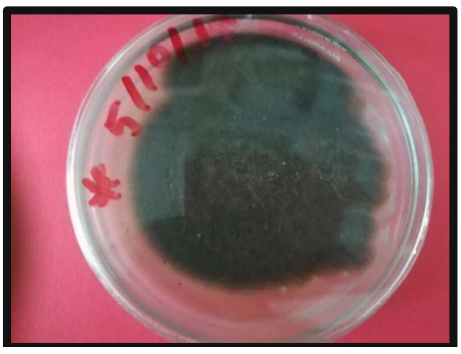
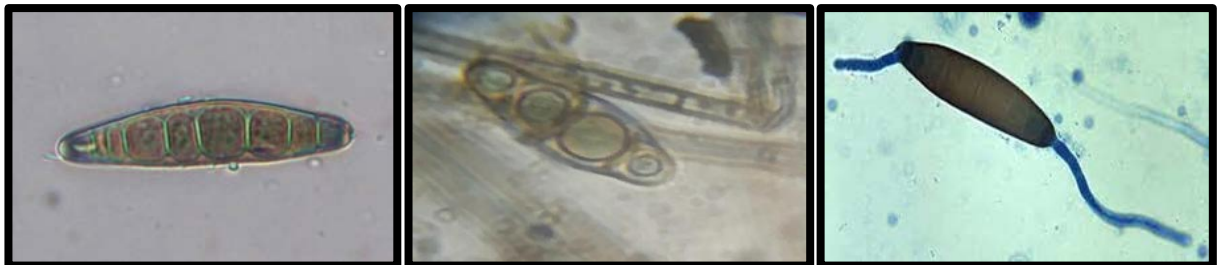


Figure 2. Pure culture of *Bipolaris Sorokiniana*.



(a) (b) (c)

Figure 3. (a) Conidia with septation, (b) Conidia with conidiophore and (c) two or bi pool germinated conidia of *B. sorokiniana* (×400).

Wheat variety BARI 33 was used for conducting experiment fungal pathogens (*Bipolaris sorokiniana*) of wheat. The botanical experimental treatments were, Control-1, T_1—Black berry Leaves, T_2-Guava Leaves, T_3—Lantena camera leaves, T_4—Eucalyptus Leaves, T_5—Turmeric Leaves, T_6—Khoksha Leaves, T_7—Papaya leaves, T_8—Gurlic Bulb, T_9—Chilli dust, T_{10}—Nigella seeds, T_{11}—Turmeric dust, T_{12}—Cloves, T_{13}—Bohera fruits, T_{14}—Black pepper, T_{15}—Neem leaves (**Table 1**).

2.4. Botanical Extract Preparation and Application

Plant parts were washed through running tap water 2 - 3 times and dried under shed for 2/3 weeks. Air dried leaves were grinded with the help of electric grinder to make fine powder [37]. For 10% leaf extracts preparation about 100 gram of plant material was dissolved in 1000 ml distilled water (w/v, 1:10) and kept in room temperature for 2-3 days, and then filtered through three-layer cheesecloth [38]. The extracts were centrifuged at 3000 rpm for 20 minutes and stored in a refrigerator at 4˚C until used. The containers were stored at room temperature for 72 hours with gentle shaking (2 - 3 times) every day. Then final aqueous extract was collected in bottle for stored and preserve in the refrigerator for future use. Once the PDA media has solidified, it is ready for use. It can be used for the cultivation and study of various microorganisms. Make sure to label the Petri dishes properly with the date and content. Store the prepared media in a cool, dark place to maintain its quality and extend its shelf life.

2.5. Preparation Plant Extracts and Amended with PDA

Here are the steps to prepare PDA media with 10% Eucalyptus leaf extract: Peel and cut two medium-sized potatoes into small pieces and wash out with distilled water by putting them into a glass beaker and add distilled water to cover the potatoes. Boil the mixture for about 30 minutes until the potatoes become soft. Strain the mixture through a cheesecloth or filter paper to obtain the potato extracts. Measure the pH of the potato extract using a pH meter or pH strips. Adjust the pH to approximately 5.6 - 5.8 by adding a few drops of hydrochloric acid (HCl) or sodium hydroxide (NaOH) solution as needed. Add 20 g of dextrose (glucose) and 15 - 20 g of agar to the potato extract. Stir the mixture using a stirring rod to dissolve the dextrose and agar completely. Measure 100 ml of Eucalyptus

Table 1. Botanicals used in this study.

Sl. No.	Plants	Local name	English name	Scientific name	Plant parts used	Active on fungal disease
1		Jam pata	Black berry	*Syzygium cumini*	Leaves	Powder of dry Leaves.
2		Payara pata	Guava leaves	*Psidium guajava*	Leaves	Powder of dry leaves
3		Lantana camera pata	Lantana camera pata	*Aegle mormelas*	Twigs and Leaves	Powder of dry leaves
4		Eucalyptus	Eucalyptus Leaves	*Eucalyptus chamadulonsis*	Twigs and Leaves	Powder of dry leaves
5		Turmeric	Turmeric Leaves	*Curcuma longa*	Leaves	Powder of dry leaves
6		Khoksha Leaves	Kakdomur	*Ficus hispida*	Leaves	Powder of dry leaves
7		Papaya	Papaya leaves,	*Carica papaya*	Leaves	Powder of dry leaves
8		Gurlic	Gurlic Bulb,	*Alliu m sativum*	Bulb	Powder of dry bulb
9		Morich	Chili dust,	*Capcicum frutescens*	Fruits	pest of fresh dry fruits.
10		Kalojira	Nigella seeds	*Nigella sativa*	Fruits	Powder of dry fruits.
11		Holud gura	Turmeric powder	*Curcuma longa*	Rhizome powder	Powder of dry Rhizome.
12		Lobongo	Cloves	*Eugenia caryophyllus*	Fruits	Powder of dry fruits.
13		Bohera	Bohera fruits	*Termenalia belerica*	Fruits	Powder of dry fruits.
14		Golmorich	Black pepper	*Piper nigram*	Fruits	Powder of dry fruits.
15		Neem	Neem Leaves	*Azadiracta indica*	Leaves	Powder of dry leaves.

leaf extract and add it to the potato extract mixture. Add distilled water to the mixture to bring the total volume to 1 liter. Stir the mixture well to ensure uni-

form distribution of the ingredients. Pour the prepared PDA media into sterile Petri dishes. Cover the dishes and place them in an autoclave or pressure cooker. Sterilize the media by subjecting it to high pressure and temperature (121˚C) for approximately 15 minutes. Allow the sterilized PDA media to cool down at room temperature until it solidifies. Avoid contamination by handling the dishes carefully.

2.6. Inoculation and Data Recording

The black pointed seeds were washed out with distilled water for 3 - 4 times. These were surface sterilized with 0.1% solution of $HgCl_2$ (Mercuric chloride) for 30 - 45 seconds to remove contamination followed by 4 - 5 washing in sterilized water. The seeds were later dried using sterilized blotters and plated on the PDA on sterilized glass Petri dishes (90 mm diameter) @ 5 seeds per plate at equal distance. These plates were incubated at 25˚C ± 1˚C in BOD incubator at 12 h day and night photoperiod cycle to stimulate sporulation in colonies. The colonies developed fully within 10 - 12 days after inoculation. Pathogens were identified by observing the colony [10] under compound stereo binocular microscope initially and later by making slides of spores and observing under compound microscope. The sub-culturing was done on PDA slants in culture tubes and stored at 4˚C in refrigerator. The growth of study of *B. sorokiniana* was done on Potato Dextrose Agar (PDA) and the plates were inoculated with 5 mm mycelia block at the middle of the plate and kept at 25˚C following the food poisoning technique [39]. After 14 days of incubation, the radial growth of the isolates on PDA (Potato Dextrose Agar) was measured by scale in centimeter. The texture, color and colony shape of the isolates were recorded according to treatments.

2.7. Data Analysis

The laboratory and Pot experiments were conducted in completely randomized design and the analysis of variance was done as per standard method [40].

3. Results and Discussion

Morphological Variability, Colony Growth Behavior and Pathogen Identification under Microscope

The range of colour of cytoplasm was witnessed in spores of different isolates of *B. sorokiniana* and the colour was light blue, blue, brown and greenish blue in the cytoplasm of spores, after staining (**Table 2**) [41]. The radial mycelial growth rate, color of the colony, surface texture of the colony, shape of the colony, conidia production ability and shape and color of conidia of *B. sorokiniana* the mycelial growth rate of the isolates ranged from 1.39 to 4.46 mm/day. On the basis of conidia morphology, the isolates were grouped into five different groups, whereas the isolates were grouped into 12 cultural groups based on cultural characteristics [42].

Table 2. Colour, shape, septation, length and breadth of conidia.

Isolates	Colour		No. of Septa		Cytoplamic colour	Shape of Spore
	Spore	Sporophore	Spore	Sporophore		
D_1	Dark Brown	Dark Brown	2 - 4	2 - 5	Brown	Oblong
D_2	Dark Brown	Dark Brown	2 - 3	2 - 5	greenish blue	Elliptical
D_3	Light Brown	Dark Brown	2 - 4	2 - 5	Light brown	Oblong

In this study media colour is watery, blackish reddish, reddish to reddish greenish, greenish, brownish to light brownish, yellowish to light blackish yellow, grayish, off white and coagulating media in different time depend upon different plant extracts. Colony colour and shapes were Gerrish blackish, Greenish blackish, Greyish blackish and shapes were round or irregular. Conidia shapes were elliptical, oblong, slightly curved and colour of conidium were light or dark brown (Table 3).

In my study, conidia colour were dark brown to light brown, conidiophore colour were brown, dark brown, grayish brown, dark olivaceous, light brown and septation of conidia were 2 - 5, highest conidial length was 17.79 µm and lowest was 6.62 µm where conidial breath was 8.27 µm and lowest was 3.79 µm.

The media colours ranged from watery, blakish redish, redish to redish greenish, greenish, brownish to light brownish, yellowish to light blakish yellow, grayish, off white, and coagulating media in different times. The colony shapes were round or irregular and colours were Gerrish blakish, Greenish blakish, Greyish blakish. The conidia and conidiophore colour ranged from dark brown to light brown, brown, dark brown, grayish brown, dark olivaceous, light brown, and the conidia shapes were elliptical, oblong, slightly curved. The septation of conidia ranged from 2 - 5, but highest septation was seen in T_4 and T_{14} treatment with the highest conidial length being 17.79 µm in T_2 treatment and the lowest being 6.62 µm in T_9 treatment, while the conidial breath was 8.27 µm in control and the lowest was 3.79 µm in T_8 treatment. Twelve cultural group of *B. sorokiniana* and observed the colony culture as smoothy and wooly blackish white regular or irregular, smoothy and wooly whitish regular or irregular, effuse and rough blackish white regular or irregular [3]. The colony colour and colony types of *B. sorokiniana* isolates were grouped in five categories on the basis of colour-olive black, olive cream, greyish cottony, black pink and black cream. Colony Colour was dirty grey with prominent creamish colour, submerged colony, olive black in colour with cream olive margin, blackish grey, Irregular colony, submerged with prominent mycelial growth with creamy and dirty grey in colour and colony types were Velvety growth with white fluffy mycelial growth on colony, Fluffy colour with no distinct center, Indistinct three sectors. Sector and margin were Irregular, smooth, Wavy margin olive in colour, Wavy margin with prominent creamy colour, 3 rings [42]. The *Bipolaris sorokiniana* colonies were velvet-like, dark olive; plane, totally covered by short conidiophores, with black conidia in their apex. Conidia reflected the light and gave the colony a

Table 3. Efficacy of botanicals on cultural and morphological characteristics of *Bipolaris sorokiniana*.

Treatments	Colony Colour	Colony shape	Conidia colour	Conidio-Phore Colour	Conidia shape	Septation of conidia	Conidial length (µm)	Conidial breath (µm)
Control	Gerrish blakish	Round	Dark Brown	Dark Brown	Elliptical	2 - 4	12.89 b-e	8.27 a
T_1	Blakish	Round	Dark Brown	Brownish	Elliptical	2 - 3	11.51 d-g	5.41 b-e
T_2	Blakish	Round	Dark Brown	Brownish	Oblong	2 - 5	17.78 a	5.95 bc
T_3	Greenish blakish	Round	Dark Brown	Brownish	Elliptical	2 - 4	14.58 bc	6.51 b
T_4	Greenish blakish	Round	Dark Brown	Grayish brown	Slightly curved	3 - 4	12.67 c-f	4.42 ef
T_5	Greenish blakish	Irrigular	Dark Brown	Dark olivaceous	Elliptical	2 - 3	11.74 c-g	4.82 c-f
T_6	Greenish blakish	Round	Light Brown	Light Brown	Elliptical	2 - 4	11.83 c-g	4.75 c-f
T_7	Greenish blakish	Round	Light Brown	Brownish	Elliptical	3 - 4	12.85 c-e	5.71 b-e
T_8	Greenish blakish	Irrigular	Dark Brown	Light Brown	Oblong	2 - 4	9.73 f-h	3.79 f
T_9	Greenish blakish	Round	Light Brown	Brownish	Slightly curved	2 - 4	6.62 i	5.06 c-f
T_{10}	Greenish blakish	Round	Dark Brown	Brownish	Elliptical	2 - 3	12.60 c-f	5.17b-e
T_{11}	Greenish blakish	Round	Light Brown	Light Brown	Elliptical	2 - 4	15.94 ab	5.46 b-e
T_{12}	Greenish blakish	Irregular	Light Brown	Brownish	Elliptical	2 - 4	10.17 e-g	4.50 ef
T_{13}	Greenish blakish	Round	Dark Brown	Brownish	Elliptical	2 - 4	6.81 hi	4.68 c-f
T_{14}	Greenish blakish	Round	Dark Brown	Light Brown	Slightly curved	3 - 5	9.25 g-i	4.56 d-f
T_{15}	Greenish blakish	Round	Dark Brown	Brownish	Oblong	2 - 3	12.14 c-g	5.91 b-d
CV			--	--		--	15.58	3.07
LSD			--	--		--	15.52	1.38

Control-1, T_1—Black berry Leaves, T_2—Guava Leaves, T_3—Lantena camera leaves, T_4—Eucalyptus Leaves, T_5—Turmeric Leaves, T_6—Khoksha Leaves, T_7—Papaya leaves, T_8—Gurlic Bulb, T_9—Chilli dust, T_{10}—Nigella seeds, T_{11}—Turmeric dust, T_{12}—Cloves, T_{13}—Bohera fruits, T_{14}—Black pepper, T_{15}—Neem leaves.

shiny appearance and the reverse side of the colony was dark olive. *Bipolaris bicolor* presented two types of colonies [30] as the type I - velvet-like, dark olive, plane colonies, totally covered by conidia, mycelium was absent, colonies were very similar to *B. sorokiniana where r*everse side presents different zones, with alternation of dark olive and light brown sections, and the type II—dark olive and slight cotton-like colonies whereas reverse side was the same color, and presents a grayish dark olive halo involving the central area this is the heterokaryotic condition of fungi in nature.

The obtained results showed that the activity of extracts depends on the plant species, method of preparation and the sensitivity of bacteria and fungi selected for testing. The results of carried investigations showed that the antimicrobial activity of the plant extracts was more effective against bacteria than fungi, similar to the results [43] [44] and [45]. The extracts of *L. nobilis*, *D. coryophyllum*, *J. oxycedrus* and *C. arborescens* showed higher inhibitory activity against the yeast *C. albicans* and the fungus *A. niger* than the standard antifungal nystatin [45]. Under tested condition, the macerate, brew, decoction and essential oils

were inhibitory to the growth of all the bacteria and fungi and also concluded that tested extracts were the source of active substances, which (in varying degree) inhibited the growth of selected strains of bacteria and fungi [46] where by using of plant extracts including essential oil both in the protection of crop and preservation of food products obtained there from and also apparent resistance of many species of fungi. Pants parts were dried in the shade, ground and extracted with water, methanol or chloroform and the antimicrobial activity of the crude extracts was tested against *E. coli*, *S. aureus*, *S. lutea*, *P. vulgaris* and *C. albicans* using the agar diffusion technique. A methanol extract of *E. polistachia* showed antimicrobial activity against all microorganisms [44].

In 27 isolates the number of cells per conidium varied from 3 - 10 septa, several celled or the number of septation varied from 2 to 8, found 2 - 13 septation in the isolates [19] [47] [48] [49] [50]. Conidiophores are 6 - 10 × 110 - 220 µm, brown, erect, unbranched, single or clustered, septate [24] and also reported that conidia are 15 - 28 × 40 - 120 µm, slightly curved, oblong, fusiform to broadly ellipsoid, olive brown to dark brown, tapered towards the end and have a prominent scar, smooth walled and having 3 - 10 thick-walled transverse septa [25], [26]. conidiophores are long, septate, simple, dark brown to olivaceous, at the base and somewhat paler at the growing tip, successive conidia are seen the regular intervals on the conidiophores [24]. The conidia are brown, several-celled (phragmosporous), elliptical, straight, or curved, germinating by one germ tube at each end [25] [26] *B. sorokiniana* has olive-brown, ovate conidia, with tapered ends and a prominent basal scar. The conidia are 15 - 28 × 40 - 120 µm and have 3- to 10- septa [51]. *B. sorokiniana* isolated from samples of wheat variety were first observed that the colony was mature within 4 - 6 days and colony was grayish black and that had suppressed type of growth, Conidiophores were unbranched, brown to dark brown, erect, single or clustered, septate and conidia were brown to olivaceous brown color, straight or slightly curved 50 - 70 µ long 15 - 20 µ wide and variation in septation from 3 - 7 [52]. Morphological Variability of *B. sorokiniana* have been reported by [2] [11] more or less of same type of conidia of *Bipolaris sorokiniana* having straight to curved, 3 - 12 septa with olive brown color. Among 20 isolates of *Helminthosporium sativum* when grown in PDA, wheat extract agar and V-8 juice agar, found a distinct difference in colony morphology due to cultural variation [16]. About 27 isolates of *Bipolaris sorokiniana* collected from 14 districts of wheat growing regions in Bangladesh has physiological and morphological variation and Colonies were ash brown, olive green, light green or dark green in color with regular or wavy margins, fluffy, spread or velvety texture and with or with or without sector [19]. He also found that number of cells per conidium varied from 3 - 10 and length and width of conidium varied from 35 - 270 µm and 15 - 65 µm depending on isolates. [19] identified seven morphological and physiological divergences among 27 isolates of *Bipolaris sorokiniana*, characterized by brown to black discoloration usually restricted to the embryonic end of the grain, but in case of severe infection, the whole grain may be discolored and shrivelled [21]. The conidia of

Bipolaris sorokiniana are fusoid, straight or curved with bipolar germination and characterized by thick walled, elliptical conidia (60 - 120 µm × 12 - 20 µm) with 4 - 8 septa [5]. The color 34 of conidia were light brown in colour though a few numbers have been recorded as deep brown in color [48]. The length and width of conidium varied from 35 - 270 µm and 15 - 65 µm and the number of cells varied from 3 - 10 septa depending on isolates [17]. The conidia are brown, several-celled (phragmosporous), elliptical, straight, or curved, germinating by one germ tube at each end [47] [50] *B. sorokiniana* has olive-brown, ovate conidia, with tapered ends and a prominent basal scar. The conidia are 15 - 28 × 40 - 120 µm and have 3- to 10-septa and *B. sorokiniana* isolated from samples of wheat variety were first observed that the colony was mature within 4 - 6 days and colony was grayish black and that had suppressed type of growth. Observation was carried out under microscope and noted that Conidiophores were unbranched, brown to dark brown, erect, single or clustered, septate and conidia were brown to olivaceous brown color, straight or slightly curved 50 - 70 µ long 15 - 20 µ wide and variation in septation from 3 - 7 [51].

In my study mycelium growth rate varies from different treatments in different times as 24 h, 48 h, 96 h, 120 h, 144 h and 168 h after inoculation. It varies from 0.50 cm to 5.50 cm. the highest mycelium growth was recorded in control-1 and lowest was in T_{13} and T_{14} that is Bohera fruits and Black piper treatment (**Table 4**).

Mycelial growth rate of *Bipolaris sorokiniana* were different in different treatments in different time as DAI (1 Days After Inoculation, 2 Days After Inoculation, 3 Days After Inoculation, 4 Days After Inoculation, 5 Days After Inoculation, 6 Days After Inoculation, 7 Days After Inoculation) where 7 DAI % Decreased growth over control was highest in Bohera and Neem Leaves T_{13} and T_{15} treatments respectively and moderately control in T_4, T_5 and T_9, the % decreased over control in T_{12} treatment. Over twelve cultural group *of B. sorokiniana* were conducted an experiment and the mycelial growth rate of the isolates ranged from 1.39 to 4.46 mm/day [2]. Colony diameter of *Bipolaris sorokiniana* after seven days of incubation and that was ranged from 20.3 mm to 63 mm and mycelial growth varied from 9.26 mm to 24.0 mm [48] [53]. Srinivas *et al.* (2009) recorded Colony diameter of *Bipolaris sorokiniana* after seven days of incubation and that was ranged from 20.3 mm to 63 mm [53] and mycelia growth rate of *B. sorokiniana* 2.77 ± 0.23 to 9.10 ± 1.09 mm/day and the differences among the radial mycelial growth of *B. sorokiniana* may be due to difference in temperature during incubation period [6]. Different plant extracts (*Azadirachta indica*, *Allium sativum*, *Eucalyptus globolus*, *Acorus calamus*, *Justicia adhatoda*) on PDA media and observed the inhibition of the growth of *B. sorokiniana* [54]. Variation was found in margin, shape, color, texture, zonation and arial mycelium in top view and in conidia and conidiophore after 12 days of inoculation. She found that at 10 days old culture give the best result but decreased gradually with the age [55]. Optimal growth of *B. sorokiniana* in four media such as the Potato dextrose agar (PDA), corn meal agar (CMA), V8 agar (V8A) and water

Table 4. Efficacy of plant extracts against mycelial growth of *Bipolaris sorokiniana*.

Treat	1 DAI (Cm)	2 DAI (Cm)	3 DAI (Cm)	4 DAI (Cm)	5 DAI (Cm)	6 DAI (Cm)	7 DAI (Cm)	% Decreased growth over control
Con	1.87 a	2.13 a	2.50 ab	3.33 a	4.10 a	4.33 a	5.58 a	----
T_1	0.80 d	1.36 bc	2.57 ab	2.63 bc	3.02 cde	4.02 ab	4.38 bc	21.51
T_2	0.77 de	1.32 bc	2.78 ab	3.04 ab	2.57 ef	2.13eg	3.21 def	42.47
T_3	1.17 c	2.27 a	2.70 ab	3.22 ab	3.63 abc	3.92 ab	4.32 bc	22.58
T_4	0.67 e	0.93 def	1.10 de	1.3667 g	1.7667 g	2.20 e	2.48 f	55.56
T_5	0.65 e	1.13 cde	1.67 c	1.80 de	2.03 fg	2.33 de	2.45 f	56.11
T_6	1.15 c	2.42 a	2.92 a	3.57 a	3.65 abc	3.87 ab	4.12 cd	26.16
T_7	0.68 de	1.08 cdef	1.33 cde	1.33 efh	0.93 ij	1.26 f	1.19 g	78.67
T_8	0.67 e	0.75 fg	0.85 ef	2.07 cd	3.50 abc	4.15 ab	4.47 bc	19.89
T_9	0.50 f	0.50 g	0.50 fi	0.57gh	2.07 f g	2.68 de	2.96 ef	46.95
T_{10}	1.50 f	1.30 bcd	1.50 cd	1.41 ef	1.31 hi	1.07 f	1.12 g	79.92
T_{11}	0.50 f	0.92 ef	1.62 de	2.42 c	2.68 def	3.03 cd	3.23 def	42.11
T_{12}	1.43 b	2.33 a	2.37 b	3.27 b	3.72 ab	4.23 ab	5.30 ab	5.01
T_{13}	0.50 f	0.77 efg	0.50 f	0.88 fg	0.50 j	0.81 fg	0.74 g	86.73
T_{14}	0.80 d	1.67 b	2.67 ab	3.23 ab	3.30 bcd	3.53 bc	3.83 cde	31.36
T_{15}	0.50 f	0.50 g	0.50 f	0.81 fg	0.73 ij	0.6 f	0.75 g	86.55
Cv	8.91	16.73	18.43	16.81	16.99	17.03	20.30	---
LSD	0.1218	0.3719	0.5376	0.6107	0.6978	0.7823	1.0580	---

Treatments: Control-1, T_1—Black berry Leaves, T_2—Guava Leaves, T_3—Lantena camera leaves, T_4—Eucalyptus Leaves, T_5—Turmeric Leaves, T_6—Khoksha Leaves, T_7—Papaya leaves, T_8—Gurlic Bulb, T_9—Chilli dust, T_{10}—Nigella seeds, T_{11}—Turmeric dust, T_{12}—Cloves, T_{13}—Bohera fruits, T_{14}—Black pepper, T_{15}—Neem leaves.

agar (WA) culture media and three light durations (0, 12 and 24 hr) for the growth diameter and colony-forming units that was 4.21 cm, 4.56 cm, and 4.36 cm [56]. *B. sorokiniana* under laboratory conditions and growth of different isolates in different period after inoculation 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h and mycelium growth was 9.9 mm, 19.8 mm, 29 mm, 42.4 mm, 52.5 mm, 61.6 mm and 69.2 mm on B-11 isolate [41]. A geographical racial differentiation of *Bipolaris sorokiniana* as he found a tendency for isolates from warm and dry regions to be less virulent and most virulent isolates came from southern and central Africa [17]. The conidia are brown, several-celled (phragmosporous), elliptical, straight, or curved, germinating by one germ tube at each end [47] [50]. *B. sorokiniana* has olive-brown, ovate conidia, with tapered ends and a prominent basal scar and the conidia are 15 - 28 × 40 - 120 µm and have 3- to 10-septa and the colony was mature within 4 - 6 days and colony was grayish black and that had suppressed type of growth [51] [52] and under microscope and noted that Conidiophores were unbranched, brown to dark brown, erect, single or clustered, septate and conidia were brown to olivaceous brown color, straight or slightly curved 50 - 70 µ long 15 - 20 µ wide and variation in septation from 3 -

7. The color 34 of conidia were light brown in colour though a few numbers have been recorded as deep brown in color [48]. The length and width of conidium varied from 35 - 270 µm and 15 - 65 µm, the number of cells per conidium varied from 3 - 10 septa depending on isolates [17]. Among 86 isolates the septation of conidia ranged from 2 - 10 septa. The conidiophores are 6 - 10 × 110 - 220 µm, brown, erect, unbranched, single or clustered, septate and also reported that conidia are 15 - 28 × 40 - 120 µm, slightly curved, oblong, fusiform to broadly ellipsoid, olive brown to dark brown, tapered towards the end and have a prominent scar, smooth walled and having 3 - 10 thick-walled transverse septa [24] [25] [26]. conidiophores are long, septate, simple, dark brown to olivaceous, at the base and somewhat paler at the growing tip, successive conidia are seen the regular intervals on the conidiophores [24].

4. Conclusion

The results of the study showed that the plant extracts had varying effects on the growth and characteristics of the fungal pathogen. The media colours ranged from watery, blakish reddish, reddish to reddish greenish, greenish, brownish to light brownish, yellowish to light blakish yellow, grayish, off white, and coagulating media in different times. The colony colour and shapes were Gerrish blakish, Greenish blakish, Greyish blakish, and shapes were round or irregular. The conidia and conidiophore colour ranged from dark brown to light brown, brown, dark brown, grayish brown, dark olivaceous, light brown, and the conidia shapes were elliptical, oblong, slightly curved. The septation of conidia ranged from 2 - 5, with the highest conidial length being 17.79 µm and the lowest being 6.62 µm, while the conidial breath was 8.27 µm and the lowest was 3.79 µm. Overall, the article provides information on the characteristics of *Bipolaris sorokiniana* under different plant extracts, which could be useful in developing strategies for managing black pointed disease in wheat.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this article.

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