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Effect of Media Composition on Vegetative and Reproductive Growth of *Alternaria brassicicola* and *Bipolaris sorokiniana*

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Abstract

Experiments were conducted to evaluate the effect of some culture media on the vegetative and reproductive growth of *Alternaria brassicicola* and *Bipolaris sorokiniana*. Twenty one (21) treatment combinations each with three replications were employed. Potato Dextrose Agar (PDA) was used as the basic growth medium in this study. Supplementation with different plant extracts with this medium produced differential growth and sporulation of the tested fungi. PDA media supplemented with mustard leaf extract showed significantly highest vegetative growth (7.8 cm) and PDA with the combination of mustard leaf, tomato fruit, carrot fruit and cabbage leaf extracts showed the highest sporulation (11×10^5 spores/ml) of *A. brassicicola*. In case of *B. sorokiniana*, the highest vegetative growth (7.4 cm) and highest sporulation (45×10^4 spores/ml) were obtained by the supplementation of mustard and wheat leaf extracts respectively. Plant extract supplementation proved better than PDA as growth medium of *A. brassicicola* and *B. sorokiniana*.

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Media composition, Vegetative growth, Reproductive growth, *Alternaria brassicicola* and *Bipolarissorokiniana*.

Introduction

The growth of microorganisms in an artificial medium is influenced by several physical and chemical factors. A nutrient material prepared for the growth of microorganisms in a laboratory is called culture medium and the nutrient composition of a culture medium plays a major role in microbial growth (Toratora *et al.*)[27]. In laboratory, fungi are isolated on specific and non-specific culture medium for cultivation, preservation, microscopic examination as well as biochemical and physiological characterization (Northolt and Bullerman; Kuhn and Ghannoum; Kumara and Rawal,)[16,13,12]. The most common growth media for bacteria are nutrient broths and for fungi are agar plates; specialized media are sometimes required for microorganism and cell culture growth (Madigan and Martinko)[14].

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In this study, two pathogens were used, *Alternaria brassicicola* and *Bipolaris sorokiniana. Alternaria* species are known as major plant pathogen. There are 299 species in the genus (Kirk *et al.*)[10], they are ubiquitous in the environment and are a natural part of fungal floral almost everywhere. The spores are airborne and found in the soil and water, as well as indoors and on objects. The club-shaped spores are single or form long chains. They can grow thick colonies which are usually green, black, or gray (Nowickiand Marcin)[17]. *Alternaria* attacks a wide range of host plants such as cole crops, crucifers, brassica etc. *Alternaria* cause black-spotting of crucifers: *Alternaria brassicae* and *Alternaria brassicicola*. Conidia of *Alternaria brassicicolaare* similar in length as *Alternaria* japonica, but have fewer longitudinal septa, are not constricted at the transverse septa, and are produced in longer chains (Tewari and Buchwaldt)[26]. *Bipolaris* is a genus of fungi belonging to the family Pleosporaceae. It was circumscribed by mycologist Robert A. Shoemaker in 1959[19]. *Bipolaris* is a large genus of *dematiaceous* hyphomycetes with more than 100 species, most of them being saprobes in soil and pathogens of plants, while some of the saprobic species are potentially able to infect humans and animals (Sivanesan)[23]. The typical morphological features of *Bipolaris* species include rapidly growing dark colonies, geniculate conidiophores with sympodial conidiogenesis, and large conidia with transversedistosepta, usually without a protuberant hilum (a basal structure scar indicating the point of attachment in the conidiogenous cell) and with bipolar germination. Morphologically similar anamorphic genera are *Drechslera*, *Curvularia*, and *Exserohilum* (Sivanesan)[23].

Fungi grow on diverse habitat in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth, colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman)[16]. However, the requirements of fungal growth are generally less stringent than for the sporulation. Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell)[25]. The experiments were performed in the laboratory condition to find out the effect of media composition on vegetative and reproductive growth of *Alternaria brassicicola* and *Bipolaris sorokiniana* on different culture media and this is the first attempt in this regard.

Materials and Methods

The experiments were conducted in the Plant Disease Diagnostic Clinic (PDDC), Department of Plant Pathology and Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensigh-2202. The experiments were performed during the period from November 2015 to December, 2016.

Collection, Isolation and identification of *Alternaria brassicicola* and *Bipolaris sorokiniana*

A. brassicicola and *B. sorokiniana* isolates were isolated from infected mustard and wheat seeds, respectively collected from farmer's own stored seeds. After collection of infected seeds, *A. brassicicola* and *B. sorokiniana* were isolated through Standard blotter method (ISTA)[9]. In this blotter method, 25 (1+8+16) mustard and wheat seeds were placed respectively on moist blotter paper (wetted with sterile water) in plastic petridishes. Then the petridishes were incubated at the incubation room 25°C to provide the conditions for the pathogen to sporulate and after 7-10 days pathogenic structures (spores and mycelia) on seeds were observed under stereo binocular microscope. Then the infested seeds were maintained on Potato Dextrose Agar (PDA) medium for 7-10 days in the incubation room and ensured about the presence of concern pathogens by preparing slide and comparing the morphological

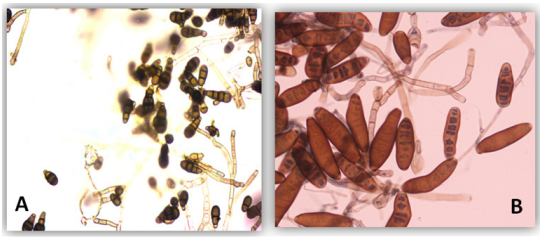

Fig. 1: Different structures of *Alernaria brassicicola* (A) and *Bipolaris sorokiniana* (B)

characters following the key outlined by Barnett[1] and Ellis[6] (Figure 1). The pure culture of both fungi were obtained by culturing fungi on PDA medium and making the fresh culture from "hyphal tip" selected from the periphery of actively growing colony under aseptic conditions. After inoculation of fresh PDA plates with fungal block, the inoculated plates were sealed with parafilm and the fungal isolates were grown under optimum growth conditions (23 ± 1°C) for 10 days.

Experimental Treatments

To study the effect of media composition on vegetative and spore production of *A. brassicicola* and *B. sorokiniana*, eleven treatments were used for *A. brassicicola* and ten treatments for *B.* sorokiniana.

Treatments for *Alternaria brassicicola* isolate

T_0= PDA (control)
T_1= PDA+ Mustard leaf extract
T_2= PDA+ Tomato fruit extract
T_3= PDA+ Cabbage leaf extract
T_4= PDA+ Bean fruit extract
T_5= PDA+ Spinach leaf extract
T_6= PDA+ Carrot fruit extract
T_7= PDA+ Sweet gourd fruit extract
T_8= T_1 (PDA+ Mustard leaf extract)+ Tomato fruit extract
T_9= T_8 (PDA+ Mustard leaf extract+ Tomato fruit extract) +Carrot fruit extract
T_{10}= T_9 (PDA+ Mustard leaf extract+ Tomato fruit extract+ Carrot fruit extract) +Cabbage leaf extract

Treatments for B. *sorokiniana* Isolate

T_0= PDA (Control)
T_1= PDA+ Wheat leaf extract
T_2= PDA+ Rice leaf extract
T_3= PDA+ Mustard leaf extract
T_4= PDA+ Tomato fruit extract
T_5= PDA+ Cabbage leaf extract
T_6= PDA+ Bean fruit extract
T_7= PDA+ Spinach leaf extract
T_8= PDA+ Carrot fruit extract
T_9= PDA+ Sweet gourd fruit extract

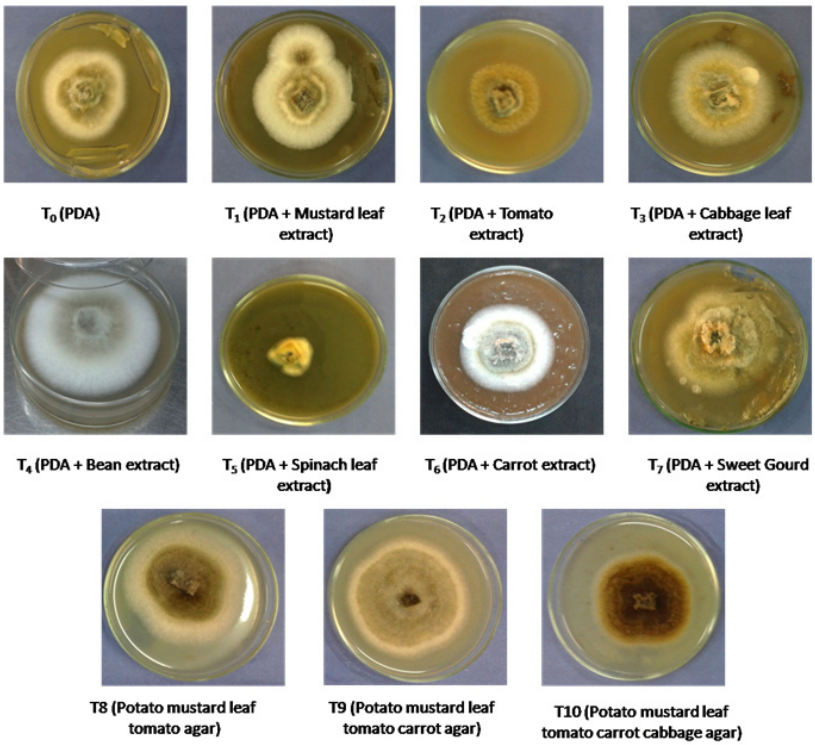

Fig. 2: Colour and appearance of pure colonies of *A. brassicicola* on PDA media supplemented with different plant extracts.

PDA Medium/Growth Medium Preparation

For this preparation 200g of clean peeled slice potato tubers were boiled in 500 ml of water. Water was separated from boiled potato slices. 20g Dextrose and 15g Agar was added to separated boiled water and made the final 1000 ml volume of PDA medium with distilled water. Then the medium was autoclaved at 121˚C , 15 PSI for 15 minutes and after proper cooling the medium, the PDA plates were prepared within laminar air flow cabinet.

Changing the Media Composition for the Growth of *Alternaria brassicicola*

To prepare a modified PDA medium, different plant extracts were added to PDA @ given in the Table 1.

After cooking, the media were poured into conical flask and the mouth of the conical flask was closed with non-absorbent cotton plug and sterilized in an autoclave at 121^{0}C under 15 PSI for 20 minutes. After autoclaving, 10-12 drops of lactic acid was added in the media for avoiding bacterial contamination and then all the media were transferred into petridishes and each of the petridishes contained 20 ml of media.

Changing the Media Composition for the Growth of *Bipolaris sorokiniana*

Media composition was changed by adding different types of host and non-host plant extracts with PDA medium. Potato wheat leaf agar medium was prepared by mixing 100ml of wheat leaf extract (1:5) with boiled water extract of 150ml sliced potato, 5g agar and 5g dextrose for 250ml of medium. On the other hand, Potato rice leaf agar medium was prepared by mixing 100ml of rice leaf extract (1:5) with boiled water extract of 150ml sliced potato, 5g agar and 5g dextrose for 250ml of medium.

Table 1: Supplemented PDA media with their composition and name

Volume of medium (ml)	PDA ingredients	Name of plant extract	Quantity (ml) of plant extract (1:5)	Name of the medium
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Mustard leaf	100	Potato mustard leaf agar (PDA+mustard leaf extract)
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Tomato fruit	100	Potato tomato fruit agar (PDA+tomato fruit extract)
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Cabbage leaf	100	Potato cabbage leaf agar (PDA+ Cabbage leaf extract)
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Bean fruit	100	Potato bean fruit agar (PDA+ bean fruit extract)
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Spinach leaf	100	Potato spinach leaf agar (PDA+ Spinach fruit extract)
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Carrot fruit	100	Potato carrot fruit agar (PDA+ Carrot fruit extract)
250	Decoction of 150 ml Potato, 5g Agar, 5g Dextrose.	Sweet gourd fruit	100	Potato sweet gourd fruit agar (PDA+ sweet gourd fruit extract)
250	Decoction of 150ml Potato, 5g Agar, 5g Dextrose.	Mustard leaf + Tomato fruit	50+50	Potato, mustard leaf tomato fruit agar (PDA+Mustardleaf+Tomato fruit extract)
250	Decoction of 150ml Potato, 5g Agar, 5g Dextrose.	Mustard leaf+ Tomato fruit+ Carrot fruit	33+33 +34	Potato mustard leaf, tomato fruit, carrot agar (PDA+Mustardleaf+Tomatofruit+Carrot fruit extract)
250	Decoction of 150ml Potato, 5g Agar, 5g Dextrose.	Mustard leaf+ Tomato fruit+ Carrot fruit +Cabbage leaf+	25+25+ 25+25	Potato mustard leaf, tomato fruit, carrot fruit, cabbage leaf agar (PDA+Mustardleaf+ Tomatofruit +Carrotfruit+Cabbage leaf extract)

Moreover, the other supplemented media such as potato mustard leaf agar medium (PDA+ Mustard leaf), potato tomato agar medium (PDA+ Tomato), potato carrot agar medium (PDA+ Carrot), potato bean agar medium (PDA+ Bean), potato spinach leaf agar medium (PDA+ Spinach leaf), potato cabbage agar medium (PDA+ Cabbage) and potato sweet gourd agar medium (PDA+ Sweet gourd) were prepared following the procedure described above (Table 1).

Inoculation of the Culture Media Plates With A. *brassicicola* and B. *sorokiniana* Isolates

PDA plates supplemented with different plant extracts were inoculated with pure culture of *A. brassicicola* and *B. sorokiniana*. In brief, mycelial blocks were cut out of 10 days old fungal colony near the margin by means of sterilized cork borer of 5 mm diameter. These blocks were transferred to the center of the petri plates by means of a sterilized inoculating needle. For each of the treatments three replications were maintained. All these were done under aseptic condition inside a laminar air flow cabinet which was sterilized previously by spraying 70% ethanol.

Observation of Vegetative Growth for Both of the Fungus Isolates on different Media

Color of colony and substrate and margin of colony were observed by naked eye for both of the fungal isolates.

Fungal Growth Measurement Technique

In case of all supplemented media, mycelial growth for both the fungi were determined directly by measuring the diameter of the colonies in the same axis. Mycelial growth of the colony was measured with the help of fine transparent plastic scale in cm and the sporulation for the both fungi were determined by spore counting using hemocytometer in spores/ml.

Measurement of Vegetative Growth Rate

After 10 days, mycelial growth of *A. brassicicola* and *B. sorokiniana* colony were recorded by measuring the average of two diameters at right angles to one

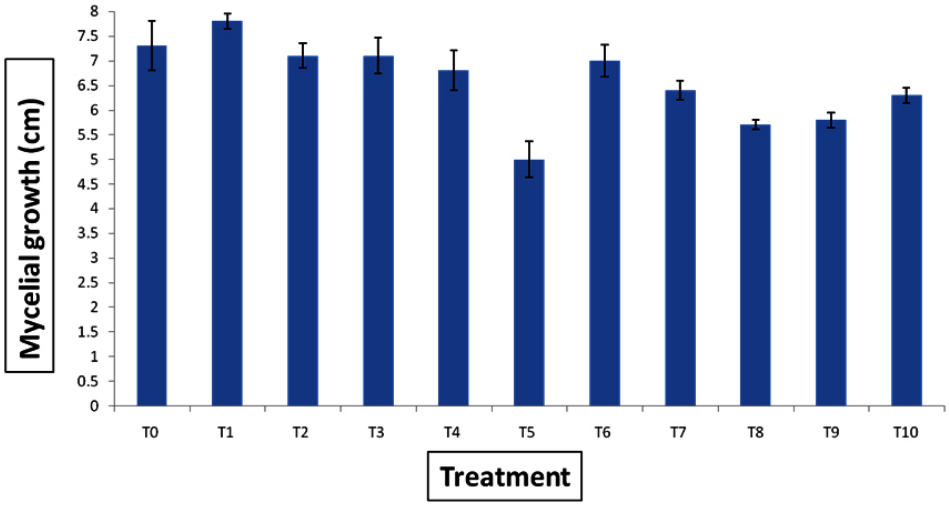

Fig. 3: Status of vegetative growth of *Alternaria brassicicola* on PDA media and PDA supplemented with different plant extracts. Here, T_0= Potato dextrose agar, T_1= PDA+ Mustard leaf extract, T_2= PDA+ Tomato fruit extract, T_3= PDA+ Cabbage leaf extract, T_4= PDA+ Bean fruit extract, T_5= PDA+ Potato spinach leaf extract, T_6= PDA+ Potato carrot fruit extract, T_7= PDA+Potato sweet gourd fruit extract, T_8= PDA+ Potato mustard leaf, tomato fruit extract T_9= PDA+ Potato mustard leaf, tomato fruit, carrot fruit extract, T_{10}= PDA+ Potato mustard leaf tomato fruit, carrot fruit, cabbage leaf extract.

another. The same procedures were repeated three times and mean number of mycelial growth was considered.

Measuring Sporulation

The degree of sporulation for both fungi were determined by adding 5ml of double distilled water and 2.5 ml of 70% ethanol in each petridish containing 10 days old pure culture of those fungi. Then the surface of the fungal growth on PDA was scraped gently with a camel hair brush to disperse the spores. After that 3 ml of the suspension and 22ml double distilled water were taken in a falcon tube and then shaken until it mixed well. From the final suspension 100 µl was placed in a hemocytometer and the number of conidia were counted under compound microscope and number of conidia/ml was estimated by using the key outlined by Tuite[28]. The same procedures were repeated three times for three replications and mean number of conidia/ml was determined.

$$\text{Measured spore density} = \frac{\text{Average spores per smalls quare} \times \text{Dilution factor}}{\text{Volume of a small square (0.0001ml)}}$$

Statistical Analysis

The experiments were done under controlled laboratory condition, and the data were analyzed following completely randomized design (CRD) by using the SPSS statistical software.

Table 2: Colony characters of *Alternaria brassicicola* on PDA media supplemented with different plant extracts

Treatments	Colony characters		
	Substrate color/ Media color	Colony color	Margin of colony
T_0= Potato dextrose agar	Light brownish	Dark brown centre and white margin	Regular
T_1= Potato mustard leaf agar	Green	Dark brown centre and white margin	Regular
T_2= Potato tomato fruit agar	Reddish	Light brown	Regular
T_3= Potato cabbage leaf agar	Light green	Dark brown centre and white margin	Regular
T_4= Potato bean fruit agar	Light green	Light brown centre and white margin	Regular
T_5= Potato spinach leaf agar	Dark green	Whitish	Irregular
T_6= Potato carrot fruit agar	Red	Brown centre and white margin	Regular
T_7= Potato sweet gourd fruit agar	Brown	Brownish	Irregular
T_8= Potato mustard leaf, tomato fruit agar	Light brown	Dark black to brown	Regular
T_9= Potato mustard leaf, tomato fruit, carrot fruit agar	Light brown	Dark black to brown along with whitish margin	Regular
T_{10}= Potato mustard leaf, tomato fruit, carrot fruit, cabbage leaf agar	Light brown	Greenish	Regular

Table 3: Spore production of *Alternaria brassicicola* by the media composition

Treatments	Spores per ml
T_0 (Potato dextrose agar)	33×10^2
T_1 (PDA+Mustard leaf extract)	64×10^4
T_2 (PDA+ Tomato fruit extract)	55×10^4
T_3 (PDA+ Cabbage leaf extract)	44×10^4
T_4 (PDA+ Bean fruit extract)	25×10^3
T_5 (PDA+Spinach leaf extract)	75×10^3
T_6 (PDA+Carrot fruit extract)	225×10^3
T_7 (PDA+ Sweet gourd fruit extract)	9×10^4
T_8 (PDA+ Mustard leaf, tomato fruit extract)	925×10^3
T_9 (PDA+ Mustard leaf, tomato fruit, carrot fruit extract)	875×10^3
T_{10} (PDA+ Mustard leaf, tomato, carrot, cabbage extract)	11×10^5

Results

This research work was conducted to select the best media composition suitable for the vegetative and reproductive growth of *Alternaria brassicicola* and *Bipolaris sorokiniana*. The tested fungal isolates were grown in PDA media supplemented with different plant extracts. The results of the experiments are given below-

Growth Characteristics of *Alternaria brassicicola on* PDA Media Supplemented with different Plant Extracts

The growth characteristics like colour of substrate/ media, color of colony and margin of colony of *Alternaria brassicicola* on different culture media were observed in this study. The colony colour of *A. brassicicola* having dark brown centre and white margin in case of PDA media and PDA supplemented with mustard leaf extract and cabbage leaf extract. Whereas, PDA media supplemented with tomato fruit, bean fruit, carrot fruit, spinach leaf and sweetgourd fruit showed light brown, light brown centre and white margin, whitish, brown centre and white margin and brownish colony respectively (Table 2; Figure 2). This result indicating that PDA media supplemented with different plant extracts have an impact on the colony color of *A. brassicicola*. On the other hand, margin of the pure colony of *A. brassicicola* on PDA medium and PDA supplemented with different plant extracts showed mostly regular form. On sweet gourd and spinach leaf extract supplemented medium, the colony appeared irregular (Table 2; Figure 2).

Effect of Media Composition on the Vegetative Growth of *Alternaria brassicicola*

PDA media supplemented with different plant extracts showed better vegetative growth. PDA media supplemented with mustard leaf extract showed significantly highest vegetative growth (7.8 cm) of the fungus, followed by supplementation with tomato fruit (7.1 cm) and cabbage leaf (7.1 cm). PDA media with no supplementation i.e. control showed better vegetative growth of *A. brassicicola* (7.3 cm) compared to the PDA media supplemented with other plant extracts. The PDA media supplemented with carrot fruit, bean fruit and sweet gourd fruit also showed better vegetative growth of *A. brassicicola* of 7.0 cm, 6.8 cm and 6.4 cm respectively. On the other hand, PDA media supplemented with the combination (treatment No. 8, 9 & 10 in Figure 2) and spinach leaf extract showed poor vegetative growth compared to the other treatments (Figure 3).

Influence on Spore Production of *Alternaria brassicicola* By The Media Composition

The data revealed that PDA media supplemented with combination of mustard leaf, tomato fruit, carrot fruit and cabbage leaf extract showed the highest sporulation (11×10^5 spores/ml) of *A.*

brassicicola followed by PDA media supplemented with combination of mustard leaf and tomato extract (925×10^3 spores/ml) and combination of mustard leaf, tomato, carrot (875×10^3 spores/ml) (Table 3). This result indicating that PDA media supplemented with more than one plant extracts produced more spores compared to the PDA media supplemented with one plant extract.

Table 4: Colony characters of *Bipolaris sorokiniana* on PDA media supplemented with different plant extracts.

Treatments	Colony characters		
	Substrate color/ media color	**Colony color**	**Margin of colony**
T_0= Potato dextrose agar	Light brownish	Dark brown to whitish at margin	Irregular, wavy margin
T_1= Potato wheat leafagar	Brown	Brownish	Irregular
T_2= Potato rice leaf agar	Brown	Dark brown at center and greenish at margin	Irregular
T_3= Potato mustard leaf agar	Green	Light brown at center and white at margin	Regular
T_4= Potato tomato fruit agar	Reddish	Dark brown at center and white at margin	Irregular, smooth wavy margin.
T_5= Potato cabbage leaf agar	Light green	Whitish colony	Irregular
T_6= Potato bean fruit agar	Light green	Whitish colony with greenish center	Thin flat, regular
T_7= Potato spinach leafagar	Dark green	Cottony White	Thin flat, irregular
T_8= Potato carrot fruit agar	Red	Dark brown at center and white at margin	smooth irregular
T_9= Potato sweet gourd fruit agar	Brown	Dark brown	Irregular, Wavy margin

Growth Characteristics of *Bipolaris sorokiniana* on PDA Media Supplemented with different Plant Extracts

The colony color of *B. sorokiniana* having dark brown centre and white margin in case of PDA media and PDA media supplemented with carrot and tomato extract whereas, PDA media supplemented with mustard, bean, cabbage, spinach and sweet gourd showed light brown at center and white at margin, whitish colony with greenish center, whitish colony, cottony white and dark brown colony of *B. sorokiniana* respectively (Table 4; Figure 4).On the other hand, PDA media supplemented with wheat and rice leaf extract had brownish and dark brown center and greenish at margin of the colony of *B. sorokiniana*. This result indicating that PDA media supplemented with different plant extracts have an impact on the colony colour of *B. sorokiniana*. On the other hand, margin of the pure colony of *B. sorokiniana* on PDA media and PDA supplemented with different plant extracts showed mostly irregular form (Table 4; Figure 4). However, colony on potato mustard leaf agar and potato bean agar was regular.

Effect of Media Composition on the Vegetative Growth of *Bipolaris sorokiniana*

The result explained that the PDA media supplemented with non-host extracts showed better vegetative growth compared to the host extracts and control. PDA media supplemented with

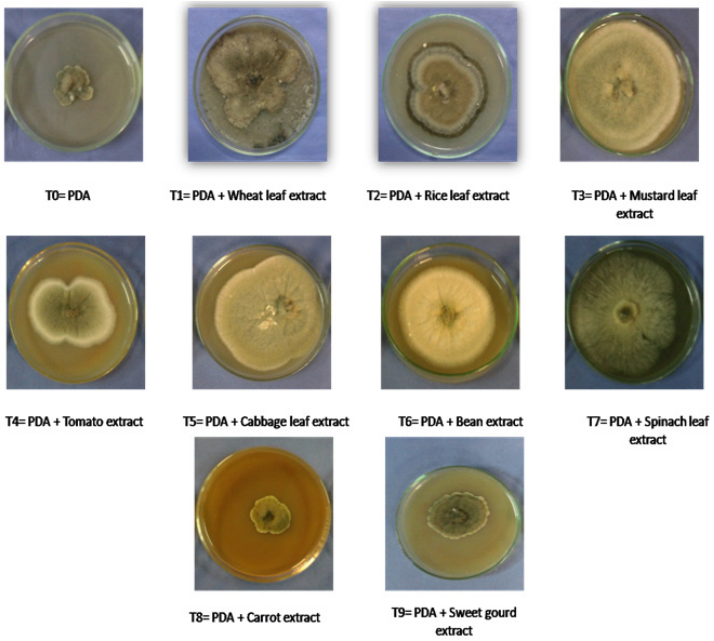

Fig. 4: Colony colour and appearance of pure colonies of *Bipolaris sorokiniana* on PDA media supplemented with different plant extracts.

mustard leaf extract showed significantly highest vegetative growth (7.4 cm) of the fungus, followed by PDA media supplemented with spinach leaf extract (7.3 cm) and bean (6.8 cm). The PDA media supplemented with host extracts such as wheat and rice showed less vegetative growth compared to the PDA media supplemented with non-host extracts. Non-host extract PDA media such as potato tomato agar medium, potato sweet gourd agar medium and potato carrot agar medium showed vegetative growth of 4.9 cm, 4.3 cm and 2.9 cm respectively. PDA media with no supplementation with host and non-host extract i.e. control showed poor vegetative growth compared to all other treatments (Fig. 5).

Influence on Spore Production of *Bipolaris sorokiniana* by he Media Composition

To know the effect of media composition on the spore production of *B. sorokiniana* isolate, 10 different PDA media composition (PDA media supplemented with different host and non-host plant extracts) were used in this research experiment. The data revealed that PDA media supplemented with wheat leaf extract showed the highest sporulation (45×104 spores/ml) of *B. sorokiniana* followed by PDA media supplemented with rice leaf extract (30×10^5 spores/ml) and PDA media supplemented with sweet gourd extract (1×10^5 ml spores/ml) (Table 5).This result indicated that PDA media supplemented with host extracts produced more spores compared to the PDA media supplemented with non-host extract.

Discussion

The experiments were conducted for the evaluation of media composition for growth characteristics of

Table 5: Spore production of *Bipolaris sorokiniana* by the media composition

Treatments	Spore per ml
T_0 (Potato dextrose agar)	5×10^4
T_1 (PDA+Wheat leaf extract)	45×10^4
T_2 (PDA+ Rice leaf extract)	3×10^5
T_3 (PDA+ Mustard leaf extract)	0
T_4 (PDA+ Tomato fruit extract)	0
T_5 (PDA+ Cabbage leaf extract)	4×10^4
T_6 (PDA+Bean fruit extract)	0
T_7 (PDA+ Spinach leaf extract)	1×10^4
T_8 (PDA+ Carrot fruit extract)	16×10^3
T_9 (PDA+Sweet gourd fruit extract)	1×10^5

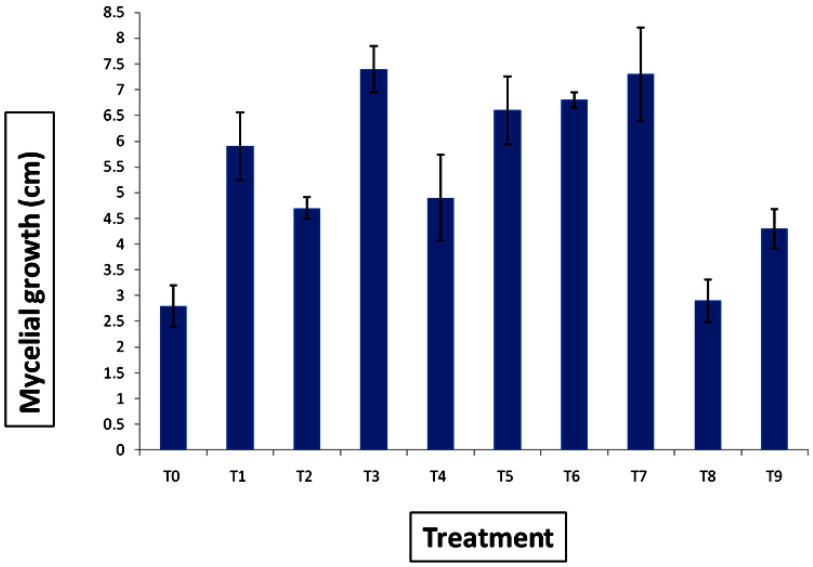

Fig. 5: Status of vegetative growth of *Bipolarissorokiniana* on PDA media and PDA media supplemented with different plant extract. Here, T_0= Potato dextrose agar, T_1=PDA+ Wheat leaf extract, T_2= PDA+ Rice leaf extract, T_3= PDA+ Mustard leaf extract, T_4= PDA+ Tomato fruit extract, T_5= PDA+ Cabbage leaf extract, T_6= PDA+ Bean fruit extract, T_7= PDA+ Spinach leaf extract, T_8= PDA+ Carrot fruit extract, T_9= PDA+ Sweet gourd fruit extract.

Alternaria brassicicola and *Bipolaris sorokiniana*, causing black leaf spot of mustard-rapeseed and leaf spot of wheat, respectively. Since the available literature on the present investigations "effect of media composition on the vegetative and reproductive growth of *A. brassicicola* and *B. sorokiniana*" is scanty, the available information pertaining to the aspects under study discussed thoroughly in the light of the some literature. In this chapter probable causes of the findings are also discussed in the aspects of the results of previous research findings.

Pathogen culture in the best suitable media is the first step of phytopathological research. For the growth study of *A. brassicicola* and *B. sorokiniana*, most of the earlier researchers used the PDA and Potato Dextrose Broth (PDB) media. Some researchers also used the Richard's broth, Czapek's dox broth, host extract agar and Sabouraud's agar medium. But the influence and comparisons among different culture media or PDA media supplemented with different host and non-host plant extracts on the vegetative and reproductive growth of *A. brassicicola* and *B. sorokiniana* are the objective of the present research work.

Studies on the growth of *A. brassicicola on* different PDA media i.e. PDA media supplemented with different plant extracts showed that PDA media supplemented with mustard leaf extract showed significantly highest vegetative growth and PDA with the combination of mustard leaf, tomato fruit, carrot fruit, and cabbage leaf extracts showed highest sporulation. However the fungus was found to grow on all the supplemented culture media tested, but PDA supplemented with mustard leaf extract was more favorable for vegetative growth of *A. brassicicola*. The present findings is in the conformity with the earlier work by Koley and Mohapatra; Saha *et al.*[11,20]. It is concluded that PDA with mustard leaf extract has the simple formulation and mustard leaf has the sufficient nutrient content, suitable for best mycelial growth of the fungus. Chand and Chandra[3] recorded maximum vegetative growth of *A. brassicicola* on host extract media. Mohapatra *et al.*[15] stated that maximum growth of *Alternaria* sesame (infecting sesame) on PDB compared to

Richard's, Czapek's dox and oat meal broth media. In addition, Somappa *et al*.[24] reported that PDB is the best medium supporting good growth of A. solani infecting tomato. These results indicated that complex formulation of the media does not allow the fast mycelial growth of *A. brassicicola*.

On the other hand, PDA media with the combinations of plant extracts (mustard leaf, tomato fruit, carrot fruit and cabbage leaf) showed the highest sporulation and lowest sporulation observed in PDA media without supplementation with plant extracts. This result indicated that the complex formulation of PDA medium with host extracts allow the fast sporulation of *A. brassicicola*. It might be due to PDA media with mustard leaf, tomato fruit, carrot fruit and cabbage leaf extracts contain all the major compounds for fungal sporulation i.e. carbon, nitrogen, phosphate, potassium, magnesium, sulphur elements including sugars. This finding also corroborates the findings of earlier researchers. Osman *et al.*[18] found Czapek's Dox Agar medium was best for the growth and sporulation of *Alternaria alternata*. Fancelli and Kimati[7] also observed that Czapek's Dox and host leaf extract medium yielded better sporulation of *Alternaria dauci* compared to other tested media. Singh[20] found that the fungus grew well on various natural media but oat meal agar proved best and excellent sporulation was detected in potato dextrose agar. Waggoner and Horsfall[29] reported that A. solani requires a carbon source (sugar) for higher sporulation, but high availability of sugar inhibits the conidia production.

In case of *Bipolaris sorokiniana*, PDA with mustard leaf showed significantly highest vegetative growth and lowest vegetative growth was found in PDA media without supplementation with plant extract. These results indicated that PDA media supplemented with plant extracts enhanced vegetative growth of *B. sorokiniana*. It might be due to PDA supplementation with plant extracts has the sufficient nutrient content, supporting the best mycelial growth of the fungus. On the other hand, PDA media supplemented with wheat leaf extract showed the highest sporulation followed by PDA media supplemented with rice leaf extract. These findings are indicating that *Bipolaris* fungus showed better sporulation on PDA media supplemented with graminaceous plant extracts compared to other plant extracts. These results corroborate the findings of former literatures such as Chattopadhyay and Dasgupta[4] found that production of conidia by *B. oryzae* was favored by media either rich in or made exclusively of plant parts. Coleman *et al*.[5] showed that methionine and ethylene both reduced mycelium growth of *B. sorokiniana* when grown on Czapek's medium or on media containing grass leaf infusion. Methionine and other amino acids reduced sporulation as did ethylene and precursors of ethylene, S-adenosylmethionine and 1-aminocyclopropane-1- carboxylic acid. Cegielko[2] examined that faster growth of *Drechslera* avenae recorded in MPA (maltose peptone agar) medium and RA (rye agar) were the best media for the sporulation of D. avanae. Garraway and Evans[8] observed xylose (1.0-10.0 g/L) stimulates sporulation but not mycelial growth of *B. maydis* when added as a supplement to a basal agar medium containing glucose, sporulation on a xylose -supplemented medium was significantly higher than on a non-supplemented control medium. Sharma and Singh[21] found that rice husk agar and near ultraviolet radiation were required for production of conidia by *Helminthosporium oryzae*.

Conclusion

In the present investigation, maximum mycelial growth of both *A. brassicocola* and *B. sorokiniana* were obtained in PDA supplemented with mustard leaf while maximum sporulation of *A. brassicocola* and *B. sorokiniana* found in PDA supplemented with all plant extracts combination and PDA supplemented with wheat leaf respectively. Based on these findings it may be concluded that PDA supplementation with extract of single or combination of host plant extracts, the sporulation of the two fungi can be enhanced.

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