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Management of *Bipolaris Sorokiniana* the Causal Pathogen of Spot Blotch of Wheat by Eucalyptus Extracts

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Abstract

The inhibitory effect of essential oil of flowering buds and potential extracts of *Eucalyptus camaldulensis* Dehn (leaf, Bark and Flowering Buds) were evaluated on the most aggressive isolate of *Bipolaris sorokiniana* from wheat crop by agar well diffusion, food poison technique and macro-dilution assay. The mycelial growth was evaluated over four periods (3; 6; 9 and 30 days from incubation) with nine concentrations of essential oils (0.5%; 01%; 2.5%; 05%; 7.5%; 10%; 15%; 50%; 100%) and 3 concentrations (01%; 05% and 10%) of effective extracts. E.oil showed a maximum zone of inhibition of 90 mm diameter and mycelia growth 0.00 ± 0.00 compares to control 40.00 ± 0.00 after 9 days of incubation at 50% and 100% concentration. The extracts of F. buds showed the strongest active values ($P<0.05$) with a 29.10 ± 0.92 ZOI compared to water extract (19.80 ± 0.33). The ethanol and methanol fruit extracts showed highest minimum inhibitory concentration (08 mg/mL) than water extracts (200 mg/ml) against *B. sorokiniana*, while minimum fungicidal concentration values for ethanol and methanol fruit extract 40 mg/mL and water extract 300 mg/mL. Extract treated hyphae were collapsed, damaged or thinner when compared with the control. This Study reveals that, F. buds were the potential part of plant. The E.oil and ethanol extract of F. buds were consider most effective that shows considerable potency (up to 97% *in-vitro* inhibition) than the bark and leaves solvents extracts, while no inhibitory effect was noticed for the aqueous extract of leaves and bark in combating the pathogen.

Keywords: Eucalyptus; Aqueous and organic solvent extracts; Essential oil; *Bipolaris sorokiniana*

Introduction

Wheat (*Triticum aestivum* L.) is one of the key cereal crops of Pakistan. It is the essential foodstuff in major part of the world compare to other crops especially that are grown in irrigated conditions [1]. In Pakistan a number of diseases affects wheat crop. However, among frequently occurring diseases of wheat crops in Pakistan induced by biotic stresses foliar blight/spot blotch are considered to contribute significantly to low average yields of the wheat crop [2]. Under warm and humid conditions wheat crop are vulnerable to infection with the spot blotch caused by *Bipolaris sorokiniana* (Sacc). The host range of *B. sorokiniana* among monocotyledonous plants are small grain cereals, like *Triticum aestivum, hordeum vulgare, Avena sativa, Sorghum bicolor* and a large number of other grasses. Several plant species other than monocotyledons including *Brassica compestris, Glycine max, Lens culinaris, Vigna radiata, Sesamum indicum, Vigna mungo and Pennisetum amaricanum* are identified as the host of *B. sorokiniana* [3]. Disease can be seed borne, soil born and airborne. The soil-borne conidia are considered the main source of inoculums, which causing secondary infection that results in severe foliar disease and yield loss. The symptoms of spot blotch are most prominent on leaves after heading and on lower leaves are more frequent, appeared as discrete, elongate, brown-black lesions [4].

This pathogen can cause the disease on whole-wheat plant (seedling blight, root rot, spot blotch lesions and black point of the grain) and results a big damage in the quantity and quality of crop yield. The most prevalent disease on five commercial varieties i.e., Bhakkhar-2001, Inqilab-91, Faisalabad-08, Lasani-2008 and Seher-2006 that were cultivated at farmer fields was spot blotch and no variety was found free of this disease. The spot blotch disease prevalence was 100% on all the Bhakkhar-2001 cultivated fields, whereas the prevalence on Inqilab -91, Faisalabad-08, Lasani-08 and Seher-2006, was 14%, 10%, 5% and 3% respectively [5]. Spot blotch of wheat is managed usually by the application of chemicals. Plant based compounds are significant sources to replaces chemicals for plant diseases management [6]. It is clear that natural products can trim down the populations of foliar pathogens and infection expansion, by reproduce thereselve as environmentally safe mechanism in integrated pest management programs [7]. Different plant species are available in records that hold natural compounds that are toxic as antimicrobial agents [8]. Al Hazmi [9] studied the effect of Neem extract on mycelia growth of *Bipolaris* and found that the Neem seed alcoholic extract was more effective (25.67%) in retarding the *Bipolaris sorokiniana* growth than the leaf alcoholic extract 15.79% and water extract 3.5%. Similarly, a slight but statistically significant difference in the number of conidia of *Bipolairis sorokiniana* was observed between the more concentrated extracts and the less concentrated extracts of *Bouhenia variegata.* The two less concentrated extracts did not control fungal growth [10]. The present study investigated the efficacy of *Eucalyptus camaldulensis* leaf bark and flowering buds extracts in the management of spot blotch of wheat under *in-vitro* conditions.

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Material and Methods

Test organism

Pathogen was isolated from diseased wheat plants. Those were infected by the mother culture of most aggressive isolate of *Bipolaris sorokiniana* in the research fields of crop disease research institute (CDRI) NARC, Islamabad and maintained on Potato dextrose agar (PDA) growth medium until used.

Collection of plant material

Different plant tissues of *Eucalyptus camaldulensis* were collected from healthy mature trees growing in the Botanical garden of Pakistan Forest Institute, Peshawar. The collected samples were identified at the Department of botany of Pakistan Forest Institute, Peshawar.

Preparation of plant samples

Samples of leaves bark and flowering buds were washed thoroughly with tap water twice to remove residual soil debris then sterilized in 10% bleach and again washed with distilled water and dried under shade at room temperature for two or three weeks to make it suitable for grinding. Then crush into fine powder by mechanical grinder. The sample obtained were store in airtight bottle until required for further analysis.

Water extracts

Powder (10 g) was put in a conical flask. Further to this were added 100 ml of sterilized water and boiled when volume of the water were concentrated to 25 ml then stopped boiling and filtered through a eight fold muslin cloth after that filter through whatman No.1 filter paper. The filtrate was further concentrated by using water bath complete dryness [11].

Organic solvent extracts

Powder (10 g) was extracted with 100 ml solvents (methanol, ethanol) by cold maceration on rotatery shaker for 3 days and filtered through Whatman No.1 filter paper. Further, the extracts were concentrated to complete dryness using rotary evaporator under reduced pressure. The volatile oil was extracted by hydrodistillation.

Antifungal bioassay

Determination of mean diameter and biomass production of the culture: Different extracts and crude oil at different concentrations were tested against the test pathogen by agar well diffusion and food poison technique [12]. For this, 5 ml of *Eucalyptus* E.oil was mixed with Tween-80 (0.05%) and further diluted with 5 ml of tripled autoclaved water thus 5 ppm stock solution was made. This stock solution was further diluted with sterilized water to give the concentrations of 0.5, 1, 2.5, 5, 7.5, 10, 15, 50, and 100 ppm. Each concentration in one ml was mixed uniformly in 25 ml of PDA (potato dextrose agar) for *Bipolaris sorokiniana*. From then on, a mycelial disc of about 6 mm diameter was inoculated in the centre of each Petri plate. The mycelia disks were cut from the periphery of a 08-day old culture. For the control treatment Tween–80 (0.05%) mixed with sterilized water instead of extracts was used. Five replicates of each treatment were used in a completely randomized design. The Petri plates were then incubated at 28 ± 2°C and results were record from the 3rd day until complete growth in control plates. Similar experiments were also performed for different solvent extracts at different concentrations. The MICs (minimum inhibitory concentrations) were determined. To determine minimum fungicidal concentration (MFC), subculture 10 µl from non turbid test tubes, Negative or blank (inoculums containing but no extract) and positive tube (extract containing but no inoculums) on PDA plates and incubates on 28 ± 2°C until growth was seen in the control subculture. If there is no growth this indicated minimum fungicidal concentration [13]. Now to determine the effect of different solvent extracts and E. oils on the dry weight of the *Bipolaris sorokiniana*, one ml of each treatment was added to 20 ml sterilized growth medium (PD broth) in 100 ml flask and inoculated with a 5 mm disc of test pathogen culture. Control treatment containing 1 ml of Tween-80 (0.05%). After 10 days of incubation fresh and dry weights of mycelia of (*Bipolaris sorokiniana*) were determined [14].

Determination of sporulation rate and Conidia characteristics of the culture: The sporulation rate measurement was done at 10 days of culture. For conidial recount the conidia from different treated plats were removed by using 10 ml of sterile distilled water containing 0.05% Tween 20 and filtered using eight fold gauze, to remove remains of culture medium and mycelia, and count it in hemocytometer.

The morphological effects caused by the oil and extract were determined by the comparative analysis of the structures (Hypea and conidia) observed in the control and in each of the treatments under an optical microscope at (100 ×).10 µl of fungal suspension were placed on microscope slides in order to determine the size of the conidia. These observations were performed by means of a Leitz- SM-Lux microscope, provided with micrometric ocular lens, under a 400 magnification (Periplan GF 10 X Leitz-Wetzlar). Thirty measurements were performed for each treatment and the greatest and smallest dimensions were study and considered as length (L) and width (W), respectively.

Results and Discussion

Effect of Eucalyptus extracts and E. oil on radial mycelial growth, percentage growth inhibition of *B. sorokiniana* at different concentrations

The inhibitory effect of different extracts of *Eucalyptus sps,* against the growth of disease causing agents have been reported previously by Rakotonirainy & Lavedrine, [15]. In the present study the inhibitory effects of Essential oil and Ethanol, Methanol and aqueous extracts of different parts of *Eucalyptus camaldulensis* were evaluated against the pathogen that causes Spot blotch of wheat crop. However, it was observed that the ethanol flowering buds extracts of *Eucalyptus camaldulensis,* produced very significant antifungal activity against *Bipolaris sorokiniana.*

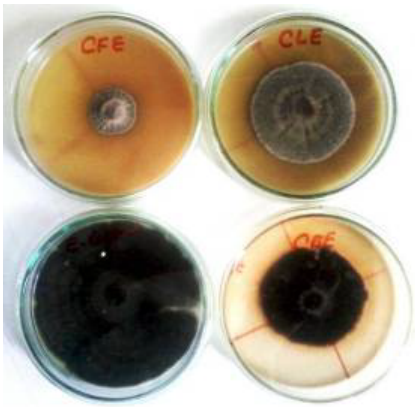

Figure 1: Treatments of Ethanol: E.control: CBE: *Camaldulensis* Bark extract; CLE; *Camaldulensis* Leave extract; CFE: *Camaldulensis* Fruit extract.

Figure 2: Zone of inhibition made by E.Oil treatments.

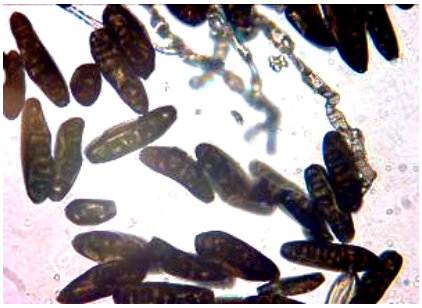

Figure 3: Conidia treated with control treatments.

Figure 4: Conidia treated with oil treatment.

In relation to diametric growth, the percentage inhibition was evaluated at four extract (ethanol, methanol water and essential oil) of leaves, flower buds and bark of *Eucalyptus camaldulensis.* Results of E.oil and flower buds extract show complete inhibition of mycelia growth until 5 days, and after 15 days of incubation the percent inhibition ranged from 97%, 91%, 67% and 98% respectively. There is only a dead protoplast observed that having no hyphea and conidia. While 100% of E.oil showed complete inhibition after 30 days of incubation. That does not agree with the results of Katooli et al. [16]. According to them 50% and 100% of *Eucalyptus camaldulensis* E.oil inhibit the growth of *Bipolaris sorokiniana* only until 5 days and after 30 day of incubation there in no inhibition of such treatments.

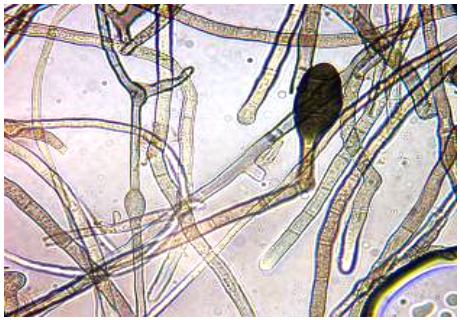

Figure 5: Hypea treated with control treatment.

Figure 6: Hypea treated with oil treatment.

B. Ext.	Extraction solvent	Part used	Color		Size				No of septa		Shape of conidia
					Length (µ)		Width (µ)				
			Conidiophore	Conidia	Conidiophore	Conidia	Conidiophore	Conidia	Conidiophore	Conidia	
E. camaldulensis Dehn.	Ethanol	Leaf	Light brown	Olivaceous brown	62-177	29-60	5-7.5	15-23	4-7	2-6	Elliptical or oval
		Flower Buds	Light brown	Olivaceous brown	58-155	30-60	3-6	12-30	2-6	1-4	Elliptical
		Bark	Light brown	Olivaceous brown	81-187	50-80	6-8.5	20-25	4-7	2-5	Elliptical or oval
	Methanol	Leaf	Light brown	Olivaceous brown	80-183	50-70	6.41-8	20-30	2-8	1-5	Oval with round ends
		Flower Buds	Light brown	Olivaceous brown	57-173	24-75	5-7.6	18-30	4-8	2-6	Oval to elliptical
		Bark	Light brown	Olivaceous brown	80-189	50-80	5.8-7.3	20-30	2-8	2-6	Oval with round ends
	Water	Leaf	Light brown	Olivaceous brown	60-170	30-70	6-7	15-25	2-9	2-6	Oval slightly curved
		Flower Buds	Light brown	Olivaceous brown	80-172	50-60	6-8	20-30	4-8	2-6	Elliptical or oval
		Bark	Light brown	Olivaceous brown	70-180	40-83	6.5-8	20-30	2-9	2-7	Oval with round ends
	Control	Ethanol	Light brown	Olivaceous brown	74-190	40-90	6-8	20-32	2-7	2-7	Oval with round ends
		Methanol	Light brown	Olivaceous brown	53.2-190	20-90	5-8	15-30	2-8	1-6	Oval with round ends
		Water	Light brown	Olivaceous brown	81.36-193.21	50-90	6.42-8.71	20-30	2-9	2-7	Oval with round ends

Table 1: Conidia characters of *Bipolaris sorokiniana* subjected to different concentrations of the of *Eucalyptus camaldulensis* Dehn extracts.

Botanical extracts	Concentration (%)	Color		Size				No of septa		Shape of conidia
				Length (µ)		Width (µ)				
		Conidiophore	Conidia	Conidiophore	Conidia	Conidiophore	Conidia	Conidiophore	Conidia	
E. essential oil	0.5%	Light brown	Olivaceous Brown	70-187	40-70	6-8	20-30	2-9	2-7	Oval with round ends
	1%	Light brown	Brown to olivaceous	66-188	36-80	5-8	15-32	2-9	2-6	Oval with round ends
	2.5%	Light brown	Olivaceous Brown	62-161	30-60	6-8	15-23	4-8	1-6	Oval with round ends
	5%	Light brown	Olivaceous to dark brown	57-164	30-60	6-8	17-30	4-8	2-5	Oval with round ends
	7.5%	Light brown	Olivaceous brown	50-130	20-32	5-7.3	11-19	2-7	2-5	Oval to elliptical
	10%	Light brown	Olivaceous Brown	50-128	20-30	4-8	10-20	2-7	1-5	oval
	15%	Light brown	Olivaceous Brown	55-166	50-70	4-7	20-22	1-6	1-4	Nearly round
	50%	Light brown	Olivaceous Brown	46-149	18-55	4-6	10-21	1-5	1-4	Oval to Nearly round
	100%	-	-	-	-	-	-	-	-	-
Control	Water	Light brown	Dark brown	81.36-193.21	50-90	6-8	20-30	4-9	2-7	Oval to elliptical
	Tween 20	Light brown	Olivaceous Brown	70.2-190	40-90	6-8	20-30	2-9	2-6	Oval with round ends
	DMSO	Light brown	Olivaceous Brown	74-190	40-90	6-8.5	20-30	2-9	2-6	Oval to elliptical

Table 2: Conidia characters of *Bipolaris sorokiniana* subjected to different concentrations of the essential oils of *Eucalyptus camaldulensis* Dehn.

Extraction solvent	*Eucalyptus camaldulensis* extracts				*Eucalyptus camaldulensis* E. oil				
	Conidial germination		Conidia recount		Conidial germination			Conidia recount	
	Part used	Germination pattern	Sporulation	No of spores/10µl	Concentration	Germination pattern	Germination %	Sporulation	No of spores/10µl
Ethanol	Leaf	Mostly unipolar sometime bipolar	+++	87-173	0.5%	Mostly unipolar, nominal bipolar	28.2	+++	5-57
	Flower Buds	Mostly unipolar sometime bipolar	+	24-83	1%	Mostly unipolar, nominal bipolar	19.45	+++	2-10
	Bark	Mostly unipolar sometime bipolar	+++	96-204	2.5%	Mostly unipolar, nominal bipolar	18.65	+++	6-23
Methanol	Leaf	Mostly unipolar sometime bipolar	++	75-170	5%	Mostly unipolar, nominal bipolar	17.45	+++	1-13
	Flower Buds	Mostly unipolar sometime bipolar	+	54-155	7.5%	Mostly unipolar, nominal bipolar	15.85	++	2-8
	Bark	Mostly unipolar sometime bipolar	+++	80-183	10%	Mostly unipolar, nominal bipolar	11.19	++	1-2
Water	Leaf	Mostly unipolar sometime bipolar	+++	115-192	15%	Mostly unipolar, nominal bipolar	10.86	++	1-3
	Flower Buds	Mostly unipolar sometime bipolar	+++	81-115	50%	Mostly unipolar, nominal bipolar	8.92	+	0
	Bark	Mostly unipolar sometime bipolar	+++	128-262	100%	Mostly unipolar, nominal bipolar	0.06	-	0
Control	Ethanol Methanol Water	Mostly unipolar sometime bipolar Mostly unipolar sometime bipolar Mostly unipolar sometime bipolar	+++ +++ ++++	101-190 73-178 171-275	DMSO	Mostly unipolar, nominal bipolar	75.04	++++	20-84

Table 3: Data on Sporulation and conidial germination of *Bipolaris sorokiniana* treated with eucalyptus sps treatments.

Although the MIC (Minimum inhibitory concentration) of water extract of flower buds against *Bipolaris sorokiniana* was higher (200 mg/ml) than that for ethanol, methanol extracts of flowers buds (08 mg/ml). While the MFC (Minimum fungicidal concentration) values for ethanol and methanol extracts of flowering buds were 40 mg/mL as compared with water extract 300 mg/mL. E.oil showed a maximum zone of inhibition of 90 mm diameter and mycelia growth 0.00 ± 0.00 compares to control 40.00 ± 0.00 after 9 days of incubation at 50% and 100% concentration. The ethanol extract of flower buds of *E. camaldulensis* showed the strongest active values ($P<0.05$) with a 29.51 ± 0.71 ZOI (Zone of inhibition) compared to methanol and water extracts 28.80 ± 0.91, 19.80 ± 0.33 respectively.

The mycelia growth inhibition and MIC studies reveal that the ethanol extract of *Eucalyptus camaldulensis* flower buds is more potent of the three extracts in inhibiting the test organism of spot blotch of wheat. It was seen in the whole study that when the concentration of extracts in the growth medium increases the inhibition of growth also increased. Similar effects of a variety of other plants products that are effective against *Bipolaris sorokiniana* were reported by several authors Hasan et al and Perello et al. [17,18].

Effect of eucalyptus extracts and E. oil on sporulation rate, conidia characteristics, fresh weight and dry weight of *B. sorokiniana* at different concentrations

The colour, length, width and number of septa of conidia and conidiophores of *Bipolaris sorokiniana* treated with different treatments were measured under the microscope. In control treatments conidial

variation were around 20-90 micron in length and 12-30 micron in width having 1-7 numbers of septa, oval with round ends or elliptical shape and olivaceous brown to dark brown in colour. Conidia affected by essential oil and flower buds extract were very small oval to nearly round and with only 1-4 or without septa compared to conidia affected by control treatments. On the other hand, the conidiophores

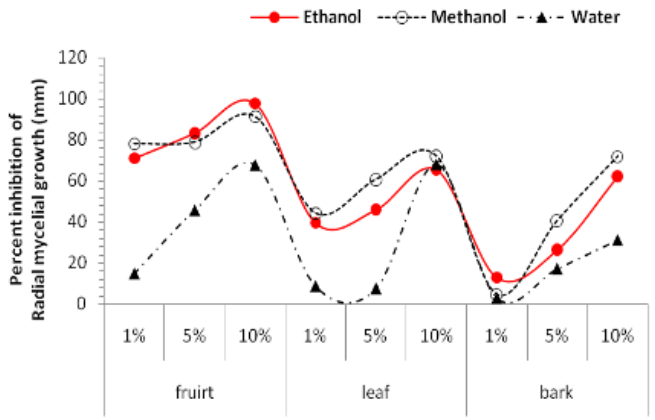

Figure 7: Effect of different Eucalyptus extracts concentration on radial mycelial growth inhibition of *B. sorokiniana*.

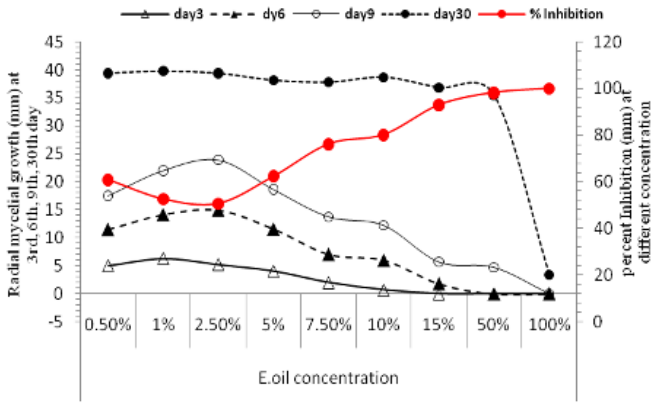

Figure 8: Effect of different E.oil concentration on radial mycelial growth and mycelial growth inhibition of *B. sorokiniana*.

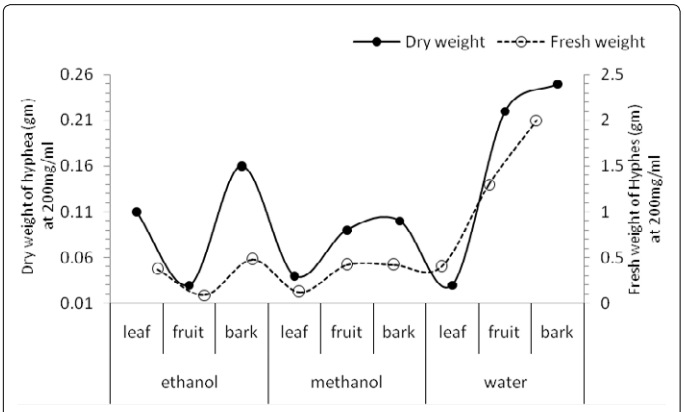

Figure 9: Relationship between different Eucalyptus extracts concentration and fresh and dry biomass of *Bipolairis sorokiniana.*

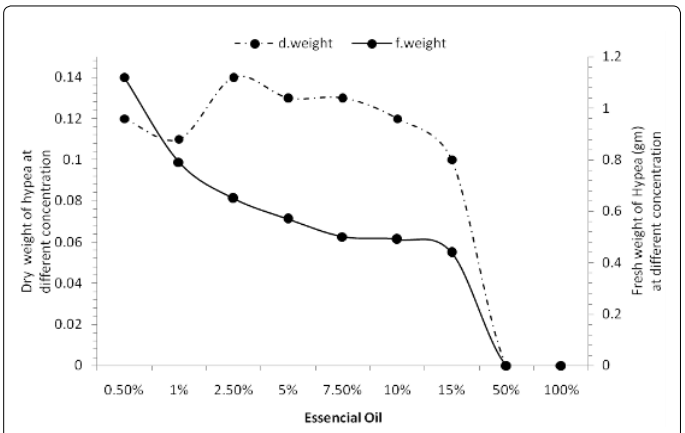

Figure 10: Relatioship between different E.Oil treatments and fresh and dry biomass of *Bipolairis sorokiniana.*

variations were around 46-189 micron in length and 3-8 micron in width having 1-9 septa and light brown in colour. Extract treated hyphae were collapsed, damaged or thinner when compared with the control treated hyphae. Only the measurements of conidia with control treatments are similar as reported by Muchovej et al. [19]. They observed that *B. sorokiniana* presented conidia more than 75 micron long and less than 25 micron wide. There were significant differences between different treatments that affect the sporulation rate of *Bipolaris sorokiniana*. Such as *E. camaldulensis* flower buds extract (ethanol) and 50% E.oil treatments have very poor sporulation rate however, there is no sporulation observed for the treatments of 100% E.oil. The sporulation rate decreases as the concentration of treatments were increases. In present study there were no biomass production observed at 100 and 50 percent Essential oil treatments. However the biomass production observed at flower buds ethanol, methanol extracts were very poor. Similarly a significant decline in the biomass production of Macrophomena *phaseolena* due to different solvent treatments of *Eucalyptus citriodora* extracts was observed by Javaid and Hafiza [20].

Conclusion

All treatment either *E. camaldulensis* different parts extracts or E.oil were effective to inhibit the *Bipolaris sorokiniana* colonies growth and spore germination. But the ethanol extract of flower buds was found more potent in combating pathogen. From the economic point of view, treatments of E.oil and flower buds extract applied at 1% and 7.5% concentration causing the management of pathogen up to 71% and 76%, were found more profitable compared to treatments of E.oil and flower buds extract applied at 10% and 50% concentration that causing the management of pathogen up to 97% and 98%.

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References

1. Bajwa MA (1985) Wheat research and production in Pakistan. In: Wheat's for more tropical environments. A proceeding of the international symposium. Mexico, DF, CIMMYT pp. 68-77.
2. Iftikhar S, Asad S, Rattu A, Munir A, Fayyaz M (2012) Screening of commercial wheat varieties to spot blotch under controlled and field conditions. Pak J Bot 44: 361-363.
3. Iftikhar S, Asad S, Munir A, Sultan A, Ahmad I (2009) Hosts of *bipolaris sorokiniana*, the major pathogen of spot blotch of wheat in pakistan. Pak J Bot 41: 1433-1436.

4. Bello GMD, Sistern MN, Monaco CI (2003) Antagonistic effect of soil rhizosphere microorganisms on *Bipolaris sorokiniana*, the causal agent of wheat seedling blight. Int J Pest Manege 49: 313-317.

5. Rattu AR, Asad S, Fayyaz M, Zakria M Iftikhar S, et al. (2011) Status of foliar diseases of wheat in Punjab, Pakistan. Mycopath 9: 39-42

6. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2004) Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Datura metel against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiological and Molecular Plant Pathology 65: 91-100.

7. Browers JH, Locke JC (2004) Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of Phytophthora blight in the greenhouse. Plant Dis 88: 11-16.

8. Goussous SJ, Abu-El-Samen FM, Tahhan RA (2010) Antifungal activity of several medicinal plants extracts against the early blight pathogen (*Alternaria solani*). Archives of Phytopathology and Plant Protection 43: 1746-1758.

9. Al-Hazmi RHM (2013) Effect of Neem (*Azadirachta indica*) leaves and seeds extract on the growth of six of the plant disease causing fungi. Global Advanced Research Journal of Microbiology 2: 089-098.

10. Elisabeth Bach E, Marcondes MCL, Patricio GF, Esquerdo KF, Cardoso V, et al. (2012) Aqueous extract of leaves from *Bauhinia variegate* used in barley plants to protect against *Bipolaris sorokiniana*. Agricultural Research and Reviews 1: 71-79.

11. Uzama D, David MB, Ahmadu R, Thomas SA (2011) Phytochemical Screening and Antibacterial Activity of *Polyalthia Longifolia* Crude Extracts. Asian J Pharm Biol Res 1: 507-511.

12. Sasode RS, Prakash S, Gupta A, Pandya RK, yadav A (2012) *In vitro* study of some plant extracts against *Alternaria brassicae* and *Alternaria brassicicola*. Journal of Phytology 4: 44-46.

13. Espinel-Ingroff A, Fothergill A, Peter J, Rinaldi MG, Walsh TJ (2002) Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for *Aspergillus* spp.: NCCLS Collaborative Study. Journal of Clinical Microbiology 40: 3204-3208.

14. Ramezani H, Singh HP, Batish DR, Kohli RK, Dargan JS (2002) Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal. New zealand plant protection 55: 327-330.

15. Rakotonirainy MS, Lavedrine B (2005) Screening for antifungal activity of essential oils and related compounds to control the bio contamination in libraries and archives storage areas. International Biodeterioration and Biodegradation 55: 141-147.

16. Katooli N, Maghsodlo R, Razavi SE (2014) Evaluation of eucalyptus essential oil against some plant pathogenic fungi. International Journal of Agroforestry and Silviculture 1: 016-017.

17. Hasan MM, Ahmed F, Islam MR, Murad KFI (2012) In vitro effect of botanical extracts and fungicides against *Bipolaris sorokiniana*, causal agent of leaf blotch of barley. J Agro for Environ 6: 83-87.

18. Perello A, Ulrike N, Slusarenko AJ (2013) *In vitro* efficacy of garlic extract to control fungal pathogens of wheat. Journal of Medicinal Plants Research 7: 1809-1817.

19. Muchovej JJ, Muchovej RMC, Ribeiro-Nesio ML (1988) Taxonomia de Drechslera, Bipolaris e Exserohilum. Fitopatologia Brasileira, Brasi´lia 13: 211-223.

20. Javaid A, Rehman HA (2011) Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*. Journal of Medicinal Plants Research 5: 2868-2872.