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# *IN-VITRO* INHIBITION OF SPOT BLOTCH DISEASE CAUSAL AGENT (*BIPOLARIS SOROKINIANA*) BY PLANT EXTRACTS

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**ABSTRACT**

The inhibitory effect of essential oil (EO) of flowering buds and potential extracts of *Eucalyptus camaldulensis* Dehn(leaf, Bark and Flowering Buds) was evaluated on colony growth of the most aggressive isolate of *Bipolari ssorokiniana* from wheat by agar well diffusion, food poison technique and macro-dilution assay. Effect on mycelial growth of test organism was evaluated at nine different concentrations of essential oils (0.5%; 01%; 2.5%; 05%; 7.5%; 10%; 15%; 50%; 100%) and 3 concentrations of aqueous, ethanolic and methanolic extracts of *E. camaldulensis* (01%; 05% and 10%) with time interval of 3, 6, 9 and 30 days. EO produced a maximum inhibition zone of 90 mm dia. and mycelial growth 0.00±0.00 compared to control 40.00±0.00after 9 days of incubation period at 50% and 100% concentrations. The ethanolic extracts of flowering budsshowed strong inhibition zone of 29.10±0.92 mm dia. that is significant values ($P < 0.05$) compared with water extract (19.80±0.33). Ethanolic and methanolic flowering buds extracts showed highest minimum inhibitory concentration (08mg/mL) than water extracts (200mg/ml) against *B. sorokiniana* while, minimum fungicidal concentration values were observed for ethanolic and methanolic flowering buds extracts at 40 mg/mL and for aqueous extract at 300 mg/mL.Hypae treated with EOand flowering buds ethanolic extract were collapsed, damaged or thinner compared to control. This Study revealed that, flowering buds extracts had the highest inhibitory effect on the growth of the pathogen. The EO and ethanolic extract of flowering buds were considered most effective that showed considerable inhibition (up to 97% *in-vitro* inhibition) than bark and leaf ethanolic and methanolic extracts, while no significant inhibitory effect on mycelia growth of the pathogen was recorded upon treatment with aqueous leaf and bark extracts.

Keywords: *Eucalyptus,* plant extracts, essential oil,Antifungal activities,*Biopolaris sorokiniana*.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the key cereal crops of Pakistan. It is an essential foodstuff in major parts of the world than other crops especially that are grown in irrigated conditions (Bajwa, 1985). Under warm and humid conditions wheat production is vulnerable to spot blotch disease caused by *Bipolaris sorokiniana.* This pathogen can attack all parts of the plant such as seedling, root and grains (seedling blight, root rot, spot blotch lesions and black point of the grain) and leads to substantial reduction in both quantity and quality of crop yield. The most frequent management practice to overcome this problem is through use of chemicals. Plant based compounds are important and environmentally safe candidate to replace chemicals in plant diseases management practices (Kagale*et al.*,2004).It is clear that natural products are biosafe having the potential to decrease the population of foliar pathogens and spread of infection. expansion, by reproduce there selves as environmentally safe mechanism in integrated pest management programs (Bowers and Locke 2004). Different plant species have been previously reported to possess strong toxic antimicrobial action. (Goussous*et al.*,2010).Al Hazmi (2013) studied the effect of *neem* extracts on mycelia growth of *B.sorokiniana*- and found that ethanolic extract of *neem* seed produced highest inhibition 25.67% of the colony growth of *B. sorokiniana* than leaf (15.79%) and water extracts (3.5%). Similarly, a slight

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but statistically significant difference in total number of conidia produced by *B. sorokiniana* was observed between more and less concentrated extracts of *Bouhenia variegata*. Less concentrated extracts did not control fungal growth (Elisabeth Bach *et al*., 2012).The study was undertaken to evaluate the efficacy of methanolic and ethanolic leaf, bark and flowering bud extracts of *E. camaldulensis* to manage spot blotch of wheat*in-vitro* condition.

MATERIALS AND METHODS

Test Organism: A culture of test fungi *B. sorokiniana* was procured from the Crop Disease Research Institute, National Agricultural Research Center (NARC), Islamabad, Pakistan which contains information regarding how they were isolated.

Plant material: Healthy plant parts i.e., leaf, bark and flowering bud of *E. camaldulensis* were collected from botanical garden of Pakistan Forest Institute, Peshawar. The collected samples were identified in the Department of Botany, Pakistan Forest Institute, Peshawar, Pakistan.

Preparation of plant extracts: Plant parts were thoroughly washed and dried under shade for four weeks and crushed into fine powder by using a mechanical grinder followed by preservation in air tight bottles until required for further analysis.

Aqueous extract: Powder (10g) of each plant part was mixed in 100ml of sterilized water and boiled until 25ml concentrated solution was left followed by filtration through eight folds of muslin cloth and Whatman No.1 filter paper respectively. The filtrate was further concentrated by using water bath till complete dryness(Uzama *et al.,* 2011).

Solvent extracts: Powder (10g) was extracted with 100 ml solvents (methanol, ethanol) by cold maceration on rotatory 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.

Extraction of essential oil:The essential oils of *E. camaldulensis* fresh leaves and flowering buds were extracted by hydro distillation method. Fresh flowering buds (500g) were grind in grinder and leaves were cut into pieces (3 cm of dia.) and transferred into 1000 mL of distilled water in volume flask that was coupled to a Clevenger-type apparatus and the solution distilled for 3 h. EO was collected and dried by anhydrous sodium sulfate and stored at 4°C prior to the analysis. Percentage yield was measured based on dry weight of the samples (Nasrabadi *et al*., 2012).

$$\text{The extraction yield} = \frac{(\text{VEO} \times 100)}{\text{D. M}}$$

(D.M: dry material; VEO: volume of essential oil)

*In-vitro* Antifungal Bioassay

Determination of mean diameter and biomass production of the culture: Extracts and EO at different concentrations were tested against the test pathogen by agar well diffusion and food poison technique (Sasode *et al*., 2012).For this, 5ml of EO was mixed with Tween-80 (0.05%) and further diluted with 5 ml sterilized water to make 5ppm stock solution. Stock solution was further diluted with sterilized water to obtain 0.5, 1, 2.5, 5, 7.5, 10, 15, 50, and 100ppm concentrations. Each concentration at the rate of 1ml was mixed uniformly in 25 mL of PDA (potato dextrose agar). A mycelial disc of approximately 6 mm in dia. was inoculated at the centre of each Petri plate. The mycelia disks were cut from the periphery of a 8-day old culture of *B. sorokiniana*. Control treatment was maintained by mixing Tween–80 (0.05%) with sterilized water instead of extract. Each treatment was replicated five times followed by incubation at 28±2°C and results were record at 24h interval started from the 3rd day until control plates were completely filled (8 days) with colony growth of the test organism. For measurement, two straight lines at right angles to one another were drawn on the bottom of each Petri dish to make four segments. They coincided in the center of 6 mm initial fungi disc. Radial mycelia growth was recorded daily from the edge of 6 mm disc until the extreme area of fungi mycelia growth, similarly data recorded for four segments that were formed by the two perpendicular lines. Data for each day equal to means of four measurements, each measurement carried out with one segment (Miyashira *et al*., 2010). Mycelial growth inhibition (MGI) percentage was calculated according to Shahi and Shahi (2011).

$$\text{MGI}(\%) = (\text{dc} - \text{dt}) \times 100/\text{dc}$$

Where, dc = mycelial growth diameter in control sets

dt = mycelia growth diameter in treatment sets.

Similar experiments were also performed for ethanol, methanol and aqueous extracts at different concentrations. The minimum inhibitory concentration (MIC) was determined for each treatment. To determine minimum fungicidal concentration (MFC), subculture 10µl from non turbid test tubes, Negative or blank (containing inoculum without extract) and positive tube (containing extract without inoculum) on PDA plates and incubated at 28±2 $^{0}$C

until growth was seen in the control subculture. If there is no growth this indicated minimum fungicidal concentration (Espinel-Ingroff *et al*. 2002). To determine the effect of different solvent extracts and EO on dry weight of the *B. sorokiniana,* one ml of each treatment was added to 20 ml sterilized growth medium (Potato dextrose broth) in 100 ml flask and inoculated with a 5 mm culture discs of test pathogen. Control treatment was maintained by adding 1 ml of Tween-80 (0.05%). After 10 days of incubation period, fresh and dry weights of mycelium were determined. (Ramezani *et al*., 2002).

Determination of sporulation rate and Conidia characteristics of the culture: The sporulation rate of 10-day-old culture of the test organism was recorded. Spore growth on medium was scrapped in 10 ml of sterile distilled water containing 0.05% Tween 20 and filtered through eight fold gauze, to remove remains of culture medium and mycelium, and total numbers of conidia were counted by using a hemocytometer.The effect of essential oils and plant extracts on colony morphology of the test organism was determined by comparative analysis of the structures (Hypae and conidia) compared to control under an optical microscope of (100 X magnifying power). Size of conidia was measured by adding 10 µl of fungal suspension on clean glass followed by observations under Leitz- SM-Lux microscope, provided with micrometric ocular lens and 400X· magnifying power (Periplan GF 10X Leitz-Wetzlar). Thirty measurements were taken for each treatment and the greatest and smallest dimensions were studied and considered as length (L) and width (W), respectively.

Analysis of Variance Table for colony growthof *B.sorokiniana*

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Parts | 2 | 15811.8 | 7905.89 | 171.26 | 0.0000 |
| Solvents | 2 | 12636.1 | 6318.05 | 136.86 | 0.0000 |
| Concentra | 2 | 15530.2 | 7765.10 | 168.21 | 0.0000 |
| Parts*Solvents | 4 | 1054.0 | 263.51 | 5.71 | 0.0002 |
| Parts*Concentra | 4 | 810.1 | 202.52 | 4.39 | 0.0017 |
| Solvents*Concentra | 4 | 587.4 | 146.85 | 3.18 | 0.0133 |
| Parts*Solvents*Concentra | 8 | 3221.3 | 402.66 | 8.72 | 0.0000 |
| Error | 621 | 28667.7 | 46.16 | | |
| Total | 647 | 78318.6 | | | |

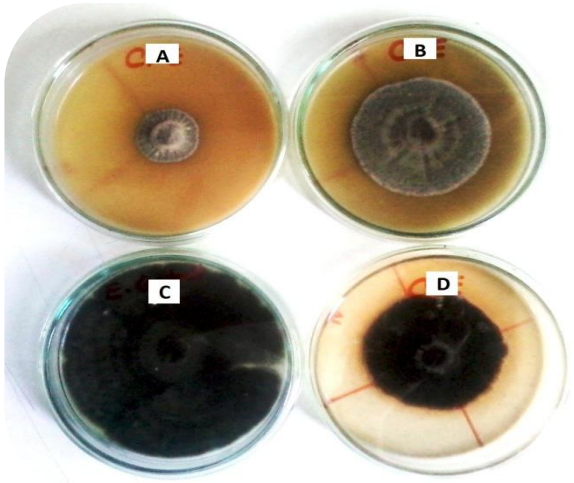


Figure **1**

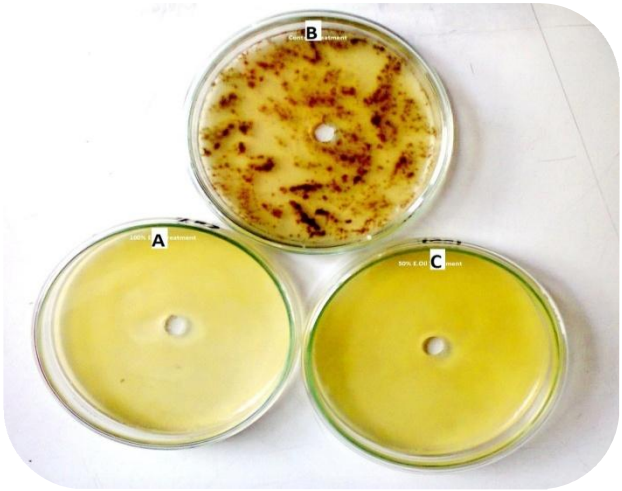


Figure **2**

Figure 1. Mycelial growth treated with A: flowering buds Concentration extracts; B: leaf extract; C: ethanol control; D: bark extract.

Figure 2. Zone of inhibition made by E.Oil A: 100%C: 50% Concentration; B: Control.

Table 1. Effect of *E. camaldulensis* extracts and EO at different concentration on colony growth, Inhibition Zone and Hypae fresh and dry weight of *B. sorokiniana*

| Botanical extracts | | Radial mycelia growth & Inhibition percentage (mm) Mean±S.E | | | | | | Zone of inhibition (mm) Mean±SE | | Hyphea weight (g) Mean±SE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Extraction solvent | Part used | Con. (%) | *E. camaldulensis* | Inhibition (%) | Con. (%) | Essential Oil | Inhibition (%) | (mg/ 100µl) | *E. camaldulensis* | *E.camaldulensis* | | Essential oil | |
| | | | | | | | | | | F.weight | D.weight | F.weight | D.weight |
| Ethanol | Leaf | 1 | 18.029±1.927 ef | 39.558 | 0.5% | 10.34±0.71cd | 60.84 | 10 | 29.10±0.92 a | 0.37±577e | 0.11±0.05 bcd | 1.122±5.7 d | 0.122±5.7 f |
| | | 5 | 16.121±1.334 f | 45.955 | | | | | | | | | |
| | | 10 | 10.304±0.927 h | 65.456 | | | | | | | | | |
| | Flower Buds | 1 | 12.208±1.124ghij | 50.073 | 1% | 12.56±0.82bc | 52.48 | 10 | 29.51±0.71 a | 0.09±5.77 f | 0.02±5.77d | 0.793±5.7 e | 0.117±5.7 g |
| | | 5 | 5.004±0.663 jk | 83.224 | | | | | | | | | |
| | | 10 | 0.975±0.229 l | 96.731 | | | | | | | | | |
| | Bark | 1 | 25.971±2.220 b | 12.933 | 2.5% | 13.08±1.02b | 50.66 | 10 | 14.5±1.18 d | 0.48±0.05 e | 0.16±0.05 abc | 0.657±5.7f | 0.099±5.7i |
| | | 5 | 21.942±1.532 cd | 26.440 | | | | | | | | | |
| | | 10 | 11.283±0.922 g | 62.174 | | | | | | | | | |
| Methanol | Leaf | 1 | 16.938±1.416 f | 44.229 | 5% | 9.94±0.81d | 62.36 | 10 | 28.75±1.97 a | 0.12±0.05 fg | 0.04±0.00 cd | 0.575±5.7 g | 0.138±5.7 d |
| | | 5 | 11.942±0.929 g | 60.679 | | | | | | | | | |
| | | 10 | 8.496±0.610 ghij | 72.025 | | | | | | | | | |
| | Flower Buds | 1 | 6.683±0.843 hij | 77.995 | 7.5% | 6.29±0.67e | 76.22 | 10 | 28.80±0.91 a | 0.42± 0.05e | 0.09±0.00bcd | 0.520±5.7h | 0.137±5.7 d |
| | | 5 | 6.421±0.668 ijk | 78.858 | | | | | | | | | |
| | | 10 | 2.646±0.341 kl | 91.287 | | | | | | | | | |
| | Bark | 1 | 28.933±1.962 ab | 4.734 | 10% | 5.22±0.60e | 80.23 | 10 | 22.60±0.99 b | 0.42±0.05 e | 0.10±0.05 bcd | 0.494±5.7h | 0.125±5.7 e |
| | | 5 | 18.008±1.339 ef | 40.706 | | | | | | | | | |
| | | 10 | 8.512±0.612 ghij | 71.932 | | | | | | | | | |
| Water | Leaf | 1 | 28.329±2.163 ab | 8.824 | 15% | 1.85±0.38f | 92.99 | 10 | 19.05±0.85 c | 1.3±0.00 c | 0.42±0.00 d | 0.442±5.7i | 0.105±5.7 h |
| | | 5 | 28.700±1.996 ab | 7.630 | | | | | | | | | |
| | | 10 | 9.875±0.532 ghi | 68.217 | | | | | | | | | |
| | Flower Buds | 1 | 26.446±1.880 ab | 14.885 | 50% | 0.45±0.23f | 98.29 | 10 | 19.80±0.33 bc | 0.40±5.77 g | 0.22±0.05ab | 0.00±0.00 j | 0±5.7 j |
| | | 5 | 16.850±1.514 f | 45.769 | | | | | | | | | |
| | | 10 | 9.967±0.923 ghi | 67.921 | | | | | | | | | |
| | Bark | 1 | 30.129±1.822 a | 3.031 | 100% | 0.00±0.00f | 100 | 10 | 20.50±1.62 bc | 2±0.00 b | 0.24±0.05 a | 0.00±0.00 j | 0±5.7 j |
| | | 5 | 25.688±2.018 bc | 17.324 | | | | | | | | | |
| | | 10 | 21.338±1.710 de | 31.325 | | | | | | | | | |
| Ethanol | Control | 5 | 29.829±1.966 | 0 | DMSO | 26.41±1.88a | 0 | 10 | 0 | 0.48±0.05 e | 0.09±0.00 bcd | 1.240± c | 0.175± c |
| Methanol | | 5 | 30.371±1.988 | 0 | | | | 10 | 0 | 0.69±0.05 d | 0.13±0.05abcd | 1.896± b | 0.258± a |
| Water | | | 31.071±1.954 | 0 | | | | 10 | 0 | 2.2±0.00 a | 0.20±0.05 ab | 2.2± a | 0.206± b |

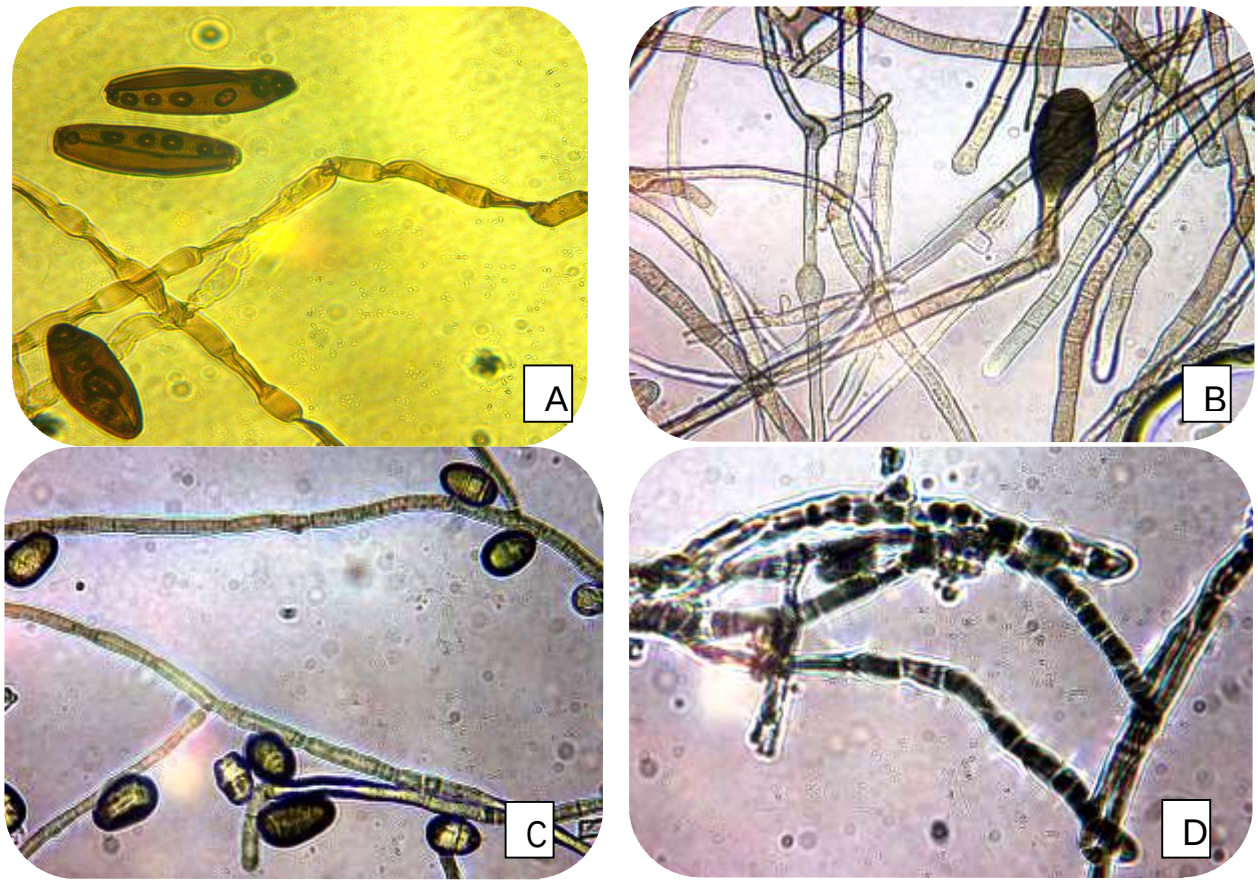


Figure 3. A: conidia treated with control treatment; B: conidia treated with oil treatment; C: Hypae treated with control treatment; D: Hypae treated with oil treatment.

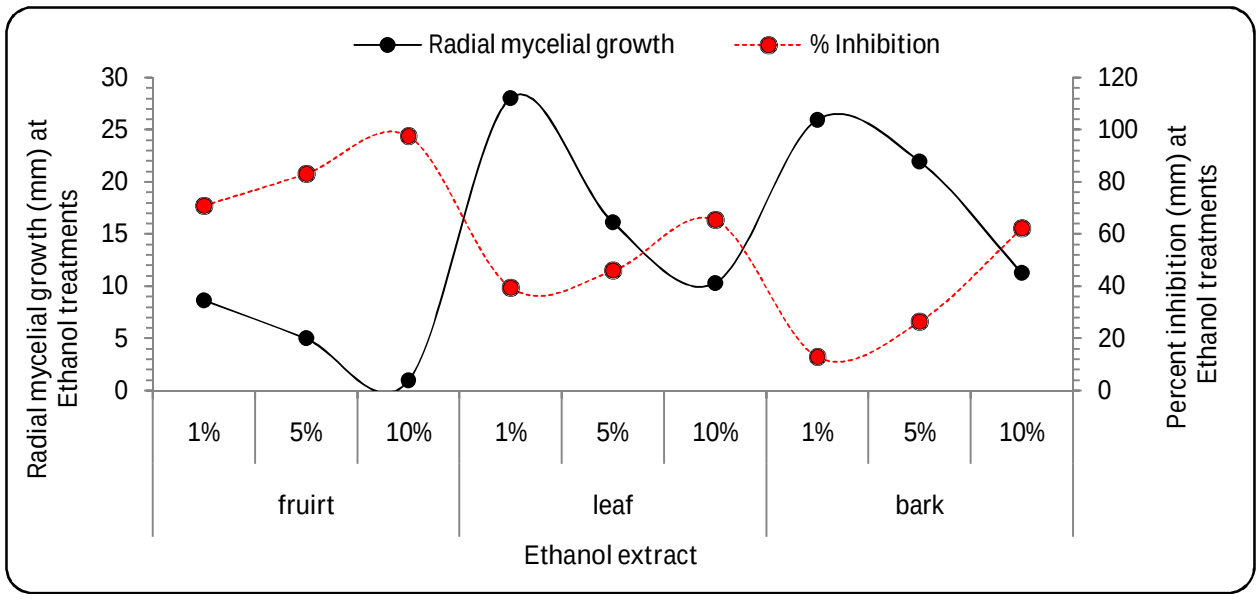


Figure 4. Effect of *E. camaldulensis* ethanol extracts on mycelia Growth and growth inhibition of *B. sorokiniana*.

Table 2. Conidia characters of *B. sorokinian a*subjected to different concentrations of *E. camaldulensis*Dehn extracts

| B. Ext. | Extraction solvent | Part used | Color | | Size MEAN±SE | | | | No of septa | | Shape of conidia |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Length (µ) | | Width (µ) | | | | |
| | | | Conidiophore | Conidia | Conidiophore | Conidia | Conidiophore | Conidia | Conidiophore | Conidia | |
| *E. camaldulensisDehn.* | Ethanol | Leaf | Light brown | Olivaceous brown | 113±1.54 | 50±0.44 | 5.60±0.32 | 22±0.28 | 4-7 | 2-6 | Elliptical or oval |
| | | Flower Buds | Light brown | Olivaceous brown | 78±1.55 | 49±0.64 | 3.63±0.42 | 19±0.10 | 2-6 | 1-4 | Elliptical |
| | | Bark | Light brown | Olivaceous brown | 126±1.74 | 63±0.49 | 7.00±0.38 | 25±0.18 | 4-7 | 2-5 | Elliptical or oval |
| | Methanol | Leaf | Light brown | Olivaceous brown | 133±1.62 | 59±0.32 | 6.98±0.29 | 21±0.33 | 2-8 | 1-5 | Oval with round ends |
| | | Flower Buds | Light brown | Olivaceous brown | 105±1.61 | 52±0.84 | 5.93±0.38 | 20±0.13 | 4-8 | 2-6 | Oval to elliptical |
| | | Bark | Light brown | Olivaceous brown | 133±1.55 | 68±0.47 | 6.60±0.24 | 25±0.20 | 2-8 | 2-6 | Oval with round ends |
| | Water | Leaf | Light brown | Olivaceous brown | 124±1.69 | 54±0.79 | 6.58±0.20 | 23±0.22 | 2-9 | 2-6 | Oval slightly curved |
| | | Flower Buds | Light brown | Olivaceous brown | 123±1.38 | 56±0.21 | 7.25±0.30 | 21±0.13 | 4-8 | 2-6 | Elliptical or oval |
| | | Bark | Light brown | Olivaceous brown | 134±1.80 | 74±0.29 | 7.16±0.21 | 28±0.31 | 2-9 | 2-7 | Oval with round ends |
| | Control | Ethanol | Light brown | Olivaceous brown | 127±1.79 | 75±0.76 | 6.87±0.30 | 27±0.18 | 2-7 | 2-7 | Oval with round ends |
| | | Methanol | Light brown | Olivaceous brown | 118±1.97 | 75±1.11 | 6.84±0.44 | 20±0.20 | 2-8 | 1-6 | Oval with round ends |
| | | Water | Light brown | Olivaceous brown | 141±1.69 | 77±0.54 | 7.33±0.44 | 25±0.15 | 2-9 | 2-7 | Oval with round ends |

Table 3. Conidia characters of *B. sorokiniana* subjected to different concentrations of *E. camaldulensis* Dehn. essential oil

| Botanical extracts | Concentration (%) | Color | | Size (MEAN±SE) | | | | No of septa | | Shape of conidia |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Conidiophore | Conidia | Length (µ) | | Width (µ) | | Conidiophore | Conidia | |
| | | | | Conidiophore | Conidia | Conidiophore | Conidia | | | |
| Essential oil of *E. camadulensisDehn.* | 0.5% | Light brown | Olivaceous Brown | 139±1.88 | 59±0.62 | 6.63±0.34 | 25±0.22 | 2-9 | 2-7 | Oval with round ends |
| | 1% | Light brown | Brown to olivaceous | 114±1.76 | 51±0.81 | 6.43±0.60 | 23±0.27 | 2-9 | 2-6 | Oval with round ends |
| | 2.5% | Light brown | Oivaceous Brown | 105±1.51 | 41±0.40 | 7.00±0.28 | 18±0.14 | 4-8 | 1-6 | Oval with round ends |
| | 5% | Light brown | Oivaceous to dark brown | 110±1.58 | 43±0.41 | 7.16±0.33 | 22±0.23 | 4-8 | 2-5 | Oval with round ends |
| | 7.5% | Light brown | Oivaceous brown | 93±1.20 | 57±0.37 | 6.60±0.37 | 20±0.03 | 2-7 | 2-5 | Oval to elliptical |
| | 10% | Light brown | Olivaceous Brown | 81±1.16 | 26±0.20 | 5.33±0.71 | 11±0.16 | 2-7 | 1-5 | oval |
| | 15% | Light brown | Oivaceous Brown | 72±1.73 | 38±0.75 | 5.90±0.58 | 17±0.23 | 1-6 | 1-4 | Nearly round |
| | 50% | Light brown | Oivaceous Brown | 62±1.64 | 25±0.22 | 4.66±0.33 | 14±0.18 | 1-5 | 1-4 | Oval to Nearly round |
| | 100% | - | - | 0.00±0.00 | 0.00±0.0 | 0.00±0.00 | 0.00±0.00 | - | - | - |
| Control | Water | Light brown | Dark brown | 137±1.72 | 70±68 | 6.75±0.30 | 23±0.16 | 4-9 | 2-7 | Oval to elliptical |
| | Tween 20 | Light brown | Oivaceous Brown | 128±1.81 | 68±87 | 7.41±0.32 | 21±0.10 | 2-9 | 2-6 | Oval with round ends |
| | DMSO | Light brown | Oivaceous Brown | 130±1.83 | 71±54 | 6.91±0.58 | 25±0.12 | 2-9 | 2-6 | Oval to elliptical |

Table 4. Data on Sporulation and conidial germination of *B. sorokiniana* treated with *E. camaldulensis* treatments

| Extraction solvent | *Eucalyptus camaldulensis* extracts | | | | *Eucalyptus camaldulensis* EO | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 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 | +++[b] | 125±8.58 | 0.5% | Mostly unipolar, nominal bipolar | 28.2 | +++[c] | 34±9.63 |
| | Flower Buds | Mostly unipolar sometime bipolar | +[a] | 44±7.71 | 1% | Mostly unipolar, nominal bipolar | 19.45 | +++[c] | 30±8.17 |
| | Bark | Mostly unipolar sometime bipolar | +++[c] | 160±11.13 | 2.5% | Mostly unipolar, nominal bipolar | 18.65 | +++[c] | 15±3.15 |
| Methanol | Leaf | Mostly unipolar sometime bipolar | ++[b] | 121±11.03 | 5% | Mostly unipolar, nominal bipolar | 17.45 | +++[c] | 8±2.26 |
| | Flower Buds | Mostly unipolar sometime bipolar | +[b] | 83±9.41 | 7.5% | Mostly unipolar, nominal bipolar | 15.85 | ++[b] | 6±1.11 |
| | Bark | Mostly unipolar sometime bipolar | +++[c] | 132±10.21 | 10% | Mostly unipolar, nominal bipolar | 11.19 | ++[b] | 6±1.46 |
| Water | Leaf | Mostly unipolar sometime bipolar | +++[c] | 146±7.58 | 15% | Mostly unipolar, nominal bipolar | 10.86 | ++[b] | 2±0.37 |
| | Flower Buds | Mostly unipolar sometime bipolar | +++[b] | 94±5.89 | 50% | Mostly unipolar, nominal bipolar | 8.92 | +[a] | 0±0.00 |
| | Bark | Mostly unipolar sometime bipolar | +++[c] | 181±12.17 | 100% | Mostly unipolar, nominal bipolar | 0.06 | - | 0±0.00 |
| Control | Ethanol<br>Methanol<br>Water | Mostly unipolar sometime bipolar<br>Mostly unipolar sometime bipolar<br>Mostly unipolar sometime bipolar | +++[c]<br>+++[c]<br>++++[d] | 146±8.29<br>124±7.62<br>181±12.36 | DMSO | Mostly unipolar, nominal bipolar | 75.04 | ++++[d] | 53±10.34 |

=No Sporulation, +[a] =Sporulation rate less than 40% compared to control , ++[b] = Sporulation rate >40% and <70%, +++[c] = Sporulation rate > 80% and <100%, ++++[d] = 100% Sporulation.

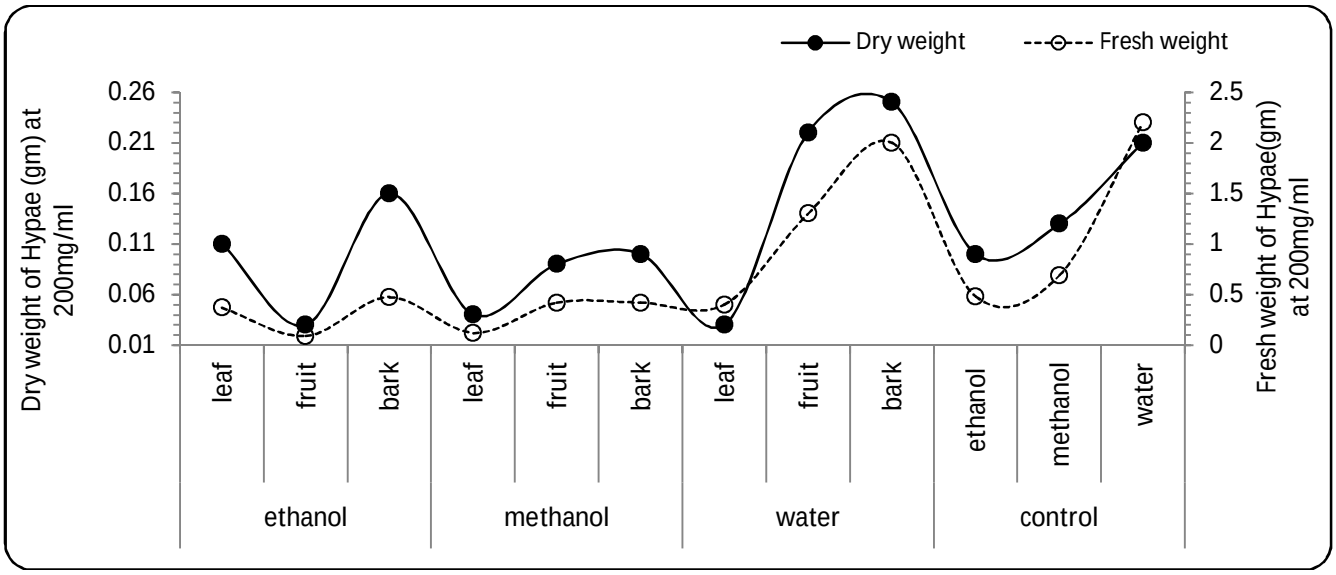


Figure 5. Relationship between *Eucalyptus* different parts extracts and fresh and dry biomass of *B. sorokiniana.*

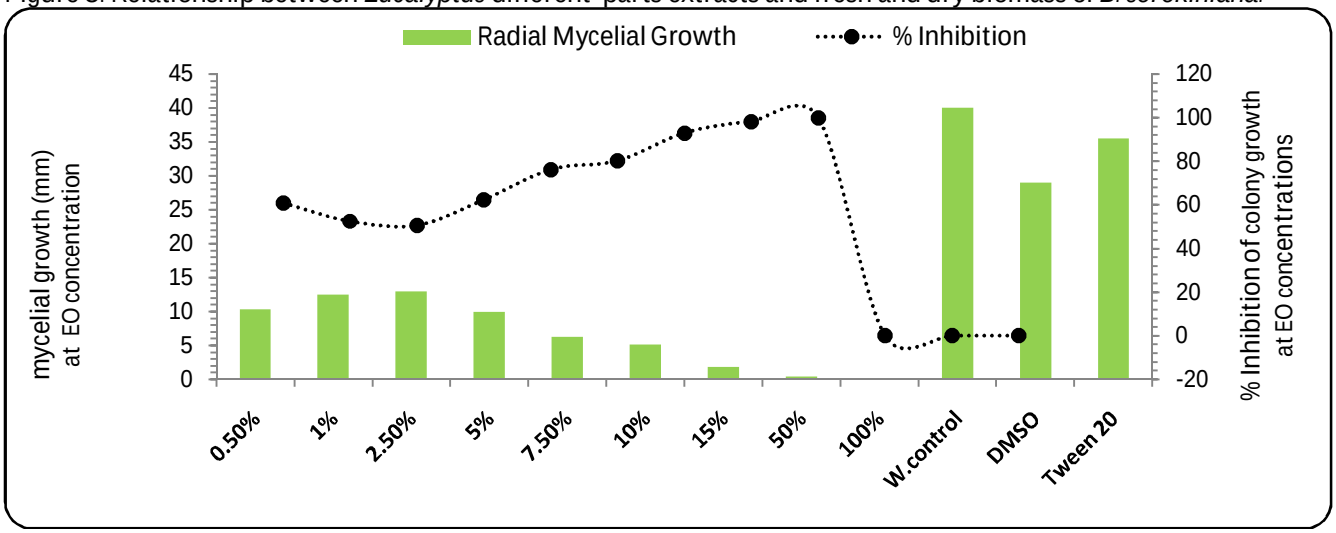


Figure 6.Relatioship between different *Eucalyptus* EO Concentrations and colony growth inhibition of *B. sorokiniana.*

RESULTS AND DISCUSSION

Effect of Eucalyptus extracts and E. oil on radial mycelial growth, mycelial growth inhibition, fresh weight and dry weight of *B. sorokiniana*at different concentrations: In the present study the inhibitory effects of Ethanol, Methanol and aqueous extracts of different parts of *E.camaldulensis* were evaluatedon colony growth of *B. sorokiniana* against Spot blotch causing pathogen of wheat crop. However, it was observed that ethanolic flowering buds extracts of *E. camaldulensis,* exhibited remarkable antifungal activity against *B. sorokiniana.* The inhibitory effect of different extracts of *Eucalyptus* spp. On colony growth of disease causing agents have been reported previously (Rakotonirainy and Lavedrine, 2005).

Colony diameter and percent inhibition was calculated. RE.oil and flower bud extracts produced complete inhibition of mycelial growth on day 5, and after 15 days of incubation the percent inhibition ranged from (50% to 98%) respectively. While, 100% EO produced complete inhibition after 30 days of incubation. That does not agree with the results of Katooli*et al*. (2014). According to their finding 50% and 100% of *E. camaldulensis* EO inhibited the growth of *B. sorokiniana*

only until 5 days and after 30 days of incubation no inhibition was noted.

Although the MIC (minimum inhibitory concentration) of aqueous flower buds extract against *B. sorokinian* awas higher (200 mg/ml) compared toethanolic, and methanolic flower buds extracts (08 mg/ml). Whereas, minimum fungicidal concentration (MFC) values for ethanolic and methanolic flowering buds extracts were 40 mg/mL as compared to water extract 300 mg/mL.EO produced best zone of inhibition of 90 mm diameter and mycelia growth 0.00±0.00 compared to control 40.00±0.00after 9 days of incubation at 50% and 100% concentrations .Flowering budsextracts of *E. camaldulensis* showed strong inhibition zone of 29.10±0.92 mm dia. that is significant values ($P < 0.05$) compared with water extract (19.80±0.33).

The mycelia growth inhibition and MIC studies revealed that ethanolic extracts of *Eucalyptus camaldulensis* flower buds are more potent than all other extracts in inhibiting the test organism. It was observed during present study that when the concentration of extracts in the growth medium was increased growth inhibition was significantly enhanced. Similar effects of a variety of other plants products that are effective against *B. sorokiniana* were reported by several authors (Hasan *et al,* 2012; Perello *et al,* 2013).

Effect of *Eucalyptus* extracts and EO on Sporulation rate and Conidia characteristics, fresh weight and dry weight of *B. sorokiniana*at different concentrations: Morphological characters of conidia and conidiophores i.e., colour, length, width and number of septa in each treatment were measured under the microscope, *B. sorokiniana* conidial variation in control treatments were around 20-90 micron in length and 12-30 micron in width having 2-7 number of septa, oval with round ends or elliptical shape and olivaceous brown to dark brown in color. E.oil flower buds extract affected conidia are very small and with only one or without any septa compared to control treatment.Hypae treated with EOand flowering buds ethanolic extract were collapsed, damaged or thinner compared to control. The conidia sizes of control treatments are similar as reported by Muchovej*et al.* (1988). They observed that *B. sorokiniana* conidia were more than 75 micron long and less than 25 micron wide. There were significant differences between different treatments that affected sporulation rate of *B. sorokiniana.* Treatments of *E. camaldulensis* flowering buds ethanolic extract and 50% EO have very poor sporulation rate however, no sporulation was observed for 100% EO treatment. The sporulation rate decreases as the concentration of treatments increases. Similarly there was no biomass production observed at 100 and 50% Essential oil treatments. However biomass production observed by ethanolic and methanolic flowering buds extracts was very poor.

## CONCLUSION

All treatment either *E.camaldulensis* leaf, bark, flowering buds extracts or EO were effective to inhibit the colony growth and spore germination of *B. sorokiniana*. But the ethanolic extract of flowering buds was found more potent in combating pathogen. From the economic point of view, treatments of EO and flowering buds extracts applied at 1% and 7.5% concentration controlled the pathogen up to 71% and 76% respectively, were more profitable compared to treatments of EO and flower buds extracts at 10% and 50% concentration that causing the management of pathogen up to 97% and 98%.

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