# *Ocimum basilicum* cures the wheat infection caused by *Bipolaris sorokiniana* by modulating the antioxidant enzymes

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## Abstract

*Bipolaris sorokiniana* (Sacc.) Shoem. is a notorious fungal pathogen that affects the wheat (*Triticum aestivum* L.) throughout the world. In this study, antifungal potential of *Ocimum basilicum* L. extract was investigated against this pathogen under both *in vitro* and green house conditions. Methanolic extract of *O. basilicum* showed significance effect and caused 100% inhibition in the mycelial growth of *B. sorokiniana* in well diffusion method. On the other hand, 100% methanolic extract significantly reduced the disease index by 97% in wheat seedlings by modulating the superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities. Furthermore, 100% concentration of methanolic extract also enhanced the dry biomass and 100-grain weight by 17% and 39%, respectively, as compared to control with pathogenic inoculation only.

**Keywords:** Antioxidant, *Bipolaris sorokiniana*, *Ocimum basilicum*, Wheat.

## Introduction

Wheat is one of the most important cereal crops of the world, serving as a staple food source of human diet around the globe (Hussein *et al*., 2023). Being a major food crop, it is cultivated on a large area and faces many production limiting factors including both biotic and abiotic stresses. Out of a total of 200 wheat diseases, 50 are major diseases that are not only widely spread but also cause significant economic losses (Al-Sadi, 2021). Overall, every year 20% of wheat losses occur due to fungal pathogens attacks (Singh *et al*., 2023). Among these, rusts, smuts and powdery mildew are utmost damaging and the most recognized diseases of wheat crop (Hussain *et al*., 2023).

*Bipolaris sorokiniana* (teleomorph: *Cochliobolous sativu*) is a fungal plant pathogen that causes leaf blotch or foliar blight and reduces the annual yield up to 44% every year and under favorable condition this loss may increase up to 100% in tropical and subtropical regions of South Asian countries (Sultana *et al*., 2018; Bibi *et al*., 2023). In Pakistan, this disease causes reduction of 56.6% in annual yield of wheat production (Iftikhar *et al*., 2008).

*Ocimum basilicum* commonly known as basil is widely used for extraction of its essential oil having strong antifungal and anti-inflammatory activities. Its oil is widely used in food as ascent and fragrance (Akpo *et al.,* 2023). Previously, it antifungal potential of *O. basilicum* has been reported under *in vitro* and *in vivo* study (Salami *et al*., 2024). As an example, 100% *O. basilicum* methanolic extract significantly inhibited the mycelial growth of *Sclerotium rolfsii* by 35% and reduced the disease incidence up to 60% in tomato seedlings (Nugroho *et al*., 2019).

Use of resistance cultivar and synthetic fungicides are the most reliable methods to control plant diseases in nowadays (Waqas *et al*., 2024). However, the use of fungicides has many ill effects on humans, animals as well as on soil microbial communities (Alkan *et al.,* 2022; Mkhtar *et al.,* 2023). Therefore, a dire need is to find out potent antifungal agents that are environment friendly (Javed *et al.,* 2021). Many recent studies have shown that natural compounds from plants can effectively control the highly problematic fungal pathogens such as *Macrophomina phaseolina* (Khan and Javaid, 2022), *Sclerotium rolfsii* (Ali *et al.,* 2020), *Fusarium oxysporum* (Naqvi *et al.,* 2022), *Rhizoctonia solani* (Rafiq *et al.,* 2022) and others. Previously, numerous reports are present to show the antifungal potential of *O. basilicum* but study regarding its antifungal activity against *B. sorokiniana* is missing both under *in* vitro and *in vivo* conditions. So, the objective of this study was to explore the antifungal potential of *O. basilicum* methanolic extract against this fungal pathogen under both *in vitro* and *in vivo* conditions.

## Materials and Methods

### Preparation of plant extract

*O. basilicum* dry leaves (200 g) were soaked in 500 mL of methanol for 7 days. After that, mixture was filtered with nelson cloth followed by

Whatman no. 41 filter paper.

**Preparation of fungal inoculum**

Fungal strain was subcultured on Potato dextrose agar (PDA) to prepare new colony for the preparation of fungal inoculum. Fungal inoculum and its desired concentration were prepared and adjusted by adopting the method described by Waqas *et al*. (2024).

***In vitro* fungicidal assay**

To check the antifungal activity of *O. basilicum* aqueous and methanolic extracts, potato dextrose broth (PDB) method was adopted as described by Waqas *et al*. (2018). For this purpose, 6 well plates were used. Five concentrations of each extract *viz.* 0, 25, 50, 75, and 100% of each extract were used. DMSO was kept as positive control.

**Pot trial**

To confirm the Kouch's postulates and antifungal potential of *O. basilicum,* a pot-based experiment was done by using completely randomized design. Seeds of wheat variety Galax-2013 were precured from market. Seeds were surface disinfected by using 1% sodium hypochlorite solution (NaOCl). Each pot with diameter 25 cm × 30 cm was filled with 10 kg of soil and 7 standard seeds were sown in each pot. After successful germination, 3 plants were retained through thinning process. In total, there were nine treatments *viz.* T1 = Control (C); T2 = *B. sorokiniana* ($2\times10^7$ CFU $mL^{-1}$) (BS); T3 = *O. basilicum* (75%) (OB1); T4 = *O. basilicum* (100%) (OB2); T5 = BS1+OB1 T6 = BS1+OB2 was assessed in this experiment each treatment has 6 replicates. Each plant received 10 mL of foliar spray of each treatment.

**Disease Index (DI)**

Disease index in wheat plant was calculated by the method described by Deng *et al*. (2022) and calculated by the formula given by (Kumari *et al*., 2023).

$$\text{DI (\%)} = \frac{\text{Sum of all disease rating}}{\text{Total no. of plants observed} \times \text{Maximum rating value}} \times 100$$

**Determination of antioxidant**

For enzyme extraction, fresh leaves of wheat were ground into poly-vinyl-polypyrrolidone (PVP) and 50 mM phosphate buffer (pH 7). The mixture was centrifuged by using ultra refrigerator centrifuge machine at 15000 rpm by adjusting the temperature at 4 ℃ for 5 min. The supernatant was used to determine the antioxidant superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities. SOD activity was evaluated by using NBT method (Beyer and Fridovich, 1987), CAT activity was determined by the procedure described by Aebi (1984) and POD activity was calculated by using the method given by Zhang *et al*. (2006).

**Yield parameters**

In yield parameters dry biomass of shoot and 100 grain weight were estimated as described by Akbar *et al*. (2023).

**Statistical analysis**

The statistical analysis was conducted using R version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria) with the assistance of packages including 'ggplot2' and 'dplyr' for data visualization and heatmap (Nguyen *et al*., 2024). Additionally, analysis of variance (ANOVA) was performed by using Minitab 21 software to compare means across multiple groups, followed by Tukey's post hoc test for pairwise comparisons and statistical significance was calculated by using a threshold of $P \leq 0.05$ (Ermergen and Taylan, 2024).

## Results and Discussion

Aqueous extract of *O. basilicum* did not show antifungal activity. Only 100% extract showed 13% reduction in mycelial growth as compared to control and DMSO. However, methanolic extract at 75% and 100% concentrations caused 77% and 100% mycelial growth inhibition, respectively (Fig. 1). Previous studies reported that *O. basilicum* ethyl acetate extract completely inhibited the spore germination of *Bipolaris hawaiensis* (Elsherbiny *et al*., 2017). Similarly, the extract reduced the radial growth of *Alternaria alternata* and *Bipolaris sorokinian* up to 80% in rice plant and also promoted the growth of plant (Sahu *et al*., 2020).

Foliar application of *B. sorokiniana* showed 87% disease index as compared to healthy control plants. Individual application of basil plant extract did not show any symptoms. However, combined treatment of extract and fungal inoculum showed 65% less disease index (DI) in 75% methanolic treated plants and 97% less DI in 100% methanolic foliar application treated plants (Fig. 2). Likewise, *O. basilicum* extract reduced the diseases intensity by 60% in tomato seedling caused by *Sclerotium rolfsii* (Nugroho *et al*., 2019). The presence of different bioactive compounds like phenol, alkaloids and flavonoids might be responsible for this antifungal potential (Sharaf *et al*., 2022).

The application of *B. sorokiniana* significantly enhanced the SOD, POD and CAT activities by 97, 78, and 69%, respectively as compared to control. On the other hand, foliar application of *O. basilicum* @ 100% concentration in methanol extract exhibited 63, 133, and 97% enhancement in SOD, POD and CAT activities as compared to control plant. Beside this, the combined application of *B. sorokiniana* + *O. basilicum* with both 75 and 100% concentrations showed remarkable increase in SOD activity by 12 and 30%, in POD activity by 49 and 73% and in CAT activity by 31 and 46% respectively, as compared to the plants received only *B. sorokiniana* inoculum foliar application (Fig. 3). Plant activates its defense mechanism when comes in contact with infectious agents. Reactive oxygen species are produced in favor of these infections and SOD is the first defense enzyme of plant that is activated and convert the hazardous ions to less toxic ions. After that POD and CAT enzymes also convert the toxic OH ions to less toxic ions and water (Hasanuzzaman *et al*., 2020).

Fungal treated plants showed remarkable reduction of 28% in dry biomass of wheat plants as compared to control healthy/untreated plants. However, the individual application of *O. basilicum* extract significantly enhanced the dry matter of wheat by 10 and 17% at 75 and 100% concentrations, respectively. On the other hand, *O. basilicum* extract with 75 and 100% concentrations together with *B. sorokiniana* synergistic treatments exhibited 19 and 39% increase in dry biomass of wheat as compared to control infected plants (Fig. 4 A). On the other hands, amalgamation of *B. sorokiniana* significantly reduced the 100-grain weight of wheat plant by 36 and 54% as compared to control group plants. While individual application of *O. basilicum* extract showed statistically non-significance results as compared to healthy plants. However, the synergistic treatment of both the fungus and basil extract significantly enhanced the 100-grain weight by 36% at 75% plant extract and 54% with 100% plant extract as compared to fungal inoculated plants (Fig. 4B).

Basil plants contain different bioactive compounds that enhance the plant growth promoting traits by modulating the growth hormones (Kosari *et al*., 2024). Beside this, due to presence of metabolites like flavonoidsx, reduce the fungal infection and make the metabolic activity to its normal route that also helps the plant to grow well under stressful condition (Dhama *et al*., 2023). Moreover, basil extract contains different kinds of nutrients like, nitrogen, phosphorus and potassium that play important roles as nutrients source for plant and also for soil microbiota that aid the plants to grow well (Song *et al*., 2024).

## Conclusion

The foliar application of *O. basilicum* not only reduced the disease intensity but also enhanced the yield of wheat. It was concluded that *O. basilicum* might contain various bioactive compounds that exhibited the promising antifungal potential against *B. sorokiniana*. These antifungal compounds need to be explored in future investigations.

## Author's contributions

HMW and MA got the concept, carried out research and analyzed the data. TK and SA collected the data. Data validation and methodology were carried out by MSI and KHB.

## Conflict of interests

Authors declare no conflict of interest.

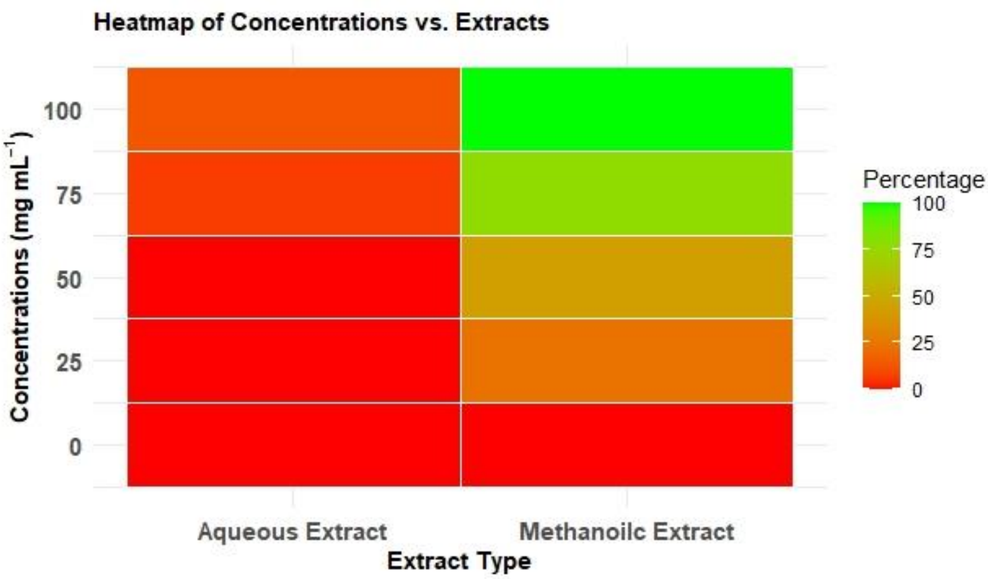


**Fig. 1:** Antifungal activity of aqueous and methanolic extracts of *Ocimum basilicum* against *Bipolaris sorokiniana*.

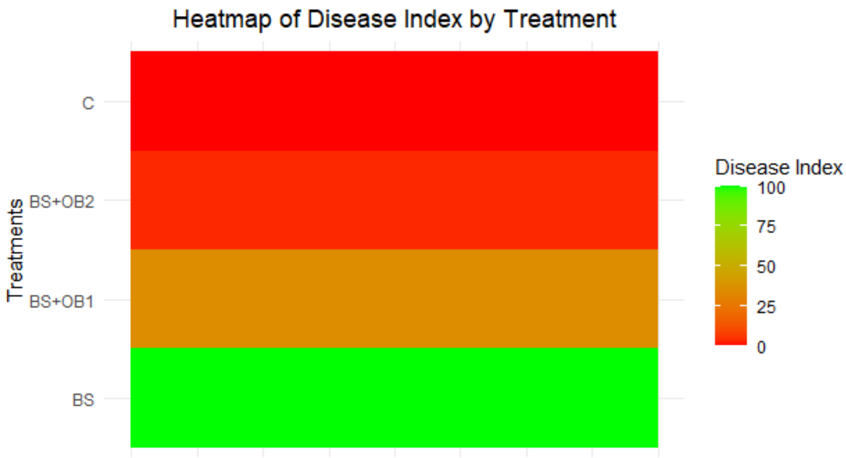


**Fig. 2:** Effect of different treatments of *Ocimum basilicum* on disease index of wheat inoculated with *Bipolaris sorokiniana*. Control (C); BS = *B. sorokiniana* ($2\times10^7$ CFU $mL^{-1}$); BS+OB1= *B. sorokiniana* + *O. basilicum.*

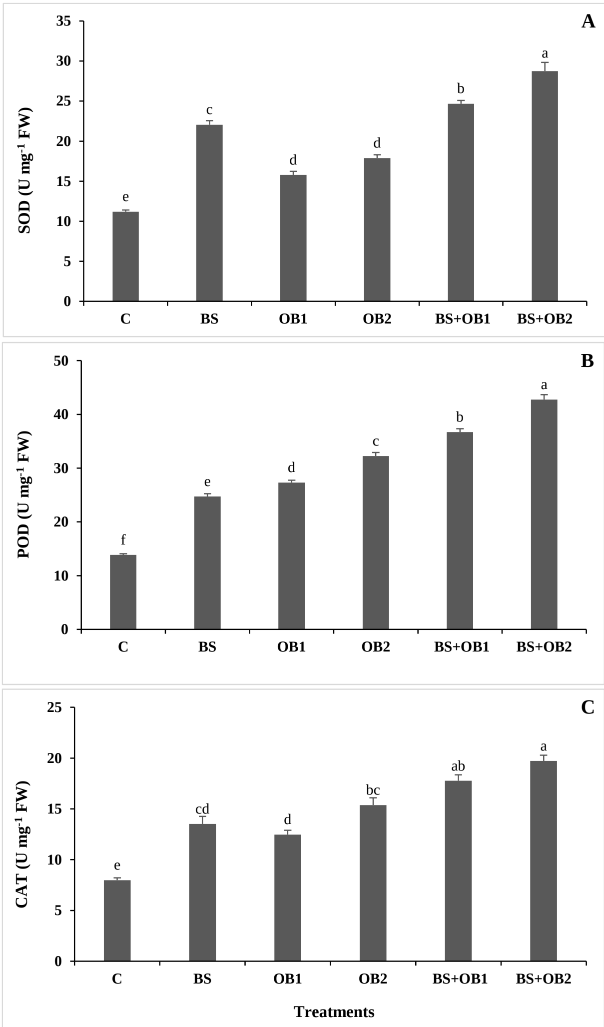


**Fig. 3:** Effect of different treatments of *O. basilicum* methanolic extract on activities of superoxide dismutase (**A**), peroxidase (**B**), and catalase (**C**) of wheat infected with *B. sorokiniana*. C = Control; BS = *B. sorokiniana* ($2\times10^7$ CFU $mL^{-1}$); OB1 = *O. basilicum* (75%); OB2 = *O. basilicum* (100%); BS+OB1 = *B. sorokiniana* + *O. basilicum* (75%): BS+OB2 = *B. sorokiniana* + *O. basilicum* (100%). Verticle bars show standard errors. Bars with different letters show significant difference.

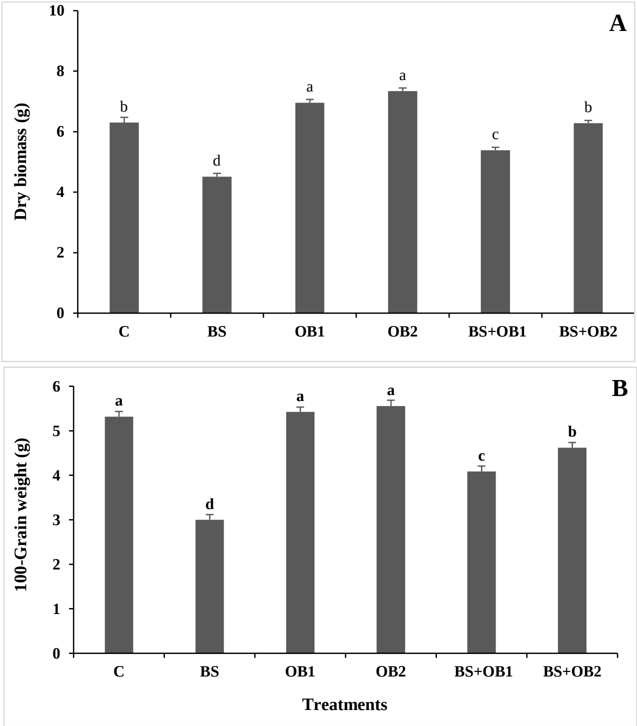


**Fig. 4:** Effect of different treatments of *O. basilicum* methanolic extract on dry biomass (**A**) and 100-grain weight (**B**) of wheat infected with *B. sorokiniana*. Verticle bars show standard errors. Bars with different letters show significant difference. For details of treatments, see Fig. 3.

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