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# MORPHOLOGICAL CHARACTERISTICS OF *Bipolaris sorokiniana* CAUSING SPOT BLOTCH OF WHEAT AND ITS IN VITRO MANAGEMENT USING DIFFERENT BOTANICALS

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## ABSTRACT

*Spot blotch disease of wheat caused by Bipolaris sorokiniana is one of the most concerning diseases of wheat, significantly constraining production over the last four decades. An estimated 25 million hectares of wheat grain are affected by spot blotch, particularly in humid and high-temperature regions. The study was conducted at Agriculture and Forestry University, Rampur, Chitwan, in 2023 with the objective to characterize the morphological traits of B. sorokiniana and manage the disease using botanicals under laboratory conditions. The research included a field survey of ten locations in Chitwan to identify the severity of spot blotch disease using double-digit scoring, morphological variations of B. sorokiniana conidia isolated from those locations, and in vitro management of the pathogen using botanical extracts (at 15 % concentration). The study revealed that the disease was most severe at Bijaynagar, Bharatpur-16, which recorded the highest number of spots per leaf (23) and the largest spot area (0.170 cm*$^2$*). Isolate of B. sorokiniana from Bijaynagar has highest radial mycelium growth (8.62 cm). Among nine botanical extracts, garlic clove and neem extract effectively inhibited pathogen mycelial growth by 63.22% and 61.15 %, respectively. The study helped to identify severely affected locations in the Chitwan district along with potential efficient management options of B. sorokiniana.*

Keywords:

*Bipolaris sorokiniana*, Botanical extracts, Disease severity, Morphological variability, Spot blotch disease

## 1. INTRODUCTION

Wheat occupies a third place after rice and maize in Nepal in terms of area under cultivation and production (MoALD, 2024). In Nepal, the majority of wheat cultivation is done in the Terai where the climate is warm and humid. As of 2022/23, the cultivated area, production, and productivity of wheat in Nepal were recorded at 697,762 hectares, 2,098,462 metric tons, and 3.01 tons per hectare, respectively (MoALD, 2024). Various diseases pose serious threats to wheat yields, among them is the foliar blight complex, being one of the most destructive fungal diseases in humid and high-temperature regions (Manandhar et al., 2016; Al-Sadi, 2021; Basnet et al., 2022). The fungus has a worldwide distribution and is aggressive under high relative humidity, elevated temperatures, and low soil fertility conditions prevalent in Nepal (Basnet et

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al., 2022). This pathogen is a causal agent of seedling blight, foliar blight/ spot blotch, common root rot, and black point of wheat, barley, and other small cereal grains and grasses (Al-Sadi, 2021). The pathogen can survive in infected crop residues and saprophytically on dead plant material (Tembo et al., 2018). Seedling infections often lead to blight, causing pre- or post-emergence seedling death (Manandhar et al., 2016; Al-Sadi, 2021). The disease becomes most severe when the crop's late post-anthesis stage coincides with periods of high relative humidity and elevated temperatures. Globally, spot blotch affects an estimated 25 million hectares of wheat fields (Gupta et al., 2022).

Considering the high yield loss due to this pathogen, knowledge of genetic diversity within the pathogen population is necessary for developing effective management strategies. *B. sorokiniana* exhibits high morphological and pathogenic variability, which complicates its identification and hampers effective disease management (Basak et al., 2024). Characterizing its morphological traits, such as colony color, growth habit, conidial size, shape, and septation, is important for accurate identification to develop effective management strategies for specific pathogen populations. Similarly, understanding the variability in disease severity in different locations can guide region-specific disease forecasting and management. In recent years, plant-based antifungal agents (botanicals) have garnered attention as eco-friendly alternatives due to their biodegradability, lower toxicity, and potential to inhibit a broad range of pathogens. However, there is limited information on the efficacy of these botanicals against *B. sorokiniana*. This study aims to investigate the morphological characteristics of *B. sorokiniana* isolated from different locations of Chitwan district, and to evaluate the potential botanical extracts for in vitro disease management.

## 2. MATERIALS AND METHODS

### 2.1 Field survey

A field survey was conducted in major wheat-growing regions of Chitwan, Nepal, during the wheat-growing season of 2023 to assess the prevalence and severity of spot blotch disease caused by *Bipolaris sorokiniana*. The survey covered a total of 10 randomly selected wheat fields of Chitwan (Table 1, Figure 1).

| Location | Ward Number |
|---|---|
| Meghauli | Bharatpur-28 |
| Jutpani | Kalika-11 |
| Patihani | Bharatpur-22 |
| Bhojad | Bharatpur-11 |
| Bhimnagar | Bharatpur-10 |
| Ratnagar | Ratnanagar-15 |
| Rampur | Bharatpur-15 |
| Shivaghat | Bharatpur-14 |
| Tandi | Ratnanagar-2 |

| Location | Ward Number |
|---|---|
| Bijaynagar | Bharatpur-16 |

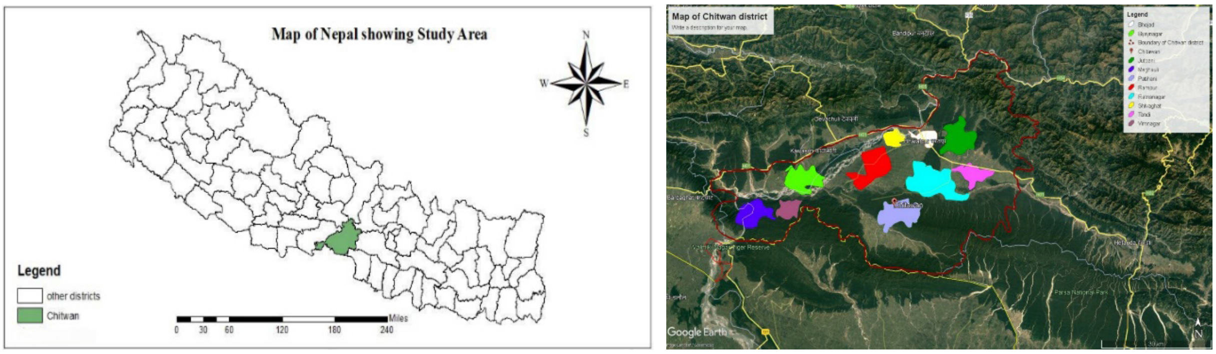


Figure 1. Map of the study site

*2.1.1 Disease scoring and severity assessment*

The double digits scale (00 to 99), developed by the modification of Saari & Prescott, 1975 was used to measures foliar infection based on two digit, where; the first digit (D1) indicates disease progress in canopy height from the ground level, and the second digit (D2) refers to severity of the disease based on diseased leaf area

Disease Severity (%) = (D1/9) × (D2/9) × 100

Where, D1 = First digit / height of infection

D2 = second digit / severity of infection

*2.1.2 Number of lesions*

The number of lesions was recorded from 10 penultimate leaves sampled from each location. The total number of spots per leaf was noted, and the average number of lesions was calculated for the sampled leaves.

*2.1.3 Measurement of the Area of spot*

The size of the necrotic spots was measured in centimeters using ImageJ software. The length and width of the lesions were recorded from 10 penultimate leaves sampled per field. For each leaf, the five largest spots were selected for measurement.

**2.2 Collection of Diseased Samples**

Wheat leaves exhibiting typical symptoms of spot blotch, such as dark brown lesions with chlorotic margins, were collected from surveyed wheat fields in Chitwan. The samples were placed in sterilized polyethylene bags, labeled, and transported to the laboratory for further study.

**2.3 Isolation and Identification of Pathogen**

Small sections (about 5 mm) were cut from the edges of the diseased leaf lesions. These sections were surface-sterilized by immersing them in 1% sodium hypochlorite solution for one minute, followed by rinsing three times with sterile distilled water. The sterilized sections were then placed on water agar plates and incubated at 25 ± 2°C for 7 days. Single spore culture was prepared by picking up single conidia of *B sorokiniana* from water agar plates and transferred to potato dextrose Agar (PDA) plates (Duveiller & Altamirano, 2000) and incubated at 25 ± 2 ºC. Emerging fungal colonies were sub-cultured to obtain pure cultures. Morphological characteristics of the isolates, including colony growth and color of 30 conidia, were observed and recorded after seven days of incubation.

**2.4 Evaluation of botanical extract against spot blotch**

*2.4.1 Preparation of Botanical Extracts*

Botanical extracts were prepared from locally available nine plants known for their antimicrobial properties, such as Neem *(Azadirachta indica)*, Mehendi (*Lawosina inermis*), Titepati (*Artemisia vulgaris*), Asuro (*Justicia adhatoda*), Abhijalo (*Drymaria diandra),* Bakaino (*Melia azedarach*), Jamun *(Syngium cumini*), Eucalyptus (*Eucalyptus globulus*), and Garlic (*Allium sativum*) (Figure 2). Fresh plant materials were washed, shade dried for three days, and ground into a paste. Extracts were obtained by homogenizing the paste with sterile distilled water (1:1 w/v) and filtering it through muslin cloth (Magar et al., 2020).


Figure 2. Preparation of botanical extract

*2.4.2 In vitro Efficacy of Botanical Extracts*

In order to evaluate the efficacy of botanical extracts against *B. sorokiniana* poisoned food technique was used. For this experiment, *B. sorokiniana* isolated from Bijayanagar was used. Botanical extracts at a concentration of 15% were added to the PDA medium and then poured into sterilized petri plates. The experiment was carried out in a completely randomized design (CRD). A 5 mm diameter mycelial plug from a 7-days-old pure culture was cut out with the help of cork borer and placed at the center of each plate. Plates without botanical extracts was used as a control treatment. All treatments were replicated three times, and the plates were incubated at 25 ± 2°C for 7 days. Colony growth diameter was measured, and the percentage inhibition of radial growth (PIRG) was calculated using the following formula:

$$PIRG = (C - T/C) \times 100$$

Where, C = Colony diameter in the control, T = Colony diameter in the treatment

**2.5 Statistical Analysis**

All the data recorded were entered in MS excel and *in vitro* data were subjected to analysis of variance (ANOVA), and mean differences were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance. Statistical analysis was performed using R studio.

## 3. RESULTS

**3.1 Field survey**

From the field survey, it was observed that the number of spots per leaf and disease scoring was found highest at Bijaynagar (23) and (77) respectively, while the number of spots and disease scoring was lowest at Bhimnagar (9.4) and (51) respectively (Table 2).

Table 2. Average number of spots per leaf and disease scoring of spot blotch disease at different locations in Chitwan, 2023

| Location | Average no of spots/leaf | Double digit scoring | Growth stage |
|---|---|---|---|
| Meghauli | 19.8 | 73 | Maturity |
| Patihani | 12.7 | 74 | Maturity |
| Bhimnagar | 9.4 | 51 | Dough |
| Rampur | 20.2 | 71 | Maturity |
| Tandi | 15.1 | 53 | Flowering |
| Jutpani | 20.2 | 76 | Maturity |
| Bhojad | 10.2 | 72 | Dough |
| Ratnanagar | 14.2 | 75 | Maturity |
| Shivaghat | 15.2 | 73 | Dough |
| Bijaynagar | 23 | 77 | Maturity |

Length of the spot was found to be highest on Bijaynagar (1.097 cm) and lowest on Tandi (0.554 cm). Breadth of spot was observed highest on Bhojad (0.164 cm) and lowest on Ratnanagar

(0.099 cm). Total area covered by spot was observed highest at Bijaynagar (0.170 $cm^2$) and lowest at Bhimnagar (0.056 $cm^2$) (Table 3).

Table 3. Average length, breadth, and area of spot blotch lesions at different locations in Chitwan, 2023

| Location | Spot length (cm) | Spot breadth (cm) | Spot area ($cm^2$) |
|---|---|---|---|
| Meghauli | 0.914 | 0.157 | 0.148 |
| Patihani | 0.855 | 0.153 | 0.131 |
| Bhimnagar | 0.557 | 0.101 | 0.056 |
| Rampur | 0.556 | 0.102 | 0.057 |
| Tandi | 0.554 | 0.107 | 0.059 |
| Jutpani | 0.724 | 0.119 | 0.086 |
| Bhojad | 0.937 | 0.164 | 0.154 |
| Ratnanagar | 0.596 | 0.099 | 0.059 |
| Shivaghat | 0.754 | 0.122 | 0.091 |
| Bijaynagar | 1.097 | 0.155 | 0.170 |

### 3.2 Morphology of *Bipolaris sorokiniana*

There was a difference in the morphology of isolated *B. sorokiniana* from different locations. The longest length of conidia was found in isolate of *B. sorokiniana* from Bhimnagar (21.083 µm) and the shortest from Bijaynagar (16.83 µm). While conidia breath was found to be almost the same for all places (Table 4).

Table 4. Average length and breadth of conidia of *B. sorokiniana* isolated from different locations of Chitwan, 2023

| *B. sorokiniana* Isolate | Conidia | |
|---|---|---|
| | Length (µm) | Breadth (µm) |
| Meghauli | 18.583 | 4.790 |
| Patihani | 20.333 | 4.750 |
| Bhimnagar | 21.083 | 5.000 |
| Rampur | 20.330 | 5.000 |
| Tandi | 19.167 | 5.000 |
| Jutpani | 19.333 | 5.000 |
| Bhojad | 21.000 | 5.000 |
| Ratnanagar | 20.750 | 5.000 |
| Shivaghat | 20.330 | 5.000 |
| Bijaynagar | 16.830 | 5.000 |

Four distinct cultural groups of *B. sorokiniana* were observed on PDA medium: Effuse Black Regular (EBR), Effuse Blackish White Regular (EBWR), Velvety Blackish White Regular (VBWR), and Effuse Whitish Regular (EWR) (Table 5 and Figure 3). The colonies varied in color, ranging from light brown to deep brown and dark brown to black.

Table 5. Cultural characteristics of *B. sorokiniana* in PDA isolated from different location of Chitwan, 2023

| Location | Cultural characteristics of *B. sorokiniana* |
|---|---|
| Meghauli | Effuse Black Regular (EBR) |
| Patihani | Effuse Blackish White Regular (EBWR) |
| Bhimnagar | Effuse Whitish Regular (EWR) |
| Rampur | Effuse Blackish White Regular (EBWR) |
| Tandi | Effuse Black Regular (EBR) |
| Jutpani | Effuse Black Regular (EBR) |
| Bhojad | Velvety Blackish White Regular (VBWR) |
| Ratnanagar | Effuse Whitish Regular (EWR) |
| Shivaghat | Effuse Black Regular (EBR) |
| Bijaynagar | Velvety Blackish White Regular (VBWR) |

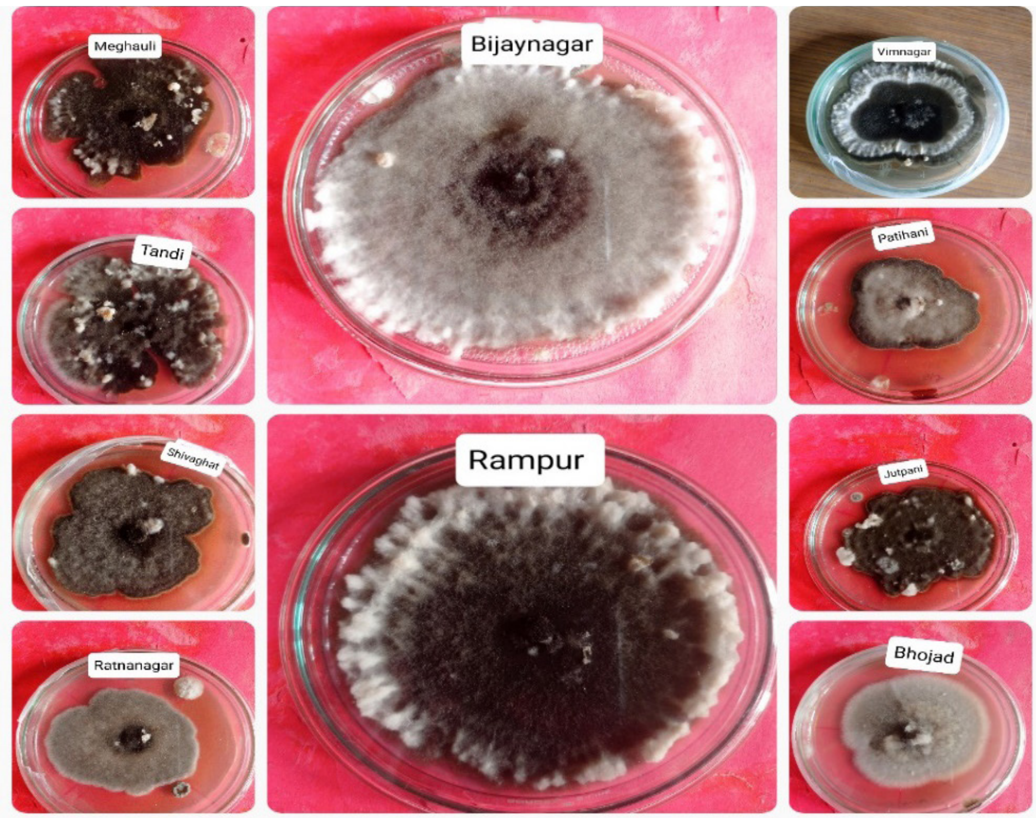


Figure 3. Cultural characteristics of *B. sorokiniana* of ten different places at Chitwan on 2023

### 3.3 Radial mycelium growth

A significant difference in the growth rate of different isolates of *B. sorokiniana* (Figure 4). The highest growth rate was observed in the Rampur isolate (1.62 cm) on the first day of culture. But from the second day highest mycelial growth was observed in the Bijaynagar isolate (2.82 cm), reaching 8.62 cm on the eighth day. The lowest growth on all days was observed in the Bhimnagar isolate, which achieved only 5.32 cm on the eighth day.

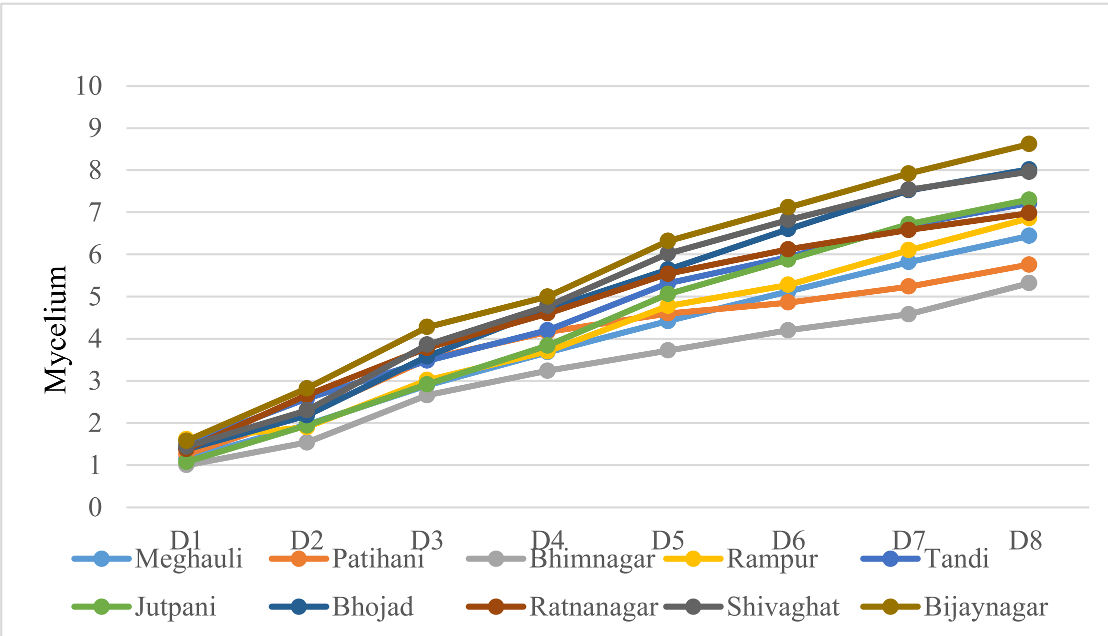


Figure 4. Radial mycelium growth of *B. sorokiniana* isolated from different location of Chitwan, 2023

### 3.4 Bioassay of botanicals against *Bipolaris sorokiniana*

The effectiveness of botanicals in inhibiting the mycelial growth of *B. sorokiniana* varied significantly. Garlic clove extract showed the highest inhibition percentage (63.22%) which was at par with neem (61.15%) and mehendi (57.93%) as shown in table 6. Garlic's efficacy can be attributed to allicin, which disintegrates cytoplasmic components and collapses fungal hyphae at a concentration of 15%, garlic clove extract completely inhibited mycelial growth (Figure 5).

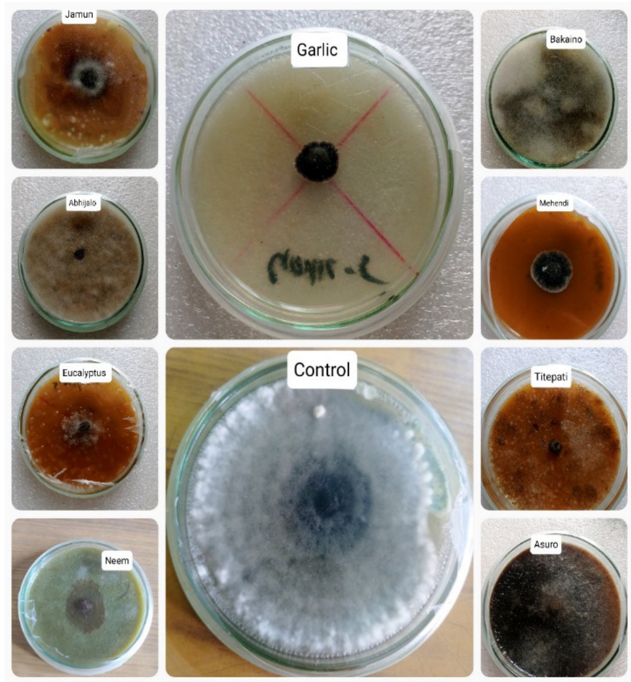


Figure 5. Radial mycelium growth of *B. sorokiniana* on PDA media containing different botanical extracts

Table 6. Effect of botanical extracts on percentage growth inhibition of the *Bipolaris sorokiniana* under the *in-vitro* conditions, 2023

| Botanical extracts/Concentration | Percentage growth inhibition over control |
|---|---|
| Asuro | $8.28^{d}$ (17.67) |
| Abhijalo | $11.03^{d}$ (18.47) |
| Bakaino | $2.07^{d}$ (12.21) |
| Eucalyptus | $47.82^{b}$ (43.55) |
| Garlic | $63.22^{a}$ (52.66) |
| Jamun | $27.36^{c}$ (30.89) |
| Mehendi | $57.93^{ab}$ (49.53) |
| Neem | $61.15^{ab}$ (51.37) |
| Titepati | $5.29^{d}$ (13.09) |
| Grand Mean | 32.16 |
| F value | *** |
| LSD | 8.36 |
| CV (%) | 20.28 |

Note: CV: Coefficient of variation, LSD: Least significant differences, mean followed by same letter in the column are not significantly different by Duncan's Multiple Range Test. *** significant at 0.1% $p<0.001$.

## 4. DISCUSSION

The field survey revealed significant variability in spot blotch severity across the surveyed locations, with Bijaynagar exhibiting the highest disease severity in terms of spot number, spot area and disease scoring. In Bijayanagar, local genotype of wheat was sown in a particular field and was at the maturity stage, this genotype might be susceptible to spot blotch. While lower spot blotch severity was observed in Bhimnagar, where variety Vijaya was sown in the field and was at the dough stage. However, varieties sown in other locations were unknown. The variations in spot blotch severity might be attributed to differences in local environmental conditions, such as temperature, humidity, and crop management practices, such as the use of resistant or susceptible varieties. Previous studies have demonstrated that warmer temperatures and high relative humidity favor the proliferation and spread of *B. sorokiniana* (Duveiller et al., 2005). This observation is in line with earlier studies that have reported increased disease susceptibility as the crop approaches physiological maturity (Duveiller et al., 2005). Hooi et al. (2023) and Chakraborty et al. (2024) identified host-pathogen interactions as major determinants of disease development in wheat-*B. sorokiniana* interactions. According to Bock et al. (2010), spot length and breadth are crucial indicators of disease severity as they reflect pathogen virulence and host susceptibility. The observed variability in spot dimensions suggests differences in pathogen aggressiveness across locations.

The isolates of *B. sorokiniana* exhibited notable variability in conidial morphology and cultural characteristics. Bhimnagar isolates had the longest conidia, while Bijaynagar isolates had the shortest. Such differences may indicate genetic diversity within *B. sorokiniana* populations. Variations in cultural traits like colony texture and color are consistent with findings by Devi et al. (2021), who observed colony color of dark brown to black and highlighted morphological diversity among *B. sorokiniana* isolates. Similarly, Mahto et al. (2012) have reported the similar observation of morphological variation among 48 isolates of Nepal and then grouped them into three categories (dark grey, light grey and white). Devi et al. (2021) also reported twelve different morphological groups of *B. sorokiniana* with conidial dimensions ranging from 125 to72 µm x 57 to25 µm (length x breadth). Singh et al (2021) and Aminuzzaman and Hossain (2005) also observed the color of pure culture to be light brown to deep brown. Significant differences in radial mycelial growth among isolates were observed highlighting the pathogenic variability of *B. sorokiniana*. The rapid growth observed in the Bijaynagar isolate might indicate a highly virulent strain. Growth trends observed over the incubation period are consistent with reports by Biswas & Das (2018) and Singh et al. (2021). The isolates having high growth rates show relatively high percent disease indices (PDIs) under controlled conditions. The rapid growth rate of *B. sorokiniana* enables them to invade host cell fast, accelerating infection and resulting in greater disease (Chakraborty et al., 2024).

The bioassay demonstrated the remarkable efficacy of garlic clove extract in inhibiting the growth of *B. sorokiniana*. This aligns with the findings of Magar et al. (2020). They observed that the highest mycelial growth inhibition percentage was recorded with the application of garlic clove extract (52.85%) at 15% concentration. The result is attributed to the inhibitory action of allicin, a compound with antifungal properties. The ability of garlic to disintegrate fungal cytoplasmic structures and cell walls underscores its potential as an eco-friendly alternative to chemical fungicides (Aala et al., 2014; Karnwal & Malik, 2024). Neem, eucalyptus, and mehendi extracts also exhibited inhibitory effects, although less potent than garlic, suggesting their supplementary role in integrated disease management strategies.

## 5. CONCLUSION

The study revealed significant variability in the occurrence, morphology, and cultural characteristics of *Bipolaris sorokiniana* isolated from different locations of Chitwan. Isolate Bijaynagar exhibited the highest disease severity, spot number, and radial mycelial growth, emerging as an important source for spot blotch infection. Morphological observations exhibited variation in conidial size and cultural characteristics of *B. sorokiniana*. Among the botanicals tested, garlic clove extract demonstrated the highest antifungal efficacy, providing a sustainable alternative for disease management.

## DECLARATION

The authors declare no conflict of interests.

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