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Research Article

Antifungal Assessment of Chemical and Biological Control Agents Against Major Seed-Borne Pathogens of Sesame

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ABSTRACT

Sesame (*Sesamum indicum* L.) is an important oilseed crop renowned for its nutritional, economic, and medicinal significance, particularly in tropical and subtropical regions. The aim of this research is to investigate the seed-borne fungal pathogens associated with sesame and evaluate the effectiveness of treatments under *in vitro* and *in vivo* conditions. The pathogens associated with the seeds were isolated by placing the infected seeds on the potato dextrose agar (PDA) media. The most frequent pathogens associated with seeds were *Aspergillus niger* and *Curvularia lunata*. These pathogens were later purified by using the hyphal tip and single-spore purification technique. Seed-associated pathogens were managed *in vitro* and *in vivo* with the help of chemical fungicides and bacterial BCAs. Statistical analyses were conducted using analysis of variance (ANOVA), a completely randomized design (CRD), and a randomized completely blocked design (RCBD). Under *in vitro* conditions, Amistar Top (Azoxystrobin + Difenoconazole) at 150 ppm proved efficient against *C. lunata* inhibited their growth up to 85% and Hombre (Imidacloprid + Tebuconazole) at 150 ppm against *A. niger* showed significant result up to 86% respectively. *Agrobacterium fabrum* showed significant antagonistic activity against both *C. lunata* and *A. niger*, Inhibited their growth up to 82% and 67% respectively. Under *in vivo* conditions, variety TH-6 was treated with most effective treatments identified in laboratory experiments. Amistar Top and Hombre, applied at 150 ppm under field conditions, resulted in 30% disease incidence against *C. lunata* and 32% against *A. niger* in comparison to untreated control with 80% disease incidence, respectively. *A. fabrum* applied against both pathogens, recorded 42% disease incidence against *C. lunata* and 45% against *A. niger*. These disease management strategies emphasize the use of standard concentrations and selective treatments for better results.

Keywords: *Sesamum indicum* L., *Aspergillus niger*, *Curvularia lunata*, seed-borne diseases, Amistar Top, Hombre, *Agrobacterium fabrum.*

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INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important oilseed crop cultivated both in the tropics and the temperate zones (Biabani and Pakniyat, 2008), mostly between altitudes of 40° N and 40° S. Sesame is an annual herbaceous species that belongs to the family *Pedaliaceae,* order *Tubiflorae*, and is grown for its flavor, oil, and edible seeds. The sesame plant is important in Nigerian agriculture due to the nutritional benefits of its leaves and the economic significance of its seeds (Muhamman and Gungula, 2008). Sesame is a source of edible oil (50%) and is enriched with protein (20%). It contains about 39% linolenic acid, 47% oleic acids (Shyu and Hwang, 2002), and 45%–55% fatty acid. Moreover, the essential minerals, including iron,

calcium, zinc, phosphorus, and magnesium are present in sesame seeds. Furthermore, they are abundant in vitamins E, B and they have high antioxidant properties (Langyan *et al*., 2022; Mostashari and Mousavi Khaneghah, 2024). It is a valuable oilseed crop, which is used in various sectors such as the food industry, agriculture, and cooking practices (Kabinda *et al*., 2022). Sesame seeds have a number of important health benefits, including those of lowering cholesterol, lowering blood pressure, anti-aging, prevent from cancer, and being antioxidant (Majdalawieh and Mansour, 2019; Mili *et al*., 2021).

In 2010, global sesame output was 3,840,000 mt from 7,800,000 ha of land. The largest sesame producer in 2013 was Burma (Myanmar), with India leading the world in sesame exports and Japan ranking first in sesame imports. Sesame has been grown worldwide on 9,398,770 ha of land. The yield was about 4.76 million tonnes (FAO, 2013), which increased from 1.12 million tonnes in the early 1960s (FAOSTAT, 2015). In Pakistan, the production of sesame increased by 34,000 tons in 2016 to 301 thousand tons in 2023. Production expanded markedly, as compared to 448 kg/ha in 2016; to 752 kg/ha in 2023, and this indicates an improvement in the efficiency of the farming techniques of sesame (FAO, 2024).

Various deteriorating microorganisms, notably fungi, have caused several problems such as poor germination, seed rot, and mortality in sesame production and storage, causing a serious risk to crop establishment and productivity (Mathur and Kabeere, 1975). These fungi can be carried by seeds either inside their tissues or externally on the surface of the seed (Nasira Altaf *et al.,* 2004). Numerous publications have documented the presence of *Aspergillus flavus* in sesame seeds along with other fungi (Mbah and Akueshi, 2001). Other fungi such as *Alternaria brassicola, Aspergillus niger, Aspergillus alba, Aspergillus viridus, Curvularia* spp*., Drechslera* spp*., Cephalosporium* spp*., Fusarium* spp*.,* and *Penicillium* spp. have also been isolated from the sesame (Nasira Altaf *et al.,* 2004) that have a serious impact on crops such as damping off, root rot, and wilting (Farhan *et al.,* 2010).

The major threat to sesame crops comes from *A. niger*, a pathogen responsible for serious damage and economic losses. This fungus effects plants and subsequently diminishes seed quality, thus impacting overall yield. It produces dark brown or black-colored spores, which are hyaline yellow; thus, these spores significantly reduce the market value. *A. niger* also cause rotting as well as leaf spots, which ultimately lead to defoliation and reduce plant quality and yield (Gautam *et al*., 2011). Numerous mycotoxins, such as fumonisin B12 and ochratoxin A, are known to be produced by *A. niger*. Seeds are contaminated by these mycotoxins during cultivation, harvest, or storage, causing serious economic losses and health hazards (Palencia *et al.,* 2010). Stem rot can cause plant death in extreme cases. Infected seeds reduce the seedlings vigor, which may appear discolored, distorted, and exhibit reduced germination rates. Additionally, when *A. niger* contaminates seed, it reduces the overall quality of the sesame oil produced from that seed, ultimately affecting the germination ability (Arun and Singh, 2023). *Curvularia* spp., particularly *C. lunata*, cause serious damage to sesame crops and produce different symptoms resulting in significant crop losses. The seeds of sesame crops in Burkina Faso were infected with this fungus and contamination rate had reached up to 76.39% (Ouali *et al*., 2023). Researchers discovered a toxin from *C. lunata* known as methyl 5-(hydroxymethyl) furan-2-carboxylate by using TLC and HPLC-MS techniques. It causes leaf spot disease, and a study shows that this pathogen is not host-specific (Liu *et al.,* 2009). *Curvularia* can produce symptoms like necrosis, chlorosis, and wilting, which reduce seed germination and affect seedling health. Discoloration and stunted growth exhibited by infected seedlings might impede development and decrease yield potential (Limtong *et al.,* 2020).

The objectives of this study were to investigate the morphological characteristics of the fungi abundantly associated with sesame seeds and to determine the *in vitro* and *in vivo* effectiveness of biological control agents (*Pseudomonas fluorescens, Bacillus megaterium*` and *Agrobacterium fabrum*) and fungicides (Score, Hombre, and Amistar Top) against the sesame-associated seed-borne fungi.

MATERIALS AND METHODS

Collection of Sesame Seeds

Seeds of the sesame were collected from different districts of Punjab, Pakistan (Faisalabad, Sheikhupura, and Toba Tek Singh). These seed samples were placed in the zipper bags and were brought to the Mycology and Biocontrol Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad.

Isolation, Purification, and Identification of Fungal Pathogens from Seed

The collected seed samples of sesame were washed with tap water to remove contamination. They were air-dried, surface sterilized with 70% ethanol, washed with sterilized water twice, and then again air-dried on blotter paper in the Laminar Air Flow Chamber. The Potato Dextrose Agar media (Potato Starch: 20 g, Dextrose: 20 g, Agar-Agar: 20 g,

and Distilled Water: up to 1000 mL) was prepared for the isolation of fungal pathogens from the seeds. The prepared media was sterilized at 121°C at 15 psi for 15 minutes along with the glassware used in the isolation. The PDA media was poured into the sterilized petri plates after adding the antibiotic at a lukewarm state (50 mg/L) to prevent the bacterial growth.

These treated seeds were placed on the PDA plates after solidification and wrapped with the parafilm. The plates were kept in the incubator for 24 hours to 48 hours to observe the fungal growth at 25±2°C. After the incubation time, the fungal growth on seeds was purified onto the new PDA plates by transferring mycelial growth and spores onto the plate with the help of sterilized inoculation needle. The inoculated fungal plate was again placed in the incubator for growth. This process was repeated to purify the fungal pathogens that appeared on the seeds. The pure fungal culture plate was identified on the basis of its morphological characters.

Microscopic Visualization of Fungal Pathogens

The microscopic examination of isolated fungi was done for confirmation of pathogens. The slides were prepared in a Laminar Air Flow Chamber using standard protocol for the temporary slides and examined under an advanced light microscope. The one-week-old cultures were selected after the morphological characterization of culture plates. A drop of distilled water was poured on the slide, and small colonies were taken from the plate and then mixed thoroughly on the slides to observe the size, shape, and structure of spores, as per standard literature (Wechter *et al*., 2007).

Pathogenicity

The isolated pathogens (*C. lunata* and *A. niger*) were confirmed by the pathogenicity of these two pathogens on the sesame plants. The sesame plant nursery was grown in the controlled conditions. As seedlings start their reproductive growth, they were inoculated with the pathogens (grown in potato dextrose broth separately) by spraying and drenching. Healthy sesame seedlings were inoculated with fungal spore suspension (1×10^6 spores mL^{-1}). Control plants were sprayed with sterile distilled water. Plants were maintained under humid conditions for disease development. The symptoms appeared on the plants after 10 days of inoculation, confirming the pathogens. Isolation was performed again from the inoculated plants to confirm Koch's postulates. Isolation from the infected part of the plant, *C. lunata* and *A. niger* were again isolated and confirmed by morphological characters and microscopy.

***In-vitro* Evaluation of Different Fungicides**

Using the poisoned food technique, three different fungicides (Score = difenoconazole, Hombre = imidacloprid + tebuconazole, and Amistar Top = azoxystrobin + difenoconazole) were tested at three different concentrations (50, 100, and 150 ppm) with 3 replicates by following Completely Randomized Design (CRD). The PDA media was inoculated with the fungicides at lukewarm condition and poured into plates as each concentration of fungicide was mixed separately in PDA media. The 5 mm mycelial discs from 1-week-old fungal cultures were placed in the center of each plate with the help of a sterilized cork borer and incubated for 7 days at 25 ± 2°C. Radial mycelial growth was recorded after 3 and 7 days. Percent Growth Inhibition (%) was calculated by using the formula:

$$\text{Percent Growth Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where C is the fungal growth in control and T is the fungal growth under treatment.

***In-vitro* Evaluation of Different BCAs**

The three identified bacterial strains (*Agrobacterium fabrum*, *Bacillus megaterium, and Pseudomonas fluorescens*) were collected from the Mycology and Biocontrol Laboratory, as they were preserved at -20 °C with the 80% glycerol preservation method. They were revived on Nutrient Agar (Beef Extract: 3g, NaCl: 5g, Glucose: 1g, Peptone: 5g, and Agar-Agar: 20g) and then multiplied in Nutrient Broth (Beef Extract: 3g, NaCl: 5g, Glucose: 1g, and Peptone: 5g) at 28 ± 2°C for 24 h. The bacterial suspension was adjusted to approximately 10^8 CFU mL^{-1} before evaluation. These multiplied bacterial strains were mixed in the media at lukewarm condition and poured into the plates. The inoculation of fungal discs procedure was repeated the same as mentioned in the fungicide evaluation with 3 replicates by following CRD. The radial mycelial growth was recorded on incubation at 28 ± 2°C after 3 and 7 days. PGI (%) was also calculated.

***In-vivo* management of sesame**

The most effective *in vitro* treatments were evaluated in the field against these two pathogens with 3 replications for their management and to reduce the losses. Amistar Top at 150 ppm was effective in controlling the *C. lunata*, Hombre at 150 ppm was effective in controlling the *A. niger,* and *A. fabrum* was effective in controlling both fungal pathogens. They were used for the management in the field, and the TH-6 variety was used to evaluate their efficacy under field conditions by using a Randomized Completely Blocked Design (RCBD). The bacterial suspension (10^8 CFU mL^{-1}) was applied as a seed treatment by soaking sesame seeds for 30 minutes before sowing. Additionally, 20 mL of bacterial

suspension was applied as a soil drench around seedlings at 7 days after emergence. Each treatment consisted of three replicates with 10 plants per replicate. The percent disease incidence (%) given by Vidhyasekaran and Muthimilan (1995) was recorded by using the disease rating scale mentioned in Table 1.

Table 1. Disease Rating Scale of Percent Disease Incidence (%).

Disease scale	Description	Disease Reaction
0	No incidence	Immune or Highly Resistant
1	1-10% incidence	Resistant
2	11-25% incidence	Moderately Resistant
3	26-50% incidence	Moderately Susceptible
4	51-70% incidence	Susceptible
5	More than 70% incidence	Highly Susceptible

$$\text{Percent Disease Incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Statistical Analysis

The collected data was statistically examined for ANOVA under a CRD and RCBD. The significance of results was assessed with a probability coefficient $P \leq 0.05$. The means were compared by using the least significant difference (LSD) test. This was done by using Statistix 8.1 software.

RESULTS

Morphological and Microscopic characterization of *C. lunata* and *A. niger*:

C. lunata forms olivaceous black cottony or velvety colonies on culture media, usually grey to dark brown, with septate, branched hyphae and curved, multiseptated conidia where the central cell is enlarged and darker. Conidia are produced on erect, geniculate conidiophores in a sympodial arrangement and often exhibit a visible hilum. *A. niger* develops fast-growing, black to dark brown colonies with a powdery texture and septate hyphae. Itproduces globose vesicles bearing metulae and phialides, forming chains of hyaline to dark conidia that give the surface a granular appearance, facilitating identification in culture. Microscopy of slides confirmed that the pathogens were *C. lunata* and *A. niger* on the basis of their colour, shape of spore, and fruiting body, as shown in Figure 1.

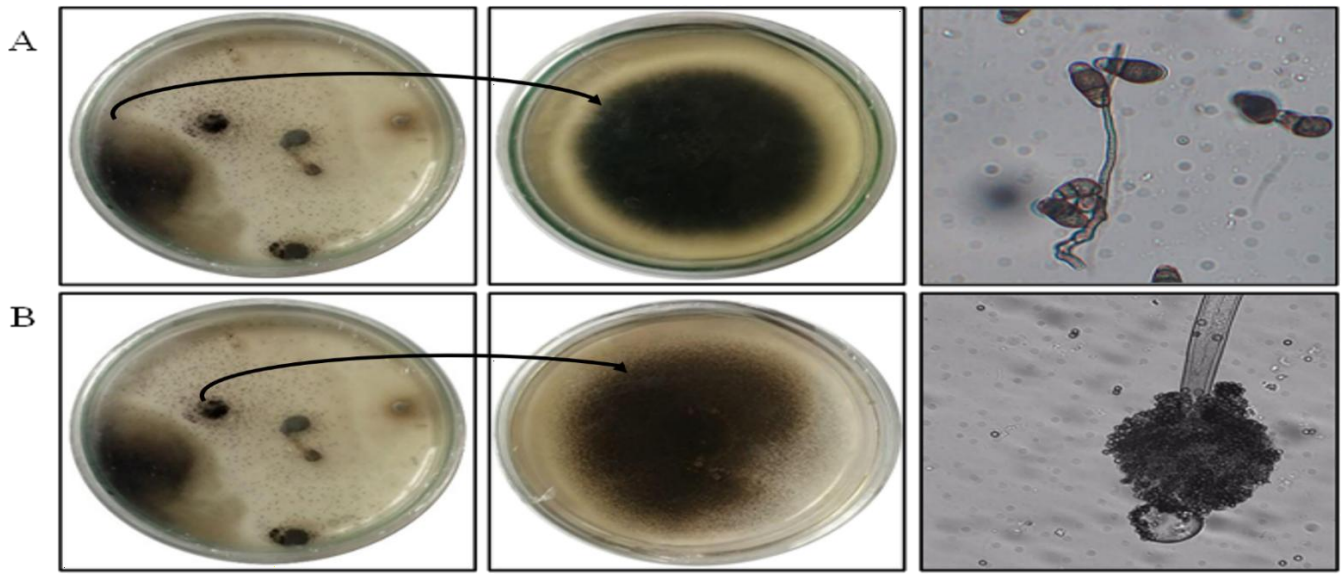

Figure 1. (A) Isolation, purification, and microscopy of *C. lunata* at 400X magnification. (B) Isolation, purification, and microscopy of *A. niger* at 400X magnification.

Effect of Fungicides on *C. lunata* Growth

The overall outcomes of the research proved the interactive influence of fungicide application, concentration, and incubation time (days) on the mycelial growth inhibition measured by the LSD All-Pairwise Comparisons Test. The combination of these three factors offered an in-depth insight into the effect of these factors on fungal development. Maximum mycelial growth was observed in the control group compared to all treatments and concentrations, with the values being equal to 3.2333 cm at 3 days and 7.3333 cm at 7 days, as shown in Table 2. Among the fungicide treatments, Amistar Top treatment suppressed mycelial growth the best at all concentrations. Amistar Top exhibited the lowest mycelial growth values at 150 ppm, 1.0 cm after 3 days and 1.1 cm after 7 days, respectively. This demonstrated strong fungicidal activity even with extended incubation time.

Score also showed good inhibitory action, as the values declined as the concentration of the chemical increased. The mycelial growth shown by Score at 150 ppm was 1.1333 cm after 3 days and 1.2667 cm after 7 days. It was only marginally more than Amistar Top. The performance of both treatments was better at higher concentrations and after a long incubation period, indicating prolonged inhibition. Hombre demonstrated moderate inhibitory activity, as it was more active than the control but not as active as Score and Amistar Top. It permitted 3.1667 cm of mycelial growth at 50 ppm after 7 days, showed an increase in mycelial growth as compared to the other fungicides, and fell within group B, significantly higher than other fungicides but lower than the untreated control. But as the concentration increased, growth was inhibited, with the least growth being experienced at 150 ppm (1.1 cm after 3 days and 1.6667 cm after 7 days). This confirmed that Hombre was dose-dependent but least effective than Score and Amistar Top.

Conclusively, the results supported the hypothesis by revealing that fungicide type, application concentration, and exposure time were all-important variables in terms of effective inhibition of mycelial growth. Amistar Top was the best fungicide, especially when used at high concentration, followed by Score, whereas Hombre exhibited intermediate activity. The high growth rate of the control group throughout confirmed the significance of fungicide application in controlling fungal pathogens. These observations qualify the suggestion of Amistar Top at 150 ppm (PGI (%) = 85) as an ideal treatment towards the management of fungal mycelial growth in equally experimental or agricultural scenarios, as shown in Figure 2.

Table 2. Efficacy of fungicides on *C. lunata* under *in vitro* conditions.

Treatments	Concentrations	3 Days		7 Days	
		Mycelial Growth (cm)	PGI (%)	Mycelial Growth (cm)	PGI (%)
Score	50 ppm	1.3667EF	57	1.5333DE	79
	100 ppm	1.2333FG	61	1.3333F	81
	150 ppm	1.1333GH	65	1.2667FG	82
Amistar Top	50 ppm	1.2667FG	60	1.3667EF	81
	100 ppm	1.1333GH	64	1.2333FG	83
	150 ppm	1H	69	1.1GH	85
Hombre	50 ppm	1.6333D	49	3.1667B	57
	100 ppm	1.3667EF	57	2C	72
	150 ppm	1.1GH	65	1.6667D	77
Control	50 ppm	3.2333B	0	7.3333A	0
LSD		0.1719			

Mean values sharing similar letters do not differ significantly

α=0.05

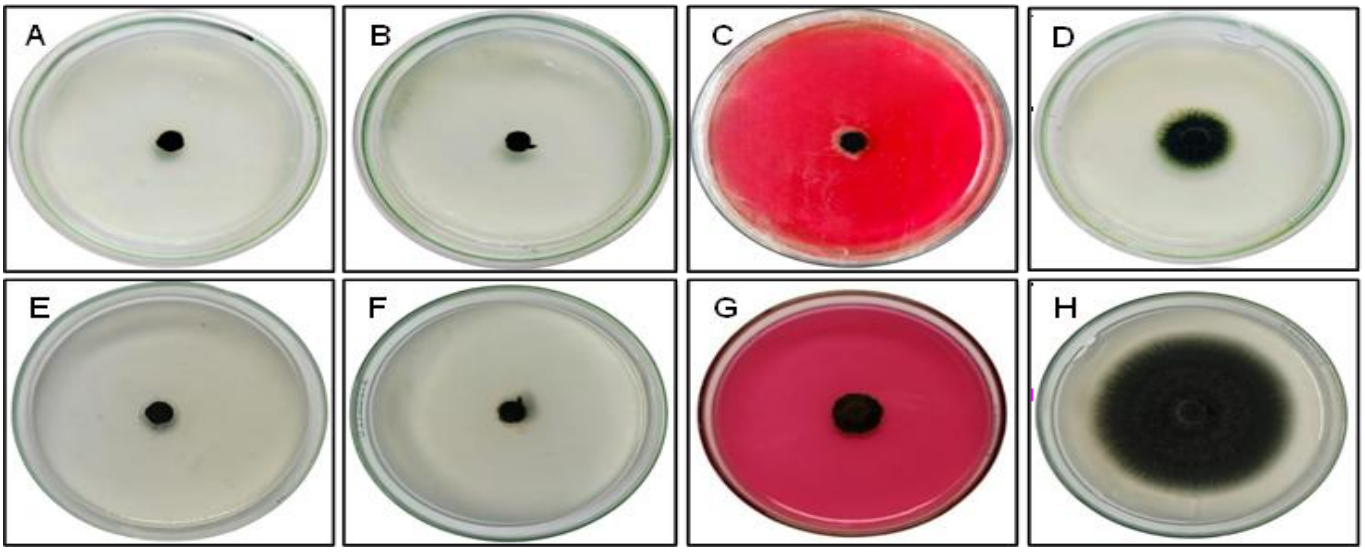

Figure 2. Pictorial demonstration of the impact of fungicide on *C. lunata* growth and their comparison with control. Score (A), Amistar Top (B), and Hombre (C) inhibited *C. lunata* growth at 150 ppm after 3 days. (D) represents the growth of *C. lunata* in the control group. Score (E), Amistar Top (F), and Hombre (G) inhibited *C. lunata* growth at 150 ppm after 7 days. (H) represents the growth of *C. lunata* in the control group.

Effect of Fungicides on *A. niger* Growth

The results showed that the growth of mycelium was greatly affected by the nature of treatment, fungicide concentration, and period of incubation. The control group, which showed no application of fungicide, had the highest mycelial growth at both 3 and 7 days, with the value of 3.3 cm at the 3rd day and 7.2 cm at the 7th day, regardless of concentration. All these statistical results falling under the letters A and B affirmed that without the chemical treatment, the fungal pathogen grew aggressively and uncontrollably. Hombre was the most effective fungicidal treatment on mycelial growth suppression. It also registered the lowest growth at both time intervals at 150 ppm with 0.9333 cm and 1.0 cm in 3 days and 7 days, respectively. At the reduced concentrations, Hombre still demonstrated significant inhibition: 1.1333 cm (3 days) and 1.2333 cm (7 days) at 100 ppm, and 1.3333 cm and 1.5333 cm at 50 ppm, proving that its efficacy was dosage-dependent yet remained very high, as mentioned in Table 3.

Score was also efficient in inhibiting fungal growth, though it had a bit lower performance compared to Hombre. Score at 150 ppm inhibited growth to 1.0667 cm and 1.4667 cm after 3 and 7 days, respectively, and this was already in good statistical groupings of “JK” and “GH.” At 100 ppm, the growth was marginally more, 1.2333 cm and 1.7333 cm, and at 50 ppm, it was further, 1.5667 cm and 2.0333 cm. As expected, the higher the concentration, the better the inhibition. Amistar Top, in contrast, demonstrated moderate activity, as the values of mycelial growth were significantly higher compared to Hombre and Score. At 150 ppm, growth was 1.3333 cm and 1.6333 cm after 3 and 7 days, respectively, which rose to 1.6667 cm and 2.3667 cm at 100 ppm and finally 1.9333 cm and 2.3667 cm at 50 ppm. These values showed that Amistar Top was the least active fungicide among the three, but it also produced a significant reduction in growth as compared to the control.

Overall, the findings of the experiment were categorical, as it was evident that fungicide treatments could inhibit mycelial growth, with Hombre at 150 ppm (PGI (%) = 86) being the most effective, followed by Score, and then Amistar Top. Also, there was an increase in fungicidal activity with a rise in concentration, and mycelial growth was mostly greater at 7 days than at 3 days, as a longer incubation time was anticipated. These results highlighted the need to employ the correct fungicide at the correct proportions in order to control the fungal pathogens, as shown in Figure 3.

Table 3. Efficacy of fungicides on the *A. niger* under *in vitro* conditions.

Treatments	Concentrations	3 Days		7 Days	
		Mycelial Growth (cm)	PGI (%)	Mycelial Growth (cm)	PGI (%)
Score	50 ppm	1.5667FG	52	2.0333D	71
	100 ppm	1.2333IJ	62	1.7333EF	75
	150 ppm	1.0667JK	67	1.4667GH	79
Amistar Top	50 ppm	1.9333DE	41	2.3667C	67

	100 ppm	1.6667FG	49	1.9DE	73
	150 ppm	1.3333HI	59	1.6333FG	77
Hombre	50 ppm	1.3333HI	59	1.5333FGH	78
	100 ppm	1.1333IJK	65	1.2333IJ	82
	150 ppm	0.9333K	71	1K	86
Control		3.3B	0	7.2A	0
LSD	0.2056				

Mean values sharing similar letters do not differ significantly
α=0.05

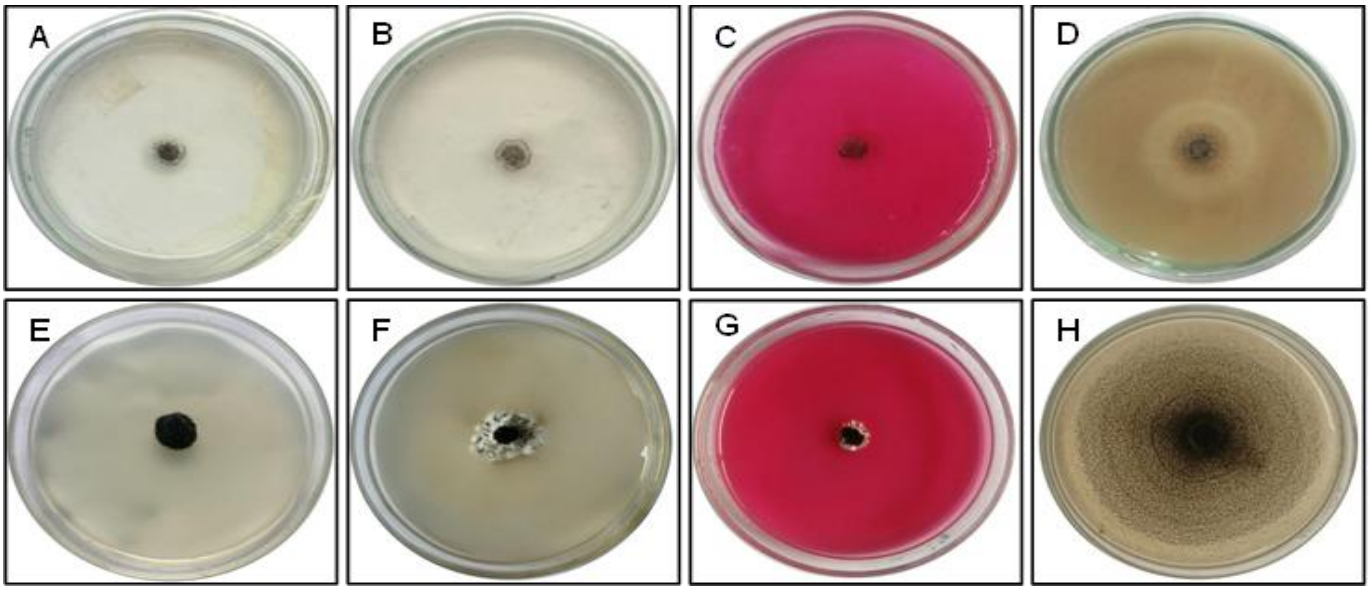

Figure 3. Pictorial demonstration of the impact of fungicide on *A. niger* growth and their comparison with control. Score (A), Amistar Top (B), and Hombre (C) inhibited *A. niger* growth at 150 ppm after 3 days. (D) represents the growth of *A. niger* in the control group. Score (E), Amistar Top (F), and Hombre (G) inhibited *A. niger* growth at 150 ppm after 7 days. (H) represents the growth of *A. niger* in the control group.

Effect of BCAs on *C. lunata* Growth

The data indicated that the kind of bacterial treatment and the days of incubation influenced the mycelial growth of the fungal pathogen. The control group, which was not subjected to any kind of bacterial treatment, showed the most mycelial growth at both 3 and 7 days after inoculation, at 3.2333 cm and 7.2333 cm. This result showed that without any biocontrol measures, the fungal pathogen could proliferate and expand very fast. *A. fabrum* showed the best inhibitory effect among the bacterial treatments, with the fungal growth being 1.1 cm and 1.2667 cm after 3 and 7 days, respectively.

These findings demonstrated the constant and excellent antifungal potential of *A. fabrum* over time. On the same note, *P. fluorescens* inhibited mycelial growth with a value of 1.2667 cm after 3 days and 2.2 cm after 7 days, which also fell in lower statistical categories, depicting moderate to strong antagonistic action. In comparison, *B. megaterium* permitted significantly more growth of the fungus, 2.4 cm after 3 days and 3.5 cm after 7 days, respectively. While this treatment did exhibit some degree of inhibition in comparison to the control, it was comparatively ineffective than *A. fabrum* and *P. fluorescens,* especially as the incubation time was increased, as mentioned in Table 4 and shown in Figure 4.

In conclusion, the experiment showed that the biocontrol agents had different suppression activity on the growth of the fungi, with *A. fabrum* (PGI (%) = 82) being the best, followed by *P. fluorescens and* then *B. megaterium*. Moreover, the extent of mycelial growth increased with time in all the treatments, which underlines the significance of timely and efficacious biocontrol intervention in effective management of fungal pathogens.

Table 4. Efficacy of BCAs on *C. lunata* under *in vitro* conditions

Treatments	3 Days		7 Days	
	Mycelial Growth (cm)	PGI (%)	Mycelial Growth (cm)	PGI (%)
A. fabrum	1.1F	65	1.2667F	82
B. megaterium	2.4D	26	3.5B	51
P. fluorescens	1.2667F	61	2.2E	69
Control	3.2333C	0	7.2333A	0
LSD	0.2022			

Mean values sharing similar letters do not differ significantly
α=0.05

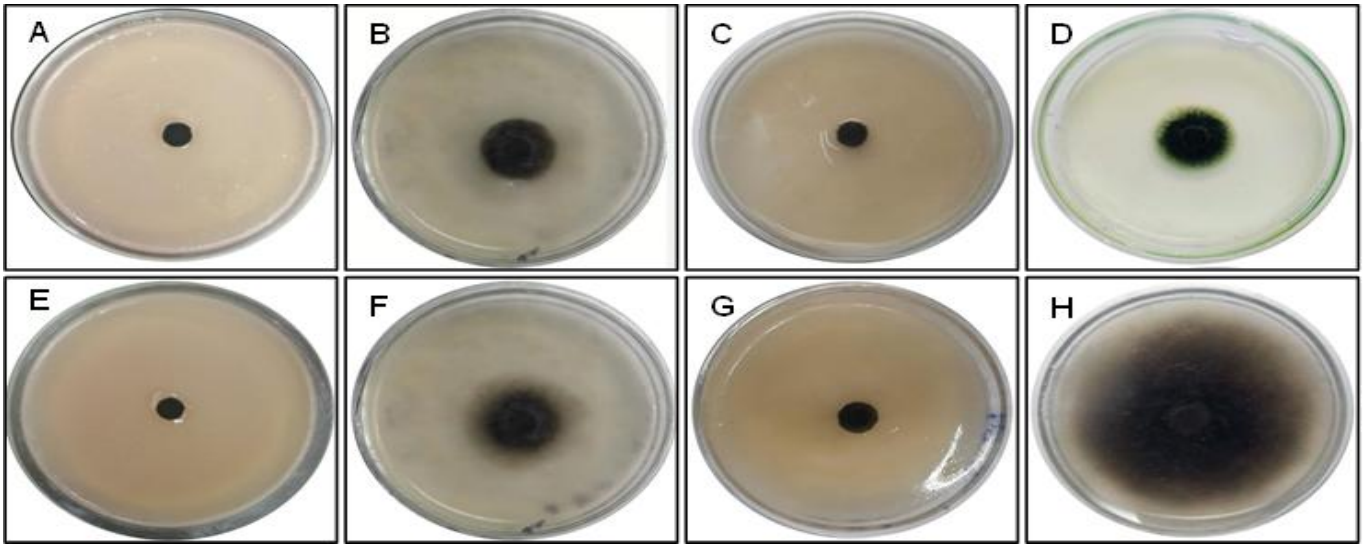

Figure 4. Pictorial demonstration of the impact of BCAs on *C. lunata* growth and their comparison with control. (A) *A. fabrum*, (B) *B. megaterium*, and (C) *P. fluorescens* inhibited *C. lunata* growth at 150 ppm after 3 days. (D) represents the growth of *C. lunata* in the control group. (E) *A. fabrum*, (F) *B. megaterium*, and (G) *P. fluorescens* inhibited *C. lunata* growth at 150 ppm after 7 days. (H) represents the growth of *C. lunata* in the control group.

Effect of BCAs on *A. niger* Growth

The results firmly indicated that the kind of bacterial treatment, as well as the period of incubation, had a great effect on the mycelial growth of the fungal pathogen. During the two observation periods, that is, after 3 and 7 days, the treatments exhibited specific differences in the growth patterns. At 3 days, the control group that was untreated with any bacteria had the highest mycelial growth of 3.2667 cm, demonstrating that the fungal growth is fast in the absence of the biocontrol agents. Conversely, the bacterial inoculations highly inhibited the growth. *B. megaterium,* limiting mycelial growth to 1.7667 cm, whereas *P. fluorescens* and *A. fabrum* restrained growth even more to 1.4667 cm and 1.4 cm, respectively, indicating a high antifungal activity, especially during the initial growth period as mentioned in Table 5.

At 7 days, the fungal growth increased in all the treatments, though the differences were still clear. Highest growth was observed in the control group with 7.1333 cm, proving that in the absence of any treatment, the fungus still grew aggressively over time. In the case of the bacterial treatments, *B. megaterium* exhibited the greatest mycelial growth of 3.0 cm, whereas *P. fluorescens* showed 2.7333 cm and *A. fabrum* 2.3333 cm. The trend indicated that *A. fabrum* remained the most effective treatment even on the 7th day, whereas *B. megaterium* was the least effective of the biocontrol agents over the days shown in Figure 5.

In a summary, it can be observed that *A. fabrum* exhibited a consistently high inhibitory effect on the fungal pathogen at day 3 and 7, followed by *P. fluorescens* (PGI (%) = 67)*,* whereas *B. megaterium* exhibited moderate inhibition. The control always promoted the greatest fungal growth as a validation that the biocontrol treatments were effective. These results showed the promise of employing certain bacterial agents, especially *A. fabrum,* as biological control measures against fungal pathogens.

Table 5. Efficacy of BCAs on *A. niger* growth under *in vitro* conditions

Treatments	3 Days		7 Days	
	Mycelial Growth (cm)	PGI (%)	Mycelial Growth (cm)	PGI (%)
A. fabrum	1.4G	57	2.3333E	67
B. megaterium	1.7667F	46	3C	58
P. fluorescens	1.4667G	55	2.7333D	61
Control	3.2667B	0	7.1333A	0
LSD	0.2564			

Mean values sharing similar letters do not differ significantly
α=0.05

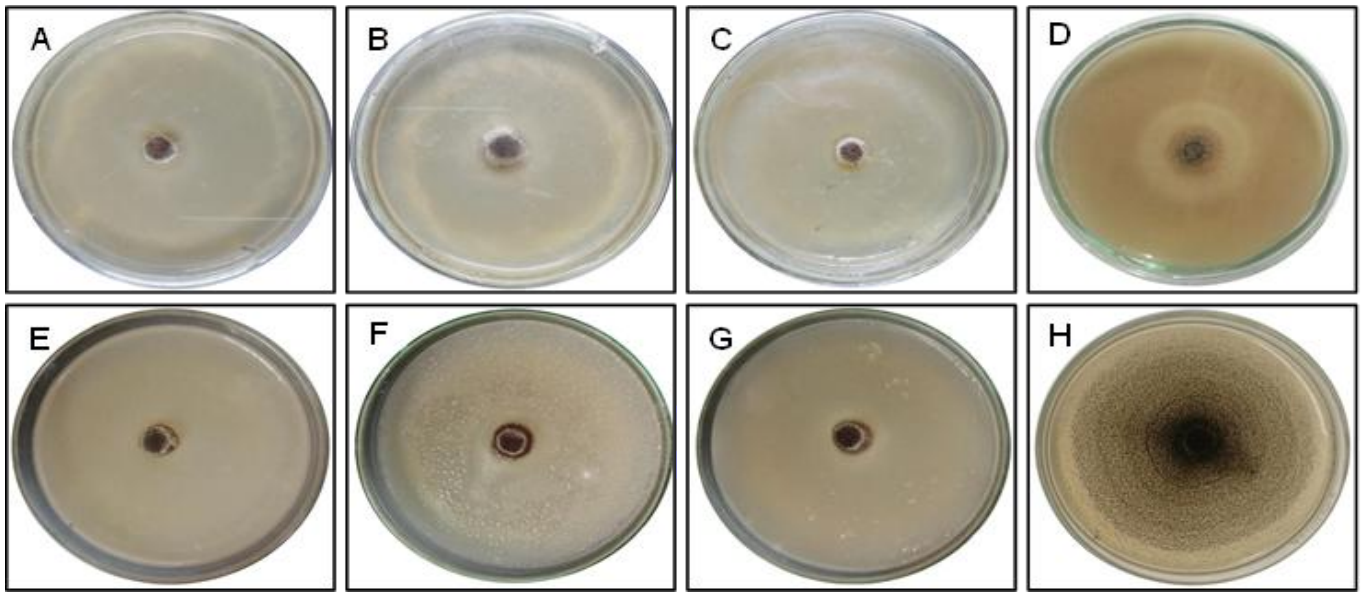

Figure 5. Pictorial demonstration of BCAs on *A. niger* growth and their comparison with control. (A) *A. fabrum*, (B) *B. megaterium*, and (C) *P. fluorescens* inhibited *A. niger* growth at 150 ppm after 3 days. (D) represents the growth of *A. niger* in the control group. (E) *A. fabrum*, (F) *B. megaterium*, and (G) *P. fluorescens* inhibited *A. niger* growth at 150 ppm after 7 days. (H) represents the growth of *A. niger* in the control group.

***In-vivo* Management of Sesame**

The results showed different treatments effected the fungal disease incidence in the sesame variety TH-6. In the control plants the disease incidence reached 80%, which indicates that the crop is susceptible when no protective action is taken, under favorable conditions. The treatment that showed the best result among all the treatments was Amistar Top, at 150 ppm applied against *C. lunata*, which showed the disease incidence up to 30%. A very similar response was obtained with Hombre, at a rate of 150 ppm against *A. niger*, which showed a disease incidence up to 32%. These fungicides represented the most effective suppression of disease, which showed the lowest disease incidence among treatments, as mentioned in Table 6.

In terms of a biological treatment with *A. fabrum*, the level of control was much more moderate. For treatment against *C. lunata* with the biocontrol agent, the disease incidence recorded was 42%, and against *A. niger,* the incidence was 45%. Although these values are not as low as the two the other two fungicides showed, but they are still effective compared to the untreated control, which shows that *A. fabrum* has potential as a biocontrol agent.

From this, it is clear that chemical fungicides gave the greatest level of disease suppression, and biological treatment was in-between chemical fungicides and no control treatment (and no control treatment showed the greatest development of disease). This demonstrates the importance of identifying appropriate management options depending on the level required, cost, and environmental factors.

Table 6. Efficacy of effective treatments on susceptible variety.

Treatments	Disease Incidence (%)
	TH-6
Amistar Top 150 ppm (*C. lunata*)	30
Hombre 150 ppm (*A. niger*)	32
A. fabrum (*C. lunata*)	42
A. fabrum (*A. niger*)	45
Control	80

Numerical values are in percent disease incidence.

DISCUSSION

The observed inhibitory trends indicate a clear dose-dependent antifungal response, with fungicide efficacy varying according to fungal species and treatment type. Score is a triazole fungicide that is systemic and has the active component as difenoconazole. It inhibits the fungal enzyme lanosterol 14α-demethylase (CYP51), required in the biosynthesis of ergosterol, an essential constituent of fungal cell membranes. Difenoconazole disturbs the synthesis of ergosterol, inducing instability in the membrane, a consequence that causes leakage of cell contents, loss of cell division, and death of fungi (Li *et al*., 2025).

Amistar Top is a broad-spectrum strobilurin fungicide that contains azoxystrobin and difenoconazole. It acts at the fungal mitochondrial cytochrome b complex (Qo site), which inhibits electron transmission and ATP synthesis. This disturbs the energy metabolism, preventing germination of spores, mycelial growth, and sporulation (Bartlett *et al*., 2002). Azoxystrobin is effective against various crop diseases. However, the risk of antimicrobial resistance, especially with single active ingredient fungicides, has been reported. Combining multiple active ingredients, such as azoxystrobin and difenoconazole, is a promising solution because pathogens find it harder to develop resistance (Hu, 2019). Studies have shown that these fungicides possess strong preventive, eradicative, therapeutic, translaminar, and systematic properties, either individually or in combined form (Marczewska *et al*., 2020). The commercial fungicide Othello®, which contains both compounds, effectively suppressed *B. bicolar*, *Colletotrichum* spp., *Curvularia* spp., and *Neopestalotiopsis* spp., which affect oil palm and coconut trees. This fungicide remains active for 28 days and shows minimal risk of resistance development even at low concentrations (Obeng *et al*., 2025).

Hombre is a mixture of seed treatment product, imidacloprid (insecticide), and tebuconazole (triazole fungicide). The fungicidal active ingredient is tebuconazole, which acts by inhibiting the lanosterol 14 alpha-demethylase and interferes with ergosterol biosynthesis, just like difenoconazole (Kovač, 2023). The study conducted in 2019 demonstrated that, among the tested systemic fungicides, tebuconazole was the most effective, providing maximum mean percent inhibition of colony growth, followed by azoxystrobin. Previous studies have also reported strong antifungal activity of tebuconazole against *A. niger* across a range of concentrations (Sekhon *et al*., 2019).

P. fluorescens synthesis antifungal metabolites, phenazines (phenazine-1-carboxamide), 2,4-diacetylphloroglucinol (DAPG), and hydrogen cyanide (HCN), which interfere with fungal cell membranes and metabolism. VOCs (HCN) suppress the germination of the spores, and cyclic polyketides (viscosinamide) kill the cells. The siderophores compete with fungi to gain access to iron, and the speedy colonization of roots increases the nutrient competition. Recent studies have shown that the five strains exhibit varying levels of antifungal activity, with the highest concentrations of *Pseudomonas* strains capable of inhibiting the growth of pathogenic microorganisms. At 5,000 µg/mL, all of the *Pseudomonas* strains (A-5, C-03, CRM-3, L-5, and Pf4-1) exhibited high resistance and the highest percentage of inhibition (81% to 100%) against the fungi *Alternaria cajani* and *C. lunata* (Shrivastava, 2008).

Agrobacterium fabrum and other rhizosphere bacteria deploy antifungal strategies by producing volatile organic compounds (dimethyl disulfide) that can disrupt fungal cell membranes and interfere with spore germination, and by synthesizing high-affinity siderophores that compete with pathogens for iron, limiting their growth and virulence (microbial VOC mechanisms in antagonistic interactions) (Almeida *et al*., 2023).

Antifungal lipopeptides (surfactin, iturin) produced by *B. megaterium* interfere with fungal cell membranes of fungi as well as VOCs (5-methyl-2-phenyl-1H-indole), which suppress spore germination and mycelial growth. It also releases chitinases and beta 1-3 glucanases, which destroy fungal cell walls, and siderophores, which restrict the access of iron. *In vivo* application reduced the fungal growth (Santos *et al*., 2014; Mannaa and Kim, 2018). The role of integrated biological approaches in suppressing fungal pathogens has also been demonstrated previously, where the combined

application of antagonistic microorganisms significantly reduced disease severity and enhanced plant growth under sustainable disease management systems (Numaad *et al*., 2025).

The data presented by research can be applied in the treatment of crop diseases like fungal diseases in sesame or even cotton and maize, where *C. lunata* infects the leaves by causing chlorosis, leaf spot, and necrosis. *A. niger* causes seed rot and damping-off as well as production of mycotoxin (ochratoxin A), resulting in yield and economic loss (Agrios, 2005; Aiswarya *et al*., 2017). The results from the study further justify the use of the integrated pest management approach that uses chemical fungicides with biological control organisms for sustainable disease management. This combined strategy significantly decreases dependence upon synthetic fungicides, reduces the environmental impact of synthetic chemicals, increase the growth rate of seedlings and slow down the development of resistance in fungal plant pathogens (Agrios, 2005; Degani *et al*., 2024).

CONCLUSIONS

This study identified *Aspergillus niger* and *Curvularia lunata* as the major seed-borne fungal pathogens of sesame. Among the chemical treatments, Amistar Top and Hombre at 150 ppm showed the highest antifungal efficacy against *C. lunata* and *A. niger*, respectively, under both *in vitro* and *in vivo* conditions. *Agrobacterium fabrum* was the most effective biological control agent, followed by *Pseudomonas fluorescens* and *Bacillus megaterium*. The combine use of effective fungicides and biological control agents will offer a sustainable and integrated approach for managing sesame seed-borne fungal diseases while reducing dependence on chemical inputs.

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AUTHOR CONTRIBUTIONS

Sobeeqa Khalid: Experiment – Conducting, data collection, and original draft preparation. Saman Arif: Data collection, manuscript review. Muhammad Usman: Conceptualization, data analysis, statistical interpretation and manuscript editing. Maham Sarwar: Methodology support and data curation. Malaika Tariq: Visualization, figure preparation. Muhammad Faheem Khan: Provision of seed material and field support; manuscript review. Amjad Abbas: Supervision, Provision of resources and lab facilities; manuscript review. Muhammad Amjad Ali: Supervision, conceptualization, project administration, critical revision, and final approval of the manuscript.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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