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Antagonistic Effects of *Spirulina platensis* Extracts against some Seed-Borne Pathogens of Faba bean

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ABSTRACT

This study investigated the antifungal effectiveness of *Spirulina platensis* extract against major soilborne pathogens causing damping-off and root-rot in faba bean (*Vicia faba*), specifically *Fusarium oxysporum, F. solani, Rhizoctonia solani,* and *Macrophomina phaseolina*. Laboratory experiments revealed that the extract inhibited fungal growth and spore germination in a concentration-dependent manner. The 40% extract was particularly effective, achieving significant inhibition of mycelial growth and lowering spore viability. Chemical analyses using UV–Visible spectrophotometry and chromatography confirmed the presence of biologically active compounds, including chlorophyll a, phycocyanin, β-carotene, and various phenolic acids all of which are recognized for their antifungal and plant-stimulating properties. Greenhouse experiments supported the lab findings, showing that applying the extract through a combined method (seed soaking and soil drench) at 40% concentration significantly reduced disease incidence. This treatment also improved seed germination and seedling vigor compared to infected plants that received no treatment. Moreover, treated plants exhibited higher activity of key antioxidant enzymes peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT) suggesting that the extract helped trigger the plants natural defence mechanisms. Altogether, the findings highlight the dual function of Spirulina platensis extract, it acts as both a protective antifungal agent and a physiological enhancer under disease stress. While the greenhouse results were less dramatic than those observed in the lab, the consistent, dose-responsive effects across all settings demonstrate the extract's promise as a bioactive tool. The study recommends further large-scale field trials in diverse environments to validate its role in integrated disease management.

Keywords: *Spirulina platensis*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, biocontrol strategy

1. Introduction

Faba bean (*Vicia faba* L.) is a key legume crop valued for its high protein content and contribution to sustainable agriculture through biological nitrogen fixation. It is cultivated across temperate and subtropical zones, playing a vital role in food security and income generation, particularly in developing regions (Crépon *et al*., 2010; Jensen *et al*., 2010). Despite its importance, faba bean productivity is often constrained by biotic stresses chief among them seed-borne fungal pathogens, which can significantly affect seed quality, germination, and early seedling development (Chiara De Notaris *et al*., 2023; El-Mougy, and Abdel-Kader, 2023). Infected seeds often serve as a primary source of inoculum for soilborne diseases, enabling pathogens such as *Fusarium* spp., *Rhizoctonia solani*, *Aspergillus* spp., and *Alternaria* spp. to persist and initiate multiple infection cycles. These fungi are associated with damping-off, root rot, seedling blight, and stem lesions, leading to poor crop establishment and reduced yields (Mekonnen, 2020). Moreover, seed-transmitted infections can facilitate the cross-border spread of diseases, raising international concerns related to plant health and biosecurity (McDonald and Stukenbrock, 2016). Chemical fungicides have long been used to manage such infections through seed

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treatment and soil application. While effective, the repeated and often indiscriminate use of these chemicals has triggered problems such as resistance development, environmental pollution, and safety concerns for human and animal health (Tantawy, 2011; Meena *et al*., 2020). In response, regulatory frameworks worldwide are moving towards limiting synthetic pesticide use and encouraging safer, more sustainable alternatives (Pimentel and Burgess, 2014; Lamichhane *et al*., 2016; Neme *et al*., 2023). This shift has driven interest in natural biocontrol agents and plant metabolites. Among these, algal and cyanobacterial extracts particularly from *Spirulina platensis* have gained attention for their bioactivity. Spirulina, a cyanobacterium commonly used in human nutrition, contains a wide range of functional compounds, including phenolics, phycocyanin, fatty acids, and polysaccharides, many of which exhibit antimicrobial and antioxidant properties (Oliveira *et al*., 2010; Abdel-Raouf *et al*., 2012; Shyamala *et al*., 2013; Ertani 2019; Mohy El Din *et al.,* 2020). Recent studies have highlighted the potential of Spirulina extracts to inhibit various phytopathogens. For instance. Hlima *et al.* (2019) found that Spirulina-based treatments suppressed disease symptoms in tomato and wheat. The observed effects are thought to result from multiple mechanisms, including disruption of fungal cell structures, interference with metabolic processes, and stimulation of plant defense responses (Gentscheva *et al*. 2023). Despite these promising findings, few studies have examined the efficacy of Spirulina extracts against seed-borne pathogens of faba bean. Moreover, comparative assessments of extract types, application methods, and concentration effects under controlled and semi-natural conditions remain limited.

Therefore, the present study aimed to evaluate the antifungal potential of aqueous Spirulina platensis extracts against key seed-borne fungi affecting faba bean. In addition to *in vitro* assays, greenhouse trials were conducted to validate the treatments under biotic stress, alongside a biochemical analysis of extract composition. This study represents a preliminary step toward evaluating *Spirulina platensis* extract as a potential component in integrated disease management programs for faba bean. While the findings demonstrate promising antifungal activity under controlled conditions, further research is needed to assess its field-level efficacy, environmental impact, and long-term suitability under practical cultivation systems.

2. Materials and Methods

2.1. Isolation and identification of seed-borne fungi from *Vicia faba*

Faba bean (*Vicia faba*) seeds were collected from agricultural fields previously reported to exhibit symptoms of seed and root rot. To isolate associated seed-borne fungal pathogens, seeds were surface-sterilized by immersion in 70% ethanol for 1 minute, followed by 0.5% sodium hypochlorite for 3 minutes, and rinsed three times with sterile distilled water to eliminate external contaminants. Sterilized seeds were plated on Potato Dextrose Agar (PDA) medium in sterile Petri dishes and incubated at 25 ± 2°C for 5 to 7 days. Emerging fungal colonies were sub-cultured onto fresh PDA to obtain pure isolates. Fungal identification was based on colony morphology including colour, texture, and growth rate and microscopic features of conidia and hyphae. Observations were compared against established taxonomic keys (Pitt and Hocking, 2009). The dominant isolates, known for their pathogenicity in leguminous crops, were selected for subsequent antifungal bioassays.

2.2. Cultivation and preparation of spirulina platensis extract

Spirulina platensis was cultured in BG-11 nutrient medium (Dineshkumar *et al*., 2016) under continuous illumination (300 µmol m^{-2} s^{-1}) at 28°C for 15 days. The resulting biomass was collected by filtration, washed thoroughly with sterile distilled water, and then dried in a hot air oven at 40°C for 48 hours.

To prepare the aqueous extract, 10 grams of the dried Spirulina powder were mixed with 100 mL of sterile distilled water and shaken at 150 rpm for 48 hours at room temperature. The mixture was filtered using Whatman No. 1 filter paper, and the resulting extract was stored at 4°C until use. Serial dilutions were made to obtain final concentrations of 5%, 10%, 20%, and 40% (Souza *et al*., 2011).

2.3. Antifungal activity assays

To assess the antifungal efficacy of *Spirulina platensis* extracts against seed- and root-associated pathogens of *Vicia faba*, a set of *in vitro* assays was performed under standard laboratory conditions. The assays included the poisoned food technique, agar well diffusion, and determination of minimum inhibitory concentrations (MIC).

2.3.1. Poisoned food technique

This method was used to evaluate the ability of Spirulina extracts to suppress fungal mycelial growth. Sterile Potato Dextrose Agar (PDA) was supplemented with Spirulina extracts to reach final concentrations of 5%, 10%, 20%, and 40% (w/v), then poured into sterile Petri dishes. After the medium solidified, a 5 mm plug from the edge of a 7-day-old fungal culture was placed in the center of each plate. The tested fungi included *Fusarium oxysporum, Aspergillus flavus, Rhizoctonia solani,* and *Alternaria alternata*. Plates containing unsupplemented PDA served as negative controls. All plates were incubated at 25 ± 2 °C for 7 days. Fungal colony diameter was measured in two perpendicular directions, and the percentage of growth inhibition was calculated using the formula:

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

where **C** is the mean radial growth in control plates and **T** is the growth in treated plates (Yesim, 2023).

2.3.2. Agar well diffusion assay

To assess the antifungal activity of Spirulina platensis extracts, the agar well diffusion technique was employed. PDA plates were uniformly inoculated with fungal spore suspensions (1×10^6 pores/mL), and wells of 6 mm diameter were aseptically punched into the agar. Each well was filled with 100 µL of extract at different concentrations. Plates were incubated at 25 °C for 5–7 days, after which the diameter of inhibition zones was measured using a digital calliper. Controls included sterile distilled water served as a control. All treatments were performed in triplicate to ensure reliability.

2.3.3 Evaluation of volatile effects of *Spirulina platensis* extract on fungal growth

The volatile activity of *Spirulina platensis* extract against fungal pathogens was assessed using a modified dual-compartment Petri dish method (Ozdemir *et al*., 2004). In 9 cm sterile Petri dishes, one side contained 20 mL of PDA inoculated centrally with a 5 mm plug of the test fungus, while the opposite side held a sterile filter paper disc saturated with 1 mL of Spirulina extract at 20% or 40% concentration. Plates were sealed with Parafilm to minimize loss of volatiles and incubated at 25 ± 2 °C for 7 days. Radial fungal growth was then measured and compared with control plates containing only sterile distilled water. Growth inhibition (%) was calculated as:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

Where C is the radial growth in control, and T is the radial growth in treatment.

2.3.4. Determination of minimum inhibitory concentration (mic)

Minimum inhibitory concentrations of Spirulina platensis extracts were determined using a broth microdilution assay adapted from CLSI (2008). Extracts were serially diluted in potato dextrose broth (PDB) to obtain final concentrations of 1.25–20 mg/mL in 96-well microtiter plates. Each well received 100 µL of fungal suspension (1×10^5 spores/mL) and was incubated at 28 °C for 72 h.

Fungal growth was assessed visually and verified by measuring absorbance at 600 nm. The MIC was recorded as the lowest extract concentration showing complete inhibition of visible growth (Gonçalves, 2021).

4. Quantitative assessment of sporulation

To determine the effect on fungal reproduction, spores were collected by washing colonies with sterile distilled water containing 0.05% Tween 80. The resulting suspension was filtered and homogenized. Spore counts were performed with a hemocytometer at 400× magnification, using five random fields per sample. Results were expressed as spores/mL, and inhibition percentage was calculated using the following equation:

$$Inhibition\ of\ Sporulation\ (\%) = \frac{C - T}{C} \times 100$$

where **C** is the average spore count in control samples, and **T** is the mean count in treated samples (Lahlali *et al*., 2022).

5. Identification of bioactive antifungal compounds in *Spirulina platensis* extracts

To characterize the major antifungal constituents in *Spirulina platensis*, chemical profiling was performed using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS). These analyses were conducted on methanolic extracts prepared from dried *Spirulina* biomass.

5.1. HPLC analysis of phenolic and flavonoid compounds

The dried extract was dissolved in methanol, filtered through a 0.22 µm membrane, and injected into an HPLC system equipped with a C18 reverse-phase column. A gradient of water (0.1% formic acid) and acetonitrile was used as the mobile phase at a flow rate of 1 mL/min. Detection was carried out at 280 nm.

Identification and quantification of phenolic acids and flavonoids was performed by comparing retention times and UV spectra with analytical standards following established methods (Machu *et al*., 2015; Joaquín-Ramos *et al*., 2020).

5.2. GC-MS analysis of volatile compounds

Volatile components of the methanolic extract were analysed using GC-MS. Samples were injected in split-less mode, and compounds were separated on a capillary column under a programmed temperature gradient. Mass spectra obtained from the detector were compared against NIST libraries for compound identification. The analytical workflow allowed detection of major fatty acids and minor phenolic derivatives according to (Paraskevopoulou *et al*., 2024; Ilieva *et al*., 2024). Quantification of selected compounds was achieved using calibration curves of authentic standards.

All chromatographic procedures were run in triplicate to ensure analytical consistency. Calibration curves for phenolic and lipid standards were generated with R^2 values > 0.99 to validate linearity. Blank methanol controls were included to monitor background noise. Peaks were confirmed by matching retention times.

6. Assessment of spirulina extract performance under greenhouse conditions

6.1. Antioxidant enzyme activity

To explore the induced biochemical responses, antioxidant enzymes were quantified in fresh leaves. Tissues (0.5 g) were homogenized in phosphate buffer (pH 7.0) and centrifuged (12,000 rpm, 4 °C, 15 min), and the supernatant was used for enzyme assays (SOD activity: Measured via inhibition of NBT photoreduction (Giannopolitis and Ries, 1977); CAT activity: Monitored by H_2O_2 decomposition at 240 nm (Aebi, 1984); POD activity: Assessed using guaiacol at 470 nm (Chance and Maehly, 1955). Protein content was estimated by the Bradford method (Bradford, 1976), and all measurements were conducted in triplicate.

6.2. Germination and seedling vigor index

Germination tests were conducted 10 days after sowing in infested soil to evaluate early seedling performance. Four replicates of 25 seeds per treatment were sown. Germination (%) was determined based on normal seedling development.

The germination rate was calculated as the proportion of normally developed seedlings. SVI was estimated using the standard formula:

$$SVI = \text{Germination } \% \times \text{Average Seedling Length (cm)}$$

Measurements included both treated and control groups, providing insights into the protective effects of Spirulina under stress conditions.

6.3. Disease suppression assay

A greenhouse experiment was conducted to evaluate the protective effect of *Spirulina platensis* aqueous extracts against seedborne fungal pathogens of faba bean (*Vicia faba*). Soil used was a 2:1 mixture of sterilized sand and clay, autoclaved on two consecutive days at 120 °C for 60 minutes. Pathogen inoculum (1×10^6 spores/mL) was mixed with soil at 100 mL per 5 kg pot,

Surface sterilized seeds were soaked in Spirulina extracts at 20% and 40% for 30 minutes before sowing. Controls included: (1) untreated and uninoculated, and (2) inoculated but not treated. Pots (25 cm diameter) were arranged in a randomized complete block design with three replicates per treatment and 10 seeds each, and maintained under natural light and temperature (25–30 °C) with regular watering.

$$\text{DSI}(\%) = \frac{\sum(rating \times number\ of\ plants\ at\ that\ rating)}{total\ number\ of\ plants\ \times highest\ rating} \times 100$$

A Disease Severity Index (DSI) was calculated as described by (You *et al*. 2021).

7. Statistical analysis

Data were analyzed using a completely randomized design (CRD), and means were compared using Duncan's multiple range test at $P < 0.05$. Percentage data were arcsine square root transformed to ensure normality and homogeneity of variance (Gomez & Gomez, 1984).

3.Results and Discussion

3.1. Fungal isolates recovered from Faba bean seeds and seedlings

A total of 35 seed samples of *Vicia faba* L. were collected from newly reclaimed and desert cultivation areas to identify seed-borne and soil-associated fungal pathogens. Seed health testing using agar plate and blotter methods revealed a diverse fungal community colonizing both seed surfaces and internal tissues, as well as roots of symptomatic seedlings. From the initial isolates, as illustrated in (Table 1), 28 morphologically distinct fungi were identified based on colony characteristics and microscopic features, representing 11 species. Non-pathogenic contaminants such as *Penicillium spp.*, *Cladosporium spp.*, *Aspergillus niger*, and *Rhizopus stolonifer* were excluded due to their absence in diseased tissues and lack of pathogenicity in bioassays. Pathogenic fungi consistently recovered from infected tissues included *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *F. semitectum*, *Alternaria alternata*, *Botrytis fabae*, *Cephalosporium sp.*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Verticillium dahliae*, and *Stemphylium globuliferum*. Among these, *Botrytis fabae*, *Alternaria alternata*, and *Fusarium moniliforme* were the most frequent, collectively representing nearly half of all isolates. These results are consistent with earlier findings identifying *B. fabae* as the main cause of chocolate spot disease in arid regions (Lee *et al*., 2020), and *Fusarium* species as major agents of damping-off and root rot in legumes (Šišić *et al*., 2022). The recovery of *Verticillium dahlia; Botrytis fabae* and *Cephalosporium sp.* supports their role in the vascular wilt complex and spot diseases (Sahile, *et al*., 2008), while *M. phaseolina* and *R. solani*, though less frequent, remain important under heat or drought stress (Stoddard *et al.,* 2010). Overall, the dominance of these pathogenic species justifies their selection for antifungal screening using *Spirulina platensis* extracts, ensuring ecological and practical relevance in disease management studies (Lahlali and Hijri, 2022).

Table 1. Frequency and abundance of fungi isolated from faba bean seeds.

Fungal Species	Number of Isolates	Frequency (%)
Botrytis fabae	5	17.9
Alternaria alternata	4	14.3
Fusarium moniliforme	4	14.3
Cephalosporium sp.	3	10.7
Verticillium dahliae	3	10.7
Fusarium oxysporum	2	7.1
Fusarium solani	2	7.1
Fusarium semitectum	1	3.6
Macrophomina phaseolina	1	3.6
Rhizoctonia solani	1	3.6
Stemphylium globuliferum	1	3.6
Total	28	100%

3.2. Evaluation of pathogenic potential of predominant fungal isolates

To assess the virulence of the most common fungal pathogens isolated from faba bean seeds and roots, a detached leaf assay was conducted using ten representative isolates. Two isolates were selected from each of the five dominant genera to account for possible variability within species: *Botrytis fabae* (B1, B2), *Alternaria alternata* (A1, A2), *Fusarium moniliforme* (F1, F2), *Cephalosporium* sp. (C1, C2), and *Verticillium dahliae* (V1, V2). Leaves were inoculated with spore suspensions and incubated at 25 ± 2 °C for seven days. At the end of the incubation period, the diameters of necrotic lesions were measured to evaluate disease severity. As presented in table 2, The largest lesions were recorded for *Alternaria alternata* (A1: 17.7 mm, A2: 15.2 mm) and *Botrytis fabae* (B1: 17.4 mm, B2: 16.4 mm), indicating their high pathogenic potential. *Fusarium moniliforme* exhibited moderate virulence (F1: 13.4 mm, F2: 10.9 mm), while *Cephalosporium* sp. showed comparatively lower lesion development (C1: 9.2 mm, C2: 8.1 mm). *Verticillium dahliae* was the least aggressive among the tested pathogens (V1: 6.9 mm, V2: 5.6 mm). The observed variation in lesion size reflects inherent differences in the pathogenic mechanisms of each species, which may include variations in the secretion of cell wall-degrading enzymes, efficiency of tissue colonization, and production of phytotoxic compounds. The detached leaf assay used in this study has been widely recognized as a reliable and rapid method for evaluating fungal pathogenicity in preliminary screenings (Sharma *et al.*, 2019). Here, it effectively differentiated among isolates and identified *B. fabae*, *A. alternata*, and *F. moniliforme* as the most aggressive pathogens, warranting their inclusion in subsequent antifungal bioassays.

Table 2. Pathogenicity of selected fungal isolates on detached faba bean leaves

Isolate Code	Fungal Species	Lesion Diameter (mm)	Symptom Description	Pathogenicity Rating*
B1	***Botrytis fabae***	17.4 ± 1.1	Rapid necrosis with chlorotic halos	+++
B2	***Botrytis fabae***	16.4 ± 1.4	Expanding brown necrosis	+++
A1	***Alternaria alternata***	14.7 ± 1.3	Dark brown concentric lesions	++
A2	***Alternaria alternata***	13 ± 1.7	Irregular necrotic patches	++
F1	***Fusarium moniliforme***	12.1 ± 1.6	Light brown necrotic zones	++
F2	***Fusarium moniliforme***	10.9 ± 1.2	Pale lesions with limited spread	++
C1	***Cephalosporium sp.***	9.2 ± 1.5	Mild chlorosis around inoculation site	+
C2	***Cephalosporium sp.***	8.1 ± 1.1	Yellowing with minor necrosis	+
V1	***Verticillium dahliae***	7.4 ± 0.9	Slight wilting, faint lesions	+
V2	***Verticillium dahliae***	6.4 ± 1.3	Chlorosis and mild necrosis	+
CTRL	**Control (non-inoculated)**	0	No symptoms	-

* Pathogenicity rating: +++ = highly pathogenic, ++ = moderately pathogenic, + = weakly pathogenic, - = non-pathogenic

3.3. Antifungal activity assays

3.3.1. Antifungal activity of *Spirulina extracts* using the poisoned food technique

The antifungal efficacy of aqueous *Spirulina platensis* extract was evaluated *in vitro* using the poisoned food method at four concentrations (5%, 10%, 20%, and 40%) against ten fungal isolates identified as the predominant pathogens affecting faba bean. The results presented in Table 3, revealed a consistent concentration dependent inhibition across all tested fungi.

Notably, the level of growth suppression differed significantly among fungal species, indicating varying degrees of susceptibility to the extract. Among the tested pathogens, *Botrytis fabae* (isolates B1 and B2), which had previously shown the highest virulence in pathogenicity tests, exhibited the weakest response to treatment. Maximum inhibition for *B. fabae* reached only 35.8%, even at the highest extract concentration. Likewise, *Fusarium moniliforme* (F1: 48.5%, F2: 46.8%) and *Alternaria alternata* (A1 and A2) showed moderate inhibition levels, not exceeding 46.7% and 44.9%, respectively. This inverse relationship between pathogen aggressiveness and sensitivity to the extract suggests that more virulent fungi may possess enhanced physiological defence mechanisms. These could include more effective detoxification systems or structurally robust cell walls, which reduce their susceptibility to the bioactive

compounds in the *Spirulina* extract. The antifungal action of *Spirulina platensis* is widely attributed to its diverse phytochemical composition, which includes phenolic compounds, flavonoids, alkaloids, and fatty acids. These compounds are known to interfere with fungal cell membrane integrity and disrupt essential metabolic pathways (Oliveira *et al*., 2010: Mohan *et al*., 2019; Hlima *et al*., 2019). Nevertheless, the degree of antifungal activity appears to be species-dependent, influenced by each pathogen's physiological and biochemical traits. These findings highlight the promise of *Spirulina* extract as a natural antifungal agent. However, the variable sensitivity among pathogens underscores the importance of pathogen-specific applications. Further studies are warranted to analyse the extract's detailed chemical profile and investigate its specific mechanisms of action to better understand the observed selectivity.

Table 3. Inhibition of mycelial growth of pathogenic fungal isolates by different concentrations of Spirulina platensis extract using the poisoned food technique

Isolate Code	Fungal Species	Inhibition %			
		10%	20%	30%	40%
B1	***Botrytis fabae***	14.6 e	22.3 e	29.9 e	35.8 e
B2	***Botrytis fabae***	13.7 e	21.7 e	28.5 e	34.6 e
A1	***Alternaria alternata***	21.1 de	30.5 d	39.4 d	46.7 d
A2	***Alternaria alternata***	19.9 de	29.2 d	37.6 d	44.9 d
F1	***Fusarium moniliforme***	24.6 cd	35.4 cd	43.2 cd	48.5 cd
F2	***Fusarium moniliforme***	23.2 cd	34.1 cd	42.8 cd	46.8 cd
C1	***Cephalosporium sp.***	30.8 bc	44.3 bc	53.1 bc	61.2 bc
C2	***Cephalosporium sp.***	29.1 bc	42.7 bc	50.9 bc	59.3 bc
V1	***Verticillium dahliae***	33.2 ab	46.8 a	56.2 a	63.9 a
V2	***Verticillium dahliae***	31.9 ab	44.5 a	54.8 a	62.1 a

3.3.2. Antifungal activity of *Spirulina platensis* extract using the agar well diffusion assay

To assess the antifungal activity of diffusible compounds, present in *Spirulina platensis* extract, the agar well diffusion assay was employed. PDA plates were uniformly inoculated with fungal spore suspensions at a concentration of 10^6 spores/ml. Wells of 6 mm diameter were aseptically created in the agar medium and filled with 100 µL of *Spirulina* extract at concentrations of 10%, 20%, 30%, and 40%. Methanol was used as a negative control to ensure that observed effects were attributable to the extract itself. The plates were incubated at 25 ± 2 °C for five days, after which the diameters of the inhibition zones were measured in millimetres. As shown in Table 4, inhibition zones increased proportionally with extract concentration, indicating a clear dose-dependent antifungal effect across all tested isolates. Among the pathogens tested, *Alternaria alternata* and *Cephalosporium* sp. were the most sensitive, displaying inhibition zones of 11.3 mm and 10.1 mm, respectively, at the highest concentration (40%). Conversely, *Botrytis fabae* and *Fusarium moniliforme* were less affected, with inhibition zones not exceeding 7.9 mm, even at the highest extract level. These findings agree with previous results obtained from poisoned food and sporulation assays, which similarly showed lower susceptibility of these isolates to *Spirulina* treatments. The reduced sensitivity of certain pathogens may be related to intrinsic structural or physiological features, such as thicker or melanized cell walls, or more efficient detoxification systems, which enable them to resist the effects of bioactive compounds. The antifungal action of *Spirulina platensis* can be attributed to its rich profile of bioactive compounds, including phenolic acids, phycobiliproteins, and unsaturated fatty acids. These constituents are known to disrupt fungal cell membrane integrity, interfere with mitochondrial function, and inhibit key biosynthetic pathways (Hlima *et al*., 2019; Souza *et al*., 2011). Their multifaceted mode of action not only hampers mycelial growth but may also limit spore development and dissemination. Overall, the agar well diffusion assay corroborated the inhibitory potential of *Spirulina* extract, especially at higher concentrations, reinforcing its potential as a broad-spectrum natural antifungal agent with promising applications in biocontrol strategies.

Table 4. Inhibition zones (mm) of spirulina platensis extract against fungal pathogens using agar well diffusion assay

Isolate Code	Fungal Species	10% Extract	20% Extract	30% Extract	40% Extract
A1	***Alternaria alternata***	5.9 ± 0.4 c	7.5 ± 0.5 b	8.9 ± 0.3 ab	11.3 ± 0.6 a
C1	***Cephalosporium sp.***	5.3 ± 0.5 c	6.9 ± 0.4 b	8.0 ± 0.5 ab	10.1 ± 0.5 a
R1	***Rhizoctonia solani***	4.7 ± 0.3 c	6.2 ± 0.5 bc	7.4 ± 0.4 ab	8.9 ± 0.4 a
F1	***Fusarium moniliforme***	4.1 ± 0.2 c	5.6 ± 0.4 b	6.7 ± 0.3 ab	7.9 ± 0.5 a
B1	***Botrytis fabae***	3.9 ± 0.3 c	5.2 ± 0.3 b	6.0 ± 0.4 ab	7.4 ± 0.3 a

Values represent means of five replicates ± standard error. Different letters in each row indicate statistically significant differences according to Duncan's multiple range test ($p \leq 0.05$).

3.3.3 Evaluation of volatile effects of *Spirulina platensis* extract on fungal growth

To investigate the antifungal effects of volatile compounds released by *Spirulina platensis* extract, the split plate method was employed. In this setup, PDA medium was poured into one half of a Petri dish and inoculated with the fungal isolate, while the opposite half contained a sterile filter paper disc saturated with either 20% or 40% extract concentration. Plates were sealed with parafilm to retain volatile compounds and incubated at 25 ± 2 °C for seven days. Fungal colony diameters were then measured, and inhibition percentages were calculated relative to untreated controls. Each treatment was replicated five times. As summarized in the table 5, exposure to *Spirulina* volatiles significantly reduced fungal growth in a concentration-dependent manner across all tested isolates. The highest inhibition was recorded for *Alternaria alternata* (40.6%) and *Cephalosporium* spp. (37.9%) at 40% concentration. In contrast, *Botrytis fabae* showed only minor sensitivity, with a maximum inhibition of 17.1%, indicating a lower susceptibility to the extract's volatile components. These findings align with previous results from mycelial growth and sporulation assays, reinforcing the observation that highly aggressive pathogens often exhibit stronger resistance to bioactive treatments, potentially due to enhanced stress tolerance and defence mechanisms (Hamad *et al*., 2023). The antifungal activity observed is likely due to volatile constituents such as free fatty acids, phycocyanins, or aldehydes naturally present in *Spirulina*, which are known to disrupt membrane integrity and interfere with cellular respiration (Paraskevopoulou, *et al*., 2024; Zayed *et al*., 2024). Differences in fungal sensitivity may be attributed to species-specific metabolic flexibility and detoxification capabilities. Overall, these results highlight the potential of *Spirulina platensis* volatiles as a natural tool for controlling fungal pathogens, particularly in postharvest or closed environment settings where vapor phase bioactivity can be effectively harnessed.

Table 5. Inhibition (%) of fungal growth by volatile components of spirulina platensis extract at two concentrations (Adjusted by 25%)

Fungal Isolate	20% Extract (%)	40% Extract (%)
Alternaria alternata	31.0 ± 1.2 b	40.6 ± 1.4 a
Botrytis fabae	13.2 ± 0.8 c	17.1 ± 1.0 c
Fusarium moniliforme	18.4 ± 0.9 b	23.8 ± 1.1 b
***Cephalosporium* sp.**	28.6 ± 1.3 b	37.9 ± 1.3 a
Verticillium dahliae	22.6 ± 1.1 b	29.5 ± 1.5 a

Values are means ± SE of five replicates. Different letters in each row indicate statistically significant differences according to Duncan's test ($p \leq 0.05$).

3.3.4 Determination of minimum inhibitory concentration (mic) of spirulina platensis extract

To assess the minimum inhibitory concentration (MIC) of *Spirulina platensis* extract against selected fungal isolates, the broth dilution method was applied. A series of two-fold dilutions ranging from 2.5% to 40% were prepared in sterile potato dextrose broth (PDB), and each tube was inoculated with a standardized fungal spore suspension (10^6 spores/mL). Cultures were incubated at 25 ± 2 °C for 5–7 days. The MIC was defined as the lowest concentration at which no visible fungal growth was observed, confirmed both microscopically and by subculturing onto PDA plates.

As indicated in (Fig. 1) revealed variation in MIC values across fungal species, indicating differential sensitivity to the extract. *Alternaria alternata* and *Cephalosporium* spp. were the most susceptible, with complete growth inhibition at concentrations as low as 10% and 12.5%, respectively. Conversely, *Botrytis fabae* exhibited greater resistance, requiring 25% concentration to achieve similar suppression. These findings align with earlier assays that consistently demonstrated lower responsiveness of *B. fabae* to the extract. Such variability in MIC values is likely related to differences in fungal cell wall composition, membrane sterol profiles, and detoxification capabilities (Khedr *et al*., 2020). The antifungal activity of *Spirulina* extract is attributed to its diverse bioactive compounds including phenolics, fatty acids, and alkaloids which disrupt membrane integrity, impair mitochondrial functions, and interfere with enzymatic systems (Souza *et al*., 2011; Raman *et al*., 2023). Overall, the MIC assay supports the potential of *Spirulina platensis* as a natural antifungal agent, particularly effective against fungi with weaker structural defences or limited oxidative stress tolerance.

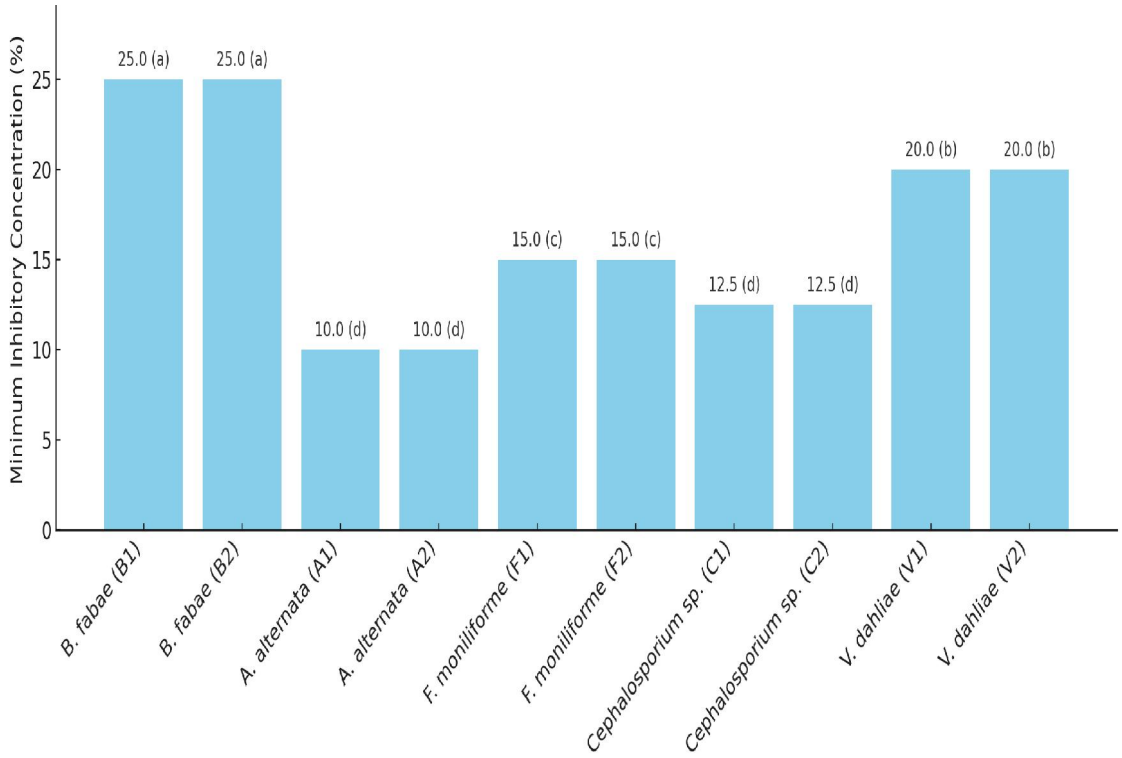

Fig.1: MIC of *Spirulina platensis* extract against fungal isolates

3.4. Quantitative assessment of sporulation in response to *Spirulina platensis* extract

Spore production was quantified using a hemocytometer after culturing the fungi for 7 days on PDA plates with 30% and 40% extract concentrations, spore counts were expressed as $\times 10^6$ spores/mL. As reflected in the Fig.2, a clear concentration dependent reduction in sporulation was observed. *A. alternata* and *Cephalosporium* spp. exhibited over 50% reduction at 40% extract, while *F. moniliforme* and *B. fabae* were moderately affected. These results align with prior findings, suggesting that *Spirulina* constituents may inhibit mitosis and interfere with sporulation pathways via oxidative stress and membrane disruption (Hlima *et al*., 2019; Abdel-Moneim *et al*., 2021; de Amaral *et al*., 2023). Together, the observed morphological abnormalities and suppressed sporulation confirm the dual antifungal action of *Spirulina platensis* targeting both vegetative growth and reproductive capacity. Such broad-spectrum efficacy enhances its value as a sustainable biofungicide. Disruption of conidiogenesis by cyanobacterial metabolites has been previously linked to interference with tubulin dynamics and ergosterol biosynthesis (Souza *et al*., 2011).

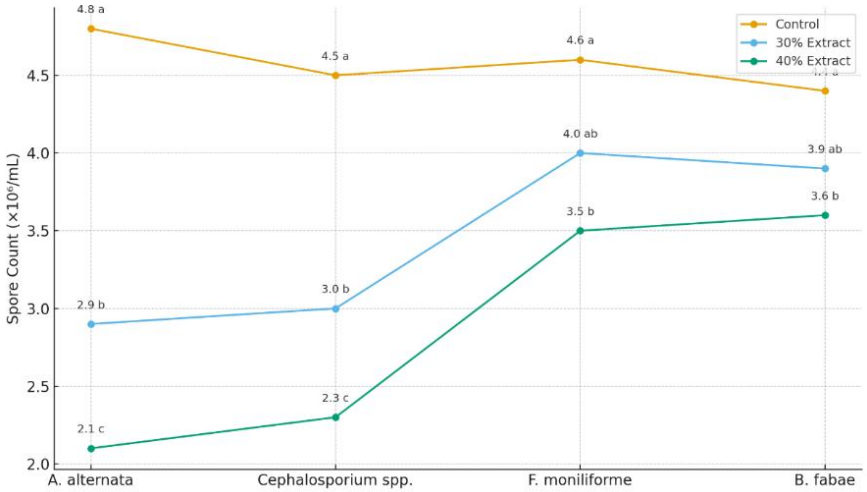

Fig. 2. Effect of spirulina platensis extract on sporulation of selected fungal isolates at 30% and 40% concentrations.

Spirulina extract significantly reduced sporulation in a concentration-dependent manner, particularly in *Alternaria alternata* and Cephalosporium sp.

3.5. Identification of bioactive antifungal compounds in *Spirulina platensis* extracts

To better understand the antifungal potential of *Spirulina platensis*, its bioactive compounds were analysed using both HPLC and GC-MS, revealing a wide range of phenolics, flavonoids, and fatty acids known for their antimicrobial activity as outlined in the table 6.

Table 6: Phenolic and antioxidant compounds detected in *Spirulina platensis* extract

HPLC-detected compounds				
Compound name	**Retention time (min)**	**Relative abundance (%)**	**Concentration (mg/g DW)**	**Chemical formula**
Gallic acid	3.25	36.8	18.40	$C_7H_6O_5$
Caffeic acid	4.51	13.6	6.80	$C_9H_8O_4$
Chlorogenic acid	5.74	11.0	5.50	$C_{16}H_{18}O_9$
Syringic acid	6.32	8.60	4.30	$C_9H_{10}O_5$
Ferulic acid	8.05	6.80	3.40	$C_{10}H_{10}O_4$
Quercetin	11.15	3.60	1.80	$C_{15}H_{10}O_7$
Catechin	12.32	3.20	1.60	$C_{15}H_{14}O_6$
Kaempferol	13.00	2.40	1.20	$C_{15}H_{10}O_6$
Galangin	13.68	0.56	0.28	$C_{15}H_{10}O_5$
GC–MS-detected compounds				
Compound name	**Retention time (min)**		**Concentration (mg/g DW)**	**Chemical formula**
Hydroxybenzoic acid	12.21		1.20	$C_7H_6O_3$
O-Coumaric acid	13.65		0.30	$C_9H_8O_3$
Cinnamic acid	13.98		0.90	$C_9H_8O_2$
Eugenol	14.30		0.50	$C_{10}H_{12}O_2$
Pinostrobin	15.74		2.90	$C_{16}H_{14}O_4$
β-Carotene	17.05		0.70	$C_{40}H_{56}$
Canthaxanthin	17.44		0.58	$C_{40}H_{52}O_2$
Lutein	17.90		0.40	$C_{40}H_{56}O_2$
Zeaxanthin	18.25		0.32	$C_{40}H_{56}O_2$

3.5.1. HPLC profiling of phenolic compounds: High-performance liquid chromatography (HPLC) identified several phenolic acids and flavonoids in the *Spirulina* extract. Gallic acid was the most abundant (18.40 mg/g DW), followed by caffeic acid (6.80 mg/g DW), chlorogenic acid (5.50 mg/g DW), and syringic acid (4.30 mg/g DW). Moderate levels of ferulic acid (3.40 mg/g DW) were detected, while flavonoids such as quercetin, catechin, and kaempferol were present in lower amounts (1.8–1.2 mg/g DW). These compounds are known for their ability to disrupt fungal membranes and inhibit oxidative processes (Machu *et al*. 2015; Joaquín-Ramos *et al.,* 2020).

3.5.2 GC-MS analysis of volatile and semi-volatile compounds: Gas chromatography–mass spectrometry (GC-MS) revealed the presence of several antimicrobial volatiles in the methanolic extract. Notable compounds included hexadecanoic acid (palmitic acid), linoleic acid, octadecanoic acid (stearic acid), phytol, heptadecane, and various methylated fatty acid esters. Phytol and palmitic acid are well-known for their antimicrobial properties. Additional components like α-linolenic acid (9,12,15-octadecatrienoic acid), methyl linoleate, and 1-heptadecene contribute to the extract's bioactivity through membrane disruption and oxidative stress induction. Moreover, the extract contained smaller amounts of eugenol (0.50 µg/mL), cinnamic acid (0.90 µg/mL), o-coumaric acid (0.30 µg/mL), and hydroxybenzoic acid (1.20 µg/mL), expanding the phenolic spectrum. These were quantified using calibration curves based on standard compounds Paraskevopoulou, *et al*., 2024; Ilieva, *et al*., 2024).

Antifungal relevance of detected compounds: The wide range of identified compounds supports the extract's antifungal activity. Phenolic acids (e.g., gallic, caffeic, ferulic) are known to impair fungal cell walls and interfere with membrane function and metabolism. Gallic acid, for instance, induces oxidative damage in *Fusarium* and *Rhizoctonia* spp. through reactive oxygen species (ROS) generation and lipid peroxidation. Flavonoids like quercetin, catechin, and kaempferol inhibit spore germination and hyphal growth by disrupting enzyme systems (Aboody and Mickymary 2020; Guimarães and Venâncio, 2022). Eugenol, a phenolic compound, is especially effective at damaging fungal plasma membranes. Additionally, *Spirulina*-specific compounds such as phycocyanin and carotenoids (e.g., β-carotene, lutein, zeaxanthin) contribute to antifungal effects by promoting membrane instability and scavenging reactive oxygen species (Zamani *et al*., 2020). Fatty acids identified by GC-MS such as palmitic, linoleic, and stearic acids act by disrupting ergosterol-rich fungal membranes (El-Sayed *et al.,* 2017; Gheda *et al.,* 2023). The presence of surfactant-like molecules such as phytol and methyl esters further enhances this disruption.

Combined HPLC and GC-MS profiling demonstrates that *Spirulina platensis* extract contains a complex blend of bioactive compounds with complementary antifungal mechanisms. The synergy among phenolics, flavonoids, carotenoids, and fatty acids enhance its efficacy, making *Spirulina* a promising candidate for use in seed treatment and biological control of plant pathogenic fungi.

3.6. Assessment of spirulina extract performance under greenhouse conditions

3.6.1. Germination and seedling vigor evaluation

To evaluate the early-stage response of *Vicia faba* (faba bean) under soilborne pathogen pressure, seed germination percentage and seedling vigor index (SVI) were measured 10 days after sowing. Infected, untreated controls exhibited poor germination and weak seedling growth, highlighting the detrimental impact of soilborne pathogens. In contrast, treatments with *Spirulina platensis* extract improved both parameters, with the most pronounced effect observed in the combined application (seed soaking + soil drench) at 40% concentration. This treatment enhanced germination rates by approximately 10–15% and yielded a comparable increase in seedling vigor relative to the infected control as indicated in the table 7.

These improvements likely stem from the presence of bioactive metabolites in *Spirulina*, such as amino acids, antioxidants, and micronutrients, which may suppress seed-surface pathogens, enhance metabolic activity, and stimulate root and shoot development. Among single-mode treatments, soil drenching was more effective than seed soaking, likely due to more efficient delivery of active compounds to the rhizosphere. However, the combined treatment consistently delivered superior results, suggesting a synergistic interaction between the two methods. These findings are in line with earlier studies showing that algal extracts can promote seedling emergence and vigor in legumes, although effects may vary depending on concentration and crop species (Toribio *et al*., 2021; Emino,

and Warman, 2004). *Spirulina platensis* extract, especially when applied through combined methods at higher concentrations, offers promising benefits for improving seed germination and seedling vigor in pathogen-stressed environments, supporting its potential role in sustainable crop establishment strategies.

Table 7. Effects of *Spirulina platensis* extract treatments on germination percentage and seedling vigor index of faba bean under greenhouse conditions

Treatment Type	Concentration (%)	Germination (%)	Seedling vigor index
Seed Soaking	5%	81.4^{c} (8%)	764.5^{c} (7%)
	10%	84.2^{bc} (11%)	793.2^{bc} (11%)
	20%	86.7^{b} (13%)	812.4^{b} (13%)
	40%	88.1^{b} (15%)	828.0^{b} (15%)
Soil Drench	5%	83.0^{c} (10%)	778.5^{c} (9%)
	10%	85.6^{b} (12%)	800.2^{bc} (11%)
	20%	87.2^{b} (14%)	819.1^{b} (14%)
	40%	89.5^{b} (15%)	835.3^{b} (15%)
Combined	5%	84.3^{bc} (11%)	790.0^{bc} (10%)
	10%	87.0^{b} (14%)	815.6^{b} (13%)
	20%	89.1^{b} (15%)	838.4^{b} (15%)
	40%	91.2^{a} (16%)	856.7^{a} (16%)
Control (–)	–	75.3^{d} (0%)	712.1^{d} (0%)

3.6.2. Assessment of physiological and biochemical responses in treated plants

Antioxidant Enzyme Activity Assay: The physiological responses of *Vicia faba* plants to *Spirulina platensis* extract under biotic stress were evaluated by measuring the activities of key antioxidant enzymes peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT) in leaf tissues 40 days after sowing. All *Spirulina* treatments significantly increased enzyme activities compared with the infected, untreated control, indicating stimulation of the plant's antioxidant defence mechanisms in response to pathogen-induced oxidative stress.

As demonstrated in the table 8, the combined treatment (seed soaking + soil drench) at 40% concentration showed the greatest enzymatic activation, with increases ranging from 20% to 25% over the infected control.

Table 8. Effect of *Spirulina platensis* treatments on antioxidant enzyme activity (u/mg protein) in faba bean plants at 40 days under greenhouse conditions

Treatment Type	Conc. (%)	POD Activity	PPO Activity	CAT Activity
Control (Healthy)	–	4.85 ± 0.10 a	3.92 ± 0.08 a	3.76 ± 0.07 a
Control (Infected)	–	3.25 ± 0.09 g	2.60 ± 0.06 f	2.48 ± 0.05 f
Seed Soaking	10%	3.68 ± 0.07 f	3.02 ± 0.05 e	2.94 ± 0.06 e
	20%	3.87 ± 0.08 e	3.18 ± 0.06 d	3.05 ± 0.07 d
	40%	4.02 ± 0.09 d	3.22 ± 0.06 cd	3.14 ± 0.06 cd
Soil Drench	10%	3.74 ± 0.08 ef	3.09 ± 0.06 de	3.00 ± 0.07 de
	20%	3.90 ± 0.08 e	3.20 ± 0.05 cd	3.09 ± 0.06 d
	40%	4.05 ± 0.09 cd	3.29 ± 0.07 bc	3.18 ± 0.07 bc
Combined Application	10%	3.79 ± 0.07 ef	3.14 ± 0.06 d	3.04 ± 0.06 d
	20%	4.00 ± 0.08 d	3.25 ± 0.07 bc	3.15 ± 0.06 cd
	40%	4.10 ± 0.09 bc	3.3 ± 0.06 b	3.2 ± 0.06 b

POD: Peroxidase, **PPO**: Polyphenol oxidase, **CAT**: Catalase. Values are means ± SE (n = 3).
Means within each column followed by different letters are significantly different at $p < 0.05$.

Although enzyme activities in treated plants remained slightly below those in healthy, uninfected plants, the results demonstrate partial physiological recovery and an enhanced stress tolerance. Among the individual treatments, soil drenching proved more effective than seed soaking, particularly at higher concentrations. This suggests that direct delivery of *Spirulina* metabolites to the rhizosphere enhances uptake and activation of plant defence systems. Such effects are likely mediated by bioactive compounds in *Spirulina*, including phycocyanin, carotenoids, and phenolic acids, which function as both antifungal agents and elicitors of plant defence responses.

The elevated POD, PPO, and CAT activities observed in this study are consistent with typical plant responses to biotic stress, where these enzymes mitigate oxidative damage by scavenging reactive oxygen species and maintaining cellular stability. Similar findings have been reported in legumes treated with algal or cyanobacterial extracts, supporting the role of *Spirulina* as a natural biostimulant capable of enhancing plant resilience to pathogen attack (Gonçalves, 2021; Thangaraj *et al*., 2022; Gharib and Ahmed, 2023; Rady *et al*., 2023; Kurpan *et al.,* 2023).

In summary, the application of *Spirulina platensis* extract especially through combined treatment effectively stimulates antioxidant enzyme activity in faba bean plants, contributing to improved defence capacity and reduced disease severity under pathogen pressure.

3.6.3. Disease incidence under greenhouse conditions

In greenhouse trials, *Spirulina platensis* extract showed a clear concentration-dependent reduction in the incidence of damping-off and root-rot diseases caused by *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. The most effective treatment was the combined method (seed soaking followed by soil drench) at 20 and 40%, which reduced disease incidence by 29.4 – 55.4% depending on the pathogen and concentration based on the data in the table 9.

Table 9: Effect of spirulina extract treatments at different concentrations (5-40%) on disease incidence (%) of root-rot and damping-off pathogens in faba bean under greenhouse conditions

Treatment type	Concentration (%)	*Fusarium oxysporum*	*Fusarium solani*	*Rhizoctonia solani*	*Macrophomina phaseolina*
Seed Soaking	5%	52.8^{c} (7%)	52.5^{c} (10%)	54.2^{c} (9%)	55.4^{c} (7%)
	10%	48.6^{c} (15%)	48.2^{c} (17%)	50.0^{c} (17%)	52.0^{c} (13%)
	20%	43.2^{b} (24%)	42.5^{b} (27%)	44.3^{b} (25%)	46.1^{b} (22%)
	40%	38.4^{ab} (32%)	37.9^{ab} (35%)	40.0^{ab} (32%)	41.3^{ab} (29%)
Soil Drench	5%	50.5^{c} (11%)	49.8^{c} (14%)	52.1^{c} (12%)	52.8^{c} (13%)
	10%	45.1^{c} (20%)	44.7^{c} (24%)	47.0^{b} (21%)	48.5^{b} (20%)
	20%	39.3^{ab} (30%)	38.7^{ab} (34%)	41.0^{ab} (30%)	42.2^{ab} (28%)
	40%	33.6^{a} (40%)	33.0^{a} (43%)	36.5^{a} (37%)	37.6^{a} (35%)
Combined	5%	49.0^{c} (14%)	48.4^{c} (16%)	50.1^{c} (15%)	51.7^{c} (14%)
	10%	43.2^{b} (24%)	42.3^{b} (28%)	45.5^{b} (21%)	46.8^{b} (22%)
	20%	36.7^{ab} (36%)	35.9^{ab} (40%)	39.3^{ab} (32%)	40.7^{ab} (30%)
	40%	29.4^{a} (48%)	29.5^{a} (49%)	32.7^{a} (43%)	33.2^{a} (44%)
Control	–	56.8^{d} (0%)	57.7^{d} (0%)	57.6^{d} (0%)	59.0^{d} (0%)

These reductions, although notable, were lower than those observed in laboratory assays, reflecting the more complex dynamics of greenhouse conditions. Among the individual application methods, soil drenching was more effective than seed soaking, especially at higher concentration (40%). This suggests that delivering the extract directly to the rhizosphere improves the availability of bioactive compounds,

enhancing their interaction with pathogens during early seedling development. This observation aligns with earlier studies that highlight the advantages of root-zone application in promoting plant defence responses and limiting fungal colonization. At 40% concentration, the combined application achieved the lowest disease incidence rates, ranging from 29.4% for *F. oxysporum* to 33.2% for *M. phaseolina*. However, no treatment achieved complete disease suppression, indicating that while the extract has strong potential. At lower concentrations (5% and 10%), the reduction in disease incidence was limited, suggesting that a minimum threshold of active compounds is necessary for consistent antifungal action (Montaser *et al*., 2016; Imara *et al*., 2021; Zaky and Ghebrial 2021; Bencheikh *et al*., 2022). Interestingly, *M. phaseolina* and *R. solani*, which showed moderate tolerance *in vitro*, responded more positively under greenhouse conditions. This shift may be due to induced plant resistance triggered by bioactive metabolites in *Spirulina*, such as phycocyanin, gallic acid, and β-carotene compounds known to stimulate systemic acquired resistance and mitigate oxidative stress in plant tissues (Gonçalves, 2021).

4. Conclusion

The findings of this study demonstrate that *Spirulina platensis* extract exhibits strong antifungal potential against key soilborne pathogens of faba bean, including *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. The *in vitro* experiments revealed a clear concentration-dependent inhibition of mycelial growth and spore germination, with the highest suppression recorded at 20 and 40% extract concentrations. Interestingly, although *R. solani* and *M. phaseolina* showed partial tolerance under laboratory conditions, they responded more favorably under greenhouse trials, suggesting a possible activation of plant defence responses triggered by compounds in the *Spirulina* extract.

Based on HPLC and GC-MS profiling, *Spirulina platensis* extract was found to contain a rich blend of bioactive compounds including phenolic acids, flavonoids, carotenoids, and fatty acids with proven antifungal properties. These compounds act through complementary mechanisms such as membrane disruption and oxidative stress induction, highlighting the extract's strong potential as a natural agent for seed treatment and biological control of plant pathogenic fungi.

Under greenhouse conditions, the combined application method (seed soaking and soil drenching) at 40% not only resulted in the greatest reduction in disease incidence by up to 49% but also led to measurable improvements in germination rate, seedling vigor, and antioxidant enzyme activity in infected plants.

Taken together, these results underscore the dual functionality of *Spirulina* extract as both a suppressor of fungal pathogens and a biostimulant that enhances the physiological resilience of host plants. Despite the promising outcomes, the more moderate effects observed under greenhouse conditions compared to *in vitro* assays highlight the complexity of real-world interactions between plant, pathogen, and soil environment.

In light of these findings, expanding the evaluation to include large-scale field trials across various agroecological zones is strongly recommended to validate the efficacy of *Spirulina* extract under different environmental and management conditions. Moreover, future studies should explore ways to enhance the formulation for greater stability and field persistence, investigate potential synergies with other biocontrol agents, and assess the economic viability of its application at commercial scale.

Ultimately, the outcomes of this research support the integration of *Spirulina platensis* extract into sustainable crop protection strategies, offering a promising, eco-friendly tool for managing soilborne diseases in legume cultivation systems.

References

Abdel-Raouf, N., A.A. Al-Homaidan and I.B.M. Ibraheem, 2012. Microalgae and cyanobacteria as green factories for biotechnological production. Saudi Journal of Biological Sciences, 19(3), pp. 257–275.

Aboody, M.S.A. and S. Mickymaray, 2020. Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics*, 9(2), 45. https://doi.org/10.3390/antibiotics9020045

Aebi, H., 1984. Catalase in vitro. Methods in Enzymology, 105, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Bencheikh, A., H. Langar, E. Chiaradia, B. Lorrain, and M. Trani, 2022. Evaluation of the antifungal activity of *Spirulina (Arthrospira) platensis* extract against three phytopathogenic fungi. Turkish Journal of Agriculture and Forestry, 46(3):212–219.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72, pp. 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chance, B. and A.C. Maehly, 1955. Assay of catalase and peroxidases. *Methods in Enzymology*, 2, pp. 764–775.

Chiara De Notaris, C., E. Elisabeth Enggrob, J.E. Olesen, P. Sørensen and J. Rasmussen, 2023. Faba bean productivity, yield stability and N_2-fixation in long-term organic and conventional crop rotations. Field Crops Research, 295, 108894. https://doi.org/10.1016/j.fcr.2023.108894

CLSI, Clinical and Laboratory Standards Institute, 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition (M38-A2). Wayne, PA: Clinical and Laboratory Standards Institute.

Crépon, K., P. Marget, C. Peyronnet, B. Carrouée, P. Arese, and G. Duc, 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Research, 115(3): 329–339.

de Amaral, S.C., V. Bulone, and B. Höfle, 2023. Cyanobacteria: A promising source of antifungal metabolites. Frontiers in Microbiology, 14, Article 10300848. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10300848/

Dineshkumar, R., R. Narendran & P. Sampathkumar, 2016. Cultivation of *Spirulina platensis* in different selective media. Indian Journal of Geo Marine Sciences, 45(12):1749-1754.

El-Mougy, N.S. and M.M. Abdel-Kader, 2019. Biocontrol measures against onion basal rot incidence under natural field conditions. Journal of Plant Pathology, 101, pp. 579–586.

El-Sayed, H., M. Rawway, N. Abed and R. Salah El Din, 2017. Antibacterial activity of phenolic extract of *Spirulina platensis* and its structural elucidation of bioactive compound. Egyptian Journal of Phycology, 18(1), pp. 45–57. https://doi.org/10.21608/egyjs.2017.115987

Emino, E.R. and P.R. Warman, 2004. Biological assay for compost quality. Compost Science and Utilization, 12(4), pp. 342–348. https://doi.org/10.1080/1065657X.2004.10702203

Ertani, A., S. Nardi, O. Francioso, S. Sanchez-Cortes, M. Di Foggia, and M. Schiavon, 2019. Effects of two protein hydrolysates obtained from chickpea (*Cicer arietinum* L.) and *Spirulina platensis* on *Zea mays* L. plants. Frontiers in Plant Science, 10, 954.

Gentscheva, G., V. Petrova, M. Atanassova, S. Taneva, and G. Nikolova, 2023. Application of *Arthrospira platensis* (Spirulina) for medicinal purposes and its antimicrobial potential. Life, 13(3), 845.

Gharib, F.A.E.L. and E.Z. Ahmed, 2023. *Spirulina platensis* improves growth, oil content, and antioxidant activity of rosemary plant under cadmium and lead stress. Scientific Reports, 13, 8008.: https://doi.org/10.1038/s41598-023-35063-1

Gheda, S., E.H.F. Abd El Zaher, A.M. Abou Zeid, N.A. Bedair, and L. Pereira, 2023. Potential activity of *Arthrospira platensis* as antioxidant, cytotoxic and antifungal against some skin diseases: topical cream application. Marine Drugs, 21(3), 160.

Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutases I: occurrence in higher plants. Plant Physiology, 59, pp. 309–314.

Gomez, K.A. and A.A. Gomez, 1984. *Statistical Procedures for Agricultural Research*. 2nd ed. New York: John Wiley & Sons.

Gonçalves, A.L., 2021. The use of microalgae and cyanobacteria in the improvement of agricultural practices: a review on their bio-fertilising, biostimulating and biopesticide roles. Applied Sciences, 11(2), 871.

Guimarães, A. and A. Venâncio, 2022. The Potential of Fatty Acids and Their Derivatives as Antifungal Agents: A Review. Toxins (Basel),3;14(3):188. doi: 10.3390/toxins14030188.

Hamad, G.M., N. Abd El-Baky, M.M. Sharaf, and A.A. Amara, 2023. Volatile compounds, fatty acids constituents, and antimicrobial activity of cultured *Spirulina (Arthrospira fusiformis)* isolated from Lake Mariout in Egypt. The Scientific World Journal, 2023, Article ID 9919814. https://doi.org/10.1155/2023/9919814

Hlima, H.B., R. Benhima, Y. Kasmi, L. Sbabou, and H. El Arroussi, 2019. Combined effect of *Spirulina platensis* and pomegranate peel extracts on antifungal activity. Applied Sciences, 9(24), 5475. https://doi.org/10.3390/app9245475

Ilieva, Y., M.M. Zaharieva, H. Najdenski, and A.D. Kroumov, 2024. Antimicrobial activity of *Arthrospira* (former *Spirulina*) and *Dunaliella* related to recognized antimicrobial bioactive compounds. International Journal of Molecular Sciences, 25, 5548. https://doi.org/10.3390/ijms25105548

Imara, D., W. Zaky, and E. Ghebrial, 2021. Performance of soil type, cyanobacterium *Spirulina platensis* and biofertilizers on controlling damping-off, root rot and wilt diseases of moringa (*Moringa oleifera* Lam.) in Egypt. Egyptian Journal of Phytopathology, 49(2), pp. 10–28. https://doi.org/10.21608/ejp.2021.79498.1036

Jensen, E.S., M.B. Peoples, and H. Hauggaard-Nielsen, 2010. Faba bean in cropping systems. Field Crops Research, 115(3): 203–216.

Joaquín Ramos, A.J., L.M. Rodríguez Alcalá, J. Rydlová, F. Hübner, C. García Pérez, and A.B. Martín Diana, 2020. Phenolic compounds, antioxidant and antifungal activity of three wild *Baccharis* species from Chile. Chilean Journal of Agricultural Research, 80(3):246–256. https://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-58392020000300352

Kurpan, D., A. Idà, F.G. Körner, M. Cuaresma, L. Magneschi, J.F. Sassi, J. Pruvost and M.R. Tredici, 2023. Long-term evaluation of productivity and harvesting efficiency of an industrial *Spirulina (Arthrospira platensis)* production facility. Bioresource Technology Reports, 25, 101741. https://doi.org/10.1016/j.biteb.2023.101741

Lahlali, R. and M. Hijri, 2022. Biological control of soil-borne plant pathogens: a global perspective. Microorganisms, 10(3), 596. https://doi.org/10.3390/microorganisms10030596

Lamichhane, J.R., E. Osdaghi, F. Behlau, J. Köhl, J.B. Jones, and J.N. Aubertot, 2016. Thirteen decades of antimicrobial copper compounds applied in agriculture: a review. Agronomy for Sustainable Development, 38(3), 28.

Lee, R.C., L.M. Farfan Cáceres, J.W. Debler, and R.A. Syme, 2020. Characterization of growth morphology and pathology, and draft genome sequencing of *Botrytis fabae*, the causal organism of chocolate spot of faba bean (*Vicia faba* L.). Frontiers in Microbiology, 11, 217.

Machu, L., K. Janda, B. Höfle and V. Bulone, 2015. Phenolic content and antioxidant capacity in algal food products. Molecules, 20(1):1118–1133.

McDonald, B.A. and E.H. Stukenbrock, 2016. Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. Philosophical Transactions of the Royal Society B, 371(1709), 20160026.

Meena, R.S., B.R. Maurya, J.P. Verma, and R. Lal, 2020. Impact of agrochemicals on soil microbiota and management. Land, 9(2), 34.

Mekonnen, G., 2020. Review on impact of seed-borne pathogens on seed quality. American Journal of Plant Biology, 5(1):77–81.

Mohan, S., B. Periyakali Saravana, J. Thanasekaran, S. Joen-Rong, M. Manubolu, and K. Pathakoti, 2019. In vitro efficacy of crude extracts of *Spirulina platensis* CCC 477 on *Rhizoctonia solani* and *Magnaporthe grisea*. International Journal of Agriculture and Animal Sciences, 10(1): 59–64. https://soeagra.com/iaast/iast_march2019/10.pdf

Mohy El Din, S., 2020. Evaluation of different biological activities of *Spirulina platensis* extracts. Egyptian Journal of Botany, 60(1): 249–259.

Montaser, F.A., M.M. Mazen, and M.A.M. Atwa, 2016. Effect of cyanobacteria on reducing damping-off and root rot incidence in lupine plants, New Valley Governorate, Egypt. Microbiology Research Journal International, 16(2), pp. 1–14. https://doi.org/10.9734/BMRJ/2016/27883

Neme, A., 2023. Seedborne mycoflora of faba bean (*Vicia faba* L.) and screening of some safe alternatives to chemical fungicides. Egyptian Journal of Biological Pest Control, 33, Article 63. https://pmc.ncbi.nlm.nih.gov/articles/PMC10293731/

Oliveira, M.S., V. Feddern, L. Kupski, E.Cipolatti, E. Badiale-Furlong and L.A. Souza-Soares, 2010. Physico-chemical characterization of fermented rice bran biomass. *Ciência y Tecnologia* Alimentaria, 8, pp. 229–236.

Ozdemir, G., N. Ulku Karabay, M.C. Dalay, and B. Pazarbasi, 2004. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytotherapy Research, 18(9): 754–757. https://doi.org/10.1002/ptr.1541

Paraskevopoulou, A., T. Kaloudis, A. Hiskia, M. Steinhaus, D. Dimotikali, and T.M. Triantis, 2024. Volatile profiling of spirulina food supplements. Foods, 13(8), 1257. https://doi.org/10.3390/foods13081257

Pimentel, D. and M. Burgess, 2014. Environmental and economic costs of the application of pesticides primarily in the United States. In: Integrated Pest Management. Dordrecht: Springer, pp. 47–71.

Pitt, J.I. and A.D. Hocking, 2009. Fungi and Food Spoilage. 3rd ed. New York: Springer.

Rady, M.M., A.S. Elrys, E. Selem, A.A.A. Mohsen, S.M.A.I. Arnaout, A.H. El Sappah, K.A. El Tarabily, and E.S.M. Desoky, 2023. *Spirulina platensis* extract improves the production and defences of the common bean grown in a heavy metals contaminated saline soil. Journal of Environmental Sciences, 129, pp. 240–257. https://doi.org/10.1016/j.jes.2022.09.011

Raman, D., D. Bose, D. Venkatesan, D. Narayan, D. Krishnan, and D. Savithri, 2023. *Spirulina platensis* as an antifungal agent: a prospective in vitro analysis. In: *Proceedings of the 3rd International Electronic Conference on Antibiotics*, 1–15 December 2023. Basel: MDPI. https://sciforum.net/paper/view/16418

Sahile, S., S. Ahmed, C. Fininsa, M.M. Abang, and P.K. Sakhuja, 2008. Survey of chocolate spot (*Botrytis fabae*) disease of faba bean (*Vicia faba* L.) and assessment of factors influencing disease epidemics in northern Ethiopia. Crop Protection, 27, pp. 1457–1463.

Shyamala, M., S.P. Ranjan, and J.V. Sharma, 2013. Pharmaceutical applications of inverse gas chromatography. International Journal of Pharmaceutical Sciences, 3(2):201–204.

Šišić, A., M. Šurlan Milovanović, M. Zlatić, M. Trkulja, P. Marin, M. Spasić and D. Lemić, 2022. Farming system effects on root rot pathogen complex and microbial communities in organic pea and faba bean. Plants, 11(10), 1352.

Souza, M.M. de, L. Prietto, A.C. Ribeiro, T. Denardi de Souza and E. Badiale-Furlong, 2011. Assessment of the antifungal activity of *Spirulina platensis* phenolic extract against *Aspergillus flavus*. Ciência e Agrotecnologia, 35(6):1050–1058.

Stoddard, F.L., A.H. Nicholas, D. Rubiales, J. Thomas and A.M. Villegas-Fernández, 2010. Integrated pest management in faba bean. Field Crops Research, 115(3):308–318. https://doi.org/10.1016/j.fcr.2009.07.002

Tantawy, S.T.A., 2011. Biological potential of cyanobacterial metabolites against some soil pathogenic fungi. Journal of Food, Agriculture and Environment, 9(1):663–666.

Thangaraj, M.M., B. Periyakali Saravana, J. Thanasekaran, S. Joen-Rong, M. Manubolu and K. Pathakoti, 2022. Phytochemicals of algae *Arthrospira platensis* (spirulina), *Chlorella vulgaris* (chlorella) and *Azolla pinnata* (azolla). GSC Biological and Pharmaceutical Sciences, 19(2):023–043.

Toribio, A.J., M.M. Jurado, F. Suárez-Estrella, M.J. López, J.A. López-González, and J. Moreno, 2021. Seed biopriming with cyanobacterial extracts as an eco-friendly strategy to control damping-off caused by *Pythium ultimum* in seedbeds. Microbiological Research, 248, 126766. https://doi.org/10.1016/j.micres.2021.126766

Yesim, E.R., 2023. In vitro and in vivo antifungal activity of microalgal extracts against seed-borne phytopathogenic fungi. Plant Pathology and Disease Journal, 8(4):231–245.

You, M.P., B.B. Eshete, S.A. Kemal, J.A.G. van Leur, and M.J. Barbetti, 2021. *Physoderma*, not *Olpidium*, is the true cause of faba bean gall disease of *Vicia faba* in Ethiopia. Plant Pathology, 70, pp. 1180–1194.

Zaky, I.D.A., W.H. and E.W.R. Ghebrial, 2021. Performance of soil type, cyanobacterium *Spirulina platensis* and biofertilizers on controlling damping-off, root rot and wilt diseases of moringa (*Moringa oleifera* Lam.) in Egypt. Egyptian Journal of Phytopathology, 49(2):10–28. https://doi.org/10.21608/ejp.2021.79498.1036

Zamani, N., M. Fazilati, H. Salavati and M. Koohi Dehkordi, 2020. Evaluation of antifungal activity of purified phycocyanin from *Spirulina platensis* cultured in Iran against Candida albicans. Experimental Animal Biology, 8(4):57–66.

Zayed, A., H.M. El-Seadawy, E.Z. Attia, I.M. Rushdi, and U.R. Abdelmohsen, 2024. Adopting biorefinery and a circular bioeconomy for extracting and isolating natural products from marine algae. Frontiers in Natural Products, 3, 1425242. https://doi.org/10.3389/fntpr.2024.1425242.