

EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND RHIZOBIUM CO-INOCULATION ON DISEASE RESISTANCE AND PHYSIOLOGICAL RESPONSES OF FABA BEAN (*VICIA FABA* L.) TO *BOTRYTIS FABAE*

ABEER HASHEM¹, MAHA ABDULLAH ALAKEEL^{1*}, TURKI M. S. DAWOUD¹ AND ELSAYED FATHI ABDALLAH²

¹Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box. 2455, Riyadh 11451, Saudi Arabia

²Department of Plant Production, College of Food & Agriculture Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia

*Corresponding author's email: 441203162@student.ksu.edu.sa

Abstract

Chocolate spot, caused by *Botrytis fabae* Sard., is a major foliar disease of faba bean (*Vicia faba* L.). A pot experiment evaluated the individual and combined effects of arbuscular mycorrhizal fungi (AMF) and *Rhizobium leguminosarum* bv. *viciae* on disease development, nodulation, pigments, osmolytes, oxidative stress markers and leaf minerals of faba bean infected with *B. fabae*. The experiment was a 2 × 2 × 2 factorial (AMF × *Rhizobium* × pathogen) in a completely randomized design with three replicates. Data were analysed by factorial analysis of variance, and means were separated by Duncan's multiple range test ($p \leq 0.05$). AMF alone reduced lesion diameter by 56.4% and disease severity by 91.4%, whereas *Rhizobium* alone reduced them by 34.5% and 22.8%, respectively. Co-inoculation produced the smallest lesions (64.5% reduction), but its effect on disease severity (92.3% reduction) did not differ significantly from that of AMF alone. Infection reduced nodule number by 88.0%, total chlorophyll by 52.4%, total soluble sugars by 70.7% and leaf K⁺, Mg²⁺, Ca²⁺ and P by 47.0 to 53.8%, and increased H₂O₂ and malondialdehyde (MDA) by 75.4% and 61.6%. In infected plants, AMF increased nodule number almost fivefold, total chlorophyll by 53.9% and leaf K⁺ by 65.1%, and lowered H₂O₂ and MDA by 28.7% and 20.9%. AMF had a much larger main effect than *Rhizobium* on most traits, and the benefit of *Rhizobium* depended on AMF status and infection. AMF-based inoculants, alone or with *Rhizobium*, show promise for managing chocolate spot, but field validation is needed.

Key words: Arbuscular mycorrhizal fungi; Chocolate spot; Co-inoculation; Induced systemic resistance; Oxidative stress; *Rhizobium leguminosarum* bv. *Viciae*; *Vicia faba*

Introduction

Plants interact with diverse microbial communities that include both beneficial and pathogenic microorganisms, and some of these microbes help to maintain plant stability and performance under stress (Pathak *et al.*, 2022). The rhizosphere is regarded as a hotspot of microbial activity. Roots release large amounts of exudates, such as amino acids, lipids, organic acids and flavonoids, which act as signaling molecules during plant-microbe interactions (Solanki *et al.*, 2023). These interactions may be symbiotic, commensal or pathogenic. In symbiotic associations, the microbial partner supplies nutrients such as nitrogen and phosphorus to the host, and the plant in return provides carbon and a habitat (Xue *et al.*, 2024a, 2024b). Arbuscular mycorrhizal fungi (AMF) are an ancient and widely studied fungal group that forms symbiotic associations with about 80% of known plant species (Slimani *et al.*, 2024). The AMF life cycle begins with spore germination, followed by hyphal growth and the formation of hyphopodia, the structures through which the fungus enters the root (Bahadur *et al.*, 2019).

Inside the root, the hyphae spread through the cortex and form tree-like arbuscules and/or vesicles (Qiu *et al.*, 2024). Arbuscules are the main site of nutrient exchange

between the plant and the fungus, and their presence in the cortex modifies host physiology and helps the plant to cope with environmental stresses (Wang, 2017; Medeiros *et al.*, 2021). Rhizobia are Gram-negative bacteria that form nitrogen-fixing symbioses with legumes such as pea, bean and clover (Wang, 2019), and the legume-rhizobia symbiosis is one of the most intensively studied plant-microbe associations (Xie *et al.*, 2020).

Faba bean (*Vicia faba* L.) is one of the most important grain legumes worldwide and ranks fourth after pea, chickpea and lentil. Its seeds contain 24 to 30% protein together with other minerals and nutrients (Akinyi *et al.*, 2022). Faba bean is one of the oldest cultivated crops and is grown throughout the world, originally in cool regions where the climate was unsuitable for soybean. Its storage proteins are mainly globulins, which are low in sulphur-containing amino acids but rich in lysine (Martineau-Côté *et al.*, 2024). Through biological nitrogen fixation, faba bean also improves soil fertility and structure and benefits crop production (Bogale *et al.*, 2022). However, large yield losses occur every year because of chocolate spot, a foliar disease caused by *Botrytis fabae*, which is one of the most common and destructive pathogens of this crop. A recent survey in Morocco documented the current status of chocolate spot and the phenotypic variability of *B. fabae* populations (Aouzal *et al.*, 2025).

AMF and rhizobia are both symbiotically associated with legumes and play important roles in the uptake of nutrients, such as nitrogen and phosphorus, and water. Their combined use has frequently been reported to improve crop productivity, and this synergy arises from their complementary roles in nutrient acquisition and stress tolerance. Rhizobia provide the plant with readily available nitrogen, while AMF improve the uptake of phosphorus and micronutrients. The two symbionts also benefit each other: AMF colonization can increase the nitrogen-fixing capacity of rhizobia in root nodules, and rhizobial inoculation can improve root colonization by AMF (Hashem *et al.*, 2018; Xie *et al.*, 2020).

AMF and rhizobia, used singly or together, are increasingly applied as biocontrol agents for plant disease management. Inoculation with either symbiont can strengthen plant defence against pathogens by activating defence enzymes, inducing systemic resistance and stimulating the production of secondary metabolites (Pozo & Azcón-Aguilar, 2007). Several studies have reported the role of AMF (Weng *et al.*, 2022) and rhizobia (Das *et al.*, 2017) in reducing pathogen infection (Akhtar *et al.*, 2011). AMF suppress pathogens through several mechanisms, the most common being the production of secondary metabolites, competition for nutrients and space, induced systemic resistance and the induction of defence proteins (Weng *et al.*, 2022).

Although the benefits of AMF and rhizobia for legume nutrition are well documented, less is known about how the two symbionts, alone and in combination, influence the response of faba bean to the foliar pathogen *B. fabae*. The present study therefore evaluated the individual and combined effects of AMF and *Rhizobium leguminosarum* bv. *viciae* on chocolate spot development in faba bean, and on nodulation, leghemoglobin, biological nitrogen fixation, photosynthetic pigments, total soluble sugars, total amino acids, proline, glycine betaine, H_2O_2 , malondialdehyde (MDA) and leaf mineral content of infected and non-infected plants. A factorial design was used so that the effect of each symbiont and their interaction could be separated.

Materials and Methods

Plant material and microorganisms: Certified seeds of faba bean (*Vicia faba* L. cv. Giza 40) were obtained from the Agricultural Research Centre (ARC), Giza, Egypt. A mixture of three AM fungi, namely *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*), *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Funneliformis mosseae* (syn. *Glomus mosseae*), previously isolated from Saudi soil and identified by Hashem *et al.*, (2016), was used in this study. Trap-culture soil containing approximately $750 \text{ spores g}^{-1}$ was used as the AMF inoculum, and soil without inoculum served as the non-mycorrhizal control. A pure, serologically characterized Egyptian strain of *Rhizobium leguminosarum* bv. *viciae* was obtained from ARC, Giza, Egypt. The strain was grown in tryptic soy broth (TSB) for 48 h, and the culture was centrifuged at 1600 rpm for 10 min. The pellet was washed with phosphate-buffered saline, and the suspension was adjusted to $10^8 \text{ cells ml}^{-1}$; each pot received 10 ml of this suspension. A virulent strain of *Botrytis fabae* was

kindly provided by Prof. Dr. Abeer Hashem, Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Growth conditions and experimental design: The pot experiment was carried out in the growth chamber of the Plant Production Department, Faculty of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. The soil was a loamy sand with the following properties: sand 85.9%, clay 8.8%, silt 5.3%, organic carbon 0.14%, total nitrogen 0.004% and pH 7.6. Pots containing sterilized soil without any AMF or *Rhizobium* inoculum served as controls. The experiment was laid out as a $2 \times 2 \times 2$ factorial in a completely randomized design, with AMF (with or without), *Rhizobium* (with or without) and *B. fabae* (infected or non-infected) as factors, giving eight treatments: (1) control; (2) *B. fabae*; (3) *B. fabae* + *Rhizobium*; (4) *Rhizobium*; (5) AMF; (6) *B. fabae* + AMF; (7) *B. fabae* + *Rhizobium* + AMF; and (8) *Rhizobium* + AMF. Each treatment had three biological replicates, and each replicate consisted of ten pots. One seed was sown per pot, and plants were grown for 12 weeks after germination under controlled temperature and watered regularly. A spore suspension of *B. fabae* ($2.5 \times 10^5 \text{ spores ml}^{-1}$) was sprayed onto 45-day-old plants (Derckel *et al.*, 1999), which were then covered with polyethylene bags for 48 h to maintain high humidity. Non-infected plants were sprayed with sterile distilled water only. At harvest, roots were carefully removed to collect nodules for the estimation of nodulation and nodule activity, and leaves from each treatment were collected for chemical and biochemical analyses. Disease was assessed on leaf samples by measuring lesion diameter and calculating the percentage severity index according to Alemayehu *et al.*, (2024).

Nodulation, leghemoglobin and nitrogen fixation: Nodule number per plant was determined following Abd Allah *et al.*, (2015). Leghemoglobin in root nodules was quantified by the method of Keilin & Wang (1945), as described in detail by Hashem *et al.*, (2014). Total nitrogen was estimated by the micro-Kjeldahl method (Allen, 1953), and biological nitrogen fixation was calculated according to Delgado *et al.*, (1994) and expressed as mg N plant^{-1} .

Photosynthetic pigments: Chlorophyll a (Chl a) and chlorophyll b (Chl b) were extracted in 80% (v/v) aqueous acetone and determined according to Arnon (1949) and Porra & Scheer (2019). The absorbance of the supernatant was read at 663, 645 and 470 nm with a spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan).

Hydrogen peroxide: H_2O_2 content was determined following Alexieva *et al.*, (2001), and the absorbance of the reaction mixture was recorded at 390 nm with a spectrophotometer (Shimadzu, Tokyo, Japan).

Malondialdehyde: MDA content was estimated according to Dhindsa *et al.*, (1981) and Rasouli *et al.*, (2023).

Glycine betaine: Glycine betaine (GB) was determined spectrophotometrically at 365 nm against a standard curve prepared with known concentrations of GB, following Grieve & Grattan (1983).

Total soluble sugars, total free amino acids and proline:

Total soluble sugars (TSS) were extracted and determined as described by Irigoyen *et al.*, (1992). Total free amino acids (TAA) were estimated according to Lee and Takahashi (1966), and proline was measured spectrophotometrically at 520 nm following Bates *et al.*, (1973) and Rasouli *et al.*, (2023).

Mineral elements: Oven-dried leaf samples were ground to a fine powder and digested with 98% H₂SO₄ and 30% H₂O₂. Total nitrogen was determined by the Kjeldahl procedure, and phosphorus (P) by the vanadomolybdophosphoric colorimetric method (Bremner & Mulvaney, 1982). K⁺ was estimated following Page *et al.*, (1982), and Mg²⁺ and Ca²⁺ as described in detail by Abd_Allah *et al.*, (2015).

Statistical analysis: Each treatment had three biological replicates (n = 3), and data are presented as mean ± standard error (SE). Data were subjected to three-way analysis of variance (ANOVA) with AMF, *Rhizobium* and *B. fabae* as fixed factors, to test the main effect of each factor and all interactions. Disease parameters, which were recorded only on infected plants, were analysed by two-way ANOVA (AMF × *Rhizobium*), and nodulation parameters by two-way ANOVA (AMF × *B. fabae*). Treatment means were compared by Duncan's multiple range test using the pooled error mean square of the ANOVA, and differences were considered significant at p ≤ 0.05. Percentage changes are expressed relative to the non-inoculated, non-infected control unless stated otherwise. Analyses were performed with SPSS version 21 (IBM Corp., Armonk, NY, USA).

Results

Chocolate spot development: All infected plants developed chocolate spot lesions, but both symbionts significantly reduced disease development (Table 1). AMF had a much stronger effect than *Rhizobium* (F = 557.7 vs 135.1 for lesion diameter and F = 1284.9 vs 27.9 for disease severity), and the AMF × *Rhizobium* interaction was significant for both traits (p < 0.01). Compared with plants infected with *B. fabae* alone, inoculation with *Rhizobium* reduced lesion diameter by 34.5% and disease severity by 22.8%, whereas AMF reduced them by 56.4% and 91.4%, respectively. Co-inoculation with AMF and *Rhizobium* produced the smallest lesions (14.5 mm, a 64.5% reduction), which were significantly smaller than those on plants inoculated with AMF alone. However, disease severity in co-inoculated plants (1.93%) did not differ significantly from that in plants inoculated with AMF alone (2.15%). The additional benefit of *Rhizobium* was therefore clear in non-mycorrhizal plants but small in mycorrhizal plants.

Nodulation, leghemoglobin and nitrogen fixation: In non-mycorrhizal plants, *B. fabae* infection sharply reduced nodulation and nitrogen fixation, decreasing nodule number by 88.0%, total nitrogen content by 72.8%, leghemoglobin by 75.7% and biological nitrogen fixation

by 69.0% compared with the control (Table 2). AMF inoculation significantly increased all four traits in both healthy and infected plants. In healthy plants, AMF increased nodule number by 73.8%, total nitrogen by 31.3%, leghemoglobin by 71.8% and biological nitrogen fixation by 52.7%. In infected plants, AMF raised nodule number from 5.0 to 24.3 per plant and total nitrogen from 10.7 to 38.6 mg N plant⁻¹, and the latter value did not differ significantly from that of the healthy non-mycorrhizal control. A significant AMF × *B. fabae* interaction was found only for total nitrogen content (p < 0.001), indicating that AMF was particularly effective in maintaining the nitrogen status of infected plants.

Photosynthetic pigments: Infection with *B. fabae* was the main factor affecting chlorophyll content (Table 3). In non-mycorrhizal plants, infection reduced Chl a by 54.1%, Chl b by 43.2% and total chlorophyll (Chl a+b) by 52.4% compared with the control. AMF significantly increased Chl a and total chlorophyll (p < 0.001), and its effect was largest in infected plants, in which Chl a and total chlorophyll were 59.5% and 53.9% higher than in infected non-mycorrhizal plants. *Rhizobium* also had a significant but smaller positive effect: it increased total chlorophyll by 10.7% in healthy plants and limited the infection-induced loss to 39.0%. The highest Chl a and total chlorophyll contents were recorded in healthy co-inoculated plants, 20.3% and 23.5% above the control. In infected plants, however, co-inoculation did not increase total chlorophyll over AMF alone, and Chl a was lower than with AMF alone. The significant three-way interaction for Chl a and total chlorophyll (p ≤ 0.008) reflects this dependence of the *Rhizobium* effect on AMF status and infection. Chl b was significantly affected only by infection (p < 0.001), and the Chl a/b ratio showed no consistent treatment pattern.

Total soluble sugars, total amino acids and proline:

Infection reduced total soluble sugars (TSS) by 70.7% and total amino acids (TAA) by 43.7% in non-mycorrhizal plants (Table 4). Of the two symbionts, AMF had the stronger effect on both traits (TSS: F = 1416.0 for AMF vs 170.6 for *Rhizobium*; TAA: F = 315.9 vs 70.4; p < 0.001; Table 5). Mycorrhizal plants contained more TSS and TAA than the corresponding non-mycorrhizal plants in every treatment, and the difference was greatest under infection, where AMF increased TSS by 141.4% and TAA by 36.7%. *Rhizobium* also increased TSS and TAA in healthy plants (by 11.4% and 13.6%) and in infected plants. Infected co-inoculated plants had significantly higher TSS and TAA than infected plants that received either symbiont alone, and their TSS content was only 6.1% below that of the control. Proline responded differently. Infection increased proline almost fourfold in non-mycorrhizal plants (294.6%), and the increase was even greater with *Rhizobium* (352.8%), AMF (396.0%) and co-inoculation (489.1%). All three main effects and all two-way interactions were significant for proline (p < 0.001; Table 5). In healthy plants, proline did not change significantly with *Rhizobium* alone (6.6%) and increased by 45.5% with co-inoculation.

Hydrogen peroxide, malondialdehyde and glycine betaine: *B. fabae* infection increased H_2O_2 by 75.4% and MDA by 61.6% in non-mycorrhizal plants compared with the control (Table 6). AMF lowered H_2O_2 and MDA in all treatments, by 18.2 to 28.7% and 13.4 to 29.6%, respectively, relative to the corresponding non-mycorrhizal plants, and *Rhizobium* also reduced both markers; the main effects of AMF, *Rhizobium* and *B. fabae* were significant for both traits ($p < 0.001$; Table 5). Among infected plants, H_2O_2 was lowest after co-inoculation (91.49 nmol g⁻¹ FW), 33.9% lower than in plants infected with *B. fabae* alone, whereas MDA did not differ significantly between co-inoculated and AMF-inoculated plants. The lowest H_2O_2 content was found in healthy co-inoculated plants (35.5% below the control), and the lowest MDA contents in healthy mycorrhizal plants with or without *Rhizobium* (29.6 to 32.9% below the control). Glycine betaine (GB) accumulated in response to infection and increased by 63.9% in non-mycorrhizal plants. Inoculation reduced this accumulation: in infected plants, GB increased by 44.8% with *Rhizobium*, 29.8% with AMF and 25.1% with co-inoculation, which is reflected in the significant AMF $\times$ *B. fabae* interaction ($F = 82.4$, $p < 0.001$). In healthy plants, GB did not differ significantly among treatments.

Mineral elements: Leaf K^+ , Mg^{2+} , Ca^{2+} and P contents decreased markedly after infection. In non-mycorrhizal plants, the reductions were 53.8%, 47.0%, 52.6% and 52.1%, respectively, compared with the control (Table 7). AMF significantly increased all four elements, and its main

effect was much larger than that of *Rhizobium* ($F = 907.2$ to 1608.7 for AMF vs 49.9 to 331.8 for *Rhizobium*; $p < 0.001$; Table 5). The benefit of AMF was greatest in infected plants, in which K^+ , Mg^{2+} , Ca^{2+} and P were 65.1%, 33.1%, 46.7% and 58.2% higher than in infected non-mycorrhizal plants, and the AMF $\times$ *B. fabae* interaction was significant for all four elements ($p \leq 0.012$). *Rhizobium* limited the losses of all four elements in infected non-mycorrhizal plants, and in infected mycorrhizal plants it further increased K^+ , Mg^{2+} and Ca^{2+} . The highest contents of all four elements were found in healthy co-inoculated plants, with increases of 23.2% (K^+), 32.3% (Mg^{2+}), 9.4% (Ca^{2+}) and 45.9% (P) over the control. Phosphorus, however, responded differently from the cations: *Rhizobium* alone reduced leaf P by 36.8% in healthy plants, and in infected mycorrhizal plants the addition of *Rhizobium* lowered P from 167.6 to 144.6 $\mu\text{g g}^{-1}$ DW.

Overview of treatment effects: Factorial ANOVA showed that AMF, *Rhizobium* and *B. fabae* each had significant main effects on most of the measured traits, and significant interactions among the factors were found for several traits (Table 5). For every trait in which both symbionts were tested, except the Chl a/b ratio, the F value for AMF was larger than that for *Rhizobium*, showing that AMF was the main contributor to the treatment effects, whereas the effect of *Rhizobium* was smaller and often depended on AMF status and infection. The results support the potential of AMF and rhizobium as complementary microbial inputs for improving faba bean performance under pathogen stress (Fig. 1).

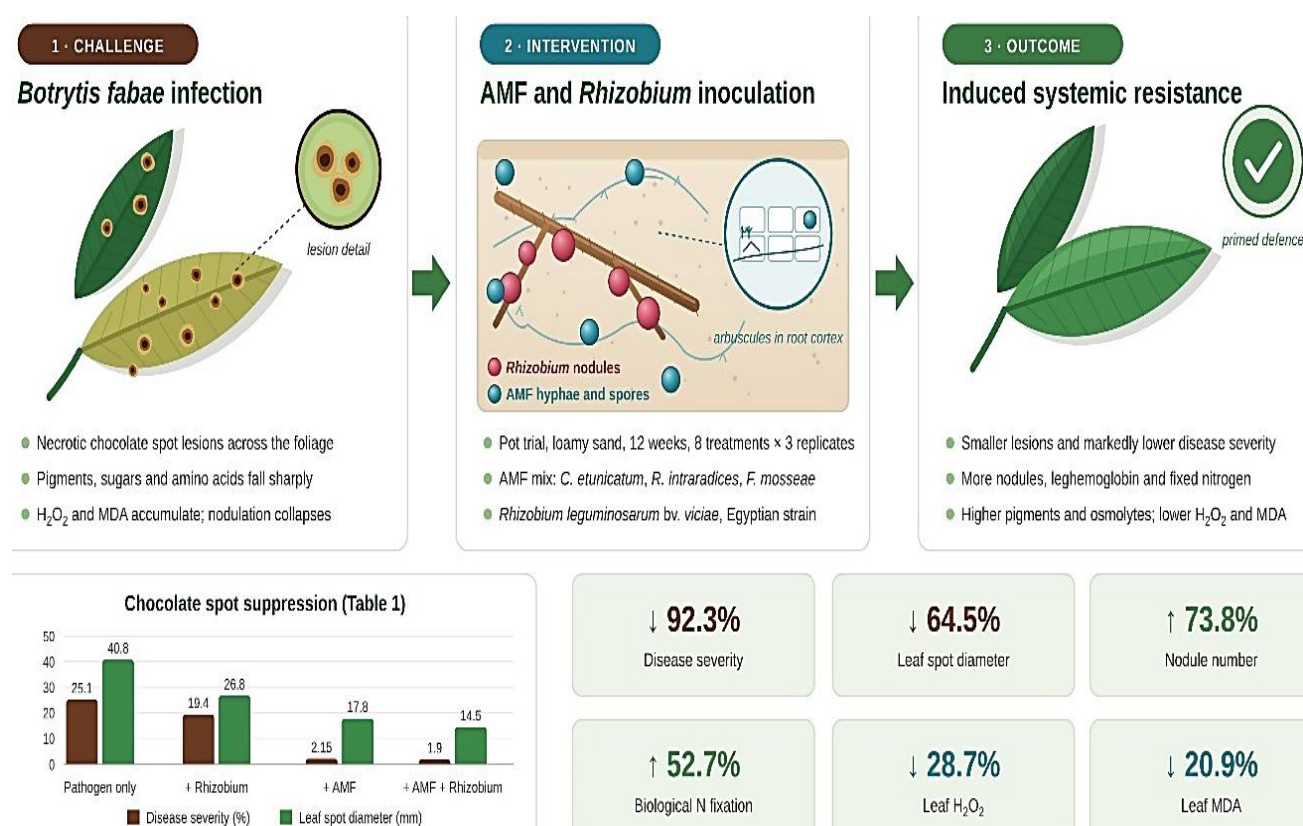


Fig. 1. A conceptual model illustrating the synergistic role of AM fungi and *R. leguminosarum* induce significant resistance against infection of *B. fabae* in faba bean.

Table 1. Effect of AMF and *Rhizobium leguminosarum* bv. *viciae* inoculation on chocolate spot (leaf spot diameter and disease severity) in faba bean infected with *Botrytis fabae*.

Treatments	Without <i>Rhizobium</i>		With <i>Rhizobium</i>	
	Leaf spot diameter (mm)	Disease severity (%)	Leaf spot diameter (mm)	Disease severity (%)
Plant + <i>B. fabae</i>	40.83 ± 0.69 ^a	25.10 ± 0.54 ^a	26.76 ± 0.96 ^b	19.37 ± 0.44 ^b
Plant + <i>B. fabae</i> + AMF	17.80 ± 0.73 ^c	2.15 ± 0.88 ^c	14.50 ± 0.55 ^d	1.93 ± 0.10 ^c

Data are mean ± SE (n = 3). Within each parameter, means followed by different letters differ significantly at p ≤ 0.05 according to Duncan's multiple range test; comparisons were made across all treatments shown for that parameter. Results of the factorial ANOVA are given in Table 5

Table 2. Effect of AMF inoculation on nodulation, total nitrogen, leghemoglobin and biological nitrogen fixation in faba bean under *Botrytis fabae* stress.

Treatments	Without AM fungi				With AM fungi			
	Nod. No.	TNC	Leg.	Biol NF	Nod. No.	TNC	Leg.	Biol NF
Plant (control)	41.6 ± 2.72 ^b	39.3 ± 0.89 ^b	10.30 ± 0.37 ^b	57.7 ± 2.40 ^b	72.3 ± 5.23 ^a	51.6 ± 0.90 ^a	17.70 ± 0.23 ^a	88.1 ± 1.53 ^a
Plant + <i>B. fabae</i>	5.0 ± 1.15 ^d	10.7 ± 0.77 ^c	2.50 ± 0.06 ^d	17.9 ± 0.54 ^d	24.3 ± 1.20 ^c	38.6 ± 0.82 ^b	8.81 ± 0.67 ^c	44.5 ± 0.70 ^c

Nod. No.: nodules (number plant⁻¹); TNC: total nitrogen content (mg N plant⁻¹); Leg.: leghemoglobin (mg g⁻¹ fresh weight); Biol NF: biological nitrogen fixation (mg N plant⁻¹). Data are mean ± SE (n = 3). Within each parameter, means followed by different letters differ significantly at p ≤ 0.05 according to Duncan's multiple range test; comparisons were made across all treatments shown for that parameter. Results of the factorial ANOVA are given in Table 5

Table 3. Effect of AMF and *Rhizobium leguminosarum* bv. *viciae* inoculation on photosynthetic pigments (μmol m⁻²) in faba bean under *Botrytis fabae* stress.

Treatments	Without AM fungi				With AM fungi			
	Chl a	Chl b	Chl a/b	Chl a+b	Chl a	Chl b	Chl a/b	Chl a+b
Plant (control)	1.49 ± 0.020 ^c	0.37 ± 0.023 ^{abc}	3.98 ± 0.18 ^a	1.87 ± 0.040 ^c	1.63 ± 0.020 ^b	0.50 ± 0.020 ^a	3.26 ± 0.09 ^b	2.13 ± 0.040 ^b
Plant + <i>B. fabae</i>	0.68 ± 0.020 ^f	0.21 ± 0.014 ^c	3.32 ± 0.11 ^b	0.89 ± 0.040 ^f	1.09 ± 0.062 ^d	0.29 ± 0.010 ^{bc}	3.83 ± 0.35 ^{ab}	1.37 ± 0.050 ^d
Plant + <i>B. fabae</i> + <i>Rhizobium</i>	0.88 ± 0.017 ^e	0.26 ± 0.011 ^c	3.39 ± 0.21 ^{ab}	1.14 ± 0.006 ^e	0.94 ± 0.010 ^c	0.38 ± 0.170 ^{abc}	2.49 ± 0.11 ^c	1.32 ± 0.020 ^d
Plant + <i>Rhizobium</i>	1.59 ± 0.020 ^b	0.48 ± 0.020 ^{ab}	3.36 ± 0.16 ^b	2.07 ± 0.020 ^b	1.79 ± 0.020 ^a	0.52 ± 0.010 ^a	3.43 ± 0.13 ^{ab}	2.31 ± 0.020 ^a

Data are mean ± SE (n = 3). Within each parameter, means followed by different letters differ significantly at p ≤ 0.05 according to Duncan's multiple range test; comparisons were made across all treatments shown for that parameter. Results of the factorial ANOVA are given in Table 5

Table 4. Effect of AMF and *Rhizobium leguminosarum* bv. *viciae* inoculation on total soluble sugars (TSS, mg g⁻¹ DW), total amino acids (TAA, mg g⁻¹ DW) and proline (mg g⁻¹ DW) in faba bean under *Botrytis fabae* stress.

Treatments	Without AM fungi			With AM fungi		
	TSS	TAA	Proline	TSS	TAA	Proline
Plant (control)	69.62 ± 1.25 ^c	22.73 ± 0.75 ^c	2.02 ± 0.070 ^g	95.92 ± 1.23 ^a	29.45 ± 0.36 ^a	2.42 ± 0.020 ^f
Plant + <i>B. fabae</i>	20.37 ± 0.78 ^g	12.79 ± 0.42 ^g	7.97 ± 0.078 ^d	49.17 ± 1.19 ^e	17.48 ± 0.51 ^e	10.02 ± 0.080 ^b
Plant + <i>B. fabae</i> + <i>Rhizobium</i>	31.50 ± 0.83 ^f	15.70 ± 0.23 ^f	9.15 ± 0.160 ^c	65.34 ± 0.91 ^d	20.13 ± 0.15 ^d	11.90 ± 0.070 ^a
Plant + <i>Rhizobium</i>	77.55 ± 1.35 ^b	25.83 ± 0.11 ^b	2.15 ± 0.038 ^g	99.01 ± 0.41 ^a	30.45 ± 0.33 ^a	2.94 ± 0.060 ^e

Data are mean ± SE (n = 3). Within each parameter, means followed by different letters differ significantly at p ≤ 0.05 according to Duncan's multiple range test; comparisons were made across all treatments shown for that parameter. Results of the factorial ANOVA are given in Table 5

Table 5. F values from the factorial analysis of variance for the effects of AMF, *Rhizobium* and *Botrytis fabae* on the measured traits of faba bean.

Parameter	Source of variation						
	AMF	<i>Rhizobium</i>	<i>B. fabae</i>	AMF × <i>Rhizobium</i>	AMF × <i>B. fabae</i>	<i>Rhizobium</i> × <i>B. fabae</i>	AMF × <i>Rhizobium</i> × <i>B. fabae</i>
Leaf spot diameter	557.7***	135.1***	n.a.	51.9***	n.a.	n.a.	n.a.
Disease severity	1284.9***	27.9***	n.a.	23.9**	n.a.	n.a.	n.a.
Nodule number	66.6***	n.a.	190.8***	n.a.	3.5 ns	n.a.	n.a.
Total nitrogen	563.6***	n.a.	603.5***	n.a.	84.9***	n.a.	n.a.
Leghemoglobin	292.6***	n.a.	433.7***	n.a.	1.8 ns	n.a.	n.a.
Biological N fixation	365.8***	n.a.	783.1***	n.a.	1.6 ns	n.a.	n.a.
Chl a	105.4***	15.3**	1364.9***	12.8**	2.7 ns	8.0*	26.0***
Chl b	4.5 ns	2.4 ns	17.3***	0.1 ns	0.0 ns	0.0 ns	0.5 ns
Chl a/b	4.0 ns	10.8**	3.7 ns	1.4 ns	0.2 ns	2.5 ns	17.7***
Chl a+b	157.6***	39.4***	1569.3***	12.0**	3.0 ns	3.8 ns	9.2**
Total soluble sugars	1416.0***	170.6***	3587.3***	0.0 ns	25.7***	30.8***	11.3**
Total amino acids	315.9***	70.4***	1354.2***	4.2 ns	3.7 ns	1.6 ns	2.6 ns
Proline	673.0***	258.0***	16322.1***	22.3***	245.3***	108.3***	1.9 ns
H ₂ O ₂	571.8***	172.5***	2004.9***	23.3***	33.5***	12.8**	22.2***
MDA	244.2***	60.1***	1457.5***	24.8***	2.3 ns	0.2 ns	0.0 ns
Glycine betaine	38.4***	12.7**	453.8***	1.9 ns	82.4***	10.1**	7.4*
K ⁺	907.2***	136.0***	3927.2***	5.4*	100.0***	1.4 ns	24.1***
Mg ²⁺	1608.7***	331.8***	7841.7***	6.3*	8.0*	6.0*	8.3*
Ca ²⁺	917.6***	221.6***	7162.4***	0.4 ns	215.5***	114.4***	1.5 ns
P	1234.4***	49.9***	2019.9***	30.5***	353.0***	38.4***	192.3***

*, ** and *** indicate significance at p ≤ 0.05, 0.01 and 0.001, respectively; ns, not significant; n.a., not applicable. Disease parameters were recorded only on infected plants and were analysed by two-way ANOVA (AMF × *Rhizobium*); nodulation parameters were analysed by two-way ANOVA (AMF × *B. fabae*); all other parameters were analysed by three-way ANOVA. Error degrees of freedom were 8 for two-way and 16 for three-way analyses

Table 6. Effect of AMF and *Rhizobium leguminosarum* bv. *viciae* inoculation on hydrogen peroxide (H₂O₂, nmol g⁻¹ FW), malondialdehyde (MDA, nmol g⁻¹ FW) and glycine betaine (GB, μmol g⁻¹ FW) in faba bean under *Botrytis fabae* stress.

Treatments	Without AM fungi			With AM fungi		
	H ₂ O ₂	MDA	GB	H ₂ O ₂	MDA	GB
Plant (control)	78.91 ± 1.88 ^e	99.15 ± 2.44 ^d	42.93 ± 1.67 ^d	60.46 ± 1.50 ^g	69.80 ± 0.76 ^f	46.12 ± 1.21 ^d
Plant + <i>B. fabae</i>	138.42 ± 1.27 ^a	160.27 ± 1.83 ^a	70.37 ± 1.06 ^a	98.74 ± 1.23 ^c	126.70 ± 2.37 ^c	55.71 ± 0.65 ^c
Plant + <i>B. fabae</i> + <i>Rhizobium</i>	111.88 ± 2.04 ^b	140.30 ± 1.46 ^b	62.16 ± 1.21 ^b	91.49 ± 0.99 ^d	121.54 ± 3.18 ^c	53.72 ± 1.34 ^c
Plant + <i>Rhizobium</i>	69.12 ± 1.06 ^f	80.24 ± 2.17 ^e	43.66 ± 0.28 ^d	50.91 ± 1.09 ^h	66.49 ± 2.20 ^f	44.82 ± 0.10 ^d

Data are mean ± SE (n = 3). Within each parameter, means followed by different letters differ significantly at p ≤ 0.05 according to Duncan's multiple range test; comparisons were made across all treatments shown for that parameter. Results of the factorial ANOVA are given in Table 5

Table 7. Effect of AMF and *Rhizobium leguminosarum* bv. *viciae* inoculation on leaf mineral elements (μg g⁻¹ DW) in faba bean under *Botrytis fabae* stress.

Treatments	Without AM fungi				With AM fungi			
	K ⁺	Mg ²⁺	Ca ²⁺	P	K ⁺	Mg ²⁺	Ca ²⁺	P
Plant (control)	235.33±4.61 ^d	1290.54±10.97 ^d	838.19±9.41 ^c	221.32±5.06 ^c	256.68±2.95 ^b	1598.42±8.33 ^b	897.80±7.30 ^b	307.50±7.13 ^b
Plant + <i>B. fabae</i>	108.66±1.73 ^h	684.47±3.58 ^h	397.72±4.16 ^g	105.97±2.24 ^g	179.45±1.69 ^f	911.05±7.32 ^f	583.30±5.60 ^e	167.60±2.31 ^d
Plant + <i>B. fabae</i> + <i>Rhizobium</i>	130.34±1.40 ^g	793.02±4.60 ^g	506.52±5.14 ^f	124.64±1.72 ^f	192.57±2.20 ^e	1096.20±5.54 ^e	677.10±4.27 ^d	144.60±0.42 ^c
Plant + <i>Rhizobium</i>	244.60±0.77 ^c	1405.28±12.28 ^c	852.57±2.06 ^c	139.84±0.65 ^c	289.95±0.88 ^a	1707.80±18.69 ^a	916.60±3.39 ^a	322.80±3.05 ^a

Data are mean ± SE (n = 3). Within each parameter, means followed by different letters differ significantly at p ≤ 0.05 according to Duncan's multiple range test; comparisons were made across all treatments shown for that parameter. Results of the factorial ANOVA are given in Table 5

Discussion

The use of beneficial microorganisms, alone or as consortia, is gaining attention as a way to improve crop productivity and reduce the reliance on agrochemicals. Consortia of bacteria and fungi are often preferred over single inoculants because they can act through several mechanisms at once, combining pathogen suppression with improved nutrient supply (Nunes *et al.*, 2024). The present study shows that AMF and *R. leguminosarum* bv. *viciae* both reduced chocolate spot in faba bean, but their contributions were unequal. AMF alone reduced disease severity by more than 90%, *Rhizobium* alone gave a modest reduction, and co-inoculation further reduced lesion size without lowering disease severity significantly below the level achieved by AMF alone. For most physiological traits, the main effect of AMF was much larger than that of *Rhizobium*, and the effect of *Rhizobium* depended on AMF status and infection. The following discussion therefore treats AMF as the main driver of the response, with *Rhizobium* acting as a complementary partner.

The strong suppression of a foliar pathogen by a root symbiont point to a systemic effect, because the AMF were confined to the roots while *B. fabae* was applied to the leaves. Similar protection has been reported in faba bean, in which AMF enhanced resistance to Fusarium wilt (Dong *et al.*, 2019) and rhizobial inoculation reduced root rot (Wang *et al.*, 2018). Mycorrhiza-induced resistance is usually explained by the priming of jasmonate-dependent defences, improved nutrition and changes in host metabolism (Pozo & Azcón-Aguilar, 2007; Weng *et al.*, 2022). A recent review likewise notes that AMF can trigger plant immune responses to pathogen attack and that induced systemic resistance operates mainly through jasmonic acid and ethylene signaling, although its effectiveness depends strongly on environmental and crop conditions (Charpe *et al.*, 2025). In the present study, lower disease was accompanied by better nutrient status, higher chlorophyll and sugar contents and lower oxidative damage, which is consistent with these explanations. However, defence-related enzymes, hormones and gene expression were not measured, so the involvement of

induced systemic resistance should be regarded as likely rather than proven.

Infection by *B. fabae* reduced nodule number, leghemoglobin and nitrogen fixation by 69 to 88%, showing that a foliar pathogen can severely restrict the root symbiosis, probably by limiting the supply of photosynthates to the nodules. AMF largely counteracted this effect and kept the total nitrogen content of infected plants at the level of the healthy control. Comparable increases in nodule number, leghemoglobin and nitrogenase activity after AMF and rhizobial inoculation of faba bean were reported by Abd-Alla *et al.*, (2014), and similar stimulation of nodulation and nitrogen accumulation was observed in *Phaseolus vulgaris* (Razakatiana *et al.*, 2020) and *Melilotus alba* (Hack *et al.*, 2019). Leghemoglobin regulates the oxygen supply to bacteroids and is essential for nitrogenase activity (Coba de la Pena *et al.*, 2018), so its higher content in mycorrhizal plants is consistent with their greater nitrogen fixation. An adequate nitrogen supply also supports the synthesis of defence-related compounds (Sun *et al.*, 2020), which may partly explain the lower disease severity in mycorrhizal plants.

Chlorophyll loss is a typical consequence of chocolate spot, as necrotic lesions reduce the functional leaf area. AMF limited this loss, and the highest chlorophyll contents were found in healthy co-inoculated plants. Higher chlorophyll in mycorrhizal plants can be related to better uptake of N and Mg, both of which are constituents of the chlorophyll molecule (Ishfaq *et al.*, 2022). Similar increases in photosynthetic pigments after AMF inoculation were reported in *Leymus chinensis* (Lin *et al.*, 2017) and *Vigna radiata* (Kumar *et al.*, 2021), and after co-inoculation with *Rhizobium laguerreae* and AMF in faba bean (Pereira *et al.*, 2019). In our study, however, co-inoculation did not raise chlorophyll above the level of AMF alone in infected plants, which suggests that under infection the two symbionts may compete for host carbon rather than act synergistically on the photosynthetic apparatus.

Soluble sugars provide energy and carbon skeletons for defence and also act as signals that induce defence genes (Bolouri-Moghaddam & van den Ende, 2012). *B. fabae* depleted leaf sugars by about 70%, whereas mycorrhizal and especially co-inoculated plants retained

much higher TSS. Increased soluble sugar content has been reported in mycorrhizal soybean (Moradi & Younesi, 2014) and in soybean co-inoculated with AMF and rhizobia (Ding *et al.*, 2016). The higher TAA content in inoculated plants probably reflects their improved nitrogen supply, in agreement with the higher protein content reported after co-inoculation with rhizobia and AMF in *Vigna unguiculata* (Pereira *et al.*, 2020) and after rhizobial inoculation in faba bean (Sánchez-Navarro *et al.*, 2020). Proline acts as an osmoprotectant, antioxidant and signalling molecule under stress (Hayat *et al.*, 2012). In the present study, proline accumulation was driven mainly by infection and was highest in infected co-inoculated plants, in line with the reports of Ashrafi *et al.*, (2014) in alfalfa and Garg and Singh (2018) in *Cajanus cajan*. Because these plants also had the lowest disease severity, their higher proline is more likely to reflect a stronger protective response than greater damage, although this interpretation needs further confirmation.

H₂O₂ acts as a signal that activates defence genes at low concentrations, but its excessive accumulation causes oxidative damage (Sahu *et al.*, 2022; Gupta *et al.*, 2023). MDA, a product of lipid peroxidation, is a widely used marker of membrane damage (Gawel *et al.*, 2004). Both markers increased after infection and were consistently lower in mycorrhizal plants, indicating that AMF reduced oxidative damage in faba bean leaves. Lower MDA after AMF inoculation has been reported in soybean (Moradi & Younesi, 2014), and after co-inoculation with AMF and rhizobia in *Sesbania cannabina* (Ren *et al.*, 2016) and *Medicago sativa* (Laouane *et al.*, 2019). AMF can also modulate H₂O₂-mediated signalling, as shown by the increased phenolic synthesis in clover roots through H₂O₂, salicylic acid and nitric oxide pathways (Zhang *et al.*, 2013). Glycine betaine is a compatible solute best known for its role in tolerance to abiotic stresses (Giri, 2011). Here, GB accumulated in response to infection, and this accumulation was smaller in inoculated plants. GB therefore appears to indicate the intensity of stress rather than to be a mechanism through which AMF and *Rhizobium* improved disease tolerance.

Pathogen infection impairs root development, nutrient uptake and translocation, and poor nutrient status in turn weakens cell walls and membranes and increases susceptibility to disease (Graham & Webb, 1991; Huber & Graham, 1999). Huber & Graham (1999) reported lower K, Ca and Mg concentrations after infection by *Fusarium oxysporum* f. sp. *vasinfectum*, and a similar decline was observed in the present study. AMF markedly increased leaf K⁺, Mg²⁺, Ca²⁺ and P, especially in infected plants. Potassium supports protein synthesis and cell wall thickening, which can restrict pathogen entry (Huber & Graham, 1999); magnesium is required for chlorophyll synthesis and enzyme activation (Ishfaq *et al.*, 2022); and calcium stabilizes membranes and the middle lamella, limiting the leakage of sugars and amino acids that pathogens exploit. Phosphorus supports energy metabolism and root growth, and its effect on disease resistance is most evident in early growth stages (Király, 1976). Our results agree with those of Abd-Alla *et al.*, (2014), who reported higher K, Fe and P in faba bean inoculated with AMF and rhizobia, and with the increases in mineral nutrients reported in *P. vulgaris* (Razakatiana *et al.*, 2020), *M. alba* (Hack *et al.*, 2019) and soybean (Thioub *et al.*, 2019). Similarly, Yilmaz (2025) reported that inoculation of faba

bean with AMF and plant growth-promoting rhizobacteria improved plant growth, biochemical constituents and mineral nutrient contents. The lower leaf P in plants inoculated with *Rhizobium* alone may reflect the high P demand of nodules and nitrogen fixation, which can draw P from the shoot when AMF are absent.

Some limitations of this study should be noted. The experiment was conducted in pots under controlled conditions, with a single faba bean cultivar and one pathogen isolate, so the results need to be confirmed in the field and with other genotypes. AMF root colonization was not quantified, and defence-related enzymes, hormones and gene expression were not measured, so the mechanisms proposed here remain to be tested directly. In addition, nodulation traits were analysed only for the AMF and *B. fabae* treatments, so the effect of *Rhizobium* inoculation on nodulation could not be separated.

Conclusion

Arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. *viciae* both reduced chocolate spot caused by *B. fabae* in faba bean, but AMF was the main contributor. AMF alone reduced disease severity by more than 90%, maintained nodulation and nitrogen status, limited the losses of chlorophyll and sugars, increased leaf K⁺, Mg²⁺, Ca²⁺ and P, and lowered H₂O₂ and MDA in infected plants. *Rhizobium* provided a smaller benefit. Co-inoculation gave the smallest lesions and the highest sugar and amino acid contents in infected plants, although it did not reduce disease severity significantly more than AMF alone. These findings support the use of AMF-based inoculants, alone or combined with compatible rhizobia, in the integrated management of chocolate spot in faba bean. Field trials across cultivars and environments, together with studies on defence signaling, are recommended before practical recommendations are made.

Acknowledgement

The authors sincerely acknowledge the support provided through the Ongoing Research Funding program (ORF-2026-356), King Saud University, Riyadh, Saudi Arabia.

Funding: The authors are sincerely thankful for the support provided through the Ongoing Research Funding program (ORF-2026-356), King Saud University, Riyadh, Saudi Arabia.

Author's Contributions: Conceptualization: A.H., M.A.A.; methodology, data collection, original data analysis and data presentation: A.H., M.A.A., E.F.A.; writing, reviewing and editing: A.H., M.A.A., T.M.S.D. and E.F.A. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper and have approved the final version of the manuscript.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Abd_Allah, E.F., A. Hashem, A.A. Alqarawi, A.H. Bahkali and M.S. Alwhibi. 2015. Enhancing growth performance and systemic acquired resistance of medicinal plant *Sesbania sesban* (L.) Merr using arbuscular mycorrhizal fungi under salt stress. *Saudi J. Biol. Sci.*, 22(3): 274-283. <https://doi.org/10.1016/j.sjbs.2015.03.004>
- Abd-Alla, M.H., A.W.E. El-Enany, N.A. Nafady, D.M. Khalaf and F.M. Morsy. 2014. Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. *Microbiol. Res.*, 169(1): 49-58.
- Akhtar, M.S., Z.A. Siddiqui and A. Wiemken. 2011. Arbuscular mycorrhizal fungi and *Rhizobium* to control plant fungal diseases. In: (Ed.): Lichtfouse, E. Alternative farming systems, biotechnology, drought stress and ecological fertilisation. Sustainable Agriculture Reviews. Vol. 6. 263-292. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-0186-1_9
- Akinyi, D.P., S. Karanja Ng'ang'a, M. Ngigi, M. Mathenge and E. Givet. 2022. Cost-benefit analysis of prioritized climate-smart agricultural practices among smallholder farmers: Evidence from selected value chains across sub-Saharan Africa. *Heliyon*, 8(4): e09228. <https://doi.org/10.1016/j.heliyon.2022.e09228>
- Alemayehu, T.Y., K. Amare, D. Belay and H. Abebe. 2024. Faba bean (*Vicia faba* L.) variety evaluation for disease resistance, yield, and agronomic traits in South Gondar, Ethiopia. *Int. J. Agron.*, 1-10. <https://doi.org/10.1155/2024/5490629>
- Alexieva, V., I. Sergiev, S. Mapelli and E. Karanov. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.*, 24(12): 1337-1344.
- Allen, M.B. 1953. Experiments in Soil Bacteriology, 1st Edn. Minneapolis: Burgess Pub. Co.
- Aouzal, S., L. Zemat, F.Z. Ibn El Mokhtar, H. Houmairi, S. Khayi, R. Mentag and S. Krimi Bencheqroun. 2025. Chocolate spot of faba bean (*Vicia faba* L.): disease status and pathogen phenotypic variability in Morocco. *Arch. Phytopathol. Plant Prot.*, 58(15): 846-864. <https://doi.org/10.1080/03235408.2025.2542584>
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24, 1. doi: 10.1104/24.1.1.
- Ashrafi, E., M. Zahedi and J. Razmjoo. 2014. Co-inoculations of arbuscular mycorrhizal fungi and rhizobia under salinity in alfalfa. *Soil Sci. Plant Nutr.*, 60(5): 619-629.
- Bahadur, A., A. Batool, F. Nasir, S. Jiang, Q. Mingsen, Q. Zhang, J. Pan, Y. Liu and H. Feng. 2019. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. *Int. J. Mol. Sci.*, 20(17): 4199.
- Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39(1): 205-207.
- Bogale, G.A., M. Mengesha and G. Hadgu. 2022. Characterization of local climate and its impact on faba bean (*Vicia faba* L.) yield in Central Ethiopia. *Adv. Meteorol.*, 2022: 8759596. <https://doi.org/10.1155/2022/8759596>
- Bolouri-Moghaddam, M.R. and W. Van den Ende. 2012. Sugars and plant innate immunity. *J. Exp. Bot.*, 63(11): 3989-3998. <https://doi.org/10.1093/jxb/ers129>
- Bremner, J.M. and C.S. Mulvaney. 1982. Nitrogen-total. In: (Eds.): Page, A.L., R.H. Miller and D.R. Keeney. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9, American Society of Agronomy, Madison, WI, USA, pp. 595-624. <https://doi.org/10.2134/agronmonogr9.2.2ed.c31>
- Charpe, A.M., B. Aglave and D.K. Ghosh. 2025. Microbial-mediated induced resistance: interactive effects for improving crop health. *Front. Microbiol.*, 16: 1660944. <https://doi.org/10.3389/fmicb.2025.1660944>
- Coba de la Pena, T., E. Fedorova, J.J. Pueyo and M.M. Lucas. 2018. The symbiosome: legume and rhizobia co-evolution toward a nitrogen-fixing organelle?. *Front. Plant Sci.*, 8: 2229. doi: 10.3389/fpls.2017.02229
- Das, K., R. Prasanna and A.K. Saxena. 2017. Rhizobia: A potential biocontrol agent for soilborne fungal pathogens. *Folia Microbiol.*, 62: 425-435.
- Delgado, M.J., F. Ligerio and C. Lluch. 1994. Effects of salt stress on growth and nitrogen fixation by pea, faba bean, common bean, and soybean plants. *Soil Biol. Biochem.*, 26: 371-376.
- Derckel, J.P., F. Baillieul, S. Manteau, J.C. Audran, B. Haye, B. Lambert and L. Legendre. 1999. Differential induction of grapevine defenses by two strains of *Botrytis cinerea*. *Phytopathology*, 89(3): 197-203. <https://doi.org/10.1094/PHYTO.1999.89.3.197>
- Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 32: 93-101. doi: 10.1093/jxb/32.1.93.
- Ding, X., S. Zhang, R. Wang, S. Li and X. Liao. 2016. AM fungi and rhizobium regulate nodule growth, phosphorous (P) uptake, and soluble sugar concentration of soybeans experiencing P deficiency. *J. Plant Nutr.*, 39(13): 1915-1925.
- Dong, Y., S. Zhao, J.X. Lv and K. Dong. 2019. Synergistic effects of intercropping with wheat and inoculation with arbuscular mycorrhizal fungi on improvement of anti-fusarium wilt and rhizosphere microbial carbon metabolic activity of faba bean. *J. Plant Nutr. Fert.*, 25(10): 1646-1656. doi: 10.11674/zwyf.18400.
- Garg, N. and S. Singh. 2018. Arbuscular mycorrhiza *Rhizophagus irregularis* and silicon modulate growth, proline biosynthesis and yield in *Cajanus cajan* L. Millsp. (Pigeonpea) genotypes under cadmium and zinc stress. *J. Plant Growth Reg.*, 37: 46-63.
- Gawel, S., M. Wardas, E. Niedworok and P. Wardas. 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiadomosci lekarskie (Warsaw, Poland)*. 57(9-10): 453-455.
- Giri, J. 2011. Glycinebetaine and abiotic stress tolerance in plants. *Plant Signal. Behav.*, 6(11): 1746-1751.
- Graham, R.D. and M.J. Webb. 1991. *Micronutrients* and disease resistance and tolerance in plants. In: (Eds.): Mortvedt, J.J., F.R. Cox, L.M. Shuman and R.M. Welch. *Micronutrients in Agriculture*. 2nd Ed. Soil Science Society of America, Madison, Wisconsin, USA, pp. 329-370. <https://doi.org/10.2136/sssabookser4.2ed.c10>
- Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70(2): 303-307.
- Gupta, A., A.N. Singh, R.K. Tiwari, P.K. Sahu, J. Yadav, A.K. Srivastava and S. Kumar. 2023. Salinity alleviation and reduction in oxidative stress by endophytic and rhizospheric microbes in two rice cultivars. *Plants*, 12(5): 976.
- Hack, C.M., M. Porta, R. Schäufele and A.A. Grimoldi. 2019. Arbuscular mycorrhiza mediated effects on growth, mineral nutrition and biological nitrogen fixation of *Melilotus alba* Med. in a subtropical grassland soil. *Appl. Soil Ecol.*, 134: 38-44. <https://doi.org/10.1016/j.apsoil.2018.10.008>
- Hashem, A., E.F. Abd_Allah, A.A. Alqarawi, G. El-Didamony, M.S. Alwhibi, D. Egamberdieva and P. Ahmad. 2014. Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. *Pak. J. Bot.*, 46(6): 2003-2013.
- Hashem, A., E.F. Abd_Allah, A.A. Alqarawi, A.A. Al-Huqail, S. Wirth and D. Egamberdieva. 2016. The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. *Front. Microbiol.*, 7: 1089. <https://doi.org/10.3389/fmicb.2016.01089>

- Hashem, A., A.A. Alqarawi, R. Radhakrishnan, A.B.F. Al-Arjani, H.A. Aldehaish, D. Egamberdieva and E.F. Abd Allah. 2018. Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. *Saudi J. Biol. Sci.*, 25(6): 1102-1114. <https://doi.org/10.1016/j.sjbs.2018.03.009>
- Hayat, S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel and A. Ahmad. 2012. Role of proline under changing environments: a review. *Plant Signal. Behav.*, 7(11): 1456-1466.
- Huber, D.M. and R.D. Graham. 1999. The role of nutrition in crop resistance and tolerance to disease. In: (Ed.): Z. Rengel. *Mineral Nutrition of Crops: Fundamental Mechanisms and Implications*. Food Products Press, New York, pp. 205-226.
- Irigoyen, J.J., D.W. Einerich and M. Sanchez-Diaz. 1992. Water stress induced changes in the concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant*, 84(1): 55-60. <https://doi.org/10.1111/j.1399-3054.1992.tb08764.x>
- Ishfaq, M., Y. Wang, M. Yan, Z. Wang, L. Wu, C. Li and X. Li. 2022. Physiological essence of magnesium in plants and its widespread deficiency in the farming system of China. *Front. Plant Sci.*, 13: 802274.
- Keilin, D. and Y.L. Wang. 1945. Haemoglobin in the root nodules of leguminous plants. *Nature*, 155: 227-229.
- Kiraly, Z. 1976. Plant disease resistance as influenced by biochemical effects of nutrients and fertilizers. In: *Fertilizer Use and Plant Health. Proceedings of the 12th Colloquium of the International Potash Institute, Atlanta, GA*, pp. 33-46.
- Kumar, A., D. Kumar Yadav and R. Ulla. 2021. Role of arbuscular mycorrhizal fungi on plant growth and photosynthetic pigments in *Vigna radiata*. *Mycopath*, 18(2): 77-83.
- Laouane, B., A. Meddich, N. Bechtaoui, K. Oufdou and S. Wahbi. 2019. Effects of arbuscular mycorrhizal fungi and rhizobia symbiosis on the tolerance of *Medicago sativa* to salt stress. *Gesunde Pflanzen*, 71(2): 135-146.
- Lee, Y.P. and T. Takahashi. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.*, 14: 71-77.
- Lin, J., Y. Wang, S. Sun, C. Mu and X. Yan. 2017. Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of *Leymus chinensis* seedlings under salt-alkali stress and nitrogen deposition. *Sci. Total Environ.*, 576: 234-241. <https://doi.org/10.1016/j.scitotenv.2016.10.091>
- Martineau-Côté, D., L. L'Hocine, F. Tuccillo, J.P. Wanasundara and F.L. Stoddard. 2024. Faba Bean as a Sustainable Plant Protein Source. In *Sustainable Protein Sources*. Academic Press. Cambridge USA. 163-184. <https://doi.org/10.1016/B978-0-323-91652-3.00001-0>.
- Medeiros, A.S., B.T. Goto and G. Ganade. 2021. Ecological restoration methods influence the structure of arbuscular mycorrhizal fungal communities in degraded drylands. *Pedobiologia*, 84: p.150690.
- Moradi, A. and O. Younesi. 2014. Influence of arbuscular mycorrhiza on membrane lipid peroxidation and soluble sugar content of soybean under salt stress. *Agric. Conspec. Sci.*, 79(4): 227-232.
- Nunes, P.S., G.V.L. Junior, G.M. Mascarin, R.A. Guimarães, F.H. Medeiros, S. Arthurs and W. Bettiol. 2024. Microbial consortium of biological products: do they have a future?. *Biol. Control*, 105439. <https://doi.org/10.1016/j.biocontrol.2024.105439>
- Page, A.L., R.H. Miller and D.R. Keeney (Eds.). (1982). Methods of soil analysis. Part 2. Chemical and microbiological properties (pp. 1159-pp).
- Pathak, P., V.K. Rai, H. Can, S.K. Singh, D. Kumar, N. Bhardwaj, R. Roychowdhury, L.C.B. de Azevedo, Kaushalendra, H. Verma and A. Kumar. 2022. Plant-endophyte interaction during biotic stress management. *Plants*, 11(17): 2203.
- Pereira, S., Â. Mucha, B. Gonçalves, E. Bacelar, A. Látr, H. Ferreira, I. Oliveira, E. Rosa and G. Marques. 2019. Improvement of some growth and yield parameters of faba bean (*Vicia faba*) by inoculation with *Rhizobium laguerreae* and arbuscular mycorrhizal fungi. *Crop Pasture Sci.*, 70(7): 595-605. <https://doi.org/10.1071/CP19016>
- Pereira, S., S. Singh, R.S. Oliveira, L. Ferreira, E. Rosa and G. Marques. 2020. Co-inoculation with rhizobia and mycorrhizal fungi increases yield and crude protein content of cowpea (*Vigna unguiculata* (L.) Walp.) under drought stress. *Landbauforschung J. Sust. Org. Agric. Syst.*, 70: 56-65.
- Porra, R.J. and H. Scheer. 2019. Towards a more accurate future for chlorophyll a and b determinations: the inaccuracies of Daniel Arnon's assay. *Photosynth. Res.*, 140(2): 215-219.
- Pozo, M.J. and C. Azcón-Aguilar. 2007. Unraveling mycorrhiza-induced resistance. *Curr. Opin. Plant Biol.*, 10(4): 393-398. <https://doi.org/10.1016/j.pbi.2007.05.004>
- Qiu, T., J. Peñuelas, Y. Chen, J. Sardans, J. Yu, Z. Xu, Q. Cui, J. Liu, Y. Cui, S. Zhao and J. Chen. 2024. Arbuscular mycorrhizal fungal interactions bridge the support of root-associated microbiota for slope multifunctionality in an erosion-prone ecosystem. *iMeta*, p.e187.
- Rasouli, F., T. Amini, S. Skrovankova, M. Asadi, M.B. Hassanpouraghdam, S. Ercisli, M. Buckova, M. Mrazkova and J. Mlcek. 2023. Influence of drought stress and mycorrhizal (*Funneliformis mosseae*) symbiosis on growth parameters, chlorophyll fluorescence, antioxidant activity, and essential oil composition of summer savory (*Satureja hortensis* L.) plants. *Front. Plant Sci.*, 14: p. 1151467.
- Razakatiana, A.T.E., J. Trap, R.H. Baohanta, M. Raherimandimby, C. Le Roux, R. Duponnois, H. Ramanankierana and T. Becquer. 2020. Benefits of dual inoculation with arbuscular mycorrhizal fungi and rhizobia on *Phaseolus vulgaris* planted in a low-fertility tropical soil. *Pedobiologia*, 83: p.150685.
- Ren, C.G., Y.J. Bai, C.C. Kong, B. Bian and Z.H. Xie. 2016. Synergistic interactions between salt-tolerant rhizobia and arbuscular mycorrhizal fungi on salinity tolerance of *Sesbania cannabina* plants. *J. Plant Growth Regul.*, 35: 1098-1107. <https://doi.org/10.1007/s00344-016-9607-0>
- Sahu, P.K., K. Jayalakshmi, J. Tilgam, A. Gupta, Y. Nagaraju, A. Kumar, S. Hamid, H.V. Singh, T. Minkina, V.D. Rajput and M.V.S. Rajawat. 2022. ROS generated from biotic stress: Effects on plants and alleviation by endophytic microbes. *Front. Plant Sci.*, 13: p.1042936.
- Sánchez-Navarro, V., R. Zornoza, Á. Faz, C. Egea-Gilabert, M. Ros, J.A. Pascual and J.A. Fernández. 2020. Inoculation with different nitrogen-fixing bacteria and Arbuscular mycorrhiza affects grain protein content and nodule bacterial communities of a fava bean crop. *Agronomy* 10(6), p.768.
- Slimani, A., M. Ait-El-Mokhtar, R. Ben-Laouane, A. Boutasknit, M. Anli, E.F. Abouraicha, K. Oufdou, A. Meddich and M. Baslam. 2024. Molecular and Systems Biology Approaches for Harnessing the Symbiotic Interaction in Mycorrhizal Symbiosis for Grain and Oil Crop Cultivation. *Int. J. Mol. Sci.*, 25(2): p.912.
- Solanki, M.K., N.C. Joshi, P.K. Singh, S.K. Singh, G. Santoyo, L.C.B. de Azevedo and A. Kumar. 2023. From concept to reality: Transforming agriculture through innovative rhizosphere engineering for plant health and productivity. *Microbiol. Res.*, 127553.
- Sun, Y., M. Wang, L.A.J. Mur, Q. Shen and S. Guo. 2020. Unravelling the roles of nitrogen nutrition in plant disease defences. *Int. J. Mol. Sci.*, 21(2): 572.
- Thioub, M., N. Ewusi-Mensah, J. Sarkodie-Addo and T. Adjei-Gyapong. 2019. Arbuscular mycorrhizal fungi inoculation enhances phosphorus use efficiency and soybean productivity on a Haplic Acrisol. *Soil Tillage Res.*, 192: 174-186.

- Wang, E.T. 2019. Symbiosis between rhizobia and legumes. In: (Eds.): Wang, E.T., C.F. Tian, W.F. Chen, J.P.W. Young and W.X. Chen. *Ecology and Evolution of Rhizobia: Principles and applications*, Springer, Singapore. pp.3-19. https://doi.org/10.1007/978-981-32-9555-1_1
- Wang, F. 2017. Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: Mechanisms and applications. *Crit. Rev. Environ. Sci. Technol.*, 47(20): 1901-1957.
- Wang, J.H., Z.L. Nie, Y.Q. Xu and Y. Zheng. 2018. The inoculation effect of rhizobia on *Vicia faba* growth and its disease resistance with root rot. *J. Southwest Forestry University*. 38 (4): 94-99. doi: 10.11929/j.issn.2095-1914.2018.04.015.
- Weng, W., J. Yan, M. Zhou, X. Yao, A. Gao, C. Ma, J. Cheng and J. Ruan. 2022. Roles of arbuscular mycorrhizal fungi as a biocontrol agent in the control of plant diseases. *Microorganisms*, 10(7): p. 1266.
- Xie, M.M., Y.N. Zou, Q.S. Wu, Z.Z. Zhang and K. Kuča. 2020. Single or dual inoculation of arbuscular mycorrhizal fungi and rhizobia regulates plant growth and nitrogen acquisition in white clover. *Plant, Soil and Environment*, 2020, 66(6): 287-294. DOI: 10.17221/234/2020-PSE
- Xue, J., L. Guo, L. Li, Z. Zhang, M. Huang, J. Cai, X. Wang, Y. Zhong, T. Dai, D. Jiang and Q. Zhou. 2024a. Effects of arbuscular mycorrhizal fungi on uptake, partitioning and use efficiency of nitrogen in wheat. *Field Crops Res.*, 306: p. 109244. <https://doi.org/10.1016/j.fcr.2023.109244>
- Xue, S., L. Kui, R. Sharifi and J. Chen. 2024b. The role of microbiome in sustainable agriculture. *Front. Microbiol.*, 15: p.1388926. <https://doi.org/10.3389/fmicb.2024.1388926>
- Yilmaz, H. 2025. Enhancements in morphology, biochemicals, nutrients, and L-Dopa in faba bean through plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi. *Sci. Rep.*, 15: 7390. <https://doi.org/10.1038/s41598-025-92486-8>
- Zhang, R.Q., H.H. Zhu, H.Q. Zhao and Q. Yao. 2013. Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signalling pathways. *J. Plant Physiol.*, 170(1): 74-79. <https://doi.org/10.1016/j.jplph.2012.08.022>