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Evaluation of the induced resistance in wheat using *Azolla pinnata*, *Bacillus velezensis* with the mycorrhizal fungus *Rhizophagus intraradices* in the control of root rot disease caused by the fungus Fusarium culmorum.

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Abstract

Six bacterial isolates, labeled B1 to B6, were isolated from healthy wheat plants grown in the wheat field at the Agricultural Research Station, College of Agriculture, Tikrit University. These isolates were differentiated based on colony texture, color, margins, and size. Results shown in Figure 1 indicated that isolate B2 exhibited the highest inhibitory effect on the growth of the pathogenic fungus, reaching an inhibition zone of 41.5 mm, while the inhibition zones of the other isolates ranged from 3.3 to 13.7 mm, except for isolates B3 and B4, which showed no inhibition of fungal growth. Molecular identification of the most potent inhibitory isolate, B2, designated as Bacillus velezensis strain Jwan-2, was recorded in the global genetic database NCBI under accession number PP320456.1. This isolate showed 99.47% sequence similarity with eight Bacillus velezensis isolates previously reported from China, Indonesia, India, and Portugal. The application of the mycorrhizal fungus Rhizophagus intraradices in combination with Bacillus velezensis and Azolla pinnata under Fusarium culmorum infection induced a higher systemic resistance in the plant compared to the individual bioagents, with peroxidase and polyphenol oxidase enzyme activities reaching 3.723 and 3.351 units/mL, respectively. Disease severity significantly decreased in all tested treatments, and the combined treatment of bacteria and Azolla recorded the lowest disease severity of 11.01%, compared to the highest severity of 88.14% observed in plants infected with the pathogen alone without mycorrhizal inoculation. The integrated treatment of bacteria and Azolla under pathogen infection and mycorrhizal inoculation also achieved the highest vegetative growth parameters, with dry weights of shoots and roots and chlorophyll content measuring 8.21 g, 4.57 g, and 45.44 SPAD units, respectively. These values were significantly higher than those recorded in plants treated only with the pathogenic fungus without mycorrhiza, which were 4.31 g, 2.07 g, and 22.4 SPAD units, respectively. Furthermore, this integrated treatment resulted in the highest wheat yield of 22.32 g per plant, compared to 5.16 g per plant in the pathogen-only treatment.

Keywords: Biological control, Wheat root rot disease, Fusarium culmorum, Bacillus velezensis, Azolla pinnata.

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I. Introduction

Wheat (Triticum aestivum) is among the world's staple cereal crops, providing approximately 55% of the daily carbohydrates and proteins for 85% of the global population. This crop is directly linked to global food security and the economy (Rebouh et al., 2022). It has been cultivated by humans for about 10,000 years, with emmer wheat—the earliest domesticated wheat species—originating in the Middle East. Over time, the importance of common wheat (Triticum aestivum L.) has increased significantly, and it is now produced on almost every continent. Numerous cultivars have been developed and improved for drought tolerance, high temperature resistance, and disease resistance. Production techniques have also advanced, enabling high grain yields. With productivity per unit area increasing, overall wheat production continues to rise (Kheiralipour et al., 2024). Wheat production is vital as it supplies energy and protein to millions worldwide, and recent advances in wheat research have led to substantial yield improvements (Yao et al., 2025). Crop production often faces challenges from plant diseases, and biological control is emerging as an effective, environmentally friendly, low-cost, and sustainable alternative to chemical control (Surovy et al., 2024). Bacteria play a crucial role in combating plant root rot diseases through various biochemical and biological mechanisms, both directly and indirectly. These mechanisms, increasingly utilized in sustainable agriculture to reduce reliance on chemical pesticides, include plant growth promotion, competition for nutrients and space, production of antibiotics, induction of systemic resistance (Induced Systemic Resistance – ISR), production of cell wall-degrading enzymes targeting the pathogen, and synthesis of compounds that inhibit pathogen virulence factors. These have been well documented by previous studies (Bonaterra et al., 2022; Riseh et al., 2025). Aiming to reduce wheat crop losses caused by root rot pathogens and to employ environmentally safe biological agents, the present study aimed to evaluate the compatibility of bacteria and Azolla with mycorrhizal fungi in controlling wheat root rot disease.

II. Materials and Methods

Biological agents

Wheat seeds of the cultivar Sham 6 were used, obtained from the Seed Technology Center – Scientific Research Authority, Iraq.

Pathogenic Fungus, *Fusarium culmorum* and mycorrhizal fungus *Rhizophagus intraradices* which was identified morphologically and molecularly, were obtained from the laboratories of the Plant Protection Department - Tikrit University, according to a previous study (Hassan and Ismail, 2025).

Isolation and Molecular Identification of Bacteria

Bacteria associated with the roots of healthy wheat plants were isolated from Salah Aldin Governorate, Iraq, using the decimal dilution method, and molecularly identified at the genus level.

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Effect of Bacteria on Pathogenic Fungal Growth

The effect of bacterial isolates on the pathogenic fungus was assessed through dual culture assay on Potato Dextrose Agar (PDA). A disc from the pathogenic fungus colony was placed in the center of a Petri dish, and bacterial streaks were inoculated with an inoculation loop 2 cm away from the fungal disc. The plates were incubated at 25°C. After incubation, the inhibition zone (the area between the bacterial streak and the fungal colony) was measured (Hassan and Ajaj, 2021).

Molecular Identification of the Most Effective Bacterial Isolate Against the Pathogenic Fungus *Fusarium culmorum*

Genomic DNA was extracted from a 100 mg sample of a freshly grown bacterial colony isolate using the ZR Fungal/Bacterial/Yeast DNA Mini Prep™ kit (ZR, USA), following the manufacturer's protocols. The 16S rRNA gene was amplified by PCR using the universal primers: forward 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse 5'-GGTTACCTTGTTACGACTT-3', which were synthesized by Integrated DNA Technologies, Canada. The PCR reaction was performed in a total volume of 25 µL, consisting of 1.5 µL genomic DNA, 5 µL Taq PCR PreMix, 1 µL of each primer (10 pmol/µL), and nuclease-free water to volume. The thermal cycling program included: (a) initial denaturation at 95°C for 5 min, (b) 37 cycles of denaturation at 95°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 45 s, followed by (c) a final extension at 72°C for 7 min. Amplifications were conducted using the GeneAmp 9700 PCR System (Applied Biosystems). PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with Intron Korea red dye and visualized under UV light at 302 nm wavelength.

The amplified 16S rRNA gene fragments were sequenced directly using an Applied Biosystems 3730XL DNA Sequencer. The obtained nucleotide sequences were compared to sequences in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) for identification. Sequence similarity percentages and accession numbers with global reference strains were recorded.

Field Experiment

Soil Sterilization

The field experiment was conducted on November 30, 2024, at the Plant Protection Research Station using a randomized complete block design (RCBD) with three replicates. Calibration irrigation was performed followed by soil plowing and establishment of a drip irrigation system. The field was sterilized by spraying 5% formaldehyde solution using a 100-liter holder sprayer to cover the entire soil surface. The soil was then tightly covered with polyethylene plastic for five days, followed by five days of ventilation.

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Treatments:

Factor 1: Combinations

- Control (healthy plants)
- Pathogenic fungus (*Fusarium culmorum*, F.c) only
- Pathogenic fungus (F.c) + Bentanol fungicide
- Pathogenic fungus (F.c) + *Bacillus velezensis* (B.v)
- Pathogenic fungus (F.c) + *Azolla pinnata* (15%)
- Pathogenic fungus (F.c) + *Bacillus* + *Azolla*

Factor 2: Mycorrhizal inoculation status

The experiment included three blocks, each block comprising 12 treatments. Treatment covered an area of 1×1 m², where wheat seeds of the Sham 6 cultivar were planted in four rows, with 20 cm spacing between each row and 20 seeds per row. A soil ridge 10 cm wide and 15 cm high was constructed between each treatment to separate them. According to the above treatments, mycorrhizal inoculum was applied at a rate of 50 spores per row before sowing. Five days after germination, plants were inoculated by adding 100 mL of pathogenic fungal spore suspension at a concentration of $10X10^8$ colony-forming units (CFU)/mL per row. Azolla was added at 15 g per row, and finally, bacterial inoculum was applied at 100 mL per row with a concentration of 10x108 CFU/mL. For the fungicide treatments with Bentanol, 100 mL per row of a 1.5% concentration was applied.

Measured Parameters:

Disease Severity

Disease severity (%) was calculated using McKinney's formula (1923) based on the infection scale of Gao et al. (1995):
0 = healthy plant with white roots,
1 = slight discoloration on roots and yellowing of a limited number of leaves,
2 = complete root discoloration with full leaf yellowing,
3 = discoloration extending from roots to stem bases,
4 = plant death.

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The disease severity index was calculated as:

Disease Severity=∑(number of plants in each grade×grade)/total plants examined×4×100

Resistance Induction Indicators

Extraction of Crude Enzyme Extracts

Three plants were taken from each replicate, and their roots were washed thoroughly with water to remove soil particles. The roots were then cut into small pieces, and 1 g of root tissue was placed in a glass mortar with 5 mL of acetate buffer solution adjusted to pH 5.6. The tissue was ground completely, and the homogenate was transferred to 10 mL test tubes and centrifuged at 5000 rpm for 5 minutes. The clear supernatant was collected into new tubes and stored at 4°C until further analysis.

Peroxidase Enzyme Activity

Peroxidase activity was estimated according to the method of Hammerschmidt et al. (1982). The absorbance of the reaction mixture containing 2.5 mL of hydrogen peroxide (H2O2) solution and 0.1 mL of enzyme extract was measured at 470 nm. One unit of enzyme activity was defined as the change in absorbance of 0.01 per minute.

Polyphenol Oxidase Activity

Polyphenol oxidase activity was determined following Mayer et al. (1968) by measuring the absorbance at 470 nm of a mixture consisting of 2.5 mL catechol solution and 0.1 mL enzyme extract using a spectrophotometer. One enzyme unit was defined as a 0.01 change in absorbance per minute.

Estimation of Dry Root and Shoot Weights

Roots were separated from shoots at the crown, thoroughly washed with distilled water, and dried in an electric oven at 50°C until constant weight. Dry weights were measured in grams using an analytical balance.

Chlorophyll Measurement

Chlorophyll content of leaves was measured post-germination using a SPAD chlorophyll meter.

Yield Estimation

Grain weight per plant: Determined at the end of the season by weighing grains from a sample of 5 plants using an analytical balance.

Statistical Analysis:

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The experiments of this study were conducted using a Completely Randomized Design (CRD). Analysis of variance (ANOVA) was performed using the SPSS software. Means were compared by the Least Significant Difference (LSD) test at a significance level of 0.05 (Al-Rawi and Khalaf Allah, 1980).

III. Results and Discussion

Bacterial Isolation and Their Effect Against the Pathogenic Fungus

Six bacterial isolates, designated B1 to B6, were isolated from healthy wheat plants grown in the wheat field at the Agricultural Research Station, College of Agriculture, Tikrit University. The differentiation among these isolates was based on differences in colony texture, color, margins, and size. Results shown in Figure 1 indicate that isolate B2 exhibited the highest inhibitory effect on the growth of the pathogenic fungus *Fusarium culmorum*, with an inhibition zone of 41.5 mm. The remaining isolates showed inhibition zones ranging from 3.3 mm to 13.7 mm, except for isolates B3 and B4, which showed no inhibition against the pathogen's growth.

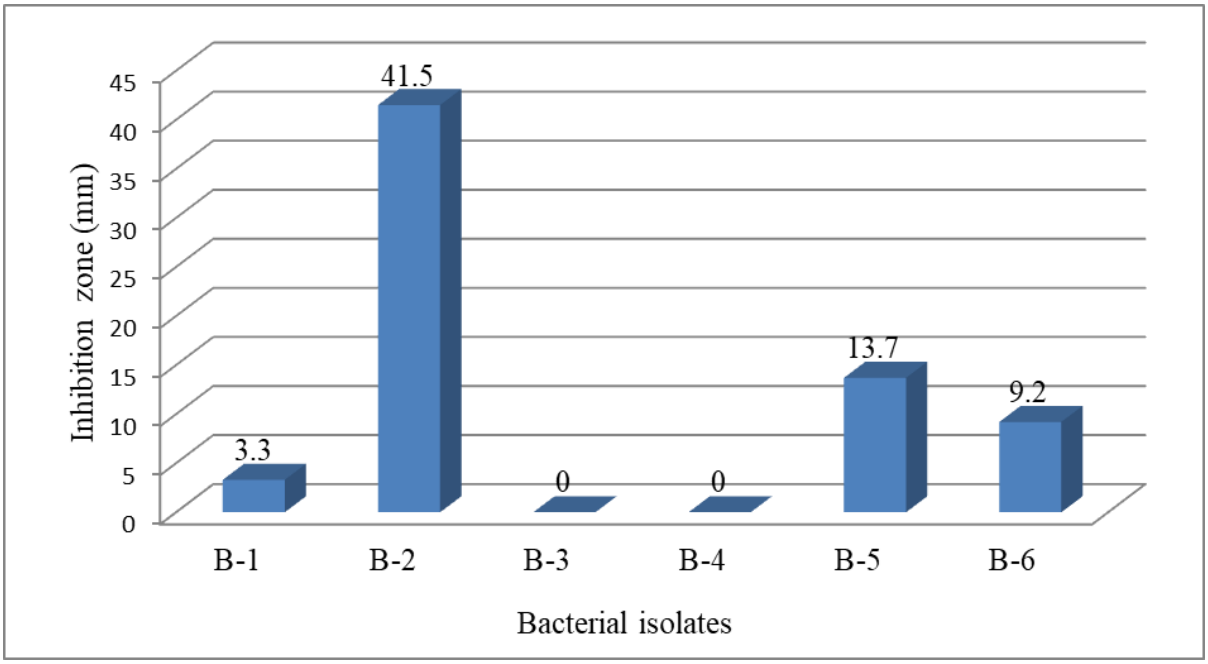

Figure 1. Effect of bacterial isolates on the growth of pathogenic fungus *Fusarium culmorum*

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The results of Table 1 showed that the highest inhibitory bacterial isolate against the pathogenic fungus, designated B-2 and identified as Bacillus velezensis strain Jwan-2, was recorded in the global genetic database NCBI with accession number PP320456.1. Its identification to the species level was confirmed by registering a 99.47% sequence similarity with eight isolates of Bacillus velezensis originating from China, Indonesia, India, and Portugal.

Table 1. The matching ratios of the bacterial isolate Bacillus velezensis strain Jwan-2 (accession number PP320456.1) isolated in the current study with some bacterial isolates recorded at the NCBI site.

Bacterial isolates recorded at the NCBI site.	Accession number	Country	Similarity (%)
Bacillus velezensis strain A-1	MW165773.1	China	99.47
Bacillus velezensis R1.1	LC414157.1	Indonesia	99.47
Bacillus velezensis strain XC1	MT649755.1	China	99.47
Bacillus velezensis strain 20180504	MH370469.1	China	99.47
Bacillus velezensis strain MB1EPC2	PQ192692.1	India	99.47
Bacillus velezensis strain KKWHNGU1	OR113010.1	India	99.47
Bacillus velezensis strain OSBR66	MN036415.1	Portugal	99.47
Bacillus velezensis strain YJC-9	MF499153.1	China	99.47
Bacterium strain BS0783	MK823971.1	China	99.47
Bacillus sp. (in: firmicutes) MA3	LC885330.1	Japan	99.47

Infection Severity

The results in Table 2 illustrate the effect of inoculation with mycorrhizal fungi, *Bacillus velezensis*, and *Azolla pinnata* on the infection severity (%) caused by the pathogenic fungus *Fusarium culmorum* on wheat cultivar Sham 6. Overall, the results show a significant reduction in infection severity in treatments inoculated with mycorrhizal fungi compared to non-inoculated controls, except for the fungicide treatment where no significant difference was observed. The lowest infection severity was recorded in the treatment combining bacteria and Azolla alongside F. culmorum, reaching 15.12%, compared to the highest severity of 61.54% in the treatment with the pathogenic fungus alone. In the mycorrhizal inoculation treatments with Rhizophagus intraradices, the lowest infection severity was 15.22% in inoculated plants versus 30.11% in non-inoculated ones. The two-factor interaction between treatments and mycorrhizal inoculation showed that infection severity decreased from 34.93% in the pathogenic fungus treatment inoculated with mycorrhiza alone, to 11.01% in the treatment combining the pathogen with Azolla, *B. velezensis*, and mycorrhizal fungus.

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Table (2) Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on the severity of infection (%) with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety.

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	0	0	0
Pathogen *F. culmorum* (f.c.) only	34.93	88.14	61.54
Pathogen F.c. + Bentanol	15.35	16.58	15.97
Pathogen F.c. + *B. velezensis* (B.v.)	14.67	29.42	22.05
Pathogen F.c. + *Azolla pinnata* (15%)	15.37	27.27	21.32
Pathogen F.c. + (B.v.) + *A. pinnata*	11.01	19.23	15.12
Inoculation rate	15.22	30.107	
LSD $_{0.05}$	Treatments;2.33, Inoculation;2.72, Intraction;3.46		

Peroxidase Induction

The results of Table 3 show the effect of inoculation with mycorrhizal fungi, *Bacillus velezensis*, and *Azolla pinnata* on the induction of peroxidase enzyme activity in wheat cultivar Sham 6 infected with the pathogenic fungus *Fusarium culmorum*. It is evident from the results that peroxidase induction significantly increased in treatments inoculated with mycorrhizal fungi compared to non-inoculated ones. The highest enzyme activity was recorded in the treatment combining bacteria and *Azolla* in the presence of *F. culmorum*, reaching 3.19 units/mL. In contrast, the lowest enzymatic activity was recorded in the pathogenic fungus treatment combined with fungicide, measuring 1.38 units/mL. Regarding mycorrhizal inoculation, the highest peroxidase activity was 2.91 units/mL in inoculated plants compared to 1.68 units/mL in non-inoculated plants. The two-factor interaction between treatments and mycorrhizal inoculation showed an increase in peroxidase induction from 2.892 units/mL in the pathogenic fungus treatment inoculated only with mycorrhizal fungi to 3.723 units/mL in the treatment combining the pathogen with *Azolla*, *B. velezensis*, and mycorrhizal fungus.

Polyphenol Oxidase Induction

The results presented in Table 4 reveal the effect of inoculation with mycorrhizal fungi, Bacillus velezensis, and Azolla pinnata on the induction of polyphenol oxidase enzyme activity in wheat cultivar Sham 6 infected with the pathogenic

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fungus Fusarium culmorum. The highest enzyme activity was recorded in the treatment combining bacteria and Azolla alongside F. culmorum, reaching 2.27 units/mL. Conversely, the lowest enzymatic activity was observed in the pathogenic fungus treatment combined with fungicide, measuring 1.14 units/mL. Concerning mycorrhizal inoculation, the highest polyphenol oxidase activity reached 2.38 units/mL in inoculated plants compared to 1.59 units/mL in non-inoculated controls. The two-factor interaction between treatments and mycorrhizal inoculation showed that polyphenol oxidase induction increased from 2.452 units/mL in the pathogenic fungus treatment inoculated only with mycorrhizal fungi to 3.315 units/mL in the treatment combining the pathogen with Azolla, *B. velezensis*, and mycorrhizal fungus.

Table (3) Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on peroxidase activity (U/ml) with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	2.671	0.058	1.365
Pathogen *F. culmorum* (f.c.) only	2.892	1.683	2.29
Pathogen F.c. + Bentanol	1.442	1.33	1.39
Pathogen F.c. + *B. velezensis* (B.v.)	3.467	2.261	2.86
Pathogen F.c. + *Azolla pinnata* (15%)	3.301	2.062	2.68
Pathogen F.c. + (B.v.) + *A. pinnata*	3.723	2.662	3.19
Inoculation rate	2.92	1.68	
LSD $_{0.05}$	Treatments;0.23, Inoculation;0.31, Intraction;0.53		

Table (4) Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on poly phenol oxidase activity (U/ml) with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	2.335	0.091	1.21
Pathogen *F. culmorum* (f.c.) only	1.243	2.452	1.85
Pathogen F.c. + Bentanol	1.164	1.111	1.14
Pathogen F.c. + *B. velezensis* (B.v.)	3.029	1.862	2.45
Pathogen F.c. + *Azolla pinnata* (15%)	3.18	1.983	2.58
Pathogen F.c. + (B.v.) + *A. pinnata*	3.351	2.092	2.72
Inoculation rate	2.38	1.59	
LSD $_{0.05}$	Treatments;0.12, Inoculation;0.16, Intraction;0.21		

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Dry weight of the shoot system

The results shown in Table 5 demonstrate the effect of inoculation with mycorrhizal fungus, *Bacillus velezensis*, and *Azolla pinnata* on the dry weight of shoot biomass in wheat cultivar Sham 6 infected with the pathogenic fungus *Fusarium culmorum*. It is observed that dry shoot weight increased significantly in treatments inoculated with mycorrhizal fungi compared to those without inoculation. The highest dry shoot weight was recorded in the treatment combining bacteria and *Azolla* in the presence of *F. culmorum*, reaching 7.68 g, compared to the lowest dry weight of 5.21 g in the treatment with the pathogenic fungus alone. Regarding inoculation with the mycorrhizal fungus *R. intraradices*, the highest dry weight reached 7.46 g in inoculated plants versus 6.39 g in non-inoculated ones. The two-factor interaction between treatments and mycorrhizal inoculation showed an increase in dry shoot weight from 6.11 g in the pathogenic fungus treatment inoculated only with mycorrhiza to 8.21 g in the treatment combining the pathogen with *Azolla*, *B. velezensis*, and mycorrhizal fungus.

dry weight of the root system

The results presented in Table 6 illustrate the effect of inoculation with mycorrhizal fungi, *B. velezensis*, and *A. pinnata* on the dry weight of root biomass in wheat cultivar Sham 6 infected with the pathogenic fungus *F. culmorum*. The highest dry root weight was recorded in the treatment combining bacteria and *Azolla* in the presence of *F. culmorum*, reaching 4.21 g, compared to the lowest dry weight of 2.59 g in the treatment with the pathogenic fungus alone. Regarding inoculation with the mycorrhizal fungus *R. intraradices*, the highest dry root weight was 3.78 g in inoculated plants compared to 3.22 g in non-inoculated ones. The two-factor interaction between treatments and mycorrhizal inoculation showed that dry root weight increased from 3.12 g in the pathogenic fungus treatment inoculated only with mycorrhiza to 4.57 g in the treatment combining the pathogen with *Azolla*, *Bacillus*, and mycorrhizal fungus. *B. velezensis*

Chlorophyll Content

The results presented in Table 7 illustrate the effect of inoculation with mycorrhizal fungus, B. velezensis, and A. pinnata on the chlorophyll content of wheat cultivar Sham 6 infected with the pathogenic fungus F. culmorum. The highest chlorophyll content was recorded in the treatment combining bacteria and Azolla in the presence of F. culmorum, reaching 42.37 SPAD units. In contrast, the lowest chlorophyll content was observed in the pathogenic fungus treatment alone, with 29.51 SPAD units. Regarding inoculation with the mycorrhizal fungus R. intraradices, the highest chlorophyll content was 41.08 SPAD units in inoculated plants compared to 35.06 SPAD units in non-inoculated ones. The two-factor interaction between treatments and mycorrhizal inoculation showed an increase in chlorophyll content from 36.62 SPAD units in the pathogenic fungus treatment inoculated only with mycorrhizal fungi to 45.44 SPAD units in the treatment combining the pathogen with Azolla, *B. velezensis*, and mycorrhizal fungus.

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Table (5) Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on dry weight of the shoot system (g) with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	7.76	6.53	7.15
Pathogen *F. culmorum* (f.c.) only	6.11	4.31	5.21
Pathogen F.c. + Bentanol	6.87	6.85	6.86
Pathogen F.c. + *B. velezensis* (B.v.)	8.07	6.89	7.48
Pathogen F.c. + *Azolla pinnata* (15%)	7.75	6.58	7.17
Pathogen F.c. + (B.v.) + *A. pinnata*	8.21	7.15	7.68
Inoculation rate	7.46	6.39	
LSD $_{0.05}$	Treatments;0.26, Inoculation;0.37, Intraction;0.41		

Table (6) Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on dry weight of the root system (g) with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	3.75	3.32	3.54
Pathogen *F. culmorum* (f.c.) only	3.12	2.07	2.59
Pathogen F.c. + Bentanol	3.38	3.35	3.37
Pathogen F.c. + *B. velezensis* (B.v.)	4.23	3.62	3.93
Pathogen F.c. + *Azolla pinnata* (15%)	3.64	3.11	3.38
Pathogen F.c. + (B.v.) + *A. pinnata*	4.57	3.84	4.21
Inoculation rate	3.78	3.22	
LSD $_{0.05}$	Treatments;0.13, Inoculation;0.15, Intraction;0.19		

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Table (7) Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on Chlorophyll Content (Spad) with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	40.09	36.53	38.31
Pathogen *F. culmorum* (f.c.) only	36.62	22.4	29.51
Pathogen F.c. + Bentanol	39.14	38.87	39.01
Pathogen F.c. + *B. velezensis* (B.v.)	42.63	36.95	39.79
Pathogen F.c. + *Azolla pinnata* (15%)	42.57	36.34	39.46
Pathogen F.c. + (B.v.) + *A. pinnata*	45.44	39.29	42.37
Inoculation rate	41.08	35.06	
LSD $_{0.05}$	Treatments;1.03, Inoculation;2.23, Intraction;2.66		

Grain Weight

The results presented in Table 8 show the effect of inoculation with mycorrhizal fungi, B. velezensis, and A. pinnata on grain weight (g/plant) of wheat cultivar Sham 6 infected with the pathogenic fungus F. culmorum. Significant differences were observed between the treatments inoculated with mycorrhizal fungi and those without inoculation. The highest grain weight was recorded in the treatment combining bacteria and Azolla in the presence of F. culmorum, reaching 20.73 g/ plant. In comparison, the lowest grain weight was observed in the treatment inoculated with the pathogenic fungus alone, which was 9.86 g/plant. For the mycorrhizal inoculation with Rhizophagus intraradices, the highest grain weight was 19.82 g/plant in inoculated plants versus 16.39 g/plant in non-inoculated ones. The two-factor interaction between treatments and mycorrhizal inoculation revealed that grain weight increased from 14.55 g in the pathogenic fungus treatment inoculated only with mycorrhizal fungi to 22.32 g/plant in the treatment combining the pathogen with Azolla, *B. velezensis*, and mycorrhizal fungus.

Table 8. Effect of inoculation with mycorrhizal fungus, *R. intraradices* , *B. velezensis* bacteria and *Azolla pinnata* on Grain Weight (g/plant)with the pathogenic fungus *F. culmorum* on wheat, Sham 6 variety

Treatments	Inoculation status with mycorrhizal fungus R. intraradices		Average of treatments
	Inoculated	Non- Inoculated	
Control (healthy plants)	22.12	19.23	20.68
Pathogen *F. culmorum* (f.c.) only	14.55	5.16	9.86

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Pathogen F.c. + Bentanol	18.44	18.37	18.41
Pathogen F.c. + *B. velezensis* (B.v.)	21.6	18.61	20.11
Pathogen F.c. + *Azolla pinnata* (15%)	19.87	17.82	18.85
Pathogen F.c. + (B.v.) + *A. pinnata*	22.32	19.14	20.73
Inoculation rate	19.82	16.39	
LSD $_{0.05}$	Treatments;0.88 , Inoculation; 1.04, Intraction;1.47		

IV. Discussion

The study's results demonstrate variability in the antagonistic effects of different bacterial isolates against the pathogenic fungus. This variation is likely due to the isolates belonging to genetically distinct species, resulting in divergent physiological behaviors. Molecular identification of the most effective isolate, classified as Bacillus velezensis, showed a 99.47% nucleotide sequence similarity with global strains recorded in the NCBI database, confirming the accuracy of the diagnosis. Despite this high similarity, minor genetic variations exist, probably due to recombination or mutations arising from diverse environmental stresses in the regions of isolation. Chemical factors such as continual pesticide use may increase mutation rates and influence fungal diversity, reflecting observed environmental variability (Hassan & Ibrahim, 2022; Hassan & Al-Qiassi, 2022).

The superiority of Rhizophagus intraradices mycorrhizal fungus in reducing infection severity in wheat cultivar Sham 6 can be attributed to its ability to enhance carbohydrate production and mineral nutrient uptake, particularly phosphorus, through roots, thereby increasing resistance against fungal pathogens (Abdelgawad et al., 2022). This study further confirmed systemic resistance induction in wheat, indicated by elevated polyphenol oxidase and peroxidase enzyme activities—likely stimulated by secondary metabolites and enzymes produced by R. intraradices. These metabolites activate the genes responsible for enzyme production as a form of systemic induced resistance, consistent with other studies reporting similar fungal-mediated resistance induction in various plant species (Hassan & Yousef, 2023; Aboud et al., 2017; Hassan & Al-Samarrai, 2018). Peroxidase activation notably contributes to strengthening cell walls by oxidizing phenolic compounds into substances like hydrogen peroxide and quinones, which release reactive oxygen species such as –OH and O2–. These ROS stimulate genes directly involved in polymerizing lignin compounds, thereby enhancing structural barriers against pathogen invasion (Chon et al., 2000; Lavania et al., 2006). A probable cause for reduced fungal infection following mycorrhizal treatments is the competitive exclusion of pathogenic fungus by R. intraradices at root infection sites, as documented by Duan et al. (2024). Furthermore, the reduced severity of fungal diseases and enhanced plant growth may be linked to mycorrhizal secretion or stimulation of growth hormones like indole-3-acetic acid (IAA), which promotes root cell division, proliferation, and enlargement (Pandino et al., 2024; Hong et al., 2024). These physiological enhancements include development of an efficient root system facilitating water and nutrient absorption, in agreement with Arabi et al. (2013), who reported similar mycorrhizal effects on nutrient uptake, plant height, biomass, and chlorophyll content. Enhanced chlorophyll content improves photosynthetic capacity by accelerating enzymatic processes, improving plant vigor and disease resistance (Pandino et al., 2024). The bacterium *Bacillus velezensis* contributes to fungal pathogen suppression by inhibiting spore germination and toxin production, thus reducing plant damage and increasing disease tolerance (Ma et al., 2024). Its role in producing siderophores, bacteriocins, and phenolic compounds with antifungal properties is well-documented (Hassan & Ismael, 2025).

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Azolla pinnata is known for enhancing plant growth by nitrogen fixation and improving soil fertility, which reflects in increased grain weight and growth parameters. The combined inoculation with mycorrhizal fungi, bacteria, and Azolla exhibits synergistic effects, surpassing the individual impacts of each, congruent with Lum et al. (2024), who demonstrated increased growth and yield in sweet corn with Azolla pinnata treatment. Interestingly, fungicide treatment with Pentanol showed no significant difference in infection severity with or without mycorrhizal inoculation, likely due to fungicide's inhibitory effect on mycorrhizal fungi themselves, thereby negating their beneficial effects. Overall, this study highlights the critical role of integrated inoculation with Rhizophagus intraradices, Bacillus velezensis, and Azolla pinnata in enhancing vegetative growth traits, systemic resistance induction, and ultimately, wheat grain yield under Fusarium culmorum infection. The significant grain weight increases in treatments combining these agents compared to pathogen-only treatments underscore the potential of such bio-inoculants in sustainable wheat disease management and productivity enhancement.

V. Conclusion

It can be concluded from this study that the enhanced resistance and growth observed in wheat inoculated with Rhizophagus intraradices, Bacillus velezensis, and Azolla pinnata are due to synergistic effects, including improved nutrient uptake, induction of systemic resistance through enzyme activation, and suppression of pathogenic fungi. The combined bioinoculants significantly enhanced physiological and yield parameters compared to treatments with the pathogen alone, demonstrating their potential as sustainable and environmentally friendly alternatives. In contrast, fungicide application diminished the beneficial effects of mycorrhizal fungi, underscoring the importance of integrated biological strategies for effective disease management in wheat cultivation.

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