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Controlling seed rot and seedling damping-off in *vicia faba* caused by *Rhizoctonia solani* using resistant cultivars and biological agents in Iraq

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Abstract

The study aimed to implement one of the integrated management strategies in controlling the seed rot and seedling damping-off disease of faba bean caused by the fungus *Rizoctonia solani*, using plant cultivars and some environmentally friendly agents. The isolation and identification results showed that 12 fungal isolates associated with the seedling damping-off disease belong to the genus *Rhizoctonia spp*., eight isolates of the fungus *F. solani*, six isolates of the fungus *F. oxysporum*, and six isolates of the fungus *Macrophomina spp*., and others. The pathogenicity test results showed that all fungal isolates were pathogenic. However, the percentage of seed germination inhibition, seedling death, and symptoms appeared varied. The isolate K.h.R1 of the fungus *Rizoctonia sp*. was outperformed in its virulence, as its infection Percentage to100%. This was molecularly identified as *R.solani* under the deposit number PV939284.1 in NCBI.Meanwhile, the results of the field experiment showed that all treatments used to control the disease caused by the pathogenic fungus *R. solani* were effective in controlling the disease; the integrated treatment of factors (*Azotobacter chrocoocum*, salicylic acid, and Zinc) was significantly outperformed in reducing the infection percentage and severity of 41.66% and 18.11%, respectively, compared to the pathogen-only treatment of 96.67% and 85.86%, respectively. By increasing the activity of the enzymes peroxidase and polyphenol oxidase and raising the total phenolic content, the integrated treatment (SA + Zn + Azo) also clearly contributed to the development of systemic resistance in the plant against the pathogenic agent. Furthermore, the plant cultivar response results revealed that the Cypriot cultivar performed significantly better than the others in terms of disease resistance, registering the lowest levels of infection and severity with mean values of 49.33% and 39.09%, respectively, in contrast to the Syrian cultivar, which recorded 66.66% and 56.15%, respectively.

Keywords: *Vicia faba* L., Root rot diseases, *Macrophomina pseudo-phaseolina*

Introduction

Faba bean (*Vicia faba* L.) is recognized as one of the important seed legume crops and belongs to the family Fabaceae. It is a staple food for many people in poor countries, due in part to its extraordinary nutritional value, particularly its protein content, which can range from 23% to 42%, as well as vitamins, minerals, and dietary fibre [1]. In addition to its importance as a food source for people, it is also a source of animal feed. Furthermore, faba bean helps to improve soil fertility by fixing atmospheric nitrogen via bacterial nodules that develop in its roots. Legume crops serve as sources of nitrogen to subsequent crops and cereal crops in particular [2].

Faba bean are grown in almost all agricultural areas in Iraq. According to the Food and Agriculture Organization (FAO) data for the year 2022, the total area sown with faba bean in Iraq was about 5203 hectares (about 20812 dunums) area with a total production of green faba bean at about 40,586 tons. As dry faba bean, the total production in the year was 1894 tons [3].

Faba bean crops are vulnerable to a wide range of pathogenic organisms, causing considerable economic losses. The abiotic and biotic factors influencing faba bean pathogens affect the severity and presence of each pathogen in different cultivated environments. Among the most important and widespread soil-borne diseases affecting faba bean are damping-off of seedlings and seed rot. The level of loss in faba bean crops due to these soil-borne pathogens primarily depends on the overall density of fungal inoculum present in the Soil, the planting time during the season, and the available biotic factors [4]. In recent years, a noticeable decrease in faba bean production in Iraq has been observed. These yield losses result from multiple factors, especially infection by a variety of soilborne pathogens, including *R. solani, Fusarium spp*., and *M. phaseolina*. These fungi infect the seeds, seedlings, and roots of the plant, causing rot and leading to significant economic yield losses [5]. Furthermore, the decrease in productivity may be attributed to a weakening genetic structure of locally grown cultivars and their susceptibility to plant diseases, additionally imported cultivars may not be as well adapted to the local environment. Variations in growth behavior and productivity also exist among cultivars due to differences in their genetic and physiological makeup, causing them to respond differently under varying environmental conditions and crop management practices. Consequently, to boost the productivity of these cultivars, it is necessary to employ diverse crop management strategies, particularly involving the application of growth regulators, nutrients and disease resistance factors [6,7].

In order to reduce the impact of pathogens, workers and specialists in the field of plant disease control have sought to find alternative means and other effective and environmentally friendly methods, including the use of biological resistance such as the use of Azotobacter spp [8,9], as *Azotobacter ssp*. bacteria are also used to control pathogens and are one of the types of nitrogen-fixing bacterial strains in the Rhizosphere region [10].

There is a wide range of methods used to reduce the spread of these diseases among them, salicylic acid holds a prominent position due to its vital role in regulating many physiological processes in plants, such as growth, photosynthesis, and flowering regulation. This acid also contributes to processes like transpiration, permeability, and ion transport. A study by [11] demonstrated that treating faba bean plants grown in Soil contaminated with *R. solani* with salicylic acid stimulated systemic resistance and improved most growth parameters in the treated plants. The addition of micronutrients such as Zinc also plays an important role in increasing plant yields and improving their quality. Zinc is an essential element for plant growth and is therefore found in all plant tissues and accumulates in varying concentrations in different parts of the plant It is considered a critical mineral element for activating many enzymes involved in carbon dioxide (CO_2) metabolism. Among the enzymes that most activate the presence of this element are fructose 1,6-biphosphatase and carbonic anhydrase, which encourage the decomposition of carbonic acid into carbon dioxide and water [12]. Based on the stated background, this study aims to investigate the efficacy of integrated management with resistant cultivars and environmentally friendly agents to decrease seed rot and damping-off of seedlings of faba bean caused by *Rhizoctonia solani* in Iraq.

Materials and Methods

Sample Collection

Faba bean plant samples containing cases of faba bean root rot were collected from different geographical locations. A vast area of agricultural sites was covered, including the governorates of Kerbala, Babylon, and Najaf. The sites in Kerbala governorate included Al-Hussainiya district, Tuwaireej district, Al-Ibrahimiya area, Ain Al-Tamr and Al-Shari'ah area, In Babil governorate, samples were collected from Al-Iskandariya and Al-Musayyib districts, while Najaf governorate included samples collected from Al-Haidariya area (Khan Al-Nas). Sampling was accomplished in October and November of 2024. Collected samples were placed in bags labelled with sample code, collection site, and date of collection, and were transported to the Plant Pathology Laboratory, Department of Plant Protection, College of Agriculture, University of Kerbala.

Isolation and identification of fungi associated with Faba bean root rot disease

Fungi associated with seed rot and seedling damping-off from infected faba bean plants were isolated. Seeds and roots from infected plants were thoroughly washed and surface-sterilized using a 2% sodium hypochlorite solution for one minute. Following sterilization, the samples were thoroughly rinsed with sterile distilled water to remove any residual disinfectant and then dried using sterile filter paper. The sterilized segments were transferred with sterile forceps into Petri dishes containing potato dextrose agar (PDA) medium, with five segments per dish. The plates were incubated at $25 \pm 2°C$. After four days, the fungal colonies that developed were examined microscopically for identification [13]. Fungal species were identified based on the taxonomic key established by [14] and the morphological characteristics described by [15,16,17].

Pathogenicity Test of Fungal Isolates from Faba Bean Roots

A pathogenicity test was conducted using faba bean plants grown on peat moss medium. After sterilization with 70% ethanol and placing them in sterilized plastic pots of 15 cm diameter, eight fungal isolates of *Rhizoctonia sp.* and three isolates of *Fusarium spp.,* and *Macrophomina sp.* were tested. The most virulent ones were selected and used in subsequent field tests and experiments. Pots containing sterile peat moss were prepared and then inoculated with fungal isolates (12 fungal isolates) individually from pure fungal cultures, with three replications for each, by adding 10 g of the incubation medium and mixing it with peat moss. Then, after 72 hours, the pots were planted with faba bean seeds (local Syrian cultivar) after sterilizing the seeds superficially with sodium hypochlorite at a concentration of 2% of the commercial Fast solution for two minutes. Then, they were washed well with sterile distilled water and dried by placing them on a sterile filter paper. Then, they were transferred using sterile forceps to plastic pots at a rate of 10 seeds in each pot in a circular manner. After 14 days, the percentage of germination, rotten seeds, and the percentage of inhibition were calculated using the following equations:

$$\text{Germination }\% = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

$$\text{Inhibition}\% = \frac{\text{Number of germinated seeds in control} - \text{Number of germinated seeds in treatment}}{\text{Number of germinated seeds in the control}} \times 100$$

Molecular identification of the most virulent *Rhizoctonia sp.* Isolate

The fungal isolate *Rhizoctonia sp.* was chosen for molecular identification based on its virulence to cause death to seedlings faba bean. The isolate was identified by DNA sequencing to explore its genetic structure and compare it with the genomes of all globally recognized isolates. The isolate was identified by sequencing the Internal Transcribed Spacer (ITS) region using PCR, then sending the resulting PCR products to Macrogen Inc. (South Korea) for sequencing .

Once the nucleotide sequences of the fungal isolates had arrived, the sequences were searched using the Basic Local Alignment Search Tool (BLAST) against the available sequence data from the National Center for Biotechnology Information (NCBI) database, while searching specifically for sequences corresponding to fungal isolates identified globally [18]. During this search, the isolate of interest did not align with 100% identity with any of the nucleotide sequences listed in the NCBI database. Using MEGA X (v. 11) software, the vectors were then used to construct a phylogenetic tree to determine how related the sequences were to the study isolate and its other closely related sequences reviewed from the NCBI database. The phylogenetic relationships were determined using the recommended "Neighbor-Joining" method using the isolate nucleotide sequences of the fungal isolate inclusively for the ITS region [19].

Seed Viability Test of Faba Bean Cultivars

A seed viability test was performed for faba bean cultivars before their planting to determine their germination potential and associated physiological quality, which was determined following the International Seed Testing Association [20] protocol. After sampling a representative random sample from each cultivar, the surface of the seeds was disinfected with a 1% sodium hypochlorite solution for one minute, rinsed with sterilized distilled water to remove any residual disinfectant [21]. Subsequently, 100 seeds of each cultivar were placed (in four replications of 25 seeds each) between strips of moist filter paper in sterilized Petri dishes and placed into an incubator at 25 ± 2 °C and appropriate relative humidity for seven days, which are optimal germination conditions for most legumes [22]. A the incubation period, total germination percentage for each cultivar was calculated using the following equation:

$$\text{Germination } \% = \frac{\text{Number of germinated seeds}}{\text{Total number of tested seeds}} \times 100$$

Seeds that had normal germination, which exhibited both the radicle and the plumule, were considered viable. This decision-making ensured that the seeds selected for use in the next agricultural experiment displayed good physiological efficiency and thus was a necessary step to maximize the quality of experimental results and standards for plant growth [20].

Evaluation of the Role of Faba Bean Cultivars and the Efficiency of Biotic and Chemical Agents, and Their Integration in the Control of *Rhizoctonia solani* Causing Seed Rot and Seedling Damping-Off in Plastic Pots

Loamy Soil was sterilized and put in plastic pots (30 × 40 cm). A selected *R. solani* strain (cultured on sorghum seeds) was placed into the oil at the rate of 1% (w/w) in treatments containing the pathogen at. The soil was then moistened and treated with the following biotic and chemical agents: Zinc - Zn, salicylic acid - SA, Azotobacter chroococcum, Azo and the fungicide Beltanol - Bel (Table 1), and where applicable as detailed in these treatments. Each pot was sown with faba bean (5 seeds per pot) 72 hours after treatment, then it was irrigated. The experiment was completed with three replications for each treatment: Control (Uninfected plants), Soil infested with *R.solani*, *R.solani* + Zinc, *R.solani* + Salicylic acid, *R.solani* + *Azo. chroococcum*, *R.solani* + Zinc + *Azo.chroococcum*, *R.solani* + Salicylic acid + *Azo. chroococcum*, *R.solani* + Zinc + Salicylic acid, *R.solani* + Zinc + Salicylic acid + *Azo.chroococcum*, *R.solani* + Beltanol fungicide.

Table (1): Concentrations and sizes of field experiment treatments

No.	Treatments	Concentration	Solution Volume / Pot
1	*R. solani*	%1	---
2	Zinc (Zn)	0.15 %	100 mL
3	Salicylic Acid (SA)	0.15 %	100 mL
4	*A. chroococcum*	4×10^8	100 mL
5	Zn + *A. chrocoocum*	0.15% + 4×10^8	50 mL + 50 mL

6	SA + *A. chrocoocum*	0.15% + 4×10^8	50 mL + 50 mL
7	Zn + SA	0.15% + 0.15%	50 mL + 50 mL
8	Zn + SA + *A.chrocoocum*	0.15% + 0.15% + 4×10^8	35 mL + 35 mL + 35 mL
9	Beltanol	0.1%	100 mL

Study of induced systemic resistance factors in faba bean plants

The effectiveness of the applied agents to induce systemic resistance against *R. solani*, the major agent of seedling damping-off in faba bean, was examined through the measure of several parameters related to plant resistance. Peroxidase (POD) activity was determined according to [23]. The measurements of polyphenol oxidase (PPO) contents in the treated plants were carried out according to [24]. Meanwhile, total phenol content was determined following [25,26].

Statistical Analysis

A randomized completely design (RCD) was used for both experiments (laboratory experiment and experiment in the shade house). The data were analyzed using the Statistical Analysis System (SAS) program, and then the significant differences between the means were compared using the least significant difference (L.S.D.) test at a significance level of 0.05 [27].

Results and Discussion

Isolation and identification of fungi associated with Faba bean root rot disease

The results of the isolation and morphological identification of fungi associated with seedling damping off disease, through the cultivation of infected root segments on PDA medium, which followed the taxonomic keys considered by [28,29,30], were 12 fungal isolates belonging to the genus *Rhizoctonia spp*., eight isolates of *F. solani*, six isolates of *F. oxysporum*, six isolates of *Macrophomina spp*. and many other fungal isolates. The fungus *Rhizoctonia spp*. had the highest occurrence, a percentage of 100% (Table 2), followed by the fungus *F. solani*, with an occurrence percentage of 66.66%, and the fungi *F. oxysporum* and *Macrophomina sp*., with an occurrence percentage of 50% and 33.33%, respectively, based on the morphological and microscopic characteristics of the growing fungal colonies.

Table (2): Fungi isolated from seed rot and seedling damping-off cases in Faba bean

No.	Fungal name	No. of isolates	Occurrence %	Frequency %
1	*Rhizoctonia* spp.	12	100	28.57
2	*F. solani*	8	66.66	19.04
3	*F. oxysporum*	6	50	14.28
4	*Macrophomina spp*	6	33.33	14.28
5	*Rhizopus* spp	5	33.33	11.90
6	*Alternaria* sp.	3	16.66	7.14
7	*Mucor spp*	2	16.66	4.76

Pathogenicity test of fungal isolates causing seed rot and seedling damping-off of Faba bean in plastic pots under shade conditions

The results of pathogenicity in plastic pots (Table 3) showed that all fungal isolates under study were able to infect faba bean seeds and seedlings, as the infection percentage ranged between 53.33-100%. These treatments differed significantly from the control treatment, free of fungi, which had an infection percentage of 0.00%. The isolate K.h.R1 of the fungus *R.solani* was outperformed in its pathogenicity, as the infection percentage reached 100% (inhibition percentage), while the isolates K.b.F1 and K.sh.R2 of the fungus *Fusarium sp*. and *R.solani* recorded an infection percentage of 90% for both isolates. The other isolates varied in their pathogenicity on faba bean seeds and seedlings, as these isolates led to the rotting of faba bean seeds before germination or the death of seedlings. Based on these results, the isolate K.h.R1 of *R. solani* was selected for molecular identification and use in subsequent experiments due to its 100% infection rate.

Table (3): Pathogenicity test of fungal isolates causing seed rot and seedling damping-off of Faba bean in plastic pots under shade conditions

No.	Fungal name	Isolate code	Germination %	Inhibition %
1	Control	----	100	0.00
2	(*Rhizoctonia sp*)1	K.h.R1	0.00	100
3	(*Rhizoctonia sp*)2	K.a.R1	20	80
4	(*Rhizoctonia sp*)3	H.s.R2	26.66	73.33
5	(*Rhizoctonia sp*)4	N.h.R1	26.66	73.33
6	(*Rhizoctonia sp*)5	K.b.R2	30	70
7	(*Rhizoctonia sp*)6	K.sh.R2	10	90
8	(*Rhizoctonia sp*)7	H.m.R1	46.66	53.33
9	(*Rhizoctonia sp*)8	K.t.R2	26.66	73.33
10	(*Fusarium sp*)1	K.b.F1	10	90
11	(*Fusarium sp*)2	H.m.F1	20	80
12	(*Fusarium sp*)3	N.h.F2	46.66	53.33
13	(*Macrophomina sp*)1	K.a.M3	26.66	73.33
14	(*Macrophomina sp*)2	N.h.M3	46.66	53.33
15	(*Macrophomina sp*)3	H.S.M3	46.66	53.33

Many species of *Rhizoctonia sp., Fusarium spp.,* and *Macrophomina sp*. are well-documented to cause seed rot and seedling damping off on a wide range of host plants. This is due to the parasitic nature of the fungi and the profusion of the hyphal networks, which consume vascular tissue, thereby blocking the delivery of water and nutrients to the leaves.in addition to the effect of enzymes and toxins produced by fungi, which play a significant role in the decomposition of plant cell walls and the entry of fungi into plant tissues of roots, seeds, and stems, which may lead to preventing germination or killing embryos [31]. The difference in fungal isolates in their pathogenicity may be attributed to the genetic difference between fungal isolates, in addition to isolates of the same type that were collected from different regions. It could also be from the differences in their ability to secrete cell wall-degrading enzymes during the first steps in the interaction. These enzymes, such as pectinase, phosphatase, cellulase, methylesterase, pectinmethylhydrase, and protease, play a strong role in the fungal pathogens. Subsequently, they could differ in the production of certain toxins, mainly phenolic or glycosidic toxins. The high pathogenic potential shown in the isolate used in this study may be due to a specific genetic profile of the isolate compared to other isolates. This is consistent with the results of other studies showing significant differences in the pathogenic potential of fungal isolates both within different species of hosts and isolate to isolate [32,33]. Given what we have discovered, the isolate that caused infection 100% of the time was selected for its molecular identification and to experiment with.

Molecular identification and nucleotide sequence analysis of the pathogenic fungus causing seed rot and seedling damping-off in Faba bean

Sequence data analysis confirmed that the fungal isolate behind root rot disease on faba bean was indeed Rhizoctonia solani, and it was deposited with the National Center for Biotechnology Information (NCBI) and given respective accession codes. The molecular nucleotide sequences had high similarities with the internal transcribed spacer (ITS) region: 98.43%~99.77% compared to the respective sequences from the NCBI GenBank, using the BLAST algorithm on each fungal isolate individually. Sequence data analysis was also conducted using MEGA software to produce a phylogenetic tree that provides an illustration of the genetic relationship between the isolate identified in this study and sequences submitted to NCBI that were closely related, based on the ITS regions of nuclear ribosomal DNA.

Nucleotide sequence analysis of the fungal isolate *R. solani* Y.N.195 Zahraa and comparison of the nucleotide sequence similarity of the ITS gene region with global fungal isolates of the same species.

By comparing the nucleotide sequence of the DNA band of the fungus *R.solani* Y.N.195 (Zahraa), which causes the bean seed rot disease, with the data available at the National Center for Biotechnology Information (NCBI), the percentage of genetic similarity was found to range between 98.44 - 99.59% (Table 4) with the isolates of the fungus R.solani isolated from China (PV661098) and from the Saudi and Egyptian isolates (OP612664 and ON259057), the Brazilian isolate (PQ627853), and the American isolate (OM045275).

Table (4): Comparison of nucleotide sequence similarity (ITS region) between the R. solani isolate Y.N.195 (Zahraa), Isolated from Al-Husseiniya district, Kerbela governorate, and other global R. solani isolates registered in the NCBI database

No.	Fungal name	Isolate code	Isolation origin	GenBank Accession No.	Sequence similarity	GenBank Sub. Year
1	*R.solani*	Y.N.195 Zahraa	Iraq	PV939284.1	100%	2025
2	*R.solani*	fng3	Brazil	PQ627853	99.59%	2024
3	*R.solani*	KAU2033	Egypt	ON259057	99.45%	2022
4	*R.solani*	YZGF05	China	PV661098	99.45%	2025
5	*R.solani*	pea/RS-1	Saudi Arabia	OP612664	99.45%	2022
6	*R.solani*	AG 4 HG-I	USA	OM045275	98.44%	2022

In Figure 1, the phylogenetic tree (Neighbor-Joining), we can see that the *R. solani* isolated as Y.N.195 (Zahraa) clustered in the same clade as the Saudi isolate (OP612664). Although it formed a different clade from the Egyptian (ON259057) and Chinese isolates (PV661098), there was no common clade with the American (OM045275) and Brazilian (PQ627853) isolates, suggesting they diverged significantly from each other.

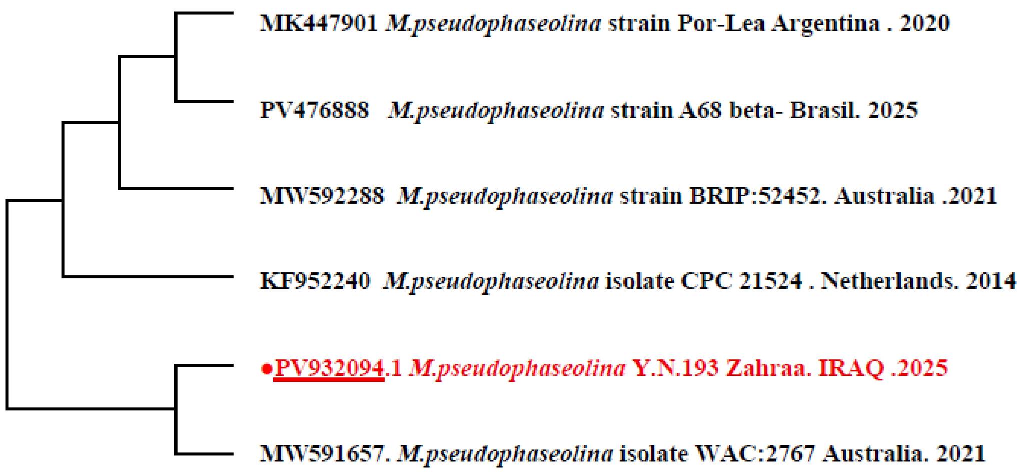

Figure (1): Phylogenetic tree of the pathogenic fungus *R.solani* Y.N.195 Zahraa (marked with a red dot), which was constructed based on the sequences of its nitrogenous bases in the ITS-rDNA region in addition to the sequences of global strains of the same pathogenic fungus obtained from the GenBank data repository.

Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in controlling seed rot and seedling damping-off of faba bean caused by *R.solani*

The results (Table 5) showed that all plant cultivars were negatively affected in their seed germination efficiency when treated with the pathogen only (R. *solani*). The results indicated that the Syrian and Iraqi cultivars were primarily susceptible to the pathogenic fungus, with germination percentages of 0.00% and 13.33%, respectively. Meanwhile, the Spanish and Cypriot cultivars demonstrated some resistance to the pathogen, with germination percentages of 40.0% and 53.33%, respectively. The results also demonstrated that all agents used to control the disease exhibited antagonistic capabilities against the pathogenic fungus and a variable increase in the percentage of seed germination compared to the pathogen-only treatment. The integration treatment between the factors (SA + Zn + Azo) significantly outperformed the other treatments in increasing the percentage of seed germination, as it reached an average of 96.67%, equivalent in its effect to the chemical pesticide treatment, as it reached 96.67% compared to the pathogen treatment only, as the average percentage of seed germination for all plant cultivars, was limited to 26.67% [34].

The high level of susceptibility exhibited by the Syrian and Iraqi cultivars indicates that they are the most susceptible cultivars to infection by this fungus [35]. Conversely, the Spanish and Cypriot cultivars seemed resistant and therefore, had some internal defenses against the pathogen's effects partly because of expression of plant defense genes, the variability in the responses exhibited by the cultivars is likely a result of variability that exists within the cultivars' genetics in regards to their pathogen recognition abilities or their abilities to activate defences against the pathogen which may have alleviated the negative impacts to germination [36].

Table (5): Evaluation of the role of plant cultivars and the efficiency of some chemical and biological factors and their integration in reducing the percentage of seed germination caused by the fungus *R.solani*.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	93.33	100	100	100	98.33
2	Pathogen (R.s)	0.00	13.33	40.00	53.33	26.67
3	Azo+(R.s)	86.66	93.33	93.33	100	93.33
4	(R.s)+Zn	66.66	80.00	86.66	93.33	81.66
5	(R.s)+SA	73.33	80.00	86.66	93.33	83.33
6	(R.s)+Zn+Azo	86.66	93.33	93.33	100	93.33
7	(R.s)+SA+Azo	86.66	93.33	93.33	100	93.33
8	(R.s)+SA+Zn	80.00	80.00	86.66	86.66	83.33
9	(R.s)+SA+Zn+Azo	93.33	93.33	100	100	96.67
10	(R.s) + Beltanol	93.33	93.33	100	100	96.67
Means		76.00	82.00	88.00	92.67	

L.S.D $_{0.05}$	Treatments: 6.897	Cultivars: 9.545	Interaction: 11.332

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Evaluation the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in reducing the percentage of infection and its severity caused by the fungus *R.solani*

The results (Tables 6 and 7) showed that all treatments used to control seed rot and seedling damping-off in the faba bean plant, caused by the pathogenic fungus R. *solani*, were effective in controlling the disease. These factors, individually or in combination, reduced the percentage and severity of infection. The integrated treatment (SA + Zn + Azo) significantly outperformed the other treatments in reducing the percentage and severity of infection, reaching 41.66% and 18.11%, respectively, compared to the pathogen-only treatment. The average percentage and severity of infection for all plant cultivars reached 96.67% and 85.86%, respectively. The results for the plant cultivar factor showed that the Cypriot cultivar significantly outperformed the other plant cultivars in its resistance to seed rot and seedling damping-off in faba bean plants, caused by the fungus *R. solani.* It recorded the lowest response to disease infection, as the infection percentage and severity rate for all experimental parameters reached 49.33% and 39.09% respectively, compared to the Syrian cultivar, which recorded an average of 66.66% and 56.15% respectively.

The research revealed significant efficacy towards controlling seed rot and the seedling damping-off disease caused by *R. solani* in faba bean using two principal measures. Firstly, there was a marked variation in the susceptibility of cultivars. The Cypriot cultivar displayed significant resistance and had the lowest percentage of infection and disease severity compared to the Syrian cultivar, which had the highest susceptibility. This study's findings are also in agreement with other studies demonstrating that cultivars differ in their resistance to fungal pathogens [37].

Furthermore, the findings also emphasized the outperformed effectiveness of using salicylic acid, Zinc, and *Azo.chroococcum* together in reducing the incidence and severity of the disease. This tri-application alone provided a significant reduction of both incidence and severity of disease, demonstrating significant control in faba bean by using both the tri-application treatment designed above and antifungal activity [38], compared to unaided or individually applied. This study's proof of effectiveness suggests not only efficacy but that there is effectiveness that is a result of the synergistic action of the three different components, which subsequently led to better defensive mechanisms in the plant and less offensive action in the pathogen [39].

Table (6): Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in reducing the percentage of infection caused by *R.solani*.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	0.00	0.00	0.00	0.00	0.00
2	Pathogen (R.s)	100	100	93.33	93.33	96.67
3	Azo+(R.s)	86.66	86.66	73.33	53.33	75.00
4	(R.s)+Zn	93.33	93.33	86.66	73.33	86.66
5	(R.s)+SA	93.33	93.33	86.66	73.33	86.66
6	(R.s)+Zn+Azo	73.33	73.33	66.66	53.33	66.66
7	(R.s)+SA+Azo	73.33	73.33	66.66	53.33	66.66
8	(R.s)+SA+Zn	80.00	80.00	80.00	66.66	76.67
9	(R.s)+SA+Zn+Azo	53.33	53.33	33.33	26.66	41.66
10	(R.s) + Beltanol	13.33	13.33	0.00	0.00	6.67
Means		66.66	66.66	58.66	49.33	
L.S.D $_{0.05}$		**Treatments: 7.276**		**Cultivars: 6.88**	**Interaction: 13.754**	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Table (7): Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in reducing the infection severity percentage caused by *R.solani*.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	0.00	0.00	0.00	0.00	0.00
2	Pathogen (R.s)	100	100	72.89	70.54	85.86
3	Azo+(R.s)	65.54	62.65	55.32	42.75	56.57
4	(R.s)+Zn	76.87	77.54	62.87	58.88	69.04
5	(R.s)+SA	76.80	76.54	62.85	58.88	68.77
6	(R.s)+Zn+Azo	66.56	62.68	57.32	44.77	57.83
7	(R.s)+SA+Azo	65.54	68.65	55.32	42.75	58.07
8	(R.s)+SA+Zn	77.80	76.54	64.85	58.48	69.42
9	(R.s)+SA+Zn+Azo	21.78	19.88	16.88	13.88	18.11
10	(R.s) + Beltanol	10.67	8.87	0.00	0.00	4.89
Means		56.15	55.33	44.83	39.09	
L.S.D $_{0.05}$		**Treatments: 9.25**		**Cultivars: 5.848**	**Interaction: 11.725**	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Evaluating the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in increasing the effectiveness of the peroxidase enzyme (POD) against infection caused by *R.solani*

The findings (Table 8) illustrated that all the treatments applied to suppress seed rot and seedling damping-off of faba bean incited by *R. solani* played a clear role in inducing systemic resistance in the plants. Management either individually or in combination produced notable variations in peroxidase (POD) enzyme levels in faba bean plants. The treatment of salicylic acid, zinc, and Azotobacter chroococcum (SA + Zn + Azo) outperformed all treatments to yield the maximum recorded peroxidase increase of 0.59 unit mg^{-1} FW, while the control obtained an average of 0.32 unit mg^{-1} FW, across all cultivars.

When considering the varietal factor, the Cypriot cultivar had statistically significant responses from the others when inducing resistance from seed rot and seedling damping-off from *R. solani*. Overall, the Cypriot cultivar produced the highest increase in peroxidase enzyme activity across all treatments average of 0.49 unit mg^{-1} FW, while the Syrian and Iraqi cultivars had a peroxidase enzyme measurement average of 0.41 unit mg^{-1} FW.

The results of this test also demonstrated the high effectiveness of the treatments used to induce systemic resistance against the fungus *Rhizoctonia solani*, which causes seed rot and seedling damping-off in faba bean plants. This was clearly reflected in the increased activity of the peroxidase enzyme (POD), an important physiological indicator associated with plant defense mechanisms. The results showed that all treatments, whether chemical (such as Zinc (Zn) and salicylic acid (SA), probiotic (*Azotobacter chroococcum*), or combinations of these treatments, contributed to stimulating the plant to produce higher levels of peroxidase compared to the control treatment. This effect is attributed to the role of these factors in activating defense pathways within plant cells. Salicylic acid acts as an important regulator of defense signals, while Zinc plays a role in stabilizing enzyme structures and enhancing their activity. Beneficial bacteria also contribute to enhancing resistance responses through the mechanism of induced systemic resistance (ISR).

Table (8): Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in increasing the effectiveness of the peroxidase enzyme against infection caused by *R.solani*.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	0.3	0.3	0.32	0.34	0.32
2	Pathogen (R.s)	---	---	0.48	0.49	0.49
3	Azo+(R.s)	0.33	0.34	0.39	0.42	0.37
4	(R.s)+Zn	0.37	0.38	0.39	0.42	0.39
5	(R.s)+SA	0.48	0.49	0.5	0.54	0.50

6	(R.s)+Zn+Azo	0.44	0.42	0.44	0.47	0.44
7	(R.s)+SA+Azo	0.46	0.46	0.52	0.55	0.50
8	(R.s)+SA+Zn	0.48	0.48	0.52	0.56	0.51
9	(R.s)+SA+Zn+Azo	0.51	0.51	0.64	0.68	0.59
10	(R.s) + Beltanol	0.35	0.32	0.36	0.39	0.36
Means		0.41	0.41	0.46	0.49	
L.S.D $_{0.05}$		**Treatments: 0.12**		**Cultivars: 0.03**	**Interaction: 0.16**	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Evaluating the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in increasing the effectiveness of the polyphenol oxidase (PPO) against infection caused by *R.solani*

The results (Table 9) showed that all treatments used to control seed rot and seedling damping-off on faba bean plants caused by the fungus (*R.solani*) had a significant effect in stimulating systemic resistance in plants against the disease. These factors, individually or in combination, led to an increase in the content of polyphenol oxidase (PPO) enzyme in faba bean plants. It was noted that the treatment of integration between the factors (SA + Zn + Azo) outperformed in raising the level of polyphenol oxidase (PPO) enzyme, as it recorded an average of 1.66 unit mg^{-1} FW, compared to the control treatment, which achieved an average of 1.34 units mg^{-1} FW. The plant cultivars also showed significant differences, as the Cypriot cultivar was outperformed in inducing resistance against seed rot and seedling damping-off in faba bean plants caused by the fungus (R.*solani*), as it recorded the highest rate of increase in the activity of the polyphenol oxidase enzyme, with an average of 1.52 unit mg^{-1} FW, compared to the Syrian and Iraqi cultivars, which recorded an average of 1.47 unit mg^{-1} FW for each of them.

The results of this test indicate that all chemical, biological and interventional treatments used in the experiment played an effective role in stimulating systemic resistance in faba bean plants against seed rot and seedling damping-off caused by the fungus *Rhizoctonia solani*, by affecting the activity of the polyphenol oxidase (PPO) enzyme, which is one of the pivotal enzymes in the plant's defense response.

Table (9): Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in increasing the effectiveness of the polyphenol oxidase (PPO) enzyme against infection caused by *R.solani*.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	1.32	1.32	1.34	1.36	1.34
2	Pathogen (R.s)	---	---	1.47	1.48	1.48
3	Azo+(R.s)	1.43	1.43	1.46	1.47	1.45
4	(R.s)+Zn	1.44	1.45	1.46	1.47	1.46

5	(R.s)+SA	1.55	1.58	1.59	1.60	1.58
6	(R.s)+Zn+Azo	1.43	1.46	1.48	1.49	1.47
7	(R.s)+SA+Azo	1.56	1.57	1.59	1.60	1.58
8	(R.s)+SA+Zn	1.52	1.52	1.56	1.57	1.54
9	(R.s)+SA+Zn+Azo	1.61	1.62	1.68	1.72	1.66
10	(R.s) + Beltanol	1.35	1.35	1.45	1.46	1.40
Means		1.47	1.48	1.51	1.52	
L.S.D $_{0.05}$		**Treatments: 0.33**		**Cultivars: 0.09**	**Interaction: 0.75**	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Evaluation the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in increasing the accumulation of total phenols and inducing resistance against infection with the fungus *R.solani*

The results (Table 10) showed that all treatments used to control seed rot and seedling damping-off in faba bean caused by the pathogenic fungus *R. solani* were highly effective in stimulating systemic plant resistance to the disease. These factors, individually or in combination, led to an increase in the total phenolic content of the faba bean. The integrated treatment (SA + Zn + Azo) significantly increased the level of total phenol accumulation by 0.61 unit mg^{-1} FW, compared to the control treatment, which recorded an average of 0.30 unit mg^{-1} FW. In contrast, the results of the plant cultivars showed significant differences in raising the level of total phenol accumulation. The Cypriot cultivar outperformed the Syrian cultivar, which recorded an average of 0.47 unit mg^{-1} FW, in increasing the total phenolic content by 0.43 unit mg^{-1} FW.

The results of this test showed that all chemical and biological treatments, whether individually or in combination, effectively contributed to enhancing the accumulation of total phenol in the faba bean plant, indicating a precise stimulation of systemic resistance against infection by the fungus *Rhizoctonia solani*. Phenolic compounds are among the most important components of plant defense, as they act as antioxidants, anti-pathogen compounds, and contribute to strengthening cell walls. The triple complementary treatment (SA + Zn + Azo) recorded the highest rate of phenolic accumulation, confirming the cumulative effectiveness of these factors in activating defense pathways within the plant. The Cypriot cultivar also demonstrated a significant outperformed in its response, indicating its genetic efficiency in producing defense compounds compared to other cultivars [40].

Table (10): Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in increasing the accumulation of total phenols and inducing resistance against infection with the fungus *R.solani*.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	0.29	0.29	0.30	0.32	0.30
2	Pathogen (R.s)	---	---	0.38	0.39	0.39
3	Azo+(R.s)	0.35	0.36	0.36	0.38	0.36
4	(R.s)+Zn	0.32	0.33	0.34	0.36	0.34
5	(R.s)+SA	0.50	0.52	0.53	0.55	0.53
6	(R.s)+Zn+Azo	0.46	0.46	0.48	0.49	0.47
7	(R.s)+SA+Azo	0.52	0.53	0.55	0.56	0.54
8	(R.s)+SA+Zn	0.52	0.53	0.55	0.58	0.55
9	(R.s)+SA+Zn+Azo	0.56	0.58	0.64	0.67	0.61
10	(R.s) + Beltanol	0.34	0.36	0.37	0.38	0.36
Means		0.43	0.44	0.45	0.47	
L.S.D $_{0.05}$		**Treatments: 0.07**		**Cultivars: 0.04**	**Interaction: 0.16**	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Evaluation the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in influencing the dry weight of shoot and root systems as affected by *R.solani*

The results (Tables 11 and 12) showed that all treatments used to control seed rot and seedling damping-off on faba bean plants, caused by the pathogenic fungus *R. solani*, were effective in increasing the dry weight of the shoot and root system. The combined treatment (SA + Zn + Azo) significantly outperformed the other treatments, with averages of 18.68 g and 4.36 g, respectively, compared to the pathogen-only treatment, which recorded averages of 7.94 g and 0.93 g, respectively. The results also showed significant differences between cultivars in the dry weight of the shoot and root system. The Cypriot cultivar outperformed, yielding averages of 16.16 g and 3.77 g, respectively, compared to the Iraqi cultivar, which recorded averages of 14.56 g and 3.06 g, respectively.

These results confirmed the positive effect of different treatments in mitigating the damage caused by the fungus *Rhizoctonia solani* on the growth of faba bean plants, specifically on the dry weight of the shoot and root system. All factors used, whether individually or in combination, showed a significant increase in growth parameters, including the fresh and dry weight of the shoot and root parts, compared to the pathogen treatment alone, which led to a significant deterioration in growth, which is consistent with what was indicated by [41], regarding the adverse effect of the fungus *R. solani* on the shoot and root growth of plants.

Table (11): Evaluation of the role of plant cultivars and the efficiency of certain chemical and biological agents and their integration in influencing the dry weight of the shoot system under *R. solani* infection.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	12.46	12.45	14.19	14.20	13.33
2	Pathogen (R.s)	----	----	7.46	8.41	7.94
3	Azo+(R.s)	15.70	14.76	16.41	17.61	16.12
4	(R.s)+Zn	14.13	13.48	15.48	15.87	14.74
5	(R.s)+SA	13.49	13.24	14.99	15.56	14.32
6	(R.s)+Zn+Azo	16.91	16.59	18.34	19.21	17.76
7	(R.s)+SA+Azo	16.89	17.05	18.25	19.19	17.85
8	(R.s)+SA+Zn	14.20	14.13	14.67	16.34	14.84
9	(R.s)+SA+Zn+Azo	17.61	17.37	19.19	20.54	18.68
10	(R.s) + Beltanol	11.26	11.97	13.32	14.69	12.81
Means		14.74	14.56	15.23	16.16	
L.S.D 0.05		Treatments: 3.24		Cultivars: 0.818	Interaction: 3.735	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

Table (12): Evaluation of the role of plant cultivars and the efficiency of specific chemical and biological agents and their integration in influencing the dry weight of root system under *R. solani* infection.

No.	Treatments	Cultivars				Means
		Syrian	Iraqi	Spanish	Cypriot	
1	Control	2.91	2.91	3.31	3.31	3.11
2	Pathogen (R.s)	---	---	1.74	1.96	0.93
3	Azo+(R.s)	3.66	3.45	3.83	4.11	3.76
4	(R.s)+Zn	3.30	3.15	3.61	3.70	3.44
5	(R.s)+SA	3.15	3.09	3.50	3.63	3.34
6	(R.s)+Zn+Azo	3.95	3.87	4.28	4.48	4.15
7	(R.s)+SA+Azo	3.94	3.98	4.26	4.48	4.17
8	(R.s)+SA+Zn	3.31	3.30	3.42	3.81	3.46
9	(R.s)+SA+Zn+Azo	4.11	4.05	4.48	4.79	4.36
10	(R.s) + Beltanol	2.63	2.79	3.11	3.43	2.99
Means		3.10	3.06	3.55	3.77	
L.S.D 0.05		Treatments: 1.45		Cultivars: 1.08	Interaction: 2.08	

Values are means of three replications. R.s = *R.solani*; Azo = *A. chroococcum*; SA = Salicylic acid; Zn = Zinc.

The field survey conducted in the governorates of Kerbala, Babylon, and Najaf indicated the occurrence of some fungal species linked to both seed rot and seedling damping off in faba bean. The most isolated fungal species was *R.solani*, and many pathogens are likely involved, but with the fungus as the dominant and clear pathogenesis agent. The new isolate of *R. solani* was identified and then deposited in the NCBI with accession number PV939284.1, with high virulence compared with the other isolates. In the field trial, all treatments were effective at managing the disease; nevertheless the integrated treatment containing *Azo.chroococcum*, salicylic acid, and Zinc was the most effective at reducing both incidence and severity, demonstrating the possibility of using eco-friendly agents as part of an integrated management of disease.

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