

Effect of some Biological and Chemical Agents and Their Interaction on Faba Bean's Growth and Protecting it From Infection with *Rhizoctonia Solani*

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ABSTRACT

The experiments were conducted at two locations, Plant Diseases laboratory –Ministry of Agriculture, Baghdad, Iraq, and College of Agricultural Engineering Sciences, Baghdad University, Al- Jadiriya, Iraq, during the seasons of 2022-2023. This study was conducted to test the effectiveness of some chemical and biological compounds, individually or in combination, against root rot disease on faba bean plants caused by the fungus *R. solani*. The greenhouse experiment showed the mixing treatments (Rh11 + Af + Se), (Rh11+ Ar + Af + Se), (Rh11 +Scn (Af) + SeNPs) and (Rh11 + Scn (Af) + Scn (Ar) + SeNPs) were the most efficient. The rate and severity of infection with the fungus *R. solani* scored 0.00 and 0.00.%, respectively. Fresh and dry weights, total chlorophyll percentage, and plant heights were significantly increased. The Rh11 + Ar + Af + Se mixture treatment could induce systemic resistance through increasing the activity of the polyphenol oxidase enzyme 79.2 and 85.2 rate change absorbance/min/ g fresh weight of plant leaves respectively.

Keywords: broad bean, Induced systemic resistance. PGPR, SeNPs, *Rhizoctonia solani*

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INTRODUCTION

Vicia faba L., which belongs to the **Fabaceae** family, is considered one of the economically important winter plants in many countries, including Iraq . It plays a critical role in improving land structure and contributing to wild pollinator maintenance. It **originated** from Algeria (Matloob & Alkaif, 2015; Dakhil & Alshimaysawe, 2023; Abou-Khater et al., 2024).;). **Faba bean** has the ability to improve soil characteristics by fixing atmospheric nitrogen in the soil through the root nodules in **coexistence** with bacteria **PGPR** (Hassan & Awad, 2017). The faba bean plant is grown **nationwide** in Iraq, as the cultivated area in 2022 was about 20,811 **dunum**, and the production was 40,586 tons, at a rate of **1,950.2 kg/dunum** . The crop is exposed to many fungal diseases, including root and stem rot. It is one of the major diseases impacting

the faba bean crop in many growing **areas** worldwide (Kadim et al., 2025). In recent years, a significant decrease in faba bean production **has** occurred due to **biotic** and abiotic factors, including those caused by many soil pathogens: mainly ***Rhizoctonia solani***, ***Fusarium spp***, ***Sclerotinia sclerotiorum***, and ***Pythium spp***. These fungi cause **severe** losses in faba bean production through rotting seeds, seedlings, and roots (Almimon , 2026). The fungus ***Rhizoctonia solani*** is **among** the most important pathogenic soil fungi that attack faba bean crops, causing seedling death and rotting of the roots and stems of plants, as well as wilting and seed rot diseases (Hassan & Awad, 2017). The intensive use of chemical pesticides and their impact on human health and the environment **have** led to the development of eco-friendly pesticide alternatives to combat

diseases (Ratto et al., 2022 ; Bharadava et al., 2026;). Bio-agents have **been** successfully used to reduce pathogen inoculum and enhance the plant **defense** system through inducing systemic resistance. These bio-factors, **also** known as Plant Growth Promoting Rhizobacteria, can improve plant growth and increase production both in quantity and quality found that the use of *Alcaligenes faecalis* bacteria demonstrated high efficiency in inhibiting the fungus *R. solani*, that causing of Rhizoctonia root rot , by 58.92%. The metabolites of rhizobacteria (PGPR), including *Pseudomonas fluorescens*, *Bacillus megaterium*, and *B. subtilis*, were found to play a pivotal role in controlling root rot disease in faba bean plants caused by the fungi *Rhizoctonia solani*, *Fusarium solani*, and *F. oxysporum* (Khalil et al., 2022). On the other hand, the use of selenium in soil decreased the infection rate of the fungus *Botrytis cinerea*, causing grey mold disease in tomatoes, from 42.19% to 25.00% (Li et al., 2022). Selenium nanoparticles (SeNPs) at a concentration of 300 µg/ml **showed** high **efficacy** in inhibiting the fungus *Bipolaris sorokiniana*, causing leaf spot disease in wheat under laboratory conditions (Shahbaz et al., 2023). Given the economic importance of the faba bean plant and to minimize the use of chemical pesticides against soil pathogens, the study aimed to control root rot disease in bean plants through testing systemic resistance-inducing agents alone or in combination with reported microbes and nanoparticles.

MATERIALS AND METHODS

Isolation and diagnosis of the pathogenic fungus: Samples of symptomatic faba bean seedlings were brought to the plant diseases laboratory of the Diagnostic Department/Crop Protection Department/Ministry of Agriculture. Then the infected parts (crown area and root system) were separated from each sample and washed well with tap water then left to dry. About 0.5 cm pieces from each sample were sterilized and transferred to petri dishes containing potato dextrose agar (PDA) (4 pieces/plate) and the plates were

incubated at a temperature of $25 \pm 2^\circ\text{C}$ for 3 days .pathogenic fungus isolates were purified and then classified to the species level based on the taxonomic keys mentioned. A culture of each isolate was preserved in form of slants medium for further tests.

Pathogenicity test of the pathogenic fungus on the germination and growth of faba bean seedlings in pots under greenhouse conditions.:

The experiment was carried out in one of the fields affiliated with the Plant Diseases Laboratory/Agricultural Protection Department at Baghdad. The inoculum was prepared from each isolate (n=19) (Table 1). Seeds of local millet *Panicum miliaceum* L., was washed, soaked in water for six hours and distributed in glass flasks at a rate of 50 g/150 ml flask and autoclaved Five discs with a diameter of 0.5 cm of each isolate grown on PDA at the age of 5 days were inoculated per flask. Inoculated flasks were incubated for 10 days at a $25 \pm 2^\circ\text{C}$ and shaken every three days to ensure the distribution of the fungus on the seeds and to avoid clumping. Ten days later, a mixture of mixed soil and peatmoss (2:1) sterilized were distributed in 2 kg plastic pots and inoculated millet seeds were placed individually in each pot. at a rate of 2% (weight/weight). Sterilized millet seeds were used for fungus free control treatment. Pots were watered and covered with perforated polyethylene bags after for 3 days. Surface sterilized seeds of a local faba bean variety were sewed at a rate of 5 seeds/pot and 3 replicates/treatment. The infection rate was calculated ten days after planting and for five consecutive days until the germination of all seeds in control treatment and continued for two weeks after germination, following the equation:

$$\text{Infectivity (\%)} = \frac{\text{No.of infected plant}}{\text{Total plant number}} \times 100$$

The experiment was performed following completely randomized design (CRD). The most pathogenic or virulent isolate (referred to as Rh11) was selected for the following experiments.

Table1. Isolates of the pathogenic fungus which collected from symptomatic faba bean plants

Sites	Symbol of Isolation	Sites	Symbol of Isolation	Sites	Symbol of Isolation
Baghdad	Rh1	Kirkuk	Rh8	Babylon	Rh14
Baghdad	Rh2				
Baghdad	Rh3	Kirkuk	Rh9	Babylon	Rh15
Baghdad	Rh4	Kirkuk	Rh10	Salahaddin	Rh16
Baghdad	Rh5	Kirkuk	Rh11	Salahaddin	Rh17
Baghdad	Rh6	Kirkuk	Rh12	Salahaddin	Rh18
Baghdad	Rh7	Babylon	Rh13	Salahaddin	Rh19

Antifungal activity of some factor against the pathogenic fungus on faba bean in pots under greenhouse conditions: The experiment was carried out in the greenhouse of the Plant Diseases Laboratory, Agricultural

Protection Department, Ministry of Agriculture. Autoclaved mixed soil and peat moss were put in plastic pots at 4 kg/pot rate. The experiment included the following treatments:

- | | |
|---|--|
| 1. soil only | 21. soil + Scn(Ar) + SeNPs. |
| 2. soil + <i>R. solani</i> (Rh 11) | 22. Sterile + Scn (Af) + Scn(Ar) + SeNPs |
| 3. soil + <i>A. faecalis</i> (Af) | 23. Rh11 + Af |
| 4. soil + <i>A. radioresistens</i> (Ar) | 24. Rh11 + Ar |
| 5. soil + normal secondary compounds Sc (Af) | 25. Rh11 + Sc (Af.) |
| 6. soil + normal secondary compounds Sc (Ar). | 26. Rh11 + Sc (Ar). |
| 7. soil + nano secondary compounds Scn(Af). | 27. Rh11 + Scn (Af) |
| 8. soil + nano secondary compounds Scn(Ar). | 28. Rh11 + Scn (Ar) |
| 9. soil + Selenium (Se) | 29. Rh11 + Se. |
| 10. soil + nano selenium (SeNPs) | 30. Rh11+ SeNPs |
| 11. soil + Ar + Af | 31. Rh11+ Ar + Af. |
| 12. soil + Af + Se | 32. Rh11 + Af + Se |
| 13. soil + Ar + Se | 33. Rh11+ Ar + Se |
| 14. soil + Ar + Af + Se | 34. Rh11 + Ar + Af + Se |
| 15. soil + (Sc (Af) + (Sc (Ar). | 35. Rh11 + Sc (Af) + Sc (Ar) |
| 16. soil + Sc (Af) + Se. | 36. Rh11 + Sc (Af) + Se |
| 17. soil + Sc (Ar) + Se | 37. Rh11 + Sc (Ar) + Se |
| 18. soil + Sc (Af) + Sc (Af) + Se. | 38. Rh11 + Sc (Af) + Sc (Ar) + Se |
| 19. soil + Scn (Af) + Scn (Af). | 39. Rh11+ Scn (Af) + Scn (Ar). |
| 20. soil + Scn (Af) + SeNPs. | 40. Rh11+ Scn(Af) + SeNPs |
| | 41. Rh11 + Scn (Ar) + SeNPs. |
| | 42. Rh11+ Scn (Af) + Scn (Ar) + SeNPs. |
| | 43. Rh 11 + Uniforme |

Surface sterilized seeds of local faba bean variety were sewn at a rate of 5 seeds/pot. The treatments of ordinary selenium, was added to the potting soil at a concentration of 100 ppm and rate of 50 ml/pot. The bacterial inoculum was added to the treatments of *A. faecalis* and *A. radioresistens* bacteria at 100 ml/pot rate and 7×10^8 and 5×10^7 (colony forming units CFU/ml) concentrations respectively, prepared from a 48 hours - old culture.. Ordinary and nano-sized secondary metabolites were added at 10 and 5 %/pot respectively, rate based on unpublished data. SeNPs treatment was added at 60 ppm concentration and 50 ml/pot rate. One ml/liter concentration of uniform pesticide was added at 50 ml/pot rate following the manufacturer's recommended

dose. The pathogenic fungal inoculum, grown on millet seeds was added to pots 14 days of sewing at 40g/pot rate. The pathogen free control pots included sterilized soil only and sterilized millet seeds. Additional fungus free treatments *A. faecalis*, *A. radioresistens*, common and nano selenium SeNPs and the regular and nano secondary compounds were included. Treatments that included more than one factor, a half the concentration of each tested materials was used. The experiment was maintained as necessary. A completely randomized design (CRD) was used including 43 treatments in three replicates. Data were analyzed using L.S.D test at 0.05 probability level. Symptoms on seedlings were observed five days of inoculation. The percentage of

infection was calculated using following the equation:

$$\text{Infectivity (\%)} = \frac{\text{No. of infected plant}}{\text{Total plant number}} \times 100$$

The percentage of chlorophyll in the leaves of the bean plants was measured using a SPAD device (Minolta SPAD 502) two months of seeding. Three leaves from each replicate, at different heights were used. Plant heights were

0= Not infected plants	1=1-25% of root is rotten
2= More than 25-50% of root is rotten	3= More than 50-75% of root is rotten
4= More than 75-100% of root is rotten or plant death	

Disease severity percent was calculated based as follows:

$$\text{Disease severity (\%)} = \frac{\text{No. of plants in class}(0 \times 0) + \dots + \text{No of plants in class}(4 \times 4)}{\text{Total no of All examined plants} \times \text{highest class}} \times 100$$

Three plants were taken from each replicate to calculate the fresh and dry weight. The root system of each plant was washed with tap water to remove soil, placed in paper bags and dried in an electric oven at a temperature of 40°C for two days until the weight was stable. Biochemical characteristics in stimulating seedling resistance to the pathogen were measured. Leaf samples were taken from plants treated with the tested factors 7, 14 and

also recorded of three plants from the surface of the soil to the top. Plants from each replicate were uprooted to estimate infection severity for 10 days. After two months of planting, the plants were uprooted from each replicate to calculate the severity of the infection following a 5-degree disease index:

21 days of adding the fungal inoculum Polyphenol oxidase (PPO).

RESULTS AND DISCUSSION

Isolation and diagnosis of the fungus *Rhizoctonia solani*, associated with faba bean rot roots and crown disease. Morphological identification confirmed the presence of *R. solani* within the symptomatic faba bean samples (Figure 1) which was identified as reported (El-Gazzar et al.,2023).

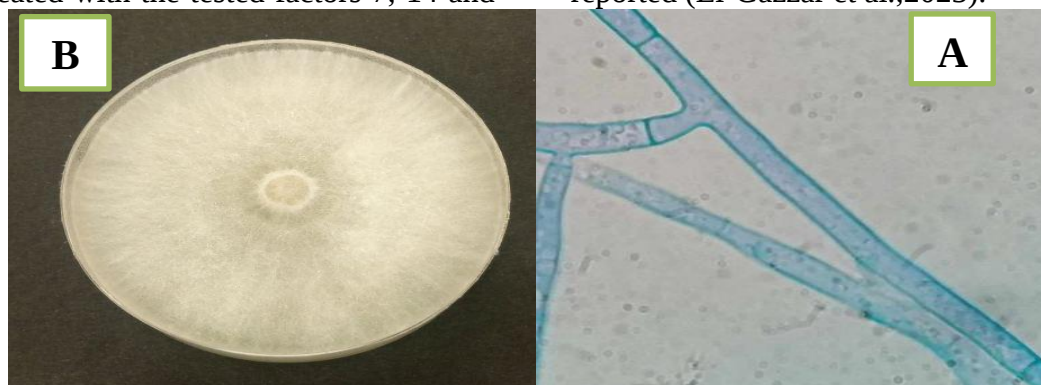


Figure 1. Morphological characteristics of *R. solani* isolate; A- The presence of shortening of branching cells in the area of emergence and the formation of transverse barriers in the branches near the point of emergence under magnification (4X); B- *R. solani* isolate grown on PDA medium germination (Table 2) and (Figure 2).The pathogenic inconsistency among the 19 isolates on faba bean seeds, may be related to the variation in the quantity and type of metabolic compounds, toxins and enzymes produced by the isolates. Besides, the genetic variability among isolates may occur as they were collected from different sources (Mousa & Hassan, 2023).

Pathogenicity of *R. solani* isolates on the germination and growth of faba bean seedlings in pots under greenhouse conditions: All 19 isolates of the fungus *R. solani* were pathogenic that reduced the seed germination of faba bean, ranged 33.33 - 96.67% compared to 0.0% which was reduction in fungus free control. Among others, Rh11 isolate scored minimum 96.67%



Figure 2. Testing the pathogenicity of *R. solani* in pots; seedlings infected with the fungus *R. solani* (left), healthy control (right).

Table 2. Detection of isolates of the pathogenic fungus *Rhizoctonia solani* on faba beans under potting condition

No.	Pathogenic fungus	Isolation symbol	Infectivity (%)
1	<i>Rhizoctonia solani</i>	Rh1	73.33
2	<i>Rhizoctonia solani</i>	Rh2	93.33
3	<i>Rhizoctonia solani</i>	Rh3	56.67
4	<i>Rhizoctonia solani</i>	Rh4	46.67
5	<i>Rhizoctonia solani</i>	Rh5	56.67
6	<i>Rhizoctonia solani</i>	Rh6	93.33
7	<i>Rhizoctonia solani</i>	Rh7	83.33
8	<i>Rhizoctonia solani</i>	Rh8	73.33
9	<i>Rhizoctonia solani</i>	Rh9	90.00
10	<i>Rhizoctonia solani</i>	Rh10	33.33
11	<i>Rhizoctonia solani</i>	Rh11	96.67
12	<i>Rhizoctonia solani</i>	Rh12	76.67
13	<i>Rhizoctonia solani</i>	Rh13	53.33
14	<i>Rhizoctonia solani</i>	Rh14	33.33
15	<i>Rhizoctonia solani</i>	Rh15	90.00
16	<i>Rhizoctonia solani</i>	Rh16	73.33
17	<i>Rhizoctonia solani</i>	Rh17	86.67
18	<i>Rhizoctonia solani</i>	Rh18	66.67
19	<i>Rhizoctonia solani</i>	Rh19	33.33
	Control		0.00
	L.S.D _{0.05}		8.78

Each infectivity data represents an average infectivity% calculated from three replicates

Inducing systemic resistance of faba bean seedlings against *R. solani* with some biological and chemical factors under greenhouse conditions: The combination treatments Rh11 + Af + Se, Rh11 + Ar + Af + Se, Rh11 + Scn (Af) + SeNPs, and Rh11 + Scn (Af) + (ScnAr) + SeNPs could decrease infection incidence and severity of the fungus *R. solani*, scoring 0.00% in both cases, compared to other treatments. This is in contrast to the fungus-only control, which scored 83.33% and 78.33%, respectively (Table 3). The antifungal activity of the factors tested against root rot disease and seedling death may involve more than one mechanism.

The first mechanism is the direct effect of the factors in inhibiting the growth of the pathogenic fungus. The second is acting as pH regulators, making the soil an unsuitable medium for fungal growth. The third is inducing the plant's systemic resistance against the pathogen by stimulating the plant to produce Pathogenesis-Related Proteins (PRPs) at the infection sites, including β -1,3-glucanase and chitinase, which break down fungal cell walls. Additionally, the accumulation of phytoalexins and enzymes, including peroxidase and polyphenol oxidase, as well as phenolic compounds, increases the strength of the plant cell wall against pathogen

penetration (Mousa. & Hassan, 2023). Alternatively, environmental conditions tolerated by the fungus enable rapid spread and development, making it more adaptable and aggressive causing diseases (Mousa and. Hassan, 2023). *A. faecalis* and *A. radioresistens* activity in reducing the incidence and severity of pathogenic fungi infections may be related to the production of secondary metabolites, including octadecyl-3, 5-di-tert-butyl-4-hydroxyphenyl-3, and antioxidants. Moreover, they improve plant growth by enhancing the production of hormones, enzymes, vitamins, and growth regulators. Hence, these bio-agents support most of the vital processes within the plant and the enzymes responsible for building the amino acid components of the cell wall, as well as secreting toxins and secondary metabolites that inhibit pathogens (El-Sayed et al., 2020; Pérez-García et al., 2023). Another advantage is their ability to produce substances that stimulate plant growth, including indole acetic acid (IAA), siderophores, and hydrogen cyanide (HCN). Their high ability to dissolve phosphate, produce ammonia, and increase the effectiveness of enzymes such as amylase, protease, xylanase, cellulase, chitinase, and catalase has been reported (Fouda et al., 2021; Patel et al., 2020). Additionally, these two species of bacteria can colonize the plant's rhizosphere, enhancing plant growth under varying conditions, as they have been isolated from the roots of different crops worldwide. The colonization of bacteria depends on their distinctive characteristics, including chemotaxis towards root secretions, metabolic production, their ability to fix nitrogen, and compete with other microorganisms. They can also provide nutrients and mineral elements, form biofilms, and include genes for Lipopeptides biosynthesis (Pérez-García et al., 2023). The efficiency of selenium nanoparticles against infection incidence and severity may be due to their activation of the plant's defenses against pathogens by regulating the levels of reactive oxygen

species (ROS) and increasing amino acid levels. It enhances cellular defenses through the accumulation of lignin, callus, and hydrogen peroxide. Selenium nanoparticles are also involved in increasing the content of jasmonic acid (JA) and the expression of JA pathway genes, as well as stimulating the activity of antioxidant enzymes (Li et al., 2023). Similarly, (Abdelsalam et al., 2023). The use of selenium nanoparticles (SeNPs) at a concentration of 20 mg/ml per 20 ml of PDA medium inhibited the fungal growth of *R. solani*, *F. oxysporum*, and *F. solani in vitro* and improved the germination of bean seeds, with soybean yield increasing by 93% and germination rate up to 19%. The antifungal activity of ordinary selenium may be through enhancing the ability of antioxidant enzymes, associated with increased activity of glutathione peroxidase and secondary compounds. It is also involved in raising the levels of defense-related enzymes, including SOD (superoxide dismutase) and CAT (catalase) thus reducing biotic and abiotic stresses in plants, stimulating metabolism, and encouraging germination and growth rates of plants (Li et al., 2023). The activity of bacterial secondary metabolites can be attributed to volatile organic compounds (VOCs) produced by bacteria, which inhibit the growth of pathogens. Additionally, these metabolites act as signaling molecules during interactions between plants and microorganisms and can spread in soil particles (Vaghela and Gohel, 2023). Furthermore, the secondary metabolites of bacteria are involved in inducing plant defenses by stimulating the activity of gallic acid and shikimic acid, which act as antifungal agents and enhance the plant defense system, similarly, confirmed that treating okra plants with secondary metabolites from *Alcaligenes faecalis* bacteria could reduce the infection severity of the fungus *Sclerotium rolfsii* in the field, as well as inhibit fungal growth in the laboratory (Ray et al., 2023).

Table 3. Effect of some chemical and biological compounds on the disease incidence and severity percentage caused by the fungus *R. solani* in pots under greenhouse.

No.	Treatments	Infectivity (%)	Disease severity (%)
1	control	0.00	0.00
2	<i>R. solani</i> (Rh 11)	83.33	78.33
3	<i>A. faecalis</i> (Af)	0.00	0.00
4	<i>A. radioresistens</i> (Ar)	0.0	0.00
5	Sc (Af)	0.00	0.00
6	Sc (Ar)	0.00	0.00
7	Scn (Af)	0.00	0.00
8	Scn (Ar)	0.00	0.00
9	Se	0.00	0.00
10	SeNPs	0.00	0.00
11	Ar + Af	0.00	0.00
12	Af+ Se	0.00	0.00
13	Ar+ Se	0.00	0.00
14	Ar + Af+ Se	0.00	0.00
15	Sc (Af) + Sc (Ar)	0.00	0.00
16	Sc (Af) + Se	0.00	0.00
17	Sc (Ar) + Se	0.00	0.00
18	Sc (Af) + Sc (Ar) + Se	0.00	0.00
19	Scn (Ar) + Scn (Af)	0.00	0.00
20	Scn (Af) + SeNPs	0.00	0.00
21	Scn (Ar) + SeNPs	0.00	0.00
22	Scn (Af) + Scn (Ar) + SeNPs	0.00	0.00
23	Rh11 + Af	13.33	10.00
24	Rh11 + Ar	33.33	25.00
25	Rh11 + Af	33.33	25.00
26	Rh11 + Sc (Ar)	46.67	36.67
27	Rh11 + Scn (Af)	20.00	15.00
28	Rh11 + Scn (Ar)	26.67	20.00
29	Rh11 + Se	13.33	10.00
30	Rh11 + SeNPs	6.67	5.00
31	Rh11 + Ar + Af	6.67	5.00
32	Rh11 + Af + Se	0.00	0.00
33	Rh11 + Ar + Se	13.33	8.33
34	Rh11 + Ar + Af + Se	0.00	0.00
35	Rh11 + Sc (Af) + Sc (Ar)	26.67	21.67
36	Rh11 + Sc (Af) + Se	26.67	20.00
37	Rh11 + Sc (Ar) + Se	33.33	25.00
38	Rh11 + Sc(Af) + Sc (Ar) + Se	13.33	8.33
39	Rh11 + Scn (Ar) + Scn (Af)	13.33	10.00
40	Rh11 + Scn (Af) + SeNPs	0.00	0.00
41	Rh11 + Scn (Ar) + SeNPs	6.67	5.00
42	Rh11 + Scn (Ar) + Scn (Af) + SeNPs	0.00	0.00
43	Rh11 + Uniforme	20.00	16.00
	L.S.D _{0.05}	11.16	8.64

*The experiments were conducted with three replicates

All treatments could enhance the growth parameters through an increase in the fresh and dry weights of the treated plants (Table 4). The treatment Rh11 + Ar + Af + Se showed the highest increase in the rate of fresh and dry weights, which were 33.5 g/plant and 11.1 g/plant, respectively, in the presence of the pathogenic fungus, followed by other treatments. A significant increase in plant height and Chlorophyll was observed 45 days

after adding the fungal inoculum, compared to the fungus-only treatment (Table 4). The combination treatment (Rh11 + Ar + Af + Se) scored 49.3 cm for plant height and 49.5 SPAD units for chlorophyll content, respectively, compared to the control treatment (fungus only), which scored 20.2 cm and 34.8 SPAD units, respectively. These factors may have improved the growth parameters of the treated plants by limiting pathogen growth,

increasing nutrient uptake by root hairs, or by producing phytochemical compounds that enhance the plant's response to stress (Pérez-García et al., 2023). It. Similarly, the bacterium *Alcaligenes faecalis* could control

chickpea blight disease caused by the fungus *Ascochyta rabiei* and improve plant growth, including fresh and dry weights (Moarrefzadeh et al., 2022).

Table 4. Effect of some chemical and biological factors on some growth parameters such as plant length, fresh and dry weight, and total chlorophyll percentage of faba bean plants in pots under greenhouse conditions

No	Treatment	Chlorophyll (SPAD)	Plant length (cm)	Fresh weight g/plant	Dry weight g/plant
1	control	47.4	44.1	26.7	8.9
2	<i>R. solani</i> (Rh 11)	34.8	20.2	19.5	7.2
3	<i>A. faecalis</i> (Af)	58.6	57.3	34.5	11.6
4	<i>A. radioresistens</i> (Ar)	55.7	55.0	32.3	11.3
5	Sc (Af)	54.6	55.8	32.1	11.3
6	Sc (Ar)	53.2	54.5	31.0	10.2
7	Scn (Af)	54.5	57.2	33.8	12.8
8	Scn (Ar)	53.7	54.8	31.8	10.6
9	Se	54.5	53.5	33.4	12.1
10	SeNPs	54.9	53.6	34.5	12.8
11	Ar + Af	58.7	57.7	36.5	13.5
12	Af+ Se	58.1	57.4	37.1	14.1
13	Ar+ Se	56.6	56.5	34.9	12.8
14	Ar + Af+ Se	58.7	59.6	37.8	14.6
15	Sc (Af) + Sc (Ar)	56.1	54.9	34.2	11.8
16	Sc (Af) + Se	55.5	55.5	35.8	13.6
17	Sc (Ar)+ Se	54.5	54.1	33.8	12.3
18	Sc (Af)) + Sc (Ar)+ Se	55.0	55.3	32.4	11.1
19	Scn (Ar) + Scn (Af)	54.9	55.7	34.6	12.6
20	Scn (Af)+ SeNPs	56.5	57.0	34.9	12.9
21	Scn (Ar)+ SeNPs	56.5	54.9	33.3	11.4
22	Scn (Af) + Scn (Ar) + SeNPs	58.8	56.3	36.9	14.1
23	Rh11 +Af	45.4	44.2	26.9	9.6
24	Rh11 + Ar	43.4	42.9	25.1	8.9
25	Rh11 + Af	41.8	42.9	23.2	8.5
26	Rh11 + Sc (Ar)	40.8	41.5	21.9	8.2
27	Rh11 +Scn (Af)	40.3	44.5	25.1	9.1
28	Rh11 + Scn (Ar)	40.0	41.7	22.0	7.5
29	Rh11 +Se	42.5	42.6	23.5	9.1
30	Rh11 + SeNPs	45.0	43.9	25.3	8.8
31	Rh11 + Ar + Af	46.4	48.3	28.7	9.8
32	Rh11+Af + Se	46.7	48.4	29.9	10.3
33	Rh11+Ar+ Se	44.0	44.3	27.9	9.6
34	Rh11 +Ar + Af + Se	49.5	49.3	33.5	11.1
35	Rh11 +Sc (Af) + Sc (Ar)	43.4	43.4	30.3	10.1
36	Rh11 + Sc (Af)+ Se	42.6	43.5	31.0	10.8
37	Rh11 + Sc (Ar) + Se	39.9	42.3	29.8	9.9
38	Rh11 +Sc(Af)) + Sc (Ar)+ Se	45.4	45.3	27.3	8.0
39	Rh11 +Scn (Ar) + Scn (Af)	44.8	44.9	26.9	9.1
40	Rh11 + Scn (Af)+ SeNPs	46.3	47.3	28.4	9.7
41	Rh11 + Scn (Ar)+ SeNPs	45.6	44.6	27.3	9.3
42	Rh11 +Scn (Ar) + Scn (Af) + SeNPs	49.1	48.7	31.8	11.0
43	Rh11 + Uniforme	38.4	38.0	23.3	7.6
	L.S.D _{0.05}	1.29	1.21	1.02	0.94

*The experiments were conducted with three replicates
Similarly, SeNPs at a concentration of 30 mg/L could increase fresh and dry weights by 17.35% and 13.43%, respectively, as well as

plant height and total chlorophyll content in wheat compared to the control treatment (Shahbaz et al., 2023). Soybean plants treated with *Actinobacterium* spp. and selenium

nanoparticles at a concentration of 25 mg/L together improved the physiological and biochemical characteristics of the plants, resulting in a higher accumulation of oxidative biomarkers, including hydrogen peroxide, enhanced the ascorbate-glutathione enzyme pathway, and increased the total chlorophyll content by 58% in the treated plants compared to the control treatment (Halawani & Aloufi, 2023). There were significant differences in the activity rate of the enzyme polyphenol oxidase (PPO), estimated based on the rate of change in optical absorption per minute per gram of fresh weight in the faba bean plant. All factors could induce systemic resistance in treated plants when the activity of the enzyme PPO increased compared to the control treatment (pathogenic fungi only) (Table 5). The highest enzyme activity was recorded as a rate of change in absorption of 79.2 per minute per gram of fresh weight, 7 days after adding the fungal inoculum in the treatment (Rh11 + Ar + Af + Se), followed by other treatments. The mixture Rh11 + Ar + Af + Se showed the highest activity, scoring 85.2 (rate of change in absorption per minute per gram of fresh weight) 14 days post-treatment, followed by other treatments. PPO activity then decreased gradually on the 21st day but remained significantly effective compared to the control treatment. Similarly, biological control agents inhibited pathogen enzymes by suppressing the action of pathogen enzymes responsible for the plant's pathogenic ability, including enzymes that degrade the plant host's cell walls and cause infection (Patel et al., 2020; Xu et al., 2020). PPO activity in the plant defense mechanism is triggered by plant

pathogens. This enzyme is one of the compounds that stimulate the formation of mechanical defenses in plants through increasing the thickness of the plant cell wall, resulting from the deposition and polymerization of infection-related proteins, lignin, and suberin. Thus, the cell wall can resist the penetration of pathogens (Abed et al., 2017). The PPO enzyme prevents pathogens from attacking the plant by oxidizing the natural phenols present in the plant tissues to produce quinone, initiating a series of polymerization reactions, resulting in the production of melanins and the formation of a hypersensitive reaction by stimulating plant resistance against pathogenic fungi (Zhang, 2023). The antagonistic bacterium *A. faecalis* could induce systemic resistance and increase PPO enzyme activity in tomato (Patel et al., 2020). Selenium at concentrations of 0.1 and 0.5 mg/kg, applied to the soil against *Sclerotinia* rot disease in turnips, demonstrated a relationship between induced resistance and some biochemical changes, including increased production of reactive oxygen species (ROS), peroxidases, and polyphenol oxidases in the treated plants (Xu et al., 2020). Moreover, foliar spraying of watermelon seedlings with SeNPs at a concentration of 10 mg/L could enhance the resistance of watermelon plants against pathogens by increasing the activity of ascorbate peroxidase, peroxidase, phenylalanine ammonia lyase, β -1,3-glucanase, and chitinase, along with increasing antioxidants, photosynthesis, and organic acids compared to the control treatment (Kang et al., 2022).

Table 5. Effect of chemical and biological compound treatments on the activity of the polyphenol oxidase enzyme (rate of change in absorption/min/g fresh weight) in faba bean plants in pots under greenhouse conditions.

No	Treatments	After 7 days	After 14 days	After 21 days
.1	control	30.5	32.4	31.4
.2	<i>R. solani</i> (Rh 11)	47.7	49.3	44.5
.3	Rh11 + Af	68.0	75.9	65.5
.4	Rh11 + Ar	64.1	71.1	61.9
.5	Rh11 + Sc (Af)	59.5	66.7	59.0
.6	Rh11 + Sc (Ar)	54.7	61.5	53.2
.7	Rh11 + Scn (Af)	64.6	71.8	63.1
.8	Rh11 + Scn (Ar)	57.4	64.4	56.4
.9	Rh11 + Se	72.1	79.8	67.0
.10	Rh11 + SeNPs	73.5	80.7	67.8
.11	Rh11 + Ar + Af	76.0	82.9	70.0
.12	Rh11 + Af + Se	76.7	83.5	71.0
.13	Rh11 + Ar + Se	73.3	80.3	68.9
.14	Rh11 +Ar + Af + Se	79.2	85.2	72.0
.15	Rh11 + Sc (Af)) + Sc (Ar)	61.3	67.6	59.1
.16	Rh11 + Sc (Af) + Se	73.7	80.1	69.0
.17	Rh11 + Sc (Ar) + Se	69.6	77.6	66.5
.18	Rh11 + Sc(Af)) + Sc (Ar) +Se	74.9	82.1	69.2
.19	Rh11 + Scn (Af) + Scn (Ar)	75.0	82.6	67.8
.20	Rh11 + Scn (Af) + SeNPs	75.5	82.8	69.2
.21	Rh11+ Scn (Ar) + SeNPs	75.1	80.5	66.6
.22	Rh11 + Scn (Af) + Scn (Ar)+ SeNPs	77.2	83.9	71.4
	L.S.D _{0.05}	1.26	1.07	1.31

*The experiments were conducted with three replicates

CONCLUSION

Scientists concerns are finding ways to reduce costs in agricultural production and to meet the sustainable development goals. This study highly suggests using the Rh11 + Ar + Af + Se mixture which significantly increased fresh and dry weights, total chlorophyll percentage, and plant heights. This mixture induced systemic resistance by enhancing the activity of the polyphenol oxidase enzyme, with respective changes in absorbance rates of 79.2 and 85.2 per minute per gram of fresh weight of plant leaves.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

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AUTHOR'S CONTRIBUTION STATEMENT

Sawsan H. A. Al-Mayahi contributed to the conceptualization, methodology, investigation, data collection, statistical analysis, and preparation of the original manuscript. Aalaa K. Hassan contributed to the supervision, experimental design, methodology, interpretation of the results, and revision of the manuscript. Salam A. H. Alamery contributed to the laboratory work, isolation and identification of the pathogenic fungus, data interpretation, and manuscript review. Deepak K. Sinha contributed to the scientific evaluation, interpretation of the findings, and critical revision of the manuscript. All authors read and approved the final version of the manuscript

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تأثير بعض العوامل الاحيائية والكيميائية وتداخلهما في نمو الباقلاء وحمايتها من الاصابة بالفطر *Rhizoctonia solani*

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المستخلص

اجريت التجارب بموقعين، مختبر امراض نبات، وزارة الزراعة، بغداد، العراق وكلية علوم الهندسة الزراعية، جامعة بغداد، الجادرية، العراق خلال الموسم ٢٠٢٢- ٢٠٢٣ لتقييم كفاءة بعض المواد الكيميائية والاحيائية بصورة مفردة او بالخلط فيما بينها في الحد من مرض تعفن الجذور الرايزوكتوني في نباتات الباقلاء المتسبب عن الفطر *R. solani*. اظهرت النتائج تحت ظروف البيت البلاستيكي ان معاملات الخلط (Se+ Af + Rh11) و (Se+ Ar + Af + Rh11) و (Se+ Ar + Af + Rh11) + Scn (Af) + Rh11 و SeNPs (SeNPs + Scn (Ar) + Scn (Af) + Rh11) مع الفطر الممرض كانت الاكفا في خفض نسبة وشدة الاصابة بالفطر *R. solani*، اذ بلغت نسبة الاصابة والشدة فيها 0.00 و 0.00% على التتابع فضلا عن تسببها في زيادة الوزن الطري والجاف ونسبة الكلوروفيل الكلي واطوال النباتات معنويا على باقي المعاملات الاخرى . كما اثبتت العوامل كفاءتها في استحثاث المقاومة الجهازية من خلال زيادة فعالية انزيم البوليفينول أوكسيديز بعد 7 و 14 يوما من اضافة الفطر الممرض فقد حققت معاملة الخلط Se+Ar + Af + Rh11 اعلى فعالية للأنزيم والتي بلغت 79.2 و 85.2 معدل التغيير بالامتصاص الضوئي / دقيقة / غم وزن طري لأوراق النبات على التتابع.

الكلمات المفتاحية: الباقلاء. استحثاث مقاومة ،البكتريا الجذرية المحفزة لنمو النبات ، SeNPs ، *Rhizoctonia solani*.