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Improving the Efficiency of Bioagents Using Certain Chemical Inducers against Root Rot and Wilt Diseases of Soybean

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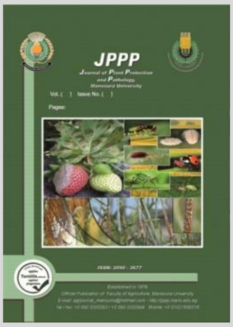

ABSTRACT

The study aimed to investigate the efficacy of *Pseudomonas fluorescens* and *Paenibacillus polymyxa* as bioagents, along with the chemical systemic resistance inducers, i.e., hydroquinone (HQ) and benzoic acid (BA) individually and in combinations, to combat root rot and wilt diseases with promoting the growth of soybean plants. Pathogenicity tests have provided that all isolates could infect soybean cv. Giza 111, resulting in varying degrees of infection. The isolates of *Rhizoctonia solani* No. 3 with accession No. (OR975921.1) and *Fusarium oxysporum* No. 2 with accession No. (OR975922.1) were the most aggressive. *In vitro, P. polymyxa* combined with HQ showed the most effective in decreasing the fungal growth of both the examined fungi. In vivo trials conducted in pots and fields revealed that *P. polymyxa* + HQ treatment ranked just behind the Topsin M-70® fungicide in terms of effectiveness. Anatomically, light microscope cross-sections of the primary roots revealed that the combinations of these agents led to protecting soybean plants from the harmful anatomical changes of both pathogens in the epidermis, cortex, and vascular cylinder structure under artificial infestation with the tested fungi. Physiological analyses indicated increased levels of peroxidase (PO) and polyphenoloxidase (PPO), along with total phenolic content (TPC), in plants treated with the combined agents compared to those treated individually. The combination treatments significantly enhanced plant growth metrics and yield components in field studies. Generally, results indicate that combining bioagents with chemical inducers proved more effective than individual applications and might be included in integrated disease management approaches.

***Keywords**:* soybean; *Rhizoctonia solani*; *Fusarium oxysporum*

INTRODUCTION

Soybean (*Glycine max* L.) is widely acknowledged as an essential source of oil and protein, with the highest protein concentration among leguminous crops (El-Abady *et al.*, 2008). However, soybean plants are susceptible to various fungal, bacterial, and viral diseases, resulting in significant yield losses. Root rot is particularly severe and widespread disease affecting soybean plants worldwide, leading to substantial reductions in the quality and quantity of soybean yield. A variety of pathogenic fungi causes these diseases. *Rhizoctonia solani*, *Fusarium solani*, and *Pythium ultimum* are the predominant pathogens responsible for damping-off and root rot in soybean (Haikal, 2008; Fayzalla *et al.*, 2009; Ghaleb *et al.*, 2021). Moreover, *Fusarium oxysporum* induced wilt disease is widely known as a destructive disease that dramatically affects soybean crops (Hashem *et al.*, 2009; Ghaleb *et al.*, 2021).

Managing soil-borne diseases like root rot and wilt diseases caused by persistent pathogens such as *R. solani* and *F. oxysporum* is a significant challenge. These pathogens can survive in the soil for extended times and infect many hosts. Traditional chemical treatments can be effective against these diseases but often come with high costs and environmental implications. Consequently, researchers are exploring alternative strategies, including induced resistance through biotic and abiotic means. Systemic-induced resistance, a process extensively researched in various plant/pathogen systems, involves boosting the resistance of plant to diseases in tissues far from the initial inducing treatment. This phenomenon has been a focus of recent reviews, such as those by (Hammerschmidt, 1999). While many of these studies have been conducted in controlled environments, there have been limited demonstrations of systemic-induced resistance under field conditions for specific plant/pathogen interactions.

Biological management is often considered a highly promising approach to effectively managing soil-borne fungal pathogens that causing root rot and wilt of soybean. Using fungicides in disease management contributes to environmental pollution and poses risks to human health (Bürger *et al*., 2012). Therefore, exploring alternate strategies is essential for efficiently managing root rot pathogens of soybean (Li and Ma, 2012). The rhizosphere, the soil area influenced by the root and its immediate vicinity, harbors diverse microbial communities. Microbial populations residing in the rhizosphere play diverse roles in plant growth, ranging from beneficial to neutral or even detrimental effects, as noted by (Whipps, 2001). Some of these microorganisms are recognized as plant growth enhancing rhizobacteria, which actively contribute to enhancing plant growth. PGPR has beneficial effects by engaging in competitive interactions with pathogens, parasitizing them, and producing antifungal compounds. Investigators have emphasized the role of these rhizobacteria in enhancing plant growth through these mechanisms, showcasing their potential to foster healthier plant development and combatting detrimental pathogens in

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the rhizosphere (Verma *et al.,* 2007; Savazzini *et al.*, 2009). Treatments with *P. fluorescens* and *P. polymyxa* significantly reduced root rot signs which caused by *R. solani* in soybean plants (Atwa *et al.*,2014; Atwa, 2018)

The controlling of soil-borne pathogens through biological methods is often associated with two primary mechanisms: improved host nutrition that increases host defenses and the direct inhibition of pathogen growth. Certain chemical agents, known as inducers, can enhance disease resistance by indirectly promoting the development of beneficial native microorganisms that support plant growth and have antagonistic characteristics against pathogens. For instance, amending soil with substances like chitin has been shown to have beneficial effects. Moreover, chitin stimulates the proliferation of chitinolytic microorganisms (De Boer *et al*., 1999). This phenomenon can lead to improved biocontrol effectiveness and the induction of plant defense proteins, as evidenced in the research carried out by (Roby *et al*., 1987). These combined impacts ultimately enhance plant protection by strengthen the plant's natural defense mechanisms and fostering a more hostile environment for pathogens in the soil.

Using abiotic inducers to trigger resistance responses is an effective strategy for managing soil borne fungal pathogens. A study by Abd EL-Hai *et al.* (2016) provided evidence of the efficacy of benzoic acid for inducing the systemic defense in soybean plants, thus offering protection against root rot disease in the field. Additionally, studies have shown the effectiveness of hydroquinone in decreasing the occurrence of root rot and wilt diseases incidence caused by pathogens like *R. solani, F. solani,* and *F. oxysporum* in lupine plants. The aforementioned agents not only reduced the incidence of diseases but also yielded notable enhancements in several aspects of lupine development, including measures related to yield components and physiological parameters. (Ali *et al*., 2009; Zian *et al*., 2019). Also, Saikia *et al.* 2003 evaluated the efficacy of *P. fluorescens* + salicylic acid in protecting chickpea to control Fusarium wilt infection. Their study showed that using *P. fluorescens* plus SA provided the most effective one in protecting seedlings of chickpea against wilting disease. Zian et al. (2019) conducted a study to evaluate the efficacy of *Trichoderma harzianum, Bacillus subtilis,* hydroquinone, and salicylic acid in managing root rot and wilt diseases and promoting the growth of lupine plants. An investigation revealed that combining bioagents with chemical inducers resulted in higher efficacy than using them separately.

The ability of biological agents and abiotic inducers to trigger defense reactions in host plants against damage caused by pathogens is well recognized. These processes involve the synthesis of pathogen-related (PR) proteins associated with disease (Nafie and Mazen, 2008), the activation of defense-related enzymes (Govindappa *et al*., 2010), and the buildup of phenolic phytochemicals (Nafie and Mazen, 2008 and El-Blasy *et al.,* 2023).

This study aims to examine the efficacy of both chemical inducers and bioagents, either separately or in combination, enhancing resistance to root-rot and wilt diseases and increasing soybean growth. Also, the anatomical changes in the root structure caused by the fungal pathogens comparing with healthy control and the best treatment were investigated. Additionally, it evaluates the efficacy of these treatments in promoting peroxidase and polyphenoloxidase activities and improving the overall phenol compounds linked to secondary plant metabolites, hence bolstering resistance against pathogen invasion.

MATERIALS AND METHODS

Origin of seeds:

The soybean variety Giza-111 (*Glycine max* L.) seeds were brought from the Legume Research Department of the Field Crops Research Institute, ARC, in Giza, Egypt.

Chemicals:

The compounds hydroquinone (HQ) and benzoic acid (BA) are used as inducers resistance in plants. These compounds were acquired from El-Nasr Co. for Intermediate Chemicals, Egypt (NCIC). Fungicide Topsin-M 70® Wp (thiophanate methyl) was acquired from Sigma Chemicals Company (St. Louis, USA) and contains the active ingredient 1-2B, which is Methoxycarbonyl.

Bioagents:

The biocontrol agents *Pseudomonas fluorescens* and *Paenibacillus polymyxa* were obtained from Dr. Mona M. Aly, who is affiliated with the Microbiology Department of the Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), located in Giza, Egypt. The aforementioned agents were previously identified in a conducted study by El-Tapey *et al.* (2019) and Aly *et al.* (2020).

Fungal pathogens:

Isolation and identification:

Sampling of soybean plants with root rot infection was conducted at many locations throughout Giza Governorate. In order to achieve surface sterilization, the affected roots were cut into sections subsequently, submerged in a 2% sodium hypochlorite solution for duration of two minutes. Following rinsing with sterilized water and drying, the sterilized parts were deposited into potato dextrose agar (PDA) medium containing streptomycin sulfate (100 µg/ml) and cultured at a temperature of 25°C. Using the techniques described by Barnett and Hunter (1998), the fungal pathogens were purified and recognized based on its microscopic, morphological, and cultural traits. Subsequently, the fungi were cultivated on PDA slants and stored at 6°C.

Preparation of fungal inoculum:

Rhizoctonia solani and *F. oxysporum* were separately grown in 500 cc glass bottles filled with 100 grams of sterilized sorghum grain medium. Each bottle received an inoculation of an actively growing disc (5 mm) from a culture of either *R. solani* and *F. oxysporum* that was four and seven days old, respectively. In order to promote a quicker and more equal colonisation of the sorghum grains, the bottles were kept in the incubated at 25 ± 1°C for 15 days.

Pathogenicity test:

The pathogenicity of the fungal isolates was assessed in the pots at the Plant Pathology Research Institute, ARC, in Giza, Egypt. The experiment utilized the soybean cultivar Giza-111. Each isolate was cultured on a sorghum-sand medium for 15 days at 25±2°C. Each 30 cm in diameter sterilized pots were filled with sterilized sandy soil and then inoculated at 2% of the weight of soil (W/W) (Abd El-Aziz *et al.,* 2013). The soil mixture was thoroughly combined, and the pots were watered and left undisturbed for a week. Equal amounts of autoclaved sorghum-sand media without fungi were placed in the control pots. Each pot contained five soybean seeds, with five replicates for each treatment. The pots were organized in a block arrangement to ensure perfect randomization. The most highly pathogenic strains of *R. solani* and *F. oxysporum* were used for all *in vitro* and *in vivo* studies. Disease incidence was assessed by measuring damping-off and root rot percentages 30 and 90 days post-planting for pots inoculated with *R. solani*. Additionally, early and late wilt percentages were recorded for plants infested with *F. oxysporum* over the same timeframes.

Molecular identification:

Extraction of DNA:

An extraction of the DNA was performed using the procedure outlined by Lee and Taylor (1990). This process included two extractions using a phenol, chloroform, and isoamyl alcohol mixture (25:24:1), followed by precipitation with an equal volume of isopropanol. After extraction, the DNA pellets were washed, resuspended in ethanol and deionized water, and stored at -20°C.

PCR Conditions and DNA Sequencing

Amplification of the rDNA ITS region was performed using ITS 1 (5′ TCC GTA GGT GAA CCT GCGG 3′) and ITS 4 (5′ TCC TCC GCT TAT TGA TATGC 3′) primers. Following an initial denaturation at 95°C for 3 minutes, the amplification procedure consisted of 20 cycles of denaturation at 95°C for 30 seconds, annealing at 68°C for 45 seconds, and extension at 72°C for 90 seconds. The last extension was conducted at 72°C for 8 minutes. Quantitative polymerase chain reaction (PCR) tests were conducted at the Plant Pathology Research Institute.

The PCR amplified results were analyzed through DNA sequencing using the ABI Prism 3130xl Genetic Analyzer. Two-directional sequencing was performed using the same primers, ITS 1 and ITS 4, following the method described by White *et al.* (1990). This sequencing process took place at Macrogen Corp., Korea. The resulting DNA sequences have been submitted to and stored in the NCBI GenBank for general accessibility and citation purposes. The phylogenetic relationship of the strain was confirmed by constructing a phylogenetic tree using the Neighbor-joining method in MEGA 11 software, with 500 bootstrap replicates.

Growing of bioagents:

A- Cultures of *Paenibacillus polymyxa* were grown in flasks filled with 50 ml of nutritional yeast dextrose broth (NYDB) medium, produced according to the following method per litre: 8 grams of nutritional broth, 5 grams of yeast extract, and 10 grams of dextrose. The culture was adjusted to a concentration of 108 cfu/ml using the technique outlined by Hino and Wilson (1958).

B- *Pseudomonas fluorescens* was grown in flasks containing King's Medium B (KMB) broth, as described by King et al. (1954),. The medium was prepared per liter with the following components: 20 grams of protease peptone, 10 millilitres of glycerol, 1.5 grams of K_2HPO_4, and 1.5 grams of $MgSO_4$. The culture was adjusted to attain a concentration of 10^8 cfu/ml.

In vitro studies:

The impact of specific bioagents and chemical inducers on the mycelial growth of the tested fungi:

Bioagents:

Rhizoctonia solani and *F. oxysporum* were cultivated on PDA medium at 25 ± 1°C for four and seven days, respectively. The PDA plates were streaked with isolates of *P. fluorescens* and/or *P. polymyxa* at the opposing edges and then incubated at the same temperature for 24 hours. After that, a 5 mm mycelial disc of the fungi being studied was positioned in the middle of each plate.

Chemical inducers:

Chemical inducers were assessed for its effect on the mycelial growth of the fungi being tested in a PDA medium. Twenty milliliters of PDA media, each containing ten millimolar amounts of HQ and BA, were separately added to the plates. Subsequently, each plate inoculated with a 5 mm mycelial disc of the tested fungi at the center.

Combination of bioagents and chemical inducers:

Individually, each 500 ml flask included 200 mL of PDA medium supplemented with ten millimolar (mM) concentrations of HQ and/or BA. The contents of each flask were then evenly distributed into ten plates. As reported, the plates were inoculated with antagonistic and pathogenic fungi. An individual agar plug containing the pathogenic isolate was positioned on the PDA plate for the control treatment. Each plate was incubated at 25 ± 1°C until the control colony filled the plate. Subsequently, the diameter of the colony was assessed using a ruler, and the percentage of growth suppression of the pathogen was determined.

In vivo studies:

Pots experiment:

The impact of bioagents (*P. fluorescens and P. polymyxa*) and chemical inducers (HQ and BA) on the occurrence of root rot and wilt diseases of soybean caused by *R. solani* and *F. oxysporum* was assessed both separately and in combination as seed soaking treatments in a pot experiment. To sterilize pots with a 30 cm diameter and drainage holes, they were soaked in a 5% formalin / 15 min. Subsequently, they were left undisturbed for a week to ensure complete evaporation. The pots were filled with sandy clay soil, using a 1:2 (V/V) ratio. Soil inoculation was accomplished by incorporating 2% inoculum of either *R. solani* or *F. oxysporum* (Abd El-Aziz *et al.,* 2013). To ensure healthy control, sterilized, un-inoculated ground sorghum grains were introduced at an equal rate.

The infested soil was mixed thoroughly and watered twice a week to encourage fungal growth and spread. Soybean seeds were planted in pots, with five seeds per pot for each treatment, replicated five times. The pots were watered as needed with. *P. fluorescens* and *P. polymyxa* cell suspensions were adjusted to 10^{8} cfu/ml after being cultured, and HQ and BA were produced as ten mM solutions. The soybean seeds were treated with Thiophanate-methyl (Topsin-M 70®) at a recommended dose of 2 g/L. The preparation of combinations of bioagents and chemical inducers involved dissolving the inducers in suspensions of the bioagents. Soybean seeds were soaked for 20 minutes in the following treatments: 1, HQ. (10 mM); 2, BA. (10 mM); 3, *P. polymyxa* (10^8cfu/mL); 4, *P. fluorescens* (10^8 cfu/mL); 5, HQ + *P. polymyxa*; 6, HQ + *P. fluorescens*; 7, BA + *P. polymyxa* ; 8, BA + *P. fluorescens*; 9, Topsin M-70® ; 10, control and 11, healthy control.

Disease assessment:

The disease incidence (DI) percentage was calculated by determining the number of plants that had root rot after 30 and 90 days of planting. The percentages of early and late wilt were recorded 30 and 90 days after crop planting using the following mathematical equations:

$$\text{Damping-off \%} = \frac{\text{No. of non-germinated seeds after 15 days + No. of dead seedlings after 30 days}}{\text{Total no. of planted seeds}} \times 100$$

$$\text{Root rotted plants \%} = \frac{\text{No. of root rotted plants after 90 days}}{\text{Total no. of planted seeds}} \times 100$$

$$\text{Early wilt \%} = \frac{\text{No. of wilted plants after 30 days}}{\text{Total no. of planted seed}} \times 100$$

$$\text{Late wilt \%} = \frac{\text{No. of wilted plants after 90 days}}{\text{Total no. of planted seed}} \times 100$$

$$\text{Survived plants \%} = \frac{\text{No. of survival plants after 90 days}}{\text{Total no. of planted seeds}} \times 100$$

Anatomical structure of soybean roots:

The anatomical alterations in the root system of soybean caused by the pathogenic fungi comparing with the healthy control and the best treatment were investigated after 30 days of sowing. Precise 5 mm sections were extracted from the permanent zone of primary root (approximately 2-3 cm below the soil surface), Immobile and kept in a solution of formalin, alcohol, and acetic acid mixture (F: A: A consists of formalin: 70% ethyl alcohol: glacial acetic acid at the rate of 18: 1: 1, respectively). Then, proceeded to wash and then dehydrate in a gradual series of 70% alcohol until absolute alcohol and then progressively purified with xylene; gradually from alcohol to xylene and embedded in paraffin wax (52-54ºC m.p.). Cross-sections, 12-15 micrometres thick, were produced using a rotary microtome. They were stained with crystal violet, cleaned with xylene, and mounted in Canada balsam (Gerlach, 1977).

The cross sections were analyzed using a light microscope at magnifications of 40x and 100x to outline and ascertain the anatomical alterations caused by pathogenic fungi in relation to healthy roots and the most effective treatment.

Impact of applying bioagents and chemical inducers to soybean seeds on the activity of oxidative enzymes and the phenol content.

An experimental study was undertaken to assess the enzymatic activity of oxidative enzymes and the total phenol content. In the pot experiment, soybean plants were cultivated according to the previously reported method. Fifteen days after planting, the peroxidase (PO) activity, polyphenol oxidase (PPO) activity, and total phenol content were quantified in fresh leaf tissue extracts of the soybean.

The peroxidase (PO) activity was extracted and analyzed following the procedures outlined by Chakraborty and Chatterjee (2007).

The extraction and testing of polyphenoloxidase (PPO) were conducted using the method defined by Sadasivam and Manickam (1996).

The quantification of phenol content in the plant leaves was performed following the strategy outlined by Zilesin and Ben-Zaken (1993).

Field experiments:

An experimental field study was conducted at two distinct locations, Giza and Etai El-Baroud Research Stations, during the summer growing season of 2022. The experiment aimed to assess the effectiveness of bioagents (*P. fluorescens and P. polymyxa*) as well as chemical inducers (HQ and BA), both alone and in combination, to combat root rot and wilt diseases. Additionally, the experiment aimed to evaluate the impact of these treatments on growth and yield metrics in the field. In the study, a fully randomized block design with three replicates was used. Each experimental unit covered a surface area of 9 m² (3 × 3 m) and consisted of six rows, each measuring 3 meters in length and 50 centimetres in width. Before planting, soybean seeds (cv. Giza-111) were soaked in treatment solutions for 20 minutes. The treated seeds were then planted in hills along both sides of the six ridges at both sites, with each hill accommodating a single seed and placed 25 cm apart. For the control treatment, soybean seeds were soaked in water for 20 minutes and planted at equal distances apart. Biometric characteristics of growth and yield were evaluated at harvest.

Statistical analysis

The collected data was analysed using the statistical program CoStat v 6.4. For this study, an analysis of variance (ANOVA) was used to assess and compare the mean values. A significance level at 1% and 5% were used for Duncan's multiple range tests.

RESULTS AND DISCUSSION

Results

Isolation of the fungal pathogens:

The isolated fungi were collected from soybean plants that demonstrated signs of root rot and wilt in their decayed roots. These plants were grown in different fields within the Giza and Beni suef Governorates. There were four isolates identified as *R. solani* and three isolates identified as *F. oxysporum.*

Pathogenicity tests:

The data presented in Table (1), clearly shows that all the isolates successfully infected the roots of soybean plants, resulting in signs of damping-off, root rot, and wilt. Among the isolates, *R. solani* isolate No. 3 that isolated from Beni suef Governorate was the most aggressive, resulting in the highest rates of damping-off (28%), root-rotted plants (20%), and the lowest survival rate (52%). Conversely, *F. oxysporum* isolate No. 2 that isolated from Beni suef Governorate also, exhibited the highest rates of early wilt (24%), late wilt (16%), and the lowest survival rate (60%). As noted in the pathogenicity tests, the fungal isolates with a high level of pathogenicity were chosen for additional investigations due to their potential to cause disease.

Table 1. Evaluating the relative pathogenicity of *R. solani* and *F. oxysporum* isolates on the suscptibale Giza-111 cultivar.

(A) ***Rhizoctonia solani:***

Isolates	Damping-off%	Root rot%	Survived Plants %
R.solani No.1	16.00 ^{c}	8.00 ^{b}	76.00 ^{b}
R.solani No.2	24.00 ^{ab}	16.00 ^{a}	60.00 ^{cd}
R.solani No.3	28.00 ^{a}	20.00 ^{a}	52.00 ^{d}
R.solani No.4	20.00 ^{bc}	16.00 ^{a}	64.00 ^{c}
Control	4.00 ^{d}	0.00 ^{c}	96.00 ^{a}

(B)***Fusarium oxysporum***

Isolates	Wilted plants %		Survived Plants %
	Early wilt	Late wilt	
F. oxysporum No.1	16.00 ^{b}	12.00 ^{a}	72.00 ^{b}
F. oxysporum No.2	24.00 ^{a}	16.00 ^{a}	60.00 ^{c}
F. oxysporum No.3	12.00 ^{b}	12.00 ^{a}	76.00 ^{b}
Control	4.00 ^{c}	0.00 ^{b}	96.00 ^{a}

Letters represent statistically significant differences between treatments within the same column, as indicated by the least significant difference test ($p \geq 0.05$).

Molecular identification of the pathogenic fungi:

The resulting sequences were submitted to the GenBank database with accession numbers OR975922.1 and OR975921.1 for *F. oxysporum* isolate No.2 and *R. solani* isolate No.3, respectively. The nucleotide sequences and BLAST evaluation show a high similarity (100% of nucleotide sequence) between the fungal isolate *F.* oxysporum No.2 and *F. oxysporum* in the GenBank under accession numbers KU377461.1 and MN560044.1 (Figure 1). In contrast, isolate No.3 of *R. solani* exhibited a complete and unaltered nucleotide sequence similarity with the closely related *R. solani* isolates KY684288.1 and KX583263.1 in the GenBank (Figure 2).

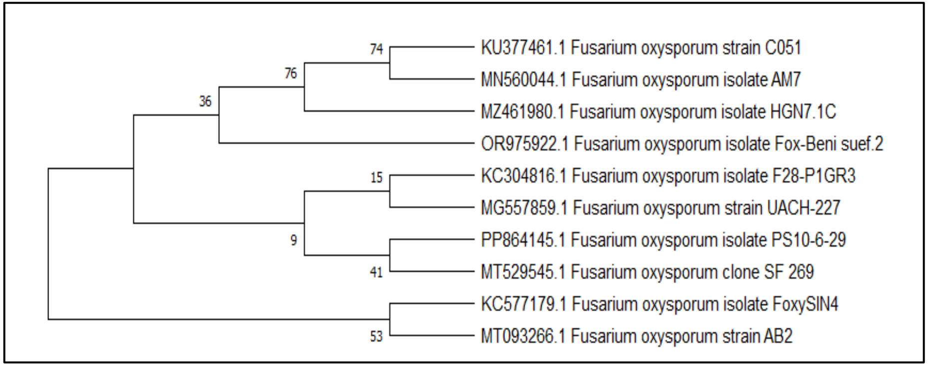


Fig.1. Phylogenetic analysis of *Fusarium oxysporum* isolate Fox-Beni suef.2 (GenBank accession No. :OR975922.1) generated in MEGA 11 using the neighbor-joining method with 500 bootstrap replicates.

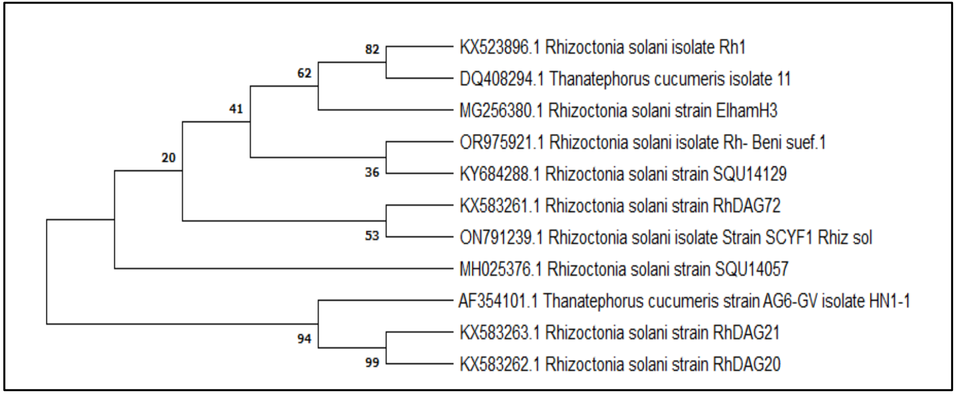


Fig.2. Phylogenetic analysis of *Rhizoctonia solani* isolate Rh-Beni suef.1 (GenBank accession No. : OR975921.1) generated in MEGA 11 using the neighbor-joining method with 500 bootstrap replicates.

***In vitro* studies:**

Impact of specific bioagents and chemical inducers against radial growth of *R. solani* and *F. oxysporium*:

The data in Table (2), show that the fungal growth of the two studied fungi was diminished to varying extents by the bioagents and chemical inducers, whether used alone or in combination.

Table 2. Impact of specific bioagents and chemical inducers against mycelial growth of *R. solani* and *F. oxysporium*:

Treatments	*R. solani*		*F. oxysporum*	
	mycelial growth (mm)	Reduction %	mycelial growth (mm)	Reduction %
Hydroquinone (HQ)	48.00 ^{c}	46.70	47.50 ^{c}	47.30
Benzoic acid (BA)	51.50 ^{b}	42.80	53.40 ^{b}	40.70
P. polymyxa	35.50 ^{d}	60.60	34.00 ^{d}	62.30
P. fluorescens	36.00 ^{d}	60.00	35.50 ^{d}	60.60
P. polymyxa + HQ	16.00 ^{g}	82.30	13.50 ^{g}	85.00
P. fluorescens + HQ	19.40 ^{f}	78.50	18.50 ^{f}	79.50
P. polymyxa + BA	21.00 ^{f}	76.70	20.00 ^{ef}	77.80
P. fluorescens + BA	23.00 ^{e}	74.50	21.50 ^{e}	76.20
Topsin M-70®	00.00 ^{h}	100.0	00.00 ^{h}	100.0
Control	90.00 ^{a}	-	90.00 ^{a}	-

Letters represent statistically significant differences between treatments within the same column, as indicated by the least significant difference test ($p \geq 0.01$).

Overall, bioagents proved to be more effective than chemical inducers in reducing the fungal growth of pathogenic fungi. Furthermore, the combination of bioagents and chemical inducers yielded better results than either treatment alone. Significantly, the combination of *P. polymyxa* and HQ showed the most efficacy in diminishing the fungal growth of tested pathogens, whereas the application of benzoic acid alone yielded the least decrease in growth.

***In vivo* studies:**

Influence of specific bioagents and chemical inducers for managing root rot and wilt diseases of soybean in the pots:

The findings shown in Table (3, A and B) indicate that all treatments, compared to the untreated control, effectively decreased root rot and wilt disease while increasing the number of surviving soybean plants, albeit to varying degrees. The application of bioagents in combination with chemical inducers proved to be more efficacious in decreasing root rot and wilt than their individual use. In cases of *R. solani*, the combination of *P. polymyxa* + HQ ranked just behind the Topsin M-70® fungicide in terms of effectiveness. Meanwhile, Topsin M-70® showed the highest efficacy against *F. oxysporum*, followed by the combination of hydroquinone (HQ) with both *P. polymyxa* and *P. fluorescens*. The lowest overall effectiveness was observed with benzoic acid (BA).

Table 3. Influence of specific bioagents and chemical inducers for managing root rot and wilt diseases of soybean in the pots (A and B).

(A) *Rhizoctonia solani:*

Treatments	Damping –off %		Root rot %		Survived Plants%	Increasing %
	Incidence%	Reduction%	Incidence%	Reduction%		
Hydroquinone (HQ)	16.00 ^{b}	42.85	8.00 ^{abc}	50.00	76.00 ^{de}	35.71
Benzoic acid (BA)	16.00 ^{b}	42.85	12.00 ^{ab}	25.00	72.00 ^{e}	28.57
P. polymyxa	16.00 ^{b}	42.85	8.00 ^{abc}	50.00	76.00 ^{de}	35.71
P. fluorescens	16.00 ^{b}	42.85	8.00 ^{abc}	50.00	76.00 ^{de}	35.71
P. polymyxa + HQ	8.00 ^{bc}	71.42	4.00 ^{bc}	75.00	88.00 ^{abc}	57.14
P. fluorescens + HQ	8.00 ^{bc}	71.42	12.00 ^{ab}	12.00	80.00 ^{cde}	42.85
P. polymyxa + BA	8.00 ^{bc}	71.42	8.00 ^{abc}	50.00	84.00 ^{bcd}	50.00
P. fluorescens + BA	12.00 ^{bc}	57.14	8.00 ^{abc}	50.00	80.00 ^{cde}	42.85
Topsin M-70®	4.00 ^{c}	85.71	4.00 ^{bc}	75.00	92.00 ^{ab}	64.28
Infested control	28.00 ^{a}	-	16.00 ^{a}	-	56.00 ^{f}	-
Healthy control	4.00 ^{c}	-	0.00 ^{c}	-	96.00 ^{a}	-

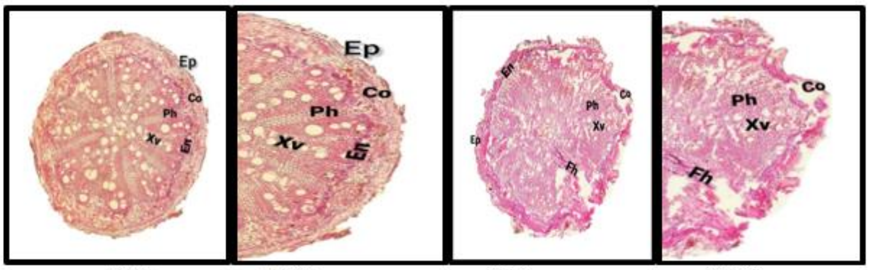
(B)*Fusarium oxysporum*

Treatments	Wilted plants %				Survived Plants %	Increasing %
	Early wilt		Late wilt			
	Incidence%	Reduction%	Incidence%	Reduction%		
Hydroquinone (HQ)	16.00 ^{ab}	33.40	4.00 ^{ab}	66.70	80.00 ^{cd}	25.00
Benzoic acid (BA)	12.00 ^{bc}	50.00	12.00 ^{a}	0.00	76.00 ^{d}	18.75
P. polymyxa	12.00 ^{bc}	50.00	8.00 ^{ab}	33.0	80.00 ^{cd}	25.00
P. fluorescens	16.00 ^{ab}	33.40	8.00 ^{ab}	33.40	76.00 ^{d}	18.75
P. polymyxa + HQ	4.00 ^{c}	83.40	4.00 ^{ab}	66.70	92.00 ^{ab}	43.75
P. fluorescens + HQ	8.00 ^{bc}	66.70	0.00 ^{b}	100.0	92.00 ^{ab}	43.75
P. polymyxa + BA	8.00 ^{bc}	66.70	4.00 ^{ab}	66.70	88.00 ^{abc}	37.50
P. fluorescens + BA	12.00 ^{bc}	50.00	4.00 ^{ab}	66.70	84.00 ^{bcd}	31.25
Topsin M-70®	4.00 ^{c}	83.40	0.00 ^{b}	100.0	96.00 ^{a}	50.00
Infested control	24.00 ^{a}	-	12.00 ^{a}	-	64.00 ^{e}	-
Healthy control	4.00 ^{c}	-	0.00 ^{b}	-	96.00 ^{a}	-

Letters represent statistically significant differences between treatments within the same column, as indicated by the least significant difference test ($p \geq 0.05$).

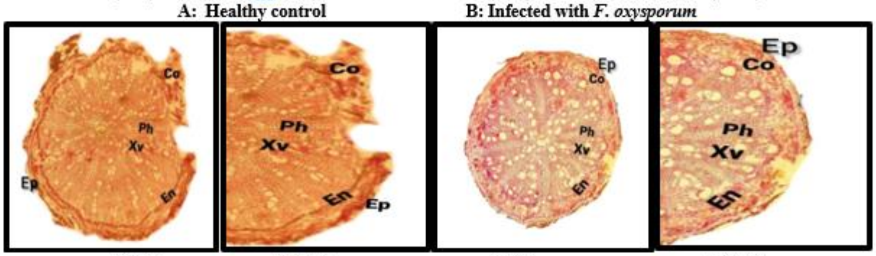
Anatomical structure of soybean root:

Fig. (3) illustrates cross sections of primary roots from healthy and infected soybean plants by each of *F. oxysporum* and *R. solani*, the causative pathogens of root rot, as well as the best treatment (*P. polymyxa* + HQ).



(X40) (X100) (X40) (X100)

A: Healthy control B: Infected with *F. oxysporum*



(X40) (X100) (X40) (X100)

C: Infected with *R. solani* D: *P. polymyxa* + HQ

Fig. 3. Cross sections of soybean roots shows the changes of anatomical structures caused by fungal pathogens comparing with healthy root and the best treatment

Ep =Epidermis Co= Cortex En= Endodermis Xv= Xylem vessels Ph = Phloem Fh = Fungal hyphae

The anatomical structure of a healthy soybean primary root (Figure 1, A) exhibits well-defined components. The epidermis (Ep) is composed of densely packed elongated cells with thin walls, typically displaying an uniseriate coloration. The cortex (Co) primarily consists of parenchyma and may exhibit a homogeneous and simple structure, along with a vascular cylinder; a uniseriate pericycle appears outside the vascular elements and is surrounded by the similarly uniseriate endodermis (En) with casparian strips and vascular cylinder which is a central part of the root that composed of the vascular system that consists of xylem (X) which forms discrete strands, alternating with the phloem (Ph) strands. The xylem is the main tissue responsible for water conduction, while the phloem is responsible for transporting nutrients. The xylem is a complex tissue made up of various types of cells, both living and nonliving. It primarily consists of treachery elements that facilitate water transport and provide structural support. There are two main types of treachery elements in the xylem: tracheids and vessel members (Xv). Vessel members connect to form long, continuous tubes known as tracheae. In contrast, the phloem is the main tissue for food transport in vascular plants, composed of sieve elements, various types of parenchyma cells, as well as fibers and sclereids.

Clear differences were observed in the primary root structure of soybean when comparing cross sections of healthy plants to those infected with both pathogenic fungi. In severely infected roots, significant changes were primarily evident in the epidermis, cortex, and vascular cylinder. As illustrated in Figure 3 (B and C), both *F. oxysporum* and *R. solani* caused extensive damage to certain epidermal and cortical cells, resulting in a brown appearance on the epidermal surface. The fungal infection affected the cortex tissue, penetrating deeper and leading to the degradation and breakdown of cell components. Additionally, some areas of the cortex displayed separation, accompanied by the hydrolysis of cell components in certain cells. The separated cells were dead and become gelatinous area (Fig. 3, C). The fungal hyphae (Fh) of *F. oxysporum* could be seen in inter- and intera-cellular cortex (Fig. 3, B). Generally, *R. solani* shows its damage to the anatomical structure of soybean root in the external tissues (epidermis and cortex), while *F. oxysporum* damage appears on the internal tissues of conducting tissues (xylem and phloem). On the other side, all treatments used led to reduce the harmful effects of both pathogens on soybean root structure. In this respect, *P. polymyxa* + HQ was more effective (Fig. 3, D).

The effects of using bioagents and chemical inducers on the enzymatic activity and total phenol content of soybean.

Oxidative enzymes

Evaluations were made of the peroxidase (PO) and polyphenoloxidase (PPO) enzymes activity in soybean plants grown from seeds that had been exposed to various bioagents and chemical inducers treatments. The results in Table (4) demonstrate that all treatments were successful in inducing the enzymes (peroxidase and polyphenoloxidase) activity. *P. polymyxa* + HQ treatment, followed by *P. fluorescens* + HQ was recognised as having the two enzymes' highest relative activity to the untreated control. Additionally, applying any of the other treatments alone was less beneficial in this regard than separately soaking seeds in hydroquinone treatment. In general, findings show that employing chemical inducers and bioagents together was more successful than using each one separately in boosting the enzyme activities of soybean plants.

Table 4. The effects of using bioagents and chemical inducers on the enzymatic activity and total phenol content of soybean.

Treatments	Peroxidase activity (absorbance at 430 nm) (Enzyme unit /mg protein/min)		Polyphenoloxidase activity (absorbance at 495 nm) (Enzyme unit /mg protein/min)		Total phenols (mg/g fresh leave weight) (absorbance at 725 nm)	
	Activity	Increasing%	Activity	Increasing%	Activity	Increasing%
			Rhizoctonia solani			
Hydroquinone (HQ)	1.962	34.93	1.352	103.61	3.232	63.23
Benzoic acid (BA)	1.793	23.31	0.987	48.64	2.550	28.78
P. polymyxa	1.812	24.62	1.235	85.99	3.211	62.17
P. fluorescens	1.806	24.20	1.221	83.88	2.981	50.60
P. polymyxa + HQ	2.721	87.13	1.623	144.42	3.654	84.54
P. fluorescens + HQ	2.678	84.18	1.510	127.40	3.553	79.50
P. polymyxa + BA	2.557	75.85	1.422	114.15	3.421	72.80
P. fluorescens + BA	2.453	68.70	1.398	110.54	2.985	50.75
Topsin M-70®	1.532	5.36	1.254	88.85	2.072	4.64
Infested control	1.454	-	0.664	-	1.980	-
			Fusarium oxysporum			
Hydroquinone (HQ)	2.010	32.93	1.414	98.87	3.441	63.08
Benzoic acid (BA)	1.856	22.75	1.110	56.11	2.756	30.61
P. polymyxa	1.945	28.63	1.321	85.79	3.410	61.61
P. fluorescens	1.841	21.75	1.243	74.82	3.112	47.48
P. polymyxa + HQ	2.812	85.97	1.645	131.36	3.859	82.89
P. fluorescens + HQ	2.711	79.29	1.523	114.20	3.741	77.29
P. polymyxa + BA	2.525	66.99	1.454	104.50	3.512	66.44
P. fluorescens + BA	2.426	60.44	1.412	98.59	3.221	52.65
Topsin M-70®	1.654	9.39	1.321	85.79	2.230	5.68
Infested control	1.512	-	0.711	-	2.110	-
Healthy control	1.291	-	0.548	-	1.651	-

Total phenol content:

An enhanced total phenol concentration was detected in soybean plants cultivated from seeds treated with several bioagents and chemical resistance inducers, either alone or in combination, compared to the control treatment that did not receive any treatment. The results shown in Table 4 indicate that the combination of *P. polymyxa* and HQ led to a notable rise in the overall phenol content. This was followed by the combination of *P. fluorescens* and HQ compared to the untreated control. The independent application of benzoic acid (BA) and/or Topsin M-70$^{®}$ resulted in less substantial increases in total phenol concentration than the other treatments. Moreover, the results indicate that the concurrent use of chemical inducers and bioagents was more efficient than their individual usage in enhancing the total phenol concentration of soybean plants.

Influence of specific bioagents and chemical inducers individually or combination for controlling root rot and wilt diseases of soybean in the field:

Field trials assessed the efficacy of chemical inducers and bioagents in Giza and Etai El-Baroud Agricultural Research Stations during the summer growing season 2022. These agents were employed as seed soaking treatments, either separately or in combination, to decrease the occurrence of damping-off and root rot/wilt diseases in soybean plants. Table (5) demonstrates that all treatments resulted in a substantial decrease in the incidence of root rot and wilt diseases compared to the control. Soaking soybean seeds in a mixture of bioagents and chemical inducers has shown superior efficacy compared to employing either alone. Research conducted by the Giza Agricultural Research Station revealed that submerging seeds in bioagents, such as *P. polymyxa* and/or *P. fluorescens*, displayed more efficacy in decreasing damping-off occurrence. and root rot/wilt diseases than any of the tested chemical inducers (HQ or BA). The Topsin M-70® treatment demonstrated the highest increase compared to the control, followed by *P. polymyxa* + HQ and *P. polymyxa* + BA, in that order. At the Etai El-Baroud Agricultural Research Station, the Topsin M-70® fungicide showed the highest increase compared to the control, followed by the treatments of *P. polymyxa* + HQ and *P. fluorescens* + HQ, in that order. Conversely, soybean seeds treated with benzoic acid (BA) alone yielded the lowest overall results.

Table 5. Influence of specific bioagents and chemical inducers for managing root rot and wilt diseases of soybean in the field.

Treatments	**Damping- off %**		**Root rotted plants %**		**Wilted plants %**		**Survived plants %**	**Increasing %**
	Incidence%	**Reduction%**	**Incidence%**	**Reduction%**	**Incidence%**	**Reduction%**		
Giza Agricultural Research Station								
Hydroquinone (HQ)	8.30^{b}	55.37	5.30^{b}	57.93	5.00^{b}	40.47	81.40^{ef}	34.76
Benzoic acid (BA)	8.60^{b}	53.76	5.30^{b}	57.93	5.40^{b}	35.71	80.70^{f}	33.60
P. polymyxa	5.60^{cd}	69.90	5.00^{bc}	60.31	4.40^{bc}	47.61	85.00^{de}	40.72
P. fluorescens	7.30^{bc}	60.75	4.70^{bc}	62.70	5.00^{b}	40.47	83.00^{ef}	37.41
P. polymyxa + HQ	3.00^{d}	83.87	2.30^{de}	81.74	2.30^{def}	72.61	92.40^{ab}	52.98
P. fluorescens + HQ	5.00^{cd}	73.11	3.00^{de}	76.19	3.00^{de}	64.28	89.00^{bc}	47.35
P. polymyxa + BA	4.60^{d}	75.26	3.00^{de}	76.19	2.00^{ef}	76.19	90.40^{abc}	49.66
P. fluorescens + BA	5.00^{cd}	73.11	3.60^{cd}	71.42	3.40^{cd}	59.52	88.00^{cd}	45.70
Topsin M-70®	3.30^{d}	82.25	2.00^{e}	84.12	1.70^{f}	79.76	93.00^{a}	53.97
Control	18.60^{a}	-	12.60^{a}	-	8.40^{a}	-	60.40^{g}	-
Etai El-Baroud Agricultural Research Station								
Hydroquinone (HQ)	5.60^{cd}	68.18	4.70^{b}	59.82	4.70^{b}	45.97	85.00^{de}	37.10
Benzoic acid (BA)	8.00^{b}	54.54	5.00^{b}	57.26	5.00^{b}	42.52	82.00^{e}	32.25
P. polymyxa	4.60^{de}	73.86	4.70^{b}	59.82	3.70^{bc}	57.47	87.00^{cd}	40.32
P. fluorescens	6.60^{bc}	62.50	5.00^{b}	57.26	5.00^{b}	42.52	83.40^{e}	34.51
P. polymyxa + HQ	2.60^{f}	85.22	2.00^{c}	82.90	2.00^{de}	77.00	93.40^{a}	50.64
P. fluorescens + HQ	4.00^{def}	77.27	2.00^{c}	82.90	2.00^{de}	77.00	92.00^{ab}	48.38
P. polymyxa + BA	4.00^{def}	77.27	3.60^{b}	69.23	3.00^{cd}	65.51	89.40^{bc}	44.19
P. fluorescens + BA	5.00^{cde}	71.60	3.60^{b}	69.23	3.00^{cd}	65.51	88.40^{cd}	42.58
Topsin M-70®	3.30^{ef}	81.25	1.00^{c}	91.45	1.30^{e}	85.00	94.40^{a}	52.25
Control	17.60^{a}	-	11.70^{a}	-	8.70^{a}	-	62.00^{f}	-

Letters represent statistically significant differences between treatments within the same column, as indicated by the least significant difference test ($p \geq 0.05$).

The effects of certain bioagents and chemical inducers on growth and productivity of soybean plants under natural infection in the field.

The data included in Table (6) demonstrate that using a mixture of chemical inducers with bioagents as seed treatments substantially affected the development and productivity of the plants at both sites, compared to the control group that did not receive any treatment. Using P. polymyxa + HQ and P. fluorescens + HQ treatments at Giza and Etai El-Baroud Agricultural Research Stations increased plant height, branch count, pod count, seed weight per plant, seed harvest weight, and seed production. The efficacy of seed treated with BA was unsatisfactory in both sites than the other treatments. The combined use of bioagents and chemical inducers significantly enhanced the growth of soybean plants compared to using them individually.

Table 6. The effects of certain bioagents and chemical inducers growth and productivity of soybean plants under natural infection in the field.

Treatments	**Plant height(cm)**	**Branches number /plant**	**Pods number / plant**	**Seed yield /plant(g)**	**100 seed Weight (g)**	**Seed yield Weight (Kg/ feddan)**
Giza Agricultural Research Station						
Hydroquinone (HQ)	71.10^{d}	3.24^{bcd}	65.40^{e}	28.85^{d}	16.60^{de}	1253^{c}
Benzoic acid (BA)	69.77^{d}	2.70^{de}	48.40^{f}	21.95^{e}	15.50^{e}	1085^{d}
P. polymyxa	77.97^{c}	3.33^{bcd}	67.40^{cde}	30.85^{cd}	17.20^{bcd}	1342^{c}
P. fluorescens	75.37^{c}	3.00^{cde}	66.40^{de}	29.86^{d}	17.10^{cd}	1275^{c}
P. polymyxa + HQ	89.70^{a}	4.33^{a}	78.40^{a}	36.70^{a}	18.70^{a}	1580^{a}
P. fluorescens + HQ	86.14^{b}	4.00^{ab}	75.70^{ab}	35.40^{ab}	18.20^{abc}	1482^{b}
P. polymyxa + BA	85.57^{b}	3.94^{ab}	73.40^{ab}	34.08^{b}	17.50^{abcd}	1347^{c}
P. fluorescens + BA	84.84^{b}	3.76^{abc}	72.70^{bc}	33.47^{b}	17.30^{bcd}	1285^{c}
Topsin M-70®	84.17^{b}	3.14^{bcde}	71.40^{bcd}	32.86^{bc}	18.40^{ab}	1541^{ab}
Control	64.87^{e}	2.40^{e}	38.40^{g}	21.36^{e}	14.30^{f}	859^{e}
Etai El-Baroud Agricultural Research Station						
Hydroquinone (HQ)	74.57^{de}	3.30^{cde}	65.40^{c}	28.42^{d}	17.20^{bc}	1312^{cd}
Benzoic acid (BA)	72.94^{e}	3.00^{e}	50.40^{d}	24.18^{e}	16.50^{cd}	1173^{e}
P. polymyxa	80.77^{c}	3.44^{bcde}	69.70^{c}	31.96^{c}	17.40^{bc}	1400^{bc}
P. fluorescens	77.27^{d}	3.14^{de}	67.70^{c}	30.87^{c}	17.30^{bc}	1304^{d}
P. polymyxa + HQ	94.74^{a}	4.55^{a}	82.70^{a}	38.68^{a}	19.20^{a}	1608^{a}
P. fluorescens + HQ	91.97^{a}	4.22^{ab}	80.70^{ab}	37.67^{ab}	18.60^{ab}	1576^{a}
P. polymyxa + BA	88.14^{b}	4.07^{abc}	78.70^{ab}	36.56^{ab}	17.80^{abc}	1540^{a}
P. fluorescens + BA	87.04^{b}	3.94^{abcd}	76.40^{b}	35.31^{b}	17.60^{abc}	1436^{b}
Topsin M-70®	85.64^{b}	3.44^{bcde}	76.70^{b}	35.16^{b}	18.70^{ab}	1584^{a}
Control	68.00^{f}	2.70^{e}	40.40^{e}	22.50^{e}	15.60^{d}	876^{f}

Letters represent statistically significant differences between treatments within the same column, as indicated by the least significant difference test ($p \geq 0.05$).

Additionally, seeds treated with bioagents showed higher efficiency in promoting plant growth compared to seeds treated with chemical inducers.

Discussion

Soybean (Glycine max (L.) Merrill) is a globally important legume crop (Patkowska and Konopinski, 2013). Various diseases have significantly affected soybean yield (Wrather and Koenning, 2006 and 2009). Microbial diseases are responsible for the majority of soybean yield losses.

Root rot are particularly severe and widespread diseases, impacting soybean plants worldwide. This results in significant reductions in the quality and quantity of soybean yield. A variety of pathogenic fungi causes these diseases. Root rot in soybean plants are primarily caused by *R. solani, F. solani* and *P. ultimum* (Haikal, 2008; Fayzalla *et al.*, 2009; Ghaleb *et al.,* 2021). Furthermore, *F. oxysporum*, causing wilt disease, is considered one of the most devastating diseases to affect soybean crops (El-Kazzaz *et al.,* 2008; Hashem *et al.,* 2009; Ghaleb *et al.,* 2021).

In the pathogenicity tests, each isolate showed the ability to infect the roots of soybean, leading to root rot and wilt signs. *Rhizoctonia solani* isolate No. 3 that was isolated from Beni suef Governorate, displayed the most aggressive behavior among the isolates, resulting in higher percentages of damping-off, root rot, and decreased plant survival rates. Conversely, *F. oxysporum* isolate No. 2 that was isolated from Beni suef Governorate also, displayed notable aggression, with the highest percentages of early wilt, late wilt, and reduced plant survival. The aggressiveness of these isolates can be attributed to the observed high rates of seedling and plant mortality. As highlighted by (Arias, 2012) who reported that understanding the morphology and genetic diversity of these pathogens could offer significant knowledge of the epidemiology and biology of Fusarium and Rhizoctonia species impacting soybean plants.

Researchers are working hard to reduce the using of synthetic fungicides and maximize alternative approaches to manage soil-borne diseases. One of the alternatives to substituting or at least reducing the using of fungicides in plant disease control is acquiring systemic resistance, which enhances the plant's resistance to subsequent pathogen attacks. This can be achieved by utilizing bioagents or chemicals as inducers. The resistance these agents elicit is long-lasting and has a broad spectrum against various pathogens. Nevertheless, achieving thorough infection control is rarely possible, as some resistance elicitors only provide disease control ranging from 20% to 85% (Walters *et al*., 2013).

The interactions among antagonistic microorganisms and plant roots in the area where plant roots grow are crucial in determining the structure of soil agroecosystems. These interactions have a far-reaching effect on crop health, productivity, and soil quality. Fungal and bacterial antagonists in the root zone can produce a diverse array of secondary compounds that positively impact plant growth. These microorganisms improve the accessibility of chemical nutrients, enhance nitrogen fixation, reduce susceptibility to frost damage, promote plant health by controlling plant diseases biologically, provide systemic disease resistance in plants, and support overall plant establishment and growth (Sturz and Christie, 2003). Chemical inducers trigger plants to activate multiple protective mechanisms to hinder the proliferation and spread of pathogens (Malolepsza and Rozalaska, 2005). According to De Gara et al. (2003), plant defense mechanisms encompass the rapid generation of reactive oxygen species, alterations in cell wall composition, the buildup of antimicrobial secondary metabolites like phytoalexins (Agrios, 2005), activation and synthesis of defense peptides and proteins play a critical role (Castro and Fontes, 2005).

The aim of this work was to assess techniques for managing root rot and wilt diseases in soybean plants. The approach involved the use of bioagents (*P. fluorescens* and *P. polymyxa*) and chemical resistance inducers (hydroquinone and benzoic acid either separately or in combination. The *in vitro* data analysis showed that both the bioagents and chemical inducers, whether used alone or in combination, significantly reduced the fungal growth of the pathogenic fungi under study. Notably, the bioagents demonstrated greater effective ess in inhibiting the fungal growth of the pathogenic fungi compared to the chemical inducers, although the degree of effectiveness varied. Moreover, the combined application of bioagents and chemical inducers demonstrated greater efficacy in inhibiting fungal growth compared to individual applications, particularly when *P. polymyxa* was used in conjunction with hydroquinone. However, when used alone, benzoic acid exhibited the lowest reduction in fungal growth among the treatments studied. These findings suggest that integrating bioagents with chemical inducers could enhance disease management in soybean, offering a more effective strategy than using either approach alone. As Wahyudi *et al.* (2011) proposed siderophores produced by bacteria play a crucial role as suppressors of phytopathogenic fungi like *F. oxysporum* or *R. solani*. In addition to the synthesis of indole-3-acetic acid (IAA), phosphate solubilization, and siderophores, biocontrol processes also include the use of antibiotic chemicals, the induction of systemic resistance (ISR) in plants, and the release of lytic enzymes (Haas and Defabo, 2005).

The findings indicate that all treatments significantly decreased damping-off and root rot/wilt diseases, enhancing plant survival *in vivo*. Co-applications of bioagents and chemical resistance inducers proved more effective in reducing disease severity and increasing plant survival than individual treatments. Notably, the combination of *P. polymyxa* and hydroquinone (HQ) ranked just below Topsin M-70® fungicide in protective efficacy. These results align with Zian *et al.* (2019), who found that combinations of HQ or salicylic acid (SA) with *Trichoderma harzianum* provided the highest protection towards root rot and wilt diseases of lupine while also enhancing growth parameters and yield components. Furthermore, Saikia *et al.* (2003) demonstrated that the combined application of *P. fluorescens* (pf4-92) and SA provided the most effective protection against Fusarium wilt in chickpea seedlings.

Our results indicate significant differences in the primary root structure of soybeans between healthy plants and those infected by pathogenic fungi. Severely infected roots exhibited notable changes in the epidermis, cortex, and vascular cylinder. Both *F. oxysporum* and *R. solani* destroyed some epidermal and cortical cells, resulting in a brown surface on the epidermis. Fungal infections primarily affected the cortex, extending deeper and leading to the degradation of cell components. This included separation in some cortex regions with hydrolysis of some cell components. Generally, *R. solani* inflicted more damage on the external tissues (epidermis and cortex), while *F. oxysporum* targeted the internal conducting tissues (xylem and phloem). All treatments applied reduced the negative impacts of both pathogens on soybean root structure, with the combination of *P. polymyxa* + HQ proving the most effective. The same results were demonstrated by several studies (Abd El-Hai and El-Saidy, 2016; Abd El-Hai and Ali, 2017)

The application of seed soaking with bioagents and chemical inducers, both separately and in combination, at the Giza and Etai El-Baroud Agricultural Research Stations considerably improved soybean growth metrics and yield components throughout the 2022 growing season. The combinations of these agents outperformed individual treatments across all measured parameters. These improvements can be attributed to the impacts of bioagents and chemical elicitors on physiological processes in plants, including ion absorption, cell elongation, cell division, and enzyme activation. For instance, low hydroquinone (HQ) doses have been shown to enhance growth and yield components in tomato (Abdel-Monaim *et al.,* 2012). Additionally, the triple combination of mycorrhizae, *P. polymyxa*, and *P. fluorescens* proved particularly effective against root rot caused by *R. solani*, leading to enhance growth and yield of soybean plants (Atwa, 2018). Furthermore, the effectiveness of *P. fluorescens* and *P. polymyxa* in biocontrol can be boosted by hydroquinone (HQ) and benzoic acid (BA), leading to a substantial increase in their population density and improved antagonistic properties against the pathogens under study. The promising potential of these combinations highlights the possibility of strengthening crop resilience and enhancing production.

Based on the provided results, the various treatments involving the application of bioagents and chemical inducers have shown effectiveness in enhancing the level of enzymes such as peroxidase and polyphenoloxidase in soybean plants. The combined treatments, particularly *P. polymyxa* + HQ and *P. fluorescens* + HQ, demonstrated the highest relative activity of these enzymes compared to the untreated control. The increase in enzyme activity is linked to enhanced resistance against various diseases according to Wang et al. (2000). The enzymes peroxidase and polyphenol oxidase participate in the process of oxidizing certain compounds produced by the host, which then function as inhibitors of the growth of the phytopathogen (Chen et al., 2000). The oxidation of phenols is facilitated by peroxidase (PO) and polyphenoloxidase (PPO), leading to the production of quinones that can inhibit certain enzymes of pathogens. Peroxidase plays a crucial role in secondary cell wall biosynthesis by contributing to lignin formation, which strengthens cell walls and impedes pathogen penetration. Polyphenoloxidase, on the other hand, is involved in plant resistance by producing antimicrobial compounds and participating in lignification during microbial attacks. Moreover, the treatments increased the total phenolic content, which has been suggested by researchers such as Nicholson and Hammerschmidt (1992) to enhance disease resistance by producing toxic quinones that act as antimicrobial agents against invading pathogens, as noted by Farkas and Kiraly (1962). These findings collectively emphasize the significance of employing a combination of chemical inducers and bioagents to boost enzyme activities and enhance the plant's defense mechanisms against diseases, highlighting the potential for improved crop health and productivity.

CONCLUSION

The results suggest that employing a combination of bioagents and chemical inducers as seed treatments leads to a substantial increase in plant resistance compared to individual treatments against infections caused by pathogens like *R. solani* and *F. oxysporum*. This combined approach enhances plant resistance and contributes to improved plant growth, increased yield, accumulation of antimicrobial substances such as total phenols, and elevated activity of defense-related enzymes. These findings indicate that utilizing such integrated treatments can be critical to comprehensive disease management strategies for field crops.

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تحسين كفاءة عوامل المقاومة الحيوية بإستخدام بعض المستحثات الكيميائية لمقاومة أمراض اعفان جذور وذبول فول الصويا

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الملخص

تهدف الدراسة الحالية إلى التحقيق في فعالية بكتيريا *Pseudomonas fluorescens* و *Paenibacillus polymyxa* كعوامل حيوية، بالإضافة إلى محفزات المقاومة الكيميائية مثل الهيدروكينون وحمض البنزويك، لمكافحة أمراض أعفان جذور وذبول فول الصويا مع تعزيز نمو النباتات. وقد أظهر اختبار القدرة المرضية أن جميع العزلات يمكن أن تصيب فول الصويا صنف جيزة ١١١ بدرجات متفاوتة من الإصابة. حيث كانت عزلة *Rhizoctonia solani* رقم ٣ والتى تم تسجيلها فى بنك الجينات برقم الوصول (OR975921.1) وعزلة *Fusarium oxysporum* رقم ٢ برقم الوصول (OR975922.1) هما الأكثر شراسة وقدرة على إحداث أمراض اعفان الجذور والذبول. وقد أظهرت معاملة *P. polymyxa* + الهيدروكينون القدرة الأكثر فعالية في تقليل النمو الفطري لكلا الفطرين قيد الدراسة تحت ظروف المعمل. وقد كشفت التجارب الحقلية التي أُجريت في الاصص والحقل أن معاملة *P. polymyxa*+ الهيدروكينون جاءت في المرتبة الثانية بعد مبيد الفطريات توبسين أم-٧٠® من حيث الفعالية. تشريحيًا، كشفت المقاطع العرضية للجذور الأولية تحت المجهر الضوئي أن التأثير المشترك لعوامل المقاومة الحيوية والمحفزات الكيميائية أدت إلى حماية نباتات فول الصويا من التغيرات التشريحية الضارة التي تسببها كلا الفطرين في تركيب خلايا البشرة، والقشرة، والاسطوانة الوعائية تحت ظروف العدوى الصناعية بالفطريات المدروسة. أظهرت التحليلات الفسيولوجية زيادة في مستويات البيروكسيديز والبولي فينول أوكسيديز، بالإضافة إلى المحتوى الكلي للفينولات في النباتات المعاملة بالعوامل المقاومة الحيوية والمحفزات الكيميائية مقارنة بتلك المعاملة بشكل فردي. وقد حسنت المعاملات المشتركة بين عوامل المقاومة الحيوية والمحفزات الكيميائية بشكل كبير مقاييس نمو النباتات ومكونات المحصول تحت ظروف العدوى الطبيعية بالحقل. بشكل عام، تشير النتائج إلى أن الجمع بين العوامل الحيوية والمحفزات الكيميائية أثبت فعالية أكثر من التطبيقات الفردية، ويمكن أن يكون جزءًا من استراتيجيات إدارة الأمراض المتكاملة.

الكلمات الدالة : فول الصويا ; *Rhizoctonia solani* ; *Fusarium oxysporu*