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Induction of Systemic Acquired Resistance against Damping-Off and Stem Rot Diseases of Chickpea Caused by *Sclerotinia sclerotiorum*

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Abstract

This study investigates the effectiveness of different resistance inducers, Bion (BTH) and salicylic acid (SA) as chemicals, dipotassium phosphate (K_2HPO_4) as a mineral salt, and ascorbic acid as a vitamin in enhancing chickpea resistance against damping-off and stem rot diseases caused by *Sclerotinia sclerotiorum* under greenhouse and field conditions. Among the treatments, Bion and SA were the most effective in reducing disease incidence and improving plant survival. In addition, these inducers enhanced growth parameters and increased seed yield. However, the highest levels in mineral content were observed in plants treated with Bion and SA. Notably, there was a marked enhancement in the activities of peroxidase (PO) and polyphenol oxidase (PPO), and an increased accumulation of total phenols compared to the untreated infested control. Dipotassium phosphate and ascorbic acid demonstrated the least effectiveness among the tested treatments, exhibiting moderate efficacy. Furthermore, their performance makes them successful enough to be included as components of integrated disease management programs.

Keywords: *Chickpea; Chemical Inducers; Sclerotinia sclerotiorum*

Introduction

Chickpea is an annual legume crop cultivated in over 50 countries [1]. It is adapted to tropical, subtropical, and temperate regional climates and considered the third most important legume crop worldwide [2,3]. Fresh green chickpea seeds are also consumed as green vegetables [4]. The crop is a rich source of nutrients, providing approximately 24% protein, 60 - 65% carbohydrates, and 6% fat. It also contains essential minerals such as calcium, phosphorus, and iron, along with vital A and B vitamins, β-carotene, and notable levels of all essential amino acids [5]. Chickpea plants fix nearly 80% of their nitrogen requirements and improve soil fertility through symbiotic relationships with rhizobacteria [6].

Of more than 70 different pathogens that attack chickpeas, *Sclerotinia sclerotiorum* is considered one of the most economically devastating diseases and is also known as *Sclerotinia* rot, *Sclerotinia* stem rot, or white mold [4,7]. This pathogen is particularly destructive in legume crops, with the potential to cause yield losses of up to 100% [8].

The management of this disease in chickpeas depends on cultural practices such as crop rotation, which can sometimes be ineffective because *S. sclerotiorum* has a wide host range, and infecting over 400 plant species [9]. In addition, it produces persistent resting structures (sclerotia), which can survive in the soil for over 7-10 years, even in the absence of host plants and under dry conditions [10,11]. Resistant

varieties are considered the most effective and practical control strategy; unfortunately, legumes generally exhibit only limited resistance to *S. sclerotiorum*. However, depending on the pathogen's nature, the breeding programs have achieved minimal success, and currently, no resistant commercial legume varieties are available [8,12].

Although disease management still relies heavily on fungicide application, the effectiveness of chemical fungicides is sometimes limited, especially under high disease pressure [13]. However, using fungicides presents several limitations, including high economic costs, chemical residues in food products, and risks to human health. Furthermore, the repeated use of fungicides can accelerate the emergence of resistant pathogen strains [7].

So, there is an increasing demand for eco-friendly alternatives that can provide effective disease management and reduce reliance on chemical fungicides, even if not completely, then at least partially. The use of plant defense elicitors (PDEs) triggers resistance in plants against a wide range of pathogens [14]. Systemic acquired resistance (SAR) is activated throughout a plant after exposure to elicitors, including pathogens and synthetic chemicals [15]. These elicitors enhance plant tolerances against subsequent pathogen infections by activating a range of defense responses, such as pathogenesis-related (PR) proteins, upregulating the expression of defense-related genes, and stimulating the production of reactive oxygen species (ROS) and phenolic compounds [16,17].

Salicylic acid (SA) is a well-established molecule that activates SAR by inducing the expression of pathogenesis-related proteins, thereby enhancing resistance to pathogen attack [18]. Similarly, benzothiadiazole (BTH), a functional analog of SA, has been shown to stimulate SAR by upregulating defense-related gene expression without causing phytotoxic effects [19]. Potassium salts, such as dipotassium phosphate, also contribute to induced resistance by strengthening the cell wall and activating defense-related pathways [20]. In addition, ascorbic acid plays a dual role as an antioxidant and a modulator of plant defense responses to suppress soil-borne diseases [21].

Aim of the Study

This study aimed to evaluate the effectiveness of various chemical inducers in managing *S. sclerotiorum* infection in chickpea plants under greenhouse and field conditions.

Materials and Methods

Plant material

Chickpea seeds (*Cicer arietinum* L.), cultivar Giza 3, were obtained from the Legume Research Dept., Field Crops Research Institute, ARC, Giza, Egypt.

The causal pathogen

Isolation and morphological identification

Diseased chickpea plants showing white cottony mycelial growth and black sclerotia on stems were collected from infected fields in Etai El-Baroud, El-Behira Governorate. The infected plant parts were surface sterilized with 1% sodium hypochlorite (NaOCl) for 2 minutes and washed 2-3 times with sterilized distilled water. Small segments from the edges of infected tissues were aseptically placed on potato dextrose agar (PDA) plates amended with streptomycin sulfate (50 mg/L) to prevent bacterial growth. Plates were incubated at 22 ± 2°C in the dark for 5 - 7 days. A pure culture of the fungus was achieved using the hyphal tip method [22]. The isolated fungus was maintained on PDA slants under a phosphate buffer at 4°C for further use [23]. Its pathogenicity was confirmed according to [24]. The isolated fungus was identified based on cultural and morphological features using a taxonomic key for *Sclerotinia* [25,26].

Molecular identification

Genomic DNA was extracted from pure fungal cultures using the cetyltrimethylammonium bromide (CTAB) method, as described by [27]. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCG

G-3′) and ITS4 (5′ TCCTCCGCTTATTGA TATGC-3′) as forward and reverse primers, respectively [28]. PCR amplification products were resolved on a 1.5% agarose gel and visualized under ultraviolet light. The obtained amplicons were then purified and subjected to sequencing. The resulting sequences were aligned and compared with those available in the NCBI GenBank database using the CLUSTAL W algorithm within the MEGA11 software [29,30]. A phylogenetic tree was constructed using the Jukes-Cantor model [31]. The analysis confirmed the identity and genetic relationship of the isolate with known *S. sclerotiorum* isolates.

Inoculum preparation

Glass bottles (500 cc) were filled with 100 g of sorghum grains and soaked in 250 ml of water overnight. The water was drained from the bottles, and 50 ml of distilled water was added, then autoclaved twice for 20 minutes at 121°C. The bottles were inoculated with 5 disks (0.5 cm) of seven-day-old *S. sclerotiorum* culture and incubated at 25°C ± 1°C until the grains were fully colonized with mycelium. The colonized grains were air-dried at room temperature for five days and crushed in a mill [32]. The crushed dried inoculum was stored in a paper bag at 4 ± 1°C until added to the soil within one week.

Seed treatments

Chemical inducers

Chickpea seeds were surface sterilized with sodium hypochlorite (1%) for 2 minutes, thoroughly rinsed with sterilized water, left to dry, then soaked in solutions of Bion® wettable granule (WG) 50%, benzothiadiazole (Syngenta Crop Protection, Inc.), [salicylic acid, dipotassium phosphate, and ascorbic acid (Sigma Aldrich, USA)], for six hours on the previous day of sowing at the rate of 3 mM for each inducer. For control plants, seeds were soaked in distilled water. Treated seeds were air-dried for 15 hours until sowing.

Fungicide treatment

Seed dressing was carried out by applying the Topsin-M 70% WP, thiophanate-methyl, at a rate of 3g/kg seeds with 1% methyl cellulose (as a sticker).

Greenhouse experiment

A greenhouse experiment was conducted at the Plant Pathology Research Institute using a completely randomized design (CRD). Thirty-centimeter-diameter pots were sterilized with a 5% formalin solution and filled with steam-disinfected sandy clay soil at a 1:2 (v/v) ratio. The inoculum of *S. sclerotiorum* was inoculated into soil at a rate of 1% of soil weight. For the healthy control, sterilized, uninoculated crushed sorghum grains were added at the same rate. The infested soil was thoroughly mixed and irrigated twice over one week before planting to promote fungal growth and ensure uniform distribution of the pathogen throughout the soil. Each treatment consisted of twelve replicate pots; each planted with five chickpea seeds. Each pot received five grams of *Mesorhizobium ciceri* (*Rhizobium* formulation) obtained from the Biofertilizer Production Unit. All pots were irrigated weekly to maintain moisture near field capacity. The treatments are as follows: (1) Bion, (2) salicylic acid, (3) dipotassium phosphate, (4) ascorbic acid at a rate of 3 mM for each inducer, (5) Topsin M-70 at 3 g/kg of seeds, and (6) soaking in distilled water served as untreated control for both infested and non-infested soil.

Plant growth assessment

Sixty-day-old plants were uprooted to record plant height (cm), fresh and dry weight of shoots and roots, numbers and dry weight of nodules, as well as macronutrient content [33] and micronutrient content [34].

Disease assessment

Damping-off disease

Disease incidence (DI) was evaluated by recording pre- and post-emergence damping-off and the percentage of surviving plants at 15, 30, and 45 days after sowing. The percentage of reduction or increase over the infected control was calculated as follows:

Reduction or increasing $\% = \frac{\text{Disease incidence (DI) of infected control} - \text{DI of treatment}}{\text{DI of infected control}} x100$

Stem rot disease

Percentage of stem rot was recorded at 75 and 90 days after sowing for green house and field experiments respectively, as follows:

$\text{Stem rot } \% = \frac{\text{No.of rotted plants}}{\text{Total No.of sown seeds}} \times 100$

Field experiments

Field trials were conducted during the 2023 winter growing season at Giza and Etai El-Baroud Agricultural Research Stations located in Giza and El-Beheira Governorates. A total of 24 plots, each measuring 10.5m^2 and comprising five rows of 3.5 x 0.6m, were arranged in a randomized block design (RBD) with four replicates per treatment. Seeds were planted at a spacing of 20 cm between hills, with one seed per hill on both sides of each ridge. Field soil was amended with 800 grams of *Mesorhizobium ciceri* formulation per feddan by thoroughly mixing it with approximately 50 kg of moist, fine sandy soil before application. Standard agricultural practices, including irrigation and fertilization, were performed following the guidelines of the Egyptian Ministry of Agriculture and Land Reclamation (MALR). At harvest, ten plants were randomly collected from the interior rows of each plot to assess growth parameters. Seed yield (tons per feddan) was calculated. The treatments were applied as mentioned before in the greenhouse experiment.

Activities of oxidative enzymes and contents of phenol

Activities of peroxidase (PO) [35], polyphenol oxidase (PPO) [36], and phenol contents [37,38] were determined 15 days after sowing.

Statistical analysis

The data obtained were analyzed using [39] computer statistical software (ASSISTAT) using one-way analysis of variance (ANOVA). The least significant difference (LSD) was used to compare the mean values at the level of $P \leq 0.05$.

Results

Morphological and molecular identification of the pathogen

According to the microscopic and morphological features of the pathogen, the isolate was identified as *S. sclerotiorum*. Blast analysis revealed that the ITS sequence of the isolate is *S. sclerotiorum* with accession number PV029721.1 (Figure 1).

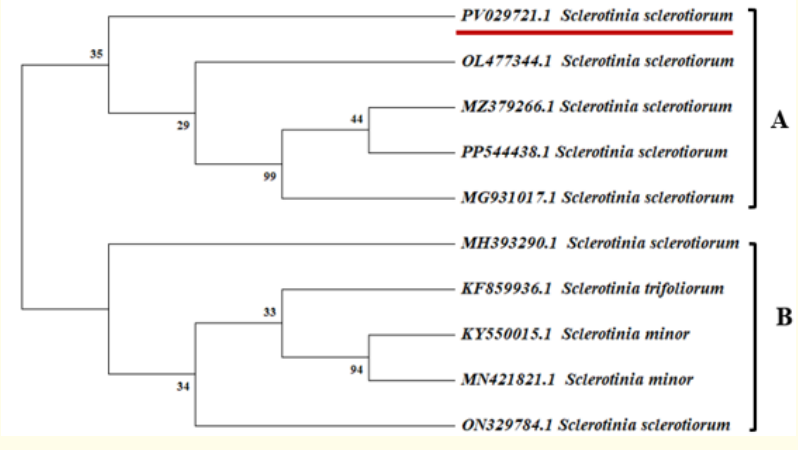

Figure 1: *Phylogenetic tree based upon CLUSTAL W alignment of ITS region of rDNA nucleotide sequences of 10 Sclerotinia isolates. A maximum likelihood was used, with bootstrap value after 1000 replications of calculated run by using MEGA11 software. The branch numbers indicate bootstrap values. Our own sequenced S. sclerotiorum isolate (ITS:PV029721.1) is underlined with red color and the tree shows its identity with the most similar S. sclerotiorum, Genbank accession numbers.*

Greenhouse experiments

Effect of some chemical inducers and Topsin-M on the incidence of damping-off and stem rot in chickpea plants grown in soil artificially infested with *S. sclerotiorum*

As shown in table 1, the fungicide Topsin M-70 proved to be the most effective in controlling damping-off in chickpea, achieving a substantial 87.5% reduction in pre-emergence incidence and the highest plant survival rate at 84%. Among the chemical inducers, Bion treatment showed the most pronounced effect, achieving a 75% reduction in pre-emergence damping-off. However, salicylic acid treatment also performed an enhanced effect, with a pre-emergence reduction of 62.5% and 68% of plants surviving. The untreated infested control had the highest pre-emergence incidence (32%), and the lowest percentage of plants survived (28%). Topsin M-70 and Bion were the most effective treatments in reducing post emergence damping off. On the other hand, the lowest stem rot incidence was observed with the fungicide and Bion treatments, each showing 8%, followed by the salicylic acid treatment that recorded 12%, compared to 20% in the untreated infested control.

Treatments	Damping-off				Survived plants %	Increasing over-infected control%	Stem rot %
	Pre-emergence		Post-emergence				
	Incidence %	Reduction %	Incidence %	Reduction %			
Bion 3 mM	8.0	75.0	4.0	80.0	76.0	171.4	8.0
Salicylic acid 3 mM	12.0	62.5	8.0	60.0	68.0	143	12.0
K_2HPO_4 3 mM	20.0	37.5	16.0	20.0	48.0	71.4	16.0
Ascorbic acid 3 mM	16.0	50.0	12.0	40.0	56.0	100	16.0
Topsin M-70	4.0	87.5	4.0	80.0	84.0	200	8.0
Control (*S. sclerotiorum)*	32.0	0.0	20.0	0.0	28.0	0.0	20.0
Healthy control (non-infested soil)	0.0		0.0		0.0		0.0
LSD ≤ 0.05	1.2		1.3		2.4		

Table 1: *Effect of some chemical inducers on damping-off incidence in chickpea plants grown in soil artificially infested with Sclerotinia sclerotiorum.*

Effect of some chemical inducers and Topsin-M on some growth parameters of chickpea plants grown in soil artificially infested with *S. sclerotiorum*

Results in table 2 present the effects of various treatments on the growth characteristics of chickpea plants grown in greenhouse conditions. Bion treatment considerably improved most growth parameters compared to the rest of the treatments. Bion treatment led to the maximum plant height (51.5 cm), shoot fresh and dry weights (13.15 and 4.15 g/plant), and root fresh and dry weights (12.6 and 2.73 g/plant), as well as the highest nodulation (31 nodules/plant; 501.6 mg/plant), followed by salicylic acid. However, dipotassium phosphate and ascorbic acid had moderate impacts compared to the untreated infested control, which recorded the lowest values across all parameters.

Treatments	Plant height (cm)	Shoot fresh weight (g/ plant)	Shoot dry weight (g/ plant)	Root fresh weight (g/ plant)	Root dry weight (g/ plant)	Nodule number / plant	Nodules dry weight (mg/ plant)
Bion 3 mM	51.5	13.15	4.15	12.6	2.73	31	501.6
Salicylic acid 3 mM	48.8	11.38	3.75	11.6	2.67	24	385.0
K_2HPO_4 3 mM	45.8	9.25	3.11	8.8	2.13	20	248.7
Ascorbic acid 3 mM	45.3	9.98	3.23	8.6	2.21	18	233.5
Topsin M-70	47.3	10.66	3.08	8.5	2.16	7	104.7
Control (*S. sclerotiorum)*	32.0	4.82	2.13	5.0	0.59	6	94.0
Healthy control (non-infested soil)	44.0	6.32	3.05	6.7	1.66	11	219.6
LSD ≤ 0.05	3.0	1.0	0.2	0.5	0.3	1.5	17.7

Table 2: *Effect of some chemical inducers on some growth parameters of chickpea plants grown in soil artificially infested with Sclerotinia sclerotiorum after 60 days of planting.*

Effect of some chemical inducers on the activity of oxidative enzymes and phenolic contents

Results in table 3 indicate that the different chemical inducers positively affected the activities of oxidative enzymes. Bion treatment resulted in the greatest enhancement of PO and PPO activities compared to the untreated, infested control. Meantime, salicylic acid treatment revealed an increase in the activity of two enzymes followed by dipotassium phosphate. Ascorbic acid treatment, however, produced the enzymes' lowest level of activity. It must be noticed that infestation of plants with *S. sclerotiorum* increased the activity of both enzymes in the absence of chemical inducers more than in healthy control treatments.

Treatments	Peroxidase activity absorbance at 430 nm Enzyme unit/mg protein/min		Polyphenol oxidase activity absorbance at 495 nm Enzyme unit/mg protein/min	
	Activity	Increasing over infected control %	Activity	Increasing over infected control %
Bion 3 mM	2.125	108.4	0.085	107.3
Salicylic acid 3 mM	1.851	81.6	0.088	114.6
K_2HPO_4 3 mM	1.574	54.4	0.076	85.4
Ascorbic acid 3 mM	1.168	14.6	0.070	70.7
Control (*S. sclerotiorum)*	1.019	0.0	0.041	0.0
Healthy control (non-infested soil)	0.741		0.021	

Table 3: *Effect of some chemical inducers on the activity of oxidative enzymes of chickpea plants grown in soil artificially infested with Sclerotinia sclerotiorum.*

Results in table 4 showed that the contents of total phenols were greatly increased in plants treated with different inducers. Bion and salicylic acid treatments resulted in the highest accumulation of total phenols, followed by treatments with dipotassium phosphate and ascorbic acid, as a similar trend was observed in PO and PPO enzymes activities. For free phenols, all treatments represented higher

figures of content in comparison with the untreated infested control. The lowest value in total and free phenolic contents was related to the healthy control treatment.

Treatments	Phenolic contents (catechol equivalents mg/g fresh weight)			
	Total phenols	Increasing over infected control%	Free phenols	Increasing over infected control%
Bion 3 mM	3.81	149	2.935	184.7
Salicylic acid 3 mM	3.78	147.1	2.509	143.4
K_2HPO_4 3 mM	3.55	132.1	2.768	168.5
Ascorbic acid 3 mM	3.46	126.1	2.832	174.7
Control (*S. sclerotiorum)*	1.53	0.0	1.031	0.0
Healthy control (non-infested soil)	1.01		0.873	

Table 4: *Effect of some chemical inducers on the phenolic contents of chickpea plants grown in soil artificially infested with Sclerotinia sclerotiorum.*

Effect of some chemical inducers and Topsin-M on the contents of macro and micro elements of chickpea plants grown in soil artificially infested with *S. sclerotiorum*

Application of different inducers improved the uptake of N, P, and K. The marked increase was observed in salicylic acid-treated plants, followed by Bion treatment (Table 5). For micronutrient Fe, Mn, Zn, and Cu uptake, there was an increase with all treatments in comparison with the untreated infested control; the most pronounced increase was observed with salicylic acid, followed by Bion and ascorbic acid treatments. The lowest values of micronutrient contents were related to dipotassium phosphate treatment. Infested control exhibited the lowest values of all nutrient contents.

Treatments	Macro elements mg/g dry weight			Micro elements ppm			
	N	P	K	Fe	Mn	Zn	Cu
Bion 3mM	2.73	2.73	2.73	356	140.1	31.3	8.1
Salicylic acid 3mM	2.91	2.91	2.91	415	143.3	33.2	8.3
K_2HPO_4 3mM	2.20	2.20	2.20	327	103.7	24.6	6.3
Ascorbic acid 3mM	2.58	2.48	2.53	331	119.2	26.5	6.9
Topsin M-70	2.11	2.11	2.11	313	111.3	21.1	5.9
Control (*S. sclerotiorum)*	1.73	1.73	1.73	192	83.4	18.0	5.1
Healthy control (non-infested soil)	2.23	2.23	2.23	242	103.6	20.4	6.0

Table 5: *Effect of some chemical inducers and Topsin-M on the contents of macro and micro elements of chickpea plants grown in soil artificially infested with Sclerotinia sclerotiorum after 60 days of planting.*

Field experiments

Effect of some chemical inducers and Topsin-M on the incidence of damping-off and stem rot of chickpea plants grown under natural infection

Results showed that the fungicide treatment effectively reduced damping off, increased plant survival rates, and lowered stem rot incidence at both locations (Table 6). For the other inducers treatments regarding pre-emergence incidence, Bion treatment consistently

decreased the disease's incidence across both locations and had the lowest pre-emergence damping-off rates, with 6.3% at Giza Research Station and 7.3% at Itai El-Baroud Research Station, respectively. This was followed by salicylic acid treatment (9.3% and 10.1%, respectively) compared with the control (35.5% and 35.3%, respectively). The reduction in post-emergence incidence was similar across both locations with the other treatments, salicylic acid treatment being the most effective. Additionally, the percentage of surviving plants was slightly higher at Giza Research Station than at Itai El-Baroud Research Station for most treatments. Meanwhile, Bion treatment showed the highest increase over the untreated control, with 69.1% and 68.9%, respectively, at the two locations. Overall, the stem rot percentages were generally lower at Itai El-Baroud Research Station compared to Giza Research Station for the same treatments.

Treatments	Damping- off				Survived plants %	Increasing over- infected control %	Stem rot %
	Pre-emergence		Post-emergence				
	Incidence %	Reduction %	Incidence %	Reduction %			
Bion 3 mM	6.3	82.2	2.5	66.2	88.8	69.1	2.4
Salicylic acid 3 mM	9.3	73.8	1.8	75.7	85.9	63.6	3.0
K_2HPO_4 3 mM	14.1	60.3	2.1	71.6	80.0	52.4	3.8
Ascorbic acid 3 mM	13.9	60.8	2.2	70.3	79.4	51.2	4.5
Topsin M-70	5.8	83.7	1.4	81.1	91.0	73.3	1.8
Untreated control	35.5	0.0	7.4	0.0	52.5	0.0	7.0
LSD ≤ 0.05	2.1		1.5		3.4		

Table 6A: *Giza agricultural research station.*

Treatments	Damping- off				Survived plants %	Increasing over -infected control %	Stem rot %
	Pre-emergence		Post-emergence				
	Incidence %	Reduction %	Incidence %	Reduction %			
Bion 3 mM	7.3	79.3	2.5	62.1	88.0	68.9	2.1
Salicylic acid 3 mM	10.1	71.4	1.8	72.7	86.2	65.5	2.9
K_2HPO_4 3 mM	14.1	60.0	2.1	68.2	80.7	54.9	3.1
Ascorbic acid 3 mM	14.9	57.8	2.6	60.1	78.4	50.5	4.1
Topsin M-70	5.8	83.6	0.9	86.4	91.9	76.4	1.4
Untreated control	35.3	0.0	6.6	0.0	52.1	0.0	6.0
LSD ≤ 0.05	2.0		1.2		3.2		

Table 6B: *Itai El-Baroud agricultural research station.*

Table 6: *Effect of chemical inducers and Topsin-M on the incidence of damping-off and stem rot of chickpea plants grown under natural infection during winter growing season 2023-2024.*

Effect of some chemical inducers and Topsin-M on some growth parameters of chickpea plants grown under natural infection

Treatments significantly enhanced all growth parameters and yield compared to the control, with slight variation between the two locations but eventually with the same trends (Table 7). In the Giza Research Station, among the tested treatments, Topsin-M and Bion

resulted in the most pronounced improvements in seed yield (1.846 tons/fed and 1.828 tons/fed, respectively). These treatments also exhibited higher values in plant height, branch and capsule numbers, and seed weight per plant. This was followed by salicylic acid with a seed yield of 1.668 tons/fed. In contrast, dipotassium phosphate and ascorbic acid recorded comparatively lower values across most parameters but significantly better than the control. In the Itai El-Baroud Research Station, Topsin-M and Bion treatments produced the highest seed yields (1.888 and 1.844 tons/fed, respectively), followed by salicylic acid (1.704 tons/fed). These treatments also recorded the highest growth parameter values. Across both locations, the untreated control treatment exhibited the lowest performance across all parameters, with notably reduced seed yield (0.503 and 0.548 tons/fed, respectively).

Treatments	Plant height (cm)	Number of branches/ plants	Number of capsules/ plant	Seed weight / Plant (g)	100-seed weight (g)	Seed yield (Tons/fed)
Bion 3 mM	70.5	3.3	41.3	28.7	33.2	1.828
Salicylic acid 3 mM	70.3	3.0	37.5	26.9	31.2	1.668
K_2HPO_4 3 mM	61.5	2.5	28.5	20.2	25.7	1.177
Ascorbic acid 3 mM	62.3	2.5	31.3	23.6	28.2	1.382
Topsin M-70	72.0	2.8	40.3	27.9	32.1	1.846
Untreated control	54.0	1.5	16.3	9.3	21.2	0.503
LSD ≤ 0.05	2.6	0.7	2.2	1.2	1.6	0.1

Table 7A: *Giza agricultural research station.*

Treatments	Plant height (cm)	Number of branches/ plant	Number of capsules/ plant	Seed weight/ Plant (g)	100-seed weight (g)	Seed yield (Tons/fed)
Bion 3 mM	72.5	3.0	45.3	29.7	34.9	1.844
Salicylic acid 3 mM	69.8	2.8	39.5	27.2	31.7	1.704
K_2HPO_4 3 mM	61.8	2.8	28.0	20.2	25.7	1.203
Ascorbic acid 3 mM	61.5	2.3	31.8	24.1	27.7	1.410
Topsin M-70	74.8	3.0	42.3	28.7	33.4	1.888
Untreated control	53.8	1.3	18.5	9.2	20.3	0.548
LSD ≤ 0.05	2.4	0.6	2.5	1.7	1.5	0.1

Table 7B: *Itai El-Baroud agricultural research station.*

Table 7: *Effect of chemical inducers and Topsin-M on some crop parameters of chickpea plants under natural infection during winter growing season 2023-2024.*

Discussion and Conclusion

Managing *S. sclerotiorum* remains challenging, and the concerns over the excessive use of fungicides have prompted interest in PDEs as eco-friendly alternatives that activate plant defense mechanisms without directly targeting the pathogen [40]. This study focused on using resistance inducers to enhance plant defense responses against *S. sclerotiorum.*

The results demonstrated that seed priming with different chemical inducers effectively mitigated damping-off, increased the survival of plants, and reduced the incidence of *Sclerotinia* stem rot in chickpea under greenhouse and field conditions. Among the tested inducers,

Bion (BTH) and salicylic acid (SA) were the most effective. The phenolic plant hormone SA plays a central role in regulating plant defense mechanisms [18,41]. Its synthetic analogue, BTH, is widely recognized for its ability to trigger systemic resistance against a broad spectrum of pathogens and is commercially available as Bion [16,42]. Both BTH and SA act as resistance inducers by activating the plant's immune system, stimulating defense-related gene expression without having direct antimicrobial effects [19].

The results also indicated that Bion treatment was more effective than salicylic acid (SA) in reducing damping-off incidence. Although SA is a well-established inducer of plant resistance, its rapid glycosylation within plant tissues often reduces its stability and limits its effectiveness. Additionally, the potential phytotoxicity of SA at higher concentrations has restricted its practical use in crop protection [7,43]. Additionally, higher concentrations lead to reduced growth due to the well-documented 'growth-defense trade-off' phenomenon, as plants divert metabolic resources to activating defense responses, which can partially suppress growth and developmental processes [44]. To overcome these limitations, several functional analogues of SA have been developed. Among them, benzothiadiazole (BTH) has demonstrated greater stability and broad-spectrum effectiveness against a wide range of plant pathogens, even at high concentrations [19,45].

In the present study, treatments with BTH and SA significantly enhanced the activities of peroxidase (PO) and polyphenol oxidase (PPO), as well as stimulated the biosynthesis of total phenolic compounds. Therefore, both BTH and SA function as downstream activators within the salicylic acid signaling pathway, directly inducing defense responses by upregulating the expression of pathogenesis-related (PR) proteins and other defense-related genes without affecting endogenous SA accumulation [19]. BTH and SA have been widely reported to increase the activity of peroxidase and polyphenol oxidase and enhance the accumulation of phenolic compounds [46,47]. Additionally, BTH stimulates the production of phytoalexins [48] and contributes to reduced fungal colonization by inducing structural defense barriers, such as cell wall appositions [49]. However, PO contributes to resistance by generating reactive oxygen species (ROS) that possess direct antifungal properties and synthesizing phytoalexins [50]. Similarly, PPO plays a defensive role by oxidizing ortho-diphenols into quinones with antimicrobial activity and promoting lignification of cell walls during pathogen attack [51]. Phenolic compounds contribute to reinforcing plant cell walls through lignin deposition, forming structural barriers that limit pathogen penetration, inhibit the activity of fungal extracellular enzymes, and reduce infection [52].

Our results confirmed that Bion and SA improved the growth of chickpea plants under greenhouse and field conditions. Consistent with earlier results, both Bion and SA have shown the ability to induce resistance and promote plant development when used at low concentrations [45]. Seed priming with SA increases pod number, enhancing nodulation and nitrogen fixation in chickpea plants [53], and improving photosynthetic efficiency and growth in mung bean plants [54]. These growth-promoting effects are likely linked to interaction with endogenous growth-regulating hormones such as auxins [55].

Likewise, the different chemical inducers have a positive effect on the uptake of macro- and micronutrients. Although chemical inducers do not directly supply nutrient uptake, evidence suggests that they may indirectly influence mineral content by modulating physiological and metabolic processes of the plants [56]. Balanced nutrition is a key factor affecting a plant's resistance or susceptibility to pathogens [57]. Ghazanfar., *et al.* [56] found that applying Bion and salicylic acid significantly enhanced the concentrations of essential micronutrients in chickpea plants. In a related study, Ghazanfar., *et al.* [58] demonstrated that Bion application reduced disease severity by 79% in chickpea, which indirectly contributed to increased manganese accumulation, suggesting a potential relation between induced resistance and improved nutrient uptake. In this respect, nitrogen enhances defense enzyme activity and systemic resistance, while potassium promotes the production of polyphenolic compounds [59]. Manganese stimulates lignin formation and phenolic compound biosynthesis [60]. Additionally, zinc supports auxin synthesis and maintains membrane integrity [61], while copper is involved in photosynthesis, respiration, and antioxidant defense, which are important for plant health [62].

This study demonstrated that dipotassium phosphate and ascorbic acid reduced the incidence of damping-off and stem rot and

increased plant survival compared to the untreated control. In addition to disease suppression, these treatments contributed to improved growth parameters and increased activities of defense-related enzymes, including peroxidase and polyphenol oxidase, as well as elevated levels of total phenolic compounds, despite being relatively less effective than Bion and SA.

In this regard, potassium phosphates offer promising, eco-friendly alternatives due to their low cost, non-toxic nature, and compatibility with food systems, making them suitable candidates for sustainable plant disease management [20]. Phosphates induce systemic acquired resistance by calcium sequestration within the pathogen, disrupting membrane integrity and activating plant hydrolytic enzymes. These enzymes release pectic fragments that act as signaling molecules, initiating host defense responses [63]. However, Arslan [20] demonstrated that a 2% solution of dipotassium phosphates completely inhibited *S. sclerotiorum* mycelial growth under both laboratory and field conditions.

Likewise, certain vitamins have emerged as effective and safe inducers of disease resistance; ascorbic acid has gained attention for its multifunctional role in enhancing plant resistance to various pathogens [64]. Ascorbic acid treatment significantly reduced the incidence of root rot and wilt in safflowers and increased fresh and dry weight [65]. More recently, ascorbic acid has been associated with the synthesis of growth-regulating compounds such as gibberellins, stimulation of cell division, and improvements in photosynthesis and flowering [66]. In addition, ascorbic acid enhances antioxidant activity and improves water and nutrient uptake, ultimately promoting plant health and productivity [67].

Although dipotassium phosphate and ascorbic acid were less effective in disease suppression, their safety, affordability, and biostimulator effects make them promising candidates for inclusion in integrated pest management (IPM) strategies. Incorporating them could reduce reliance on fungicides and limit the development of fungicide-resistant pathogen strains in agricultural systems [20,64].

Eventually, the integration of systemic acquired resistance inducers into crop management programs not only enhances plant health and productivity but also aligns with the goals of sustainable and ecologically responsible agriculture.

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