

Plant defense activation and down-regulation root rot and wilt in chickpea diseases by some abiotic substances

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Abstract. *Rhizoctonia solani* in addition to *Fusarium oxysporum* f. sp. *ciceris* have been recorded the most closely related fungi to root rotted as well as wilted chickpea plants assembled from various locations in Ismailia Governorate, Egypt. In the pathogenicity tests, all isolates were capable of infecting chickpea plants cv. Giza 2 and caused signs of root rot in addition to wilt diseases to variable degrees. The isolate of *Rhizoctonia solani* number 4 with accession No. (OR074128) and *F. oxysporum* isolate number 3 with accession No. (OR074126) were the more virulent ones. In the present study, abiotic substances viz hydroquinone, salicylic acid, benzoic acid, and chitosan were evaluated compared with Topsin M-70® fungicide for controlling these diseases under *in vitro* in addition to *in vivo* conditions. The application of abiotic substances demonstrated fungicidal action was inhibited the mycelia outgrowth of the two examined fungi by varying degrees. Chitosan was the greatest efficacy accompanied by hydroquinone and salicylic acid. In the pots and field experiments, all treatments significantly decreased root rot in addition to wilt diseases incidence consequently raised the number of survival plants. The highest protection from the infection of both the tested pathogenic fungi occurred under chitosan treatment followed by hydroquinone and salicylic acid. showed the best increase over the control, hydroquinone came next followed by salicylic acid. In physiological aspects, the activities of defense-related enzymes, exactly (peroxidase “PO”, polyphenol oxidase “PPO”, total phenol content “TPC”) and photosynthetic pigments enhanced in all abiotic substances applications as contrasted with untreated inoculated plants in addition to untreated uninoculated plants.

1 Introduction

Chickpea (*Cicer arietinum* L.) is considering the most significant high-protein legume crops worldwide. Chickpeas are regarded as one of the main staple meals, especially for the underprivileged, because of their high nutritional content and reasonable pricing compared to other sources of animal proteins. However, it sensitivity to disease infections due to cultural and genetic practices, farmer delays in planting, so chickpea productivity is declining

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annually (Anonymous, 1989). Many crops grown in various soil types are susceptible to root rot in addition to wilt diseases resulting from various soil-borne fungi. Wherever a crop is grown, a variety of several complex pathogenic fungi can infect chickpea. Both of *Fusarium oxysporum* f.sp. *ciceri* as well as *Rhizoctonia solani* are the primary pathogens affecting chickpea (Stanislaw *et al.*, 2004 and Singh *et al.*, 2013). *Rhizoctonia solani* is a destructive pathogen that leads to substantial damages in various crops around the world. (Domsch *et al.*, 2007). *Rhizoctonia solani* isolates infect more than 500 host species as plant pathogens and saprophytes in the soil (Ogoshi, 1996). Due to the lack of totally resistant cultivars at this time, there are few methods for controlling *Rhizoctonia* diseases (Li *et al.*, 1995). The ecological behavior of *R. solani*, which has very widely different host and a high sclerotia survival rate under the optimum various environmental conditions, its control is very challenging. There is a need to develop improved safe alternatives to fungicides due to the adverse impacts on human health in addition to the environment when expresses to manage soil-borne fungi in the soil [1-27].

Another strategy with the potential to control soil-borne pathogens caused root rot diseases is the use of abiotic inducers to generate resistance. Antioxidants have been effectively used to increase plant resistance to fungal infections (Galal *et al.*, 2000; Shahda, 2001). Plants increase their potential for defense by assembling appropriate cellular defenses response before pathogen infection (Palmieri *et al.*, 2016). Under controlled conditions, salicylic acid use increases systemic resistance against chickpea wilt disease (Saikia *et al.*, 2003; Sarwar *et al.*, 2010). Moreover, Salicylic acid may also play a crucial component in the local in addition to systemic activation of host defense responses under the abiotic stress (Fawzy, 2013), it has been demonstrated that the accumulated salicylic acid is crucial for systemic acquired resistance in plants. Additionally, hydroquinone has been shown to improve lupine plant crop parameters, yield components, and physiological studied while reducing both root rot incidence as well as wilt diseases reasoned by *R. solani*, *F. solani*, in addition to *F. oxysporum* (Ali *et al.*, 2009; Zian *et al.*, 2019). Hydroquinone, a safe antioxidant chemical, has shown promise for the management of seed-borne fungi in ornamental plants (Yousef, 2001), peanuts (Elwakil, 2003), and onions and peanuts (Hanafi, 2003). *Fusarium* wilt disease is controlled in numerous plant species by chitosan treatment of seeds or soil (Badawy *et al.*, 2005). In this regard, several studies have revealed that antioxidants, viz, citric acid, benzoic acid and salicylic acid increase plant resistance against soybean root rot in addition to stalk rot as antimicrobial agents (Abd EL-Hai *et al.*, 2016). According to Elwagia or Algam (2014) in addition to Mishra *et al.* (2014), the application of chitosan increases the plant resistance against a few pathogens in many plants variety [28-49].

According to Sarhan *et al.* (2018) and Elsis (2019), enzymatic activities of host response viz peroxidase “PO” and polyphenol oxidase “PPO” are ISR elicitors that promote defense responses associated with the regulation of plant diseases. Several plant metabolites, including phenolics, flavonoids and tannins as well as lignin, are produced by these enzymes in the phenylpropanoid pathway (Govindappa *et al.*, 2010; Abd El-Rahman *et al.*, 2012). According to Ahlbrock and Scheel (1989), these metabolites help defend plants against pathogenic attacks [50-79].

The present study aimed to evaluate various abiotic chemicals' ability to trigger plant defensive responses against chickpea root-rot in addition to wilt diseases and to improve growth characteristics in both potted plants and outdoor plants. Additionally, we discuss how these treatments can increase the efficacy of polyphenol oxidase enzyme or peroxidase enzyme, as well as the total phenol content and photosynthetic pigments of plants, which can then express resistance to fungal pathogenic infection.

2 Materials and methods

Plant material:

Chickpea seeds cv. Giza-2 were acquired from the Legume Crops Res. Department, Field Crops Research Institute, ARC, Giza, Egypt.

Abiotic substances:

Hydroquinone, salicylic acid, benzoic acid and chitosan were supplied by El-Nasr Company Intermediate Chemicals, Egypt (NCIC), while chitosan, which had M.Wt of 150 kDa as well as a degree of deacetylation ranging from 75-85%, was acquired from Sigma-Aldrich Chemicals Company.

Pathogenic fungi

Isolation and purification of the pathogens:

Chickpea plants that exhibited common signs of root rot and wilt disease were gathered via multiple locations within Ismailia government. Infectious samples were thoroughly rinsed with tap water before being cut into tiny pieces then dried with disinfected filter paper. The infested dried parts had been then disinfected on the surface using NaClO two percent for two minutes. The disinfected pieces re-washed multiple times with sterilized distilled water, and dried with disinfected filter paper, situated on potato dextrose agar (PDA) medium with penicillin antibiotic (20 IU/mL) then incubated at twenty five $\pm$ one degree Celsius, Hyphal tips or single spore techniques were taken from the developed colonies then recognized by Barnett, Hunter (1972) and Both (1985) in accordance with their morphological in addition to microscopic properties and transferred to PDA slants and incubated for identification and further studies in a refrigerator at five degree Celsius for future researches of their molecular characteristics as described by Dhingra and Sinclair (1985).

Pathogenicity tests

To identify the most virulent isolate from each species for further studies, four isolates from *R. solani* and 4 isolates of *F. oxysporium* f.sp. *Ciceris* had been tested for pathogenicity on chickpea cv. Giza-2 grown in pots filled up sterilized soil artificially infested with individual isolates at the Ismailia Agricultural Research Station. The preparations of *R. solani* as well as *F. oxysporum* f. sp. *ciceris* inoculum isolates had done by cultivating every isolate individually on sorghum-sand medium for fifteen d at twenty five $\pm$ two degree Celsius. Disinfected pots (30 cm in diam.) filled up with disinfected soil then artificially infested with the examined isolates at a rate of 2% (W/W) before being thoroughly mixed. The pots were irrigated as needed for seven days. Disinfected chickpea seeds cv. Giza 2 had sown at the rate of five seeds pot⁻¹. Four replicates were used, with the same set serving as a control. The most virulent isolate from the tested isolates was picked for utilize *in vitro* in addition to *in vivo* further investigations. Disease prevalence was estimated as damping-off %, or root-rot % at 30 in addition to 90 days after planting, respectively, in pots under infested with *R. solani*. While, the pots under infested with *F. oxysporum*; the early and late wilt percentages were estimated at the same time points.

Molecular Characterization of both tested Pathogens:

Extraction of DNA:

DNA extraction was performed outlining the technique by Lee as well as Taylor (1990). Extraction was performed twice using phenol, chloroform, in addition to isoamyl alcohol mixture in the rate of (25:24:1), then DNA resulted was precipitated by adding one isopropanol volume. After extraction, the pellets of DNA washed, dissolved in ethyl alcohol and double-distilled water, and kept at negative twenty degree Celsius till use.

PCR Conditions and DNA Sequencing

ITS zone of rDNA was amplified utilizing the ITS 1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS 4 (5' TCC TCC GCT TAT TGA TATGC 3') primers, with the following thermocycler program: ninety five degree Celsius for 3 minutes, accompanied by 40 cycles of

ninty five degree Celsius for thirty s, 68 degree Celsius for 45 s, as well as seventy two degree Celsius for ninety s. The latter elongation stage carried out at seventy two degree Celsius for 8 min. PCR reactions were carried out at the Plant Pathol. Res. Instit., A R C, Giza, Egypt. The sequences of DNA were obtained by sequencing the amplified PCR products utilizing an ABI Prism 3130xl Genetic Analyzer with the same primers utilized for PCR amplification, as described by White *et al.* (1990). The sequencing was conducted at Macrogen Corp., Korea, and the resulting sequences of DNA were placed in the NCBI GenBank database.

Multiple Sequences Alignment:

For the multiple sequence alignment, CLUSTALW was used, and the function "build" of ETE3 v3.1.1, as carried out on GenomeNet (<https://www.genome.jp/tools/ete/>), was employed to perform the alignment and phylogenetic reconstructions, following Huerta-Cepas *et al.* (2016) method.

***In vitro* - studies:**

Effect of some abiotic substances on fungal growth:

Under laboratory conditions, the antifungal potential of hydroquinone, salicylic acid, benzoic acid, and chitosan was assessed and compared with that of Topsin-M 70® towards *R. solani* or *F. oxysporum* f.sp. *ciceris* pathogens after its molecular identified. Abiotic substances were mixed separately with sterilized PDA medium, and the resulting solutions were poured into sterilized Petri plates. Salicylic acid, benzoic acid, and hydroquinone were each added to 20 mL of PDA medium at a concentration of 10 mM per plate. Chitosan was dissolved by acetic acid 1%, distilled water solution (v/v) at pH 5.6, sterilized in an autoclave, and introduced to the disinfected PDA medium prior to solidification to achieve a concentration of 4 mg ml⁻¹ according to Benhamou *et al.*, (1998) method. A 5-mm-diameter plug from the outside of a 7-day-old colony of *F. oxysporum* f.sp. *ciceris* or a four-day-old colony of *R. solani* were used for inoculation of four plates of each treatment; then incubated at twenty five± two degree Celsius. The average fungal growth diameter was determined while the control attained the plate's edge.

***In vivo* - studies:**

Pots experiment:

The efficacy of hydroquinone, salicylic acid, benzoic acid, in addition to chitosan as seed soaking agents against chickpea root rot in addition to wilt diseases brought on by infection of *R. solani* or *F. oxysporum* f.sp. *ciceris* comparing with Topsin-M 70® fungicide was conducted at Ismailia Agricultural Research Station, ARC. Sterilized pots (30 cm diam.) stuffed with a 1:2 (v/v) mixture of disinfested sandy clay soil. The artificially infested soil separately with both fungi inoculum at 2% (w/w), while sterilized un-inoculated soil were used as a healthy control. Artificially infested soil with fungi inoculums were watered as needed for a week before sowing to facilitate hyphal growth and to enhancing fungal growth distribution in the soil. Chickpea seeds were drenched for 2 h in every one of the ensuing treatments: 1) 10 mM salicylic acid; 2) 10 mM hydroquinone; 3) 10 mM benzoic acid; 4) 4 g/L chitosan; 5) 1 g/L Topsin M-70®; 6) sterilized water as the control; and 7) sterilized water as the healthy control. Five seeds in every pot in addition to four pots had utilized as a replicate for every treatment, four replications were used.

Disease assessment:

The disease prevalence was estimated by observing the number of plants affected by emergence damping-off at 30 d and root rot at 90 days of planting. Similarly, the percentage of early and late wilt was determined by recording the number of affected plants after thirty in addition to ninety d of planting, respectively, as stated by the latter formulas:

$$\begin{aligned} \text{Damping-off \%} &= \frac{\text{Number of non-germinated seeds after fifteen days + dead seedlings number at 30 days}}{\text{planted seeds total number}} \times 100 \\ \text{Root rotted plants \%} &= \frac{\text{No. of root rotted plants after ninety days}}{\text{planted seeds total number}} \times 100 \\ \text{Early wilt \%} &= \frac{\text{No. of wilted plants after 30 days}}{\text{planted seed total number}} \times 100 \\ \text{Late wilt \%} &= \frac{\text{wilted plants number after 90 days}}{\text{Total no. of planted seed}} \times 100 \\ \text{Survived plants \%} &= \frac{\text{Existence plants number after 90 days}}{\text{Total no. of planted seeds}} \times 100 \end{aligned}$$

Effects of chickpea seed treatment with abiotic substances on enzymes activity, total phenol compound and photosynthetic pigments.

In the pot experiment with chickpea plants, an additional experiment was conducted to measure enzyme activity and total phenol content. Fresh leaves of chickpea plants were collected 15 d after planting, and the extracts were prepared. Fresh leaves extract was utilized to determine the efficacy of peroxidase “PO” or polyphenol oxidase “PPO”. Additionally, total phenolic content was measured in fresh chickpea leaves.

Peroxidase efficacy was assessed based to the methodology outlined by Chakraborty and Chatterjee (2007).

Polyphenoloxidase enzyme efficacy was assayed according to Sadasivam and Manickam (1996).

Total phenols (mg catecole / 100 g fresh weight) in chickpea plant fresh leaves was determine as described using Folin – ciocalteau reagent according to Zilesin or Ben-Zaken (1993)

Photosynthetic pigments content i.e. chlorophyll a, chlorophyll b, total chlorophyll, as well as carotenoids were assessed in the blades of chickpea plant leaves 60 day after planting. The second leaf from the plant tip, specifically the third leaflet, was used for the analysis following the methodology described by Arnon (1949).

Field experiments:

Field experiments were conducted in Ismailia and Etai El-Baroud Agri during the winter growing season of 2021–2022. Res. Stat. farms to assess the effectiveness of several abiotic substances (salicylic acid, benzoic acid, hydroquinone, and chitosan) against soil born fungi and boosting chickpea cultivation in addition to yield parameters, as compared to Topsin-M 70® fungicide. Randomized Complete Block Design with three replicates per treatment was utilized in these experiments. Every replicate covering an area of 6 m² (2 × 3m) containing four rows of 3 m in length, separated by fifty cm in width. Chickpea seeds of cv. Giza-2 were drenched for 2 h in the same treatments as those used in the pot experiment before sowing. Seeds were sown on hills spaced 25 cm apart, one seed per hill on both sides of the row; for

the control treatment, chickpea seeds were drenched in distilled water for 2 h. The normal cultural practices for cultivating chickpea were done as a suggested by Ministry of Agricultural or Land Reclamation, Egypt. Damping-off, root-rotted, wilted, in addition to surviving plants percentage were estimated as previously described. Growth parameters *i.e.* plant height, branches number/plant, or yield components (number of capsules /plant, 100 seeds weight and total seed yield plot⁻¹) were listed at the harvest stage.

Statistical analysis
Analysis of variance (ANOVA) was utilized for the statistical analyzed of all obtained data with the help of computer statistical software (COSTAT ver. 6.4). The compare among mean values was done as mentioned by Duncan’s multiple range test at P ≤ 5%.

3 Results

Isolation in addition to identification of the pathogens:

Information in Table (1) shows that, eight fungal isolates were isolated from samples of chickpea plants exhibiting signs of root rot or wilt from naturally infected samples collected in the Ismailia Governorate. The isolated fungi included four isolates for each of *R. solani* and *F. oxysporum*.

Pathogenicity tests:
The isolated as well as recognized fungi (8 isolates) had tested to determine their pathogenicity of chickpea cv. 2 in a pot experiment. Data in Table (1) mention that the eight examined isolates were capable of infecting chickpea plants and cause signs of emergence damping-off in addition to root rot, or wilt diseases. Among tested isolates, the isolate number four of *R. solani* was extremely virulent; leading to the highest percentage of damping-off (20%), plants infected with root rot (20%), and the lowest percentage of surviving plants (60%). In contrast, *F. oxysporum* isolate No. 3 was found to be the most aggressive among all the tested isolates, causing the highest percentages of early wilt (20%), late wilt (35%), and the lowest percentage of surviving plants (45%). Therefore, these highly pathogenic fungal isolates were selected after its molecular identified for further studies according to their pathogenic abilities.

Table (1) Pathogenicity test of various *R.solani* and *F. oxysporum* isolates isolated from root-rotted chickpea plants on Giza-2 cultivar

(A) *Rhizoctonia solani*:

Isolates	Damping –off %	Root-rot %	Survived Plants %
<i>R.solani</i> No.1	10.00 b	10.00 b	80.00 bc
<i>R.solani</i> No.2	20.00 a	10.00 b	70.00 cd
<i>R.solani</i> No.3	10.00b	5.00 b	85.00 b
<i>R.solani</i> No.4	20.00 a	20.00 a	60.00 d
Chick	0.00 c	0.00 b	100.00 a

(B)*Fusarium oxysporum* f.sp. *ciceris*

Isolates	Wilted plants %		Survived Plants %
	Early wilt	Late wilt	
<i>F. oxysporum</i> No.1	20.00 a	10.00 b	70.00 b
<i>F. oxysporum</i> No.2	20.00 a	25.00 a	55.00 c
<i>F. oxysporum</i> No.3	20.00 a	35.00 a	45.00 c
<i>F. oxysporum</i> No.4	5.00 b	25.00 a	70.00 b
Chick	0.00 c	0.00 b	100.00 a

Means within every column accompanied by diverse letter significantly differ as stated by Duncan’s multiple range test at p ≤ 5 %.

Molecular Properties of the Examined Pathogens:

The ensuing sequences were entered into GenBank database under accession no: OR074126 for *F. oxysporum* isolate No.3 and accession no: OR074128 for *R. solani* isolate No.4. The nucleotide sequences and the blast assessment indicate that the fungal isolate (*F. oxysporum* No.3) had a high similarity (100% of nucleotides sequence) with *F. oxysporum* in the Gene Bank under accession no: MK594680.1 (figure:1) .On the other hand, *R. solani* isolate No.4 had 100% identity of nucleotides sequence with the closely *R. solani*, (accession no: MH025376.1) in Gene Bank (figure :2).

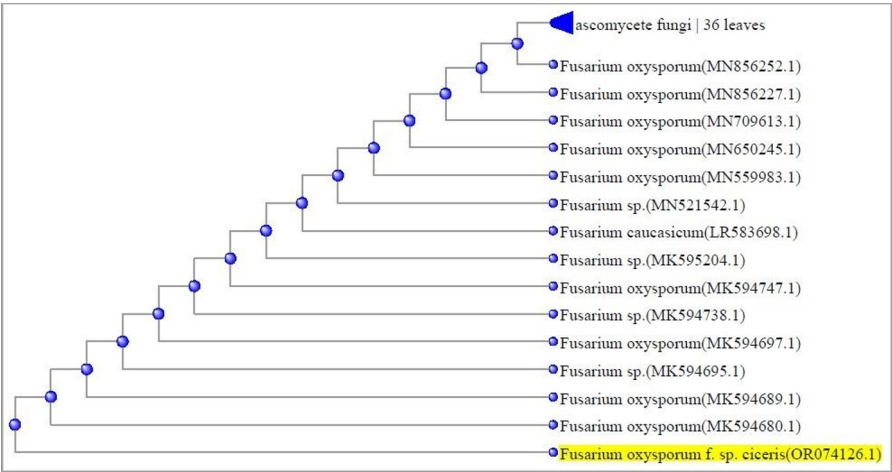


Fig. 1: Phylogenetic tree and Multiple Sequence Alignment of *Fusarium oxysporum* GenBank (ITS: OR074126), showing its identity with the most similar *F. oxysporum*, GenBank accession Nos. MK594680.1, MN856252.1 and MK594697.1

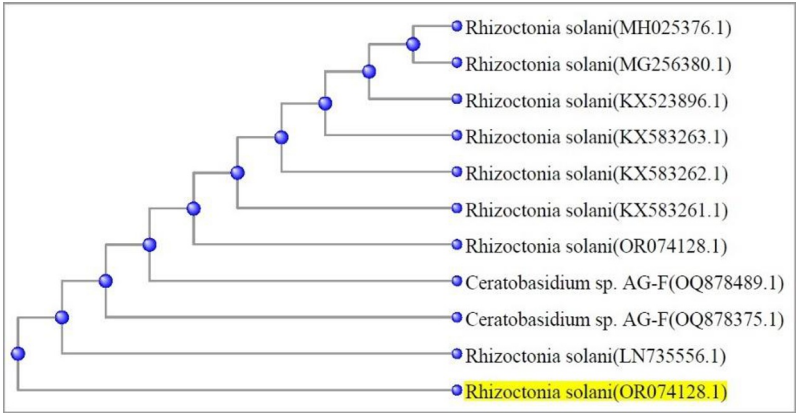


Fig. 2: Phylogenetic tree and Multiple Sequence Alignment of *Rhizoctonia solani* GenBank (ITS: OR074128), showing its identity with the most similar *R. solani*, GenBank accession Nos. MH025376.1, KX523896.1 and KX583261.1

Fungal growth as affected by abiotic substances:

The inhibitory effects of hydroquinone, salicylic acid, benzoic acid, and chitosan, with regard to Topsin-M 70® fungicide, toward the mycelia radial outgrowth of both examined pathogenic fungi were evaluated *In vitro* testes. As could be observed in Table (2), all tested abiotic substances led to a significantly lowering of the mycelia radial outgrowth of the two examined fungal pathogens, but to different degrees. Topsin-M 70% was found to be the

most effective treatment, with a 100% reduction in the mycelia radial growth for both fungi. Chitosan treatment was also effective, with an 86.40% and 92.07% reduction in the mycelia radial growth for *R. solani* No. 4 or *F. oxysporum* f.sp. *ciceris* No. 3, respectively. However, benzoic acid treatment recorded the lowest reduction percentages. The isolate of *F. oxysporum* f.sp. *ciceris* No. 3 was more sensitive to abiotic substances than *R. solani* No. 4.

Table 2. Mycelia radial growth of the most offensive pathogenic fungi isolates as a response of abiotic substances

Treatments	<i>R. solani</i> No. 4		<i>F. oxysporum</i> f.sp. <i>ciceris</i> No. 3	
	Mycelial growth (mm)	Reduction %	Mycelial growth (mm)	Reduction %
Chitosan	12.24 d	86.40	7.14 d	92.07
Salicylic acid	15.60 c	82.70	11.24 c	87.52
Benzoic acid	24.54 b	72.74	18.10 b	79.90
Hydroquinone	15.40 c	82.90	9.14 d	89.85
Topsin M-70®	00.00 e	100.0	00.00 e	100.0
Control	90.00 a	-	90.00 a	-

Means within every column accompanied by diverse letter substantially distinct as stated by Duncan’s multiple range test at $p \leq 5\%$.

Disease prevalence of root-rot or wilt as a response of abiotic substances of chickpea plants:

Tables (3, A and B) summarized the effects of various abiotic substances on the incidence of damping-off or root rot/wilt diseases, in addition to the existence plants of chickpea cultivated in artificially infested potted soil with both the most aggressive fungal isolates. It can be easily notice that all examined abiotic substances significantly lowered the disease impact of these diseases and raised the number of survival plants in regard to the control treatment, with varying degrees of effectiveness. The application of artificial infestation with *R. solani* No. 4, Topsin-M 70® was the most effective treatment, providing 90% protection, followed by chitosan and hydroquinone treatments, which provided 85% protection each. Similarly, the artificial infestation with *F. oxysporum* f.sp. *ciceris* No. 3, Topsin-M 70® was the most effective treatment, providing 95% protection, followed by chitosan treatment, which provided 90% protection. In contrast, seeds soaked in benzoic acid provided the lowest level of protection.

Table 3. the effects of various abiotic substances on the impact of root rot or wilt diseases in chickpea under artificially infected with the most aggressive fungal isolates.

(A) *R.solani* No. 4:

Treatments	Damping –off %		Root rotted plants %		Survived Plants %	Increasing %
	Incidence %	Reduction %	Incidence %	Reduction %		
Chitosan	10.0 c	75.0	5.0 b	75.0	85.0 bc	112.50
Salicylic acid	15.0 bc	62.5	5.0 b	75.0	80.0 bc	100.00
Benzoic acid	20.0 b	50.0	5.0 b	75.0	75.0 c	87.50
Hydroquinon e	10.0 c	75.0	5.0 b	75.0	85.0 bc	112.50
Topsin M-70®	10.0 c	75.0	0.0 b	00.0	90.0 ab	125.00
Infested control	40.0 a	-	20.0 a	-	40.0 d	-

Healthy control	0.0 d	-	0.0 b	-	100.0 a	-
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(B)*Fusarium oxysporum* f. sp. *ciceris* No. 3:

Treatments	Wilted plants %				Survived Plants %	Increasing %
	Early wilt		Late wilt			
	Incidence %	Reduction %	Incidence %	Reduction %		
Chitosan	5.0 cd	83.4	5.0 b	75.0	90.0 abc	80.0
Salicylic acid	10.0 bc	66.7	5.0 b	75.0	85.0 bc	70.0
Benzoic acid	15.0 b	50.0	15.0 a	25.0	70.0 d	40.0
Hydroquinone	15.0 b	50.0	5.0 b	75.0	80.0 cd	60.0
Topsin M-70®	5.0 cd	83.4	0.0 b	100.0	95.0 ab	90.0
Infested control	30.0 a	-	20.0 a	-	50.0 e	-
Healthy control	0.0 d	-	0.0 b	-	100.0 a	-

Means within every column accompanied by diverse letter substantially distinct as stated by Duncan’s multiple range test at p ≤ 5 %.

Oxidative enzymes activity in addition to phenol content as response of chickpea seeds treating with some abiotic substances:

A- Oxidative enzymes activity

The efficacy of some enzymes related plant defense was estimated in chickpea plants cultivated in soil infested with *R. solani* in addition to *F. oxysporum* f.sp. *ciceris* after treating the seeds with abiotic substances. Table (4) show that any of abiotic substances used stimulated both enzymes activity. Chitosan treatment listed the highest activity of the peroxidase enzyme, accompanied by hydroquinone or salicylic acid treatments, in regard to the control under infested soil with *R. solani*. The maximum activity of the polyphenol oxidase enzyme has observed also with chitosan, but accompanied by hydroquinone and salicylic acid treatments. In the case of artificial infestation with *F. oxysporum* f.sp. *ciceris*, chitosan treatment resulted in the highest activity of both peroxidase or polyphenol oxidase enzymes, followed by hydroquinone then salicylic acid treatments, compared with control.

B- Total phenol content:

As shown in Table (4) pre sowing treatments of chickpea seeds with various abiotic substances increased total phenol compounds of the resulting plants in regard to control. The highest average in total phenol content has shown under artificial infestation with *R. solani* in addition to *F. oxysporum* f. sp. *ciceris* by application with chitosan, followed by hydroquinone and salicylic acid. However, the lowest impact on total phenol content was observed when using of benzoic acid followed by Topsin M-70® fungicide comparing to the untreated infested control.

Table 4. Enzymatic activity and total phenol as a response of chickpea seeds treating with some abiotic substances

Treatments	Peroxidase activity (at wave length 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) (at wave length 725 nm)	
	Activity	Increasing %	Activity	Increasing %	Activity	Increasing %
<i>R. solani</i> No.4						
Chitosan	1.543	162.86	1.611	125.31	4.623	132.66
Salicylic acid	1.365	132.53	1.532	114.26	3.412	71.71
Benzoic acid	0.875	49.06	1.083	51.46	2.898	45.84
Hydroquinone	1.465	149.57	1.422	98.88	3.656	83.99

Topsin M-70®	1.166	98.63	1.198	67.55	2.825	42.17
Infested control	0.587		0.715		1.987	
<i>F. oxysporum</i> f. sp. <i>ciceris</i> No.3						
Chitosan	1.578	152.48	1.648	117.12	4.524	114.20
Salicylic acid	1.457	133.12	1.488	96.00	3.568	68.93
Benzoic acid	1.125	80.00	1.195	57.44	3.231	52.98
Hydroquinone	1.568	150.88	1.583	108.56	3.623	71.54
Topsin M-70®	1.183	89.28	1.213	59.81	2.953	39.82
Infested control	0.625		0.759		2.112	
Healthy control	0.496		0.533		1.867	

Effect of treating chickpea seeds with abiotic substances on the chlorophyll and carotenoids contents:

During photosynthesis, sugar is produced from carbon dioxide (CO₂) and water (H₂O) by photosynthetic pigments inside the chloroplasts and with the help of light. Sugar is stored in plant organs to produce chemical energy required for plant growth and productivity. Therefore, chlorophyll content in the green leaves of angiosperm flowering plants is a good parameter that reflects plant health. Hence, plants are able to resist the different stresses. Moreover, carotenoids are considered antioxidants that protect chlorophylls from light oxidation.

Chlorophyll a and b contents in chickpea plant leaves under *R. solani* infestation were higher than that of *F. oxysporum* f.sp. *ciceris* infestation, whereas the carotenoid content was inversely correlated (Table 5). In general, chitosan treatment maximized the chlorophyll a and b contents, followed by hydroquinone. The healthy control came next, followed by salicylic acid and benzoic acid, while Topsin M-70® came the last order after in relation to other treatments. Moreover, low chlorophyll content was observed in the infested control. In contrast, the highest values of carotenoids were obtained for hydroquinone, chitosan, and salicylic acid. Benzoic acid came next, followed by Topsin M-70® for both pathogenic infections.

Table 5. Impact of treating chickpea seeds with abiotic substances on the photosynthetic pigments

Treatments	Chlorophyll, A content (mg/gram fresh leaves weight)	Chlorophyll, B content (mg/gram fresh leaves weight)	Carotenoids content (mg/gram fresh leaves weight)
<i>Rhizoctonia solani</i>			
Chitosan	1.362	0.946	0.403
Salicylic acid	1.170	0.831	0.318
Benzoic acid	1.125	0.760	0.298
Hydroquinone	1.355	0.940	0.457
Topsin M-70®	0.988	0.562	0.247
Infested control	0.908	0.531	0.189
<i>Fusarium oxysporum</i> f.sp. <i>ciceris</i>			
Chitosan	1.214	0.921	0.408
Salicylic acid	1.163	0.818	0.351
Benzoic acid	1.104	0.741	0.311
Hydroquinone	1.204	0.912	0.472
Topsin M-70®	0.918	0.546	0.268
Infested control	0.862	0.516	0.218
Healthy control	1.178	0.842	0.402

Field experiments:
Impact of some abiotic substances on root rot in addition to wilt diseases

The purpose of these experiments was to assess the efficacy of various abiotic substances as seed treatments for reducing the impact of damping-off, root rot, or wilt diseases in chickpea crops during the winter growing season of 2021–2022. Field trials were conducted in Ismailia or Etai El-Baroud Agric. Res. Stations, and the outcomes are described in Table (6). Generally, the impact of damping-off or root rot/wilt significantly reduced under any tested treatment compared to the control. At Ismailia Agric. Res. Station, Topsin M-70® fungicide was the best treatment and came first, followed by chitosan, hydroquinone and salicylic acid. However, chickpea seeds treated with benzoic acid showed the least lowering in the prevalence of these diseases. The same trend was spotted for the Etai El-Baroud Agric. Res. Station.

Table 6. Root rot in addition to wilt diseases in chickpea plants as affected by seed treatment with abiotic substances under field conditions during 2021/2022 season.

Treatments	Damping- off %		Root rotted plants %		Wilted plants %		Survived plants %	Increasing %
	Incidence %	Reduction %	Incidence %	Reduction %	Incidence %	Reduction %		
Ismailia Agricultural Research Station								
Chitosan	6.3 bc	62.9	1.3 c	79.3	2.0 c	83.4	90.4 ab	39.7
Salicylic acid	8.0 b	52.5	3.6 b	42.8	3.3 c	72.5	85.0 bc	31.3
Benzoic acid	7.3 b	57.0	3.3 bc	47.6	8.0 b	33.4	81.4 c	25.8
Hydroquinone	6.0 bc	64.7	3.0 bc	52.3	3.3 c	72.5	87.7 ab	35.5
Topsin M-70®	4.0 c	76.4	2.6 bc	58.7	1.3 c	89.2	92.0 a	42.2
Control	17.0 a	-	6.3 a	-	12.0 a	-	64.7 d	-
Etai El-baroud Agricultural Research Station								
Chitosan	2.6 cd	74.0	3.0 c	70.0	3.0 bc	75.6	91.4 ab	35.0
Salicylic acid	4.3 bc	57.0	3.7 bc	63.0	4.6 bc	62.6	87.4 c	29.0
Benzoic acid	5.0 b	50.0	5.0 b	50.0	5.6 b	54.4	84.4 d	24.6
Hydroquinone	3.6 bcd	64.0	4.0 bc	60.0	3.4 bc	72.3	89.0 bc	31.4
Topsin M-70®	2.3 d	77.0	2.3 c	77.0	2.4 c	80.4	93.0 a	37.3
Control	10.0 a	-	10.0 a	-	12.3 a	-	67.7 e	-

Means accompanied by variable letters in every column are substantially distinct as stated by Duncan’s multiple range test at $p \leq 5 \%$.
Impact of seed treatment with abiotic substances on germination and crop parameters of chickpea plants in the field:

Table (7) cleared that any of abiotic substances used occurs a significant improve in chickpea cultivation then yield parameters in both locations. In terms of plant height, the greatest increase observed when using Topsin M-70® fungicide and/or chitosan treatments at Ismailia and Etai El-Baroud Agric. Res. Stations. Similarly, resulted in both locations cleared the same effects on the number of branches in regard to the control. At Ismailia Agric. Res. Station, chitosan treatment came first followed by the Topsin M-70® fungicide or hydroquinone treatments. At Etai El-Baroud Agric. Res. Station, Topsin M-70® fungicide, accompanied by chitosan treatment was more effective in this regard.
Yield parameters were also significantly in all treatments comparing to the control. Topsin M-70® fungicide treatment give the highest number of capsules /plants, plant seed weight, and weight of 100 seeds, followed by chitosan and hydroquinone treatments, respectively, at both locations. Furthermore, all treatments result to a substantially rise in seed yield compared with that of the untreated control. At Ismailia, Topsin M-70 % fungicide came the first order followed by chitosan and hydroquinone treatments, while at Etai El-Baroud, Topsin M-70 fungicide followed by both chitosan and hydroquinone treatments resulted in

the highest seed yield. However, benzoic acid recorded least effective among other treatments in both locations.

Table 7. growth and crop characters in chickpea plants as affected by seed treatment with abiotic substances under field conditions during 2021/2022 season

Treatments	Plant hight (cm)	Branches number plant ⁻¹	Capsules number plant ⁻¹	Seed weight /plant (g)	100 seed weight (g)	Seed yield /plot (Kg)
Ismailia Agricultural Research Station						
Chitosan	82.00 ab	6.10 a	28.20 a	12.16 b	23.21 a	0.944 b
Salicylic acid	69.54 c	5.06 b	26.94 b	11.82 b	22.10 ab	0.919 b
Benzoic acid	61.84 d	3.30 c	23.40 c	10.36 c	21.43 b	0.743 c
Hydroquinone	76.94 b	5.44 ab	26.76 b	11.83 b	22.24 ab	0.937 b
Topsin M-70®	83.50 a	5.87 ab	29.20 a	12.95 a	23.31 a	1.084 a
Control	52.84 e	3.10 c	19.54 d	7.23 d	20.65 b	0.367 d
Etaï El-baroud Agricultural Research Station						
Chitosan	86.50 a	6.54 a	29.20 b	12.58 b	23.65 a	1.018 b
Salicylic acid	74.84 c	5.40 b	26.64 c	11.79 b	22.50 b	0.896 c
Benzoic acid	63.47 d	4.54 c	26.54 c	10.76 c	21.81 bc	0.839 c
Hydroquinone	78.40 b	5.54 b	27.20 c	12.26 b	22.41 bc	1.018 b
Topsin M-70®	84.00 a	6.84 a	31.40 a	14.53 a	23.84 a	1.251 a
Control	58.40 e	3.77 c	19.97 d	8.84 d	21.25 c	0.496 d

Means accompanied by variable letters in every column are substantially distinct as stated by Duncan’s multiple range test at $p \leq 5 \%$.

4 Discussion

Chickpea, scientifically known as *Cicer arietinum* L., is a widely cultivated fabaceous crop and it is considered a vital constituent of human food in addition to animal feed worldwide (Anonymous, 2005). In addition, it also plays an essential role in improving soil fertility. However, chickpea plants are vulnerable to various soil-borne fungi, including *Fusarium oxysporum* f. sp. *ciceris*, *F. eumartii*, *F. solani*, *Rhizoctonia solani*, *Pythium ultimum*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, or *Verticillium albo-atrum*, which can cause damping-off, root-rot, stem rot, or wilt diseases (Nene as well as Reddy, 1987).

Isolation trials were done from chickpea roots collected from various locations in Ismailia Governorate. Eight fungal isolates were recognized as *Rhizoctonia solani* (4 isolates) and *Fusarium oxysporum* (4 isolates). Pathogenicity tests were conducted, and all the isolated fungal isolates were determined to be pathogenic capability with different degrees to chickpea cv. Giza-2. Among the fungal isolates, isolate number 4 of *R. solani* was discovered to be extremely virulent, resulting in the highest percentages of damping-off, root-rotted plants, and fewer surviving plants. While, *F. oxysporum* isolate No. 3 was the most virulent, causing a highly percentages of early as well as late wilt and fewer surviving plants. These finding were in accordance with Kaur et al., (2007); Khalil, (2007) and Abdel-Monaim, (2011).

According to the findings of *in vitro* experiments, the examined abiotic substances give diverse degrees of suppression to the fungal linear outgrowth of the examined fungi. Topsin-M 70® was discovered to be the most efficient of the substances examined, followed by chitosan, hydroquinone, and salicylic acid treatments, in that order. These results are agreed

with other studies that have shown that *F. oxysporum* f. sp. *ciceris* or *R. solani* linear outgrowth has inhibited by chitosan, hydroquinone, and salicylic acid (Wade and Lamondia, 1994; Sarwar *et al.*, 2005; Veladi *et al.*, 2013). Chitosan has been consistent several of antimicrobial actions towards several plant pathogens (Benhamou, 2004; Badawy *et al.*, 2005; El-Mohamedy *et al.*, 2013). Chitosan also can permeabilize the *Neurospora crassa* fungus' plasma membrane and it leads to inhibition of cells growth (Palma-Guerrero *et al.*, 2009). Chitosan's mode of action against fungi has been the subject of numerous hypotheses (El Ghaouth *et al.*, 1992; Ait Barka *et al.*, 2004). In fact, according to Hirano as well as Nagao (1989), chitosan antifungal properties are largely dependent on the polycationic form of the substance and polymer chain length. Chitosan can cause notable morphological modifications, structural changes, in addition to molecular disarrays of the fungal cells in addition to being effective at suppressing the cultivation of the pathogens (El Ghaouth *et al.* 1992, El Ghaouth *et al.* 1999, in addition to Ait Barka *et al.* 2004). Salicylic acid (SA), is a naturally occurring phenolic compound that contains monohydroxybenzoic acid with ortho or para positions of the OH- group (Cherif *et al.*, 2007; Huang *et al.*, 2009) and has suppression impact as an antimicrobial in addition to antifungal compound (Abad *et al.*, 2007; Ansari *et al.*, 2013), was found to be capable of significantly suppress *R. solani* mycelial outgrowth at two, four and eight mg/ml concentrations.

All of the abiotic substances under investigation dramatically decreased the diseases assessment of chickpea plants (damping-off, root rot, in addition to wilt) in both pots and field studies. The Topsin M-70® treatment showed the greatest level of protection, followed by each of (chitosan and hydroquinone) and salicylic acid treatments, in that order. Badawy *et al.*, (2005) proved that chitosan has been shown to possess the capacity to dominate *Fusarium* wilts in a set of plant species when used as a soil or seed treatment. In fact, the powerful elicitor chitosan is commonly used to manage plant disease. When used on plant tissues, it can bind a range of substances and initiate a speedy wound healing process. To occur the infections of healthy tissues; at first it is the causal organism prevented from spreading then infecting other tissues by agglutinating at infection sites and forming a physical barrier. Chitosan in addition to its derivatives are widely recognized for acting as potent inducers, promoting a range of plant responses both locally at the locations of infection in addition to systemically alerting hygienic areas of the plant. Also, it induces and activities of some proteins and metabolites which related to plant defense, like phytoalexins in addition to PR-proteins, as well as early signalling pathways (Liu *et al.*, 2007; Badawy and Rabea, 2011). The present study's outcomes are also correspond with those of prior studies which demonstrate chitosan has a dual function during host-pathogen interactions, acting as an antibacterial abiotic agent as well as activating several of plant defense mechanisms. The combined effects of these factors lead to reduced disease severity. Furthermore, HQ was the most successful at lowering the prevalence of disease occurred by *S. sclerotiorum* and *R. solani*. However, hydroquinone has been shown to function as an antioxidant by preventing other molecules from oxidizing and causing oxidative loss to lipids, proteins, nucleic acids, in addition to carbohydrates (Al-Askar *et al.*, 2013), these findings corroborate our researches. This is due to its capacity to give electrons to reactive oxygen species, which helps to stabilize them and prevent them from causing damage to other molecules in the cell (Anbudhasan *et al.*, 2014). Additionally, root treatment with salicylic acid resulted in systemic resistance in chickpea plants toward wilt disease as well as decreased the disease incidence by 23–40% (Saikia *et al.* 2003). In alongside that, the activation of phytoalexins in addition to Protection Related proteins i.e. chitinase and -1, 3-glucanase could be related to a decrease in chickpea prevalence of root rot and wilt diseases (Kuc, 2006).). From findings in this work it can be suggest that chitosan, hydroquinone, and salicylic acid treatments have the capability to generate systemic resistance towards *F. oxysporum* f. sp. *ciceris* as well as *R. solani* in chickpea under both pot and field trials.

In this investigation, photosynthetic pigment content in chickpea leaves was determined because it provides a clear indicator of the health of the plant, in addition to the role of carotenoids as antioxidants. The concentration of chlorophyll a and b in chickpea plant leaves under artificial infestation by *R. solani* was higher as compared with *F. oxysporum* f. sp. *ciceris* infestation. Chitosan treatment maximized chlorophyll a and b content followed by hydroquinone. While, the low chlorophyll content occurs under *F. oxysporum* infested control, comparing with *R. solani* infested control. While, the highest values of carotenoids occurred under hydroquinone treatment followed by chitosan, then salicylic acid.

The reduction in photosynthetic pigments by infection of both pathogenic fungi may be due to killing or breakdown of the lateral roots followed by decreasing the water absorption surface, which in turn causes chlorosis leaves, leading to disruption in physiological processes, including photosynthetic pigments formation (Abd El-Hai or El-Saidy, 2016 and Abd El-Hai and Ali, 2017). *Fusarium oxysporum* f. sp. *ciceris* was extremely aggressive due to occlusion of vascular bundles, especially xylem vessels, by fungal mycelium, while the damage of *R. solani* is clearly in the external tissues of epidermis and cortex.

Chitosan overcame the harmful effects of both pathogens on photosynthetic pigment content due to its antifungal activity (Ali et al., 2009), enhancing plant physiological processes (Farouk, 2005) and increasing phenolic content as well as specific phytoalexins synthesis (Ben-Shalom et al., 2003).

Obtained results demonstrated that, all the treatments lead up to raise in photosynthetic pigments are expected to increase yield and the seeds protein amount, because a relationship between photosynthesis process and protein amount (Smith et al., 1989). Where, sucrose translates from leaves to seeds and is used as a precursor for protein biosynthesis. In general, it can be said that any treatment can lead to raises photosynthetic pigments will lead to raise in carbohydrate content which includes components of the cell wall, including pectin, which serves as a mechanical obstacle towards overrun of fungal pathogens (Hamideh et al., 2013)

Seed soaking applications of abiotic substances (chitosan, hydroquinone and salicylic acid) in both Ismailia and Etai El-Baroud Research Station Farms during the winter cultivating season 2021–2022 led to a significant response that appeared to raise in chickpea germination in addition to yield parameters. These effects were visually observed and were comparable to the lowering of disease, which may be result to induction of systemic resistance in chickpea by these treatments. These findings are consistent with earlier reports by Saikia *et al.* (2003), Sarwar *et al.* (2005), Veladi *et al.* (2013), and Zian *et al.* (2019), which have shown that chitosan, hydroquinone and salicylic acid treatments can enhance vegetative growth and induce plants systemic resistance. Elwakil (2003) also discovered that hydroquinone increased peanuts growth and raised their yield up to 50%. The impact of abiotic elicitors on physiological plant processes like ion absorption, cell elongation, cell division, and enzyme activation may be responsible for these increases. In this case, low doses of salicylic acid have been shown to improve tomato cultivation in addition to yield components (Abdel-Monaim *et al.*, 2012).

According to the current research, peroxidase (PO) and polyphenol oxidase (PPO) enzymes can be activated by soaking seeds in the investigated abiotic substances. All of the treatments improved enzyme activity; chitosan had the largest effects on PO and PPO enzymes as well as total phenol content, followed by hydroquinone and salicylic acid treatments, respectively comparing with untreated control under both fungal infections. A rise in enzyme action has observed connected with a rise in resistance to infection by a number of diseases (Wang *et al.* 2000). In particular, peroxidase has been shown to participate in the secondary cell wall manufacturing process by acting polymerizing process of hydroxy or lignin by incorporating methoxycinnamic alcohols as well as establishing powerful cross-links among cellulose, hemicellulose, and other plant materials (Grisebach, 1981). This observation led to suggests that the increase in Peroxidase may be directly linked

to the greater capacity of tissue and it acts a lignification process, in turn, limit the pathogens penetration (Gross, 1979).

Meanwhile, the enzyme polyphenol oxidase (PPO), which is located in the thylakoid membranes of chloroplasts, is widely found in plant cells. It is essential to the ability of plants to resist microbial invasion. Although it has the greatest activity in the hydroxylation of mono-phenols to di-phenols, it is also capable of lignifying plant cells during microbial invasion and dehydrating o-di-phenols to generate o-quinones, which are antimicrobial compounds. Following their interaction with tryptophan, these o-quinones can create IAA. It is a plant hormone which activities a variety of physiological processes, including growth and development (Mayer and Harel, 1979).

Comparing the tested applications with untreated control, it was discovered that the treatments raised the total phenolic content. Phenolic chemicals have a main role in disease resistance by a rapid accumulation at the infection site (Nicholson and Hammerschmidt, 1992). According to their findings, phenols can be converted into quinones or semi-quinones, that are extremely hazardous as well as have a considerable antibacterial impact on the pathogen that has invaded (Farkas and Kiraly, 1962).

According to the findings of this study, pre sowing seed treatments of chickpea with abiotic substances lead to a significantly protection towards the infection by *R. solani* as well as *F. oxysporum* f.sp. *ciceris* and increase plant resistance, as well as elevated plant cultivation, yield parameters, and the cumulating of certain antimicrobial agents like total phenol compound. These treatments also promoted chlorophyll synthesis and elevated the action of oxidative enzymes involved plant defense concerning enzymes, like peroxidase in addition to polyphenol oxidase.

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