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Efficacy of Chemical Inducers of Resistance for Controlling Rhizoctonia Damping-Off and Root-Rot Diseases in Sugar Beet Crop

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ABSTRACT

Rhizoctonia solani was isolated from sugar beet plants showing damping-off and root-rot symptoms, collected from various fields located in several governorates of Egypt. All the obtained isolates were able to attack sugar beet plants causing damping-off and root-rot diseases. In the pathogenicity test, *R. solani* isolate No.3 isolated from Malawi was the most virulent. The efficacy of six different chemical inducers of resistance was evaluated *in vitro* and *in vivo* for reducing damping-off and root-rot diseases in sugar beet. All the tested resistance inducers significantly reduced damping-off and root-rot severity and increased survived plants under greenhouse conditions. The reduction in damping-off and root-rot severity was increased with increasing chemical inducer concentration, with 400 ppm was the most effective in reducing damping-off and root-rot severity. The most effective treatments were salicylic acid and coumarin at 400 ppm conc., being the most effective in reducing damping-off and root-rot incidence compared to the control treatment. Meanwhile, xanthan was the least effective treatment. Salicylic acid, coumarin and benzoic acid were the most effective treatments on the macerating enzymes produced by the pathogen *i.e.*, total pectinase, pectin lyase and cellulase and reducing *R. solani* growth and dry weight. In physiological studies, the activity of oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO), as well as phenolic content were higher in plants inoculated with *R. solani* and treated with chemical inducers than in untreated plants. Salicylic acid had the highest level of total phenols content and activity of the tested oxidative enzymes, followed by coumarin. Whereas benzoic acid showed the least enzyme activity. Under field conditions, during 2020/2021 and 2021/2022 growing seasons, all tested resistance inducers significantly reduced damping-off, root-rot and disease severity in addition to increasing the percentage of survived plants as well as improved sugar beet plant growth parameters (weight of sugar beet, total soluble solid (T.S.S), sucrose content and yield) compared to control. Meanwhile, xanthan recorded the lowest effects in both growing seasons. These findings suggested that these chemicals may play an important role in the control of sugar beet damping-off and root-rot diseases by inducing systemic resistance in sugar beet plants, in addition to their effect on reducing the pathogen growth and suppressing production of macerating enzymes *in vitro* and *in vivo*, consequently affect the compatibilities of the pathogen and its ability to colonize the host.

Keywords: Sugar beet, Rhizoctonia damping-off, root-rot, chemical inducers, macerating enzymes, oxidative enzymes, phenolic content.

1. Introduction

Sugar beet (*Beta vulgaris* L, Fam. Chenopodiaceae) is one of the world's most important sugar crops. The cultivated area of the sugar beet crop in Egypt was approximately 584.58 thousand feds, the average sugar production was approximately 18.7 tons per fed (Anonymous, 2019). Sugar beets are ranked second only to sugar cane in terms of resistance to plant diseases that reduce productivity (Sehsah, *et al.,* 2022).

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Sugar beet is an industrial crop grown in 48 countries with a total land area of over 9 million hectares and is the second largest source of sugar after sugar cane. Sugar beets account for approximately 30% of global annual sugar production (Jafarnia, *et al.,* 2013).

Sugar beet has become the first crop for sugar production, accounting for 59.5% of Egyptian sugar production in recent years (Anonymous, 2019). Sugar beet is the most important, accounting for nearly 40% of global sucrose production. However, due to infections by various plant pathogens, this production has gradually decreased over the last few decades (Mabrouki *et al.,* 2020).

In addition to reducing sugar extraction from juice and thus sugar percentage, the roots of infested plants are unable to store sugar in the amount and concentration present in healthy roots, resulting in a significant loss for farmers. Increases impurity concentrations while decreasing extractable and root sucrose yields, resulting in higher processing losses (Lamey *et al.,* 1996, Smith and Campbell, 1996 and Abdel-Monaim and Atwa, 2015).

Sugar beet in Egypt is attacked by several root-rot pathogens, the most serious of which are *Rhizoctonia solani* Kühn. Sugar beets are vulnerable to diseases that have a significant impact on sugar productivity (Windels *et al.,* 2005). *R. solani* is one of the most common fungal root pathogens in beet fields around the world. The pathogen, in conjunction with other causal organisms, is responsible for a significant decrease in root yield, as well as a decrease in sucrose content and juice purity in the affected roots (El-Kazzaz *et al.,* 1987, Abada, 1994, Aly *et al.,* 2010 and Elwakil, *et al.,* 2018).

Some of these methods, such as fungicide use, have negative effects on the environment and human health. A great deal of effort has gone into developing safe non-traditional management methods that are less hazardous to humans and animals (Wang, *et al.,* 2015 and Zhang, *et al.,* 2010). The use of antioxidants as chemical inducers for improving plant resistance against various diseases is highly recommended as fungicide alternatives due to their low risk to human health (Eliwa *et al.,* 2021). One of the potential management strategies for these diseases is the use of antimicrobial compounds to increase plant resistance. Chemical inducers like salicylic acid and ascorbic acid have all been shown to induce resistance in plants (Thabet, 2008).

antioxidants as plant pathogen resistance inducers (SAR) are associated with metabolic and structural changes within plants. According to Vallad and Goodman (2004), SAR may be induced by both biotic and abiotic elicitors, resulting in the accumulation of salicylates such as salicylic acid and the expression of pathogen-related genes according to Van Loon and Bakker (2005) and Elwakil, *et al.,* (2018), SAR, is a distinct signal transduction pathway that plays an important role in plants' ability to defend against plant pathogens. The recognition of a plant pathogen immediately initiates a cascade of molecular signals and gene transcription, resulting in the production of defense molecules by the plant.

The present study was conducted to throw light on the effectiveness of chemical inducers of resistance on *R. solani* and sugar beet plants and their role in reducing sugar beet root-rot and damping-off diseases and how they affected growth parameters, sugar content, and total soluble solids.

2. Materials and Methods

2.1. Plant material

Sugar beet seeds (*Beta vulgaris*) Kwamera cv. were obtained from Maize and Sugar Crops Diseases Research Dept., Plant Pathology Research Institute, ARC, Giza.

2.2. Isolation of fungi associated with sugar beet root-rot

Naturally diseased sugar beet roots showing typical symptoms of root-rot were collected from various locations at different governorates, Egypt. The diseased materials were thoroughly washed with tap water several times. Surface disinfection was performed by immersing samples in 0.1% sodium hypochlorite solution for 3 minutes. Small pieces of the disinfested roots were plotted in Petri dishes on potato-dextrose agar (PDA) medium. At 25^{o}C, the plates were incubated. The isolated fungi were purified using the hyphal tip technique and identified according to their cultural and morphological features at the Unit of Identification of Microorganisms, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt according to Singh (1982) and Barnett and Hunter (1987).

2.3. Pathogenicity test

In this study, apparently healthy sugar beet seeds (Kwamera cv.) were used. The experiment was carried out in clay pots (35 cm diameter) at the Plant Pathology Research Institute, ARC, Giza's Maize, and Sugar Crops Diseases Research Department.

Glass bottles with a capacity of 500 ml, each contains 200 gm sorghum grains medium were autoclaved for 30 minutes at 1.5 atm, then inoculated with a 3-day-old disc bearing growth of the pathogenic fungus and incubated at 25± 2°C for 15 days. Sterilized pots (35 cm diameter), each was filled with 5 kg of disinfested soil. The soil was infested with the pathogen at a ratio of 3% of potted soil in the study, and the pots were moistened with water for one week before sowing to stimulate the fungal growth and ensure its establishment in the soil (Abo-Elnaga ,2014).

The pots were then planted with disinfested sugar beet seeds, cv., Kawamera, (10 seeds /pot), watered and fertilized as usual. Each treatment consisted of six pots and other pots with uninfested soil were planted with seeds to serve as control.

2.4. Disease assessments

At 15 and 45 days after planting, seedlings and plant stand readings were taken. Disease assessment was performed by recording the percentages of pre-, post-emergence damping-off after 15 and 45 days, as well as survived plants after sowing, according to El-Shafey *et al.,* (1988) as follows:

$$\% \text{ Pre-emergence} = \frac{\text{Number of non germinated seeds}}{\text{Total number of sown seeds}} \times 100$$

$$\% \text{ Post-emergence} = \frac{\text{Number of dead seedling}}{\text{Total number of sown seeds}} \times 100$$

$$\% \text{ Root rotted plants} = \frac{\text{Number of root rotted plants}}{\text{Total number of sown seeds}} \times 100$$

$$\% \text{ Survived plants} = \frac{\text{Number of survivals}}{\text{Total number of planted seeds}} \times 100$$

2.5. Disease severity

The beets were rated in the field using a disease classes scale modified by Büttner *et al.,* (2004) from (0 to 9), with 0 indicating healthy beets and 9 indicating highly infected beets (Figure, 1). For each replicate a disease severity index (DS%) was calculated as follows:

$$DSI = \frac{\Sigma d}{d\,max \times n} \times 100$$

Whereas:
d is the disease rating possible,
d max is the maximum disease rating
n is the total number of plants examined in each replicate.

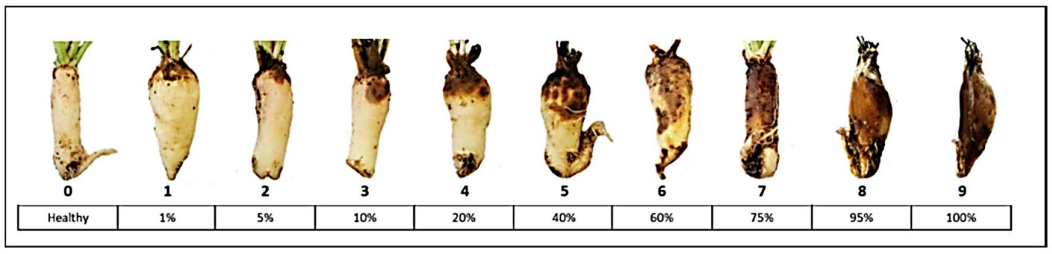

Fig. 1: Disease scale used to score Rhizoctonia root and crown rot in sugar beet based on the percentage of rotten root surface area.

2.5. Factors affecting fungal growth

The effect of six chemicals (Ascorbic acid, benzoic acid, salicylic acid, coumarin, xanthan, and L-Lysine) at different concentrations (100, 200, and 400 ppm) on *R. solani* growth reduction and dry weight in Czapek's solid and liquid media incubated at 25±2°C for 5-7 days, respectively was tested *in vitro*.

To achieve the desired concentration, the tested chemicals (Ascorbic acid, benzoic acid, salicylic acid, and xanthan) were dissolved in warmful water but coumarin and the amino acid L-lysine were dissolved in ethyl alcohol and added to Czapek's solid and liquid media (Abdou, 1998). As a control, media without the tested chemicals were used. After sterilization, media in plates or flasks were inoculated separately with discs of 5-day-old cultures of *R. solani* (Galal and Abdou, 1996). As previously stated, growth of *R. solani* in petri plates was recorded after 5 days of incubation at 25±2°C but dry weight of the fungus was recorded after 7 days of incubation the flasks at 25±2°C. The two-growth parameters of *R. solani* i.e., growth reduction and dry weight (gm) were estimated.

2.5.1. Linear growth

Disks (5 mm in diameter) were cut from a fungal culture's actively growing edge and plotted in the center of Petri dishes containing Czapek's agar medium. After a 5-day incubation period, the mean of two perpendicular diameters was calculated for *R. solani*. Three dishes were used as replicates (Tzeng and DeVay, 1989). The plates were examined, and mycelial growth in each treatment was measured to determine the activity of the tested chemical as a reduction in linear growth. Following the formula proposed by Yeh and Sinclair (1980):

$$G = C\text{-}T/C \times 100$$

Where:
G = Denotes the percentage reduction in fungal growth.
C = Fungal growth in the control (Pathogen alone)
T = Fungal growth in the treatment (Pathogen with the tested chemical).

2.5.2. Dry weight

A disc (5 mm in diameter) was cut from the actively edge of a 5-day-old culture and placed in 100 ml flasks with 50 ml of autoclaved Czapek's liquid medium. After 7 days of incubation at 25ºC, the culture medium was filtered through filter paper (Whatman No 1), and the fungal mass was dried for 12 hours at 85º C before being weighed. In this study, three replicates were used.

2.6. Scanning Electron Microscopy (SEM) studies

The effect of salicylic acid and coumarin on the morphology of *R. solani* hyphae was examined using a (JSM-200 IT) Scanning Electron Microscope (SEM) at Alexandria University's Faculty of Science.

After fixation in a modified (Karnovsky, 1965) solution (2.5% buffered glutaraldehyde + 2% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4), the samples were washed three times in 0.1 M sodium phosphate and 0.1M sucrose. For 90 minutes, the tissues were post-fixed in 2% sodium phosphate with osmium tetroxide pH 7.4. The samples were washed three times with sodium phosphate buffer PH 7.4 (0.1M). Dehydration of samples was accomplished using serial dilutions of ethanol (El-Kazzaz, *et al.,* 2022). The samples were dried to a critical point, and the specimens were coated with gold-palladium membranes before being examined in a JSM-200 IT) scanning electron microscope at Alexandria University's Faculty of Science.

2.7. Evaluation of chemical inducers on sugar beet under greenhouse conditions

The experiment was carried out in clay pots at the Plant Pathology Research Institute, ARC, Giza's Maize, and Sugar Crops Diseases Research Department.

Chemical inducers, ascorbic acid, benzoic acid, salicylic acid, xanthan, coumarin and L-Lysine at (100, 200 and 400 ppm) were evaluated under greenhouse conditions against sugar beet root-rot caused by *R. solani* compared with maxim 3.5% at the rate of 2 ml/kg seeds as a fungicide control. Sugar beet seeds, cv. Kwamera, were soaked for 6 hours in the solution of the desired chemical inducer. Treated seeds were sown in infested pots (30 cm diameter, 10 seeds per pot).

This experiment was carried out on September 15th, 2020. As replicates, six pots were used. Disease incidence of pre- and post-emergence damping-off and root-rot diseases at 15, 45, and 150 days after planting, respectively as well as survived plants were determined as mentioned before.

2.8. Physiological studies on Rhizoctonia sugar beet interaction

Macerating enzymes, i.e., total pectinase, pectin lyase, and cellulase were investigated. In each 250 ml Erlenmeyer flask, 50 ml of Czapek's medium with 0.33% sucrose, 0.33% carboxy methyl cellulose (CMC), and 0.33% pectin as carbon source were distributed and autoclaved at 121°C for 10 minutes (Shihata *et al.,* 1995). Flasks were inoculated with a 5-mm diameter disc removed from the edge of actively grown cultures of the tested fungus (5 days old) after cooling and incubated at 25°C for 7 days.

Culture filtrates were obtained by filtration the media through filter papers (Whatman No. 44) followed by centrifugation at 6000 rpm for 10 min at 4ºC. The supernatants (crude enzymes) were used immediately or stored at -20ºC till using.

2.9. Enzyme assay

The collective pectolytic activity was measured viscometrical using the 3 mL crude enzyme and 3 mL 1% citrus pectin dissolved at room temperature in 0.1M phosphate buffer pH 7.0 were used in the reaction. (Mahadevan and Sridhar, 1982). As a control, heat-inactivated culture filtrate was used. For 90 minutes, the reaction mixture was incubated at a constant water bath temperature of 30ºC. The reduction in viscosity of the substrate was calculated using the Fenske-Ostwad viscosimeter and the following formula:

$$V = \frac{To-T}{To-TH_2O}$$

Where:
V= percent of loss in viscosity.
To= Flow time in seconds at zero second.
T= Flow time in seconds after incubation.
TH_2O = Flow time of distilled water.

Cellulase activity was determined using a viscometrical method. The reaction mixture included 3 ml of 1% CMC in 0.1 acetate buffer pH 5.2 plus 3 ml of crude enzyme preparations and was incubated at 30ºC for 1 hour. As previously stated, the reduction in viscosity of the substrate was calculated.

The unit of enzyme activity was defined as a square of decreasing percentage in viscosity per min. (McCarroll and Thor, 1998).

Pectin lyase was measured spectrophotometrically at 30ºC using the procedure developed by Fogarty and Ward (1974), modified by Saleh (1987). 1 ml of 0.1% pectin dissolved in 0.05 M Tris-hydrochloric acid buffer pH 8.0- and 1-mM calcium chloride was added to the reaction mixture, along with 0.5 ml culture filtrate. After 5 minutes, the absorbance at 235 nm was measured in comparison to the control. Enzyme activity was measured in enzyme units (Δ/min).

2.10. Activity of macerating enzymes *in vitro*

Czapek's liquid media were prepared in 250 ml flasks with 400 ppm salicylic acid or Benzoic acid or Coumarin, 100 ml media in each, and sterilized. Each flask was inoculated with a 0.5 cm disc of the tested fungus's and incubated at 25°C. The culture filtrate was obtained after 10 days and used to determine total pectinase, cellulase and pectin lyase enzyme activity.

2.11. Activity of macerating enzymes *in vivo*

Beta seeds (cv. Kawamera) were soaked for 6 h in salicylic acid, benzoic acid and coumarin at 400 ppm, and evaluated under greenhouse conditions against sugar beet root-rot caused by *R. solani*. The developed roots were taken after 150 days, homogenized in cold phosphate buffer 0.1 M, pH 7.0 (1 g: 5 ml) using pre-cold mortar and pestle. The homogenates were centrifuged at 5000 rpm for 10 min to obtain the supernatant (crude enzyme) which was used as crude enzyme extract after dialyzing against phosphate buffer for 24 h at 4ºC. Activity of macerating enzymes i.e., pectinase, cellulase and pectin lyase were determined as mentioned before.

2.12. Detection of oxidative enzymes and phenolic compounds

The effect of sugar beet treatment with the most efficient chemical inducers on the activity of oxidative enzymes (peroxidase and polyphenoloxidase) and phenolic compounds, related to plant defense against infection with soil-borne pathogen of damping-off and root-rot diseases was determined in the leaves of Sugar beet plants (cv. Kwamera) after application of the tested inducers.

2.12.1. Enzymes extraction and bioassay

Each treatment's Sugar beet leaves tissues (1g) were homogenized separately in a mortar with 0.1 M sodium phosphate buffer at pH 7.1 at a rate of 2 ml/g fresh weight leaves for 1 minute. The triturated tissues were then strained through four layers of cheesecloth, with the filtrates centrifuged at 3000 rpm for 15 minutes at 6°C.

2.12.2. Polyphenoloxidase activity

Polyphenoloxidase activity was determined according to Esterbaner *et al.,* (1977). The reaction mixture contained 1.0 mL of enzyme extract, 1.0 mL of 0.2 M sodium phosphate buffer at pH 7.0, and 1.0 mL of catechol. With distilled water, the reaction mixture was reduced to a final volume of 6.0 ml. The activity of polyphenoloxidase was measured as a change in absorbance/min at 495 nm.

2.12.3. Peroxidase assay/activity

Peroxidase activity was measured at 425 nm by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 according to Allam and Hollis (1972). The reaction mixture contained 0.5 mL of 0.1 M sodium phosphate buffer at pH 7.0, 0.5 mL of enzyme extract, 0.3 mL of pyrogallol, and 0.1 mL of 1.0% H2O2 distilled to a final volume of 3.0 mL.

2.12.4. Determination of phenolic compounds

Phenolic compounds were determined using the colorimetric method according to Snell and Snell (1953).

The free phenols were determined by adding one ml of the phenol reagent and three ml of a 20% sodium carbonate solution to a 0.5 ml sample of isopropanol, which was then diluted to ten ml with warm distilled water (30-35°C). After 20 minutes, the mixture was read at 520 nm against a reagent blank with a spectrophotometer.

To determine total (free and conjugated) phenols, ten drops (approximately 0.25 ml) of concentrated HCl were added to the isopropanol samples (0.5 ml) in a test tube, rapidly heated to boiling over free flame with provision for condensation and placed in a boiling water bath for 10 minutes. 1 ml of the folin-ciocalteu reagent and 3 ml of 20% sodium carbonate were added after cooling. The mixture was diluted to 10 mL with warm distilled water, and after 20 minutes, readings at 520 nm were taken against a reagent blank using a spectrophotometer. Subtracting free phenols from total phenols yielded conjugated phenols. Based on the catechol standard curve, all phenolic compound determinations were expressed as mg catechol/g fresh weight of plant sample.

2.13. Field experiments

Field Trails were conducted at Giza Agricultural Research Station, Agricultural Research Center, Egypt, during the winters of 2020-2021 and 2021-2022 to study the effect of different chemical inducers of resistance. In a completely randomized design, the experiments were set up in both growing seasons. There were eight treatments with three replications. In each treatment, fifteen seeds were planted (5 seeds x 3 replicates). Each replicate consisted of 5 seeds spaced 70 cm apart in a 3.5 m-long row (Shawky, *et al.,* 2020). Beta seeds (cv. Kawamera) were soaked in the desired treatment solution for 6 hours before planting. The treatments were as follows: (Ascorbic acid, benzoic acid, salicylic acid, **x**anthan, coumarin and L-Lysine, maxim as a fungicide and control) to study the effect of different chemicals on growth, yield, chemical composition, and quality control of sugar beet. The number of alive seedlings was listed after 15 days (to calculate the pre-emergence damping off %), and after 45 days (to calculate the post-emergence damping off, and the survival plants %) (Dahiphale, 2006).

2.14. Plant growth characters

At harvest time, roots were harvested, and ten randomly selected roots were taken for determination of yield /plot Kg. According to Elwakil *et al.,* (2017). The following sugar beet plant characteristics were measured after 180 days: root weight (Kg)/plant and yield/plot.

2.15. Determination of total soluble solids (TSS)

In fresh roots T.S.S. was determined using a hand refractometer according to McGinnis, (1982) and Hozayen, (2002).

2.16. Determination of sucrose content

According to Anonymous (1990), sucrose content was determined using a saccharometer.

2.17. Statistical analysis:

Data obtained were statistically analyzed by the analysis of variance (ANOVA) using Computer Statistical Package (Assistat V.7.6 beta) originated by Silva and Azevedo (2009). Mean comparisons were made using the least significant differences (LSD) at 0.05 % level of significance (Snedecor and Cochran, 1989).

3. Results and Discussion

3.1. Isolation and Identification of the Causal Pathogens

The isolated fungi from sugar beet roots and plants showing damping-off and root-rot symptoms were purified and identified as *Rhizoctonia solani* Kühn based on their cultural and morphological characteristics.

3.2. Pathogenicity test

3.2.1. Pathogenicity test of *R. solani* isolates on Sugar beet plants under greenhouse conditions

Results in Table (1) show that the six tested *R. solani* isolates were pathogenic and varied significantly in their virulence on Sugar beet plants (Coomera cv.) under greenhouse conditions. Malawy-R_3 isolate was the most aggressive, with the highest percentages of pre- and post-emergence damping-off and the lowest survived plants (55.0, 25.0 and 20.0%), respectively. Meanwhile, Giza-R_6 was the least virulent isolate, being 28.33, 6.67, and 65.0% at pre- and post-emergence damping-off and survived plant, respectively.

These findings are consistent with those reported by El-Kazzaz *et al.,* (2000), El-Kholi (2000), Hussein (2005) and El-Wakil *et al.,* (2018) who reported that the most important diseases affecting fodder and sugar beet production in Egypt are damping-off and root-rot caused by a variety of pathogens including *R. solani, Macrophomina phasolina*, *Scleorotium rolfsii*, and *Fusarium* sp. All the isolates were capable of attacking sugar beet plants and causing damping-off and root rot/wilt diseases. In the pathogenicity tests, *R. solani* isolate FB1, *F. solani* isolate FB7, and *F. oxysporum* isolate FB11 were the most virulent (Mahmoud *et al.,* 2007; Abdel-Monaim and Atwa, 2015).

Table 1: Pathogenicity test of six *Rhizoctonia solani* isolates on Sugar beet (Coomera cv.) under greenhouse conditions.

Isolate No.	Source		Damping- off (%)		Survived Plants (%)
	Governorate	Locations	Pre*	Post**	
R1	Beni-sweef	Beni-sweef	35.00	23.33	41.67
R2	Beni-Sweef	Biba	45.00	21.67	33.33
R3	Menia	Malawi	55.00	25.00	20.00
R4	Qaloubiya	Toukh	40.00	8.33	51.67
R5	Menuofia	El-Bagour	48.33	13.33	38.33
R6	Giza	Giza	28.33	6.67	65.00
Control (Uninfected)* **	-	-	0.00	0.00	100.00
L.S.D. at 5%			9.3	8.1	11.1

* 15 days after sowing. ** 45 days after sowing.
*** plants grown in uninfested soil.

3.3. Laboratory studies

3.3.1. Effect of different chemical inducers on *R. solani* growth reduction *in vitro*.

Data in Table (2) reveal that all tested chemical inducers at different concentrations significantly increased the growth reduction of *R. solani* compared with control. This reduction was gradually increased by increasing the concentration of the tested chemical inducers from 100, 200 and 400 ppm, being (29.21, 33.96 and 55.31%) growth reduction, respectively. Salicylic acid and coumarin were the most effective treatments in reducing *R. solani* growth reduction, being (76.42 and 71.85%), respectively. Meanwhile Xanthan recorded the lowest percentage of growth reduction (9.01%).

Such finding agrees with those reported by Abdelaziz (2017) and Kasem (2018) who mentioned that salicylic acid, catechol, citric acid, and ascorbic acid at different concentrations were the most effective antioxidants in inhibiting the linear growth of *F. solani*, *R. solani* and *M. phasolina*. Also, Abdel-Monaim and Atwa (2015) recorded that at different concentrations, all the tested potassium salts significantly suppressed the growth of pathogenic fungi *in vitro*. Morsi and El-Bana (2000) also reported the role of antioxidant toxicity toward several pathogens.

Table 2: Effect of various chemical inducers at different concentrations on *R. solani* growth reduction on Czapek's solid media incubated at 25±2°C for 5 days *in vitro*.

Treatments	Reduction (%) in the linear growth of *R.solani* after 5 days of incubation			Mean
	Concentrations ppm			
	100	200	400	
Ascorbic Acid	9.26	15.92	37.78	20.99
Benzoic Acid	42.59	59.25	80.37	60.74
Salicylic Acid	67.78	79.26	82.22	76.42
L-lycine	12.968	9.25	34.82	19.01
Coumarin	60.74	72.22	82.59	71.85
Xanthan	11.11	1.85	14.07	9.01
Control	-	-	-	0.0
Mean	29.21	33.96	55.31	-
L.S.D. at 5%	Treatments-T = 2.49	Concentration-C = 1.28	TXC = 3.84	

3.3.2. Effect of the tested chemicals on *R. solani* dry weight in Czapek's liquid medium *in vitro*.

Data in Table (3) indicate that all tested chemicals at different concentrations (100, 200, and 400 ppm) significantly decreased *R. solani* dry weight gradually by increasing the concentration of the tested chemicals, being 0.421, 0.395 and 0.288 gm dry weight, respectively.

Table 3: Effect of tested chemicals at different concentrations on *R. solani* dry weight (gm) using Czapek's liquid medium at 25±2°C for 7 days *in vitro*.

Treatments	Dry weight growth of *R. solani* on Czapek's liquid medium			Mean
	Concentrations ppm			
	100	200	400	
Ascorbic Acid	0.493	0.357	0.304	0.384
Benzoic Acid	0.396	0.340	0.289	0.342
Salicylic Acid	0.299	0.219	0.174	0.231
L-lycine	0.293	0.447	0.225	0.322
Coumarin	0.295	0.229	0.158	0.227
Xanthan	0.604	0.606	0.298	0.503
Control	0.569	0.569	0.569	0.569
Mean	0.421	0.395	0.288	-
L.S.D. at 5%	Treatments-T = 0.045	Concentration-C = 0.023	TXC= 0.07	

Coumarin and salicylic acid were the most effective treatments in reducing *R. solani* dry weight, being (0.227 and 0.231gm), respectively. Meanwhile, xanthan and ascorbic acid recorded the highest dry weights (0.503 and 0.384 gm), respectively.

These results are in agreement with those reported by Serghini *et al.,* (2001), Arslan, *et al.,* (2009), Ordonez, *et al.,* (2009) and Abdel-Monaim and Atwa (2015) who reported that chemical inducers also have wide spectrum antimicrobial properties. Salicylic acid and coumarin are shown to be effective antioxidants as growth inhibitors of some soil borne fungal pathogens i.e., *R. solani* and *F. solani.*

3.3.3. Efficacy of tested chemicals against *R. solani* damping-off and root-rot diseases in sugar beet under greenhouse conditions.

Data presented in Table (4) indicate that treated sugar beet seed with each of the tested chemicals alone at different concentrations significantly decreased *R. solani* damping-off and root-root diseases and increased number of the survived plants under greenhouse conditions.

Coumarin and salicylic acid at 400 ppm were the most effective treatments against Rhizoctonia damping-off and root-rot being (6.67 and 13.33%), (8.33 and 16.67%), respectively. Meanwhile, xanthan was the lowest effective treatment for controlling damping-off, being (43.33%) at 400 ppm conc. compared with the control plants grown in infested soil.

Table 4: Efficacy of the tested chemicals at different concentrations against Rhizoctonia damping-off and root-rot diseases in sugar beet under greenhouse conditions.

Chemical inducers	Conc.	Damping-off %	Root rot %	Survived plant %
Ascorbic Acid	100	30.00	13.33	56.67
	200	26.67	13.33	60.00
	400	23.33	15.00	61.67
Benzoic Acid	100	20.00	16.67	63.33
	200	23.33	10.00	66.67
	400	15.00	13.33	71.67
Salicylic Acid	100	20.00	8.33	71.67
	200	16.67	13.33	70.00
	400	8.33	16.67	75.00
L-lycine	100	26.67	11.67	61.67
	200	40.00	6.67	53.33
	400	16.67	15.00	68.33
Coumarin	100	26.67	6.67	66.67
	200	10.00	13.33	76.67
	400	6.67	13.33	80.00
Xanthan	100	26.67	13.33	60.00
	200	36.67	10.00	53.33
	400	43.33	10.00	46.67
Maxim	-	5.00	8.33	86.67
Infected control*	-	46.67	21.67	31.67
Uninfected control**	-	0.00	0.00	100.00
L.S.D. at 5%		9.197	9.486	11.472

* Plants raised from seeds planted in soil infested with *R. solani*.
** Plants raised from seeds planted in uninfested soil with *R. solani*.

These results are in harmony with those recorded by Sharma, *et al.,* (1986) Abbas (2004), Abdel-Monaim and Atwa (2015), Khalifa *et al.,* (2016) and Elwakil *et al.,* (2018) who found that soaking seeds in a solution of chemical inducers i.e., Ascorbic, potassium salts and salicylic acids before planting significantly reduced damping-off and root-rot severity and increased plant survival % under

greenhouse conditions. Barreto *et al.,* (2021) and Eliwa *et al.,* (2021) found that catechol, ascorbic and salicylic acids achieved the best disease control followed by citric acid and potassium silicate against *R. solani* and *F. oxysporum*.

3.4. Effect of sugar beet treatment with the most effective chemicals in the presence of *R. solani* on the macerating enzymes *in vitro* and *in vivo*.

Data presented in Table (5 a & b) show the effect of some chemicals (Coumarin, benzoic acid and salicylic acid) on the activity of pectinase, cellulase and pectin lyase in czapek's liquid medium and in sugar beet tissues as a percent of control *in vivo* and *in vitro*, respectively.

Data in Table (5 a) and Fig. (2) indicate that all tested chemicals decreased viscosity and the percentage of pectinase activity. Salicylic acid was the most effective treatment followed by coumarin and benzoic acid, being (71.1, 82.2 and 80.7%) and (63.8, 75.2 and 85.1%) *in vitro* and *in vivo*, respectively.

Results in Table (5 a) and Fig. (3) reveal that all chemical treatments decreased viscosity and the percentage of cellulase activity compared to the control. Coumarin was the most effective treatment followed by salicylic acid and benzoic acid, being (69.3, 76.3 and 81.1%) and (71.3, 82.2 and 85.9%) *in vitro* and *in vivo*, respectively.

Data in Table (5 b) and Fig. (4) indicate that all chemicals decreased the percentage of Pectin lyase activity. Salicylic acid was the most effective treatment followed by benzoic and coumarin, being (74.7, 82.5 and 90.0 %) and (73.0, 84.9 and 88.4%) *in vitro* and *in vivo*, respectively.

The obtained results are in harmony with those recorded by Braga, *et al.,* (1998) and Faure, (2002) who reported that polysaccharides, pectin form a major structural component of primary cell wall and are the main constituents of the middle lamella, which is responsible for cell-to-cell adhesion. The Plant or pathogen cell walls contain signaling components that elicited by microbial attack induce the production of defense molecules against attack by pathogens and insects (Abdel-Massih, *et al.,* 2003). The pectic enzymes are major weapons by which microorganisms invade their hosts successively (Bateman and Miller, 1966). Microorganisms produce these enzymes inductively rather than constitutively. Plants produce polygalacturonase-inhibiting proteins as part of their defense against disease (Chimwamurombe, *et al.,* 2001). Its role in tissue maceration by plant pathogenic microorganisms has been demonstrated using mutants that lack PL activity (Beaulieu *et al.,* 1993).

Table 5a: Effect of sugar beet treatment with the most effective chemicals in the presence of *R. solani* on pectinase and cellulase activity as macerating enzymes *in vitro* and *in vivo*.

Treatments	Pectinase activity				Cellulase activity			
	In vitro		*In vivo*		*In vitro*		*In vivo*	
	Loss in viscosity	% of control	Loss in viscosity	% of control	Loss in viscosity	% of control	Loss in viscosity	% of control
Coumarin	0.544	82.2	0.582	75.2	0.225	69.3	0.320	71.3
Benzoic acid	0.534	80.7	0.659	85.1	0.260	81.1	0.228	85.9
Salicylic acid	0.471	71.1	0.494	63.8	0.245	76.3	0.275	82.2
Control	0.662	-	0.774	-	0.321	-	0.263	-
L.S.D. at 5%	0.010		0.011		0.005		0.007	

Table 5b: Effect of sugar beet treatment with the most effective chemicals in the presence of *R. solani* on pectin lyase activity as a macerating enzyme *in vitro* and *in vivo*.

Treatments	Pectin lyase activity			
	In vitro		*In vivo*	
	Enzyme units	% of control	Enzyme units	% of control
Coumarin	0.334	90.0	0.351	88.4
Benzoic acid	0.306	82.5	0.337	84.9
Salicylic acid	0.277	74.7	0.290	73.0
Control	0.371	-	0.397	-
L.S.D. at 5%	0.016		0.018	

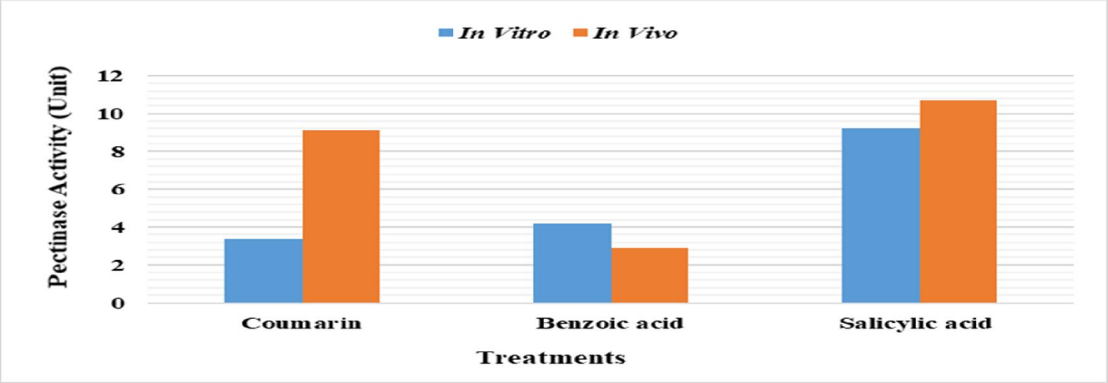

Fig. 2: Pectinase activity (expressed as enzyme units) in culture filtrate of *R. solani* 7 days after incubation at 25°C in Czapek's medium amended with 400 ppm concentration of coumarin, benzoic acid and salicylic acid *in vitro*. Also, activity of pectinase (expressed as enzyme units) in sugar beet roots raised from seeds planted in soil infested by *R. solani* after seed soaking (6h) *in vivo*.

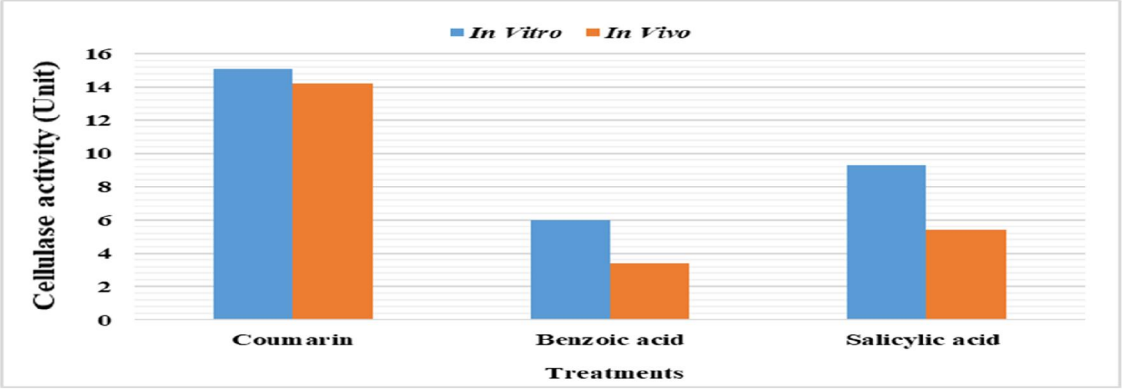

Fig. 3: Cellulase activity (expressed as enzyme units) in culture filtrate of *R. solani* 7 days after incubation at 25°C in Czapek's medium amended with 400 ppm concentration of coumarin, benzoic acid and salicylic acid *in vitro*. Also, activity of the total cellulase (expressed as enzyme units) in sugar beet roots raised from seeds planted in soil infested by *R. solani* after seed soaking (6h) *in vivo*.

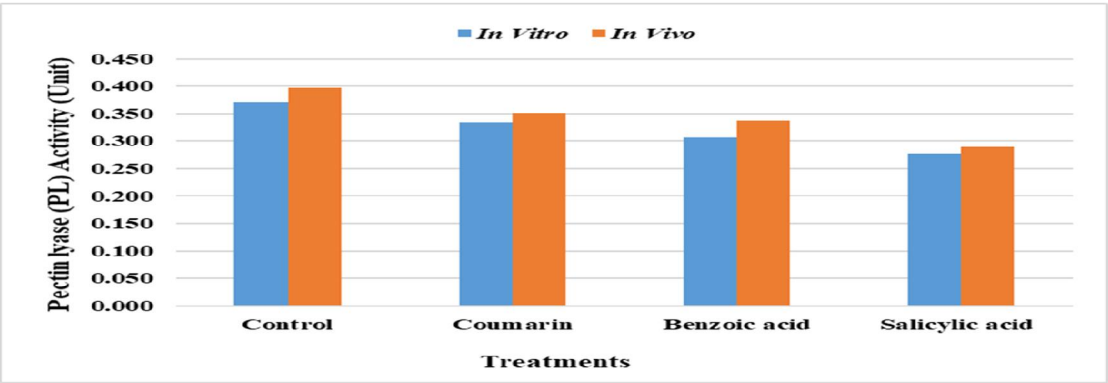

Fig. 4: Pectin lyase (PL) activity (expressed as enzyme units) in culture filtrate of R. solani 7 days after incubation at 25oC in Czapek's medium amended with 400 ppm concentration of coumarin, benzoic acid and salicylic acid in vitro. Also, activity of pectin lyase (expressed as enzyme units) in sugar beet roots raised from seeds planted in soil infested by R. solani after seed soaking (6h) in vivo.

3.4. Scanning Electron Microscopy (SEM) studies

In respect of the activity of the two effective chemical inducers, salicylic acid (S) and coumarin (C) compared with normal hyphae of *R. solani* (N) was studied and examined by Scanning Electron Microscopy (SEM). This technique is a very useful tool provides a clear view for the effect of the selected chemical inducers on the pathogenic fungus malformation or lysis. The morphological changes in the hyphal growth of the pathogenic fungus due to the effect of the tested chemical inducers are illustrated in Fig. (4).

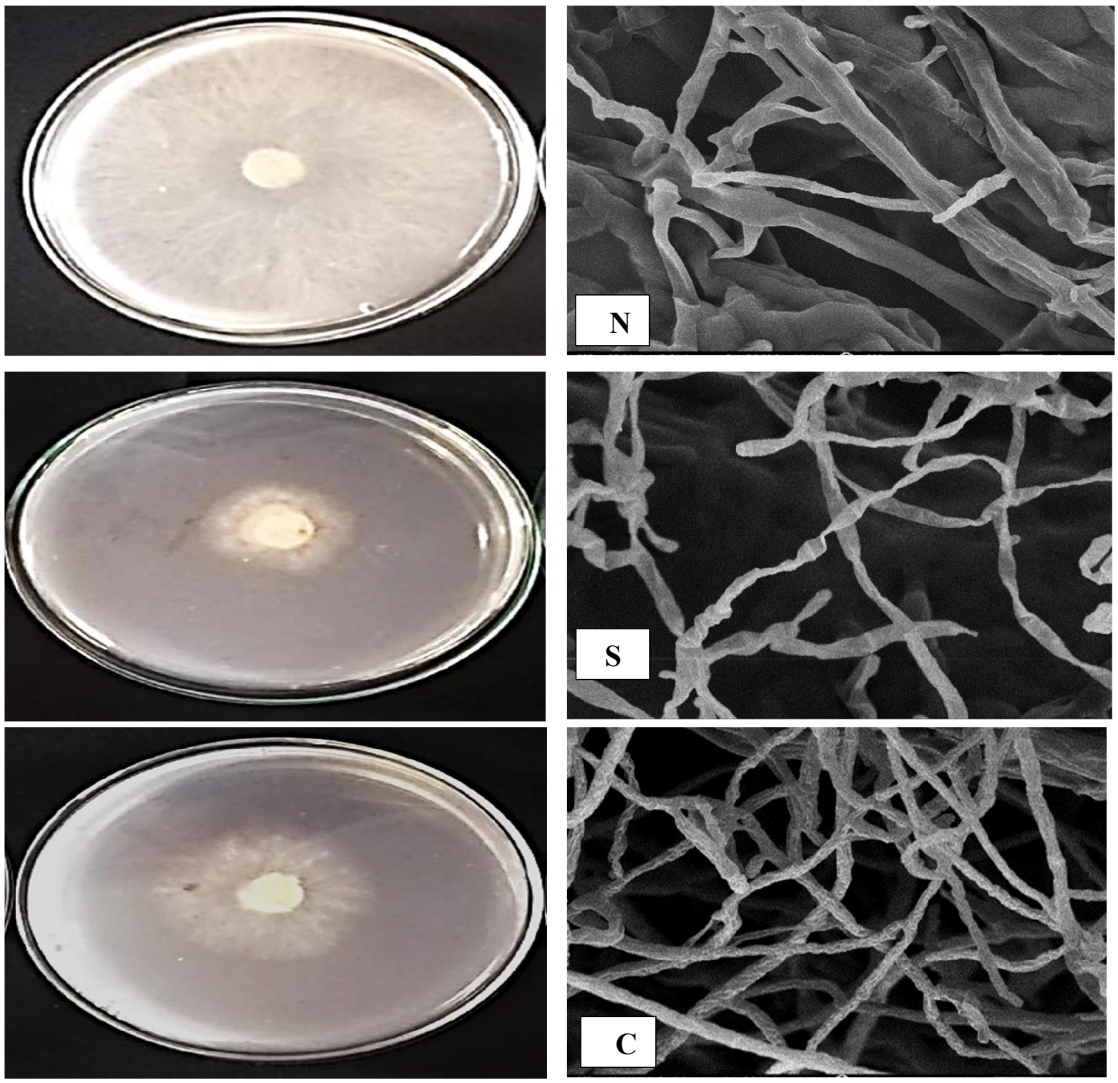

Fig. 4: Scanning electron microscope (SEM) Images show morphological alteration, deformation and damage on hyphae of *R. solani* due to the effect of salicylic acid in (S) and coumarin (C) compared with normal hyphae of *R. solani* (N).

Scanning electron microscope images show morphological alteration, wrinkling, deformation, and damaging the hyphae of *R. solani* due to the effect of salicylic acid and coumarin compared to the hyphae of untreated control. These data suggest a potential interaction between salicylic acid and coumarin and *R. solani* cell wall constituents, which could result in cell structural damage. Alternately, the information could point to the activation of specific intracellular signaling pathways, which would lead to the structural destruction of the fungal cell.

Many researchers have been interested by biotic and abiotic agents which can induce synthesis, and lytic enzyme production, developing systemic resistance, and encouraging plant development (Velivelli *et al.,* 2014 and Xiang *et al.,* 2018). Using a scanning electron microscope, they noticed

overgrowth and lysis of *R. solani* in dual cultures with *T. longibrachiatum*, as well as morphological anomalies such as atrophy and lysis using *P. polymyxa* in both fungal mycelia. Parasitism of pathogenic fungi was reported by *Trichoderma* species in other studies (Melo and Faull, 2000). Scanning electron microscopic analysis revealed that *T. harzianum* strains antagonist with *R. solani* (Vijayakumar and Sivaram, 1997 and Melo & Faull 2000). *T. harzianum* overgrew and coiled around the *R. solani* cells, invading and damaging the host hypha. Through the mechanical activity, the host cells are penetrated. Secretion of antifungal compounds has been found to prevent the growth of different plant pathogens (Hassan *et al.,* 2014).

3.5. Effect of sugar beet treatment with the most effective inducers in the presence of *R. solani* on oxidative enzymes and phenolic compounds *in vivo*.

Data in Table (6) indicate that treatment of sugar beet seeds before planting with the effective chemical inducers i.e., salicylic acid, coumarin and benzoic acid resulted an increase in peroxidase and polyphenol oxidase activity, as well as phenolic compounds in sugar beet leaves at 30 days after sowing date compared with untreated ones (check). The highest increase in peroxidase and polyphenol oxidase activity was recorded due to treatment by salicylic acid followed by coumarin and benzoic acid treatments, being (1.041, 0.898 and 0.757) and (0.659, 0.713 and 0.547) in both enzymes, respectively compared to uninfected and infected control treatments.

Table 6: Effect of seed treatment with effective chemical inducers in the presence of *R. solani* on peroxidase, polyphenol oxidase activities and phenolic content (mg/g fresh weight) in sugar beet leaves at 30 days after application.

Treatments	Enzymes activity* (min/g fresh weight of sugar beet leaves)		Phenolic content (mg/g fresh weight in sugar beet leaves)		
	Peroxidase	Polyphenol-oxidase	Free	Conjugated	Total
Salicylic acid	1.041	0.659	6.67	12.12	18.79
Benzoic acid	0.757	0.547	4.97	8.68	13.65
Coumarin	0.898	0.713	5.55	10.32	15.87
Infected control*	0.559	0.315	3.39	7.00	10.39
Uninfected control**	0.417	0.296	2.76	5.38	8.14
L.S.D. at 5% for	0.016	0.085	0.387	0.306	0.175

* Plants raised from seeds planted in soil infested with *R. solani*.
** Plants raised from seeds planted in uninfested soil with *R. solani*.

Results in Table (6) also, show that all effective chemical inducers increased the phenolic compounds compared to infected and uninfected control. Salicylic acid was the most effective treatment followed by coumarin and benzoic acid which recorded the highest accumulation of phenolic compounds (6.67, 12.12 and 18.79), (5.55, 10.32 and 15.87) and (4.97, 8.68 and 13.65) of free, conjugated and totals, respectively compared with infected and uninfected control treatments.

These results are supported by the results obtained by Barakat *et al.,* (1979), Sharma and Dubey (2007), El-kazzaz *et al.,* (2015), Khalifa *et al.,* (2016) and Sehsah *et al.,* (2022) who showed that the activity of oxidative enzymes has increased in sugar beet leaves infected with the pathogen and treated with biotic and abiotic inducers. Oxidation enzymes, such as polyphenol oxidase, peroxidase, and phenol compounds inducing plants to resist pathogens which play an important role in the cellular defense against the oxidative stresses (Radjacommare *et al.,* 2004 and Sharma and Dubey, 2007). Peroxidases are oxidoreductive enzymes that have a critical role in several metabolic processes such as oxidation of phenols (Reuveni *et al.,* 1991 and Swami *et al.,* 2018), reinforcement of cell walls in plants (Dean and Kuc, 1987 and Bernards *et al.,* 1999) and enhancing lignification in response to infection (Hammerschmidt and Kuc, 1982), which may restrict the penetration of the pathogen (Ride, 1983). Polyphenoloxidase is involved in the formation of melanin compounds in the necrotic tissues (Mayer, 1987). In addition, many studies have shown that PPO is induced in response to mechanical wounding, fungal and bacterial infection and by treatment with signaling molecules such as jasmonic acid, methyl jasmonate (MeJA), systemin and salicylic acid (Constabel *et al.,* 2000 and Stewart *et al.,* 2001). These enzymes play important roles in oxidizing phenols to produce quinones (the more fungi toxic

compounds) and subsequently control diseases (Bi and Zhang, 1993). Phenol is one of the most stress-responsive plant (Rodrigues *et al.,* 2003 and El-Argawy *et al.,* 2016). Similar findings were recorded by Gouda (2001), EL-Sayed (2007) and Abdalla *et al.,* (2019) who found that all seed treatment with selected plant extracts and chemical inducers, significantly increased phenolic compounds (free, conjugated and total phenols). According to Matern and Kneusal (1988), accumulation of phenols at the infection site, which acts as defense system that can be translocated by plants and converted enzymatically into defensive substance at the site of the attack.

3.6. Field experiments

Data presented in Tables (7 & 8) reveal that all tested chemical inducers, L-lycine, coumarin, salicylic acid, benzoic acid and xanthan in addition to Maxim as a recommended fungicide significantly decreased damping-off, root-rot, disease severity and increased the survived plants compared to control treatment, Also, all chemical inducers significantly increased yield and yield components (Total soluble solid, sucrose content and Purity) of sugar beet under field conditions, during the two growing seasons 2020/2021 and 2021/2022.

Table 7: Effect of different chemical inducers on root rot and disease severity and yield and yield components (Total soluble solid, Sucrose content and Purity) under field conditions, during 2020/2021.

Treatments	2020/2021 growing season								
	Damping-off (%)	Root-rot %	Survived Plants %	D.S.* %	Root weight (Kg)	Yield/plot (Kg)	T.S.S** (%)	Sucrose content%	Purity %
Ascorbic acid	17.86	9.52	72.62	23.33	1.67	51.57	18.73	14.20	75.79
Benzoic acid	16.67	5.95	77.38	20.56	1.93	59.32	20.93	16.77	80.10
Salicylic acid	9.53	4.76	85.71	17.78	2.79	85.89	25.43	20.60	81.00
L-lycine	13.11	13.09	73.80	25.00	1.39	42.88	19.87	13.30	66.97
Coumarin	9.53	7.14	83.33	13.89	2.17	66.87	24.30	19.20	79.01
Xanthan	19.05	10.71	70.23	26.67	1.34	41.20	18.80	11.50	61.21
Maxim	3.57	2.38	94.05	7.22	1.97	60.68	23.60	17.00	72.04
Control	19.05	15.48	65.47	35.56	1.03	31.59	13.47	8.03	59.66
L.S.D. at 5%	6.35	8.25	6.93	5.67	0.32	6.78	0.92	0.84	4.7

D. S.*= Disease severity T.S.S** = Total soluble solid

Table 8: Effect of different chemical inducers on root rot and disease severity and yield and yield components (Total soluble solid, Sucrose content and Purity) under field conditions, during 2021/2022.

Treatments	2021/2022 growing season								
	Damping-off (%)	Root-rot %	Survived Plants %	D.S.* %	Root weight (Kg)	Yield/plot (Kg)	T.S.S** (%)	Sucrose content %	Purity %
Ascorbic acid	14.28	8.33	77.39	19.44	2.000	61.60	22.43	17.30	77.12
Benzoic acid	10.71	9.52	79.77	16.67	2.24	68.93	23.53	18.63	79.18
Salicylic acid	10.71	2.38	86.91	10.00	3.21	91.74	26.23	21.97	83.74
L-lycine	15.47	9.52	75.00	22.00	1.43	43.98	15.57	11.00	70.67
Coumarin	9.52	5.95	84.53	12.22	2.89	89.04	25.77	21.03	81.63
Xanthan	16.67	11.90	71.43	28.33	1.66	51.06	16.27	10.70	65.80
Maxim	7.14	2.38	90.48	5.00	2.47	76.21	19.57	14.13	72.23
Control	17.85	13.09	69.06	31.11	1.14	35.01	14.63	9.33	63.80
L.S.D. at 5% for	6.82	7.61	5.44	3.72	0.26	8.12	0.63	0.90	4.46

D. S.*= Disease severity T.S.S** = Total soluble solid

Data in Table (7) reveal that salicylic acid and coumarin were the most effective treatments where they recorded (9.53, 4.76, 17.78 & 85.71%) and (9.53, 7.14, 13.89 & 83.33%) damping-off, root-rot, disease severity and survived plants, respectively. On the other hand, xanthan showed the lowest effect on damping-off, root-rot, disease severity and survived plants, being (19.05, 10.71, 26.67 & 70.23%), respectively. Also, salicylic acid and coumarin were more effective where they recorded the highest increase in sugar beet yield (2.79 & 85.89 Kg) and (2.17 & 66.87 Kg) yield/plant and yield/plot and enhancement sugar beet yield components (25.43, 20.60 & 81.0%) and (24.30, 19.20 & 79.01%) (T.S.S), sucrose content and purity, respectively. On the other hand, xanthan and L-lycine were the lowest effective treatments compared with the control treatment.

Data concerning 2021/2022 growing season (Table, 8) show that salicylic acid and coumarin were the most effective treatments, being (10.71, 2.38, 10.0 & 86.91%) and (9.52, 5.95, 12.22 & 84.53%) damping-off, root-rot, disease severity and survived plants, respectively.

Meanwhile, xanthan treatment showed the lowest effect treatment on damping-off, root-rot, disease severity and survived plants, being (16.67, 11.90, 28.33& 71.43%), respectively. Also, salicylic acid and coumarin were more effective treatments and recorded the highest increase in sugar beet yield (3.21 & 91.74 Kg) and (2.89 & 89.04 Kg) yield/plant and yield/plot and increased yield components which recorded (26.23, 21.97 & 83.74%) (25.77, 21.03 & 81.63%), respectively. On the other hand, xanthan and L-lycine were the lowest effective treatments.

These results are in harmony with those recoded by Abbas (2004), Khalifa *et al.,* (2016), Elwakil, *et al.,* (2018) and Barreto *et al.,* (2021) who found that soaking seeds in some antioxidant solutions (Ascorbic and salicylic acids) decreased *F. oxysporum* and *R. solani* disease severity. Also, Gouda (2001), EL-Sayed (2007), Abdalla *et al.,* (2009) and Abdel-Monaim and Atwa (2015) found that all plant extracts and antioxidants, i.e., potassium salts significantly reduced damping-off and root rot/wilt severity and increased plant survival in field conditions and increased the yield parameters. Moreover, antioxidants induced systemic or local resistance (Mostafa, 2006). Salicylic acid is considered to mediate plant responses to pathogens which associated with induced systemic acquired resistance (Delanay *et al.,* 1994; Galal and Abdou, 1996 and Galal *et al.,* 1997). Also, Abdel-Monaim and Atwa (2015) found that potassium salts at different concentrations significantly contributed to various growth and yield parameters such as root length, root diameters and root wights compared to the control. Also, El-Sayed and Farrag (2011), Abd El-fattah and El-Geddawy (2015), Eid and EL-Sayed (2017), Ghazy, *et al.,* (2021) and Sehsah, *et al.,* (2022) reported that some induced compounds gave the highest percentages of total soluble solids (T.S.S.), sucrose percentages in sugar beet roots and purity. Sharma *et al.,* (1986) reported that the use of specific antioxidants improved plant growth parameters due to the role of antioxidants in stimulating physiological processes and reflecting an improvement in vegetative growth.

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