Egyptian Journal of Phytopathology,Vol. 52, No.1, pp 109-151 (2024)
DOI 10.21608/EJP.2024.362774

ORIGINAL PAPER

Induction of Resistance and Defense Mechanisms of Geranium Plants Against Root Rot Disease Using Gallic Acid and 3,5-dinitrobenzoic Acid.

Imara, D.A*[1] ; El-Mohammady,M.M.S[2].; Hanafy,S.A.H[2]. and Yousef, R.S[3].

Received: 7 June 2024 / **Accepted**: 28 June 2024 / **Published online**: 30 June 2024

ABSTRACT

Root rot is a serious disease, causing considerable losses in essential oil production every year. In addition, the time of planting has a substantial impact on the severity and rate of disease development, combining the best strategies for applying exogenous polyphenolic compounds, plays a critical role in lowering disease-induced oxidative stress. This study focused on the role of gallic acid (GA) and 3,5-dinitrobenzoic acid (3,5-DNBNZA) acid in regulating resistance against geranium root rot pathogens *i.e.*, *Fusarium semitectum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Pythium irregulare* via a multifunctional defense system. GA (600 mgL^{-1}) and 3, 5-DNBZA (300 mgL^{-1}) showed optimal antifungal activity by affecting the pathogen's cell wall integrity and capacity to produce oxidative enzymes at various levels *in vitro*. The pathogens' mixture has a synergistic action since the infection percentages significantly increased than each pathogen alone in the pathogenicity test. The quadratic regression analysis confirmed that disease incidence (%) was gradually increased from winter to summer season. The dual application approach of GA and 3, 5-DNBZA was superior to the single application method for protection against root rot incidence and disease severity, coinciding with delaying the symptoms, improving the plant biomass and oil yield. Along with their favourable effects on biochemical traits, defense phenolics, enzymatic activities, and reduced leaf senescence. The present study recommended the dual application of GA and 3, 5-DNBZA as a sustainable and safe alternative management strategy for root rot disease and enhanced oil production. Also, elucidate the physiological and biochemical mechanisms behind their protective role.

Keywords: Induced resistance, *Pelargonium graveolens*, soil-borne phytopathogens, antioxidant enzymes, biotic stress.

*Correspondence: **Doaa Abd El-Samie Imara**
E-mail: dr.doaaimara@arc.sci.eg
Imara, D.A*[1]
0000-0001-9960-811X

El-Mohammady,M.M.S[2]

Hanafy,S.A.H[2].

Yousef, R.S[3].
0009-0006-2919-7726

[1]Ornamental, Medicinal and Aromatic Plant Diseases Res. Dept., Plant Pathology Research Institute, A R C, Giza, Egypt.

[2]Plant Physiology Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

[3]Biochemistry Department, Faculty of Agriculture, Cairo University, Giza ,Egypt.

INTRODUCTION

Rose-scented geranium (*Pelargonium graveolens* L'Her Ex Ait.) is a valuable industrial crop belonging to the family Geraniaceae. It is grown worldwide for its essential oil (EO) and absolutes, which rank among the top twenty oils in the world (Mazeed *et al*., 2022). Its strong rose-like scent makes it popular in the grade perfumery, cosmetic, food, and pharmaceutical industries (Bergman *et al*., 2021). The essential oil of geranium is effective as an antibacterial (Asgarpanah and Ramenzanloo, 2015 and Pradeepa *et al*., 2016), antioxidant, and antifungal qualities, as well as its immune-modulating and metabolic effects (Ali *et al.,* 2020 and Neague *et al*.,2018). China and Egypt are the primary producers, accounting for 150 tonnes of the total 300 tonnes available on the market, followed by Algeria, Morocco, and India (Mazeed *et al*., 2022 and Blerot *et al*., 2016).

Geranium plants are liable to attack by a variety of soil-borne phytopathogens. Root rot is one of the most serious diseases, Fusarium cutting rot (*Fusarium* spp.), Rhizoctonia root and crown rot (*Rhizoctonia solani*), Macrophomenia root and stem rot (*Machrophomina phaseolina*), Pythium root rot (*Pythium* spp.), which causes considerable losses in the quantity and quality of essential oil in geranium plants and yield production annually and hence are a serious agronomic concern in Egypt and worldwide (Prasad and Singh, 2014; Munera and Hausbeck, 2015; Adolf, 2016 and Dewidar *et al.*, 2019). The quality of EO components, particularly terpenoid composition is affected by the genetics of the plant, the age of the leaf, the planting date, season, and harvesting dates. However, the impact of these factors on this role is poorly understood (Malatova *et al.*, 2011; Prasad *et al*., 2010 and Kumar *et al*., 2021).

Exogenous elicitors, such as polyphenol constituents, are one of the main strategies that can induce resistance to subsequent pathogen attacks by activating various defense-related enzymes, acting as specific receptors/ion channels in the plasma membrane, triggering gene expression, and enhancing the production and accumulation of bioactive compounds (secondary metabolites) in plant organs to impart protection to the plant against stress by utilizing both locally and systemically (SAR) (Arya *et al*., 2021; Li *et al*.,2021 and Š´cepanovi´c *et al*., 2022). These elicitors are presented as ecologically friendly, as their positive effect is sustainable and safe for use as a promising alternative for managing soil-borne fungal diseases and improving plant biomass yield production and quality. Generally, these elicitors are the chemical compounds that stimulate the biosynthesis pathway by activating specific

transcriptional factors and upregulating genes (Frąckowiak *et al*., 2019 ; Bhashar *et al*., 2022 and Asadollahei *et al*., 2023). Credible research has shown that the physicochemical properties of elicitors, the concentration used, the application methods and timing, the rate and frequency of application, as well as the plant species and pathogens, all affect whether an elicitor has a positive or negative effect (Li *et al*., 2021; Asadollahei *et al*., 2023 and Riahi *et al*., 2020).

Gallic acid (GA) and 3, 5-dinitrobenzoic acid (3,5-DNBZA) are phytochemical phenolic compounds that occur naturally in the leaves, and roots of diverse plant species via the shikimic acid and phenylpropanoid pathways that have defense mechanisms in plant and provide protection against pathogens (De La Rosa *et al*., 2019 and Deepmala, 2019). GA has shown strong antioxidant capacities and antifungal activity against *Fusarium* spp.*; Alternaria* spp. and *Pythium* sp. (Osorio *et al*., 2010; Zhao *et al.,* 2018 and El-Nagar *et al.,* 2020). BZA derivatives have been evidenced to activate resistance in various plant species against diverse phytopathogens, including viruses, fungi, and bacteria, based on their biological characteristics and structural activity (Frąckowiak *et al*., 2019 and Nehela *et al.,* 2021). GA and BZA derivatives also play protective roles in plant adaptation to biotic and abiotic stresses (De La Rosa *et al*., 2019). Therefore, investigations targeting the impact of elicitors to protect plants against pathogenic fungi and enhance the production of industrially valuable essential oils and phenolic secondary metabolites as natural sources of antioxidants in aromatic and medicinal plants are still finite (Riahi *et al*., 2020 and Cappellari *et al*., 2020). Finding efficient novel strategies to improve the production of high-value secondary metabolites (SMs) due to their potent antioxidant properties and credible effects in enhancing plants' ability to withstand biotic and abiotic stress is necessary and an ongoing challenge for researchers worldwide (Arya *et al*., 2021 ; Bhashar *et al*.,2022 and Asadollahei *et al*., 2023).

The objectives of this study were to: (1) investigate how planting time affects the severity and rate of disease development, (2) estimate the impact of the antifungal properties of GA, and 3, 5-DNBZA on the morphological, physiological, and biochemical changes against the four root rot phytopathogens of geranium *i.e*., *Fusarium semitectum, Macrophomina phaseolina, Rhizoctonia solani* , and *Pythium irregulare,* in lab assay, (3) assess the effect of synthetic plant-resistance inducers; GA and 3,5-DNBZA at three concentrations on the development of root rot disease incidence, (4) determine the application method, timing, frequency, and appropriate proportion dose of GA and 3,5-DNBZA for controlling root rot disease, improving the production of

high-value secondary metabolites, and essential oil production in the geranium plant and decreasing the usage of fungicides totally or fractionally.

MATERIALS AND METHODS

1. *In Vitro* experiments:

Isolation, purification and Identification of the isolated microorganisms:

Plant samples of geranium plants showing typical root rot, stem rot, and wilt symptoms were collected from heavily naturally infected field in El-Qanater El-Khayria Agricultural Research Station at Qalubiya Governorate, Egypt. The technique for isolating and purifying the causal pathogens is described by Imara *et al.*, (2021). The identity of the isolates was carried out according to their morphological and cultural characteristics as described by Nelson *et al*., (1983) for *Fusarium* species and Domsch *et al*., (1980) for *Pythium* species , *Macrophomina phseolina*, and *Rhizoctoinia solani*. Data on the frequency of isolated pathogenic microorganisms from roots and basal stem of geranium plants were recorded using the following formula.

$$\text{Frequency (\%)} = \frac{\text{Number of colonies of each isolated fungus}}{\text{Total number of colonies of all isolated fungi}} \times 100$$

The antifungal effect of gallic acid (GA) and 3, 5dinitrobenzoic acid (3, 5-DNBZA) on suppression the radial growth of the tested pathogens.

Four isolated pathogens *i.e. Fusarium semitectum, Macrophomina phaseolina*, *Rhizoctonia solani* , and *Pythium irregulare* were grown on PDA medium to use under *in vitro* tests. Gallic acid (99.5%) and 3,5-dinitrobenzoic acid (97%) were obtained from EL-Gomhoria for Trading Chemicals and Medical Appliances (GOMAC). Each was dissolved in ‘2ml” 100% dimethyl sulfoxide (DMSO) to obtain a stock solution of 10000-ppm of each. Three concentrations of gallic acid (GA) and 3,5-dinitrobenzoic acid (3,5-DNBZA) *i.e.* 300, 600, and 900 mgL^{-1} were used in this investigation. This experimental technique was carried out as described by Nehela *et al.,* (2021). The sterilized PDA medium, which included 2ml of "DMSO" and without any treatments for each pathogen, was used as control. Five plates were used as replicates for each particular treatment and then incubated at $25\pm2^{o}C$. The average radial mycelial growth “colony diameter (cm)” of all treatments was recorded once the controls were fully grown. Following the primary screening results, the selected GA at 600 mgL^{-1} and 3, 5-DNBZA at 300 mgL^{-1} were further tested. To investigate the mechanisms by which GA and 3, 5-DNBZA suppress the tested pathogens, we measured their effects on mycelial dry weight (gm), the tested pathogens' membrane integrity, and these pathogen's ability to produce extracellular oxidative

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

enzymes in culture filtrates, such as the activity of peroxidase and carboxymethyl cellulase enzymes.

Evaluation of the antifungal activity of GA at 600 $mgL^{-1}$and 3,5-DNBZA at 300 mgL^{-1} on mycelial dry weight (MDW) (gm), the membrane integrity of the four tested pathogens, and their capacity to produce extracellular oxidative enzymes in culture filtrates.

Individually, the best doses of GA at 600 mgL^{-1} and 3, 5-DNBZA at 300mgL^{-1} were added to conical flasks (100 ml) containing 50 ml of sterilized potato dextrose broth (PDB) medium. The control treatment was separate PDB flasks free of either GA or 3, 5-DNBZA as described by Nehela *et al.,* (2021). Each treatment and each parameter that was measured underwent three replications in this test. The mycelial mats were collected after the required incubation period of each pathogen (incubated at $25\pm2^{o}C$) and filtered through a sterilized two-filter papers Whatman filter (No. 1). Evaluation of the antifungal activity of GA and 3,5-DNBZA on different tested parameters of the four tested pathogens, was carried as follow:

1- Assess the effect of the tested organic acids on mycelial dry weight:

Mycelial dry weight (MDW), was determined after drying the mycelial mats at $60^{o}C$ to obtained a constant weight (for 72h).

2- Utilizing the membrane stability index (MSI %) to evaluate the tested pathogens' membrane integrity as measured by the conductivity meter:

The obtained mycelial mat was measured in a conductivity meter to clarify the changes in membrane permeability and the release of the cellular material. According to the methods of Premachandra *et al*., (1991) which modified by Sairam, (1994), MSI was calculated using the formula: MSI = [1-(C1 / C2)] X 100 where, C1 and C2 are the electric conductivities recorded at 40°C for 20 min and 100°C for 10 min, respectively.

3- Detect enzymes secreted by the tested pathogens:

Furthermore, the tested pathogens' culture filtrate was used to detect enzymes secreted by the tested pathogens. The detection of peroxidase and Carboxy methyl cellulase enzymes activity in the culture filtrates of pathogens was measured as follows:

Peroxidase enzyme activity (POD) was measured in different samples as described by Worthington, (1977). The change in absorbance of the reaction mixtures was measured against a blank at 425 nm wave length. Enzyme activity was

expressed as 425/min/ml. The carboxymethyl cellulase enzyme activity was determined in different samples by measuring the amount of reducing sugars liberated from carboxymethyl cellulose (CMC) using the DNS (3, 5-dinitrosalicylic acid) method (Ladeira *et al*., 2015). One unit of enzyme activity (U) was defined as the amount of enzyme that released mg/ml of glucose per minute. The reaction was measured by spectrophotometry at 540 nm. Enzyme activity was expressed as 540/min/ml. The sample readings for the two investigated enzymes have taken using a UV-Vis spectrophotometer.

2. *In Vivo* experiments:

These experiments were accomplished at the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt, under pot conditions.

Pathogenicity test:

The pathogenic ability of the four isolated pathogens (*Fusarium semitectum, Macrophomina phaseolina, Rhizoctonia solani,* and *Pythium irregulare*) was evaluated under artificial conditions at the pot experiment using the pathogenicity test technique according to Imara *et al*., (2021). Disinfested clay pots (30 cm) were filled with previously formalin-disinfested clay and sand soil (3:1 v/v). The potted soil was infested with a 2% (w/w) inoculum of each pathogen alone and/ or with a mixture of the four pathogens prepared by mixing equal percentages of each pathogen (1:1:1:1 w/w). Three healthy un-rooted terminal cuttings of *Pelargonium graveolens* L'Herit. cv. 'Local variety' (25 cm. long), were transplanted in each pot. A randomized complete block design with three replicates each consisted of three pots and three cuttings in each pot for each treatment under natural environmental conditions was used. Re-isolation from plant roots was performed to complete the step of the etiology study as per Koch's postulates. Disease assessment was calculated as disease incidence% (DI %) was performed at 60, 90, and 120 days after transplanting according to the following formula: DI (%) = No. of infected plants due to root rot/ Total No. of plants X100. The pathogens' mixture was selected as the most virulent to complete the study.

Follow up the development of root rot disease incidence of geranium plants grown in infested soil with the pathogens' mixture during the two growing seasons:

The percentage of disease incidence was examined at the two growing seasons during periods from the winter to the summer season in 2017/2018 to 2018/2019 to study the impact of the season's conditions on the progression % of root rot disease incidence due to the pathogens' mixture. The experimental technique was

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

carried out as previously described under the pathogenicity test. The terminal cuttings of geranium were planted on November during both seasons. Soil infestation was achieved by mixing the previously prepared mixture of the four pathogens (inoculum) with the soil at the rate of 2% of soil weight. Un-rooted terminal cuttings were prepared and immediately transplanted in soil artificially infested with the mixture of four pathogens using the similar technique previously used in the pathogenicity test. All agricultural practices *i.e*., irrigation and fertilization, were carried out as the recommendation of Agric. Ministry for the production of geranium crop. The disease incidence was measured six times at monthly intervals beginning ninety days after planting by calculating the disease incidence percentage as mentioned before. The plants were harvested twice (May and September of each season), respectively. The experiment was arranged as a randomized complete block design with three replicates, each replicate was represented by three pots and three cuttings in each pot. Also, the quadratic regression analysis (response curve model) was carried out between disease incidence (%) as a response variable and time of the season (months) as an explanatory variable to determine the relationship between them.

Evaluation the effect of GA and 3, 5-DNBZA at three concentrations on development of root rot disease incidence:

Three concentrations (300, 600, and 900 mg L^{-1}) of the two tested compounds *i.e.*, gallic acid and 3,5-dinitrobenzoic acid were used *in vivo*. Un-rooted terminal geranium cuttings (cv. Local variety) (25 cm long) taken from visually healthy mother plants were separately dipped in the desired doses of the tested treatments for 30 min and transplanted in infested and non-infested soil with the four pathogens' mixture as described before. Geranium cuttings were dipped in Topsin-M fungicide at the recommended dose (2 g L^{-1}) for ninety seconds as the comparison treatment, and non-treated geranium cuttings (dipped in sterilized water, involving "DMSO") were transplanted in infested and non-infested soil (positive and negative control, respectively) according to (Nehela *et al.*, 2021). This experiment was arranged as a randomized complete block design in triplicate (each replicate was represented by three pots and three cuttings in each pot) and included nine treatments, under natural environmental conditions , and geranium cuttings were planted on November during the 2018/2019 season. All agricultural practices were similar to the technique previously mentioned. Disease incidence estimation was carried out just before the first and second cuts (Mayand September) respectively, during the growing season in

2018/2019 by calculating the percentage of the disease incidence as mentioned before.

Effectiveness of the exogenous application techniques (single and dual) of GA at 600 mgL^{-1} and/or 3, 5-DNBZA at 300 mgL^{-1} to manage root rot disease of geranium plants, under pot experiment conditions during the growing season (2020/2021).

GA at 600 mgL^{-1} and 3, 5-DNBZA at 300 mgL^{-1} were chosen as the best-tested treatments for geranium plants to investigate the effectiveness of exogenous application methods (single and dual) on root rot incidence and disease severity, as well as, some growth parameters and biochemical constituents of geranium plants. All experimental techniques employed in the experiment were carried out as mentioned before in previous experiment. The experiment consisted of two groups. In the first group, single application procedure, the cuttings were individually soaked in either of 600 mgL^{-1} GA or 300 mgL^{-1} 3, 5-DNBZA for 30 minutes before transplanting in the infested and un-infested soil. The second group employed a dual application technique in which the cuttings were individually soaked in each of 600 mgL^{-1} GA acid and/or 300 mgL^{-1} 3, 5-DNBZA for 30 minutes before transplanting in the infested and un-infested soil. Following fifteen days after transplanting, two hundred ml. for each of the 600 mgL^{-1} GA and/or 300 mgL^{-1} 3, 5-DNBZA individually were administered to each pot around the roots of plants as a soil drench therapy and applied five times throughout the trial, with thirty-days intervals. Untreated- geranium plants were grown in infested soil (positive control) and untreated- geranium plants were grown in un-infested soil (negative control). The experiment was set in a randomized complete blocks design with triplicate for each treatment (each replicate consisted of one pot and three cuttings/pot). The first factor is allocated to the tested elicitors and the second one to application methods. In May of the growing season (2021), geranium plants had harvested at the harvest (cutting) stage (six months old plants). Disease incidence and severity % were estimated at the end of the experiment six months after planting as follows:

Root rot disease incidence and severity on geranium plants.

The disease incidence (DI) % was measured as mentioned before.

Disease severity (DS)% was estimated based on the progress of yellowing and root rot and rotting using the rating scale according to a 0- 5 scale of (Dewidar *et al*.,2019) with minor modification where 0=0, l=> 0 -10, 2=>10-25, 3=>25-50, 4=>50- 75 and 5=>75-100%. Disease severity % = [Σ (n x c)]/ (N x C) x 100

Where: n = Number of infected plants, c = Category number, N = Total number of examined plants, and C = the highest category number of infections.

Plant growth measurements:

Plant height (cm), herb and root biomass (g/plant), as well as dry weights of herb and roots (g/plant), leaf area (cm^2), and dry weight of shoot/root ratio were recorded at the harvest stage of the growing season 2020/2021. The volatile oil percentage was determined according to (British Pharmacopeia , 1963) .

Biochemical constituents' analyses of geranium leaves:

Plant biochemical measurements*:*

For biochemical analysis, randomized samples of fresh geranium leaves (5 gram) were collected from each treatment (GA at 600 mgL^{-1} and/or 3, 5-DNBZA at 300 mgL^{-1}) in the single application method after 90 days from planting, as well as after 48 hours from the last soil drench treatment in the case of the dual application technique. All parameters were examined in tissue extracts of geranium plants from the following treatments: GA-and 3, 5-DNBZA -treated plants grown in infested and un-infested soil under the application technique's (single and dual treatments) and the control treatments (positive and negative).

Determination of total and reducing sugars:

Total sugars were determined in ethanol extract of geranium leaves. Absorbance was measured at 490 nm for technical replicates; the amount of sugars (mg/g FW) measured reference to a standard curve previously constructed for the particular sugars under examination (Dubois *et al*., 1951). However**,** reducing sugars were determined in leaves by 3, 5-dinitro salicylic acid DNS method (Miller, 1959) and then measured at 520 nm using a spectrophotometer, in the presence of a blank, the amount of reducing sugars (mg/g FW) measured reference to a standard curve of glucose constructed for the particular sugars under examination.

Determination of total soluble protein:

Total soluble protein was determined in leaves according to the method described by Bradford (1976). The absorbance was measured at 595 nm after 2 minutes. The weight of the protein (mg/g FW) was plotted against the corresponding absorbance resulting in a standard curve of Albumin bovine serum.

Determination of total and free phenolic compounds:

Total phenolic contents were determined in each extract using a Folin-Ciocalteu method described by Singleton, (1974). The absorbance was measured at 765 nm using a spectrophotometer. Phenolic contents were calculated based on the standard curve of gallic acid used as standard. Whereas, free phenolic compounds were extracted by the

method of Hartzfeld *et al.,* (2002) and Zhao *et al.,* (2006). The fresh leaves of geranium were extracted with 80% aqueous acetone for 20 min at room temperature. The mixture was centrifuged at 3000 rpm for 10 min, and then the supernatant was collected. The extraction was repeated three times. The supernatants were evaporated to dryness at 45 °C, and finally collected with methanol to a known volume. The extracts were stored by avoiding light at − 20°C until use. We added hexane to the residues after extraction of free phenolic. After centrifugation at 1500 rpm for 5 min, methanol /H_2SO_4 (90:10, v/v) was added to the residues at 70 °C for 1 h. The final solution was extracted with ethyl acetate three times. The organic fractions were combined and evaporated at 45 °C, before being collected with methanol and stored at −20 °C until use.

The variation in phenolic compounds content in geranium leaves by HPLC analysis

After 48 hours of treatment, in the case of the dual application technique under infested and un-infested soil conditions, randomized samples of the fresh geranium leaves were taken from each treatment (GA & 3,5-DNBZA) as well samples from each of the control (positive and negative) to quantify the phenolic compounds in geranium plants using HPLC analysis. Phenolic compounds were determined according to Goupy *et al.,* (1999) as follows; five grams of each tissue sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml were collected in vials for injection into HPLC Agillant 1260 equipped with auto sampling injector, solvent degasser, ultraviolet (U.V) detector set at 280 nm and the column temperature was maintained at 35°C. Separation and determination of phenolic acids were performed by HPLC, the solvent systems used were gradient of A (8% CH_3COOH/ H_2O), and B (acetonitrile). The separation was done with the following gradient at 0 - 20 min 5% B, 95% A, at 20 - 50 min,10% B, 90% A, at 50 - 55 min, 30% B, 70% A, at 55 - 100 min, 50% B, 50% A, at 100 - 120 min, 100% B the solvent flow rate was 1 ml/min and the separation was at 35°C, and the injection volume was 10 µl of the standards and extracts. Phenolic compounds were assayed by external standard calibration at 280 nm.

Antioxidant enzymes activities:

Glutathione peroxidase activity was measured according to Paglia and Valentine, (1967). Record the decrease of absorbance was at 340 nm/ min. Enzyme Activity (U/g) = (A_{340} / min ÷ 0.00622) x 121.

Catalase activity was assayed Spectrophotometrically according to Aebi, (1984) and Fossati., (1980), read sample

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

(A_{Sample}) against sample blank and standard ($A_{Standard}$) against Standard blank at 510 nm.

Catalase Activity: In Tissue (U/g) = (($A_{standard} - A_{Sample}$) ÷ $A_{standard}$) x (1÷ gm tissue).

Statistical analysis:

The analysis of variance (ANOVA) was conducted and the means of the treatments were compared using L.S.D. at 5% of the statistical analysis according to Snedecor and Cochran**, (**1980), using the SPSS program version 16. Data of the disease incidence (%) were transformed to angles using arcsine transformation before statistically analyzing.

$$\text{Angle} = \frac{\text{acrsin}}{\sqrt{\text{percentage}}}$$

RESULTS

1. *In Vitro* experiments:

Isolation, identification and frequency of the isolated microorganisms:

Isolation trials from infected geranium plants collected from EL-Qanater EL-Khayria Agrcultural Research Station at Qalubiya governorate yielded three different fungi species, in addition to one fungal-like organism. These were morphologically identified as *Fusarium semitectum* Berk & Rav., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Khun and *Pythium irregulare* Braun. The frequency of the isolated microorganisms from infected geranium plants is shown in Table (1), *F. semitectum* was the most frequent fungus (57.02%), followed by *R. solani* (22.81%). However, *M. phaseolina* and *P. irregulare* showed the lowest frequency, being 6.14 and 14.04%, respectively.

The antifungal effect of gallic acid and 3, 5-dinitrobenzoic acid on suppression the radial growth of *F. semitectum, M. phaseolina, R. solani* and *P. irregulare*.

Data in Fig. (1) demonstrate that the isolated microorganisms varied in their sensitivity to the tested organic acids, namely gallic acid (GA) and 3, 5-dinitrobenzoic acid (3, 5-DNBZA) at (300, 600 and 900 mg L^{-1}) when compared to the control. For the two organic acids, 3, 5-DNBZA, was the most effective one particularly when used at 600 and 900 mgL^{-1}, while, GA was the least effective at the tested concentrations.

Overall, the effect was more evident when the concentration of the organic acids was increased. *F. semitectum* was the most sensitive one, followed by *P. irregulare*. On the other hand, *M. phaseolina* and *R. solani* scored the lowest ones.

Table (1) Frequency of isolated microorganisms from diseased geranium plants collected from Qalubiya governorate.

Microorganisms	No. of isolates	Frequency (%)
Fusarium semitectum	65	57.02
Macrophomina phseolina	7	6.14
Pythium irregular	16	14.04
Rhizoctonia solani	26	22.81
Total	114	100.0

Our findings demonstrated that 3, 5-DNBZA has more antifungal efficacy than GA against the tested microorganisms under the investigation and that the antifungal activity of the examined organic acids was dosage-dependent.

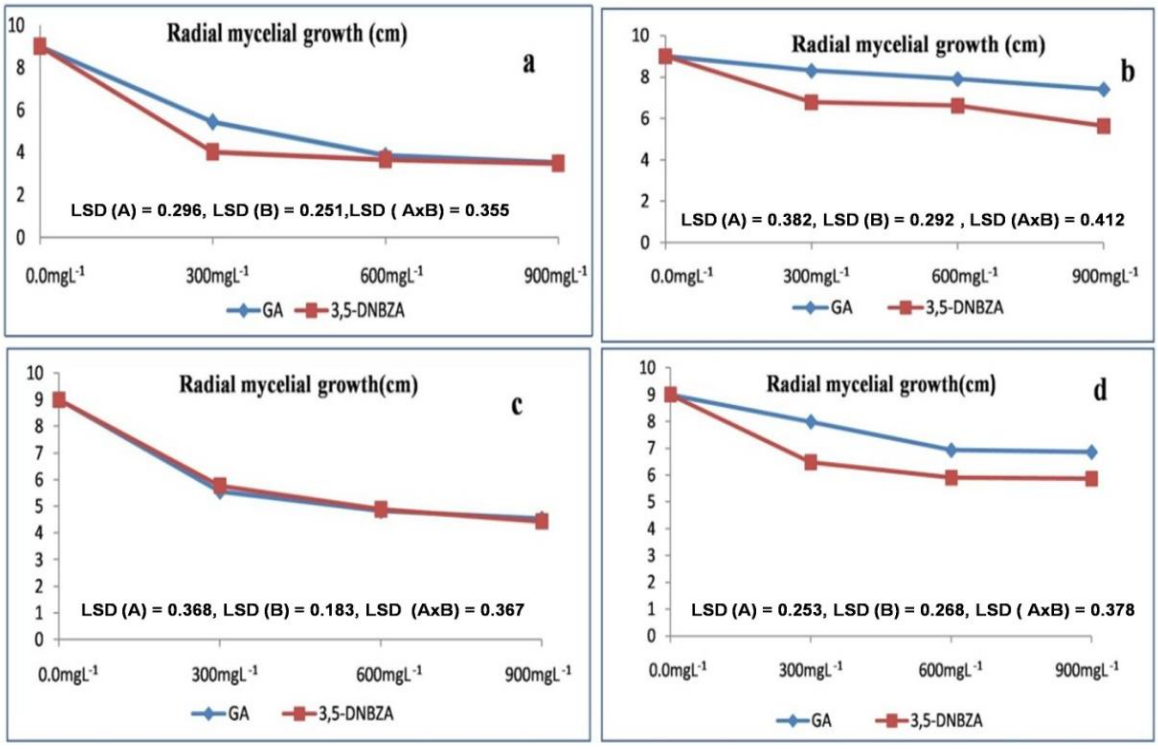

Fig. 1 Effect of organic acids (A). "gallic acid (GA) and 3, 5 dinitrobenzoic acid (3,5-DNBZA)" at three concentrations (B) on mycelial radial growth of (a): *F. semitectum*, (b): *M. phaseolina*, (c): *P. irregulare*, and (d): *R. solani*, respectively.

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

Evaluation of the antifungal activity of GA at 600 mgL^{-1} and 3,5-DNBZA at 300 mgL^{-1} on mycelial dry weight (MDW) (gm), the membrane integrity of the four tested pathogens, and their capacity to produce extracellular oxidative enzymes in culture filtrates.

The mycelial dry growth of the tested pathogens resulted in the liquid medium containing either GA or 3, 5-DNBZA was statistically suppressed at varying degrees compared with the control Fig.(2a). Remarkably, organic acids lengthened the period before the mycelium begins to develop. Regarding the tested pathogens, GA and/or 3, 5-DNBZA demonstrated the highest antifungal activity against *R. solani*, resulting in a considerable decrease in mycelial dry weight, in comparison to the other tested pathogens. It was evident that 3, 5-DNBZA had a stronger effect on decreasing the mycelial dry weight of pathogens than GA, except for *P. irregulare*.

Data illustrated in Fig. (2b) indicate that a significant variation was recorded in cell membrane integrity expressed as the membrane stability index (MSI%) for the tested pathogens in the presence or absence of GA and 3, 5-DNBZA. Results showed that 3, 5-DNBZA reduced the membrane potential of *R. solani* then *M. phaseolina* and *P. irregulare*, while *F. semitectum* was the most resistant fungus and retained its intact membrane. On the other hand, GA caused the highest strong disruption of *F. semitectum* membrane integrity compared with the other tested pathogens Fig. (2b). It was interesting to observe that *M. phaseolina*, *R. solani* and *P. irregulare* recorded the highest maintained intact membrane (permeability reached 98.280, 96.023 and 93.501%, respectively) in the treatment of GA compared with the control (permeability reached 60.868, 66.122, and 69.195%, respectively). Noteworthy results in Fig.(2a,b) showed that *R. solani* was more susceptible to the toxic effect induced by 3, 5-DNBZA than GA, as indicated by a marked inhibition of mat production and increased cell membrane permeability reaching 33.354 - 96.023%, respectively.

As shown in Fig. (2c,d), all isolates of pathogens tested were able to secret peroxidase (POD) and carboxymethyl cellulase (CMCase) enzymes in culture filtrates; however, their production potential was variable and was less active in *M. phaseolina* and *R. solani*, respectively than control (untreated). The results in Fig. (2c,d) exhibited clearly that GA and 3, 5-DNBZA significantly exerted an effect on the ability of these pathogens to secret peroxidase and CMCase enzymes compared with control, with no always significant difference ($P<0.05\%$) between GA and 3, 5-DNBZA. Results in Fig. (2c) show that GA and 3, 5-

DNBZA significantly had the highest influence in inhibition secretion of peroxidase for *R. solani* (0.012-0.018 mg/ml) followed by *P. irregulare* (0.023-0.079 mg/ml) compared with control (0.387-0.151 mg/ml), respectively. 3, 5-DNBZA was scored significantly as the most effective in inhibiting the secretion of peroxidase and CMCase enzymes for *F. semitectum* (0.005-1.929 mg/ml) compared with control (0.082-3.786 mg/ml), respectivelyFig. (2c,d).. When compared to the control, *P. irregulare* scored the lowest secretive of CMCase enzyme in culture filtrate under GA stress conditions followed by 3, 5-DNBZA Fig. (2d).

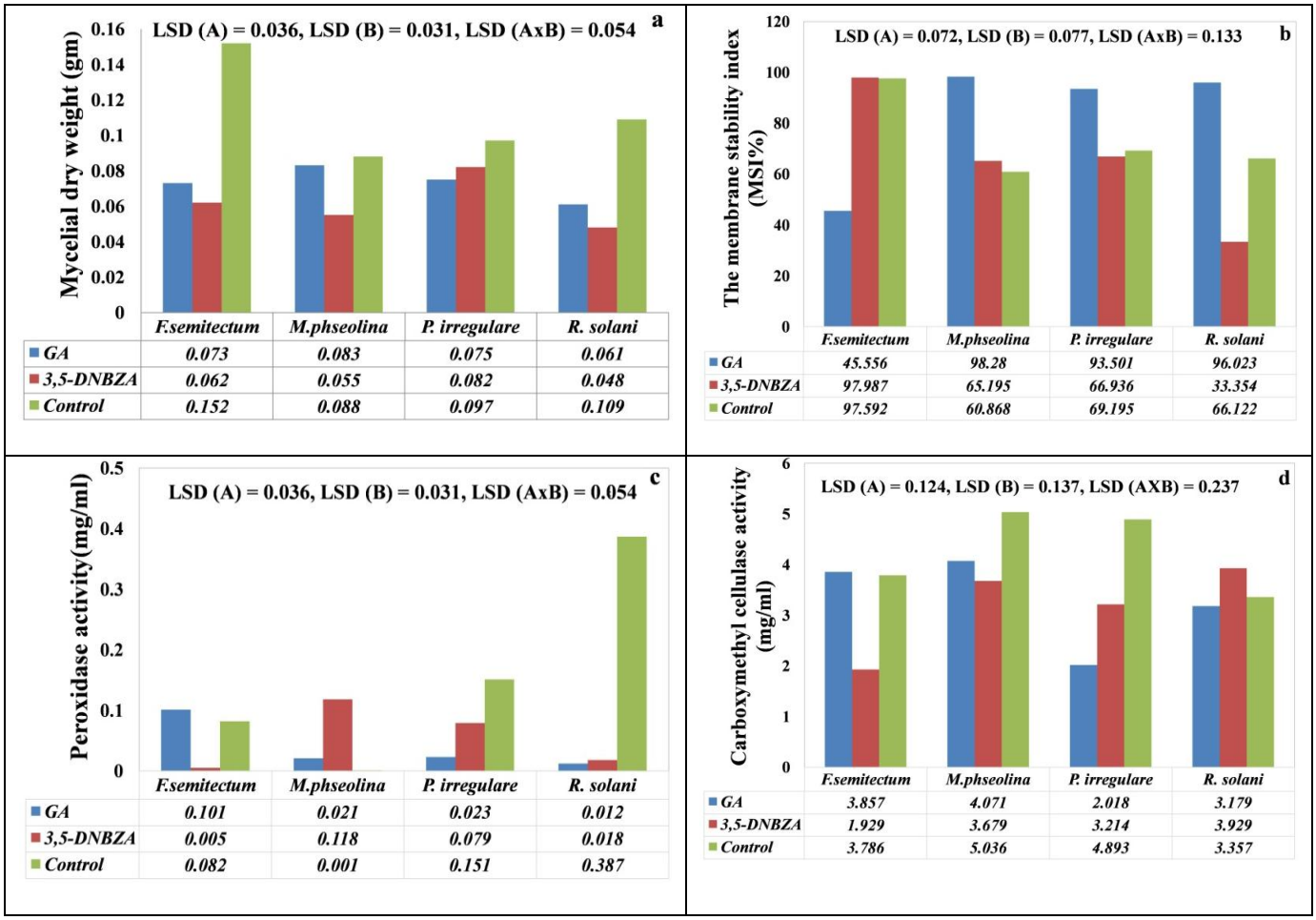

Fig.2 Effect of organic acids (A) "gallic acid (GA) and 3,5dinitrobenzoic acid (3,5-DNBZA)" on changes in some physiological and biochemical characteristics of the four tested pathogens (B). (a): Mycelial dry weight (gm), (b): The membrane stability index (MSI%), (c): Peroxidase activity(mg/ml), and (d): Carboxymethyl cellulase activity (mg/ml).

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

2. *In Vivo* experiments:

Pathogenicity test:

Data presented in Table (2) indicate that all isolates of microorganisms either alone or in a mixture were able to infect geranium plants at a wide variation in the percentages of infection and cause root rot symptoms compared with negative control under artificially infested soil conditions, it was observed that infection levels were increased gradually over time. Significant differences were obtained between percentages of infection caused by the pathogens' mixture and those recorded from using each pathogen alone. The pathogens' mixture was more virulent (34.31%), than each pathogen alone followed by *F. semitectum* (22.36%) then *M. phaseolina* and *R. solani* (19.47%), each alone however, *P. irregulare* showed the lowest infection (12.98%) at 120 days after planting. From the obtained data Table (2), the pathogens' mixture recorded a high root rot incidence of geranium plants (22.36, 24.73, and 34.31%) at different ages of growth (60, 90, and 120 days) compared to the tested pathogens each alone. Accordingly, it was selected to complete further studies based on their pathogenic ability.

Table (2): Pathogenicity test of the tested pathogens in artificially infested soil of geranium plants 60, 90, and 120 days after planting.

Pathogens	Disease incidence % after					
	60 days		90 days		120 days	
	*O	**T	*O	**T	*O	**T
F. semitectum	7.41	12.98	11.11	19.47	14.81	22.36
M. phaseolina	3.70	6.49	7.41	12.98	11.11	19.47
P. irregulare	3.70	6.49	7.41	12.98	7.41	12.98
R. solani	7.41	12.98	11.11	19.47	11.11	19.47
pathogens' mixture	14.81	22.36	18.52	24.73	33.33	34.31
+Negative control	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D. at 5%	1.381	0.694	1.209	0.712	0.781	0.834

*O: original results, (percentage), **T: Transformed results (arcsine), + Negative control (geranium plants grown in un-infested soil).

Follow up the development of root rot disease incidence of geranium plants grown in infested soil with the pathogens' mixture during the two growing seasons

Geranium plants were found to be severely affected by the root rot disease Fig.(.3**)** during the winter and summer seasons in 2017/2018 and 2018/2019. Figure 3(i) shows

that the disease incidence (%) was progressively increased with increasing time and just after the cut. The maximum percentage of the disease incidence was recorded in the summer season, ten months after planting (the second cut) in the seasons being, 77.78% in the first season and 81.48% in the second season, respectively. Meanwhile, the minimum percentage of the disease incidence was recorded in the winter season, six months after planting (the first cut) being, 48.14% in the first season and 51.85% in the second season, respectively. Generally, the infected plants showed gradual yellowing of the lower leaves margins, then dark brown dry rotting on the roots, lack of a lateral root system, and stunting followed by wilting and dryness of some branches, leading to premature drying of plants and then often death Fig. 3(ii). The previous results are confirmed by quadratic regression analysis (response curve model) between disease incidences (%) and time of the season (months). Results revealed that disease incidence (%) was significantly explained ten months across the two cuts in the two seasons where the coefficients of determination (R^2%) recorded 0.9943 and 0.9824, respectively. The highest values of coefficients of determination (R^2%) indicated the goodness of fit of the response surface model. This result pointed out that the disease incidence (%) was gradually increased from winter to summer Fig. 3(i).

Evaluation the effect of GA and 3, 5-DNBZA at three concentrations on development of root rot disease incidence:

Results in Table (3) show that the tested organic acids at the three concentrations (300, 600, and 900 mgL^{-1}) and Topsin-M 70% fungicide caused a significant reduction in the disease incidence (%) compared with the positive control during the growing season (2018/2019) for the first and second cuts. The efficacy of the two tested organic acids was found to be variable according to the concentration and the type of the acids tested. GA was more effective than 3, 5-DNBZA in controlling the root-rot in both cuts. GA at 600 mgL^{-1} was the superior treatment where it gave the lowest disease incidence for both the first and the second cut (6.49, 27.62% respectively), followed by Topsin-M 70% fungicide only in the first cut. Meanwhile, 3, 5-DNBZA at 300 mgL^{-1} gave the best results for controlling root-rot (22.36, 29.80%, respectively) in this concern.

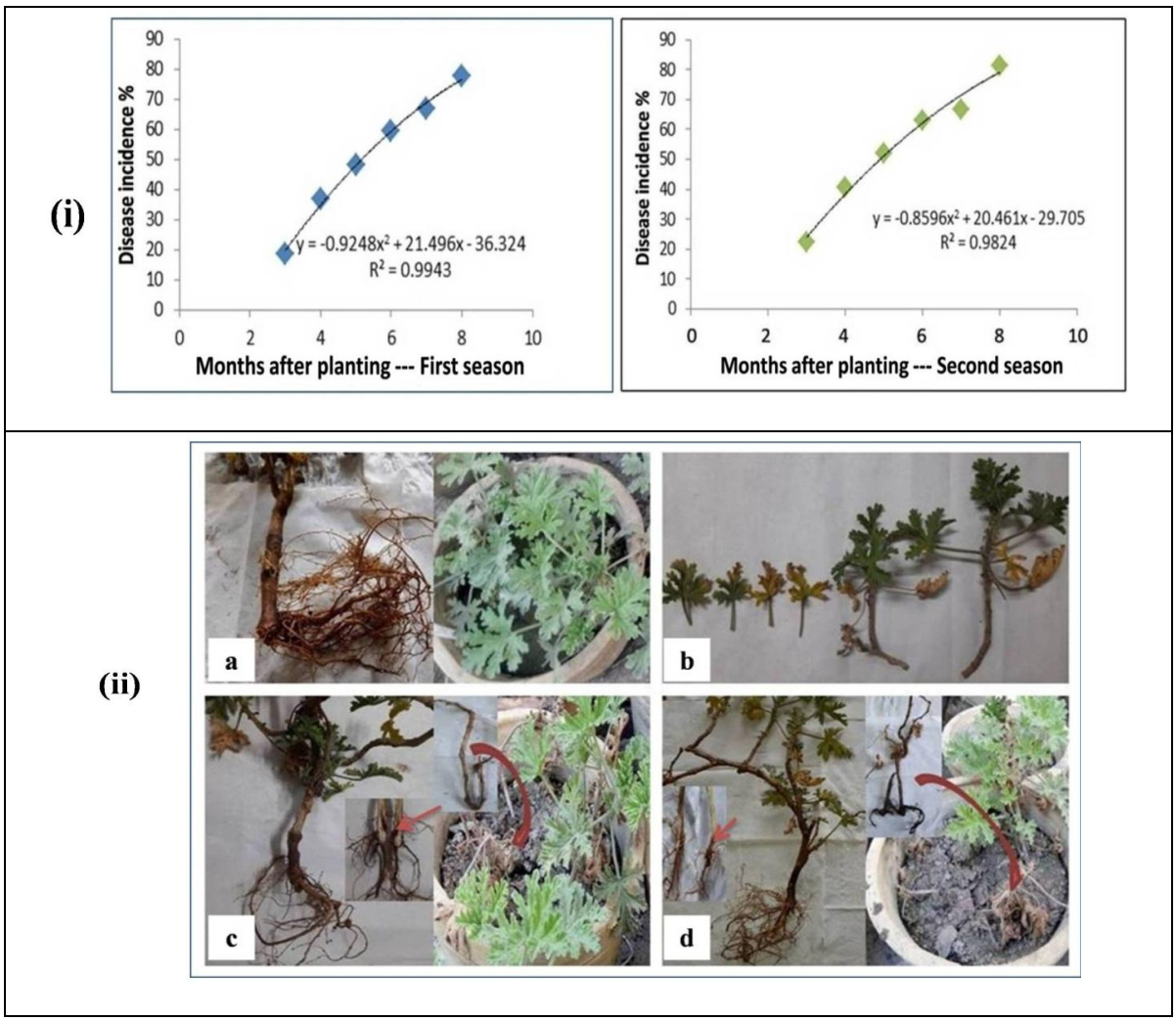

Fig. 3 (i): Follow up the root rot incidence and progress on geranium plants grown in artificially infested soil with the pathogens' mixture during the two growing seasons, (ii): Observation of pathological symptoms in infected plants (geranium plants at six- and ten-months age), (a): Healthy geranium plants, (b): Development stages of symptoms on infected leaves, (c): Typical symptoms of root rot on geranium plants at six-month-old under the pathogens' mixture stress, and (d): Typical symptoms of root rot on geranium plants at ten-month-old under the pathogens' mixture stress.

Table (3): Effect of GA and 3, 5-DNBZA at three concentrations on the root-rot disease incidence of geranium plants grown in artificially infested soil with the pathogens' mixture.

Treatments	Conc. (mgL^{-}	Disease incidence %			
		1st cut		2nd cut	
		*O	**T	*O	**T
GA	300	22.22	27.62	40.74	38.87
	600	3.70	6.49	22.22	27.62
	900	14.81	22.36	25.92	29.80
3,5-DNBZA	300	14.81	22.36	25.92	29.80
	600	25.92	29.80	48.14	43.94
	900	33.33	35.07	48.14	43.94
Topsin –M70%	2 g/L	11.11	19.47	33.33	35.07
$^{+}$Positive control	-	48.14	43.94	74.06	59.30
$^{++}$Negative control	-	0.0	0.0	0.0	0.0
L.S.D.at 5%:		0.818	0.739	0.136	3.762

*O: original results, (percentage), **T: Transformed results (arcsine), $^{+}$ Positive control (un-treated geranium plants grown in infested soil), $^{++}$Negative control (un-treated geranium plants grown in un-infested soil).

Effectiveness of the exogenous application techniques (single and dual) of GA at 600 mgL^{-1} and 3, 5- DNBZA at 300 mgL^{-1} to manage root rot of geranium plants, under pot experiment conditions during the growing season (2020/2021).

Development of root rot incidence and disease severity (%) of geranium plants.

According to the results shown in Table (4), the exogenous GA 600 mgL^{-1} and 3, 5-DNBZA 300mgL^{-1} treatments played a vital role in enhancing geranium plant resistance without any phytotoxicity symptoms. In general, dipping cuttings alone or combined with soil drench treatments with GA or 3, 5-DNBZA were statistically significant and had a remarkable impact in reducing the incidence and severity of root rot, being 27.78, 22.22%, and 12.22 ,8.89% , respectively, compared to a positive control (untreated plants), being 44.44-20.00%, respectively under artificially soil infestation conditions, during the growing season 2020/2021Table (4) and Fig. (4). In the single application technique, GA was more effective than 3, 5-DNBZA in reducing the percent of disease incidence and severity (%), actually delaying the progression of root rot symptoms,

reaching 22.22, 11.11% and 33.33, 13.33%, respectively. Except for the 3, 5-DNBZA treatment, all exogenous application techniques (single and dual) showed equal pattern protection against root rot incidence. The results revealed a positive relationship between disease severity and the dual application method, which recorded the lowest disease severity % due to GA and 3, 5-DNBZA (8.89%), respectively. The final results demonstrated that the dual application approach was superior to the single application method at providing potent preservation against root rot incidence and disease severity of geranium (22.22, 27.78%) and (8.89, 12.22%), respectively. This strategy also slowed the spread of the disease symptoms throughout the growing season and relieved the harmful effects of the phytopathogens' mixture on geranium plants Fig.(4).

Growth parameters of geranium plants

The experiment's data in the 2020–2021 season indicated that the single and dual application techniques have statistically influenced the improvement of the morphological characteristics and oil yield (ml/plant) of geranium plants cultivated in infested or un-infested soil compared to the control (positive and negative) Figures (5 and 6), the differences were not always statistically significant between organic acids treatments under infested soil stress.

Table (4): Effect of the exogenous application techniques of GA and 3, 5-DNBZA on the changes in resistance induction and severity (%) for geranium plants in infested soil.

Parameters / *A.M. / Treat.	Disease assessment								
	Disease incidence% $(O)^{+}$			Disease incidence% $(T)^{++}$			Disease severity%		
	GA	3,5 DNBZA	Mean (B)	GA	3,5-DNBZA	Mean (B)	GA	3,5-DNBZA	Mean (B)
**The single method	22.22	33.33	27.78	28.13	35.26	31.70	11.11	13.33	12.22
***The dual method	22.22	22.22	22.22	28.13	28.13	28.13	8.89	8.89	8.89
****Positive control	44.44	44.44	44.44	41.81	41.81	41.81	20.00	20.00	20.00
Mean(A)	29.63	33.33	---	32.69	35.07	---	13.33	14.07	---
L.S.D.	A=0.170; B=0.073; AXB=0.103			A=0.064; B=0.042; AxB=0.060			A=0.509; B=0.440; AXB=0.622		

*A.M.: Application methods; ** The single method: dipping cuttings; ***The dual method: dipping cuttings plus soil drench; ****Positive control (un-treated geranium plants grown in infested soil); O^{+}: original results (percentage); T^{++}: transformed results (arcsine). (A): Treatments "GA and 3, 5-DNBZA", and (B): Application methods "A.M".

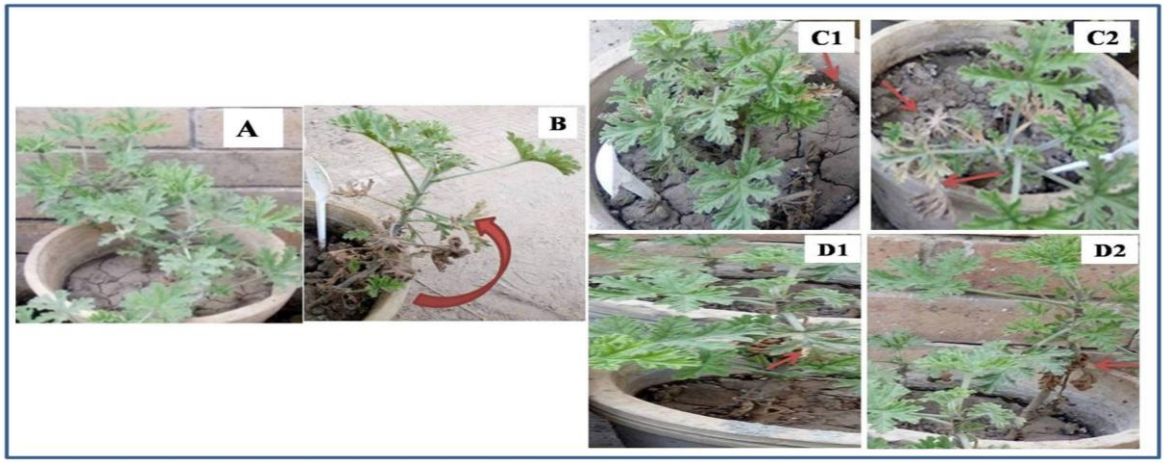

Fig. 4 Effect of the exogenous application techniques of GA and 3, 5-DNBZA against root rot of geranium plants grown in infested soil with the fungal mixture, significantly reduced the disease severity (%), and displayed minimal symptoms throughout the experiment under single and/or dual conditions, respectively, A): Healthy geranium plant, B): Infected plants (positive control);un-treated geranium plants grown in infested soil, (C1 and C2): Geranium plants treated with GA and 3, 5-DNBZA grown in infested soil under single technique conditions, respectively, and (D1 and D2): Geranium plants treated with GA and 3, 5-DNBZA grown in infested soil under dual technique conditions, respectively.

Despite some differences in their effects, the dual application technique was significantly more efficient than the single technique for increasing the fresh and dry weights of herbs and roots per plant and fresh and dry weights per plant, especially in the GA-treated plants grown under infested soil conditions, according to the results in Fig. (5), respectively. In the dual application method, GA was the superior treatment for the highest plant height and the largest leaf area in this regard since it gave 40.03cm^2 , 458.86 cm^2, respectively, compared to 3, 5-DNBZA treatment, which gave 35.35cm^2 , 416.27cm^2, under infested soil conditions Fig. (6a,b). With a few exceptions, gallic acid treatment substantially increased all plant growth parameters under single and dual-application techniques, whereas 3,5-DNBZA treatment only showed a similar pattern in the case of the dual-application method. Overall, the information in Figures (5 and 6) indicate that under treatments (GA or 3, 5-DNBZA) and pathogens stress conditions, leading to changes in biomass allocation will influence the equilibrium between root and shoot growth.

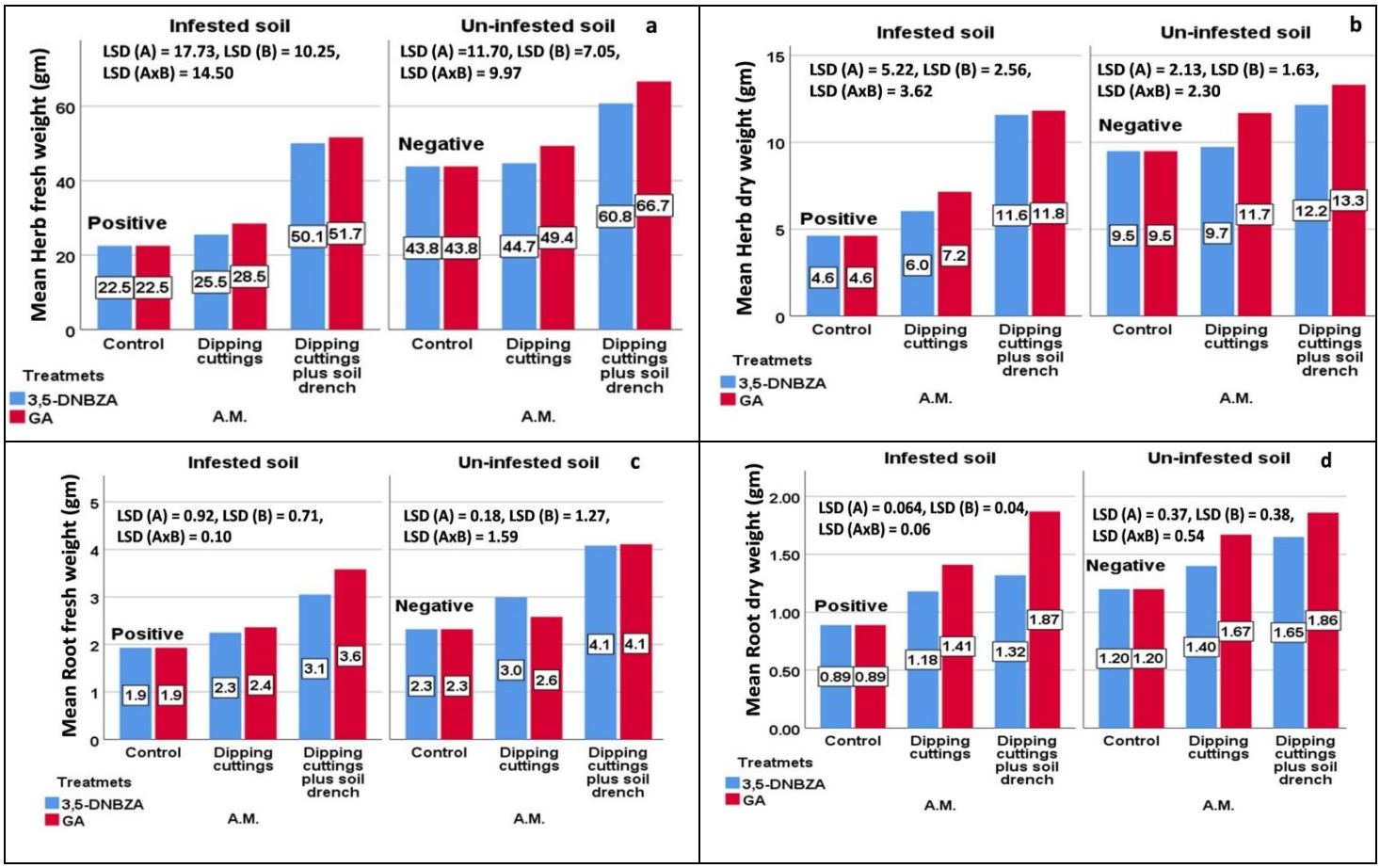

Fig. 5 Interaction effect between elicitors "Treatments" (A). "gallic acid (GA) and 3, 5-dinitrobenzoic acid (3, 5-DNBZA)" and application methods "AM" (B) *i.e.*"single and dual" on improving some growth parameters of geranium plants under infested and un-infested soil conditions. (a,b): Mean fresh and dry weights of herbs, respectively, and (c,d): Mean fresh and dry weights of roots ,respectively. Dipping cuttings; (the single application method), Dipping cuttings plus soil drench; (the dual application method), Positive control (un-treated geranium plants grown in infested soil), and Negative control (un-treated geranium plants grown in un-infested soil).

According to the results in Fig. (6c,d),, under infested soil stress conditions, the dry weight of shoot/root ratio, and the oil yield (ml/plant) were superior in 3, 5-DNBZA - treated plants than GA-treated plants under application techniques (single and dual) conditions. These results indicate that the variation in the morphological characteristics of geranium plants may be due to the type of acid, their concentrations used, as well as methods and timing of application and causative microorganisms of root rot disease.

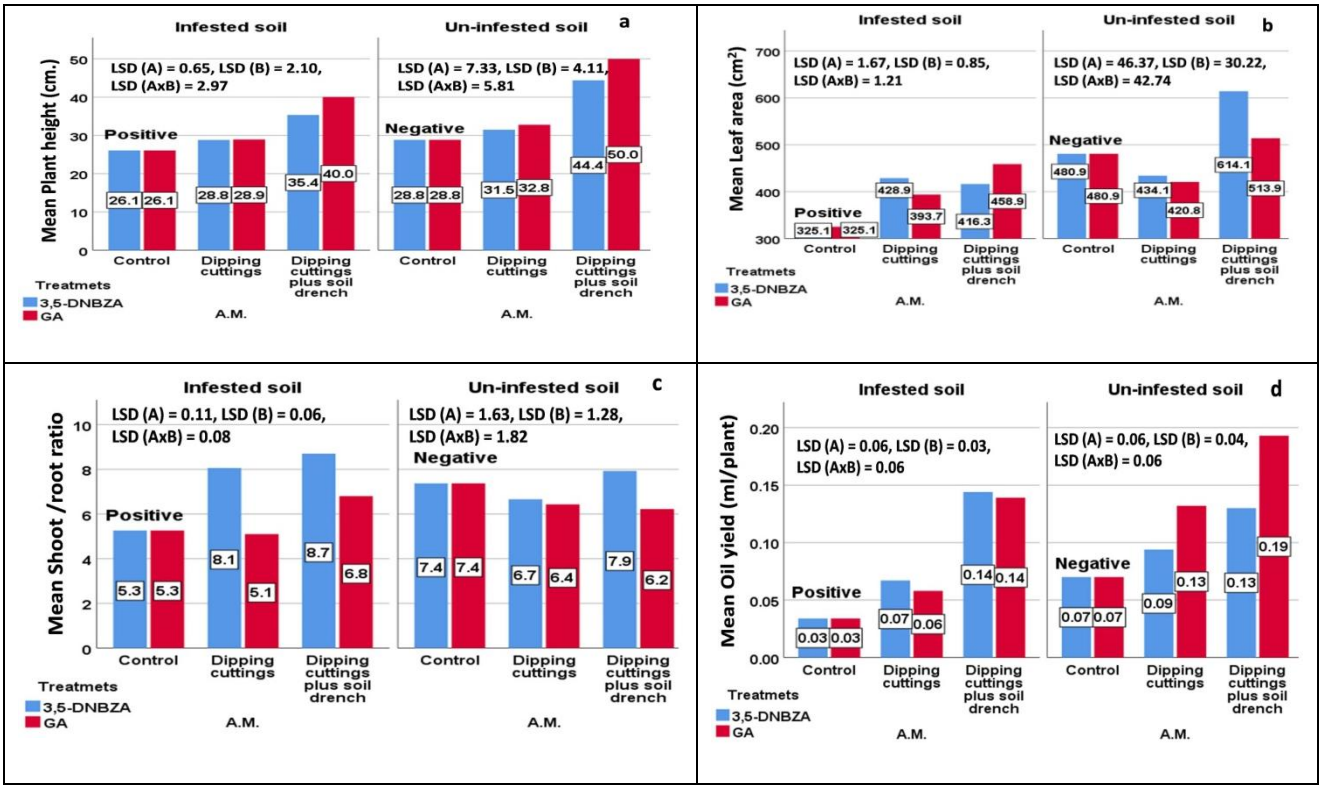

Fig. 6 Interaction effect between elicitors "Treatments" (A) "gallic acid (GA) and 3, 5 dinitrobenzoic acid (3, 5-DNBZA)" and application methods "AM" (B) *i.e.* "single and dual" on improving some growth parameters and enhancing oil yield (ml/plant) of geranium plants under infested and un-infested soil conditions. (a): Mean plant height (cm), (b): Mean leaf area (cm^2), (c):Mean shoot/root ratio, and (d): Mean oil yield (ml/plant). Dipping cuttings; (the single application method), Dipping cuttings plus soil drench; (the dual application method), Positive control (un-treated geranium plants grown in infested soil), and Negative control (un-treated geranium plants grown in un-infested soil).

Effectiveness of the exogenous application techniques (single and dual) of GA and /or 3, 5-DNBZA on stimulation of geranium plants defense mechanisms.

1- On total, reducing sugars and total protein.

Data presented in Table (5) show an increase in total, reducing sugars and total protein in the single and dual application of GA and /or 3, 5-DNBZA in geranium plants compared to the negative control under un-infested soil conditions. On the other hand, the positive control was the highest when the plants were grown in infested soil; the dual method consistently demonstrated a significant increase when compared to the single method. In the dual application method, GA treatment caused increment in this regard since it gave 10.63 and 7.73 mg/g FW for total and reducing sugars,

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

respectively, compared to 3, 5-DNBZA treatment, which gave 10.11and 7.38 mg/g FW under fungal mixture stress conditions. Total protein showed a similar pattern in the case of the dual-application method in un-infested soil but no significant increase among GA, 3, 5-DNBZA, and positive control was recorded when plants were grown in infested soil conditions. The results in Table (5) show a correlation between leaf senescence and sugar status under pathogens stress and that GA and 3,5-DNA are more effective at reducing leaf senescence compared with the positive control.

Table (5) Changes in total, reducing sugars, and total protein in leaves of geranium plants treated with GA and 3, 5-DNBZA grown in infested and un-infested soil under single and dual application.

Parameters / *A.M. / Treat.	Plants grown in infested soil								
	Total sugars (mg/g FW)			Reducing sugars (mg/g FW)			Total protein (mg/g FW)		
	GA	3,5-DNBZA	Mean (B)	GA	3,5-DNBZA	Mean (B)	GA	3,5-DNBZA	Mean (B)
**The single method	9.32	8.87	9.09	7.31	6.50	6.90	5.50	4.66	5.08
***The dual method	10.63	10.11	10.37	7.73	7.38	7.55	5.10	5.57	5.33
+Positive control	15.11	15.11	15.11	9.77	9.77	9.77	5.35	5.35	5.35
Mean(A)	11.68	11.36	--	8.27	7.88	--	5.31	5.19	--
L.S.D.	A=0.257; B=0.163; AXB=0.231			A=0.333; B=0.307; AXB=0.434			A=0.064; B=0.126; AXB=0.179		
Plants grown in un-infested soil									
**The single method	6.58	5.47	6.02	4.92	2.33	3.62	4.46	4.58	4.52
***The dual method	8.13	6.39	7.26	6.53	4.57	5.55	3.98	4.31	4.14
++Negative control	4.17	4.17	4.17	2.10	2.10	2.10	3.82	3.82	3.82
Mean(A)	6.29	5.34	--	4.51	3.00	--	4.08	4.23	--
L.S.D.	A=0.231; B=0.119; AXB=0.168			A=0.444; B=0.206; AXB=0.292			A=1.465; B=0.924; AXB=1.307		

*A.M.: Application methods; ** The single method: dipping cuttings; ***The dual method: dipping cuttings plus soil drench, +Positive control (un-treated geranium plants grown in infested soil), ++Negative control (un-treated geranium plants grown in un-infested soil), FW: Fresh weight. (A): Treatments "GA and 3, 5-DNBZA"and (B): Application methods "A.M".

2- On total and free phenols.

Data illustrated in Table (6) show a significant increase in both total and free phenol compounds in treatments with GA and 3,5-DNBZA compared with positive and negative control in single and dual applications. Thus, the highest accumulation of total phenols was recorded with positive control then GA and 3, 5-DNBZA (7.77, 7.32, and 7.23 mg/g FW, ,respectively). Free phenols showed a similar pattern in the case of

the dual-application method in infested soil conditions (6.21, 6.00, and 4.98 mg/g FW, respectively) whereas the lowest were recorded with negative control.

Table (6) Changes in total phenols and free phenols in leaves of geranium plants treated with GA and 3, 5-DNBZA grown in infested and un-infested soil under single and dual application

Parameters *A.M. / Treat.	Plants grown in infested soil					
	Total phenols (mg/g FW)			Free phenols (mg/g FW)		
	GA	3,5-DNBZA	Mean(B)	GA	3,5-DNBZA	Mean(B)
**The single method	6.76	6.04	6.40	4.61	4.40	4.50
***The dual method	7.32	7.23	7.27	6.00	4.98	5.49
+Positive control	7.77	7.77	7.77	6.21	6.21	6.21
Mean(A)	7.28	7.01	--	5.60	5.19	--
L.S.D.	A=0.176; B=0.246; AXB=0.347			A=0.257; B=0.352; AXB=0.498		
Plants grown in un-infested soil						
**The single method	5.63	4.85	5.24	4.33	2.43	3.37
***The dual method	5.67	5.49	5.58	4.35	4.32	4.33
++Negative control	4.10	4.10	4.10	2.35	2.35	2.35
Mean(A)	5.13	4.81	--	3.67	3.03	--
L.S.D.	A=0.170; B=0.292; AXB=0.413			A=0.184; B=0.119; AXB=0.168		

*A.M.: Application methods; ** The single method: dipping cuttings; ***The dual method: dipping cuttings plus soil drench, +Positive control (un-treated geranium plants grown in infested soil), ++Negative control (un-treated geranium plants grown in un-infested soil), FW: Fresh weight. (A): Treatments "GA and 3, 5-DNBZA"and (B): Application methods "A.M".

3-The variation in phenolic compounds content in geranium leaves

According to HPLC analysis, the contents of phenolic compounds, *i.e.,* quinic acid, tannic acid, gallic acid, benzoic acid, and resorcinol acid, were found in varying proportions in untreated geranium plants, GA and 3, 5-DNBZA-treated plants under the dual application technique planted in infested and/or un-infested soil Table (7). Under pathogens stress, GA - treated plants recorded the highest concentrations of quinic acid, tannic acid, and benzoic acid compared to 3, 5-DNBZA-treated plants under the dual application technique condition. In the dual application method, the gallic acid level was considerably lower in 3, 5-DNBZA-treated plants (3.65µg/g) than in GA-treated plants (5.57µg/g) grown under soil infestation conditions. Furthermore, the amount of gallic acid, quinic acid, tannic acid, and benzoic acid was more in geranium plants cultivated under

pathogens stress than in healthy ones. The results of the dual application method presented in Tables (4 and 7), reveal a strong positive correlation between the root rot disease incidence and the phenolic compounds in GA- and 3, 5-DNBZA-treated geranium plants, which indicates a defense function.

Table (7) Changes in the concentration of phenolic compounds in leaves of geranium plants treated with GA- and 3, 5-DNBZA grown in infested and un-infested soil during the growing season (2020/2021), under dual application.

Compounds / Treatments	Phenolic compounds (µg/g FW)							
	Apegenin	Gallic acid	Quinic Acid	Tannic Acid	Benzoic acid	Chlorgenic Acid	Cinnamic Acid	Resorcinol acid
Plants grown in infested soil								
GA	NA	5.57	459.0	166.4	368.9	NA	NA	2600.8
3,5-DNBZA	NA	3.65	415.3	162.6	357.7	NA	NA	NA
*Positive control	NA	5.61	420.8	154.7	335.9	NA	NA	975.3
Plants grown un infested soil								
GA	NA	16.5	1054.6	440.3	80.0	NA	NA	8127.0
3,5-DNBZA	NA	7.13	699.5	340.1	153.1	NA	NA	3901.2
** Negative control	NA	2.8	349.7	120.5	211.7	NA	NA	NA

*Positive control **(**un-treated geranium plants grown in infested soil**),** **Negative control (un-treated geranium plants grown in un-infested soil**).**

4- On peroxidase and catalase activities

Results in Table (8) reveal that treatments with GA and 3, 5-DNBZA reflected a significant increase in peroxidase and catalase activities compared with negative control which support the ability of GA and 3, 5-DNBZA in dual application to promote the plant defense mechanisms as signaling molecules. Data indicated that the oxidative action of pathogens on plants (positive control) grown in infested soil as an increase in peroxidase and catalase activities at 0.098 and 0.105 $U/g^{-1}FW$, respectively. On the other hand, data showed that the single and dual treatments with GA and 3, 5-DNBZA represented a decrease in peroxidase and catalase activities compared with positive control plants grown in infested soil but more than those grown in un-infested soil.

Table (8) Changes in peroxidase, catalase activities in leaves of geranium plants treated with GA and 3, 5-DNBZA grown in infested and un-infested soil under single and dual application.

Parameters / *A.M. / Treat.	Plants grown in infested soil					
	Peroxidase ($Ug^{-1}FW$)			Catalase (Ug^{-1} FW)		
	GA	3,5-DNBZA	Mean(B)	GA	3,5-DNBZA	Mean(B)
**The single method	0.027	0.026	0.026	0.082	0.066	0.074
***The dual method	0.048	0.039	0.043	0.095	0.088	0.091
+Positive control	0.098	0.098	0.098	0.105	0.105	0.105
Mean(A)	0.057	0.054	--	0.055	0.086	--
L.S.D.	A=0.064; B=0.042; AXB=0.060			A=0.064; B=0.042; AXB=0.060		
Plants grown in un-infested soil						
**The single method	0.023	0.012	0.022	0.048	0.038	0.043
***The dual method	0.025	0.014	0.019	0.052	0.040	0.046
++ Negative control	0.01	0.01	0.01	0.037	0.037	0.037
Mean(A)	0.019	0.012	--	0.045	0.038	--
L.S.D.	A=0.064; B=0.042; AXB=0.060			A=0.064; B=0.042; AXB=0.060		

*A.M.: Application methods; ** The single method: dipping cuttings; ***The dual method: dipping cuttings plus soil drench, +Positive control (un-treated geranium plants grown in infested soil), ++Negative control (un-treated geranium plants grown in un-infested soil), FW: Fresh weight. (A): Treatments "GA and 3, 5-DNBZA"and (B): Application methods "A.M".

Discussion

One of the promising approaches in this area is enhancing plant resistance by using the best strategies for applying exogenous abiotic elicitors to maximize the application of alternative management techniques to control soil-borne pathogens (Frackowiak *et al*., 2019 and Li *et al*., 2021). Polyphenolic compounds and their derivatives are a class of the most significant and common plant allelochemicals in the ecosystem due to high biological activities, and protective effect against diseases-induced oxidative stress, which are a safe alternative to chemical fungicides for managing phytopathogenic fungi that don't harm the environment or soil microbes (De La Rosa *et al*., 2019; Deepmala, 2019 and Nehela *et al*., 2021).

This study showed that *F. semitectum, M. phaseolina, P. irregulare*, and *R. solani*, which cause the basal stem and root rot diseases, with variation among them, were the most dominant pathogens threatening geranium plant cultivation in El-Qanater El-Khayria Agricultural Research Station Qalubyia governorate. This result coincided with previous studies that confirmed the danger of *Fusarium* spp., *M. phaseolina, Phytophthora cinnamomi,*

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

Pythium splendens, Verticillium dahlia, and *R. solani* as dominant soil-borne pathogens attacking geranium plants at five governorates in Egypt (Hilal, 1985 and Imara, 2000). The same results were also obtained by (Prasad and Singh, 2014 and Adolf, 2016). The pathogens' mixture had a synergistic action since the infection percentages were significantly increased more than that caused by each pathogen alone in the pathogenicity test, which resulted in the highest plant death percent and similar typical symptoms in naturally infected fields. A similar trend was found by (Imara, 2000 and Dewidar *et al.,* 2019). The current study indicated that the growing season played an influential role in the emergence and severity of diseases, as well as in the host plants. The disease incidence (%) was gradually increased from winter to summer in 2017 /2018 and 2018/2019, as confirmed by the quadratic regression analysis, and the increasing incidence of the root rot disease coincided with the periods just after the first and second cuts in both seasons. This could be due to a relatively higher temperature, which could increase the percentage of disease incidence and coinciding with the temperature increase could be more optimum for penetration followed by infection and disease development as reported by (Hilal,1985 and Prasad and Singh, 2014), that the disease intensity was high in the old plants, with severe rotting on the apical shoots and wilting on the young plants, which could be due to high temperature during the summer and/or after the cut, the new growth may become more susceptible. Several studies have found that temperature and pH can affect a pathogen's ability to secrete effecter proteins (Louis *et al.,* 2014). The effector proteins secreted by phytopathogens play a significant role in the interaction between pathogens and host-plant, as well as disease progression. Effector proteins can alter the cellular environment of the host once they have entered it, working in the extracellular matrix to facilitate infection and colonization, while also suppressing the host's immune response (Białas *et al.,* 2018). This result is in line with the reports of (Prasad *et al*., 2010) that root rot incidence was the highest in the summer, whereas stem rot incidence was maximal in the winter. The timing of planting and the season significantly impact the type, severity, and rate of disease spread (Kalra *et al.*, 1992).

Based on the chemical structure of phenolic compounds, hydroxyl groups' number, location, and presence have been believed to be related to their relative toxicity to microorganisms, confer antioxidant characteristics, and anti-microbial activity (Cowan, 1999; Kumar and Goel, 2019 and Parvin *et al*., 2022). In this study, analyzing the effect of gallic acid and 3, 5-dinitrobebzoic acid showed potent dose-

dependent fungistatic activity against the four tested phytopathogens, which varied in their sensitivity *in vitro*. Noteworthy, organic acids have also lengthened the time before the mycelium begins to grow. GA at 600 mgL^{-1} and 3, 5-DNBZA at 300 mgL^{-1} exhibited optimal fungistatic activity. With a few notable exceptions, 3, 5-DNBZA at 300 mgL^{-1} effectively altered the physiological and biochemical characteristics of the pathogens, roughly similar to GA at 600 mgL^{-1}, which was not always a significant difference between them. In convention with these results, GA, a tri-phenolic molecule with a low molecular weight, is emerging as an efficient at inducing apoptosis (Gharib *et al.*, 2018). The antibacterial and other biological activities of gallic acid appeared to be linked with the hydrolysis of ester linkage between gallic acid and polyphenols such as tannins hydrolyzed, inhibit fungal membrane ergosterol biosynthesis, as well GA possessed antifungal activities and inhibit the mycelial radial growth against a wide range of phytopathogenic fungi in a dose-dependent manner (Osorio *et al.*, 2010; Li *et al.*, 2017 and EL-Nagar *et al.*, 2020). Furthermore, GA significantly suppressed the growth of *Trichophyton rubrum*, and destroys its structure by changing the gene expression profile of *T rubrum*, specifically (Fan *et al.*, 2023), and suppressed *Aspergillus flavus* growth and aflatoxin formation by significantly inhibiting the expression of *far B* gene (Zhao *et al.*, 2018). Previous reports have suggested that based on their biological characteristics and structural activity of BZA derivatives contribute to their antifungal action, which exhibited potent dose-dependent fungistatic activity against *Alternaria solani* which causes early blight in tomato plants (Nehela *et al.*, 2021). According to a study on the relationship between structure and activity, adding a methyl, methoxyl, or chloro group to the aromatic ring's position four or esterifying the carboxylic acid with an alkyl group, respectively, improved the antifungal activity of benzoic and gallic acids against *Aspergillus* spp. (Kim *et al.*, 2010). However, this antifungal activity is related to the structural characteristic of BA derivatives, which affect the fungal cell cycle by preventing cell separation during cell division against *Alternaria solani* (Laborda *et al.*, 2019 and Nehela *et al.*, 2021). Another mechanism of the primary action for antifungal activity of benzoic acid and its derivatives against pathogenic fungi and yeast depends upon is to cause energy loss and ATP depletion as well as concentrations used (Warth, 1991 and Nehela *et al.*, 2021). We found that the cell membrane integrity of the tested pathogens varied significantly in the presence of GA and 3, 5-DNBZA compared with the control. According to our findings, preceding investigations reported that acetic acid significantly inhibited the

growth of *Colletotrichum* spp., thus the fungus can regrowth when acetic acid has been removed, suggesting that the growth inhibition was more closely related to the inhibition of respiration than structural damage of the cell (Kang *et al*., 2003).

Omar *et al.,* (2018) also mentioned that, the degree of inhibition may vary from one fungus to another, depending on the fungal species and their structural features and susceptibility. Furthermore, it is known that anions that interfere with the dissociation of the acid molecule enhance the action of organic acids as antimicrobial agents; certain specific cations may also significantly increase the effectiveness of organic acids by increasing the acid's solubility in the microbial cell membrane (Leon *et al.,* 1993). However, we presume that 3, 5-DNZA has antifungal activity due to their chemical structure, which involves as yet unidentified mechanism. Polyphenolic acids affect the fungal cell's acidification, stop or modify metabolic activities of various cell components, and impair fungal ability to produce oxidative enzymes by inhibiting respiration, which obstructs energy metabolism (Krebs *et al.,* 1983; Kang *et al.,* 2003 and Omar *et al.,* 2018). Phenolic compounds possess redox-active and could serve as potent antifungal candidates, which target cellular antioxidant and cell wall integrity systems in fungi (Kim, *et al.,* 2020). Enzyme inhibition by the oxidized compounds, possibly by reacting with sulfhydryl groups or with different proteins, is one of the mechanisms hypothesized to be responsible for phenolic toxicity to microorganisms (Mason and Wasserman, 1987).

Several studies elucidated that organic acids' placements of hydroxyl groups on the benzene ring, along with their redox properties, have significant antioxidant activity and high radical scavenging activity, quench active oxygen species, and break down peroxides, which are related to reducing oxidative stress. (Lattanzio *et al*., 2006; Vondráková *et al*., 2020; Nehela *et al*., 2021 and Xu *et al*., 2021). Moreover, we found that GA at 600 mgL^{-1} and 3, 5-DNBZA at 300 mgL^{-1} were the most effective in enhancing the resistance of geranium plants against root rot without any phytotoxicity symptoms compared to the tested other concentrations during the growing season 2018/2019 for the two cuts. The synthetic chemical elicitors might emulate natural inducers of resistance against causative microorganisms of root rot disease of geranium plants. In line with findings, according to El-Nagar *et al*., (2020) and Nehela *et al*., (2021) , the exogenous application of gallic acid, benzoic acid and their derivatives increased the endogenous phenolic content, which was strongly correlated with the disease progression without any phytotoxic

symptoms. Additionally, they found that these compounds directly inhibited the growth of *Alternaria solani in vitro* and impede *A. solani* from colonizing leaf tissues. Recent studies have shown that the exogenous application of ρ-hydroxybenzoic acid dramatically increased the production of phenolic acids in the root border cells of grapevine seedlings (Liu *et al*., 2019). Benzoic acids (BZAs) are C6-C1 aromatic carboxylic acids that serve as precursors for building blocks and pivotal structural components for many primary and specialized metabolites related to plant fitness and play a significant role in plant responses to biotic and abiotic stress (Widhalm and Dudareva, 2015). In this regard, the exogenous application of BZA and its derivatives enhanced the accumulation of endogenous BA produced directly by plants, which in turn elevated endogenous SA levels and induced pathogenesis-related proteins to reduce the detrimental effect of *Alternaria solani* on tomato plants through a complex multilayered defense system that involved major mechanisms (enzymatic and non-enzymatic) (Nehela *et al*., 2021). Salicylic acid is known to function as an endogenous signal that mediates plant defense against pathogens and induces systemic acquired resistance (SAR). Additionally, it stimulates the production of pathogenesis-related (PR) proteins that significantly contribute to pathogen resistance (Abdel-Rahman *et al.*, 2021). These findings suggest that the exogenous application of GA and DNBZA, which are phenolic chemicals, have antioxidant effects that suppress the development of disease symptoms (Thakur and Sohal, 2013).

Nowadays, numerous studies explain how different application techniques can help manage plant diseases successfully by promoting the expression of defense-associated genes, inducing "SAR", activating various defense-related enzymes, and enhancing the biosynthesis of secondary metabolites (SMs) (Riahi *et al*., 2020; El-Garhy *et al.,* 2020; Asadollahei *et al*.,2023 and Wang *et al*., 2023). In this study, GA was more effective in protecting geranium plants, delaying symptoms while concurrently enhancing growth characteristics when applied using techniques (single and dual), followed by 3, 5-DBZA in the case of the dual technique application. In agreement with these findings, gallic acid and its derivatives have antioxidant properties that increase tomato plants' resistance to *Alternaria solani* infection by reducing oxidative stress, where they can act as reactive oxygen species (ROS) scavengers and shield cells from oxidative stress (El-Nagar *et al*., 2020), as well, enhancing the antioxidant defense system, and improving leaf membrane stability for sunflower

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

seedlings exposed to Cd stress (Saidi *et al.*, 2021). A study by (Singh *et al.*, 2017) revealed that gallic acid had a favorable impact on total chlorophyll, total carotenoids, total flavonoid content, rice seedling growth, and the plant's defensive condition by increasing TPC, TFC, and a decrease in total ROS. Moreover, we presume the high efficacy of gallic acid in comparison to 3, 5-dinitrobenzoic acid may be due to their chemical structure. Our findings showed that the dual application strategy of GA at 600mgL^{-1} and 3, 5-DNBZA at 300mgL^{-1} was superior to the single application method for protection against the severity of root rot disease, delaying the progression of the symptoms throughout the growing season (2020/2021) while concurrently improving plant biomass and oil yield. According to (El-Nagar *et al*., 2020), the fungistatic effect of gallic acid and its derivatives begins to wear off around fifteen days after treatment. They also observed that after 21 days, the early blight disease progress curves on tomato leaves slightly increased in gallic acid. We believe that combining the dual method with the tested polyphenolic compounds plays a critical role in reducing oxidative stress. As a result, the technique proved to be effective in suppressing the development of root rot disease symptoms due to (i) Dipping geranium cuttings in each of GA and 3, 5-DNBZA before planting might cause the activation or inactivation of many defense-related genes, transitory phosphorylation/dephosphorylation of proteins, and secondary metabolite synthesis (Narayani and Srivastava 2017). Induction of systemic acquired resistance (SAR) before contact with pathogens stimulate plants efficacy and efficiency in their defense against pathogens via the production of phytoalexins, PR proteins, accumulation of salicylic acid, and active oxygen species (AOS) (Thakur and Sohal, 2013; Frąckowiak *et al*., 2019 and Abdel-Rahman *et al*., 2021). (ii) Duplicated treatments at intervals of GA and 3, 5-DNBZA around the roots of geranium plants (as soil drench) might activate various defense biosynthetic pathways, inducing the production of more endogenous polyphenolic compounds, higher quantities and quality of secondary metabolites, and increased yield production (EL-Garhy *et al*., 2020; El-Nagar *et al*., 2020; Li *et al*., 2021 and Asadollahei *et al*., 2023). Accordingly, the authors suggest these chemicals, depending on their efficacy and manner of application, to be used in fields alone or in combination with fungicides. Thus, they could be utilized as alternatives to fungicides in plant protection (Li *et al*., 2021). We found that GA and 3,5DNBZA affected the distribution of dry matter and carbohydrates (reducing sugars, total sugars) between shoots and roots. Under single and dual application techniques, with their various impacts, there was a strong

correlation between the shoot/root dry weight ratios and the proportional distribution of total carbohydrates in shoots and roots. The shoot/root ratio essentially represents the net product of the differential in carbohydrate distribution between the root and shoot. Although this distribution is genetically determined, extrinsic variables like nutrition and biotic and abiotic stress can affect it (Pandey and Chikara, 2014 and Soundy *et al*., 2005). Sugars play a significant role in signaling the regulation of plant metabolism, and their ability to regulate senescence may be essential to the success of these various stress-response mechanisms (Wingler and Roitscg, 2008). In this study, we showed that the dual application of GA and 3, 5-DNBZA improved the biochemical traits, defense phenolics, and enzymatic activities in geranium leaves. Moreover, plant biomass and oil yield production were significantly correlated with enzymatic activity and level of endogenous phenolic compounds (EL-Garhy *et al.*, 2020 and Li *et al*., 2021). The relationship between induced resistance and some biochemical changes in plant tissues has become a model of plant disease resistance. The obtained results confirmed the ability of 3, 5-dinitrobenzoic acid and gallic acid to promote the synthesis of total phenols accumulation through the shikimic acid pathway activation, thus could be due to the signaling action of gallic acid and 3, 5-dinitrobenzoic acid as external addition on the metabolism as a defense molecule or the effect of these treatments as chemical inducers of plant immunity through secondary metabolites synthesis and their accumulation as mediators in plant defense against pathogen infection when subjected to be oxidized with hydrogen peroxide content to form direct fungicidal active compounds against pathogen infection, that mechanism is not subjected in fungicidal treatment which suppresses the pathogen infection directly which reflects lower content of phenols compared with 3, 5-dinitrobenzoic acid and gallic acid. In addition, total phenol contents was significantly increased in positive control as compared to negative control may be due to the defense strategy of plants consisting of two stages. The first stage is assumed to involve the rapid accumulation of phenols at the infection site, which function slows the growth rate of the pathogens and allows for activation of the secondary stage that will more thoroughly restrict the pathogen. The secondary responses contain the activation of specific defense mechanisms which includes the synthesis of molecules associated with pathogen strain (Nehela *et al.*, 2021 and Abdel-Rahman *et al.*, 2021). The antioxidant enzyme activity was significantly increased in the positive control compared to the negative control to scavenge ROS generated by oxidative stress, enhancing plant tolerance to biotic stress. Obtained results may be due to that ROS

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

played an important role in induced resistance, cell signaling, and regulating the gene expression of antioxidant defense mechanisms (Nehela *et al.,* 2021). Catalase and glutathione peroxidase were evaluated under the above-mentioned treatments. The response of these enzymes was variable at different concentrations of GA and 3, 5-DNBZA. In general, some investigators have found that peroxidase and polyphenol oxidase might catalase the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure (Avdiushko *et al*.,1993). Also, lignin is considered an important structural component of plant cell walls to prevent pathogen ingress. Phenols accumulate near infected tissues, thus inhibiting the development of pathogens in the tissue (Thakur and Sohal, 2013; Vondráková *et al*., 2020; Parvin *et al*., 2022; and Lavanya *et al*., 2022).

A hypothetical model of the potential defensive roles of GA at 600 mgL^{-1} and 3, 5-DNBZA at 300 mgL^{-1} against root rot caused by the pathogens' mixture is proposed and presented in Figure (5). These findings demonstrated that GA and 3, 5-DNBZA mitigate the harmful effect of a mixture of phytopathogens on geranium plants via a complex multifunctional defense system involving at least three major mechanisms. (a) GA and 3, 5-DNBZA exhibit a potent concentration-dependent antifungal activity against *Fusarium semitectum, Macrophomina phaseolina, , Rhizoctonia solani,* and *Pythium irregulare,* and affect the integrity of pathogens cells' walls and their capacity to produce peroxidase and carboxymethyl cellulase enzymes. (b) GA and 3,5-DNBZA stimulate the activation of antioxidant defense mechanisms to neutralize the harmful effect of oxidative stress (ROS) and preserve their equilibrium inside infected plants. Two main pathways enable the enhancement of the antioxidant system by GA and 3, 5-DNBZA: The enzymatic antioxidant defense mechanism is the first line of antioxidant defense response, while the non-enzymatic antioxidant defense mechanism is the second line of defense against disease-induced oxidative stress damage (ROS). (c) GA and 3,5-DNBZA enhance the accumulation of polyphenolic compounds content (also known as allelochemicals) in higher quantities in geranium leaves, which is associated with the defense response against phytopathogens infection of geranium. (d) GA and 3, 5-DNBZA play a role in the regulation of leaf senescence by modulation of the sugar status. Furthermore, the dual application technique has a favorable influence on the successful management of root rot disease in geranium plants.

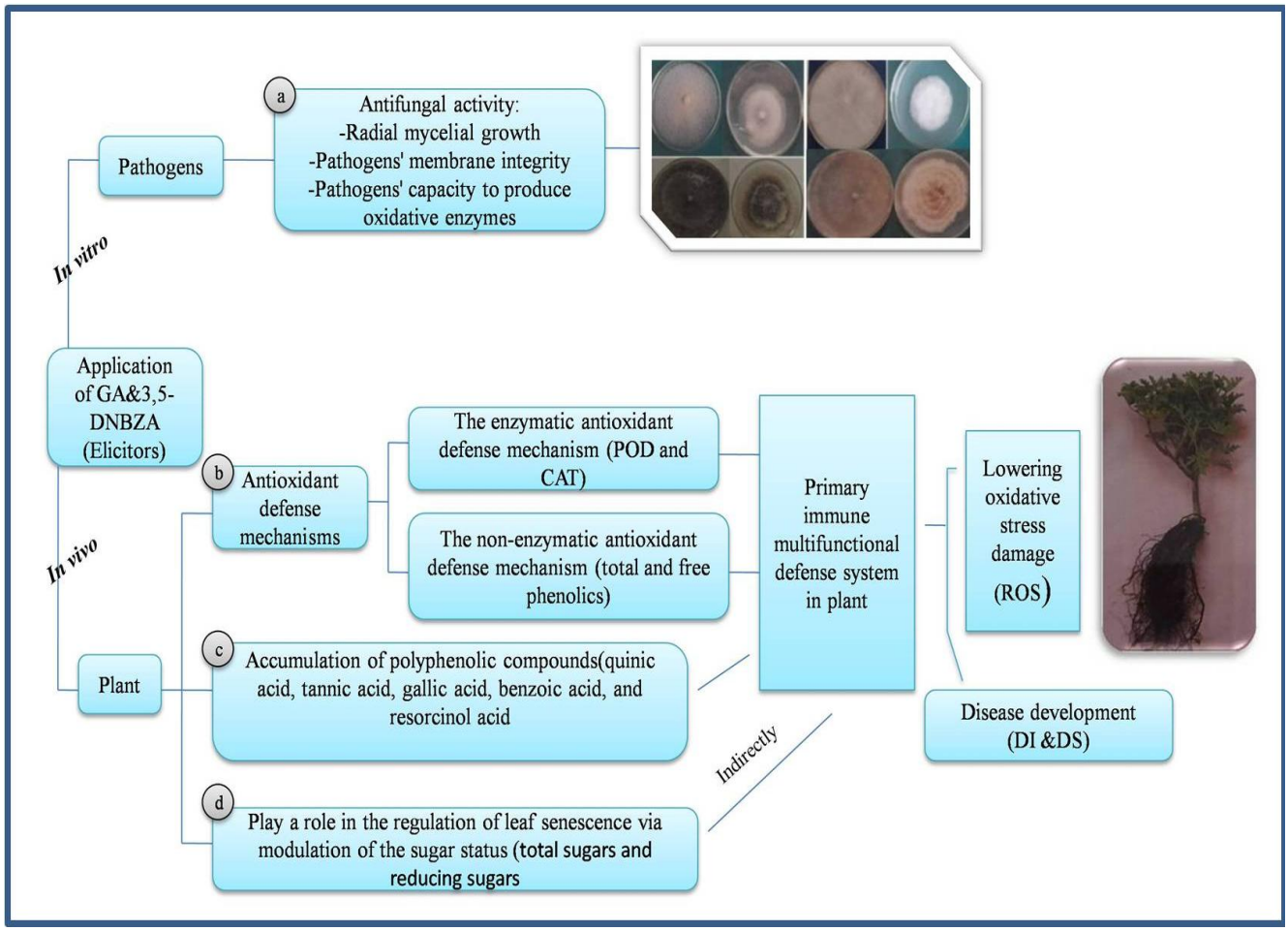

Fig.5 Schematic illustrated that elicitors function as signal chemicals at low concentrations, providing information for the plant to trigger defense. Blue rectangles with lines represent the various defense mechanisms supplied by GA and 3, 5-DNBZA against root rot disease in geranium plants (*in vitro* and *in vivo*).

Conclusion

Results of the *in vitro* and the *in vivo* trials in this study suggest the dual technique application of GA at 600 mgL^{-1} and 3, 5-DNBZA at 300 mgL^{-1} are sustainable substitutes and effective strategies for controlling root rot disease of the geranium plant caused by (*Fusarium semitectum*, *Macrophomina phaseolina*, *Pythium irregulare*, and *Rhizoctonia solani*) that target may decrease the usage of fungicides totally or fractionally, as well improving plant biomass and essential oil yield production, through enhancement the accumulation of polyphenolic compounds and antioxidant defense mechanisms. So to obtain good results in geranium under biotic stress, it is preferable to cultivate geranium plants

Egypt J. of Phytopathol.,Vol. 52, No.1, pp 109-151

in the winter season. Furthermore, further research and more studies are needed to support this hypothesis and to fully comprehend the molecular and cellular mechanisms behind the antifungal properties of GA and 3, 5DNZA against phytopathogens of geranium.

Abbreviations:

SAR – systemic acquired resistance, BZA – benzoic acid, 3,5-DNBZA- 3,5 dinitro benzoic acid, GA- gallic acid, MSI- membrane stability index, EO- essential oil, PhCs -Phenolic compounds, DI%- disease incidence %, DS%- disease severity%, SMs-secondary metabolites, MDW- mycelial dry weight, POD- peroxidase enzyme, and CMCase- carboxymethyl cellulase.

Authors' contributions

D. A. and R.S. conceived of this study and designed the experiments, D. A., M. M., S. A. and R.S. prepared figures and drafted the manuscript, D. A. and R.S. analysed the data and discussed the results. All authors have read and agreed to the published version of the manuscript.

Acknowledgments:

The authors would like to express their appreciation to Prof. Waleed Mohamed Fares. Central Laboratory for Design and Statistical Analysis Research. ARC, Egypt, for his valuable and constructive suggestions during the planning and development of this research work.

References:

Abdel-Rahman, F.A.; Khafagi, E.Y.; Soliman, M.S.; Shoala, T. and Ahmed, Y. 2021. Preharvest application of salicylic acid induces some resistant genes of sweet pepper against black mold disease. European Journal of Plant Pathology, 159:755-768.

Adolf, K.M. 2016. Root rot of geranium transplants and its biological control. Int. J. Agric. Technol., 12(5): 899-914.

Aebi, H. 1984. Methods Enzymol., 105:121-126.

Ali, I.B.E.; Tajini, F.; Boulila, A.; Jebri, M.A.; Boussaid, M.; Messaoud, C. and Sebai H. 2020. Bioactive compounds from Tunisian *Pelargonium graveolens* (L'Hér) essential oil and extracts:α-amylase and acethylcholinesterase inhibitory and antioxidant, antibacterial and phytotoxic activities. Industrial Crops and Products,158,112951.

Arya, S.S.; Lenka, S.K.; Cahill, D.M. and Rookes, J.E. 2021. Designer nanoparticles for plant cell culture systems: mechanisms of elicitation and harnessing of specialized metabolites. Bioessays, 43, 2100081. https://doi.org/10.1002/bies.202100081.

Asadollahei, M.V.; Tabatabaeian, J.; Yousefifard, M.; Mahdavi, S.M.E. and

Nekonam, M.S. 2023. Impact of elicitors on essential oil compositions and phytochemical constituents in *Lavandula stoechas* L. Plant Physiology and Biochemistry, 194: 722-730.

Asgarpanah, J. and Ramenzanloo, F. 2015. An overview of phytopharmacology of *Pelargonium graveolens* L. Ind. J. Tradit Know, 14(4): 558-563.

Avdiushko, S.A.; Ye, X.S. and Kuc, J. 1993. Detection of several enzymatic activities in leaf prints of cucumber plants. Physiological and Molecular Plant Pathology, 42: 441- 454.

Bergman, M.E.; Bhardwaj, M. and Phillips, M.A. 2021. Cytosolic geraniol and citronellol biosynthesis require a Nudix hydrolase in rose-scented geranium (*Pelargonium graveolens*).The Plant Journal, 107: 493–510.

Bhaskar, R.; Xavier, L.S.E.; Udayakumaran, G.; Kumar, D.S.; Venkatesh, R. and Nagella, P. 2022. Biotic elicitors: a boon for the in-vitro production of plant secondary metabolites. Plant Cell Tissue Organ Cult., 149(2):7–24. https://doi.org/10.1007/s11240- 021-02131-1.

Białas, A.; Zess, E.K.; De, J.L.C.; Franceschetti, M.; Pennington, H.G.; Yoshida, K.; *et al.*, 2018. Lessons in effector and nlr biology of plant-microbe systems. Molecular Plant-Microbe Interactions, 31(1):34-45. MPMI08170196FI.

Blerot, B.; Baudino, S.; Prunier, C.; Demarne, F.; Toulemonde, B. and Caissard, J.C. 2016. Botany, agronomy and biotechnology of Pelargonium used for essential oil production. Phytochem Rev., 15: 935–960.

Bradford, M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

British Pharmacopeia, 1963. Determination of Volatile Oil in Drugs. The Pharmaceutical Press, London.

Cappellari. L. R.; Santoro, M.V.; Schmidt, A.; Gershenzon, J. and Banchio, E. 2020. Improving phenolic total content and monoterpene in mentha x piperita by using salicylic acid or methyl jasmonate combined with rhizobacteria inoculation. International Journal of Molecular Sciences, 21(1), 50:1-22.

Cowan, M.M.1999. Plant products as antimicrobial agents. Clin. Microbial. Rev., 12: 564- 582.

De La Rosa, L.A.; Moreno-Escamilla, J.O.; Rodrigo-García, J.; Alvarez-Parrilla, E. 2019. Phenolic Compounds. In "*Postharvest Physiology and Biochemistry of Fruits and Vegetables*" Yahia E, Carrillo-Lopez A. (Eds.),

Elsevier, Cambridge, UK., 253–271. ISBN 9780128132784.

Deepmala, S. 2019. Allelochemical stress, ROS and plant defence system. International Journal of Biological Innovations, 1 (1): 33-35. https://doi.org/10.46505/IJBI.2019.1107

Dewidar, A.A.; Kenawy, A.G.M. and Ghebrial, E.W.R. 2019. Influence of different garlic treatments on controlling basal stem rot, root rot and infection by broomrape in geranium plants. Egypt J. Phytopathol., 47 (1): 347 -366. ISSN 1110-0230.

Domsch, K.H.; Gams, W. and Anderson, T.H. 1980. Compendium of Soil Fungi. *Academic Press, London,* 1: 887.

Dubois, M.K.; Gilles, J.K.; Hamiton, P.; Roberts, A. and Smith, F. 1951. A colorimetric method of determination of sugars. Nature, 166-167.

El-Garhy, H.A.S.; Abdel-Rahman, F. A.; Shams, A.S.; Osman, G.H. and Moustafa, M.M.A. 2020. Comparative analyses of four chemicals used to control black mold disease in tomato and its effects on defense signaling pathways, productivity and quality traits. Plants, 9(7):808.

El-Nagar, A.; Elzaawely, A.A.; Taha, N.A. and Nehela, Y. 2020. The antifungal activity of gallic acid and its derivatives against *Alternaria solani*, the causal agent of tomato early blight. Agronomy, 10(9), 1402:1-23.

Fan, G.F.; Xu, Z.G.; Liu, X.S.; Yin, W.; *et al*., 2023. Antifungal efficacy of gallic acid extracted from pomegranate peel against *Trichophyton rubrum*: *in vitro* Case study. Natural Product Communications, 18(1):1-9.

Fossati,P. 1980.Clin. Chem., 26: 227-231.

Frąckowiak, P.; Pospieszny, H.; Smiglak, M. and Obrępalska-Stęplowska, A. 2019. Assessment of the efficacy and mode of action of benzo (1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) and its derivatives in plant protection against viral disease. Int. J. Mol. Sci., 20(7), 1598: 1-27.

Gharib,F A.EL.; Zeid,I.M.; Ghazi,S.M. and Ahmed, E.Z.(2018). Physiological effects of ascorbic and gallic Acids on growth and metabolic activities of cowpea (*Vigna unguiculata* L.) plants. J Plant Physiol Pathol., 6(4):1-9. doi: 10.4172/2329-955X.1000188.

Goupy, P.; Hugues, M.; Biovin, P. and Amiot, M. J. 1999. Aantioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric., 79:1625-1634.

Hartzfeld, P.W.; Forkner, R.M.; Hunter, D. and Hagerman, A.E. 2002. Determination of hydrolyzable tannins (Gallotannins and Ellagitannins) after reaction with potassium iodate. J. Agric. Food Chem., 50:1785–1790. doi: 10.1021/jf0111155

Hilal, A.A. 1985. Pathological Studies on Some Medicinal and Aromatic Plants in Egypt (Pelargonium and Chamomile). Ph.D. Thesis Fac. Agric. Suez. Canal. Univ.

Imara, D.A. 2000. Studies on Some Root rot Diseases of Pelargonium. M.Sc. Thesis Fac. Agric. Cairo. Univ. 134.

Imara, D.A.; Zaky, W.H. and Ghebrial, E.W.R. 2021. Performance of soil type, cyanbacterium S*pirulina platensis* and biofertilizers on controlling damping-off, root rot and wilt diseases of Moringa (*Moringa oleifera* Lam.) in Egypt. Egyptian Journal of Phytopathology, 49(2):10-28.

Kalra, A.; Parmeswaran, T.N.; Ravindra, N.S. 1992. Influence of planting date on losses and yield responses of geranium (*Pelargonium graveolens*) to root rot and wilt. The Journal of Agricultural Science (Cambridge), 118(3): 309-314.

Kang, H.C.; Park, Y.H. and Go, S.J. 2003. Growth inhibition of a phytopathogenic fungus, *Colletotrichum* species by acetic acid. Microbiol. Res., 158: 321

Kim, J.H.; Campbell, B.C.; Mahoney, N.; Chan, K.L.; Molyneux, R.J. and Balajee, A. 2010. Augmenting the activity of antifungal agents against aspergilli using structural analogues of benzoic acid as chemosensitizing agents. Fungal Biol., 114: 817–824. [CrossRef]

Kim, J.H.; Chan, K.L.; Tam, C.C.; Cheng, L.W. and Land, K.M. 2020. Crosstalk between the antioxidant and cell wall integrity systems in fungi by 2-hydroxy-4-methoxybenzaldehyde.Cogent Food & Agriculture, 6(1):1-7. 1823593, DOI: 10.1080/23311932.2020.1823593

Krebs, H.A.; Wiggins, D.; Stubbs, M.; Sols, A. and Bedoya, F.1983. Studies on the mechanism of the anti-fungal action of benzoate. Biochem. J., 214: 657–663.

Kumar, D.; Padalia, R. C.; Suryavanshi, P. *et al*., 2021. Essential oil yield, composition and quality at different harvesting times in three prevalent cultivars of rose-scented geranium. Journal of Applied Horticulture, 23(1): 19-23.

Kumar. N. and Goel, N. 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep., 24, e00370. [Google Scholar] [CrossRef]

Laborda, P.; Li, C.; Zhao, Y.; Tang, B.; Ling, J.; He, F. and Liu, F. 2019. Antifungal metabolite *p*-aminobenzoic acid (pABA): Mechanism of action and efficacy for the biocontrol of pear bitter rot disease. J. Agric. Food Chem., 67(8): 2157– 2165.

Ladeira, S.A.; Cruz, E.; Delatorre, A.B.; Barbosa, J.B. and Martins, M.L.L. 2015. Cellulase production by thermophilic *Bacillus* sp. SMIA-2 and its detergent compatibility. Electron. J. Biotechnol., 18:110–115. https://doi. org/10.1016/J.EJBT.2014.12.008

Lattanzio, V.; Lattanzio, V.M.T. and Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In "*Phytochemistry: Advances in Research*" Imperato F (Ed.), Research Signpost, Trivandrum-695 023, Kerala, India., 23–67. ISBN 813-080-034-9

Lavanya, S.N.; Raj, S.N.; Jadimurthy, R.; Sudarsan, S.; Srivastava, R.; Tarasatyavati, C.; Rajashekara, H.; Gupta, V.K. and Nayaka, S.C. 2022. Immunity elicitors for induced resistance against the downy mildew pathogen in pearl millet. Scientific Reports, 12, 4078. https://doi.org/10.1038/s41598-022-07839-4.

Leon, S.P.; Inove, N. and shinano, H. 1993. Effect of acetic and citric acids on the growth and activity (VB-N) of *Pseudomonas* sp. and *Moraxella* sp. Bull. Fac. Fish. Hokkaido Univ., 44(2):80-85.

Li, Z. J.; Liu, M.; Dawuti, G.; Dou, Q.; Ma, Y.; Liu, H. G. and Aibai, S. 2017. Antifungal activity of gallic acid *in vitro* and *in vivo*. Phyther. Res., 31:1039–1045.

Li, J.; Kolbasov, V.G.; Pang, Z.; Duan, S. *et al*., 2021. Evaluation of the control effect of SAR inducers against citrus Huanglongbing applied by foliar spray, soil drench or trunk injection. Phytopathology Research, 3(2): 2-15.

Liu, Q.; Li, K.; Guo, X.; Ma, L.; Guo, Y. and Liu, Z. 2019. Developmental characteristics of grapevine seedlings root border cells and their response to ρ-hydroxybenzoic acid. Plant Soil, 443:199–218. [CrossRef].

Louis B.; Waikhom, S.D.; Roy, P.; Bhardwaj, P.K.; Singh, M.W.; Goyari, S.; Sharma, C.K. and Talukdar NC. 2015. Erratum: Secretome weaponries of *Cochliobolus iunatus* interacting with potato leaf at different temperature regimes reveal a CL[xxxx] LHM-motif. BMC Genomics, 16:347. DOI 10.1186/s12864-015-1337-3

Malatova ,K.; Hitimana, N.; Niyibizi, T.; Simon, J.E. and Juliani, H.R. 2011. Optimization of harvest regime and post-harvest handling in geranium

production to maximize essential oil yield in Rwanda. Ind. Crops Prod., 34:1348–1352.

Mason, T.L .and Wasserman, B.P. 1987. Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. Phytochemistry, 26:2197 -2202. Crossref. ISI.

Mazeed, A.; Maurya, P.; Kumar, D.;Yadav, S. and Suryavanshi, P. 2022. Efficient nutrient management for rose scented geranium (*Pelargonium graveolens* L′Herit ex Ait). Journal of Applied Research on Medicinal and Aromatic Plants, 31: 100409. https://doi.org/10.1016/j.jarmap. 2022. 100409

Miller, G.L. 1959. Use of Dinitrosalicylic Acid Reagent for the Determination of Reducing Sugars. Analytical Chemistry, 31:426-428. http://dx.doi.org/10.1021/ac60147a030

Munera, J.D.C. and Hausbeck, M.K. 2015. Integrating host resistance and plant protectants to manage Pythium root rot on Geranium and Snapdragon. Hort Science, 50(9):1319–1326.

Narayani, M. and Srivastava, S. 2017. Elicitation: a stimulation of stress in *in vitro* plant cell/tissue cultures for enhancement of secondary metabolite production. Phytochem. Rev., 16:1227–1252.

Neagu, A.F.; Costea, T. and Nencu, I. *et al*., 2018. Obtaining and characterization of selective *Pelargonium graveolens* LHer.dry extract with potential therapeutic activity in metabolic diseases. Farmacia, 66(4): 592-596.

Nehela, Y.; Taha, N.A.; Elzaawely, A.A.; Xuan, T.D.; Amin, M.A.; Ahmed, M.E. and EL-Nager, A. 2021. Benzoic acid and its hydroxylated derivatives suppress early blight of Tomato (*Alternaria solani*) via the induction of salicylic acid biosynthesis and enzymatic and nonenzymatic antioxidant defense machinery. Journal of Fungi, 7(8), 663:1-26.

Nelson, P.E.; Toussoum, T.A. and Morasas, W.F.O. 1983. *Fusarium* species, *An Illustrated Manual for Identification*. The Pennsylvania State Univ. Press, 193.

Omar, M.S.; Kordali, S. and Korkmaz, M. 2018. Evaluation of the effect of benzoic acid on some plant pathogenic fungi. International Journal of Agricultural and Natural Sciences, 1(1): 03-05.

Osorio, E.; Flores, M.; Hernández, D.; Ventura, J.; Rodríguez, R. and Aguilar, C.N. 2010. Biological efficiency of polyphenolic extracts from pecan nuts shell (*Carya Illinoensis*), pomegranate husk (*Punica granatum*) and creosote bush leaves (*Larrea tridentata* Cov.)

against plant pathogenic fungi. Industrial Crops and Products, 31(1):153-157.

Paglia, D. E. and Valentine, W. N. 1967. J. Lab. Clin. Med., 70: 158-169.

Pandey, M. and Chikara, S.K. 2014. *In vitro* regeneration and effect of abiotic stress on physiology and biochemical content of *Stevia rebaudiana* 'Bertoni'. Journal of Plant Science & Research, 1(3):113.

Parvin, K.; Nahar, K.; Mohsin, S.M.; Mahmud, J.A.; Fujita, M. and Hasanuzzaman, M. 2022. Plant Phenolic Compounds for Abiotic Stress Tolerance. In "*Managing Plant Production Under Changing Environment"* Hasanuzzaman M, Ahammed GJ, Nahar K (Eds.), Springer Nature Singapore Pte Ltd.,193-237.

Pradeepa, M.; Kalidas, V. and Geetha, N. 2016. Qualitative and quantitative phytochemical analysis and bactericidal activity of *Pelargonium graveolens* L'Hér. Int. J. Appl Pharm, 8(3): 7-11.

Prasad, D. and Singh, K.P. 2014. Integrated Management of Root Rot and Wilt Complex in Scented Geranium In "*Approaches and Trends in Plant Disease Management* " Gupta SK, Sharma M.(Eds.), 5A, New Pali Road ,Jodhpur-342001(India), 315-334.

Prasad, D.; Singh, A.; Singh, K.P.; Bist, S.; Tewari, A. and Singh, U.P. 2010. The role of phenol compounds in disease resistance in geranium. Archives of Phytopathol. and Plant Protection, 43(7):615_623.

Premachandra, G.S.; Saneoka, H.; Kanaya, M. and Ogata, S. 1991. Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. J. Exp. Bot., 42(2): 167-171.

Riahi, L.; Cherif, H.; Miladi, S.; Neifar, M.; Bejaoui, B.; Chouchane, H.; Masmoudi, A.S. and Cherif, A. 2020. Use of plant growth promoting bacteria as an efficient biotechnological tool to enhance the biomass and secondary metabolites production of the industrial crop *Pelargonium graveolens* L'Hér. under semi-controlled conditions. Industrial Crop and Products, 154,112721.

Š´cepanovi´c, M.; Koš´cak, L.; Šoštarˇci´c,V.; Pismarovi´c, L.; Milanovi´c-Litre, A. and Kljak, K. 2022. Selected Phenolic Acids Inhibit the Initial Growth of *Ambrosia artemisiifolia* L. Biology, 11, 482:1-11.

Saidi, I.; Guesmi, F.; Kharbech, O.; Hfaiedh, N. and Djebali, W. 2021. Gallic acid improves the antioxidant ability against cadmium toxicity: Impact on leaf lipid composition of sunflower (*Helianthus annuus*) seedlings. Ecotoxicology and Environmental Safety, 210, 111906.

Sairam, R.K. 1994. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. Indian J. Exp. Biol., 32: 584- 593.

Singh, A.; Gupta, R. and Pandey, R. 2017. Exogenous application of rutin and gallic acid regulate antioxidants and alleviate reactive oxygen generation in *Oryza sativa* L. Physiol. Mol. Biol. Plants, 23(2):301–309.

Singleton, V. I. 1974. Analytical fractionation of the phenolic substances of grapes and wine and some practical uses of such analyses. In. "*Chemistry of Winemaking*" Webb A. (Ed.), Advances in Chemistry, American Chemical Society, 184-211.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 6th Ed. Iowa State Univ. Press, Ames, Iowa, USA.

Soundy, P.; Cantliffe, D.J.; Hochmath, G.J. and Stoffella, P. 2005. Management of nitrogen and irrigation in lettuce transplant production affect transplant root and shoot development and subsequent crop yield. Hort. Science, 40(3):607-610.

Thakur, M. and Sohal, B.S. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A Review. ISRN Biochemistry, 1-10. Article ID 762412, http://dx.doi.org/10.1155/2013/762412

Vondráková, Z.; Malbeck, J.; Trávníčková, A.; Černý, R. and Cvikrová, M. 2020. Phenolic acids in selected scab-resistant and mildew-tolerant apple cultivars. Acta Physiologiae Plantarum,42(43):1-10.

Wang, R.; Li. H.; Qin, Z.; Wang, Y.; Yang, Q.; Zhang, H. and Li, M. 2023. Antifungal activity and application of *Bacillus tequilensis* A13 in biocontrol of *Rehmannia glutinosa* root-rot disease. Chemical and Biological Technologies in Agriculture, 10(20):1-12.

Warth, A.D. 1991. Mechanism of action of benzoic acid on *Zygosaccharomyces bailii*: effects on glycolytic metabolite levels, energy production, and intracellular pH. Applied and Environmental Microbiology , 57(12):3410–3414. [CrossRef] [PubMed]

Widhalm, J.W. and Dudareva, N.A. 2015. Familiar Ring to It: Biosynthesis of plant benzoic acids. Molecular Plant, 8: 83-97.

Wingler, A.; and Roitscg, T. 2008. Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. Plant Biology, 10 (Suppl. 1): 50–62. ISSN 1435-860.

Worthington, 1977. Enzyme Manual. Worthington Biochemical Corportion, Freehold, New Jersey, USA., 66-70.

Xu, Y.; Tang, G.; Zhang, C.; Wang, N. and Feng, Y. 2021.Gallic Acid and Diabetes Mellitus: Its Association with Oxidative Stress. Molecules, 26, 7115

Zhao, H.F.; Dong, J.J.; Lu, J.; Chen, J.; Li, Y.; Shan, L.; Lin, Y.; Fen, W. and Gu, G.X. 2006. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). J. Agric. Food Chem., 54:7277–7286. doi: 10.1021/jf061087w.

Zhao, X.; Zhi, Q.Q.; Li, J.Y.; Keller, N.P. and He, Z.M. 2018. The antioxidant gallic acid inhibits aflatoxin formation in *Aspergillus flavus* by modulating transcription factors FarB and CreA. Toxins, 10, 270:1-17.