

Original Research Article

Effect of Foliar Humic Acid on Water Productivity, Biochemical Parameters and Biotic Stress on Geranium Yield

ABSTRACT

Two-year experiment was conducted during 2017/2018 and 2018/2019 to investigate the effect of humic acid treatments [1.0, 1.5 and 2.0 cm/l] on water productivity, biochemical parameters [essential oils and proline content] and biotic stress [Cucumber Mosaic Virus (CMV)] on geranium yield. The aim of the study was to determine the appropriate concentration of humic acid that attain the highest water productivity (WP), highest production of essential oils and proline content beside decreasing Cucumber Mosaic Virus (CMV) concentration. The results season under (120, 100, and 80% ETo), respectively. The highest values of WP were attained under irrigation with 80% ETo and application of humic acid with 2.0 cm/L, namely 9.31 kg/ha in both growing seasons. While, the highest percentage of geranium essential oil and proline contents (68.16 and 3.73 %), respectively were found under application of 2.0 cm/L humic. On the contrary the results showed a significant reduction in CMV accumulation in plants treated with 2.0 cm/L humic acid compared to untreated infected plants (challenge control). Thus, it could indicate that the highest values of fresh yield were obtained under foliar application of 2.0 cm/L humic acid with 120% ETo (evapotranspiration). The results also showed that the amount of applied irrigation water were (15155, 12168 and 9334 m³/ha) in first growing season and were (15218, 12298, and 9678 m³/ha) in second growing season. It is recommended to irrigate geranium plants with 80% ETo under spraying with 2.0 cm/L humic acid to conserve irrigation water, increase the efficiency of water unit, attain highest production of essential oils and increase productivity of endogenous osmoprotectants (proline) which mainly acts against biotic stress (CMV).

Keywords: Water productivity, Oil contents, Proline, Cucumber Mosaic Virus (CMV), Virus concentration and biotic stress.

1. INTRODUCTION

Ornamental plants are important export crops in Egypt. Planted in about 103306 hectare, in 2020 Ministry of Agriculture and Land Reclamation, Economic Affairs Sector (EAS) [1]. Out of which, geranium is planted in about 2234 hectare producing 17975 ton (EAS, 2020). Geranium is perennial aromatic herb commonly cultivated for production of valuable volatile oil [2].

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A humic acid (HA) is an organic molecule that improves soil properties, plant growth, and agronomic traits. Numerous authors indicated that soil physical, chemical, and biological characteristics, including texture, structure, and water holding capacity are affected positively by HA [3] & [4]. HA application enhanced the drought resistance and provides support for crop production in face of water shortage [5]. Sustainable agriculture is used to increase productivity of crops and reduces soil pollution using alternatives natural sources of fertilizers [6]. Although fertilizer is the critical factor affects the production of geranium, it should be wisely used to prevent oil degradation and environmental pollution [7].

Water is a limiting factor in geranium production, in the cultivation of aromatic plants, abiotic factors, such as water deficit, influence the yield and composition of essential oils [8]. Water productivity is one of the methods to increase management practices on understanding the effects of water deficit [9]. Water stress affects plant growth and productivity but plants have different and often multiplex mechanisms to react to water shortages [9]. Abdel-Ghany and Abd El-Aleem [10] indicated that irrigation water requirements and the sensitivity of the growing geranium plants to water deficits is of a great interest to horticultural producers whenever planning irrigation strategies. Sánchez-Blanco [11] indicated that deficit irrigation induces different morphological and physiological responses in ornamental plants, but the application of irrigation management can obtain quality plants adapted to the environment. One procedure used to evaluate the effectiveness of the application of different amounts of irrigation water is the calculation of water productivity. Water productivity increases under application of deficit irrigation, relative to its value under full irrigation [12]. Water productivity is evaluating the effectiveness application of different irrigation amounts is, which is a quantitative term used to define the relationship between crop produced and the amount of water involved in crop production [13]. Essential oil increased under drought stress in two *Salvia* species and Sweet Basil [14] & [15].

Geranium (*Pelargonium* spp.) faces many biotic stresses (e.g., fungal diseases, bacterial diseases) [16] & [17] as well as, over 20 viruses have been found in *pelargonium* cultivars including Beet curly top virus (leaf hopper transmitted), Cucumber mosaic virus, (CMV), mechanical and aphid transmitted), Impatiens necrotic spot virus (INSV, thrips transmitted), Tomato bushy stunt virus (TBSV), Tomato ringspot virus (nematode transmitted), Tomato spotted wilt virus (TSWV, thrips transmitted), Tobacco mosaic virus (TMV, mechanical transmitted), Tobacco necrosis virus (TNV), Tobacco rattle virus (nematode transmitted), Arabis mosaic virus (nematode transmitted), *Pelargonium* flower break virus (PFBV, mechanically transmitted), and *Pelargonium* line pattern virus. All can be transmitted to the next crop through propagation procedures [18]. "Viruses do not kill geranium plants, but some can as Cucumber Mosaic Virus (aphid transmitted) severely reduce vegetative growth by affecting leaves and can reduce flower quality and marketability by deforming blooms and causing color breaks or streaks. The economic losses caused by these diseases are difficult to assess since infected plants are often symptomless and because both the cultivar and the environment in which the plants are growing can influence the severity of the symptoms. The problem is compounded by the diverse sources of stock plants and the fact that most geranium cultivars, especially those that are fieldgrown, are virus-infected [19]; [20]; [21].

Proline is considered important approaches to battle the abiotic stresses affecting physiological, biochemical, and molecular processes in plants. Proline can ameliorate morphological and physiological parameters as well as stress tolerance under counteractive environmental factors [22] & [23]. Foliar application of Humic acid enhance proline accumulation, induce synthesis of proline performance and induced systemic resistance against CMV biotic stress tolerance in geranium plants [24]; [25]; [26].

Thus, the aim of the current work was to determine the appropriate humic acid concentration with suitable irrigation amounts that attain the highest essential oil of geranium, the highest water productivity under surface irrigation and highest proline content production to increase biotic stress tolerance and reduce CMV concentration in geranium.

2. MATERIALS & METHODS

The current study was conducted on naturally CMV infected geranium plants to investigate the effect of foliar spraying with humic acid under different irrigation treatments to enhance vegetative growth yield, essential oil and proline production under biotic stress (CMV). The experiment was performed during 2017/2018 and 2018/2019 at the Experimental Farm of Medicinal and Aromatic Plants Research Department in El Kanater El Khairiya, Horticultural Research Institute, Agriculture Research Center in Egypt. The average meteorological values during the experimental period were obtained from the following: <https://power.larc.nasa.gov/data-access-viewer/>. Evapotranspiration (ET_o, mm/day) was calculated using BISm model [27]. The values of ET_o in 2017 for November and December were 2.94 and 2.60 (mm/day). As for 2018 ET_o average values from January till December were 2.83, 3.46, 5.16, 6.54, 9.01, 9.46, 9.20, 8.50, 7.20, 5.82, 3.16 and 3.18, respectively.

The physical and chemical analyses of the soil were determined according to [28]. The soil texture was clayey, having a physical composition average over the two growing seasons as follows: 23.35% sand, 21.93% silt, 50.41% clay, and 1.43% organic matter. The soil chemical analysis average over the two growing seasons was as follows: pH = 7.35; E.C (dS/m) = 0.63; and total nitrogen = 35.78 ppm; available phosphorus = 27.66 ppm; available potassium = 0.93 ppm. Soil moisture constants in the experimental site according to [29] are presented in Table 1.

Table 1: Soil moisture parameters of the experimental site.

Soil moisture constants							
Depth	Field capacity		Wilting point (WP)		Available water		Bulk density (BD) Mg/m ³
	%	Cm	%	cm	%	cm	
0-15	38.9	6.94	18.2	3.25	20.7	3.69	1.19
15-30	36.5	6.57	17.1	3.11	19.4	3.49	1.20
30-45	33.9	6.46	16.5	3.14	17.4	3.31	1.27
45-60	32.8	6.84	16.4	3.42	16.4	3.42	1.39
Total		26.81		12.92		13.91	

2.1. Experimental design:

The experimental design was split plot with three replicates, three irrigation amounts were assigned to the main plots and four foliar applications of humic acid were assigned to the sub-plots as followed:

- Three irrigation treatments:
 - a) Application of 120% ET_o (full irrigation, FI control);
 - b) Application of 100% ET_o (deficit irrigation, DI₁) and;
 - c) Application of 80% ET_o (deficit irrigation, DI₂).
- Four application of humic acid:
 - a) CMV infected geranium plants without application of humic acid (H1= control);
 - b) Foliar applications of 1.0 cm/L humic acid (H2= 1.0 cm/L);
 - c) Foliar applications of 1.5cm/L humic acids (H3= 1.5 cm/L) and;
 - d) Foliar applications of 2.0 cm/L humic acid (H4= 2.0 cm/L).

Humic acid treatments were sprayed three times, 15, 45 and 75 days after planting. Geranium was planted on the 1st of November in both seasons. Each plot included 3 rows with distance of 60 cm between rows and 25 cm between plants within the rows. The

plot area was 4.5 m² (1.80 × 2.5), included 27 plant/plot. Cattle manure (36 m³/ha) and calcium super phosphate (15.5% P₂O₅) at the rate of 600 kg/ha were added during land preparation; two weeks before planting. Whereas, ammonium sulphate (20.5%N) at 960 kg/ha, and potassium sulphate (48% K₂O) at 240 kg/ha were added in three equal doses. The first dose was applied 45 day after planting, the second dose applied after one month after the first dose and the third dose after the first cut. Geranium plants were harvested twice by cutting the vegetative parts, 10-15 cm above the soil surface. The first cut and second cut (harvest) were done on 20th May and 15th October at the first season, respectively. In the second season, the first and second cut (harvest) was done on 15th May and 5th October, respectively.

2.2. The following parameters were measured:

2.2.1. The total fresh weight (ton/ha).

2.2.2. Essential oil percentage (%) was determined in fresh plants of the two cuts according to the method described by [30]. In addition, essential oil samples of the 2nd cut during the 2nd season were subjected to gas liquid chromatography (GLC) according to the methods of [31] & [32].

2.2.3. Chemical analysis of proline contents in dry leaves was determined according to Bates *et al.*, [33].

2.2.4. Water relations

Soil moisture content was gravimetrically determined in soil samples taken from consecutive depths of 15 cm down to 60 cm. Soil samples were collected just before each irrigation, and 48 hours after irrigation to calculate the following:

2.2.4.1 Amount of applied irrigation water (AIW):

Submerged flow orifice with fixed dimension was used to measure the amount of water applied, according to Michael [34] as follows:

$$Q = CA \sqrt{2gh}$$

Where:

Q = discharge through orifice, (1/sec); C = coefficient of discharge, (0.61); A = cross-sectional area of the orifice, cm²; G = acceleration due to gravity, (981 cm/sec.²); H = pressure head, causing discharge through the orifice, (cm).

2.2.4.2. Crop water productivity (WP):

WP is defined as crop yield per unit of applied irrigation water, which determines the efficient use of applied irrigation water Zhang [35] and is given as follows:

$$WP = \frac{\text{Yield (ton/ha)}}{\text{Seasonal AIW (m}^3 \text{ water applied/ha)}}$$

2.2.5. Effect of different treatment of humic acid on CMV Concentration:

Enzyme-Linked Immunosorbent Assay (ELISA) Indirect ELISA was used to detect the Concentration of CMV in naturally infected geranium plants according to the method described by [36]. Leaves showed viral like symptoms (mosaic, chlorosis and mottling) were collected from the top of the geranium plant to detect virus infection. 200 µl of CMV specific immunoglobulin G (IgG) at concentration 1 µg/ml were diluted in coating buffer pH 9.6 and incubated in the microtitre plate at 4°C overnight. The wells were washed three times with washing buffer pH 7.4 [phosphate buffer saline (0.15 M NaCl) containing 0.1% tween 20 and 0.01% sodium azide]. 200 µl of each sample were diluted 1 to 20 (w/v) in extraction buffer

H1(Control)	63.50	54.67	47.89	55.35	71.1	60.3	53.8	61.73
H2(1.0 cm/L)	82.69	72.17	64.60	73.15	88.4	75.6	70.8	78.27
H3(1.5 cm/L)	88.13	77.27	70.13	78.51	94	82.6	75.9	84.17
H4(2.0 cm/L)	106.43	94.80	86.79	96.01	115.3	102.6	95.6	104.50
Mean	85.2	74.7	67.4	75.76	92.2	80.3	74.0	82.17
LSD at 5 %								
Irrigation (I)	0.71				0.16			
Humic acid (H)	0.43				0.28			
I X H	0.88				0.49			

FI: Irrigation 120%ETo; DI1: Irrigation 100%ETo; DI2: Irrigation 80% ETo.

3.2. Effect of humic acid and irrigation on geranium essential oil yield (liter/ha) and essential oil percentage (%)

3.2.1. The essential oil yield (liter/ha)

Table (3) indicated significant relationships between irrigation treatments, spraying with humic acid and their interaction in both growing seasons. Application of full irrigation (120% ETo) and spraying with 2.0 cm/L humic acid gave the highest value of geranium oil yield, namely 60.93 liter/ha average over the two growing seasons, compared to no application of humic acid, which attained 29.02 liter/ha. Furthermore, under deficit irrigation treatments, application of 2.0 cm/L humic acid gave the highest value under irrigation 80% ETo namely, 64.33 liter/ha average over the two growing seasons, compared to no application of humic acid, which attained 26.14 liter/ha averaged over the two growing seasons (Table 3). This result is in agreement with the findings of [44] who reported that the highest essential oil yield was obtained from plants irrigated every 5 days and fertilized with 1.2 g N/pot and sprayed with 1% K-humate. Also, [45] & [46] reported that as fresh herb yield was decreased under low water application, the oil yield was also decreased.

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Table 3: Essential oil yield (liter/ha) as affected by interaction between irrigation treatments and humic acid in both growing seasons.

Treatments	2017/2018				2018/2019			
	FI	DI1	DI2	Mean	FI	DI1	DI2	Mean
H1 (Control)	28.38	26.95	25.53	26.95	29.65	28.16	26.74	28.18
H2 (1.0 cm/L)	38.86	36.81	36.63	37.43	38.63	39.84	41.77	40.08
H3 (1.5 cm/L)	43.80	43.81	43.69	43.77	45.78	45.68	47.59	46.35
H4 (2.0 cm/L)	59.60	58.11	60.49	59.40	62.26	60.84	68.16	63.76
Mean	42.66	41.42	41.59	41.89	44.08	43.63	46.07	44.59
LSD at 5 %								
Irrigation (I)	0.13				0.75			
Humic A (H)	0.34				0.56			
I X H	0.6				0.98			

FI: Irrigation 120%; DI1: Irrigation 100%; DI2: Irrigation 80 % of evapotranspiration (ETo).

3.2.2. Percentage of essential oil of geranium

The results in Table (4) indicated significant relationships between irrigation treatments, spraying with humic acid and their interaction in the first growing seasons and between irrigation treatments, spraying with humic acid in the second season on geranium essential oil percentage. The results also showed that application of full irrigation decreased geranium essential oil percentage under all humic acid treatments in both growing seasons. The

highest value of geranium essential oil percentage was found under application of 80% ETo and spraying with 2.0 cm/L humic acid namely 0.71% average over the two growing seasons.

In this context, [43] reported that humate application lead to increase oil content in Thujaoorientalis plants. Said-Al Ahlet al.,[44] stated that foliar application of K- humate promoted growth and possessed the best oil percentage in oregano plants.

Table 4: Essential oil percentage (%) at affected by interaction between irrigation treatments and humic acid in both growing seasons.

Treatments	2017/2018				2018/2019			
	FI	DI1	DI2	Mean	FI	DI1	DI2	Mean
H1 (Control)	0.45	0.49	0.53	0.49	0.42	0.47	0.50	0.46
H2 (1.0 cm/L)	0.47	0.51	0.57	0.52	0.44	0.53	0.59	0.52
H3(1.5 cm/L)	0.50	0.57	0.62	0.56	0.49	0.55	0.63	0.56
H4(2.0 cm/L)	0.56	0.61	0.70	0.62	0.54	0.59	0.71	0.62
Mean	0.49	0.55	0.61	0.55	0.47	0.54	0.61	0.54
LSD at 5 %								
Irrigation (I)	0.00				0.01			
Humic A (H)	0.01				0.01			
I X H	NS				0.02			

FI: Irrigation 120%; DI1: Irrigation 100%; DI2: Irrigation 80 % of evapotranspiration (ETo).

3.2.3. Effect of irrigation and humic acid on proline content

Significant relationships between irrigation treatments, spraying with humic acid and their interaction in both growing seasons on proline contents in geranium tissues were found (Table 5). The results also indicated that the highest contents of proline in geranium plants was found under application of 80% ETo and application of 2.0 cm/L humic acid namely, 3.72 mg/100g proline average over two growing seasons, compared no application of humic acid, which attained 3.01 mg/100g proline average over the two growing seasons. Whereas, the lowest value namely, 2.75 mg/100 g proline was obtained under irrigation with 120% ETo and application of 2.0 cm/L humic acid average over the two seasons. It was reported that proline play a role as osmotic preservation, free radicals scavenger, and second role in maintaining cellular stability and induction of systemic acquired resistance against biotic stresses [47].

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Table 5: Proline content (mg/100 g) at affected by interaction between irrigation treatments and humic acid in both growing seasons.

Treatments	2017/2018				2018/2019			
	FI	DI1	DI2	Mean	FI	DI1	DI2	Mean
H1 (Control)	2.07	2.74	3.03	2.61	2.12	2.72	2.98	2.61
H2 (1.0 cm/L)	2.29	2.73	3.07	2.7	2.3	2.86	3.03	2.73
H3(1.5 cm/L)	2.47	2.95	3.25	2.89	2.55	3.02	3.15	2.91
H4(2.0 cm/L)	2.69	3.01	3.73	3.14	2.81	3.3	3.70	3.27
Mean	2.38	2.86	3.27	2.84	2.45	2.98	3.22	2.88
LSD 5%								
Irrigation (I)	0.08				0.10			
Humic (H)	0.11				0.06			
I X H	0.19				0.18			

FI: Irrigation 120%; DI1: Irrigation 100%; DI2: Irrigation 80 % of evapotranspiration (ET_o).

3.2.4. Water requirements for geranium plants

3.2.4.1. Applied irrigation water to geranium

The results in Table (6) indicated that the amount of applied irrigation water for geranium plants 15155, 12168 and 9334 m³/ha in first growing season and were 15218, 12298, and 9678 m³/ha in second growing season under 120, 100, and 80 ET_o, respectively. Application of 100% ET_o resulted in 20% water saving, whereas application of 80% ET_o saved 38% of the applied irrigation water, compared to 120% ET_o average over the two seasons.

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Table 6: Effect of irrigation treatment on the amounts of applied irrigation water (AIW, m³/ha) and percentage of saved water (IWS %) for geranium plants in the two growing seasons.

Irrigation treatments	AIW(m ³ /ha)	IWS%
2017/2018		
FI	15155	-
DI ₁	12168	20
DI ₂	9334	38
2018/2019		
FI	15218	-
DI ₁	12298	19
DI ₂	9678	36

FI: Irrigation 120%; DI1: Irrigation 100%; DI2: Irrigation 80 % of evapotranspiration (ET_o).

3.2.4.2. Geranium yield losses under deficit irrigation and humic acid treatments

The results in Table (7) showed that geranium yield losses were lower in the first season, compared to its counterpart values in the second season which can be attributed to climate variability between the two seasons. The lowest values of geranium yield reduction percentage under 100% and 80% ET_o were 11 and 17%, respectively, under application of 2.0 cm/L humic acid averaged over the two growing seasons. Furthermore, application of 2.0 cm/L humic acid resulted in lower yield losses under both deficit irrigation treatments, compared to yield loss under application of 120% ET_o and spraying with 2.0 cm/L humic acid.

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Table 7: Geranium yield losses (YR%) as a result application of deficit irrigation treatments under foliar applications of humic acid in both growing seasons.

Humic treatments	Fresh yield (ton/ha)			YR%	
	FI	DI1	DI2	DI1	DI2
2017/2018					
H1 (Control)	63.5	54.7	47.9	14	25
H2 (1.0 cm/L)	82.7	72.2	64.6	13	22
H3 (1.5 cm/L)	88.1	77.3	70.3	12	20
H4 (2.0 cm/L)	106.4	94.8	86.9	11	18

2018/2019					
H1 (Control)	71.1	60.3	53.8	15	24
H2 (1.0 cm/L)	88.4	75.6	70.8	14	20
H3(1.5 cm/L)	94	82.6	75.9	12	19
H4(2.0 cm/L)	115.3	102.6	95.6	11	17

FI: Irrigation 120%; DI1: Irrigation 100%; DI2: Irrigation 80 % of evapotranspiration (ETo).

3.2.4.3. Water productivity (WP) to geranium

Table (8) showed that the highest values of WP were attained under irrigation with 80% ETo, namely 9.31 and 9.88kg/hain both growing seasons under applicationhumicacid with 2.0 cm/L.While, the lowest valueswere found under irrigation 120% ETowith no application of humic acid namely4.19 and 4.67kg/ha in the both growing seasons.

Table 8: Water productivity (WP, kg/ha) forgeranium plantsunder different irrigation treatments and humicacidapplicationin two growing seasons.

Treatments	2017/2018			2018/2019		
	FI	DI1	DI2	FI	DI1	DI2
H1 (Control)	4.19	4.50	5.13	4.67	4.90	5.56
H2 (1.0 cm/L)	5.46	5.93	6.92	5.81	6.15	7.32
H3(1.5 cm/L)	5.81	6.35	7.53	6.18	6.72	7.84
H4(2.0 cm/L)	7.02	7.79	9.31	7.58	8.34	9.88

FI: Irrigation 120%; DI1: Irrigation 100%; DI2: Irrigation 80 % of evapotranspiration (ETo).

3.2.5. Effect of different treatment of humic acid on CMV Concentration:

Cucumber Mosaic Virus accumulation (biotic stress) in systemically naturally infected geranium plants was determined by ELISA (Table 9). The results declared that CMV Concentration decreased significantly in plants treated with humic acid compared to control (C). The most pronounced effect was achieved by applying foliar applications of (2.0 cm /L Humic acid). The highest percentage of CMV Concentration in infected untreated plants was (2.42 and 3.54)in both growing seasons. However, the results showed negative significant correlation between virus concentration and spraying with humic acid in both growing seasons on geranium plants. It was found that under foliar applications of 2.0 cm /L humic acid, virus concentration was decreased to (0.48 and 0.69nm) over two growing seasons respectively. It was reported that humic acid play a role in decreasing virus concentration,thus induce of systemic acquired resistance against biotic stresses [47]; [48]; [49] &[50].

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Table 9:Effect of different treatment of humic acid on CMV Concentration (nm) in both growing seasons.

Treatments	Virus Concentration (405 nm)	
	2017/2018	2018/2019
H1 (Control)	2.42 a	3.54 a
H2 (1.0 cm/L)	1.89 b	2.02 b
H3(1.5 cm/L)	1.01c	1.56 c
H4(2.0 cm/L)	0.48 d	0.69 d

Means (\pm SE standard error) followed by different letters (a, b, c, d) are significantly different from each other as indicated by Tukey's test ($p < 0.05$).

4. Conclusions

The results of this research indicated that application of 120% under spraying with 2.0 cm/L humic acid to geranium increase its fresh yield. However, application of 80% ET under spraying with 2.0 cm/L humic acid attained the highest values of geranium essential oil, water productivity and proline production. Whereas, pronounced decrease in CMV Concentration. Therefore, it is recommended to irrigate geranium plants with 80% ET under spraying with 2.0 cm/L humic acid to conserve irrigation water, increase the productivity of water unit and may be a hopeful strategy to overcome the biotic stresses by triggering the antioxidant defense system to induce systemic acquired resistance.

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Novelty Statement:

The novelty of our work entitled "Effect of foliar humic acids on water productivity, biochemical parameters and biotic stress in Geranium yield" highlighted on application of humic acid on geranium plants to improve essential oil, water productivity under surface irrigation and proline content production to increase biotic stress tolerance and reduce CMV Concentration that may be a hopeful strategy to overcome the biotic stresses by triggering the antioxidant defense system to induce systemic acquired resistance
Thank you for your consideration!

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