..

Original Research Article

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

ABSTRACT

Two-year experiment was conducted during 2017/2018 and2018/~~2019to~~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 indicated 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 m^3/ha) in first growing season and were (15218, 12298, and 9678 m^3/ha) in second growing 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 ~~of2~~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 be 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).

Formatted: Font color: Red

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 valuable volatile oil [2].

A humic acid (HA) is an organic molecule that improves soil properties, plant growth, and agronomic treats. 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.) facieses 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 field grown, are virus-infected [19]; [20]; [21].

Proline is considered important approaches to batter the a biotic 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].

Formatted: Font color: Text 2

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 (ETo, mm/day) was calculated using BISm model [27]. The values of ETo in 2017 for November and December were 2.94 and 2.60 (mm/day). As for 2018 ETo 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
	% weight	Cm	% weight	cm	% weight	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 spilt 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% ETo (full irrigation, FI control);
b) Application of 100% ETo (deficit irrigation, DI_1) and;
c) Application of 80% ETo (deficit irrigation, DI_2).

- 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 1.5 cm/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 1thof 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 m^2 (1.80 × 2.5), included 27 plant/plot. Cattle manure (36 m^3/ha) and calcium super phosphate (15.5% P_2O_5) 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% K_2O) 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 on15th 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 pplied, 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, cm^2; 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 (0.01M PBS pH 7.4 containing 0.05% Tween-20, 2% polyvinyl pyrrolidine, M. wt. 40,000) and then incubated at 4°C overnight. The plate was washed three times with washing buffer (PBS-T). 200 µl of IgG alkaline phosphate conjugate diluted at 1/100 in conjugate buffer pH 7.0 (phosphate buffer saline, bovine serum albumin (BSA) 1%, Tween 0.25%) was added to each well and incubated for 3 hrs at 37°C. The conjugate was removed and the plate washed three times with washing buffer (PBS-T). 200 µl of freshly prepared substrate (P-nitro phenylphosphate in substrate buffer (10% diethanol amine, NaN3 0.01%) at concentration 0.75 mg/ml was added to each well. The reaction was readed spectrophotometrically at 405 nm. After incubation at 37°C for 30 and/or 60 mins using vnusken ELISA reader. The reaction was stopped by adding 50

Formatted: Font color: Text 2

µl of 3M Na OH. The absorbance was measured at a wavelength of 405 nm using a Sunrise™ TECAN, Switzerland microplate reader. The ELISA values were indicated to be positive for more than twice of healthy plant values. ELISA experiment was conducted three times and three replicates (two leaves for each replicate) were used for each test.

2.2.6. Statistical analysis

All collected data were analyzed with analysis of variance (ANOVA) with Tukey's test ($p < 0.05$) procedure using MSTAT-C Statistical Software Package [37]. Differences between means were compared by using Duncan multiple range tests at 0.05 [38].

Formatted: Font: Font color: Dark Red

3. RESULTS AND DISCUSSION

3.1. Effect of humic acid and irrigation amount on geranium fresh yield

The results in Table (2) indicated that there were significant relationships between irrigation treatments, spraying with humic acid and their interaction in both seasons. The results also revealed that application of full irrigation (120% ETo) gave the highest value of geranium fresh yield with humic acid 2.0 cm/L, namely 110.9 ton/ha average over the two growing seasons, compared to no application of humic acid, which attained 67.3 ton/ha. Similar trends were obtained under deficit irrigation treatments in both growing seasons. The table also showed that spraying geranium plants with humic acid 2.0 cm/L under application of 100% or 80% ETo produced fresh yield higher than the value obtained under full irrigation without spraying with humic acid, namely 98.7 and 91.3 ton/ha average over the two growing seasons. This result implied that foliar application humic acid help geranium plants to withstand the effect of water deficiency and maintain reasonable yield.

Similar results were obtained by [39] & [40] who found that herb fresh weight and volatile oil yield of *Pelargonium graveolens* plants were gradually increased with increasing the amounts of the applied irrigation water. Furthermore, [41] indicated that drought stress had a significant effect on the decrease of most of the studied characteristics of aromatic geraniums. The promoting effects of humic acid may be attributed to its role of improving photosynthesis and consequently increasing of the secondary metabolites production [42]. Zaghloul et al., [43] indicated that spraying *Thujaorientalis* plants with humic acid increased its growth compared with no sprayed plants due to the direct effect of humic acid on solubilization and transport of nutrients.

Table 2: Geranium yield (ton/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)	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 agreements ~~withthe~~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 decrease under low water application, the oil yield was also decreased.

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 Thujaorientalis plants. Said-Al Ahl et 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 onproline 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/100gprolineaverage over two growing seasons, compared no application of humic acid, which attained 3.01 mg/100 g 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].

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 (ETo).

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 m^3/ha in first growing season and were 15218, 12298, and 9678 m^3/ha in second growing season under 120, 100, and 80 ETo, respectively. Application of 100% ETo resulted in 20% water saving, whereas application of 80% ETo saved 38% of the applied irrigation water, compared to 120% ETo average over the two seasons.

Table 6: Effect of irrigation treatment on the amounts of applied irrigation water (AIW, m^3/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 (ETo).

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% ETo 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% ETo and spraying with 2.0 cm/L humic acid.

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 9.88 kg/ha in both growing seasons under application humic acid with 2.0 cm/L. While, the lowest values were found under irrigation 120% ETo with no application of humic acid namely 4.19 and 4.67 kg/ha in the both growing seasons.

Table 8: Water productivity (WP, kg/ha) for geranium plants under different irrigation treatments and humic acid application in two growing seasons.

Formatted: Font: Font color: Red

Formatted: Font: Font color: Red

Formatted: Font: Font color: Dark Red

Treatments	FI	DI1	DI2	FI	DI1	DI2
2017/2018				2018/2019		
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 0.69 nm) 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].

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 (±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% ETo 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% ETo 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.

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!

5. References

1- Economic Affairs Sector, (EAS). Agricultural Economics Research Institute (AERI), Ministry of Agriculture and Land Reclamation, 2020.

2- Kumar D, Padalia R C, Suryavanshi P, Chauhan A, Pratap P, Verma S, Venkatesha K T, Kumar R, Singh S, and Tiwari A K. Essential oil yield, composition and quality at different harvesting times in three prevalent cultivars of rose-scented geranium. J. Appl. Hortic. 2021; 23:19–23.

3- Ampong K, Thilakaranthna M, and Gorim L Y. Understanding the role of humic acids on crop performance and soil health. Sec. Plant-Soil Interactions. 2022; 4:1-14, https://doi.org/10.3389/fagro.2022.848621.

4-Yang F and Antonieti M. Artificial humic acids: sustainable materials against climate change. Adv. Sci. 2020; 7:1-7. DOI: 10.1002/advs.201902992.

5-Chen Q, Zhaoming Q, Guohua M, Wang W, Dai J, Zhang M, Wei Z, Liu Z. Humic acid modulates growth, photosynthesis, hormone and osmolytes system of maize under drought conditions. Agricultural Water Management. 2022;263(1):107447. https://doi.org/10.1016/j.agwat.2021.107447.

6-Seleiman M, and Hafez E M. Optimizing Inputs Management for Sustainable Agricultural Development. Mitigating Environmental Stresses for Agricultural Sustainability in Egypt. 2021; P: 487–507. DOI: 10.1007/978-3-030-64323-2_18.

7-Gadallah L S, El-Ghadban M A E, Soliman A Sh, Mehasen H R A, Abbas M S. Effect of foliar application of anti salinity substances on geranium (*Pelargonium graveolens*) plants under salt stress. The Journal of Animal & Plant Sciences. 2020; 30(3): 723-729.

8- Mendoza J D S, Lilian C C, João S C C, Walter J S, Lin C M, Campos F G, Boaro C S F, and Marques M O M. Effect of irrigation depth on biomass production and metabolic profile of *Lippia alba* (linalool chemotype) essential oil. Agricultural Water Management. 2022; 262(107393), https://doi.org/10.1016/j.agwat.2021.10739.

9-Akbarzadeh A and Shahnazari A. The effect of deficit irrigation strategies on the efficiency from plant to essential oil production in peppermint (Mentha piperita L.). Frontiers in Water, 2021; 3:682640, https://doi.org/10.3389/frwa.682640

10- Abdel-Ghany G G and Abd El-Aleem W H. Effect of trickle irrigation and salicylic acid applications on quality and productivity of *pelargonium graveolens* plant; Egypt. J. of Appl. Sci. 2020; 35(5):63-74.

11- Sánchez-Blanco M J , Ortuño M F , Bañon S and Álvarez S. Deficit irrigation as a strategy to control growth in ornamental plants and enhance their ability to adapt to drought conditions. The Journal of Horticultural Science and Biotechnology. 2019; 94(2):137-150. https://doi.org/10.1080/14620316.2019.1570353.

12-Mila A J, Ali M d H, Akanda A R, Rashid M d, Rahman M d A, and Moral M T. Effects of deficit irrigation on yield, water productivity and economic return of sunflower. Cogent Food & Agriculture. 2017; 3: 1287619. https://doi.org/10.1080/23311932.2017.1287619.

13-Greaves G E and Yu-Min W. Yield response, water productivity, and seasonal water production functions for maize under deficit irrigation water management in southern Taiwan. Plant Production Science. 2017; 20(4):33-365. https://doi.org/10.1080/1343943X.2017.1365613

14-Farouk S and Omar M M. Sweet basil growth, physiological and ultrastructural modification, and oxidative defense system under water deficit and silicon forms treatment. J. Plant Growth Regul. 2020; 39:1–25.

15-Bidabadi S S, VanderWeide J and Sabbatini P. Exogenous melatonin improves glutathione content, redox state and increases essential oil production in two Salvia species under drought stress. Sci. Rep. 2020; 10(1):1–12.

16- Sofy A R and Soliman A M. Molecular identification of a Cucumber mosaic virus subgroup I Egyptian isolate from geranium based on bioinformatics analysis of CP gene sequence Egypt. Egypt. J. Virol. 2011; 8:178–194.37.
17- Faoro F, Gozzo F. Is modulating virus virulence by induced systemic resistance realistic? Plant Sci. 2015; 234:1–13. [CrossRef] [PubMed].
18- El-Borollosy A M and Oraby M M. Induced systemic resistance against Cucumber mosaic cucumovirus and promotion of cucumber growth by some plant growth-promoting rhizobacteria. Ann. Agric. Sci. 2012; 57:91–97. [CrossRef].
19- Derbalah A S H and Elsharkawy M M. A new strategy to control Cucumber mosaic virus using fabricated NiO-nanostructures. Journal of Biotechnology. 2019; 306:134-141. DOI:10.1016/j.jbiotec.2019.10.003 Corpus ID: 203984868
20- Gallitelli D. The ecology of Cucumber mosaic virus and sustainable agriculture. Virus Res. 2000; 71: 9-21.
21- Sofy A R, Dawoud R A, Sofy M R, Mohamed H I, Hmed A A and El-Dougdoug N K. Improving regulation of enzymatic and non-enzymatic antioxidants and stress-related gene stimulation in cucumber mosaic cucumovirus-infected cucumber plants treated with glycine betaine, chitosan and combination, Molecules. 2020;25: 2341.
22- Ashraf M and Foolad M R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 2007; 59:206–216. [CrossRef].
23- Hosseinifard M, Stefaniak S, Javid M G, Soltani E, Wojtyla L and Garnczarska M. Contribution of Exogenous Proline to Abiotic Stresses Tolerance in Plants: A Review. Int. J. Mol. Sci. 2022; 23(9):5186. https://doi.org/10.3390/ijms23095186.
24- Sadeghipour O. Cadmium toxicity alleviates by seed priming with proline or glycine betaine in cowpea (Vigna unguiculata (L.) Walp.) Egypt. J. Agron. 2020; 42:163–170.
25- El-Beltagi H S, Sofy M R, Aldaej M I, Mohamed H I. Silicon alleviates copper toxicity in flax plants by up regulating antioxidant defense and secondary metabolites and decreasing oxidative damage. Sustainability. 2020; 12:4732. doi: 10.3390/su12114732.
26- Qirat M, Shahbaz M and Perveen S. Beneficial role of foliar-applied proline on carrot (Daucus carota L.) under saline conditions. Pak. J. Bot. 2018; 50:1735–1744.
27- Snyder R L, Orang M, Bali K and Eching S. Basic irrigation scheduling BIS. http://www.waterplan.water.ca.gov/landwateruse/wateruse/Ag/CUP/Californi/Climate_Data_010804.xls. 2004.
28- Stackman W P. Determination of pore size by the air bubbling pressure method. Proceedings of the Wageningen Symposium on water in the unsaturated zone, Netherlands, UNESCO. 1966; 366- 372.
29- Jackson M L. Soil Chemical Analysis. Pub.Prentice Hall of Indian Pvt. Ltd, New Delhi, 1973.
30- Pharmacopoeia B. British Approved Names. A Dictionary of Drug Names for Regulatory Use in the UK. Stationary Office Press, London, UK, 2002.
31- Hoftman E. Chromatography.2nd ed., Reinhold Publ. Corp. 1967; 208-515.
32- Bunzen J N , Guichar J, Labbe P, Prevot J, Seprpinet J, and Tranchant J. Practical Manual of Gas Chromatography Journal Tranchant, Ed., El-Seivier Publ. Co., Amesterdam-London. 1969; 189-206.
33- Bates L S, Waldren R P and Teare I D. Rapid determination of free proline for water stress studies. Plant and Soil. 1973; 39:205-207.
34- Michigan State University. MSTAT-C Micro Computer Statistical Program, Version 2. Michigan State University, East Lansing, 1983.
35- Zhang Cun-Hui. Compound decision theory and empirical Bayes methods: invited paper. The Annals of Statistics. 2003; 31(2):379-390.
36- Zehnder G W, Yao C, Murphy J F, Sikora E R and Kloepper J W. Induction of resistance in tomato against Cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. BioControl. 2000; 45:127–137.
37- Michigan State University. MSTAT-C Micro Computer Statistical Program, Version 2. Michigan State University, East Lansing. 1983.

38- Duncan B D. Multiple ranges and multiple F test. Biometrics. 1955;11:1- 42.
39- Hammam K A, AwadAlla S S S, and Noreldin T. Response of growth, yield and essential oil of geranium plants to surface irrigation and humic acid treatments. Asian Plant Research Journal. 2021; 7(2): 39-56.
40- Abd El-Kafee M O, El-Mogy E E A M, and Ashour A M A N. Response of *Pelargonium graveolens* (L.) plants to treatments of irrigation, chemical, organic and bio-fertilization under sandy soil conditions. J. Plant Production, Mansoura Univ. 2014; 5(7):1345-1373.
41- Osman A S and Adil A M. The Role of Proline and Nano-Fertin Growth of Pelargonium (*Pelargonium graveolens* L.) Under Different Irrigation Period. East African Scholars J Agri Life Sci. 2021; (4)1:15-24, DOI:10.36349/easjals.2021.v04i01.003.
42- Wani A B, Chadar H, Wani A H, Singh S and Upadhyay N. Salicylic acid to decrease plant stress. Environ ChemLett. 2017; 15(1):101- 123.
43- Zaghloul S M, El-Quesni F E M and Mazhar A A M. Influence of Potassium Humate on Growth and Chemical constituents of Thuja Orientalis L seedlings. Ozean Journal of Applied Sciences. 2009; 2(1):73-78.
44- Said-Al Ahl H A H, Ayad S H and Hendawy S F. Effect of potassium humate and nitrogen fertilizer on herb and essential oil of oregano under different irrigation intervals, Journal of Applied Sciences. 2009; 2(3).
45- Singh M and Ramesh S. Effect of irrigation and nitrogen on herbage, oil yield and water- use efficiency in rosemary grown under semi- arid tropical conditions. J. Med. Aromatic Plant Sci. 2000; 22(IB):659-662.
46- Zehtab-Salmasi S, Javanshir A, Omidbaigi R, Aly-Ari H and Ghassemi-Golezani K. Effects of water supply and sowing date on performance and essential oil production of anise (Pimpinella anisumL.). Acta Agronomica Hungarica. 2001; 49(1):75-81. https://doi.org/10.1556/AAgr.49.2001.1.9
47- Abbas M F, Jasim A M and Al-Zubaidy B H. The effect of proline on growth and iconic composition of embryogenic callus and somatic embryos of the date palm (*Phoenix actylifera* L. Cv. Ashkar) under Na Cl stress. Inter. J. of Farming and Allied Sci. 2012; 1(30):82-87.
48- Sofy A R, Dawoud R A, Sofy M R, Mohamed H I, Hmed A A and El-Dougdoug N K. Improving regulation of enzymatic and non-enzymatic antioxidants and stress-related gene stimulation in cucumber mosaic cucumovirus-infected cucumber plants treated with glycine betaine, chitosan and combination, Molecules. 2020; 25: 2341.
49- Zehnder G W, Yao C, Murphy J F, Sikora E R and Kloepper J W. Induction of resistance in tomato against Cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. BioControl. 2000; 45:127–137.
50- Zhang X, Zhang G, Li P, Yang Q, Chen K, Zhao L, Apaliya M T, Gu X, Zhang H. Mechanisms of glycine betaine enhancing oxidative stress tolerance and biocontrol efficacy of Pichia caribbica against blue mold on apples. Biol. Control. 2017;108:55–63. [CrossRef].