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EFFECT OF CITRIC ACID ON SOME MORPHOLOGICAL CHARACTERISTICS AND THE CONTENT OF MAJOR NUTRIENT OF CHAMOMILE (*MATRICARIA CHAMOMILLA* L.) AT INCREASING CONCENTRATIONS OF KINETIN

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Abstract

A field biological experiment was carried out in the Botanical Garden of the Science Department / College of Basic Education / University of Mustansiriya for the winter growth season 2018-2019 in order to find out the effect of concentrations increasing of Citric acid and growth regulator kinetin on some morphological characteristics and the major nutrient content of Chamomile (*Matricaria chamomilla* L.). The experiment was designed according to Randomized Complete Block Design (RCBD) with three replicates, the results showed an increasing has been occurred in plant height, number of vegetative branches, shoot dry weight, root length, root volume, dry root weight and the content of major elements as nitrogen, phosphorus and potassium content in the plant compared to control groups.

Key words: Chamomile (*Matricaria chamomilla* L.), Citric acid, kinetin.

Introduction

Chamomile (*Matricaria chamomilla* L.) is one of the most important medicinal plants that commonly used in medicine since ancient times, because of its flowers contain a volatile oil reached to approximately 1% of the dry weight of flowers. Furthermore, chamomile oil is a blue-colored, heavy, viscous liquid because it contains the substance (Azulene), as well as containing other substances such as: Terpenoids, Flavonoids, Tanines, α-bisabolol, Chamazulene and Glycosides (Lozykowska, 2003), which belongs to Asteraceae family and it was formerly named as Compositae and Stellar (Al-Djui, 1996; Al-Nazareth, 2001; Abu Zaid, 2001). Moreover, it is important in the food and pharmaceutical industries, where Chamomile is widely used in most countries as a medication for many diseases and as a treatment for inflammation and antibacterial (Salamon, 2004). Citric acid is an organic acid that has an important role in the Krebs cycle and is the most available and effective in releasing energy that occurring in the mitochondria with the presence of oxygen. Citric acid produces from the reaction Acetyl CoA with Oxalacetate and releasing CoA as part of the aerobic respiration reactions that include Glycolysis, Krebs cycle and the electro transport system (Dvllan and Francis, 1991). Citric acid is an antioxidant and can behave similar to Auxins in terms of increasing cell division and volume, also is considered a healthy substance as an environmental friendly (Ibrahim *et al.*, 2007). Cytokinins are the main stimulate and regulator of cell division, a derivative of the Nitrogen base of Adenine (Al Khafaji, 2014). Cytokinins also have a physiological effect on the plant, including apical dominance, senescence, nutrient movement, effectiveness and activity of promeristem apical, flowering development, dormancy in seeds and buds, distinguishing and developing chloroplast, furthermore the Cytokinins are able to overcome the effect of Abscisic acid inhibiting Gibberellic action (Wasfi, 1995).

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Materials and Methods

The experiment was designed according to Randomized Complete Blocks Design (RCBD), where the experiment field was divided into 27 experimental units and the area of one experimental unit was‘ (1×1) m^2. The seeds were planted in a swarm for each line and the quantity of seeds were 12 $kg.h^{-1}$ at 8 of November

2018, the plants were sprayed after reaching the stage of 4-5 leaves and the treatments were as follows: control treatments sprayed with distilled water only, plants sprayed with a concentration of 10 mg.L^{-1} of Citric acid, plants sprayed with a concentration of 20 mg.L^{-1} of Citric acid, plants sprayed with a concentration of 50 mg.L^{-1} of kinetin, plants sprayed with a concentration of 100 mg.L^{-1} of kinetin. The following characteristics were studied as listed below:

• Plant height (cm): The plant height of the vegetative stage for five plants from each experimental unit was measured from the soil surface to the highest point in the plant by a graduated ruler.

• Number of vegetative branches (branch.Plant^{-1}): The number of vegetative branches was calculated for five plants randomly taken from each experimental unit.

• Root length (cm): The length of the root was measured from the zone of its contact with the stem at the end of the root a graduated ruler.

• Root volume (cm^3): The root volume was calculated by taking the volume of displacement water from the graduated cylinder.

• Shoot and root dry weight (gm.Plant^{-1}): Five plants and their roots were randomly lifting from each experimental unit, as water was added to the experimental units a day before the plants were lifted and the root is separated from shoot and dried in an electric oven machine at a temperature of 65-70^{o}C till the weight of each treatment is stable.

• Estimation of Nitrogen concentration (mg. g plant^{-1}): The percentage of Nitrogen for the shoot was estimated in the digested samples, where the total nitrogen was estimated according to the modified Kjeldahl analysis using the Micro Kjeldahl device depending on the method of A.O.A.A.C., (1975).

• Estimation of Phosphorus concentration (mg. g plant^{-1}): The Phosphorus content of the shoot was estimated by the spectrophotometer at the wavelength of 882 nm according to the Matt method, (1970).

• Estimation of Potassium concentration (mg. g plant^{-1}): The potassium ratio of the shoot was estimated by the flame photometer according to the (page *et al*., 1982) method, as the principle of the device's operation is based on the thermal emission of the combustion of compounds and as a result of this emission absorbs wavelength without the other.

Finally, the data were statistically analyzed and the averages were compared with the lowest significant difference at 0.05 (SAS, 2004).

Table 1: Effect of Citric Acid and Kinetin Spray on Chamomile Plant height (cm).

Citric Acid Concentrations (mg.L^{-1})	**Kinetin Concentrations (mg.L^{-1})**			**Citric Acid average**
	0	**50**	**100**	
0	40.01	36.60	30.55	35.72
10	49.32	38.22	48.72	45.42
20	53.40	44.61	47.96	48.66
Kinetin average	47.57	39.81	42.41	-
LSD (0.05)	Citric Acid Concentrations = 2.44			
	Kinetin Concentrations = 2.44			
	Interaction = 4.22			

Results and Discussion

The results in table 1 showed a significant increase in plant height as the concentration of 10 mg.L^{-1} of Citric acid was characterized by giving the highest rate of plant height reached to 48.66 cm with an increasing percentage of 36.22%. The results of the same Table also indicate that the treating of the Chamomile plants by Kinetin resulted in a significant decrease in plant height at the concentration of 50 mg. L^{-1} which gave the lowest height ratio of 39.81 cm and a declining percentage of 16.31% compared to control treatments.

The results in table 2 also showed a significant increase in the number of vegetative branches for Citric acid spraying treatments, where the concentration of 10 mg.L^{-1} superior by giving the highest average number of branches amounted to 28.71 branches.Plant^{-1}, while the control treatment gave the lowest average number of branches reached 23.42 branches.Plant^{-1} with a decrease percentage of 18.42% compared with the concentration of 10 mg.L^{-1} for this trait. The results of the same table also show a significant difference in the average number of vegetative branches under the effect of Kinetin concentrations and the concentration 50 mg.L^{-1} was superior by giving the highest average of 29.75 branches. Plant^{-1}, with an increase percentage of 32.99% compared to control treatments.

Table 2: Effect of Citric acid and Kinetin spraying on the number of vegetative branches in chamomile plant (branch.Plant^{-1}).

Citric Acid Concentrations (mg.L^{-1})	**Kinetin Concentrations (mg.L^{-1})**			**Citric Acid average**
	0	**50**	**100**	
0	19.09	27.70	23.47	23.42
10	25.46	29.90	30.75	28.71
20	22.55	31.64	26.86	27.02
Kinetin average	22.37	29.75	27.03	-
LSD (0.05)	Citric Acid Concentrations = 1.58			
	Kinetin Concentrations = 1.58			
	Interaction = 2.74			

Table 3: Effect of Citric acid and Kinetin spraying on the dry weight of shoot (g).

Citric Acid Concentrations ($mg.L^{-1}$)	Kinetin Concentrations ($mg.L^{-1}$) 0	50	100	Citric Acid average
0	4.11	6.29	5.33	5.24
10	5.38	7.16	7.37	6.64
20	4.92	7.58	5.94	6.15
Kinetin average	4.92	7.58	5.94	-
LSD (0.05)	Citric Acid Concentrations = 0.41			
	Kinetin Concentrations = 0.41			
	Interaction = 0.71			

The results of table 3 also showed a significant increase in the dry weight, where the concentration of 10 $mg.L^{-1}$ was superior by giving the highest average reached to 6.64g with an increasing percentage of 26.71%. The same Table also shows that plants, spraying with various concentrations of Kinetin resulted in a significant increase in dry weight of plants, where the concentration 50 $mg.L^{-1}$ was superior by giving it the highest average of 7.01 g with an increase percentage of 46.04% compared to the control treatment.

The results of table 4 showed the superiority of spray treatment with concentration of 10 $mg.L^{-1}$ of citric acid in the root length trait, which gave an average of 13.33 cm with an increase percentage of 40.16%. The same table also shows that the concentration of 50 $mg.L^{-1}$ of the two Kinetin was characterized by giving the highest root length rate amounted to 13.10 cm and an increase percentage of 30.08% compared to control treatments.

The results of table 5 showed a significant increase in the root volume as the concentration 10 $mg.L^{-1}$ of Citric acid was superior by achieving the highest rate amounted to 1.987 cm^3 with an increasing percentage amounted to 42.53% and the results of the same table indicate that there was a significant increase at foliar spraying of Kinetin, where the concentration 50 $mg.L^{-1}$ was characterized by achieving the highest rate reached to 1.919 cm^3, with a difference of an increase amounted 30.27% compared to the control treatments.

Table 4: Effect of Citric acid and Kinetin spraying on the root length of Chamomile (*Matricaria chamomilla* L.) (cm).

Citric Acid Concentrations ($mg.L^{-1}$)	Kinetin Concentrations ($mg.L^{-1}$) 0	50	100	Citric Acid average
0	8.07	10.67	9.77	9.51
10	11.91	13.67	14.42	13.33
20	10.22	14.95	12.37	12.51
Kinetin average	10.07	13.10	12.19	-
LSD (0.05)	Citric Acid Concentrations = 0.77			
	Kinetin Concentrations = 0.77			
	Interaction = 1.33			

Table 5: Effect of Citric acid and Kinetin spraying on the root volume of Chamomile (*Matricaria chamomilla* L.) (cm^3).

Citric Acid Concentrations ($mg.L^{-1}$)	Kinetin Concentrations ($mg.L^{-1}$) 0	50	100	Citric Acid average
0	1.145	1.515	1.387	1.394
10	1.773	2.014	2.175	1.987
20	1.500	2.228	1.674	1.801
Kinetin average	1.473	1.919	1.745	-
LSD (0.05)	Citric Acid Concentrations = 0.120			
	Kinetin Concentrations = 0.120			
	Interaction = 0.207			

Also the results of table 6 showed that the Citric acid spraying treatments had a significant effect on the root dry weight, where the concentration 10 $mg.L^{-1}$ was characterized by giving the highest rate amounted to 4.27 g with an increasing percentage of 47.33% and it can be observed from the same table the significant effect of the Kinetin spraying treatments, where the concentration 50 $mg.L^{-1}$ was superior by giving the highest rate of 4.27 kg and an increase percentage of 29.78% compared to the control treatments.

The results of table 7 showed that the addition of different levels of Citric acid has led to a significant increase in plant content of Nitrogen, the level of 10 $mg.L^{-1}$ was superior by giving the highest average reached to 15.21 $mg.g^{-1}$ by an increase percentage of 39.92%, the same table indicated that there were significant differences when spraying the plant at different concentrations of Kinetin, where the concentration 50 $mg.L^{-1}$ was significantly superior by giving the highest value amounted 14.69 $mg.g^{-1}$ with an increase percentage of 23.96% compared to the control treatments.

The results in table 8 showed a significant effect of

Table 6: Effect of Citric acid and Kinetin spraying on the root dry weight of Chamomile (*Matricaria chamomilla* L.) (g).

Citric Acid Concentrations ($mg.L^{-1}$)	Kinetin Concentrations ($mg.L^{-1}$) 0	50	100	Citric Acid average
0	2.57	3.37	3.07	3.00
10	3.96	4.42	4.89	4.42
20	3.34	5.01	3.74	4.03
Kinetin average	3.29	4.27	3.90	-
LSD (0.05)	Citric Acid Concentrations = 0.27			
	Kinetin Concentrations = 0.27			
	Interaction = 0.48			

Table 7: Effect of Citric acid and Kinetin spraying on the root content of Nitrogen (mg.g^{-1}).

Citric Acid Concentrations (mg.L^{-1})	Kinetin Concentrations (mg.L^{-1}) 0	50	100	Citric Acid average
0	9.89	11.86	10.85	10.87
10	13.87	15.76	16.01	15.21
20	11.80	16.44	13.09	13.78
Kinetin average	11.85	14.69	13.32	-
LSD (0.05)	Citric Acid Concentrations = 1.01			
	Kinetin Concentrations = 1.01			
	Interaction = 1.75			

the Citric acid using concentrations, where the concentration 10 mg.L^{-1} was characterized by achieving the highest rate of phosphorus shoot content reached 8.15 mg.g^{-1} and by an increase percentage of 41.49%, Kinetin also had a positive and significant effect on the shoot content of phosphorus as shown in the same table, where the concentration 50 mg.L^{-1} was superior by giving the highest average amounted 7.93 mg.g^{-1} with an increase percentage of 26.88% compared to the control treatments.

The results in table 9 indicated the significant superiority of Citric acid spraying treatments in the Potassium vegetable growth content, where the concentration 20 mg.L^{-1} gave a rate of 17.97 mg.g^{-1} by an increase percentage amounted 37.17%, the same table showed the significant effect of the spraying treatments by Kinetin towards increasing the level of use concentrations where the concentration 100 mg.L^{-1} achieved the highest rate of this trait was 17.50 mg.g^{-1} with an increase percentage of 26.26% compared to the comparison treatments.

The increase in plant height with the increase of Citric acid concentrations may be attributed to its role in stimulating cell division and elongation, this was positively reflected in the increase in plant height, which is agreed with what (Ahmed *et al*., 1997) findings, where that Citric acid at certain concentrations may be having a similar behavior in auxins action in increasing cell division and elongation. Furthermore, the reason for the decrease of the plant height under various concentrations of Kinetin may be due to the role of Kinetin in stimulating the growth of axillary buds by breaking the apical dominance (Lynrah *et al*., 2002), which leads to a decrease the main stem height of the plant (Srivastava, 2001). The increase in the number of vegetative branches may be due to the role of Citric acid in stimulating photosynthesis processes in plant and using its outputs in the building and development process, resulting in an increase in the number of vegetable branches. The significant effect of Kinetin may be due to the role it takes through releasing the growth of inhibited axillary buds by the auxins effect, which effects of the decrease of the apical dominance during stimulating and activating the tissues adjacent to the buds and stem vascular tissues, this facilitates the transfer of water and nutrients that encourage the emergence and development of axillary buds (Mohammed and Younis, 1991). Moreover, the reason for increasing the shoot dry weight may be due to the increase in the number of branches as shown in table 2 for the same treatment, resulting in an increase in the number of leaves, which may be positively reflected in increasing the efficiency of photosynthesis and thus increasing the shoot dry weight, as well as the increase in root length at 10 mg.L^{-1} it may be due to the role of Citric acid in increasing the biochemical and physiological processes inside the plant (Shadeed *et al*., 1990), including the division and elongation of root cells. In addition to that, the increase in root length at a concentration of 50 mg.L^{-1} of Kinetin was also due to its role in stimulating the apical meristem cell division which increases the root length (Wasfi, 1995). The increase in the root volume at 10 mg.L^{-1} may be attributed to the role of Citric acid in increasing the root length as shown in table 4, which was positively reflected in increased root volume of the plant. The increase in the root volume at the concentration of 50 mg.L^{-1} of Kinetin may be due to the role it plays by controlling the growth

Table 8: Effect of Citric acid and Kinetin spraying of phosphorus shoot content (mg.g^{-1}).

Citric Acid Concentrations (mg.L^{-1})	Kinetin Concentrations (mg.L^{-1}) 0	50	100	Citric Acid average
0	5.09	6.43	5.77	5.76
10	7.39	8.41	8.65	8.15
20	6.26	8.96	6.99	7.40
Kinetin average	6.25	7.93	7.14	-
LSD (0.05)	Citric Acid Concentrations = 0.56			
	Kinetin Concentrations = 0.56			
	Interaction = 0.98			

Table 9: Effect of Citric acid and Kinetin spraying of Potassium shoot content (mg.g^{-1}).

Citric Acid Concentrations (mg.L^{-1})	Kinetin Concentrations (mg.L^{-1}) 0	50	100	Citric Acid average
0	11.21	13.34	14.68	13.10
10	14.02	18.46	17.87	16.78
20	16.36	17.59	19.95	17.97
Kinetin average	13.86	16.49	17.50	-
LSD (0.05)	Citric Acid Concentrations = 0.89			
	Kinetin Concentrations = 0.89			
	Interaction = 1.55			

and development of plant organs. Kinetin also has a role in the plant response to the external factors, including the reception of light in modern vegetative branches, also food and water provision to the roots through its role in increasing vegetative growth, resulting in increased plant portability to photosynthesis and increase its output, which positively reflects on the root volume (Werner and Schmulling, 2009). Furthermore, the increasing root dry weight may be attributed to the positive role shown by treatment of 10 $mg.L^{-1}$ of Citric acid and treatment of 50 $mg.L^{-1}$ of Kinetin in stimulating vegetative growth, which leads to an increase in the efficiency of photosynthesis and then the accumulation of the resulting material in plant parts, including the root. As well as, the superiority of the same treatments in the root length trait as shown in table 4 and root volume in table 5, this was positively reflected in increasing the root dry weight. The increase in plant content from Nitrogen and Phosphorus elements at a concentration of 10$mg.L^{-1}$ may be due to the role of Citric acid in increasing biochemical and physiological processes within the plant (Shadeed *et al*., 1990), which increased the element absorption from the soil solution, as well as the role of Citric acid by increasing the root growth (Tables 4 and 5). This has led to an increase in the element absorption by the roots and its accumulation in the plant, the significant effect of Kinetin may be due to the existence of a direct correlation between the vegetative growth indicators and the Nitrogen and Phosphorus concentrations (Halabi, 2012). This was confirmed by the results of the current study as the treatment that led to the increase of vegetative growth itself has achieved a superior in increasing the concentration of Nitrogen and Phosphorus. In addition to the increase in Potassium at the concentration of 20 $mg.L^{-1}$ of Citric acid and concentration of 100 $mg.L^{-1}$ of Kinetin may be due to the occurrence of food balance status within the plant tissue, as noted increasing the Nitrogen and Phosphorus elements at a concentration of 10 $mg.L^{-1}$ of Citric acid and 50 $mg.L^{-1}$ and the Potassium decreased while the Potassium increased at concentration of 20 $mg.L^{-1}$ of Citric acid and concentration of 100 $mg.L^{-1}$ of Kinetin and decreased the Nitrogen and Phosphorus elements.

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