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ORIGINAL ARTICLE

EFFECT OF FOLIAR APPLICATION WITH KT-30 AND ACTIVE DRY YEAST IN GROWTH AND CHEMICAL CONTENT OF NAGAMI KUMQUAT (*FORTUNELLA MARGARITA* SWINGLE) SAPLINGS

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Abstract: The experiment was carried out in the lath house of Horticulture and Landscape Gardening Department, College of Agriculture, University of Anbar, Iraq, during the 2020 growing season, on three-year-old nagami kumquat saplings to study the effect of foliar spraying with KT-30 at four concentrations (0 and 2, 4 and 8 mg.L^{-1}), active dry yeast at three levels (0, 0.4 and 0.8%) and their interactions in some vegetative growth traits and chemical content. The results indicated that the two study factors showed a significant effect in all of the studied characteristics especially the foliar application treatment of KT-30 at a concentration of 8 mg.L^{-1} and the spraying with dry yeast at a level of 0.8% where achieved a significant superiority in the number and length of secondary shoots increment, leaves number increment, leaves area, percentage of nitrogen, phosphorus, potassium in leaves and their total chlorophyll content compared to the control treatment which achieved the lowest values. Bilateral interference treatments had a significant effect on all of the studied traits especially K3XY2 treatment.

***Key words*:** Foliar application, Cytokinins, Active dry yeast, Citrus, Vegetative growth.

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1. Introduction

Nagami or Oval Kumquat (*Fortunella margarita* Swingle) is an evergreen small tree or shrub belonging to the Rutaceae family. The trees are slow-growing reach heights of 1.5 to 2.5 m and have a vase-like or rounded canopy, grow well in areas with hot summer (25-38^{o}C), but are also able to overcome stormy winters with temperatures as low as -10^{o}C, this ability to tolerate temperatures below 0^{o}C for a long time is due to the long state of dormancy occurring in winter, which is discontinued only after several weeks of warm weather when new shoots or blossoms occur. Despite this, the trees grow better and produce best quality fruits in warmer regions [Abobatta (2018)]. The fruits are small ovals with a diameter of about (1.5-3 cm) and an average weight of 12g [Ladaniya (2008)], not very juicy, ranging from acid to subacid, and pleasantly flavored, they can be eaten along with the peel, which is rich in essential oils, antioxidants, and fiber. Fruits contain anti-oxidant vitamins such as vitamin A, C, E, and B-complex. It also contains minerals like calcium, copper, potassium, manganese, iron, selenium and zinc [Tan *et al*. (2016)].

Phytohormons are the most important endogenous substances for moderating physiological and molecular responses, a critical requirement for plant survival, Phytohormons act at their site of synthesis or elsewhere in plants following their transport [AL-Taey (2017), AL-Taey and Majid (2018)].

Cytokinins encourage cell division and regulate budding. It affects the polarization of mineral elements and nutrients that are produced by the plant and prevent flowering, fruiting and leaves from falling and

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senescence [AL-Taey *et al*. (2018), Hamza and AL-Taey (2020)].

One of the major issues with citrus seedlings grown in nurseries is their slow growth and the relatively long time it takes to reach the appropriate stage of transport to a sustainable location, which drives up production costs and necessitates the use of alternative methods to accelerate sapling growth, including foliar spraying with plant growth regulators such as CPPU or called KT-30 [N-phenyl-N-(2-chloro-4-pyridyl) urea], which is a substituted phenylurea, showing a cytokinin-like activity exceeding that of the adenine-type derivatives [Shudo (1994)], due to its high stability in the plant tissues where it is not degradation by the enzyme cytokinin oxidase (CKO*x*) that catalyse the degradation of adenine-based cytokinins [Mok and Mok (2001)], this synthetic growth regulator involved in many essential developmental processes, such as cell division and differentiation, overcome of apical dominance, nutrient uptake and assimilation, maintain chlorophyll concentration, enhance photosynthesis activity, delay senescence, abiotic stress response, as well as hormonal regulation of plant morphogenesis and other influences [Davies (2004), AL-Taey and Saadoon (2012)].

Biostimulants called the attention upon vegetables and in the last few decades, they subsequently became a positive alternative to chemical fertilizer. These tend to decrease even the severe environmental pollution [AL-Taey *et al.* (2017), AL-Taey and Majid (2018)] biostimulus activated the cell metabolism which reflected positively on growth and yield of crops [Al-Khafajy *et al*. (2020), AL-Taey and Burhan (2021)].

Active dry yeast considered bio-stimulant is characterized by its high content of minerals, vitamins (B1, B2 and B6), amino acids, enzymes, sugar and plant hormones namely cytokinins [Manea *et al*. (2019), Loai and Al Dabagh (2020)]. Application of active dry yeast is very effective in releasing CO_2, which reflect on improving net photosynthesis [Idso *et al*. (1995)]. It also has stimulatory effects on cell division and expansion, protein and nucleic acid biosynthesis and the formation of chlorophyll [Sinha and Pant (2014), Alalaf (2020)].

2. Materials and Methods

This study was conducted in lath house, Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq from April 2020 to December 2020 to study the effect of foliar spraying with KT-30 and active dry yeast in vegetative growth and chemical content of Nagami Kumquats (*Fortunella margarita* Swingle). At the age of three years, 108 uniform saplings were chosen in their growth as much as possible budded on the rootstock of Rough Lemon (*Citrus jambhiri* Lush.) and cultivated in plastic pots weighing 8 kg, all service operations were conducted for all saplings from fertilization, weeding, control of insects and diseases when needed. Samples were also gathered from the soil for the conduct of some chemical and physical analyzes before the implementation of experiment as demonstrated in Table 1.

A factorial experiment was conducted with two factors, first factor included foliar spraying with KT-30 solution at four concentrations: K0 (sprayed with distilled water), K1 (2 $mg.L^{-1}$), K2 (4 $mg.L^{-1}$) and K3 (8 $mg.L^{-1}$). The plant growth regulator produced by AlzChem group AG/Germany (I.A. 98%) was prepared after the weighed of growth regulator powder according to each concentration and dissolved into 20 ml of ethanol (90 percent) and then complete the volume to 1 liter with distilled water.

The second factor included spraying at three concentrations with active dry yeast: Y0 (spraying with distilled water), Y1 (0.4%) and Y2 (0.8%), the dry pure yeast (*Saccharomyces cerevisiae*) powder was activated by using sources of carbon and nitrogen with the ratio of 6:1. This ratio is appropriate to achieve the highest vegetative production of yeast (each ml yeast contained about 12000 of yeast cells).

The foliar applied of KT-30 was conducted according to the following dates: (5/4, 5/5, 5/9 and 5/10), as for spraying with the active dry yeast, it was sprayed after one day of spraying with the growth regulator and for each date using a backpack sprayer

Table 1: Physical and chemical properties of experimental soil.

Particle size distribution ($g.kg^{-1}$ soil)						Available nutrients ($mg.kg^{-1}$ soil)		
Sand	**Loam**	**Clay**	**Texture**	**pH**	**EC (1:1) $ds.m^{-1}$**	**N**	**P**	**K**
591.4	194.1	214.5	Sandy loam	7.2	2.23	69.7	14.6	194.3

of (16 liters) until drip point with the addition of a wetting agent (Triton B) to the spray solution (0.1%).

2.1 Statistical analysis

The experiment was conducted based on Randomized Complete Block Design with two-factor (4 × 3). Each treatment was represented in three replicates. Data were analyzed according to the statistical software of GeneStat and the least significant difference test (L.S.D) at 5% level was used to compare the arithmetic averages.

2.2 Studied traits

Number and length of the secondary shoots increment: The number of shoots ($shoot.sapling^{-1}$) and shoots length (cm) were calculated on the main shoots (before conducting the treatments) in April of 2020, it was calculated at the end of the experiment in the December of 2020, the average increase was calculated by the difference between the two readings.

Leaves number ($leaf.saplings^{-1}$): At the end of the experiment the number of leaves has been calculated.

Leaves area (dm^2): Ten leaves were selected per experimental unit from the node of the fifth-eighth of the shoots tips in December of 2020, then taken from each leaf a disk by cork borer (disk diameter 1 cm or with area of 0.786 cm^2), the leaves and disks putted in oven at a temperature of 65°C until weight stability, the average of leaf area was calculated according to Dvornic (1965).

Leaves area of the sapling = number of leaves per seedling x leaf average area (cm^2).

Leaves minerals content: Ten leaves were randomly selected per experimental unit. These samples were washed several times with tap water and distilled water then oven dried at 70°C till a constant weight then 0.2 gm. of each ground sample was digested. The digested solution was used for the determination of N, P and K.

Total nitrogen %: Total nitrogen % was determined using microkjeldahl methods according to Chapman and Pratt (1978).

Phosphorus (%): It was determined by using spectrophotometer 882 Mu according to the method outlined by Murphy and Riely (1962).

Potassium (%): It was determined photometrically using the method recommended by Horneck and Hanson (1998).

Total chlorophyll content in leaves (mg. $100g^{-1}$ fresh weight): Ten fully expanded leaves samples were taken at the sixth to eighth node of the shoots tips for the extraction of chlorophyll a and b, which was estimated according to method of Bajracharya (1999).

3. Results and Discussion

Number ($shoot.sapling^{-1}$) and length (cm) of the secondary shoots increment: Table 2 shows the significant effect of foliar application with KT-30 in increasing the number and length of secondary shoots, especially the K3 concentration, which achieved the highest increment, amounted to 13.44 $shoot.sapling^{-1}$, 13.12 cm compared to the rest of the concentrations, while the lowest mean was at K0, which was 9.89 $shoot.sapling^{-1}$, 8.42 cm, respectively. The spray with active dry yeast at a concentration of Y2 significantly outperformed the rest of the treatments, as it gave a 13.17 $shoot.sapling^{-1}$, 12.24 cm, while the lowest average increase in the number of secondary shoots and their length at Y0 level, was 9.92 $shoot.sapling^{-1}$, 9.89 cm, respectively. As for the bilateral interaction between the two study factors, the K3XY2 treatment achieving the highest values for these two traits, reaching 15.33 $shoot.sapling^{-1}$, 14.96 cm, respectively, while the treatment K0XY0 recorded the lowest values, reaching 8.33 $shoot.sapling^{-1}$, 7.14 cm, respectively for both traits.

Number of leaves ($leaf.sapling^{-1}$) and leaves area (dm^2): The data in Table 3 shows that the number of leaves and leaves area increased as a result of treatment with KT-30, especially the concentration of K3, which was significantly excelled on the rest of the treatments, achieved a values of 324.3 $leaf.sapling^{-1}$, 61.67 dm^2, while the lowest values were 204.8 $leaf.sapling^{-1}$, 38.62 dm^2 at the K0 concentration. The foliar spraying with dry yeast showed a significant increase, especially the Y2 level which achieved the highest values of 316.2 $leaf.sapling^{-1}$, 56.73 dm^2, while the lowest values was at the level of T0 which reached 212.0 $leaf.sapling^{-1}$, 40.17 dm^2. The interaction between the growth regulator and dry yeast had a significant effect in increasing the number of leaves and leaf area, where the treatment K3XY2 achieved the highest values reached 366.5 $leaf.sapling^{-1}$, 68.81 dm^2 while K0XY0 recorded the lowest values, which amounted

Table 2: Effect of spraying with KT-30 and active dry yeast and their interaction in the number and length of secondary shoots of nagami kumquat saplings.

Number of secondary shoots (shoot.sapling^{-1})					Length of secondary shoots (cm)			
KT-30 (K) mg.L^{-1}	**Dry yeast (Y) %**			**Means (K)**	**Dry yeast (Y) %**			**Means (K)**
	Y0	**Y1**	**Y2**		**Y0**	**Y1**	**Y2**	
K0	8.33	10.00	11.33	9.89	7.14	8.75	9.38	8.42
K1	10.67	11.67	12.00	11.45	9.63	10.26	11.47	10.45
K2	9.00	12.33	14.00	11.78	10.92	10.93	13.16	11.67
K3	11.67	13.33	15.33	13.44	11.86	12.54	14.96	13.12
Means (Y)	9.92	11.83	13.17	**Means (Y)**	9.89	10.62	12.24	
LSD 0.05	**K**	**Y**	**K × Y**		**K**	**Y**	**K × Y**	
	0.85	0.74	1.48		1.13	0.98	1.97	

Table 3: Effect of spraying with KT-30 and active dry yeast and their interaction in the leaves number increment and leaves area of nagami kumquat saplings.

Leaves number increment (leaf.sapling^{-1})					Leaves area (dm^2)			
KT-30 (K) mg.L^{-1}	**Dry yeast (Y) %**			**Means (K)**	**Dry yeast (Y) %**			**Means (K)**
	Y0	**Y1**	**Y2**		**Y0**	**Y1**	**Y2**	
K0	178.0	191.5	245.0	204.8	30.64	39.28	45.96	38.62
K1	211.5	297.0	322.5	277.0	40.95	55.56	55.43	50.64
K2	195.5	306.0	331.0	277.5	40.03	58.18	56.72	51.64
K3	263.0	343.5	366.5	324.3	49.27	66.34	68.81	61.67
Means (Y)	212.0	**284.3**	**316.2**	**Means (Y)**	40.17	54.84	56.73	
LSD 0.05	**K**	**Y**	**K × Y**		**K**	**Y**	**K × Y**	
	23.27	19.62	39.24		8.53	7.39	14.78	

to 178.0 leaf.sapling^{-1}, 30.64 dm^2 respectively for both traits.

The positive effect of foliar spraying with KT-30 in increasing the vegetative growth traits such as number and length of shoots may be attributed to its role in breaking of apical dominance and stimulating the growth of lateral buds, as well as stimulating the cellular division in apical meristems and adding new cells to the plant [Davies (2004)]. As for the reason increasing the number and area of leaves treated by KT-30 may be attributed to its role in stimulating the growth of leaves primordia and chloroplast development, in addition, KT-30 affects the allocation of nutrients and assimilates in the plant towards treated tissues with it [Mok and Mok (2001)] and their use in the building of vegetative parts. These results are consistent with the results of Al-Ahbaby (2016), where the number of leaves and their area, as well as branch length, increased significantly for two cultivars of grape (*Vitis vinifera* L.) saplings when spraying with KT-30 at a concentration of 10 mg.L^{-1}, it also agreed with Al-Hayali and Al-Janabi (2019), who reported a significant increase in the number and length of shoots as well as the number and area of leaves when sprayed with thidiazuron (TDZ) on marumi kumquat (*Fortunella japonica* Swingle) saplings at a concentration of 20 mg.L^{-1}.

The increase in vegetative growth traits as a result of spraying with active dry yeast may be attributed to its effective components such as major and minor elements, amino acids, vitamins and, natural hormones which enhanced cell division and expansion, metabolism and other biological reactions **[**Sinha and Pant (2014)]. In addition, the application of dry yeast is very effective in releasing CO_2 which reflects on improving photosynthesis efficiency [Idso *et al*. (1995)]. So all

Table 4: Effect of spraying with KT-30 and active dry yeast and their interaction in the leaves content of nitrogen and phosphorus of nagami kumquat saplings.

N%					P%			
KT-30 (K) mg.L^{-1}	Dry yeast (Y) %			Means (K)	Dry yeast (Y) %			Means (K)
	Y0	Y1	Y2		Y0	Y1	Y2	
K0	1.99	2.03	2.05	2.02	0.14	0.19	0.23	0.18
K1	2.05	2.08	2.09	2.07	0.18	0.22	0.27	0.22
K2	2.06	2.10	2.11	2.09	0.19	0.25	0.30	0.24
K3	2.13	2.16	2.18	2.15	0.24	0.27	0.33	0.28
Means (Y)	2.05	2.09	2.10	**Means (Y)**	0.18	0.23	0.28	
LSD 0.05	**K**	**Y**	**K × Y**		**K**	**Y**	**K × Y**	
	0.03	0.02	0.05		0.04	0.03	0.06	

these functions may be supplied the saplings with their growth requirements. These results agree with the results of Haggag *et al.* (2015) where the plant height, shoots number, and the number of leaves increased significantly for the olive (*Olea europaea* L.) saplings when spraying with the active dry yeast at a concentration of 1%, it also agreed with Kamel (2015), who reported a significant increase in shoot length, number of leaves, and leaf area when sprayed with a dry yeast on pomegranate (*Punica granatum* L.) transplants at a concentration of 0.5%.

Leaves content of nitrogen and phosphorus (%): Table 4 indicates a significant differences in the percentage of nitrogen and phosphorus due to the spraying with KT-30 where the K3 concentration was significantly excelled on the other treatments by giving the highest values reached 2.15%, 0.28% while K0 concentration showed the lowest values reached 2.02%, 0.18%. The foliar application with the active dry yeast showed a significant effect where the treatment of Y2 achieved the highest values which amounted to 2.10%, 0.28%, while the Y0 level gave the lowest leaves content of nitrogen and phosphorus which amounted to 2.05%, 0.18%. The interaction between the two factors had a significant effect in increasing the leaves content of nitrogen and phosphors, where the treatment K3XY2 achieved the highest values reached 2.18%, 0.33%, while the interaction treatment K0XY0 recorded the lowest values which amounted to 1.99%, 0.14%.

Leaves content of potassium (%) and total chlorophyll (mg.100gm^{-1} fresh weight): The study treatments affected the leaves content of potassium as well as its content of total chlorophyll. Table 5 shows that the spraying with KT-30 at a concentration of K3 significantly excelled on the rest of the concentrations by giving it the highest values of 1.65%, 1.38 mg.100gm^{-1} fresh weight compared to the K0 concentration, which showed the lowest values, which reached 1.38%, 1.17 mg.100gm^{-1} fresh weight.

The spraying with active dry yeast led to a significant increase in these two traits by increasing concentrations of spraying, especially the Y2 level which achieved the highest values reached 1.68%, 1.39 mg. 100gm^{-1} fresh weight while the lowest values were 1.36%, 1.14 mg. 100gm^{-1} fresh weight at Y0 level, The binary interaction achieved significant differences in the leaves content of potassium and total chlorophyll, the treatment K3XY2 achieved the highest values reached 1.82%, 1.49 mg.100gm^{-1} fresh weight compared to the treatment K0XY0, which recorded the lowest value amounted to 1.24%, 1.04 mg.100gm^{-1} fresh weight.

The positive effect of KT-30 on leaves content of NPK might be attributed to its stimulating role in the uptake and transport of nutrient elements in the plant towards the treated tissues with it [Mok and Mok (2001)]. In addition, the foliar spray with active dry yeast had a positive increase in NPK concentration in almond saplings leaves, may be due to its content of nutrients and amino acids [Sinha and Pant (2014)] that were absorbed directly by leaves, on the other hand, the increase in the leaves content of nutrients can be attributed to the effect of the two study factors in increasing the vegetative growth of the saplings (Tables

Table 5: Effect of spraying with KT-30 and active dry yeast and their interaction in the leaves content of potassium and their total chlorophyll of nagami kumquat saplings.

K%					Total chlorophyll (mg.100g^{-1} fresh weight)			
KT-30 (K) mg.L^{-1}	**Dry yeast (Y) %**			**Means (K)**	**Dry yeast (Y) %**			**Means (K)**
	Y0	**Y1**	**Y2**		**Y0**	**Y1**	**Y2**	
K0	1.24	1.39	1.53	1.38	1.04	1.15	1.33	1.17
K1	1.35	1.49	1.68	1.50	1.11	1.20	1.35	1.22
K2	1.37	1.52	1.69	1.52	1.16	1.26	1.42	1.28
K3	1.51	1.63	1.82	1.65	1.28	1.37	1.49	1.38
Means (Y)	1.36	1.50	1.68	**Means (Y)**	1.14	1.24	1.39	
LSD 0.05	**K**	**Y**	**K × Y**		**K**	**Y**	**K × Y**	
	0.05	0.04	0.08		0.06	0.05	0.11	

2, 3), which positively affects their ability to absorb the relatively available minerals in the soil as shown in Table 1 to achieve nutritional equilibrium in plant. These results are consistent with Al-Hayali and Al-Janabi (2019), where the percentage of NPK in leaves significantly increased when spraying the marumi kumquat saplings by thidiazuron, it also agrees with Haggag *et al*. (2015), who obtained a significant increase in the concentration of NPK in leaves of olive saplings when spraying it with dry yeast.

The reason for increased leaves content of total chlorophyll as a result of foliar application with KT-30 may be due to its role in promoting chlorophyll biosynthesis by activating the enzyme NADH-Protochorophyll, in addition to its role in stimulating chloroplast development [Davies (2004)]. The reason for the increased total leaf chlorophyll content as a result of treatment with active dry yeast may be due to its role in biosynthesis this pigment. These results correspond with Al-Ahbaby (2016) where the leaves content of grape saplings significantly increased from total chlorophyll when spraying it with KT-30, it also agreed with the results of Al-Dulaimi and Al-Janabi (2021) when spraying young olive trees with the Promalin at a concentration of 50 ml.L^{-1}, which significantly increased the concentration of total chlorophyll in leaves, it also agrees with Kamel (2015), who obtained a significant increase in the concentration of chlorophyll in the leaves of pomegranate transplants when spraying it with the active dry yeast.

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