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EFFECT OF YEAST, KINETIN AND SALICYLIC ACID ON THE GROWTH OF *ALOE VERA* L. PLANT AND ON THE PRODUCTION OF SOME MEDICINALLY ACTIVE COMPOUNDS

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Abstract

Aloe vera considered as one of medicinal plant and the most applied world wide. It is a useful source of active medicinal compounds such as Aloin, Alo- Emodine, Cenamic acid and Anthranol additional to vitamins; B12, Vitamin A, B-Group vitamins, Vitamin C, Vitamin E and folic acid. It's gel contains important amino acids needed by the human body and seven of the eight essential ones that just cannot be made.

The present study aimed to improve the quantitative and qualitative production of medically active substances for *Aloe vera* L. through the use combination of dry yeast suspension and plant growth regulators "Salicylic acid" and Kinetin.

The experiment was carried out under lath condition (50% light) of the Medical Plants Unit / College of Agricultural Engineering Sciences-University of Baghdad 2017/2018. For kinetin (50, 100 mg/l) and yeast suspension (5, 10 gm/l) as well as comparison treatment according to RCBD design with three replicates.

The results showed superiority significant for the treatments of $S_1K_2Y_1$, $S_1K_1Y_1$, and $S_2K_2Y_1$ in the number of leaves amounted to 18.15, 17.16 and 17.03 leave.plant^{-1}respectively, the highest weight of the leaves was at the treatment of $S_1K_1Y_1$(4600 gm.plants^{-1}). The wet and dry gel weight was significantly affected by the experimental treatments with the highest gel weight at the two treatments $S_1K_1Y_1$, $S_2K_1Y_1$(2643,2592 gm.plants^{-1}respectively).For active substances, the treatment $S_2K_2Y_1$ was higher in the ratio of Aloin and Aloe-Emodin which reached to 58.33 and 88.90 micrograms respectively and the two treatments $S_2K_2Y_1$ and $S_2K_2Y_1$in the ratio of anthranol. The two treatments $S_2K_1Y_1$ and $S_2K_2Y_2$ gave the highest rate of cenamic acid which reached to 18.13 and 18.10ìg.gm^{-1}respectively.

Key words : *Aloe vera*, HPLC, yeast, kinetin, salicylic acid, active medicinal compound.

Introduction

About 80 percent of the world's population relies on medicinal herbs as an initial medicine to treat a number of diseases as WHO mentioned, including *Aloe vera* L. (Mamedov, 2012) from the family of Asphodelaceae (Bhuvana *et al.*, 2014). The leaves are considered as a medical part of plant that the gel is extracted from which containing the anthraguinins derivatives included glycosides such as Aloin and Aloe-Emodin that have medicine effect (Roy *et al.*, 2012 and Patidar *et al.*, 2012).

Due to the importance of medicinal plants, several studies have been carried out to increase quantitative and qualitative production. Among these methods used are the leaves feeding, which is one of the methods for sprinkling of nutritious solutions on the vegetative parts by adopting concentrations that provide the maximum benefit and to be careful of plant damage. This requires determining the appropriate concentration of the nutritious solutions according to plants types, their ages, and stages of growth (Martin, 2012). The leaves tissues can absorb the nutrients through the cracks in the cuticle layer and the Ectodesmata moving to the rest of leave and pores (Shanika, 2015).

Among these substances that have clear effect on plant growth are growth regulators; including Kinetin which is one of the cytokines, which in turn plays an

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important role in promoting the movement and transport of nutrients towards areas treated as high metabolites, as well as in promoting cell division (Taiz and Zeiger, 2010).

Salicylic acid is a vegetarian hormone of phenolic nature. It is an organic chemical inducer that gives indication for the plants to direct their energy towards the formation of defensive compounds against diseases and insects (Lichi *et al.*, 2012). Yeast is a single-celled, unicellular organism that follows one of the people of fungi kingdom. It is one of the richest sources of organic iron, natural mine of trace elements in the body, source of zinc and vitamins, amino acids and proteins (Khafaji, 1990; N.RP, 1977; Ahmed *et al.*, 1997). So this study aimed to improve the quantitative and qualitative production of medically active substances of *Aloe vera* plant through the use of dry yeast suspension and plant growth regulators.

Alam *et al.* (2012) found that salicylic acid sprinkling on the *Catharanthus roseus* plant at the concentration of 10^{-7}M increased the branch length, dry and wet weight of the vegetative and root group, as well as increased photosynthetic rate and the nitrate reductase activity. Tala *et al.* (2014) found that salicylic acid spraying significantly increased wet and dry weight, number of branches, and plant height in municipal palms. In their study, (Ghasemzadeh and Jaafer, 2012) noted that the effect of salicylic acid spraying at different concentrations of (0, 90, 140mg.L^{-1}) in *Zingiber officinale* showed that increasing of acid concentration from 90 mg.l^{-1} to 140 mg.L^{-1} led to increased plant content of flavonoids and dissolved carbohydrates.

Youssif and Iman (1998) reported that the spray of kinetin at different concentrations(40, 60,and 80 mg.L^{-1}) on *Lavandula officinalis* resulted in a significant increase in the pilot oil content and the effective medicinal substance (Lenalol Acetate).

Lynrah (2002) noted that the *Turmeric curcuma* Longa spraying with concentration of 10, 20 mg.l^{-1} achieved significant differences in plant height, total yield and percentage of the active substance curcumin in oil when compared with treated plants by only distilled water. Jassim (2009) found that the freesia plant was sprayed with dry bread yeast at a concentration of (8-10 kg.L^{-1}) has improved all vegetative and synovial growth characteristics. Safaa *et al.* (2011) found that the most significant increase were in plant height, number of branches, number of leaves, leave area, soft and dry weights and quantity of base oil when sprayed dry yeast extract on *Pelargonium graveolens* in concentration of (4gm.L^{-1}).

Materials and Methods

Experimental study location

The experiment was carried out in the wooden canopy of the Medical Plants Unit, College of Agricultural Engineering Sciences-University of Baghdad for the spring season for the research year of 2017/2018.

The *Aloe vera* plant seedlings were obtained in two years age from the plantation of the medicinal plant unit in the horticulture section. The seedlings were planted in 35 cm diameter and 35 cm high vessels and filled with mixed-soil mixed with Peat Moss by 1:3 and studied its characteristics as shown in table 1.

These seedlings were placed in the canopy in three replicates resulted in 27 experimental units (including the experimental unit on 4 plants) and conducted the service operations throughout the experiment (watering, weeding, fighting and feeding). Leaves spray was at night using the materials disseminated at the highest concentration possible, Salicylic acid S and Kinetin K and the number of yeast Ye suspension three times during the season which ended two weeks before the end of the agricultural season.

Table 1 : The separation conditions of active compounds.

Compound	Mobile Phase	Column	Detector	Flow Rate
Aloin	Acetonitrile: D.W 80:20 ml	C18-ODS (30cmx4.6mm)	UV=280	1 ml.min^{-1}
Alo-emodin				
Anthranol	Methanol: D.W: Formic Acid 60:35:5 ml		UV=360	
Cenamic acid	Acetonitrile: D.W 60:40 ml		UV=254	

Treatments and experimental design

The experiment consisted of a combination formula of 9 treatments according to RCBD design. The combinations were as follows:

1. Control.

2. Combination of Salicylic acid 100 mg.l^{-1} + 50 Kinetin mg.l^{-1} + yeast suspension 5 gm.l^{-1}.

3. Combination of Salicylic acid 100 mg.l^{-1} + 50 Kinetin mg.l^{-1} + yeast suspension 10 gm.l^{-1}.

4. Combination of Salicylic acid 100 mg.l^{-1}+ 100 Kinetin mg.l^{-1}+ yeast suspension 5 gm.l^{-1}.

5. Combination of Salicylic acid 100 mg.l^{-1} + 100 Kinetin mg.l^{-1} + yeast suspension 10 gm.l^{-1}.

6. Combination of Salicylic acid 200 mg.l^{-1} + 50 Kinetin mg.l^{-1} + yeast suspension 5 gm.l^{-1}.

7. Combination of Salicylic acid 200 mg.l^{-1}+ 50 Kinetin mg.l^{-1} + yeast suspension 10 gm.l^{-1}.

8. Combination of Salicylic acid 200 mg.l^{-1} + 100 Kinetin mg.l^{-1} + yeast suspension 5 gm.l^{-1}.

9. Combination of Salicylic acid 200 mg.l^{-1}+ 100 Kinetin mg.l^{-1} + yeast suspension 10 gm.l^{-1}.

Vegetative properties were measured as follow: number of leaves (leaf.plant^{-1}), leaf weight (gm), wet and dry gel weight (gm), medicinal properties: content of chromatin, aloeodine, anthraol and cinnamic acid.

Preparation of standard materials

Weight of 0.1 gm of standard material and then dissolved in 100 ml of high-purity methanol so that the final concentration of the standard solution was 100 µg.ml^{-1} (100 ppm) and thus we had the stock solution. A number of concentrates (1, 2, 3 and 5) were prepared from the original prepared stock solution for calibration curve.

Active compound measurement

The gel was taken by cutting the leave lengthwise into two parts, then scouring the gel and adding 96% ethyl alcohol and then placing it in the blender. After that it was cleaned the impurities and fibers and stored in sealed plastic containers until time of the analysis.

The active materials were estimated by using high performance liquid chromatography (HPLC) -Shimadzu 2010 model Japanese origin- to provide optimal conditions for the separation of standard compounds at a concentration of50 ìg.ml^{-1}.The separation was done by using reversed phase column with rapid separation "fast liquid chromatography" (FLC) (Lu Hong-mei *et al*., 2006; Yuegang Zuo *et al*., 2008).

After analysis of the standard material and samples: Identification of active compound depended on Retention Time while the estimation of active compound concentration depended on Area under the peak for samples according to the following equation :

$$C_{sample} = \frac{C_{standard} \times A_{sample}}{A_{standard}} = \frac{D.F.}{Wt.\ or\ V}$$

$C_{standard}$: Concentration of standard (µg.ml^{-1}).

A_{sample}: Area under the peak of sample.

$A_{standard}$: Area under the peak of standard.

D.F: Dilution Factor

Wt. or V : Weight or Volume of taken sample.

Results and Discussion

Characteristics of vegetative growth

The present study showed that there were significant differences in weight and leaves number by the induction of plant treatment. So it has been achieved highest number of leaves in the treatments $S_1K_2Y_1$, $S_1K_1Y_1$ and $S_2K_2Y_1$amounted to 18.15, 17.16 and 17.03 leave.plant^{-1}, respectively, while the highest weight of the leaves was at the treatment of $S_1K_1Y_1$(4600 gm.plants^{-1}) when compared with the control treatment, which gave 5.56 leave.plant^{-1}, 1439 gm.plants^{-1} respectively as it shown in table 3.

The results of table 4 showed that wet and dry gel weight was affected by the experimental treatments which gave the highest weight of wet gel in treatments $S_1K_1Y_1$, $S_2K_1Y_1$ (2643, 2592 gm.plants^{-1}) comparing with the lowest weight of control treatment which gave 686 gm.plants^{-1}. In the dry weight of the gel in table 4, the treatment $S_2K_1Y_1$ and $S_1K_1Y_1$ gave the highest weight of 24.34 and 22.74 gm.plants^{-1} comparing with the control treatment which gave the lowest rate at 6.76 gm.plants^{-1}.

The researchers believe that the reasons for the plants' response to the SA treatment may be due to it's role in increasing the efficiency of photosynthesis, carbonic anhydrase, and nitrate oxidase enzyme (which is the main attributed reason for salicylic acid prevention from oxins oxidation and increasing the internal content of it in the plant). The SA also reduces the effectiveness of the proteinase enzyme as well as increasing nutrient uptake and cellular membrane protection, thereby improving growth rates and metabolism (Ahmed *et al*., 2001 and Fariduddin *et al*., 2003; Akbarpour *et al*., 2014), resulting in an increase in construction and growth, dry, wet and number of leaves. The role of Salicylic acid in improving vegetative growth indices may be attributed to its stimulating effect on green growth. It is an active growth hormone (Raskin, 1992).

The effect of kinetin may be attributed to increased vegetative growth by increasing the age of leaves and preventing their aging, which has positively affected the weight of the vegetative population (Kim *et al*., 2006 and Sakakibara, 2006) due to increased chlorophyll content in leaves through protein synthesis. Kinetin is encouraged to synthesize the RNA and thus increases the plant metabolic rate (Devlin and Wetham, 1998). The effect of positive yeast on all studied characteristics is

Table 2 : Effect of salicylic acid, kinetin and yeast suspension on the number of leaves (leave. Plant^{-1}) of *Aloe cera*.

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	17.16	15.84	16.5
	K2	18.15	15.56	16.86
S2	K1	14.93	15.01	14.97
	K2	17.03	15.62	16.33
LSD		N.S		N.S
Mean of yeast		16.82	15.51	
LSD		0.762		
S*Y				
		Y1	Y2	S mean
S1		17.66	15.7	16.68
S2		15.98	15.31	15.65
LSD		0.98		0.592
K * Y				
		Y1	Y2	K mean
K1		16.04	17.59	15.73
K2		15.42	15.59	16.59
		0.957		0.586
Comparison				
Comparison Mean		5.56		
LSD		1.31		

Table 3 : The average of soft leave weight (gm.plant^{-1}).

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	4600	4448	16.5
	K2	4062	3552	16.86
S2	K1	4261	3341	14.97
	K2	3922	3271	16.33
LSD		109.1		77.2
Mean of yeast		4211	3653	
LSD		54.6		
S*Y				
		Y1	Y2	S mean
S1		4331	4000	4165
S2		4092	3307	3699
LSD		77.2		54.6
K * Y				
		Y1	Y2	K mean
K1		4431	3895	4136
K2		3992	3412	3702
		N.S		54.6
Comparison				
Comparison Mean		1439		
LSD		104.6		

Table 4 : Mean of wet Gel weight (gm.plant^{-1}).

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	2643	2480	2561
	K2	2319	2237	2278
S2	K1	2592	1980	2286
	K2	2372	2141	2257
LSD		109.1		77.2
Mean of yeast		4211	3653	
LSD		122.6		86.7
S*Y				
		Y1	Y2	S mean
S1		2481	2358	2420
S2		2482	2061	2271
LSD		86.7		61.3
K * Y				
		Y1	Y2	K mean
K1		2618	2230	2424
K2		2346	2189	2267
LSD		86.7		61.3
Comparison				
Comparison Mean		686		
LSD		114.8		

Table 5 : Mean of dry Gel weight (gm.plant^{-1}).

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	22.74	20.46	21.60
	K2	20.13	19.30	19.71
S2	K1	24.34	17.57	20.96
	K2	17.82	18.64	18.23
LSD		1.417		N.S
Mean of yeast		21.26	18.99	
LSD		0.708		
S*Y				
		Y1	Y2	S mean
S1		21.43	19.88	20.66
S2		21.08	18.10	19.59
LSD		1.002		0.708
K * Y				
		Y1	Y2	K mean
K1		23.54	19.01	21.28
K2		18.98	18.97	18.97
LSD		86.7		0.708
Comparison				
Comparison Mean		6.76		
LSD		1.315		

Table 6 : Effect of salicylic acid, kinetin and yeast suspension on Aloin compound (µg.gm^{-1}) for *Aloe vera* plant.

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	7.47	11.63	9.55
	K2	12.93	15.37	14.15
S2	K1	14.17	20.23	17.20
	K2	58.33	33.60	45.97
LSD		0.66		0.47
Mean of yeast		23.23	20.21	
LSD		0.33		
S*Y				
		Y1	Y2	S mean
S1		10.20	13.50	11.85
S2		36.25	26.92	31.58
LSD		0.47		0.33
K * Y				
		Y1	Y2	K mean
K1		10.82	15.93	13.38
K2		35.63	24.48	30.06
LSD		86.7		0.33
Comparison				
Comparison Mean		2.77		
LSD		0.67		

Table 7 : Effect of salicylic acid, Kinetin and yeast suspension on Aloe-emodin (µg.gm^{-1}) for *Aloe vera* plant.

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	25.50	55.30	40.40
	K2	71.73	74.67	73.20
S2	K1	71.67	82.10	76.88
	K2	88.90	86.33	87.62
LSD		1.32		0.93
Mean of yeast		64.45	74.60	
LSD		0.66		
S*Y				
		Y1	Y2	S mean
S1		48.62	64.98	56.80
S2		80.28	84.22	82.25
LSD		0.93		0.66
K * Y				
		Y1	Y2	K mean
K1		48.58	68.70	58.64
K2		80.32	80.50	80.41
LSD		0.93		0.66
Comparison				
Comparison Mean		22.30		
LSD		1.31		

due to the production of Auxin, Gibberellins and cytokinine (Ahmed *et al.*, 2004; El-Tohamy and El-Greadly, 2007, El-Tohamy *et al.*, 2008).

These plant hormones are important in the growth and differentiation of plant tissues, their role in cell division and expansion, and as polarizing factors for processed foods that balance the physiological and biological processes and increase photosynthesis processes and improve growth properties (Attia and Jadou, 1999 and Jensen, 2004). As well as containing carbohydrates, protein, amino acids, and many vitamins (Mahmoued, 2001).

Characteristics of chemical growth

There was superiority significant in treatments $S_2K_2Y_1$, which gave the highest rate from Aloin and Aloe-Emodin (58.33, 88.90 µg.gm^{-1}) comparing with the control treatment which gave (2.77, 22.30 µg.gm^{-1}) as it shown in tables 6, 7.

The results of table 8 for anthranol indicated that the treatment by $S_2K_2Y_2$ and $S_2K_2Y_1$ were significantly higher which reached to 13.127 and 13.50 µg.g^{-1} compared to control treatment that gave 6.96 µg.g^{-1}. $S_2K_1Y_1$, $S_2K_2Y_1$ and $S_2K_2Y_2$ gave the highest ratio of cenamic acid to 18.13, 18.10 and 18.07 respectively, compared with the control treatment that gave 11.07 µg.g^{-1}. Salicylic acid (SA) contributes in activation of certain important enzymes such as amylase, which improve and increase the vegetative growth for plants.

This improves growth, photosynthesis, absorption and transport of nutrient elements within the plant, which results in increased secondary metabolites (Khan *et al*., 2003; Zahwan *et al*., 2010 and Serhed, 2012).

The increase in active medical compounds after the treatment is attributed to the containment of cytokines such as kinetin and yeast which include various mineral elements, especially nitrogen, which has an effect in increasing the concentration of medically active substances in leaves by increasing vegetative growth, number of leaves, width and thickness, leading to an increase in carbonation and thus an increase in the production of secondary compounds within the plant where nitrogen enters directly into the composition of these compounds or indirectly through the construction of some enzymes responsible for the composition of these compounds (Allen and Pilpeam, 2006).

Conclusion

This study suggests the high benefit of using this combination in improving, enhances and increase the production of active medical compounds by increasing

Table 8 : Effect of salicylic acid, Kinetin and yeast suspension on Anthranol (µg.gm^{-1}) for *Aloe vera* plant.

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	10.543	10.200	10.372
	K2	10.047	11.287	10.667
S2	K1	11.833	12.853	12.343
	K2	13.127	13.350	12.238
LSD		0.438		0.310
Mean of yeast		12.80	10.80	
LSD		0.219		
S*Y				
		Y1	Y2	S mean
S1		10.295	10.743	10.519
S2		12.480	13.102	12.791
LSD		N.S		0.219
K * Y				
		Y1	Y2	K mean
K1		11.188	11.527	11.358
K2		11.587	12.318	11.953
LSD		N.S		0.219
Comparison				
Comparison Mean		9.69		
LSD		0.406		

Table 9 : Effect of salicylic acid, Kinetin and yeast suspension on Cenamic acid (µg.gm^{-1}) for *Aloe vera* plant.

Salicylic acid	Kinetin	Yeast		S*K
		Y1	Y2	
S1	K1	11.87	12.83	12.35
	K2	13.43	14.43	13.93
S2	K1	18.13	14.77	16.45
	K2	18.10	18.07	18.08
LSD		0.39		0.27
Mean of yeast		15.38	15.03	
LSD		0.19		
S*Y				
		Y1	Y2	S mean
S1		12.65	13.63	13.14
S2		18.12	16.42	17.27
LSD		N.S		0.19
K * Y				
		Y1	Y2	K mean
K1		15.00	13.80	14.40
K2		15.77	16.25	16.01
LSD		0.27		0.19
Comparison				
Comparison Mean		11.07		
LSD		0.37		

vegetative growth, number of leaves, width and thickness, photosynthesis, absorption and transport of nutrient elements, as well as the activation of certain important enzymes.

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