

# Effect of Gibberellic, Salicylic Acids, and NPK Fertilizers on growth and chemical constituents of Rosemary plants (*Rosmarinus officinalis* L.).

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## Abstract

This experiment conducted on rosemary plant (*Rosmarinus officinalis* L.), aimed to investigate the effect of gibberellic acid (GA3), salicylic acid (SA), and NPK fertilizer on growth and chemical constituents as well as determining the best concentration. After two weeks from planting the rooted cuttings in plastic pots were sprayed with the SA at three concentrations (0, 150 and 300 mg l<sup>-1</sup>), GA3 Sprayed with two concentrations (0, 400 mg l<sup>-1</sup>), and NPK sprayed as Nano NPK at concentrations (3 and 6 g l<sup>-1</sup>), the control plants were fertilized with regular fertilizer recommendation of NPK (200-N, 100-P, 50-K mg l<sup>-1</sup>). The results showed: The triple interaction among the three factors studied indicated that the plants which sprayed with 400 mg l<sup>-1</sup> GA3, 300 mg l<sup>-1</sup> SA, and 6 g l<sup>-1</sup> nano NPK fertilizers gave the highest value number of branches 19.97 branch plant<sup>-1</sup>, volatile oil percent 1.626 %, and total chlorophyll content 32.70 mg g<sup>-1</sup> f.w, whereas plant height increased to highest value at 400 mg l<sup>-1</sup> GA3, 300 mg l<sup>-1</sup> SA and 3 g l<sup>-1</sup> nano NPK which reached to 38.30 cm, also production of volatile oil per plant increased at 400 mg l<sup>-1</sup> GA3, 150 mg l<sup>-1</sup> SA, and 6 g l<sup>-1</sup> nano NPK fertilizers 0.947 ml plant<sup>-1</sup>. It appears that using those levels gave the best growth and development results on rosemary plant. Further research is needed to investigate whether the higher concentrations than used in this research will give the better parameters.

**Keywords:** Rosemary plant, Gibberellic acid, Salicylic acid, Nano NPK fertilizers, Vegetative biomass, and volatile oil.

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## INTRODUCTION

*Rosmarinus officinalis* L. (Rosemary) is a common household plant which belongs to the family Lamiaceae that originates from the Mediterranean region (Begum et al., 2013). It is a fragrant evergreen shrub reaching up to 2 m height, leaves are strongly curved, leathery, opposite, linear and aromatic with prominent midrib (Arnold et al., 1997; & Sasikumar, 2012). Rosemary is commonly used as a flavoring agent in food processing and spice (Saito et al., 2004). It has therapeutic properties and has been used in the folk medicine, pharmaceutical, and cosmetics industries (Baker & Grant, 2018 Macedo et al., 2020).

There are many plant growth regulators used for inducing plant growth and development. Gibberellic acid (GA3) is a crucial plant growth regulator because it works and activates the processes of dividing and expanding cells, stimulates a number of vital enzymes, and increases the activity of numerous biological and physiological processes of the cell by promoting the synthesis of DNA, RNA, and proteins. (Hopkins & Hüner, 2009). The

simulative effect of GA3 on growth of medicinal plants was studied by (Hasan & Sultan, 2020) using GA3 at 150 mg l<sup>-1</sup> on Rosemary plant lead to increase plant height, number of branch, volatile oil percentage, production of volatile per plant, and total chlorophyll content in leave. Also (Abdel-Hamid, 2020) indicated that spraying the sweet basil (*Ocimum sanctum* L.) plants with GA3 at 200 mg l<sup>-1</sup> increase vegetative growth, and volatile oil percentage of plant.

Salicylic acid (SA) is one of the most important plants phenolics, its function as a regulation of physiological and biochemical processes such as, pigment accumulation, photosynthesis, enzyme activities, nutrient uptake, and response plants to biotic and abiotic stresses, enhances the production then accumulation of secondary metabolites in plants (Rivas-San Vicente & Plasencia, 2011; & Ali, 2021). Ali & Attia, (2015) found that the application of salicylic acid (0, and 0.2 mM) on two cutting (*Rosmarinus officinalis*) plant for two seasons, the results indicated that SA at level 0.2 mM caused the increase plant height, number of branches per plant. Fetouh, (2016) is reported to foliar spray SA at 0.4 Mm of Rosemary plants were

increase plant height, number of branches, total chlorophyll content, volatile oil percent and yield.

Nano fertilizers are the important tools in agriculture to progress crop growth, yield and quality parameters with increase nutrient use efficiency, high surface area, reduce wastage of fertilizers and cost of cultivation, as well as increase the global food security (Mandal, 2021; & Shilpa, et al., 2022). Spraying sweet basil (*Ocimum basilicum* L.) plants to nano NPK fertilizers at 2.5 g l<sup>-1</sup> increase the total chlorophyll content in leaves, essential oil percent and production volatile oil per plant (Alhasan et al., 2021). While Mahmoud & Swaefy, (2020) indicated that nano NPK fertilizers increased plant height, number of branches, and chlorophyll content of sage (*Salvia officinalis*) plants when spraying plant at 1000 mg l<sup>-1</sup> nano NPK presence in nano-zeolites fertilizers.

The present study is designed to obtain the best level of SA and GA3 to supporting potted plants from extreme climatic effects through the use of SA and to push the vegetative growth through the use GA3. In addition, to compare between the use of regular and nano chemical fertilizers to determine which one is the best are effects on vegetative growth, and volatile oil on rosemary plant.

## MATERIAL AND METHODS

This research was carried out during the period between 18th Dec 2021 to 1st Jul 2022, at the nursery of Duhok–University, Kurdistan region, Iraq. The rooted cuttings rosemary was planted in the medium consisted of (three parts of river soil + one part of sand + two part of peat moss) in 17 cm diameter plastic pots. The experiment was conducted to study three factors which include: first Gibberellic acid (GA3), the plants sprayed with two levels of GA3 (0, 400 mg l<sup>-1</sup>) obtained from (Bio world Company) for two time at 20th Feb and 20th March. The

second, Salicylic acid (SA), after two weeks from planting rooted cuttings in pots were sprayed with three levels of salicylic acid (0, 150 and 300 mg l<sup>-1</sup>) obtained from (Oxford Lab chm Company) for three times, at 1st Jan, 15th Feb, and 15th March. The third factor is Nano NPK Fertilizer, the plants sprayed with Nano NPK at concentrations (3 and 6 g l<sup>-1</sup>) obtained from (Khazra Company), which add at monthly interval started from 1st Feb till 1st June, the control plants were fertilized with regular fertilizer recommendation of NPK (200-N, 100-P, 50-K mg l<sup>-1</sup>) (Hemdan et al., 2020) by using urea (46% nitrogen), triple super phosphate (48-50 % P2O5), and Potassium sulphate (50-52 % K2O). The experiment was consisted of three factors 2 GA3 x 3 SA x 3 NPK fertilizers= 18 treatment with three replicates and five plants for each one. The factorial experiment was conducted by using RCBD design, The following parameters were recorded at the end of the experiment in 30th Jun 2022. Plant height (cm), branches number (branch plant-1), volatile oil percentage (%), and its amount per plant (ml plant-1) according (Clevenger, 1928), and total chlorophyll content in leaves (mg g<sup>-1</sup> f.w) according (Bruinsma, 1963). The data analyzed by using SAS (2002). Means comparison was done by Duncan's Multiple Ranges Test under 5%.

## RESULTS

Plant height (cm): The results in Table (1) indicated that spraying Gibberellic acid on Rosemary plants with a concentration of 400 mg l<sup>-1</sup> caused a significant increase in plant height which reached 37.03%. On the other hand, increasing salicylic acid concentrations significantly increased plant height. The plant height also significantly increased when sprayed with 6 g l<sup>-1</sup> of nano NPK fertilizers reaching 29.18 cm when compared with recommended NPK treatment of fertilizers as control which gave 26.33 cm.

**Table (1): Effect of Gibberellic, Salicylic acids, and NPK fertilizers on the plant height (cm) of *Rosmarinus officinalis* plants.**

Gibberellic acid (mg l <sup>-1</sup> )	Salicylic acid (mg l <sup>-1</sup> )	NPK Fertilizers			GA <sub>3</sub> * SA	GA <sub>3</sub> effect
		Recommended (200-100-50 mg l <sup>-1</sup> )	Nano 3 (g l <sup>-1</sup> )	Nano 6 (g l <sup>-1</sup> )		
0	0	21.40 <sup>g</sup>	24.27 <sup>e-g</sup>	22.10 <sup>fg</sup>	22.59 <sup>c</sup>	23.49 <sup>b</sup>
	150	24.07 <sup>e-g</sup>	23.67 <sup>e-g</sup>	22.40 <sup>fg</sup>	23.38 <sup>c</sup>	
	300	24.33 <sup>e-g</sup>	21.87 <sup>g</sup>	27.30 <sup>d-f</sup>	24.50 <sup>c</sup>	
400	0	24.63 <sup>e-g</sup>	28.37 <sup>c-e</sup>	34.80 <sup>ab</sup>	29.27 <sup>b</sup>	32.19 <sup>a</sup>
	150	30.90 <sup>b-d</sup>	31.53 <sup>b-d</sup>	33.17 <sup>bc</sup>	31.87 <sup>b</sup>	
	300	32.67 <sup>bc</sup>	38.30 <sup>a</sup>	35.30 <sup>ab</sup>	35.42 <sup>a</sup>	
GA <sub>3</sub> * NPK	0	23.27 <sup>c</sup>	23.28 <sup>c</sup>	23.93 <sup>c</sup>	SA effect	

<b>Fertilizers</b>	<b>400</b>	29.40 <sup>b</sup>	32.73 <sup>a</sup>	34.42 <sup>a</sup>	
<b>SA* NPK Fertilizers</b>	<b>0</b>	23.02 <sup>d</sup>	26.32 <sup>cd</sup>	28.45 <sup>a-c</sup>	25.93 <sup>b</sup>
	<b>150</b>	27.48 <sup>bc</sup>	27.60 <sup>a-c</sup>	27.78 <sup>a-c</sup>	27.62 <sup>b</sup>
	<b>300</b>	28.50 <sup>a-c</sup>	30.08 <sup>ab</sup>	31.30 <sup>a</sup>	29.96 <sup>a</sup>
<b>NPK Fertilizers effect</b>		26.33 <sup>b</sup>	28.00 <sup>ab</sup>	29.18 <sup>a</sup>	

Means with same letter for each factor and interactions are not significantly different at 5% level based on DMRT.

The highest increase in plant height reached 35.42 cm for the interaction between 400 mg l-1 GA3 and 300 mg l-1 SA, with increasing percentage reached 56.67%. In addition, the interaction between GA3 and NPK fertilizers showed a significant effect, with the highest plants 34.42 cm for the plants that were sprayed with GA3 at 400 mg l-1 and 6 g l-1 of nano NPK fertilizers. The results showed that the plants sprayed with SA at 300 mg l-1 interacted with 3 and 6 g l-1 nano NPK fertilizers gave the highest significant values of plant height which reached 31.30 and 30.08 cm respectively, in comparison with the lowest value 23.02 cm for SA at 0 mg l-1 interact with recommended NPK fertilizers as control.

The triple interaction among GA3, SA and NPK fertilizers factors indicated that the plants which sprayed

with 400 mg l-1 GA3, 300 mg l-1 SA and 3 g l-1 nano NPK fertilizers gave the highest value of plant height reached 38.30 cm, this treatment increased 79.97% compared with control.

Number of branches (branch plant-1): The outcomes in Table (2) indicated that spraying Gibberellic acid on Rosemary plants at a concentration 400 mg l-1 caused a considerably increased in the number branches reaches 14.96 branch plant-1. Furthermore, the increasing salicylic acid concentrations sprayed on the plants caused an increase in this trait significantly, from 13.45 to 13.93 and 15.16 branch plant-1 for 0, 150, and 300 mg l-1 respectively. Whereas spraying the rosemary plants with nano NPK fertilizers at 6 g l-1 increased this characteristic significantly to 15.11 branch plant-1.

**Table (2): Effect of Gibberellic, Salicylic acids, and NPK fertilizers on the number of branches (branch plant<sup>-1</sup>) of *Rosmarinus officinalis* plants.**

Gibberellic acid (mg l <sup>-1</sup> )	Salicylic acid (mg l <sup>-1</sup> )	NPK Fertilizers			GA <sub>3</sub> * SA	GA <sub>3</sub> effect
		Recommended (200-100-50 mg l <sup>-1</sup> )	Nano 3 (g l <sup>-1</sup> )	Nano 6 (g l <sup>-1</sup> )		
<b>0</b>	<b>0</b>	13.43 <sup>cd</sup>	12.08 <sup>d</sup>	14.17 <sup>b-d</sup>	13.23 <sup>b</sup>	13.39 <sup>b</sup>
	<b>150</b>	11.73 <sup>d</sup>	14.57 <sup>b-d</sup>	13.83 <sup>b-d</sup>	13.38 <sup>b</sup>	
	<b>300</b>	13.13 <sup>b-d</sup>	14.30 <sup>b-d</sup>	13.30 <sup>cd</sup>	13.58 <sup>b</sup>	
<b>400</b>	<b>0</b>	14.20 <sup>b-d</sup>	13.74 <sup>cd</sup>	13.07 <sup>cd</sup>	13.67 <sup>b</sup>	14.96 <sup>a</sup>
	<b>150</b>	13.10 <sup>cd</sup>	14.07 <sup>b-d</sup>	16.30 <sup>bc</sup>	14.49 <sup>b</sup>	
	<b>300</b>	13.20 <sup>cd</sup>	17.03 <sup>b</sup>	19.97 <sup>a</sup>	16.73 <sup>a</sup>	
<b>GA<sub>3</sub>* NPK Fertilizers</b>	<b>0</b>	12.77 <sup>c</sup>	13.65 <sup>bc</sup>	13.77 <sup>bc</sup>	<b>SA effect</b>	
	<b>400</b>	13.50 <sup>bc</sup>	14.95 <sup>ab</sup>	16.44 <sup>a</sup>		
<b>SA* NPK Fertilizers</b>	<b>0</b>	13.82 <sup>b-d</sup>	12.91 <sup>cd</sup>	13.62 <sup>b-d</sup>	13.45 <sup>b</sup>	15.16 <sup>a</sup>
	<b>150</b>	12.42 <sup>d</sup>	14.32 <sup>b-d</sup>	15.07 <sup>a-c</sup>	13.93 <sup>b</sup>	
	<b>300</b>	13.17 <sup>cd</sup>	15.67 <sup>ab</sup>	16.63 <sup>a</sup>	15.16 <sup>a</sup>	
<b>NPK Fertilizers effect</b>		13.13 <sup>b</sup>	14.30 <sup>ab</sup>	15.11 <sup>a</sup>		

Means with same letter for each factor and interactions are not significantly different at 5% level based on DMRT.

The highest number of branches per plant increased to 16.73 branch plant-1 when sprayed the plant with 400 mg

l-1 GA3 interact with 300 mg l-1 SA which differ significantly from all other treatments studied. Spraying

plants with GA3 at 400 mg l-1 and nano NPK fertilizers at 6 g l-1 gave the largest value 16.44 branch plant-1. The third dual interaction among SA and NPK fertilizers exposed that the best significant interaction was for 300 mg l-1 SA and 6 g l-1 nano NPK fertilizers, which recorded 16.63 branch plant-1 in comparison with the lowest value 12.42 branch plant-1 for 150 mg l-1 SA and recommended NPK fertilizer.

The triple interaction among the concentration of GA3, SA and NPK fertilizers factors, indicated that the plants which sprayed with 400 mg l-1 GA3, 300 mg l-1 SA, and 6 g l-1 nano NPK fertilizers concentration gave the

highest value of number of branch reach 19.97 branch plant-1.

Volatile oil percentage (%): The results in Table (3) clarified that spraying Rosemary plants with Gibberellic acid at 400 mg l-1 caused a significantly increase in the volatile oil percentage reaching 1.429 %. On other hand increased Salicylic acid concentration sprayed on the plants to 300 mg l-1 caused to increase volatile oil percentage significantly 1.417 % while the control treatment record 1.251%. Using nano NPK fertilizers at 6 g l-1 increased significantly the volatile oil to 1.423%.

**Table (3): Effect of Gibberellic, Salicylic acids, and NPK fertilizers on Volatile oil percentage (%) of *Rosmarinus officinalis* plants.**

Gibberellic acid (mg l <sup>-1</sup> )	Salicylic acid (mg l <sup>-1</sup> )	NPK Fertilizers			GA <sub>3</sub> * SA	GA <sub>3</sub> effect
		Recommended (200-100-50 mg l <sup>-1</sup> )	Nano 3 (g l <sup>-1</sup> )	Nano 6 (g l <sup>-1</sup> )		
0	0	1.186 <sup>c-e</sup>	1.214 <sup>b-e</sup>	1.162 <sup>de</sup>	1.187 <sup>b</sup>	1.244 <sup>b</sup>
	150	1.095 <sup>e</sup>	1.178 <sup>de</sup>	1.309 <sup>a-e</sup>	1.194 <sup>b</sup>	
	300	1.298 <sup>a-e</sup>	1.315 <sup>a-e</sup>	1.440 <sup>a-d</sup>	1.351 <sup>ab</sup>	
400	0	1.193 <sup>c-e</sup>	1.286 <sup>a-e</sup>	1.462 <sup>a-d</sup>	1.314 <sup>ab</sup>	1.429 <sup>a</sup>
	150	1.527 <sup>a-c</sup>	1.406 <sup>a-e</sup>	1.541 <sup>ab</sup>	1.492 <sup>a</sup>	
	300	1.417 <sup>a-e</sup>	1.405 <sup>a-e</sup>	1.626 <sup>a</sup>	1.482 <sup>a</sup>	
GA <sub>3</sub> * NPK Fertilizers	0	1.193 <sup>c</sup>	1.236 <sup>bc</sup>	1.304 <sup>bc</sup>	SA effect	
	400	1.379 <sup>bc</sup>	1.366 <sup>ab</sup>	1.543 <sup>a</sup>		
SA* NPK Fertilizers	0	1.190 <sup>b</sup>	1.250 <sup>b</sup>	1.312 <sup>ab</sup>	1.251 <sup>b</sup>	
	150	1.311 <sup>ab</sup>	1.292 <sup>b</sup>	1.425 <sup>ab</sup>	1.343 <sup>ab</sup>	
	300	1.358 <sup>ab</sup>	1.360 <sup>ab</sup>	1.533 <sup>a</sup>	1.417 <sup>a</sup>	
NPK Fertilizers effect		1.286 <sup>b</sup>	1.301 <sup>b</sup>	1.423 <sup>a</sup>		

Means with same letter for each factor and interactions are not significantly different at 5% level based on DMRT.

The result indicated that the volatile oil percent increased to 1.492 % for 400 mg l-1 GA3 and 150 mg l-1 SA, whereas the lowest percentage was 1.187 for control. In addition to that, the result showed a significantly increased in volatile oil percentage 1.543 % when sprayed the plants with 400 mg l-1 GA3 and 6 g l-1 nano NPK fertilizers. On the other hand, the result showed that the plants sprayed with 300 mg l-1 SA and fertilized with 6 g l-1 nano NPK fertilizers gave the highest significant value 1.533 % of volatile oil percentage in comparison with the least value 1.190 % for 0 mg l-1 SA and fertilized with recommended NPK fertilizers.

The triple interaction among the three factors studied indicated that the plants which sprayed with 400 mg l-1 GA3, 300 mg l-1 SA, and 6 g l-1 nano NPK fertilizers gave the highest value 1.626 % of volatile oil percentage compared with the lowest value 1.095 % for the plants

sprayed with 0 mg l-1 GA3, 150 mg l-1 SA, and fertilized with recommended NPK fertilizers.

Volatile oil production per plant (ml plant-1): The results in Table (4) showed that the production of volatile oil was significantly highest 0.741 ml plant-1, when sprayed the plants with GA3 at 400 mg l-1, with increased percentage 33.75%. Additionally, increased concentrations of SA sprayed on plants to 150 and 300 mg l-1 caused a significantly increase of volatile production to 0.658 and 0.701 ml plant-1 respectively. Fertilizing the plants with nano NPK fertilizers at 6 g l-1 increased the volatile oil production to 0.714 ml plant-1.

The results showed that plants treated with 400 mg l-1 GA3 interact with 150 and 300 mg l-1 SA gave a significantly highest values 0.787- and 0.786-ml plant-1 respectively. In addition to that, the interaction between

the concentrations of Gibberellic acid and NPK fertilizers showed a significantly increase in volatile oil production, the highest value reached 0.886 ml plant<sup>-1</sup> for spraying

400 mg l<sup>-1</sup> of GA<sub>3</sub> and fertilized with 6 g l<sup>-1</sup> of nano NPK fertilizers compared with the other interactions.

**Table (4): Effect of Gibberellic, Salicylic acids, and NPK fertilizers on volatile oil production per plant (ml plant<sup>-1</sup>) of *Rosmarinus officinalis* plants.**

Gibberellic acid (mg l <sup>-1</sup> )	Salicylic acid (mg l <sup>-1</sup> )	NPK Fertilizers			GA <sub>3</sub> * SA	GA <sub>3</sub> effect
		Recommended (200-100-50 mg l <sup>-1</sup> )	Nano 3 (g l <sup>-1</sup> )	Nano 6 (g l <sup>-1</sup> )		
0	0	0.532 <sup>de</sup>	0.549 <sup>de</sup>	0.466 <sup>e</sup>	0.516 <sup>c</sup>	0.554 <sup>b</sup>
	150	0.535 <sup>de</sup>	0.481 <sup>de</sup>	0.568 <sup>c-e</sup>	0.528 <sup>c</sup>	
	300	0.641 <sup>b-e</sup>	0.614 <sup>b-e</sup>	0.595 <sup>b-e</sup>	0.617 <sup>bc</sup>	
400	0	0.454 <sup>e</sup>	0.697 <sup>b-d</sup>	0.796 <sup>ab</sup>	0.649 <sup>b</sup>	0.741 <sup>a</sup>
	150	0.641 <sup>b-e</sup>	0.774 <sup>a-c</sup>	0.947 <sup>a</sup>	0.787 <sup>a</sup>	
	300	0.803 <sup>ab</sup>	0.642 <sup>b-e</sup>	0.914 <sup>a</sup>	0.786 <sup>a</sup>	
GA <sub>3</sub> * NPK Fertilizers	0	0.569 <sup>c</sup>	0.548 <sup>c</sup>	0.543 <sup>c</sup>	SA effect	
	400	0.632 <sup>bc</sup>	0.704 <sup>b</sup>	0.886 <sup>a</sup>		
SA* NPK Fertilizers	0	0.493 <sup>c</sup>	0.623 <sup>a-c</sup>	0.631 <sup>a-c</sup>	0.582 <sup>b</sup>	
	150	0.588 <sup>bc</sup>	0.628 <sup>a-c</sup>	0.758 <sup>a</sup>	0.658 <sup>a</sup>	
	300	0.722 <sup>ab</sup>	0.628 <sup>a-c</sup>	0.754 <sup>a</sup>	0.701 <sup>a</sup>	
NPK Fertilizers effect		0.601 <sup>b</sup>	0.626 <sup>b</sup>	0.714 <sup>a</sup>		

Means with same letter for each factor and interactions are not significantly different at 5% level based on DMRT.

Generally, the plants which sprayed with 400 mg l<sup>-1</sup> GA<sub>3</sub>, 150 mg l<sup>-1</sup> of SA, and fertilized with 6 g l<sup>-1</sup> of nano NPK fertilizers gave the highest value of volatile oil production 0.947 ml plant<sup>-1</sup>, which record increase percentage 108.59% compared with the least value 0.454 ml plant<sup>-1</sup> for the plants sprayed with 400 mg l<sup>-1</sup> GA<sub>3</sub>, 0 mg l<sup>-1</sup> SA and fertilized with recommended NPK fertilizers.

Total chlorophyll content in leaves (mg g<sup>-1</sup> fresh weight): The outcomes in Table (5) demonstrated that spraying Rosemary plant with Gibberellic acid at 400 mg l<sup>-1</sup> caused significantly increased in chlorophyll content in leaves reaching 20.25 mg g<sup>-1</sup> f.w. Furthermore, increased salicylic acid concentrations sprayed on the plants increased the values of this characteristic significantly

from 22.08 to 23.65 and 25.72 mg g<sup>-1</sup> f.w for 0, 150, and 300 mg l<sup>-1</sup> SA respectively. Whereas spraying plants with 6 g l<sup>-1</sup> of nano NPK increased chlorophyll content in leaves to 25.26 mg g<sup>-1</sup> f.w.

The chlorophyll content in leaves recorded a significantly highest value of 28.43 mg g<sup>-1</sup> fresh weight when plants were treated with 400 mg l<sup>-1</sup> GA<sub>3</sub> and 300 mg l<sup>-1</sup> SA. In addition to that, plants that were sprayed with GA<sub>3</sub> at 400 mg l<sup>-1</sup> and fertilized with 6 g l<sup>-1</sup> nano NPK fertilizers have a significantly higher value of the chlorophyll content in leaves reaching 28.24 mg g<sup>-1</sup> f.w. The third interaction showed that the plants which sprayed with SA at 300 mg l<sup>-1</sup> and fertilized with 6 g l<sup>-1</sup> of nano NPK fertilizers gave the highest significant value of chlorophyll content in leaves reached 28.06 mg g<sup>-1</sup> f.w.

**Table (5). Effect of Gibberellic, Salicylic acids, and NPK fertilizers on total chlorophyll content in leaves (mg g<sup>-1</sup> fresh weight) of *Rosmarinus officinalis* plants.**

Gibberellic acid (mg l <sup>-1</sup> )	Salicylic acid (mg l <sup>-1</sup> )	NPK Fertilizers			GA <sub>3</sub> * SA	GA <sub>3</sub> effect
		Recommended (200-100-50 mg l <sup>-1</sup> )	Nano 3 (g l <sup>-1</sup> )	Nano 6 (g l <sup>-1</sup> )		
0	0	20.20 <sup>e</sup>	24.41 <sup>cd</sup>	21.42 <sup>de</sup>	22.01 <sup>c</sup>	22.44 <sup>b</sup>
	150	21.33 <sup>de</sup>	23.54 <sup>de</sup>	21.99 <sup>de</sup>	22.29 <sup>c</sup>	
	300	23.75 <sup>de</sup>	21.86 <sup>de</sup>	23.43 <sup>de</sup>	23.01 <sup>bc</sup>	

<b>400</b>	<b>0</b>	21.07 <sup>de</sup>	21.87 <sup>de</sup>	23.50 <sup>de</sup>	22.14 <sup>c</sup>	25.20 <sup>a</sup>
	<b>150</b>	23.66 <sup>de</sup>	22.89 <sup>de</sup>	28.51 <sup>b</sup>	25.02 <sup>b</sup>	
	<b>300</b>	24.57 <sup>cd</sup>	28.01 <sup>bc</sup>	32.70 <sup>a</sup>	28.43 <sup>a</sup>	
<b>GA<sub>3</sub>* NPK Fertilizers</b>	<b>0</b>	21.76 <sup>c</sup>	23.27 <sup>bc</sup>	22.28 <sup>bc</sup>	<b>SA effect</b>	
	<b>400</b>	23.10 <sup>bc</sup>	24.25 <sup>b</sup>	28.24 <sup>a</sup>		
<b>SA* NPK Fertilizers</b>	<b>0</b>	20.63 <sup>c</sup>	23.14 <sup>bc</sup>	22.46 <sup>bc</sup>	22.08 <sup>c</sup>	
	<b>150</b>	22.50 <sup>bc</sup>	23.21 <sup>bc</sup>	25.25 <sup>b</sup>	23.65 <sup>b</sup>	
	<b>300</b>	24.16 <sup>b</sup>	24.94 <sup>b</sup>	28.06 <sup>a</sup>	25.72 <sup>a</sup>	
<b>NPK Fertilizers effect</b>		22.43 <sup>b</sup>	23.76 <sup>b</sup>	25.26 <sup>a</sup>		

Means with same letter for each factor and interactions are not significantly different at 5% level based on DMRT.

The triple interaction among the concentrations of GA<sub>3</sub>, SA and NPK fertilizers clarified that the plants which sprayed with 400 mg l<sup>-1</sup> GA<sub>3</sub>, 300 mg l<sup>-1</sup> SA, and fertilized with nano NPK fertilizers at 6 g l<sup>-1</sup> gave the highest value of chlorophyll content in leaves 32.70 mg g<sup>-1</sup> f.w.

## DISCUSSION

As shown in Tables (1, 2, 3, 4, and 5) sprayed Rosemary plants with 400 mg l<sup>-1</sup> gibberellic acid caused a considerably increased in plant height, number of branches, volatile oil percent, production of volatile oil per plant, and total chlorophyll content when compared with control. These findings can be attributed to gibberellic acid's physiological function in plants, which involves simulating and activating the processes of dividing and expanding cells. Gibberellic acid also activates numerous enzymes that are essential for plant growth and development and raises the activity of numerous biological and physiological processes of the cell by enhancing the synthesis of DNA, RNA, and proteins. Gibberellins also influence the permeability of cell walls and raise the water content of cells (Hopkins & Hüner, 2009). For increase plant height It is possible to interpret the gibberellic acid's ability to enhance auxin levels, which in turn causes an increase in cell division and cell elongation (Kuraishi & Muri, 1964; & Taiz & Zeiger, 2010), or this could be explained by the ability of GA<sub>3</sub> to stimulate the synthesis of mRNA for hydrolytic enzymes and by the fact that larger cells eventually result in longer internodes. (Baliah et al., 2018). Moreover, increased volatile oil percentage, and its amount per plant when treated with GA<sub>3</sub> is my refer to plant growth regulators can influence on essential oil production through effects on plant growth, essential oil biosynthesis and the number of oil storage structures (Sharafzadeh & Zare, 2011). On the other hand, may be due to their effects on enzymatic pathways of terpenoids biosynthesis (Sangwan et al., 2001; & Prins et al., 2010). The increase

in chlorophyll content in leave could be possibly attributed to the enhancement of ultra-structural morphogenesis of plastids by GA<sub>3</sub>, as suggested by (Arteca, 1996). Additionally, gibberellin works to protect the destruction chlorophyll by activating the chlorophyllase enzyme. (Jacob-Wilk et al., 1999). The result was in conformity with the finding of (Hasan & Sultan 2020) on *Rosmarinus officinalis* L. plant, (Dadkhah, 2016) on *Satureja hortensis* plant, (Rashad et al., 2009) on *Calendula officinalis* L. plant.

Increasing salicylic acid concentrations sprayed on plants to 300 mg l<sup>-1</sup> caused an increase in plant height, branches number, and volatile oil percentage, total chlorophyll content in leaves, as showed in Tables (1, 2, 3, and 5). Whereas, increasing the concentrations of SA sprayed on plants to 150 and 300 mg l<sup>-1</sup> caused a significantly increase of production volatile oil per plant as shown in Tables (5). These results can be explained by the stimulating effect on regulating plant physiological and biochemical processes such as cell division, differentiation and elongation, as well as, it stimulates the absorption of elements and control of their transition, increase the permeability of cellular membranes of ions, accelerate the process of carbon and protein synthesis and increases the activity of enzymatic processes of photosynthesis (Raskin, 1990; & El-Tayeb, 2005), the increase in plant height possibly as a result of SA ability to increase the amount of internal hormones like gibberellin, auxin, and cytokinin in plants, which in turn promotes cell division and elongation and, eventually, plant growth and development (Hayat & Ahmed, 2007). While the increase in volatile oil percentage and yield might be due to increase in nutrients uptake, growth rate, or alterations in the leaf oil gland population, carbohydrates content, and also due to the beneficial effect of SA on metabolism and enzymes activities responsible for mono or sesquiterpene biosynthesis (Gharib, 2006; & Pirbalouti et al., 2014). SA may be inducing gene expression related to the biosynthesis and production of some classes of secondary metabolites in plants (Taguchi et al., 2001). On other hand SA regulates

the PAL Phenylalanine ammonia-lyase enzyme activity, which act as a biosynthetic enzyme catalyzes for forming the defensive compounds such as phenolic compounds and terpenoids (Dong et al., 2010). Plants treated with SA increased in leaves chlorophyll content which could be attributed to its stimulatory effects on Rubisco activity and photosynthesis (Idrees et al., 2010). Or it could be as a result of SA capacity to boost the activity of particular enzymes, promoting chlorophyll biosynthesis or decreasing chlorophyll breakdown, increasing net photosynthesis. (Li et al., 2014). The results of present study agreed with Fetouh (2016) on *Rosmarinus officinalis* L. plants, Mohammed Saeed, (2020) on *Gazania rigens* L. plants, and Saharkhiz & Goudarzi, (2014) on *Mentha piperita* L. plant.

Nutrition plays a key role in plant growth and development. In the case of medicinal plants, nutrients increases will stimulate plants to growth and increase their yield and synthesize essential oils and active substances (Aziz et al., 2010; & Zheljzkov et al., 2011). In present study fertilizing the plants with 6 g l<sup>-1</sup> of nano NPK fertilizers caused an increase the value of plant height, branches number, volatile oil percentage, production volatile oil per plant, and total chlorophyll content in leaves, as shown in Tables (1, 2, 3, 4, and 5). These outcomes can be explained by the fact that nano fertilizers increase nutrient use efficiency, have a large surface area, and release slowly, which aids in quickly absorbing nutrients and speed of penetration, representation, and movement, leading to increased growth speed and quality (e.g., protein and starch) by promoting photosynthesis. (Valiki et al., 2015; & Sajyan et al., 2020). The higher essential oil productivity in rosemary under integrative application of fertilizer could be explained by the increasing other plant parameters such as plant height, and number of branches which may be correlated with essential oil yield (Amoaghaie & Golmohammadi., 2017). Or may be due to the presence nitrogen effect of the compound fertilizer on enzymatic activity and metabolism for the production of essential oils (Khalid, 2013). The reason for the increase total chlorophyll content may be due to the rapid arrival of nutrients added by the foliar spray method to reached the cells at a faster time, which helps in the speed and continuity of the supply of nutrients necessary for metabolic processes in the plant, including the construction of chlorophyll pigments (Rajasekar et al., 2017; & Al-Mohammad et al., 2021). The results of present study agreed with Alhasan et al., (2021) on *Ocimum Basilicum* L. Plant, AL-Qasy & Al-Shammari, (2022) on *Trigonella foenum-gracecum* L. plant, and Mahmoud & Swaefy, (2020) on *Salvia officinalis* plant.

## CONCLUSION

Thus, it was concluded that the results gained from this experiment exposed that *Rosmarinus officinalis* L. plants positively responded to GA<sub>3</sub>, SA, and NPK fertilizers. The plants treated with 400 mg l<sup>-1</sup> of GA<sub>3</sub>, 300 mg l<sup>-1</sup> SA, and 6 g l<sup>-1</sup> nano NPK fertilizers had significantly influenced on plant height, number of branches, volatile oil percentage, production of volatile oil per plant, and total chlorophyll content in leaves. Furthermore, the triple interaction between three factors studied 400 mg l<sup>-1</sup> GA<sub>3</sub>, 300 mg l<sup>-1</sup> SA, 6 g l<sup>-1</sup> nano NPK fertilizers increase most studied parameter. It appears that using those levels gave the best growth and development results on rosemary plant. Further research is needed to investigate whether the higher concentrations than used in this research will give the better parameters.

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