# Effect of Foliar Application by Nano-Amino Acids and NPK Fertilizer on the Content of Salvia Rosmarinus Leaves of Volatile Oils

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## Abstract

The study aims to investigate the effect of foliar apllication of nano-amino acids and NPK fertilizer in the rates of volatile oils, chlorophyll, Nitrogen, Phosphorus and Potassium in the leaves of Salvia rosmarinus. The experiment was factorial with two factors, namely nano-amino acids at three concentrations (0, 2 and 3) ml.L-1 , and the second factor is NPK compound with three concentrations (0, 3 and 4) g.L-1 and the interaction between them. The results showed that mostly the higher concentrations of foliar spraying treatments with nano-amino acids and NPK compound( 3 ml/L and 4gm/L respectively) gave the highest of the volatile oils rates and the interaction between the two gave the highest rates within the experiment's treatments.

Keywords: Salvia rosmarinus leaves; nano-amino acids; NPK fertilizer

## 1. Introduction

Salvia rosemarinus, commonly known as rosemary, belongs to the Lamiaceae family, and its native to the Mediterranean, from southern Europe to western Asia.It is cultivated all over the world. Nano-fertilizers differ greatly in physical and chemical properties compared with chemical fertilizers due to their small size being 1-100 nm in diameter (Rajput et al., 2021; Verma et al., 2022) which helps their rapid uptake by plants and facilitates the beneficial functions of the nitrogen cycle, promoting Enzymes activities and increase the photosynthesis rate. One of the most important components of the rosemary plant is the essential volatile oils as an aromatic plant. The volatile oil contains a complex mixture of 95.10 % of monoterpenes and 4.77% of sesquiterpenes (Rašković et al., 2014). Several studies indicated that amino acids cause a significant increase in the percentage of volatile oils of different plants, as in the study conducted by Eid et al. 2010, and Safa et al. 2011, who all confirmed that the external addition of amino acids caused a significant increase in the rate of oils, also. EL-Zefzafy et. al.,2016 showed that the external addition of amino acids resulted in improving quantity and quality of volatile oil components.

The flyer for the leaves of the studied plants. It had been found that the NPK compound had an effect on the properties of the volatile oils of Salvia rosmarinus, as its addition led to an increase in the proportion of α-Thujone oil and camphor, and it also led to an increase in the chemical properties of the plant. Therefore, the research aimed to study the effect of the foliar application of nano amino acids and NPK fertilizer on the rates of the volatile oils such as cinol, Cymene and others in Salvia Rosmarinus. Nitrogen (N), phosphorous (P), and potassium (K) are considered essential nutrients for plant growth and development. Nitrogen is important for plants, as it is involved in the formation of chlorophyll and increases the efficiency of photosynthesis. It also enters the representation of proteins and helps in the manufacture of vitamins, the lack of nitrogen causes a decrease in plant growth. (Al-Sahhaf, 1989)

## 2. Materials and Methods

The plant leaves were sprayed with solutions of nano-amino acids in three concentrations (0,2, 3 ml.L-1) and NPK solution in three concentrations also (0, 3, 4) g.L -1 in addition to the interaction between them. The experiment was carried out according to a Completely Randomized Design (C.R.D) with this two factors.The manual sprayer was used to spray the leaves in the early morning until the plants were completely wet, While the control plants were sprayed with distilled water only, taking into account the separation of treatments with a barrier to avoid the flying mist. The spraying was done three times, once every ten days.Three plants has been randomly taken from each experimental unit, as they were cut from the crown area to separate the vegetative part from the roots, then weighed with a sensitive balance and then left to dry at room temperature (25-30) oC for several days, until the weight was stable, oven was not used in order to avoid loss of the volatile oils. Then sent to analysis, (20 g) of the fresh vegetative part was taken and placed in a beaker, (100 ml) of distilled water was added to it and placed in the Clevenger apparatus for 3 hours, then the oil was collected and (20 ml) of hexane was added to it to separate the oil from the water droplets collected with the oil . The oil was collected and kept in the refrigerator until the analysis process. Gas chromatography-mass spectrometry was used for the identification of components in rosemary essential oil (Hcini et al.,2012).

### Measuring the chlorophyll content of the leaves (mg/g fresh weight)

The content of leaves of chlorophyll a, chlorophyll b and total chlorophyll in the leaves was estimated

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based on the method presented by the two scientists (Dere and Gunes, 1998) by taking (1 g) of the plant and cutting it into small pieces and crushing it in a ceramic mortar with (10 ml) 80% of acetone, then the filtrate was separated from the sediment using a centrifuge at a speed of 3000 rpm for 15 minutes. Ultraviolet spectrophotometer at two wavelengths (645 and 663 nm), the following equations were applied to calculate the ratio of chlorophyll a, chlorophyll b and total chlorophyll:

Total chlorophyll (mg/g) = 20.2 (D 645) – 8.02 (D 645) X (V/1000 x W)

Where

(V) = final filtrate volume (ml)

(D) = device reading

(W) = Weight of the model

## Determination of nitrogen content in leaves

Keldahl's method was used to estimate the protein percentage in the samples and based on the method mentioned by (van Dijk et al., 2000) and others, where a known weight of the sample was taken within (0.2 g) and placed in a beaker, then (5 ml) of acid was added. Concentrated sulfuric and an appropriate amount of a mixture of potassium sulfate and copper sulfate. The mixing process was carried out by heating the contents. After the mixing was finished, the mixture was transformed into a clear, blue-colored liquid. The liquid was quantitatively transferred to the distillation flask of the Kildall device, which contains a concentrated solution (40%) of sodium hydroxide. It is connected to a condenser distillation flask that ends with a test tube immersed in a receiving flask containing a known volume of boric acid (20%) in addition to drops of methyl guide red and dye (bromocresol blue). 25 ml) and then the liquid collected with hydrochloric acid (0.1) standardized and a control solution (Blanck) of the chemicals in the above was prepared except for the model and the nitrogen percentage is calculated according to the following equation.

Nitrogen % = volume of HCl consumed x standard x 0.014 / sample weight x 100

## Estimation of the percentage of phosphorous in the leaves

The phosphorous content of the plant was estimated by taking a weight of (0.5 g) from the crushed and dried sample and dissolving it with (5 ml) sulfuric acid and (2 ml) of perchloric acid. Aluminum molybdate and ascorbic acid were used (colorimetric method) and then measured using a spectrophotometer (Spectrophotometer) at a wavelength (700 nm).

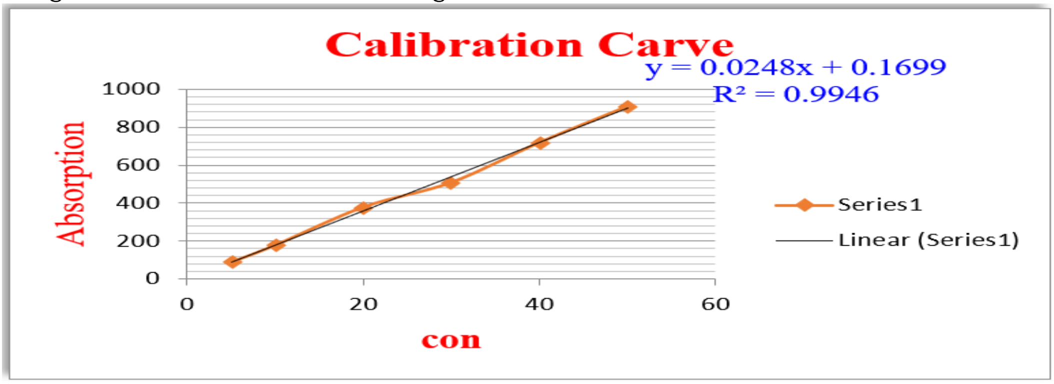


*Figure 1: shows the absorbance curve of phosphorous*

## Determination of potassium content in leaves

Potassium was estimated in the plant samples (which were collected, dried and ground), then (3 g) of the plant sample powder was put into a (25 ml) Griffin beaker cup, then (3 ml) of concentrated perchloric acid solution was added to it and covered. The cup using a watch bottle ((watch glass, and it was heated quietly and on an electric hot plate)), and the temperature was gradually raised in order to complete the mixing process and when the mixture reached the dryness stage, the cup was left to cool, and it was added again (3 ml) From the concentrated nitric acid solution, cover the cup and continue heating until the mixing process ends, where we get a light colored digestate mixture, then the evaporation process was carried out until it approached the stage of drying, and (5 ml) was added. of diluted hydrochloric acid solution with water in a ratio of (1:1), then the heating process was carried out, in order to dissolve the remaining sample after the mixing process, then distilled water was added, and the filtration process was carried out in order to get rid of the remaining and insoluble substances, and the volume of the solution was adjusted according to the concentration Expected in samples to a volume (100 ml or 50 ml or less), as The sample is ready for analysis. The absorbance of these mixed samples was measured using an atomic absorption device of the type (SHEMADZU AA 7000).

# 3. Experimental Treatments

T1: Distilled water

T2: NPK (3 g.L-1 )

T3: NPK (4 g.L-1 )

T4: Nano amino acids (2 ml.L-1 )

T5: Interaction between Nano amino acids (2 ml.L-1 ) + NPK (3 g.L-1 )

T6: Interaction between Nano amino acids (2 ml.L-1 ) + NPK (4 g.L-1 )

T7: Nano amino acids (3 ml.L-1 )
T8: Interaction between Nano amino acids (3 ml.L-1 ) + NPK (3 g.L-1 )
T9: Interaction between Nano amino acids (3 ml.L-1 ) + NPK (4 g.L-1 )

## 4. Results

The results in the table below show that foliar application with the nano-amino acids had a significant effect on the leaf content of the volatile oils, as the concentration of (3 ml.L-1 ) had the most effect on the rate of the volatile oils and recorded the highest rates in the compounds (cinol, cymene, limon, terpinen, camphor, apinene and camphene) which were ( 35.35, 3.98, 5.08, 7.99, 14,63, 9.71 and 5.78 )% respectively compared with plants treated with distilled water only, which gave the lowest rate. Also, the foliar application with NPK fertilizer had also a significant effect on the leaf content of volatile oils, as the concentration of (4 g.L-1 ) was superior to the rest of the treatments in giving the highest rate in the(cinol, cymene, limon, terpinen, camphor, apinene and camphene) compounds and recorded ( 35.35, 3.97, 5.12, 8.04, 14.69, 9.69, 5.83)% respectively compared with plants treated with distilled water only, which gave the lowest rate. The interaction between the two experimental factors, the nano amino acid at a concentration of (3 ml.L-1 ) and the NPK compound at a concentration of (4 g.L-1 ), had the greatest significant effect, as it gave the highest rates within the experimental treatments.

Table (1): The effect of nano-amino acids and NPK compound on the rates of volatile oils in Salvia rosmarinus leaves

| No | | Cinol % | Cymene % | Limonene% | Terpinen % | Camphor % | a-pinene % | Camphene% |
|---|---|---|---|---|---|---|---|---|
| T1 | | 33.16i | 2.29i | 3.37i | 6.52i | 13.06i | 8.20i | 4.17i |
| T2 | | 33.87g | 2.82g | 3.88g | 7.05g | 13.56g | 8.60g | 4.70g |
| T3 | | 34.71e | 3.33e | 4.60e | 7.59e | 14.26e | 9.06e | 5.16e |
| T4 | | 33.64h | 2.53h | 3.74h | 6.84h | 13.26h | 8.36h | 4.37h |
| T5 | | 34.91d | 3.74d | 4.84d | 7.84d | 14.49d | 9.29d | 5.48d |
| T6 | | 35.39c | 3.96c | 5.15c | 8.09c | 14.74c | 9.73c | 5.92c |
| T7 | | 34.4f | 3.08f | 4.26f | 7.27f | 13.93f | 8.80f | 4.84f |
| T8 | | 35.73b | 4.26 | 5.38b | 8.24b | 14.89b | 10.05b | 6.09b |
| T9 | | 35.94a | 4.62a | 5.61a | 8.45a | 15.08a | 10.29a | 6.41a |
| The average of Nano amino acideffect | control | 33.91 c | 2.81 c | 3.95 c | 7.06 c | 13.63 c | 8.62 c | 4.67 c |
| | 2 ml.L$^{-1}$ | 34.64 b | 3.41 b | 4.58 b | 7.59 b | 14.16 b | 9.13 b | 5.26 b |
| | 3 ml.L$^{-1}$ | 35.35 a | 3.98 a | 5.08 a | 7.99 a | 14.63 a | 9.71 a | 5.78 a |
| The average of NPK effect | control | 33.73c | 2.63c | 3.79c | 6.88c | 13.41c | 8.45c | 4.46c |
| | 3 g.L-1 | 34.84b | 3.60b | 4.70b | 7.71b | 14.31b | 9.31b | 5.42b |
| | 4 g.L-1 | 35.35a | 3.97a | 5.12a | 8.04a | 14.69a | 9.69a | 5.83a |

Also there was a significant effect on the chlorophyll, N, P, and K levels which also increased with the increase of the concentrations at each experimental treatment, while the interaction between them gave the highest effect, as shown in table (2).

Table (2): The effect of nano-amino acids and NPK compound on the rates of chlorophyll,N,P and K in Salvia rosmarinus leaves

| No | | Chlorophyll % | N % | P% | K % |
|---|---|---|---|---|---|
| T1 | | 25.16i | 0.46i | 0.52dc | 4.65i |
| T2 | | 25.52g | 0.55g | 0.40f | 4.75g |
| T3 | | 25.98e | 0.61e | 0.44fe | 4.83e |
| T4 | | 25.38h | 0.51h | 0.40f | 4.70h |
| T5 | | 26.24d | 0.64d | 0.48de | 4.88d |
| T6 | | 26.40c | 0.67c | 0.58bc | 4.93c |
| T7 | | 25.73f | 0.58f | 0.44fe | 4.79f |
| T8 | | 26.68b | 0.70b | 0.64ba | 4.98b |
| T9 | | 26.88a | 0.75a | 0.68a | 5.07a |
| The average of Nano amino acideffect | control | 25.55c | 0.54c | 0.46c | 4.75c |
| | 2 ml.L$^{-1}$ | 26.00b | 0.61b | 0.49b | 4.84b |
| | 3 ml.L$^{-1}$ | 26.43a | 0.68a | 0.59a | 4.94a |
| The average of NPK effect | control | 25.42c | 0.52c | 0.46c | 4.71c |
| | 3 g.L$^{-1}$ | 26.14b | 0.63b | 0.51b | 4.87b |
| | 4 g.L$^{-1}$ | 26.42a | 0.68a | 0.57a | 4.94a |

## 5. Discussion

It has been noted from the tables above that foliar application with nano-amino acids and NPK compound had a significant effect on increasing the percentage of volatile oils, chlorophyll, Nitrogen, Potassium and phosphorus in the plant leaves.Nano amino acids contribute to the development of biochemical and metabolic processes as they are easy to absorb and move within the plant, as well as being ready components for building and manufacturing proteins and enzymes (El-Shabasi et al., 2005 and Kamal et al., 2008). The increase in the oil content in the leaves may explain the fact that amino acids, after their structural and vital role inside the plant cell, enter the catabolic pathway in cytosol to produce the pyruvate compound, and this in turn turns into two units of Acetyl-CoA to enter Acetyl-CoA in The Acetate Mevalonate pathway to produce Isopentenyl pyrophosphate, the active form of building volatile oils (Batla and Lalm,2019, Asadi et. al. 2018). Some studies mentioned that external addition of amino acids significantly increased the leaves content of essential oil percentage ( Omer et.al.2013) , the element nitrogen in NPK compound may be important in the manufacture and increase of the percentage of chlorophyll in the leaves and thus has affected the efficiency of the photosynthesis process, which led to an increase in the manufacture of nutrients within the plant such as carbohydrates (Larimi et al., 2014), which led to an increase in the production of turbines through The entry of carbohydrates into the process of glycolysis and the production of pyruvic acid and then Mevalonic acid later, which is the main key to the production of isoprene units, which represent the basic unit for the formation of turbine oils (Murarikova et al., 2017). Bidwell,(1979) demonstrated that phosphorus has a vital role in energy compounds and translocation the elements and tissues formation, as well as potassium is very important for translocation the carbohydrates, water balance and increasing the elements in plants part.

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