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Original Article

The Effect of Putrescine Foliar Application on the Macronutrient Elements of Sage Shoots (*Salvia officinalis* L.)

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ABSTRACT

Sage is a plant belonging to the Labiatae family. It is native to Middle East and Mediterranean areas, but today has been naturalized throughout the world. In folk medicine, *Salvia officinalis* has been used for the treatment of different kinds of disorders including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, and hyperglycemia. This study was conducted to evaluate the impact of the foliar application of putrescine on the macro elements of sage leaves. The treatments used included four levels of putrescine, Put (Control: 0, Put1: 500, Put2: 1000, and Put3: 1500 ppm) with five replications.

According to the results of this research, putrescine had significant effects on the amount of nitrogen, phosphorus, potassium, calcium, magnesium and sulphate. The results showed that the amount of potassium and calcium in sage increased significantly when putrescine was used at 1500 mg/l. The highest magnesium and sulphate content was observed in 1000 mg/l. The findings of the present study can be used to manage the production of medicinal plants and the quality of their products. In addition, in sustainable agriculture, using polyamine substances can reduce the application rate of chemical fertilizers, thereby maintaining the environment. Also, the polyamine putrescine (Put) plays an essential role in controlling the innate immune response.

INTRODUCTION

Salvia officinalis (Sage) is one of the most valuable plants of this genus, which has been widely used in traditional medicine, cosmetics, perfumery, and health industries since long ago [1]. Also, among the valuable benefits of this medicinal plant, it has been reported to treat high fever and some digestive disorders. It is a strong antiseptic, antibacterial, anti-inflammatory, and anti-syphilis [2]. Polyamines are a group of organic compounds known as aliphatic hydrocarbons that require the growth and development of prokaryotic and eukaryotic organisms are necessary. In plant cells, putrescine, triamine spermidine, and tetraamine are the main polyamines. In plants, polyamines are widespread in all living organs, and the concentration of these substances increases in proliferating cells. Polyamines have a vital role in the transcription process of protein synthesis and the balance of cellular enzyme activity [3]. Polyamines also have many significant effects on the physiological functions of plants. They play an essential role in growth and development, cell differentiation, response to biotic and abiotic stresses, organogenesis, flower development, breaking the dormancy of tubers, and many other vital cellular activities [4].

In plant cells, putrescine, triamine spermidine, and tetraamine are the main polyamines. The simplest known polyamine is putrescine. It is obtained directly from ornithine or arginine by the enzyme ornithine and arginine decarboxylase (Fig1). Recently, researchers proved that putrescine could be introduced as a growth material because it has played a very influential role in many processes of plant growth and development [5]. This study aimed to investigate the effect of putrescine foliar application on the macronutrients of *S. officinalis* (Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Sulphate).

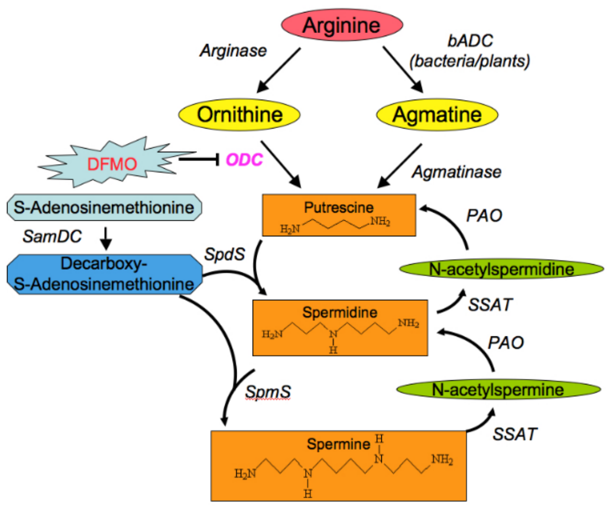

Fig. 1 Biosynthetic pathways of polyamines in plants.

MATERIALS AND METHODS

Cultivation of Seeds and Preparation of Seedlings

First, Pure sage seeds were obtained from Zar Ghata Fars Company, then the seeds were placed in distilled water for 24 hours to germinate. In the culture medium, sage seeds were placed in a ratio of 1:1, cocopeat and perlite. Germination occurred ten days after transferring the seeds to the planting trays. Sage seedlings were transferred to prepared pots after five leaves. It filled the rectangular pools with coco peat: perlite: soil in a ratio of 1: 1: 1 and 12 identical and healthy seedlings of the same size of sage were placed in each pot.

The soil used in the pots was sandy loam soil, which was air-dried, homogenized, and sieved with less than 2 mm sieve before use in the banks. It evaluated some physicochemical characteristics of the earth according to standard soil science techniques: 1. the percentage of clay, 2. sand, and 3. obtained silt through the hydrometer. It measured the pH of the saturated pulp through a glass electrode pH meter. So, it measured the carbon percentage through the wet oxidation method. Other macro elements and microelements were calculated from the standard techniques of element determination. The physicochemical parameters of the soil in the horizon from 0 to 30 cm are as follows: loamy soil with medium grain size, C/N ratio less than 10, organic carbon content 3.8%, nitrogen content 0.42, available P content 200 ppm, available iron content 65.9 ppm, available zinc content 45.2 ppm, available manganese content 61, available copper content 1.4 ppm and pH of 6.8- 7.

Greenhouse Experiment, Suitable Growth Conditions, and Putrescine-applied Treatments

It conducted this research in a controlled greenhouse in Yasuj city, Iran. With a relative humidity of 60% and a temperature of 25°C during the day and 18°C at night. (28˚30' North and 36˚51' East, 1870 meters above sea level). During the experimental period, we tried to control the environmental conditions of the greenhouse until the plants were harvested and all the environmental conditions were relatively stable. This experiment was a completely randomized design with five replications and four treatments. Foliar spraying of putrescine in 4 concentrations was zero (as control: Put1), 500, 1000, and 1500 mg/l (Put2, Put3, and Put4, respectively). Four weeks after transferring the uniform seedlings to the pots (which were subjected to unification pruning), the first foliar spraying of putrescine was done. At the time of foliar spraying, spray the control plants with distilled water. Foliar spraying was done in 2 stages; the second foliar spraying was done three weeks after the first foliar spraying. About four weeks after the second foliar application, plants were harvested to measure macronutrients of sage leaves.

Plant Harvesting and Determination of Nutrient Contents

After the second foliar application, plants were harvested, rinsed with distilled water, dried at 65 °C for 48h, weighed, ground, and dry ashed at 550°C. Different methods were used for different nutrients:

Nitrogen: The Kjeldahl method or combustion method was commonly used to convert nitrogen in the sample into ammonia, which could then be quantified. Phosphorus: Digestion with strong acids was often used to convert phosphorus into a form that could be measured colorimetrically. Potassium: Potassium was usually extracted using water or other suitable solvents, and its concentration was determined through various analytical techniques like flame photometry or atomic absorption spectroscopy. Calcium and Magnesium: These were also typically extracted using acid digestion, and their concentrations were measured using atomic absorption spectroscopy or other suitable methods.

Sulfate: Sulfate could be determined by precipitation with a barium solution and then measuring the remaining barium ions using titration or other techniques [6-10].

Statistical Analysis

The experiment was conducted in a completely randomized design with five replications. Data were analyzed statistically using ANOVA in SAS software (v. 9.1), and Duncan's test was employed to compare the means (P≤ 0.05). GraphPad Prism was used to design figures.

RESULTS AND DISCUSSION

Potassium and Calcium Content

The effect of putrescine (Put) was significant on the potassium and calcium content of the sage (Fig 2, 3). Furthermore, as can be seen in Fig. 2, putrescine showed a significant positive effect in increasing the performance of potassium, so that increasing the concentration up to 1500 mg/l, a significant increase in the percentage of potassium was observed. Total calcium showed a significant increase with putrescine (Fig. 3). It is like the potassium content; the best economic growth was 1500 mg/l of putrescine. Potassium (K+) is of paramount importance in plant cell physiology. K+ is an essential macronutrient that fulfills critical functions related to enzyme activation, osmotic adjustment, turgor generation, cell expansion, regulation of membrane electric potential, and pH homeostasis [11]. There is a steep curvilinear relationship between the tissue concentration of K+ and plant growth, from which a critical concentration of K+ supporting 90% of maximum yield can be determined. Above this concentration, growth has no correlation with the increased K+ content, but at lower K+ concentrations, growth declines rapidly. Consequently, K+ fertilization is common practice in modern agriculture and about 40–60% of crop yields are attributable to commercial fertilizer use [12]. However, agricultural fertilization is far from being fine-tuned with nutritional requirements. The main constituents of leaves essential oil (1,8-cineole, borneol, camphor, α-terpineol and myrtenal) and mineral accumulation were affected by K treatments [13].

Researchers also showed the positive effects of calcium in medicinal plants, calcium significantly differentiated the quantity of particular compounds (1.8-cyneole, geranyl, α-terpinolene and β-pinene) in basil oil [14]. Lee and Yang (2005) reported an increase in the plant growth and yield of *Chrysanthemum boreale* with increasing of CaCO3 levels [15].

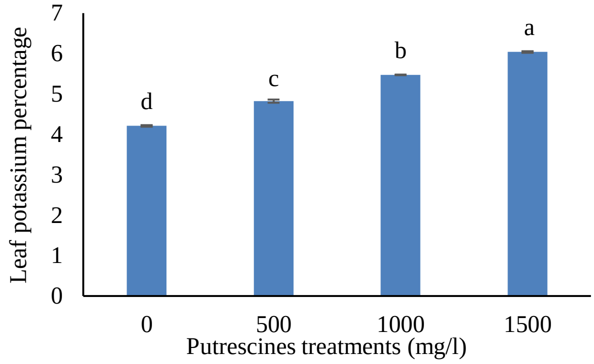

Fig. 2 Effect of different concentrations of putrescine (Put) on potassium content of sage (*S. officinalis* L.).

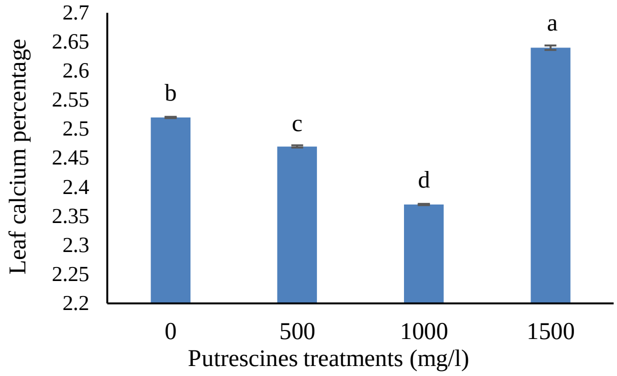

Fig. 3 Effect of different concentrations of putrescine (Put) on the calcium content of sage (*S. officinalis* L.).

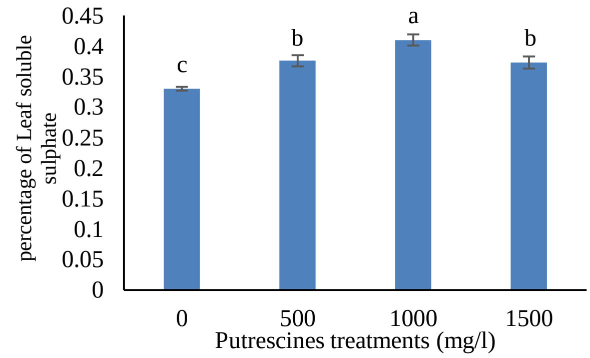

Fig. 4 Effect of different concentrations of putrescine (Put) on sulphate content of sage (*S. officinalis* L.).

Magnesium and Sulphate Content

The amount of magnesium and sulphate content increased with foliar spraying of putrescine. So, the most significant increase of these elements was seen at the concentration of 1000 mg/l of putrescine .The rise in the concentration of this polyamine showed the reverse trend (Fig. 4, 5). Magnesium (Mg) has a positive effect on the growth of organized cultures and also on the quality and quantity of essential oil production [16].

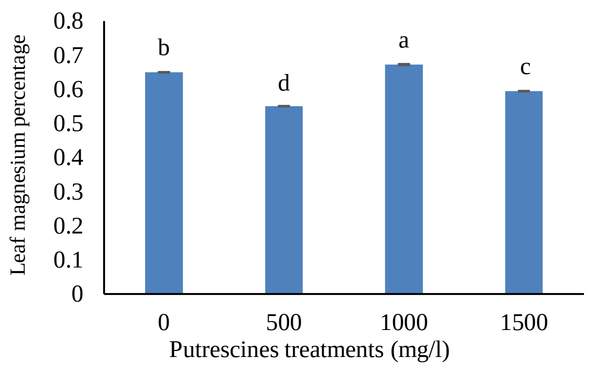

Fig. 5 Effect of different concentrations of putrescine (Put) on magnesium content of sage (*S. officinalis* L.).

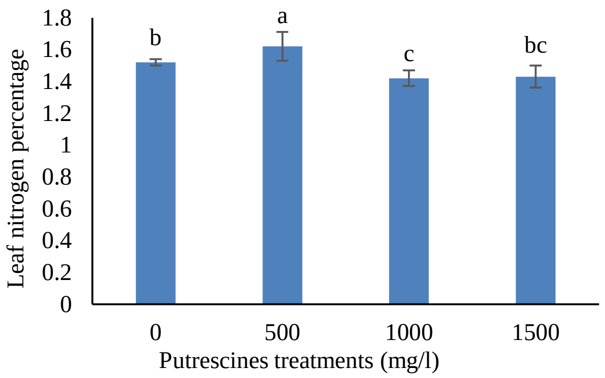

Fig. 6 Effect of different concentrations of putrescine (Put) on the nitrogen content of sage (*S. officinalis* L.).

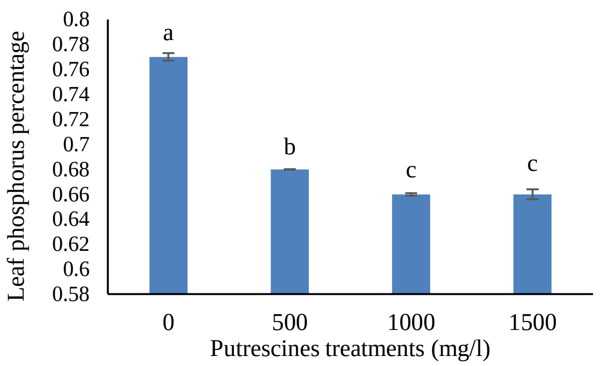

Fig. 7 Effect of different concentrations of putrescine (Put) on phosphorus content of sage (*S. officinalis* L.).

Nitrogen and Phosphorus Content

Like other elements, nitrogen content was affected by putrescine foliar application, so the best concentration of putrescine for increasing nitrogen content was found to be 500 mg/l, and with increasing concentration, the amount of nitrogen content decreased (Fig. 6). On the other hand, the amount of phosphorus showed a completely different trend with foliar spraying of putrescine so that the highest amount of phosphorus was observed in the control plants (Fig. 7). Nitrogen is essential for plants to synthesize nucleic acids, proteins, phytohormones, coenzymes, and chlorophyll; it can promote plant growth and increase plant branches and green leaves [17]. Phosphorus is the main component of the plant nucleus and protoplast and is an essential component of nucleic acid, phosphoester, and adenosine triphosphate and it can assist plant root development [17].

putrescine is an organic compound that can have various effects on nutrient availability in different contexts. Its role can be complex, and the specific effect it has on nutrient levels depends on the environmental conditions and the organisms involved. Here are some general factors that can explain how putrescine might increase or decrease nutrient availability:

1. Microbial Decomposition: Putrescine is often associated with decomposition processes. When organic matter, such as dead plants or animals, decomposes, it releases putrescine. Microorganisms involved in decomposition, such as bacteria and fungi, may use putrescine as a nitrogen source. This can lead to an initial increase in nutrient availability, particularly nitrogen.
2. Nitrogen Cycling: Putrescine contains nitrogen, and it contributes to the nitrogen cycle in ecosystems. When putrescine is metabolized by microbes, it can be converted into ammonia or other nitrogen-containing compounds. Ammonia, in particular, can be further transformed into nitrate or nitrite, which are forms of nitrogen that plants can take up as nutrients. This can enhance nutrient availability for plants.
3. Toxic Effects: On the flip side, high concentrations of putrescine can have toxic effects on organisms, including microorganisms and plants. Excessive putrescine levels can disrupt cellular processes and inhibit the growth of certain organisms, leading to a decrease in nutrient cycling and availability.
4. Plant-Microbe Interactions: Putrescine can influence the interactions between plants and soil microbes. Some plants can release putrescine or related compounds into the soil as a way to shape their root microbiome. This can either promote the growth of beneficial microbes that enhance nutrient uptake by the plant or inhibit the growth of harmful pathogens, thus indirectly affecting nutrient availability to the plant.

In summary, the effect of putrescine on nutrient availability is context-dependent and can vary widely depending on factors such as the concentration of putrescine, the types of organisms present, and the specific environmental conditions. It can both increase and decrease nutrient availability, with its role in nutrient cycling and microbial interactions being particularly important in understanding its impact.

CONCLUSION

In sustainable agriculture, the use of polyamine materials can reduce the consumption of chemical fertilizers and thus preserve the environment. Polyamine can also have a positive effect on high-consumption elements. As a result, the findings of the present research can be used to manage the optimal production of medicinal plants and the quality of their products.

Fig. 8 Graphical abstract: a figure that explains the message of a research paper clearly and attractively. It's generally published together with other elements of the paper.

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