Effect of organic fertilization on yield and quality of rosemary (*Rosmarinus officinalis* L.) essential oil

Efecto de la fertilización orgánica sobre rendimiento y calidad del aceite esencial de romero (*Rosmarinus officinalis* L.)

Jeimmy Alexandra Cáceres[1], Jairo Leonardo Cuervo A.[1], and Javier Leonardo Rodríguez C.[1]

ABSTRACT

Rosemary production (*Rosmarinus officinalis* L.) in Colombia is destined mainly for international markets (2.898 t in 2006), Although the national demand is low, this is a promising crop in some areas of the country, having potential to enhance producers life quality through the implementation of sustainable crops allowing the decrease of non-beneficial conditions in agriculture labors. Studying the response to the application of biofertilizers as an alternative to implement rosemary organic crops has become an important tool for the integrated crop management. In this research three commercial biofertilizer applied to the soil were evaluated (*Azotobacter chroococcum*, *Pseudomonas fluorescens*, humic and fulvic acids) facing a control treatment, significant differences were found regarding the number of stems growth per plant, however variables as oil extract volume and plant height did not present significant differences when compared with control treatment.

Key words: biofertilizer, PGPR bacteria, nutrient solubilisation.

RESUMEN

La producción de romero (*Rosmarinus officinalis* L.) en Colombia está destinada principalmente a la exportación (2.898 t para el 2006) pues la demanda a nivel nacional es baja, sin embargo este es un cultivo promisorio en ciertas zonas del país, siendo potencial para algunos productores debido al mejoramiento de la calidad de vida a través de la implementación de cultivos sostenibles que permitan disminuir la nocividad de las labores agrícolas. Es así que conocer la respuesta de esta especie frente a la aplicación de biofertilizantes como alternativa para implementar cultivos de romero orgánicos se convierte en una herramienta base para el manejo integrado del cultivo. En esta investigación se evaluaron tres productos biofertilizantes comerciales aplicados al suelo (*A. chroococcum, P. fluorescens*, ácidos húmicos y fúlvicos) frente a un testigo, se encontraron diferencias significativas en el número de tallos generados por las plantas, sin embargo variables como el volumen de aceite esencial extraído y la altura de la planta no presentaron diferencias significativas con respecto al testigo.

Palabras clave: biofertilzantes, bacterias PGPR, solubilización de nutrientes

Introduction

Rosemary (*Rosmarinus officinalis* L.) is a species member of the Lamiaceae family, is a woody herb (Avila *et al*., 2011) cultivated mainly for essential oil production; in Colombia the commercialization of this product is performed through exporting trade, this activity had a growth of 6.5% between 2000 and 2006 (Conpes, 2008) due to the low internal demand of aromatic herbs either the lack of fresh consume or essential oil production, about 5 t per year (Barrientos *et al*., 2012).

Aromatic crop area (basil, thyme, rosemary, chives, oregano, mint, tarragon, marjoram, sage, calendula, chamomile, peppermint) in Colombia was about 1200 ha by 2008 and it had a production of 72.8 t year^{-1}, being Cundinamarca department the most productive department with 62.8% of the total volume produced (MADR *et al*., 2009). Actually, the commercial and academic interest on rosemary (*R.* officinalis L.) oil, lays on its antioxidant and liposoluble capacity, cosmetic and pharmaceutic use, and food industry potential (Peng *et al*, 2005; Commission Regulation (EU), 2011; Yang *et al*., 2016).

By the above, the investigation in this species has increased, with the objective of know more about the agronomic requirements to improve the yield in this crop. Otherwise, it has been state that the production of secondary metabolites is highly related to genetic and environmental conditions, been affected by several abiotic factors as types of soil, water availability, nutrients solubility, light, UV radiation, among others (Hamilton *et al*., 2001; Ormeño *et al*., 2008; Pavarini *et al*., 2012; Nogués *et al*., 2015).

Received for publication: 7 March, 2017. Accepted for publication: 20 July, 2017

Doi: 10.15446/agron.colomb.v35n2.63155

[1] Faculty of Agricultural Sciences, Universidad Nacional de Colombia. Bogota (Colombia). jacaceresz@unal.edu.co

Actually, new alternatives for agriculture fertilization has been developed and researched, leading to the use of bio-stimulants with several benefits, among which are, tolerance to stress caused by biotic and abiotic factors, easy nutrient assimilation, efficient water use. The substance correspond to microorganisms, humic acids, fulvic acids, hydrolyzed proteins, amino acids and algae (Calvo *et al*., 2014); the application of these substances has been converted into an important strategy for agriculture sustainability, since its properly use allow the combination of either pesticides and/or fertilizers of chemical synthesis without decreasing the crops yield (Cordovilla *et al*., 1999; Aseri *et al*., 2008; Ambrosini *et al*., 2015).

Associated rhizosphere microorganisms play an important role over soil biodiversity, since they can influence positively the plants growth due to the provision of nutrients, antibiotics and phytohormones around the roots (Vrieze, 2015). Actually associated rhizosphere microorganisms are used frequently to solubilize compounds, to enhance element fixation, to promote growth through secondary metabolites or phytohormones induction, and to induce systematic resistance in plants of interest (Aseri*et al*., 2008). This activity is a product of microbial decomposition, humic substances (humic and fulvic acids) (Asli y Neumann, 2010) wich play an important role in soil, through nutrient availability, soil/atmosphere oxygen and carbon interchange and toxic chemical transport and transformation (Piccolo and Spiteller, 2003).

Humic acids present in soils affect plant physiology and the composition and function of rhizosphere microorganism (Varnini and Pinton, 2001), additionally these substances comprise more than 60% of organic matter of the soil and are the lead component of organic fertilizers with a high nutrient content (Stevenson, 1994). However it cannot be recommended as the only source of nutrients, as the plant response to these substances is associated to interactions between membrane transportations responsible of nutrient absorption from humic and fulvic acids (Canellas *et al*., 2015).

Bacteria inoculation can generate a plant growth increase, germination percentage increase, benefic response to external stress factors and protection of plant diseases (Lugtenberf *et al*., 2002). The most used fungi and bacteria as bio-stimulants are *Glomus* (mycorrhizae), *Azotobacter*, *Pseudomonas*, *Bacillus*, *Azospirrilum* (Wu *et al*., 2005; Egamberdiyeva, 2007; Aseri *et al*., 2008; Cappellari *et al*., 2013).

Azotobacter, is a genus of aerobic bacteria which fixate atmosphere nitrogen (Kizilkaya, 2008), decreasing the nitrogen loss by natural biochemical process and increasing its availability to the crop, several species of this genus are reported to be employed as biofertilizers, been the most recognized *A. chroococcum*, as being a plant growth promoting rhizobacteria (PGPR) through phytohormone production like auxins and gibberellins, It is recognized by its relations with other microorganisms like mycorrhizal fungi (Kilam *et al*., 2015).

Other commonly used bacteria is *Pseudomonas fluorescenses*, species reported as growth promotor, responsible for iron consumption increase and plant growth stimulator under drought conditions (Sharma *et al*., 2013; Calvo *et al*., 2014; Gopalakrishnan *et al*., 2015).

The objective of this research was evaluate the effect of solubilizing bacteria and humic substances on rosemary (*Rosmarinus* officinalis L.) essential oil yield and production, with the end of stablish the best option of organic fertilization to rosemary production in Guasca Cundinamarca municipality.

Materials and methods

Cultivar of rosemary (*Rosmarinus* officinalis L.) plants known as Israeli were used as vegetal material , at the beginning of the experimental period plants with one year after seeding and seetled with drip irrigation tape system were employed. This assay was located in Guasca-Cundinamarca municipality (4°51’57.624” N and 73°52’9.919” W) at an altitude of 2,962 m a.s.l. average temperature of 20.9°C and relative humidity of 83% (IDEAM, 2016), the research was carried out in an area of 500 m^2 and the soil was clasified to the taxonomic subgroup Typic Dystrudepts (IGAC, 2000).

A completely randomized design (CRD) was followed with four treatments and 6 repetitions, the experimental units correspond to a single plant. The four treatments correspond to: T0, organic soil conditioner (total N: 1%; P_2O_5: 1%; K_2O: 1.5%; CaO: 3%; MgO: 1.3%; C/N 11%; pH 8; CE: 5 dS m^{-1}; CIC: 70 meq/100 g); T1, organic soil conditioner + *Azotobacter chroococcum* (7 × 105 CFU); T2; organic soil conditioner + *Pseudomonas fluorescens* (9 × 105 CFU); T3, organic soil conditioner + humic and fulvic acids (1%).

Organic soil conditioner was applied in crown shape at the base of the plant accordingly to the fertilization used previously in the allotment, the treatments were applied four times every two weeks, using drench application with bug bomb leading the spear directly to the plant root, seeking homogeneity in the application.

Data were taken 15, 30, 45, 60, 81 and 94 d after beginning the treatment application, considering variables like height (measured with a measuring tape ±1 cm) and stem number per plant, at 94 d after treatment application a 20-25 cm stem cuttings harvest was realized, to extract essential oils and evaluate the effect of treatments, For the steam distillation method, 266 g of *R. officinalis* L. fresh vegetal material was used, making the distillation during 120 min after obtaining the first distillate drop (Cassel et al., 2009; Yahya and Mohd, 2013), after the oil extraction procedure the sample was retired with a Pasteur pipette and left to decant during 2 d to eliminate the hydrolates of the sample and finally it was weighted with a Denver scale (±0.1 mg), accomplishing percentages of oil yielding, Finally, humidity data were taken founding vegetal material humidity of T0, 52.15%; T1, 53.834%; T2, 50.829%; T3, 52.494%.

Data analysis were analyzed with the statistical software SAS 9.1.6, to ensure data normality and homogeneity and finally we use the Tukey's range test to conclude significant differences.

Results and discussion

In general aromatic herbs have culinary, medicine, cosmetic and decorative uses, they are used in fresh, dehydrated and in some cases its essential oil is extracted (Cardona and Barrientos, 2011), for all those uses the aerial component of the plant (leafs) are very important. This is why during crop lifespan, management focused to reach plant vigor, aiming to achieve the most number of new branches for the next harvest. It was observed that plants presented a positive response to treatments 1, 2 and 23. The height of the plants was evaluated during the sampling observing significant differences only 60 d after treatment was applied, being the application of *A. chroococcum* the one that presented the best response with respect the other treatments (Fig. 1.), however, there were not contrasting differences with control test.

Maheshwari *et al*. (2012) in researches conducted with *Sesamum indicum* L., showed how the applications of *A. chroococcum* present results comparable with chemical synthesis substances fertilization, in parameters like, protein content and the essential oil yield and content. However Abdel *et al*. (2014) reports how microbial colonization by *A. chroococcum* is significantly affected by factors like nitrogenous fertilization, plant growth state and soil moisture content, among others. For *P. flourescens* it is reported how its application increases the roots elongation and the aerial component of canola, lettuce and tomato (Hall *et al*., 1996; Dadrasan *et al*., 2015), however the results of this research allow to conclude that for the *R. officinalis* var. Israeli species at this agroclimatic and edaphic conditions, the treatments did not contribute to increase the plant height.

Referring the number of stems, the evaluation of plant branching as consequence of hormone production for *A. chroococcum* and *P. fluorescens* and how it affects the number of branchesis recorded in table 1, 30 d after treatment apply the effects of the applications can be observable, at the end of the experimental cycle the treatment with humic

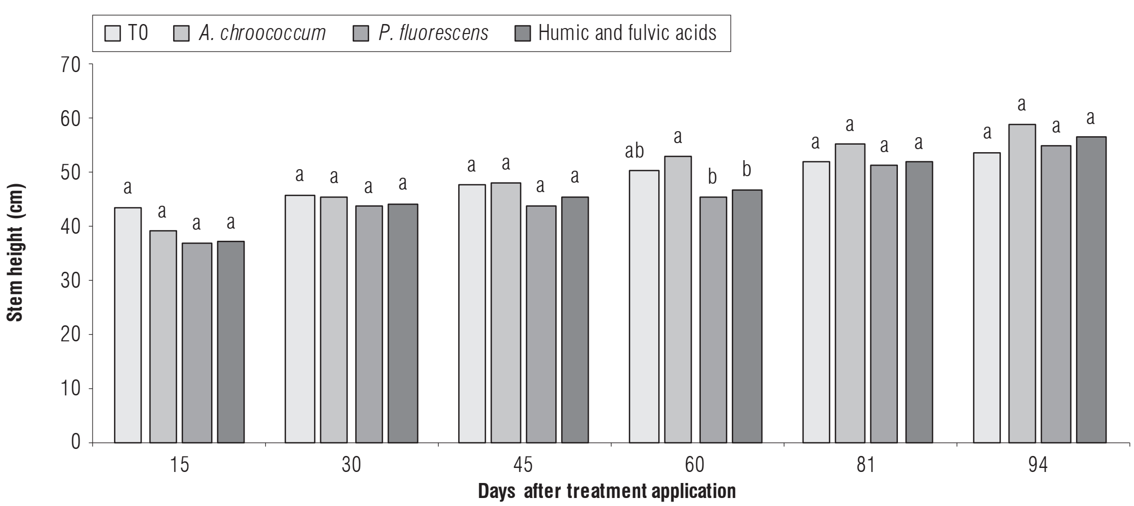

FIGURE 1. Stem height of *R. officinalis* after application of biofertilizers. Means with different letters indicate significant difference according to Tukey test ($P \leq 0.05$).

TABLE 1. Stem number of *R. officinalis* L. after application of biofertilizers.

	Days after treatment application											
	15		30		45		60		81		94	
T0	6	a	6	c	7	b	9	b	9	b	10	c
A. chroococcum	7	a	7	bc	9	b	10	b	11	ab	12	bc
P. fluorescens	8	a	8	ab	10	ab	11	ab	12	ab	13	ab
Humic and fulvicacids	8	a	10	ab	12	ab	14	a	14	a	15	a

Means with different letters indicate significant difference according to Tukey test ($P \leq 0.05$).

and fulvic present an increment in the number of stems per plant of 55.5 % compared to control.

This response can be related with the effects that humic and fulvic acids in the soil, on characteristics as nutrient bioavailability (principally phosphorus) and microbial population (Delgado *et al*., 2002; Canellas *et al*., 2015). Puglisi *et al*. (2008) report an increase on the exudate productions through plant roots, as a consequence of substances application including humic and fulvic acids and the use of soil conditioner as compost, increasing the activity of some beneficial microorganism present naturally in cultivated soil.

Also it have been reported that an application effect of this substances is the production of phytohormones which promotes the growing (Smolen *et al*., 2014), all this due to the capacity of this substances to generate soil aggregates, proteins, carbohydrates, aliphatic biopolymers and lignin content, thus favoring the microbial activity, improving soil structure and the development of several beneficial microorganism (Calvo *et al*., 2014).

Chemical analysis conducted on rosemary plants show the presences of terpens and terpenoids, including components as camphor, 1,8-cineole, α-pinene, camphene, α-terpineol and borneol, with an average density of 0.877 g cm^{-3} (Atti-Santos *et al*., 2005; Moncada *et al*., 2016), secondary metabolites production in plants occur principally through the Shikimate pathway (Narwal and Sampietro, 2009); the biosynthesis of terpenes occur through two pathways, Mevalonate pathway and the non-mevalonate pathway also called the MEP/DOXP pathway (Zuzarte and Salgueiro, 2015), having as precursors compounds like isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), These compounds are originated from metabolic pathways in the plant that occurred in cell organelles as chloroplast, cytoplasm and mitochondria. However this results (Tab. 2) don´t show the effect of treatments on the production of rosemary oil.

TABLE 2. Production of essential oil of *R. officinalis* L. after application of biofertilizers.

Treatment	Volume (mL)		Weight (g)		Density (g mL^{-1})	
T0	1.00	a	0.98	a	1.04	a
A. chroococcum	1.07	a	0.94	a	0.90	a
P. fluorescens	0.70	a	0.75	a	1.17	a
Humic and fulvicacids	0.60	a	0.58	a	0.98	a

Means with different letters indicate significant difference ($P \leq 0.05$), according to Tukey test.

It is how the photosynthesis and nutrient efficiency absorption by plants are the first characteristic to accomplish an adequate working of the other metabolic pathways, where the availability and movement of the elements can activate and increase yield.

Conclusions

The application of humic and fulvic acids did not show a positive effect in the increase of the height of the plants, however, we observed an increase of rosemary yield, due to the increase in the number of stems per plant, additionally changes were not observed regarding physical characteristics of the essential oil related to the fertilization treatments used conventionally, for which it is considered useful for the production of this aromatic plant.

The health of the soil and the fertility base, depends of the food web in which the bacteria, micro-fauna (nematodes and protozoa) and earthworms plays a major role in the nutrient cycle (Warlde*et al*., 2004), it is why the application of biofertilizers isn't enough to a sustainable management of aromatic crops, it's necessary the implementation of integral management plans that allow gradually reactivate such food web and allows the fertility and health of the soil.

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