Effects of Different Limestone Rates on Yield and Yield Indicators of a Sage (*Salvia officinalis* L.) Ecotype

Qatip Doda [a], Nikollaq Bardhi [b*], Zyhdi Teqja [c], Gjergji Mero [d], Irena Kallço [d], Bujar Zeka [e]

[a] Regional Directorate of Agriculture, Dibër, Albania

[b] Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Plant Production, Kodër Kamëz, 1032, Tirana, Albania

[c] Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Horticulture, Kodër Kamëz, 1029, Tirana, Albania

[d] University "Fan S. Noli", Faculty of Agriculture, Department of Plant Production, Korçë, Albania

[e] Regional Directorate of Agriculture, Berat, Albania

Corresponding author: Nikollaq Bardhi

Abstract

The study on the effects of different limestone rates on yield and yield indicators of sage (*Salvia officinalis* L.), was conducted during four consecutive years, 2010-2013, in Maqellara, Dibra District, at the north-eastern part of Albania. A randomized complete block design (RCBD) with four different rates of limestone [V1 = control (0 q ha^{-1}), V2 = 40 q ha^{-1}, V3 = 80 q ha^{-1}, V4 = 120 q ha^{-1}] and four replications for variant, with a plot size of 60 m^2 or 333 sage plants for variant in each replication, was used. For planting, there were used prepared seedlings from a wild Albanian sage ecotype, grown in Dibra District. During the vegetative period and at the harvest, there were measured and evaluated several mean yield indicators at the blooming stage (before harvest), such as number of shoots per plant, plants height (main shoot length) (cm), lateral shoot length (cm), leaf length (cm), leaf width (cm), length of petiole (cm), yield of the 1st mowing, yield of the 2nd mowing, and total yield (q ha^{-1}). First year there was applied one mowing, while during the other three consecutive years, there were applied two mowing, at the full blooming stage. Obtained results showed that limestone use significantly affected yield and yield indicators of sage under Maqellara, Dibër, climate conditions. There was observed a yield raise of 7.5-35.4% for variants treated with limestone. The highest yearly and total yield was obtained using 120 q ha^{-1} limestone, with a four year mean yield of 26.7 q ha^{-1} or 7 q ha^{-1} (35.4%) higher than control. Recommended limestone rate for Maqellara, Dibër, conditions was 80-120 q ha^{-1} and keeping the sage up to five years under cultivation.

KEYWORDS: effect, limestone rate, sage, *Salvia officinalis* L., yield, yield indicator.

INTRODUCTION

Sage (*Salvia officinalis* L.) (also called common sage, garden sage, golden sage, kitchen sage, true sage, culinary sage, Dalmatian sage, and broadleaf sage) is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a member of the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world (Baricevic *et al.*, 2002). In Albania, it is a very sprout medicinal plant, grown naturally as wild plant (ARIFP, 1988; Demiri, 1971; Paparisto *et al.*, 1988; Vangjeli *et al.*, 1995; Hyso and Çobaj, 2005) and as cultivated plant (Bardhi, 2008; Bardhi *et al*., 2001; Ahmetaj and Çeku, 1988), recently, with a high importance for the Albanian export and economy (WBA, 2002; Marko and Dishnica, 2002). It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant. The common name "sage" is also used for a number of related and unrelated species. Cultivated forms include purple sage and red sage. In Turkey, *Salvia officinalis* is widely known as *adaçayı*, meaning "island tea". The specific epithet *officinalis* refers to the plants with a well-established medicinal or culinary value - the *officina* was the traditional storeroom of a monastery where herbs and medicines were stored plants with a well-established medicinal or culinary value (Lorraine, 2012; Stearn, 2004; Asllani, 2002; Asllani, 2000). *Salvia officinalis* was described by Carl Linnaeus in 1753. It has been grown for centuries in the Old World for its food and healing properties, and was often described in old herbals for the many miraculous properties attributed to it (Clebsch and Barner, 2003). *S. officinalis* has been classified under many other scientific names over the years, including six different names since 1940 alone (Sutton, 2004). Cultivars are quite variable in size, leaf and flower colour, and foliage pattern, with many variegated leaf types (Bardhi, 2008). The Old World type grows to approximately 2 ft (0.61 m) tall and wide, with lavender flowers most common, though they can also be white, pink, or purple. The plant flowers in late spring or summer. The leaves are oblong, ranging in size up to 2.5 in (6.4 cm) long by 1 in (2.5 cm) wide. Leaves are grey-green, rugose on the upper side, and nearly white underneath due to the many short soft hairs. Modern cultivars include leaves with purple, rose, cream, and yellow in many variegated combinations (Bardhi, 2008; Haska *et al.*, 2005; Clebsch and Barner, 2003). With over 500 species, from colored varieties to dwarfs to non -lowering varieties, sage is grown throughout the world. The plant is in bloom from June to August. The seeds are smooth, and like the Garden Clary, produce a great quantity of soft, tasteless mucilage, when moistened. If put under the eyelids for a few moments the tears dissolve this mucilage, which envelops any dust and brings it out safely. *Salvia officinalis* has been used since ancient times for warding off evil, snakebites, increasing women's fertility, and more. Theophrastus wrote about two different sages, a wild undershrub he called *sphakos*, and a similar cultivated plant he called *elelisphakos*. Pliny the Elder said the latter plant was called *salvia* by the Romans, and used as a diuretic, a local anaesthetic for the skin, a styptic, and for other uses. Charlemagne recommended the plant for cultivation in the early Middle Ages, and during the Carolingian Empire, it was cultivated in monastery gardens (Watters, 1901).

Strabo described it in his poem "*Hortulus*" as having a sweet scent and being useful for many human ailments - he went back to the Greek root for the name and called it *lelifagus* (Kinziotis, 2000). The plant had a high reputation throughout the Middle Ages, with many sayings referring to its healing properties and value. It was sometimes called *Salvia salvatrix* (sage the saviour), and was one of the ingredients of Four Thieves Vinegar, a blend of herbs which was supposed to ward off the plague. Dioscorides, Pliny, and Gallen all recommended sage as a diuretic, homeostatic, emmenagogue, and tonic (Kinziotis, 2000). Common sage is grown in parts of Europe for distillation of an essential oil, though other species, such as *Salvia fruticosa* may also be harvested and distilled with it. In Britain sage has for generations been listed as one of the essential herbs, along with parsley, rosemary and thyme. It has a savoury, slightly peppery flavour. It appears in many European cuisines, notably Italian, Balkan (Asllani, 2002) and Middle Eastern cookery. In British and American cooking, it is traditionally served as sage and onion stuffing, an accompaniment to roast turkey or chicken at Christmas or Thanksgiving Day. In Traditional Tamil Siddha medicine, sage is used for respiratory ailments like asthma and alleviating nasal discharge associated with Upper respiratory infections. Sage leaves are crushed in boiling water and the fumes are inhaled. In the traditional Austrian medicine *Salvia officinalis* herb has been used internally (as tea or directly chewed) for treatment of disorders of the respiratory tract, mouth, gastrointestinal tract, and skin (Vogl *et al.*, 2013). *Salvia* and "sage" are derived from the Latin *salvere* (to save), referring to the healing properties long attributed to the various *Salvia* species (Kinziotis, 2000). It has been recommended at one time or another for virtually every ailment by various herbals. Modern evidence shows possible uses as an anti-sweating agent, antibiotic, antifungal, astringent, antispasmosdic, estrogenic, hypoglycemic, and, and tonic. In a double blind, randomized and placebo-controlled trial, sage was found to be effective in the management of mild to moderate Alzheimer's disease. The strongest active constituents of sage are within its essential oil, which contains cineole, borneol, and thujone. Sage leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, corsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides, and estrogenic substances (Bardhi2008; Haska *et al.*, 2005). Investigations have taken place into using sage as a treatment for Alzheimer's disease patients (Akhondzadeh *et al.*, 2003; Dos Santos-Neto *et al.*, 2006; Perry *et al.*, 2007). Sage leaf extract may be effective and safe in the treatment of hyperlipidaemia. In favourable conditions in the garden, *Salvia officinalis* can grow to a substantial size (1 m^2 or more), but a number of cultivars are more compact. As such they are valued as small ornamental flowering shrubs, rather than for their herbal properties. Some provide low ground cover, especially in sunny dry environments. Like many herbs they can be killed by a cold wet winter, especially if the soil is not well drained. Sage cultivars are easily propagated from summer cuttings, in vitro cultures (Kongjika *et al.*, 2002; George, 1996), and some cultivars are produced from seeds (Vangjeli *et al.*, 1995).

Since sage is a very important medicinal plant in Albania, which is going to occupy a huge cultivation area, especially in hilly and mountainous areas (Bardhi *et al.*, 2001), there is a need to find the most appropriate cultivation technology in specific areas of the country. The aim of the study was to observe the effect of limestone on yield and yield indicators of the wild sage ecotype of Dibra District.

MATERIAL AND METHODS

Experimental design. The study on the effects of different limestone rates on yield and yield indicators of sage (*Salvia officinalis* L.), was conducted during four consecutive years, 2010-2013, in Maqellara, Dibra District, at the north-eastern part of Albania. A randomized complete block design (RCBD) with four different rates of limestone [V1 = control (0 q ha^{-1}), V2 = 40 q ha^{-1}, V3 = 80 q ha^{-1}, V4 = 120 q ha^{-1}] and four replications for variant, with a plot size of 60 m^2 (or 333 sage plants) for variant in each replication, was used. The plot was situated in a hilly land with a sloping gradient of 10-15% and planting density was (60 cm x 30 cm) or 55550 plant ha^{-1}. The total experiment size was 960 m^2.

Soil analyses before experimental set up showed the below mentioned results: pH = 6.95, humus content = 2.2%, N = 0.14%, P_2O_5 = 11.6 mg/100 g soil, P_2O = 13.27 mg/100 g soil, and $CaCO_3$ = 1.64%. Limestone was used at the last land preparation process (milling).

Plant material. For planting, there were used prepared seedlings from a wild Albanian sage ecotype, grown naturally in Dibra District. Seedlings' planting was carried out on 5th April 2010. First year was applied only one mowing, while during the other three consecutive years, there were applied two mowing, at the full blooming stage. First year there was applied one mowing, which was carried out on September 10, 2010, while during the other consecutive years, there were applied two mowing, at the full blooming stage, corresponding on June 5-15 and September 5-15, each year.

Measurements and observations. During the vegetative period and at the harvest, there were measured and evaluated several mean yield indicators at the blooming stage, such as:

- number of shoots per plant
- plants height (main shoot length) (cm)
- lateral shoot length (cm)
- leaf length (cm)
- leaf width (cm)
- length of petiole (cm)
- yield of the 1st mowing, yield of the 2nd mowing, and total yield (q ha^{-1}).

Measurements for plant height, leaf length, leaf width and petiole, and counting for the number of shoots per plant and the number of leaves per shoot, were carried out in a representative sample of 20 sage plants for each variant on each replication, which were labelled with unmoved plastic labels during the study period. Measured data were recorded, analyzed and were calculated yearly and overall mean values.

Statistical analyses. The obtained data were subject of ANOVA and differences between variants were tested using LSD test (0.05 and 0.01) (Papakroni, 2001).

RESULTS AND DISCUSSION

Effect of limestone rates on morphological (vegetative) indicators of sage. The obtained data showed that the use of limestone significantly affected the main morphological indicators of sage. There were found significant differences between variants with treated with limestone and control for the main vegetative characters, but there were found significant differences between different rates of limestone.

Number of shoots per plant. Use of limestone affected significantly the number of shoots per plant. For the first year of the study, the shoot number was increased from 10.7 to 38.5% in variants treated with limestone compared to control. The lowest shoot number was observed the fourth year for control, while the highest shoot number was observed for the highest limestone rate (120 q ha^{-1}) of 40.3 shoots per plant. The limestone rate of 120 q ha^{-1} did not show any significant difference for the first three years. The decrease of shoot number per plant was significant for all variants in the fourth year, but this number was higher for limestone treated variants. Differences were significant for both probability levels (Table 1).

Table 1. Number of shoots per plant of *Salvia officinalis* L, according to different years of cultivation and different limestone rates (** significant at $p<0.01$ and * significant at $p<0.05$).

Variants	Years of the study (2010-2013) I	II	III	IV	Four years mean
Control (0 q ha^{-1} limestone)	28	28.3	32.25	22.75	27.83
40 q ha^{-1} limestone	31	32.5	34.5	26.0	31.0

80 q ha^{-1} limestone	36.5*	34.7*	37.5*	27.8*	34.1*
120 q ha^{-1} limestone	38.8 **	38.7**	40.3**	29.0**	36.7**
LSD 0.05 *	2.73	2.94	3.013	1.24	1.34
LSD 0.01 **	3.13	3.53	3.63	1.88	1.82

***Plants height** (main shoot length)* **(cm).** The obtained data showed that use of limestone significantly affected the main shoot length at the blooming stage since the first year. Observed results showed a significant raise the third year for all variants, but there were significant differences between limestone rates (variants). The highest mean value of plants height (cm) was measured for the highest limestone rate (120 q ha^{-1}) by 93.5 cm, and the four years mean of plants height, as well (85.5 cm) (Table 2).

Lateral shoot length (cm). Lateral shoot length is a very important yield indicator linked to herb yield and quality. The obtained data showed that use of limestone significantly affected the lateral shoot length since the first year. There were observed significant differences through the years of the study for lateral shoot length. In the first year, raise of the length of lateral shoots was significant for the highest limestone rate (120 q ha^{-1} limestone) for both probability levels (95% and 99%), and for 80 q ha^{-1} limestone for 95% probability level. For both the 2nd and 3rd years of the study, the highest limestone rate (120 q ha^{-1} limestone) showed the most significant high value for both probability levels (95% and 99%).

Table 2. Plants height (cm) of *Salvia officinalis* L., according to different years of cultivation and different limestone rates (** significant at p<0.01 and * significant at p<0.05).

Variants	Years of the study (2010-2013)				Four years
	I	II	III	IV	mean
Control (0 q ha^{-1} limestone)	77.3	78.0	81.8	70.5	76.9
40 q ha^{-1} limestone	82.5	82.3	83.8	73.0**	80.4
80 q ha^{-1} limestone	84.8 *	84.8 *	87.5 *	73.0**	82.5
120 q ha^{-1} limestone	87**	87.8**	93.5**	73.5**	85.5**
LSD 0.05 *	1.342	1.351	1.4201	1.487	1.671
LSD 0.01 **	1.673	1.742	1.652	1.675	2.103

The highest mean value of lateral shoot length (cm) was measured for the highest limestone rate (120 q ha^{-1}) in the 2nd year by 14.8 cm, as well as the highest four years mean of lateral shoot length (12.1 cm). In the 4th year, both limestone rates (80 and 120 q ha^{-1}), differed significantly with other variants for both probability levels (95% and 99%) (Table 3).

Table 3. Lateral shoot length (cm) of *Salvia officinalis* L, according to different years of cultivation and different limestone rates (** significant at p<0.01 and * significant at p<0.05).

Variants	Years of the study (2010-2013)				Four years
	I	II	III	IV	mean
Control (0 q ha^{-1} limestone)	6.4	11.8	11.9	10.4	10.1
40 q ha^{-1} limestone	7.1	12.4	12.3	10.9	10.7
80 q ha^{-1} limestone	7.4 *	13.6*	13.5 *	11.4**	11.5

120 q ha^{-1} limestone	7.7**	14.8**	14.6**	11.8 **	12.1**
LSD 0.05 *	0.354	0.376	0.524	0.502	0.452
LSD 0.01 **	0.465	0.528	0.671	0.641	0.635

Leaf length (cm). The observed data showed that use of limestone significantly affected the leaf biometrical parameters, such as leaf length, leaf width and petiole, since the first year. Limestone rates of 80 and 120 q ha^{-1} showed the highest leaf length values for all years of the study, and there were not significant differences between them (Table 4).

Table 4. Leaf length (cm) of *Salvia officinalis* L, according to different years of cultivation and different limestone rates (** significant at $p<0.01$ and * significant at $p<0.05$).

Variants	Years of the study (2010-2013)				Four years mean
	I	II	III	IV	
Control (0 q ha^{-1} limestone)	6.2	6.5	6.4	6.5	6.4
40 q ha^{-1} limestone	7.6**	7.2	7.3 **	7.0	7.3 *
80 q ha^{-1} limestone	7.7**	7.4 *	7.4**	7.4 **	7.5**
120 q ha^{-1} limestone	7.9**	7.7**	7.7**	7.6 **	7.7**
LSD 0.05 *	0.664	0.568	0.561	0.437	0.316
LSD 0.01 **	0.956	0.768	0.713	0.527	0.437

Leaf width (cm), length of petiole (cm), and number of leaves per shoot. The observed data showed that use of limestone in different rates significantly affected the leaf width, length of petiole, and number of leaves during four years of the study, compared to control. Limestone rates of 40 and 80 q ha^{-1} did not show any significant difference between them for leaf width and length of petiole, but these indicators were increased significantly compared to control. The highest values of leaf width and length of petiole were measured and observed using 120 q ha^{-1} limestone (Figure 1). Number of leaves per shoot was increased with the use of limestone and with the increase of limestone rate. Number of leaves was significantly increased form 1st to the 3rd year, followed by a significant decrease in the 4th year for the same limestone rate. In the 3rd year there was observed the highest number of leaves for all variants (Figure 1).

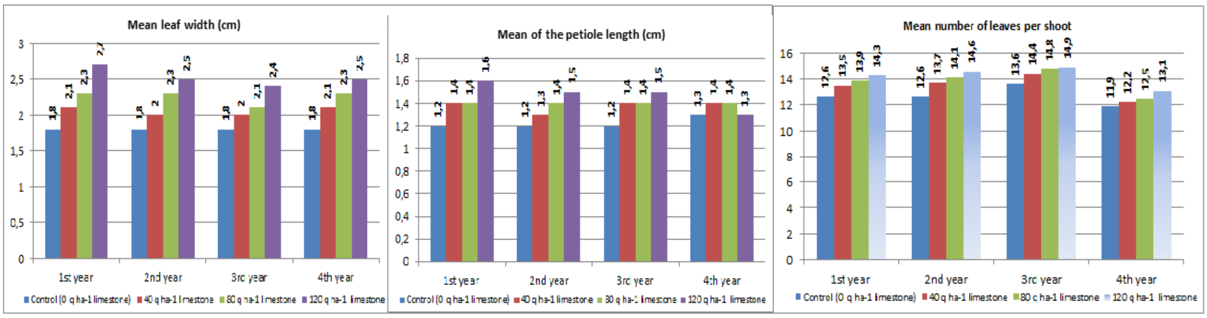

Figure 1. Leaf width (cm), petiole length (cm), and number of leaves per shoot (mean values) of Salvia officinalis L, grown under Maqellara, Dibër, Albania, climate conditions.

Yield of the 1st mowing yield (q ha^{-1}). During the first year, the sage plants were mowed only once and a little bit later than the other consecutive years in order to create well developed plants which can survive easily during the winter time. There was observed that the yield of the 1st mowing of sage was significantly different from year to year for the same variant and for different limestone rates. Use of limestone was followed with a significant yield increase, but the highest yield was observed for limestone rate of 80 and 120 q ha^{-1}, by 15.5 and 15.9 q ha^{-1}, respectively. Maximum first mowing yield for the other three years was observed for the highest limestone rate (120 q ha^{-1}) for both probability levels. During the 4th year, first mowing yield was decreased significantly for control and other variants, but yield decrease was 43% for control and only 16.4% for 120 q ha^{-1} limestone (Table 5), which means that limestone affects significantly sage degradation. This is the reason that, in soils with high limestone content, sage can survive and produce very well up to five years.

Table 5. First mowing yield of *Salvia officinalis* L (q ha^{-1}) according to different years of cultivation and different limestone rates (** significant at p<0.01 and * significant at p<0.05).

Variants	Years of the study (2010-2013)				Four years
	I	II	III	IV	mean
Control (0 q ha^{-1} limestone)	13.0	11.2	11.9	7.4	10.9
40 q ha^{-1} limestone	13.42	11.6	12.9	8.525	11.6
80 q ha^{-1} limestone	15.5**	13.1	14. 7	11.77	13.8
120 q ha^{-1} limestone	15.9**	15.0 **	15.9 **	13.3 **	15.0 **
LSD 0.05 *	0.63	0.41	0.366	0.68	0.786
LSD 0.01 **	0.91	0.60	0.526	0.98	0.985

Yield of the 2nd mowing yield (q ha^{-1}). The highest second mowing yield was observed during the second year for all variants, where the yield varied from 13.1 q ha^{-1} (control) to 16.4 q ha^{-1} (120 q ha^{-1} limestone). Yield was significantly increased for all treated variants compared to control, but the yield increase was significantly different (from 9.1% (V2) to 20.1% (V4). Maximum second mowing yield for three years was observed for the highest limestone rate (120 q ha^{-1}) for both probability levels. During the 4th year, the 2nd mowing yield was decreased significantly for control and other variants, but yield decrease was 27.5% for control and only 10.4% for 120 q ha^{-1} limestone (Table 6).

Table 6. Second mowing yield of *Salvia officinalis* L (q ha^{-1}) according to different years of cultivation and different limestone rates (** significant at p<0.01 and * significant at p<0.05).

Variants	Years of the study (2010-2013)				Four years
	I	II	III	IV	mean
Control (0 q ha^{-1} limestone)	-	13.1	12.8	9.5	11.8
40 q ha^{-1} limestone	-	14.4	13.5	10.6	12.8
80 q ha^{-1} limestone	-	16.1 **	14.9	13.8	14.9 *
120 q ha^{-1} limestone	-	16.4**	15.8**	14.7**	15.6**
LSD 0.05 *	-	0.47	0.43	0.47	0.547
LSD 0.01 **	-	0.68	0.62	0.68	0.786

Total yield (q ha^{-1}). There was observed that limestone use and limestone rate significantly affected the total yield of *Salvia officinalis* L, grown under Maqellara, Dibër, climate conditions. The highest yields for all variants were observed during the third year of sage cultivation. There were observed not significant differences between 2nd and 3rd year of cultivation for the same variant. Maximum total yield for four years was observed for the highest limestone rate (120 q ha^{-1}), by 106.8 quintals, or a mean yield of 26.7 q ha^{-1}. During the 4th year, the total yield was decreased significantly for control (43%) and the other variants, by 28.03% (V2), 10.8% (V3), and 11.1% (V4), respectively (Table 7). Although the sage yield decreases after the third year, using high limestone rate can be a possibility keeping the sage until the 5th year.

Table 7. Total yield (q ha^{-1}) of *Salvia officinalis* L, according to different years of cultivation and different limestone rates (** significant at p<0.01 and * significant at p<0.05).

Variants	Years of the study (2010-2013)				Total	4 years
	I	II	III	IV	yield	mean
Control (0 q ha^{-1} limestone)	13.0	24.1	24.8	16.9	78.7	19.7
40 q ha^{-1} limestone	13.4	26.0	26.4	19.0	84.8	21.2
80 q ha^{-1} limestone	15.5 **	29.2	29.6	25.6	99.8	24.9 **
120 q ha^{-1} limestone	15.9 **	31.4 **	31.5 **	28 **	106.8	26.7 **
LSD 0.05 *	0.63	0.653	0.61	0.69	------	1.634
LSD 0.01 **	0.91	0.936	0.95	0.99	------	1.837

Sage yield was significantly different for four years of the study and for different limestone rates, which emphasize the importance of land liming for sage cultivation.

CONCLUSIONS

The observed data showed that limestone use and limestone rates significantly affected yield and yield indicators of sage (*Salvia officinalis* L). The highest performance of yield and yield indicators were observed using 120 q ha^{-1} limestone. The highest yearly and total yield was obtained using 120 q ha^{-1} limestone, with a four year mean yield of 26.7 q ha^{-1} or 7 q ha^{-1} (35.4%) higher than control. Recommended limestone rate for Maqellara, Dibër, conditions was 80-120 q ha^{-1} and keeping the sage up to five years under cultivation.

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