ISSN 2236 - 4420

Mineral nutrition, planting density, biometric and phenological characterization of the lamb´s ear

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Abstract: The lamb's ear (*Stachys byzantina* K. Koch) is an example of underutilized food species, characterized in Brazil as an unconventional vegetable. Currently, there is enough government incentive for the rescue and reintroduction of this species in the Brazilian food, however, there is little information available about the suitable techniques for its cultivation and for large-scale production. Thereon, we aimed to evaluate the lamb's ear plants development under different levels of fertilization, supplied by NPK formulations determined by variations obtained from adjustments to the recommendations for fertilization of leafy crops, besides evaluating the behavior of the crop, when conducted at different planting densities, as well as to determining the characterization of the phenological stages of this species in the region southern Minas Gerais. Two experiments were conducted, one to determine the phenological stage, which was arranged in a design of randomized blocks with four replications, and the other for evaluation of the phytotechnical handling of the crop, which was arranged in the randomized blocks with split plots. The production rate varied significantly when the crop was subjected to different levels of fertilizer and different spacing, and the best result was 48.05 Mg.ha^{-1} by handling in a less dense spacing of 50 cm between plants and 30 cm between lines under the fertilization of 300 kg.ha^{-1} N, 240 kg.ha^{-1} of K_2O and 800 kg.ha^{-1} P_2O_5. The crop cycle, from planting to harvest, was set at 140 days after the planting.

Keywords: Unconventional vegetables, Crop cycles, *Stachys byzantina* K. Koch.

Nutrição mineral, densidade de plantio e caracterização biométrica e fenológica do peixinho

Resumo: O peixinho (*Stachys byzantina* K. Koch) é um exemplo de espécie alimentícia subutilizada, caracterizada no Brasil como hortaliça não convencional. Atualmente, tem-se bastante incentivo governamental em prol do resgate e reintrodução dessa espécie na alimentação do brasileiro, entretanto, existem poucas informações disponíveis no que se referem às técnicas indicadas para o cultivo e para a produção em larga escala. Diante isso, objetivou-se avaliar o desenvolvimento de plantas de peixinho sob diferentes níveis de adubação, supridos por meio de formulações de NPK determinados por variações obtidas a partir de adaptações das recomendações para a adubação de culturas folhosas, além de avaliar o comportamento da cultura, quando conduzida em diferentes densidades de plantio, e também determinar a caracterização dos estádios fenológicos dessa espécie na região no sul de Minas Gerais. Dois experimentos foram conduzidos, um para determinar o estádio fenológico, que foi arranjado em um delineamento de blocos casualizados com quatro repetições, e o outro para avaliar o manejo fitotécnico da cultura, que foi disposto em blocos casualizados com parcelas subdivididas. O índice produtivo variou significativamente quando a cultura foi submetida aos diferentes níveis de espaçamentos e adubações, sendo que o melhor resultado obtido foi de 48,05 Mg.ha^{-1}, mediante condução em espaçamento menos adensado, de 50 cm entre plantas e 30 cm entre linhas, sob a adubação de 300 kg.ha^{-1} de N, 240 kg.ha^{-1} de K_2O e 800 kg.ha^{-1} de P_2O_5. O ciclo da cultura, do plantio à colheita, foi estabelecido em 140 dias após o plantio.

Palavras chave: Hortaliças não convencionais, Ciclo produtivo, *Stachys byzantina* K. Koch.

Introduction

Much has been said about the wide availability of plant and floristic resources in Brazil, however, only few species are currently being used as food by people. In the history of mankind, there is a record that more than seven thousand species of plants have been used as food, although currently it is true that only a few crops are responsible for the world's nourishment, since 75% of the food produced matches only 12 plant species and five animal species Food and Agriculture Organization of the United Nations [FAO] (1999). Together, the crops of rice, corn and wheat are responsible for over 50% of the calories ingested by men (FAO, 2014). There is a lack of scientific studies on most of the plant species considered food and much of the information on the possible uses of the techniques for each crop is generated by popular knowledge, without deeper scientific research.

Popularly known as lamb’s ear or woolly hedge nettle, the *Stachys byzantina K. Koch* (syn. *Stachys lanata*) is an herbaceous plant, perennial, belonging to the Lamiaceae family, found spontaneously in the soft climates of Europe and Asia in shaped clumps (Brasil, 2013, Kinupp & Lorenzi, 2014). In Brazil, it is considered an unconventional vegetable species, and there are reports of consumption of their fresh leaves, breaded, fried, or in the form of tea or infusion. It hardly flowers in Brazilian conditions, and therefore, their multiplication is usually performed by division of the clumps (Brasil, 2010a, 2010b).

There is a great pharmacological interest in the species, especially for presenting anti-inflammatory and anticancer effect. There are also reports of good nutritional potential in this species, mainly for presenting in its constitution high levels of vitamin C, vitamin K, carbohydrates and antioxidant potential (Asnaashari et al., 2010, Brasil, 2010a, 2013, Kinupp & Lorenzi, 2014).

Despite increasing government incentives to the rescue and use of this unconventional vegetable in national feeding, there is little information available for its cultivation and production in Brazilian soil and climate conditions, such as crop cycle, propagation, plant density by land area in a proper spacing, fertilization and nutrition, need for irrigation, pest and diseases control and harvesting. Neither there are available cultivars and propagative materials for the planting or the characterization of some varieties already grown (Silva et al., 2018).

Usually, this species is grown using the already defined indications set for the crops of conventional vegetables, such as the fertilizer levels indicated for the lettuce crop (Brasil, 2013).

To ensure the quality and productivity of a culture, phytotechnical knowledge should be available to the farmer, mainly when related to the appropriate management of each crop (Filgueira, 2006, Borém & Miranda, 2009). Because they have great ability to adapt to different environments, plants with the same genotype can have productive differences when conducted in different conditions of luminosity, soil and environments (Radin et al., 2004, Lima et al., 2013, Moraes et al., 2013 & Ziech et al., 2014). With that in mind, we aimed to evaluate the lamb’s ear crop at different levels of fertilization and planting density, through the productive and biometrics features of its leaves, as well as to characterize the phenological stages of the species in southern Minas Gerais.

Material and methods

Two experiments were conducted, one to determine the phenological stage and the other for evaluation of the phytotechnical handling of the crop. The experiments were set up in June 2014 in the experimental field of the Vegetable Crops sector, in Lavras, Minas Gerais (21º 14 'South latitude, 40 ° 17' West longitude and altitude of 918.80 m). The climate in the region is classified as humid temperate, with hot summers and dry winters, being, therefore, classified as the Cwb type in Köppen (Brasil, 1992).

The soil of the experimental area is classified as Dystroferric Red Latosol and has the following characteristics in the topsoil layer of 0 to 20 cm: pH (in H_20) = 6.1; exchangeable Al = 0.10 cmol dm^{-3}; Ca $^{2+}$ = 1.70 cmol dm^{-3}; Mg $^{2+}$ = 0.40 cmol dm^{-3}; P- Mehlich = 4.81 mg dm^{-3}; K $^{+}$ = 46 mg dm^{-3}; Organic matter = 1.18 dag Kg $^{-1}$; V = 48.85% ; Base Sum = 2.22 cmol dm^{-3}; CTC = 4.54 cmol dm^{-3}; Zn^{2+} = 1.32 mg dm^{-3}; Fe^{2+} = 93.60 mg dm^{-3}; Mn^{2+} = 26.60 mg dm^{-3}; Cu^{2+} = 1.18 mg dm^{-3}; B= 0.33 mg dm^{-3}; S=14.55 mg dm^{-3}; Clay = 36 dag Kg^{-1}; Silt = 18 dag Kg^{-1}; Sand = 46 dag Kg^{-1}; Soil texture = Clayey.

The soil preparation was done in a conventional way with a plowing and two harrows and the beds were raised with a bed tiller in the dimensions of 1.25 meters wide by 0.25 meters high. Based on the soil chemical analysis, liming was performed using 0.50 t ha^{-1} of limestone (PRNT 100%). Farming practice made according to the need of crop (Brasil, 2013).

Propagation of the plant material and evaluation of the phenological stages

The lamb's ear seedlings were grown from the best plants presents *in vivo* originated from Federal University of Lavras Non-Conventional Vegetables Germplasm collection. From each clump the propagules were taken, which were prepared to give place to new plants of the species being directly transplanted to field conditions under the spacing of 20 x 25 cm plots with dimensions of 1.25 meter wide by 0.25 meters tall.

The experiment consists of a total of 90 plants, which were arranged in a design of randomized blocks with four replications. The data was obtained from the central plants of each plot, with the rest being considered as the border.

The classes for the phenological stages were obtained according to adaptations of the unified coding, from the phenological stages of crop development (BBCH-code), which is based on the binary classification of phenological stages, according to the macro stages (first digit) and the micro stages (second digit) of the cultures (Lancashire et al., 1991). The data collected were related to the days after the planting (DAP), inferred in the following characters in the field: the beginning and the end of the swelling of the propagative organs, the counting from one to nine or more visible leaves, and the counting of the number of developed leaves with plants on field, on different days after the planting. After the leaves harvesting it was assessed the total number of leaves and the total number of developed leaves of each plant.

The data related to the beginning and the end of the swelling of the propagules and the early formation of the first roots were estimated by visual observations. When it was found that, apparently, more than 80% of the seedlings exhibited these conditions in certain days after the planting (DAP), according to which the first 10 days after planting irrigation was supplied so that the soil moisture remained close to field capacity by the drip irrigation system.

The other evaluations were performed every two days accounting the apparent leaves up to 30 DAP. Subsequently to 30 DAP, it was recorded the total number of leaves per plant, and the total number of leaves developed by plant species at different stages of the cycle. Taking into account that the developed lamb's ear leaves, larger than 8 cm, are considered suitable for the market (Brasil, 2010a, 2010b), it was carried out the counting of the number of marketable leaves at 45 DAP and 90 DAP. After harvesting the leaves, at 140 DAP, it was estimated the total number of leaves and the total number of marketable leaves (larger than 8 cm length) in each plant. The leaf dimensions were estimated with a digital pachymeter (Shopcal 150 mm, TESA).

From each set of data we obtained values for the mean, the median, the mode and the standard deviation and, after confirming the normality of the data, for the regression analyzes, seeking to relate the amount of marketable leaves observed in the days after the planting (DAP).

Evaluation of the influence of different spacing and fertilization on the productivity and on the biometric character of the culture

The statistical design adopted was the randomized blocks with split plots. Thus, in the field each block contained two main plots each receiving one of the levels of spacing to be studied and each portion of these was subdivided into four subplots, each receiving a level of fertilization.

The chemical formulations adopted as different fertilizer treatments were made using ammonium sulphate (18% N), single superphosphate (18% P_2O_5) and potassium chloride (60% K_2O) and as sources of nutrients nitrogen, phosphorus and potassium respectively.

We used two levels of planting density corresponding to the spacing between plants of 20 cm and 25 cm between lines (E1) (Brasil, 2010a), resulting in a 0.05 m² ground area occupied by the plant (20 plants per m²), and the spacing of 50 cm between plants and 30 cm between lines (E2), about 6 plants per m² (Silva, 1997), resulting in a 0.15 m² ground area occupied by each plant.

We used four different fertilizer levels adapted from the signs to vegetables crops (Ribeiro et al., 1999), with a low availability of P and K verified, prior to the chemical analysis of soil. The following treatments were adopted for fertilization: A1 = no chemical fertilizer; A2 = 75 $kg.ha^{-1}$ N, 60 $kg.ha^{-1}$ of K_2O and 200 $kg.ha^{-1}$ of P_2O_5; A3 = 150 $kg.ha^{-1}$ N; 120 $kg.ha^{-1}$ of K_2O and 400 $kg.ha^{-1}$ of P_2O_5; A4 = 300 $kg.ha^{-1}$ N, 240 $kg.ha^{-1}$ of K_2O and 800 $kg.ha^{-1}$ P_2O_5. About 140 days after the planting the crop has been harvested and the evaluation of the following characters: productivity of fresh material (Mg ha^{-1}), total number of leaves, total number of marketable leaves (> 8 cm long) and total number of unmarketable leaves (<8 cm length) was made.

The statistical analysis was performed using the R platform via the Action software (ESTATCAMP, 2014). The data with abnormal distribution were transformed according to a specific technique (square roots quiz), and the data with normal distribution were directly subjected to an analysis of variance and regression, when appropriate.

Results and discussion

The lamb's ear (*Stachys byzantina* K. Koch) crop's phenological stages

The *Stachys byzantina K. Koch* harvest was carried about 140 days after the planting, in the moment determined by the observations, when most of the leaves in each plant had a marketable size and some of the older leaves demonstrate some yellowness. The crop does not flourish in the climatic conditions of the southern region of Minas Gerais, and therefore it was only defined the macro stage 0 (roots and shoots of the propagative organs), the macro stage 1 (sprouting of the leaves) and the macro stage 2 (growth / development of the leaves), as described in Table 1.

Table 1 - Lamb's ear (*Stachys byzantina K. Koch*) crop phenological stages.

Phenological Stage	Definition	Mean (DAP*)	Median	Mode	Standard Deviation
00	Reproductive organ in dormant stage	0	0	0	0
01	Beginning of the swelling of the reproductive organs	3	3	3	0
02	End of the swelling of the reproductive organs	7	7	7	0
03	Growth / formation of the first lateral roots	11	11	11	0
11	First visible leave	11	11	11	1,57
12	Second visible leave	13	13	13	1,34
13	Third visible leave	14	14	13	1,59
14	Fourth visible leave	16	15	15	1,53
15	Fifth visible leave	17	17	17	1,49
16	Sixth visible leave	18	19	17	1,79
17	Seventh visible leave	21	21	21	2,38
18	Eighth visible leave	25	25	25	1,96
19	Nine or more visible leaves	27	27	27	1,89
		Mean (nº leaves)	**Median**		**Standard Deviation**
21	Marketable-sized leaves (45 DAP)	14	15		3,56
22	Marketable-sized leaves (90 DAP)	30	29		7,10
23	Marketable-sized leaves (140 DAP)	65	65		13,41
23	Total number of leaves (140 DAP)	129	133		20,48

* DAP = days after the planting

At the macro stage 0 the means of the observations regarding the roots and shoots of propagative organs were defined. The initial stage was defined when the propagative organs were in a dormant stage before being transplanted to the field in 0 DAP. From that moment, it was observed the moment when the overall average of propagules began their swelling, which occurred about 3 DAP, and when most of them did not have more variation in their size or turgidity, marking this date as the end of the swelling, which occurred about 7 DAP. The first roots and leaves produced in each plant were visible around 11 DAP.

At the macro stage 1, in the first 30 days of the development of the crop, the average DAP in relation to the number of visible leaves were taken as a result. It can be observed that the plants can take about 30 days to provide nine or more visible leaves. It is noteworthy that at this stage, the number of leaves, regardless of size and development of each leave, from the first to nine or more visible leaves, were recorded, observed at some time after the planting (DAP).

In terms of the macro stage 2, the crop presented an average of 14 marketable leaves at 45 DAP, which corresponds to 11% of the leaves in marketable size produced after the harvest. At 90 DAP, the plants showed an average of 30 leaves of marketable size, corresponding to 24% of the total leaf production. At harvest time, held at 140 DAP, it was found that the plants produced an average total of 129 leaves, of which 65 had a marketable size. With this, it is inferred that the crop had after the harvest about 50% of its leaves with a marketable size.

The regression analysis for the number of marketable leaves produced by the crop on different days after planting was carried out (Table 2), whereby it was obtained the following mathematical model: y = 0,42x; where y = number of marketable leaves and x = DAP.

Table 2 - Linear regression for the number of marketable leaves in relation to the days after the planting in the lamb's ear (*Stachys byzantina K. Koch*) crop.

	Coefficient	**Standard Deviation**	**Significance and adjustment**
Predictor (DAP)	0,42	0,01	p-valor = 0,00
Waste	-	10,87	R^2 = 94%
Model	*y=0,42x*	-	-

The lamb's ear is considered a vegetable because it has similar characteristics to the conventional vegetables such as the herbaceous set of the plants and the annual or biennial cultivation, and also because they demand a similar crop management, intensive as the one used in lettuce crops (*Lactuca sativ*a L.), arugula (*Eruca sativa* Mill.), cabbage (*Brassica oleracea* L.), among others. However, the biggest difference of this crop when compared to the crops of conventional vegetables is that despite the fact that the lamb's ear is cultivated as an annual or biannual vegetable, it is a perennial species, although the ideal would be performing a renewal of the crop every six months as a result of phenological responses of this species when cultivated in the Brazilian climate (Brasil, 2013). It is noteworthy that the characterizations of the phenological stages were carried out taking into account that the species does not flourish in the climatic condition of the southern region of Minas Gerais and, therefore, it only presents its vegetative growth phase and it's propagated by vegetative propagation.

The production cycle of the crop (about 140 DAP), compared with the crops of conventional vegetables, such as lettuce, endive, shallots and cabbage (with the beginning of the harvest in approximately 70 to 100 DAP) (Filgueira, 2006), has around 40 days more to the harvest.

In order to meet the objectives of this study, the harvest was carried out in a way of picking up all the leaves of the plants at the same time. Observing the results of the number of marketable leaves and in development submitted after the harvesting, it was possible to observe that part of the leaves was still in development resulting in about 50% of the total leaves harvested in the lamb's ear.

Currently, the baby vegetables market is growing and it is characterized by the product trade before it completes its full development, therefore, it is in the category of trade *in nature* of small leaves (Vasconcelos et al., 2011; Calori et al., 2014). If the farmer's option is to harvest the entire plant, smaller leaves could be marketed in this new market niche. Another option would be to carry out the staggered crop, leaving the smaller leaves on plants, thus allowing the clumps to regenerate aiming at a future harvest. However, there is a clear demand for new studies to assess the most appropriate managements of the harvest and post-harvest of the crop.

The influence of different spacing and fertilization on the production and biometric characters of the *Stachys byzantina K. Koch*

There were different productive responses to the treatments of different levels of fertilizer and planting densities, with the interaction of the effects of these treatments being significant (Table 3), and, therefore, a general mathematical model was obtained for both of the planting densities used (Table 4): y = 10x, where y refers to the amount produced and to the formulated fertilization for each treatment (1, 2, 3 and 4) corresponding to the formulated fertilizer A1, A2, A3 and A4 used in both of the adopted planting densities.

Table 3 - Analysis of the variance for production levels obtained in the different treatments of spacing and fertilization on the lamb's ear (*Stachys byzantina K. Koch*) crop.

Factor	DF	SS	MS	P-value
Block	3	1,056798	1,018584352	0,3262
Spacing (S)	1	1,004725	1,004724517	0,5496
Error 1	3	1,031799	1,010489953	-
Fertilization (F)	3	69,97163	4,120728487	0,000
S x F	3	1,071238	1,02320346	0,0172
Error 2	18	1,098249	1,00521972	-
CV1 (%)	5,08	-	-	-
CV2 (%)	3,59	-	-	-

* DF – Degrees of Freedom; SS – Sum of Squares; MS – Mean Square; CV – Coefficient of Variation.

Table 4 - Linear regression of the production rates of lamb's ear (*Stachys byzantina* K. Koch) in relation to the levels of fertilizer used.

	Coefficient	Standard Deviation	P-value/adjustment
Spacing			
Fertilization	10,08	1,29	0,00
Waste	-	7,07	R^2 = 95%
Model*	*y=10x*	-	-

* With y equivalent to the production (t ha^{-1}) and x the level of fertilizer used (treatments 1, 2, 3 and 4).

Within the two planting densities used, the effects of the addition of the nutrients N, P and K, responded in production averages of 10.12 $Mg.ha^{-1}$; 20.74 $Mg.ha^{-1}$; 20.44 $Mg.ha^{-1}$ and 48.05 $Mg.ha^{-1}$ according to the increased doses of the nutrients A1 to A4 respectively.

Plants under a less dense spacing (E2) generally produced a larger number of leaves (298) and marketable leaves (162), which were present in larger numbers with the additions of nutrients N, P, and K, while plants under the largest spacing (E1) produced on average 131 leaves, of which 65 were marketable.

The response of the number of leaves in development was also positive to the addition of the nutrients N, P and K, and the plant density E2 was also more productive for this character. Plants under the E2 planting density produced an average of 136 developing leaves, while under the E1 planting density the plants produced an average of 66 leaves. However, even with a lower production per plant in E1 the total production per hectare was like that observed in E2.

Usually, in vegetables crops there is a great demand for nutrition, and generally the production rates of these crops vary according to the fertilizer levels and planting densities (Ribeiro et al., 1999, Radin et al., 2004 & Ziech et al., 2014). In general, the different planting and fertilization density levels used showed significant influences in the production index.

The vegetable species have different productive responses when conducted in different managements. In the lettuce crop, for example, when handled in greenhouse it is expected to produce an average of 56 $Mg.ha^{-1}$ (Ribeiro et al., 1999), however, when grown in handling conditions in the field, it is expected to produce about 20 $Mg.ha^{-1}$ and, for each type of crop, there is a specific fertilization recommendation (Ribeiro et al., 1999 & Ziech et al., 2014.). The best production results with the *Stachys byzantina* were similar to those expected to be obtained with the lettuce in a greenhouse (Ribeiro et al., 1999), however, those results were observed in the culture field, thus demonstrating the great productive potential of this species.

Conclusion

The lamb's ear crop was more productive when conducted in a less dense spacing, of 50 cm between plants and 30 cm between rows, under the fertilization of 300 $kg.ha^{-1}$ N, 240 $kg.ha^{-1}$ of K_2O and 800 $kg.ha^{-1}$ P_2O_5. The crop cycle, from planting to harvest, was established in about 140 DAP.

Acknowledgements

Thank to the Fundação de Amparo à Pesquisa de Minas Gerais [FAPEMIG] and to the CNPq/Conselho Nacional de Desenvolvimento Científico e Tecnológico [MCT] for project financing. To the Universidade Federal de Lavras [UFLA)] for the technology available.

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Recebido em: 13/07/2017
Aceito em: 18/04/2018