ECOLOGIA BALKANICA
2020, Special Edition 3 pp. 41-50

Effect of Mixed Cropping Systems on Yield and Quality of Lettuce (Lactuca sativa L.)

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Abstract. The purpose of this work is to determine the quantitative and qualitative changes in biometric and biochemical parameters of lettuce plants when grown mixed with medicinal and vegetable species. The experiments were carried out in a growing house. The plants, used to create mixed crops, were lettuce (*Lactuca sativa* L.), cutivar "Bohemia", tagetes (*Tagetes* sp. L.), basil (*Ocimum basilicum* L.), calendula (*Calendula officinalis* L.) summer savory (*Satureja hortensis* L.) and arugula (*Eruca sativa* L. Cav.). The results of the analyses showed that the lettuce plants developed together with calendula had a significantly greater leaf and root mass. The calendula plants were 66.5% higher than the average height of the lettuces in the experiment and those developed together with savory and basil respectively 15.3% and 14.5%. The lettuce plants with the best vegetative development also had a high chlorophyll content. The highest value of the indicator Ch a + Ch b was measured in the variant of lettuce grown together with arugula. The lettuce in the containers with arugula had the highest solids content (10.68%) and total sugars (9.8%) in their leaves, while the vitamin C content was highest in the lettuce grown together with calendula (8.57 mg/100g fresh weight).

Key words: mixed farming, *Lactuca sativa*, container experiment.

Introduction

For most of the history of plant growing, the food has been produced from polyculture cropping systems. In nature, the plants always grow in a community, they support each other and complement each other. Combinations of plants prevent the emergence and development of potential pests, as well as increase yields and quality (Sunulahpašić et al., 2017). In their diversity, joint farming systems are close to natural phytocenoses and represent an attempt to create agrocenoses on the principle of differentiation of ecological niches. These complex non-natural systems have not yet been studied in detail. It has been empirically established that joint crops give a significantly higher yield per unit area than in separate cultivation (Poltoretskyi et al., 2019; Prykhodko et al., 2019), without sufficiently clarifying the mechanism of this phenomenon. It is generally accepted that the increased productivity of mixed crops is probably due to the differences between the components, in the way they use the growth factors of the habitat and in their competition for them. The relationships between the components of joint crops affect different aspects of plant life activity. The positive interaction between crops in joint crops is largely determined by the different timing and different activity of nutrient, uptake by their root system. Particularly favourable are those plants in which the minimum and maximum absorption of the elements of mineral nutrition are in different stages of the growing season (Kannan, 2010; Manolov & Manolova, 2013). To be biologically useful, mixed components must be carefully selected. The scientific principles for the selection of components for growing crops at the same place and the same time suitable for intensive farming conditions are not well developed. In this regard, research on interspecific and intraspecific relationships in the agrocenosis is essential, as individual crops at different stages of their ontogenetic development differ from each other in their needs for environmental factors - light, temperature, water, elements of mineral nutrition and other. The production of lettuce aims to increase yields and improve the quality of products that are environmentally friendly. The share of organically grown leafy vegetables is also increasing compared to conventional products. The study of the relationship between growing together cultivated species at different stages of their existence helps to find appropriate technologies for the creation and management of mixed crops, ensuring the sustainability of ecosystems and the potential of the respective habitat. Currently, there are numerous examples proving the higher efficiency of heterogeneous agrocenoses in intensive management conditions (Bevz & Toshkina, 2020; Zhulanova et al.; 2020). The joint cultivation of vegetables is also an old practice in Bulgarian horticulture.

The aim of the present work was to establish the quantitative and qualitative changes of biometric and biochemical parameters in lettuce plants in its joint cultivation with medicinal and vegetable species.

Materials and Methods

Characteristics of soil substrates and plant material

The experiment was planted in the second half of April in a growing house under controlled conditions, using a lettuce (*Lactuca sativa* L.) cultivar "Bohemia". The plants tagetes (*Tagretes* sp. L.), basil (*Ocimum basilicum* L.), calendula (*Calendula officinalis* L.), summer savory (*Satureja hortensis* L.) and arugula (*Eruca sativa* (L.) Cav.) were used to create mixed crops. The experiment was embedded in 3 L containers and contained 8 variants with 3 replicates. The soil was an alluvial-meadow soil (Fluvisol), suitable for growing leafy vegetables. The starting soil was poorly humus and was characterized by a neutral reaction, a very low content of mineral nitrogen and very good supply with mobile forms of phosphorus and potassium (Table 1). The used manure was bovine, well decomposed, meeting the phytosanitary and biological requirements of the crop (Shaban et al., 2014). The soil to manure weight ratio in the experiment is 5:1. The study also included a compost substrate variant (Rindstrup Group) with 28% organic "C" and a balanced macronutrient content.

The following variants were tested: 1. control variant: soil with lettuce plants (SL); 2. control variant: soil + manure with lettuce plants (SML); 3. soil + manure with lettuce plants + arugula (SML+ A); 4. soil + manure with lettuce plants + calendula (SML + C); 5. soil manure with lettuce plants + tagetes (SML + T); 6. soil + manure with lettuce plants + savory (SML + H); 7. soil + manure with lettuce plants + basil (SML + B); 8. compost substrate with lettuce plants (CSL). In the test containers, 3 lettuce plants and 3 tagetes, calendula, savory, basil or arugula plants were planted. In variants-1, 2 and 8 containing only soil, soil + manure and substrate, only lettuce plants are planted.

Growth and phenological observations

The studied vegetative parameters and biometric measurements of lettuce plants were performed: plant height (cm), root weight (g/plant), leaf mass (g/plant), total plant mass (g/plant), number of leaves (number) and stem diameter (cm).

Analysis of soil and plant samples

The soil samples from all variants of the experiment were taken and tested before setting the experiment and at the end of the growing season. The following indicators were reported - pH_{H2O}, pH_{KCl}, mobile forms of N, P, K, Ca, Mg. The hydrogen index (pH) was determined potentiometrically in H_2O and KCl (Arinushkina, 1962); the mineral N content was measured by the Bremner and Keeney method (Bremner & Keeney, 1965); the content of K_2O or P_2O_5 was determined in lactate extract (DPS-AL) (Ivanov, 1984); the soil organic C (humus) was determined by the Turin method (Vorobyova, 1998). The plant samples were analysed for N, P, K, Ca and Mg during the growing season and at the end of the active growing season. The content of macronutrients in plants was determined by the following methods: the content of K_2O and P_2O_5 spectrophotometrically by the method of Milcheva & Brashnarova (1975) and total N according to Kjeldahl (Horneck & Miller, 1998). The content of plastid pigments (chlorophyll-a, chlorophyll-b) was determined in fresh plant samples by the method of Zelenskiy & Mogileva (1980). The leaf dry matter content of lettuce was determined by drying at 60 ° C for 3 hours and then at 105 ° C to constant weight (Tomov et al., 1999); the content of vitamin C in lettuce leaves was determined reflectometrically by a method based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex.

After averaging and straining the samples, the resulting juice was diluted with oxalic acid and filtered. To the filtrate thus obtained was added polyvinylpolypyrrolidone (Divergan® RS) at pH >1. After mixing, the sample was filtered and the Vitamin C content was read on an RQflex® reflectometer. The sugar content of the lettuce leaves was determined using an RQflex reflectometer and test strips.

Statistical analysis

Statistical processing of the results obtained was prepared with the statistical product Statgraphics-18 (2017). Analysis of variance (ANOVA) was used to determine the influence of the test parameters. The mean values were compared by a Tukey test.

Results

Table. 2 shows the values of the studied biometric indicators of lettuce plants in different variants.

The lettuce plants were well developed after planting them into the pots with arugula and tagetes. The average survival of the lettuce plants picked in the experiment was very good - 2.54 pieces/pot. The lowest number of 1.67 lettuce plants was reported in the pots with the substrate variant. There are no statistically proven differences in the number of lettuce plants between the experimental variants.

The tallest were the plants in the variant with calendula – 21.33 cm, followed by those developed together with savory (14.77 cm) and basil (14.67 cm). At an average height of 12.81 cm for the experimental plants, the lettuce of the calendula variant were 66.5% higher, and those developed in the vicinity of savory and basil were 15.3 resp. 14.5%. The differences in heights were statistically proven between variant 4 (SML + C) and variants 6 (SML + S) and 7 (SML + B), while between the variants with savory and basil the differences in the heights of the lettuce plants were not significant. Except for the control variants 1 (SL), the variant 3 with arugula were with the lowest lettuce plants (9.67 cm). There were statistically proven differences in plant heights between lettuce plants in the control variant and all other variants except variant 3 with arugula.

At the expense of the lower height, the lettuce plants grown together with arugula were the most leafy (13.07 leaves/plant) or 25.6% more formed leaves than the average number of leaves for the experiment. A large number of leaves were formed by lettuce plants in pots with calendula (17.5% more than the average number for all experimental variants).

Table 1. Content of nutrients in soil, manure and compost before the experiment.

Substrate	pH		NH_4-N+NO_3-N	P_2O_5	K_2O	Ca	Mg	Humus
	H_2O	KCI	(mg/кg)	mg /100g				%
Soil	6.7	5.9	18.9	24.7	37.4	-	-	1.72
Manure	8.0	7.2	107.7	365.2	482.9	-	-	44.02
Compost	5.5	-	98.5	110	192	-	10	-

Table 2. Growth parameters of lettuce plants.

Variant	Number of plants per pot	Plant height/ cm /	Number of leaves per plant	Total plant mass (g/plant)	Leaf mass (g/plant)	Root weight (g/plant)
1.SL	2.33	8.30	8.97	6.65	5.46	1.19
2.SML	2.67	12.53	9.47	10.80	9.66	1.13
3.SML+A	3.0	9.67	13.07	13.06	11.24	1.82
4.SML+C	2.66	21.33	12.23	14.18	11.22	2.96
5.SML+T	3.00	10.97	10.27	7.80	6.84	0.95
6.SML+S	2.67	14.77	9.53	9.63	8.33	1.30
7.SML+B	2.33	14.67	9.23	6.41	5.78	0.63
8.CSL	1.67	10.27	10.50	10.05	8.71	1.34
Mean	**2.54**	**12.81**	**10.41**	**9.82**	**8.41**	**1.42**
F- ratio	2.26	23.96	5.34	13.69	7.10	10.83
P- value	0.0336	0.0000	0.0027	0.0000	0.0006	0.0000
LSD ≤ 95	0.865	2537	1.933	2.299	2.540	0.648
LSD ≤ 99	-	3.495	2.664	3.168	3.499	0.893

The lettuce leaves were similar in number in the variants with savory and basil, as well as in the containers of variant 2 (soil + manure). The differences in the number of leaves were significant between variant 3 (SML+A) and the variants with tagetes, savory and basil. The total average weight of plants in the study was 9.82 g/plant. The variant with calendula, which formed the highest plants, also had the largest total measured mass (leaves+root) 14.18 g or with 44.4% heavier plants compared to the average total mass of lettuce plants in the experiment. In pots grown with arugula, lettuces have large masses of 13.06 g (33% more than the average total weight), regardless of the small height, thanks to the large number of developed leaves. The lettuces in pots with co-growing arugula weighed 13.06 g (33% more than the average total weight) despite the small height, thanks to the large number of developed leaves. The lowest weight was of the lettuces from the version with basil 6.41 g (34.7% less than the average total weight for the experiment). The total weight of these plants was lower than that of lettuces (6.65 g) in the control version without manure. There were no proven differences between the variants, in which the lettuce plants is grown together with arugula and calendula. The differences (LSD ≤ 99) between the marigold variant and the peat, tagetes, savory and basil variants were statistically very well represented. The plants developed together with arugula (11.24 g/plant) and calendula (11.22 g/plant) had the highest leaf mass, significantly exceeding that in the other variants. With an average above-ground mass for the experiment of 8.41 g/ plant, the excess in variants 3 (SML+ A) and 4 (SML + C) was 33.7 and 33.4%. The leaf mass of the lettuces from the other mixed cultivation variants was between 5.78 and 8.71 g/plant. The differences between the variant with arugula and those with tagetes and savory were with a high degree of evidence (LSD ≤ 99), as well as between the pots of lettuces grown together with calendula and those with tagetes and savory. In accordance with the well-developed above-ground part, the lettuces of the variant grown together with calendula also had the best developed root system of 2.96 g/plant. The measured root mass of these plants was more than twice the average (1.42 g/plant) for the experiment. The lettuce plants from pots with basil had the least developed roots 0.63 g/plant. There were very strong differences (LSD ≤ 99) in the root masses of lettuces between the pots with calendula and the variants with tagetes, savory and basil.

The plastid pigments are involved in photosynthesis and play a role in plant growth and development (Table. 3). In the study, the chlorophyll content (Ch a + Ch b) varied between 6.943 and 10.425 mg%. The highest value of the indicator Ch a + Ch b was measured in the variant of lettuce grown together with arugula.

The values of the quality parameters of the lettuce production are presented in table (Table 4). In the experimental variants, the dry matter in the lettuces varied from 6.53 to 0.68%. The high dry matter content of the plants grown with arugula corresponded to both the high plastid content and the reported high yields of vegetative and root mass in this variant. The dry matter content of plants is genetically determined. Vegetable crops are characterized by a relatively low dry matter content, but their characteristic feature is their high content of vitamins (Stancheva et al., 2004). The content of ascorbic acid in the lettuce leaves, with the exception of the control variant (soil with lettuce plants, SL), varied in a narrow range from 6.26 to 8.57 mg/100g. The lettuce leaves grown next to calendula had the highest vitamin C content. The low vitamin content in the plants of the control variant (SL) corresponds to their weak vegetative development.

The nitrate content in the experimental variants ranged between 183.6 and 858.6 mg per 1000g of fresh mass and was significantly lower than the permissible contents (Stancheva et al., 2004; European Commission (2006). The high content of nitrates in lettuce leaves grown in containers with arugula corresponds to low yields and disturbed plant development. No nitrites were detected in the rest of the production, with the exception of lettuce plants grown alone on soil + manure in which were measured 8.77 NO_2 mg per 1000g of fresh mass. This probably has a direct or indirect connection with the mixed cultivation of crops.

The content of total nitrogen in the vegetable mass of lettuce varied between 0.86% and 1.45 % (Table 5). The high nitrogen content in lettuce pants of control variant (soil + manure with lettuce plants SML) is probably due to the lack of competing species, while the relatively high levels of total nitrogen in arugula and calendula variants could be explained by optimal nutrition conditions and consequently good vegetative development of lettuce plants. Despite the good mineral nitrogen supply of the substrate of variant 8 (compost substrate with lettuce plants, CSL), the lettuce plants had the lowest nitrogen content. The measured phosphorus contents in lettuces were between 0.89% and 0.46%. Apart from the high values of absorbed phosphorus in the plants in the variants without concomitant culture, high levels of absorbed phosphorus were present in lettuce plants grown together with arugula and calendula. In the plants of the variant with basil, where the reported vegetative mass was the lowest, there were obviously problems in the absorption of phosphorus as well as potassium.

Table 3. Content of plastid pigments. Legend: different letters = stat. difference; $p<0.05$; Tukey test.

Variant	Ch a [mg%]	Ch b [mg%]	Ch a+ Ch b	Ch a/Ch b	C car. [mg%]
1.SL	5.940bc	2.478a	8.418c	2.40b	2.157b
2.SML	6.042c	2.547a	8.589c	2.37b	2.182b
3.SML+A	6.744c	3.681b	10.425f	1.83a	2.155b
4.SML+C	6.308c	2.714a	9.022d	2.32b	2.183b
5.SML+T	4.802a	2.318a	7.120b	2.07ab	1.787a
6.SML+S	5.429b	2.324a	7.753b	2.34b	1.941a
7.SML+B	5.198b	2.789a	7.987b	1.86a	1.841a
8.CSL	4.826a	2.117a	6.943a	2.28b	1.886a

Table 4. Quality parameters in lettuce cultivar "Bohemia". Legend: different letters = stat. difference; $p<0.05$; Tukey test.

Variant	Dry matter (%)	total sugars (%)	Ascorbic acid mg/100 g f. m.	NO_3 mg/ 1000 g f. m.	NO_2 mg/ 1000g f.m.
1.SL	8.24b	6.2b	5.11a	397.8b	0.00
2.SML	6.53a	5.0a	6.26b	788.5f	8.77
3.SML+A	10.68d	9.8f	7.43a	539.7c	0.00
4.SML+C	8.50b	6.3b	8.57a	724.3f	0.00
5.SML+T	9.13c	9.8f	8.449a	692.9d	0.00
6.SML+S	7.72ba	5.0a	7.90a	657.2d	0.00
7.SML+B	8.40b	7.1c	7.02a	858.6e	0.00
8.CSL	8.20b	8.1d	6.83b	183.6a	0.00

Table 5. Nutrient content in 30-day-old lettuce plants. Legend: different letters = stat. difference; $p<0.05$; Tukey test.

Variant	Total N (%)	P (%)	K (%)	Ca (%)	Mg (%)
1.SL	1.17b	0.89c	6.3b	1.46c	0.35a
2.SML	1.45c	0.88c	6.2b	1.00b	0.38a
3.SML+A	1.24c	0.84c	6.3b	1.33c	0.37a
4.SML+C	1.13b	0.72b	6.4b	1.20bc	0.33a
5.SML+T	0.98b	0.67b	6.0b	1.02b	0.30a
6.SML+S	0.99b	0.70b	6.1b	0.95b	0.28a
7.SML+B	0.98b	0.46a	5.3a	0.72a	0.24a
8.CSL	0.86a	0.65b	6.1b	1.04b	0.25a

Table 6. Content of nutrients in the soil after the end of the experiment. Legend: different letters = stat. difference; $p<0.05$; Tukey test.

Variant	pH		NH_4-N+NO_3N	P_2O_5	K_2O	Ca	Mg	Hummus
	H_2O	KCI	(mg/κg)	mg/100 g				%
1.SL	6.9a	6.0a	16.1a	15.5b	33.3a	290b	42.5a	1.67a
2.SML	7.3a	6.5a	20.7b	99.0a	164.0f	365c	48.0a	4.43c
3.SML+A	7.3a	6.6a	16.7a	103.1a	138.4c	363c	58.0b	3.37b
4.SML+C	7.3a	6.6a	17.7a	103.8a	123.0b	368c	60.0c	3.78b
5.SML+T	7.2a	6.6a	20.2b	102.6a	135.0c	360c	61.0c	4.00c
6.SML+S	7.2a	6.5a	23.6b	103.3a	132.7c	343c	55.0b	3.54b
7.SML+B	7.2a	6.4a	23.6b	90.6a	129.5b	360c	59.0bc	3.45b
8.CSL	6.6a	5.7a	66.2c	17.1b	38.4a	175a	42.0a	41.37d

The soil analysis after harvesting the lettuce plants and the end of the experiment showed no differences in the soil reaction of the control variant: soil + manure with lettuce plants (SML), i. e. the crops associated with lettuce did not have a different effect on the soil reaction (Table 5). With the exception of a variant 8 (compost substrate with lettuce plants CSL), in which the mineral nitrogen content was already higher in the initial samples, in all other variants, the NH_4-N + NO_3-N content at the end of the study was very low regardless of the addition of manure. In the variants with arugula or calendula, the residual nitrogen contents in the soil were lower, probably due to the higher nitrogen export with the vegetative mass. The high residual levels after the completion of the experiment of mobile forms of phosphorus, potassium, calcium and magnesium in the soil of the manure variants apparently did not have a differentiated effect on the studied indicators (Table 6).

Discussion

The results indicate that the lettuce plants developed together with arugula and calendula were with the statistically proven highest rates of leaf and root mass of the plants from the variant with calendula were 66.5% taller, and those developed together savory and basil were 15.3% resp. 14.5% higher, than the average height in the experiment.

The lettuce plants that had the best vegetative development also had a high chlorophyll content. The highest value of the indicator Ch"a"+Ch"b" was measured in the variant of lettuce grown together with arugula. The obtained contents of plastid pigments in the performed study were higher than the values obtained in our other studies (Dinev & Mitova, 2011; Mitova & Marinova, 2012; Mitova et al., 2017) under similar controlled conditions (container experiment). The probable cause for this may be the cultivar characteristics, as well as favourable meteorological conditions in April and May. By comparing the data with those obtained from the biometric analysis, it can be seen that the plants (arugula, calendula) that have the best vegetative development also have a high chlorophyll content. Despite the small number of plants (1.67) in the containers with compost substrate with lettuce plants (variant 8, CSL), which implies better conditions for the development of lettuce plants, as well as the balanced content of macro-and micronutrients, the chlorophyll content of lettuces in this variant is the lowest. In this case, this low content of plastid pigments is probably related to the mixed culture. It is known that in conditions of mixed crop, significant changes in the chlorophyll content of maize leaves were found under the influence of grown together annual legumes, which had a significant impact on the intensity of photosynthesis and productivity of maize (Stancheva, 2011). According to some authors (Pochinok, 1976) the normal ratio of Ch a/Ch b should be 3:1. Berova et al. (2007) consider that the ratio between chlorophylls is in the range of 2-3:1, but it is not constant, but depends on a number of factors. In the present study, it was found that the ratios of Ch a/Ch b reported when harvesting the plants were close to those indicated in the literature as optimal. Only in the variants of mixed cultivation of lettuce with arugula and basil were the values of this ratio lower.

The results highlight that the lettuce plants in the pots with arugula had the highest dry matter content (10.68%) and total sugars (9.8%) in their leaves, while the vitamin C content was highest in lettuce leaves grown together calendula. The study confirmed the data obtained in other works with vegetable crops, namely that mixed cropping favours the synthesis of ascorbic acid (Wierzbicka & Majkowska-Gadomska 2012). The total sugars in the experimental variants ranged between 5.0 and 9.8%. The plants in the arugula and tagetes pots had the most total sugars in their leaves. The values obtained in the study for total sugars were higher than those in similar experiments with lettuce (Dinev & Mitova, 2011; Mitova & Marinova, 2012) and comparable with those obtained by Mitova et al. (2017) in experiments with fertilizing of lettuces with under increasing nitrogen fertilization norms.

The nitrogen contents of the plants obtained in the study are lower than those cited in the literature (Mitova & Marinova, 2012), which is probably due to both cultivar characteristics and the fact that the initial soil has a very low content of mineral nitrogen, and nitrogen from manure is more difficult to digest.

The amounts of potassium absorbed by lettuce plants were completely comparable with those indicated by other authors (Mitova & Marinova, 2012) and correlate with the values of phosphorus absorbed by plants in all variants. The variants in which lettuces were grown together with arugula (6.3%) and calendula (6.4%) also had high potassium content. The amounts of calcium and magnesium absorbed by the plants were comparable to calcium and significantly lower than those cited in the literature for magnesium. Absorption disturbances were observed in both elements in the basil variant. The variants with arugula and calendula were again with the greatest intake of calcium and magnesium in the plants.

The fact that lettuce plants in the compost substrate variant, which has a neutral soil reaction and a more balanced ratio of nutrients compared to the soil and manure variants, did not form high yields and quality indicators, could be explained with a favourable influence of the growth of mixed crop plants. The introduction into the crop of components with different rates of leaf formation and duration of the vegetation period significantly changes the photosynthetic potential of the agrophytocenoses. It, in turn, affects the growth of biomass and the net productivity of photosynthesis.

Our results are in line with information obtained by other authors (Ijoyah, 2012; Maseko et al., 2018), who prove that the effect of mixed intercropping of production and yield potential and quality in vegetables and leafy vegetables. In the study carried out by Guvenc & Yildirim (2006), cabbage was used as a main crop, and cos lettuce (*Lactuca sativa* L. var. *longifoila*), leaf lettuce (*Lactuca sativa* L. var. *crispa*) were used as intercrops under field conditions. The production was increased significantly when cabbage was intercropped with cos lettuce, leaf lettuce. This cropping systems did not significantly affect nitrogen, phosphorus, potassium, calcium, magnesium and iron content of cabbage.

The results obtained in this study pointed out that intercropping systems increase total yield, productivity and quality of the lettuce plants. The results obtained could be used as a basis of a wider study to determine the effectiveness and sustainability of the lettuce production under mixed cropping system.

References

Arinushkina, E.V. (1962). *Guide for chemical analysis of soils*: Publishing House of Moscow University, 95-113. (In Russian).

Berova, M., Stoeva, N., Vassilev, A., & Zlatev, Z. (2007). *Practice Guide for Physiology of Plants*: Academic Publishing House, Plovdiv University, 42- 45. (In Bulgarian).

Bevz, S., & Toshkina, E. (2020). Cultivation of legume-grain agrocenoses for the purpose of resource saving in forage production. *IOP Conference Series: Earth and Environmental Science*, *422*(1), 012034. doi: 10.1088/1755-1315/422/1/012034.

Bremner, J.M., & Keeney, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. *Analytica chimica acta*, 32, 485-495. doi: 10.1016/S0003-2670(00)88973-4.

Dinev, N., & Mitova, I. (2011). Effects of mineral and organic fertilization on accumulation of pollutants in spinach plants. *Soil Science, Agrochemistry and Ecology*, XLV, (1-4): 104-110.

European Commission. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, *364*, 365-324. Retrieved from: eurlex.europa.eu.

Guvenc, I., & Yildirim, E. (2006). Increasing productivity with intercropping systems in cabbage production. *Journal of Sustainable Agriculture*, *28*(4), 29-44. doi: 10.1300/J064v28n04_04.

Horneck, D.A., & Miller, R.O. (1998). Determination of total nitrogen in plant tissue. *Handbook of reference methods for plant analysis*, *2*, 75-83.

Ijoyah, M.O. (2012). Review of intercropping research: Studies on cereal-vegetable based cropping system. *Scientific Journal of Crop Science*, *1*(3), 55-62.

Ivanov, P. (1984). A new acetate-lactate method for determination of plant available phosphorus and potassium in soil. *Soil Science and Agrochemistry*, *4*: 88-98. (In Bulgarian).

Kannan, S. (2010). Foliar fertilization for sustainable crop production. *In Genetic engineering, biofertilisation, soil quality and organic farming*. (pp. 371-402). Springer, Dordrecht, Netherlands.

Manolov I., & Manolova, V. (2013). Organic farming - a good perspective for the small farmer. *Operational Program "Human Resources Development", 2007-2013*, (pp. 66). Midion Ltd. (In Bulgarian).

Maseko, I., Mabhaudhi, T., Tesfay, S., Araya, H.T., Fezzehazion, M., & Plooy, C.P.D. (2018). African leafy vegetables: A review of status, production and utilization in South Africa. *Sustainability*, *10*(1), 16. doi: 10.3390/su10010016.

Mitova, I., & Marinova, S. (2012). Effects of enriched vermiculite on the formation of yield and quality in lettuce. *Bulgarian Journal of Soil Science, Agrochemistry and Ecology*, *46*(2). (In Bulgarian).

Mitova, I., Nenova, L., Stancheva, I., Geneva, M., Hristozkova, M., & Mincheva, J. (2017). Lettuce response to nitrogen fertilizers and root mycorrhization. *Bulgarian Journal of Agricultural Science*, *23*(2), 260-264.

Pochinok, H. N. (1976). *Methods of biochemical analysis of plants.* Kiev: Naukova dumka (In Russian).

Poltoretskyi, S., Prykhodko, V., Poltoretska, N., Prokopenko, E., & Demydas, H. (2019). Agro-ecological and biological aspects of the components selection for mixed sowings of forage crops. *Ukrainian Journal of Ecology*, *9*(3), 31-36.

Prykhodko, V.O., Poltoretskyi, S.P., & Bilonozhko, V.Y. (2019). Ecological and biological basis of selection of components for mixed crop sowing. *Cherkasy University Bulletin: Biological Sciences Series*, *2*: 63-73. doi: 10.31651/2076-5835-2018-1-2019-2-63-73. (In Ukrainian).

Shaban, N., Bistrichanov, S., Moskova, Ts., Kadum, E., Mitova, Iv., Tityanov, M., & Bumov, P. (2014). *Vegetable production,* Published by House at University of Forestry, Sofia, 490 p. (In Bulgarian).

Stancheva, I., Mitova I., Atanasova E., & Toncheva, R. (2004). Influence of sources and norms of nitrogen fertilization on yield and quality of lettuce. *Ecology and Industry*, *6*(1), 82-83. (In Bulgarian).

Stancheva, Y. (2011). *Methods for practical use of the relationship between plants and other organisms I and II*. Retrieved from trayanabio.wordpress.com. (In Bulgarian).

Statgraphics Technologies. (2017). Statgraphics 18. Retrieved from: statgraphics.com.

Sunulahpašić, A., Čekić, S., Golijan, J., & Hamidović, S. (2017). The Ecological

Role of Interactions between Plants in Agroecosystems. *Agrosknowledge*, *18*(4), 293-305. doi: 10.7251/AGREN1704293S.

Tomov, T., Rachovsky, G., Kostadinova, S., & Manolov, I. (1999). *Manual for Agrochemistry,* Plovdiv, Bulgaria, 110 p. (In Bulgarian).

Vorobyova, L. A. (1998). *Chemical analysis of soils.* Moscow: Publishing house of Moscow State Univ., 272 p. (In Russian).

Wierzbicka, B., & Majkowska-Gadomska, J. (2012). The effect of biological control of the carrot fly (*Psila rosae*) on the yield and quality of carrot (*Daucus carota* L.) storage roots. *Acta Scientiarum Polonorum Hortorum Cultus*, *11*(2), 29-39.

Zelenskiy, M., & Mogileva, G. (1980). *Comparative evaluation of plant photosynthetic based on a photochemical activity of chloroplasts.* Methodical instructions: VIR, Leningrad, 36 p. (In Russian).

Zhulanova, V. N., Ulyanova, O. A., Rylova, O. V., & Saaya, C. S. (2020). Rapeseed productivity in mixed crops of Tuva. *IOP Conference Series: Earth and Environmental Science, 421*(6), 062020. doi: 10.1088/1755-1315/421/6/062020.

Received: 17.07.2020
Accepted: 08.12.2020