



Impact of green manure plants on the yield and bioactive compounds content of lettuce

Gayane Martirosyan*, Gayane Sargsyan, Karine Sarikyan, Gohar Adjemyan, Anna Hakobyan, Alvina Avagyan, Laura Tadevosyan and Armenuhi Pahlevanyan

Scientific Centre of Vegetable and Industrial Crops, Ministry of Economy of the Republic of Armenia, Darakert, Ararat Region, 0808, Republic of Armenia

***Corresponding Author:** Gayane Martirosyan, PhD, Head of Department of Seed Production and Primary Products Processing, Scientific Centre of Vegetable and Industrial Crops, D. Ladoyan 38, Darakert, Ararat region, 0808, Armenia.

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ABSTRACT

Background: Lettuce (*Lactuca sativa* L.) is a nutrient-rich vegetable that delivers a variety of essential vitamins, minerals, and bioactive compounds, including polyphenols, carotenoids, and chlorophyll. These compounds contribute to health benefits, particularly through their antioxidant properties, while keeping the vegetable low in calories. Organic farming practices, which exclude synthetic pesticides and fertilizers, have been demonstrated to enhance the levels of beneficial compounds like antioxidants and polyphenols in vegetables. Additionally, practices like green manuring improve soil health and fertility, contributing to the overall quality of the produce.

Objective: The primary objective of the research was to assess the impact of different green manure plants on the bioactive component content, including polyphenols, carotenoids, and ascorbic acid, of various lettuce varieties. Additionally, the productivity of the various green manure plants was measured with the aim of enhancing food functionality.























Materials and Methods: Green manuring plants for fertilization in crop rotation with lettuce, bean (*Phaseolus vulgaris* L.), green pea (*Pisum sativum* L.), and soybean (*Glycine max* (L.) Merr.) were used. Soil analysis for nitrogen (N), phosphorus (P), and potassium (K), were performed using a Gallery Aqua Master Discrete Analyzer. Soil analyses were conducted both before the sowing of green manure plants and after the harvesting of lettuce. The content of ascorbic acid, polyphenols and carotenoids were identified with spectrophotometer.

Results: The agrochemical analysis showed that the use of green manure reduced the soil's bulk density by 9.2% to 14.4%. Green manuring plants demonstrated the most nitrogen accumulation, exceeding the control by 1.69-2.39 mg/100g, potassium by 1.2-3.1 mg and phosphorus by 0.1-0.05mg. The lettuce variety 'Manushak' from soybean-treated

plots had the highest phenolic acid content, 1.68 times more than the control, and the greatest Vitamin C increase (13 mg/%). In 'Veradarz' lettuce, leaves from plot manured with soybean Vitamin C increased by 11.5 mg/%, and β-carotenoids increased by 1.28 times. Green manuring contributed to increasing the yield of lettuce by 8.1%-30.0%. The highest lettuce yield (358.4 c/ha) was from the Veradarz variety obtained in the soybean-fertilized field.

Conclusion: Applying green manure plants, specifically soybean, enhanced the lettuce leaves' functional properties by improving soil quality and increasing nutrients and bioactive compounds like phenols, carotenoids, and Vitamin C. This technology boosts lettuce quality, productivity, and functionality, making it a recommended method for producers.

Keywords: lettuce, green manuring, soil quality, carotenoids, phenolic acid, vitamin C, productivity

Soil improvement						Biological compounds						
Standard	Volumetric weight, g/cm ³	pH	Plant-available nutrients, in mg per 100 g of soil			Veradarz	Manushak	Kanach gangur	Veradarz	Manushak	Kanach gangur	
			N	P ₂ O ₅	K ₂ O							Total phenolic acid, mg CAE
	1.19	8.2	4.11	1.50	47.0				25.1	1.8	2.1	9.7
	1.04	7.3	6.50	1.60	50.1				42.3	3.4	2.7	21.2
	1.08	7.5	6.00	1.55	49.2				34.3	2.1	2.5	19.5
	1.09	7.6	5.80	1.52	48.2				36.4	2.4	2.2	15.6
									63.5	2.1	1.0	18.8
									36.5	1.9	1.0	14.8

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a highly valued vegetable due to its nutritional qualities. It can be cultivated year-round, in any season, and in various systems such as greenhouses, hothouses, and open fields. The cultivation and demand for lettuce are increasing worldwide due to its many dietary properties and functional value [1-4].

Overall, lettuce is a nutrient-dense vegetable that offers a variety of essential vitamins, minerals, and bioactive compounds, including polyphenols, carotenoids, and chlorophyll, which contribute to its antioxidant-related health benefits while being low in calories [5-6].

Research indicates that red lettuce contains higher levels of phenolics than green lettuce, making it a superior source of antioxidants in the diet. Additionally, lettuce is rich in Vitamin C [7-8].

Epidemiological studies have demonstrated that higher vegetable consumption is associated with a lower risk of chronic diseases, such as cancer, cardiovascular disease, and age-related functional decline. These health benefits are believed to be linked to the macronutrients, micronutrients, and bioactive compounds found in vegetables (9-10). Food rich in nutrients like vitamins, minerals, fatty acids, certain polysaccharides, and non-nutrients such as polyphenols have demonstrated therapeutic potential against viral activity and can boost immune function. Therefore, polyphenols play a crucial role in preventing cardiovascular and cancerous diseases in human health [11-12].

Studies have shown that organic farming, which forgoes synthetic pesticides and fertilizers, can enhance the concentration of beneficial compounds like antioxidants and polyphenols in vegetables [13-14]. The use of green manure plants has been found to have a positive impact on the bioactive compounds of lettuce. Green manure plants, which are grown specifically to be plowed back into the soil to improve its fertility and structure, can significantly influence the nutritional quality and health benefits of subsequent crops,

including lettuce [15-20].

Therefore, adopting advanced and sustainable cultivation technologies is essential for producing high-quality vegetables that offer maximum health benefits. These practices not only enhance the nutritional value of vegetables but also contribute to environmental sustainability and food security.

MATERIAL AND METHODS

Experimental site and plots: The experiment was conducted from 2023 to 2024 at the Scientific Centre of Vegetable and Industrial Crops of the Ministry of Economy of the Republic of Armenia (40.10583330N, 44.41388990E; altitude 650-700 m). The average temperature varies from -2.6 °C in January to 26.2 °C in July, with an annual precipitation of 289 mm. As green manuring plants for fertilization in crop rotation for lettuce plants were used bean (*Phaseolus vulgaris* L.), green pea (*Pisum sativum* L.), and soybean (*Glycine max* (L.) Merr.). Accessions from the Genebank of the Scientific Centre of Vegetable and Industrial Crops were used [27]. Green manure plants were sown in mid-April and mowed at the end of May during peak flowering. The green manure was incorporated into the soil at a depth of 10-15 cm, and lettuce seedlings were planted at the end of May. The experimental field covered an area of 4000 m².

The following experimental plots were laid: 1. control- N₁₂₀P₆₀K₁₂₀; 2. soybean; 3. bean; 4. pea. On each plot were planted 3 locally bred varieties of lettuce ('Veradarz', 'Manushak' and 'Kanach gangur'). 'Veradarz' is medium-early maturity variety. Leaves are green. Head weight is 400-550 g. Yield is 40-45 t/ha. 'Manushak' is an early maturity red loose-leaf variety with head of 180-200 g weight and yield capacity of 25-30 t/ha. 'Kanach gangur' is mid early maturity green loose leaf variety head weight of which is 200-250g and productivity of 25-30 t/ha.

The planting scheme for lettuce seedlings: the width of the meadow is 40 cm, the width of the streams

is 30 cm, and the distance between the plants is 25 cm. The experiment was conducted using a block-randomized design with four replications.

Analyses: Soil analyses for nitrogen (N), phosphorus (P), and potassium (K) were carried out using a Gallery Aqua Master Discrete Analyzer (Thermo Fisher Scientific, USA), both before sowing the green manure plants and after harvesting the lettuce. The concentrations of ascorbic acid, polyphenols, and carotenoids were determined using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA).

Statistical Analysis: The experimental data were statistically analyzed using the ANOVA method. Results were presented as means \pm standard deviations (SD). An F-test at a 5% significance level was employed to assess variance among the tested variants, examining their effects on lettuce yield and the biochemical elements in both the soil and lettuce leaves.

RESULTS

Research evaluating the levels of bioactive compounds and their functionality in vegetables has shown a direct correlation with soil quality. Our experiments, consistent with findings from other studies [16-20], revealed that employing green manure plants substantially enhanced soil quality, increased crop yields, and improved the quality of the harvested produce.

The soil agrochemical analysis indicated that the application of green manure significantly decreased the

soil's bulk density by 9.2% to 14.4% compared to the control. The greatest improvement, 14.4%, was observed when soybeans were utilized as the green manure crop. Using soybean as green manure resulted in the highest nitrogen accumulation, exceeding the reference variant (control) by 2.39 mg per 100 g of soil. When beans and peas were used, nitrogen levels exceeded the control by 1.89 mg and 1.69 mg, respectively. Phosphorus content increased by 0.1 mg with soybeans and by 0.05 mg with beans, while peas did not significantly increase phosphorus levels. Potassium content rose by 3.1 mg with soybeans, 2.2 mg with beans, and 1.2 mg with peas (Table 1).

Lettuce is rich in phenols, carotenoids, and vitamin C. The quantity of these bioactive components varied by lettuce variety and green manure used. The laboratory analysis of fresh leaves of lettuce showed that in the control option the concentration of phenolic acids in the fresh foliage of 'Manushak' variety is 1.6 times higher than in 'Veradardz' and 1.5 times higher than in 'Kanach gangur'. This result is most likely due to the color of the lettuce. Studies have shown that applying green manure to lettuce significantly increases the levels of phenolic acids, carotenoids, and vitamin C in the leaves in all varieties compared to the control (Table 2). Relative to the control, leaves of the 'Manushak' variety from plots treated with soybean and pea exhibited the highest levels of phenolic acids, surpassing the control by 1.63 and 1.62 times respectively, and in case of treatment with beans by 1.52 times.

Table 1. Agrochemical properties of the soil in the experimental plot

Test variants	Volumetric weight,		Plant-available nutrients,		
	g/cm^3	pH	in mg per 100 g of soil		
			N	P ₂ O ₅	K ₂ O
Reference variant (N ₁₂₀ P ₆₀ K ₁₂₀)	1.19 \pm 0.01	8.2 \pm 0.02	4.11 \pm 0.00	1.50 \pm 0.00	47.0 \pm 0.02
Soybean	1.04 \pm 0.02	7.3 \pm 0.00	6.50 \pm 0.00	1.60 \pm 0.01	50.1 \pm 0.01
Bean	1.08 \pm 0.00	7.5 \pm 0.00	6.00 \pm 0.02	1.55 \pm 0.02	49.2 \pm 0.00
Pea	1.09 \pm 0.01	7.6 \pm 0.02	5.80 \pm 0.00	1.52 \pm 0.00	48.2 \pm 0.01
LSD _{0.05}	0.03	0.05	0.05	0.04	0.02

The concentration of β -carotene and lutein varies depending on the variety and the plants used as green manure. The highest amount of lutein was found in the ‘Veradarz’ variety, which exceeded ‘Manushak’ variety 1.68 times and ‘Kanach gangur’ variety 2.0 times. Green manuring increased lutein by 0.2-1.5 mg across all varieties. The highest increase was in the ‘Veradarz’ variety with soybean manure, and the lowest in ‘Kanach gangur’ with bean manure (Table 2).

The content of β -carotenoids in the control plants ranged from 0.9 mg to 2.1 mg. The highest level was

observed in the ‘Veradarz’ variety, while the lowest levels were found in the ‘Manushak’ and ‘Kanach gangur’ varieties, as shown in Table 2. The difference in β -carotenoid content between the ‘Manushak’ and ‘Kanach gangur’ varieties was not significant. Green manuring with soybean and bean significantly increased β -carotenoid content in the ‘Veradarz’ variety by 1.28 times and 1.19 times, respectively. However, this technology did not support an increase in β -carotenoid content in the leaves of lettuce in the ‘Kanach gangur’ variety.

Table 2. Effects of green manure plants on bioactive compound levels in lettuce (in 100g fresh weight (FW))

Test variants	Variety	Total phenolic acid, mg CAE	Major Carotenoids, mg		Vitamin C, mg/ %
			Lutein	β carotenoids	
Reference variant	Veradarz	25.1	1.8	2.1	9.7
	Manushak	38.2	1.1	0.9	12.3
	Kanach gangur	23.6	0.9	1.1	8.7
Soybean	Veradarz	42.3	3.4	2.7	21.2
	Manushak	62.3	2.6	1.5	25.3
	Kanach gangur	42.0	2.2	1.3	19.2
Bean	Veradarz	34.3	2.1	2.5	19.5
	Manushak	58.2	1.5	1.1	22.3
	Kanach gangur	34.3	1.1	0.9	18.0
Pea	Veradarz	36.4	2.4	2.2	15.6
	Manushak	63.5	2.1	1.0	18.8
	Kanach gangur	36.5	1.9	1.0	14.8
LSD _{0.05}		1.06	0.13	0.38	0.62

The impact of green manure on the total phenolic acid content in the ‘Veradarz’ variety demonstrated an increase of 1.68 times in soybean variant, 1.36 times with beans, and 1.45 times in pea application variant. In the

‘Kanach gangur’ variety, the increases were 1.78, 1.45, and 1.54 times, respectively. Similar changes were recorded in the Vitamin C content. The ‘Veradarz’ lettuce from the soybean-treated plot contained 11.5 mg/%

more Vitamin C compared to the control, while the bean and pea plots showed increases of 9.8 mg/% and 5.9 mg/%, respectively. In the 'Manushak' variety, green

manuring with soybean resulted in a 13 mg/% increase in Vitamin C, and the bean and pea treatments contributed to increases of 10 mg/% and 9.1 mg/%, respectively.

Table 3. Influence of green manuring on morphological and yield characteristics of different varieties

Test variants	Varieties	Leaves, psc	Head mass, g	Yield, c/ha	Marketable yield, c/ha	% of marketable yield from the total	Increase in yield, %
Reference variant	Veradarz	35.0	425.0	297.5	266.2	89,5	-
	Manushak	26.0	250.5	175.4	143.8	82.0	-
	Kanach gangur	30.0	372.4	260.6	228.3	87.6	-
Soybean	Veradarz	42.0	512.0	358.4	345.8	96,5	30.0
	Manushak	33.0	287.3	201.1	173.7	86.4	20.7
	Kanach gangur	38.0	446.2	312.3	293.0	93.8	28.3
Pea	Veradarz	36.0	468.0	327.6	301.6	92.1	13.3
	Manushak	28.0	265.0	185.5	155.4	83.8	8.1
	Kanach gangur	34.0	413.0	289.0	259.3	89.7	13.6
Bean	Veradarz	39.0	497.0	348.0	328.1	94.3	23.3
	Manushak	30.0	271.3	190.0	163.1	85.8	13.4
	Kanach gangur	36.0	428.0	299,6	272.6	91.0	19.4
LSD _{0.05}		3.5	3.21	2.1	2.13		

The use of green manure crops (soybean, beans, peas) in lettuce cultivation significantly impacts the total and marketable yield of the three experimental lettuce varieties by increasing the number of leaves per head and head mass, as confirmed by our studies. Analysis of Table 3 data shows that, compared to the control, green manure application positively affects the mass of lettuce heads. In the soybean treatment the mass of lettuce heads exceeded the control by 20.5% in 'Veradarz', 14.7% in 'Manushak', and 19.0% in 'Kanach gangur'. The bean treatment resulted in respective increases of 16.9%, 8.5%, and 14.9%, while the pea treatment saw increases of 10.1%, 5.7%, and 10.9%. These changes in head mass due to green manure also affected the yield. All three green manure treatments contributed to increased yields in the lettuce fields. The soybean treatment resulted in an increase of total yield, with 60.9 c/ha in 'Veradarz', 25.7 c/ha in 'Manushak', and 51.7 c/ha in 'Kanach gangur'

compared to the control.

DISCUSSIONS

Organic agriculture promotes increased product functionality and improved human health outcomes [28]. Studies conducted by many researchers have shown that green fertilization, which is one of the organic cultivation technologies, supports the increase in soil nutrients, which directly enhances the quality of cultivated crops [21-23]. However, there are few data available on the impact of green manure plants (soybean, bean, pea) on qualitative and quantities properties of lettuce.

As several publications indicate, legumes are among the most effective green manure crops for improving soil quality and nutrients. In this study, soybean, bean, and pea crops were used as green fertilizers for lettuce cultivation [16, 29, 30-31]. The findings suggested that soybean provided the best results among the green fertilizers tested. It reduced the soil's volumetric weight

by 14.4%, increased nitrogen content to 6.50 mg, phosphorus to 1.60 mg, and potassium to 50.1 mg per 100g of soil, exceeding the control by 2.39 mg, 0.1 mg, and 3.1 mg, respectively. Our findings align with Gurjinder S. Baath et al. [32], confirming that soybean is more effective at accumulating nitrogen in the soil, which leads to increased crop productivity.

Many studies have investigated the composition of different (poly)phenol subgroups, including phenolic acids, flavones, flavanols, and anthocyanins, across various cultivars, highlighting their importance as sources of functional food components (24- 27). Quantity of bioactive components depends, not only on soil fertility, but also on crop variety. Thus, we investigated three different types of lettuce with varying color and type. In the control plants, the content of phenolic acids in the fresh red loose leaves of the 'Manushak' variety is 1.6 times higher than in the green Romaine type 'Veradarz' variety and 1.5 times higher than in the green loose type 'Kanach gangur'. Our research results are similar to experimental data by various authors, confirming that red-colored varieties are richer in phenolic acids than green lettuce types [18, 19]. These findings may be attributed to the tendency of red lettuce varieties to channel more carbon into polyphenol production instead of growth. This surplus of carbohydrates also contributes to the synthesis of secondary metabolites, including phenols [20].

Carotenoids, a class of lipid-based phytochemicals, play a crucial role in plant development and defense mechanisms, primarily due to their antioxidant properties, which also confer various health benefits to humans. These compounds act as secondary pigments in photosynthesis, protecting plants from photo-oxidative damage. Carotenoids are the main dietary source of provitamin A, with β -carotene being particularly significant. In lettuce, the predominant carotenoids include β -carotene, β -cryptoxanthin, and lutein, with their levels differing among various lettuce varieties [34,

36]. Our research found that the 'Veradarz' variety exhibited the highest levels of lutein (1.8 mg) and β -carotene (2.1 mg) per 100g of fresh weight. The studies show that green manuring increases carotenoids concentration in three studied lettuce varieties compared to the control. The 'Veradarz' variety manured with soybean had the highest lutein content with 88.8% more compared to the control. The 'Kanach gangur' variety demonstrated the largest increase in concentration of 144.4% compared to the control. Additionally, the 'Kanach gangur' variety manured with beans had a lutein content increase of 22.2%.

The content of β -carotenoid also varied depending on the applied green manure crop and lettuce variety. It is important to consider that green manuring technology did not demonstrate a significant increase in β -carotenoid content in the leaves of 'Kanach gangur' variety. High concentration of β -carotenoid was recorded in 'Veradarz' variety.

Green manures, particularly legumes, play a crucial role in sustainable agriculture by fixing atmospheric nitrogen, which becomes available to subsequent crops like lettuce. Our experiments demonstrated that incorporating legumes as green manure significantly improved the growth and yield of lettuce, confirming the essential role of nitrogen in its optimal development. Green manuring increased the yield of lettuce by 8.1%-30.0%. The highest yield (358.4 c/ha) was obtained in the soybean-fertilized field from the 'Veradarz' variety. The yield increased due to the greater head mass and a higher number of leaves. This enhancement in yield supports the potential use of legumes in crop rotations to naturally boost soil fertility and reduce dependence on synthetic fertilizers.

While our findings align with the conclusions of previous studies [27, 31-34], they also open new avenues for further research. For instance, the long-term impact of different legume species on soil health and their effectiveness across various lettuce varieties warrant

deeper investigation. Additionally, exploring the integration of legumes with other organic amendments could provide more comprehensive strategies for achieving sustainable high yields. These insights not only validate existing knowledge but also contribute original perspectives that could be vital for advancing sustainable agricultural practices.

CONCLUSION

In conclusion, the integration of green manure plants into agricultural practices represents a sustainable approach that not only improves soil health and fertility but also enhances the nutritional value of crops such as lettuce by elevating their content of bioactive compounds. This practice contributes to a more resilient agricultural system, reducing the dependency on synthetic fertilizers and promoting environmental sustainability.

The technology employed in our study has shown a significant positive impact on both the yield and quality of lettuce, establishing a strong foundation for extending its application to other crops. The observed increase in phenolic acids, carotenoids, and vitamin C further enriches the functional properties of lettuce, reinforcing its status as a functional food. This underscores the potential for lettuce not only as a dietary staple but also as a preventive health measure. These findings warrant further clinical trials to explore the broader health benefits of such enriched crops in reducing the risk of chronic diseases.

Furthermore, the broader implications of our research suggest that integrating green manure and other sustainable practices into crop production could play a critical role in addressing global food security challenges. By enhancing both the nutritional and functional quality of crops, such practices offer a viable pathway toward healthier food systems, supporting both human health and environmental stewardship. Future research should explore the long-term impacts of these practices across different agricultural contexts and crop varieties to fully realize their potential.

List of Abbreviations: CAE: meaning chlorogenic acid equivalent, N: nitrogen, P: phosphorus, K: Potassium.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: GM, AP and KS designed the research. GM, AP, AH, LT and GS conducted the research. AP and GA performed biochemical analysis. GM performed statistical analyses. GM and AA wrote the manuscript. GS edit the article. All authors read and approved the final version of the manuscript.

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