

RESEARCH ARTICLE

Effect of vermicompost on yield, quality, and antibacterial activity of garlic

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For the purpose of improving the quality of garlic organic products, the influence of humus and different norms of vermicompost on the yield, nutritional value and antibacterial properties of common garlic (*Allium sativum* L.). The use of the vermicompost for garlic gives an increase in the yield at the level of 1.7–3.9 t ha of the garlic cultivar 'Prometei' compared to the control 'Sofiiskyi' and 2.2–5.2 in relation to the control of the garlic cultivar 'Prometei'. It was established that the caloric content of the product with the fertilizer of vermicompost may increase to 22.68 g 100 g⁻¹ fresh weight (FW), with the fertilize of 5 t ha⁻¹ of vermicompost, and the total content of sugar is increasing by 21.52–40.81% depending on the cultivar. The content of vitamins increased in parallel with increasing in the rate of vermicompost. As a result of laboratory studies, the influence of varietal features on the accumulation of vitamins was revealed. The antibacterial effect of the garlic essential oils against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* was significant; the diameters of the inhibition zone were *S. aureus* is 21.35–27.10 mm, *E. coli* – 16.97–26.46 mm, *B. subtilis* – 16.42–25.36 mm, and the number of *Mycobacterium smegmatis* colonies decreased by 23.96–43.44%. This study has been proved that garlic juice had played a very important role in struggling with the studied bacteria (*S. aureus*, *E. coli* and *B. subtilis*); therefore it can replace chemical antibiotics that have always had unwanted side effects on the body such as allergy and antimicrobial resistance. The obtained data shows that using of vermicompost was more effective than fertilizer of humus.

Key words: Antibacterial activity; Garlic; Nutritional value; Vitamins

Introduction

Garlic is one of the most effective phytoncidic-medicinal plants, characterized by bactericidal, fungicidal, anti-acidic, analgesic, and other properties (Viger, 2001; Martins et al., 2016). On its basis the medicinal products are established and widely used such as allacin, sativin, alistat, alohol and others.

It is known that garlic cloves contain about 41.3% of dry matter, 6.7% of protein, 29.3% of carbohydrates, as well as vitamins C, B1 and substances of hormonal nature. Garlic is especially rich in mineral compounds. Its ash contains potassium (260 mg per 100 g of raw matter), phosphorus (140), sodium (120), calcium (90) and iron (1.5 mg). Garlic bulbs contain about 0.1% essential oils, which include organic sulphur compounds (allylpropyl disulfide, allyl, etc.) (Mardomi, 2017).

It has been proved that garlic extract at low concentration (1:32–1:40) inhibits the growth of pathogenic fungi such as *Coccidioides immitis*, *Auxarthron zuffianum* and *Uncinocarpus*, which are agents of coccidiosis. Other studies have shown that allacin is effective against *Histoplasma capsulatum*, a fungus that similar to the tuberculosis illness (Egbobor, 2007; Singh, 2008; Pure et al., 2017). Scientists (Degwale et al., 2016; Kenea, Gedamu, 2018) have noticed a positive effect of vermicompost that it has been shown not only on the productivity of garlic, but also on its quality. Thus, the content of essential oil in garlic bulbs for introducing vermicompost in norms from 5 to 25 t ha⁻¹ increased by 14.4–41.1% (Golmohammadzadeh et al., 2015).

A number of other scholars have got a significant positive impact on the quality of seedlings vegetables and other crops (Atiyeh et al., 2000; Zaller, 2007; Arancon et al., 2008; Lazcano, Domínguez, 2010; Pour et al., 2013; Kwan et al., 2015).

Therefore, all given above are important to improve the quality of the products used in the pharmaceutical industry, due to the reduction of the use of fungicides and the improvement of the phytosanitary state of garlic crops. For this purpose, it is important to study the influence of vermicompost on the quality of garlic varieties in organic farming technology.

Materials and Methods

The study of influence of vermicompost on food quality and antibacterial activity of common garlic (*Allium sativum* L.) was taking place in 2017–2019. The total area for the experiment –400 m², for plot –100 m², for sampling –10 m². The plots were arranged in a systematic order with four replication. The predecessor – early vegetables. Planting was carried out by the scheme of 45 × 6 cm at the end of the 5–10 of October. The location of the plots was systemic.

Determination of B-complex vitamins content

The sample preparation: all the vegetables and fruits were washed and dried, weighed 50 gm and cut into small pieces and extracted with 0.1 NHCL (sodium chloride) on the water bath at suitable temperature and time period. All extracts were filtered through 0.40 micron filter and taken into 100 ml volumetric flask and volume was add up for mobile phase (McCormick, 1989; 1996).

The standard preparation

stock of standard (Sigma Aldrich Analytical grade Reagent) prepared by dissolving 0.01g of each standard in 100 ml of mobile phase followed by successive dilutions.

High-performance liquid chromatography (HPLC) analysis

HPLC (Shimadzu, Model Prominence 20A) equipped with UV detector and Supelco Discovery Cis18 column (25 cm in length and 0.45 internal diameter) was used for analysis. Mobile phase was 50 m MK₂HPO₄ and MeOH (70:30) at 1 ml min⁻¹ flow rate and 10µL of each sample / standard was injected and monitored at UV 254 nm. The antimycobacterial activity of garlic oil was estimated by the colony count method (Dibua et al., 2010). Solutions and extracts of the sterile medicinal product were prepared by dissolving in sterile water and then filtering through 0.22 µm nylon filter. The inoculum containing 1.5 × 10⁷ CFU (colony-forming unit) ml⁻¹ of bacteria *Mycobacterium smegmatis* was used for research and was prepared by breeding 1:10 bacterial suspension having turbidity comparable to McFarland No. 0.5. One hundred micro litres of suspension of the test microorganism were sown and distributed using sterile cotton on a surface on an agar medium to obtain uniform growth (Moghaddam et al., 2011). The paper discs of 6 mm in size. Under aseptic conditions, empty sterilized disks were impregnated with a solution of rifampicin (3 mg ml⁻¹) and garlic oil (50 mg ml⁻¹). These disks are placed on an agar medium. The appropriate growth control, sterility and solvent were also maintained left for 30 minutes at room temperature to allow for oil diffusion and then incubated at 37°C for 21 days. The antimycobacterial activity was estimated by colony counting method. The studies were conducted in three replicates, while taking into account the average of three indications. The antibacterial activity of garlic oil was evaluated relative to *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* in the inhibition zone by the use of amoxicillin as a standard preparation (El-Mahmood, 2009; Ross et al., 2009). The studies were conducted in three replicates, while taking into account the average of three indications.

Nutritional value

Proteins, fats, carbohydrates and ash content were determined by using standard methods described in the procedures of the American Organization of Analytical Chemists (International Organization of International, AOAC International) (Horwitz, Latimer, 2005). The crude fat was determined using a Soxhlet apparatus (Behr R 106 S, Germany) with petroleum ether, according to the AOAC 920.85 methodology (Horwitz, Latimer, 2016). The content of ash was determined by burning at 600°C to constant mass in accordance with procedures AOAS 923.03 (Horwitz, Latimer, 2016). The energy was calculated by the formula:

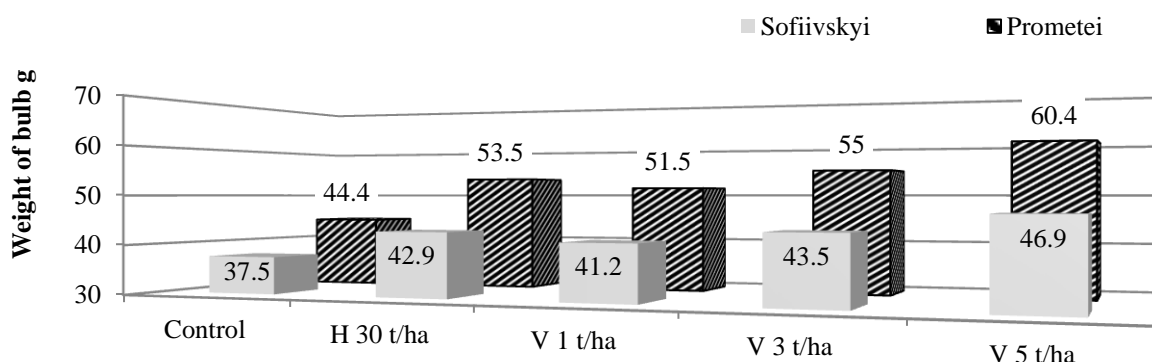
$$\text{Energy (kcal)} = 4 \times (\text{protein}) + 4 \times (\text{carbohydrate}) + 9 \times (\text{fat}) \quad (1).$$

The free sugar was determined by using HPLC, coupled with a refractive index detector using the internal standard methodology (mesostiosis) (Guimarães et al., 2013). For the food and chemical composition, three samples were analysed for each variety, and all analyses were performed in three replicates. The results were expressed as averages and standard deviation. The chemical composition were analysed using a one-way dispersion analysis, followed by the Tvyxi's Honesty Difference (TQ) test with=0.05 using statistical analysis program SAS, version 9.1.3 statistical program (IBM Corp., Armonk, NY, USA). All results are expressed as g 100⁻¹ g fresh weight (FW).

Results

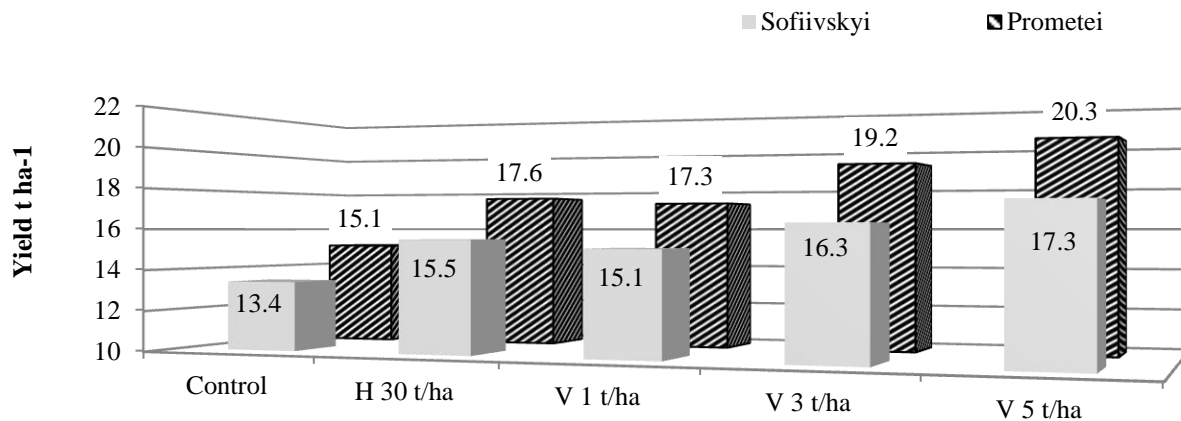
The weight of the bulb of the garlic cultivar 'Sofiivskiyi' with the fertilizer of 30 t ha⁻¹ of humus increased by 5.4 g, with the fertilizer of vermicompost 1, 3 and 5 t ha⁻¹ the bulb's weight increased by 3.7, 6.0 and 9.4 g (Figure 1). The garlic cultivar 'Prometei' without fertilizer was dominated over the control by 6.9 g. With the fertilizing of 30 t ha⁻¹ of humus the indicator increased by 16.0 g, with the fertilizer of vermicompost norms 1 3 and 5 t ha⁻¹ the weight of the bulb was increased by 14.0, 17.5 and 22,9 g.

The use of the vermicompost for garlic gives an increase in the yield at the level of 1.7–3.9 t ha of the garlic cultivar 'Prometei' compared to the control 'Sofiivskiyi' and 2.2–5.2 in relation to the control of the garlic cultivar 'Prometei' (Figure 2).



H – Humus, V – Vermicompost

Figure 1. The bulb's weight of the garlic cultivars depending on the fertilizer by vermicompost (2017–2019).



H – humus, V – vermicompost

Figure 2. The yield commodity of the garlic cultivars depending on the fertilizer by vermicompost (2017–2019).

The changes of the nutritional value of the studied garlic cultivars with the fertilizer of vermicompost are presented in Table 1. The significant differences were observed not only between cultivars, but also between fertilizer variants, indicating that the conditions of cultivation have a significant effect on the quality of garlic. Thus, the content of ash depending on the cultivar and the norm of vermicompost increased by 0.05–0.37 g 100⁻¹ g FW, the content of the protein increased significantly from 0.18 to 0.99 g 100 g⁻¹ FW (up to 16.26% relatively to control), the fat content increased by 0.02–0.16 g 100 g⁻¹ FW, the carbohydrate content increased by 0.70–4.32 g 100 g⁻¹ FW (3.50–17.85%). The calorie content with the fertilizer of vermicompost was increased from 3.70 g 100 g⁻¹ FW, with the fertilizer of 1 t ha⁻¹ of vermicompost in the cultivar 'Sofiivskiy' up to 22.68 g 100 g⁻¹ FW, with the fertilizer of 5 t ha⁻¹ of vermicompost in the cultivar 'Prometei'.

Table 1. The nutritional value of the garlic cultivars depending on the fertilizer (t ha⁻¹) by vermicompost.

Cultivar (factor A)	Fertilization (factor B)	Dry weigh (%)	Ash	Proteins	Fat	Carbohydrates	Energy kcal 100 g ⁻¹ FW
Sofiivskiy	Control	36.49	1.15	6.2	0.18	20.00	106.42
	Humus 30	37.11	1.22	6.31	0.22	22.40	116.82
	Vermicompost 1	37.62	1.20	6.38	0.20	20.70	110.12
	Vermicompost 3	37.93	1.26	6.54	0.24	22.90	119.92
	Vermicompost 5	38.45	1.27	7.02	0.25	23.08	122.65
Prometei	Control	41.53	1.55	6.15	0.26	24.20	123.74
	Humus 30	42.51	1.70	6.32	0.32	26.80	135.36
	Vermicompost 1	42.78	1.65	6.35	0.30	26.00	132.10
	Vermicompost 3	43.09	1.84	6.6	0.39	27.70	140.71
	Vermicompost 5	43.94	1.92	7.14	0.42	28.52	146.42
LSD _{0.01}	A	0.89	0.023	0.085	0.004	0.38	1.83
	B	1.41	0.037	0.134	0.006	0.61	2.89
	A×B	1.48	0.053	0.190	0.009	0.86	4.10

FW – Fresh Weight.

The vitamins are indispensable, vital organic compounds that do not produce calories, but they are important for cellular metabolic responses. The vitamins are required in small amounts for normal growth and function of the body (Lee et al., 2018). The vitamin of fruits and vegetables can vary widely across the globe due to various environmental factors, soils, etc. (Food and Nutrition Board, 1998; Scognamiglio et al., 2015; Combs, McClung, 2016).

The studies founded a significant difference between the garlic cultivars and the fertilizer variants (Table 2). The obtained data shows that using of 1 t ha⁻¹ of vermicompost was more effective than the fertilizer of 30 t ha⁻¹ of humus. The content of vitamins increased in parallel with increasing in the rate of vermicompost. The most significant increase of the whole complex of vitamins was noted in vitamin B₉, the content of which was at the lowest level. Thus, in the cultivar of Sofiivskiy the amount of this vitamin with the fertilizer of vermicompost increased by 166.7–266.7%, in the cultivar 'Prometei' without fertilizer this indicator was lower than the control by 25.0%. With the fertilizer of vermicompost, the content vitamin B₉ increased by 75.0–150.0% relative to the control and by 133.3–233.3% relative without fertilizer variant.

Table 2. The B-complex of vitamins of the garlic cultivars depending on the fertilizer (t ha⁻¹) by vermicompost.

Cultivar (factor A)	Fertilization (factor B)	Content of vitamins, mg / mcg 100 g ⁻¹ FW						
		B ₃ mg	B ₆ mg	B ₅ mg	B ₄ mg	B ₁ mg	B ₂ mg	B ₉ mcg
Sofiivskiy	Control	16.07	2.56	0.97	0.49	0.60	0.06	0.12
	Humus 30	18.12	3.09	1.16	0.61	0.72	0.10	0.27
	Vermicompost 1	18.16	3.14	1.18	0.58	0.65	0.10	0.27

Prometei	Vermicompost 3	20.05	3.19	1.22	0.64	0.74	0.12	0.32
	Vermicompost 5	23.03	3.19	1.26	0.72	0.77	0.12	0.44
	Control	15.11	2.36	0.86	0.43	0.86	0.05	0.09
	Humus 30	17.45	2.60	0.94	0.60	0.94	0.10	0.24
	Vermicompost 1	18.00	2.85	1.09	0.60	1.09	0.11	0.21
	Vermicompost 3	19.27	3.08	1.15	0.62	1.15	0.11	0.28
	Vermicompost 5	20.20	3.13	1.19	0.68	1.19	0.13	0.30
LSD _{0.01}	A	0,34	0,056	0,018	0,013	0,022	0,003	0,009
	B	0,54	0,089	0,029	0,021	0,035	0,005	0,014
	A×B	0,77	0,126	0,041	0,030	0,050	0,007	0,020

FW – Fresh Weight.

As a result of laboratory studies, the influence of varietal features on the accumulation of vitamins was revealed. The cultivar 'Prometei' varied more in vitamin B₁, but less in relation to the cultivar 'Sofiivskiy' of all other vitamins (B₃, B₆, B₅, B₄, B₂ and B₉).

Table 3. The free sugars of the garlic cultivars depending on the fertilizer (t ha⁻¹) by vermicompost.

Cultivar (factor A)	Fertilization (factor B)	Free sugars g 100 g ⁻¹ FW			
		fructose	glucose	sucrose	total sugars
Sofiivskiy	Control	0.12	traces	1.98	2.10
	Humus 30	0.16	0.15	2.15	2.46
	Vermicompost 1	0.21	0.18	2.27	2.66
	Vermicompost 3	0.22	0.22	2.34	2.78
	Vermicompost 5	0.25	0.23	2.71	3.19
Prometei	Control	0.10	traces	2.13	2.23
	Humus 30	0.13	0.10	2.35	2.58
	Vermicompost 1	0.19	0.15	2.37	2.71
	Vermicompost 3	0.27	0.24	2.46	2.97
	Vermicompost 5	0.31	0.29	2.54	3.14
LSD _{0.01}	A	0.004	–	0.043	0.048
	B	0.006	–	0.068	0.076
	A×B	0.008	–	0.096	0.146

FW – Fresh Weight.

Among the sugars (Table 3), the bulk is sucrose, where its content is within 1.98–2.54 g 100 g⁻¹ FW, and the increase by using of vermicompost is 8.59–19.25% depending on the cultivar. The most significant is the increase in the amount of fructose in the experimental variants, where the increase in the control with the fertilizer of 30 t ha⁻¹ of humus was 30.0–33.3%, and with the fertilizer of vermicompost in different norms, increased the content of fructose by 75.0–210.0%. Also, with the fertilizer of organic fertilizers, the content of glucose increased significantly, while in the control variants only traces were observed. The content of total sugar with the fertilizer of vermicompost increased by 21.52–40.81%. According to the obtained data (Table 4), it is evident that the essential oil of the garlic growth with the fertilizer of the organic fertilizers, and especially of vermicompost, it has a more depressing effect relative to the control, which can be explained by increasing in the content of allicin and an improvement in its quality. In the study of antibacterial activity, it was found that the diameter of the inhibition zone *E. coli*, *S. aureus* and *B. subtilis* was significant but less weighty than amoxicillin in the standard.

Table 4. The antibacterial activity of the garlic cultivars depending on the fertilizer of vermicompost.

Cultivar (factor A)	Fertilization (factor B)	Colony count	Decrease in colony count (%)	Diameter of zone inhibition mm		
				<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
	Amoxicillin (St.)	–	–	35.71	27.09	36.55
Sofiivskiy	Control	38.05	0	17.20	14.08	15.55
	Humus 30	27.92	26.62	25.34	17.55	18.45
	Vermicompost 1	28.75	32.34	21.85	16.97	22.00
	Vermicompost 3	24.61	35.32	26.35	22.30	24.25
	Vermicompost 5	21.52	43.44	27.10	26.46	25.36
Prometei	Control	39.42	0	15.43	14.00	15.12
	Humus 30	30.65	22.24	21.35	16.76	17.10
	Vermicompost 1	31.80	23.96	21.67	16.05	16.42
	Vermicompost 3	28.44	27.85	22.00	18.42	18.94
	Vermicompost 5	24.30	38.35	22.09	18.59	19.66

St. – Standard.

Discussion

In general, the investigated garlic varieties showed a significant difference in their quality indicators and nutritional value not only between genotypes but also between the variants of vermicompost fertilization. This fact indicates that, in addition to the genotype, growing conditions and methods of cultivation can greatly affect the chemical composition and nutritional value, and therefore the quality of the final garlic products. The antibacterial effect of garlic against *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* was studied in this research. It was found that the sensitivity of bacteria gradually increased with increasing the rate of vermicompost, which indicates its positive effect on the quality of allicin (Table 4). The resistance to the bacterial drugs is a global problem, because many bacterial species have become resistant to the antibacterial agents (Garau et al.,

1994). Thus, there is a need to evaluate the efficacy of plant chemicals concerning to the growth of bacteria by extracts of plants to be used with dichloromethane extraction (Laenger et al., 1996), maceration and Soxhlet fluid extraction with hexane (Vilegs et al., 1997). Garlic is known to have antibacterial, antifungal and antiviral activity (Bakri et al., 2005). The present results are in direct correlation with previous studies and research (Reuter et al., 1996), which reported that garlic inhibits the growth of *Staphylococcus* and many other species. Another study found that the raw garlic juice is highly active against *Escherichia coli* and *Typhoid salmonella* (Abdon et al., 1972). (Sasaki et al., 1999) found the activity of garlic against methicillin of *Staphylococcus aureus* and *Candida albicans*. The extract of garlic has the antibacterial activity against *Helicobacter pylori* in a moderate concentration, so it has a protective effect against gastric ulcers (Satiawane et al., 2005).

Conclusion

The use of the vermicompost for garlic gives an increase in the yield at the level of 1.7–3.9 t ha⁻¹ of the garlic cultivar 'Prometei' compared to the control 'Sofiivskiyi' and 2.2–5.2 in relation to the control of the 'Prometei'. The studied cultivars of garlic and the effect of vermicompost on their quality showed that the cultivars and the conditions of cultivation (fertilization) greatly affect the chemical composition and nutritional value, and hence the quality of the final product of garlic. The studies have shown that the use of organic fertilizers, especially vermicompost, improves significantly the antifungal and antibacterial properties of garlic essential oils, so the chemical antibiotics that can have unwanted side effects, such as allergy and antimicrobial resistance, it can be replaced by natural herbal sources.

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