International Journal of Plant & Soil Science
3(12): 1531-1537, 2014; Article no. IJPSS.2014.003

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Effects of Different Manures on Quantity of Sulfur Compounds in the Garlic

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OM and ES designed the study. Authors OM, AB and SI carried out the analysis. Authors MP and OM wrote the manuscript. All authors read and approved the final manuscript.

Short Research Article

Received 19th June 2014
Accepted 29th July 2014
Published 16th August 2014

ABSTRACT

Aims: Study investigates relation of sulfur compounds content in garlic and different type of manures that were used for rearing of this vegetable. Measured parameter was total sulfur which could be a valid parameter of content of aromatic and medicinal sulfur compounds in garlic (alliin etc.).
Study Design: Quality of garlic mainly depends from content of sulfur compounds, there are articles related to influence of mineral fertilizer on the quality of garlic bulbs but effect of manures hasn't been enough investigated. This research should give an estimation of effect of some manures on the quantity of sulfur compounds in garlic.
Place and Duration of Study: Sarajevo University - Department of Chemistry, Educons University - Department for Ecological Agriculture, Laboratory of Public Enterprise „Vodno podrucje sliva rijeke Save", between Mart 2012 - June 2013.
Methodology: Garlic plants were treated separately with: sheep, poultry, cow manures and mineral fertilizer. Total sulfur analysis of garlic bulbs was performed by the Ion

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Chromatography method, with the sample first being treated in a strong oxidizing media by mixture of perchloric and nitric acid. Total sulfur quantity in the manures was quantified by ICP-OES, samples previously have been treated with nitric acid.
Results: The highest level of total sulfur was found in the fresh bulb of garlic that has been treated with poultry manure - it was 30% higher than in garlic which has been cultivated with cow manure and 40% higher compared with garlic that has been cultivated with sheep manure. The total sulfur content in the manures was different: The highest was in sheep manure, followed by poultry and the lowest level was found in cow manure.
Conclusion: Application of poultry manure has resulted in best quality garlic. The most important parameter that can increase the content of sulfur compounds in garlic is the possibility of mineralizing the manure.

Keywords: Garlic; sulfur compounds; manures.

1. INTRODUCTION

Garlic (*Allium sativum* L.) could be considered a “sulfur plant“, because sulfur compounds dominate in the chemical composition of secondary metabolites. These sulfur compounds have the most important position in defining aromatic and medicinal effects of garlic [1,2]. Primary organosulfur ingredients of a fresh garlic bulb are alliin and γ-glutamilcysteines which comprise about 80% of total sulfur [3]. The well-established ratio of these compounds in the total sulfur content in the fresh bulbs provide the possibility to use the total sulfur content as an important nutritional and medicinal parameter of species in the Alliaceae family and a valid prediction factor of the alliin/allicin content. Another reason for focusing on total sulfur is the increase of alliin contents during the first few months of storage, which can be explained by its formation from γ-glutamylcysteines [3]. To date, a dozen organosulfur compounds have been isolated and identified from garlic, some as primarily present and most of them as products of decomposition due to different ways of bulb processing.

As mentioned, an increase of sulfur compounds in garlic would positively impact the quality of this plant, especially in the context of organic production. Garlic that is to be used for primarily medicinal purposes many producers prefer using manures, not artificial fertilizers. On the other hand, agronomists recommend using mineral fertilizers, not only in intensive production, but also in non-professional production [4]. The influence of mineral fertilizers on the sulfur-compound concentration in garlic bulbs has been investigated in many works and is well known. Special attention has been drawn to the ratio content of nitrogen and sulfur in such fertilizers and the interaction and competition of these elements [5]. The same influence and interaction in the case of using manures is not well known. Application of different manures during garlic cultivation could produce different contents of sulfur compounds in the garlic bulbs, and hence different aromatic and therapeutic effects of this plant.

The present study investigates the possibility of increasing total sulfur compounds in garlic by utilizing different manures. Garlic has been treated with sheep, poultry and cow manures. Additionally, in one assay it was treated with a mineral fertilizer. The control sample was garlic, free of any fertilizer. Analysis of total sulfur in garlic bulbs was performed using the Ion Chromatography method (HPIC) [6,7]. Sulfur content in the manures was determined by the Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) method. Samples were previously treated with a mixture of nitric and perchloric acid [7,8].

2. MATERIALS AND METHODS

2.1 Samples

Samples of garlic were cultivated in perforated containers and treated separately with sheep, poultry and cow manures. Every manure was dosed in three containers with six seedlings of garlic. Additionally, two assays of garlic had been performed, one with a mineral fertilizer in order to crop cultures (6:12:5) and another as the control sample without any fertilizer. Total number of containers was 15 (5 assays, 3 containers per assay). Quantities of added manures were the same, according to agronomic standards [4], and for mineral fertilizers according to the producer's recommendation. Sowing period was the end of March and samples were collected in the middle of August. All samples of garlic were reared in the same condition and in the same soil, previously homogenized. Containers were embedded in the level of land, and plants were reared in natural conditions, free of any agro-technical measure (irrigation etc.) except previously adding of fertilizers. Reason for this way of rearing was to compare effects of manures, so obtained results of total sulfur were lower than usually. Sample for analysis of total sulfur included a randomly picked three bulbs from one container, all three bulbs have been homogenized by blender before analysis.

2.2 Chemicals and Equipment

The total sulfur analysis was performed using the following chemicals and equipment: nitric acid Suprapur® (Merck), perchloric acid Suprapur® (Merck), magnesium oxide p.a. (Fluka), magnesium carbonate p.a. (Merck), sodium carbonate p.a. (Merck), deionized water, HPIC system (Shimadzu: CDD-10A, LC-10AD, SIL-10Ai, DGU-14A, CTO-10A, SCL-10A), column (IC SI-90G; Shodex), balance (AB 104; Mettler Toledo), ICP-OES system (Thermo 6000 iCAP), Microwave oven (Sineo MDS-8).

2.3 Procedure for the Total Sulfur Determination in the Garlic

Approximately 0.15 g of a fresh sample was weighed at analytical precision. The sample was mixed with 2 ml of 65% nitric acid and 1 mL of 70% perchloric acid (nitric acid contained a magnesium oxide at the concentration of 3 mg/mL). The mixture was stored for 24 h and then gently heated on a plate (avoiding boiling) for approximately four hours until a dry residue almost appeared (the heat of the plate was adjusted so that the water reached the temperature of 50ºC). The residue, once cooled, was dissolved in deionized water up to the 50 mL mark. This solution was filtrated and its sample has been analyzed by the HPIC system. The blank was prepared in the same way, but without the sample.

Measurement: 990 µL of solution (sample/blank/standard) and 10 µL of carbonate buffer solution (0.17 M $NaHCO_3$ and 0.18 M Na_2CO_3) were mixed in a vial of HPIC and 10 µL of the vial solution was injected into the HPIC system. Chromatographic parameters were as follows: mobile phase: carbonate buffer (0.0017 M $NaHCO_3$ and 0.0018 M Na_2CO_3); flow-rate: 1 mL/min; temperature: 40ºC; time: 20 minutes; column: strong anion exchange (SAX). Under these conditions retention time for the sulfate anion was 10.7 minutes.

2.4 Procedure for the Total Sulfur Determination in Manures

Approximately 0.3 g of a homogenized sample of wet manure and 0.07 g of mineral fertilizer were weighed in microwave digestion vessels at analytical precision. Then, 3 ml of a mixture

of HNO_3 and $HClO_4$ (8:1 vol.) was added to the sample. Vessels have been warmed up in the following way: to 175ºC - 6 min., 175ºC - 15 min. The heating power was 600 W. After thirty minutes of cooling, digested samples were poured into a 50 ml flask and filled up with deionized water. Samples from flasks were filtrated and injected into the IPC-OES system. In the samples sulfur was measured at 182.16 nm. The blank was prepared in the same way, without adding fertilizer.

2.5 Statistical Analysis

All results were expressed as mean value ± standard deviation (SD) of triple measurements. Considering that containers were filled previously homogenized soil, it is expecting that SD between three containers, treated with same manure, should be lower than rearing in field. Results were statistically analyzed using ANOVA, and means of assays were compared by Tukey test (SPSS). Differences were considered significant at $p<0.05$.

2.6 Additional Analysis

In order to provide answers based on results, additional analysis were performed: total nitrogen and sulfur content in the manures and moisture of garlic bulbs. Determination of water in garlic bulbs was done by maintaining samples at 105℃ for 2.5 hours. Total nitrogen in the manures was quantified automatically by the Total Nitrogen Analyzer. Available sulfate in the soil (on two months period) was determined using the HPIC method, and sulfate from the soil was extracted by water [9].

3. RESULTS AND DISCUSSION

Values of total sulfur in garlic bulbs are shown in Table 1 and they are ranging from 1854 mg/kg for garlic bulbs cultivated with poultry manure to 1289 mg/kg when using sheep manure.

Table 1. Content of total sulfur and water in the tested garlic bulbs

Fertilizer	Cow	Sheep	Poultry	Mineral	Control sample
Total sulfur in garlic bulbs (mg/kg fresh sample)	1422±31	1289±39	1854±38	1660±65	1327±19
Moisture of garlic bulbs (%)	58.2±0.01	65.3±0.03	56.1±0.00	57.4±0.02	60.7±0.03

Results of total sulfur in garlic are mean of triple analysis ± expressed SD. Values are significantly different at $p<0.05$ using Tukey test, except two comparison: sheep manure - control sample and cow manure - control sample. Results and SD of moisture determination were obtained by double analysis.

Table 2 contains the results of total sulfur and nitrogen in the used manures and available sulfate in the soil in the second and fourth months. Results of total sulfur and nitrogen in manures were obtained as mean value of two replicates. Results of available sulfate in soil are mean of triple analysis, related to garlic samples. Means of available sulfate for II month are significantly different ($p<0.05$) for comparisons poultry - cow manure and sheep - cow manure. Means of available sulfate for IV month are significantly different ($p<0.05$) only for comparison sheep - other manures.

Table 2. Total sulfur and nitrogen content in the used fertilizers and sulfate content in the samples of soil treated with different fertilizers

Fertilizer	Total sulfur content in the fertilizer (mg/g)	Total nitrogen content in the fertilizer (mg/g)	Available sulfate in the soil treated with related fertilizers (mg/kg)	
			II month of vegetation	IV month of vegetation
cow	1.01±0.06	1.43±0.21	60.84±2.03	54.25±2.31
sheep	2.55±0.11	3.64±0.19	72.65±2.42	76.20±2.57
poultry	1.20±0.06	4.86±0.18	80.22±2.01	52.00±1.50
mineral	21.05±0.23	60.0±0.43	65.92±10.90	55.00±9.53

The concentration of sulfur compounds in garlic varies among different species (genetic factors) and depends on external factors such as: climate, soil, light intensity, agro-technical measures, etc. Available sulfur (sulfate) depends on the nature of soil and applied manures. The obtained results (Table 1) could be explained by the fact that sulfur compounds have a higher tendency for mineralization in poultry than in other manures [10,11]. This tendency could be seen from monitoring the sulfate concentration in the soil during the vegetation period (Table 2). During the first period of growth, the highest level of accessible sulfate was found in the soil treated with poultry manure. The first period of vegetation is mainly responsible for the assimilation of sulfates, because it is that period during which plants take up considerable amount of nitrogen which is followed by the assimilation of sulfur. Reduction of sulfur atoms from sulfate (+6 oxidation state) to organosulfide (-2 oxidation state) needs 8 electrons by atom, and this great quantity electron only could be provided during period of strong photosynthetic activity in the first period of vegetation. This approach (sulfate in the soil) cannot explain those results obtained using sheep and cow manure. However, it could help shed light on the nitrogen content of the manures. It is well known that a high nitrogen (nitrate) content in the soil reduces sulfate assimilation in plants [12,5], and one of reason is the reduction of nitrogen atoms, from nitrate to amino form, so this process is competitive to reduction of sulfur. Nitrogen content has been found to be 2.5 higher in sheep manure than in cow manure (Table 2) and such a high level of nitrogen can remarkably limit the assimilation of sulfate. Also great assimilation of nitrogen increases bulb mass, and growth of mass reduces concentration of some secondary metabolites by mass unit. The level of total sulfur content in garlic treated with a mineral fertilizer is not comparable to the levels found when using other fertilizers, considering the different nature, origin and dosage. Except for poultry manure, the mineral fertilizer has proved to be better for producing quality garlic. The concentration of sulfur compounds was higher in the control sample than in the garlic cultivated with sheep manure. The bulb of this sample had a significantly lower mass, and as the bulb mass decreases the concentration of sulfur per gram increases. Low mass is directly correlated with the lack of nitrogen nutrients in the soil. Also, this comparison (sheep manure – control sample) doesn't show statistically significant differences at $p<0,05$ and could be concluded that sheep manure hasn't positive effect on the concentration of sulfur compounds in garlic. Results show that moisture in the bulbs increases with the decrease of sulfur compounds concentration, and that was also noted in previous studies [13]. This statement is valid for the same garlic species, which is cultivated in the same location. Furthermore, according to some studies, a higher level of water in the bulbs correlates with a high concentration of nitrogen in the used fertilizers, confirming the competitive mutual relation of sulfur and nitrogen [13,14]. It is interesting to note that total sulfur and moisture have an impact on the resistance of garlic during storage. Higher level of water in the bulb indicates a faster loss of quality (taste, freshness etc.), and a higher content of sulfur compounds has the opposite impact, it boosts the quality of bulbs [15,1].

4. CONCLUSION

Poultry manure proved to be as the best for the purpose of producing garlic with a high aromatic and medicinal potential, even better than mineral fertilizers with sulfate. The content of sulfur in the manure is not the main factor in increasing total sulfur compounds in the garlic, it is rather a potential of mineralization. The nitrogen content in the manure has a significant impact on the content of sulfur compounds in the garlic, too. Mineral fertilizer, used for vegetable cultures, (6:12:5), has proven very advantageous in the production of quality garlic, but of course not in the organic production. Fertilizers with high nitrogen content, on the other hand, should be avoided.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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