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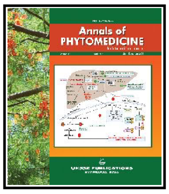

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Biochemical analysis of garlic (*Allium sativum* L.) under integrated nutrient management in North Western Himalayas

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Abstract

The present investigation was carried out at the Experimental Farm, Department of Vegetable Science, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh in rabi 2020-2021. The experiment was set up in a Randomized Complete Block Design comprising three replications of thirteen organic and inorganic fertilizer treatment combinations. The treatment combination which comprised 75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2 found best for the biochemical characteristics. The combined application of 75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2 significantly influenced biochemical attributes of garlic and resulted significantly maximum TSS (36.30 °B), dry matter (44.84%), oleoresin content (1.68%), allicin content (4.87 mg/kg), total protein (1.48%), pyruvic acid (39.21µmole g^{-1}), total phenol (765 mg GAE/kg) and allyl methyl thiosulfinate (AMThs) content (4.91 mg/kg). While the maximum sulphur (1.64%) and zinc content (0.37 mg/kg) were recorded with the combined application of 100% RDN + S @ 50 kg/ha and 100% RDN + Zn @ 7.5 kg/ha. Our findings suggested that using organic fertilizers in combination with inorganic nutrients improves the bulb quality of garlic.

1. Introduction

Garlic *(Allium sativum* L.) is a member of the family Alliaceae and originated in Central Asia. It is used for both culinary as well as medicinal purposes (Chauhan *et al.,* 2022). Garlic is derived from the old English word "gar," which means "spear" and perhaps refers to "clove." The edible underground stem of garlic is made up of multiple smaller bulbs called cloves (Chotaliya and Kulkarni, 2017). The nutritional value of garlic is considerably higher than that of other bulbous crops. It contains a considerable amount of carbohydrates (29%), proteins (6.3%), minerals (0.3%), and essential oils (0.1-0.4%), as well as fat, vitamin C, and sulphur (Memane *et al.,* 2008). It has considerable antibacterial, antifungal, antiviral and antiprotozoal properties. Garlic is beneficial for cardiovascular and immune health due to its antioxidant and anticancer properties (Rani *et al.,* 2023; Reddy *et al.,* 2023). Oil acts as a powerful antiseptic primarily due to the presence of allicin content. It has long been recommended for the treatment of various ailments, including sores, wounds, ulcers, pneumonia, bronchitis and gastrointestinal disorders (Chauhan *et al.,* 2022). Plant nutrition is one of the most important variables affecting plant growth and ultimate output. As heavy feeders, bulbous crops need sufficient levels of nitrogen, phosphorus, potassium and sulphur in addition to other nutrients that could jeopardize the nutritional value of the bulb.

After the green revolution, an increase in vegetable production and quality has been noticed as a result of the excessive usage of chemical fertilizers; however, this widespread application has resulted in soil degradation, ecological risks and the depletion of non-renewable energy sources (Shamna and Poyil, 2023; Timbidaya *et al.,* 2023). The growing costs of chemical fertilizers, together with their detrimental effects on soil health, the environment and human health, compelled farmers to switch to alternate nutrition sources in place of growing vegetables. The switch from the conventional technique to the integrated nutrient management system occurred as a result of knowledge of the deterioration of soil health and excessive application of chemical fertilizers in modern farming (Kumar and Srivastava, 2006). After nitrogen, phosphorus and potassium, sulphur is the fourth most important nutrient for plants. It is essential for the synthesis of amino acids such as cystine (27%) and cysteine (26%) and a component of vitamin A, as well as for the activation of certain enzyme systems in plants (Havlin *et al.,* 2004). A deficiency of sulphur in soils has often led to low bulb crop yields due to poor utilization of macro and micronutrients. Sulphur when present in sufficient amounts in the soil, stimulates plant growth and also reduces the buildup of cancer causing chemicals such as nitrates in vegetables (Thomas *et al.,* 2000). Garlic cultivation has been shown to have a significant impact on zinc application (Lal and Maurya, 1991). Zinc has several functional roles, including the metabolism of auxin,

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impacting the activity of the enzymes carbonic anhydrase and dehydrogenase, synthesizing cytochrome and stabilizing ribosomal fractions. This study was conducted in order to ascertain which combination of nutritional sources could most effectively maintain the bulb quality of garlic.

2. Materials and Methods

2.1 Experimental site

The present investigation was conducted at the Experimental Farm of the Department of Vegetable Science, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP), India, in the years 2020-2021, with the garlic cultivar Kandaghat Selection. At an elevation of 1270 m, it is located at 3550 N latitude and 77110 E longitude. Agroclimatically, it is situated in Himachal Pradesh's mid-hill zone, with a sub-temperate to sub-tropical climate and annual rainfall of 1000-1300 mm.

2.2 Treatments and experimental details

The experiment was laid out in a randomized complete block design (RCBD) with three replications comprising thirteen treatments. The recommended doses of fertilizers for garlic are: N 125, p 75 and k 60 kg ha^{-1}. Nitrogen, phosphorus, potassium, sulphur and zinc were used as inorganic fertilizers in the form of urea, single superphosphate (SSP), murate of potash (MOP), bentonite sulphur, and zinc (EDTA) respectively (Table 1).

Table 1: Details of the treatments

Treatment code	Treatment details
T_1	Absolute Control
T_2	100% RDN (125:75:60 kg per hectare of NPK)
T_3	90% RDN + 10% RDN through VC
T_4	80% RDN + 20% RDN through VC
T_5	100% RDN + Zn @ 5 kg/ha
T_6	100% RDN + Zn @ 7.5 kg/ha
T_7	75% RDN + Zn @ 5 kg/ha + 5% Jeevamrit @ 1 l/m^2
T_8	75% RDN + Zn @ 7.5 kg/ha + 5% Jeevamrit @ 1 l/m^2
T_9	100% RDN + S @ 40 kg/ha
T_{10}	100% RDN + S @ 50 kg/ha
T_{11}	75% RDN + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2
T_{12}	75% RDN + S @ 50 kg/ha + 5% Jeevamrit @ 1 l/m^2
T_{13}	75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2

Note: RDN: recommended dose of nutrients; VC: vermicompost; NPK: nitrogen, phosphorus and potassium; Zn: zinc; S: sulphur

2.3 Total soluble solids (TSS)

Total soluble solids (TSS) content was determined using an 'Ermah and Refractrometer' at room temperature by putting a drop of juice on the prism and taking the reading (AOAC, 1970).

2.4 Dry matter content

For dry matter content (%) estimation, ten fresh plants were taken at harvest from each plot. After initial air drying, the samples were placed in the oven at 65 ± 2ºC for another 45-48 h of drying (AOAC, 1970). When the cloves reached a constant weight, they were considered dried. The following formula was used to calculate the dry matter content:

Dry matter content of the bulb (%) =

$$\frac{\text{Weight of cloves after drying}}{\text{Weight of cloves before drying}} \times 100$$

2.5 Oleoresin content

For the oleoresin content (%) of garlic, powdered dried cloves were used and sieved to the finest particles. The oleoresin content was determined using a sample of 10 g of the finest particles. The solvent was ethanol, and the oleoresin content was determined using a standard procedure (AOAC, 1970). The solution was collected in the beaker and baked at 70°C overnight to evaporate the ethanol and leave the oleoresin in the beaker. Before collecting the solution and after the solution had evaporated, the weight of the beaker was recorded. The oleoresin content is determined by the weight difference:

Oleoresin content (%) =

$$\frac{\text{Weight of oleoresin in the sample (g)}}{\text{Weight of the powdered sample (g)}} \times 100$$

2.6 Sulphur and zinc content

The sulphur content of the bulb (%) was determined by Wet digestion method (Chesnin and Yien, 1950), whereas zinc content of the bulb (mg/100 g) was estimated using Agilent 5110 ICP-OES.

2.7 Pyruvic acid

Without the use of water, garlic cloves were crushed and skinned in a pestle and mortar. Equal parts of crushed cloves and water were combined for 10 min and the homogenate was then filtered through

Whatman No. 4 filter paper. As per the instructions provided (Schwimmer and Weston, 1961), the filtrate was employed for the pyruvic acid analysis using dinitro phenyl hydrazine.

2.8 Total proteins

The amount of total protein in garlic bulbs was determined. 1 g of peeled garlic cloves had been crushed in a mortar and pestle with ten milliliters of phosphate buffer, and the extract was centrifuged at 20,000 g for 15 min to get the extract. Alkaline copper solution was added to the supernatant solution, which had been divided into batches of 0.1 ml each, to increase the volume to 1 ml. After giving the mixture a little stir, it was left to sit at room temperature for 15 min. The test tube was then filled with 0.2 ml of Folin-Ciocalteu (FC) reagent and carefully mixed. For 30 min, the mixture was incubated at room temperature in the dark. Following incubation, the amount was diluted to 5 ml, and the absorbance at 660 nm was recorded. The protein content was subsequently calculated as a percentage (Lowry *et al.,* 1951).

2.9 Total phenol

The Folin-Ciocalteu (FC) reagent was used to calculate the concentration of total phenol. Garlic cloves that had been peeled were crushed and the liquid within was extracted using 80% methanol. 100 l of FC reagent (0.25 N) was added to each test tube containing 20 l of garlic extract and the mixture was thoroughly combined using a vortex mixer. The mixture was subsequently provided with a 300 l addition of sodium carbonate solution and mixing was maintained. The combination was then kept for 2 h at room temperature with no light. A UV-visible spectrophotometer was used to detect the absorbance at 765 nm. Standard solutions with concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg ml^{-1} were used to create standard curves for gallic acid and the findings were represented in mg of gallic acid equivalent (GAE) kg^{-1} of fresh garlic (Yoo *et al.,* 2012).

3. Results

3.1 Total soluble solids (°Brix)

Total soluble solids are one of the most important traits of garlic and these are influenced by various nutrients and soil characteristics. An inquisition of Table 2 clearly shows that total soluble solids are influenced by different treatments. The highest TSS (36.30 °Brix) was recorded in the treatment T_{13} (75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) which was followed by the treatment T_{10}, *i.e.,* 100% RDN + S @ 50 kg/ha (35.46 °Brix), whereas the lowest TSS (31.12 °Brix) was recorded under the treatment T_1.

3.2 Dry matter content (%)

The dry matter content of the bulbs is one of the most important parameter. It is directly related to yield. Dry matter refers to material remaining after removal of water and includes carbohydrates, fats, proteins, vitamins, minerals and antioxidants. The results revealed that the highest (44.84 %) dry matter content was recorded in T_{13} which was statistically at par with the treatment T_{10}.

3.3 Oleoresin content (%)

Oleoresin is a highly concentrated product that contains all of the flavoring ingredients that are soluble in the solvent. The amount of oleoresin in a variety determines its quality and market value. Maximum oleoresin content (1.68 %) in T_{13} (75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) was statistically at par with the treatment T_{10}, *i.e.* (100% RDN + S @ 50 kg/ha (1.61%).

3.4 Sulphur content (%)

The maximum sulphur content of bulb (1.64%) was recorded in the treatment T_{10} (100% RDN + S @ 50 kg/ha) which was followed by the treatment T_9 (100% RDN + S @ 40 kg/ha).

3.5 Zinc content (mg/100 g)

Maximum zinc content in bulb (0.37 mg/100 g) was recorded in the treatment T_6 (100% RDN + Zn @ 7.5 kg/ha) which was statistically at par with the treatment T_8, *i.e.* (75% RDN + Zn @ 7.5 kg/ha + 5% Jeevamrit @ 1 l/m^2 (0.36 mg/100 g).

3.6 Allicin content (mg/kg)

An introspection of data in Table 3 showed that the maximum allicin content (4.87 mg/kg) was observed in T_{13} (75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) which was followed by the treatment T_{10} (100% RDN + S @ 50 kg/ha).

3.7 Total protein content (%)

An appraisal of data in Table 3 revealed that highest protein content (1.48%) was recorded in T_{13} (75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) which was statistically at par with the treatment T_{10} (100% RDN + S @ 50 kg/ha).

3.8 Pyruvic acid (µmole g^{-1})

A cursory glance of Table 3 revealed that pyruvic acid content was maximum (39.21µmole g^{-1}) in T_{13} (75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) which was followed by the treatment T_{10}, *i.e.* (100% RDN + S @ 50 kg/ha).

3.9 Total phenol (mg GAE/kg)

A perusal of the data presented in Table 3 showed that significant differences were observed for total phenol. Maximum total phenol content in bulb (765 mg GAE/kg) was recorded in the treatment T_{13} (75% RDN + Zn @ 5kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) which was significantly at par with the treatment T_9, *i.e.* (100% RDN + S @ 40 kg/ha (0.33 mg/100 g), T_{10} 100% RDN + S @ 50 kg/ha T_{11} 75% RDN + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2 and T_{12} (75% RDN + S @ 50 kg/ha + 5% Jeevamrit @ 1 l/m^2). While the minimum total phenol was recorded under the treatment T_1.

3.10 Allylmethyl thiosulfinate (AMThs mg/kg) content

The maximum AMThs content of bulb (4.91 mg/kg) was recorded in the treatment T_{13} (75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2) which was followed by the treatment T_{10} (100% RDN + S @ 50 kg/ha). Minimum AMThs content of bulb (3.10 mg/100 g) was recorded in T_1.

Table 2: Effect of integrated nutrient management on total soluble solids, dry matter content, oleoresin content, sulphur content and zinc content

Treatment code	Treatment details	TSS (°B)	Dry matter content (%)	Oleoresin content (%)	Sulphur content (%)	Zinc content (mg/100 g)
T_1	Absolute Control	31.12	36.10	1.02	1.09	0.10
T_2	100% RDN (125:75:60 kg per hectare of NPK)	33.58	37.68	1.15	1.20	0.15
T_3	90% RDN + 10% RDN through Vermicompost	33.84	38.02	1.17	1.22	0.19
T_4	80% RDN + 20% RDN through Vermicompost	33.66	38.72	1.23	1.25	0.18
T_5	100% RDN + Zn @ 5 kg/ha	34.12	40.86	1.30	1.29	0.33
T_6	100% RDN + Zn @ 7.5kg/ha	34.24	41.76	1.36	1.32	0.37
T_7	75% RDN + Zn @ 5 kg/ha + 5% Jeevamrit @ 1 l/m^2	34.01	39.23	1.20	1.24	0.27
T_8	75% RDN + Zn @ 7.5 kg/ha + 5% Jeevamrit @ 1 l/m^2	34.03	40.03	1.21	1.26	0.36
T_9	100% RDN + S @ 40 kg/ha	35.28	43.26	1.50	1.57	0.19
T_{10}	100% RDN + S @ 50 kg/ha	35.46	43.88	1.61	1.64	0.21
T_{11}	75% RDN + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2	35.02	42.26	1.39	1.40	0.17
T_{12}	75% RDN + S @ 50 kg/ha + 5% Jeevamrit @ 1 l/m^2	35.13	42.75	1.42	1.48	0.18
T_{13}	75% RDN + Zn @ 5 kg/ha + S @ 40kg/ha + 5% Jeevamrit @ 1 l/m^2	36.30	44.84	1.68	1.37	0.34
CD		**0.55***	**1.53***	**0.05***	**0.05***	**0.01***

* Significant at 5 % level

Table 3: Effect of integrated nutrient management on Allicin content, total protein content, pyruvic acid content, total phenol content and allyl methyl thiosulfinate (AMThs) content

Treatment code	Treatment details	Allicin content (mg/kg)	Total protein (%)	Pyruvic acid (µmole g^{-1})	Total phenol (mg GAE /kg)	AMThs (mg/kg)
T_1	Absolute Control	3.16	0.82	20.21	507	3.10
T_2	100% RDN (125:75:60 kg per hectare of NPK)	3.34	0.90	20.27	525	3.32
T_3	90% RDN + 10% RDN through Vermicompost	3.61	1.11	22.50	594	3.39
T_4	80% RDN + 20% RDN through Vermicompost	3.69	1.15	22.91	604	4.12
T_5	100% RDN + Zn @ 5 kg/ha	3.81	1.21	26.23	651	4.20
T_6	100% RDN + Zn @ 7.5 kg/ha	3.85	1.24	27.14	693	4.25
T_7	75% RDN + Zn @ 5 kg/ha + 5% Jeevamrit @ 1 l/m^2	3.71	1.34	28.17	708	4.36
T_8	75% RDN + Zn @ 7.5 kg/ha + 5% Jeevamrit @ 1 l/m^2	3.74	1.37	29.07	715	4.41
T_9	100% RDN + S @ 40 kg/ha	4.45	1.41	33.14	749	4.59
T_{10}	100% RDN + S @ 50 kg/ha	4.62	1.44	35.67	760	4.62
T_{11}	75% RDN + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2	4.51	1.40	31.12	731	4.50
T_{12}	75% RDN + S @ 50 kg/ha + 5% Jeevamrit @ 1 l/m^2	4.58	1.42	32.45	738	4.54
T_{13}	75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2	4.87	1.48	39.21	765	4.91
CD		**0.16***	**0.04***	**1.41***	**36.72***	**0.14***

*** Significant at 5 % level**

4. Discussion

Enhancing crop quality, including vegetables and other crops, may need better control of soil inputs such as potassium, phosphorus and nitrogen (Nai-hua *et al.,* 1998). An effective and well-balanced application of organic and inorganic components can result in high-yield, high-quality garlic. Because prolonged and intensive agriculture without proper fertilizer management would cause soil health and productivity to decline, the need for an integrated supply of nutrients to maintain productivity is thus emphasized.

Improved garlic quality was shown to be mostly dependent on applying micronutrients like zinc and B combined with the prescribed fertilizer dosage (Gorana, 2015). Sulphur is essential for plant growth and development. It contributes to the production of several amino acids, including methionine, cystine and cysteine (Singh *et al.,* 2018; Chattoo *et al.,* 2018). It is also responsible for giving garlic its distinctive flavor and aroma, which includes mustard and onion (Tisdale *et al.,* 1985). Significant increases in bulb results, TSS, volatile oil content and sulphur content were observed when 50 kg of sulphur was applied individually to garlic bulbs. Higher amounts of chlorophyll, photosynthesis and protein content in crop plants all of which are fouund in garlic bulbs as well as the role that sulphur plays in enhancing nutrient absorption by root systems, might all contribute to an increase in the TSS content of the bulb (Patidar *et al.,* 2017).

The combining sulphur with NPK increased the bulb dry matter content significantly. This may be due to the role of sulphur in improving amino acids, as well as the fact that nutrient uptake increases dry matter accumulation in the bulb (Damse *et al.,* 2014; Anand *et al,*. 2017). The application of nitrogen and phosphorus @ 92 kg N ha^{-1} + 40 kg P ha^{-1}, as well as 30 and 60 kg S ha^{-1}, resulted in significantly higher bulb dry matter content in garlic (Diriba *et al.,* 2013). Sulphur had a significant effect on dry matter content, which increased with increasing sulphur levels. This could be due to increased photosynthesis and the accumulation of more dry matter as a result of maximum vegetative growth (Zaman *et al.,* 2011).

The use of organic amendments, which showed increasing trends in volatile and fatty oil content, could be attributed to garlic's significant increase in oleoresin content (Gowda *et al.,* 2007; Hari *et al.,*2009; Singh *et al.,* 2012). According to Sidhu and Sekhon (2000), the physico-chemical and biological characteristics of soil, which permit roots to proliferate more, have a direct relationship with the improvement in quality attributes as a result of different fertilizer treatments. This leads to better uptake and utilization of nutrients necessary to improve crop quality.

A sufficient supply of nutrients, especially in the crop root zone, may have enhanced the chemical and biological characteristics of the soil, facilitated the proliferation of plant roots, and improved the crop's ability to utilize nutrients, as evidenced by the bulb's higher sulphur content (Kumar and Kamboj, 2019).

Using micronutrients like zinc or boron can lead to increased vegetative growth, yield, and quality parameters because these are the most vital nutrients for cell division, the metabolism of nitrogen and carbohydrates and the relationship between water and growth in plants (Thakur and Kumar, 2021).

A rise in AMThs concentration and allicin and other thiosulfinates might be attributed to S's role in the synthesis of non-protein S amino acids called S alk(en)yl cysteine sulfoxides. The current research's observation of a positive correlation between S dose and allicin, AMThs and ATPThs concentrations in garlic bulbs clarifies the role of S in the synthesis of S-alk (en)yl cysteine sulfoxides. There was a significant correlation between the S dose and the alliin content of onion and garlic leaves (Bloem *et al.,* 2005).

In this present study, S fertilization led to a rise in pyruvic acid content, a valid indication of pungency and taste intensity. The positive correlation between pyruvic acid concentration and S dose observed in this study provides illumination on the increased pyruvic acid concentration associated with S fertilization. In a prior investigation on onions, pyruvic acid and S dosage were found to have a similar beneficial connection (Thangasamy *et al.,* 2013).

The present study's rise in total phenol content at high S dosages may have been caused by the suppression of polyphenol oxidase, a phenolic enzyme and peroxidase activity (Vallejo *et al.,* 2003). Additionally, it was noted that after applying S to mustard leaves, the total phenol content and antioxidant activity increased (Shamloo *et al.,* 2017; Li *et al.* 2008). The elevated levels of total phenolic acids and antioxidant activity, as well as the lower yield, may be connected to the plant's defense system against temperature stress. The application of S increases the level of these beneficial chemicals, which not only helps the plant withstand challenges like high temperatures but also enhances the nutritional value of the garlic.

In contrast to yield, increasing sulfur resulted in an increase in total protein concentration. S is found in proteins and other biomolecules (Atmaca, 2004). It is also integrated into proteins as the redox-active cysteine residue and in essential antioxidant molecules such as glutathione, thioredoxin, and glutaredoxin (Mukwevho *et al.,* 2014). Consequently, it is anticipated that S will enhance protein and antioxidant activity.

5. Conclusion

According to the results of the study, the treatment of 75% RDN + Zn @ 5 kg/ha + S @ 40 kg/ha + 5% Jeevamrit @ 1 l/m^2 showed the most positive impact on various parameters, including TSS, dry matter, oleoresin, allicin, total protein, pyruvic acid, total phenol and allyl methyl thiosulfinate (AMThs) content in garlic. For increasing the quality of garlic, the integrated strategy proved much more advantageous than pure organic treatments. Our research may shed light on the relevance of combining organic manure with synthetic fertilizer for increasing garlic crop yield and quality.

Conflict of interest

No potential conflict of interest was reported by the author(s)

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