RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (3) 1308-1315, 2024
http://doi.org/10.54910/sabrao2024.56.3.38
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MINERAL AND NAN0-SULFUR FERTILIZERS EFFECTS ON THE MORPHOLOGICAL AND BIOCHEMICAL COMPONENTS 0F THE GINGER (*ZINGIBER OFFICINALE* L.)

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SUMMARY

An experiment on ginger (*Zingiber officinale* L.) commenced in 2020 using the plant growth incubator of the Laboratories, Department of Biological Sciences, College of Sciences, University of Wasit, Iraq. The study aimed to investigate the effects of mineral sulfur fertilizer at concentrations of 0, 2, 4, 6, and 8 ml/l, and nano-sulfur with concentrations of 0, 1, 2, 3, and 4 ml/l on the plant height, the number of leaves, leaf content of total chlorophyll, leaf area, the tannin of plant, phytic acid, and essential oil of the ginger rhizome. It was evident that a single use of the previously mentioned variables significantly impacted ginger's vegetative characteristics and chemical composition, particularly at high concentrations. Using the two-way interactions obtained distinct results. The treatment design was a factorial experiment (5 m × 5 m × 3 m) in a completely randomized design with three replicates. The study revealed significant enhancement in all attributes, whether the morphological and biochemical content achieved the highest mineral sulfur and nano-sulfur fertilizer concentrations alone, or in combinations.

Keywords: Ginger (*Zingiber officinale* L.), mineral sulfur and nano-sulfur fertilizers, morphological traits, biochemical components, volatile oil

Key findings: In the ginger (*Z. officinale* L.) plants, adding nano-sulfur fertilizer had a superior effect when in the nano state than in the metal ion sulfur and significantly improved the morphological and biochemical components.

Communicating Editor: Dr. Samrin Gul

Manuscript received: October 13, 2023; Accepted: December 19, 2023.

Citation: Al-Shammeryi WHM (2024). Mineral and nan0-sulfur fertilizers effects on the morphological and biochemical components 0f the ginger (*zingiber officinale* L.). *SABRAO J. Breed. Genet.* 56(3): 1308-1315. http://doi.org/10.54910/sabrao2024.56.3.38.

INTRODUCTION

The Zingiberaceae family contains approximately 53 genera and over 1200 species. Zingiberaceae is widespread throughout southern and southeastern Asia, including plants such as ginger (*Zingiber officinale* L.), *Zingiber zerumbet* (bitter ginger), and *Curcuma longa* (turmeric), which are frequent ingredients in herbal medicine. Some members of the Zingiberaceae family are currently the subject of intense research interest due to their widespread use as spices and herbal ingredients in traditional medicine. *Zingiber officinale* is one of the most well-known members of the Zingiberaceae family (Ali *et al*., 2019; Styawan *et al*., 2022).

Red ginger (*Zingiber officinale* var. rubrum) is one of the extensively used rhizome-based medicinal plants. The rhizome of the red ginger plant contains numerous beneficial essential oils. Red ginger has the maximum essential oil concentration compared with elephant ginger and empiit ginger (Pertiwi *et al*., 2013; Setiawan *et al*., 2022). Ginger is a 2-to-4-feet-tall perennial with grass-like leaves up to one foot long. The underground root or rhizome serves medicinal and culinary purposes (Zadeh and Kor, 2014).

The subterranean pungent and aromatic rhizome, serving culinary purposes, is an economic component. Ginger is an ingredient in gingerbread, cookies, cakes, puddings, stews, and relishes. Ginger often trades in three fundamental forms: green (fresh), pickled or preserved, and dried. Only dried ginger (whole, peeling, or sliced) is a known spice; green or fresh ginger comes primarily as a vegetable, and pickled or preserved ginger has become a prime trade for Chinese and Japanese food. Moreover, ginger oil and oleoresins become exchangeable. Although several countries produce ginger, India and China are the only significant exporters of powdered ginger (Kandiannan *et al*., 1996), followed by Nigeria, Sierra Leone, Australia, Fiji, Bangladesh, Jamaica, Nepal, and Indonesia.

Sulfur is an element required by all living organisms, including plants. Methionine, cysteine, glutathione, vitamins (biotin and thiamine), phytochelatins, chlorophyll, coenzyme A, and S-adenosylmethionine contain sulfur. Sulfur is also a component of several secondary metabolites (SMs) and is essential for the plant's physiological functions, growth, and development. The sulfur requirements of plants vary by species and developmental stage (Narayan *et al*., 2012). Liquid Sulfur is an essential plant nutrient. Previously considered a secondary nutrient, it now prevails as the 'fourth macronutrient' alongside nitrogen, phosphorus, and potassium. Green methods for synthesizing nanoparticles with plant extracts are superior to physical, chemical, and microbial methods due to their simplicity, portability, environmental friendliness, and reduced reaction time (Mayegowda *et al.,*2023).

Sulfur has numerous applications in various agricultural fields. Utilized in the cultivation of grapes, vegetables, strawberries, and numerous other cultivated plants; however, sulfur can be regarded as a pesticide with a high level of effectiveness in agriculture, where it is effective against a variety of powdery mildew maladies and black spot due to their unique quantum size properties and large surface areas, thus, sulfur nanoparticles have many advantages over micro-sulfur (Salem *et al*., 2016a). Given the significance of nanocomposites in agriculture, in general, and medicinal plants, in particular, a nanosulfur substance attained assessment for its influence on vegetative growth and the active chemicals in ginger plants.

MATERIALS AND METHODS

The presented experiment materialized in 2020 in the plant growth incubator of the Department of Biology Sciences, College of Sciences, University of Wasit, Iraq. According to ISTA (1985), a typical plant growth incubator (model LGC5101 of Korean origin) enclosed seven, with a temperature of 25 °C ± 2 °C, relative humidity of 70%, and illumination of 12 h per 24 h. The randomized complete block design (RCBD) with factorial arrangement had three replications. The seeds were grown in containers divided into compartments and filled with peat moss (Van Egmond), which contains a high concentration of essential elements, pH of 6.5, and electrical conductivity between 1.3 and 1.5.

With the experimental blocks split into three, each block contained 15 units, totaling 75 experiment units, each holding 10 seeds. The ginger plant incurred spraying with sulfur fertilizer ***(***Afesol ***)***AL-Sanabel Company at 0, 2, 4, 6, and 8 ml/l concentrations and nanosulfur at 0, 1, 2, 3, and 4 ml/l concentrations, SPAD-502 (KMS, Inc., Osaka, Japan). After 14 days of sowing, spraying treatment ensued on studied characters: plant height, number of leaves, chlorophyll content of the leaves, and leaf area (Laing *et al*., 1973); determination of tannin contents (Cabral *et al*., 2010), phytic acid in leaves (Alkarawi and Zotz, 2013), and essential oil of ginger rhizome (A.O.A.C.1980). Sulfur nanoparticle preparation proceeded according to Salem *et al*. (2016b).

Statistical analysis

Data recording and statistical analysis employed the analysis variance, and comparing the differences among the treatment means used the least significant differences (LSD) test at a 0.05 probability level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Plant height

As shown in Table 1, sulfur fertilizer significantly affected the plant height. An increase in sulfur fertilizer concentration from 2 to 8 mL/L also increased the plant's mean height from 27.33 to 35.80 cm. The highest plant height was prominent in plants sprayed with 8 mL/L of sulfur fertilizer. The nanosulfur also substantially impacted the plant height. Similarly, the mean of plant height increased gradually as the concentration of nanosulfur increased. The 4 ml/L of nanosulfur gave the tallest plants (40.40 cm) compared with the 1 ml/L (29.35 cm) and the control (25.27 cm), respectively. The interaction between sulfur fertilizer and nanosulfur appeared significant (Table 1). The tallest plants were those sprayed with 6 mL/L of nanosulfur and 4 mL/L of sulfur fertilizer.

Table 1. Effect of sulfur fertilizer and nano sulfur and their interactions on plant height.

Nano sulfur	Sulfur fertilizer					Means (cm)
	0	2	4	6	8	
0	19.67	23.33	24.33	28.33	30.67	25.27
1	24.00	28.00	31.33	32.33	32.00	29.53
2	25.67	30.00	35.00	27.00	32.67	30.07
3	34.33	38.67	39.00	32.67	40.33	37.00
4	33.00	38.33	43.33	44.00	43.33	40.40
Means (cm)	27.33	31.67	34.60	32.87	35.80	

$LSD_{0.05}$ Sulfur fertilizer = 1.084, Nano Sulfur = 1.084, Interaction = 2.423

Table 2. Effect of sulfur fertilizer and nano sulfur and their interactions on the number of leaves.

Nano sulfur	Sulfur fertilizer					Means (#)
	0	2	4	6	8	
0	20.00	21.00	24.00	26.00	28.67	23.93
1	23.67	25.00	25.67	28.67	30.33	26.67
2	26.00	28.00	33.00	34.33	37.33	31.73
3	30.00	32.67	35.67	42.00	46.33	37.33
4	30.67	32.33	38.00	44.00	48.33	38.67
Means (#)	20.07	27.80	31.27	35.00	38.20	

$LSD_{0.05}$ Sulfur fertilizer = 0.759, Nano Sulfur = 0.759, Interaction = 1.698

Leaves per plant

The results indicated that sulfur fertilizer considerably impacted the number of leaves (Table 2). An increase in sulfur fertilizer concentration from 2 mL/L to 8 mL/L caused the number of leaves to increase consistently from 20.07 to 38.20. The plants sprayed with 8 mL/L of sulfur fertilizer produced more leaves. The application of nanosulfur exhibited a significant impact on the number of leaves. The concentration of nanosulfur gradually increased the number of leaves. In comparison to the 1 ml/L (26.67) and the control (23.93), the 3 ml/L (37.33) of nanosulfur produced more leaves. The interaction between sulfur fertilizer and nanosulfur was significant (Table 2). A plant sprayed with 8 mL/L nanosulfur and 4 mL/L sulfur fertilizer produced more leaves.

Chlorophyll content

The findings suggest that applying sulfur fertilizer had a notable effect on the mean chlorophyll content, as presented in Table 3. The augmentation of sulfur fertilizer concentration from 2 to 8 mL/L resulted in a rise in the mean chlorophyll content of leaves from 34.90 to 43.26. Plants sprayed with 8 mL/L of sulfur fertilizer contained the highest levels of total chlorophyll in their leaves. The nanosulfur significantly affected the average chlorophyll content of leaves. Similarly, the increase in nanosulfur led to a steady rise in the average chlorophyll concentration of leaves. Comparing the 1 ml/L (33.62) and the control (29.38), the 3 ml/L (44.44) of nanosulfur produced the leaves' maximum total chlorophyll content. The interaction effect between the sulfur fertilizer and nanosulfur on the average chlorophyll content of leaves was significant (Table 3). The maximum leaf content of total chlorophyll emerged in plants sprayed with 8 mL/L nanosulfur and 4 ml/L sulfur fertilizer.

Leaf area

The leaf area increased regularly from 21.49 to 33.31 cm^2 when raising the sulfur fertilizer concentration from 2 to 8 mL/L. Plant spraying with 8 mL/L of sulfur fertilizer resulted in the broadest leaf area. The nanosulfur significantly influenced the leaf area. Similarly, the increase in nanosulfur led to a gradual growth in the leaf area. Comparing 3 ml/L (35.09 cm^2) of

Table 3. Effect of sulfur fertilizer and nano sulfur and their interactions on the total chlorophyll content of leaves.

Nano sulfur	Sulfur fertilizer					Means (mg/g)
	0	2	4	6	8	
0	22.10	26.31	29.52	33.56	35.42	29.38
1	26.62	29.45	34.63	38.25	39.18	33.62
2	32.00	36.44	39.45	41.37	42.26	38.30
3	36.93	42.17	46.15	48.34	48.59	44.44
4	37.44	40.14	42.25	43.14	50.85	42.76
Means (mg/g)	31.02	34.90	38.40	40.93	43.26	

$LSD_{0.05}$ Sulfur fertilizer = 0.891, Nano Sulfur = 0.891, Interaction = 1.99

Table 4. Effect of sulfur fertilizer and nano sulfur and their interactions on the leaf area.

Nano sulfur	Sulfur fertilizer					Means (cm^2)
	0	2	4	6	8	
0	15.53	16.73	18.80	21.30	22.14	18.90
1	19.92	22.52	25.96	28.77	29.10	25.26
2	19.62	26.22	29.03	32.62	32.12	27.92
3	29.59	32.89	35.55	37.71	39.70	35.09
4	22.77	28.89	31.75	37.66	43.47	32.91
Means (cm^2)	21.49	25.45	28.22	31.61	33.31	

$LSD_{0.05}$ Sulfur fertilizer = 1.158, Nano Sulfur = 1.158, Interaction = 2.590

Table 5. Effect of sulfur fertilizer and nano sulfur and their interactions on the tannin content of leaf.

Nano sulfur	Sulfur fertilizer					Means (%)
	0	2	4	6	8	
0	0.1367	0.1733	0.2033	0.2067	0.2200	0.1880
1	0.1967	0.2233	0.2433	0.2367	0.2600	0.2320
2	0.2133	0.2300	0.2200	0.2633	0.2767	0.2407
3	0.2167	0.2500	0.2700	0.3067	0.3333	0.2753
4	0.2600	0.2967	0.3300	0.3700	0.3700	0.3253
Means (%)	0.2047	0.2347	0.2533	0.2767	0.2920	

$LSD_{0.05}$ Sulfur fertilizer = 0.00891, Nano Sulfur = 0.00891, Interaction = 0.01993

nanosulfur with the 1 ml/L (25.26 cm^2) and the control (18.90 cm2) showed the nanosulfur producing the largest leaf area. The interaction effect between the sulfur fertilizer and nanosulfur was significant (Table 4). The plant sprayed with 8 mL/L nanosulfur and 4 mL/L sulfur fertilizer had the supreme leaf area.

Leaf tannin content

The results indicated that sulfur fertilizer substantially impacted the leaves' tannin content (Table 5). When boosting sulfur fertilizer concentrations from 2 mL/L to 8 mL/L, the leaf tannin content increased sustainably from 0.2347% to 0.2920%. Plants sprayed with 8 mL/L of sulfur fertilizer had the maximum tannin concentration in their leaves. The nanosulfur had a significant impact on the tannin content of leaves. Similarly, the leaves' tannin content rose steadily as the level of nanosulfur increased. Maximum tannin content was notable using the 4 ml/L (0.3253%) nanosulfur compared with the 1 ml/L (0.2320%) and the control (0.1880%). The interaction effect of sulfur fertilizer and nanosulfur on the leaves' tannin content was significant (Table 5). The crop sprayed with 6.8 mL/L nanosulfur and 4 mL/L sulfur fertilizer provided the highest tannin content.

Leaf phytic acid

The outcomes showed that sulfur fertilizer significantly affected the phytic acid content (Table 6). Raising sulfur fertilizer concentrations from 2 mL/L to 8 mL/L enhanced the phytic acid concentration consistently from 27.83% to 33.77%. Plants sprayed with 8 mL/L of sulfur fertilizer contained the maximum levels of phytic acid. The nanosulfur had a significant impact on the phytic acid concentration. Similarly, increasing nanosulfur caused the phytic acid content to rise consistently. Maximum phytic acid was evident at 4 ml/L concentrations of nanosulfur compared with the 1 ml/L concentrations (27.21%) and the control (22.58%). The interaction between sulfur fertilizer and nanosulfur significantly affected the phytic acid concentration (Table 6). Plants sprayed with 8 mL/L nanosulfur and 4 mL/L sulfur fertilizer comprised the highest levels of phytic acid.

Essential oil

The results showed that sulfur fertilizer remarkably impacted the essential oil content in the ginger rhizome (Table 7). Increasing sulfur fertilizer concentration from 2 to 8 mL/L boosted the phytic acid concentration consistently, with the essential oil of ginger rhizome rising from 2.267% to 3.427%. The maximum levels of ginger rhizome essential oil were notable in plants sprayed with 8 mL/L of sulfur fertilizer. The nanosulfur also significantly affected the ginger rhizome essential oil. The amount of ginger rhizome essential oil increased when increasing the nanosulfur concentration. Maximum ginger rhizome essential oil appeared with the 4 ml/L (3.940%) nanosulfur compared with the 1 ml/L (2.053%) and the control (1.127%). The interaction effect between the sulfur fertilizer and nanosulfur fertilizer was significant (Table 7). Plants sprayed with 8 mL/L nanosulfur fertilizer and 4 mL/L sulfur fertilizer resulted in the highest levels of ginger rhizome essential oil.

Table 6. Effect of sulfur fertilizer and nano sulfur and their interactions on the phytic acid.

Nano sulfur	Sulfur fertilizer					Means (%)
	0	2	4	6	8	
0	20.72	21.63	22.22	23.67	24.34	22.51
1	22.85	25.51	25.84	29.73	32.10	27.21
2	24.84	34.88	34.57	37.07	38.33	33.94
3	25.70	27.36	28.88	30.74	34.63	29.46
4	27.18	29.77	34.73	35.77	39.43	33.38
Means (%)	24.26	27.83	29.25	31.40	33.77	

$LSD_{0.05}$ Sulfur fertilizer = 1.138, Nano Sulfur = 1.138, Interaction = 2.544

Table 7. Effect of sulfur fertilizer and nano sulfur and their interactions on the essential oil of ginger rhizome.

Nano sulfur	Sulfur fertilizer					Means (%)
	0	2	4	6	8	
0	0.467	0.833	1.233	1.500	1.600	1.127
1	1.367	1.700	1.967	2.633	2.600	2.053
2	1.633	2.467	2.867	3.667	3.500	2.827
3	1.933	3.067	3.300	3.700	4.433	3.287
4	2.467	3.267	4.267	4.700	5.000	3.940
Means (%)	1.573	2.267	2.727	3.240	3.427	

$LSD_{0.05}$ Sulfur fertilizer = 0.2292, Nano Sulfur = 0.2292, Interaction = 0.5125

The effects of sulfur fertilizer on the vegetative properties of the plant incurred positive influences (Tables 1, 2, 3, and 4). As an essential plant growth index, leaf area determines the plant's ability to absorb solar energy, ultimately affects plant growth, development, and yield (Jarallah and Abbas, 2019; Chowdhury *et al*., 2020). The application of sulfur fertilizer modifies the physicochemical properties of the plants, increasing the availability of nutrients and promoting plant growth and development. It, in turn, increases the translocation of nutrients to the reproductive organs and positively affects photosynthesis, which can substantially increase yield and yield components (Aspel *et al*., 2021; Gowacka *et al*., 2023). The effects of sulfur fertilizer on the plant’s active compounds, lipids, and mineral nutrients are available in Tables 5, 6, and 7. Crucial plant metabolites contain sulfur, i.e., iron-S clusters, amino acids, lipids and polysaccharides, disulfides, peptides, co-factors, glucosinolates, and hormone precursors. Metabolites containing sulfur govern various physiological and biochemical processes in plants. In addition, it is a fact that the foliar application of sulfur affects the growth, productivity, and quality parameters of oilseed crops (Abido, 2018; Shah *et al*., 2022).

The results revealed that nanosulfur had a positive impact on the vegetative properties of the plant (Tables 1, 2, 3, and 4), with the same also reported in past studies (Salem *et al*., 2016a; Burkitbayev *et al*., 2021). The results demonstrated that sulfur release from nanosulfur reached protraction for 42 days, whereas sulfur released from conventional sulfur fertilizer (gypsum) terminated after 35 days. Compared with gypsum-fertilized plants, nanosulfur-fertilized plants had substantially higher dry matter production (11%–12%), seed yield (15%), and oil content (14.7%). (Subramanian *et al*., 2022). According to the data, using nanosulfur has proven to enhance the available sulfur in soil. It may be due to the maximum availability of sulfur in the soil from applying SMNZ-based sulfur fertilizer, which increases crop absorption. Given the gradual and consistent discharge of sulfur, the crop absorbed more sulfur even after 816 h, whereas the conventional sulfur reached depletion within 384 h. It indicates that surface-modified nanozeolite-based sulfur fertilizer may be advantageous to modulate the efficient release of nutrients commensurate with crop needs (Thirunavukkarasu *et al*., 2018). Plants treated with sulfur-containing nanofertilizers gave the same outcome (Tables 5, 6, and 7). There was a substantial increase in the production of efficacious compounds (Abyaneh and Varkeshi, 2014).

CONCLUSIONS

A significant increase was evident in all attributes, including morphological characteristics and biochemical content, with the highest mineral sulfur and nanosulfur fertilizer concentrations alone or in combination.

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