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Yield and Quality Attributes of Nasturtium as Affected by Nitrogen and Sulfur Supply

R. M. Sabry[1*], A. A. A. Elsayed[1], and S. S. Ahmed[1]

ABSTRACT

Two field experiments were conducted during two successive seasons to evaluate growth and glucotropaeolin content of nasturtium (*Tropaeolum majus*) under different levels of nitrogen and sulur supply. Three nitrogen rates (0, 60 and 120 kg N ha^{-1}) as ammonium nitrate and three sulfur rates (0, 50 and 100 kg S ha^{-1}) as elemental sulfur were used. Results indicated that nitrogen and sulfur supply increased biomass accumulation of *T. majus* including plant height, branches number, plant fresh weight, leaf fresh and dry weights, and stem fresh and dry weights. The highest values of growth traits were recorded with the highest nitrogen rate (120 kg ha^{-1}) and the medium or the highest sulfur rate (50 and 100 kg S ha^{-1}) at all growth stages in both seasons. Glucotropaeolin content ranged from 23.45 to 45.26 µmol g^{-1} DW in leaves and from 117.92 to 156.31 µmol g^{-1} DW in seeds. The response of glucotropaeolin content to nitrogen supply varied between plant parts and growth stages. Glucotropaeolin content in seeds was unaffected by nitrogen supply. The highest glucotropaeolin content in leaves was found in plants that received 120 kg N ha^{-1}. Glucotropaeolin content in leaves and seeds significantly increased with sulfur supply in both seasons at all growth stages.

Keywords: Benzyl glucosinolate, Glucotropaeolin content, Medicinal plant, *Tropaeolum majus*.

INTRODUCTION

Nasturtium (*T. majus*) is an annual herbaceous herb that belongs to tropaeolaceae family and native to the Andes in South America, from Bolivia to Colombia. It is used as a medicinal, edible and ornamental plant. The plant contains significant amounts of one type of glucosinolate called glucotropaeolin or benzyl glucosinolate, which accumulates in mature plants (Kleinwächter *et al*., 2008). Nasturtium is a source of natural coloring that can be very important in organic food processing. Besides, it can be used as food ingredient or in culinary and salads for seasoning (Das *et al*., 2019). Nasturtium possess a great medicinal importance worldwide because it has anti-spasmodic, antineoplastic, hepatoprotective, anti-inflammatory, anti-scorbutic, digestive, laxative and dermatologic activities (Chevallier and Dorling, 1996; Butnariu and Bostan, 2011; Bazylko *et al*., 2013). Nasturtium also exhibited antiseptic, stimulant, demulcent, antibiotic, antifungal, aperient, expectorant, antimycotic, antioxidant, antimicrobial, and antibacterial properties (Matallana et al., 2006; Tiwari et al., 2014; Jakubczyk et al., 2018; Valsalam et al., 2019). T. majus extract may be a potential therapeutic agent for preventing and treating obesity (Kim et al., 2017), curing cancer and sore throat (Pintão et al., 1995; Das et al., 2019). Recent studies have shown that its seeds and leaves contain fatty acids, flavonoids and phenolics including anthocyanin and ascorbic acid (Jakubczyk *et al*., 2018). Higher content of nitrogen and sulfur-containing compounds accounting for 86.4% of the volatile fraction composition of

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[1] Medicinal and Aromatic Plants Research Department, National Research Center, Dokki, Cairo, Egypt.
*Corresponding author; e-mail: rehamsabry2000@hotmail.com

essential oil from *T. majus* aerial parts were quantified (Benyelles *et al*., 2015). Adequate level of mineral nutrients is crucial for optimum plant growth and productivity. Nitrogen is, by far, the most needed mineral nutrients in agroecosystems mainly due to its dynamic in the soil profile (Souri *et al*., 2018; Souri and Dehnavard, 2017; Hatamian *et al*., 2020). Among essential plant nutrients, Nitrogen (N) and Sulfur (S) are known to be essential elements for growth, development, and various physiological functions in plants, especially those that require higher amounts of S and N for optimum growth and yield. Glucosinolate-containing species need strategies for N and S fertilization more than other non-glucosinolate species, since glucosinolates contain both N and S in their constituents (Rosen *et al*., 2005; Aires *et al*., 2006; Omirou *et al*., 2009). The few studies dealing with nasturtium focused on the N and S supply effect on glucotropaeolin content. It has been shown that benzyl glucosinolate content of *T. majus* was increased over 50-fold by fertilizing a particular cultivar with 8.3 mM sulfate (Matallana *et al*., 2006). Nitrogen fertilization had no consistent influence on the glucotropaeolin content while sulfur fertilization with 100 kg S ha^{-1} significantly enhanced the glucotropaeolin content in leaves and seeds (Bloem *et al*., 2007).

Overall, there is little information regarding the N and S requirements of nasturtium. Thus, we aimed to enhance or manipulate biomass and glucotropaeolin content of nasturtium plants by supplying different amounts of sulfur and nitrogen.

MATERIALS AND METHODS

Site Description and Soil Properties

Two field experiments were performed during 2018 and 2019 growing seasons at the Experimental Station of the Faculty of Agriculture, Cairo University, Egypt (30° 28′ 05″ N, 31° 22′ E), to study the effect of nitrogen and sulfur supply on biomass and glucotropaeolin content of nasturtium (*T. majus*).

The properties of the soil are shown in Table 1.

Layout and Design of the Experiment

Seeds of *T. majus* were planted in the field soil at the first week of November. All plots received a basic P dose as triple superphosphate (120 kg P_2O_5 ha^{-1}) and K (240 kg K_2O ha^{-1}) before planting. The experimental design was a randomized complete block with three replicates. Three nitrogen rates (0, 60 and 120 kg N ha^{-1}) as ammonium nitrate and three sulfur rates (0, 50 and 100 kg S ha^{-1}) as elemental sulfur were assigned randomly in plots. The fertilization was applied as split in two applications; half was before planting and the remaining was top-dressed after 6 weeks. Each plot consisted of four rows, 3 m long, 60 cm apart and the distance between plants in the row was 60 cm. Irrigation, plant protection, and weed control were carried out when necessary. The aerial parts (leaves, stems and flowers) were sampled at three stages: The vegetative stage (70 days after sowing), flowering stage (140 days after

Table 1. Physical and chemical analysis of the soil.

Clay (%)	Silt (%)	Sand (%)	OM (%)	CaCO3 (%)		EC (dS m^{-1})		pH	
35.5	28.8	36.7	1.5	2.7		1.7		7.8	
Available nutrients (ppm)				Soluble cations mmolc L^{-1}			Soluble anions mmolc L^{-1}		
N	P	K	Fe	Ca	Mg	Na	HCO_3	Cl	SO_4
5	10	100	14	6.5	2.3	6.2	4.0	4.6	7.0

sowing) and maturity stage (180 days after sowing). At each sample, plant height, number of branches, plant fresh weight, fresh and dry weights of leaves and stems were determined. Plants were cut at the ground level and separated into leaves and stems to determine their fresh weights. Leaf and stem samples were immediately deep-frozen (-20°C), then freeze-dried, and finely ground before determination of their dry weights. Glucotropaeolin content was determined in leaves and stems of the samples at the three stages, while in seeds only in the third stage. (Christ, BETA 1-8 LD plus, Osterode am, Harz, Germany).

Glucosinolates Analysis

HPLC determined glucotropaeolin content according to the EU official method (ISO 9167-1) for desulfoglucosinolates. Glucotropaeolin (Merck, Darmstadt, Germany) was used as the internal standard for quantification. Briefly, 0.3 g of fine powder freeze-dried material was extracted by adding 5 mL of 70% MeOH solvent and 50 µL of a 5 mM gluctropaeolin as internal standard. The extracts were centrifuged and the residue was re-extracted twice. The supernatants were combined and the extract volume was adjusted to 10 mL. The extract was loaded onto a small column containing DEAE-Sephadex A-25 (Sigma Aldrich, USA) and glucosinolate was desulfated by adding aryl sulfatase and incubated at room temperature overnight. Desulfoglucosinolates were analyzed with an HPLC unit using ZORBAX Eclipse Plus C18 column (4.6×150 mm, 5 µm PN 883952-702, Agilent Technologies, Inc., USA) with UV detection at 229 nm using a water/acetonitrile gradient (Doheny-Adams *et al*., 2017). The column temperature was maintained at 35°C with a flow rate of 1 mL min^{-1}. Sample injection volume of 30 µL. The mobile phase consisted of: (A) Water and (B) 20% (v/v) acetonitrile, and the gradient elution program was 1% B for 1 minute, linear gradient to 99% B for 20 minutes, 99% B for 3 minutes, linear gradient to 1% B for 5 minutes, then, 1% B for 10 minutes. The amounts of glucotropaeolin were calculated based on the peak areas of glucotropaeolin and the content was expressed as µmol g^{-1} DW.

Statistical Analysis

In each season, normality distributions were checked out by the Wilk Shapiro test (Neter *et al*., 1996). Then, the data were subjected to the analysis of variance in Randomized Complete Block Design (RCBD) by using MSTAT-C V.2.1 software package (Freed *et al*., 1989) for each season separately according to procedures reported by Gomez and Gomez (1984). Differences among means were compared for each trait using Least Significant Differences test (LSD) (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Effect of Nitrogen Supply on the Vegetative Growth Characters of *T. majus*

Significant effects of N supply were observed on biomass accumulation including plant height; branches number plant^{-1}, plant fresh weight, leaf fresh and dry weights, stem fresh and dry weights (Table 2). The highest increase in these parameters was observed in the highest N rate (120 kg ha^{-1}) at all growth stages in both seasons, while the lowest was recorded in the control plants. These results agree with previous studies reporting that high doses of nitrogen supply significantly enhanced growth and yield of different species such as broccoli plants (Schonhof *et al*., 2007), winter rapeseed (Öztürk 2010), and cabbage (Rosen *et al*., 2005). The positive response of *T. majus* to lower and higher N supply was expected since N is the primary limiting factor for plant growth and yield that determines the production of plant biomass

Table 2. Effect of nitrogen supply on plant height, branches number, fresh weight, leaf fresh and dry weights, stem fresh and dry weights of *T. majus* during first and second seasons.^{a}

Nitrogen supply	Plant height (cm)	Branches no/Plant	Fresh weight (g plant^{-1})	Leaf FW (g)	Leaf DW (g)	Stem FW (g)	Stem DW (g)
1st Season							
1st Sample							
N1	32.3 ^{c}	2.7 ^{c}	68.7 ^{c}	23.4 ^{c}	5.0 ^{c}	36.1 ^{c}	5.8 ^{c}
N2	35.5 ^{b}	3.4 ^{b}	78.0 ^{b}	28.8 ^{b}	5.4 ^{b}	40.4 ^{b}	6.4 ^{b}
N3	38.0 ^{a}	3.6 ^{a}	98.8 ^{a}	32.9 ^{a}	6.1 ^{a}	56.5 ^{a}	7.4 ^{a}
2nd Sample							
N1	65.1 ^{c}	5.8 ^{c}	439.1 ^{c}	46.4 ^{c}	8.4 ^{c}	174.6 ^{c}	11.7 ^{c}
N2	72.5 ^{b}	6.9 ^{b}	589.6 ^{b}	55.8 ^{b}	10.4 ^{b}	233.7 ^{b}	13.3 ^{b}
N3	85.9 ^{a}	7.7 ^{a}	705.6 ^{a}	63.5 ^{a}	11.7 ^{a}	264.8 ^{a}	15.1 ^{a}
3rd Sample							
N1	70.0 ^{c}	10.1 ^{c}	651.9 ^{c}	71.5 ^{c}	13.1 ^{c}	199.8 ^{c}	40.2 ^{c}
N2	77.0 ^{b}	11.6 ^{b}	840.6 ^{b}	88.1 ^{b}	17.3 ^{b}	282.3 ^{b}	57.6 ^{b}
N3	90.0 ^{a}	12.9 ^{a}	969.2 ^{a}	101.2 ^{a}	20.3 ^{a}	342.1 ^{a}	67.3 ^{a}
2nd Season							
1st Sample							
N1	32.3 ^{c}	3.7 ^{c}	86.2 ^{c}	23.3 ^{c}	4.8 ^{c}	44.8 ^{c}	5.0 ^{c}
N2	34.8 ^{b}	4.6 ^{b}	101.7 ^{b}	30.7 ^{b}	6.5 ^{b}	53.5 ^{b}	5.9 ^{b}
N3	38.8 ^{a}	5.5 ^{a}	126.4 ^{a}	40.0 ^{a}	8.2 ^{a}	58.1 ^{a}	6.8 ^{a}
2nd Sample							
N1	70.3 ^{c}	7.8 ^{c}	925.0 ^{c}	75.8 ^{c}	13.9 ^{c}	171.2 ^{c}	17.8 ^{c}
N2	78.0 ^{b}	9.1 ^{b}	1107.0 ^{b}	99.1 ^{b}	18.2 ^{b}	265.8 ^{b}	27.6 ^{b}
N3	87.3 ^{a}	11.2 ^{a}	1250.0 ^{a}	141.9 ^{a}	26.1 ^{a}	367.5 ^{a}	38.1 ^{a}
3rd Sample							
N1	71.67 ^{c}	10.1 ^{c}	938.3 ^{c}	79.7 ^{c}	13.9 ^{c}	208.3 ^{c}	21.6 ^{c}
N2	79.92 ^{b}	11.3 ^{b}	1216.2 ^{b}	106.2 ^{b}	18.2 ^{b}	328.3 ^{b}	34.1 ^{b}
N3	89.67 ^{a}	13.7 ^{a}	1395.0 ^{a}	138.5 ^{a}	26.1 ^{a}	462.9 ^{a}	47.7 ^{a}

[a] Values with different letters in the same column are significantly different at P< 0.05. N1: 0, N2:, N3: 120 kg N ha^{-1}, and S1: 0, S2: 50, S3: 100 kg S ha^{-1} .

(Souri and Neumann, 2018). It acts as building block in the plant tissues and genetic, and metabolic and physiological processes. Moreover, N is an essential component in synthesizing nucleic acids, amino acids, coenzymes, amides and chlorophyll. It participates in the transport of protons and electrons in photosynthesis, respiration and the signal transduction path between organs (Rathke *et al*., 2005; Skubij *et al*., 2020).

Effect of Sulfur Supply on Vegetative Growth Characters of *T. majus*

Vegetative traits of *T. majus* increased with S supply in both seasons (Table 3). Plants that received the highest supply were significantly taller and had more branches than other treatments, whereas the control plants were shorter and had lower number of branches. The fresh weigh, fresh leaves and stems per plant were significantly greater in plants supplied with 100 kg S ha^{-1}, followed by 50 kg S ha^{-1} compared to non-supplied plants. The more significant plant fresh weight was attributable to the significantly greater plant height and branches number.

Leaf and stem dry weights reached maximum values at 100 kg S ha^{-1} followed by 50 kg S ha^{-1}, whereas the lowest values were recorded for non-supplied plants in both seasons. However, the differences were

Table 3. Effect of sulfur supply on plant height, branches number, fresh weight, leaf fresh and dry weights, stem fresh and dry weights of *T. majus* during first and second seasons.

Sulfur fertilization	Plant height (cm)	Branches no/Plant	Fresh weight (g plant^{-1})	Leaf FW (g)	Leaf DW (g)	Stem FW (g)	Stem DW (g)
1st Season							
1st Sample							
S1	34.0 ^{b}	3.13 ^{b}	74.8 ^{c}	24.8 ^{c}	5.1 ^{c}	40.3 ^{c}	6.0 ^{c}
S2	35.7 ^{a}	3.27 ^{b}	81.8 ^{b}	28.3 ^{b}	5.5 ^{b}	44.2 ^{b}	6.4 ^{b}
S3	36.3 ^{a}	3.44 ^{a}	88.8 ^{a}	32.1 ^{a}	6.1 ^{a}	48.5 ^{a}	7.3 ^{a}
2nd Sample							
S1	71.1 ^{c}	6.5 ^{b}	528.3 ^{c}	52.2 ^{b}	9.6 ^{b}	206.5 ^{b}	12.4 ^{b}
S2	74.3 ^{b}	6.9 ^{a}	587.2 ^{b}	55.8 ^{a}	10.2 ^{a}	231.0 ^{a}	13.7 ^{a}
S3	77.5 ^{a}	6.9 ^{a}	618.9 ^{a}	57.8 ^{a}	10.5 ^{a}	235.5 ^{a}	13.9 ^{a}
3rd Sample							
S1	75.2 ^{c}	10.9 ^{c}	778.7 ^{c}	80.9 ^{c}	15.7 ^{b}	247.2 ^{c}	51.1 ^{c}
S2	79.1 ^{b}	11.6 ^{b}	814.5 ^{b}	87.5 ^{b}	17.1 ^{a}	277.3 ^{b}	54.7 ^{b}
S3	82.6 ^{a}	12.1 ^{a}	868.3 ^{a}	92.3 ^{a}	17.9 ^{a}	300.0 ^{a}	59.2 ^{a}
2nd Season							
1st Sample							
S1	34.0 ^{c}	4.2 ^{b}	97.9 ^{c}	28.5 ^{c}	6.1 ^{c}	49.5 ^{c}	5.6 ^{b}
S2	35.5 ^{b}	4.6 ^{b}	105.6 ^{b}	31.8 ^{b}	6.4 ^{b}	51.7 ^{b}	5.8 ^{b}
S3	36.3 ^{a}	4.9 ^{a}	110.7 ^{a}	33.5 ^{a}	6.9 ^{a}	55.0 ^{a}	6.2 ^{a}
2nd Sample							
S1	75.0 ^{c}	8.4 ^{b}	1002.0 ^{c}	91.6 ^{c}	16.6 ^{c}	224.5 ^{c}	23.3 ^{c}
S2	79.1 ^{b}	9.5 ^{a}	1096.0 ^{b}	104.5 ^{b}	19.2 ^{b}	275.0 ^{b}	28.4 ^{b}
S3	81.6 ^{a}	10.2 ^{a}	1167.0 ^{a}	120.5 ^{a}	22.2 ^{a}	305.0 ^{a}	31.7 ^{a}
3rd Sample							
S1	76.7 ^{c}	10.8 ^{b}	1064.2 ^{c}	98.0 ^{c}	16.8 ^{c}	290.0 ^{c}	30.1 ^{c}
S2	80.9 ^{b}	11.7 ^{a}	1206.7 ^{b}	108.2 ^{b}	19.2 ^{b}	333.3 ^{b}	34.3 ^{b}
S3	83.7 ^{a}	12.3 ^{a}	1278.8 ^{a}	118.3 ^{a}	22.2 ^{a}	376.7 ^{a}	39.1 ^{a}

[a] Values with different letters in the same column are significantly different at $P< 0.05$. N1: 0, N2: 60, N3: 120 kg N ha^{-1} and S1: 0, S2: 50, S3: 100 kg S ha^{-1}.

not significant between plants supplied with 100 and 50 kg S ha^{-1} in some cases mostly in the flowering stage. The growth response of *T. majus* to S supply at all stages of growth and during the two seasons reached an ideal degree in 100 kg S ha^{-1} treatment, although the magnitude of response to N was better. The results are supported by the findings of Rosen *et al.* (2005) who revealed that increasing sulfur rate improved yield and tissue S concentration of cabbage but decreased N concentration. Application of S significantly increased fresh weight of the main root of horseradish (Rivelli *et al.*, 2016). Studies have shown that significant increase in protein concentrations with application of S has been reported in canola and broccoli. Reducing yield, protein and enzyme synthesis are adversely affected with limiting S supply (Schonhof *et al.*, 2007; Jan *et al.*, 2010). High sulfur fertilization appeared to increase chlorophyll and protein contents in leaves, suggesting a superior photosynthetic activity in comparison with plants grown without S (Anjum *et al.*, 2012). The positive impact of sulfur may be due to its role as an essential element for plant growth and its presence in proteins, glutathione, phytochelatins, thioredoxins, chloroplast membrane lipids, and certain coenzymes and vitamins (Jamal *et al.*, 2010). Another study showed that forage brassicas often respond more to N than S fertilization (Wilson *et al.*, 2006).

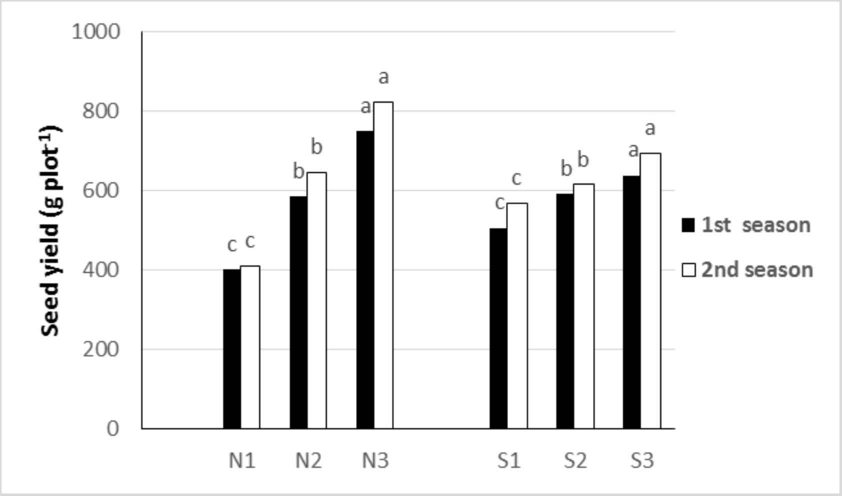

Figure 1. Effect of nitrogen and sulfur supply on seed yield of *T. majus* during two seasons. N1: 0, N2: 60, N3: 120 kg N ha^{-1}, and S1: 0, S2: 50, S3: 100 kg S ha^{-1} .

Effect of Nitrogen and Sulfur Supply on Seed Yield of *T. majus*

Seed yield ranged from 400 to 750 g plot^{-1} in the first season and from 710 to 821 in the second season (Figure 1). Seed yield gradually increased with nitrogen supply in both seasons. The highest seed yield was observed at the maximum N supply (120 kg ha^{-1}) whereas the lowest value was recorded in non-supplied plants. Seed yield of plants supplied with 120 kg N ha^{-1} were significantly higher by 28%, 27% than those supplied with 60 kg N ha^{-1} in the first and second seasons, respectively. Besides, in these two seasons, when N supply increased from 0 to 60 kg ha^{-1}, significant increases of, respectively, 46 and 57% in seed yield were observed, and further increases of 87 and 100% were recorded for 120 kg N ha^{-1} compared to N_1.

Significant differences occurred with S supply exhibiting increases in seed yield plot^{-1} (Figure 1) to reach the maximum at 100 kg S ha^{-1}, whereas the lowest value was recorded in the control plants. Seed yield of plants supplied with 100 kg S ha^{-1} were significantly higher by 7 and 13% than 50 kg S ha^{-1}, in the first and second seasons, respectively. In these two seasons, when sulfur supply increased from 0 to 50 kg ha^{-1}, significant increases of, respectively, 17% and 8% in seed yield were observed, and further increases of 26 and 22% were recorded for 100 kg S ha^{-1} compared to S_1. Results obtained here are in agreement with the results reported by Jackson (2000) on canola and by Nuttall *et al.* (1987) on brassica, who indicated that seed yield significantly responded to nitrogen and sulfur.

Effect of Nitrogen on Glucotropaeolin Content

Glucotropaeolin content was quantitatively determined in leaves, stems and seeds of *T. majus* plants grown under N and S supply (Figure 2-7). Seeds showed higher yields of glucotropaeolin followed by leaves and dropped to very low amount in stems. Glucotropaeolin content ranged from 23.45 to 45.26 µmol g^{-1} DW in leaves and from 117.92 to 156.31 µmol g^{-1} DW in seeds. The response of glucotropaeolin content to N supply varied between plant parts and growth stages. While glucotropaeolin content in seeds was unaffected by N supply, there were significant increases in glucotropaeolin content of leaves, especially

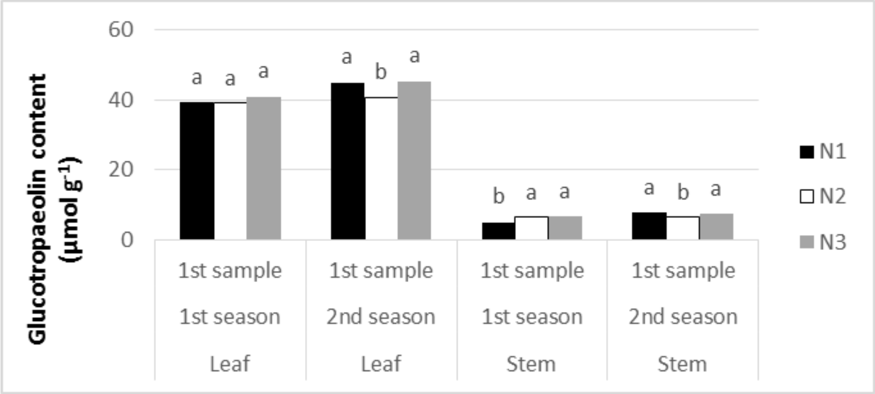

Figure 2. Effect of nitrogen on glucotropaeolin content in leaves and stems of *T. majus* in the first sample during two seasons. N1: 0, N2: 60, N3: 120 kg N ha^{-1}

at later stages (the second and third samples) compared to the early one.

The highest glucotropaeolin content in leaves was found in plants that received 120 kg N ha^{-1} (Figures 2, 3, and 4). Nitrogen supply with 120 kg N ha^{-1} enhanced the glucotropaeolin content in leaves by a range of 8-18% in the different samples compared to the other rates. There were no apparent trends in how glucotropaeolin content in stems were influenced when nitrogen supply increased since the stems have only a small proportion of this glucosinolate.

Several studies have focused on the positive effect of N on total glucosinolates. In a study by Schonhof *et al.* (2007), broccoli plants grown with an insufficient N supply showed an increase in total glucosinolate concentrations independent of the sulfur level, whereas, Bilsborrow *et al.* (1993) revealed that under constant sulfur supply, increasing nitrogen up to 150 kg ha^{-1} increased seed glucosinolates of oilseed rape. However, there was no effect at higher nitrogen rates. In summary, previous results showed that glucosinolate content differ in response to nitrogen since nitrogen supply may increase, decline, or have no impact on their content and composition. (Kim *et al.*, 2002; Schonhof *et al.*, 2007; Omirou *et al.*, 2009). That may be varied with plant species, growth stage and organs, and the rate of nitrogen supplied (Falk *et al.*, 2007).

Effect of Sulfur on Glucotropaeolin Content

Data illustrated in Figures 5, 6, and 7 demonstrate that glucotropaeolin content in leaves clearly responded to sulfur supply in both seasons at all growth stages. As sulfur supply increased, glucotropaeolin content in leaves and seeds increased.

Increasing S supply from 0 to 50 and further to 100 kg S ha^{-1} significantly increased the glucotropaeolin content in leaves by 3 to 21% and by 4 to 17% in the first and second seasons, respectively. On the other hand, the greatest glucotropaeolin content in seeds was recorded from plants that received 100 kg S ha^{-1} and increased the glucotropaeolin content by about 11% in both seasons compared to the control. The differences between 50 to 100 kg S ha^{-1} failed to be significant in the first season. Several studies have focused on the positive effect of S on total glucosinolates since it is essential constituent of glucosinolates. Glucosinolate concentrations of *Brassica rapa* can be increased by S fertilization (Schonhof *et al.*, 2007). In addition, an increased sulfur supply has been shown to result in higher total glucosinolates up to 20-fold of Brassica (Falk *et al.*, 2007) and sinigrin, glucobrassicanapin, gluconapin and

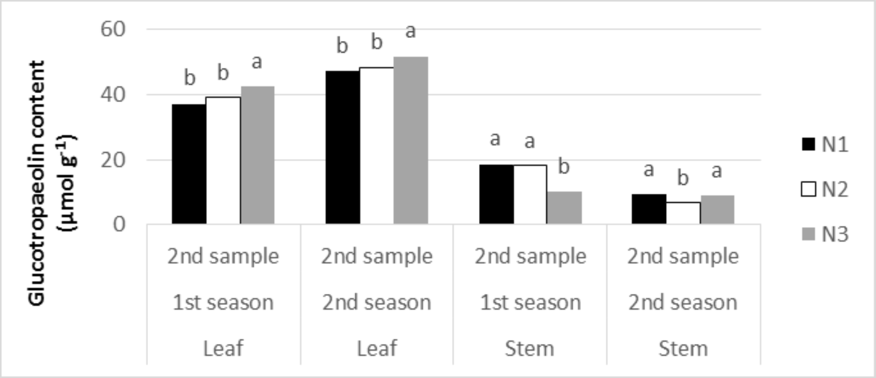

Figure 3. Effect of nitrogen on glucotropaeolin content in leaves and stems of *T. majus* in the second sample during two seasons**.** N1: 0, N2: 60, N3: 120 kg N ha^{-1}

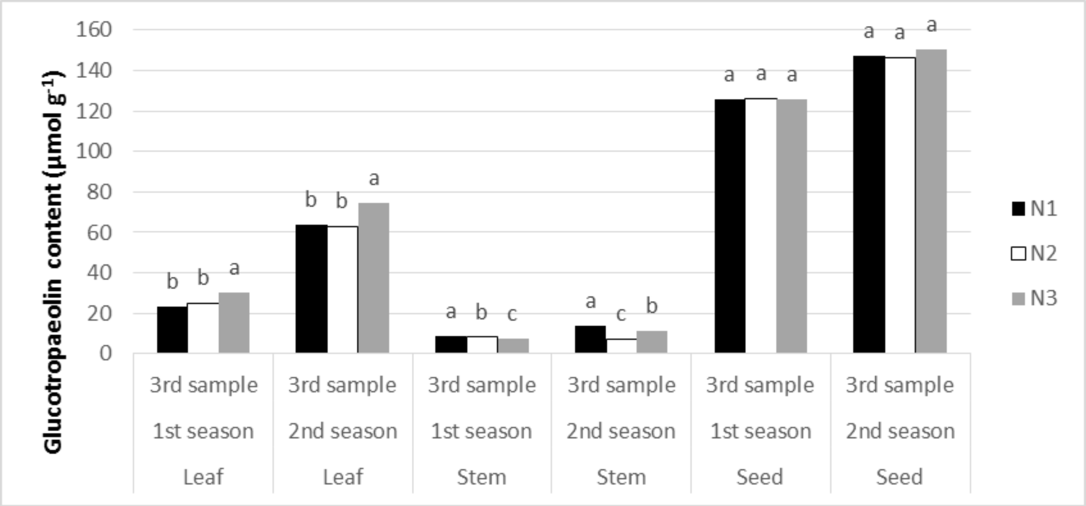

Figure 4. Effect of nitrogen on glucotropaeolin content in leaves, stems and seeds of *T. majus* in the third sample during two seasons. N1: 0, N2: 60, N3: 120 kg N ha^{-1}

progoitrin in *Brassica juncea* L. (Kaur *et al*., 1990). Similarly, higher sulfur fertilization increased the alkyl and alkenyl glucosinolate concentrations in broccoli and radish, respectively (Krumbein *et al*., 2001). In contrast, higher nitrogen fertilization declined the alkyl glucosinolate concentration in broccoli, whereas, Vallejo *et al*. (2003) reported that sulfur supply, in general, did not affect total glucosinolates in edible portions of broccoli on a clay soil. This may be because the soil S content was already sufficient.

Effect of Nitrogen and Sulfur Interaction on Vegetative Growth of *T. majus*

Results presented in Tables 4 and 5 show that interaction between nitrogen and sulfur supply had a significant effect on plant height, branches number, plant fresh weight, fresh leaves and stems as well as dry leaves and stems plant^{-1} and seed yield plot^{-1}.

These results indicated that the maximum of the above-mentioned parameters were found at the combination between 120 kg N ha^{-1} and 100 kg S ha^{-1}, while the lowest

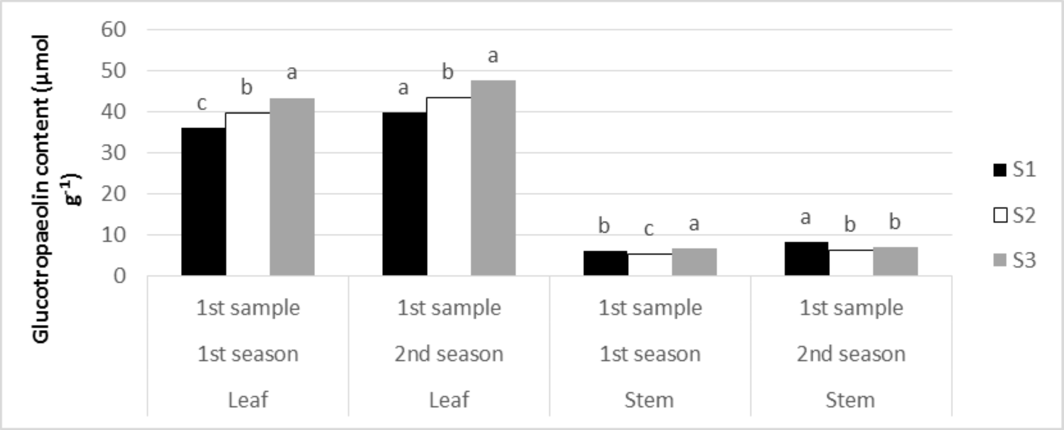

Figure 5. Effect of sulfur on glucotropaeolin content in leaves and stems of *T. majus* in the first sample during two seasons. S1: 0, S2: 50, S3: 100 kg S ha^{-1}.

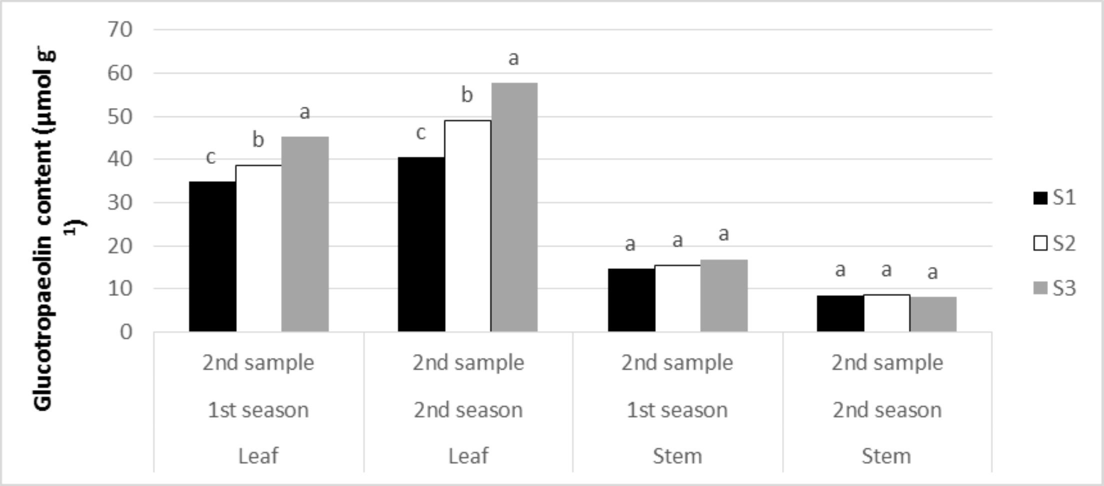

Figure 6. Effect of sulfur on glucotropaeolin content in leaves and stems of *T. majus* in the second sample during two seasons**.** S1: 0, S2: 50, S3: 100 kg S ha^{-1}.

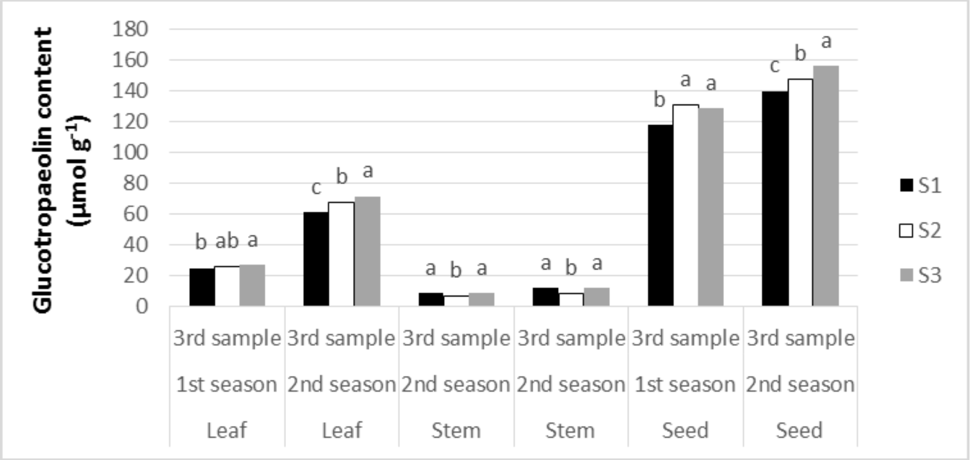

Figure 7. Effect of sulfur on glucotropaeolin content in leaves, stems and seeds of *T. majus* in the third sample during two seasons. S1: 0, S2: 50, S3: 100 kg S ha^{-1}.

values were obtained without sulfur and nitrogen supply at all growth stages, except the second sample of the first season.

Improvement in the growth, yield attributes and nitrogen accumulation with the combined application of N and S was also observed by Fazili *et al*. (2010). Plants grown with nitrogen and sulfur supply had lower nitrate-N content and higher nitrate reductase activity in leaves than those grown with only N, allowing nitrate to be reduced to nitrogen (Fismes *et al*., 2000). The balance between them (N:S ratio) affect assimilation of sulfate ions regulating the plant S requirements concerning its N demand. The narrowing N:S ratio leads to the accumulation of inorganic sulfur compounds, while the expansion of the N:S ratio increases the uptake of non-protein N forms. (Skubij *et al*., 2020).

Interaction of Nitrogen and Sulfur Supply on Glucotropaeolin Content

The interaction between nitrogen and sulfur supply on glucotropaeolin content in leaves, stems, and seeds was investigated (Table 6). Nitrogen and sulfur supply significantly improved the glucotropaeolin content in leaves and seeds at all growth stages and in both years. Leaf glucotropaeolin content in the higher supply of N associated with the lower or the higher supply of S was significantly greater than that in the lower supply of N. On the other hand, with no S supply, leaf glucotropaeolin content was the lowest at all growth stages in both years. Glucotropaeolin content in stems was changed with both N and S supply, however, it did not show a consistent general trend and no explainable variation was detected.

Based on several reports, glucosinolates content of many plants mainly depends on the balance between nitrogen and sulfur status or the N:S ratio (Zhao *et al*., 1993). In oilseed rape (*Brassica napus* L.) and *Brassica nigra* L., studies have reported that high N with low or insufficient S supply declined glucosinolate content of the seed. In contrast, plants with low N and adequate S had greater allyl isothiocyanate levels (Wolfson, 1982, Zhao *et al*., 1993). As nitrogen to sulfur supply decreased, glucosinolate content tended to increase (Falk *et al*., 2007). However, Palaniswamy *et al*. (1995) noticed that increasing N:S ratio in nutrient solution declined the phenylethyl isothiocyanate concentration in watercress plants. Interestingly, N supply increased glucosinolate concentration or did not affect when the S-sufficient soil was used.

CONCLUSIONS

In general, N and S supply proved to be key factors in determining higher biomass accumulation of *Tropaeolum majus*, whether used as herb or seed. Seeds of *Tropaeolum majus* showed higher yields of glucotropaeolin followed by leaves and stems. Glucotropaeolin content ranged from 23.45 to 45.26 μmol g^{-1} DW in leaves and from 117.92 to 156.31 μmol g^{-1} DW in seeds. An increased N or S supply resulted in higher total glucotropaeolin content. Finally, from our results, a rate of 120 kg ha^{-1} of N and 100 kg S ha^{-1} determined the optimal response in terms of both yield and quality.

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اثر نیتروژن و گوگرد کاربردی برعملکرد و صفت های کیفی گل لادن

ر. م. صبری، ا. ا. ا. السید، و س. س. احمد

چکیده

برای ارزیابی رشد گیاه گل‌لادن (*Tropaeolum majus*) و محتوای گلوکوتروپائولین (glucotropaeolin) آن گیاه در اثر کاربرد مقادیر مختلف نیتروژن و گوگرد، دو آزمایش مزرعه‌ای در دو فصل پیاپی اجرا شد. در این آزمون ها از سه میزان نیتروژن (0، 60 و 120 کیلوگرم نیتروژن در هکتار) به شکل نیترات آمونیوم و سه میزان گوگرد (0، 50 و 100 کیلوگرم در هکتار) به شکل گوگرد عنصری استفاده شد. نتایج نشان داد که کاربرد نیتروژن و گوگرد باعث افزایش تجمع زیست توده *T. majus* ، ارتفاع بوته، تعداد شاخه، وزن تر بوته، وزن تر و خشک برگ و وزن تر و خشک ساقه شد. در هر دو فصل رشد و در تمام مراحل رشد، مقادیر بیشینه صفات رشد در مورد تیمار بیشترین میزان نیتروژن (120 کیلوگرم در هکتار) و مقدار متوسط یا بالاترین میزان گوگرد (50 و 100 کیلوگرم در هکتار) ثبت شد. محتوای گلوکوتروپائولین در برگها از 23/45 تا 45/26 میکرومول در گرم ماده خشک، و در دانه ها از 117/92 تا 156/31 میکرومول در گرم ماده خشک بود. در قسمت‌های مختلف گیاه و مراحل مختلف رشد، پاسخ مقدار گلوکوتروپائولین به مقدار نیتروژن کاربردی متفاوت بود.محتوای گلوکوتروپائولین در دانه ها تحت تأثیر نیتروژن کاربردی قرار نداشت.

بیشترین میزان گلوکوتروپئولین در برگهای گیاهانی مشاهده شد که 120 کیلوگرم نیتروژن در هکتار دریافت کردند. مقدار گلوکوتروپائولین در برگ و دانه با کاربرد گوگرد در هر دو فصل و در تمام مراحل رشد به طور معنی‌داری افزایش یافت.