

## The Effect of Manganese and Sulfur Applications on the Rocket (*Eruca vesicaria subsp. Sativa*) Plant Grown in Lime Soils

Ayşen AKAY<sup>1\*</sup>

<sup>1</sup>Selçuk Üniversitesi, Ziraat Fakültesi, Toprak Bilimi ve Bitki Besleme Bölümü, 42130, Konya

<sup>1</sup><https://orcid.org/0000-0002-2541-0167>

\*Sorumlu yazar: aakay@selcuk.edu.tr

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### ABSTRACT

Due to the high lime and pH in the soil, sulfur and micro element deficiencies are observed in green vegetable plants. Rocket, one of the green vegetables and a member of the Brassicaceae family, is widely produced in our country. It is noteworthy that rocket is grown by producers almost everywhere and that this plant grown in our region also has a deficiency of microelements. For this reason, in this study were investigated that the sulfur and manganese requirement of rocket, which is one of the vegetable plants grown in Konya calcareous soil, and the effect of the interaction between these two elements on plant growth and yield. In our study, sulfur (S) fertilizer was applied in three doses (0- 40- 80 kg S da<sup>-1</sup>) and manganese (Mn) fertilizer in three doses (0- 1- 2 kg Mn da<sup>-1</sup>) was applied to the rocket plant. The experiment was carried out in the greenhouse conditions according to the randomized plots factorial design. At the end of the experiment, the average leaf height, chlorophyll SPAD value, plant fresh weight, plant height and plant Mn concentration did not differ with different doses of fertilizer applications in the rocket plant. On the other hand, the sulfur concentration in the plant and soil increased significantly with sulfur fertilization (p<0.05). It has been determined that if there is sufficient sulfur in the soil for rocket, there is no need for additional sulfur fertilization.

## Kireçli Topraklarda Yetiştirilen Roka (*Eruca vesicaria subsp. Sativa*) Bitkisine Mangan ve Kükürt Uygulamalarının Etkisi

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### ÖZ

Topraklardaki yüksek kireç ve pH' dan dolayı yeşil sebze bitkilerinde kükürt ve mikro element noksanlığı gözlenmektedir. Yeşil sebzelerden biri olan ve Brassica familyasının bir üyesi olan roka ülkemizde yaygın şekilde üretilmektedir. Rokanın hemen her yerde yetiştirilmesi ve bölgemizde yetiştirilen bu bitkide de mikro element noksanlığı görülmesi dikkati çekmiştir. Bu nedenle yürütülen çalışmada, Konya kireçli topraklarında yetiştirilen rokanın kükürt (S) ve mangan (Mn) ihtiyacının belirlenmesi ve bu iki element arasındaki etkileşimin bitki gelişimi ve verimine etkisi araştırılmıştır. Çalışmamızda; kükürtlü gübre üç dozda (0- 40- 80 kg S da<sup>-1</sup>) ve manganlı gübrede üç dozda (0- 1- 2 kg da<sup>-1</sup> Mn) roka bitkisine uygulanmıştır. Deneme sera şartlarında tesadüf parselleri faktöriyel deneme desenine göre yürütülmüştür. Deneme sonunda roka bitkisinde ortalama yaprak boyu, klorofil SPAD değeri, bitki

yaş ağırlığı, bitki boyu ve bitki Mn konsantrasyonu farklı dozlarda gübre uygulamaları ile farklılık göstermemiştir. Buna karşın bitkide ve toprakta kükürt konsantrasyonu kükürt gübrelemesi ile önemli artış göstermiştir ( $p<0.05$ ). Roka için toprakta yeterli seviyede kükürtün bulunması halinde ek kükürtlü gübrelemeye ihtiyaç olmadığı belirlenmiştir.

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## 1. Introduction

Existing agricultural lands in our country contain relatively high amounts of  $\text{CaCO}_3$  and have extremely poor organic matter, resulting in high pH of the soils. This situation directly affects the availability of plant nutrients necessary for plant growth. Elemental sulfur (S) can be used as a nutrient and a soil acidifier (Lindemann et al., 1991; Neilsen et al., 1993). Acidity produced during elemental S oxidation, it increases the chemical and physical properties of alkaline and sodic soils (Wainwright, 1984) and also this acidity increases the availability of nutrients such as P, Mn, Ca and  $\text{SO}_4$  in soils (Lindemann et al., 1991).

Sulfur is one of the six macroelements essential for plants, plays an important role in catalysis and regulation functions in plants (Marshner, 2005). For many years, it has been thought that sulfur does not limit plant productivity. Sulfur deficiency is observed in some agricultural areas in the world due to the recent restrictions on sulfur air pollutant emissions, which are components of acid rain (Leustek and Kazuki Saito, 1999). Sulfur is also a powerful pesticide and fungicide. It is used in large quantities to combat powdery mildew for crops such as grapes and sugar beets. This creates another source that leaves a residual effect on the soil, apart from the use of sulfur as a fertilizer (Hinckley et al., 2020).

It is found in sulfur, cystine and methionine amino acids and various metabolites in the plant. The product after sulfate assimilation is cysteine, then other compounds such as methionine, S-adenosylmethionine, glutathione, thiamine, coenzyme-A, iron-sulfur centers, phytochelatins and glucosinolates are produced (Leustek et al., 2000; Lewandowska and Sirko, 2008). Sulfur is involved in many events such as regulation of enzyme activities and biosynthesis, protection of cells, photosynthesis, and respiration in plants (Davidian and Kopriva, 2010). In recent studies, it has been stated that sulfur has become a limiting factor in plant growth (Scherer, 2001). Almotory et al. (2020) stated that agricultural sulfur application increases the availability of  $\text{Fe}^{+2}$  and  $\text{Mn}^{+2}$  in the soil, but it decreases the availability of  $\text{Zn}^{+2}$  and  $\text{Cu}^{+2}$ . In this study, the application of sulfur at  $2000 \text{ kg ha}^{-1}$  level increased up to 21.43% the amount of the available  $\text{Mn}^{+2}$  in the soil. In another study, sulfur increased the amount of available Fe, Mn, and Zn in the soil (Saleh 2001).

Sulfur is important for various agricultural products as well as vegetables such as rocket. Low availability of sulfur may affect the quality of cultivated plants and especially the Brassicaceae family due to their high sulfur requirement (Houhou et al., 2018). Sulfur deficiency affected the biochemical quality and morphological parameters of *Eruca sativa* and decreased the total dry weight of the plant and the chlorophyll concentration. It was also observed that both sulfate and total sulfur concentrations

in the leaves decreased rapidly in response to the reduced sulfur supply to the plant (Houhou et al., 2018).

Rocket (*Eruca sativa*) is in the Brassicaceae family, it is an endemic, one-year species that is extensively produced in our country and in Mediterranean countries such as Italy, Greece, Portugal, and Egypt, and has a spicy sharp taste (Bianco and Boari, 1997; Morales and Janick, 2002). Rocket, which dates back to the Middle Ages, is also known as a medicinal plant and is used as an aphrodisiac, eye infections, digestive and kidney problems (Yaniv et al., 1998); it has cell protective and anti-ulcer activities against stomach lesions (Alqasoumi et al., 2009). Similar to other Brassica species, rocket contains glucosinolates such as allyl sulfocyanate, and compounds very rich in sulfur such as isothiocyanates (Bennet et al., 2002; Martinez-Sanchez et al., 2006), and erucic acid in the seed oil. Rocket (*Eruca sativa*) has consumed as a vegetable (leaves) and a spice (leaves, seeds, flowers) (Bermejo and León, 1994).

According to the data of 2021, the annual production of rocket in Turkey is 27.350 tons (Anonymous, 2022). However, the production data of individual gardeners are not known exactly, except for the officially announced values. In addition to nitrogen, phosphorus, and potassium at the nutrition of the rocket plant should not be ignored the effect of sulfur and manganese. Manganese acts as a cofactor for many enzymes, including lignin synthesis, as is known (Burnell, 1988). This function of manganese assumes a key role in disease resistance. In soil experiments, it was stated that fertilizers containing sulfur in reduced (thiosulfate) form had a strong mobilizing effect on Mn, and plants accumulated large amounts of Mn in the biomass compared to oxidized S (sulphate). Thus, fertilization with sulfur in the form of thiosulfate can be very effective in correcting Mn deficiency in soils formed on ancient marine sediments where Mn availability limits plant growth (Husted et al., 2005). The plant's tolerance to soluble Mn can also be affected by the concentration of S, Al, Zn and Cu in the medium. In addition, S can be lower the pH of the growth medium and increase the availability of Mn to plants (El-Jaoual and Cox, 1998). Wallace et al. (1974) reported that S significantly reduced the yield of soybeans suffering from Mn toxicity.

Studies on the sulphurous and manganese fertilizer demand of plants on calcareous soils in our region are insufficient. For this reason, the effect of sulphurous and manganese fertilizer application on the yield, plant growth, chlorophyll content, product quality and element concentration of rocket, which is one of the green vegetable plants, was investigated in this study. In the study, suitable and economical sulfur and manganese doses were tried to be determined for the plant grown in calcareous soil.

## **2. Material and Method**

### **Material**

The soil used in the experiment was taken from Konya - Selcuk University Faculty of Agriculture, Saricalar farmland from a depth of 0-30 cm. After drying in the air, it was passed through a 4 mm

sieve and filled into pots (2.5 kg pot<sup>-1</sup>). Rocket seeds used in the experiment are commercial seeds produced and sold to farmers.

### **Method**

The experiment was set up in the greenhouse according to the factorial experimental design at randomized plots with 4 replications. In the study carried out to determine the effect of sulfur and manganese fertilization on the development and some yield characteristics of rocket, three doses of S (0- 40- 80 kg da<sup>-1</sup>) (S0- S1- S2) using bentonite-S containing 90% S and three doses of Mn (0- 1- 2 kg da<sup>-1</sup> Mn) (Mn0- Mn1- Mn2 ) Mn EDTA form was applied to the experiment pots.

The experiment was carried out in 36 pots in total. After rocket seeds were planted in pots in a greenhouse environment, their development was followed until the beginning of flowering. Additional fertilization was applied to all pots, according to the soil analysis results (Fe-EDDHA (0.5 kg Fe/da) and urea fertilizer (10 kg N/da) was applied to the pots. After these processes, the pots were regularly watered considering the field capacity of the soil. Chlorophyll spad value (with Minolta-502 SPAD meter) in mature leaves in each pot at the beginning of flowering, and plant leaf height, plant height, and plant above-ground weight values were determined at the end of the experiment.

### **Plant Analyzes**

The harvested plants were pre-washed, dried at 65 degrees Celsius and ground in a mill made of steel material. The ground plant samples were wet burned with HNO<sub>3</sub> on the hot plate, and then S and Mn were determined. For this purpose, the Mn contents of the solutions obtained were determined by reading in AAS (Bayraklı, 1987; Kacar and Inal, 2010) and S concentrations were determined by reading in a spectrophotometer according to the turbidimetric method adapted from Chesnin and Yien (1951).

### **Soil analyzes**

Before the experiment, soil samples were taken from 0-30 cm from the field and some physical and chemical properties were determined by analyzing (Table 1). Soil texture, pH, salinity, CaCO<sub>3</sub> by Scheibler calcimeter method, organic matter, available phosphorus, exchangeable cations, plant useful Fe, Zn, Cu, Mn, field capacity and wilting point (Kacar, 2016) analyzes were made. In addition, soil samples were taken from the pots at the end of the experiment and sulfur determined by spectrophotometer using potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) according to turbidimetric method (Fox et al., 1964).

The soil used in the experiment, which was carried out under greenhouse conditions, has a slightly alkaline pH and is in the salt-free soil class. Clay loam is textured, poor in organic matter and has a high lime content. The soil contains sufficient K, moderate levels of P, Mn and Cu, and low levels of Fe and Zn (Table 1).

**Table 1.** Some physical and chemical properties of the experiment soils

Experiment Soil Properties	Texture class	EC(ds/m)	pH	CaCO <sub>3</sub> (%)	Organic Matter (%)	Field capacity	Fading point
	Clay loam	0.93	7.90	12.94	1.98	26	14
		Cu	Mn	Zn	Fe	P	K
		(mg kg <sup>-1</sup> )					
		0.96	1.30	0.58	2.46	5.15	831.88

### Statistical evaluation

The results obtained from the greenhouse experiment were subjected to analysis of variance using MINITAB package programs in four replications. The results expressed as mean  $\pm$  standard deviation (SD) (significant differences between mean at 95% level of confidence). Significant differences were determined by Anova test.

### 3. Results

Different doses of Mn applications in the experiment caused significant differences on leaf chlorophyll spad value, plant fresh weight, plant height, the S concentration in the plant, the Mn concentration in the plant and the S concentrations remaining in the soil at the end of the experiment ( $p < 0.05$ ). While increasing Mn dose caused a decrease in chlorophyll spad value, plant fresh weight, plant height, S concentration in the plant compared to the control applications. The Mn concentration in the plant and the S concentration in the soil increased compared to the control.

There was no significant difference in leaf length values. Mn contents in the plant are in the range of the sufficiency limit values given for vegetables (30-250 mg Mn kg<sup>-1</sup> per leaf). Sulfur contents in the plant are slightly above the sufficiency limit values (0.30-1.00% S per leaf) (Kacar and Katkat, 1998). The S content in the soil showed a significant increase in the 2 kg da<sup>-1</sup> Mn application compared to the control and 1 kg da<sup>-1</sup> Mn application (Table 2).

**Table 2.** The effect of sulfur fertilizer applications on the data obtained from the rocket plant and the results of variance and Tukey test analysis  
(\*Different letters show the comparison of the mean values according to Tukey test;  $p < 0.05$ )

Application doses (kg S da <sup>-1</sup> )	Leaf length (cm)	Leaf chlorophyll spad value	Plant fresh weight (g pot <sup>-1</sup> )	Plant height (cm)	S in soil (mg kg <sup>-1</sup> )	S in the plant (mg kg <sup>-1</sup> )	Mn in the plant (mg kg <sup>-1</sup> )
0	10.14 $\pm$ 0.41 a	41.73 $\pm$ 2.08 a	58.28 $\pm$ 4.21 a	19.55 $\pm$ 1.11 b	5.20 $\pm$ 1.14 c	9306 $\pm$ 795 b	46.55 $\pm$ 2.27 b
40	8.83 $\pm$ 0.34 b	37.43 $\pm$ 0.73 b	44.13 $\pm$ 4.14 b	19.25 $\pm$ 3.72 b	33.34 $\pm$ 6.79 b	11548 $\pm$ 1696 a	48.67 $\pm$ 4.40 a
80	8.71 $\pm$ 0.23 b	35.46 $\pm$ 1.22 c	43.99 $\pm$ 2.35 b	23.15 $\pm$ 2.53 a	58.94 $\pm$ 25.66 a	10965 $\pm$ 587 a	49.03 $\pm$ 1.52 a

**Table 3.** The effect of manganese fertilizer applications on the data obtained from the rocket plant and the results of variance and Tukey test analysis  
 (\*Different letters show the comparison of the mean values according to Tukey test;  $p < 0.05$ )

S doses (kg S da <sup>-1</sup> )	Mn doses (kg Mn da <sup>-1</sup> )	Leaf length (cm)	Leaf chlorop. spad value	Plant fresh weight (g pot <sup>-1</sup> )	Plant height (cm)	S in the plant (mg kg <sup>-1</sup> )	Mn in the plant (mg kg <sup>-1</sup> )	S in soil (mg kg <sup>-1</sup> )
0	0	9.95 ± 0.49	43.07 ± 0.69 a	62.95 ± 1.41 a	18.82 ± 0.84 b	8594 ± 503 e	44.21 ± 1.62 c	5.75 ± 0.96 d
	1.0	10.17 ± 0.41	43.03 ± 0.58 a	56.86 ± 2.89 b	19.88 ± 1.42 b	10183 ± 505 cde	47.81 ± 2.01 bc	4.68 ± 1.21 d
	2.0	10.32 ± 0.35	39.07 ± 0.98 b	55.03 ± 2.96 b	19.96 ± 0.87 b	9140 ± 270 de	47.62 ± 1.15 bc	5.16 ± 1.26 d
40	0	9.07 ± 0.11	36.96 ± 0.72 cd	49.31 ± 2.73 c	23.37 ± 2.543 a	12517 ± 1317 a	44.45 ± 3.91 c	35.26 ± 7.17 bc
	1.0	8.45 ± 0.34	37.26 ± 0.62 bc	41.28 ± 1.23 e	19.04 ± 0.77 b	12265 ± 1679 ab	48.65 ± 2.08 abc	36.56 ± 6.31 bc
	2.0	8.98 ± 0.06	38.07 ± 0.43 bc	41.79 ± 0.29 e	15.34 ± 0.69 c	9862 ± 519 cde	52.92 ± 1.90 a	28.20 ± 4.90 c
80	0	8.67 ± 0.28	36.37 ± 0.34 cd	46.23 ± 0.32 cd	23.92 ± 0.62 a	11527 ± 219 abc	49.49 ± 2.32 ab	42.19 ± 8.63 b
	1.0	8.67 ± 0.17	35.06 ± 1.57 d	41.11 ± 0.91 e	19.93 ± 0.53 b	10837 ± 706 a-d	48.95 ± 1.31 abc	41.83 ± 5.62 b
	2.0	8.80 ± 0.27	34.94 ± 1.09 d	44.62 ± 1.02 de	25.60 ± 0.34 a	10531 ± 144 b-e	48.66 ± 0.98 abc	92.79 ± 3.97 a

In the experiment, increasing doses of S applications caused significant decreases in leaf length, leaf chlorophyll spad value, and plant fresh weight values. The plant height, S concentration in the plant, Mn concentration in the plant and the S concentration remaining in the soil at the end of the experiment increased significantly with increasing sulfur doses. It was observed that sulfur application had a positive effect on plant Mn uptake (Table 3).

When the combined effect of increasing doses of S and Mn is examined, while there was no significant change in leaf length, leaf chlorophyll spad value showed a significant decrease compared to control applications ( $p < 0.05$ ). A similar situation was observed in the fresh weight of the plant. Plant height values gave positive results especially when Mn0 dose were applied together with S40 and S80 doses ( $p < 0.05$ ).

**Table 4.** The effect of sulfur and manganese fertilizer applications on the data obtained from the rocket plant

Application doses (kg Mn da <sup>-1</sup> )	Leaf length (cm)	Leaf chlorophyll spad value	Plant fresh weight (g pot <sup>-1</sup> )	Plant height (cm)	S in soil (mg kg <sup>-1</sup> )	S in the plant (mg kg <sup>-1</sup> )	Mn in the plant (mg kg <sup>-1</sup> )
0	9.23 ± 0.63	38.80 ± 3.21 a	52.83 ± 7.76 a	22.04 ± 2.78 a	27.73 ± 17.52 b	10879 ± 1893 a	46.05 ± 3.58 b
1.0	9.09 ± 0.85	38.45 ± 3.63 a	46.42 ± 7.90 b	19.62 ± 0.98 b	27.69 ± 17.71 b	11095 ± 1341 a	48.47 ± 1.73 a
2.0	9.36 ± 0.74	37.36 ± 2.01 b	47.15 ± 6.17 b	20.30 ± 4.42 b	42.05 ± 38.89 a	9844 ± 672 b	49.74 ± 2.71 a

(\*Different letters show the comparison of the mean values according to Tukey test;  $p < 0.05$ )

S concentration in the plant increased with increasing S doses but it decreased with increasing Mn doses ( $p < 0.05$ ). Although the Mn concentration in the plant increased partially with increasing Mn doses, increasing S doses increased the S concentration in the plant compared to the control. Especially in S40 -Mn2 application, the plant Mn concentration is the highest (52.92 mg kg<sup>-1</sup>). Mn concentrations in rocket samples collected from Aydın region in Turkey were between 25.60 and 79.30 mg kg<sup>-1</sup>, with an average of 40.58 mg kg<sup>-1</sup> Mn (Barlas et al., 2011).

At the end of the experiment, the available S concentration remaining in the soil at the end of the experiment increased with increasing S applications, especially at S80 applications, compared to the control. In fact, the S concentration, which was below the limit value given for S in the soil, reached the highest value at the S80-Mn2 dose in control applications (92.79 mg kg<sup>-1</sup>) ( $p < 0.05$ ) (Table 4).

#### 4. Discussion

Considering the S and Mn concentration values in the plant, it was determined that the dose application of 40 kg S da<sup>-1</sup> and 1 kg Mn da<sup>-1</sup> affected these data positively. However, S and Mn applications are ineffective in the growth parameters obtained at the end of the experiment. As is known, rocket is rich in iron, potassium and sulfur, and also contains high levels of protein and vitamin A (Porto et al., 2013).

The use of nano Fe and Zn applications on rocket, alone or in combination with chicken manure, significantly increased almost all growth parameters (such as fresh weight and seed yield, photosynthetic rate, transpiration rate, water use efficiency) compared to the control (Mahmoud et al., 2018). Organic fertilizers applied integrated with inorganic fertilizers provided higher yields than single chemical fertilizers (Sarwar et al., 2008). Fe, Mg and Mn deficiency causes the appearance of leaf chlorosis in rocket grown in hydroponic environment; It has been observed that lack of oxygenation and excess macronutrients affect the chlorosis and necrosis of rocket (Ramirez et al., 2022).

The plants in the Brassicaceae family, it has the highest demands for sulfur fertilization as they need sulfur for isothiocyanate synthesis (mustard oils). Plants such as horseradish and radish from root vegetables, onions and garlic from the Liliaceae family, spinach from the leafy vegetables and pepper from the fruit and vegetable group produce essential oil (Smatanová et al., 2004).

Although the sulfur concentration in the plant increased significantly ( $p < 0.05$ ) with the increasing sulfur dose in the study, no difference was observed between the sulfur doses of 40 and 80 kg da<sup>-1</sup>. An increase in soil sulfur was observed with increasing sulfur doses compared to the control. In a study on pepper, the S content of pepper increased with increasing S doses, and it was reported that there was no effect when the S content in the soil was 30.6 mg kg<sup>-1</sup> (Smatanová et al., 2004).

Gülser and Ayaş (2016) stated that, the application of increasing doses of sulfur in the spinach plant caused a significant decrease in the Mn content of the plant. The lowest Mn content was obtained with 124 mg kg<sup>-1</sup> in S3 (375 g m<sup>-2</sup> elemental sulfur) application, while the highest Mn content was obtained as 135 mg kg<sup>-1</sup> in S0 (control) application.

## **5. Conclusion**

Considering the S and Mn concentration values in the plant, it was determined that 40 kg S da<sup>-1</sup> and 1 kg Mn da<sup>-1</sup> doses affected these values positively. However, S and Mn applications are ineffective in the growth parameters examined. According to the data obtained, it has been concluded that if there is sufficient sulfur in the soil for rocket, there is no need for additional sulfur fertilization, and also unnecessary fertilizer consumption should be avoided considering the economic difficulties that the farmer will experience. Considering the results obtained, it is thought that it will be beneficial to conduct field trials and investigate the effects of sulfur and manganese on other growth parameters.

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## **Author Contribution**

All intellectual rights in the article belong to the author. The author completed the study alone in the stages of obtaining the results of the experiment, laboratory analysis and writing the article.

## **Conflict of Interest**

The author declared that there is no conflict of interest.



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