



Effect of different sulfur levels plus *Thiobacillus* on yield and yield components of Canola (*Brassica Napus L.*) cultivars in Gorgan, Iran

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ABSTRACT

In an effort to determine the effect of different sulfur levels plus *Thiobacillus* (*Thiobacillus* sp.) bacteria on yield, yield components and protein content of canola cultivars, a factorial experiment was conducted in a randomized complete block design with three replications in 2014-2015. The experimental treatments consisted of three hybrid genotypes of winter canola (Hyola401, RGS003 and Gerry) and sulfur at five levels (0, 250, 500, 750 and 1000 kg ha⁻¹, plus *Thiobacillus*). The results indicated that various cultivars of canola and different values of sulfur plus *Thiobacillus* had a significant effect on studied factors. As Hyola 401 contained the highest number of pods per plant, 1000 grain weight, grain yield and shoot dry matter. Gerry genotype also had the maximum height to the first branch and number of branches, while the largest diameter of main stem was observed in RGS003 and Gerry. Moreover, the 1000 kg S ha⁻¹ treatment achieved maximum height to first branch and harvest index, while the highest number of pods per plant, number of branches, grain yield and shoot dry mass were found in 750 and 1000 kg S ha⁻¹ treatments. In the interactions, the highest grain yield was observed in the interaction of Hyola401 in 1000 kg S ha⁻¹, while the highest protein content in the interaction of Gerry cultivar was obtained in 750 and 1000 kg S ha⁻¹. In conclusion, sulfur and thiobacillus supplementation could be lead to better crop performance and significant increment in protein level of canola seeds.

INTRODUCTION

As the world population grows, there is an increasing demand for food products on a daily basis. Although the global food reserves in terms of wheat, rice, corn and beans are discussed as main dishes, oilseeds play an undeniably important role in this goal. Containing more than 40% oil and around 40% protein meal canola (*Brassica napus* L.) has been known as a major type of oilseed in the world over recent

decades (FAO 1995). As one of the most important oilseeds, canola accounts for about 12% of the total global production of oilseeds, amounting to nearly 377.6 million tons (FAO 2007).

One of the key agricultural management practices is fertilizing, since it affects the quantity and quality of crop yield (Farzbod et al. 2014). Sulfur is the fourth basic element needed for crop production and the third major element required by canola (Grant and Bailey 1993). Involved in chlorophyll production, insufficient amounts of sulfur will turn the leaves yellow and reduce photosynthesis in plants. Sulfur is also applied in the construction of a few important amino acids such as methionine and cysteine, the lack of which particularly in young plant organs would disrupt growth. Involved in cell division, sulfur can affect fertility and regeneration in canola. Sulfur deficiency affects root growth more than shoot growth. In this scenario, root activity decreases to absorb water and nutrients from the soil (Khodbarin and Eslam Zadeh 2001; Mohammadi Nick Pour et al. 2005).

Cruciferous plants, including canola, need higher levels of sulfur compared to other important

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crop species such as maize and wheat. For instance, 16 kg of sulfur is required to produce one ton of canola (per hectare), while 2-3 kg of sulfur is required to produce one ton of wheat (Blake Kalf et al. 2001).

A few researchers have reported that sulfur deficiency delays flowering. Moreover, it can reduce the number of siliques toward the plant end as well as the number of grains in each silique. In soils suffering sulfur deficiency, application of 50 kg S ha⁻¹ can lead to maximum crop reaction. Any higher amounts of sulfur are not recommended because of the possible adverse impact on the quality of canola seeds, e.g. lowered amount of oil and intensified glucosinolates (Malakuti et al. 2000).

The biological oxidation of sulfur in the soil is mainly handled by Thiobacillus the population of which in Iranian soils is insignificant due to low amounts of organic matter, previous use of sulfur and their inoculants (Kariminia and Shahrestani 2003). Therefore, if elemental sulfur is dispersed on the soil surface and replaced immediately along with organic matter underneath the soil, the sulfur oxidation will take place faster in the vicinity of moisture and Thiobacillus (Hamedi and Jafari, 2007).

This study attempted to examine the effect of different levels of sulfur plus Thiobacillus on yield and yield components.

MATERIALS AND METHODS

The experiment was conducted in crop year 2014-2015 in Gorgan at latitude of 36° 50' N, longitude of 54° 22' E, height above sea level of 77 and annual average rainfall of 600-650 mm.

The experiment was conducted in facrotrial based randomized complete block design with three replications. Three hybrid genotypes of winter canola, including Hayola 401, RGS and Gerry, were employed as the first factor and sulfur levels of 0, 250, 500, 750 and 1000 kg S ha⁻¹ plus Thiobacillus as the second factor (1kg for all plots according to factory instructions). The seeds were planted in November 2014 at a rate of 5 kg per hectare (density: 100 plants per square meter).

Prior to treatments and tillage, a combined sample was obtained from the farm soil at depth of Table 1. The results of soil analysis

Soil depth (cm)	Lime (%)	pH	electrical conductivity (ds m ⁻¹)	Clay (%)	Silt (%)	Sand (%)	Total nitrogen	Sulfur (ppm)	Phosphorus (ppm)	Potassium (ppm)
0-30	13	7.7	0.8	41	45	11	0.13	25	1.0	380

0-30 cm, on which physical and chemical analyses were carried out. The results of soil analysis demonstrated loam texture (Table 1).

Phosphate fertilizers (triple super phosphate) at a rate of 100 kg per hectare and potassium (potassium sulfate) at a rate of 100 kilograms per hectare were inoculated into the soil entirely prior to planting. The required 150 kg per hectare of nitrogen fertilizer was given to the soil in three stages. After preparing the ground, the planting map was implemented. Each experimental plot for each genotype involved four 5-meter planting lines at 25-cm spaces. Two lateral rows together with 50 cm from the beginning and the end of each row were considered margins, while the second and third rows were used for seasonal sampling from grain yield and yield components. There were a total of 45 experimental plots at three replications.

Having been established, the plant intervals were thinned out to achieve a density of 100 plants per square meter. For this purpose, the planting distance on the line was set to be 5 cm. The plants were thinned manually over January and February. Irrigation took place in two stages, one after planting and the other within 20 days after the first irrigation. After harvesting, the number of pods per plant, seeds per pod, 1000 grain weight, yield, plant height, height to the first branch, number of branches, diameter of main stem, shoot dry matter, harvest index and protein content (%) were determined.

The plants were harvested based on 95% browning of the pods, which was converted into kilograms per hectare after measuring the grain yield.

The protein content was measured by pouring 1 g of milled sample inside a protein digestion tube. Then, 7.5 g of potassium sulfate and 0.5 g of copper sulfate (CuSO₄.5H₂O) as a catalyst were added. At the next stage, 20 ml of concentrated sulfuric acid was added and the tubes were heated for 4 hours until full digestion and the solution turning green. Then, the tubes were completely cooled down and then 50 ml of distilled water was added to each tube. The digested tubes were connected to a distillation apparatus and approximately 90 cc of 40% soda solution was added to it. In the opposite side, 50 ml of 4% boric acid solution was poured into a 500 ml flask to

collect the ammonia fumes and then heated until full distillation.

A few drops of reagent (red methyl + green bromocresol) were added to about 200 ml of collected solution, which was then titred by normal HCl solution 0.1 until red was solid

Protein content (%) = $0.8755 \times$ consumption volume / g sample (Iranian National Standards Organization)

The variance analysis of data involved SAS 9.2, while the mean values were compared through LSD at probability level of 5%. At the next stage, several charts were drawn in MS EXCEL.

RESULTS AND DISCUSSION

Analysis of variance on traits

As indicated in Table 2, the number of pods per plant, height to the first branch, number of branches, diameter of main stem, grain yield and shoot dry matter involved probability level of 1%, while 1000 grain weight involved a probability level of 5%. Furthermore, the number of pods per plant, height to the first branch, number of branches, grain yield, shoot dry matter and harvest index were affected by sulfur content at probability level of 1%, whereas the number of seeds per pod, grain weight, plant height, diameter of main stem and protein were not affected by sulfur levels. Interaction of genotype and sulfur was also significant only on grain yield and protein content at probability levels of 1% and 5%, respectively.

Results of comparing the average values of traits

Number of pods per plant

The results variance of data analysis suggested that there was a significant difference between cultivars in terms of the number of pods per plant at probability level of 1%. In fact, the highest number of pods per plant was achieved by Hayola 401 at 64.31, while the lowest number of pods per plant was found in RGS at 48.98 and Gerry at 54.52.

Moreover, there was a significant difference between sulfur levels in terms of the number of pods per plant at probability level of 1%. The highest number of pods per plant was found in sulfur levels of 1000 and 750 kg ha⁻¹ at 62.4 and 61.17, whereas the lowest number of pods per plant was found in 0 kg ha⁻¹ at 47.77. However, treatments AB and A indicated no statistically significant differences.

Number of pods per plant is counted as one of the most important yield components. Basically, number of pods per plant is the defining characteristic of canola yield, because the pods cover the seeds on the one hand and supply photosynthetic material required by seeds while

determining their weights on the other hand (Clarke and Simpson 1978).

The cultivars producing lower numbers of pods and yet have larger pod length are preferred over other cultivars with shorter pods, because longer pods tend to hold a larger number of seeds (Azizi et al. 1999). Diepenbrock (2000) reported that the number of pods per plant was effective in grain yield, i.e. a feature affected by the survival of branches, buds and flowers. Increased green surface of pods would lead to higher seed growth and lower abortion.

(Mostafavi Rad et al. 2012) reported that Licord variety achieved the highest number of pods per plant. In the early stages of grain filling, the pods contribute to growth and development through photosynthesis. Grain yield strongly depends on pods since they play a major role in photosynthesis after flowering. (Sina and Ramea 2012) reported that cultivars indicated a considerable difference in the production of pods per plant at probability level of 1%. The maximum and minimum numbers of pods per plant were achieved in Sarigol and Hayola 401, respectively.

Sulfur intake in canola increases the number of pods per plant mainly due to the important role of sulfur in improvement of photosynthesis Marshner (1995). A few other researchers have reported that sulfur deficiency delays flowering (Malakuti and Tehrani 2000). Moreover, it can reduce the number of pods toward the plant end as well as the number of grains in each pod. In soils suffering sulfur deficiency, application of 50 kg S ha⁻¹ can lead to maximum crop reaction. Any higher amounts of sulfur are not recommended because of the possible adverse impact on the quality of canola seeds, e.g. lowered amount of oil and intensified glucosinolates (Malakuti and Tehrani 2000).

1000 grain weight

The results of data variance analysis suggested that there was a significant difference between cultivars in terms of 1000 grain weight at probability level of 5%. In fact, the highest 1000 grain weight was achieved by Hayola 401 at 86.3 g, whereas the lowest was found in Gerry and RGS at 3.49 and 3.51 g, respectively.

As one of the yield components of canola, 1000-seed weight varies less frequently than other yield components (Azizi et al. 1999).

Sina and Ramea (2012) reported that 1000 grain weight was significant ($p \leq 0.01$), where the highest 1000 grain weight was obtained in Hayola 401. Aien (2008) also reported that the highest 1000 grain weight was achieved by Hayola 401. Mostafavi Rad et al. (2012) reported similar results,

Table 2. The results of variance analysis (mean square) for the effect of cultivar, sulfur and interaction of cultivar*sulfur on the traits under study

S.O.V	DF	Number of pods per plant	Number of grains per pod	1000 grain weight	Plant height	Height to first branch	Number of branches	Height of main stem	Grain yield	Shot dry matter	Harvest Index	Protein
Replication	2	140.10 ^{ns}	89.23 ^{**}	25.78*	4580.7 ^{**}	9.12 ^{ns}	0.18*	5.20 ^{**}	47625.3 ^{ns}	7188457.9 ^{ns}	0.71 ^{ns}	6.68 ^{ns}
Cultivar	2	903.58 ^{**}	21.13 ^{ns}	9.66*	1283.84 ^{ns}	2359.74 ^{**}	0.88 ^{**}	11.29 ^{**}	279840.5 ^{**}	32089170.5 ^{**}	0.77 ^{ns}	9.09 ^{ns}
Sulfur	4	333.01 ^{**}	7.93 ^{ns}	0.98 ^{ns}	1051.8 ^{ns}	289.26 ^{**}	1.46 ^{**}	1.46 ^{ns}	279380.98 ^{**}	16959824.9 ^{**}	23.28 ^{**}	4.80 ^{ns}
Cultivar*Sulfur	8	76.16 ^{ns}	9.35 ^{ns}	3.17 ^{ns}	379.4 ^{ns}	24.6 ^{ns}	0.28 ^{ns}	0.74 ^{ns}	248788.85 ^{**}	343403.74 ^{ns}	3.11 ^{ns}	9.21 ^{**}
Error	28	45.40	13.75	1.25	441.92	14.77	0.05	1.12	17566.2	255295.5	2.31	3.93
CV%	-	13.3	19.67	24.11	17.63	5.91	4.7	14.36	7.87	11.81	11.76	9.73

* and ** represent the significance of data at probability levels of 5% and 1%, respectively. Moreover, ns stands for not significance of data.

Table 3. The results of comparing mean effects of cultivars and sulfur on several traits under study

DF	Number of pods per plant	Number of grains per pod	1000 grain weight	Plant height	Height to first branch	Number of branches	Height of main stem	Grain yield	Shot dry matter	Harvest Index	Protein
Cultivars											
Hayola 401 (a1)	64.31A	20.06	3.86A	111.84	52.28C	4.53C	6.4B	3471.41A	11026.71A	31.48	19.52
RGS (a2)	54.52B	18.3	3.51B	116.13	65.3B	4.84B	7.6A	3350.71B	10743.12B	31.19	20.52
Gerry (a3)	48.98B	17.8	3.49B	129.57	77.36A	5.01A	7.8A	3355.1B	10518.02B	31.90	21.06
Sulfur levels											
0 (b1)	47.77C	18.5	3.63	124.1	55.77D	4.42B	6.94	2597.5C	10123.87B	30.11C	18.71
250 (b2)	52.44BC	18.3	3.56	125.44	64.10C	4.64B	7.69	2712.2BC	10729.12AB	31.4B	19.90
500 (b3)	55.9AB	20.4	3.65	131.44	65.86BC	4.57AB	7.15	2989.9B	10753.13AB	32.90AB	20.13
750 (b4)	61.17A	18.3	3.57	137.02	69.14AB	4.87A	7.18	3312.2A	10734.29A	32.58AB	20.53
1000 (b5)	62.4A	18.08	3.8	147.5	69.97A	5.45A	7.97	3495.8A	11095.9A	33.7A	22.55

stating that Licord obtained the highest 1000 grain weight.

Height to first branch

The results of variance analysis suggested that there was a significant difference between cultivars in terms of height to first branch at probability level of 1%. In fact, the maximum height to the first branch was found in Gerry at 77.36 cm, whereas the minimum height to the first branch was found in Hayola 401 at 52.28 cm. Moreover, there was a significant difference between sulfur levels in terms of height to first branch at probability level of 1%. In fact, the maximum height to the first branch was found in sulfur level of 1000 kg ha⁻¹ at 69.97 cm, while the minimum height to the first branch was found in 0 kg ha⁻¹ at 55.77 cm. However, there was no statistically significant difference between treatments AB and A.

This trait is important for harvesting, and genotypes whose first branches form at a higher point tend to be more suitable for mechanized harvesting.

The results obtained by Svecnjak and Rengel (2006) and Walton (2004) showed the greatest height to the first branch was achieved by Licord.

Mostafavi Rad et al. (2012) stated that the application of 40 kg S ha⁻¹ achieved the maximum height to the first branch.

Number of branches

The results of variance analysis suggested that there was a significant difference between cultivars in terms of the number of branches at probability level of 1%. In fact, the highest number of branches was found in Gerry at 5.01, whereas the lowest number of branches was found in Hayola 401 at 4.53.

Moreover, there was a significant difference between sulfur levels in terms of the number of branches at probability level of 1%. The highest number of branches was achieved by sulfur levels of 1000 and 750 kg ha⁻¹ at 5.45 and 4.87, while the lowest number of branches was found in 0 and 250 kg ha⁻¹ at 4.42 and 4.64. However, there was no statistically significant difference between treatments AB and A.

Different cultivars have involve varying genetic potentials to produce lateral buds and thus produce lateral shoots. In fact, this potential is affected by different conditions such as plant density. The cultivars generated under different environmental conditions and densities probably regulate the production of lateral buds through switching on and off certain genes related to the production of lateral buds as well as activating/deactivating the hormonal system. The

branching rate in canola depends on the cultivar, environment, plant nutrition and farm management practices (Azizi et al. 1999). Overall, researchers have reported the different branching capacities between Brassica species as well as several cultivars of a species (Rao et al. 1991). Sina and Ramea (2012) reported that the effect of cultivar on the number of branches was significant at probability level of 1%. In their study, Hayola 401 outperformed RG4504 and Sarigol.

Mostafavi Rad et al. (2012) stated that the application of 40 kg S ha⁻¹ achieved the largest number of branches.

Diameter of main stem

The results of variance analysis suggested that there was a significant difference between cultivars in terms of diameter of main stem at probability level of 1%. The highest diameter of main stem was found in Gerry and RGS at 8.7 and 6.7 mm, whereas the minimum diameter of main stem was found in Hayola 401 at about 6.4 mm.

A larger diameter of main stem brings about several advantages and disadvantages. One of the advantages can be accumulation of nutrients in the crown and stem, which in turn leads to better overwintering under inclement weather conditions, while one disadvantage can be a severe loss of grains during the mechanized harvest.

Grain yield

The results of variance analysis suggested that there was a significant difference between cultivars in terms of grain yield at probability level of 1%. The highest grain yield was achieved by Hayola 401 at 3471.41, while the lowest grain yield was found in RGS and Gerry at 3350.71 and 3355.1.

Moreover, there was a significant difference between sulfur levels in terms of grain yield at probability level of 1%. The highest grain yield was achieved at sulfur levels of 1000 and 750 kg ha⁻¹ at 3495.8 and 3312.2, whereas the lowest yield was found in 0 kg ha⁻¹ at 2597.5.

Sina and Ramea (2012) and Aien (2008) reported that the effect of cultivar on grain yield was significant at probability level of 1% Sina and Ramea (2012) also, compared the average values of grain yield among three genotypes, revealing that Hayola 401 achieved the largest and Sarigol achieved the smallest grain yield. Omidi et al. (2007) reported that canola cultivars showed significant differences in terms of yield and yield components. Furthermore, Mostafavi Rad et al. (2012) reported that Licord achieved the highest grain and oil yield.

McGrath and Zhao (1996) stated that the application of appropriate sulfur level in areas

suffering sulfur deficiency can enhance the canola yield four times. In a study on the effect of sulfur on canola in India, it was reported that the application of different sulfur sources before flowering can enhance grain and oil yield. In their experiments, Mostafavi Rad et al. (2012) found that the treatment of 40 kg sulfur per hectare outperformed the other treatments in terms of traits such as grain and the oil yield. In a study on the effect of sulfur on canola oilseed yield, the maximum yield per hectare was reported in 20 kg Jackson (2000). Several researchers have reported that canola cultivars and species reacted differently to sulfur fertilization in terms of several traits such as yield components, oil and protein concentration (Grant et al. 2003, Malhi and Gill 2006).

Shoot dry matter

The results of variance analysis suggested that there was a significant difference between cultivars in terms of shoot dry matter at probability level of 1%. The highest shoot dry matter was achieved by Hayola 401 at 11026.71, whereas the lowest shoot dry matter was found in RGS and Gerry at 10518.02 and 10743.12, respectively.

Moreover, there was a significant difference between sulfur levels in terms of shoot dry matter at probability level of 1%. The maximum shoot dry matter was achieved at sulfur levels of 1000 and 750 kg ha⁻¹ at 11095.9, while the minimum shoot dry matter was found at 0 kg ha⁻¹ at 10734.29. However, there was no statistically significant difference between treatments AB and A.

Mendham et al. (1981) and Rao et al. (1991) stated that cultivars with larger leaf area at the

Table 4. The results of comparing mean interaction effects of cultivars and sulfur on several traits under study

Treatment	Grain yield	Protein
Hayola 401×0	2710C	19.65C
Hayola 401×250	2991.6BC	19.20C
Hayola 401×500	2980.5BC	20.94B
Hayola 401×750	3601.4AB	21.78AB
Hayola 401×1000	3670.1A	22.09AB
RGS×0	2790.4C	19.15C
RGS×250	2994.6BC	19.61C
RGS×500	3101.7B	21.42B
RGS×750	3289.6AB	22.3AB
RGS×1000	3371.5AB	23.01AB
Gerry×0	2801.4C	18.22C
Gerry×250	2980.4BC	19.61BC
Gerry×500	3001.2BC	21.99B
Gerry×750	3119.5B	23.2A
Gerry×1000	3401.9AB	25.2A

beginning of growth stage receive more light and produce more dry matter.

It is critical to strike a balance between the intake of nitrogen and sulfur fertilizers for better control of canola seed quality (Fismes et al. 2000). Theoretically, 16 tons per hectare of elemental sulfur is required to 1% of soil lime to a depth of 30 cm (Alaei Yazdi and Barzegar Firoozabadi 2004). McGrath and Zhao (1996) and Rudy et al. (2000) showed that sulfur can enhance the ratio of reproductive organs to the total dry matter. Moreover, sulfur deficiency inhibits the growth of reproductive organs and even leads to sterility in pods.

Harvesting index

The results of variance analysis showed that there was a significant difference between sulfur levels in terms of harvest index at probability level of 1%. The maximum harvest index was achieved by 1000 kg ha⁻¹ at 33.7%, whereas the minimum harvest index was found in 0 kg ha⁻¹ at 30.11%. However, there was no statistically significant difference between treatments AB and A.

The harvest index reflects the distribution ratio of photosynthetic materials between economic yield and of biological yield Shariati (2000).

Mostafavi Rad et al. (2012) stated that increasing sulfur levels enhanced the harvest index.

Results of comparing the average interaction effects of traits under study

Grain yield

The interaction effects of treatments left a significant effect on grain yield at probability of 1%. The highest grain yield was achieved by 1000×Hayola 401 at 3670.1, whereas the lowest grain yield was found in treatments 0×Hayola 401, 0×RGS and 0×Gerry at 2710, 2790.4 and 2801.4, respectively. However, there was no statistically significant difference between treatments AB and A.

Protein

The interaction effects of treatments left a significant effect on protein content (%) at probability of 1%. The highest protein content was achieved by treatments 1000×Gerry and 750×Gerry at 25.2 and 23.2%, while the lowest protein content was found in 0×Gerry, 0×RGS, 250×Hayola 401, 250×RGS and 0×Hayola 401 at 18.22, 19.15, 19.20, 19.61 and 19.65%, respectively. However, there was no statistically significant difference between treatments AB and A.

CONCLUSION

The results indicated that various cultivars of canola and different values of sulfur plus

Thiobacillus had a significant effect on studied factors. As Hyola 401 contained the highest number of pods per plant, 1000 grain weight, grain yield and shoot dry matter. Gerry genotype also had the maximum height to the first branch and number of branches, while the largest diameter of main stem was observed in RGS003 and Gerry. Moreover, the 1000 kg S ha⁻¹ treatment achieved maximum height to first branch and harvest index, while the highest number of pods per plant, number of branches, grain yield and shoot dry mass were found in 750 and 1000 kg S ha⁻¹ treatments. In the interactions, the highest grain yield was observed in the interaction of Hyola401 in 1000 kg S ha⁻¹, while the highest protein content in the interaction of Gerry cultivar was obtained in 750 and 1000 kg S ha⁻¹. In conclusion, sulfur and Thiobacillus supplementation could be lead to better cropperformance and significant increment in protein level of canola seeds.

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