Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Effects of Thiobacillus, Sulfur and Micronutrient Spray on Some Traits of Green Beans

Morteza Motamed*[1], Mehdi Baradaran Firoozabadi[2], Jafar Masoud Sinaki[1]

[1] *Department of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran*

[2]*Faculty Member, Shahrood University of Technology, the Agriculture Department, Shahrood, Iran*

(Received: 6 January 2018 Accepted: 17 March 2018)

KEYWORDS

Zinc sulfate;
Green beans;
Sodium molybdate;
Sulfur powder;
Yield

ABSTRACT: A factorial experiment with the split plot arrangement using the complete randomized block design with three replications was carried out adjacent to the Agriculture School of Shahrood University of Technology in 2010-2011 to study the effects of sulfur and *Thiobacillus* application and zinc and molybdenum sprays on some traits of green beans. The treatments included three levels of sulfur (zero, 100 kg/ha of sulfur powder, and 100 kg/ha of sulfur powder + *Thiobacillus* bacteria) as the main factor, and three levels of zinc spray (0, 5, and 10g/l) and two levels of molybdenum spray (0 and 0.5 g/l) as the sub-main factors. The treatments that included sulfur were applied before seeding. Zinc (using zinc sulfate) and molybdenum (using sodium molybdate) were sprayed during the growing season and 5 weeks after planting. Results showed the treatment of applying sulfur at 100 kg/hectare and *Thiobacillus* bacteria increased leaf fresh and dry weights by 57 and 54%, mean pod length by 14%, and number of lateral branches by 25% compared to the control. Moreover, this treatment increased pod fresh and dry weights by 41.6 and 42.11%, respectively, compared to the treatment of not applying sulfur, and improved yield by 42% compared to the control. Considering the results concerning yield in response to sulfur, zinc, and molybdenum, it can be said that application of sulfur and *Thiobacillus* together with zinc spray can be very useful in growing green beans.

INTRODUCTION

Green beans (*Phaseolus vulgaris* L.), the second most important legume after peas, are of worldwide nutritional importance [1], and are among plans that can be grown at various times as a spring or summer crop [2]. Of the important field crops, beans respond the most to application of micronutrients and deficiencies of elements such as zinc, boron, iron, molybdenum, and copper disrupt their natural growth and development and reduce their yield. *Thiobacillus* are a group of gram-negative bacteria that derive their energy needs through oxidizing inorganic sulfur compounds, play a very important role in preventing leaching of mineral substances, especially sulfur, and lead to recovery of metallic compounds [3]. Among the 16 nutrients required by plants, seven (Fe, Zn, Mn, B, Cu, Mo, and Cl) are needed in slight amounts, and that is why they are called micronutrients [4]. Sulfur is one the structural constituents of the amino acids cysteine and methionine, and plays an essential role in the synthesis of proteins and enzymes. Therefore, insufficient availability of sulfur can influence yield and quality of crops [5]. Zinc is one of the essential micronutrients in plant nutrition [6], and molybdenum is another element that must be paid attention to in nitrogen nutrition, is an essential element for the activity of Mo-nitrogenase and, therefore, is important in the nutrition of pulses [7]. Studies on beans have shown increased application rates of the sulfur ferti-

* Corresponding author: motamedmorteza@yahoo.com (M. Motamed).

lizer magnesium sulfate improve leaf weight and surface area [8]. Sulfur fertilizers (potassium sulfate) increased stem fresh weight in mung beans [9]. Results indicated improved growth indices, increased yield of green beans through increased plant height, greater number of leaves, larger fresh weight of plants and of pods, and longer pods caused by zinc spray [10]. Praveen Kumar et al [11] stated increased sulfur content of mung beans improved leaf surface area. Abdul-Latif et al [12] showed iron and zinc foliar sprays, singly or in combination, significantly increased plant height and number of branches per plant and pod weight in mung beans. Karimi et al [13] conducted research on colza and reported the maximum number of pods per plant was achieved by simultaneous application of sulfur and *Thiobacillus* bacteria. Zinc sprays significantly increased the number of fertile pods in green beans [10].

Absorption of all the required elements is usually not possible in second crops and, hence, complementing soil micronutrients through foliar sprays can improve plant growth under stress conditions. Green beans have a short growing season (because their green pods are consumed), are insensitive to planting date, are not a demanding crop, are adapted to harsh conditions, and planting them as a second crop will improve soil fertility(because of nitrogen fixation) in addition to increasing farmers' income.

This research intended to study whether green beans could be used as a second crop after cereals in Shahrood, and especially in Bastam (which is one of the centers of bean production in Shahrood).

MATERIALS AND METHODS

This study was conducted in a field adjacent to the Agriculture School of the Shahrood University of Technology (in Bastam) in an area of about 900 m^2 on green beans in the crop year 2010-2011. The region has a semi-arid to arid climate, an altitude of 1349 meters, latitude of 36˚ and longitude of 54˚, average rainfall of 200 mm, and clay loam soil.

The experimental project was performed in the form of a factorial experiment with the split plot arrangement based on complete randomized blocks with three replications each consisting of six blocks. The treatments included three levels of sulfur (zero, 100 kg/ha of sulfur powder, and 100 kg/ha of sulfur powder + *Thiobacillus* bacteria) as the main factor, and three Zn spray levels (zero, 5, and 10 g/l) and two Mo spray levels (0, and 0.5 g/l) as the sub-main factors.

Before testing the depths of zero to 10 and 10 to 30 cm of soil sampling and soil profile was determined (Table 1). In order to see the effects of treatments of chemical fertilizers were used.

Table 1. The results of the study soil

SAR 2.9	Total Nitrogen (%) 0.056	Organic Carbon (%) 0.08	The Percentage of Neutralizing 25.5	Soil Acidity 7.97	The electrical Conductivity (dSm-1) 2.45	Saturation (%) 33.2
S.S.P 33.7	Moisture Content 3.1	Sand (%) 41.0	In Between (%) 30.0	Clay (%) 29.0	Available Potassium (p.p.m) 220.0	Phosphorus (p.p.m) 18.0
Clare (p.p.m) 7.25	Sulfate (p.p.m) 17.0	Total Anions (p.p.m)24.25	Calcium (p.p.m) 10.0	Magnesium (p.p.m) 6.5	Sodium (p.p.m) 8.4	Total Cations (p.p.m) 24.9

Seeds were planted in rows 50 cm apart with a distance of 10 cm between adjacent seeds (20 plants/m^2). In all, the experiment consisted of 42 plots each 10 m long and with 4 rows. The two side rows were considered the borders, and the two middle ones were used for applying the treatments and for making the measurements. Before planting, all seeds were inoculated with the rhizobial inoculant specifically prepared for beans. To make sure the seeds were impregnated with the bacteria, a 20% solution of the white substance in the inoculant package that looks like was prepared first, and the seeds were put in the solution to become completely wet and sticky. The inoculant was then poured on the seeds and the container was rotated like a rolling cylinder so that the seeds were completely exposed to the sticky material and the inoculant.

During the experiment, drip tape irrigation was carried out at 72-h intervals. The irrigation system was similar to that is common in pressurized irrigation systems and included a pump, a disc filter, water transfer lines, and drip tapes. Weeds were controlled manually twice, and no pests or diseases were observed during the growing season. Treatments that included sulfur were applied before planting. Since moisture is one of the necessary conditions for the start of *Thiobacillus* activity, the field was first irrigated and, at field capacity, 8- cm deep holes were dug on the rows. The required amounts of sulfur powder plus *Thiobacillus* were then poured in, and the holes were filled up to 3 cm from the soil surface (which was the planting depth). After a week, the seeds were planted in the same holes in one day. Zinc (zinc sulfate) and molybdenum (sodium molybdate) were sprayed in the vegetative growth stage five weeks after planting.

After eliminating border effects, 6 plants were randomly harvested in each plot and their fresh weights were recorded, they were then put in a drying oven at 70 ˚C for 48 h, and were weighed again to determine their dry weights [14]. To determine the number of pods per plant, six plants were randomly selected (after eliminating border effects) and the number of pods in each was counted and the numbers were added up and divided by six to determine [14]. To measure the average pod length, 6 plants were randomly selected in the field and a ruler was used to measure and record the lengths of the pods. At full maturity, and after eliminating border effects, plants were randomly harvested and the average number of lateral shoots per plant was calculated [15]. At the end of the growing season and after eliminating border effects, whole plants were harvested at ground level, and a precision balance with one- gram accuracy was used to measure yield, which was converted to yield per hectare [16].

SAS 9.1 and MSTATC were employed for statistical analysis of the data, Excel was used for drawing the diagrams, and the LSD test was performed at the 5% probability level for comparison of the means.

RESULTS AND DISCUSSION

Leaf fresh and dry weights

Table 2 shows results of ANOVA (mean squares) of leaf fresh and dry weights. Rates of sulfur application had significant effects on leaf fresh and dry weights, and the maximum leaf dry weights of 9.02 and 9.52g/plant were observed in the treatments of applying 100 kg/ha of sulfur powder and 100 kg/ha of sulfur + *Thiobacillus*, respectively. Moreover, the treatment of applying 100 kg/ha of sulfur powder and *Thiobacillus* yielded the maximum fresh leaf weight of 35.28 g/plant, which was not significantly different from that of the treatment in which 100 kg/ha of sulfur powder was applied (Table 3).

Different plants require various amounts of sulfur for their desirable growth, and sulfur constitutes 0.2-0.5% of their dry weights. Among plant families, the Leguminosae family requires large quantities of sulfur. Although sulfur contents of plants are mainly similar to their phosphorous contents, the care and sensitivity farmers show to applying phosphorous fertilizer in crop plants are not observed in relation to sulfur application [17]. Increased levels of sulfur in mung beans increased leaf surface area [18]. Sulfur increased leaf dry weight in mung beans [19]. It seems that increased levels of sulfur absorption have synergistic effects on nitrogen absorption leading to greater number of leaves in green beans [20]. Use of fertilizers containing sulfur had positive effects on leaf fresh and dry weights [21]. Salem et al [22] reported increased sulfur levels improved leaf surface area in spring wheat. Sulfur increased leaf weight in colza [23], and Oliveira et al [8] noticed increased rates of sulfur fertilizer (magnesium sulfate) increased leaf weight and surface area.

Pod fresh and dry weights

Results of ANOVA indicated various rates of sulfur application and zinc sprays had significant effects on pod fresh and dry weight in green beans at the 1 and 5% levels, respectively. The treatment of applying 100 kg/ha of sulfur powder+ *Thiobacillus* had the maximum effect with the average pod fresh and dry weights of 185.96 and 35.18 g/plant, respectively. In treatments that received 100 kg/ha of sulfur powder but no *Thiobacillus*, pod dry weights were not significantly different from that of the control. However, the fresh weight of plants in these treatments were significantly greater compared to that of the control, which showed plants in these treatments received greater quantities of water because of sulfur application (Table 2). Among the levels of zinc spray, the treatment of spraying 10 g/l achieved the largest pod fresh and dry weights with the averages of 168.06 and 31.45 g/plant, respectively. The increased pod dry weight at this level of zinc application was 12.8% higher than that of the control, while the lower rate of zinc application (5 g/l) improved this trait by only 7.6 % (Table 3).

Use of sulfur at 30 kg/ha improved plant height, number of pods per plant, straw yield, seed yield, and dry matter yield by 14, 56, 25, 20, and 26% in soybean plants [24]. Since sulfur is a constituent of ferredoxins in chloroplasts, it plays an important role in photosynthesis [25]. Moreover, sulfur has an important role in the quality of seeds in pods so that its deficiency causes abortion of seeds in the pods [26]. Furthermore, Karaman et al [27] showed dry matter production increased in bean plants with increases in zinc concentration. Moreover, Abdul-Latif et al [12] noticed foliar sprays of Fe and Zn, singly or in combination, significantly increased plant height, number of branches in plants, and pod weight in mung beans, and sulfur treatments improved fresh pod yield and growth features of green beans [10].

Number of pods per plant

Results of ANOVA indicated the effects of sulfur and zinc, and the mutual effects of sulfur and zinc, on the number of pods per plant were significant at the 1 and 5% levels, respectively (Table 2). Application of 100 kg/ha of sulfur powder increased the number of pods per plant by 1.5 on average. Of course, this increase was not significant. When *Thiobacillus* bacteria were used together with this rate of sulfur application, the number of pods per plant increased by 4.33, which was statistically significant. It placed the treatment in a separate statistical group (Table 3). The higher level of sulfur application increased the number of pods per plant in green beans because of the positive effects of sulfur on Rhizobia bacteria, which was due to improved nitrogen absorption and was caused by the effects of sulfur on lateral branches (Table 2). Zinc spray at 10g/l resulted in the maximum number of pods per plant (20.77 0n average), and the higher level of zinc application increased the number of pods per plant at all levels of sulfur application. Among the mutual effects of sulfur and zinc sprays, the combination of 100 kg/ha of sulfur powder* zinc spray at 10g/l had the greatest effect (16.9 pods per plant).

According to Rinaldo et al [28], application of sulfur in colza increased the number of pods per plant through contributing to greater absorption of nitrogen. Results of research conducted by [29] also indicated increasing rates of sulfur application up to 50 kg/ha improved the number of pods per plant in jute. Applying agricultural sulfur at 400 kg/ha together with *Thiobacillus* had positive effects on the number of pods per plant in beans [30]. Karimi et al [13] conducted studies on colza and stated that simultaneous application of sulfur and *Thiobacillus* bacteria resulted in the maximum number of pods per plant. Sulfur improved the ratio of reproductive organs to total dry matter of plants, and that sulfur deficiency stopped growth of reproductive organs and even led to pod abortion [31, 32]. Mostafavi Rad et al [33] reported application of net sulfur at 40 kg/ha yielded the best results with respect to the number of siliques per plant in colza.

Zinc sprays at five and 10g/l increased the number of pods per plant by 9.2 and 11.7%, respectively. Tammy and Gridley [10] reported zinc sprays had significant effects on the number of fertile pods in green beans. In this relation, Pandi et al [34] noticed zinc sprays increased the number of pods, the number of seeds, and seed yield in black mung bean.

Pod length

Sulfur powder application and levels of zinc spray significantly influenced average pod length at the 5 and 1% level, respectively (Table 2). Sulfur powder at 100 kg/ha together with *Thiobacillus* significantly increased pod length compared to the control, but there were no significant differences between the treatments themselves in this respect. Zinc sprays at 10 g/l had the maximum effect on pod length (with average pod length of 16.63 cm): this treatment increased pod length by about 3 cm and was placed in the superior statistical group. Pod length with zinc sprays at 5 g/l was 14.82 cm on average, which was still significantly longer compared to the control (Table 3).

In the same relation, Monjurulalam et al [29] stated applying higher rates of sulfur application increased pod length compared to the control in Indian flax. Results of Tammy and Gridley obtained indicated growth indices and yield of green beans improved through increases in plant height, number of leaves, plant fresh weight, pod fresh weight, and pod length caused by zinc sprays, application of zinc sulfate and borax in black-eyed peas caused the greatest increase in pod length [37]. Syed Hamdan et al [38] believed zinc had positive effects on pod length in peas.

Yield

Different levels of sulfur application and zinc and molybdenum sprays had significant effects on yield at the 1% level (Table 2). Results obtained from comparison of the means of the main effects (Table 3) indicated the 100 kg/ha sulfur+

Thiobacillus treatment with 16.50 t/ha of fresh pods in green beans had the highest yield: it increased yield of green beans by 42% compared to the treatment that did not receive sulfur. Application of sulfur at 100 kg/ha without *Thiobacillus* also increased yield by 17.3%, which was significantly higher than that of the control. Moreover, zinc spray at 10 g/l resulted in maximum yield (14.90 t/ha), which was 15.8% higher than that of the control. It is worth mentioning that zinc sprays at 5 g/l also significantly increased yield compared to the control. Molybdenum sprays also increased yield by 4.9% compared to the control, which was statistically significant (Table 3).

Increases in the final yield of crop plants result from improvement in each yield component. As shown in this research, the components of yield were influenced by the treatments and, eventually, increases in each component led to improved total yield of green beans. Sulfur availability is involved in regulating nitrate reductase and ATP sulfurylase, and in increasing proteins and chlorophyll concentration, in pulses [39, 40].

Zinc sprays can be a suitable method for compensating for nutrient deficiencies, especially zinc, in light sandy soils. Most semi-arid regions have sandy soils that have problems on mobility of stimulating compounds and absorption by roots [41]. Zinc sprays had considerable effects on yield and yield components and quality of peanut [42].

Kholbarin and Eslamzadeh [43], showed molybdenum application influenced, and increased, dry weight and yield of beans.

Number of lateral branches

Among the sources of variation, only the effects of sulfur on the average number of lateral branches were significant at the 1% level (plants in the treatments of 100 kg/ha sulfur, and 100 kg/ha sulfur + *Thiobacillus* bacteria had 8.79 and 8.74 lateral branches, respectively). These numbers were significantly higher compared to 6.99 lateral branches in plants of the control treatment) (Table 3). It seems that increases in the number of lateral branches had a positive effect on the percentage of polyphyllous plants in green beans resulting from the application of sulfur. Moreover, more pods per plant may be produced because of the increase the number of lateral branches. In this relation, increased sulfur content in mung beans increased the number of lateral branches [19]. Results obtained on mung beans showed greater leaf surface area, number of lateral branches, number of nodes, dry weight of nodes, and plant height resulted from sulfur application [18]. Research conducted on various colza cultivars indicated raising the rate of sulfur application to 40 kg/ha increased the number of lateral branches by 10% compared to the control [33]. Raising the rate of sulfur application to 150 kg/ha (by using 45% bentonite) significantly increased the number of lateral branches in canola [44]. Raviv et al [45] also reported raising rate of sulfur application increased the number of lateral branches in spring safflower. Increased number of lateral branches in plants resulting from sulfur application could be due to improved levels of the hormone auxin and chlorophyll synthesis and increased photosynthesis [46].

Table 2. ANOVA (mean squares) of morphological and agronomic features in green beans influenced by sulfur and Thiobacillus bacteria application, and by zinc and molybdenum sprays

S.O.V	df	Leaf dry weights	Leaf fresh weights	Pod fresh weights	Pod dry weights	Number of pods per plant	Pod length	Yield	Number of lateral branches
Block	2	222.86	718.75	136881.32	399.14	40.48	1.66	5.93	25.57
Sulfur	2	707.53**	665.80*	638284.16**	1861.23**	86.83**	5.02*	243.12**	18.87**
Error	4	75.31	419.38	48110.91	140.29	17.58	4.02	6.65	1.58
Zn	2	70.40 ns	460.60 ns	311247.47*	907.59*	23.81**	35.25**	41.51**	2.39 ns
Mo	1	81.66 ns	247.39 ns	74816.99 ns	218.16 ns	1.67 ns	0.49 ns	13.58**	0.25 ns
S× Zn	4	69.80 ns	389.56 ns	26520.25 ns	77.33 ns	9.08*	0.24 ns	1.61 ns	1.87 ns
S×Mo	2	22.42 ns	431.84 ns	82469.32 ns	240.48 ns	3.08 ns	1.22 ns	0.66 ns	2.32 ns
Zn×Mo	2	15.90 ns	347.28 ns	11034.47 ns	32.17 ns	0.87 ns	1.50 ns	0.29 ns	2.73 ns
S× Zn×Mo	4	79.88 ns	440.23 ns	58910.56 ns	171.28 ns	4.79 ns	0.97 ns	1.14 ns	2.84 ns
Error	30	53.87	3.66	74843	218.24	3.19	1.06	1.28	2.31

ns,*,**:non-significant, significant at $P<0.05$ and $P<0.01$, respectively

Table 3. Comparison of the means of morphological and agronomic traits of green beans as influenced by various levels of sulfur application and zinc and molybdenum sprays

Sulfur (kg/ha)	Leaf dry weights (gr per plant)	Leaf fresh weights (gr per plant)	Pod fresh weights (gr per plant)	Pod dry weights (gr per plant)	Number of pods per plant	Pod length (cm)	Yield (T/ha)	Number of lateral branches
0	6.29 b	22.34 b	130.85 c	24.83 b	17.94 b	14.54 b	11.61 c	6.99 b
100	9.02 a	31.08 a	152.79 b	28.81 b	19.46 b	15.20 a	13.62 b	8.79 a
100 + Thiobacillus bacteria	9.52 a	35.28 a	185.96 a	35.18 a	22.27 a	15.58 a	16.50 a	8.74 a
Zn (gr/lit)								
0	7.58 a	25.53 a	147.61 c	27.88 b	18.59 b	13.87 c	12.87 c	8.11 a
5	8.56 a	32.82 a	156.64 b	30.01 ab	20.31 a	14.82 b	13.89 b	8.56 a
10	8.18 a	26.80 a	168.06 a	31.45 a	20.77 a	16.63 a	14.90 a	7.84 a
Mo (gr/lit)								
0	8.30 a	31.27 a	152.76 a	29.23 a	19.72 a	15.02 a	13.55 b	8.10 a
0.5	7.91 a	27.33 a	155.30 a	30.24 a	20.07 a	15.20 a	14.22 a	8.24 a

In each column, there is no significant difference between treatments with common letters according to Duncan test.

CONCLUSIONS

Sulfur and *Thiobacillus* application together with zinc sprays had promising effects in increasing yield of green beans, and these results are confirmed in the few studies that have been conducted on sulfur and *Thiobacillus* application together with zinc sprays. Considering results on responses of yield to sulfur application and zinc and molybdenum sprays in this research, it can be said that application of sulfur and thiobacillus together with zinc sprays has been considerably useful in green beans because, firstly, sulfur reduces pH in soils of arid regions thereby increasing availability of elements such as nitrogen, phosphorous, and other nutrients. Secondly, zinc application makes suitable quantities of this element available to plants without polluting the rhizosphere environment or the soil. Finally, application of sulfur together with *Thiobacillus* causes the maximum increase in fresh pod weight compared to the other treatments, and the valuable elements of nitrogen, phosphorous, and sulfur are utilized with suitable efficiency.

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