Role of Rhizobium, Organic fertilizer rates and Sulfur on Soil fertility, Nitrogen Balance and productivity of Broad Bean under South Sinai Conditions

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ABSTRACT

Soil fertility is one of the major limiting factors for crop’s productivity in Egypt and the world in general. Biological nitrogen fixation (BNF) has a great importance as a non-polluting and a cost-effective way to improve soil fertility through supplying N₂ to different agricultural systems. Faba bean (Vicia faba L.) is one of the most efficient nitrogen-fixing legumes that can meet all of their N needs through BNF. Therefore, understanding the impact of rhizobial inoculation and contrasting soil rhizobia on nodulation and N₂ fixation in faba bean is crucial to optimize the crop yield, particularly under low fertility soil conditions. The efficiency of Rhizobium leguminosarum in enhancing nitrogen fixation and nutrient uptake in Faba bean and improving soil fertility. the field experiment were carried out at privet farm at tour city South Sinai Governorate, during the two consecutive winter seasons of 2018/2019 and 2019/2020. The experiments were conducted to study the effect of Rhizobium leguminosarum inoculation, organic manure, soil amendment i.e., 0; 25; 30 and 35m³/fed., and four rates of sulfur application i.e., 0; 100; 150 and 200 kg/fed., on growth, productivity, nodulation, soil microbial counts, enzymatic activities, soil total N, P, K, Fe, Zn and Mn, soil chemical properties (pH and EC) and Nitrogen balance of broad bean, Aspany F1 cv. grown under sandy soil conditions. Results revealed that organic manure at the rate of 30m³/fed which is superior significantly plant yield and total yield (ton/fed) followed by organic manure at the rate of 35m³/fed. No significant differences occurred between these two organic treatments in both seasons. Sulfur amendment at the rate of 200 kg/fed followed by the rate of 150 kg/fed had the highest values and significant increases on plant weight; number of branches/plant; plant yield; total yield (ton/fed), there are no significant differences between both treatments in both growing seasons. While, each of the study factors had an individual significant effect on total microbial counts, enzymatic activities (Dehydrogenase and Nitrogenase), nodule No., nodule N%, nodule dry weigh, soil total nitrogen, phosphorus, potassium, Fe, Zn and Mn, soil chemical properties (pH and EC) and Nitrogen balance. But combined treatment organic manure 35m³/fed + sulfur at the rate of 200 kg/fed with rhizobial inoculation gave highest values on all most traits in both seasons.

Keywords: Rhizobium, Soil Fertility, Nitrogen Balance, Broad bean, organic manure, sulfur, growth, yield

Introduction

Desert soils are known with poor fertility that considered as main challenges in crop production. Symbiotic relationship between faba bean (Vicia faba L.) and rhizobium, faba bean called host that provides rhizobia with nutrients while rhizobia provide its host with nitrogen in the form of ammonia through nitrogen fixation. Legumes can improve soil fertility in nitrogen deficient area. Biological nitrogen fixation (Rhizobia-legumes symbiosis) is a promising technologies which play an important role in reducing the consumption of chemical N-fertilizers, increasing soil fertility, decreasing the production cost, and reducing pollution effect of chemical fertilizers in the environment, N₂ fixed by legumes is assessed to 77% annually to global agricultural systems (Herridge et al. 2008). Faba bean is one of the most efficient nitrogen-fixing legumes and faba bean plants can meet all of their N needs through biological nitrogen fixation (BNF), N₂-fixed by faba bean were ranged from 45 to 300 kg N·ha⁻¹. Under different Egyptian field conditions, the amount of N₂-fixed by this legume ranged between 121 and 171 kg N·ha⁻¹ (Lindemann, and Glover, 2003)

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Broad bean fits sustainable agriculture model due to symbiosis with Rhizobium, the seeds being a good source of energy, proteins, polyphenols, and fiber. The large amount of broad bean biomass residues can be employed for biofuel production, thus valorizing the overall production process (Amalfitano et al., 2018).

Faba bean (Vicia faba L.) is a major leguminous crop a one of the main important source of protein for the Egyptian people (Nassib et al. 1991). Also, it is good source of energy and amino acids (Nalle et al. 2010), as well as, its beneficial effects on soil quality and productivity. At the same time it has faba bean offers ecosystem services such as renewable inputs of nitrogen into crops and soil via biological ambient N2 fixation (Köpke et al. 2010) and is thought to be the third important feed grain worldwide (Singh et al. 2013). Moreover, Faba bean cropping might save about 100–200 kg N ha−1 in N fertilizers needed for crops which are grown after broad bean (Jensen et al. 2010). In Egypt, it is considered a strategic crop (Hegab et al. 2014).

The horizontal expansionism of agricultural area one of the solutions to faces a growing imbalance between agriculture production and population increase in Egypt so about 2.38 million feddans of sandy desert soil could be added to the cultivated area (Ministry of Agriculture and Land Reclamation, 2006). The sandy reclaimed soil of the coarsest texture is hard to be productive because of the lower water holding capacity, the higher aeration, the rapid drain, the lower content of the organic matter and the higher fertilizer leaching (El Banna, 1998). In commercial agriculture, the use of chemical fertilizers cannot be ruled out completely. However, there is a need for integrated application for alternate sources for nutrient for sustaining the desired crop productivity (Tiwari, 2002). Recently, the agricultural technology introduced natural agricultural materials as soil conditioners.

Organic inputs include organic manures, crop residues and Biofertilizers. These are low-cost and ecofriendly inputs have tremendous potential for supplying nutrients which are reduce the dependence of chemical fertilizers. The use of organic soil amendments has been associated with desirable soil properties including higher plant available water holding capacity and cation exchange capacity and lower bulk density and can foster beneficial microorganisms (Doran, 1995 and Drinkwater et al. 1995). Benefits of compost amendments to the soil include pH stabilization and faster infiltration rate due to enhance soil aggregation (Stamatiadis et al., 1999). Organic fertilizers contain organic matter and include a diverse group of materials. Organic manures improve the behaviors of several elements in soils through that active group (fulvic and humic acids) which are increase the ability to retain the elements in complex and chelate form. Organic manures release the elements over a period of time and are broken down slowly by soil microorganisms (Ali et al. 2014). Uses of organic materials are safe for human health and environmental elements. Also, the recycling of organic wastes can also increase soil fertility on the long run through increasing soil organic carbon and the storage of nutrients (Herencia et al. 2008). Also, even the poor soil structure of the sodic soils (Farid et al. 2014b and Kamel et al. 2016).

Moreover, manure is an excellent source of major plant nutrients such as N, P and K, also provides many of the secondary nutrients that plants require. Manure also has a liming effect and neutralizes the acid characteristic of most smallholder soils (Nzuma 1998). Also, the improvement in the nodulation, growth and yield characters of faba bean and other legumes from organic amendments has been reported by different researchers. (Singh 2005 and Tadele et al. 2016). In the same line Osama (2015) indicated that adding of organic substances either alone or in combinations has an effective role on enhancement of studied characters of plant growth; total chlorophyll content; leaf chemical constitutes; flowering pattern; yield components and seed chemical composition of faba bean as compared to the untreated plants. Also, applications of the organic extracts, as well as the compost/biogas tea increased significantly NPK uptake by the growing plants and consequently improved the dry matter yield of faba bean plants (Farid et al. 2018).

Sulphur is one of the most macronutrients for the plant, so it plays an important role in growth and development of plants. Sulphur is usually required by legumes for protein synthesis as a constituent of three amino acids; cystein, cystine and methionine, contributes in the conformation of enzyme protein and some coenzyme essential for metabolism promotes reproductive development and nitrogen fixation and is called a master nutrient for oil seed production (El-Hamzawi, 2001).

Concerning the information showed that the requirement of sulfur for symbiotic nitrogen fixation of legume crops, however, is still scarce. Scherer and Lange (1996) observed yield reduction and a lower N accumulation of grain and fodder legumes under sulfur deficiency conditions. For this reason
Lange (1998) stated that growth of leguminous plant species may be affected by sulfur through its effect on N2 fixation by Rhizobium bacteria. Also, the role of sulfur in legumes growth is important from the point of view that deficiency of the S-containing amino acids cysteine and methionine may limit the nutritional value of food and feed (Sexton et al. 1998). Sulfur has occupied an important place – after nitrogen, phosphorus, and potassium in balanced fertilization programs due to use of sulfur-free fertilizers (Mukherjee and Singh 2002).

As numerous research studies show, shortage of sulfur component in the soil had to reduce in the yield and seed quality of pulses (Tabe and Higgins, 1998 and Glowacka et al. 2019). Sulfur also plays a vital role in N2 fixation (Kaiser et al. 2005 and Mendel and Bittner, 2006). Elemental S is a relatively new fertilizer used in agriculture, therefore its impact on the yield and chemical composition of plants is not yet fully known. Fertilizer not only is a source of S for plants after oxidation, but also considerably changes soil properties by acidifying (Zhou et al., 2009). Thus, it can alter the availability of minerals, including K. Elemental S, as a nutrient carrier and a component initiating many microbiological processes in soil, it is a significant factor in metabolic and physiological changes in legume yields (Niewiadomska et al., 2015).

Materials and Methods

The field work was carried out at privet farm at tour city South Sinai Governorate, during the two consecutive winter seasons of 2018/2019 and 2019/2020. The physical and chemical soil characteristics of the studied site and soil samples after harvest were determined according to Page et al. (1982) and Klute (1986) respectively, as recorded in Table (1). The chemical analysis of irrigation water was carried out using the standard method of Page et al. (1982) and presented in Table (2). In addition, the organic manure analysis presented in Table (3).

| Soil depth (cm) | Texture class | Soluble anions (me/l) | pH soil paste | E.C dSm-1 | Soluble cations (me/l) |
|----------------|---------------|-----------------------|---------------|-----------|------------------------|
| 0 – 30         | Sandy loam    | HCO₃⁻ 2.10 SO₄²⁻ 17.65 Cl⁻ 23.64 | 7.24          | 4.36      | Ca²⁺ 8.23 Mg²⁺ 12.56 Na⁺ 20.20 K⁺ 2.4 |
| pH: Acidity    | E.C.: Electrical conductivity |

| Soluble anions (me/l) | pH paste | E.C dSm⁻¹ | Soluble cations (me/l) |
|-----------------------|----------|-----------|------------------------|
| HCO₃⁻ 1.44            | SO₄²⁻ 1.89 | Cl⁻ 4.37 | Ca²⁺ 2.6 Mg²⁺ 3.86 Na⁺ 0.78 K⁺ 0.46 |
| pH: Acidity, E.C.: Electrical conductivity, dSm⁻¹: deceime per meter, |

| Season | Moisture (%) | Organic matter (%) | Total C % | C/N ratio | Total N % | Total P % | Total K % |
|--------|--------------|--------------------|-----------|-----------|-----------|-----------|-----------|
| 2019   | 11.59        | 22.07              | 14.25     | 9.69      | 1.47      | 0.85      | 1.34      |
| 2020   | 10.40        | 20.65              | 13.47     | 10.44     | 1.29      | 0.87      | 1.45      |

The experiments were performed to investigate the effect of rhizobium, organic manure amendment rates and agriculture sulfur on soil fertility, nitrogen balance and productivity of broad bean plants (Vicia faba) which were grown under sandy soil conditions and drip irrigation system. Sixteen treatments were used which were the combination of four organic manure rates i.e., 0, 25, 30 and 35 m³/fed (cattle manure). Also, four rates of agricultural sulfur at the rate of 0, 100, 150 and 200kg/fed., were added during prepare soil for planting.

Calcium super phosphate (15.5% P₂O₅) at the rate of 300 kg /fed., were added during land preparation. Potassium sulphate (48% K₂O) at the rate of 150 (kg/fed.) and ammonium sulphate (20.5% N) at the rate of 100 (kg/fed.) fertilizer quantities were divided and applied within drip irrigation system starting after 30 days from planting to end of maturity. While, broad bean seeds Aspany F1 cv. were sown in the first week of October through the two growing seasons respectively.
Experimental plot area was 32 m² (4m wide * 8m long), which is consisted of four ridges, each ridges width is 60 Cm and had one drip irrigation line, broad bean seeds were soaked in warm water 6 hour before planting and treated by Rhizobium bacteria, then planted within two lines for each ridge 25 cm apart between seeds. All agricultural practices for broad bean crop were followed according to the recommendation of Egyptian Ministry of Agriculture.

1. **Inocula Preparation and Seed Inoculation**

Heavy cell suspension of *Rhizobium leguminosarum* 1 × 10⁹ colony-forming unit/ml were grown in YEM medium (Vincent, 1970) and injected into the carrier (60% water holding capacity), vermiculite supplemented and peat 300gm in polyethylene bags previously sterilized by gamma irradiation (Saleh *et al.*, 2001), At sowing, broad bean seeds were coated with rhizobia at a rate of 10 g of inoculant/1 kg seeds, using Arabic gum solution (16%) as the adhesive agent for seed coating (Yousef *et al.*, 2014).

2. **Nodulation**

After 50 days, sampling for nodulation was performed by digging the roots of five randomly selected plants in each plot around 20 cm depth which is approximately the rooting depth of faba bean, and the radius of approximately 12 cm extending out from the central stem containing entire root system of the faba bean. Roots was washed from surrounding soil, nodules from crown region and lateral roots subsequently were removed from the roots and collected in plastic bag for counting. The total number of nodules were counted and the effective and non-effective nodules were counted separately (visual observation) in those five sample plants by taking the intensity of the pink color into consideration (i.e. pink color nodules are considered as effective nodules) and the effective nodules undergo further analysis like for nodule number, nodule nitrogen content and nodule dry weight. The mean values of effective nodules from the five plants were recorded as number of nodules per plant. For nodule dry weight the collected nodules were dried in an oven for 65 h at 75°C to a constant weight to determine nodule dry weight per plant. The average from five plants was taken as nodule dry weight per plant.

3. **Microbial determinations**

Rhizosphere soil sample were analyzed for: total microbial counts on Bunt and Rovira medium Nautiyal (1999). Soil samples were analyzed for: dehydrogenase activity using a standard acetylene reduction assay as described by Casida *et al.* (1964). Nitrogenase activity was determined according to Haahetela *et al.* (1981).

4. **Growth and yield parameters**

After 90 days from planting, nine plants of each replicate were randomly taken for recording vegetative growth and yield characteristics, *i.e.*, plant weight; number of branches/plant, plant yield. In addition, all harvest times were weighted and calculated to calculate total yield (ton/fed) from each treatment.

5. **Soil nutrient contents**

Before applying the treatments and at the end of experiment of work, soil samples were taken from each treatment at major root zone (0–60 cm depth). Soil samples were prepared for analysis, dried, sieved (through 2 mm) and analyzed for total macronutrients by Page *et al.* (1982). Micronutrients content were determined according to Cottenie *et al.* (1982). Nitrogen balance was estimated by using the equation of Hashem and Bouthaina (1992).

6. **Experimental design and statistical analysis**

The experimental treatments were arranged in split plot design with three replicates, the main plots were assigned for organic manure, whereas, agriculture sulfur treatments rates were randomly arranged in the sub plots. Statistical analyses of obtained data were analyzed according to Thomas and Hills (1975).
Results and Discussion

1. Plant Growth and yield Parameters:

Growth and yield parameters results, i.e., plant weight; number of branches/plant; plant yield and total yield (ton/fed) Table (4&5) indicated that there were significant positive effects for rhizobia inoculation, organic manure and sulfur application on all investigated growth parameters. From the data, it could remark the following:

Generally all organic manure treatments showed increase in all growth, yield characters when compared with control in both growing seasons. Organic manure at the rate of 35m³/fed superior significantly on plant weight; number of branches/plant when compared with control treatment. Also, organic manure at the rate of 30m³/fed are superior significantly on plant yield and total yield (ton/fed). There are no significant differences occurred between these two organic treatments in both seasons. Improvement effect of organic manure may be due to the role of organic manure increasing soil organic carbon and the storage of nutrients (Herencia et al. 2008); improve the chemical, physical and biological properties of the soil (Souza, 2010); increased available water holding capacity, cation exchange capacity; lower bulk density and improve the behaviors of several elements in soils (Ali et al. 2014). The results obtained in the same line with those reported by (Singh 2005; Osama 2015; Tadele et al. 2016 and Farid et al. 2018 ) they found that organic amendments showed improvement in the nodulation, growth and yield characters of broad bean and other legumes.

Sulfur treatments showed improvement on values in all growth and yield parameters when compared with control treatment. Sulfur amendment at the rate of 200 kg/fed followed by the rate of 150 kg/fed had the highest values and significant increase on plant weight; number of branches/plant; plant yield and total yield (ton/fed.) there are no significant differences between both treatments in both seasons. The role of Sulfur in legumes growth is important from the point of view that deficiency of the S-containing amino acids cysteine and methionine may limit the nutritional value of food and feed (Sexton et al. 1998). Also sulfur plays a vital role in N2 fixation (Kaiser et al. 2005 and Mendel and Bittner, 2006). Thus, it can alter the availability of minerals, including K. Elemental S, as a nutrient carrier and a component initiating many microbiological processes in soil, is a significant factor in metabolic and physiological changes in legume yields (Niewiadomska et al. 2015).These results agree with those reported by Scherer and Lange (1996) observed yield reduction and a lower N accumulation in grain and fodder legumes under S deficiency conditions. Also, Lange (1998) stated that growth of leguminous plant species may be affected by S through its effect on N2 fixation by Rhizobium bacteria. Moreover, the combination treatment between organic manure at the rate of 30m³/fed + sulfur at the rate of 100 kg/fed and organic manure at the rate of 35m³/fed + sulfur at the rate of 200 kg/fed showed the heights values significantly on plant yield and total yield respectively, in the first season only.

2. Total microbial counts and enzymatic activities:

Order to evaluate the effect of rhizobial inoculation organic manure rates and sulfur levels on the determination, interaction of soil microbial groups and their activities in rhizosphere of broad bean plants, the following were determined:

3. Total microbial counts:

Initial total microbial counts before cultivation were 49 ×10^5 cfu/g dry soil. Represented data in Table (6) showed that microbial counts greatly affected by organic matter levels, microbial counts increased as organic matter level increased, organic matter at 35m³ recorded 100% increase in microbial population compared to control. However, sulfur levels slightly affected microbial population. Highest concentration caused 35% increase in microbial population over control. The highest total microbial counts were recorded with organic manure 35m³/fed + sulfur at the rate of 200 kg/fed being 139 and 143×10^5 cfu/g dry soil at first and second seasons respectively. This might be due to the simulative effect of added biofertilizers on microbial community in broad bean plant rhizosphere which leads to increase total microbial counts. The enhancement effect of microbial activity is a good parameter for many soil improvement indicators (Abd El-Gawad, 2013).
Table 4: Effect of organic manure and sulfur on plant weight and number of branches/plant of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Treatments                  | Characters | Plant weight (gm) | Number of branches /plant |
|-----------------------------|------------|-------------------|---------------------------|
|                             |            | Control           | Org 25m³ | Org 30m³ | Org 35m³ | Control           | Org 25m³ | Org 30m³ | Org 35m³ |
| Organic application         |            |                   |          |          |          |                   |          |          |          |
| Sulfur add.                 |            |                   |          |          |          |                   |          |          |          |
| Control                     | 403.0      | 575.6             | 534.5    | 700.7    | 553.4    | 2.8               | 3.2      | 3.2      | 3.6      | 3.2      |
| Sulfur 100 Kg               | 481.2      | 645.9             | 663.7    | 741.3    | 633.0    | 3.2               | 3.4      | 4.0      | 3.7      | 3.6      |
| Sulfur 150 Kg               | 564.3      | 689.2             | 711.3    | 737.0    | 675.5    | 2.7               | 3.6      | 4.5      | 4.7      | 3.9      |
| Sulfur 200 Kg.              | 518.7      | 681.7             | 732.3    | 781.0    | 678.4    | 2.9               | 3.2      | 4.8      | 5.1      | 4.0      |
|                             | **X−**     | **648.1**         | **660.5**| **740.0**| **2.9**  | **3.3**           | **4.1**  | **4.3**  |          |
|                             |            |                   |          |          |          |                   |          |          |          |
| Control                     | 425.0      | 592.3             | 553.2    | 674.0    | 561.1    | 2.6               | 3.0      | 4.2      | 4.1      | 3.5      |
| Sulfur 100 Kg               | 507.7      | 635.7             | 671.4    | 761.2    | 644.0    | 2.5               | 3.3      | 4.6      | 4.6      | 3.8      |
| Sulfur 150 Kg               | 534.7      | 660.9             | 693.9    | 803.3    | 672.0    | 2.9               | 3.8      | 4.6      | 4.6      | 4.0      |
| Sulfur 200 Kg.              | 592.3      | 658.9             | 742.4    | 772.0    | 691.4    | 2.8               | 3.8      | 4.6      | 5.0      | 4.1      |
|                             | **X−**     | **636.9**         | **664.1**| **752.6**| **2.7**  | **3.5**           | **4.5**  | **4.6**  |          |
| L. S. D. (0.05) for:        | **Sea. 1** | **Sea. 2**        |          |          |          |                   |          |          |          |
| Organic rate                | 56.9       | 110.9             |          |          |          |                   |          |          |          |
| Sulfur add                  | 40.3       | 78.41             |          |          |          |                   |          |          |          |
| Interaction                 | NS         | NS                |          |          |          |                   |          |          |          |

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Table 5: Effect of organic manure and sulfur on plant yield and total yield of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Seasons   | Treatments     | Characters | Plant yield (g) | Total yield (ton/fed) |
|-----------|----------------|------------|-----------------|-----------------------|
|           | Organic application |            |                 |                       |
|           | Sulfur add.     |            |                 |                       |
|           | Control         | Org 25m³  | Org 30m³        | Org 35m³              | X^-        | Control | Org 25m³ | Org 30m³ | Org 35m³ | X^- |
| 1st season| Control         | 45.9       | 93.6            | 108.8                 | 103.7      | 88.0     | 1.5       | 3.1       | 3.7       | 3.5 |
|           | Sulfur 100 Kg   | 68.3       | 106.8           | 195.4                 | 143.9      | 128.6    | 2.3       | 3.6       | 6.1       | 4.8 |
|           | Sulfur 150 Kg   | 76.2       | 119.6           | 181.9                 | 174.0      | 137.9    | 2.6       | 4.0       | 6.1       | 5.8 |
|           | Sulfur 200 Kg   | 72.0       | 121.8           | 180.7                 | 190.3      | 141.2    | 2.4       | 4.1       | 6.1       | 6.4 |
|           | X^-             | 65.6       | 110.5           | 166.7                 | 153.0      | 2.2      | 3.7       | 5.5       | 5.1       |     |
| 2nd season| Control         | 47.3       | 86.8            | 126.8                 | 110.8      | 92.9     | 1.6       | 2.9       | 4.3       | 3.7 |
|           | Sulfur 100 Kg   | 58.7       | 106.6           | 158.3                 | 147.7      | 117.8    | 2.0       | 3.6       | 5.3       | 5.0 |
|           | Sulfur 150 Kg   | 67.3       | 130.0           | 190.7                 | 162.8      | 137.7    | 2.3       | 4.4       | 6.4       | 5.5 |
|           | Sulfur 200 Kg   | 68.7       | 136.2           | 195.1                 | 196.5      | 149.1    | 2.3       | 4.6       | 6.6       | 6.6 |
|           | X^-             | 60.5       | 114.9           | 167.7                 | 154.5      | 2.0      | 3.9       | 5.6       | 5.2       |     |

L. S. D. (0.05) for:

|                  | Sea. 1 | Sea. 2 |                  | Sea. 1 | Sea. 2 |
|------------------|--------|--------|------------------|--------|--------|
| Organic rate     | 14.68  | 13.95  | 0.42             | 0.47   |        |
| Sulfur add       | 11.11  | 13.57  | 0.31             | 0.46   |        |
| Interaction      | 22.22  | NS     | 0.61             | NS     |        |
Table 6: Effect of organic manure and sulfur on total microbial counts and enzymatic activities on rhizosphere soil of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Seasons | Characters | Total microbial counts | Dehydrogenase | Nitrogenase |
|---------|------------|------------------------|---------------|-------------|
|         |            | Control                | Org 25m³  | Org 30m³  | Org 35m³  | X | Control | Org 25m³  | Org 30m³  | Org 35m³  | X |
| Organic application & Sulfur add. |           |                        |             |             |             |   |         |             |             |             |   |
| Control | Org 100 Kg | 51.0                   | 76.0       | 93.0       | 102.0      | 80.5 | 0.78   | 0.93   | 1.02   | 1.08   | 0.95 | 31.6  | 33.6  | 35   | 38   | 34.6 |
| Control | Sulfur 150 Kg | 58.0                  | 79.0       | 97.0       | 117.0      | 87.8 | 0.83   | 0.97   | 1.12   | 1.19   | 1.03 | 31.9  | 39.2  | 42.9 | 44.1 | 39.5 |
| Control | Sulfur 200 Kg | 62.0                  | 80.0       | 104.0      | 124.0      | 92.5 | 0.92   | 1.13   | 1.28   | 1.35   | 1.17 | 32.2  | 41.2  | 44.8 | 47.3 | 41.4 |
| Control | X | 60.0                  | 79.5       | 101.5      | 120.5      | 0.78 | 0.93   | 1.02   | 1.08   | 0.95   | 32   | 39.4  | 42.4  | 44.7 |
| Sulfur 100 Kg | 56.0 | 81.0 | 97.0 | 108.0 | 85.5 | 0.84 | 0.94 | 1.05 | 1.17 | 1 | 32.5 | 33.8 | 36.2 | 39.3 | 35.5 |
| Sulfur 150 Kg | 64.0 | 84.0 | 103.0 | 123.0 | 93.5 | 0.89 | 1.01 | 1.06 | 1.20 | 1.04 | 32.5 | 40.3 | 43.2 | 45.3 | 40.3 |
| Sulfur 200 Kg | 69.0 | 89.0 | 109.0 | 131.0 | 99.5 | 0.95 | 1.17 | 1.26 | 1.30 | 1.17 | 32.7 | 41.8 | 46.8 | 48.9 | 42.6 |
| Sulfur 200 Kg | 76.0 | 92.0 | 118.0 | 143.0 | 107.3 | 0.96 | 1.19 | 1.31 | 1.37 | 1.21 | 32.9 | 44.5 | 47.2 | 50.4 | 43.8 |
| X | 66.3 | 86.5 | 106.8 | 126.3 | 0.91 | 1.08 | 1.17 | 1.26 | 32.7 | 40.1 | 43.4 | 46.0 |

L. S. D. (0.05) for:

|          | Sea. 1 | Sea. 2 |          | Sea. 1 | Sea. 2 |
|----------|--------|--------|----------|--------|--------|
| Organic treatment | 1.00   | 0.87   | 0.02     | 0.01   |        |
| Sulfur add | 0.84   | 1.29   | 0.01     | 0.01   |        |
| Interaction | 1.69   | 2.57   | 0.02     | 0.03   |        |

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4. Soil enzymes:

Dehydrogenase and nitrogenase enzymes were measured to clarify the effect of rhizobia inoculation, organic manure rates and sulfur levels on soil enzymatic activity at the harvesting stage. Obtained results showed that both dehydrogenase and nitrogenase enzymes were varied within the different treatments as shown in Table (6). Combined treatment organic manure 35m³/fed + sulfur at the rate of 200 kg/fed with rhizobial inoculation surpassed all individual treatments. Shalaby (2018) observed an improved dehydrogenase and CO₂ evolution in the rhizosphere zone of wheat plants due to bio-fertilizers application. Ewais et al. (2017), reported that, combined inoculation of bio and organic fertilization gave the highest nitrogenase and dehydrogenase activities and CO₂ evolution in rhizosphere.

5. Nodulation

Represented data Table (7) and Fig (1) both the main effect and the combined effects of organic manure rates and sulfur levels significantly affected nodule number plant with rhizobium inoculation, combined treatment organic manure 35m³/fed + sulfur at the rate of 200 kg/fed with rhizobial inoculation gave highest nodule number/ plant, Highest nitrogen content and nodule dry weight compared to all other treatments. These results compatible with the finding of Nagwa et al. (2012) they reported that inoculation of strain to faba bean significantly increased nodule number. Fekadu et al. (2018) reported that faba bean plants that were not inoculated with rhizobium but treated with FYM, lime and P, formed nodules with a higher number as compared with plants inoculated and received the same treatment. Also, EL-Wakeil and EL-Sebai (2007) who stated that application of rhizobium strains brought significantly higher nodule dry weight and nodule number.

6. Macro and micronutrients, in rhizosphere

Results in Tables (8 and 9) showed that soil total nitrogen, phosphorus, potassium, Fe, Zn and Mn concentrations were increased with increasing organic manure and sulfur rates. Data revealed that the values of nutrients significantly increased by addition sulfur concentrations. The combined treatment organic manure 35m³/fed + sulfur at the rate of 200 kg/fed recorded significantly the highest soil N, P, K, Fe, Mn and Zn concentrations for the two seasons. In general, the obtained data presented in Tables 9 and 10 showed that, the beneficial effect of the applied treatments, particularly elemental sulphur at the applied rates of 100, 150 and 200 kg/fed. That was commonly achieved by lowering soil pH and in turn encouraging the availability of plant essential nutrients, especially phosphorus and sulphur as macronutrients as well as Fe, Mn, and Zn as micronutrients. The superiority of combined effect of added elemental sulphur with organic manure rates as soil application and bio-fertilizer as Rhizobia (Rhizobium leguminosarum) for the noticeable increment in soil total nutrient contents could be attributed to the pronounced decreases in the values of soil pH and EC vs the favorable amelioration in soil biological conditions that encouraging the released nutrients from soil native sources in the available forms as well as easier mobility towards plant roots, and in turn their uptake by plants. In addition, the application of elemental sulphur tend to accelerate the released active inorganic acid (H₂SO₄) that leads to controlling soil availability and mobility of nutrients, which are more sensitive to the undesirable effects of alkaline soil media. Consequently, the applied elemental sulphur to the soil plays an important role for its nutritional status 9 and 10. In this connection, the integrated role of organic matter, applied elemental sulphur plus bio-fertilizer (Rhizobia), which resulted in more pronounced nutrients availability in the soil, on the basis of lowering soil pH and microbial activity that enhances the solubilization of nutrient from the native and added sources. Moreover, such prevailing conditions enhance the slow release of nutrients during the mineralization processes as well as minimizing their possible lose by leaching. These finding are also in agreement with Kaplan et al. (2005), who reported that, a potential strategy to enhance nutrients availability is the lowering soil pH that can be achieved through application of acid-producing fertilizers like sulphure-containing materials.
Table 7: Effect of organic manure and sulfur on nodule No., nodule N% and nodule dry weight of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Seasons | Characters | Organic application | Sulfur add. | 1<sup>st</sup> season | Nitrogenase μMC₃H₄/kg/h |
|---------|------------|---------------------|-------------|------------------------|------------------------|
|         |            | Control             | Org 25m³    | Org 30m³               | Org 35m³               | X⁻ | Control | Org 25m³ | Org 30m³ | Org 35m³ | X⁻ | Control | Org 25m³ | Org 30m³ | Org 35m³ | X⁻ |
| Control |            | 25.0                | 27.0        | 27.5                   | 28.0                   | 26.6 | 2.08    | 2.18     | 2.23     | 2.35     | 2.35 | 0.31    | 0.34     | 0.37     | 0.39     | 0.35 |
| Sulfur 100 Kg |    | 25.5                | 28.0        | 29.5                   | 30.7                   | 28.7 | 2.13    | 2.21     | 2.29     | 2.38     | 2.25 | 0.31    | 0.35     | 0.38     | 0.41     | 0.36 |
| Sulfur 150 Kg |    | 26.0                | 28.5        | 32.0                   | 32.5                   | 30.8 | 2.17    | 2.25     | 2.31     | 2.44     | 2.29 | 0.32    | 0.38     | 0.393    | 0.41     | 0.39 |
| Sulfur 200 Kg |    | 26.0                | 29.7        | 32.5                   | 35.0                   | 31.8 | 2.24    | 2.28     | 2.39     | 2.47     | 2.35 | 0.35    | 0.42     | 0.43     | 0.44     | 0.422  |
| X⁻      |            | 25.6                | 28.3        | 30.4                   | 31.5                   | 2.16  | 2.22    | 2.29     | 2.39     | 2.35     | 0.35 | 0.37    | 0.39     | 0.41     |            | 0.41 |
| control |            | 27.0                | 30.5        | 31.2                   | 31.4                   | 29.3  | 2.1     | 2.32     | 2.34     | 2.39     | 2.413| 0.34    | 0.363    | 0.37     | 0.397    | 0.365  |
| Sulfur 100 Kg |    | 27.9                | 31.1        | 31.2                   | 31.9                   | 30.5  | 2.16    | 2.38     | 2.6      | 2.68     | 2.76 | 0.35    | 0.413    | 0.43     | 0.443    | 0.411  |
| Sulfur 150 Kg |    | 28.0                | 31.7        | 31.6                   | 32.2                   | 31.4  | 2.24    | 2.41     | 2.6      | 2.74     | 2.84 | 0.39    | 0.44     | 0.473    | 0.477    | 0.445  |
| Sulfur 200 Kg |    | 29.0                | 31.0        | 32.7                   | 32.9                   | 24.2  | 2.36    | 2.58     | 2.67     | 2.73     | 2.93 | 0.413   | 0.467    | 0.503    | 0.53     | 0.478  |
| X⁻      |            | 29.0                | 23.3        | 31.2                   | 31.9                   | 2.16  | 2.43    | 2.55     | 2.63     | 2.63     | 0.37 | 0.42    | 0.45     | 0.45     |            | 0.46 |

L. S. D. (0.05) for:

| 1<sup>st</sup> season |
|------------------------|
| Organic treatment      |
| 0.75 0.36 0.03 0.04    |
| Sulfur add             |
| 0.62 0.40 0.02 0.35    |
| Interaction            |
| 1.25 0.79 0.04 0.70    |

- 0.04 0.04
Table 9: Effect of organic manure and sulfur on Fe, Mn and Zn on rhizosphere soil of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Treatments | Characters | Fe | Mn | Zn |
|------------|------------|----|----|----|
| **Organic application** | **Org 25m³** | **Org 30m³** | **Org 35m³** | **Mean** | **Org 25m³** | **Org 30m³** | **Org 35m³** | **Mean** | **Org 25m³** | **Org 30m³** | **Org 35m³** | **Mean** |
| Control     | 11.7       | 13.2 | 14.9 | 16.5 | 14.1 | 26.5 | 27.7 | 28.3 | 28.9 | 27.9 | 1.68 | 1.82 | 1.99 | 2.13 | 1.91 |
| Sulfur 100 Kg | 12.4       | 14.3 | 15.8 | 16.8 | 14.8 | 26.7 | 29.6 | 30.8 | 31.2 | 29.58 | 6.77 | 1.95 | 2.01 | 2.23 | 3.24 |
| Sulfur 150 Kg | 12.4       | 15.5 | 17.3 | 18.9 | 16.0 | 26.9 | 29.8 | 31.3 | 31.8 | 29.95 | 1.73 | 1.96 | 2.04 | 2.26 | 1.99 |
| Sulfur 200 Kg | 12.5       | 15.6 | 14.7 | 19.9 | 15.7 | 27.8 | 30.1 | 32 | 34.2 | 32.1 | 1.76 | 2.1 | 2.09 | 2.27 | 2.06 |
| X⁻          | 11.7       | 13.2 | 14.9 | 16.5 | 14.1 | 26.5 | 27.7 | 28.3 | 28.9 | 27.85 | 2.98 | 1.96 | 2.03 | 2.22 |      |

L. S. D. (0.05) for: | Sea. 1 | Sea. 2 | Sea. 1 | Sea. 2 | Sea. 1 | Sea. 2 |
|------------------|------|------|------|------|------|------|
| Organic treatment | 0.01 | 0.04 | 0.11 | 0.07 | 0.02 | 0.03 |
| Sulfur add        | 0.03 | 0.44 | 0.08 | 0.13 | 0.03 | 2.13 |
| Interaction       | 0.06 | 0.88 | 0.17 | 0.26 | 0.05 | 4.27 |
Table 8: Effect of organic manure and sulfur on N, P and K on rhizosphere soil of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Seasons | Characters | N ppm | P ppm | K ppm |
|---------|------------|-------|-------|-------|
|         |            | 1st season |       |       |
| Treatments | Organic application | Org 25m³ | Org 30m³ | Org 35m³ | X^- | Org 25m³ | Org 30m³ | Org 35m³ | X^- | Org 25m³ | Org 30m³ | Org 35m³ | X^- |
| Control | Control | 132 | 184 | 199 | 202 | 179.3 | 9.4 | 9.5 | 9.6 | 9.6 | 255.0 | 265.0 | 271.0 | 279.0 | 267.5 |
| Sulfur 100 Kg | Sulfur add | 136 | 185 | 208 | 222 | 187.8 | 9.7 | 9.8 | 10.2 | 10.4 | 10.0 | 256.0 | 269.0 | 274.0 | 281.0 | 270.0 |
| Sulfur 150 Kg | | 136.7 | 191 | 214 | 227 | 192.2 | 10.2 | 10.4 | 10.9 | 11.2 | 10.4 | 263.0 | 273.0 | 280.0 | 289.0 | 276.3 |
| Sulfur 200 Kg. | | 139 | 194 | 217 | 228 | 194.5 | 10.4 | 11.1 | 10.7 | 12.3 | 10.6 | 265.0 | 275.0 | 283.0 | 294.0 | 279.3 |
| X^- | | 135.9 | 188.5 | 209.5 | 219.8 | 194.5 | 9.9 | 10.1 | 10.2 | 10.4 | 10.2 | 259.8 | 270.5 | 277.0 | 285.8 |
| Control | control | 135.3 | 187.7 | 205 | 219 | 186.8 | 9.5 | 9.7 | 10.3 | 11.1 | 10.2 | 272.3 | 275.0 | 279.0 | 282.0 | 277.1 |
| Sulfur 100 Kg | Sulfur add | 142 | 189.3 | 209.3 | 222.7 | 190.8 | 9.7 | 11.2 | 10.9 | 11.5 | 10.82 | 276.0 | 279.3 | 284.7 | 287.0 | 281.8 |
| Sulfur 150 Kg | | 142.7 | 196 | 218.7 | 223.7 | 195.3 | 10.3 | 11.4 | 12.6 | 13.7 | 12 | 280.0 | 284.0 | 289.0 | 289.3 | 285.6 |
| Sulfur 200 Kg. | | 143.7 | 209 | 223 | 235 | 202.7 | 10.5 | 11.7 | 12.8 | 13.9 | 12.2 | 283.0 | 287.3 | 289.0 | 293.3 | 288.2 |
| X^- | | 140.9 | 195.5 | 214 | 225.1 | 225.1 | 10 | 11 | 11.65 | 12.55 | 277.8 | 281.4 | 285.4 | 287.9 |
| L. S. D. (0.05) for: | Sea. 1 | Sea. 2 | Sea. 1 | Sea. 2 | Sea. 1 | Sea. 2 |
| Organic treatment | 0.87 | 1.66 | 0.02 | 0.02 | 1.75 | 1.36 |
| Sulfur add | 1.78 | 2.02 | 0.04 | 0.04 | 1.68 | 3.00 |
| Interaction | 3.55 | 4.04 | 0.08 | 0.08 | 3.36 | 6.00 |

D.W: Dry weight
**Organic application**

|         | Control | Org 25m³ | Org 30m³ | Org 35m³ |
|---------|---------|----------|----------|----------|
| control |         |          |          |          |
| Sulfur 100 Kg |        |          |          |          |
| Sulfur 150 Kg |        |          |          |          |
| Sulfur 200 Kg |        |          |          |          |

**Fig. 1**: Effect of organic manure and sulfur on root growth of broad bean

7. **PH and EC**

Data illustrated in Table 10 showed the changes of some soil chemical properties as affected by the studied treatments.

Soil (pH and EC): Results revealed in Table 10 showed that pH values of soil after plant harvesting were slightly affected by the studied treatments; the values of pH were slightly decreased for both tested seasons. In respective of elemental sulphur, data in Table (10) showed a clearly response of some soil properties, i.e., pH and EC (Electrical conductivity) to the applied treatments, particularly those treated with the highest rates of elemental sulphur of 150 or 200 kg. That was true, since elemental sulphur can oxidized by many soil microorganisms forming sulphuric acid, leading to frequent reactions
Table 10: Effect of organic manure and sulfur on PH and EC on rhizosphere soil of broad bean plants during 2018/2019 and 2019/2020 growing seasons.

| Seasons | Characters | 1<sup>st</sup> season | 2<sup>nd</sup> season |
|---------|------------|------------------------|----------------------|
| Treatments | Organic application | pH | EC | pH | EC |
| | Sulfur add. | Control | org 25m³ | org 30m³ | Org 35m³ | X¯ | Control | org 25m³ | org 30m³ | Org 35m³ | X¯ |
| | control | 8.1 | 7.9 | 7.7 | 7.6 | 7.8 | 2.6 | 2.5 | 2.2 | 1.5 | 2.2 |
| | Sulfur 100 Kg | 8.0 | 7.8 | 7.6 | 7.5 | 7.7 | 2.6 | 2.5 | 2.2 | 1.2 | 2.1 |
| | Sulfur 150 Kg | 7.8 | 7.7 | 7.6 | 7.4 | 7.6 | 2.6 | 2.5 | 1.9 | 1.0 | 2.0 |
| | Sulfur 200 Kg. | 7.7 | 7.6 | 7.5 | 7.5 | 7.6 | 2.5 | 2.5 | 1.9 | 1.0 | 2.0 |
| | X¯ | 7.9 | 7.8 | 7.6 | 7.5 | 2.6 | 2.5 | 2.0 | 1.2 | |
| | control | 8.2 | 8.0 | 7.7 | 7.8 | 7.9 | 2.5 | 2.5 | 2.3 | 2.2 | 2.4 |
| | Sulfur 100 Kg | 8.0 | 8.0 | 7.7 | 7.7 | 7.8 | 2.6 | 2.5 | 2.3 | 1.7 | 2.2 |
| | Sulfur 150 Kg | 7.9 | 7.8 | 7.6 | 7.2 | 7.6 | 2.5 | 2.5 | 2.0 | 1.1 | 2.0 |
| | Sulfur 200 Kg. | 7.8 | 7.7 | 7.5 | 7.4 | 7.6 | 2.4 | 2.3 | 2.0 | 1.1 | 1.9 |
| | X¯ | 8.0 | 7.9 | 7.6 | 7.5 | 2.5 | 2.4 | 2.1 | 1.5 | |

L. S. D. (0.05) for:
- Organic treatment: Sea. 1 = 0.06, Sea. 2 = 0.05
- Sulfur add: Sea. 1 = 0.01, Sea. 2 = 0.25
- Interaction: Sea. 1 = 0.04, Sea. 2 = 0.25
Table 11: Effect of organic manure and sulfur on Nitrogen balance in rhizosphere soil of broad bean.

|                     | Control       | 100 kg/fed. sulfur | 150 kg/fed. sulfur | 200 kg/fed. sulfur |
|---------------------|---------------|--------------------|--------------------|--------------------|
|                     | Org (o)      | Org (25)           | Org (30)           | Org (35)           |
| Before cultivation first season |               |                    |                    |                    |
| N in soil           | 118          | 118                | 118                | 118                |
| N in seed           | 1.54         | 1.54               | 1.54               | 1.54               |
| N in fertilizer     | 31           | 31                 | 31                 | 31                 |
| Total Nitrogen kg/fed | 150.5       | 150.54             | 150.54             | 150.54             |
| After Harvesting first season |               |                    |                    |                    |
| N in soil           | 132          | 184                | 199                | 202                |
| Total N. uptake     | 43.4         | 132.5              | 144.4              | 146.8              |
| Total N. Kg/fed     | 175.4        | 316.5              | 343.4              | 348.8              |
| N. Balance          | 24.86        | 165.96             | 192.86             | 198.26             |
| Net gain over control kg/fed. | 141.1       | 168                | 173.4              | 13.2               |

Net gain over control kg/fed.
with chemical compounds resulting from the microbial activity of Rhizobia (*Rhizobium leguminosarum* ) itself, consequently such acidic media led to lowering soil pH value. Also, the created sulphuric acid reacts with the Ca$^{2+}$ native soil CaCO$_3$ and resulting in CaSO$_4$. The latter can be ionized to SO$_4^{2-}$ which was also reduced soil pH. These results are in agreement with those obtained by Awadalla *et al.*, (2003).

8. Nitrogen balance:
Table (11) showed the nitrogen balance before and after cultivation the broad bean with using rhizobium seed inoculation, organic manure and sulfur rates. There was definite gain in all treatments with the exception of control. The difference in nitrogen balance of the various treatments and the control (Table 12) could be taken to represent mainly the difference between the amount of atmospheric nitrogen added by fixation and nitrogen loss either by volatilization and/or de-nitrification. The present data are in complete agreement with the data recorded by Abd El-Gawad and Omar (2014).

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