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# Effect of soil and foliar fertilizers on alleviation of salt injury on *Vicia faba* L. in terms of enzymatic & non-enzymatic antioxidants

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**Abstract :** A field experiment was conducted during 2014/2015 growth season to study the effect of three soil of fertilizers: control, 200 kg/ha compound fertilizer NPK 18-18-18 and organic (10 ton/ha of sheep manure) and their interaction with three foliar fertilizers:control, high potash and silicon on alleviation of salt stress injury on broad bean plants in silt-clay soil with 7.8 acidity and 9.4 dS/m salinity by estimating Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione (GSH), Ascorbic acid and proline. The results showed that soil fertilizer caused a significant increase in the activity of CAT,APX, GSH, but it had no significant effect on SOD activity. While it caused a significant decrease in proline and a changeable effect on ascorbic acid content whereas compound fertilizer caused a significant reduction in ascorbic acid compared to control in opposite of that, organic fertilizer caused a significant increase in ascorbic acid compared to control. Foliar fertilizers caused a significant increase in the activity of CAT, SOD, GSH and proline, but it had no significant effect on APX and ascorbic acid compared to control. The interactions had a significant effect on all parameters.
**Keywords :** Broad bean , Salinity, antioxidants, organic fertilizer, silicon.

## Introduction:

Salinity is the main problem on plant production in many countries all over the world[1]. The main factor for increasing soil salinity is irrigation of plant with saline water, poor cultural practices, and low precipitation. It causes various effects on plant physiology such as increasing respiration rate, ion toxicity, changes in plant growth, mineral distribution, and membrane instability resulting from calcium displacement by sodium[2], and decreased photosynthetic rate[3]. Similar to other environmental stresses, salt stress leads to the generation of reactive oxygen species (ROSs), such as superoxide radical ($O_2^-$), hydroxyl radical (OH), singlet oxygen ($^1O_2$) and hydrogen peroxide ($H_2O_2$)[4].The accumulation of ROSs damages critical organelles via lipid peroxidation and is capable of inducing damage to almost all cellular macromolecules, including DNA, proteins and carbohydrates[6]. The activity of anti-oxidative enzymes as the most important components in scavenging and the prevention of ROSs damage usually increases under salt stress conditions[6]. Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger[7]. Faba bean (*Vicia fab a*L.) is the most important leguminous crops used for human. Exogenous application of fertilizers have been successfully employed to mitigate the salt-induced losses[8]. Fertilizers offer the best means of increasing yield and of maintaining soil fertility at a level sufficiently high to ensure that good yields can be obtained consistently, year after year. It has been observed that supplemental silicon improves yield and reduce the plant biotic and abiotic stresses[9]. Possible mechanisms of the Si-mediated alleviation of salt stress in higher plants include: 1. the stimulation of enzymatic and non-enzymatic anti-oxidative defense systems[10] and the reduction of oxidative membrane damage[11], 2. the improvement of water uptake via the increasing volume and weight of roots[12], the prevention of water loss via the reduction of both cuticular[13] and stomatal transpiration, and 3. The reduction of $Na^+$ uptake[14]with an increasing of K:Na ratio[15] and/or the alteration of $Na^+$ distribution and other ions within plants. The aim of this experiment was to determine the effect of soil and foliar fertilizers on alleviation of salt injury on broad bean plants in terms of enzymatic and non-enzymatic antioxidants.

## Materials & Methods

A field experiment was conducted during 2014/2015 growth season to study three treatments of soil fertilizers (control, 200 kg/ha compound fertilizer (NPK, 18-18-18) and organic (10 ton/ha of sheep manure) with three treatments of foliar fertilizers (control, high potash, silicon)in silt-clay soil in which the soil acidity was 7.8 and the salinity was 9.4 dS/m on broad bean (*Vicia faba* L.). Randomized complete block design with three replicates was used. The experimental unit contained 3 ridges (2.4 x 3 $m^2$) seeded on two sides (25 cm apart) with broad bean seeds (local variety) after soaked in water for 24 hours at 6/10/2014.At seeding time, the organic fertilizer was added according to the treatments as line down the planting line. Two weeks after germination, complete fertilizer (NPK) was added according to the treatment in line10 cm down the plant line. Foliar fertilizer was added two times, first at one month after germination, and the second at flowering stage.

The data were recorded during the flowering stage, which included SOD (super oxide dismutase) activity according to Marklund and Marklund[16], Catalase activity according to Aebi[17], ascorbate peroxidase (APX)according to Asada and Chen[18]. The content of GSH according to Ellman[19], Proline content according to Bates *et al.*[20]and Ascorbic acid content according to Shalata and Neumann[21].The data were analyzed and the means were compared according to Least Significant Difference ($LSD_{0.05}$)[22].

## Results

Table 1 demonstrated that the plants supplemented with soil fertilizers (compound or organic) showed significant increase in catalase activity (7.2 and 7.4) unit respectively compared to control (2.9 unit), as well as catalase activity increased in plants supplemented with foliar fertilizers (high potash and silicon) to (7.6 and 5.3) unit respectively compared to control 4.6 unit. Whereas the supplement of soil fertilizers, simultaneously with foliar fertilizers which caused increases of enzyme activity. The highest CAT activity was obtained when supplementation with soil compound fertilizer simultaneously with foliar application of high potash (9.3 units).

**Table (1): Effect of fertilizers on catalase activity (U).**

| soil fertilizers | foliar fertilizers | | | Average of soil fert. |
|---|---|---|---|---|
| | Without spray | High potash | Silicon | |
| **Control** | 1.5 | 4.4 | 2.7 | **2.9** |
| **Compound** | 6.6 | 9.3 | 5.6 | **7.2** |
| **Organic** | 5.6 | 9.1 | 7.6 | **7.4** |
| **Average of foliar fert.** | **4.6** | **7.6** | **5.3** | |
| **$LSD_{(0.05)}$** | **Soil f.=0.662 foliar=0.662 interaction=1.146** | | | |

Table 2indicated that application of soil fertilizers (compound or organic) had no significant effect on plant content of superoxide dismutase activity (15.4 and 14.8) units respectively compared to control (14.4 units), while SOD activity increased in plants supplemented with silicon, which reached to 18.1 units compared to control (13.5 units). Whereas, the supplement of soil fertilizers simultaneously with foliar fertilizers caused changeable enzyme activity at spraying high potash, while it caused significant increase at spraying silicon.

**Table (2): Effect of fertilizers on Superoxide dismutase activity (U).**

| soil fertilizers | Foliar fertilizers | | | Average of soil fert. |
|---|---|---|---|---|
| | Without spray | High potash | Silicon | |
| **Control** | 14.1 | 10.4 | 18.8 | **14.4** |
| **Compound** | 11.9 | 16.4 | 17.9 | **15.4** |
| **Organic** | 14.5 | 12.2 | 17.7 | **14.8** |
| **Average of foliar fert.** | **13.5** | **13** | **18.1** | |
| **$LSD_{(0.05)}$** | **Soil f.=n.s. foliar=1.300 interaction=2.252** | | | |

Table 3 showed that APX activity increased in plants supplemented with soil fertilizers (organic) and reached **$3.6\times10^{-4}$** compared with control (**$3.0\times10^{-4}$**), while foliar fertilizer had no significant effect on plant content of APX activity. Whereas, the supplement of soil fertilizers simultaneously with foliar fertilizers caused changeable enzyme activity and soil supply with compound fertilizer + spraying high potash or organic fertilizer + spraying of silicon had a significant effect in increasing APX activity in plants($3.8\times10^{-4}$ and $4.2\times10^{-4}$units respectively) compared to control ($2.8\times10^{-4}$unit).

**Table (3): Effect of fertilizers on Ascorbate peroxidase activity (U).**

| soil fertilizers | Foliar fertilizers | | | Average of soil fert. |
|---|---|---|---|---|
| | Without spray | High potash | Silicon | |
| **Control** | $2.8\times10^{-4}$ | $3.1\times10^{-4}$ | $3\times10^{-4}$ | **$3.0\times10^{-4}$** |
| **Compound** | $3.4\times10^{-4}$ | $3.8\times10^{-4}$ | $2.8\times10^{-4}$ | **$3.3\times10^{-4}$** |
| **Organic** | $3.3\times10^{-4}$ | $3.3\times10^{-4}$ | $4.2\times10^{-4}$ | **$3.6\times10^{-4}$** |
| **Average of foliar fert.** | **$3.2\times10^{-4}$** | **$3.4\times10^{-4}$** | **$3.3\times10^{-4}$** | |
| **$LSD_{(0.05)}$** | **Soil f.=$0.36\times10^{-4}$ foliar=n.s. interaction=$0.63\times10^{-4}$** | | | |

Table 4 showed that soil fertilizer caused a significant effect in increasing glutathione compared to control and organic fertilizer was superior significantly. Foliar fertilizer caused a changeable effect, that high potash decreased GSH significantly with a percentage reduction of 33% while silicon cased a significant increase with a percentage increase of 37.2% compared with control. The interaction had a significant effect that low GSH obtained from silicon (1217) , while high value obtained from organic fertilizer + silicon spraying (7627).

**Table (4): Effect of fertilizers on glutathione GSH (µg/g.F.W).**

| soil fertilizers | Foliar fertilizers | | | Average of soil fert. |
|---|---|---|---|---|
| | Without spray | High potash | Silicon | |
| **Control** | 2807 | 3005.3 | 1217 | **2343.1** |
| **Compound** | 4080.3 | 2152 | 6092 | **4108.1** |
| **Organic** | 3999.5 | 2134.5 | 7627 | **4587** |
| **Average of foliar fert.** | **3628.9** | **2430.6** | **4978.7** | |
| **$LSD_{(0.05)}$** | **Soil f.=205.9 foliar=205.9 interaction=356.7** | | | |

Table 5 showed that soil fertilizers had a changeable effect, in which compound fertilizer caused a reduction in ascorbic acid content significantly compared with control with a percentage reduction of (20.8%), while organic fertilizer caused a significant increase with a percentage increase of (52%). Foliar fertilizer had no significant effect. The interaction had a significant effect in which compound fertilizer + silicon spraying gave lowest content of ascorbic acid (91.6 mg/g.f.w.) ,while organic fertilizer + silicon spraying gave highest content of ascorbic acid (276.7 mg/g.f.w.).

**Table (5): Effect of fertilizers on ascorbic acid (mg/g.f.w)..**

| soil fertilizers | foliar fertilizers | | | Average of soil fert. |
|---|---|---|---|---|
| | Without spray | High potash | Silicon | |
| **Control** | 166.3 | 165.5 | 173.9 | **168.6** |
| **Compound** | 159.2 | 149.9 | 91.6 | **133.6** |
| **Organic** | 251.1 | 240.5 | 276.7 | **256.1** |
| **Average of foliar fert.** | **192.2** | **185.3** | **180.7** | |
| **$LSD_{(0.05)}$** | **Soil f.=15.73 foliar=n.s. interaction=27.25** | | | |

Table 6 showed that soil fertilizers had a significant effect in decreasing proline content and compound fertilizer caused high reduction in proline content significantly compared with control with a percentage reduction of (51.2%), while organic fertilizer caused a percentage reduction of (7%). Foliar fertilizer caused an increase of proline content and silicon spraying was significant compared to control only with a percentage reduction of (9.1%). The interaction had a significant effect in which high proline content results from no soil fertilizer + high potash spraying and organic fertilizer application + silicon spraying, while low proline content results from compound fertilizer application + silicon spraying.

**Table (6): Effect of fertilizers on proline (mg/g.D.W)**

| Ground fertilizers | foliar fertilizers | | | Average of soil fert. |
|---|---|---|---|---|
| | Without spray | High potash | Silicon | |
| **Control** | 3.5 | 5.0 | 4.4 | **4.3** |
| **Compound** | 3.4 | 1.7 | 1.3 | **2.1** |
| **Organic** | 3.1 | 3.8 | 5.0 | **4** |
| **Average of foliar fert.** | **3.3** | **3.5** | **3.6** | |
| **$LSD_{(0.05)}$** | **Soil f.=0.225 foliar=0.225 interaction=0.394** | | | |

## Discussion

Faba bean (*Vicia faba* L.) is a one of the most important legume. It is cultivated for human consumption, cattle feeding and it is also used as green manure for the poor soils. Soil salinity is one of the most important constraints that limit crop production in arid and semi arid regions. High concentrations of soluble salts in the root zone impose physiologic stresses on plants growth. These stresses may be caused by a salt present in soluble (or free) form (osmotic stress). They may also be due to toxic or specific-ion effects, or to nutritional imbalances.

The results showed that using high potash and organic fertilizers treatment led to a significant increase in CAT and SOD activity. This results are agreed with[23] that high potash supplementation caused a significant increase in broccoli antioxidants both in broccoli leaves and flowers respectively and that high potash treatment led to increase plant tolerance to salt stress by increasing antioxidant mechanisms .

The effect of Si on the antioxidant enzymes activity under salt stress has been reported by[24]who has described an increase in SOD activity in salt-stressed barley leaves and increases in SOD and CAT activity in salt-stressed barley roots[25].Silicon improved activity of antioxidant enzymes, resulting in enhanced crop resistance to oxidative stress induced by *Phytophoramelonis* infection and improved cucumber growth[26].It could be concluded that higher activities of SOD and APX in salt-stressed leaves induced by Si addition may protect the plant tissues from membrane oxidative damage under salt stress, thus mitigating salt toxicity and improving the growth of cucumber plants[27]. As well, the redox status of glutathione was also improved with a significant increase in reduced glutathione (GSH) in 2 mM Si treated plants at 30 mM NaCl[28]. These results are in agreement with[29], reporting that GSH increases with applications of Si in NaCl stressed wheat. The highest antioxidant activity was obtained in plants treated with compost fertilizer[30]. Organic fertilizer treated plants have higher antioxidant activity[31].

In broccoli, high potash caused a significant increase of antioxidant activity both in leaves and flowers[23]. It may be related to the role of K in survival mechanism of the plant.[32] shows clearly the specific effect of potassium on the antioxidant level which is increase at 0.05 mM $KNO_3$ or because its role in enzymes activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance[33,34] who demonstrated that potassium treatment increased antioxidant activity in millet plants.

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