



# Interactive Effect of *Trichoderma viride* on Broad Bean (cv. *Vicia faba* L.) Genotypes Grown Under Different Salinity Stress Conditions

Abdel Kareem S. H. Mohamed<sup>1,\*</sup>, Mahmoud G. Mahmoud<sup>1</sup>, Abd El-Monem M. Sharaf<sup>2</sup>

<sup>1</sup>Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt

<sup>2</sup>Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

## Email address:

Kshm76@yahoo.com (A. K. S. H. Mohamed)

\*Corresponding author

## To cite this article:

Abdel Kareem S. H. Mohamed, Mahmoud G. Mahmoud, Abd El-Monem M. Sharaf. Interactive Effect of *Trichoderma viride* on Broad Bean (cv. *Vicia faba* L.) Genotypes Grown Under Different Salinity Stress Conditions. *International Journal of Ecotoxicology and Ecobiology*. Vol. 1, No. 3, 2016, pp. 141-151. doi: 10.11648/j.ijee.20160103.21

Received: November 23, 2016; Accepted: December 7, 2016; Published: January 5, 2017

**Abstract:** An investigation was undertaken to evaluate the interaction effect of *Trichoderma viride* for their possible role in imparting stress resistance and provide insight in to the potential of broad bean (cv. *Vicia faba* L.) genotypes to adapt to saline conditions. For this, broad bean genotypes (Assiut1, Assiut16 and Assiut159) were treated with different salinity stress levels (00, 75, 150 and 250 mM NaCl) singly or in combination with *Trichoderma* in the presence of salinity. In the obtained results, the overall plant growth parameters such as shoot fresh, dry weight and physiological, bio-chemical activities and antioxidant enzyme activities (catalase and peroxidase) were measured after 27 days of plant harvest. The interaction results showed that the effect of salinity stress was significantly reduced due to application of *Trichoderma* in terms of plant growth or in the case of Na<sup>+</sup> accumulation in plant cells. In defense related physiological, biochemical and antioxidant enzyme activity also showed marked increase due to single or in combination of *Trichoderma* with salinity. Moreover, the interactive effects of *Trichoderma* were more pronounced in increasing overall growth, reducing transport of Na<sup>+</sup> from root to shoot to save cytoplasm from the toxic effect of salinity and bringing about defense related physiological, biochemical, antioxidant enzyme activities in the tested-broad bean genotypes.

**Keywords:** Broad Bean, *Trichoderma viride*, Antioxidant Enzymes, H<sub>2</sub>O<sub>2</sub>, MAD, Salinity Stress

## 1. Introduction

Most of Egyptian crop plants such as broad bean are glycophytes and have to be considered as salt-sensitive [1]. The most salt-sensitive growth stage of broad bean is the vegetative shoot growth, whereas seed germination and reproductive stage are rarely affected by salinity [2]. Na<sup>+</sup> has been proven to be the major toxic ion for broad bean and other plant species such as maize [3, 4, 5]. Fortmeier and Schubert, [3] reported that, the reduced Na<sup>+</sup> accumulation in leaves of salt-resistant plant was the result of two better strategies of Na<sup>+</sup> exclusion; low uptake of Na<sup>+</sup> at the root surface and lower root-shoot translocation of Na<sup>+</sup>. As such, all mechanisms which lead to reduce Na<sup>+</sup> translocation to the shoot contribute to salt resistance [6]. While reactive oxygen species (ROS)

cause damage to plant cells by peroxidation of unsaturated fatty acids in membranes, desaturation of proteins and disrupting carbohydrates and DNA in cells [7, 8, 9, 10], the enzymes antioxidants activities were increased in different cellular compartments especially in chloroplast and mitochondria [11, 12, 13] which play a great role in avoiding cytoplasm from the toxic effect of salinity by lowering Na<sup>+</sup> concentration in leaf cells.

Little is known about the important role of *Trichoderma viride* in salt stress-resistant of plant species. *Trichoderma*-induced plant responses have been well investigated for biotic stresses; however its role in plant responses to salt stress is not well understood. Recently, some

of *Trichoderma* species are commonly found in soil especially near the roots of plants. Some species form colonizations with the roots and some may live as parasites on other fungi [14]. In general the interaction effect of *Trichoderma* with plants accelerates their growth, yield production, and provide tolerance to different stresses such as salinity and drought. Also, Harman *et al.*, [15], reported that under biotic and abiotic stresses, *Trichoderma* spp enhanced the uptake of the major important nutrients, production of some antioxidant defense system enzymes such as peroxidases (POD), and Catalase (CAT), [16, 17], other biochemical activity (chitinases,  $\beta$ -1,3-glucanases, lipoxxygenase- pathway hydroperoxidelyase), and non-enzymatic antioxidant compounds (e.g.; phenols, ascorbate and flavonoids) to promote stress tolerance in plant leaf cells [18, 19, 20] and it is involved in a variety of physiological mechanisms [21].

Also, these microorganisms act as the alternatives to enhance the crop production under the salt-affected soils. The used of *Trichoderma* as a biologically control strategy to alleviate the adverse effect of salinity is of such alternatives. However, Hermosa *et al.* [22] and Rawat *et al.* [23] investigated the roles of other *Trichoderma* species on the alleviation of toxic effects of salinity stress. Whereas, they found that some *Trichoderma* strains can interact directly with roots, increasing plant growth potential, and tolerance to abiotic stresses. In this way, Hemed *et al.* [24] and Egberongbe *et al.* [25] also reported the bio-vital role of *Trichoderma* spp in alleviating the toxicity induced by salt stress; thus normalizing the uptake mechanism in plants by supplying the essential nutrients. The strategies of *Trichoderma* in the alleviation of salinity stress in the broad bean plants could be similar to those mechanisms of *arbuscular mycorrhizal* (AM) fungi in ameliorating salt stress in plants [26]. Similarly, *Ochradenus baccatus* plants were studied to observe the ability of *T. hamatum* to alleviate the negative effects of salinity stress, [14]. *Trichoderma* supply mineral nutrients to plants, especially phosphorous, which is precipitated by ions like Ca, Mg, and Zn [27].

Because high levels of salinity in the field, plant tolerance to these soil constraints needs to exist in combination with *Trichoderma* [28]. Where salinity is the dominant toxicity, production and yield improvements to crops are likely to be small, unless cultivars are tolerant to salinity [29]. The combined effect of *Trichoderma* and salt on germination and early growth has not been looked at in the previous work, since it has only recently been discovered that they appear to commonly coexist in the field. There have also been few studies on the mobility of these salts over the growing season. Crop growth and yield is highly dependent on the success of germination, establishment and seedling growth. Hence, the objectives of this study are to quantify the effects of *Trichoderma viride* and salinity as well as to investigate and assess the interactions on the germination and early growth stages of broad bean in an effort to improve the production potential for farmers and provide knowledge of suitable tolerant cultivars to constrained soil.

## 2. Material and Methods

### 2.1. Plant Growth and Inoculation Conditions

*Trichoderma viride* used in this investigation was obtained from the Botany and Microbiology Dept., Faculty of Science, Al Azhar University Assiut, Egypt. Cultures of fungi were grown on potato dextrose agar and incubated at 28.5°C for 3 days. 1cm<sup>2</sup> plugs of 3 day-old potato dextrose agar grown cultures of actively growing *Trichoderma* were placed in 500 ml flasks containing 50 gm of rice, and 50 ml of sterile distilled water. Colonized rice grains were air dried and milled to fine powder. The formulation was prepared by diluting spore powder with talcum powder (mesh 350 with 95% whiteness) and 1% carboxy methyl cellulose (CMC) to get desired concentration of bio-control agent in the formulation. Seeds of broad bean (cv. *Vicia faba* L.) were surface sterilized with 1 percent sodium hypochlorite for 3 min. Sterilized seeds were classified into two groups; the first one was sown in glass jar as control, and the second group was inoculated with the above *T. viride* formulation for 24 hours. Then the seed were cultivated in plastic pots contain 4 Kg of clay soil, these pots were irrigated with tap water every 6 days until the fourth leaf appearance after that the plants were treated with different NaCl concentrations (0, 75 mM, 150 mM and 250 mM NaCl). From the inoculated seeds only ten seeds per pot of each broad bean genotypes (Assiut 1, Assiut 16 and Assiut 159) were sown and then the seedlings were thinned to five plants per pot. The experiment was carried out in air open under natural conditions with 26±2°C at light and 18±2°C at night. After harvesting, shoots and roots were separated and freshly weight then oven-dried at 80°C for 24 hours for dry weight measurements.

### 2.2. Parameters of Na<sup>+</sup> Exclusion Strategies

According to Sümer *et al.* [30], strategies of Na<sup>+</sup> exclusion were calculated as following equations:

$$\text{Na}^+ \text{ exclusion at the root surface} = \frac{\text{Total plant Na}^+ \text{ content}}{\text{Root dry weight}} \quad (1)$$

$$\text{Na}^+ \text{ translocation from root to shoot} = \frac{\text{Shoot Na}^+ \text{ content}}{\text{Root Na}^+ \text{ content}} \quad (2)$$

### 2.3. Cation Analysis

Cation analysis was measured by flame-photometer model (M7D). About 200 mg ground plant materials (e.g. shoots and roots) was used for analysis of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> ion concentrations. Plant material was dry-ashed at 105°C over night in a forced-air oven. After cooling, plant materials were digested in 5 M HNO<sub>3</sub> by heating prior to boiling. Then filtration was measured to determine the final concentrations of Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> ions.

### 2.4. Biochemical Analysis

#### 2.4.1. Catalase Activity (EC 1.11.1.6)

Catalase (CAT) activity was determined spectrophotometrically by following the consumption of H<sub>2</sub>O<sub>2</sub>

for 1 min according to Aebi [31]. The decrease in absorbance at 240 nm was monitored and the resulted of catalase activity as ( $\Delta$  abs. 240 mg/ dry weight).

#### 2.4.2. Peroxidase Activity (EC 1.11.1.7)

Peroxidase activity was determined spectrophotometrically with some modifications. The assay medium contained 2.5 ml of 100 mM K-phosphate buffer at (pH 5.5), 100  $\mu$ l of 1 mM guaiacol and 0.1ml of enzyme extract. The reaction was started by addition of 300  $\mu$ l of 1.3 mM  $H_2O_2$ . The increase in absorbance at 470 nm was recorded. The resulted of peroxidase activity as ( $\Delta$  abs. 470 mg/ dry weight).

#### 2.4.3. Determination of Hydrogen Peroxide

Hydrogen peroxide ( $H_2O_2$ ) content of leaf samples was spectrophotometrically measured as described by [32]. About 0.05 g of leaf sample was extracted with 4 ml cold acetone. An aliquot (3 ml) of the extracted solution was mixed with 1 ml of 0.1% titanium dioxide in 20% (v/v)  $H_2SO_4$  and the mixture was then centrifuged at 6000 rpm for 15 min. The intensity of yellow color of the supernatant was measured at 415 nm.

#### 2.4.4. Determination of Lipid Peroxidation

The lipid peroxidation (MDA) was determined in leaf samples by measuring malondialdehyde formation using the thiobarbituric acid reaction as described by Madhava Rao and Sresty, [33] with some modifications. The concentration of MDA was calculated by using an extinction coefficient ( $155 \text{ mM}^{-1} \text{ cm}^{-1}$ ) and the results expressed as MDA/g fresh weight.

### 2.5. Statistical Analysis

All data obtained were subjected to one-way analysis variance (ANOVA), using the SPSS statistical package. For comparison of the means, the Duncan's multiple range tests ( $p < 0.05$ ) were used.

## 3. Results

### 3.1. Trichoderma Viride Promote Growth and Confer Salt Tolerance in Broad Bean

To investigate the role of *Trichoderma viride* in the conformation of salt tolerance in plants, broad bean genotypes were grown either under salt stress alone or in combination with *Trichoderma* and salt stress. Plants inoculated with *T. viride* showed enhanced shoot growth of Assiut 1 when grown with or without 250 mM + *Trichoderma* in compared with salt-treated plants, (Table 1). While, the genotype cv. Assiut 16 showed slightly improvement of growth at 250 mM + *Trichoderma*, the genotype 159 showed no significantly change in shoot fresh weight after application of *T. viride* and salt stress in compared to salt-treated plant. Saline stress also had a negative effect on shoot dry weight of all broad bean (cv. *Vicia faba* L.) genotypes. Shoot dry weight in Assiut 1 grown with 250 mM NaCl decreased by 43.64% when compared with the control treatment. However, the shoot dry weight of cv. Assiut1 and cv. Assiut 16 was slightly increased after inoculated with *T. viride* in compared to cv. Assiut 159 which had no significantly change. The difference in shoot fresh and dry weights clearly demonstrated the beneficial effects of the fungus (*T. viride*) under salt stress (Table 1).

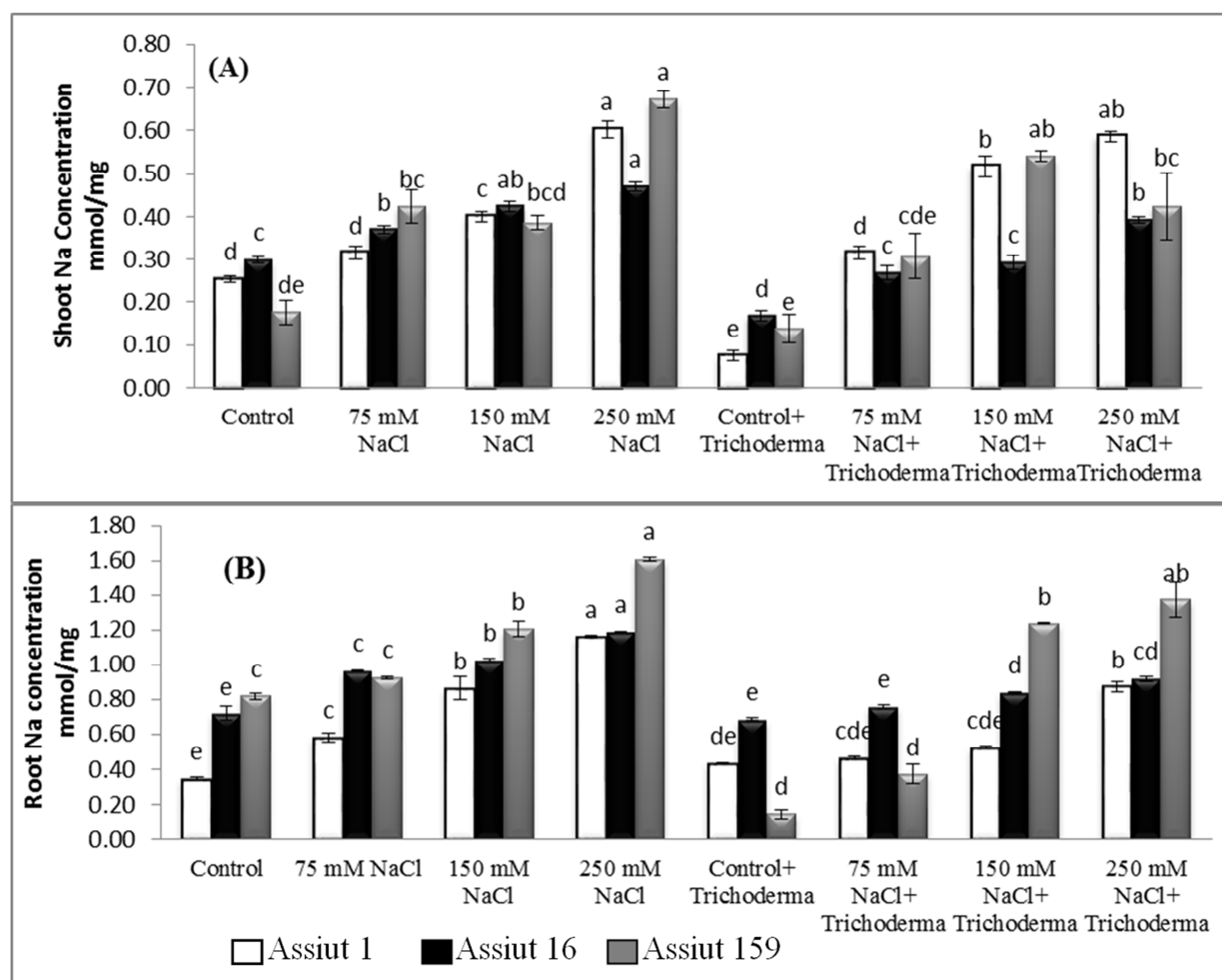
**Table 1.** Shoot fresh and dry weights of broad bean (cv. *Vicia faba* L.) genotypes cultivated under control and salt stress levels (75mM, 150 mM and 250 mM NaCl) treated with *Trichoderma viride*. Data are means of four replicates  $\pm$  SE. Broad bean plants were harvested 27 d after the beginning of plant cultivation. Significant differences ( $P \leq 5\%$ ) between treatments and genotypes are indicated by different letters.

Treat.	Shoot fresh weight (g Pot <sup>-1</sup> )			Shoot dry weight (g Pot <sup>-1</sup> )		
	Asyut 1	Asyut 16	Asyut 159	Asyut 1	Asyut 16	Asyut 159
Control	85.50 $\pm$ 3.1 <sup>a</sup>	83.46 $\pm$ 1.4 <sup>a</sup>	64.84 $\pm$ 0.6 <sup>ab</sup>	8.18 $\pm$ 0.1 <sup>a</sup>	9.61 $\pm$ 0.5 <sup>a</sup>	7.99 $\pm$ 0.4 <sup>a</sup>
75 mM NaCl	52.68 $\pm$ 3.8 <sup>c</sup>	62.49 $\pm$ 3.1 <sup>bc</sup>	59.98 $\pm$ 0.9 <sup>ab</sup>	6.64 $\pm$ 0.2 <sup>bc</sup>	8.02 $\pm$ 0.6 <sup>ab</sup>	5.83 $\pm$ 0.0 <sup>bc</sup>
150 mM NaCl	18.87 $\pm$ 0.6 <sup>d</sup>	44.31 $\pm$ 0.6 <sup>d</sup>	58.42 $\pm$ 0.9 <sup>ab</sup>	4.76 $\pm$ 0.1 <sup>d</sup>	4.14 $\pm$ 0.5 <sup>cd</sup>	5.48 $\pm$ 0.2 <sup>cd</sup>
250 mM NaCl	16.49 $\pm$ 0.4 <sup>d</sup>	36.30 $\pm$ 0.5 <sup>d</sup>	47.22 $\pm$ 0.7 <sup>c</sup>	4.06 $\pm$ 0.3 <sup>d</sup>	3.39 $\pm$ 0.1 <sup>d</sup>	4.37 $\pm$ 0.3 <sup>de</sup>
Control + <i>T. viride</i>	70.44 $\pm$ 0.2 <sup>b</sup>	69.61 $\pm$ 0.9 <sup>b</sup>	66.74 $\pm$ 3.9 <sup>a</sup>	7.74 $\pm$ 0.1 <sup>ab</sup>	9.00 $\pm$ 0.7 <sup>a</sup>	8.04 $\pm$ 0.4 <sup>a</sup>
75 mM NaCl+ <i>T. viride</i>	66.14 $\pm$ 3.7 <sup>b</sup>	57.52 $\pm$ 3.5 <sup>c</sup>	63.50 $\pm$ 4.2 <sup>ab</sup>	8.94 $\pm$ 0.3 <sup>a</sup>	8.50 $\pm$ 0.3 <sup>ab</sup>	7.15 $\pm$ 0.3 <sup>ab</sup>
150 mMNaCl + <i>T. viride</i>	61.09 $\pm$ 2.9 <sup>bc</sup>	57.02 $\pm$ 1.5 <sup>c</sup>	55.42 $\pm$ 0.6 <sup>bc</sup>	6.31 $\pm$ 0.2 <sup>c</sup>	6.30 $\pm$ 0.2 <sup>bc</sup>	6.30 $\pm$ 0.3 <sup>bc</sup>
250 mMNaCl + <i>T. viride</i>	36.30 $\pm$ 0.2 <sup>c</sup>	39.08 $\pm$ 0.4 <sup>d</sup>	35.23 $\pm$ 0.3 <sup>d</sup>	4.61 $\pm$ 0.4 <sup>d</sup>	5.09 $\pm$ 0.2 <sup>cd</sup>	3.88 $\pm$ 0.1 <sup>c</sup>
LSD=0.05	=12.973487	=9.6125719	=10.945869	=1.269826	=2.21969	1.424338

### 3.2. Effect of Trichoderma Application on Na<sup>+</sup> Ion Toxicity of Salt-Stressed Plants

In this study analysis of Na<sup>+</sup> ion concentrations showed that cv. Assiut 1 and cv. Assiut 16 accumulated low shoot Na<sup>+</sup> concentration as compared to cv. Assiut 159, (Fig. 1). However, in combination with bio-control agent of *T. viride* and salt stress, the opposite results were observed, whereas, the genotype cv. Assiut 159 showed low shoot Na<sup>+</sup> concentration (0.42 mmol mg<sup>-1</sup> DW) as compare to the genotype cv. Assiut 1 (0.59 mmol mg<sup>-1</sup> DW) which did not differ when plant treated with either

salt stress alone or in combination of *Trichoderma* and salt stress. On the other hand, the broad bean genotypes cv. Assiut 1 and cv. Assiut 16 exhibited a lower Na<sup>+</sup> concentration in the roots at 250 mM NaCl (1.16 and 1.19 mmol mg<sup>-1</sup> DW, respectively) compared to cv. Assiut 159, which had the highest Na<sup>+</sup> concentration (1.61 mmol mg<sup>-1</sup> DW). Accordingly, the combination of *Trichoderma* with salt stress decreased the concentration of Na<sup>+</sup> in the roots of all plant species as compared to high salt stress levels. However, the lowest root Na<sup>+</sup> concentration was in the genotype cv. Assiut 1 and cv. Assiut 16 as compared to cv. Assiut 159 especially at 150 mM and 250 mM NaCl as shown in Figure (1).



**Figure 1.** Sodium concentration in the shoot (A) and root (B) dry weights of various broad bean genotypes under control and salinity treatment (250 mM NaCl). Data are means of four replicates  $\pm$  SE. Broad bean plants were harvested 27 d after the beginning of plant cultivation. Significant differences ( $P \leq 5\%$ ) between treatments and genotypes are indicated by different letters.

Opposing results were found for  $K^+$  concentrations in shoots of the three tested broad bean genotypes. The plants contained sufficient concentrations of  $K^+$  under stress treatments as shown in Table (2). In this concept, all broad bean genotypes showed no significantly change in shoot  $K^+$  concentration either under salt stress alone or in combination with *Trichoderma* and salt stress as compared to control plants. However, Application of *Trichoderma* to the salt-treated  $Ca^{2+}$

concentrations in shoots was comparable for all broad bean genotypes. The genotype Assiut 1 showed the highest concentrations of  $Ca^{2+}$  compared to other two genotypes after application of *Trichoderma* with salt stress as compared to control plants. So, the application of *Trichoderma* to the salt-treated plants showed improvement in the concentrations of  $Ca^{2+}$  in plant tissues as compared to salt-treated plants, (Table 2).

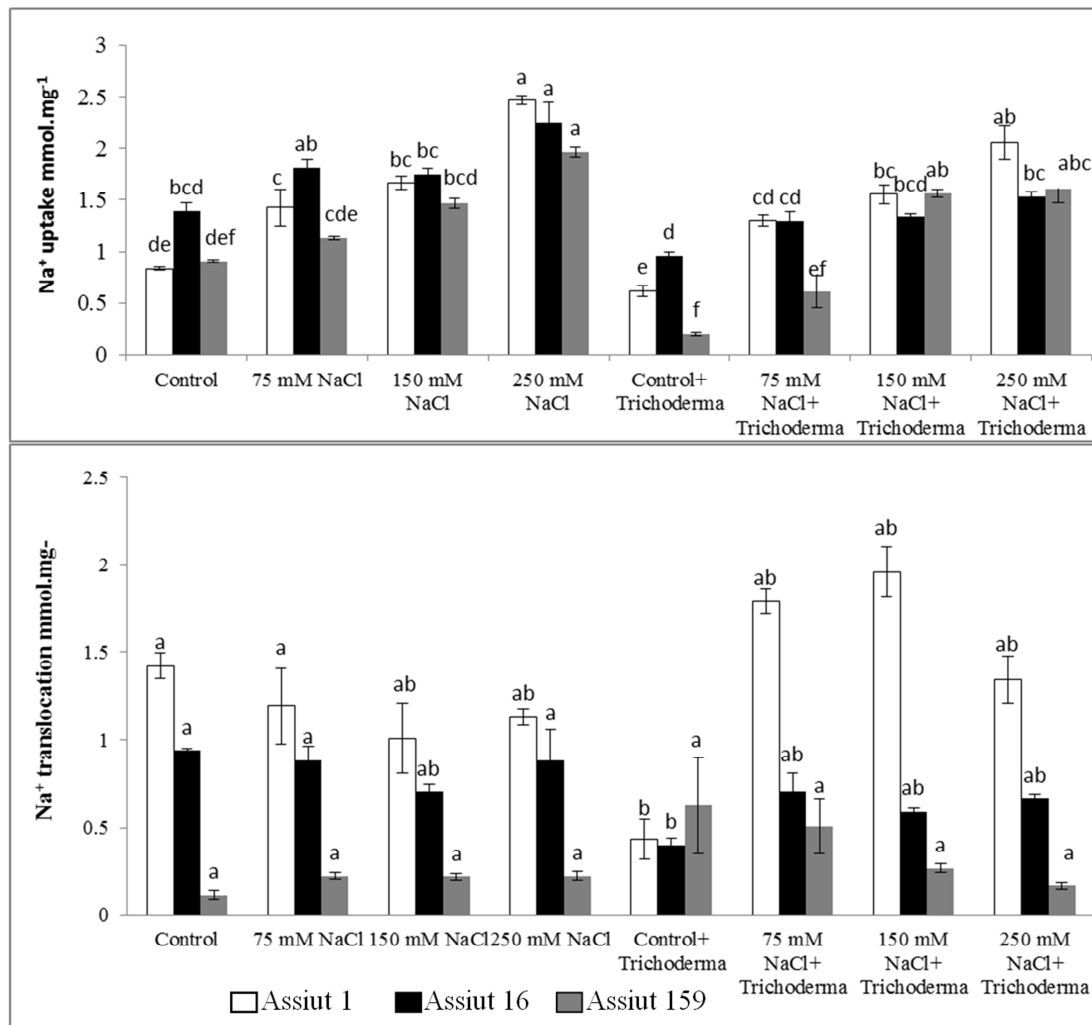
**Table 2.** Shoot  $K^+$  and  $Ca^{2+}$  concentrations of broad bean (cv. *Vicia faba* L.) genotypes cultivated under control and different salt stress levels treated with *Trichoderma viride*. Data are means of four replicates  $\pm$  SE. Broad bean plants were harvested 27 d after the beginning of plant cultivation. Significant differences ( $P \leq 5\%$ ) between treatments and genotypes are indicated by different letters.

Treatment	Shoot $K^+$ concentration (g Pot <sup>-1</sup> )			Shoot $Ca^{2+}$ concentration (g Pot <sup>-1</sup> )		
	Asyut 1	Asyut 16	Asyut 159	Asyut 1	Asyut 16	Asyut 159
Control	0.11 $\pm$ 0.00 <sup>bc</sup>	0.11 $\pm$ 0.00 <sup>bc</sup>	0.11 $\pm$ 0.00 <sup>c</sup>	2.50 $\pm$ 0.00 <sup>d</sup>	2.20 $\pm$ 0.09 <sup>b</sup>	2.77 $\pm$ 0.08 <sup>b</sup>
75 mM NaCl	0.11 $\pm$ 0.01 <sup>bc</sup>	0.12 $\pm$ 0.00 <sup>a</sup>	0.11 $\pm$ 0.00 <sup>c</sup>	3.67 $\pm$ 0.10 <sup>d</sup>	3.08 $\pm$ 0.05 <sup>ab</sup>	4.75 $\pm$ 0.08 <sup>b</sup>
150 mM NaCl	0.11 $\pm$ 0.01 <sup>bc</sup>	0.12 $\pm$ 0.00 <sup>a</sup>	0.12 $\pm$ 0.00 <sup>ab</sup>	6.83 $\pm$ 0.25 <sup>c</sup>	3.55 $\pm$ 0.25 <sup>a</sup>	6.50 $\pm$ 0.17 <sup>b</sup>
250 mM NaCl	0.10 $\pm$ 0.01 <sup>c</sup>	0.12 $\pm$ 0.00 <sup>bc</sup>	0.11 $\pm$ 0.00 <sup>c</sup>	11.33 $\pm$ 0.42 <sup>a</sup>	5.66 $\pm$ 0.42 <sup>ab</sup>	9.83 $\pm$ 0.25 <sup>a</sup>
Control + <i>T. viride</i>	0.13 $\pm$ 0.00 <sup>a</sup>	0.12 $\pm$ 0.00 <sup>a</sup>	0.13 $\pm$ 0.00 <sup>a</sup>	3.25 $\pm$ 0.22 <sup>d</sup>	2.18 $\pm$ 0.22 <sup>b</sup>	2.35 $\pm$ 0.19 <sup>b</sup>
75 mM NaCl+ <i>T. viride</i>	0.12 $\pm$ 0.00 <sup>ab</sup>	0.10 $\pm$ 0.00 <sup>c</sup>	0.10 $\pm$ 0.00 <sup>d</sup>	6.83 $\pm$ 0.54 <sup>c</sup>	2.52 $\pm$ 0.52 <sup>b</sup>	3.7 $\pm$ 0.08 <sup>b</sup>
150 mMNaCl+ <i>T. viride</i>	0.11 $\pm$ 0.00 <sup>bc</sup>	0.12 $\pm$ 0.00 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>cd</sup>	8.17 $\pm$ 0.25 <sup>c</sup>	3.01 $\pm$ 0.25 <sup>ab</sup>	4.17 $\pm$ 0.25 <sup>b</sup>
250 mMNaCl+ <i>T. viride</i>	0.10 $\pm$ 0.00 <sup>c</sup>	0.11 $\pm$ 0.00 <sup>bc</sup>	0.12 $\pm$ 0.00 <sup>bc</sup>	8.58 $\pm$ 0.17 <sup>b</sup>	3.77 $\pm$ 0.17 <sup>ab</sup>	7.23 $\pm$ 0.08 <sup>b</sup>
LSD=0.05	=12.973487	=9.6125719	=10.945869	=1.269826	=2.219697	1.4243386

### 3.3. $\text{Na}^+$ Uptake and $\text{Na}^+$ Translocation from Root to Shoot

$\text{Na}^+$  uptake by the roots and translocation of  $\text{Na}^+$  from root to shoot was studied in the three broad bean genotypes under different salinity levels (75 mM, 150 mM and 250 mM NaCl), (Fig. 2). Treatment with *Trichoderma* of salt-stressed plants significantly reduced the  $\text{Na}^+$  uptake at the root surface in all broad bean genotypes, when compared to the plants grown under salt stress alone. However, root-to-shoot  $\text{Na}^+$

translocation was increased after the application of *Trichoderma* to salt stressed plants. As a result, application of *Trichoderma* to salt-stressed plants decreased the  $\text{Na}^+$  concentrations in the plant tissues. These results indicate that *Trichoderma* application improves  $\text{Na}^+$  exclusion either by decreasing passive influx or by increasing the active efflux of  $\text{Na}^+$ , (Fig. 2).



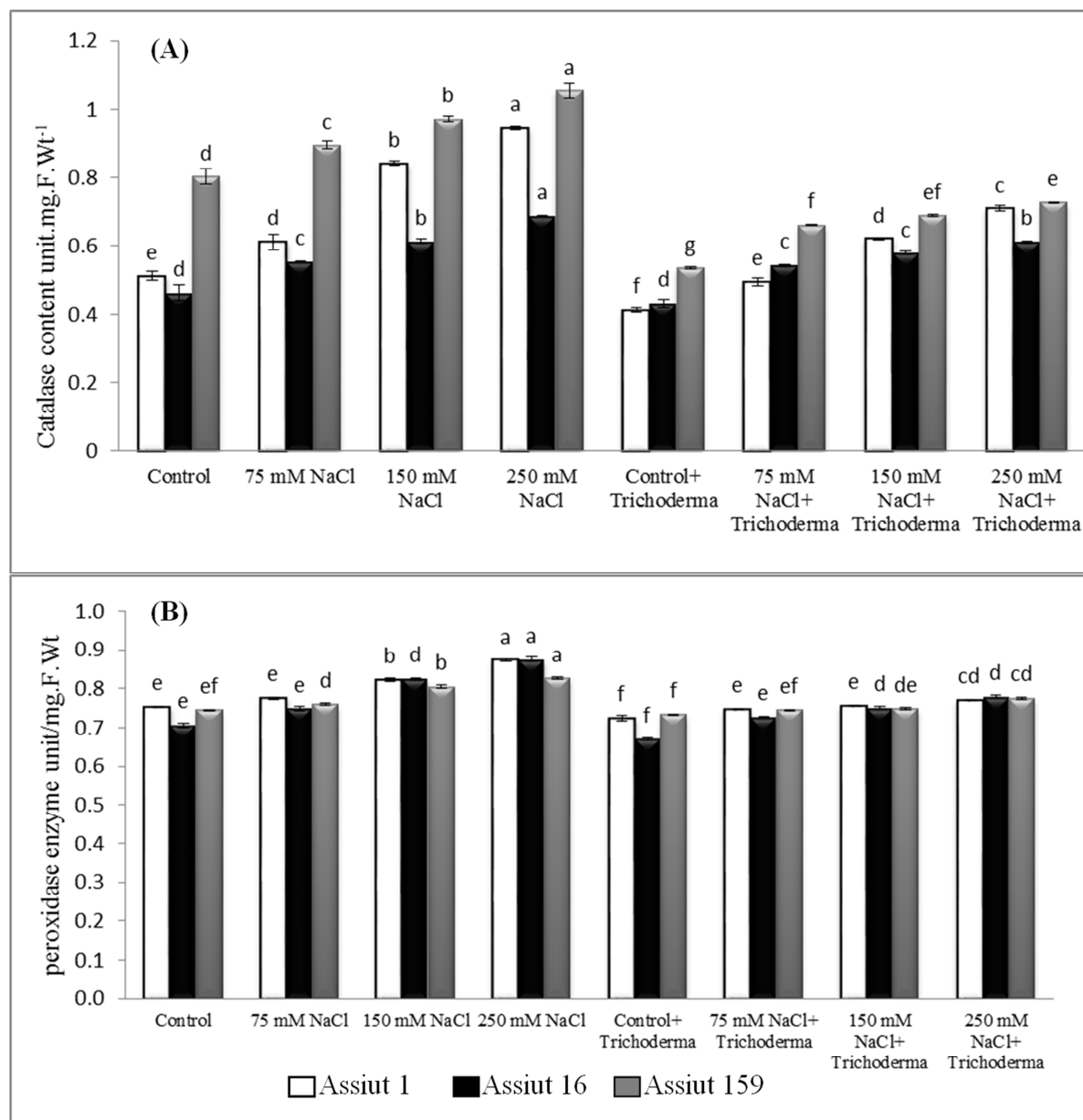
**Figure 2.** (A) Sodium uptake at root level (plant  $\text{Na}^+$  content/ root dry weight) and (B) sodium translocation from root to shoot of three broad bean genotypes under control and different salinity levels in combination with *Trichoderma* and salt stress. Data are means of three replicates  $\pm$  SE. Broad bean were harvested 27 d after the beginning of plant cultivation. Significant differences ( $P \leq 5\%$ ) between treatments and genotypes are indicated by different letters.

### 3.4. Catalase and Peroxidase Enzymes

Generally, salt stressed plants have ability to protect themselves from the oxidative stresses by synthesis of antioxidant enzymes. The results cleared that, catalase activity was significantly enhanced by increasing salinity levels in all plant species in compared to control. However, plant salt-treated in combination with *Trichoderma* reduced the activity of catalase antioxidant enzyme of broad bean (cv. *Vicia faba* L.) plants compared to salt stress plants. As shown in Figure (3A) salinity stress increased the catalase activity in

cv. Assiut 1 and cv. Assiut 159 (0.95 and 1.05 unit  $\text{mg}^{-1}$  FW, respectively) in comparison to cv. Assiut 16 which showed the lowest catalase antioxidant activity (0.68 unit  $\text{mg}^{-1}$  FW).

On the other hand, the POD activity was slightly increased by the treatment of broad bean genotypes with salt stress. Whilst, the application of plant with *Trichoderma* caused significant decrease in the peroxidase activity compared with salt-treated plants. These results indicate that, increase the activity of antioxidant enzymes (catalase and peroxidase) reduced the deleterious effect of salinity on plant growth in the broad bean (cv. *Vicia faba* L.) genotypes (Fig. 3B).



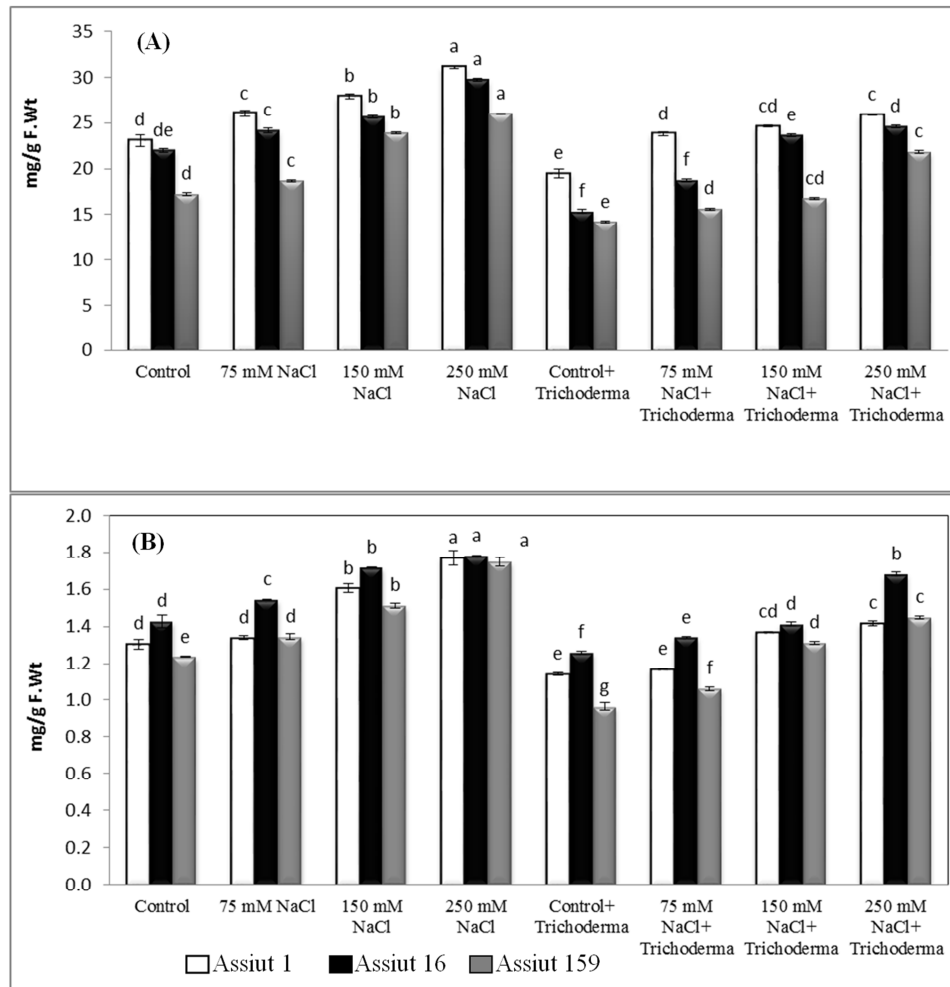
**Figure 3.** Catalase (CTA), (A) and peroxidase (POD) enzyme (B), activities of broad bean plants cultivated under control and salt stress levels (75mM, 150 mM and 250 mM NaCl) treated with *Trichoderma viride*. Data are means of four replicates  $\pm$  SE. Broad bean plants were harvested 27 d after the beginning of plant cultivation. Significant differences ( $P \leq 5\%$ ) between treatments and genotypes are indicated by different letters.

### 3.5. MDA and $H_2O_2$ Contents

The changes in lipid peroxidase represented as MDA accumulation in the plant cell under high salt stress. MDA content in all broad bean genotypes was increased when plant treated with high salinity levels as compared to control. cv. Assiut 1 and cv. Assiut 16 showed high MDA content (31.16 and 29.74 mg g<sup>-1</sup> FW, respectively) as compared to cv. Assiut 159 which showed low MDA content (26.0 mg g<sup>-1</sup> FW) when plant grew under the high salinity level (250 mM NaCl), (Fig. 4A). Application of *Trichoderma* and salt stress decreased MDA content in all tested broad bean genotypes compared to salt-stressed plant, however, the broad bean plant cv. Assiut 159 showed lower MDA content compared to cv. Assiut 1 and

cv. Assiut 16.

As shown in Figure (4B), hydrogen peroxide ( $H_2O_2$ ) content was increased in all tested broad bean plant species under salt stress. Application of *Trichoderma* with salt stress showed a significantly decreased in the hydrogen peroxide ( $H_2O_2$ ) content in all tested plant genotypes compared with salt stress. However, there are a significant differences between all tasted broad bean plants when treated with *Trichoderma* and salt stress, whereas, cv. Assiut 16 showed higher  $H_2O_2$  content as compared to cv. Assiut 1 and cv. Assiut 159, (Fig. 4B). These results cleared that, applied of *Trichoderma* helped broad bean (cv. *Vicia faba* L.) plants to deleterious effect of salt stress by decreasing the  $H_2O_2$  contents in its leaf cells.



**Figure 4.** MDA content (A) and H<sub>2</sub>O<sub>2</sub> content (B) of broad bean (cv. *Vicia faba* L.) grown under control and different salinity levels in combination with *Trichoderma* and salt stress application for 27 days. The results represent means  $\pm$  SE of at least three independent values. Significant differences ( $P \leq 5\%$ ) between values are indicated by different letters.

## 4. Discussion

High salinity levels inhibited the shoot growth of all broad bean genotypes. Although severe effects of Na<sup>+</sup> toxicity on shoot fresh weight by 250 mM NaCl treatment have been shown in broad bean genotype cv. Assiut 1, no significant differences were observed in shoot biomass between cv. Assiut 16 and cv. Assiut 159 under the high salinity stress (250 mM NaCl). While, shoot growth of genotype cv. Assiut 1 showed high significantly increased after application of *T. viride*, the genotype cv. Assiut 159 had severe reduction, suggesting that *Trichoderma* confers tolerance to the genotype Assiut1 but it was not suitable for the genotype cv. Assiut 159 under salt stress.

Shoot growth can possibly be reduced due to reduced uptake of water under high salinity stress. Many experiments have confirmed that inhibition of shoot growth often occurs without any change in water relations of plant cells [35, 36]. In the current study, shoot and root fresh weights were significantly reduced in cv. Assiut 1 but only slightly reduction was observed in Assiut 16 and Assiut 159.

Comparison of genotypes showed that cv. Assiut 1 was able to maintain better shoot and root growth than cv. Assiut 159 after using *Trichoderma* as a bio-agent control, indicates that cv. Assiut 1 is a relatively osmotic-resistant genotype and cv. Assiut 16 is moderately salt resistant while cv. Assiut 159 identify as salt sensitive. In this sense, application of *Trichoderma* as a bio-control maintained and stimulated the growth biomass in broad bean cv. Assiut 1 under salinity stress conditions as compared to other broad bean plants (cv. Assiut 16 and cv. Assiut 159). These data agree with the previous data reported by Schwob et al. [37] and Saleh, [38], which found a significant difference between both plant organs (shoot and root biomass), when treated with mycorrhiza in combination with salinity stress. Application of *Trichoderma* in combination with a low shoot Na<sup>+</sup> concentration can be used to identify salt-resistant plants in the both phase growth of salt stress. The observed severe growth reduction may be due to Na<sup>+</sup> accumulation which finally resulted in disorders of protein synthesis and enzyme activation [40].

The broad bean Assiut 1 showed a high stunting shoot

growth compared to the other genotypes, especially cv. Assiut 159. Similar observations were reported by Eker *et al.*, [41], who found a significant difference between maize varieties due to effect of salt stress. Furthermore, results in this work showed that  $\text{Na}^+$  concentration was higher in the root compared to the shoot which has also been observed for other plant species genotypes [42, 43, 44]. Our results showed that, both salt sensitive and tolerant broad bean cv. Assiut 159 and cv. Assiut 1, respectively have high  $\text{Na}^+$  concentration in leaves during vegetative stages while the broad bean cv. Assiut 16 had medium concentration of Na. Differential distribution of  $\text{Na}^+$  and  $\text{K}^+$  was observed in broad bean leaves [2], rice [45; 46; 47] and concentrations were much higher in leaves of other plant species [48].

According to our results, the high accumulation of  $\text{K}^+$  in broad bean cv. Assiut 1 more than in broad bean cv. Assiut 159 did not beneficial in this plant species, thus confirming the problematic role of  $\text{K}^+$  under stress conditions reported by Maathuis and Amtmann, [49] who suggested that due to physicochemical similarities between  $\text{Na}^+$  and  $\text{K}^+$ , excess  $\text{Na}^+$  antagonistically competes with  $\text{K}^+$  uptake leading to  $\text{Na}^+$  will passively influxes into the cytosol of plant cell. Calcium concentration plays an important role in the maintenance of high growth criteria under salt stress conditions, [50]. Song *et al.* [51], reported that high levels of external  $\text{Ca}^{+2}$  are essential for the maintenance of high root uptake and shoot accumulation of  $\text{Ca}^{+2}$  and  $\text{K}^+$  on saline soils and thus for avoiding salinity damage in plants as shown in rice plants. However, in the present study, after *Trichoderma* application, the shoot concentrations of  $\text{Ca}^{+2}$  were contributed to salt-stress tolerance especially in cv. Assiut 1 and cv. Assiut 159.

The results presented in this study show that the lower  $\text{Na}^+$  exclusion from the shoots of cv. Assiut 1 was due to higher uptake of  $\text{Na}^+$  at the root surface and higher root-to-shoot translocation of  $\text{Na}^+$ . In contrast to salt resistant, salt sensitive cv. Assiut 159 showed good  $\text{Na}^+$  exclusion at the root surface and also from the shoot. These results are in agreement with the observations of Pitann *et al.* [5], they described that these two different strategies of  $\text{Na}^+$  exclusion are not suitable parameters to classify the plant salt resistance. Our results further show that treatment with *Trichoderma* of salt-stressed plants decreased  $\text{Na}^+$  uptake at the root surface and hence improved  $\text{Na}^+$  exclusion from the shoots of all broad bean genotypes. Although the shoot  $\text{Na}^+$  concentrations in comparison to NaCl were high in NaCl + *Trichoderma* treatment, shoot growth was improved by *Trichoderma*. These results confirm that shoot  $\text{Na}^+$  did not reach toxic levels during the short period of salt stress. However, *Trichoderma* treatment increased the root-to-shoot  $\text{Na}^+$  translocation, which indicates that the higher shoot  $\text{Na}^+$  in *Trichoderma* + NaCl plants (cv. Assiut 1) was mainly due to increased  $\text{Na}^+$  transport from root to the shoot.  $\text{Na}^+$  exclusion at the root surface can be controlled by passive influx through selective cation channels [22] or by active efflux of  $\text{Na}^+$  from root cells. The active efflux of  $\text{Na}^+$  through plasmalemma-localized  $\text{Na}^+/\text{H}^+$  antiporters has been reported for many plant species [52, 53, 54, 55, 56 and 5], a similar

mechanism in broad bean has not been identified.

To further test *Trichoderma*-induced plant response to salt stress, the three broad bean genotypes cv. Assiut 1, cv. Assiut 16 and cv. Assiut 159 previously identified as NaCl tolerant or sensitive and related to osmo-protection, or to general response to oxidative stress, was determined in this study in control plants and plants subjected to different salt stress levels (75, 150 and 250 mM NaCl) with or without *Trichoderma* pre-treatment in combination with salt stress.

In the absence of *Trichoderma*, high concentration of NaCl influence antioxidant enzyme activities that scavenge reactive oxidative species (ROS) which had a sever effects on the biochemical reaction in plant cells. Alscher *et al.*, [57] reported that scavenging ROS through the increased activity of antioxidant enzymes improved salt tolerance as in broad bean plants in this study (Fig. 3). Also, highly increased in the activity of antioxidant defense system of broad bean and wheat plants at 150 mM NaCl were obtained by Mohamed and Shaddad [2]. The high activity of antioxidant enzyme indicates that CAT play vital role in scavenging the ROS, which affect the stability of membrane and cause partial up-regulation of cell membrane. The experimental evidence reviewed here indicates that in the presence of *Trichoderma*, both antioxidant defense system catalase (CAT) and peroxidase (POD) had a slightly decreased in all broad bean plants under salinity stress compared to salinity stress alone. However, the activity of peroxidase (POD) enzyme was less reduction in all plant species rather than the activity of catalase (CAT) enzymes.

There is no significantly difference was observed in the activity of the antioxidant defense system between all broad bean genotypes either under salt stress or in combination with *Trichoderma* and salt stress, indicates that antioxidant oxidative stress not are suitable parameters to identify plant salt resistance. This work also supports the hypothesis that using of *Trichoderma* as a bio-control agent is not contribute to improve the osmotic stress-resistant of broad bean genotypes (*Vicia faba* L. cv. Assiut) under different salinity stress levels.

Salinity stress significantly increased concentration of MDA (lipid peroxidation). Measurement of  $\text{H}_2\text{O}_2$  and MDA concentrations in leaves of salt treated plants that are oxidative stress indicator.  $\text{H}_2\text{O}_2$  caused membrane damage that fasten the Haber-Weiss reaction, by production of hydroxyl radical ( $\text{OH}^\cdot$ ) and lipid peroxidation. These parameters were significantly decreased after *Trichoderma* application with salinity stress in this study; however there are contradictory results in different studies. Dionisiases and Tobita [58] proposed that there is a good correlation between lipid peroxidation and  $\text{H}_2\text{O}_2$  concentration under high salinity level and *Trichoderma* application in rice but in other study both  $\text{H}_2\text{O}_2$  and MDA concentrations were increased in Tomato [59] by using the bio agent control. Interestingly, MDA content in *V. faba* plants was significantly decreased in response to *Trichoderma* application, which reinforced the suggestion that *Trichoderma* application can ameliorate the stressful condition by increasing the stability of membranes in *V. faba*.



## 5. Conclusion

Broad bean genotypes Assiut1 is relatively more salt resistant compared to cv. Assiut 159 and cv. Assiut 16. In this context, the *Trichoderma* application may play a major role in the development of salt resistance. Increasing translocation of Na<sup>+</sup> ions in shoot of salt resistant cv. Assiut 1 more than salt sensitive cv. Assiut 159 increased Na<sup>+</sup> sequestration into leaf vacuoles and thus contributed to tissue tolerance of cv. Assiut 1 under high salt stress. Also, in this study *Trichoderma* application induced the activity of enzymatic antioxidant defense system (CAT and SOD), reducing reactive oxygen species (ROS) in the presence of salt stress conditions. Thus, our data provide good evidence for the stimulatory effects of application of *Trichoderma* to induce salt tolerance in the newly developed of broad bean genotypes.

## Acknowledgment

The authors wish to thank Dr. Ahmed M Abdel-Hadi (Botany and Microbiology Department Faculty of Science, Al-Azhar University, Assiut, Egypt) for helping in the identification of marine fungal strains. The authors thank Dr. Ahmed M M Kassem (Botany and Microbiology Department Faculty of Science, Al-Azhar University, Assiut, Egypt) for scientific advice and Dr. Mahmoud Ewais (Faculty of Agriculture, Al-Azhar University, Assiut, Egypt) for helping in analysis and determination of cations. We are also grateful for Dr. Mahmoud Samy (Botany and Microbiology Department Faculty of Science, Al-Azhar University, Cairo, Egypt) for helping in statistical analysis.

## References

- [1] M. Ashraf and M. R. Foolad "Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycinebetaine and prolin", *Env. Exp. Bot.*, 2007, 59: 206-216.
- [2] A. S. H Mohamed and M. A. K. Shaddad "Effect of salinity on dry matter production, ion accumulation, some metabolites and antioxidant enzymes in wheat and broad bean", *Al Azhar Bull.*, 2013, 24, No. 2: 149-164.
- [3] R. Fortmeier and S. Schubert "Salt tolerance of maize (*Zea mays* L.): The role of sodium exclusion", *Plant Cell Environ.*, 1995, 18: 1041-1047.
- [4] C. Zörb, A. Noll, S. Karl, K. Leib, F. Yan, and S. Schubert "Molecular characterization of Na<sup>+</sup>/H<sup>+</sup> antiporters (*ZmNHX*) of maize (*Zea mays* L.) and their expression under salt stress", *J. Plant Physiol.*, 2005, 162: 55-66.
- [5] B. Pitann, A. Mohamed, A. Neubert and S. Schubert "Tonoplast Na<sup>+</sup>/H<sup>+</sup> antiporters of newly developed maize (*Zea mays*) hybrids contribute to salt resistance during the second phase of salt stress", *J. Plant Nutr. Soil Sci.*, 2013, 176 (2): 148-156.
- [6] R. Munns "Genes and salt tolerance: bringing them together", *New Phytol.*, 2005; 167: 645-663.
- [7] H. X. Zhang and E. Blumwald "Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit", *Nat. Biotech.*, 2001; 19, 765-768.
- [8] R. K. Sairam., K. V. Rao and G. C. Srivastava "Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration", *Plant Sci.*, 2002, 163, 1037-1046.
- [9] A. Sabra, F. Daayf and S. Renault "Differential physiological and biochemical responses of three *Echinacea* species to salinity stress", *Scientia Hort.*, 2012, 135 (24): 23-31.
- [10] F. Eyidogan and M. T. Öz "Effect of salinity on antioxidant responses of chickpea seedlings", *Acta Physiol Plant.*, 2007, 29:485-493.
- [11] K. Apel and H. Hirt "Reactive oxygen species: metabolism, oxidative stress, and: metabolism, oxidative stress, and signal transduction", *Annu. Rev. Plant Biol.*, 2004, 55: 373-399.
- [12] S. Masood, L. Saleh, K. Witzel C. Plieth and K. H. Mühling "Determination of oxidative stress in wheat leaves as influenced by boron toxicity and NaCl stress", *Plant Physiol. Biochem.*, 2012, 56: 56 - 61.
- [13] A. Parida, A. B. Das and P. Das "NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures", *J. Plant Biol.*, 2002, 45: 28-36.
- [14] A. Hashem, E. F Abd-Allah, A. A. Alqarawi, A. A. Al Huqail and D. Egamberdieva "Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier", *J. Plant Interact.*, 2014, 9: (1) 857-868.
- [15] G. E. Harman, C. R. Howell, A. Viterbo, I. Chet, and M. Lorito "*Trichoderma* species-Opportunistic, avirulent plant symbionts", *Nat. Rev. Microbiol.*, 2004, 1, 43-56.
- [16] A. Fini, L. Guidi, F. Ferrini, C. Brunetti, M. Di Ferdinando, S. Biricolti, S. Pollastri, L. Calamai, and M. Tattini "Drought stress has contrasting effects on antioxidant enzymes activity and phenylpropanoid biosynthesis in *Fraxinus ornus* leaves: an excess light stress affair?", *J. Plant Physiol.*, 2012, 169: 929-939.
- [17] F. Eyidogen, and M. T. Oz "Effect of salinity on antioxidant responses on chickpea seedlings", *Acta. Physiol. Plant.*, 2007, 29, 485-493.
- [18] U. Deinlein, A. B. Stephan, T. Horie, W. Luo, G. Xu and J. I. Schroeder "Plant salt-tolerance mechanisms". *Trends in Plant Sci.*, 2014, 19, 371-379.
- [19] M. L. Dionisio-sese and S. Tobita "Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance", *J. Plant Physiol.*, 2000, 157, 54-58.
- [20] M. Shores, G. E. Harman and F. Mastouri "Induced systemic resistance and plant responses to fungal biocontrol agents", *Annu. Rev. Phytopathol.*, 2010, 48: 21-43.
- [21] V. Balbi and A. Devoto "Jasmonate signaling network in *Arabidopsis thaliana*: crucial regulatory nodes and new physiological scenarios", *New Phytol.*, 2008, 177, 301-318.
- [22] R. Hermosa, A. Viterbo, I. Chet and E. Monte "Plant-beneficial effects of *Trichoderma* and of its genes", *Microbiol.*, 2012, 158, 17-25.

- [23] K. D. Rawat, M. Chahar, P. V. Reddy, P. Gupta, N. Shrivastava, U. D. Gupta, M. Natrajan, V. M. Katoch, K. Katoch, and D. S. Chauhan "Expression of CXCL10 (IP-10) and CXCL11 (I-TAC) chemokines during *Mycobacterium tuberculosis* infection and immune prophylaxis with *Mycobacterium indicus pranii* (Mw) in guinea pig", *Infect Genet Evol.*, 2013, 13, 11-17.
- [24] I. Gal-Hemed, L. Atanasova, Z. M. Komon, I. S. Druzhinina, A. Viterbo, and O. Yarden "Marine Isolates of *Trichoderma* spp. as Potential Halotolerant Agents of Biological Control for Arid-Zone Agriculture", *Appl Environ Microbiol.*, 2011, 77: (15), 5100-5109.
- [25] H. O. Egberongbe, A. K. Akintokun, O. O. Babalola, and M. O. Bankole "The effect of *Glomus mosseae* and *Trichoderma harzianum* on proximate analysis of soybean (*Glycine max* (L.) Merrill.) Seed grown in sterilized and unsterilized soil", *J. Agri. Ext. Rural Devel.*, 2010, 2: (4), 54-58.
- [26] H. Evelin, R. Kapoor and B. Giri "Arbuscular mycorrhizal fungi in alleviation of salt stress", a review, *Annals of Bot.*, 2009, 104: 1263-1280.
- [27] G. N. Al-Karaki "Growth of mycorrhizal tomato and mineral acquisition under salt stress", *Mycorrhiza.*, 2000, 10: 51-54.
- [28] P. R. John, R. D. Tyagi, D. Prévost, B. Satinder, P. K. Stéphan, and R. Y. Surampalli "Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. adzuki and *Pythium arrhenomanes* and as a growth promoter of soybean Crop Protection", 2010, 29: 1452-1459.
- [29] A. N. Shahzad "The Role of Jasmonic Acid (JA) and Absciscic Acid (ABA) in Salt Resistance of Maize (*Zea mays* L.)", 2011, www.doktorverlag.de; VVB *Laufersweiler Verlag* Staufenberggring 15 D-35396 GIESSEN; ISBN: 978-3-8359-5829-6.
- [30] A. Sümer, C. Zörb, F. Yan, and S. Schubert "Evidence of sodium toxicity for the vegetative growth of maize (*Zea mays* L.) during the first phase of salt stress", *J. Appl. Bot.*, 2004, 78, 135-139.
- [31] H. Aebi "Catalase in Vitro. Methods in Enzymology" 1984, 105: 121-126.
- [32] S. P. Mukherjee and M. A. Choudhuri "Implications of water stress-induced changes in the level of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings", *Physiol. Plant.*, 1983, 58: 166-170.
- [33] R. K. V. Madhava and T. V. S. Sresty "Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses", *Plant Sci.* 2000, 157: 113-128.
- [34] R. Munns "Physiological process limiting plant growth in saline soil: some dogmas and hypothesis", *Plant Cell Environ.*, 1993, 16: 15-24.
- [35] J. B. Passioura "Water transport in and to roots". *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.*, 1988, 39:245- 265.
- [36] Q Gong, P. Li, S. Ma, S. I. Rupassara and H. Bohnert "Salinity stress adaptation competence in the extremophile *T. halophila* in comparison with its relative *A. thaliana*". *Plant J.* 2005, 31: 826-839.
- [37] I. Schwob, M. Ducher, H. Sallanon, and A. Coudret "Trees Struct. Funcr", 1998, 12: 236-240.
- [38] M. S. A. Saleh Increased heavy metal tolerance of cowpea plants by dual inoculation of an *arbuscular mycorrhizal* fungi and nitrogen-fixer *Rhizobium* bacterium", *Afr. J. Biotechnol.* 2006, 5: 132-144.
- [39] A. A. Radi, F. A Faraghal and M. A. Hamada "Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity", 2012, 78: 135-139.
- [40] M. Tester and R. Davenport "Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plant", *Annal. Bot.*, 2003, 91: 523-527.
- [41] S. Eker, G. Cömertpay, Ö. Konufikan, A. ÜLGER, L. Öztürk, and I. Çakmak "Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties", *Turkish J. of Agricult. Forest.* 2006, 30: 365-373.
- [42] G. J. Alberico, and G. R. Cramer "Is the salt tolerance of maize related to sodium exclusion? I. Preliminary screening of seven cultivars", *J. Plant Nutr.* 1993, 16: 2289-2303.
- [43] L. Erdei and E. Taleisnik "Changes in water relation parameters under osmotic and salt stresses in maize and sorghum", *Physiol. Plant.* 1993, 89: 381-387.
- [44] A. D. Neto and J. N. Tabosa "Salt stress in maize seedlings: I. Growth analysis", *Revista Brasileira de Engenharia agricola* Ambiental., 2004, 159-164.
- [45] A. R. Yeo and T. J. Flowers "Accumulation and localization of sodium ions within the shoot of rice (*Oryza sativa*) varieties differing in salinity resistance", *Physiol. Plant.*, 1982, 56: 343-348.
- [46] A. R. Yeo, S. J. M. Capom and T. J. Flowers "The effect of salinity upon photosynthesis of rice (*Oryza sativa* L.): gas exchange by individual leaves in relation to their salt content", *J. exp. Bot.* 1985, 36: 1240-1248.
- [47] Z. Aslam, M. Salim, R. H. Quresh, and G. R. Sandhu "Salt tolerance of *Echinochloa crusgalli*", *Biol. Plant.*, 1987, 29: 66-69.
- [48] H. Wang, Z. Wu, Y. Zhou, J. Han and D. Shi "Effects of salt stress on ion balance and nitrogen metabolism in rice", *Plant Soil Environ.*, 2012, 58: 62-67.
- [49] F. J. M. Maathuis and A. Amtmann "K<sup>+</sup> nutrition and Na<sup>+</sup> toxicity: The basis of cellular K<sup>+</sup>/Na<sup>+</sup> ratios", *Annal. Bot.*, 1999, 84: (2), 123-133.
- [50] A. Wakeel, S. Hanstein, B. Pitann and S. Schubert "Hydrolytic and pumping activity of H<sup>+</sup>-TPase from leaves of sugar beet (*Beta vulgaris* L.) as affected by salt stress", *J. of Plant Physiol.*, 2010, 167: 725-731.
- [51] J. Q. Song, X. R. Mei, and H. Fujiyama "Adequate internal water status of NaCl-salinized rice shoots enhanced selective calcium and potassium absorption", *Soil Sci. Plant Nutr.*, 2006, 52: 300-304.
- [52] E. Blumwald, S. A. Gilad, and P. M. Apse "Sodium transport in plant cells", *Biochimica et Biophysica Acta.*, 2000, 140-151.
- [53] C. Zörb, A. Noll, S. Karl, K. Leib, F. Yan, S. Schubert "Molecular characterization of Na<sup>+</sup>/H<sup>+</sup> antiporters (*ZmNHX*) of maize (*Zea mays* L.) and their expression under salt stress", *J. Plant Physiol.*, 2005, 162: 55-66.
- [54] A. Wakeel, M. Gul, and M. Sanaullah "Potassium Dynamics in three alluvial soils differing in Clay Contents", *Emir. J. Food Agric.*, 2013, 25: 39-44.

- [55] M. P. Apse, G. S. Aharon, W. A. Snedden and E. Blumwald "Salt tolerance conferred by overexpression of a vacuolar  $\text{Na}^+/\text{H}^+$  antiport in *Arabidopsis*", *Science*, 1999, 285: 1256–1258.
- [56] M. P. Apse, J. B. Sottosanto and E. Blumwald "Vacuolar cation/ $\text{H}^+$  exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of *AtNHX1*, the *Arabidopsis* vacuolar  $\text{Na}^+/\text{H}^+$  antiporter", *J. Plant.*, 2003, 36: 229–239.
- [57] M. L. Dionisio-sese, and S. Tobita "Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance", *J. Plant. Physiol.*, 2000, 157: 54-58.
- [58] F. Mastouri, T. Bjorkman and G. E. Harman "*Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit", *Mol. Plant Microbe Interact.*, 2012, 25: 1264–1271.
- [59] R. G. Alscher, N. Erturk and L. S. Heath "Role of superoxide dismutases (SODs) in controlling oxidative stress in plants", *J. Exp. Bot.*, 2002, 53: 1331–1341.