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EFFECT OF ATONIK AND BORON SPRAY ON ANTIOXIDANTS OF BER FRUITS (*ZIZIPHUS MAURITIANA* LAM.) CV. "TUFAHI"

Majid Abdulhameed Ibrahim*, Manal Zibari Sabti and Hussein Lafta Al-Seadi

University of Basrah, College of Agriculture, Department of Horticulture and Landscape Design, Basrah, Iraq

Abstract

The results showed that 6+15 mg.L^{-1} (atonik+boron) gave the highest significant value in SOD and APX activities at maturation stage of fruit, reached 17.380 unit.mg^{-1} proteins and 0.830 µmol.mg^{-1}.min^{-1}, respectively. The 9+20 mg.L^{-1} (atonik+boron) gave the highest significant value in ascorbic acid and carotenoids content in fruit at maturation stage, reached 155.7 and 0.69 mg.100^{-1} fresh weight, respectively. While the control treatment gave the lowest value in the SOD and APX activities, ascorbic and carotenoids content during maturation stage of fruit (9.430 unit.mg^{-1} proteins, 0.577 µmol.mg^{-1}.min^{-1}, 132.30 and 0.56 mg.100^{-1} fresh weight, respectively). But during the ripening stage of fruits, the 6+15 mg.L^{-1} (atonik+boron) gave the highest significant value in SOD activity and ascorbic acid content, reached 29.380 unit.mg^{-1} proteins and 196.30 mg.100^{-1} fresh weight, respectively. The 9+20 mg.L^{-1} (atonik+boron) gave the highest significant value in APX activity in fruit at ripening stage (1.08 mg.100^{-1} fresh weight). While the control treatment gave the lowest value in SOD and APX activities, and ascorbic acid content in fruit at the ripening stage, recorded 14.580 unit.mg^{-1} proteins, 0,760 µmol.mg^{-1}.min^{-1} and 163.30 mg.100^{-1} fresh weight, respectively.

Key words: Ascorbic acid, Carotenoids, Free radicals, Maturation, Ripening

Introduction

Ber tree belongs to *Ziziphus* genus of the Rhamnaceae family. This tree is an evergreen fruit tree that grows in tropical, subtropical and warm temperate regions (San & Yildirim, 2010; Williams, 2006). The ber plant is of economic importance and has many therapeutic benefits. The ber fruits have a wonderful taste and come in attractive colors. They are consumed on a wide commercial scale and are used in various areas of the food industry such as drinks, jams, cakes, pickles, and others due to their high nutritional value (Guo *et al.*, 2013; Nazni & Mythili, 2013; Najafabadi *et al.*, 2017). The fruits and leaves of ber are vital in the fight against diseases such as cancer and liver and in the protection of the digestive system and antioxidants (Shen *et al.*, 2009; Cheng *et al.,* 2012; Plastina *et al.*, 2012). The cultivation of ber trees spread in the southern regions of Iraq, which contain many cultivars. The "Tufahi" variety is one of the most important economic cultivars of high and early productivity and with an excellent quality of fruits (Attaha *et al.*, 2006). Many orchards in southern Iraq suffer from weak growth, low productivity and bad quality due to their exposure to many different stresses such as salinity and nutrient deficiencies. Most of the agricultural soils in southern Iraq tend to have a basic pH (7.5-8.5). Their high lime content coincides with a significant reduction in the amount of boron ready for the plant due to its deposition in a complex insoluble form with aluminum and silicon due to high concentration of hydroxyl ions (Al-Douri & Al-A'areji, 2014). This leads to oxidative stress and increases the production of Reactive Oxygen Species (ROS) within the plant cells (Gill & Tuteja, 2010; Cui & Zhao, 2011). ROS is a powerful oxidant in living cells. It causes cell membrane damage and can oxidize fats, proteins, and pigments and lead to genetic changes in DNA, leading to dysfunction in the metabolic processes and death of cells (Jaspers & Kangasjarvi, 2010; Kusvuran, 2010).

***Author for correspondence :** E-mail: majidalbassiri@yahoo.com

Abbreviations:

Atonik : Bio-stimulator based on 3 synthetic nitro phenols

SOD : Superoxide dismutase

APX : Ascorbate peroxidase

ROS : Reactive oxygen species

Plants have developed an antioxidant system to withstand oxidative stresses that protect cells from oxidative effects and reduce them. This system includes enzymatic and non-enzymatic antioxidants. The most important enzymes that remove active oxygen are the Superoxide dismutase (SOD) and the Ascorbate peroxidase (APX). Non-enzymatic antioxidants include ascorbic acid, carotenoids, and phenols, which play an important role as antioxidants (Sharma *et al.*, 2012; Shivanna *et al.*, 2013). Bio-stimulators improve plant growth and produce safe, high-quality food in an environment-friendly manner. Atonik is manufactured by Asahi Chemical Manufacturing Company Limited, Osaka, Japan. The active components of it are Sodium para-nitrophenolate, Sodium ortho-nitrophenolate and Sodium 5-nitroguaiacolate (Arysta Life Science, 2016). Atonik is one of the most important bio-stimulators that positively affect the plant's bio-processes. This compound increases the plant's tolerance to stresses resulting from inappropriate conditions by increasing the effectiveness of plant antioxidants, as well as the ability to improve the growth and productivity of different plant species (Calvo *et al.*, 2014; Przybysz *et al.*, 2014). Boron is one of the minor nutrients, and its deficiency results in the inhibition of plant growth. The most important activity of boron is to facilitate the movement and transfer of the products of photosynthesis from the leaves (source) to the active areas (sink) in the plant. This element has a role in regulating cell membrane activity and gene expression (Shaaban, 2010). It also stimulates the biosynthesis of proteins through its effect in the process of DNA synthesis as well as its role in increasing vitamin C and B. Boron is not part of the enzyme system but has a role in stimulating certain enzymes (Verma & Verma, 2010). The present study was conducted due to the lack of studies on the use of bio-stimulators and boron element on ber trees in southern Iraq. The aim of the study was determining the effect of atonik (bio-stimulant) and boron spraying on enzymatic and non-enzymatic antioxidants. The purpose was improving the growth of trees and the increase in the production of fruits in quantity and quality.

Materials and Methods

This study was conducted during the 2015-2016 growth season on the ber trees (*Ziziphus mauritiana* Lam.) cv. "Tufahi", in one of the private orchards in Qurna District, north of Basrah. Twelve ber trees similar in vegetative growth at about 7 years old were selected, and planted in silty-clay soil. The trees were sprayed twice, the first time in the fruit set stage. The second time of spraying is three weeks after the first time. The trees were sprayed early in the morning using a 16-liter sprayer pump and even full wetness. H_3BO_3 was used as a source of boron. It is a highly soluble boron fertilizer containing 17% boron. Tween 20 was added with 0.1% concentration of prepared solutions to reduce the surface tension of the water and facilitate the absorption of the material by the surface of the leaves.

The study included the following combinations:

1. (0 + 0) mg / L^{-1} treatment of control (Spraying with distilled water).
2. (3 + 10) mg. L^{-1} atonic* + Boron.
3. (6 + 15) mg. L^{-1} atonic + Boron.
4. (9 + 20) mg. L^{-1} atonic + Boron.

*Atonik is a unique, residue-free bio-stimulator based on 3 synthetic nitrophenols (naturally occurring compounds in living cells).

The studied traits

Enzyme and non-enzymatic antioxidants were estimated in the fruits of ber trees ("Tufahi" cultivar) in two stages: Maturation and ripening fruits.

1. Determination of enzymatic antioxidants

Extract enzymes: Take 0.5 g of the pulp of fresh fruits and mash in a ceramic mortar. After the mashing process, add 5 ml of the extract solution and mix. The mixture is then placed in fine tubes for the cold centrifuge. The centrifuge was then carried out at 10,000 cycles/min and 4 °C for 10 minutes. Then take the 2.5 ml solution and put in special tubes (2.5 ml), and store at 80 ° C until the enzymatic activity is estimated.

Enzymatic activity of Superoxide dismutase (SOD)

The enzymatic activity of Superoxide dismutase (SOD) was estimated according to the method described by Marklund & Marklund (1974). This method is based on the ability of SOD to inhibit Pyrogallol oxidation.

Enzymatic activity of Ascorbate Peroxidase (APX)

The enzymatic activity of the Ascorbate Peroxidase (APX) was estimated in fruits according to the method described by Nakana & Asada (1981).

2. Determination of non-enzymatic antioxidants
Ascorbic acid content in pulp of fruit

Ascorbic acid content was estimated by titration to a certain volume of fruit juice with 2, 6-Dichloro phenol indophenols on the basis of the number of milligrams per 100 g of fresh pulp weight according to the method described in A.O.A.C. (1980).

Total carotenoids in the outer shell of fruit

Total carotenoids were estimated in the fruit shell according to the method described by Goodwin (1976).

The Phenolic compounds in pulp of fruit

Phenolic compounds were extracted from dried fruits by distillation with distilled water according to the Folin-Denis method mentioned in Dalali & Al-Hakim (1987).

Experimental design and statistical analysis

The experiment was designed according to the Randomized Complete Block Design (RCBD). Each treatment included three replicates and one tree per experimental unit. A revised least significant difference (R-LSD) was used to compare between the means of treatment at the probability level of 0.05 according to Al-Rawi & Khalaf Allah (2000).

Results and Discussion

Effect of atonik and boron on activity of antioxidant enzymes

Superoxide dismutase (SOD)

The combination between atonik and boron application at 6+15 mg.L^{-1} gave the highest significant value in enzymatic activity of SOD during maturation and ripening stages of fruit, reached 17.38 and 29.38 unit.mg^{-1} protein, respectively. The two combinations between atonik and boron applications (6+15 and 9+20 mg.L^{-1}) did not produce any significant difference in enzymatic activity of SOD during maturation and ripening stages. While the control treatment gave the lowest significant value in enzymatic activity of SOD during maturation and ripening stages of fruit, reached 9.43 and 14.58 unit.mg^{-1} protein, respectively (fig. 1). The increase in the activity of SOD enzyme in fruits was attributed to the direct role of atonik and boron in increasing its activity. Atonik is a bio-stimulant that increases the activity of enzymes, including its effect on the activity of the enzyme SOD, which leads to the reduction of ROS groups in plant cells. Increasing the activity of the SOD enzyme can increase the tolerance of plants to stress. The ability of cells to withstand stress conditions, especially oxidative stress, is measured by maintaining a high level of antioxidant enzymes (Alwan *et al.*, 2014; Djanaguiraman *et al.,* 2004). As well as their role in increasing the content of proteins in ber fruits (Al-Seadi, 2017). The results of the study are similar to those of many researchers (Djanaguiraman *et al.,* 2010; Kaya & Ashraf, 2015).

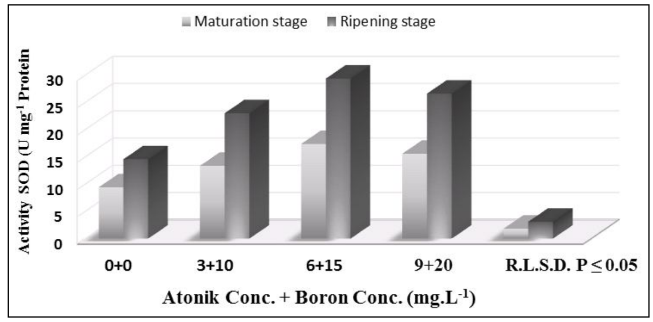

Fig. 1: Effect of Atonik and boron in Superoxide dismutase (SOD) activity in ber fruits cv. "Tufahi".

Ascorbate peroxidase (APX)

Fig. 2 shows the significant effect of spraying with atonik and boron in increasing APX activity in fruits during the maturation and ripening stages. The combination between atonik and boron applications at 6+15 mg.L^{-1} recorded the highest significant increase in the activity of the APX enzyme in fruits at the maturation stage, reached 0.830 µmol. mg^{-1} min^{-1}. While the combination of 9+20 mg.L^{-1} (atonik + boron), was a significant superior in the activity of APX enzyme during ripening stage (1.08 µmol. Mg^{-1} min^{-1}). The control treatment (Without spraying) recorded the lowest activity of the APX enzyme in the two stages of maturation and ripening of fruits was 0.577 and 0.760 µmol. mg^{-1} min^{-1}, respectively. The spray of atonik and boron contributed to the increased production of hormones and enzymes, which included APX enzyme (Gunes *et al.*, 2006; Djanaguiraman *et al.*, 2010). The APX enzyme stimulates the degradation of hydrogen peroxide into water and oxygen (Gill & Tuteja, 2010; Sergio *et al.*, 2012). The results of the study are similar to what many researchers found when studying cotton and tomato plants (Djanaguiraman *et al.*, 2004; Djanaguiraman *et al.*, 2010).

Effect of atonik and boron on non-enzymatic antioxidants

Ascorbic acid content in fruit pulp

The results of Figure 3 indicate the significant effect

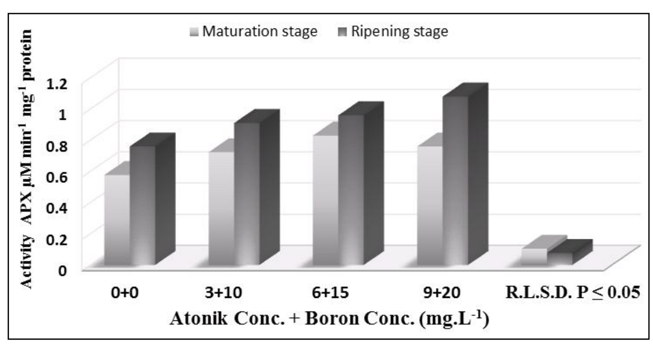

Fig. 2: Effect of Atonik and boron in Ascorbate peroxidase (APX) activity in ber fruits cv. "Tufahi".

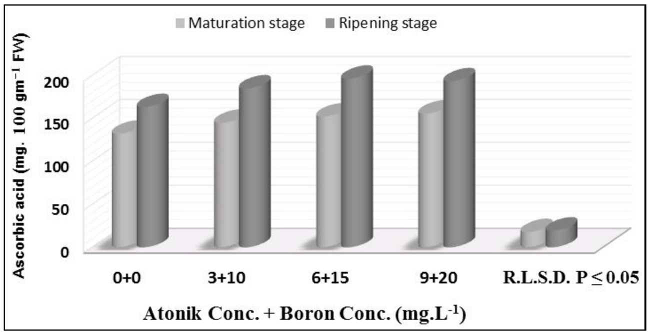

Fig. 3: Effect of Atonik and boron in Ascorbic acid content in ber fruits cv. "Tufahi".

of atonik and boron on the content of ascorbic acid during the maturation stage of fruits. Both the two combinations between atonik and boron (6+15 and 9+20 mg.L^{-1}) were significantly superior to control treatment. The combination between atonik and boron at 9+20 mg.L^{-1} gave the highest increase in the ascorbic acid content of the fruits at 155.7 mg.100 g $^{-1}$ fresh weight. While the control treatment gave the lowest ascorbic acid content in the fruit which was 132.3 mg.100 g^{-1} fresh weight. During the ripening stage of fruits, the 6 mg.L^{-1} atonik + 15 mg.L^{-1} boron treatment gave the highest significant value in ascorbic acid content that reached 196.3 mg.100 g^{-1} fresh weight. While the control treatment gave the lowest ascorbic acid content in the fruit which was 163.3 mg.100g^{-1} fresh weight (fig. 3). The increase in the content of ascorbic acid in fruits is due to the combined role of atonik and boron in the activation of metabolic processes that lead to an increase in the level of glucose, which has an important role in increasing the content in fruits (Djanaguiraman *et al.*, 2010). Ascorbic acid is one of the strongest non-enzymatic antioxidants in the main cell components and the inhibitory force of cell membrane oxidation and has the ability to suppress ROS and reduce hydrogen peroxide (Blokhina *et al.*, 2003; Gill & Tuteja, 2010). The results of the study were similar to those obtained by many researchers when studying other plants (Marie & Toma, 2010; Szot *et al.*, 2014).

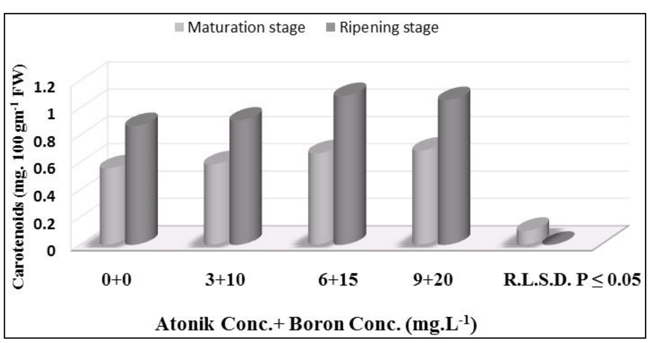

Fig. 4: Effect of Atonik and boron in carotenoids content in ber fruits cv. "Tufahi".

The content of carotenoids

Treatment of the 9 mg.L^{-1} atonik + 20 mg.L^{-1} boron recorded the highest concentration of carotenoids at 0.69 mg.100g^{-1} fresh weight during the maturation stage of ber fruits. While the control treatment recorded the lowest value of carotenoids as 0.56 mg.100g^{-1} fresh weight. Fig. 4 shows no significant differences between the means of treatments in the content of the carotenoids in the fruit shell during the ripening stage. Increasing the content of carotenoids in fruits is of great importance as it plays a role in many functions in plant metabolism and oxidative stress, which plays an important role in the elimination of active oxygen groups (Faize *et al.*, 2010). The results of the study are similar to those of other studies that used atonik application (Abbas *et al.*, 2009; Haroun *et al.*, 2011). But these results are not in agreement with another study when boron was applied (Saadati *et al.*, 2013).

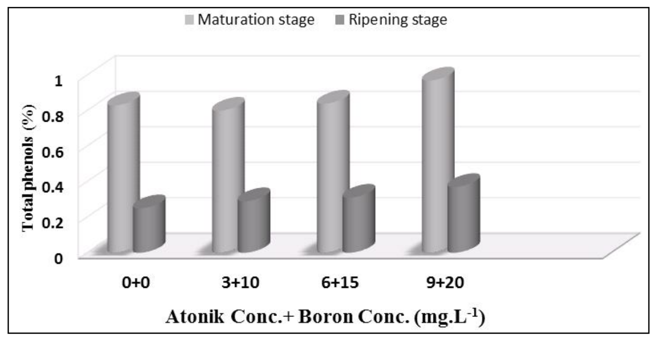

Fig. 5: Effect of Atonik and boron in phenolic compounds in ber fruits cv. "Tufahi".

The phenolic compounds in fruit pulp

The results from fig. 5 show no significant differences between the phenol content in the pulp of fruit during maturation and ripening stages. The 9 mg.L^{-1} atonik and 20 mg.L^{-1} boron combinations gave the highest value in phenol content in the pulp of fruit during maturation and ripening stages which reached 0.97% and 0.37%, respectively. While the combination between atonik and boron at 3+10 mg.L^{-1} gave the lowest value in phenol content during maturation stage of fruit (0.80%). Also, the control treatment gave the lowest value in phenol compounds during ripening stage which reached 0.25% (fig. 5). The increase in phenolic compounds is particularly important as antioxidants and in the protection of ROS systems (Bendini *et al.*, 2007). The results of this study are similar to Saadati *et al.* (2013), which found that the spraying of boric acid twice on three varieties of olive trees, did not significantly affect the content of fruits of total phenols. Also these results are not in agreement with another study about atonik application on bean plant (Kocira *et al.*, 2017).

Conclusion

Ber fruits have a high content of enzymatic and non-enzymatic antioxidants. The spraying of trees with atonik and boron gave positive results in the increase of enzymatic and non-enzymatic antioxidants.

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