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BIO STIMULANT ACTIVITY OF CHITOSAN DISSOLVED IN DIFFERENT ORGANIC ACIDS ON TOMATO PLANT

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ABSTRACT: This study aims to evaluate and compare the stimulant capabilities of the foliar application of chitosan, dissolved in aqueous solutions of four organic acids (acetic acid, ascorbic acid, citric acid, and malic acid), on tomato (*Solanum lycopersicum* L.). A pot experiment was carried out under greenhouse conditions during the summer season. Morphological traits, photosynthetic pigments, ascorbic acid, total phenol, osmolytes, antioxidant enzyme activities (peroxidases, polyphenol oxidases, Superoxide dismutase, and catalase), and yield parameters were evaluated. The results indicated that chitosan treatments showed improvements in almost all aforementioned parameters, these responses were different according to the associated organic acid. Moreover, chitosan treatments, compared to control, significantly increase all the studied yield parameters in tomato plants, especially chitosan dissolved in an aqueous solution of malic acid and ascorbic acid that gave the most potent effect regarding the number of flower/ plant, the number of fruits, as well as the weight of fruits/ plant in comparison with plants treated with chitosan dissolved in an aqueous solution of citric acid, or acetic acid, respectively. Since it is a non-toxic, biocompatible, biodegradable, and available material in the global market, the foliar application of chitosan, especially that dissolved in aqueous solutions of ascorbic or malic acids, are recommended to be used as commercial applications under normal conditions (not stressed) as pre and after flowering biostimulants to improve tomato plant health and thus increasing the yield. The results can be extended to all the plants of the family Solanaceae after further investigations.

Key words: Tomato, biostimulant, chitosan, organic acids, antioxidant enzymes.

INTRODUCTION

The dramatic shift in climatic conditions and the increased food demand leading to inefficient use of synthetic chemicals in the agriculture sector to overcome the various challenges that overburden food production. This approach leads to the deterioration of the quality of both agricultural lands and food, which in turn had prolonged catastrophic impacts on the environment and human health. Natural-based plant biostimulants are used as a safe, eco-friendly alternative for plant health improvement to mitigate different stresses, and thus to increase the vegetative growth and yield when applied in small quantities (Sinha *et al.,* 2014; Zulfiqar *et al.,* 2020). Chitosan is among the promising natural-based plant biostimulants (Pichyangkura and Chadchawan, 2015), well-known for its ability to alleviate different abiotic and biotic stresses (Malerba and Cerana, 2015; Hidangmayum *et al.,* 2019; Malerba and Cerana, 2020).

Chitosan is a natural-based linear polysaccharide derived from chitin, which considers the second abundant biopolymer in nature after cellulose, and can be obtained from insect exoskeletons, crustacean shells, and fungal cell walls (Younes and Rinaudo, 2015). After suitable processing of raw chitin, a partial (at least 50%) or complete alkaline deacetylation process is carried out to obtain chitosan. The degree of deacetylation becomes higher if a larger amount of N-acetyl-D-glucosamine units are turned into D-glucosamine (Younes and Rinaudo, 2015). The molecular weight as well as the degree of deacetylation usually determines the physical properties of the produced chitosan including solubility, adsorption capability, and biodegradability (Alvarenga, 2011). The applicability of chitosan is expanding over years in the industrial, environmental, agricultural, and medical fields, due to its affordable price, availability in the market with different grades, eco-friendly (non-toxic), besides its outstanding properties including; for instance, biocompatibility, biodegradability, antimicrobial, anticancer properties. Currently, Chitosan is produced over the globe with an exponentially increasing global demand, which is estimated to be over 8000 tons per year in 2017 (Grand view research, 2020).

Tomato (*Solanum lycopersicum* L.) is a short-lived perennial cropped as annual. It is part of the Solanaceae (nightshade family) and is usually grown for its edible fruits. Tomato considers among the important crops grown over the world for their economic and nutritional value (Abdel Latef and Chaoxing, 2011; Zhang *et al.,* 2017), which considers a reason beyond choosing it as a model plant in our study.

As mentioned before, Chitosan is well-known as an elicitor with a large prospect of resolving stress adaptation issues due to abiotic and biotic stresses (Malerba and Cerana, 2015). Its ability to scavenge ROS and eventually enhance stress resistance efficiency has attracted researchers to explore more applications of chitosan on plants. For instance, studies of Mahdavi and Rahimi (2013), Mahdavi (2013), Ray *et al.* (2016), and Al-Tawaha *et al.* (2018) on *Trachyspermum ammi*, *Plantago ovata*, *Vigna radiate*, and *Zea mays*, respectively, showed that the treatment with chitosan reduced the effects of abiotic stress by increasing the activity of antioxidant enzymes, which led to a decrease in the content of oxidative damage indicators, such as malondialdehyde (MDA). Moreover, several reports have shown that the application of chitosan improved morphological characteristics (Shoot length, root length, number of laterals branches per plant, and number of leaves) in the case of maize (Guan *et al.,* 2009; Lizárraga-Paulín *et al*., 2011), rice (Songlin and Qingzhong, 2002; Boonlertnirun *et al.,* 2017), and common beans (Zayed *et al.,* 2017).

The intrinsic property of chitosan that it is not dissolved in neutral aqueous solutions, but rather in acidic solutions of weak carboxylic acids, such as; acetic, ascorbic, citric, lactic, and malic acids. Using acetic acid, as the associated organic acid to facilitate the dissolution of chitosan, is common in commercial formulations in the agricultural sector. Although, acetic acid was reported as the best associated organic acid in the case of coating fruits to prevent fungal growth (Romanazzi *et al.,* 2009), the effect of the associated organic acid on plant biostimulant activity was not evaluated. Using other organic acids such as citric and ascorbic, which are known for their stimulant activities on plants (for instance Talebi *et al.,* 2014), could lead to synergetic effects, which might increase the performance of the biostimulant.

To the best of our knowledge, there is no published work concerning the best associated organic acid on the biostimulant activity of chitosan. Therefore, this study aims to evaluate and compare the biostimulant activity of the foliar application of chitosan, dissolved in aqueous solutions of four different organic acids (acetic acid - ascorbic acid - citric acid - malic acid), as a stimulant strategy for improving the health, growth, physio-biochemical characteristics, and the yield of tomato plants. Suggesting, through the results, the best associated organic acid that might give synergetic effects with chitosan and could incorporate within the biostimulant formulations on the commercial scale.

MATERIALS AND METHODS

1- Used Materials

A low molecular weight Chitosan (M_w = 50 – 150 KDa) was purchased from National Research Center, Giza, Egypt. The organic acids were obtained from Sigma Aldrich (Merk). All chemicals were used without further purification.

2- Characterization of chitosan sample

Fourier transform infrared (FTIR) analysis was carried out for chitosan using an FTIR spectrometer (Thermo Scientific Nicolet iN10, National Research Center, Cairo, Egypt). The measurements were performed within a spectral range of 400 – 4000 cm^{-1} and a spectral resolution of 4 cm^{-1}. The degree of acetylation (DA) of chitosan was estimated based on the infrared spectra, according to Boukhlifi (2020). DA was calculated using the absorbance ratio (A1655/A3450) by the following equation: DA (%) = (A1655/A3450) ×100/1.33 and the degree of deacetylation (DDA) percentage was calculated via subtraction of DA percentage out of 100% according to the following equation: DAA (%) =100 - DA (%). The viscosity of chitosan (1% w/v) dissolved in an aqueous solution of acetic acid (1% v/v) was measured using Brookfield RVDVE230 Medium-range viscometer at 100 rpm at room temperature.

3- Pot experiment

Four weeks age tomato seedlings (cultivar 023) were obtained from the Agricultural Research Center (ARC), Giza, Egypt at the beginning of the summer season. Uniform seedlings were transplanted into plastic pots (40 cm in diameter) contain a mixture of sand and clay (1:3 W/W), total 7 kg, in a plastic greenhouse, at Smart Land company for agriculture development, 6th of October, Egypt. The pots were arranged in a completely randomized design with six replicates to study the biostimulant activity of chitosan dissolved in aqueous solutions of the four different organic acids. Four treatments and one control were included in this experiment as following; control (absolute) irrigated with tap water, treatment with chitosan dissolved in aqueous acetic acid solution (Ch ACE), a treatment with chitosan dissolved in aqueous ascorbic acid solution (Ch ASC), a treatment with chitosan dissolved in aqueous citric acid solution (Ch CIT), and treatment with chitosan dissolved in aqueous malic acid solution (Ch MAL). For all treatments, the concentration of both the chitosan and the associated carboxylic acids was 100 ppm individually. The chitosan was foliar applied three times (once per week), before and after the flowering period. The experiment was carried out under normal conditions, i.e., no external stresses (such as salinity) were added. The samples were collected for measuring different growth traits (shoot length, root length, number of leaves, and number of lateral branches per plant), yield characteristics, and biochemical analysis after 60 days from transplanting.

4- Photosynthetic pigments estimation

A previously mentioned method, in Vernon and Seely (1966), was used to estimate chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a + b (Chl a + b), and carotenoids contents in fresh tomato leaves.

5- Osmolytes content estimation

The soluble sugar and soluble protein content were estimated in the dried shoot according to the methods described in Irigoyen *et al*. (1992) and Lowry *et al*. (1951). The proline content was estimated in the dry shoot according to Bates *et al*. (1973).

6- Ascorbic acid and total phenol contents estimation

The method of Jagota and Dani (1982) was used to estimate the ascorbic acid of dry shoot. Total dry shoot phenol content was estimated according to Dai *et al*. (1993).

7- Assay of antioxidant enzymes

Peroxidase (POD), Polyphenol oxidase (PPO), Superoxide dismutase (SOD), and Catalase (CAT) were assayed according to the methods of Bergmeyer (1974), Matta and Dimond (1963), Marklund and Marklund (1974), and Aebi (1984), respectively. The activities of POD, PPO, SOD and CAT were assayed in fresh tomato leaves.

8- Statistical analyses

Two-way variance analysis (ANOVA) applied to the resulting data. The least significant difference (LSD test) using CoStat (CoHort, Monterey, CA, USA) was used to demonstrate statistically relevant differences between treatments at $p < 0.05$. Results are shown as mean ± standard deviation (n = 3).

RESULTS

1- Chitosan characterization

The molecular weight of chitosan, as mentioned before, is ranging from 50 to 150 kDa, and can be considered as low molecular weight chitosan. This was confirmed by the low viscosity value (14.7 ± 0.4 CP) obtained for chitosan (1% w/v) dissolved in an aqueous solution of acetic acid at 100 rpm (Kasaai *et al.,* 2000). The degree of deacetylation (DDA) was about 65%. Characteristic FTIR bands of chitosan were illustrated in (Fig.1). A strong band at around 1088 Cm^{-1} corresponds to C-O stretching. The absorption band centered on 2880 Cm^{-1} can be attributed to C-H asymmetric stretching. The presence of bands at around 1425 and 1382 Cm^{-1} could confirm CH_2 bending and CH_3 symmetrical deformations. The absorption band at 1155 Cm^{-1} can be related to the asymmetric stretching of the C-O-C bridge. The signal at 891 Cm^{-1} may be corresponding to the CH bending out of the plane of the ring of monosaccharides. These bands are characteristic of chitosan as reported before (Queiroz *et al.,* 2014). The presence of N-acetyl groups was confirmed by the bands around 1626 Cm^{-1} (C=O stretching of amide I) and 1594 Cm^{-1} (N-H bending of primary amine). The beak around 1250 Cm^{-1} was assigned as the bending vibrations of hydroxyls present in chitosan. The band at 3446 Cm^{-1} corresponds to N-H and O-H stretching, as well as the intermolecular hydrogen bonds.

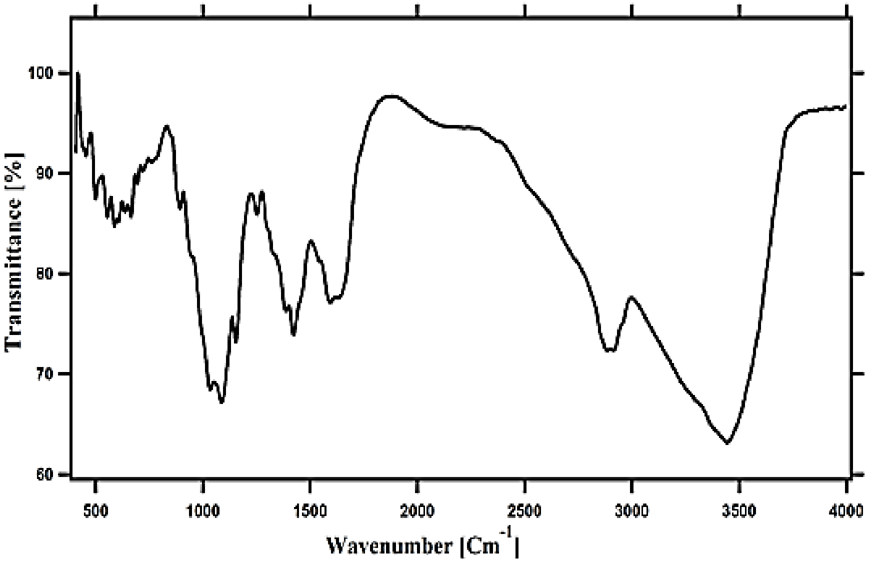

Figure 1: The Fourier transform infrared (FTIR) spectrum of the used Chitosan.

2- Growth parameters

The results showed that the foliar application of chitosan solutions (Ch ACE, Ch ASC, Ch CIT, and Ch MAL) had a positive effect on all vegetative growth parameters of tomato plants compared with the control plants (Fig. 2). However, different responses were observed, which could be related to the associated organic acid. A significant increase in the shoot length and number of laterals was noticed on Ch ASC, Ch CIT, and Ch MAL treatments comparing with either Ch ACE treatment or the control. Moreover, the application of Ch ASC gave the highest significant increase in root lengths, followed by Ch MAL, Ch ACE, and Ch CIT treatments. Furthermore, it was found that Ch ACE, Ch MAL, and Ch ASC treatments showed a significant increase in the number of leaves compared to Ch CIT treatment and the control.

3- Photosynthetic pigments

Statistical analysis of results in (Fig.3) revealed that there were significant increase in Chlorophyll a, b, and Carotenoids. Different contents of chlorophyll a, b, as well as carotenoids were observed between the different treatments. The increase in Chlorophyll a content was significantly higher in Ch CIT compared to control, followed by Ch ASC, Ch MAL, and Ch ACE respectively. Moreover, the results showed that the application of Ch ACE and Ch ASC treatments gave the most potent effect regarding the total chlorophyll content in comparison with plants treated with Ch MAL and Ch CIT, with the aforementioned treatments not significantly different from the control. Furthermore, the obtained results illustrated that the contents of carotenoids in chitosan treated plants were significantly increased compared to the control. Ch MAL treatment had higher carotenoids content, followed by Ch ASC, Ch ACE, and Ch CIT, respectively.

4- Osmolytes

Results in (Fig.4) show that soluble sugars, total soluble protein, and proline contents of tomato plants significantly enhanced due to the foliar application of chitosan solutions (Ch ACE, Ch ASC, Ch CIT, and Ch MAL) compared to the untreated plants.

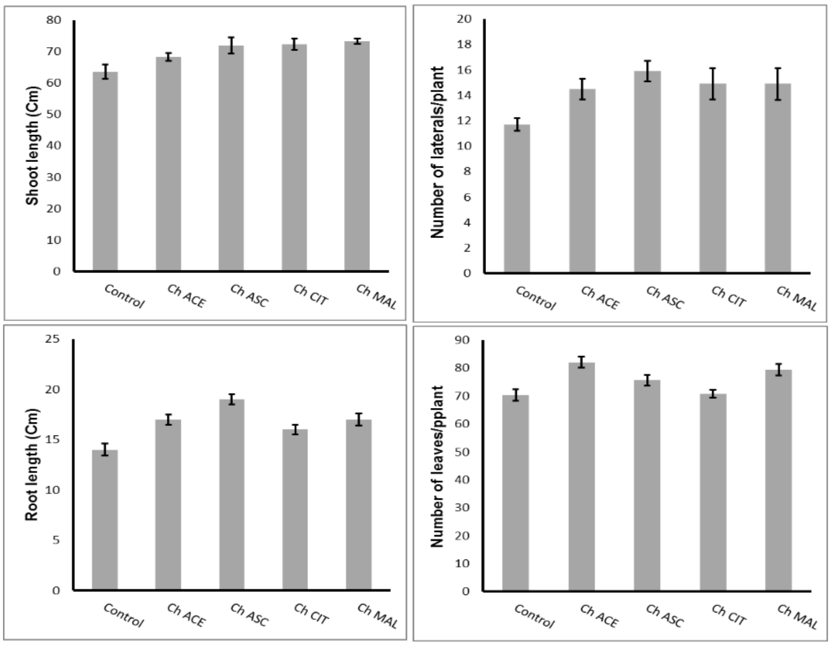

Figure 2: Effect of foliar application of chitosan dissolved in organic acids (Ch ACE, Ch ASC, Ch CIT, Ch MAL) on shoot length (cm), root length (cm), number of laterals and number of leaves of tomato plants. Data presented as means ± SE (n=3).

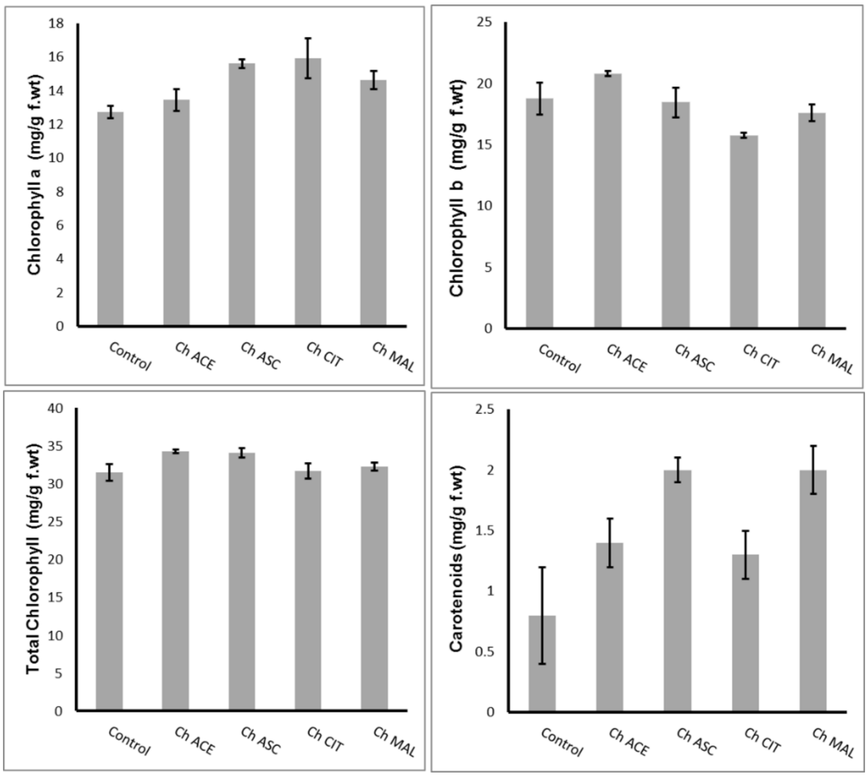

Figure 3. Effect of foliar application of Chitosan dissolved in (Ch ACE, Ch ASC, Ch CIT, Ch MAL) on chlorophyll a (mg g^{-1} FW), chlorophyll b (mg g^{-1} FW), chlorophyll a+b (mg g^{-1} FW) and carotenoids (mg g^{-1} FW) of tomato plants. f.wt: fresh weight. Data as means ± SE (n=3).

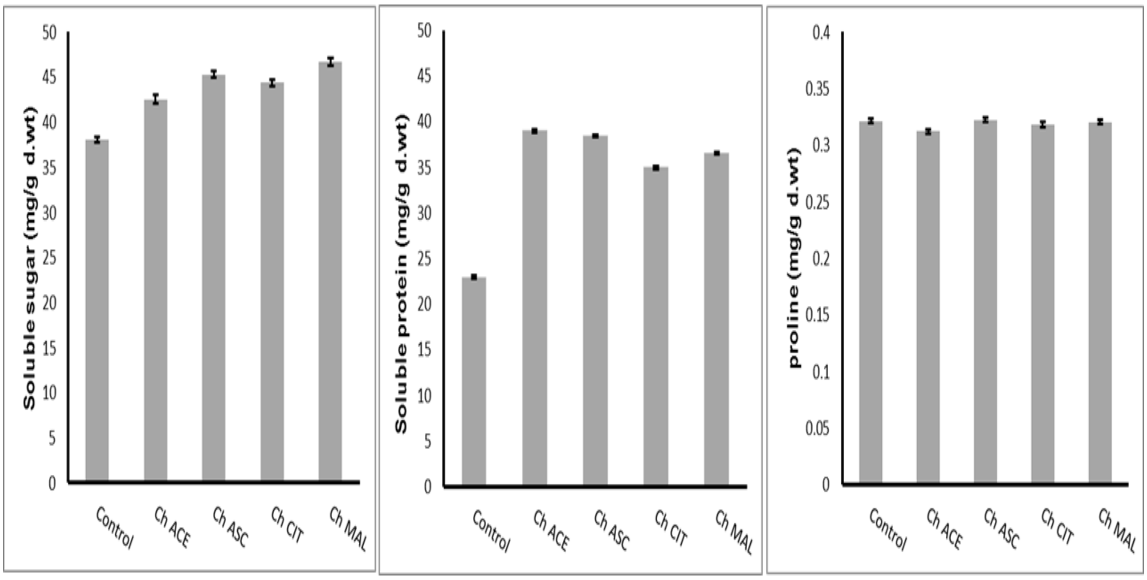

Figure 4: Effect of foliar application of chitosan dissolved in organic acids (Ch ACE, Ch ASC, Ch CIT, Ch MAL) on the content of Soluble sugars, Soluble protein and Proline (mg g^{-1} DW) of tomato plants. Data presented as means ± SE (n=3). d.wt : dry weight.

The obtained results revealed that the application of Ch MAL gave the most potent effect as regards the Soluble sugars comparing with plants treated with Ch ASC and Ch CIT, followed by Ch ACE as compared to the control. Moreover, it was found that tomato plants treated with Ch ACE and Ch ASC gave the most potent effect as regards the soluble protein content comparing with plants treated with Ch MAL, followed by Ch CIT as compared to the control. Furthermore, the obtained results illustrated that in chitosan treated plants, the contents of Proline were increased in response to the treatment with chitosan solutions Ch ASC, Ch CIT, Ch MAL, and Ch ACE, respectively as compared to control. The increases in proline were found to be statistically, insignificant (Fig.4).

5- Phenols and ascorbic acid contents in tomato plant leaves

Data illustrated in (Fig. 5) revealed that tomato plants treated with chitosan, dissolved in the aqueous solutions of acetic acid, ascorbic acid, citric acid, and malic acid, exhibited significant increases in contents of total phenols and ascorbic acid compared to the control plants, however still variable between the four treatments. The highest average content of Ascorbic acid was accounted for Ch ASC (58.5 %), followed by Ch MAL (31.73 %), Ch ACE (26.8 %), and Ch CIT (21.8 %), while the highest average content of total phenols was accounted for Ch ACE (30 %) and Ch ASC (25%), followed by Ch MAL (20%) and Ch CIT (15%), respectively comparing with untreated plants.

6- Antioxidant enzymes activity

The activities of PPO, POD, SOD, and CAT illustrated in (Fig. 6) were increased in the treated plants comparing with the control. The treatments of Ch ACE, Ch ASC, Ch CIT, and Ch MAL increased the activity of PPO by 0.816, 0.745, 0.607, and 0.607 unit/g. f.wt/ hour, respectively

compared to control (0.317 unit/g. f.wt / hour). Moreover, the treatments of Ch CIT, Ch ASC, Ch MAL, and Ch ACE increased the activity of POD by 0.113, 0.077, 0.072, and 0.07 unit/g. f.wt / hour, respectively compared to the control (0.038 unit/g. f.wt / hour). Furthermore, the treatments of Ch CIT, Ch MAL, Ch ACE, and Ch ASC increased the activity of SOD by 0.161, 0.103, 0.010, and 0.010 unit/g. f.wt / hour, respectively compared to the control (0.054 unit/g. f.wt / hour). While the foliar application of chitosan solutions Ch CIT, Ch ASC, Ch MAL, and Ch ACE increased the activity of CAT by 0.324, 0.222, 0.208, and 0.201 unit/g. f.wt / hour, respectively comparing with corresponding controls (0.109 unit/g. f.wt / hour).

7- Yield characters

The obtained results in (Fig. 7) show significant increases in all studied yield parameters in tomato plants. The application of Ch CIT and Ch ASC gave the most potent effect regarding the average number of flowers per plant (26.9 and 26.3) in comparison with the plants treated with Ch MAL (24.9), followed by Ch ACE (20.2), compared to the control (17.6). Moreover, tomato plants treated with Ch MAL gave the most potent effect regarding the average number of fruits per plant (19.3) in comparison with plants treated with Ch ACE (18.8) and Ch ASC (18.4), followed by Ch CIT (16.6), compared to the control (11.7). Furthermore, results in (Fig. 7) showed that the application of Ch MAL and Ch ASC gave the most potent effect regarding the average weight of fruits per plant (314 g and 301 g, respectively) in comparison with the plants treated with Ch CIT (282 g), followed by Ch ACE (270 g) compared to the control (210 g).

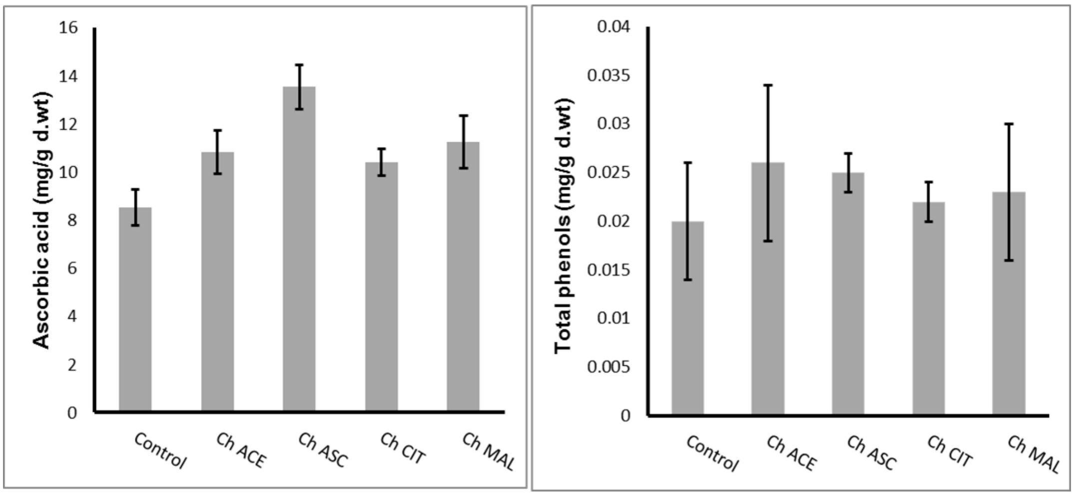

Figure 5. Effect of foliar application of chitosan dissolved Ch ACE, Ch ASC, Ch CIT, Ch MAL) on total phenols and ascorbic acid of tomato plants.
Data as means ± SE (n=3). d.wt: dry weight.

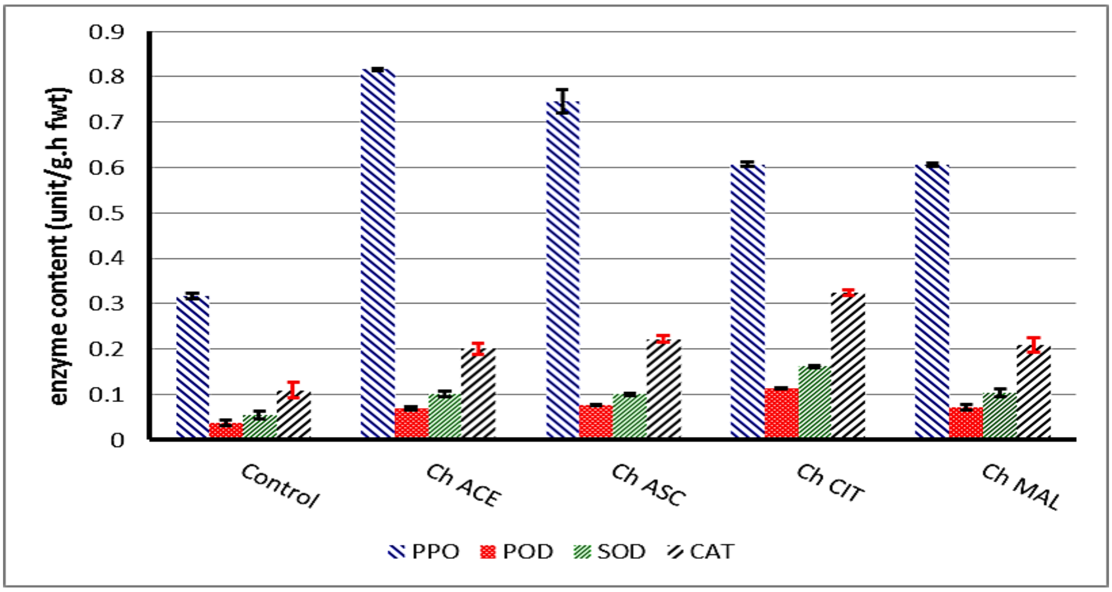

Figure 6. Effect of foliar application of Chitosan dissolved in different organic acids (Ch ACE, Ch ASC, Ch CIT, Ch MAL) on POD activity ($U\ g^{-1}$ FW) , PPO activity ($U\ g^{-1}$ FW), SOD activity ($U\ g^{-1}$ FW) and CAT activity ($U\ g^{-1}$ FW) of tomato plants. Data presented as means ± SE (n=3).

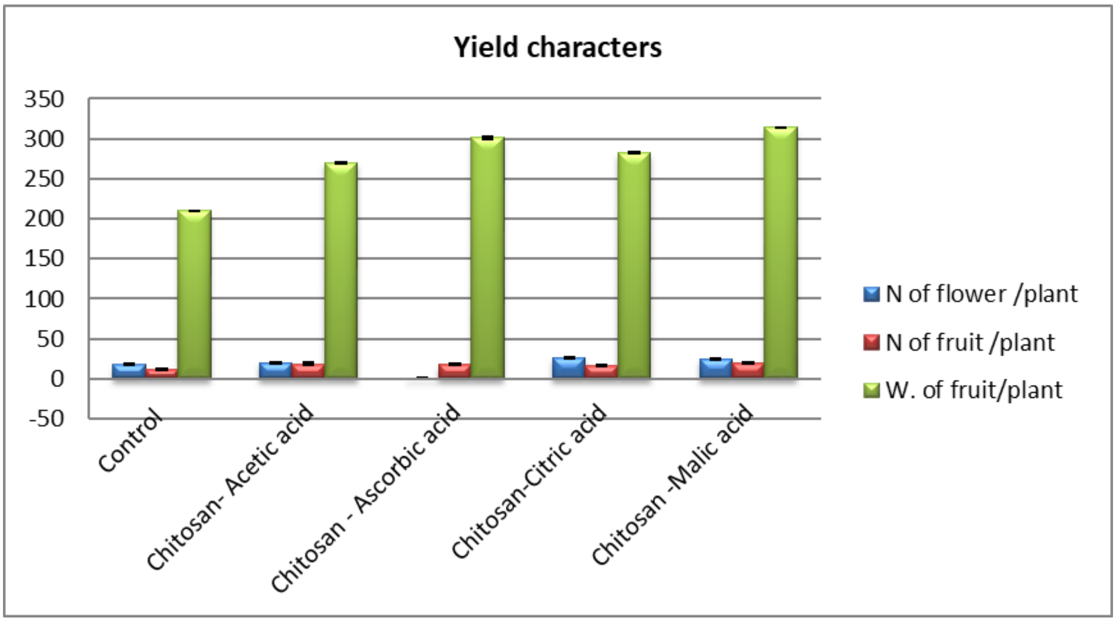

Figure 7. Effect of foliar application of Chitosan dissolved at Ch ACE, Ch ASC, Ch CIT, Ch MAL) on yield parameters of tomato plants. Data as means ± SE (n=3). Data followed by different letters are significantly different LSD P ≤ 0.05.

DISCUSSION:

The essential role of a successful plant biostimulant is that it can help the crops withstand difficult stresses, which still present even under normal conditions, including deficiency of

nutrients in the agricultural land and/or the limited absorption via plant roots while increasing productivity. Using of natural products has been discussed in the literature to enhance the growth of different crop plants as wheat (Lizárraga-Paulín *et al.,* 2011; Ma *et al.,* 2012), fenugreek, chickpea, and maize (Abdel Latef *et al.,* 2017 a; Abdel Latif *et al.,* 2017 b; Abdel Latif *et al.,* 2019), respectively. As illustrated in the results, morphological aspects (Shoot length, root length, the number of laterals branches per plant, and the number of leaves) were increased due to chitosan application, especially which dissolved in the aqueous solutions of ascorbic acid, followed by malic and citric acids, respectively. Reports have shown that application of inducers such as chitosan improved morphological characteristics, for instance, in the case of maize (Guan *et al.,* 2009; Lizárraga-Paulín *et al.,* 2011), rice (Songlin and Qingzhong, 2002; Boonlertnirun *et al.,* 2017), and common beans (Zayed *et al.,* 2017). Additionally, the use of chitosan dissolved in some organic acids such as citric acid, ascorbic acid, and malic acids improves the plant's ability to ameliorate biotic and abiotic stress (Hidangmayum *et al.,* 2019). Plants treated with chitosan dissolved in acetic acid haven't shown phytotoxicity in different crops as Japanese pear (Du *et al.,* 1997) kiwifruit, or table grape (Romanazzi *et al.,* 2002), however, it did not convey the best enhancement in growth parameters in this study.

In the current study, results showed that tomato plants treated with chitosan solutions, especially dissolved in acetic and ascorbic acids, improved significantly photosynthetic pigments that will eventually lead to enhancement of photosynthetic rates. These results are consistence with Phothi and Theerakarunwong (2017), which reported that chitosan application enhanced photosynthetic rates of *Oryza sativa* plants throughout enhancement photosynthetic pigments. This increase might be attributed to enhanced stomatal conductance, transpiration rate and/or cell size and number (Khan et al., 2002). This may also be since chitosan has been reported to trigger plant defense reactions (Thakur and Sohal, 2013), and it may trigger NADPH oxidase activity, thereby stimulating the production of H_2O_2. Thus, chitosan could activate ROS scavenging systems in plants (Vidhyasekaran, 2016).

The accumulation of osmolytes serves as a common phenomenon that plays an important role in ROS scavenging, supply plant cell with energy as well as modulating cell redox homeostasis (Szabados and Savoure, 2010; Sharma *et al.,* 2011; Abdel Latef *et al.,* 2019). Foliar application of chitosan solutions, especially the dissolved in malic and ascorbic acids, enhanced osmolytes in shoots of tomato plants. These results are in harmony with Zou *et al.* (2015). A study by Safikhan *et al.* (2018) reported a significant increase in osmolytes contents in chitosan treated milk thistle (*Silybum marianum* L.) plants. Chitosan caused an enhancement in the contents of soluble sugars, soluble protein throughout its role in increasing the expression of enzymes involved in glycolysis (Chamnanmanoontham *et al.,* 2015; Rabêlo *et al.,* 2019). Proline accumulation in tomato shoots safeguards the photosynthetic process throughout preventing damage of photosynthetic pigments caused by ROS (Silva-Ortega et al., 2008; Soshinkova *et al.,* 2013). The results did not show a favorable associated acid regarding the increasing of proline content. This is in accordance with the fact that the plants in the experiment of this study were free from external abiotic stresses.

ROS scavenging in plants occurs in two ways, enzymatically and non-enzymatically, to safeguards plant cells from oxidative damage. Non-enzymatically pathway includes phenolic compounds and ascorbic acid, which can overcome ROS production (Gill and Tuteja, 2010; Kubalt, 2016; Abdel Latef *et al.,* 2019). The accumulation of plant secondary metabolite acts as a plant adaptive mechanism in response to biotic and abiotic stress. In this study, plants treated with chitosan dissolved in ascorbic, acetic, malic, and citric acids, respectively, increased the contents of total phenols and ascorbic acid in shoots of tomato plants. The significant increase in ascorbic acid content in the plants treated with chitosan dissolved in aqueous solution of ascorbic acid is due to the direct incorporation of ascorbic acid inside the cells of the plant shoot. Our results are in accordance with other investigators (Tiwari *et al.,* 2010; Kasim *et al*., 2016; El-Shourbagy *et al.,* 2017). Phenolic compounds and ascorbic acid support antioxidant roles by scavenging the free radicals, reducing their reactivity to the membrane components (Akram *et al.,* 2017; Abdel Latef *et al.,* 2019). Moreover, Phenolic compounds are also able to stabilize cell membranes by reducing membrane fluidity, which results in reduced mobility of free radicals across membranes, thus limiting membrane peroxidation (Kubalt, 2016). The obtained results are consistent with Bakhoum *et al.* (2020) and Farouk *et al.* (2013). Also, a study of Abdallah *et al*. (2020) showed that treatment with chitosan increased significantly contents of phenolic compounds which directly decline lipid oxidation throughout transferring a phenolic hydrogen atom to a radicle.

SOD, CAT, POD and POO provide a large number of defensive enzymes associated with various stresses including salinity (Kordrostami *et al.,* 2017; Abdel Latef *et al.,* 2020). These enzymes act as initial steps in increasing plant resistance to various stresses as well as the formation of phenolic compounds (Van Loon et al., 1998; Rios-Gonzalez et al., 2002). Our results showed different strategies to improve plant health as they increase the activity of certain antioxidant enzymes to keep ROS at the lower level in the cell. Antioxidant enzymes as SOD helps in conversion of $O2^{\cdot -}$ to H_2O_2, which act as the first line in facing oxidative stress, while CAT and POD help in the conversion of H_2O_2 to H_2O (Gill and Tuteja, 2010). SOD, CAT, POD and PPO activities were greater in the plants treated with chitosan solutions, compared to un-treated plants. Application of chitosan was reported to increase the activity of catalase and peroxidase in tomato (Ortega-Ortiz *et al.,* 2007), eggplants (Mandal, 2010) and milk thistle (Safikhan *et al.,* 2018). Chitosan chemical composition includes (uridine diphosphate N-acetyl-d-glucosamine (UDP-GlcNAc) as nucleotide sugars which when applied in plants recognized by cell throughout chitin synthase chitin deacetylase enzymes and caused the formation of chitosan oligomers that's involved in plant cell signals (Hadwiger, 2015; Malerba and Cerana, 2016).

CONCLUSION

From the obtained results, it can be concluded that the foliar application of chitosan dissolved in aqueous solutions of the four different organic acids, especially ascorbic and malic acids, can promote the health of tomato plants. That, in turn, stimulated the vegetative growth as well as the yield, probably through the enhancement of osmoprotectant compounds, antioxidant system, and non-enzymatic system of reactive oxygen species (ROS) scavenging. In conclusion, the synergetic effects of ascorbic and malic acids could

be more advantageous for plants under normal conditions, thus we recommend both acids for the chitosan-based pre and after flowering bio stimulant formulations in the agriculture commercial sector. Further confirmation is required to compare these results with the results of different types of chitosan, and the results of other plants cultivated in different conditions as well as to investigate these synergetic effects under different biotic and abiotic stresses, which might be different from the current results. Understanding the true mechanisms is also required, but left for future work.

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تأثير المنشط الحيوي الشيتوزان المذاب في الأحماض العضوية المختلفة على نبات الطماطم

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الملخص العربي

تهدف هذه الدراسة إلى تقييم ومقارنة التأثيرات المنشطة للشيتوزان المذاب في محاليل مائية لأربعة أحماض عضوية مختلفة هى (حمض الخليك وحمض الأسكوربيك وحمض الستريك وحمض الماليك) عند رشه على أوراق نباتات الطماطم (*Solanum lycopersicum* L.).

أجريت تجربة أصص في صوبة بلاستيكية خلال فصل الصيف ، حيث تم تقييم الصفات المورفولوجية ، أصباغ التمثيل الضوئي ، حمض الأسكوربيك ، الفينول الكلي ، السكريات الكلية والبروتين الكلى والبرولين ، أنشطة إنزيم مضادات الأكسدة (البيروكسيداز ، أوكسيديز بوليفينول ، ديسموتاز الفائق ، والكاتلاز) ، والمحصول لنباتات الطماطم المعاملة .

أشارت النتائج إلى أن معاملات الشيتوزان أظهرت تحسنًا في جميع الصفات والمكونات المذكورة أعلاه تقريبًا، وكانت هذه الاستجابات مختلفة وفقًا للحمض العضوي المذاب فيه الشيتوزان .

علاوة على ذلك ، أدت معاملات الشيتوزان ، مقارنة مع الكنترول، إلى زيادة معنوية في جميع معاملات المحصول المدروسة في نباتات الطماطم ، وخاصة الشيتوزان المذاب في محلول مائي من حمض الماليك وحمض الأسكوربيك الذي أعطى التأثير الأكثر فعالية فيما يتعلق بعدد الأزهار / نبات وعدد الثمار ، وكذلك وزن الثمار / نبات مقارنة بتلك النباتات المعالجة بالشيتوزان المذاب في محلول مائي من حامض الستريك ، أو حمض الخليك. ونظرًا لأنه مادة غير سامة ومتوافقة حيويًا وقابلة للتحلل الحيوي ومتوفرة في السوق العالمية .

توصى نتائج البحث بفاعلية التطبيق الورقي للشيتوزان ، خاصةً المذاب في المحاليل المائية من أحماض الأسكوربيك أو الماليك ، للتطبيقات الحقلية في ظل الظروف العادية (غير مجهدة) وذلك للاستفادة من تأثيرها كمنشطات حيوية قبل وبعد الإزهار لتحسين جودة نبات الطماطم وبالتالي زيادة المحصول ، ويمكن أن تمتد النتائج للتطبيق لتشمل جميع نباتات عائلة Solanaceae بعد مزيد من الدراسات.

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