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Full Length Article

Response of Tomato Growth to Foliar Spray and Root Drenching of Aqueous Garlic Extract: A Cocktail of Antioxidative Defenses, Chlorophyll, Carotenoid and Soluble Sugar Contents

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Abstract

In our previous work, aqueous garlic bulb extracts (AGE) with 100-200 µg mL^{-1} have shown considerable effects on the growth of tomato and cucumber plants, however, to investigate the effects for repetition of these applications more than once in a growing period, a pot experiment in a plastic tunnel was conducted to evaluate whether a single or a repeated application of AGE would be inducible for enhanced tomato growth. AGE was applied at the rate of 100 µg mL^{-1}as foliar spray and root drenching applications and their respective effect was determined on the growth and physiological conditions of tomato plants. Findings of the study revealed that AGE can modulate the antioxidant enzymes, chlorophyll content and soluble sugar content of the treated tomato plants. The effect however, depends upon the method of application and moreover, the repetition of treatment as root irrigation may cause adverse effects on the treated plants. AGE foliar and root application influenced plant growth parameters such as plant height, shoot fresh weight, root length, fruit size and weight. Similarly, differences were found in the antioxidative response of the treated plants; nonetheless, these treatments affected the abundance of chlorophyll, carotenoid, and the total soluble sugar contents in the plants. Current findings indicate that garlic extracts bear priming effects which could be useful to enhance certain physiological aspects of the receiver plants; the bioactive role of aqueous garlic extracts is hereby addressed to postulate a botanical stimulator for enhanced vegetable production under specialized horticultural situations.

Keywords: Aqueous garlic extract; Foliage and root application; Antioxidative response; Chlorophyll content

Introduction

In specialized horticultural situations such as plastic tunnel farming system, though production of vegetables could be facilitated (Eriksen *et al.,* 2003), yet the enclosed environment therein, sometimes has limitations such as high humidity and temperature (Pfeufer *et al.,* 2016) which often favor pathogenic growth, posing a significant threat to the production. To prohibit such problems and facilitate enhanced production, synthetic chemicals are often utilized, however, the growing awareness about the hazardous consequences due to the overuse of these chemicals and the increasing demand for greener produce among the consumers, it is becoming mandatory to explore and find environment friendly entities to sustain agriculture production and maintain environmental safety (Seiber *et al.,* 2014; Malo *et al.,* 2017). Therefore, scientific communities are now turning their research focus towards exploring and utilization of biostimulants to enhance the agricultural production (Zaller, 2006; Portz *et al.,* 2008; Martins *et al.,* 2016). These botanicals can be characterized as stimulators, promoters or inducers, depending upon their chemical composition and bioactivity (Andresen *et al.,* 2015; Hayat *et al.,* 2016; Mukerji, 2006). To ensure the bioactivity of a proposed chemical of interest, it is however vital to consider that how the receiver plant will respond to it. To solve this question, various physiological aspects of the receiver plants could be of significance such as photosynthesis, antioxidative response, chlorophyll contents, total soluble sugars and carotenoid contents etc. Nevertheless, morphological examination of the plant is inevitable and should be taken into consideration to understand and validate the effects of a bioactive compound (Belguith *et al.,* 2009; Rodrigues *et al.,* 2013; Khan *et al.,* 2016).

To date, various medicinal plants have been studied for their active compounds and respective effects upon the biological activities of both the receiver plants and the neighboring environment (Dixon, 2001; Von *et al.,* 2005; Hanafy *et al.,* 2012; Lanzotti, 2012; Perelló *et al.,* 2013; Sheren *et al.,* 2015). Garlic, is one among these medicinal plants having a marvelous repute of strong antimicrobial potential (Ankri and Mirelman, 1999; Afzal *et al.,* 2000; Hayat *et al.,* 2016). In the past decade, advances have been made to evaluate and understand the allelopathic potential

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of garlic on the neighboring crops and soil (Xiao *et al.*, 2012; Wang *et al.*, 2015). Furthermore, a plethora of research studies have shown the antioxidant effects of garlic derived compounds demonstrating their active role as an anticancer entity and as remedy for various cardiological complications in human health (Oommen *et al.*, 2004; Gorinstein *et al.*, 2008; Pan and Wu, 2014). However, in plant productions side, very few researches have been conducted and the application of garlic-based botanicals as growth promoter and the particular responses of the receiver plants to understand the bioactive mechanism is less understood. In our previous studies, it has been indicated that garlic aqueous extracts (AGE) can actively participate in the activation of antioxidant enzymes of cucumber plant exerting variable effects on its growth depending on the concentration of the extracts (Hayat *et al.*, 2016). Both of its direct action against pathogens and involvement in the physiological responses of the receiver plants, garlic is therefore a subject of interest to evaluate as a biostimulant. Therefore, we attempted to study the effects of AGE applied at various durations on tomato plants grown under plastic tunnel conditions. Our study includes the primal morphological observations coupled with the significant physiological indications which are often associated to the stress sensing and growth promoting responses such as the antioxidative response, chlorophyll, soluble sugars and carotenoid contents to establish the bioactivity of AGE on the receiver plants under plastic tunnel farming system.

Materials and Methods

Garlic Extract Preparation

Garlic cultivar G025 was obtained from the garlic germplasm unit, college of Horticulture Northwest A&F University China. The aqueous extract was prepared according to method previously described in our article (Hayat *et al.*, 2016). Briefly, randomly selected 10 g of sample was ground in a sterile mortar and pistil and then homogenized in 100 mL distilled water. The homogenate was further centrifuged at 10,000 rpm and the supernatant was collected and filtered through 0.24 µL pore filter. From the supernatant, dilution was carried out to obtain a final concentration of 100 µg mL^{-1}. Distilled water was take as control treatment.

Tomato Growth Conditions

The experiment was conducted at the plastic tunnel facility, college of Horticulture, Northwest A&F University, China in the year 2016. Tomato cultivar Dong Ya-Fen Guan was used to perform the experiment. Seeds were sown in plastic trays. After fourth true leaf (about a month-old seedling), uniform sized tomato seedlings were selected and transferred to plastic pots containing growing substrate purchased from Shanghai Fuang agrochemical co., China and were maintained under plastic tunnel condition with natural daylight.

Treatment Application

After one-week post transplantation, the garlic extracts were applied as foliage application and root drenching method. For foliar application, spray automizer was used. The plants were sprayed with 50 mL till dipping. To apply the treatments into roots, same amounts were used as root drenching. The treatments were repeated for a second time after 15 days for one half of the plants. Water was used as control treatment. Each set of replicates contained 10 seedlings and each treatment was replicated three times having a total of 30 seedlings per treatment.

Morphological Traits

To evaluate the morphological traits, six plants were randomly selected from each replicate and total of 18 plants were accounted for each treatment to measure the morphological parameter. The average for each of the selected 6 plants was then used to analyze the data for one replicate.

Plant Growth and Fruit Measurements

Plant height and root length were measured using a measuring tape. The plant height was recorded after fifteen days of each treatment and then average data of these two times were used for analysis. The stem diameter was measured in mm using Vernier calipers. Stem diameter was measured at three different points and the average was used. The data of stem diameter were taken in same pattern to that of plant height. Total number of branches per plant was recorded by counting.

Shoot fresh weight was measured after fruit picking. To measure fruit fresh weight, the plants were immediately weighed using an electronic balance. Number of flowers were recorded following the method described (Rab and Haq, 2012). Briefly, the total number of flower clusters per plant were recorded and then divided by total number of plants in each replicate.

The fruit size was measured using Vernier calipers. The size was determined by measuring the width and length of fruit and then the average from both was calculated. Fruit weight was recorded by using an electronic weight balance.

Physiological Assessment

To evaluate the physiological indices, we selected five plants from each replication and a total of fifteen plants were evaluated to account the physiological parameters. The mean data were used to analyze the data statistically.

Antioxidant Enzymes and MDA Content Analysis

The antioxidant enzyme analysis was done using the protocol mentioned in our previous article (Hayat *et al*., 2016). Briefly, leaf samples (0.500 g) were ground with 2 mL of cold extraction buffer (0.05 M phosphate buffer, pH 7.8) and the entire mixture was transferred to centrifuge tubes with another 6 mL of the same extraction buffer and centrifuged for 20 min at 10,000×*g* and 4◦C (Gao, 2006). The supernatant was used to determine the content of MDA and enzyme activities for each treatment; the measurements were performed in triplicate.

The MDA content was measured using the thiobarbituric acid (TBA) reaction (Zhang, 2004). 2 mL of the extract supernatant were mixed with 2 mL 0.6% (w/v) TBA solution dissolved in 5% (v/v) trichloroacetic acid (TCA), heated in boiling water for 10 min, and then cooled to allow the flocculate to sediment. The supernatant was used for the spectrophotometric determination of MDA. The absorbance at the wavelength of 450 and 532 nm was measured and subtracted from the absorbance at 600 nm. MDA content was expressed as the amount of substance per gram of fresh leaves (nmol·g^{-1} Fw).

Total SOD activity was estimated by the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) (Gao, 2006). The reaction mixture contained 1.5 mL 0.05 M phosphate buffer (pH 7.8), 0.3 mL 0.1 mmol·L−1 EDTA-Na_2, 0.3 mL 0.13 mol·L^{-1} methionine, 0.3 mL 0.75 mmol·L^{-1} NBT, 0.3 mL 0.02 mmol·L^{-1} riboflavin, 0.05 mL enzymatic extract, and 0.25 mL distilled water in a total volume of 3 mL for the reaction mixture. After exposure to fluorescent light (86.86 μmol·m^{-2}·s^{-1}) for 10–20 min (endpoint determined by the color of the reaction solution), the absorbance was recorded at the wavelength of 560 nm. SOD activity was determined as 50% inhibition of the NBT reduction caused by the superoxides generated from the reaction of photo-reduced riboflavin and oxygen. The total SOD activity was expressed in units per gram of fresh leaves.

The guaiacol method was used for the determination of POD activity (Bestwick *et al.*, 1998). A reaction mixture was prepared using 50 mL 0.05 M phosphate buffer (pH 7.8), 28 μL guaiacol, and 19 μL 30% H_2O_2 (v/v); 3.5 mL of the reaction mixture solution was placed into a cuvette with a 1 cm path length. The increase in absorbance at the wavelength of 470 nm was recorded over 3 min at 30 s intervals after the addition of 0.5 mL enzyme extract. The results were presented as D470 per minute per gram of fresh leaves (U·g^{-1}·min^{-1}).

Chlorophyll a, b, Total Chlorophyll and Carotenoid Contents Determination

The chlorophyll contents and carotenoid contents in fresh leaves of tomato were determined using 0.2 g leaf sample in 25 mL of 80% acetone and placing at room temperature for 48 h in dark. The absorbance of the extract was recorded on spectrophotometer (UV-3802, UNICO, MDN, USA) on 663, 645 and 652 nm wave length (Lichtenthaler and Wellburn, 1983; Dere *et al.*, 1998)

Chlorophyll a b and total chlorophyll content was determined by the following formula:

Chlorophyll a = (12.7*A663) - (2.69*A645)
Chlorophyll b = (22.9*A645) - (4.68*A663)
Chlorophyll a+b = (Ca + Cb)

Total carotenoid content (C x+c) = (1000A470 – 1.82Ca –85.02Cb)/198.

The quantification in terms of (mg/gFW), the following equation was used

$$Q = (CV/W) *100$$

Where, C is the concentration (mg/L), V is the volume of solvent and W is fresh weight of the sample.

Soluble Sugar Content

To determine the soluble sugar contents, a standard procedure stated by Gao (Gao, 2006) was followed with appropriate adaptations necessary for the plant samples. Briefly, 0.5 g leaf sample was homogenized in 5 mL of ethanol (80%) and heated in a water bath for 30 min maintained at 80^0C. The samples were cooled at room temperature and centrifuged at 3500 rpm for 10 min. Total soluble sugars were determined by calculating the absorbance of samples against glucose and anthrone (dissolved in H_2SO_4) with a spectrophotometer at 620 nm.

Statistical Analysis

The experiment was designed in a randomized complete block with split plot arrangement and the data were subjected to analysis of variance (ANOVA). Comparison among the means was performed using LSD with a 0.05 level of significance. Figures were drawn using sigma plot version 10.

Results

AGE Foliage and Root Application Exerts Diverse Effects on Morphology of Tomato

The effect of foliage application and root drenching of AGE on the morphology of tomato plants is presented in Table 1. Variable effects on the morphological aspects of tomato were observed after the treatments. Growth promoting effect was observed in the plants to which AGE was applied as foliage application or as single dose in the form of root drenching and most of the parameters reached a statistical significance compared to those of control plants. However, the repetition of root drenching method caused adverse

Fig. 1: AGE (aqueous garlic extract) application influence the plant height of tomato

Plant height of tomato effected by AGE application. The pictures were taken at the start of flowering. Ck represents control treatment, Root1 and Root 2 represents single and repeated root drenching of AGE respectively whereas Spray represents foliar application of AGE

effects in some parameters comparatively to other treatments. Maximum plant height was recorded in the plants where AGE was applied as foliage application followed by single application of AGE as root drenching (Fig. 1). The effect was statistically different to those of control plants, however, no statistical difference among the AGE treatments was recorded, whereas the repeated dose of root drenching exerted adverse effect resulting in restricted plant height. Similarly, shoot fresh weight was also significantly higher in plants treated with AGE single time foliage and root drenching than those of control treatments. However, repeating the same application adversely affected the shoot fresh weight and there was significant reduction in shoot fresh weight of the plants applied with repeated root drenching. The number of branches per plant was maximum in the plants applied with AGE single time root drenching (9.33) followed by the repeated dose of foliar AGE application but there was no statistical difference between these treatments. Minimum number of branches was recorded for the plants applied with repeated dose of AGE as root drenching and the effect was significantly lowered to those of other AGE treated plants. For flower number, the maximum data was recorded for plants applied with single foliar application of AGE with an average of 27.8 while minimum number of flowers was recorded for the plants applied with repeated root drenching of AGE. The stem diameter however, interestingly increased in the plant applied with AGE root drenching method followed by the repeated application of same treatment. The lowest stem diameter was recorded in control plants. AGE foliar application gave statistically significant increase in root length of 23.827 cm as compared to control plants. However, the repeated application of AGE as root drenching resulted in a significantly reduced root length of 17.3 cm indicating an adverse effect on the plant growth. The fruit size and fruit weight were significantly altered in both the foliage spray and root drenching methods. As compared to control treatments, the fruit size showed statistical significance and maximum fruit size was recorded for the plant applied with the repeated dose of AGE as foliar application. Similarly, all the treatments significantly differed as compared to those of control for the fruit weight and maximum fruit weight was recorded for the plants applied with single time root drenching with AGE whereas the repetition of the same treatment reduced the fruit weight compared to other treatments.

Antioxidant Enzymes as Influenced by AGE Treatments

The effect of AGE on the antioxidant enzymes and MDA content are depicted in Fig. 2. As it can be observed in Fig. 2, application of AGE caused alteration in the activity of SOD and compared to the control, AGE foliage spray in a single dose lowered the activity, however, the repetition of same dose resulted in a significantly higher activity of the SOD. Applied AGE as root drenching revealed a vice versa situation and the single dose caused a significantly higher activity while the repetition resulted in a reduction of SOD activity as compared to control plants. Fig. 2 represents the effect of AGE on the POD activity and it can be observed that both the foliar and root drenching of AGE altered the activity of this enzyme comparative to that of the control plants. The single dose of AGE resulted in lowering the activity of POD compared to that of the control plants with the foliar application bearing the strongest effect. However, the repetition of same treatments after 15 days, the effect was observed otherwise, where both the root drenching and foliar spray increased the POD activity.

In Fig. 2 the effect of AGE application on the lipid peroxidation i.e., the MDA content of the tomato seedlings is presented. It can be noticed that both the foliar application and root drenching have no significant effects on the MDA content of the treated plants when compared to the control plants. The single application of AGE as foliar spray however, increased the MDA content comparatively. When the same applications were repeated, no significant difference was observed however, the effect was comparatively higher to those of the initial application.

AGE Affects Chlorophyll Contents of the Treated Plants

The effect of AGE on the chlorophyll contents of tomato plants is depicted in Fig. 3. As it can be observed that the AGE application both as foliar spray as well as root drenching, considerably influenced the chlorophyll contents of the tomato plants and interestingly, contrasting effect was observed based on the method of application. Foliage spraying of AGE

Table 1: Influence of AGE application on the morphology of tomato plants

Application method	Times of AGE treatment	Plant height (cm)	Shoot fresh weight (g)	Number of branches	Number of Flowers	Stem diameter (mm)	Root length (cm)	Fruit size (cm^2)	Fruit weight (g)
Water treatment	single	46.40±1.83 b	58.82±2.54 b	6±1.2 c	16±0.5 bc	9.07±2.11 b	19.82±0.83 b	14.65±1.50 b	62.91±2.60 c
(Control)	double	44.23±3.08 b	57.85±2.30 b	6±1.0 c	16±0.4 b	9.12±1.41 b	19.62±0.80 b	13.33±2.01 b	64.32±1.14 c
Foliar Spray	single	55.87±5.15a	68.16±2.56 a	8±1.5 b	28±2.1 a	9.55±1.90 b	23.83±0.94 a	18.66±3.00a	88.54±2.32 a
	double	47.30±3.47 b	57.54±2.50bc	9±0.6 ab	26±1.1 a	10.12±2.50ab	20.21±0.94 b	20.00±2.62 a	89.58±4.70 a
Root drenching	single	50.53±5.31ab	65.70±1.95 a	9±0.8 a	27±1.5 a	10.71±2.61 a	22.70±1.01 a	18.77±1.50 a	92.99±5.71 a
	double	44.73±6.13 b	53.25±2.53 c	5±1.0 c	14±0.5 c	9.48±1.50 b	17.30±0.88 c	12.66±1.73 b	76.90±3.27 b

Means and standard errors are presented. Data were subjected to analysis of variance. Means sharing same letter case are statistically similar at P= 0.05

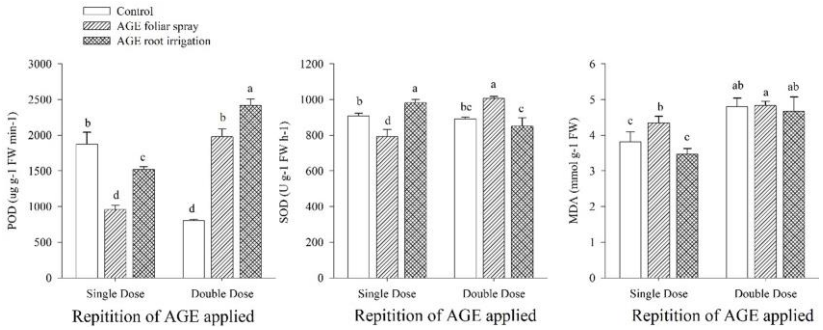

Fig. 2: Influence of AGE (aqueous garlic extract) foliar spray and root irrigation on the antioxidative enzymes and lipid peroxidation levels of tomato plants

Means and standard errors are represented. Means followed by same letter are not significantly different from each other (P=0.05)

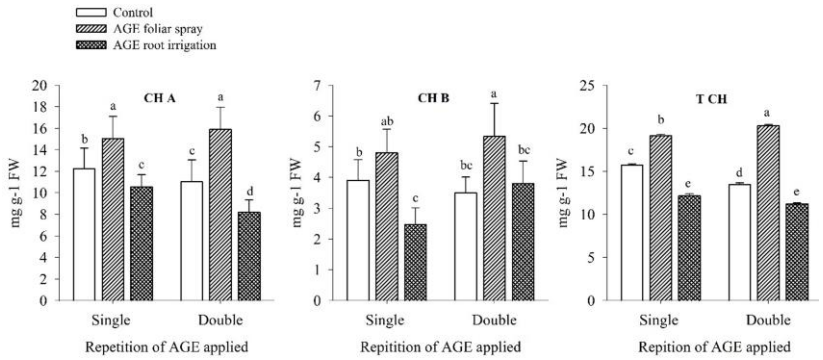

Fig. 3: Effect of foliar spray and root irrigation of AGE (aqueous garlic extract) on chlorophyll a, b and total chlorophyll content of tomato plants

Means and standard errors are represented. Means followed by same letter are not significantly different from each other (P=0.05)

significantly enhanced the chlorophyll a, b and total chlorophyll content both as the single dose or the repeated dose. However, the root drenching with AGE gave the opposite results decreasing the chlorophyll a, b and total chlorophyll content of the treated plants comparatively to the other treatments.

The Soluble Sugar and Carotenoid Contents of Tomato are Altered Due to AGE Application

Fig. 4 shows the influence of AGE on the carotenoid and soluble sugar content of tomato plants. Regarding the carotenoid content of tomato plants applied with AGE, the same effect was observed as to that of chlorophyll content. The foliar application of AGE enhanced the carotenoid content and the effect was significantly higher than those of the control plants (both in the first and repeated application). Root drenching method, however, resulted in a lowering effect on the carotenoid content and the effect is significantly different than those of the control as well as the AGE foliar application method.

The influence of AGE on the total soluble sugars of tomato is also of interest as the foliar application for the first time did not alter a significance effect in the soluble sugars but the repeated application gave a rise in the soluble sugars compared to those of control plants. AGE as root drenching,

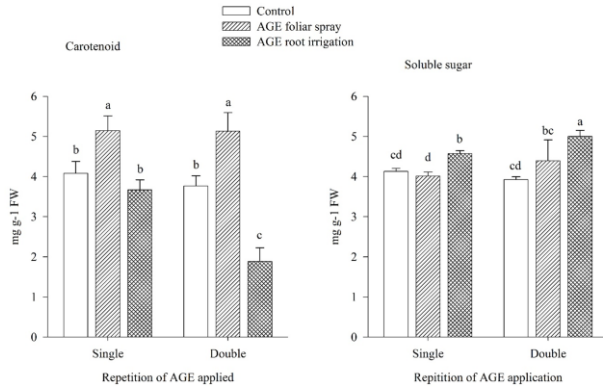

Fig. 4: AGE (aqueous garlic extract) foliar spray and root irrigation reveals alterations in the soluble sugar and carotenoid contents of the tomato plants

Means and standard errors are represented. Means followed by same letter are not significantly different from each other (P=0.05)

on the other hand, resulted an increase in the total soluble sugars both in the initial as well as repeated application and the effect reached to a level of significance compared to the other treatments.

Discussion

AGE application exerted diverse effects on the treated plants and the effects in some parameters varied when the same treatments were repeated. These findings indicate the approximate duration of AGE efficacy and offer an insight into the biological activity of AGE in the growth of tomato plants, allowing us to modulate the application for AGE as a growth promoting botanical for plastic tunnel growing facilities. The primal morphological traits such as plant height, shoot fresh weight, number of branches, fruit size and weight etc., were influenced with the application of AGE, but the effect was dependent on the method and repetition of application. AGE has been reported to contain complex growth promoting substances such as vitamins, saponins, carbohydrates, proteins, alkaloids, sugars such as fructose (Martins *et al.,* 2016). A well-maintained dose of these compounds can actively participate in the promoted growth conditions of the receiver plants. Furthermore, some studies report the presence of micro nutrients in garlic such as Zn, Mn and other elements (Jones *et al.,* 2007) which are likely to participate in the enhanced growth traits of treated plants in the current research findings. Moreover, AGE contains vital organosulfur compounds such as allicin, DADS, DATS etc., (Avato *et al.,* 2000; Jones *et al.,* 2007), shown to be antioxidant in nature (Banerjee *et al.,* 2003) and can actively react to the lipid bilayers (Gruhlke *et al.,* 2015) therefore, might involve in signaling and altering the physicochemical properties of the receiver plants.

The enhanced growth of tomatoes in the current study suggests that AGE can act as plant growth promoter. However, the repetition of AGE as root drenching, hinders the growth of the plants which maybe the result of frequency of application and hence, can be understood as the concentration threshold for AGE. Previously, we observed that at higher concentrations, AGE results in inhibition of cucumber growth and current findings therefore strongly corroborate our previous work (Hayat *et al.,* 2016). Similar growth promoting and inhibiting effects based on the concentration of garlic derived compounds have been reported in earlier studies and are therefore in close agreement to current study findings (Ting-ting *et al.,* 2011; Han *et al.,* 2012; Han *et al.,* 2013).

The antioxidative response of the treated plants showed variation due to the method of application as well as the time of application of AGE. This variation reflects important bioactivity of AGE to stimulate the growth of the receiver plants. Under normal conditions, plant cellular activities are kept in balance with the help of constitutive measures of the antioxidant enzymes such as SOD balance the excessive oxygen radical by converting it into H_2O_2 and O_2 whereas, the POD further stabilizes the H_2O_2 into H_2O and O_2 (Gapper and Dolan, 2006; Gill and Tuteja, 2010; Baxter *et al.,* 2014). Therefore, these antioxidants are considered to be the first line of defense and the alteration in these enzymes suggest that the plants are alarmed due to a possible stress or stress like stimulus (Tian *et al.,* 2012; Siddique and Ismail, 2013; Liu *et al.,* 2014). It was observed that these antioxidants were altered variably depending upon the method and repetition of application. It is likely that AGE applied to the plants was perceived as stimulator and the stimulus was therefore dependent upon the method and duration of application. These variations in the antioxidative responses of the receiver plants due to the AGE application strongly confirm our previous work (Hayat *et al.,* 2016). The active state of antioxidants is often reported to be a sign of stress response in the plants (Bhattacharjee, 2005; Ara *et al.,* 2015; Wan *et al.,* 2015), current results therefore suggest the priming capacity of the AGE to stimulate stress like responses in the receiver plants. Interestingly however, the observed MDA content, which is an indicator of lipid peroxidation (Candan and Tarhan, 2003), revealed that there was no severe stress condition on the plants except the repeated root drenching with AGE, where a restricted growth condition was observed. The situation of altered levels of antioxidants with low or non-significant lipid peroxidation allow us to hypothesize a primed or induced state of defense response in the tomato plants due to AGE application, causing speedy physiological responses related to these antioxidants and resulting in enhanced growth conditions. Moreover, another plausible explanation is the involvement of ROS in enhanced metabolic activities, growth and division on the cellular levels (Gapper and Dolan, 2006; Grace, 2007). However, to balance these ROS, the antioxidative role is inevitable in order to ensure protection from excessive ROS and maintain cellular homeostasis. The primed tomato plants are therefore supposed to have a speedy growth as compared to the control plants.

The chlorophyll content of the plants treated with foliage application of AGE increased both times it was applied whereas in root drenching method, a decreasing trend was observed. The chlorophyll contents, in particular the chlorophyll a of the plants was enhanced due to the foliar application of AGE.A higher chlorophyll content maybe involved in the growth promotion and the primary metabolic responses of the treated plants compared to the control (Shalaby and El-ramady, 2014). However, the decline of chlorophyll contents suggests the inhibitive effects exerted on the receiver plants due to application of AGE beyond threshold levels. Various studies report that chlorophyll content is effected under stresses or the influence of various treatment and may sometimes enhance the growth and physiological conditions (Candan and Tarhan, 2003; Siddique and Ismail, 2013; Chai *et al*., 2016; Khan *et al.,* 2016; Yuyan *et al.,* 2016). These reports therefore support our results about the alteration in the chlorophyll drawn by AGE application through various methods and repetitions. It was also observed that the total carotenoid contents were significantly influenced with application of AGE particularly applied as foliar spray. Carotenoids are integral structural components of the photosynthetic antenna and protect plants from photooxidative damages (Pessarakli, 1999; Hanafy *et al.,* 2012; Racchi, 2013) and could be considered as active players of various plant physiological responses such as plant defenses against various abiotic and biotic stresses. Previously, garlic extracts have been reported to increase the carotenoid and chlorophyll contents enhancing the growth of *Scheflerra arboricola* plants (Hanafy *et al.,* 2012). It is important to consider that due to foliar application, a rise in the carotenoid contents might be helpful not only to increase the growth, but also, it could be of potential to cope with the sudden environmental fluctuations inside the plastic tunnel such as temperature. However, more detailed approaches may clarify our hypothesis if the alterations in the carotenoids and chlorophyll could be modulated into plant resistance. The soluble sugars of the treated plants were also inclined with a statistical difference indicating the active role of AGE in the physiology of tomatoes. Particularly for the root drenching with AGE, the soluble sugars were significantly increased. Sugars are important for the fruit quality of tomatoes and increasing sugar levels, specifically glucose and fructose create a higher ratio of sugar to organic acids making the fruit sweeter and tastier (Radzevičius *et al.,* 2009). In our study, it was observed that the fruit weight and size was significantly increased due to application of AGE which can be correlated to the inclined concentration of the total soluble sugars content of the treated plants. Furthermore, soluble sugars and biomass enhancements under saline conditions reported earlier (Shafi *et al.,* 2015), suggest that the growth improvement in current study is also in relation to these physiological activities such as chlorophyll, carotenoids and soluble sugar contents.

Nevertheless, the observed effect in various parameters differed based on the mode of application. It is likely be due to the fact that during foliar application, although the plants are capable to utilize the receiving chemical quite efficiently, yet there is risk of loss in the form of evaporation or transpiration. In the root irrigation however, there are more chances of uptake of the applied chemical and thus may result more prominent in some cases of current study. These findings are supported by earlier report where garlic intercropping in soilless medium actively altered the growth of pepper plants (Ding *et al.,* 2016). However, considering the active antimicrobial potency with effective improvement in the growth of the receiver plant, the foliage application of garlic may be preferable in the plastic tunnel in order to ensure protection and production.

Nonetheless, the altered primary metabolic responses of tomato plants applied with AGE and the resulting promoted morphological growth also offers a plausible explanation to the biological stimulation capacity of AGE as growth promoting and priming stimulator. As reported earlier, an induced state of primary metabolism can be interpreted as priming of the defense responses of the plants (Smirnoff, 1996; Aghdam *et al.,* 2016; Dawood, 2016; Khan *et al.,* 2016; Malo *et al.,* 2017). It is suggested that AGE application altered the primal metabolic activities of tomato plants that probably lead to activation of ROS, for which the constitutive antioxidants were activated. Thus, priming of these responses alternatively enhanced the metabolic activities and plants showed better growth conditions as compared to those of control plants. The restriction of the growth due to overdose or repetition of the AGE root drenching further supports this hypothesis that the receiving plants might be influenced from the stimulus of AGE perceived as stress or stress like response. This presumption is supported by various reports about the inhibitive effect of garlic and garlic derived compounds on the growth of plants (Djurdjevic *et al.,* 2004; Ting-ting *et al.,* 2011; Talukdar, 2013; Ding *et al.,* 2016). Current findings are vital and provide future perspectives to explore the molecular aspects of these phenomena and explain the mechanics of these pathways. Therefore, our future concern will be to study the molecular patterns related to these responses in order to confirm our findings and validate our hypothesis.

Conclusion

The effect of 100 µg mL^{-1} of AGE as foliar spray as well as root drenching ensures the better yield and growth performance of tomato plants grown under plastic tunnel conditions. These findings strongly confirm our previous reports and further identify that AGE can be utilized as a growth promotor, where the foliar application at 15-days interval may result in enhanced growth conditions and larger fruits size of the tomato plants. Furthermore, the preparation is handy and bares less or no hazardous side effects and offers ecofriendly and greener production of the horticultural

produce. The influence on primary metabolites of the treated plants provide a platform to elaborate and study the biological activity of the AGE inside the treated plants to identify and later confirm the molecular patterns involved in the bioactive mechanism of these extracts. Future research study is needed to evaluate the major bioactive constituents of AGE such as Allicin and DADS to identify the potent organosulfur component responsible for priming the defense and growth aspects of the receiver plants.

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