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Aqueous garlic extract stimulates growth and antioxidant enzymes activity of tomato (*Solanum lycopersicum*)

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ABSTRACT

A glasshouse bioassay was conducted to assess the efficacy of aqueous garlic extract (AGE) as a biostimulator for the growth improvement of tomato seedlings. Application of AGE as foliar spray and/or fertigation, resulted in morphological and physiological stimulation of tomato seedlings. The plant height, leaf area, stem diameter, plant fresh/dry weight were significantly improved due to the applied treatments, however, higher concentrations applied as fertigation negatively influenced some parameters. The antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD) showed increased activity in response to variable concentrations of AGE. The highest concentration administered as fertigation resulted in increased malondialdehyde (MDA) content accumulation in the leaves indicating lipid peroxidation or possible oxidative stress in these seedlings and the effect was correlating to the hindered growth of the seedlings. Our findings provide basis for understanding the functional bioactivity of allicin containing AGE in the receiver plants' physiology. We therefore suggest AGE as a botanical for bio-priming and stimulation of an oxidative response in tomato which might be helpful in various stressful situations.

1. Introduction

With the course of agricultural expansion, the use of synthetic chemicals though satisfies farming community both in crop production and protection aspects but due to the hazardous outcomes in a long term cropping systems have made the utility of these chemicals questionable (Aktar et al., 2009; Hadi et al., 2014; Sugeng et al., 2013). Moreover, the target pathogens are increasingly developing resistance to these synthetic chemicals, thereof threatening the sustainability of agriculture production (Ayazi et al., 2011; Kahmann and Basse, 2001; Talukdar et al., 2013). To cope with these challenges, the trend to identify and formulate chemicals of organic origin as alternatives, is interestingly increasing. Plant derived secondary metabolites have thus became the center of focus for many scientists to understand their chemistry (Gorinstein et al., 2008; Grace, 2007; Jones et al., 2007; Li et al., 2010), origin (Cantor et al., 2011; Mukerji, 2006). Furthermore, studies show their active influence in various aspects such as allelopathy (Ambika, 2013; Gniazdowska and Bogatek, 2005), biological control studies (Guleria and Kumar, 2009; Gurjar et al., 2012; Mukerji, 2006) and as biofertilizers (Andresen et al., 2015; Hafez et al., 2013; Hanafy et al., 2012). Nonetheless, some botanicals have been recognized as biostimulants (Liu et al., 2012; Oracz et al., 2007). Therefore, identification and understanding of functional bioactivity of botanicals can be potentially useful for the farming and consumer communities. Among many useful plant species, garlic (*Allium sativum)* has been practiced as a potent remedy since the dawn of civilization (Bayan et al., 2014; Tripathi, 2009; Rivlin, 2001). Garlic contains beneficial chemical ingredients such as enzymes, vitamins, flavonoids (Block, 1985), organosulfur compounds such as allicin, diallyl- disulfide and thiosulfinates (Bhuiyan et al., 2015; Borlinghaus et al., 2014; Khar et al., 2011). It has a strong antimicrobial potential against a variety of bacteria (Ayazi et al., 2011; Bakri and Douglas, 2005; Fujisawa et al., 2009; Iwalokun et al., 2004; Shobana et al., 2009), fungi (Aala et al., 2014; LowChen et al., 2008; Muhsin et al., 2001) and viruses (Afzal et al., 2000; Ankri and Mirelman, 1999). Moreover, the antioxidant potential of garlic is reported to be beneficial in various cardiovascular complexities (Afzal et al., 2000; Agarwal, 1996; Chan et al., 2013) and possess antitumor properties (Agarwal, 1996; Miron et al., 2003; Thomson and Ali, 2003). Studies suggest significant allelopathic role of garlic plantation in cropping systems to have improved the growth of neighboring crops (Djurdjevic et al., 2004; Han et al., 2013; Wang et al., 2015; Xiao et al., 2012; Zhi-hui, 2011). Nevertheless, its allelopathy has been suggested to alter the ecology of the cropping systems such as improving the soil conditions which ultimately enhance the receiver plants' growth. (Ahmad et al., 2013; Han et al., 2012; Wixted and McGraw, 2010).

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Possessing strong antimicrobial property and reputed allelopathic potential, the use of garlic derived compounds seem an interesting area of study. However, plants being sessile organisms, have evolved specialized mechanics to sense and process different stimuli (Balderas-Hernández et al., 2013; Dawood, 2016; Noctor et al., 2012; Shah and Zeier, 2013) and therefore, to study the function of a botanical compound inside the receiver plant may require specific observations. During the last few decades, studies have elaborated and explained various biological processes in plants during interactions with the ultimate environmental cues (Halliwell, 2009; Kunkel and Brooks, 2002; Levy-Booth et al., 2008; Rodrigues et al., 2013; Sandhu et al., 2009). The role of antioxidant enzymes is a good example of such specialized mechanisms which offer an insight into plant biology during a stress or stress like situations (Arora et al., 2002; Siddique and Ismail, 2013; Wu et al., 2014). Reactive oxygen species (ROS) are the key players to interact with stress conditions (Ahmad et al., 2008) and their overproduction sometimes may result in cellular destructions and DNA manipulation (Arora et al., 2002; Baxter et al., 2014; Bhattacharjee, 2005; Das and Roychoudhury, 2014). Thus, in order to constitute these ROS, plants possess sophisticated mechanism of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) etc. (Arora et al., 2002; Bartosz, 1997; Das and Roychoudhury, 2014; Gill and Tuteja, 2010).To understand the biological activity of a chemical in the growth and physiology of a plant species, it is therefore imperative to consider these antioxidant enzymes to indicate the threshold levels of these chemicals required for sustainable growth (Abogadallah et al., 2010; Alscher et al., 2002; Gupta et al., 1993; Nagy, 2013).

With increasing knowledge of the chemistry of garlic aqueous extracts to contain various growth promoting factors such as vitamins, flavonoids, phenolic compounds, carbohydrates, enzymes etc., (Afzal et al., 2000; Gorinstein et al., 2008; Hafez et al., 2013; Hussein et al., 2014; Lanzotti, 2006), the use of garlic extracts or garlic derived compounds can possibly stimulate the biology of receiver plants. However, much is required to understand and specify the exact chemical constituent governing the biological activity of raw garlic extracts in order to formulate a next generation, ecofriendly botanical for sustainable agriculture production. Current research work is therefore an effort to understand the biostimulation functions of allicin containing aqueous garlic extracts (AGE) in the growth and defense related physiology of tomato seedlings.

2. Materials and method

2.1. Aqueous garlic preparation

Fresh, uniform sized bulbs of garlic cultivar G025 (based on previous findings) were selected from the garlic germplasm NWSUAF Yangling, Shaanxi, China and stored at -20 °C until further use. Aqueous extracts were prepared according to (Ting-ting et al., 2011) with slight modifications. 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, serial dilutions were carried out with distilled water to make final concentrations of 0 (control), 50 µg mL^{-1}, 100 µg mL^{-1} and 200 µg mL^{-1} respectively.

2.2. Plant material

Tomato cultivar Dong Ya-Fen Guan was sown in plastic trays until germination. At four leaves emergence, the seedlings were transferred to plastic pots (10*12 cm) containing growing mix Youmiao Shangzhang Jizhi, purchased from Shanghai Fuang agrochemical co., China and were maintained at optimum temperature (28/20 °C, day/night) and natural daylight under glasshouse. One week later, treatments were applied. AGE with three concentrations (50, 100 and 200 µg mL^{-1}) were applied both as foliar spray and fertigation. Distilled water was taken as control treatment. Each replicate set contained 10 seedlings and the experiment had three replications. 20 days after treatment, samples were collected to determine the physiological indices.

2.3. Morphological indices

2.3.1. Plant height

Plant height was recorded in cm using a measuring tape in cm.

2.3.2. Stem diameter

Stem diameter was measured using an electronic Vernier caliper. Data was recorded for measuring three points in seedling stem and means were calculated accordingly.

2.3.3. Fresh weight/dry weight

Plant fresh weight was measured immediately after rooting up the plants using electronic balance. To determine the dry weight, samples were oven dried for 24 h at 80 °C so that the moisture content could be evaporated. Weight of samples was recorded in grams (gm).

2.3.4. Leaf area

For leaf area measurement, images were captured using Nikon D3400 (Nikon Co. Thailand) which was mounted on a tripod and fixed at 20 cm from the leaf. Fully expanded, mature leaves were selected from each seedling and to avoid error, five leaves were considered as one sample size. The leaf area was measured using ImageJ (ImageJ National institute of health USA) (https:imagej.nih.gov) following the protocol of Abràmoff et al. (2005).

2.4. Physiological indices

2.4.1. Antioxidant enzymes and MDA content

A standard procedure stated by Wang et al., (Wang et al., 2015) was followed to measure the antioxidant enzymes and MDA content. 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). Two milliliters 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 (u·g^{-1}Fw).