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Original Research Article

Potential of Wood Vinegar in Enhancing Fruit Yield and Antioxidant Capacity in Tomato

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Abstract - Tomatoes are considered as one of the main components of daily meals in most households. Thus, it is important to invest in studies enhancing their yield and nutritional value. The study evaluated the effect of wood vinegar (WV) on tomato under greenhouse conditions. Data on fruit number, fruit weight, and plant height were recorded. Total phenolic content (TPC) and antioxidant activity of tomato were determined using the Folin-Ciocalteu reagent and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, respectively. FRR-CF+500x-WV significantly increased the fruit number (86.11%) and fruit weight (81.78%) over the control. Results were comparable to HRR-CF+250x-WV, indicating that conventional fertilizer and WV may have synergistic effect on each other. TPC and scavenging effect on DPPH radical was improved by 250x-WV application. The increase in the phenolic compounds can be attributed to WV as a direct source or as a factor triggering the plants to produce more secondary metabolites with the purpose of increasing natural defenses. The significant effect obtained by applying the full recommended rate of conventional fertilizer on the carotenoid content was due to the availability of the major nutrients needed by the plant. Correlation analysis showed that carotenoids have negative correlation with TPC and DPPH. However, TPC and DPPH showed that these are positively correlated with each other. Tomatoes are excellent source of antioxidants associated with the reduction of some human diseases and improved health. The results provided evidence that WV alone and/or its combination with conventional fertilizers has favorable effects on the quality of tomato.

Key words - Antioxidant activity, Carotenoids, DPPH radical scavenging assay, Total phenolic content, Wood vinegar

Introduction

Tomatoes (*Solanum lycopersicum* L.) and their products are excellent sources of nutrients and secondary metabolites. The fruits are rich in food components that have antioxidant properties and are good sources of carotenoids, particularly lycopene, ascorbic acid and phenolic compounds (George *et al*., 2004; Sahlin *et al*., 2004; Ilahya *et al*., 2011; Pinela *et al*., 2012). Clinton (1998) mentioned that dietary intake of tomatoes has been related with reducing the possibility of having cancer and cardiovascular diseases. This beneficial effect is attributed to the antioxidants, and specifically to the carotenoids and some phenolic compounds.

Tomatoes can be easily grown from backyard to commercial farming provided with proper care and management. Extensive use of inorganic fertilizers has been widely used in conventional farming. However, the practice of applying inputs from organic sources to increase production and improve crop quality has gained research interest. Composts and manures are the commonly used organic inputs in tomato production. But, the potential of wood vinegar (WV) can be taken into consideration as an organic amendment.

Pyroligneous acid is the technical term referring to wood vinegar. It is produced from condensation of smoke during charcoal production (Yoshimoto, 1994; Kishimoto, 1997). Wood vinegar is mainly composed of water (80-90%) with sufficient amount of organic compounds (10-20%) including more than 200 chemical substances having acetic acid as the main component. Other chemicals in WV include different kinds of phenol, carbonyl and alcohol. It is extensively used in production of agricultural crops to stimulate plant growth, seed germination, as soil disinfectant and pest and disease management (Kim *et al*., 2000; Kim *et al*., 2001; Lee and Huh, 2002; Mu *et al*., 2003; Yatagai and Unrinrin, 1989;

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Rico *et al*., 2007). Several studies have been conducted citing the importance of wood vinegar in agriculture (Benzon *et al*., 2015; Rui *et al*., 2014; Kang *et al*., 2012; Prasertsit *et al*., 2011; Yatagi *et al*., 2002). Beneficial effects of WV to soil as an organic amendment have been cited in many studies such as in rice (Ichikawa and Ota, 1982; Tsuzuki *et al*., 2000), sorghum (Esechie, 1998), sweet potato (Shibayama, 1998), sugarcane (Uddin *et al*., 1995), and melon (Du *et al*., 1997; Tsuzuki *et al*., 1993).

To our knowledge, little or no information has been published yet regarding the effect of WV on nutritional value of tomatoes. Thus, the purpose of the study is to investigate on the effect of WV application on the yield and antioxidant capacity of tomato grown in greenhouse conditions.

Materials and Methods

A greenhouse experiment on tomato was conducted at Kyungpook National University, Daegu, South Korea during summer of 2015. The experimental set up consists of three replications arranged in a Completely Randomized Design. The treatments were: Control - no fertilizer and no wood vinegar; 250 times dilution of wood vinegar (250x-WV); 500 times dilution of wood vinegar (500x-WV); Half Recommended Rate of conventional fertilizer (HRR-CF); Full Recommended Rate of conventional fertilizer (FRR-CF); HRR-CF + 250x-WV; HRR-CF + 500x-WV; FRR-CF + 250x-WV; and FRR-CF + 500x-WV.

Experimental set-up

Tomato seeds were sown in seedling trays and allowed to grow for one month. Uniform seedlings were selected then transplanted onto pots containing 10 ㎏ soil. Fertilizer rate was computed based on the area occupied per pot (0.09 m^2). The full recommended rate was 1.37 g N - 2.38 g P_2O_5 - 1.28 g K_2O/pot. Basal application of fertilizer was done and side dressing at 3 (0.68 g N - 0.00 g P_2O_5 - 0.75 g K_2O), 6 (0.68 g N - 2.65 g P_2O - 0.75 g K_2O), and 9 (0.68 g N - 0.00 g P_2O_5 - 0.75 g K_2O) weeks after transplanting. The experiment was terminated three months after transplanting.

Data Gathering

Harvesting of tomato fruits commenced about two months after transplanting when fruits turned orange-red. The data on fruit number and fruit fresh weight were recorded. Continuous harvesting was done as the fruits become mature. The data on plant height was gathered at termination of the experiment.

Determination of total phenolic content (TPC)

Tomato extracts were prepared following the method of Genovese *et al.* (2003) using the Folin-Ciocalteu reagent with some modifications. The samples were extracted in proportions of 4:20 (m/v) with methanol. The residue was re-extracted in the same proportions using the same solvent. The extracts that were obtained were filtered using Whatman No. 1 filter paper and the volume was made up to 50 ㎖. The total phenolics determination was carried out in accordance with Ham *et al*. (2013). The extract (0.5 ㎖) was mixed with 0.5 ㎖ of 4% sodium carbonate (Na_2CO_3) and 0.5 ㎖ of 25% Folin-Ciocalteu’s reagent. The solution was mixed then incubated under room temperature for 30 minutes. Absorbance readings were measured at 750 ㎚ (Thermo Scientific, Multiskan GO UV-Vis spectrophotometer) and results were expressed in ㎎ of gallic acid equivalent/g of residue.

Scavenging effect on DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical

The antioxidant activity of tomato extracts was determined through DPPH radical scavenging activity assay. This method is quick and inexpensive, and frequently used for the evaluation of the anti-oxidative potential of various natural products (Soler-Rivas *et al*., 2000). The principle behind is that DPPH having an odd electron, gives a strong absorption band at 516 ㎚). In the presence of a free radical scavenger, this electron becomes paired, which results to absorption loss and consecutive stoichiometric decolorization with reference to the number of electron acquired. The difference in absorbance resulting in this reaction is assessed to evaluate the antioxidant potential of the samples. Scavenging effect on DPPH radical was determined following the method cited by Kang *et al.* (2015) with some modifications. Sample extracts (1 ㎖) were mixed with 3 ㎖ of 0.004% methanolic solution of DPPH.

The mixture was shaken vigorously and left to stand for 30 minutes at 30°C under dark condition. The absorbance was measured using UV-Vis spectrophotometer (Thermo Scientific, Multiskan GO) at 517 nm against the corresponding control. The inhibition percentage of the absorbance of the DPPH solution was calculated using the following equation:

$$\text{Inhibition \%} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Determination of total carotenoids

Estimation of total carotenoids was done based on the method of Thimmaiah (1999) using acetone extract of tomato fruits. Two grams of sample was homogenized in a mortar and pestle to make a fine paste. Acetone (20 ㎖) was added and allowed to settle for 2 minutes. The mixture was then filtered using Whatman No. 1 filter paper. The filtrate volume was made up to 40 ml with acetone. The filtrate was mixed and incubated overnight at 4°C. Absorbance was read at 450 ㎚ and carotenoids were estimated using the formula:

$$C = D \times V \times f \times 10/2500$$

Where: C = amount of carotenoids (㎎/100 g)
D = absorbance at 450 ㎚
V = volume of original extract (㎖)
f = dilution factor
2500 = average extinction coefficient of the pigments

Statistical Analysis

The statistical analyses were carried out using the IBM SPSS Statistics ver. 21 for Windows software. The differences between treatments were determined by applying Tukey's honestly significant difference test at $P \leq 0.05$.

Results and Discussions

The plant height of tomato was measured at termination of the experiment. There was no statistically noticeable difference observed in plant height (Fig. 1). However, WV application alone and in combination with conventional fertilizers was able to enhance plant height compared to the control. The absence of significant differences in plant height could be due to the time and age of plant when the measurement was done. Usually, noticeable differences are recognized at an early stage when the plants are more responsive to any treatment applications. Wood vinegar is used in crop production towards soil quality improvement, pest elimination and plant growth stimulation (FFTC, 2005). Wood vinegar applied in the soil helps increase the population of beneficial microbes and promote plant root growth (Tancho, 2008). The nutritional components in the WV attract microbes including bacteria and fungus to roots of plants where symbiotic relationship occurs (Narwal, 2000; Tsuzuki *et al*., 1989). Symbiosis leads to the improvement of the nutritional status due to nitrogen supplied by bacteria and phosphorous coming from mycorrhizae. This results to increased photosynthetic rates and plant growth enhancement (Kaschuk *et al*., 2009).

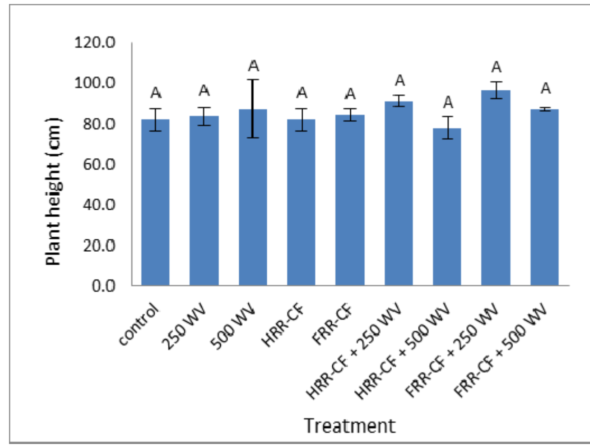

Fig. 1. Plant height of tomato as influenced by conventional fertilizer and wood vinegar application under greenhouse conditions. Means with the same letter(s) are not significantly different from each other based on Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate standard error.

The fruits were harvested regularly for a period of about one month. The number and weight were recorded and then the fruits were stored in the refrigerator prior to analysis. Highly significant differences were observed in the total number of fruits and fruit weight. Summarized in Table 1 are the fruit number and fruit weight of tomato as influenced by WV application. The treatment combination FRR-CF+500x-WV had the most significant effect on fruit number and weight. Nonetheless, this treatment was comparable to the lower rate fertilizer combined with either of the WV concentrations. The percentage increase in number of fruits obtained from FRR-CF+500x-WV over the control was 86.11%. On the other

Table 1. Effect of conventional fertilizer and wood vinegar application on fruit number, fruit weight and total carotenoid content of tomato under greenhouse conditions[z]

Treatment	Variables		
	Fruit number	Fruit fresh weight (g)	Total carotenoids (mg g^{-1})
Control	5 d	35.95 b	5.75 b
250 WV	8 cd	62.49 ab	6.60 b
500 WV	6 cd	42.73 b	6.90 b
HRR-CF	10 bcd	60.39 ab	6.75 b
FRR-CF	25 abc	137.34 ab	15.29 a
HRR-CF+250 WV	29 ab	174.66 ab	7.09 b
HRR-CF+500 WV	23 abcd	117.48 ab	5.88 b
FRR-CF+250 WV	30 a	166.06 ab	7.15 b
FRR-CF+500 WV	36 a	197.32 a	7.05 b

[z]Means within a column followed by the same letter(s) are not significantly different based on Tukey's HSD test at 5% level of significance.

hand, about 83.33% increase in fruit number was observed when HRR-CF+250x-WV was applied. This means that lower rate of conventional fertilizer combined with a more concentrated wood vinegar solution would yield statistically comparable results. The data on fresh weight of fruits revealed almost similar results. As mentioned previously, the FRR - CF + 500x - WV treatment performed best in enhancing this parameter. Moreover, the percentage increase over the control was 81.78%. Again, it was comparable to the increase obtained by the HRR - CF + 250x - WV treatment (78.35%). This indicates that conventional fertilizer and WV might probably have synergistic effect on each other. The components of WV might aid in the dissolution of inorganic substances in the fertilizers, rendering nutrients more available for plant uptake. The acidic nature of WV mainly due to its acetic acid content contributed to dissolution and uptake mechanism. Increase in fruit number and weight can be attributed to the beneficial effects of WV, which enhanced fruit production and quality. According to Gifford and Evans (1981) the large amounts of assimilates produced and the associated high sink strength are the important factors to consider in determining high yields. These were manifested in the WV treated plants and/or its combination with conventional fertilizers. Wood vinegar might have enhanced the fruit number and weight of tomato due to the improved root system, flowering and plant growth. In addition, sink stimulated-photosynthesis could possibly lead to an increased phase of leaf activity or delaying senescence (Paul and Peliny, 2003). This could increase the potential period for plant growth and fruit weight or probably yield. Harris *et al.* (1985) suggested that carbon sink strength of symbioses stimulated the photosynthetic rate. The components of WV (esters, alcohols, acids and some metals) might also have contributed to better growth, production, and quality of tomatoes.

Tomatoes are excellent source of phenols especially in the Indian and American diet. These compounds also contribute to the antioxidant capacity of tomatoes. The total phenolic content of tomato extracts were calculated as mg of gallic acid equivalent/g of residue (Fig. 2). Similar to fruit number and weight, results revealed that the TPC of tomato were highly influenced by the different treatments. The application of WV diluted 250 times greatly affected the total phenolic content of tomato. Wood vinegar increased the total phenolic content of tomato which could be attributed to several reasons. Wood vinegar having different kinds of organic substances can be the direct source of phenolic compounds resulting in the increase in the total phenolic content. Wood vinegar functions as a control agent against pest and disease occurrence. The increase in the phenolic compounds might also be due to the plant's secondary metabolism. A number of secondary metabolites act as fungicides and antibiotics to protect the plants from fungi and bacteria. Since no pesticides were applied, WV might have triggered the plants to produce more of these compounds with the purpose of increasing

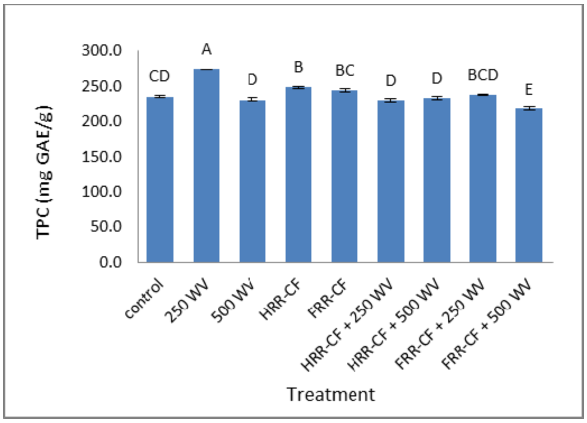

Fig. 2. Effect of conventional fertilizer and wood vinegar application on the total phenolic content of tomato grown in greenhouse conditions. Means with the same letter(s) are not significantly different from each other based on Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate standard error.

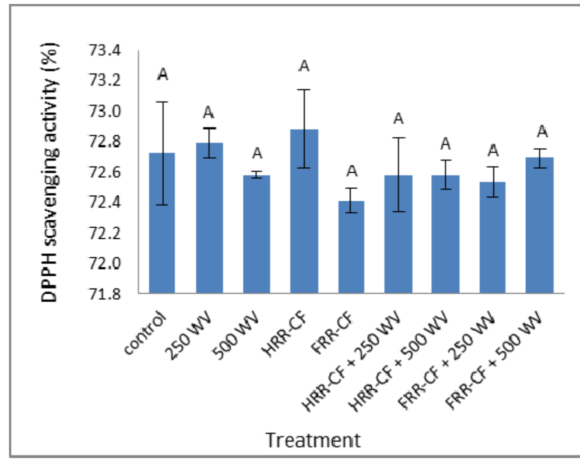

Fig. 3. DPPH radical scavenging activity of tomato as influenced by conventional fertilizer and wood vinegar application under greenhouse conditions. Means with the same letter(s) are not significantly different from each other based on Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate standard error.

natural defenses. The results were in agreement with those in the studies conducted by Borguini *et al.* (2013) and Toor *et al.* (2006). They showed that the total phenolics of the organically grown tomatoes resulted to higher content than those that received conventional fertilization. The absence of any chemical substance sprayed on organically grown tomatoes triggered the production of secondary metabolites such as phenols to combat pests and diseases. The organic amendments might have contributed to the production of these compounds.

There were no noticeable differences observed on the DPPH scavenging activity of tomato extracts. However, the scavenging effect on DPPH radical was improved by 250x - WV application (Fig. 3). The antioxidant activity is the potential effect of certain substances that are extracted from the food matrix to capture free radicals. The DPPH test was used in order to evaluate the in vitro antioxidant activity of the tomato extracts, so as to estimate the potential health benefits provided by tomato consumption. The scavenging potential of the tomato extract is indicated by the degree of discoloration due to the hydrogen donating ability (van Gadow *et al.*, 1997). Presented in Fig. 3 are the results relating to the capture of free radicals by the sample extracts through the DPPH assay. As also seen in the total phenolic content, the scavenging effect on DPPH radical was improved by 250x-WV application. Moreover the values obtained in all treatments were comparable to the artificial antioxidant, butylated hydroxy toluene (BHT). The scavenging activity on free radicals can be attributed to the phenolic compounds containing hydroxyl groups (Hemi *et al.*, 2002).

The total carotenoid content of tomato was estimated using the acetone extract of fruits. Statistically significant effect was attributed in the application of the full recommended rate of conventional fertilizer (Table 1). Although insignificant, other treatments were able to enhance the total carotenoid content of tomato compared to the control treatment. Considerable increase was observed when application of either high or low fertilizer was combined with 250x - WV. The yellow, orange, and red colors of many fruits and vegetables are attributed to the carotenoids. These are naturally occurring fat-soluble pigments synthesized mainly by plants. Carotenoids are a group of phytochemicals that plays an important role in the prevention of diseases (cancer, cardiovascular and other chronic diseases) and maintaining good health (Rao and Rao, 2007). The noticeable effect incurred by the application of the full recommended rate of conventional fertilizer was due to the availability of the major nutrients needed by the plant. As cited by Dumas *et al.* (2003), the carotenoid content in tomato grown in pots tends to increase as the supplied nitrogen increase. Moreover, increasing application of phosphorous under hydroponic growth condition resulted in improved color development and carotenoid content of the fruit. In terms of potassium, the carotenoid content of tomato under growth chamber condition also increased as the potassium levels in the nutrient solution increased. The carotenoid content in fruits and vegetables, and particularly in tomatoes, depends on the factors such as exposure to light, temperature and degree of fruit ripeness (Abushita *et al*., 2000). The absence of any difference among other treatments could be due to the control over the ripening. It is quite difficult to determine the exact stage of ripeness, even though all samples may be described as ripe.

To determine the relationship between the carotenoid content, total phenolic content, and DPPH radical scavenging potential of tomato, correlation analysis was performed. Results revealed that carotenoids have negative correlation between the two variables. However, TPC and DPPH showed that these are positively correlated with each other. As mentioned previously, DPPH scavenging activity is associated with the phenolic compounds. Synergism between the two as enhanced by WV application at lower concentration is a good indication of improving the antioxidant activity of tomato.

The mechanism on how WV affects plant growth and development is not yet clearly understood. Although it was recommended that the active principles in WV behaves in a manner similar to that of other plant growth regulators (Senaratna *et al*., 1999; Gardner *et al*., 2001). Wood vinegar application may affect the endogenous hormonal pattern of the plant in several ways. This can be through supplementation of sub-optimal levels or by interaction with their synthesis and translocation or inactivation of existing hormone levels (Arshad and Frankenberger, 1993).

Fruits and vegetables are the main sources of antioxidants, which makes these foods essential to human health. The presence of antioxidants in tomato has been proven to be the major reason by which tomato and its products are associated with the decrease of some human diseases. Considering that tomatoes are one of the main components of daily meals in most households, it becomes increasingly important to invest in studies enhancing their quantity and quality in terms of yield and nutritional value. From these results it can be concluded that WV application alone and/or its application with conventional fertilizers has favorable effects on tomato production as reflected in the yield and antioxidant properties. Further research would be desirable to assess the effect of WV on specific compounds with nutritional importance.

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