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Antioxidant content of Tomatoes (*Lycopersicon esculentum* cv MT1) treated by different type of Pesticide, Fertilizer and growth medium in Compost

Aishah Elias, Sahilah Abd. Mutalib*, Wan Aida Wan Mustapha, Safiyyah Shahimi, Norhidayu Mohamed, Rul Aisyah Mat Repin

School of Chemical Science and Food Technology, Faculty of Science and Technology Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Abstract : Antioxidant of tomato (Lycopersicon esculentum Mill. cv MT1) that have been pla nted according to split split plot experimental design and subjected to sixteen (n=16) treatment s namely as T1 to T16 were evaluated. The antioxidant of tomato extracts were determined by three methods namely total phenolic content (TPC), free radical scavenging activity (DPPH) a nd Ferric Reducing Antioxidant Power (FRAP). The highest mean efficiency for TPC and DPP H values in tomato were from T7, 1163.6 mg Gallic acid equivalent (GAE)/100 g and 55.7 % (chemical pesticide, mixture of organic and chemical fertilizer; and growth medium in cow ma nure compost). However T3, 54.2 % (chemical pesticide, organic fertilizer and growth mediu m in cow manure compost) showed significantly highest for DPPH only. FRAP values for T2, 9.00 µmol trolox equivalent (TE)/100 g (chemical pesticide, without fertilizer and growth med ium in EFB compost) treatment showed significantly higher ($p<0.05$) than other treatment. Pea rson coefficient correlation test showed positive correlation ($p<0.05$) between TPC and DPPH assay ($r=0.933$) and FRAP assay ($r=0.874$), respectively showed that the phenolic compounds was a contributor of the antioxidant activity in tomato. Thus, the finding of this study demonst rated that pesticide, fertilizer and growth medium in compost factor and their interaction did n ot show any specific patterns content toward TPC, DPPH and FRAP; while the TPC was the m ain contributor of antioxidant activity in tomat

Keywords : Antioxidant; tomato; pesticide; compost; fertilizer.

Introduction

Tomatoes are one of the functional food that benefit towards health and play role in disease risk prevention [1]. Tomatoes can be sources of micronutrient especially polyphenol compound that contribute to the antioxidant content [2]. Phytochemical compounds in tomatoes such as lycopene and β-carotene are fat soluble as well as vitamin C which is water soluble and intermediate hydrophobicity compound such as quercetin, glycocydes, naringenin, chalcone dan chlorogenic acid that have been known to contribute in antioxidant activities in tomatoes [3]. The use of antioxidant can lowering oxidative stress, DNA mutations, malignant transformation, as well as other parameters of cell damage [4].

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The usage of compost to rejuvenate soil had been long known by community such as manure and agricultural waste [5,6]. Cow manure were one of the waste that had been composted to be used in planting while empty fruit bunch (EFB) is a wet, cellulose rich oil palm mill residue that is used as an organic fertilizer and mulch substrate[7]. The use of compost from plant sources such as EFB as a mix of growing medium can promote plant growth and increase fruit quality[8,9,10]. Interestingly, there are a lot of studies about the effect of compost on phenolic compounds and antioxidant activities in plants except in tomato[5, 11].

In this study we investigate the effect of pesticide, fertilizer and compost on the phenolic compounds and antioxidant activities in tomato. Pesticide is always being used to control the pest. Whereas, fertilizer is used to support plant growth and yield. Application of organic manures along with chemical fertilizer was possible and reported by several researchers and co-workers [12, 13]. While, application of EFB along with chemical, or organic or both chemical and organic fertilizer has not reported in tomato. Thus, in this study we are looking on the antioxidant content of tomatoes (*Lycopersicon esculentum* cv MT1) treated by different type of pesticide, fertilizer and growth medium in compost.

Materials and Methods

Samples Collection

Tomato fruits were planted in UKM Plant House Complex, Selangor, Malaysia during April to December 2015 according to split-split plot experimental designed as in Table 1, with three replication. Ripening tomato samples were collected at month 5 after planting. Samples were cut into cubes and kept into -20°C freezer. Frozen tomato was freeze dried using freeze dryer (Labconco, Czech Republic) to maintain its quality and kept in -10°C freezer until used.

Antioxidants Extraction

Freeze dried tomato were ground using food processor (Warring, USA). The extraction procedure was conducted with 0.1 g samples and 10 mL methanol as extracting solvent was shaking for 2 hours. Solutions then were centrifuged using centrifuge (Kubota, Japan) for 10 minutes at 3,000 x g. The supernatant were collected and kept in universal bottle at 4°C in refrigerator for further analysis. All tests were performed at room temperature.

Table 1 Treatment for tomato plant (n=T1-T16) and details of the factors and its level.

Type of pesticides	Type of fertilizers	Type of composts	Treatment
Chemical pesticide (Malathion and Confidor)	Without fertilizer	Cow manure	T1
		Empty fruit bunch	T2
	Organic fertilizer (Agroplus, MY))	Cow manure	T3
		Empty fruit bunch	T4
	Chemical fertilizer (NPK 15:15:15)	Cow manure	T5
		Empty fruit bunch	T6
	Organic and chemical fertilizer	Cow manure	T7
		Empty fruit bunch	T8
Organic pesticide	Without fertilizer	Cow manure	T9
		Empty fruit bunch	T10
	Organic fertilizer (Agroplus, MY)	Cow manure	T11
		Empty fruit bunch	T12
	Chemical fertilizer (NPK 15:15:15)	Cow manure	T13
		Empty fruit bunch	T14
	Organic and chemical fertilizer	Cow manure	T15
		Empty fruit bunch	T16

Determination of Total Phenolic Content

An aliquot of 0.1 mL tomato extract was added with 1.0 mL 7.5 % (w/v) sodium carbonate ($NaCO_3$) (BDH, Germany) and was left for 5 minutes. About 0.5 mL 0.1% (v/v) Folin – Ciocalteu (FC) (Merck, Germany) reagent were then added to the mixture and then stored at dark room for 2 hours. The absorbance was read at 765 nm using spectrophotometer (BMG Labtech, Germany) at room temperature. Standard calibration curve was done similar as above except the sample was replaced with gallic acids (Sigma, USA) concentration range from 0 to 200 mg/mL. The equation obtained from the gallic acid calibration curve was y = 0.0047x + 0.0897 (R^2 =0.9969). Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g dried sample (mg GAE/100 g of DW) [14].

Determination of Ferric Reducing Antioxidant Power (FRAP)

FRAP reagent was prepared using 300 mM acetate buffer, pH 3.6 (A total of 3.1 g sodium acetate trihydrate (Sigma, USA) added with 16 mL glacial acetic acid and made up to 1 L with distilled water), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) (Sigma, USA) was made by dissolved it in 40 mM hydrochloric acid (HCl) (Merck, Germany); and 20 mM iron chloride hexahydrate ($FeCl_3.6H_2O$)(Merck, Germany). The reagent mixture was done in the ratio of 10:1:1. Freshly prepared FRAP reagent (1 mL) were mixed into tomato extract (0.1 mL). The mixture was stored at dark room for 30 min and the absorbance was measured at 595 nm using spectrophotometer (BMG Labtech, Germany). Standard calibration curve was done similar as above except the sample was replaced with different concentrations of trolox (Sigma, USA) range from 0 to 2.0 µmol/mL. The equation obtained from the trolox calibration curve was y = 1.2645x + 0.2625 (R^2 =0.9995). The results were expressed as µmol of trolox equivalents per 100 g of dried sample (µmol TE/g of DW) [14].

Determination of Radical Scavenging Activity (DPPH)

Stock solution of methanol DPPH (Sigma, USA) was prepared by adding 0.04 g DPPH into 100 mL methanol and was kept in -20°C until being used. About 350 ml stock solution was added into 350 mL methanol to achieve reading range from 0.7 to 1.0 unit at 516 nm. An aliquot of 0.1 mL sample were pipetted and added with 1 mL of methanol DPPH solution that have been prepared into suitable reading as mentioned. The absorption was read at 516 nm using spectrophotometer (BMG Labtech, Germany) after 30 minutes incubation in the dark [14]. The blank solution was made by replacing sample with extraction solvent which is methanol. The percentage of DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH scavanging activity } (\%) = \left[\frac{\text{Absorbance blank} - \text{Absorbance sample}}{\text{Absorbance blank}}\right] \times 100$$

Statistical Analysis

Data collected were analyzed statistically using SAS 9.2 software by General Linear Model (GLM). The mean separations were carried out by the least significant difference (LSD) method at a 5% significance level.

Results and Discussion

Phenolic compounds are important in terms of the nutritional and commercial properties of agricultural products[15]. Other than that it also contribute to fruit quality and nutritional value by modifying color, taste, aroma, and flavor, and also by providing beneficial health effects. These compounds also play a role in plant defensive mechanisms by counteracting reactive oxygen species (ROS), thus minimizing molecular damage due to microorganisms and insects[16].

In this study, three factors which were pesticide, fertilizer and growth medium in compost and its interaction of each factors were the source of variation (Pesticides x Fertilizer; Pesticides x Compost; Fertilizer x Compost; Pesticide x Fertilizer x Compost). The analysis of variance using split-split plot experimental design showed that all factors and their interaction did not give significant ($p>0.05$) values. Which means there was no contribution of the factors either pesticide, fertilizer or growth medium in compost towards phenolic content as indicated in Table 2 and Table 3. Though other researchers and co-workers showed that chicken dung [17] and sludge [18] can resemble soil conditioner and able to improve the nutrient supply in the soil for sunflowers, our

finding showed the compost was not able to contributed towards TPC, DPPH and FRAP analysis. Our findings were also in contrast with [12] which showed the usage of fertilizer could enhance the production of secondary metabolites and improve antioxidant activity (DPPH and FRAP) of *Labisia pumila* herb. This may be due to different species of plants, fertilizer and the origin of the compost applied in the growth of plant.

Table 2 Mean square value of analysis of variance (ANOVA) for the effects of treatments to the antioxidant of tomato plants per plot.

Source of variation	DF	TPC	DPPH	FRAP
Pesticide	1	7313.7	176.3	9.6
Fertilizer	3	60422.7	26.1	6.3
Compost	1	39483.6	4.0	0.8
Pesticides x Fertilizer	3	71054.2	141.8	2.0
Pesticides x Compost	1	33469.9	140.6	1.9
Fertilizer x Compost	3	268448.9	468.3	17.7
Pesticide x Fertilizer x Compost	3	57693.2	120.9	4.6

DF = degree of freedom, *significant at $p<0.05$.

Table 3 Mean value for post hoc LSD of total phenolic content (TPC), free radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) in tomato of each factor level.

Factor	Level of factor	DF	TPC	DPPH	FRAP
Pesticide	Chemical pesticide	24	822.17^a	44.34^a	6.81^a
	Organic pesticide	24	797.48^a	40.51^a	5.91^a
Fertilizer	Without fertilizer	12	889.3^a	44.48^a	7.44^a
	Chemical fertilizer	12	753.2^a	40.99^a	5.89^a
	Organic fertilizer	12	746.8^a	42.01^a	6.09^a
	Chemical and organic fertilizer	12	849.9^a	42.23^a	6.01^a
Compost	Cow manure compost	24	838.51^a	42.71^a	6.23^a
	EFB compost	24	781.15^a	42.14^a	6.49^a

DF = degree of freedom; Different alphabet showed significant difference at $p<0.05$.

The total phenolic content (TPC) was measured in terms of gallic acid equivalent using the Folin Ciocalteu (FC) reagent. The method was measuring the reducing capacity of the sample with the FC reagent by reducing the color of FC reagent from yellow to dark blue[19]. The DPPH assay method is based on reduction of DPPH which is a stable free radical. Moreover, DPPH assay has been used widely to determine the ability of antioxidant in sample to reduce the DPPH by donating hydrogen to form neutral DPPH[20]. The FRAP assay is based on the ability of an antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ) where it will form an intense blue Fe^{2+}-TPTZ complex. The absorbance decrease is proportional to the antioxidant content in the sample extracts[21].

Result of phenolic content (TPC) and antioxidant activity of tomato extract using DPPH and FRAP from planting medium treated with different pesticide, fertilizer and growth medium in compost were as in Table 4. The results showed that TPC, DPPH and FRAP varies within treatments. The mean efficiency for TPC and DPPH values in tomato showed that tomato from T7, 1163.6 mg GAE/100 g and 55.7 % (chemical pesticide, organic and chemical fertilizer, and growth medium in cow manure compost) were showed significantly higher ($p<0.05$) than other treatments. While T3, 54.2% (chemical pesticide, organic fertilizer, medium growth in cow manure compost) tomatoes showed significant ($p<0.05$) value for DPPH only. Results of these studies (T3 and T7) were similar as previous study by[22]. In their study the tomato fruits were showed significantly high antioxidant activity with DPPH method but not significantly in FRAP method. While, treatment T2 (chemical pesticide, without fertilizer and growth medium in EFB compost) showed significantly higher ($p<0.05$) in antioxidant activity using FRAP method rather than DPPH. FRAP values was 9.00 µmol TE/100 g for T2 and it showed significantly higher ($p<0.05$) than other treatments. This result may due to no fertilizer apply which can cause stress to plant. Thus, the plant will produce high antioxidant biomolecule[23]. Antioxidant levels can be increased and activated by plant defense systems against pests and diseases or other stress factors[24]. Besides using compost to enhance the antioxidant level, compost also reported able to

rejuvenate the soil condition. Similar observation was also reported by other researchers [5] who planted the *Mesembryanthemum edule* into mixture compost with soil leading to the enhancement of antioxidant content.

In the present study, TPC value were lowest significantly for tomato T4, 550.4 mg GAE/100 g (chemical pesticide, organic fertilizer, growth medium in EFB compost), T5, 581.9 mg GAE/100 g (chemical pesticide, chemical fertilizer, growth medium in cow manure compost) and T13, 596.3 mg GAE/100 g (organic pesticide, chemical fertilizer, growth medium in cow manure compost). While, the lowest values DPPH were shown by T12, 31.7% (organic pesticide, organic fertilizer, growth medium in EFB compost) and T13, 32.3% (organic pesticide, chemical fertilizer, growth medium in cow manure compost). For FRAP, lowest value was T15, 4.26 µmol TE/100g (organic pesticide, organic and chemical fertilizer, growth medium in cow manure compost). The TPC, DPPH and FRAP analysis showed no specific patterns for the lowest value in all treatments (Treatment T1 to T16) and the results are inconclusive.

Table 4 Mean value for each TPC, DPPH and FRAP for each treatment T1 to T16.

Treatment	TPC (mg GAE/100 g)	DPPH (%)	FRAP (µmol TE/100 g)
T1	748.89 ± 311.83^{ab}	38.73 ± 14.06^{ab}	$6.15 \pm 1.88^{a-d}$
T2	881.81 ± 184.33^{ab}	46.15 ± 9.63^{ab}	9.00 ± 2.06^{a}
T3	1014.58 ± 464.57^{ab}	54.20 ± 20.28^{a}	8.18 ± 3.96^{ab}
T4	550.42 ± 286.04^{b}	35.70 ± 8.98^{ab}	$4.77 \pm 1.54^{b-d}$
T5	581.94 ± 296.73^{b}	36.73 ± 16.55^{ab}	$5.22 \pm 2.73^{b-d}$
T6	882.36 ± 252.90^{ab}	47.76 ± 15.61^{ab}	$7.18 \pm 2.52^{a-d}$
T7	1163.6 ± 656.30^{a}	55.69 ± 24.21^{a}	$7.94 \pm 4.07^{a-c}$
T8	753.75 ± 146.60^{ab}	39.75 ± 6.10^{ab}	$5.99 \pm 0.84^{a-d}$
T9	1005.55 ± 142.76^{ab}	47.65 ± 13.57^{ab}	$7.14 \pm 2.35^{a-d}$
T10	921.11 ± 376.86^{ab}	45.38 ± 18.92^{ab}	$7.48 \pm 3.22^{a-d}$
T11	875.42 ± 302.23^{ab}	42.30 ± 10.16^{ab}	$6.55 \pm 2.01^{a-d}$
T12	572.50 ± 11.73^{b}	31.74 ± 5.36^{b}	4.06 ± 0.62^{d}
T13	596.3 ± 61.81^{b}	32.32 ± 1.30^{b}	4.37 ± 0.24^{cd}
T14	926.67 ± 206.47^{ab}	51.21 ± 7.58^{ab}	$7.59 \pm 1.29^{a-d}$
T15	721.81 ± 164.98^{ab}	34.06 ± 5.80^{ab}	4.26 ± 0.83^{d}
T16	760.56 ± 134.20^{ab}	39.39 ± 7.92^{ab}	$5.82 \pm 1.22^{a-d}$

Different alphabet in same row shows significant different at ($p<0.05$). Combination of each factor level for treatment T1 to T16 were as followed:

T1: Chemical pesticide, without fertilizer, cow manure compost ,
T2: Chemical pesticide, without fertilizer, EFB compost,
T3: Chemical pesticide, organic fertilizer, cow manure compost,
T4: Chemical pesticide, organic fertilizer, EFB compost,
T5: Chemical pesticide, chemical fertilizer, cow manure compost,
T6: Chemical pesticide, chemical fertilizer, EFB compost,
T7: Chemical pesticide, chemical and organic fertilizer, cow manure compost,
T8: Chemical pesticide, chemical and organic fertilizer, EFB compost,
T9: Organic pesticide, without fertilizer, cow manure compost,
T10: Organic pesticide, without fertilizer, EFB compost,
T11: Organic pesticide, organic fertilizer cow manure compost,
T12: Organic pesticide, organic fertilizer, EFB compost,
T13: Organic pesticide, chemical fertilizer, cow manure compost,
T14: Organic pesticide, chemical fertilizer, EFB compost,
T15: Organic pesticide, chemical and organic fertilizer, cow manure compost,
T16: Organic pesticide, chemical and organic fertilizer, EFB compost.

The linear Pearson's correlation coefficient was performed to explore the trend of association between total phenolic content (TPC) and two antioxidant activities (DPPH and FRAP) of *Lycopersicon esculentum* Mill.

cv MT1 (Table 5). From Table 5 it showed that TPC and antioxidant activities were significantly ($p<0.05$) correlated with DPPH assay (r=0.933) and FRAP assay (r=0.874). Therefore, it can be suggested that the active compound found in sample extracts are mainly from phenolic substances. In this study, DPPH assay showed strongly positive correlation with FRAP assay (r=0.911). Study by [25] also showed linear correlation between DPPH and FRAP assay in tomato. This may be caused by phenolic compound in tomatoes were flavonoids (quercetin, kaempferol and naringenin) and hydroxynnamic acids (caffeic, chlorogenic, ferulic and φ-coumaric acids) which possess strong antioxidant activity[2]. However, the quercertin and chlorogenic acid did not high antioxidant activity in tomato[2].

Table 5 Pearson's correlation coefficient of antioxidant activities of *Lycopersicon esculentum* Mill. cv MT1.

Correlation coefficient (r)	TPC	DPPH	FRAP
TPC	1	0.933*	0.874*
DPPH		1	0.911*
FRAP			1

* Significant at the $p<0.05$.

Conclusion

In conclusion, the finding of this study demonstrated that factors; pesticide, fertilizer and growth medium in compost and their interaction did not contribute to phenolic content and antioxidant activity of tomato. Whereas, total phenolic compound (TPC) was the contributor of the antioxidant activity in tomato.

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