

The Effects of Soil Temperature From Soil Mulching and Harvesting Period On Phenol, Flavonoid And Antioxidant Contents of Java Tea (*Orthosiphon Aristatus* B.)

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Abstract

Purpose: The secondary metabolites of Java tea (*Orthosiphon aristatus* B) are effective to treat kidney inflammation, kidney stones, and other diseases. Abiotic factors have been known to determine the secondary metabolites contents of Java tea. This study aimed at evaluating the effects of soil temperature resulted from soil mulching and harvesting period on total phenol, flavonoid and antioxidant contents of Java tea.

Methods: The research was carried out using nested with completely randomized design under a screen house at Karanganyar, Indonesia from July to December 2019. The first factor was soil mulching (control; black plastic mulch, transparent plastic mulch, biodegradable mulch and rice straw mulch) with three repetitions. The first factor was nested in second factor, namely harvesting period which consisted of two levels, i.e.: 80 and 100 days. The observation parameters were soil temperature of 10-minutes (maximum; mean; minimum and soil temperature based- growing degree days, GDD) with sensors and logger, plant growth (plant height, number of leaves, fresh and dry weight); and secondary metabolites including phenol, flavonoids and antioxidant.

Results: The results confirmed the order of the highest to the lowest mean soil temperature was resulted under the transparent plastic mulch> straw > black plastic mulch >control > biodegradable plastic mulch (26.69 > 26.29 > 26.10 > 26.07 > 25.68 °C, respectively). The total phenol, flavonoids and antioxidant produced ranging from 193.75 – 412.50 mg GAE/ 100 g DW; 81.13 – 141.47 mg QE/ 100 g DW; and 1875.5 – 2144.4 µmol TE/g DW.

Conclusion: Higher maximum soil temperature resulted into lower phenol content, while higher minimum soil temperature and shorter harvest period increased total flavonoid. Longer harvest period produced more total phenol and antioxidant due to bigger soil temperature based- Growing Degree Day (GDD).

Introduction

Java tea (*Orthosiphon aristatus* B.) is a plant from the Lamiaceae family that is widely gown in Indonesia. *Orthosiphon aristatus* is a medicinal plant used traditionally (Koay & Gam., 2011). The part of the Java tea that is often used and believed to have medicinal properties is the leaves. *Orthosiphon aristatus* spread throughout Southeast Asia and Australia (Di et al., 2013). In Indonesia, there are about 207 ha of cultivated land for Java tea. There are many centers for Java tea cultivation in West Java. Based on the National Plantation Agency of West Java (2017), the productivity data for Java tea in 2017 at Sukabumi was 56 tones ha⁻¹. This production rate can be increased through improved cultivation techniques. Environmental conditions are an important factor besides genetics in determining the productivity of secondary metabolites of medicinal plants (Yang et al., 2018). Many environmental factors affect the growth of a Java tea, such as drought stress, lack of certain nutrients, temperature, and pests and diseases. Radušienė et al., (2012) also added that environmental factors that influence include light intensity, temperature, climate, water availability, soil type and composition, and several other things that can affect the quality and productivity of medicinal plants.

The efficacy of Java tea is determined by the presence of secondary metabolites, including diterpenoids, phenolics, flavonoids, rosmarinic acid, and sinensetin. Java tea contain flavonoids (sinensetin and eupatorin), caffeic acid, and rosmarinic acid. (Muhammad et al., 2011). *Orthosiphon aristatus* (Blume) Miq. has many benefits, such as stimulating urine output (diuretic) and dissolving kidney stones (Faramayuda et al., 2020). Java tea can be used as an alternative for the production of bioactive compounds with antioxidant activity (Lim et al., 2013). Java tea are used to treat kidney inflammation, kidney stones, diabetes, hypertension, rheumatism, tonsils, gout, and menstrual disorders (Chai et al., 2014).

Unpredictable climate change causes the growth of Java tea to be less than optimal. To minimize this problem, a solution is needed to improve the microclimate around the Java tea plant. One of the microclimate modification techniques is to apply mulch (Komariah et al., 2008). The use of mulch can increase the levels of phenolic compounds in gapes (Osrecak et al., 2015). Mulch affects the higher content of epicatechin and gallic acid in gapes (Osrecak et al., 2016). Abdillah et al. (2015) found the increase in light stress given is in line with the increasing phenolic and flavonoid content, as well as their antioxidant activity. Light is a physical factor that can affect the production of metabolites, where the increased light intensity was positively correlated with phenolic levels.(Akula & Ravinshankar, 2011). The studies regarding the effects of soil temperatures on secondary metabolites are rarely found, hence this study aimed at evaluating the effects of soil temperature resulted from soil mulching and harvesting period on total phenol, flavonoid and antioxidant contents of Java tea. The results of this study are expected to provide insight into strategies for optimizing the production of certain compounds in plants through soil mulching and harvesting period.

Materials And Method

This research was conducted from July to December 2019 at Wonosari Village, Gondangejo District, Karanganyar Regency (7°29'33" S; 110°51'22" E), Indonesia. The soil type was Inceptisols with the soil characteristics presented in Table 1 (soil pH, C-Organic, total nitrogen, available phosphorus and potassium were 6.55; 3,78%; 4.06%; 1.39 ppm and 10.08 mg/ 100mg; respectively). The soil analysis was carried out at the Laboratory of Chemistry and Soil Fertility, Faculty of Agriculture, Sebelas Maret University. Secondary metabolite content analysis was carried out at the Laboratory of Biopharmaca Tropical Study Center, IPB University, Bogor.

Table 1. Selected soil characteristics for research media

pH	C-Organic (%)	Total Nitrogen (%)	Available Phosphorus (ppm)	Available Potassium (mg/100g)
6.55	3.78	4.06	1.39	10.08

This experiment was prepared using the completely randomized in nested design with two factors, namely harvest period as an independent factor and variation of soil mulching as the nested factor. Harvest period consists of two treatments, namely the harvest period of 80-days and 100-days. The soil mulching consisted of five treatments, namely control, black- plastic, transparent plastic, biodegradable plastic, and straw. Each treatment was repeated 3 periods, hence there were total of 30 experimental units.

Java Tea Planting

Java tea was stem cutting seedling from mother plant of approximately 10 cm length. Preparation for transplanting started with soil preparation. Soil was air-dried, crushed and sieving with 2mm sieves. The sieved soil was then mixed with manure compost and roasted rice husks with volume proportion of 20 (soil) : 3 (roasted rice husks) : 2 (manure compost). The mixed planting media was then inserted into 40 cm diameter and 35 cm that pot until the pot is full of the media. The media were then saturated with water and incubated for 24 hours. The 2 weeks old java tea seedling (stem cutting seedling) were transplanted into the pot, each pot occupied by one stem seedling. Soon after that, the polyethylene transparent plastic mulching of 0.06 mm; polyethylene -black plastic mulching of 0.08 mm; biodegradable plastic from cassava starch of 0.22 mm, and 3 cm height of dry rice straw were applied covering the pot surface completely, except the stem at the middle of the pot. The irrigation used drip irrigation at each pot with a volume of 1,500 ml per day using 2.2 mm transparent hose connected to a 1,500 ml mineral water bottle, which was refilled every day. Maintenance activities including weeds, pests and plant diseases control was intensively carried out manually, without the usage of pesticide.

Soil Temperature Measurement

Soil temperature observations are carried out using waterproof soil temperature sensor probe DS18B20 connected to the Arduino and logger, self-assembled by researchers. The actual soil temperatures data were recorded every 10 minutes. The Growing Degree Day (GDD) observed in this study was the soil temperature-based GDD, which used the soil temperature to calculate the GDD. The GDD was calculated as the accumulation of daily soil temperature differences.

Agronomic and Harvest observations

Observation of agronomic characters was carried out once a week including plant height (cm), and number of leaves. The harvesting of the Java tea was carried out when Java tea were 80 days and 100 days old. After harvested, each plant was weighed to get the fresh weight. To observe the dry weight, each plant was dried in the oven at a temperature of 50 °C for 2 x 24 hours.

Sample Extraction

Extraction was carried out using 96% technical ethanol solvent. Simplicia was weighed as much as 2 g, then put in Erlenmeyer and added with the solvent with a ratio of 1:10 and sonicated for 30 minutes. The extract was filtered using Whatman filter paper, then the filtrate obtained was concentrated with a rotary evaporator until the volume became 10 mL.

Determination of Total Phenol

Determination of total phenol according to Stoilova et al., (2007) with a modified Folin-Ciocalteu method. Determination of the total phenol content of Java tea leaf extract was carried out spectrophotometrically using the Folin-Ciocalteu reagent and gallic acid as a comparison. A total of 1 mL of sample was inserted into the test tube, then added 5 mL of 7.5% Folin Ciocalteu reagent then vortexed until homogeneous and allowed to stand for 8 minutes in a dark room. The mixture was then added with 4 mL of 1% NaOH and then vortexed until homogeneous and placed in a dark room for 1 hour. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 730 nm. Quantitative analysis of total phenol was carried out by making standard curves of 0, 10, 30, 50, 70, and 100 ppm concentrations of gallic acid. Total phenol is reported as gram of Gallic Acid Equivalent (GAE) / g of extract.

Determination of Total Flavonoids

Determination of total flavonoids refers to the method of Lee et al., (2012). The total flavonoid content was determined using aluminum chloride. A total of 10 µL of extract was mixed with 60 µL of ethanol, 10 µL of AlCl₃ (10% w/v), 10 µL of CH₃COOK (1M), and 110 µL of distilled water on 96-well plates and incubated at room temperature for 30 minutes. The absorbance was measured at a wavelength of 415 nm. Quercetin was used as a standard with a concentration range of 20, 40, 60, 80, 100, 120, and 140 µg / mL. Total flavonoids are reported as gram of quercetin equivalent (QE) / gram of extract.

Determination of Antioxidant Content

The determination of antioxidant content refers to the method of Re et al., (1999). ABTS radical solution was prepared by mixing 10 mL of 7 mM ABTS with 5 mL of 2.45 mM K₂S₂O₈ for 12-16 hours in a dark place. A total of 180 µL of ABTS radicals were reacted with 20 µL of extract and incubated at room temperature for 15 minutes. The absorbance of the mixture was then measured at a wavelength of 734 nm. Trolox was used as standard and its antioxidant activity was reported in mg of Trolox equivalent (TE) / gram of extract.

Data analysis

The data from the observations were tested using ANOVA and if the variance at the 5% level had a significant effect, a further test was carried out with the Duncan Multiple Range Test (DMRT) at the 5% level. Pearson correlation test and multiple regression analysis were conducted to test the correlation between the secondary metabolites content with soil temperatures and growth parameters.

Results

Soil temperature

Figure 1 presents the dynamics of the mean soil temperature of various mulch materials. From Figure 1 it can be seen the mean soil temperature fluctuated, with the highest mean soil temperature was found in the transparent plastic mulch. The mean soil temperature in the transparent plastic mulch ranged from 25.19-28.19 °C (mean 26.69 °C). While the mean soil temperature below the straw, black plastic and control ranged from 24.88-27.69 °C (mean 26.29 °C); 24.80-27.39 °C (mean 26.10 °C); and 24.86-27.28 °C (mean 26.07 °C), respectively. The lowest mean soil temperature was found in the biodegradable plastic mulch ranging from 24.38-26.97 °C (mean 25.68 °C).

Effects of treatments on all observed parameters

Table 1. Anova of nested plot design on each parameter

Parameter	Sig.	
	Harvest age	Variation of mulch (Harvest Age)
Minimum soil temperature	<0.01**	<0.01**
Mean soil temperature	.387 ^{ns}	.213 ^{ns}
Maximum soil temperature	.014*	<0.01**
Plant height	.706 ^{ns}	.683 ^{ns}
Number of leaves	.037*	<0.01**
Fresh weight	<0.01**	.017*
Dry weight	<0.01**	<0.01**
Phenol	<0.01**	.280 ^{ns}
Flavonoids	.011*	.380 ^{ns}
Antioxidant	.014*	.746 ^{ns}
GDD	<0.01**	.150 ^{ns}

Note: $\alpha = 0.05$; ns = not significant; * = significant; and ** = highly significant

Table 1 shows the analysis of variance (ANOVA) of the treatments on all observed parameters. From Table 1 it can be seen that the harvest period affected the minimum soil temperature ($P < 0.01$ **), maximum soil temperature ($P = 0.014$ *), number of leaves ($P = 0.037$ *), fresh weight ($P < 0.01$ **), dry weight ($P < 0.01$ **), total phenol ($P < 0.01$ **), total flavonoids ($P = 0.011$ *), Antioxidant ($P = 0.014$ *), and GDD ($P < 0.01$ **) very significantly. Table 1 also shows the type of mulch nested in harvest period affected the minimum soil temperature ($P < 0.01$ **), maximum soil temperature ($P < 0.01$ **), number of leaves ($P < 0.01$ **), fresh weight ($P = 0.017$ *), and dry weight ($P < 0.01$ **) significantly.

Table 2 shows the effects of each treatment on all observed parameters. It can be seen the treatments significantly influenced all parameters except the mean soil temperature and plant height. Table 2 also depicts the minimum soil temperature under black plastic mulch at 100 days harvest period was the lowest (18.66 °C) compared to other treatments. Even though the minimum soil temperature at 80 days of harvest period was quite high (21.5 °C) but did not differ significantly among mulching treatments. This is thought to be due to drip irrigation applied, which causes the soil temperature to be low and stable because crop water was enough and low evaporation, coupled with the long harvest age. The minimum soil temperature in the 80 days of harvest ranged from 20.43-21.70 °C, while in the treatment, the 100 days of harvest age ranged from 18.66-21.81 °C. In general, biodegradable plastic mulch produced the same minimum soil temperature (not significantly different) as the control. From Table 2 it can also be seen that the maximum soil temperature under black plastic mulch was higher than the control, especially at the 80 days of harvest. Meanwhile, the GDD presented in Table 2 also shows that in general, 100 days of harvest period produced higher GDD (1505.90-1643.25 °C) compared to 80 days (1293.52-1362.03 °C).

Overall, Table 2 shows that various of mulch nested at harvest period had a significant effect on the number of leaves, where M5(P2), M4(P1), and M1(P1) treatments showed the highest number of leaves, namely 698, 627, and 566, respectively. Furthermore, the fresh weight was significantly different, 100 days harvest treatment was higher (171.56-337.45 g) than the 80 days (84.43-178.36 g). Furthermore, dry weight was generally greater at 100 days harvest (28.23-77.58 g) than 80 days (15.46-30.83 g), however, transparent plastic mulch and biodegradable plastic showed lower dry weight than control.

Table 2. Effects of mulch nested at harvest period on all parameters

Parameter	Unit	Treatment										S
		M1(P1)	M2(P1)	M3(P1)	M4(P1)	M5(P1)	M1(P2)	M2(P2)	M3(P2)	M4(P2)	M5(P2)	
Minimum soil temperature	°C	21.25 ^{cde}	21.50 ^{de}	21.70 ^e	21.31 ^{cde}	20.43 ^{bc}	20.56 ^{bcd}	18.66 ^a	21.48 ^{de}	19.66 ^b	21.81 ^{es}	s
Mean soil temperature	°C	26.46	26.85	27.24	26.70	25.70	26.61	26.11	27.11	25.69	26.98	n
Maximum soil temperature	°C	33.08 ^a	37.81 ^{cd}	33.98 ^{ab}	34.50 ^{ab}	39.81 ^d	34.25 ^{ab}	36.72 ^{bc}	34.94 ^{ab}	32.63 ^a	32.94 ^a	s
GDD	°C	1300.15 ^a	1331.17 ^a	1362.03 ^a	1319.07 ^a	1293.52 ^a	1594.92 ^{bc}	1547.05 ^{bc}	1643.25 ^c	1505.90 ^b	1629.84 ^c	s
Plant height	Cm	83.67	80.67	77.33	71.00	83.00	78.75	86.10	82.13	74.63	79.83	n
Number of leaves		566 ^{def}	486 ^{cde}	252 ^a	627 ^{ef}	509 ^{cde}	266 ^a	449 ^{bcd}	329 ^{ab}	376 ^{abc}	698 ^f	s
Fresh weight	Gram	178.36 ^{ab}	151.92 ^{ab}	84.43 ^a	122.04 ^{ab}	165.83 ^{ab}	316.52 ^d	337.45 ^d	212.67 ^{bc}	171.56 ^{ab}	274.32 ^{cd}	s
Dry weight	Gram	30.83 ^{ab}	30.22 ^{ab}	15.46 ^a	21.93 ^{ab}	30.67 ^{ab}	72.10 ^d	77.58 ^d	36.47 ^{bc}	28.23 ^{ab}	49.81 ^c	s
Phenol	mg GAE/ 100 g DW	294.128 ^{abc}	246.46 ^{ab}	241.41 ^{ab}	247.73 ^{ab}	193.75 ^a	399.24 ^{de}	369.57 ^{cde}	308.83 ^{bcd}	399.24 ^c	412.50 ^e	s
Flavonoids	mg QE/ 100 g DW	84.63 ^{abc}	128.36 ^{bc}	141.47 ^c	97.30 ^{abc}	89.80 ^{abc}	64.13 ^a	82.24 ^{ab}	94.97 ^{abc}	90.30 ^{abc}	81.13 ^{ab}	s
Antioxidant	μmol TE/g DW	1922.5 ^a	1998.4 ^a	1875.5 ^a	1959.8 ^a	1946.2 ^a	2144.4 ^b	2067.8 ^b	2136.6 ^b	2103.0 ^b	1949.3 ^b	s

Note: s= significant; ns= not significant; a=0.05; means followed by the same letter in the same row are not significantly different; M1= control (no mulching); M2= black- plastic mulch; M3= transparent plastic mulch; M4= biodegradable plastic mulch; M5= rice straw mulch; P1= harvest period 80 days; P2= harvest period 100 days

Table 2 shows 100 days of harvest period resulted in higher phenol content than 80 days. The 100-day harvest period showed higher phenol levels ranging from 308.833-412.500 mg GAE / 100 g dry extract compared to the 80-day harvest period (193.750-294.128 mg GAE / 100 g dry extract). While the highest total flavonoid content was found in the P1M3 and P2M3 treatments, namely 141.467 and 94.967 mg QE / 100 g dry extract, respectively. Table 2 also shows that the harvest period has a significant effect on Antioxidant. The amount of Antioxidant in the 100-day harvesting age treatment showed higher yields (1949.3-2144.4 μmol TE / g dry extract) than 80 days (1875.5-1998.4 μmol TE / g dry extract).

The agronomic characters and soil temperature effects on secondary metabolites

Table 3. Pearsons' correlation of agronomic characters and soil temperature with secondary metabolites of Java tea

Agronomic characters	Correlation coefficient & P-value		
	Phenol	Flavonoids	Antioxidant
Plant height	0.198	-0.067	0.015
	(0.353)	(0.780)	(0.940)
Number of leaves	0.070	-0.122	-0.230
	(0.768)	(0.665)	(0.303)
Fresh weight	0.792	-0.524	0.353
	(<0.01)	(0.026)	(0.083)
Dry weight	0.735	-0.484	0.283
	(<0.01)	(0.068)	(0.202)
Minimum soil temperature	-0.453	0.523	-0.185
	(0.045)	(0.015)	(0.366)
Mean soil temperature	-0.460	0.394	-0.125
	(0.031)	(0.063)	(0.517)
Maximum soil temperature	-0.520	-0.177	-0.032
	(0.016)	(0.455)	(0.876)
GDD	0.674	-0.237	0.374
	(<0.01)	(0.276)	(0.042)

Note: The confidence level is 95% ($\alpha = 0.05$); the number in brackets is the P-value; the grey cell means significant correlation

Table 3 shows the Pearson's correlation analysis between agronomic characters and soil temperature with secondary metabolites of Java tea (Phenols, Flavonoids, and Antioxidant). Table 3 shows that phenol correlates with fresh weight ($P < 0.01$), dry weight ($P < 0.01$), minimum soil temperature ($P = 0.045$), mean soil temperature ($P = 0.031$), maximum soil temperature ($P = 0.016$), and GDD ($P < 0.01$). Table 3 also shows that flavonoids correlated with fresh weight ($P = 0.026$) and minimum soil temperature ($P = 0.015$). Table 3 shows that Antioxidant also only correlated with GDD ($P = 0.042$).

Table 4 shows the multiple regression analysis of secondary metabolites of Java tea with soil temperature. It shows the maximum soil temperature can be used to predict phenol content, marked by the significance value of the ($p = 0.015$) and the largest determinant coefficient compared to flavonoids and Antioxidant ($R^2 = 0.492$). Soil temperature could not predict the flavonoid and antioxidant contents because the significances (p) of the model were higher than α , i.e.: 0.132 and 0.732, respectively.

Table 4. Multiple regression analysis to estimate the secondary metabolites of Java tea from soil temperatures

Parameter	Sig.	B-coefficients						Model Sig. (p)	R	R ²
		Tmin	Tmean	Tmax	Tmin	Tmean	Tmax			
Phenol	.189	.355	.015	-.058	-.066	-.035	.015	.015	.702	.492
Flavonoids	.465	.326	.454	2.359	4.730	-.742	.132	.132	.552	.305
Antioxidant	.641	.831	.540	-22.503	-15.043	8.850	.732	.732	.247	.061

Note: number in the grey cell means significant at $\alpha = 0.05$

Figure 2 presents the effects of mulch nested at harvest period on phenol and flavonoid levels of Java tea. It can be figured out in general, the 100-days harvest period produced higher phenol content of Java tea (308.833-412.500 mg GAE / 100 g dry extract) compared to the 80-days harvest period (193.750-294.128 mg GAE / 100 g dry extract). However, it generally shows the different results on the levels of flavonoids, whereas the treatment of 80-days harvest period shows higher yields (84.633-141.467 mg QE / 100 g dry extract) when compared to 100-days harvest period (64.133-94.967 mg QE / 100 g dry extract).

Figure 3 presents the effect of mulch nested at harvest period on soil temperature. From Figure 3 it can be seen the minimum soil temperature in general at 100-days harvest period shows higher yields (18.65-21.81 °C) compared to 80-days harvest (20.43-21.7 °C). From Figure 3 it can also be seen that in general, the mean soil temperature in the 80-day harvest treatment shows higher yields (25.7-27.24 °C) compared to 100-days harvest (25.69-27.12 °C). From Figure 3 it can also be seen that in general, the maximum soil temperature at the 80-day harvest treatment shows higher yields (33.08-39.81 °C) compared to the 100-day harvest period (32.63-36.72 °C).

Figure 4 presents the effect of soil mulching nested at harvest period on GDD and phenol content of Java tea. From Figure 4 it can be seen GDD affects the phenol content of Java tea. Figure 4 also shows that in general, the 100-day harvest resulted in a higher phenol content of Java tea (308.833-412.500 mg GAE

/ 100 g dry extract) compared to the 80-day harvest treatment (193.750-294.128 mg GAE / 100 g dry extract).

Figure 5 presents the effect of mulch nested at harvest period on GDD and Antioxidant content of Java tea. Figure 5 figures out GDD affects the Antioxidant content of Java tea. Figure 5 also shows that in general, the 100-day harvest resulted in higher levels of Antioxidant content (1949.35-2144.41 $\mu\text{mol TE} / \text{g dry extract}$) compared to the 80-day harvest treatment (1875.59-1998.43 $\mu\text{mol TE} / \text{g dry extract}$).

Discussion

Soil temperatures affected the total phenol due to minimum, mean, and maximum soil temperatures correlated with the total phenol (Table 3). However, the total phenol was more specifically determined by the maximum soil temperature (Table 4). The maximum soil temperature varied according to mulch type (Table 1; $p < 0.01$). The higher maximum soil temperature led to the lower of total phenol content, as shown in Table 3 indicated by the negative correlation ($r = -0.520$). This is in line with research conducted by Aidoo et al. (2016) where high soil temperatures associated with specific changes in secondary metabolites and physiological behavior. The abiotic stress affected the growth and production of secondary metabolites (Akula & Ravinshankar, 2011). The responses of plant secondary metabolites such as phenolics, flavonoids, terpenoids, and alkaloids are generated from various biochemical processes by environmental stress (Yang et al., 2018). Abiotic stress significantly affects the production of plant secondary metabolites (Ashraf et al., 2018).

However, Table 3 also confirms that the mean soil temperature negatively correlated with phenol ($r = -0.040$), meaning that the higher mean soil temperature reduced the total phenol content of java tea. If seen from Figure 1, the dynamics of soil temperature under transparent plastic mulch resulted in the highest mean soil temperature during planting, while the lowest mean soil temperature was produced under biodegradable plastic mulch followed by black plastic mulch then straw. Thus, from Figure 1 it can be assumed that cultivating Java tea with biodegradable plastic mulch can produce high total phenols, due to the low mean soil temperature. Similar results were also reported by You et al. (2020), where low soil temperature under biodegradable plastic mulch increased the secondary metabolite of tomatoes, namely the ratio of vitamin C. Androgapholide, which is the major secondary metabolite of a medicinal property of Kiriya, also resulted greater yield when planted with straw mulch rather than black plastic mulch due to lower soil temperatures, as reported by Sreethu et al. (2018). This certainly proved that environmental factors determined the biosynthesis of plant secondary metabolites (Verma & Shukla, 2015).

However, in general, total phenol was also largely determined by the harvest period. The longer harvest period resulted in higher GDD (Table 2). This means that the higher the GDD, the higher the phenol content (Figure 5). Thus, the phenol content can be increased by the accumulation of the mean soil temperature, called as soil temperature-based GDD. This is nearly similar with research conducted by Stagnari et al. (2018) who found that GDD affected the total phenolic content and antiradical activity in basil, and the phenolic content in the *Sphallerocarpus gacilis* roots (Gao et al., 2011). GDD has an effect on increasing the total phenolic content of lettuce in Vulcan and Crispino cultivars (Bunning et al., 2010). Beans with the Jaki-9281 genotype that accumulated higher GDD also resulted in higher phenol content (Ba & Chimmad, 2018).

The total phenol content produced due to the soil temperature states was opposite to the produced total flavonoid (Figure 2), whereas the total flavonoids of 100-days were lower than 80-days harvest period. The total flavonoids were more influenced by the minimum soil temperature (Table 3), indicated by the positive correlation ($r = 0.523$). The minimum soil temperatures were also influenced by the various of mulch (Table 1), where black plastic mulch resulted the lowest minimum soil temperature (18.66 °C). Zhang et al. (2017) and Yaghi et al. (2013) and also found minimum soil temperature under black plastic mulch was lower than that of control and transparent mulch, respectively.

The total flavonoids were also controlled by the plant weight (Table 3). The negative correlation value ($r = -0.548$) means that the higher the fresh weight caused the lower total flavonoids content. Indirectly, total flavonoids were also controlled by the harvest period: the longer harvest period led to higher fresh weight (Table 2). This means the shorter harvest period may increase the total flavonoids. This is in line with the research conducted by Zeng et al., (2020), who confirmed some flavonoids and flavonoid glycosides are affected by the harvest period. Elmastas et al., (2017) also found the content of flavonoids and phenolic acids can change according to the harvest time. In the contrary, the longer harvest period may cause several components of antioxidants such as ascorbic acid to decrease (Ghasemnezhad et al., 2011).

The antioxidant contents were strongly regulated by the soil temperature based-GDD (Table 3), with correlation value ($r = 0.374$), meaning the bigger the GDD, the higher the antioxidant content produced. This is confirmed in Figure 6 where the longer the harvest period, the higher the GDD and antioxidant. GDD, which is the accumulated mean soil temperature, is largely determined by the harvest period (Table 1, $P < 0.01$). This is in line with research conducted by Fuchs et al., (2017) which explained there was a significant increase in alkaloid concentrations with an increase in GDD. Red wine produced higher levels of phenolic compounds and antioxidants in the late harvest period (Šćepanović, 2019).

Therefore, in general, it is verified that the total phenol contents in Java tea were low at high mean soil temperatures. However, the high minimum soil temperatures would increase the total flavonoids content. The high soil temperature based -GDD might increase the phenol and antioxidant contents but possibly would decrease the flavonoid levels.

Conclusion

The higher mean soil temperature under transparent plastic mulch resulted into lower total phenol content of the Java tea, where the higher minimum soil temperatures under black plastic much produced higher total flavonoids contents. Higher soil temperature-based - GDD due to the longer harvesting period increased the phenol and antioxidant contents. Further study to confirm more detail mechanism regarding soil temperature effects on secondary metabolites regulation is required.

Abbreviations

GDD
Growing Degree Day
g
gram
mL
milli liter
nm
nano meter
GAE
Gallic Acid Equivalent
μL
micro liter
QE
Quercetin Equivalent
w/v
weight/volume
M
Molar
ABTS
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
TE
Trolox Equivalent
°C
degree Celcius
DW
Dry Weight

Declarations

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Conflict of Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

Ethics Approval

The authors certify that no risks arouse in conducting this research project. We confirm that the research was conducted in line with all national rules, legal and local ethical standards.

Consent for Publication

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Figures

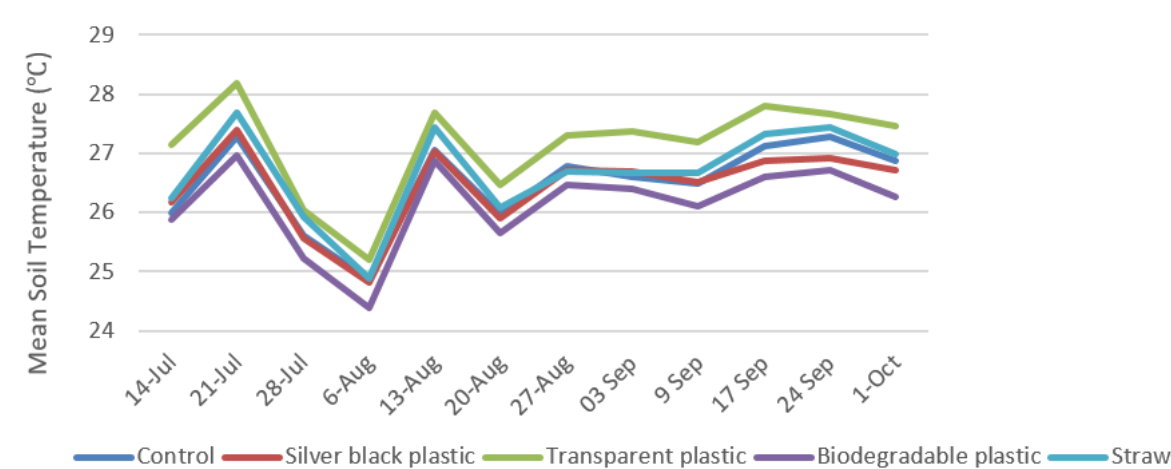


Figure 1
Dynamics of mean soil temperature under various mulch materials

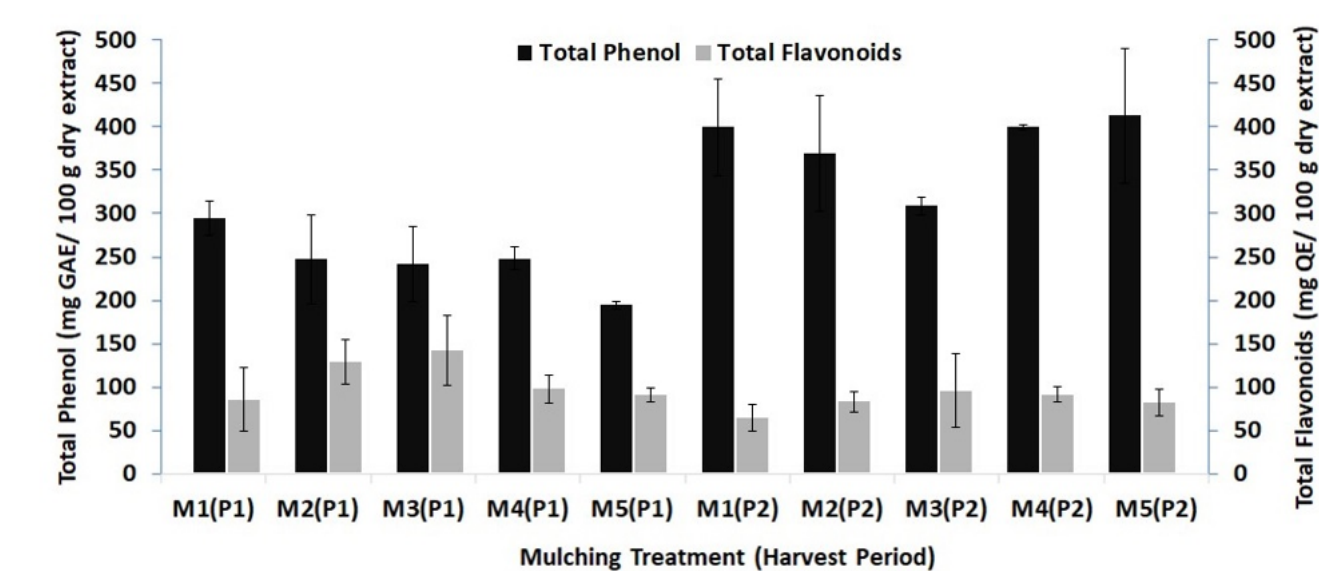


Figure 2

Effects of soil mulching on Phenol and Flavonoid of Java tea (P1= harvest period 80 days; P2= harvest period 100 days; M1= control (no mulching); M2= black- plastic mulch; M3= transparent plastic mulch; M4= biodegradable plastic mulch; M5= rice straw mulch; error bars represent standard deviation)

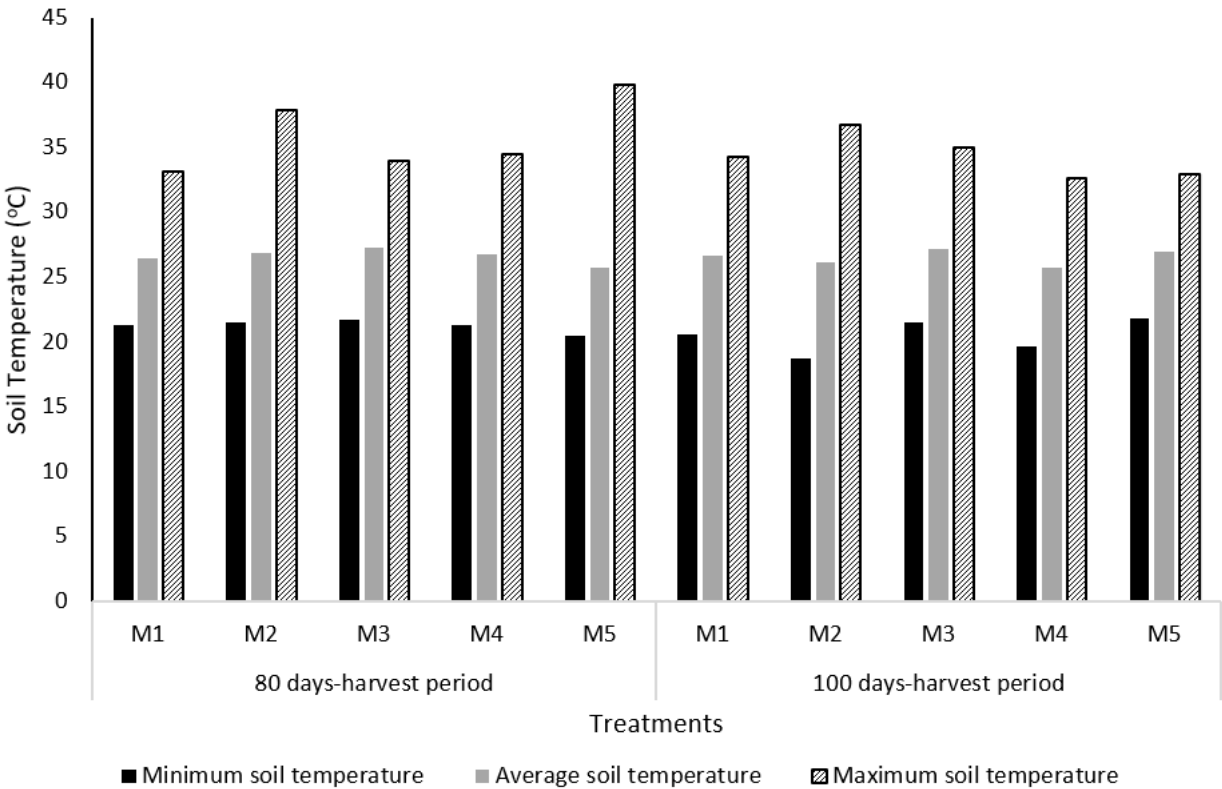


Figure 3
Effect of Mulch nested at Harvest period on Soil Temperature (P1= harvest period 80 days; P2= harvest period 100 days; M1= control (no mulching); M2= black- plastic mulch; M3= transparent plastic mulch; M4= biodegradable plastic mulch; M5= rice straw mulch)

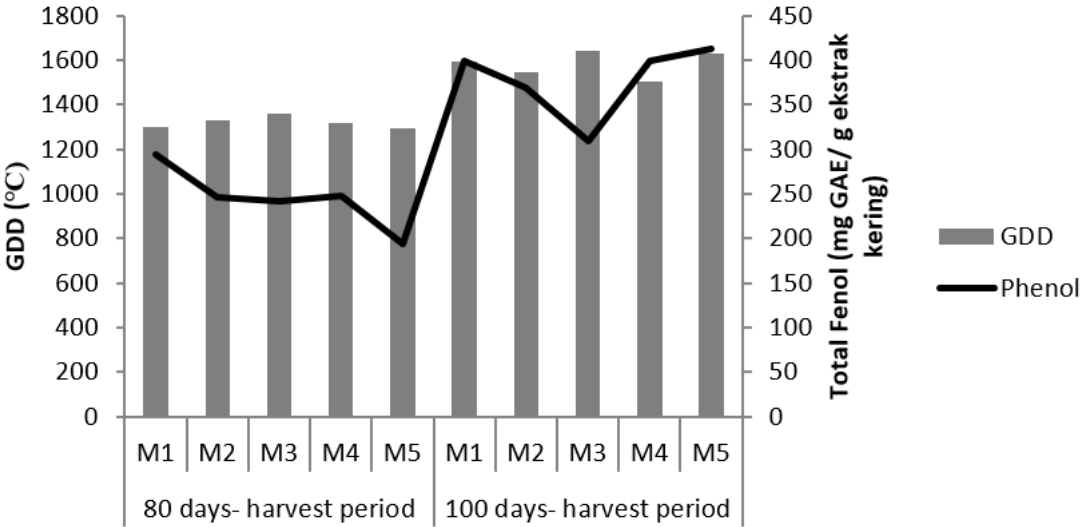


Figure 4
Effect of Mulch nested at Harvest Period on GDD and Phenol Content of Java tea (P1= harvest period 80 days; P2= harvest period 100 days; M1= control (no mulching); M2= black- plastic mulch; M3= transparent plastic mulch; M4= biodegradable plastic mulch; M5= rice straw mulch)

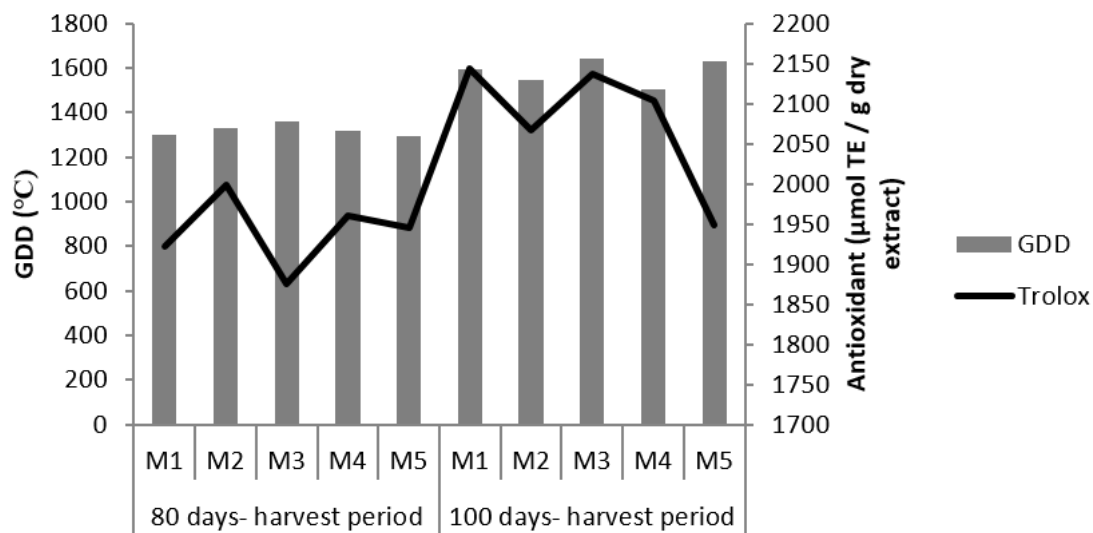


Figure 5

Effect of Mulch nested at Harvest Period on soil temperature based-GDD and Antioxidant content of Java tea (P1= harvest period 80 days; P2= harvest period 100 days; M1= control (no mulching); M2= black- plastic mulch; M3= transparent plastic mulch; M4= biodegradable plastic mulch; M5= rice straw mulch)