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The effects of soil temperature from soil mulching and harvest age on phenol, flavonoid and antioxidant contents of Java tea (*Orthosiphon aristatus* B.)

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Abstract

Background: The environmental conditions resulted by the agronomic management practices may govern the secondary metabolite contents of medicinal plants, including Java tea (*Orthosiphon aristatus* B). Abiotic factors such as temperatures have been known to determine the secondary metabolite contents of Java tea. This study aimed at evaluating the effects of soil temperature resulting from soil mulching and harvest age on total phenol, flavonoid and antioxidant contents of Java tea.

Methods: The research was arranged using nested (hierarchy design) with completely randomized design under a screen house at Karanganyar, Indonesia, from July to December 2019. The main factor was soil mulching (control; black plastic mulch, transparent plastic mulch, biodegradable mulch and rice straw mulch) with three replicates. The main factor was nested in the temporal hierarchy factor, namely harvest age which consisted of two levels, i.e., 80 and 100 days. The observation parameters were soil temperature of 10 min (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). Overall, the harvest age 100 days resulted in higher plant growth, indicated by the higher fresh and dry weight of biomass, higher phenol and antioxidant contents than 80 days. Soil mulching, especially with plastic and biodegradable plastic mulches with long harvest age (100 days) effected into lower fresh and dry weight of plants. On the other hand, soil mulching indirectly resulted in lower phenol but higher flavonoid contents through higher soil temperature, while antioxidant contents were higher under the big soil temperature-based Growing Degree Day (GDD). The total phenol, flavonoids and antioxidant produced ranging from 193.75 to 412.50 mg GAE/ 100 g DW; 81.13 to 141.47 mg QE/ 100 g DW; and 1875.5–2144.4 µmol TE/g DW.

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

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Keywords: Medicinal plants, Secondary metabolite, Microclimate, Biodegradable plastic mulch

Introduction

Java tea (*Orthosiphon aristatus* B.) is a plant from the Lamiaceae family that is widely grown in Indonesia and spread throughout Southeast Asia and Australia [15]. In Indonesia, there are about 207 ha of cultivated land for Java tea, with many centers for Java tea cultivation in West Java area. Based on the National Plantation Agency of West Java (2017), the productivity data for Java tea in 2017 at Sukabumi were 56 tones ha^{-1}. Java tea has many benefits, such as stimulating urine output (diuretic) and dissolving kidney stones [17], also can be used as an alternative for the production of bioactive compounds with antioxidant activity [32]. The efficacy of Java tea for health benefits is determined by the presence of secondary metabolites [10, 23], while the environmental conditions are the important factor in determining the productivity of secondary metabolites of medicinal plants besides genetics [56].

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. [39] reported that environment factors such as light intensity, temperature, climate, water availability, soil type and composition, and others can affect the quality and productivity of medicinal plants. Abdillah et al. [2] and Akula & Ravinshankar [4] found the increase in light stress applied was in line with the increasing phenolic and flavonoid content, as well as their antioxidant activity. The changes in the climate affects the physiological and morphological status of plant growth and production [24], as well as the secondary metabolite contents in plants [4]. Modification of microclimate, as the artificial control of climate in the micro-level is the future trend to maintain and increase crop growth and productivity [53]. One of the microclimate modification techniques is by applying soil mulch [30]. The use of mulch can increase the levels of phenolic compounds in grapes [37], also You et al. [57] demonstrated the increased of the Vitamin C, soluble sugar, and sugar–acid ratio in tomato by the application of red and blue plastic polyethylene mulches. The antioxidant contents of perennial wall rocket were the highest under biodegradable and low-density polyethylene (LDPE) black plastic mulches [13]. The plastic mulch also increased the essential oil of *Melissa officinalis* L. as reported by Biasi et al. [9]. Soil mulching is the common practice in agriculture which is widely implemented by Indonesian farmers. Unfortunately, the studies regarding the effects of soil mulching in Indonesia are mostly regarding with soil properties and soil–water conservation [18], [29]; [42, 51], but the studies regarding the effects of soil temperatures on secondary metabolites have not been found.

Harvesting age played an important role on phenolic content and antioxidant in coffee [14], also the concentration of antioxidant agents in soybean, oyster and sea cucumber [5]. Thounaojam et al. [52] demonstrated that short harvesting age during flowering stage of *Ocimum basillicum* L. resulted in the significantly higher linalool content. However, Hadjipieri et al. [25] found no differences in free phenolic content in loquat fruit with different harvest date. Till date, little attention has been paid to the effects of harvest age of Java Tea on active compounds such as phenol, flavonoids and antioxidant. Therefore, this study aimed at evaluating the effects of soil temperature resulting from soil mulching and harvest age 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 harvest age.

Materials and methods

This research was conducted from July to December 2019 at Wonosari Village, Gondangejo District, Karanganyar Regency (7º29′33″ S; 110º51′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/100 mg, 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.

This experiment was conducted in a greenhouse, and was prepared using the completely randomized in nested design with two factors, namely harvest age as an independent factor and soil mulching as the nested factor. Harvest age consists of two levels, namely the harvest age of 80 days and 100 days. The soil mulching consisted of five levels, namely control, black plastic, transparent plastic,

Table 1 Selected soil characteristics for research media

pH	C-organic (g kg^{-1})	Total nitrogen (g kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})
6.55	21.9	4.06	1.39	100.8

biodegradable plastic, and straw. Each treatment was replicated 3 times, hence there were total of 30 experimental units. The black plastic mulch used was made from low-density polyethylene, the thickness of 0.08 mm with black color at both sides. The material of transparent plastic mulch applied was high-density polyethylene (HDPE) with 0.06 mm thickness and transparentcolor. The biodegradable plastic mulch made from cassava starch with 0.22 mm thickness and light-green transparent color, was bought from the producer telobag.com. Finally, the straw mulch employed was fresh straw with the application height approximately 3 cm, CN ratio was 54.0, and N concentration was 0.7%

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 2-mm 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 pot until the pot was filled with the media. The media were then saturated with water and incubated for 24 hours. The 2-week-old Java tea seedlings (stem cutting seedlings) were transplanted into the pot, each pot occupied by one stem seedling. Soon after that, the polyethylene transparent plastic mulch, polyethylene black plastic mulch, biodegradable plastic, and 3-cm height of dry rice straw were applied covering the pot surface completely, except the stem at the middle of the pot. Drip irrigation was used in 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 were carried out using waterproof soil temperature sensor probe DS18B20 connected to the Arduino and logger, self-assembled by researchers. The actual soil temperatures of 0–10 cm depth data were recorded every 10 min. The cumulative Growing Degree Day (GDD) formula [34, 47] is presented in Eq. (1):

$$GDD = \sum_{i}^{m} (T_i - T_{\text{base}}), \tag{1}$$

$$T_i = \frac{(T_{\max} - T_{\min})}{2} \tag{2}$$

where Ti is the mean air temperature (°C) on the ith day of the growing season, where $i = 1, 2, \ldots$ m days with a temperature higher than the base or threshold temperature (T_{base}, °C) during the growing season, and T_{max} and T_{min} are the daily maximum and minimum air temperatures (°C), respectively. According to Ritchie & Nesmith [41], the base temperature for a wide variety of crops is 20–28 °C, hence the base temperature (T_{base}) assumed in this study is 24 °C. Since no formula employing the soil temperature information on phenological stages of plant have been found, this study adopted Eqs. (1) and (2) by replacing air temperatures with soil temperatures data, hence the GDD formulated in this study was the soil temperature-based GDD, which was calculated as stated in Eq. (3):

$$GDD_{ST} = \sum_{i}^{m} (ST_i - T_{\text{base}}), \tag{3}$$

where GDD_{ST} is soil temperature-based Growing Degree Days (the Growing Degree Days calculated by employing soil temperatures); ST_i is the mean daily soil temperature recorded by the sensor and logger on the i^{th} day of the growing season, $i = 1, 2, \ldots$ m days with a temperature higher than the base temperature; T_{base} is soil temperature base (24 °C).

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 was 80 days and 100 days old. After harvesting, 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 ℃ for 2 × 24 h.

Sample extraction

Extraction was carried out using 96% technical ethanol solvent. The dry leaves were powdered and 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 min. 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 was according to Stoilova et al. [50] using a modified Folin–Ciocalteau 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 taken in a test tube, then added 5 mL of 7.5% Folin–Ciocalteu reagent, vortexed until homogeneous and allowed to stand for 8 min 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 h. The absorbance of the solution was measured using the *Hitachi U-2800 Double Beam UV/VIS 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. [31]. The total flavonoid content of the leaf extract was determined using aluminum chloride. A total of 10 µL of extract was mixed with 60 µL of ethanol, 10 µL of $AlCl_3$ (10% w/v), 10 µL of CH_3COOK (1 M), and 110 µL of distilled water on 96-well plates and incubated at room temperature for 30 min. 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 of the leaf extract refers to the method of Re et al. [40]. ABTS radical solution was prepared by mixing 10 mL of 7 mM ABTS with 5 mL of 2.45 mM K2S2O8 for 12–16 h 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 min. The absorbance of the mixture was then measured at a wavelength of 734 nm using the *Hitachi U-2800 Double Beam UV/VIS Spectrophotometer*. Trolox was used as standard and its antioxidant activity was reported in mg of Trolox equivalent (TE)/g 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, with the mean separation was performed through 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 temperatures

Figure 1 presents the dynamics of the minimum, mean and maximum soil temperatures under various mulch materials. From Fig. 1 it can be seen the soil temperature fluctuated, with the highest fluctuation observed at maximum soil temperature. In general, minimum, mean and maximum soil temperatures fluctuation was 20.5–30, 24.5–31 and 27.2–34.5 C, respectively. The soil temperature under transparent plastic mulch dominated the highest temperature, especially the minimum and mean soil temperatures. Biodegradable plastic mulch experienced the lowest minimum, mean and maximum air temperatures almost at the whole periods, but initially the maximum soil temperatures were higher than rice straw mulch. Overall, soil mulching except plastic biodegradable increased minimum and mean soil temperatures than control, but decreased the maximum soil temperatures. The mean soil temperature in the transparent plastic mulch ranged from 25.19 to 28.19 ℃ (mean 26.69 °C). While the mean soil temperature below the straw, black plastic and control ranged from 24.88–27.69 ℃ (mean 26.29 °C); 24.80–27.39 ℃ (mean 26.10 °C); and 24.86–27.28 ℃ (mean 26.07 °C), respectively. The lowest mean soil temperature was found in the biodegradable plastic mulch ranging from 24.38 to 26.97 ℃ (mean 25.68 °C).

Effects of treatments on all observed parameters

Table 2 shows the analysis of variance (ANOVA) of the treatments on all observed parameters. From Table 2 it can be seen the harvest age 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 2 also shows the type of mulch nested in harvest age 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 3 presents the significance of harvest age on the mean of each observed parameter. It is confirmed that harvest age significantly influenced all observed parameters except mean soil temperature. All parameters, i.e., minimum and maximum soil temperature, plant height, number of leaves, fresh and dry weight, phenol, flavonoids and antioxidant contents, as well as GDD were significantly higher in the 100 days harvest age than 80 days.

Table 4 shows the effects of each treatment on all observed parameters. It can be seen that the treatments are significantly influenced all parameters except the mean soil temperature and plant height. Table 4 also depicts the minimum soil temperature under black plastic mulch at 100 days harvest age was the lowest (18.66 °C) compared to other treatments. Even though the minimum soil temperature at 80 days of harvest age was quite high (21.5 ℃), it did not differ significantly

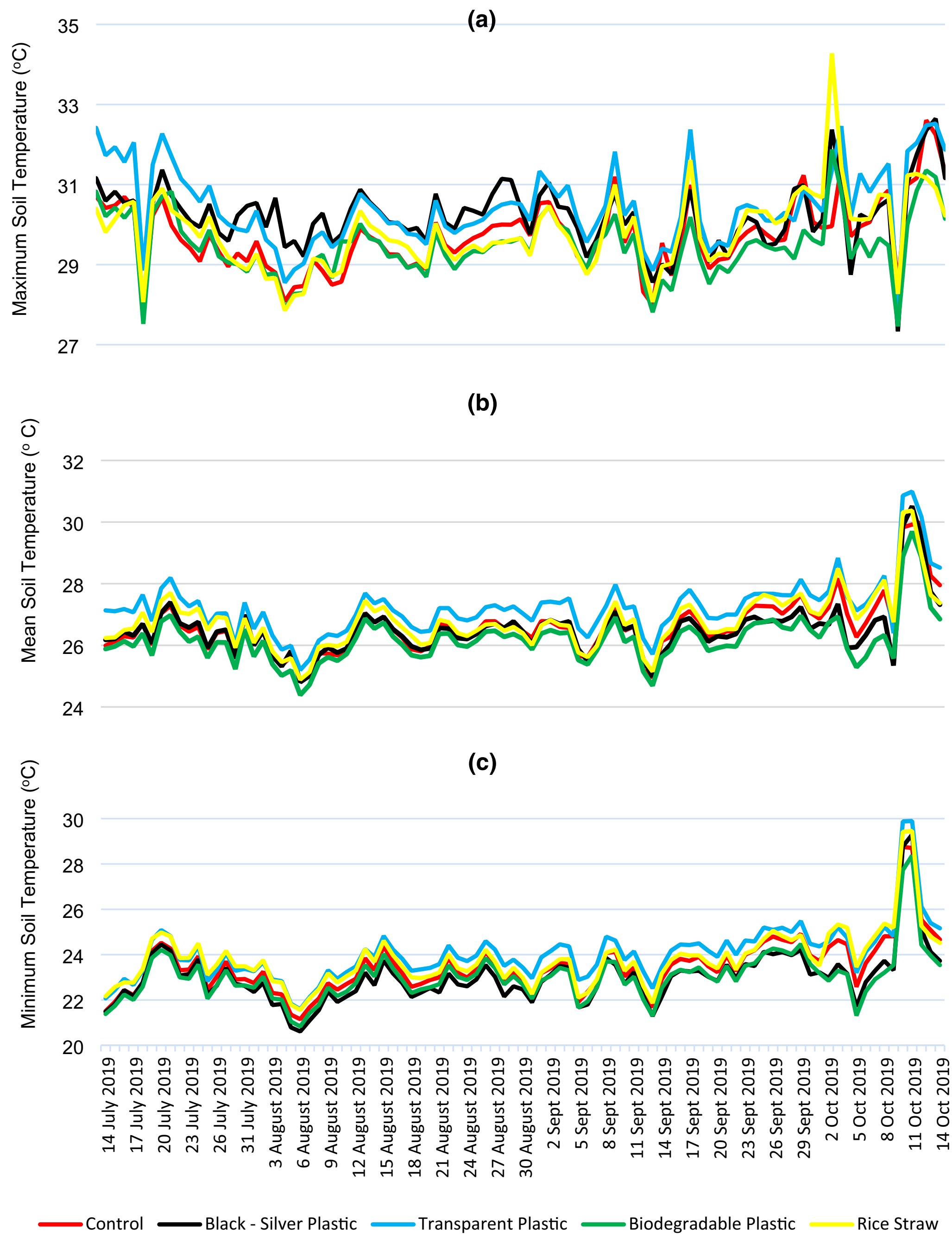

Fig. 1 Dynamics of maximum (**a**)/mean (**b**) and minimum soil temperature under various mulch materials

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 to 21.70 ℃, while in the treatment, the 100 days of harvest age ranged from 18.66 to 21.81 ℃. In general, biodegradable plastic mulch produced the same minimum soil temperature (not significantly different) as the control. From Table 4, 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 age produced

Table 2 ANOVA of variation of mulch nested in harvesting age on each parameter

Parameter	Sig	
	Harvesting age	Variation of mulch (harvesting age)
Minimum soil temperature	< 0.01**	< 0.01**
Mean soil temperature	0.387 ns	0.213 ns
Maximum soil temperature	0.014*	< 0.01**
Plant height	0.706 ns	0.683 ns
Number of leaves	.037*	< 0.01**
Fresh weight	< 0.01**	0.017*
Dry weight	< 0.01**	< 0.01**
Phenol	< 0.01**	0.280 ns
Flavonoids	0.011*	0.380 ns
Antioxidant	0.014*	0.746 ns
GDD	< 0.01**	0.150 ns

α 0.05, *ns* not significant

* = significant; and ** = highly significant

higher GDD (1505.90–1643.25 ℃) compared to 80 days (1293.52–1362.03 ℃).

Overall, Table 4 shows that different mulches combined with harvest age 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 4 also shows 100 days of harvest age resulted in higher phenol content than 80 days. The 100 days harvest age showed higher phenol levels ranging from 308.833–412.500 mg GAE/100 g dry extract compared to the 80 days harvest age (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 4 also confirms that the harvest age has a significant effect on antioxidant. The amount of antioxidant in the 100 days 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 5 shows the Pearson's correlation analysis between agronomic characters and soil temperature with secondary metabolites of Java tea (phenols, flavonoids, and antioxidant). Table 5 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 5 also demonstrates that flavonoids correlated with fresh weight ($P=0.026$) and minimum soil temperature ($P=0.015$), while antioxidant also only correlated with GDD ($P=0.042$).

Table 6 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

Table 3 Significance of harvesting age on the mean of each observed parameter

Parameter	Unit	Harvesting age	
		80 days	100 days
Minimum soil temperature	℃	21.29 ± 0.63 a	20.63 ± 1.24 b
Mean soil temperature	℃	26.65 ± 0.69	26.50 ± 0.75
Maximum soil temperature	℃	35.38 ± 2.92 a	34.12 ± 1.61 b
Plant height	cm	79.71 ± 10.78	80.40 ± 5.73
Number of leaves		487.82 ± 141.83 a	425.45 ± 153.56 b
Fresh weight	Gram	142.88 ± 41.94 a	273.32 ± 79.54 b
Dry weight	Gram	25.91 ± 7.12 a	52.84 ± 21.65 b
Phenol	mg GAE/100 g DW	244.57 ± 42.49 a	368.86 ± 61.53 b
Flavonoids	mg QE/100 g DW	110.13 ± 31.70 a	82.52 ± 19.21 b
Antioxidant	µmol TE/g DW	1940.5 ± 155.79 a	2080.3 ± 107.02 b
GDD	℃	1321.2 ± 56.91 a	1.584 ± 72.13 b

Means followed by the same letter in the same row are not significantly different at $\alpha=0.05$

Table 4 Effects of mulch nested at harvest age on all parameters

Parameter	Unit	Treatment										Sig
		M1(P1)	M2(P1)	M3(P1)	M4(P1)	M5(P1)	M1(P2)	M2(P2)	M3(P2)	M4(P2)	M5(P2)	
Minimum soil temperature	℃	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	℃	26.46	26.85	27.24	26.70	25.70	26.61	26.11	27.11	25.69	26.98	ns
Maximum soil temperature	℃	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	℃	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	ns
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

S significant, *ns* not significant; $\alpha = 0.05$; 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 age 80 days, *P2* harvest age 100 days

Table 5 Pearson's correlation of agronomic characters and soil temperature with secondary metabolites of Java tea

Agronomic characters	Correlation coefficient and *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)**

The confidence level is 95% (α = 0.05); the number in brackets is the P-value; the bold cell means significant correlation

antioxidant contents because the significances (*P*) of the model were higher than α, i.e., 0.132 and 0.732, respectively.

Figure 2 presents the effects of mulch nested with harvest age on phenol and flavonoid levels of Java tea. It can be figured out in general, the 100 days harvest age produced higher phenol content of Java tea (308.833–412.500 mg GAE/100 g dry extract) compared to the 80 days harvest age (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 age shows higher yields (84.633–141.467 mg QE / 100 g dry extract) when compared to 100 days harvest age (64.133–94.967 mg QE/100 g dry extract).

Figure 3 presents the effect of mulch nested with harvest age on soil temperature. From Fig. 3 it can be seen the minimum soil temperature in general at 100 days harvest age shows higher yields (18.65–21.81℃) compared to 80 days harvest age (20.43–21.7 ℃). From Fig. 3 it can also be seen that in general, the mean soil temperature in the 80 days harvest age shows higher yields (25.7–27.24 ℃) compared to 100 days harvest age (25.69–27.12 ℃). Figure 3 also confirms that in general, the maximum

Table 6 Multiple regression analysis to estimate the secondary metabolites of Java tea from soil temperatures

Parameter	Sig			B-coefficients			Model Sig. (p)	*R*	R^2
	T_{min}	T_{mean}	T_{max}	T_{min}	T_{mean}	T_{max}			
Phenol	.189	.355	**.015**	− .058	− .066	− .035	**.015**	.702	.492
Flavonoids	.465	.326	.454	2.359	4.730	-.742	.132	.552	.305
Antioxidant	.641	.831	.540	− 22.503	− 15.043	8.850	.732	.247	.061

Number in the bold means significant at α = 0.05

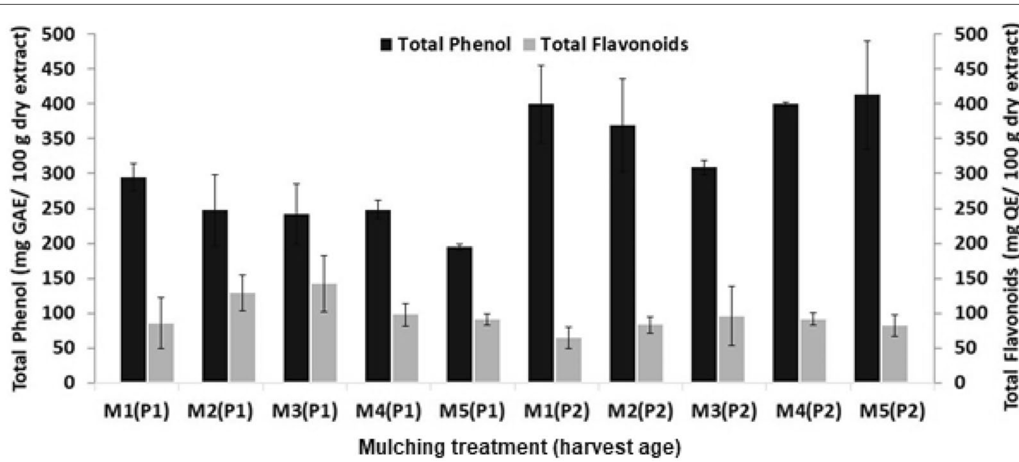

Fig. 2 Effects of soil mulching on phenol and flavonoid of Java tea (*P1* harvest age 80 days, *P2* harvest age 100 days, *M1* control (no mulching), *M2* black plastic mulch, *M3* transparent plastic mulch, *M4* biodegradable plastic mulch, *M5* rice straw mulch; error bars represent standard deviation)

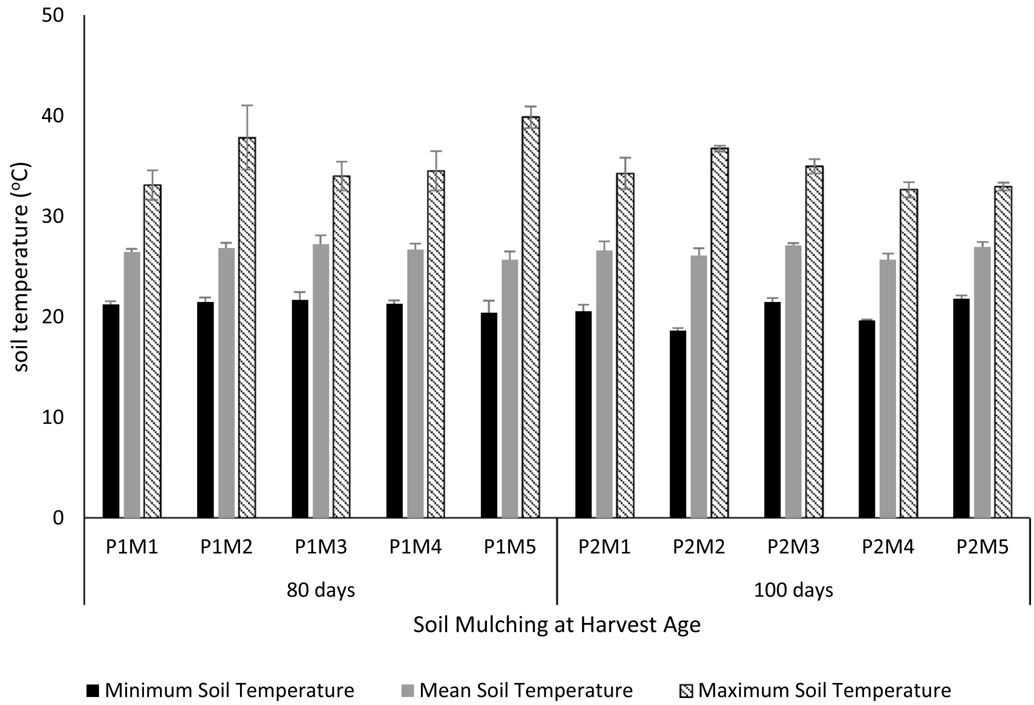

Fig. 3 Effect of mulch nested at harvest age on soil temperature (*P1* harvest age 80 days, *P2* harvest age 100 days; *M1* control (no mulching), *M2* black plastic mulch, *M3* transparent plastic mulch, *M4* biodegradable plastic mulch, *M5* rice straw mulch)

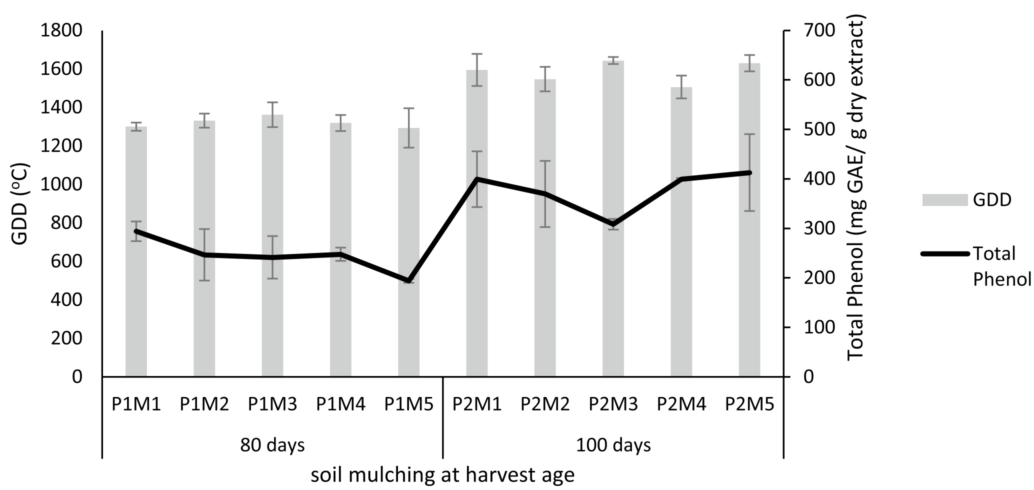

Fig. 4 Effect of mulch nested at harvest age on GDD and phenol content of Java tea (*P1* harvest age 80 days, *P2* harvest age 100 days, *M1* control (no mulching), *M2* black plastic mulch, *M3* transparent plastic mulch, *M4* biodegradable plastic mulch, *M5* rice straw mulch)

soil temperature at the 80-day harvest treatment shows higher yields (33.08–39.81 ℃) compared to the 100 days harvest age (32.63–36.72 ℃).

Figure 4 presents the effect of soil mulching nested with harvest age on GDD and phenol content of Java tea. From Fig. 4 it can be seen GDD affects the phenol content of Java tea. Figure 4 also shows that in general, the 100 days harvest age resulted in a higher phenol content of Java tea (308.833–412.500 mg GAE/100 g dry extract) compared to the 80 days harvest age (193.750–294.128 mg GAE / 100 g dry extract). Figure 5 presents the effects of mulch nested at harvest age on GDD and antioxidant content of

Java tea. Figure 5 demonstrates GDD affected the antioxidant content of Java tea. Figure 5 also shows that in general, the 100 days harvest age resulted in higher levels of antioxidant content (1949.35–2144.41 µmol TE/g dry extract) compared to the 80 days harvest age (1875.59–1998.43 µmol TE/g dry extract).

Discussion

Soil temperatures affected the total phenol due to minimum, mean, and maximum soil temperatures correlated with the total phenol (Table 5). However, the total phenol was more specifically determined by the maximum soil temperature (Table 6). The maximum soil temperature varied according to mulch type (Table 2; $P < 0.01$). The higher maximum soil temperature led to the lower of total phenol content, as shown in Table 5 indicated by the negative correlation ($r = -0.520$). This is in line with research conducted by Aidoo et al. [3] 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 [4]. The responses of plant secondary metabolites such as phenolics, flavonoids, terpenoids, and alkaloids are generated from various biochemical processes by environmental stress [56]. Abiotic stress significantly affects the production of plant secondary metabolites [6].

However, Table 5 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. As seen from Fig. 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 Fig. 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. [57], 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 Kiriyath, 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. [48]. This certainly proved that environmental factors determined the biosynthesis of plant secondary metabolites [54].

However, in general, total phenol was also largely determined by the harvest age. The longer harvest age resulted in higher GDD (Table 5). This means that the higher the GDD, the higher the phenol content (Fig. 4). 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. [49] who found that GDD affected the total phenolic content and antiradical activity in basil, and the phenolic content in the *Sphallerocarpus gracilis* roots [20]. GDD has an effect on increasing the total phenolic content of lettuce in Vulcan and Crispino cultivars [11]. Beans with the Jaki-9281 genotype that accumulated higher GDD also resulted in higher phenol content [7].

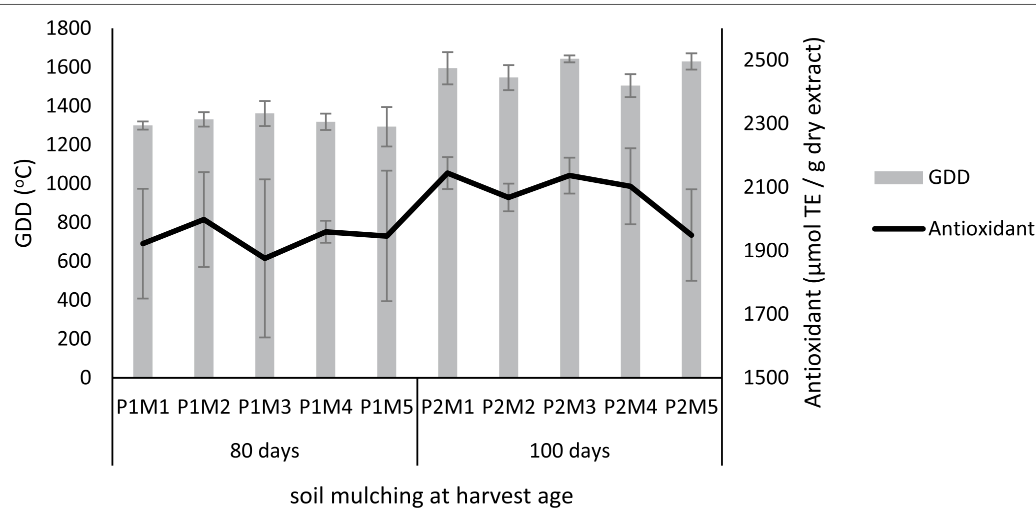

Fig. 5 Effect of mulch nested at harvest age on soil temperature-based GDD and antioxidant content of Java tea (*P1* harvest age 80 days, *P2* harvest age 100 days, *M1* control (no mulching), *M2* black plastic mulch, *M3* transparent plastic mulch, *M4* biodegradable plastic mulch, *M5* rice straw mulch)

The soil temperatures were influenced by the various of mulch (Table 2), where black plastic mulch resulted in the lowest minimum soil temperature (18.66 ℃), in line with Zhang et al. [59] and Yaghi et al. [55] that also found minimum soil temperature under black plastic mulch was lower than that of control and transparent mulch, respectively. The total phenol content produced due to the soil temperature states was opposite to the produced total flavonoid (Fig. 2), whereas the total flavonoids of 100 days were lower than 80 days harvest age. The total flavonoids were regulated by the minimum soil temperature (Table 5), indicated by the positive correlation ($r = 0.523$). It is shown that the warmer minimum soil temperature would enhance the flavonoids content. Meanwhile, the decrease in soil temperatures (maximum, mean and minimum) likely to increase phenol content (significant negative correlation: − 0.453, − 0.460 and − 0.520, respectively). Generally, it can be said that the stress due to high soil temperature resulted in higher flavonoids contents but lower phenol contents. The fresh weight of plants may indicate the stress intensity [26, 33], whereas higher fresh weight indicates lower stress intensity on plant. The response of flavonoids content to fresh weight was positive, while it was negative to phenol content. On the other hand, flavonoids content was bigger under higher minimum soil temperature, while phenol content was bigger under higher all soil temperature properties (minimum, mean and maximum). These results indicate that high minimum soil temperature would be preferable for plant growth and minimize stress, hence the flavonoids would be increased. On the other hand, high soil temperature properties would lead to plant stress, but increase the phenol contents. The elevated phenol content due to abiotic stress have been confirmed by many studies [28, 35, 36, 43, 45], but the elevated flavonoids content due to low level of abiotic stress level was only reported by few studies [1, 21, 27]. However, further investigation on specific response of flavonoids to abiotic stress in Java tea is suggested to confirm the results.

The total flavonoids were also controlled by the harvest age (Table 2), this demonstrates the shorter harvest age may increase the total flavonoids, or longer harvest age decrease the flavonoids content. That is because the concentration of flavonoids decreased in the mature leaves probably due to physiological processes [8]. The lower concentration of linalool compound in the mature leaves were also reported by Thounaojam et al. [52] in *Ocimum*. Similarly, Singh et al. [46] also reported the decrease in oil content in the mature leaves of Indian basil at longer harvest age. This is in line with the research conducted by Zeng et al. [58], who confirmed some flavonoids and flavonoid glycosides decreased by the longer harvest age. Elmastas et al. [16] also found the content of flavonoids and phenolic acids can change according to the harvest time. On the contrary, the longer harvest age may cause several components of antioxidants such as ascorbic acid to decrease [22].

The antioxidant contents were strongly regulated by the soil temperature-based GDD (Table 5), with correlation value (r) = 0.374, meaning the bigger the GDD, the higher the antioxidant content produced. This is confirmed in Fig. 5 where the longer the harvest age, the higher the GDD and antioxidant. GDD, which is the accumulation of the diurnal mean soil temperature differences, is largely determined by the harvest age (Table 2, $P < 0.01$). GDD relates to the accumulation of heat unit or thermal time and energy [34], which involves several physiological processes in plants [38], including antioxidant capacity [12]. This study confirms higher GDD enhanced the antioxidant content due to higher accumulation of thermal time and energy. This is in line with research conducted by Fuchs et al.[19] 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 age [44].

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. This study shows the evidence of the benefits of implementing soil mulching in the Java tea cultivation, hence suggests Indonesia farmers to adopt this technology to enhance the active compounds production. That is because soil mulching can create a suitable microclimate condition that supports secondary metabolites production.

Conclusion

The higher mean soil temperature under transparent plastic mulch resulted in a lower total phenol content of the Java tea, whereas the higher minimum soil temperatures under black plastic mulch produced higher total flavonoids contents. Higher soil temperature-based GDD due to the 100 days harvest age increased the phenol and antioxidant contents than 80 days. Further study is required to confirm a more detailed mechanism regarding soil temperature effects on secondary metabolites regulation.

Abbreviations

GDD: Growing Degree Day; g: Gram; mL: Milliliter; nm: Nanometer; GAE: Gallic acid equivalent; μL: Microliter; QE: Quercetin equivalent; w/v: Weight/volume;

M: Molar; ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TE: Trolox equivalent; °C: Degree Celsius; DW: Dry weight; P1: 80 Days harvest age; P2: 100 Days harvest age; M1: Control (no mulching); M2: Black plastic mulch; M3: Transparent plastic mulch; M4: Biodegradable plastic mulch; M5: Rice straw mulch.

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Authors' contributions
All authors have contributed to the manuscript and the common agreement has been reached before submission. All authors participated in the work in a substantive way and they are prepared to take full public responsibility for their work. All authors read and approved the final manuscript.

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Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate
The authors certify that there were no risks in conducting this research project. We confirm that the research was conducted in line with all national rules, legal and local ethical standards.

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

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