



Original Article

Photosynthesis and Antioxidative Systems of *Andrographis paniculata* as Affected by Compost Tea Rates

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Abstract

Andrographis paniculata (Burm.f.) Nees is a medicinal herb in the family Acanthaceae. The leaves of the mature plant contain abundant diterpenoids of medicinal properties. The objectives of the present study were to investigate the impacts of compost tea rates on photosynthesis parameters and antioxidant reactions of *A. paniculata*. The plant was propagated using seed and planting in a polybag. After four weeks, the plant was transplanted into polybag sized 20 cm×25 cm. Compost tea was used at rates of (0, 25, 50, 75 and 100%). Treatments were arranged in randomized completely block design (RCBD) with three replicates. Before flowering stage, the plants were harvested. The results revealed that rates of compost tea significantly affected total dry weight, leaf area, chlorophyll, stomatal conductance, FRAP and total flavonoid contents (P 0.01), while there were no significant difference on DPPH reduction, and total phenolic contents and photosynthetic rate. Soil amendment with compost tea gave a favorable effect on growth and improved soil pH and CEC. Total flavonoid was highly significantly differences among the treatments. The greatest TDW (26.15 g), FRAP (96.03% of inhibition) and flavonoid (1943.7 mg/g) were observed at the 70% rate of compost tea. The highest rates of compost tea (100%) significantly decreased the phenolic and flavonoid compounds. These results suggested that *A. paniculata* could reach high biomass and quality characteristics with 50 and 70% rate of compost tea. However, in general compost tea had a good potential to improve soil pH and increase plant growth and antioxidant contents.

Keywords: *Andrographis paniculata*, Antioxidant, Compost tea, Photosynthesis.

Introduction

Andrographis paniculata (Burm.f.) Nees (2n=50) known as Naein-e Havandi in Iran, is a medicinal herb commonly known as “King of Bitters” in the family Acanthaceae that rich in active compounds of therapeutic importance. The plant extract contains three major compounds namely diterpenes, flavonoid and stigmaterols with the most active compound identified as andrographolide. The herb exhibited a wide scope of pharmaceutical properties such as anti-HIV [1], anti-H1N1, anticancer, immuno-stimulant [2], antipyretic, anti-inflammatory, and anti-diarrhea [3] and other related diterpenoids constituents including andrographolide, neoandrographolide

and 14-deoxy-11, 12-didehydroandrographolide, neoandrographolide, andrographiside, etc that possess various medicinal properties [4,5]. The plant is an erect annual herb with a dark-green quadrangular stem; lanceolate and pinnate leaves; small and white flowers; linear-oblong capsules; and tiny yellowish brown seeds [6]. It grows abundantly in tropical climates [4].

Compost tea is one of the top organic fertilizers that undergo biotechnological process, which is rich in essential plant nutrients in forms that are readily taken up by the plants [7]. The negative effect of inorganic fertilizers on the environment and their future cost make it useful to assimilate a greater use of organic materials in cultivation practices to improve crops production. There are

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serious efforts worldwide to use organic manures to provide the same amount of food with less fossil fuel-based inorganic fertilizer [8]. Siddiqui *et al.* [9] reported that integrating nutrient management with organic manures and inorganic fertilizers increased yields and chemical constituents in *Centellaasiatica*. The application of compost tea for plant metabolism is becoming popular, and new systems are being developed to meet the requirements of different crops systems. Several studies have reported the efficiency of compost teas obtained from agro-wastes as organic substrate to enhance the growth and yield of *Centellaasiatica* [8].

Addition of organic soil amendments to the soil stimulates microbial activity by increased in population of actinomycetes, algae, bacteria, fungi, and other organisms such as nematodes [10]. Organic amendments contain high proportion of organic matter can decrease the heavy metals bioavailability in the soil and thus helps to improve vegetation on contaminated soil [11,12]. Furthermore, soil amendments were able to be pH dependent. Soluble organic complexes were formed in a high pH situations and it can increase metal solubility [13].

Our understanding of the quantitative effects of compost tea rates on important physiological traits like photosynthesis parameters and antioxidant reactions were not determined for *A. paniculata*. Yield is the direct criterion to assess the compost tea rates, but the physiological responses are complex and need to consider photosynthesis parameters and antioxidant reactions. The objectives of the present study were to investigate the photosynthesis and antioxidant of *A. paniculata* as affected by compost tea rates to determine the optimum level of fertilizer rates required for optimum growth and quality of *A. paniculata*.

Material and Methods

Genetic Material and Germination Conditions

The seeds of *Andrographis paniculata* (Burm.f.) Nees accession No 11314 provided from the Medicinal Plants Research Center, Shahed University, Tehran, Iran. The seeds were surface sterilized with 10% sodium hypochlorite (NaOCl) solution for 10 minutes [14] and thoroughly rinsed with distilled water. The seeds were germinated in Petri dishes containing filter papers moistened with sterile water under controlled growth chamber

(light/dark regime of 14/10h at 28–30 °C, relative humidity 60- 75%). The seedlings at two leaves stage were transferred into the jiffy media.

Experimental Technique

The experiment was carried out as arandomized complete block design (RCBD) with four rates of compost tea (0, 25, 50, 75 and 100%) five and three replicates. The 30-days seedlings were transferred from jiffy media into the pot with different rates of compost tea. Compost tea was prepared described by Naidu *et al.* [15]by preparing commercial compost and water at the ratio of 1:5 (w/v, compost:water) supplemented with microbial starter. Compost teawas applied every two weeks until before flowering of the plant. Before flowering stage, the plants were harvestedand data on total dry weight (TDW), total chlorophyll (T.Chl), net photosynthetic rate (Pn), stomatal conductance (COND), transpirationrate, 2, 2-diphenyl-1-picrylhydrazyl scavenging potential (DPPH), ferric reducing antioxidant power assay (FRAP), total phenol and flavonoid content were measured.

Dry Weight

Leaves, stems and roots were dried up by anoven at 68 °C for 48 hours. The total dry weight refers to total biological yield which includes leaves, stems, and roots.

Chlorophyll Content

Chlorophyll content of leaves was measured using Chlorophyll-Meter-XT-SPAD-502 equipment. Measurements were made on three different leaves that is young leaf, mature leaf and older leaf. The average of three leaves reading was determined. The chlorophyll content was taken in the morning at 8 am to 11 am.

Photosynthetic Parameters

The net-photosynthetic rate (Pn) and stomata conductance (gs) were measured using the Infra-red Gas Analyser (IRGA; Model Portable Photosynthesis System LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). Before measurements, the LI-6400 portable photosynthesis system was calibrated and the measurements were taken at 1000 $\mu\text{molm}^{-2}\text{s}^{-1}$ of photosynthetically active radiation (PAR), 400 $\mu\text{mol/mol}$ carbon dioxide, 30°C leaf temperature, 60% relative humidity with air flow rate was set at 500 cm^3/min [16]. The measurements of gas exchange were carried out between 9 to 11 am from leaf number four from the

shoot tip of plants in each treatment under transparent shade condition. The leaf surfaces were cleaned and dried before being enclosed in the leaf cuvette. Data for net photosynthesis rate ($\mu\text{moles m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{m}^{-2} \text{ s}^{-1}$) and transpiration rate in were simultaneously recorded.

Extraction of Antioxidant Compounds

A total of 0.5 g of fresh leaves of each treatment were cut into small pieces and placed in 150 mL conical flask. 25 mL of distilled water was added and the flask was covered with aluminum foil. The samples in the conical flask were placed in the shaker at room temperature for one hour in the dark. After one hour, the samples were filtered using Whatman No.2 filter paper and the extracts were stored at -80°C freezer.

DPPH Free Radical Scavenging Assay

Forty μL of extract was added to 3 mL of 0.1 mM methanolic DPPH solution. The mixture was incubated in room temperature for 30 minutes and the initial absorbance of DPPH in methanol was measured on a Shimadzu UV-1201 spectrophotometer at 515 nm until the absorbance constant [17].

Ferric reducing antioxidant power assay (FRAP)

Two hundred microliter of extract were added with 3 mL of FRAP reagent that were prepared with mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM Iron (III) Chloride Hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) at 10:1:1 ratio. The mixture was incubated in the water bath for 30 minutes at 37°C [17].

Determination of Total Phenolic Content

Total phenolic content was determined by Marinova *et al.* method [18] using Folin-Ciocalteu reagent. One mL of extract was diluted with 9 mL distilled water. One mL of Folin-Ciocalteu's phenol reagent was added and then 10 mL of the mixture is mixed. Ten mL of 7% Na_2CO_3 were added after 5 minutes. The mixture was diluted to 25 mL with adding 4 mL of distilled water and incubated in room temperature for 90 minutes. After 90 minutes, the absorbance was measured using spectrophotometer at 750 nm.

Determination of Total Flavonoids

The total flavonoid determination was conducted by Marinova *et al.* method [18] using Aluminium Chloride Colorimetric method. First, 1 mL extract was added with 4 mL distilled water in a flask. Then, 0.3 mL of 5% NaNO_2 was added. After 5 minutes, 0.3 mL of 10% AlCl_3 was added and the mixture was diluted to 10 mL by adding 2.4 mL of distilled water. The absorbance of the mixture was measured at 510 nm.

Results

The analysis of variance revealed a significant difference between different rates of compost tea in terms of TDW, chlorophyll, COND, transpiration nrate, FRAP, total phenolic and flavonoid contents in the plants ($P < 0.01$), while there were no significant differences on net photosynthetic rate and DPPH content ($P > 0.05$).

Table 1 Analysis of variance of compost tea rates on photosynthesis and antioxidative systems in *Andrographis paniculata* (Burm.f.) Nees.

| Source of variation | df | Mean square | | | | | | | | |
|---------------------|----|---------------------|--------------------|-------------------|---------------------|--------------------|---------------------|--------------------|---------------------|----------------------|
| | | TDW | T. Chl | Pn | COND | Trans | DPPH | FRAP | T. Phenol | Flavonoid |
| R | 2 | 2.18 ^{ns} | 62.1 [*] | 18.7 [*] | 0.00 ^{ns} | 0.07 ^{ns} | 31.99 [*] | 2.15 ^{ns} | 0.03 ^{ns} | 346.67 ^{**} |
| Compost tea rates | 4 | 31.92 ^{**} | 96.3 ^{**} | 7.0 ^{ns} | 0.003 ^{**} | 0.89 ^{**} | 12.85 ^{ns} | 13.80 [*] | 0.001 ^{**} | 227.07 [*] |
| Error | 8 | 3.21 | 12.2 | 4.2 | 0.000 | 0.07 | 6.50 | 2.53 | 0.000 | 35.67 |

** , * and ns, refer to 1%, 5% and not significant, respectively.

TDW; Total dry weight, T. Chl; Total chlorophyll, Pn; Net photosynthetic rate, COND; Stomatal conductance, Trans; Transpiration rate, DPPH; 2, 2-diphenyl-1-picrylhydrazyl, FRAP; Ferric reducing antioxidant power assay, T. Phenol; Total phenol.

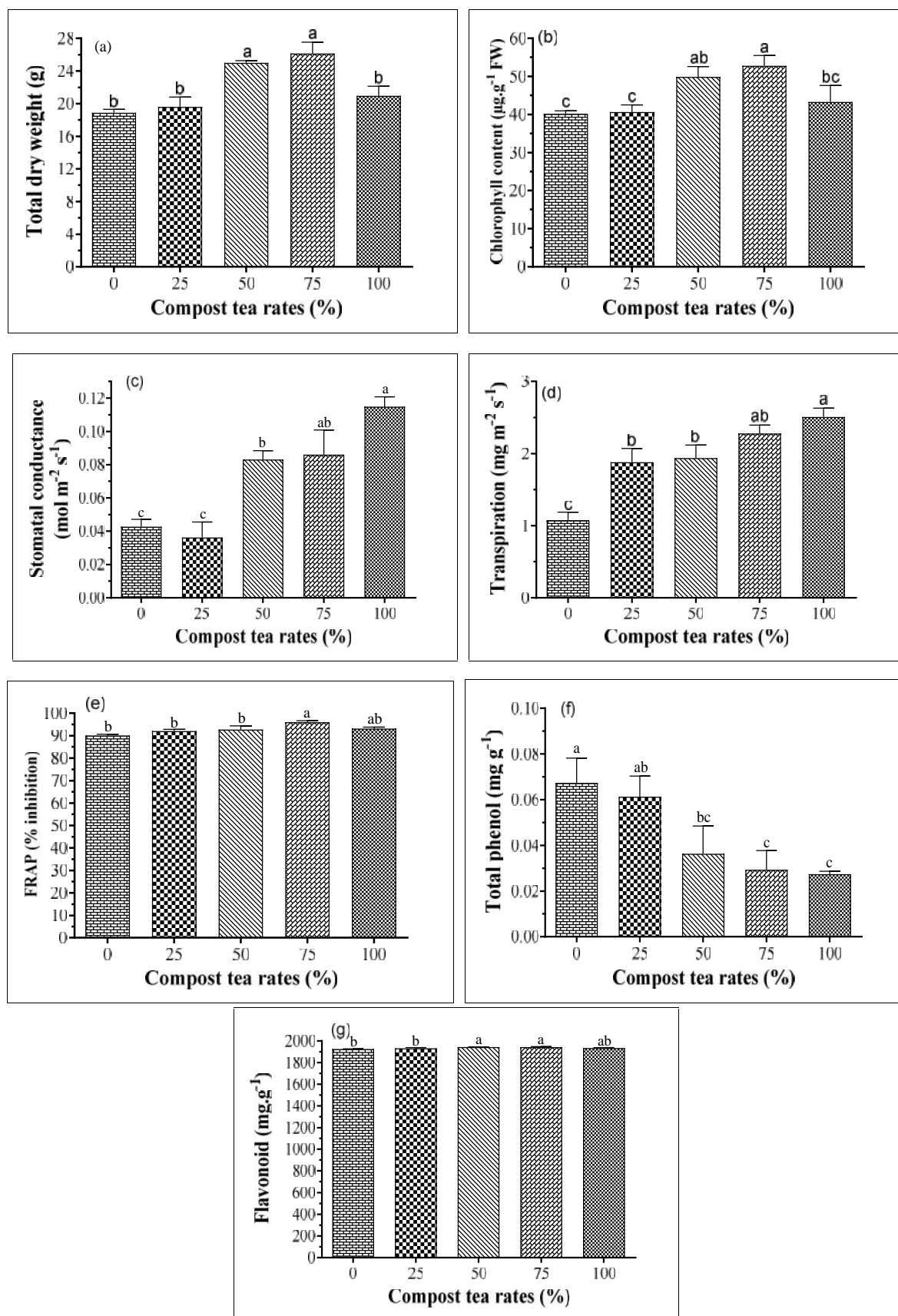


Fig. 1 Comparison of the photosynthesis and antioxidative systems in *Andrographis paniculata* (Burm.f.) Nees under various compost tea rates using Duncan's multiple range test ($P < 0.01$). Different letters indicate the significant difference between the values of pair of treatments. TDW (a), Chlorophyll content (b), Stomatal conductance (c), Transpiration rate (d), FRAP (e), Total Phenol (f) and Flavonoid compounds (g).

The plant reached the lowest (18.89 ± 0.42 g) and highest (26.15 ± 1.40 g) TDW at the 0% and 25% compost tea rate, respectively (Fig. 1a). Chlorophyll variation due to different rates of compost tea was highly significant. The plant at the 75% rate of compost tea reached relatively higher chlorophyll content ($52.80 \pm 2.51 \mu\text{g}\cdot\text{g}^{-1}$ FW), while at the 0% rate of compost tea the chlorophyll content was the lowest ($40.23 \pm 0.74 \mu\text{g}\cdot\text{g}^{-1}$ FW) (Fig. 1b). There were no significant differences among different rates of compost tea in terms of Pn, nevertheless the plant reached the highest Pn ($25.52 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at the 75% rate of compost tea. The results revealed that there was high significant difference among different rates of compost tea in terms of COND. The highest ($0.115 \pm 0.006 \text{ mol m}^{-2} \text{ s}^{-1}$) and lowest ($0.036 \pm 0.009 \text{ mol m}^{-2} \text{ s}^{-1}$) COND were found at the 100% and 25% compost tea rate, respectively (Fig. 1c). The results showed that there was high significant difference among different rates of compost tea in terms of transpiration rate. The plant reached the highest ($2.51 \pm 0.12 \text{ mg m}^{-2} \text{ s}^{-1}$) and the lowest ($1.08 \pm 0.11 \text{ mg m}^{-2} \text{ s}^{-1}$) transpiration rate at the 100% and 0% compost tea rate, respectively (Fig. 1d). There were no significant differences among different rates of compost tea in terms of DPPH, however the plant reached the highest DPPH (8.2%) at the 0% rate of compost tea. The results revealed that there was significant difference among different rates of compost tea in terms of FRAP (P 0.05). The highest ($96.03 \pm 0.04\%$) and lowest ($90.10 \pm 0.56\%$) FRAP were found at the 75% and 0% compost tea rate, respectively (Fig. 1e). The total phenol content among different rates of compost tea significantly varied from ($0.067 \pm 0.01 \text{ mg/g}$) to

($0.027 \pm 0.006 \text{ mg/g}$) at the 0% and 100% compost tea rate, respectively. The results showed that there was significant difference among different rates of compost tea in terms of total flavonoid content. The highest ($1943.67 \pm 3.71 \text{ mg/g}$) and the lowest ($1923.00 \pm 4.93 \text{ mg/g}$) total flavonoid content were obtained at the 75% and 0% compost tea rate, respectively (Fig. 1f) (P 0.05). The highest rates of compost tea (100%) significantly decreased the phenolic and flavonoid compounds.

The correlation among most of the measured traits was significant at P 0.01 (Table 2 and Fig. 2). The correlation between total dry weight and total chlorophyll as well as net photosynthetic rate, FRAP and flavonoid were positively significant, while a negative correlation was determined between total phenol content and stomatal conductance as well as FRAP. The highest positive correlation ($r=0.794$) was found between total chlorophyll and flavonoid content, and the lowest positive correlation was found between net photosynthetic rate and DPPH ($r=0.002$).

Discussion

The basic purpose of organic fertilizers is to improve the soil quality by amending it with nutrients it may lack. Organic fertilizers provide nutrients in a usable form, which will help improve plant growth. Compost teas as plant organic matter due to the presence of essential micro and macronutrients and other bioactive compounds are reported for enhancing plant growth by improving the physical, chemical and biological properties of the soil and nutrients content [19,20,21].

Table 2 Correlation coefficient (r) among measured traits of *Andrographis paniculata* (Burm.f.) Nees under compost tea treatments.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-------------------|---------|---------|---------|---------|--------|--------|---------|--------|---|
| (1) TDW | 1 | - | - | - | - | - | - | - | - |
| (2) Chl | 0.719** | 1 | - | - | - | - | - | - | - |
| (3) Pn | 0.528* | 0.673** | 1 | - | - | - | - | - | - |
| (4) COND | 0.478 | 0.428 | 0.032 | 1 | - | - | - | - | - |
| (5) Transpiration | 0.507 | 0.432 | 0.084 | 0.644** | 1 | - | - | - | - |
| (6) DPPH | -0.232 | -0.062 | 0.002 | -0.254 | -0.371 | 1 | - | - | - |
| (7) FRAP | 0.672** | 0.513 | 0.086 | 0.420 | 0.600* | -0.236 | 1 | - | - |
| (8) Phenol | -0.491 | -0.312 | 0.203 | -0.637* | -0.486 | 0.169 | -0.554* | 1 | - |
| (9) Flavonoid | 0.680** | 0.794** | 0.756** | 0.303 | 0.487 | 0.100 | 0.432 | -0.317 | 1 |

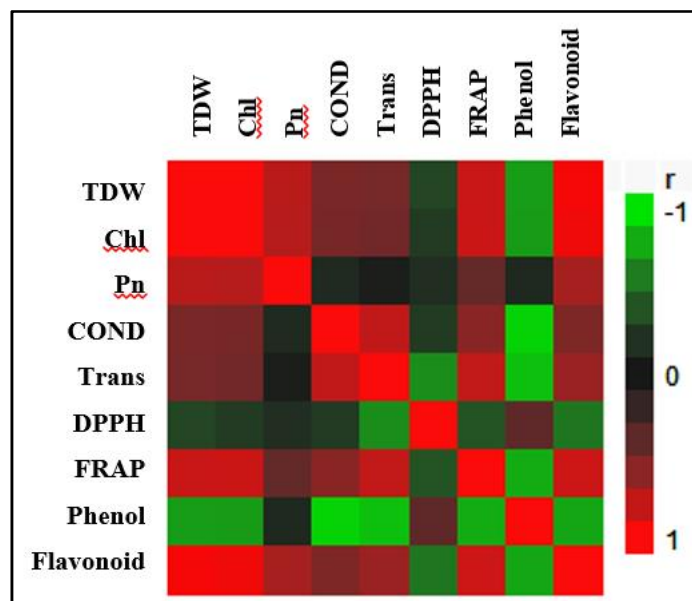


Fig. 2 Correlations between measured traits of *Andrographis paniculata* (Burm.f.) Nees under compost tea treatments. The strength and direction of the correlations among the different traits are indicated by the color (red indicates positive correlations while green indicates negative correlations, and the shading represents the strength of the correlation).

Furthermore, the favorable effect of compost tea may be related to the good equilibrium of nutrients and water in the root medium [22] or the valuable effects of microbes on vital hormones and enzymes in the plant growth cycle [20]. It has been reported that use of compost tea or biofertilisers has led to an increase in growth indices and macronutrient content of *Plantago arenaria* plant [23]. In this study, applying compost tea increased plant growth and gave significantly high yield. This increase might be related to the positive effect of compost tea and micro organisms in increasing the root surface area per unit of soil volume, water use efficiency and photosynthetic activity, which directly affect physiological processes. In agreement with the reports of Gayler *et al.* [24] on Juvenile trees, Siddiqui *et al.* [21] on *Abelmoschus esculentus* and Yasmeen *et al.* [25] on *Centella asiatica*, the findings of this study indicated that the use of compost tea noticeably increased the yield, photosynthetic parameters and antioxidant contents stomatal conductance of the plant. This is a remarkable finding of the study. In particular, the availability of key macronutrients in organic manures during plant growth has significant potential to increase antioxidant accumulation and produce secondary metabolites in some plants [26]. The application of compost and microorganisms was also demonstrated to significantly improve growth and essential oil production in *Rosmarinus officinalis* [22]. The

positive relationships between total dry weight and antioxidant content and photosynthetic parameters were observed in the present study. The results of studies on a number of plants also confirm that the yield and production of bioactive components in medicinal plants were influenced by the sufficient supply of nutrients [9,21,24].

Conclusion

The plant growth, chlorophyll content, net photosynthesis rate and antioxidant compounds were poor at the extreme rate of compost tea (100%). In general compost tea had a good potential to increase plant growth and antioxidant contents. The highest plant growth and antioxidant contents were reached at the 75% rate of compost tea. The content of the pharmacologically active components in medicinal plants is influenced both genetically and environmentally, depending on cultivation conditions. Thus, cultivation conditions can be optimised to obtain maximum pharmacologically active components. The findings of this study suggest that application and management of compost tea could be an effective procedure to increase herbage yields as well as bioactive components in the cultivation of *Andrographis paniculata*. However, the production of bioactive components of the plant is depending on the combination of beneficial microbes, micro

and macronutrients and chemical compounds in compost tea.

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