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Effects of treatments on production of useful natural products of *Pelargonium graveolens* L'Hér.

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ABSTRACT

We evaluated the stimulatory effects of aqueous extracts of *Posidonia oceanica* L. Delile (Aquatic Plant), *Ulva rigida* C. Agardh (Algae), coffee spent residues and conidial suspensions of two isolates of *Trichoderma* spp. (A and B) to increase its plant biomass, methanolic extract yield, phenolic compounds content and the antioxidant power in *Pelargonium graveolens* L'Hér. The aqueous extracts and conidial suspensions were applied in irrigation water to plants of *Pelargonium graveolens* during vegetative growth (autumn) and flowering (spring). All applied treatments increased the vegetative biomass of plants, content of chlorophyll, carotenoids and phenolic compounds (including flavonoids, flavonols and condensed tannins), and their antioxidant power except isolate (A) of *Trichoderma sp*. The highest IC_{50} values (0. 35 mg/mL and 0.40 mg/mL) were recorded for plants treated with *P. oceanica* L. and *U. rigida* C. extracts, respectively. These values were calculated at the vegetative stage in autumn, which remained low compared to that of ascorbic acid (0.09 mg/mL). The infrared spectroscopic analysis of *P. graveolens* L. dry weight showed the presence of chemical groups characteristic to phenolic compounds and the transmittance values of the spectra revealed a better absorption of the radiation in plant samples treated with the above mentioned extracts. These results confirm the presence of higher content of phenolic compounds responsible for increased antioxidant activity compared to the other treatments. The bio-stimulatory effect of these treatments can be recommended as biological inputs for industrial-scale production of *P. graveolens* L.

Key words: Antioxidant activity, bio-stimulants, coffee, *Pelargonium graveolens* L'Hér, plant biomass, *Posidonia oceanica*, secondary metabolites, *Ulva rigida*

INTRODUCTION

Pelargonium graveolens L'Hér. (family Geraniaceae, Figure 1) native from Cape, South Africa and was introduced in Algeria in 1850 to extract its essential oil for use in the cosmetics and perfumes (13). It is rich in secondary metabolites, hence, used in food, cosmetics, pharmaceuticals, agriculture as insecticide and veterinary as antioxidants, antimicrobials and antitumors. Its essential oil is in great demand in perfume industry. Many studies have focussed on the antioxidant activity of this oil, but little work was done to identify the phenolic compounds, flavonoids and tannins (5).

Checkouri *et al*. (4) described *Pelargonium graveolens* L. among 8-medicinal plants (*Aphloia theiformis*, *Ayapana triplinervis*, *Dodonaea viscosa*, *Hubertia ambavilla*, *Hypericum lanceolatum*, *Psiloxylon mauritianum* and *Syzygium cumini*) mentioned in the French Pharmacopeia. These plants possess very high antioxidant activity.

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The conidial suspension of *Trichoderma* and *P. oceanica* L. leaf extracts are stimulatory to tomato, potato and strawberry (15). The coffee residues (left after coffee preparation) stimulates the lettuce growth and increased its phenolic contents (3). The application of seaweed extracts increased the vegetative biomass in *P. graveolens* (22).

Figure 1. *Pelargonium graveolens* L'Hér, (A) the vegetative stage and (B) the flowering stage.

This study aimed to optimize the vegetative biomass, pigments and phenolic content and antioxidant activity of *P. graveolens* L. using treatments [conidial suspensions of two *Trichoderma* spp (A & B), aqueous extracts of coffee residues, *U. Rigida and P. oceanica* L. These treatments were based on availability and ecofriendly nature

MATERIALS AND METHODS

Pelargonium graveolens L. plants were grown from cuttings in field from mid-March 2018 to end April 2019 in a field at EHEV Staoueli, west Algiers (Altitude: 36 m, Latitude : 36.75, Longitude: 2.89 36 ° 45 ′ 21 ″ North, 2 ° 53 ′ 25 ″ East). During study period, the mean temp was 18° C, rainfall: 605 mm and 446 mm in 2018 and 2019.

The cuttings of healthy 6-month-old *Pelargonium graveolens* L. plants were provided by Horticultural and Green Space Company (EHEV), Staoueli, Algiers. These were planted in Multi-cells plates containing sterilized soil + peat (1:1 ratio) in greenhouse. From these plants after 45 days, cuttings were used for transplanting in the experimental plot of 20 m x 60 m asper scheme of Eiasu et *al*. (7). The plot was divided into 4 Blocks (i.e. 4 replicates), each block was further divided into 7-Sub-blocks (4.2m x 3m) one for each treatment. Each sub-block (1.5 m. apart) had 4 rows 1.0 m apart, plant to plant spacing was 60 cm, each with 8 plants. Irrigation was done by drip system.

Experimental treatments

The 7-experimental treatments were T1: NPK fertilizer (20:20:20), T2: Conidial suspension of *Trichoderma sp* (A), T3: Conidial suspension of *Trichoderma sp* (B) (15), T4: Aqueous extracts of coffee residues (left after coffee preparation), T5: *U. rigida* (Algae) extract, T6: *P. oceanica* L (Sea plant). extract and C: Control (Untreated) (Table 1).

Table 1. Experimental treatments

Code	Treatment	State	Source
C	Control		
T1	Fertilizer NPK ***	Liquid	EHEV Staoueli
T2	*Trichoderma sp* (A)	Conidial suspension	Mycology lab. National Plant Protection Research Institute, Blida city (20)
T3	*Trichoderma sp* (B)		Mycology lab. National Plant Protection Research Institute, Blida city (20)
T4	Coffee spent residue	Aqueous extract	Cafeterias in Koléa.Tipaza city
T5	*Ulva rigida* *	Aqueous leaf extract	Fouka Marine Beach,Tipaza city
T6	*Posidonia oceanica***		Cherchell Beach, Tipaza city

*: Algae, **: Sea plant, Control : No treatment applied, ***: Applied at 100 Kg/ha

Aqueous Extracts of coffee residues, *U. rigida* and *P. oceanica*: These were prepared from 100 g air-dried material of each source (T4 to T6), boiled separately in 1.0 L water for 1.0 h and filtered through fine mesh cloth.

Conidial suspensions of *Trichoderma*: These were prepared by adding sterile distilled water to 15-days-old pure cultures of each isolate (grown on PDA medium and incubated at 28 °C). The suspensions were collected separately in sterile test tubes and the conidia were counted by Malassez cell under a light microscope at magnification (X125). The resulted concentrations were adjusted with sterile distilled water to 10^7 spores ml^{-1}.

All treatments were sprayed with 250 ml extract(s) or water (control) per plant, 15 days after transplanting (April 2018). Afterwards, treatment were applied 6-times at interval of 15 days. All treatments were compared with NPK fertilizer (20:20:20) applied at 100 Kg/ha and the control plants (No treatment). The plants were harvested twice, after 7-months in Autumn (during vegetative phase, late October 2018) and thereafter 5-months in Spring (during flowering phase, April 2019.) The same treatments were applied 15 days after the first harvest in October 2018.

Growth parameters

The plant biomass was evaluated by measuring the length and number of stems, area and number of leaves and dry weight of plants in vegetative phase. The number of umbels and flowers, main roots fresh weight and roots length were recorded in flowering stage.

Plant physiological and biochemical parameters

I. Leaf pigments content

The leaf pigments were extracted, 50 mg fresh leaves were finely cut and homogenized in 0.5 ml of 80 % acetone and centrifuged for 3 min at 3000 rpm. The absorbance of supernatants was measured using a UV-VIS spectrophotometer. The chlorophyll and carotenoid contents were calculated by the formulae of Lichtenthaler (8):

$$Chl_a \ (mg.g^{-1}\ fw) = 12.25\ A_{663.2} - 2.79\ A_{646.8}$$
$$Chl_b \ (mg.g^{-1}\ fw) = 21.50\ A_{646.8} - 5.10\ A_{663.2}$$
$$Chl_{a+b} \ (mg.g^{-1}\ fw) = 7.15\ A_{663.2} + 18.71\ A_{646.8}$$
$$C_{x+c} \ (mg.g^{-1}\ fw) = (1000\ A_{470} - 1.82\ _{Chla} - 85.02\ _{Chlb})/198.$$

II. Methanolic extracts

The methanolic extracts were prepared by taking separately 20 g leaf powder in shaded flasks containing 200 mL of 80 % methanol. After maceration, the extracts were filtered and evaporated under vacuum at 40 °C using a rotary evaporator. The flasks were subjected to rotary magnetic stirrer at room temperature for 72 h. The resulting dry extract was stored at 4 °C (9) until use. The yield of extract was calculated as under:

Yield (%) = [weight of extract (g) / weight of plant material (g)] x 100.

III. Phenolic compounds

The contents of total polyphenols, total flavonoids, total flavonols and condensed tannins were determined at vegetative and flowering stages, from air dried leaves, ground into powder and stored in brown glass bottles to protect from light and moisture.

(i). Total polyphenols: The content of total polyphenols was determined using Folin-Ciocalteu method (10). Extract sample (0.2 mL) was mixed with 1 mL of 10-folds diluted Folin-Ciocalteu reagent and 0.2 mL of 7.5 % Na_2CO_3, incubated for 30 min at room temperature. Absorbance was recorded at 765 nm with a UV-VIS spectrophotometer. The concentration of the sample was calculated from the equation of gallic acid calibration curve and the results were expressed as mg gallic acid equivalent per g dry weight (mg GAE/g dw).

(ii). Total flavonoids: The total flavonoids content was determined by mixing 0.5 mL extract with 0.5 mL 2 % $AlCl_3$ in ethanol. After 1.0 h incubation at room temperature, the absorbance was measured at 420 nm. The sample concentration was calculated from the equation of the quercetin calibration curve and the results were expressed as mg quercetin equivalent per g dry weight (mg EQ /g dw) (18).

(iii). Total flavonol : The total flavonol content was determined by mixing 2 mL extract with 2 mL of 2 % $AlCl_3$ in ethanol and 3 mL of $C_2H_3NaO_2$ (50 g/L). After 1.0 h incubation at 20 ^{0}C, the absorbance was taken at 440 nm. The sample concentration was calculated from the quercetin calibration curve and the results were expressed as mg quercetin equivalent per g dry weight (mg QE/g dw) (11).

(iv). Total condensed tannins: The total condensed tannins (proanthocyanidins) content was determined by mixing0.5 mL of the extract with 3 mL of 4% vanillin-methanol solution and 1.5 mL HCl. After incubation for 15 min at room temperature, the absorbance was recorded at 500 nm. The sample concentration was calculated from a catechin calibration curve and the results were expressed as mg catechin equivalent per g dry weight (mg CE/g dw) (11).

IV. Antioxidant activity

Antioxidant activity was determined by mixing 0.2 mL of methanolic extract at various concentrations (0.2, 0.4, 0.6, 0.8, 1 mg/mL) with 1.8 mL of methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.04 mg/mL). After incubation for 30 min in dark at room temperature, the Absorbance was measured against a blank at 517 nm. The results were expressed as inhibition (%) of DPPH radical asper the following formula of Dzamic *et al* (12):

Inhibition percentage (%) = $(\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}) \times 100 / \text{Absorbance}_{\text{blank}}$

Antioxidant activity was evaluated against ascorbic acid as standard. The IC_{50} values were expressed in mg/mL and determined graphically by linear regression.

V. Spectroscopic analysis by FTIR

The dry samples of *P. graveolens* L. harvested at the vegetative stage had the highest content of phenolic compounds; thus, spectroscopic analysis by FTIR of these samples was done using the potassium bromide (KBr) pellet technique. Dry sample (1 mg) was ground with 75 mg of KBr and then pelletized by pressuring for 8 min in hydraulic press. The sample holder containing the pellet was placed in the measuring chamber. All IR spectra were recorded in the infrared region (4000-400 cm^{-1}) using the Opus 6.5 software of the spectrometer (Bruker Tensor 27 FTIR). The allocation of functional groups was done as per the correlation table of infrared spectroscopy in the literature.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Significance of the differences among means was evaluated by Tukey's test using the MINITAB software vers.19, with $P \leq 0.05$ (20). The results are presented as mean ± standard deviation of four replicates.

RESULTS

Biomass

The application of aqueous extracts of *P. oceanica* L., *U. rigida* C. and ground coffee residues and conidial suspension of *Trichoderma sp*. (B) significantly increased the vegetative biomass of *P. graveolens* L. than control and fertilizer. These treatments also increased the length and number of stems, number of leaves, leaf area and aerial dry weight at both phenological stages (Vegetative and flowering). The number of umbels and flower fresh weight as well as the length of roots, at the flowering stage, were increased by these treatments (Tables 2 and 3). The alphabetical classification in groups was done for the statistical analysis of Data. Extracts of *P. oceanica* L. and *U. rigida* C. were the best stimulators. But, the conidial suspension of *Trichoderma sp*. (A) was not stimulatory.

Table 2. Effect of the treatments on biomass of *P. graveolens* L. at vegetative stage.

Code	Length stems (cm)	Number of stems	Number of leaves	Leaf area (cm^2)	Dry weight (kg)
C	84.06 ± 2.94 c	10.04 ± 1.12 b	1849.67 ± 7.14 d	50.56 ± 4.54 bc	0.57 ± 0.01 d
T1	88.60 ± 6.71bc	10.76 ± 1.20 ab	1895.95 ± 6.39 c	53.54 ± 3.73 ab	0.73 ± 0.04 c
T2	76.41 ± 5.69 d	09.35 ± 1.03 bc	1835.12 ± 7.55 e	44.83 ± 2.11 c	0.54 ± 0.03 de
T3	91.51 ± 3.82 bc	10.76 ± 1.12 ab	1894.71 ± 7.19 c	53.11 ± 3.55 ab	0.76 ± 0.01 bc
T4	95.28 ± 6.86 ab	11.13 ± 1.23 ab	1919.28 ± 6.98 b	54.78 ± 3.24 ab	0.79 ± 0.01 b
T5	101.94 ± 4.06 a	11.84 ± 0.95 ab	1939.33 ± 5.03ab	57.77 ± 3.50 ab	0.88 ± 0.01 ab
T6	104.10 ± 5.94 a	13.06 ± 1.08 a	1954.28 ± 5.45 a	60.66 ± 3.31 a	0.91 ± 0.03 a

C: Control A, T1: fertilizer NPK, T2: *Trichoderma* (A), T3: *Trichoderma* (B),
T4: Coffee residues extract, T5: *U.rigida* extract*, T6: *P.oceanica* extract**
Values followed by the same letter in the same column do not differ significantly (P = 0.05).

Table 3. Effects of treatments on growth parameters of *P. graveolens* L. at flowering stage.

Code	Length stems (cm)	Number of stems	Number of leaves	Leaf area (cm²)	Dry weight (kg)	Number of umbels/plant	Number of flowers/umbel	Fresh root weight (g)	Main root length (cm)
C	57.70 ± 2.37 c	08.69 ± 0.87 bc	756.24 ± 8.64 e	48.35 ± 3.67 bc	0.28 ± 0.01 c	28.96 ± 1.40 bc	05.95 ± 0.48 bc	550.24 ± 9.77 f	50.67 ± 0.90 b
T1	63.26 ± 4.23 bc	09.75 ± 0.70 b	884.42 ± 8.35 d	52.01 ± 4.31 abc	0.32 ± 0.02 bc	33.52 ± 2.70 b	06.46 ± 0.41 b	885.37 ± 9.73 a	56.80 ± 0.53 a
T2	54.50 ± 3.36 d	08.22 ± 0.78 c	690.79 ± 9.48 f	43.89 ± 3.79 c	0.26 ± 0.02 c	24.66 ± 1.92 c	05.47 ± 0.56 d	505.79 ± 7.45 g	47.17 ± 0.62 c
T3	64.12 ± 4.66 bc	10.09 ± 0.94 ab	883.03 ± 8.33 d	52.22 ± 3.11 abc	0.35 ± 0.04 b	33.78 ± 1.92 b	06.51 ± 0.25 b	678.45 ± 6.74 e	57.65 ± 0.59 a
T4	66.89 ± 4.82 b	10.24 ± 1.14 ab	907.16 ± 9.55 c	53.58 ± 3.42 ab	0.36 ± 0.03 b	33.77 ± 1.92 b	06.56 ± 0.50 b	740.16 ± 6.29 d	58.07 ± 0.57 a
T5	74.24 ± 2.42 a	10.75 ± 0.60 ab	965.92 ± 6.41 b	56.24 ± 3.85 ab	0.40 ± 0.03 ab	36.01 ± 1.90 ab	07.14 ± 0.34 ab	777.53 ± 8.61 c	56.77 ± 0.89 a
T6	75.46 ± 1.94 a	11.61 ± 0.98 a	996.07 ± 7.98 a	60.24 ± 3.65 a	0.44 ± 0.02 a	39.07 ± 2.04 a	07.92 ± 0.36 a	820.09 ± 8.61 b	57.87 ± 0.83 a

C: Control A, T1: fertilizer NPK, T2: *Trichoderma* (A), T3: *Trichoderma* (B), T4: Coffee residues extract, T5: *U.rigida* extract*, T6: *P.oceanica* extract**
Values followed by the same letter in the same column do not differ significantly (P = 0.05).
*: Algae, **: Sea plant, Control : No treatment applied,

Leaf pigments content

Application of aqueous extracts significantly increased the total chlorophyll (Chl a+b) and the total carotenoid contents of *P. graveolens* L. than control at both phenological stages (Figure 2). On the other hand, the conidial suspension of *Trichoderma sp*. **(**A**)** recorded slightly lower values than the control.

The total chlorophyll contents ($Chl_a + Chl_b$) were 12.45, 10.27, 2.97 and 2.11 $mg.g^{-1}fw$ in plants treated with aqueous extracts of *P. oceanica* and *U. rigida* C, fertilizer and control plants, respectively during the flowering stage. However, the total carotenoid contents with these treatments were 5.38, 4.83, 1.6 and 1.3 mg. $g^{-1}fw$. The contents of foliar pigments at the two phenological stages were similar.

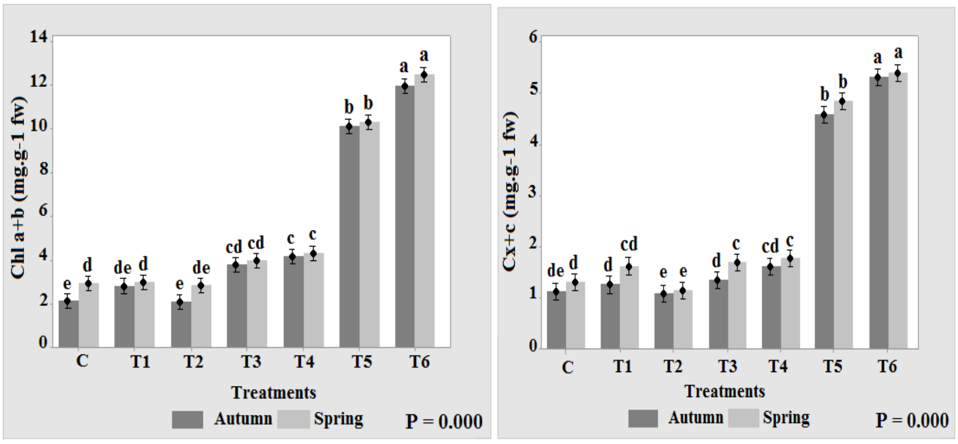


Figure 2. Effects of treatments on leaf pigments at vegetative and flowering stages of *P. graveolens* L.

Methanolic extracts yield

The applied treatments significantly increased the yield of methanolic extracts from *P. graveolens* L. (Figure 3). The higher yield of methanolic extract was recorded for plants treated with *P. oceanica* L. (14.77 %) and *U. rigida* C. (12.35 %) extracts compared with control (8.78 %) and fertilizer (8.71 %). However, the conidial suspension of *Trichoderma sp*. (A) gave lower yield than control (7.67 %). The higher yields were obtained at the vegetative stage in autumn.

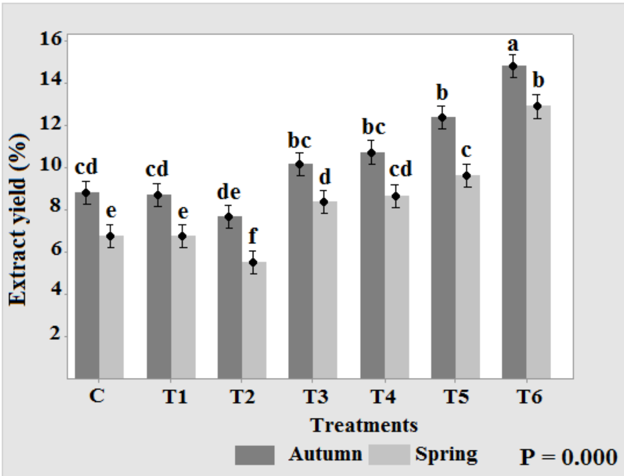
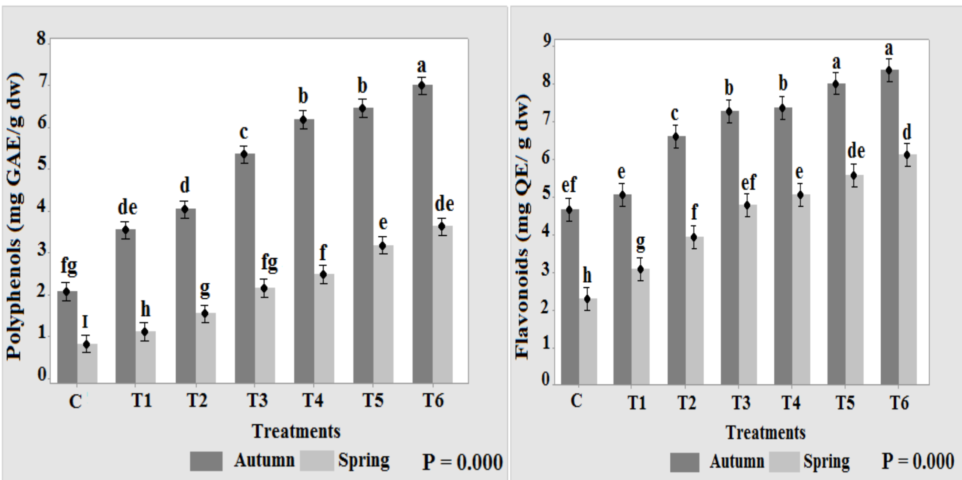


Figure 3. Effects of treatments on the yield of methanolic extracts during the vegetative and flowering stages of *P. graveolens* L.

Phenolic contents

All treatments including the conidial suspension of *Trichoderma sp.* (A) increased the contents of total polyphenols, total flavonoids, total flavonols and condensed tannins (Figure 4) over control and fertilizer at both phenological stages. The higher phenolic contents were recorded at the vegetative stage in autumn. Flavonoids were the most abundant compounds. Their highest contents were found in plants treated with aqueous extracts from *P. oceanica* (8.34 mg GAE/g dw) and *U. rigida* C. (8.0 mg SAG/g dw) than fertilizer (5.05 mg SAG/g dw) and control (4.65 mg SAG/g dw).



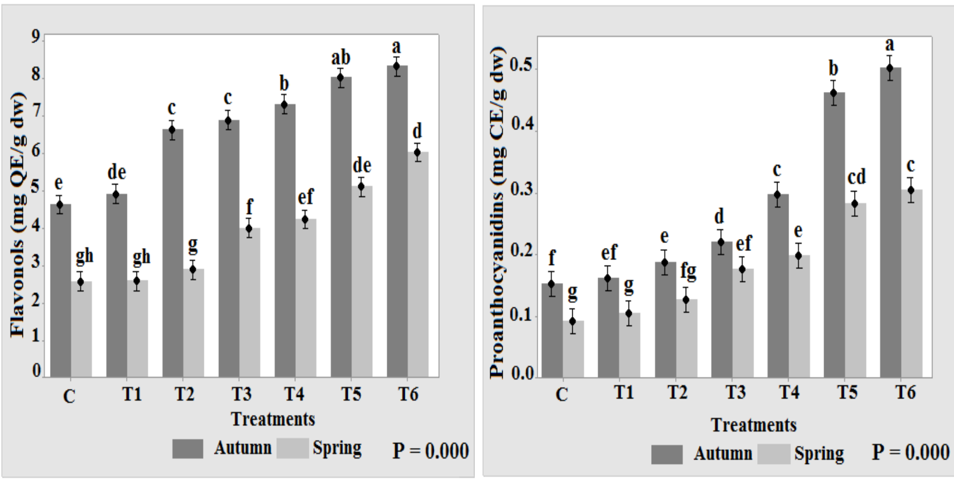


Figure 4. Effects of treatments on phenolic contents at the vegetative and flowering stages of *P. graveolens* L.

Antioxidant activity

The methanolic extract of *P. graveolens* L. leaves had high antioxidant activity (Figure 5). The IC_{50} values showed significant variability (P=0.00) between the treatments at both phenological stages. The plants treated with aqueous extracts of *P. oceanica* had highest IC_{50} values (0.35 mg/mL) and *U. rigida* C. (0.40 mg/mL) than control (0.73 mg/mL) and fertilizer (0.70 mg/mL), these also had highest phenolic contents at the vegetative stage in autumn. However, the ascorbic acid used as standard had IC_{50} value of 0.09 mg/mL.

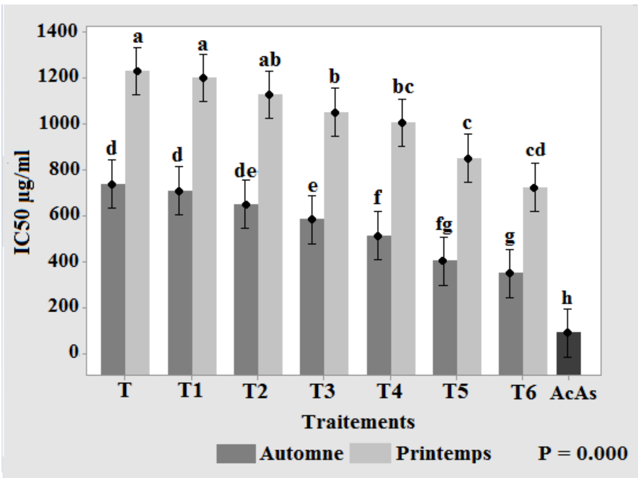


Figure 5. Effects of treatments on IC_{50} of antioxidant activity of methanolic extracts from *P. graveolens* L. in different seasons.

FTIR analysis

FTIR offers quantitative and qualitative analysis of organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument to detect the functional groups and characterizing the covalent bonding. FTIR spectroscopic analysis of 7-samples in the infrared region (4000-400 cm^{-1}) showed 14-bands (Table 4) for each spectrum due to the vibrations of different chemical groups (Figures 6 to 12). Generally, the spectra consist of several bands arising from the vibrations of different groups viz., proteins, lipids, carbohydrates, phenols and nucleic acids, indicating that the leaves are rich in biochemical compounds.

Band 1 (3800-3600 cm^{-1}) is characteristic of O–H elongation vibrations of hydroxyl group due to the presence of free alcohol (27). Band 2 (3400-3200 cm^{-1}) signifies the O–H and N–H elongation vibrations of bond alcohol from phenolic compounds (23) and aliphatic primary amine from proteins and polysaccharides (28). Besides, this association is more specifically attributed to tannins with their interaction with polysaccharides and proteins (21). Band 3 (2935-2845 cm^{-1}) represents the symmetrical and asymmetrical C–H elongation vibrations of methylene (CH_2) which originates from lipids, carbohydrates and nucleic acids. The same vibrations were present in band 8 (1470-1370 cm^{-1}) characteristic of methyl (CH_3) due to lipids, flavonoids and aromatic rings (16). Band 4 (2400-2000 cm^{-1}) is attributed to the O=C=O elongation vibration of carbon dioxide (9). Band 5 (1750-1650 cm^{-1}) denotes C=O elongation vibration of flavonoids and their derivatives (23) as well as the vibrations of the amide group I and II of proteins, which is done with a weak coupling of band 6 (1650-1550 cm^{-1}) of N–H bending and band 10 (1280-1150 cm^{-1}) which implies C–N elongation vibrations (28).

Band 7 (1615-1450 cm^{-1}) is attributed to the elongation vibrations of C=C double bond due to aromatic compounds (8), flavonoids, condensed tannins and amino acids (21). Band 9 (1350-1260 cm^{-1}) signifies O–H elongation vibrations of primary and secondary alcohols. Band 11 (1200-1050 cm^{-1}) shows C–O elongation vibrations of primary and secondary alcohols (17) and condensed tannins (21). Band 12 (900-670 cm^{-1}) reveals C–H deformation vibrations of the aromatic compounds. Band 13 (700-600 cm^{-1}) and 14 (600-500 cm^{-1}), respectively characterize the elongation vibrations of bromo-aliphatic and aliphatic iodinated alkyl halide compounds, respectively (17).

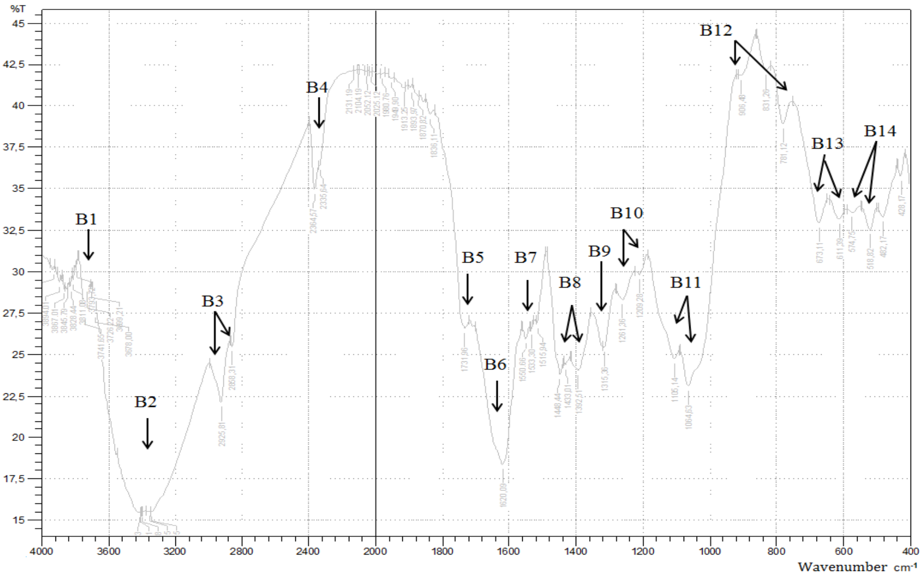


Figure 6. The infrared spectrum of the control of *P. graveolens* L., B- Band

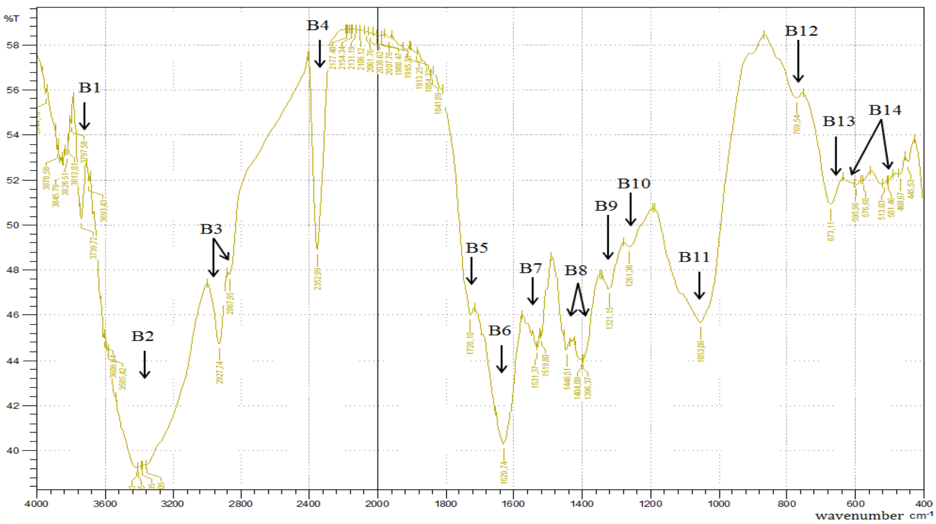


Figure 7. The infrared spectrum of the fertilizer control of *P. graveolens* L, B- Band

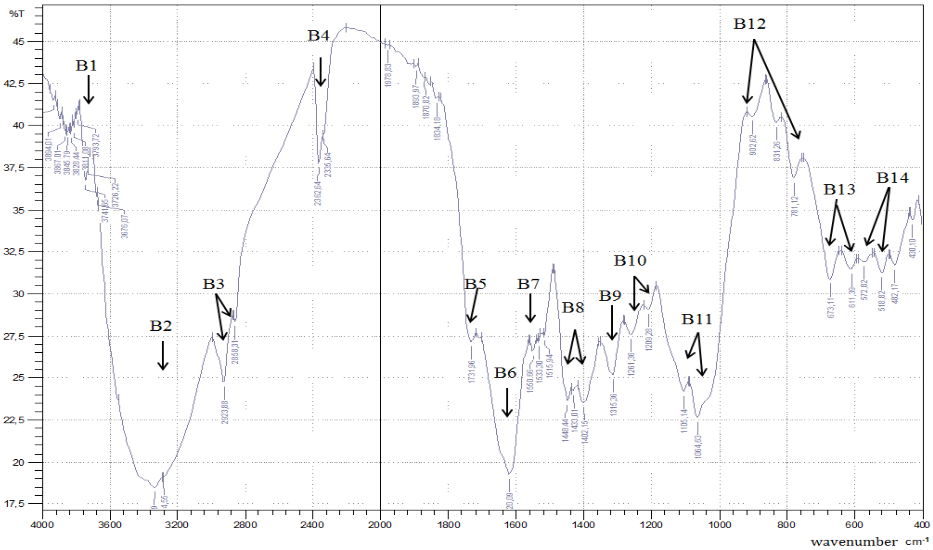


Figure 8. The infrared spectrum of *P. graveolens* L. sprayed with conidial suspension of *Trichoderma* (A), B- Band.

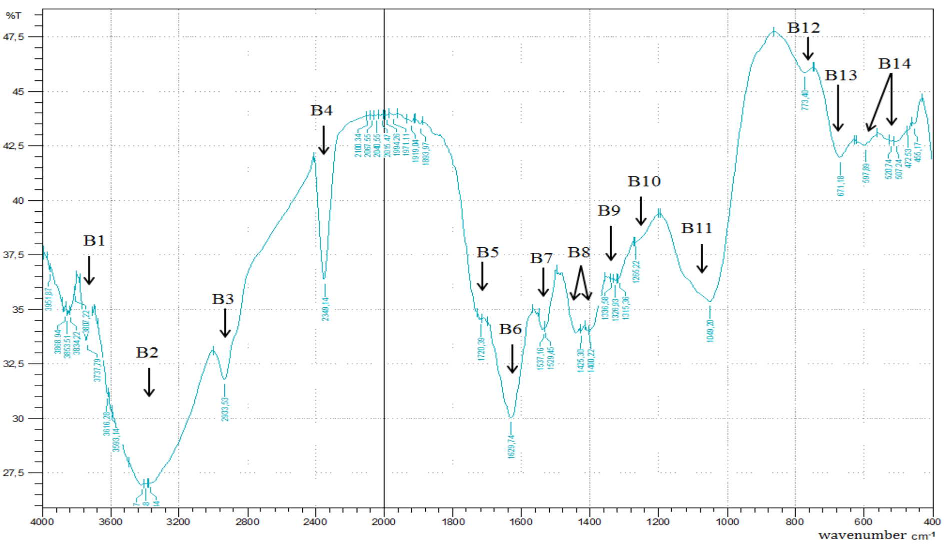


Figure 9. The infrared spectrum of *P. graveolens* L. sprayed with conidial suspension of *Trichoderma* (B), B- Band.

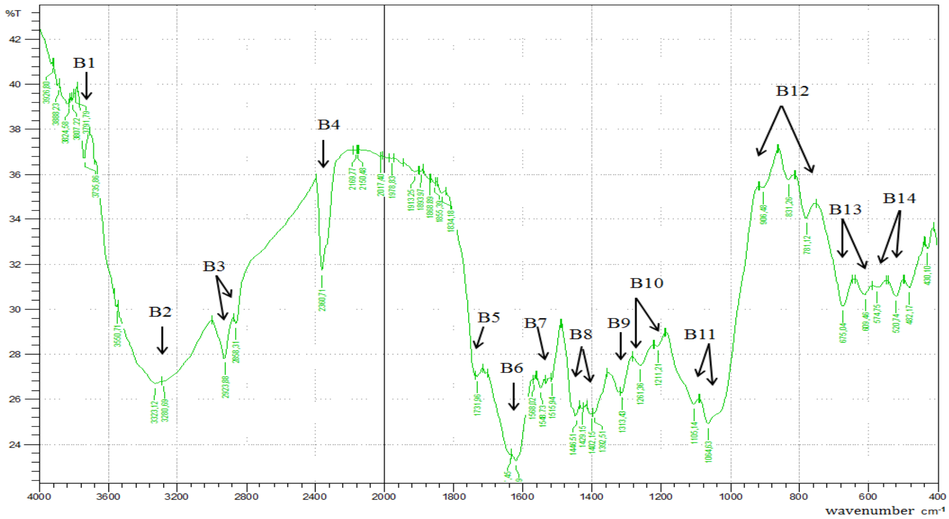


Figure 10. The infrared spectrum of *P. graveolens* L. sprayed with coffee residues extract, B- Band.

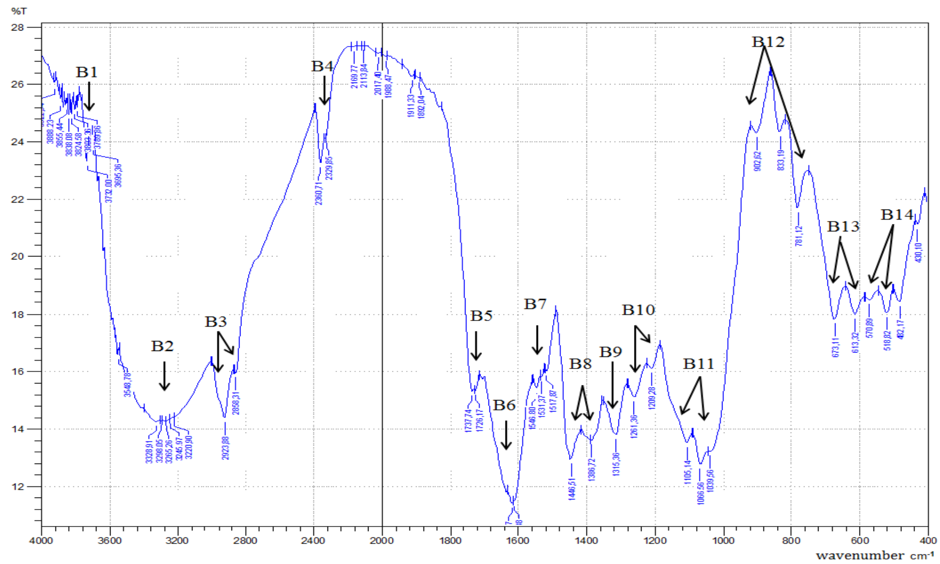


Figure 11. The infrared spectrum of *P. graveolens* L. sprayed with *U. rigida extract*, B-Band.

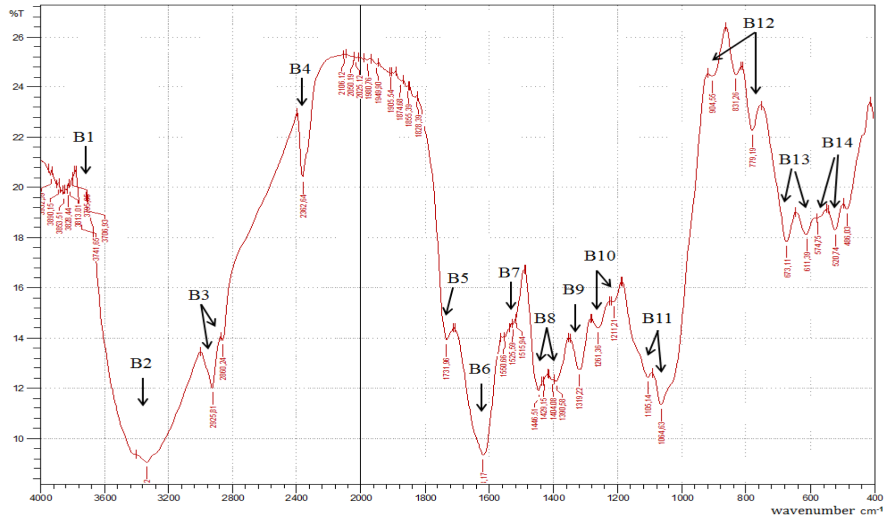


Figure 12. The infrared spectrum of *P. graveolens.* sprayed with *P. oceanica extract*.

This variability did not show change in the allocation of functional groups of the samples. This variability was observed in the 7 spectra in the elongation vibrations OH-NH (Band 2), C-H (Band 3 and 8), O=C=O (Band 4) and C-I (Band 14). However, the spectral similarity was observed in the O-H (Band 1) elongation vibrations for the control, the samples were grown with the conidial suspension of the (A) isolate of *Trichoderma* sp. and the aqueous extract prepared from *P. oceanica*. The C=O elongation (band 5) was for the control, treatments such as *Trichoderma* sp. (A) isolate, the extract of coffee residues and *P. oceanica*. The N-H elongation (Band 6), C=C (Band 7) and C-Br (Band 13) were for the control and (A) isolate. While, O-H elongation (Band 9) was for the control, the (A) isolate and the extract of *U. rigida*. The C-N elongation (Band 10) was for the control, the (A) isolate, the extracts of coffee residues and *U. rigida*. The C-O elongation (Band 11) was for the control, the (A) isolate, the extract of coffee residues and that of *P. oceanica* L. The O-H deformation (Band 12) was for the control and the extract of coffee residues.

There was variability in the band absorption intensity across the spectra. The spectrum of (A) isolate was similar to control, unlike the other spectra in terms of peak and transmittance values. The lowest transmittance was recorded in the spectrum of plants treated with the extract from *P. oceanica*, followed by *U. rigida*, indicating better absorption of radiation. This explains their high levels of phenolic compounds, which provides better antioxidant activity than other treatments, which is consistent with results of our previous research.

DISCUSSION

These results showed that spraying of *P. graveolens* plants with conidial suspensions of *Trichoderma* spp. improved its vegetative growth, which might be due to the presence of cytokinins, proteins, volatile organic compounds with activity similar to auxins (21). The stimulatory effects of coffee residues extract on *P. graveolens* L. could be attributed to its contents of nitrogen, carbohydrates, cellulose, hemicellulose, lipids, proteins and phenolic compounds. The coffee residues were used as cultivation substrate. Its application stimulated the plants growth and increased the contents of phenolic compounds (5).

The application of algal extracts increased the vegetative biomass of *P. graveolens*. These result are in harmony with (6). Also, an increase of total polyphenol was reported in wheat, after treatment with sea weeds and the increase in growth biomass was attributed to their contents of proteins, carbohydrates, lipids, polysaccharides, pigments, vitamins, coenzymes, mineral elements, polyphenol and growth hormones present in *U. rigida* (22).

Few studies have been done on the role of *P. oceanica* in agriculture as bio-stimulant. These are applied as compost in lettuce, basil, melons and tomato plants to increase their growth and yield, due to its richness in mineral elements, lipids, proteins, carbohydrates and phenolic compounds (23). The phenolic contents of *P. graveolens* varies with the geographical distribution and the type of solvent used. This variability was reported by other researchers **(**24)**,** who showed that the phenolics yield of *P. graveolens* extract in Tunisia was 17.25 % using methanol, 14.25 % with ethyl acetate and 5.70 % for hexane. Thus, increase in the phenolic compounds in *P. graveolen* with treatments makes it suitable for pharmaceutical, nutritional, cosmetics as source of antioxidants.

The antioxidant activity of *P. graveolens* methanolic extract was measured by the electron donation ability of its antioxidant constituents in the extract under test, to bleach the purple-coloured solution of DPPH radical. The degree of colour change was proportional to the concentration and potency of its antioxidants. The decrease in the absorbance of the reaction mixture indicated significant DPPH radical scavenging activity of plant extracts **(**12**).** The higher inhibition (%) and higher contents of polyphenols, flavonoids, flavonol and tannins revealed their roles as antioxidants in *P. graveolen*.

The infrared analysis results showed spectral variability characterized by a shift between the spectra in terms of band positions with disappearance or appearance of new peaks and the absorption intensity was compared to control. Nevertheless, this variability did not change the allocation of functional groups of samples. The observed lowest transmittance values recorded in the spectrum of treated plants with extracts from *P. oceanica* L. and *U. rigida* indicated better absorption of radiation. This probably explains their high contents of phenolic compounds, which provides better antioxidant activity than other treatments. The intensity of absorption band (transmittance) provides information about the concentration of its characteristic chemical group (13). The similarity of infrared spectra indicated the similarity in chemical composition. Differences in the band shapes and absorption intensities of the spectra can be explained by the variability in the profile of phenolic compounds or their concentrations (25) and/or the change in protein, lipid and phospholipid concentrations of the samples (15).

CONCLUSIONS

This study confirmed that treatments based on natural products optimized the production of biomass, yield of methanolic extracts, leaf pigments, phenolic compounds and antioxidant activity of *P. graveolens* L. Aqueous extracts of *P. oceanica* and *U. rigida* were most stimulatory to growth, biomass and the antioxidant activity. FTIR analysis, proved that the presence of phenolic compounds was responsible for this antioxidant activity.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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PUBLISHER NOTE