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CHEMICAL COMPOSITION OF ESSENTIAL OILS AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF TWO ENDEMIC PLANTS FROM ALGERIA

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Keywords:

Lavandula antineae; *Thymus algeriensis*; Essential oils; phenolics; flavonoids; the antioxidant activities

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ABSTRACT: Essential oils extracted from two Algerian endemic plants *Lavandula antineae* and *Thymus algeriensis* were analyzed by the GC, the results showed the presence of a large amount of oxygenated sesquiterpenes in both species. Total phenolics and flavonoids content were determined according to the Folin-Ciocalteu method and the aluminum trichloride method, the antioxidant activities of two types of extracts (ethyl acetate and n-butanol) from the plants were tested with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The rates of total phenolics and flavonoids present in *L. antineae* equal to 2,013± 0,009 mg GAE / g of dry plant and 0.587± 0,003 mg QER / g of dry plant material respectively. *Thymus algeriensis* has presented values of phenolics and flavonoids equal to1.337± 0,001 mg GAE / g and 0.339± 0,001 mg QER / g of the dry plant. The ethyl acetate extract of *L. antineae* have presented the higher EC50 value (0047mg/ml).

INTRODUCTION: The use of plants as food and medicinal remedies since ancient times is partially attributed to the biological efficacy of secondary metabolites that possess antioxidant activities such as phenolic compounds, Vitamins C and E, and carotenoids. Phenolic compounds constitute the most abundant class of antioxidants with an estimated total dietary intake as high as 1 g/day, which is 10 times higher than the intake of vitamin C and 100 times that of Vitamin E [1].

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The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects [2].

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 1, 1-Diphenyl-2- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [3]. Two algerian endemic plants from the Lamiaceae family are chosen in this study: *Lavandula antineae* witch is used by locals as an

antiseptic, bacteriostatic, sedative, vulnerary, anti cold and anti rheumatism and *Thymus algeriensis*, known since ancient times by its medical properties as a diuretic, mucolytic, analgesic, vulnerary, as a tonic lung, liver and spleen, digestive disorders, in the treatment of superficial wounds [4].

We conducted our study in the analysis of the chemical composition of the essential oils, determination of total phenolics and flavonoids and test the antioxidant activity of extracts (ethyl acetate and n-butanol) from the two plants by the DPPH assay.

MATERALS AND METHODS:

Plant Material: The identification of *Lavandula antineae* was conducted in the center of scientific and technical research in arid regions (CRSTRA) of Biskra-Algeria and *Thymus algeriensis* species was identified in the national park of Bellazma (Batana-Algeria). The harvesting of desert lavender has been done at the flowering period (end of February to the beginning of April), *Thymus algeriensis* sample was harvested in the tow moths of March and April. The aerial parts (flowers, leaves and stems) were dried outdoors in the shade for later extraction of essential oils and flavonoids.

Extraction of essential oils: According to the protocol described by Mesplede (2004) [5] and Rodrigues and *al.* (2012) [6], 150 g of the dry plant undergoes steam distillation during 3 hours, the organic phase is extracted with di ethyl ether, and the essential oil is recovered after evaporation of the solvent using a rotary evaporator.

Analysis of the Chemical Composition of the Essential Oils by GC: Determining the chemical composition of essential oils was performed by gas chromatography (GC).

Principe: In GC, the sample is vaporized at the inlet of a column containing a solid or liquid active substance called stationary phase, then it is conveyed therethrough by means of a carrier gas. The different molecules of the mixture will separate out of the column depending on the affinity to the stationary phase [7].

Operating –Conditions: GC analyzes were performed using a Perkin Elmer Autosystem instrument, equipped with a divider injector, two columns (50 x 0.22 mm id, film thickness: 0.25 microns) nonpolar (BP -1, polymethylsiloxane), polar (BP-20, polyethylene glycol) and two flame ionization detectors. The operating conditions are: pressure helium carrier gas 20 psi column head; temperature of the injector and detector 250°C; temperature program: 60 to 220 °C (80 min) at 2°C/min, with a bearing 20 min at 220 °C; 1/60 split injection mode.

Identification of compounds:
The identification of compounds in each essential oil is based:

- On the comparison o f their retention index (Ir), determined relative to reference compounds of retention indices.

Preparation of Methanolic Extracts: A leaf powder 2.5g of each test sample was macerated in 25 ml 80% methanol. The extract was then stored at 4°C for 24 h, filtered and the solvent evaporated to dryness under reduced pressure at 50°C using a rotary evaporator [8].

Extraction of Flavonoids: The extraction method was made by organic solvents according to Hossain and *al.* (2013) [9], Bakht and *al.* (2014) [10], Andersen and Markham (2005) [11] and Macheix (2005) [12]. Methanolic maceration (2 L) is made from 200 g of the dry powder of the arial parts of *Lavandula antineae* and *Thymus algeriensis.* The extracts were concentrated on a rotavapor at 40 °C under a reduced pressure. The residue is taken up with boiling water, defatted with chloroform then exhausted successively with hexane, ethyl acetate and n-butanol.

Determination of Total Phenolic Content: The determination of total phenolic was made according to the method of Folin Ciocalteu described by Djeridane (2006) [13]: A volume of 100 µl of the crude methanolic extract of the plant is introduced into the test tubes, the mixture (500 µl of reagent of Folin Ciocalteu diluted 10 times and 1000 µl of distilled water) is added, stirred and incubated for 1 min at room temperature. After 1 min in 1500 µl of sodium carbonate 20% was added. The tubes are stirred and kept for 1 hour at room temperature in the dark. The absorbance is measured at 765 nm against a blank using a spectrophotometer.

A standard curve is performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed in milligrams (mg) gallic acid equivalent per gram of the dried plant material (mg GAE / g).

Determination of Flavonoids Content: The total flavonoids were assayed using the protocol described by Zheizen (1999) [14]: 500 µl of the methanolic extract is mixed with 1500 µl distilled water followed by 150 µl of sodium nitrite 5 % at 5 min 150 µl of aluminum trichloride at 10% is added to the mixture after 6 min of incubation at room temperature, 500 µl of sodium hydroxide at 4% is added immediately, the mixture is thoroughly stirred in order to homogenize the content, the absorbance of the solution pinkish color was determined at 510 nm against a blank using a spectrophotometer. A standard curve is performed in parallel under the same operating conditions using quercetin as a positive control. The results are expressed in milligrams (mg) of quercetin equivalent per gram of the dry plant material.

Antioxidant Activity:

Scavenging free radical DPPH test: The antioxidant test was performed with the DPPH radical scavenging method, DPPH is a stable radical, with a strong absorption maximum at 517nm (purple color) in the UV spectrum. In the presence of an antioxidant which acts as a hydrogen donor, DPPH radical is reduced to 1,1-diphenyl-2-picrylhydrazyl by accepting an electron from the antioxidant and accompanied by loss of purple color. 50µl of each extract (ethyl acetate and n-butanol) at various concentrations (0.0125 to 5 mg / ml) were added to 2 ml of the methanol solution of DPPH (0,025g/l).

In parallel, a negative control was prepared by mixing 50 µl of methanol with 2 ml of the methanol solution of DPPH. The absorbance reading is made against a blank prepared for each concentration at 515nm after 30 min of incubation in the dark at room temperature. The positive control is represented by a standard solution of an antioxidant; ascorbic acid, the absorbance was measured in the same conditions as the samples for each concentration and the test is repeated 3 times. The results were expressed as percent inhibition (I %).

I% = [(Abs control - Abs test) / Abs control] x 100.

The EC_{50} (the effective concentration of the antioxidant extract necessary for the trapping of the 50% mole of DPPH) values were determined graphically by linear regression[15].

RESULTS AND DISCUSSION:

Yield and Chemical Composition of the Essential Oils: The essential oil of *L. antineae* extracted by the method of hydrodistillation was a pale yellow color and has represented a yield of 0.1%.

The chemical analysis of essential oil of *L. antineae* showed 11 compounds representing 95.73% of eluted components (**Table 1**). The chromatographic profile showed that the oil is rich in oxygenated sesquiterpenes with a percentage of 38.22% as thymol (15.33%), caryophyllene oxide (10.46%), Spathulenol (7.41%), Hanamanthagouda and *al.* (2010) [16] found values of thymol and caryophyllene oxide, which equals 2.35% and 3.68% respectively in the essential oil of *L.bipinnata*. Pala-Paul and *al.* (2004) [17] obtained a value of Spathulenol equal to 2.6% in Lavandula canariensis essential oil which are a small values comparatively with ours. The hydrocarbon sesquiterpenes represented a percentage of 32.65% as beta-bisabolene (24.36%) and caryophyllene (6.9%).

The essential oil of *Thymus algeriensis* obtained was a pale yellow color and in yield of 1.54%. Chemical analysis of the essential oil of *T. algeriensis* revealed 30 compounds representing 99.96% of the total composition of this essential oil (**Table 2**) with the predominance of oxygenated sesquiterpenes (40.32%) as elemol (18.38) and the β -eudesmol (11.50%), followed by a considerable percentage of oxygenated monoterpenes such as camphor (14.22%) and the hydrocarbon sesquiterpenes (23.61%) as alpha-caryophyllene (9.68%).

A study reported by Ben Ali Elhadj and *al.* (2012) [18], done on the essential oils of eight samples of northern Africa of *T. algeriensis* species, found that β-eudesmol has a percentage ranging from 0.1 to 0.9%, the camphor takes values from 0.2% to 12.7 % and considerable value in thymol that can reach

54.9% in some samples. El Ouariachi and *al.* (2014) [19] marked the absence of thymol in the essential oil of the Moroccan *T. algeriensis*. The essential oil yield difference can be explained by climatic factors such as drought or heat stress that can affect photosynthesis in plants that will react by increasing the production of secondary metabolites [20]. The variability in the chemical composition of essential oils can be caused by biotic and abiotic factors which act on the genes that code the biosynthetic pathways terpenes [21, 22, 20].

TABLE 1: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *LAVANDULA ANTINEAE*

S. no	Name of the compound	Retention time	Area (%)
1	2,4,6-Octatriene, 2,6-dimethyl	19.211	1.06
2	Thymol	25.127	15.33
3	Caryophyllene	29.531	6.90
4	Isocaryophillene	30.131	1.39
5	beta-bisabolene	31.901	24.36
6	Eudesma-3,7(11)-diene	33.255	1.08
7	Spathulenol	34.337	7.41
8	Caryophyllene oxide	34.577	10.46
9	tau-Cadinol	36.025	2.06
10	alpha-Cadinol	36.438	1.88
11	Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-	41.124	23.80

TABLE 2: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *THYMUS ALGERIENSIS*

S. no	Name of the compound	Retention time	Area (%)
1	Eucalyptol	16.043	2.84
2	Linalol	18.154	1.39
3	Camphor	20.154	14.22
4	Borneol	20.832	6.44
5	Carvomenthenol	21.170	1.04
6	p-menth-1-en-8-ol	21.581	2.11
7	beta-Myrcene	23.511	0.79
8	Thymol	24.746	0.70
9	Bornyl acetate	24.862	2.41
10	Terpinyl acetate	26.867	1.50
11	Copaene	27.995	0.61
12	alpha-Bourbonene	28.370	1.77
13	Gurjunene	29.150	0.75
14	alpha-Caryophyllene	29.518	9.68
15	beta-Cubebene	29.757	1.00
16	Germacrene D	30.247	0.67
17	alpha-Bisabolene	30.559	0.63
18	Gurjunene isomer	30.827	0.86
19	Germacrene isomer	31.397	4.55
20	τ-Cadinene	32.322	0.88
21	δ-Cadinene	32.499	2.21
22	Elemol	33.288	18.38
23	Caryophyllene oxide	34.564	3.51
24	Cubenol	35.339	1.57
25	τ-Eudesmol	35.820	2.19
26	Cadinol	36.019	2.23
27	β-Eudesmol	36.451	11.50
28	Phthalic acid, mono-(2-ethylhexyl) ester isomer	36.902	1.34
29	Phthalic acid, mono-(2-ethylhexyl) ester	37.006	1.25
30	Isoaromadendrene epoxide	37.376	0.94

Content of total phenolic and flavonoids: The total phenolic was estimated by the Folin Ciocalteu colorimetric method, based on the protocol of Djeridane (2006) [13], using gallic acid as standard. A

linear calibration curve was plotted from gallic acid concentrations ranging from 0.1 to 1.2 mg / ml, with a value of r^2 = 0.996. The total phenolic composition was expressed by mg of gallic acid equivalents per g of dried plant material (**Fig. 1**). The rate of total phenolic present in *L. antineae* equal to 2,013± 0,009 mg GAE / g of dry plant, Gülcin and *al.* (2004) [23] found a yield of phenolic compounds equivalent to a value of 226.76 mg from 25g of an ethanolic extract of *L. stoechas*, a rate of polyphenols which equal to 3.78 mg/g was estimated by Costa and *al.* (2013) [24] from an extraction with an ethanol-water mixture of phenolic compounds of *L. viridis*. The rate of total polyphenols presents in *T. algeriensis* equal to 1.337± 0,001 mg GAE / g of dry plant.

The flavonoids assay was performed by the colorimetric method using the aluminum trichloride (Zheizen, 1999) [14], a linear calibration curve was established using quercetin as standard at different concentrations ranging from 0.01 to 0.25 mg / ml (**Fig. 2**) with r^2 = 0.996, flavonoids rate was determinated at a value of 0.587± 0,003 mg QER / g dry plant material for *L. antineae* and with a rate equal to 0.339± 0,001 mg QER / g of the plant material dried to *T. algeriensis*. Zeghad and merghem (2013) [25] found a polyphenol rate, equal to 9.07 mg / g of tannic acid in the ethanol extract of *T. vulgaris* and flavonoids a rate equivalent to a value of 8.56 mg quercetin / g.

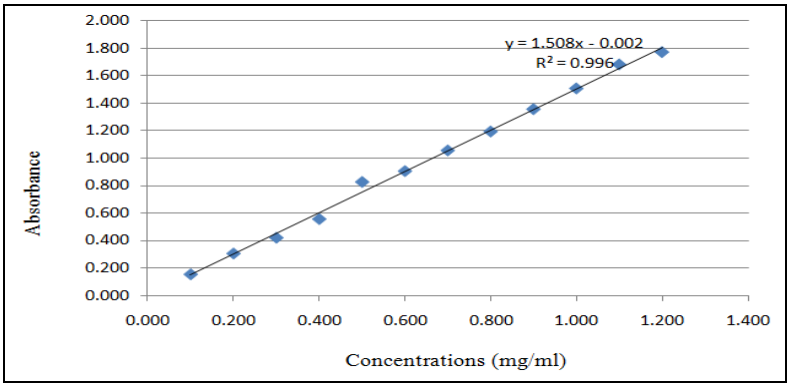

FIG. 1: CALIBRATION CURVE OF TOTAL PHENOLIC COMPOUDS BY GALLIC ACID

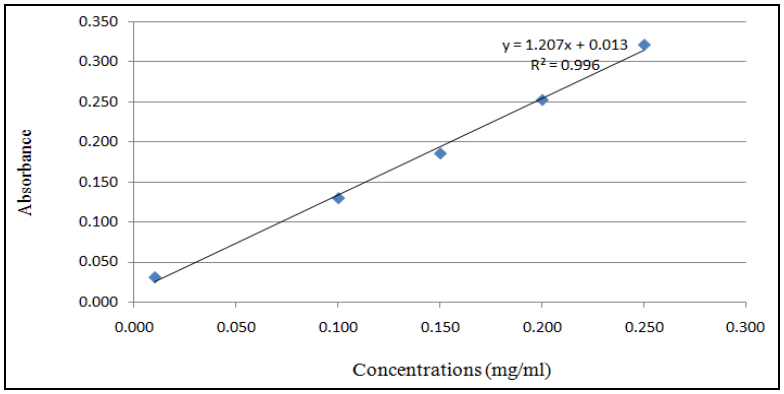

FIG. 2: CALIBRATION CURVE OF FLAVONOIDS BY QUERCETIN

Results of the antioxidant activity by the DPPH radical scavenging: Each extract of each plant was characterized by its color and its yield (**Table 3**)

TABLE 3: CHARACTERIZATION OF EACH EXTRACT

Extract		Yield	Color
L. antineae	Ethyl acetate	0.15%	yellowish
	Butanol	2.23%	Brown
T. algeriensis	Ethyl acetate	0.27%	Yellow
	Butanol	1.57%	Light brown

Scavenging free radical DPPH test: The antioxidant activity of ethyl acetate extracts and butanol of the tow plants and standard (ascorbic acid) against the radical DPPH was evaluated using a spectrophotometer following the reduction of this radical which is accompanied by passage of the violet color (DPPH•) to yellow (DPPH -H) measured at 515nm. This reduction in capacity is determined by a decrease in absorbance induced by radical-scavenging substances.

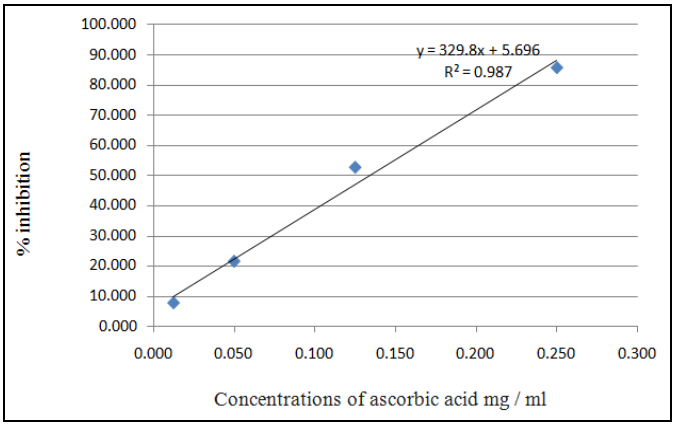

FIG. 3: INHIBITORY PERCENTAGES AGAINST DPPH DEPENDING OF THE ASCORBIC ACID CONCENTRATIONS

According to the results obtained, ascorbic acid, ethyl acetate and n-butanol extracts of *L. antineae* have presented EC_{50} (the effective concentration of the antioxidant extract necessary for the trapping of the 50 % mole of DPPH) values equal to 0.134, 0047, and 0,385 mg /ml successively, ethyl acetate extract has a high antioxidant capacity than ascorbic acid (**Fig.3, 4, 5**).

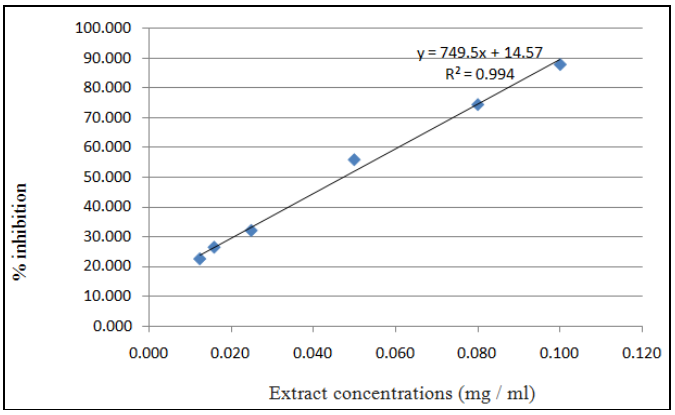

FIG. 4: INHIBITORY PERCENTAGES AGAINST DPPH DEPENDING OF CONCENTRATIONS OF ETHYL ACETATE EXTRACT OF *L. ANTINEAE*

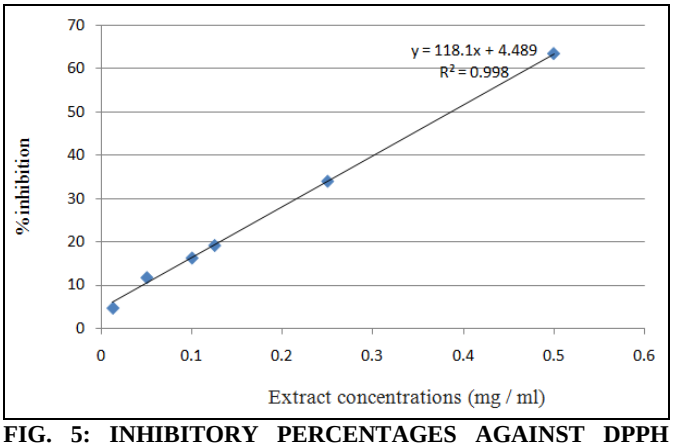

FIG. 5: INHIBITORY PERCENTAGES AGAINST DPPH DEPENDING OF CONCENTRATIONS OF N-BUTANOL EXTRACT OF *L. ANTINEAE*

Thymus algeriensis was marked by an EC_{50} = 0.290 mg / ml (extract ethyl acetate) and EC_{50} which equal to 1.45 mg / ml (extract n-butanol) (**Fig. 6** and **7**), Khled Khoudja and *al.* (2014) [26] found that EC50 values equal to 0.048 and 0.987 mg/ml successively from the extracts of ethyl acetate and n-butanol of the same plant.

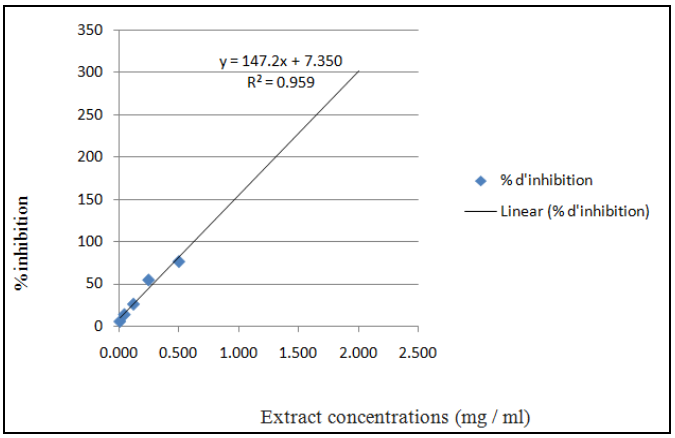

FIG. 6: INHIBITORY PERCENTAGES AGAINST DPPH DEPENDING OF CONCENTRATIONS OF ETHYL ACETATE EXTRACT OF *T. ALGERIENSIS*

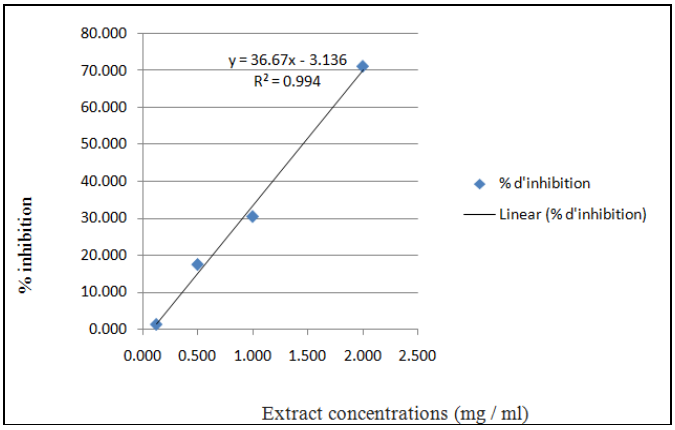

FIG. 7: INHIBITORY PERCENTAGES AGAINST DPPH DEPENDING OF CONCENTRATIONS OF N-BUTANOL EXTRACT OF *T. ALGERIENSIS*

CONCLUSION: Our results showed the presence of a large amount of oxygenated sesquiterpenes in the essential oil of *L. antineae* and the essential oil of *T. algeriensis.* The ethyl acetate extract of *L. antineae* have presented EC_{50} value higher than that of ascorbic acid where the possibility of use in the pharmaceutical field, much remains to be done and extract from this endemic species and not studied yet. The differences obtained in the results of *T. algeriensis*, comparing with other studies, may be due to soil and climate conditions and the genetic diversity within a same species.

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CONFLICT OF INTEREST: The authors have no conflict of interest.

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