Effect of Gamma Ray Irradiation on Chemical Composition, Antioxidant, Antimicrobial, and Insecticidal Activities of *Thymus pallescens* Essential Oil

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The essential oils isolated by hydrodistillation from *Thymus pallescens* de Noé dried leaves exposed to γ -irradiation at dose levels of 0, 5, 10, 20, and 30 kGy were analyzed by gas chromatography–flame ionization detector (GC–FID) and GC–mass spectrometry (MS) and tested for their antioxidant, antimicrobial, and insecticidal activities. No qualitative change was observed in the chemical composition. Carvacrol (81.8–85.7%) was the most prominent component. Gamma-irradiation at 20 kGy affects quantitatively some components. Antioxidant activity was evaluated by four different test systems, namely, inhibition of lipid peroxidation (thiobarbituric acid reactive substance, TBARS), ferric reducing power, and scavenging of radicals DPPH[•] and ABTS^{•+}. In all systems, irradiated oils at 20 and/or 30 kGy showed the most antioxidant efficiency. Overall, the antimicrobial activity conducted against seven microorganisms revealed no significant changes according to the radiation dose. Fumigation bioassays and contact method against confused flour beetle *Tribolium confusum* revealed that the oil irradiated at 20 kGy had highest insecticidal activity. The results showed that gamma-irradiation of *T. pallescens* could be not only beneficial safe decontamination perspective but also as an improvement factor of some of its properties.

Keywords: *T. pallescens*, irradiation, essential oil, GC–MS, antioxidant activity, antimicrobial activity, insecticidal activity

Introduction

Thymus (Lamiaceae) is a large genus divided in eight sections, comprising more than 250 species particularly prevalent in the Mediterranean area [1]. The plants of *Thymus* genus are among the most popular plants throughout the world, commonly used as herbal teas, flavoring agents (condiment and spice), and aromatic and medicinal plants [2]. *Thymus pallescens* de Noé (synonym *Thymus fontanesii* Boiss. et Reut.) common and endemic species in Northern Algeria is widely used by local population as food additive and in Algerian folk medicine for its antitussive, antiseptic, expectorant, anti-helminthic, and anti-spasmodic properties [3].

The herbal plant, like other production, can be contaminated by microorganisms that originate from soil, animals, storage, or postharvest treatment and primary processing or dry process [4, 5]. Extended shelf-life is a key factor for making any food commodity more profitable and commercially available for long periods of time at the best possible quality. Thus, it is important to apply useful decontamination procedures for dried spices and herbs to reduce the level of contamination. Several decontamination methods exist but the most versatile treatment among them is processing with ionizing radiation [6]. The ionizing radiations originated from gamma rays are produced by radioactive substances (radioisotopes). The approved sources of gamma rays for food irradiation are the radionuclides cobalt-60 (⁶⁰Co; the most common) and cesium-137 (¹³⁷Cs) [7]. Radiation processing with gamma radiation or electron beam is in an exceptional position

among the most recent non-thermal methods for post-harvest decontamination of food [8]. Gamma radiation from Co60 is applied at standard conditions to spices and herbs [9]. About 50% of the total amount of irradiated food worldwide is dry herbs and vegetables [10]. The gamma irradiation method is allowed for the decontamination of dried aromatic herbs, spices, and vegetable seasonings with a maximum overall average absorbed dose of 10 kGy, but this limitation has been raised by the US Food and Drug Administration (FDA) to doses up to 30 kGy for these products [11, 12].

Since every step of essential oil production has an influence on the final result of the product, the question arises if the irradiation of the starting material using ionizing radiation could possibly affect the composition of the essential oil obtained and therefore its biological activities. *T. pallescens* is one of the most widespread and probably the most abundant Algerian *Thymus* species. The main goal of the herein reported study is to institute the influence of gamma ray irradiation of dried leaves of *T. pallescens* at different doses on the chemical composition of essential oils and their antioxidant, antimicrobial, and insecticidal activities.

Experimental

Plant Material and Irradiation. *T. pallescens* was collected before the flowering stage. The plant was dried in the shade at room temperature. Then, the dried leaves were separated from the plant and packed in four batches polyethylene (100 g). The samples were irradiated at room temperature (25 °C) using gamma rays from a cobalt-60 radiation source type COP-4

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(ORIS industries, France). The process of irradiation was performed by Nuclear Research Centre of Algiers (CRNA). Doses of gamma radiation from Co^{60} applied to plant material were 5, 10, 20, and 30 kGy ($\pm 20\%$) at the dose rate of 6.91 Gy/min as determined with a Fricke dosimeter. A portion of non-irradiated plant material was kept and used as a control.

Isolation of Essential Oils. After irradiation, the samples of dried leaves were immediately submitted to hydrodistillation in a Clevenger-type apparatus for 3 h and the collected oils were dried with anhydrous Na_2SO_4 , measured and transferred to colored glass flasks and kept at a temperature of 4 °C and analyzed within 7 days post-irradiation.

Analysis of the Essential Oils

GC Analyses. Gas chromatography (GC) analysis was performed with a Hewlett-Packard 6890 GC-FID equipped with an HP 5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm film thickness). Program temperature of the column was 60 °C for 8 min, increasing at 2 °C/min toward 280 °C and held isothermal at 280 °C for 15 min. Injection at 250 °C of diluted samples (0.2 μL ; 1/10 hexane, v/v) was achieved by splitting, and the split ratio was 1:25. N_2 was used as carrier gas at a flow rate of 0.5 mL/min. Flame ionization detection was performed at 320 °C. The percentage composition of the each oil was computed by the normalization method from the GC peak areas, calculated as the mean value of three injections, without using correction factors.

GC-MS Analyses. Gas chromatography-mass spectrometry (GC-MS) analysis was performed with a Hewlett-Packard computerized system comprising a 6890 gas chromatograph coupled to a quadrupole mass spectrometer (model HP 5973) equipped with an HP5 MS capillary column (5% phenyl methylsiloxane, 30 m $\times$ 0.25 mm, 0.25 μm film thickness). Helium was the carrier gas at flow rate of 0.5 mL/min; 0.2 μL (1/10 in hexane, v/v) as injected volume; split mode (1:25); and 250 °C as injection temperature. Temperature program of the oven is described above for GC analysis. For detection, we used an ionization mode with electron ionization at 70 eV over a scan range of 30–550 atomic mass units.

Compound Identification. The oils components were identified by matching their recorded mass spectra with the data bank mass spectra (Wiley 7N and NIST 2005 libraries) and literature mass spectra [13] and by comparison of their retention indices relative to C_8 – C_{16} *n*-alkane [3, 13, 14]. Some structures were further confirmed by available authentic standards analyzed under the same conditions described above.

Insecticidal Activity

Insect Cultures. Colonies of confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), were maintained in the laboratory without exposure to any insecticide. They were reared in five glass containers (20 cm diameter $\times$ 30 cm height) covered by a fine mesh cloth for ventilation. Each container contained 250 g of wheat flour mixed with 5% (w/w) of baker's yeast which was infested by insects. The rearing glass containers were maintained in the dark cabinet of an incubator at a constant temperature of 30 ± 1 °C and 70% relative humidity. Only adults were used for the test.

Contact Toxicity. Five stocks solutions of the essential oils from the irradiated and unirradiated dried leaves were prepared in acetone at the concentrations of 8, 16, and 32 $\mu\text{L}/\text{mL}$. One milliliter of each solution or acetone (control) was spread on the surface of the filter paper (Whatman N°1, 9.0 cm diameter) and placed into a Petri dish (9 cm diameter, 1.5 cm height) which is left open in order to evaporate the solvent. Once the solvent had been evaporated, twenty unsexed adults of *T. confusum* were introduced (three replicates for essential oils and control). Mortality was recorded daily for 4 days. Mortality was recorded after 24 h of exposure to oil, and insects were considered as dead when if

at this time they showed no signal of any movement. Bioassays are designed to evaluate lethal concentrations LD_{50} and LD_{90} values, which represent doses needful to kill 50% and 90% of the exposed insects, respectively, of the same dish determined by probit analysis tested using the method of Finney [15].

Fumigation Assay. The fumigant toxicity of the essential oils was evaluated in sealed glass vial (volume 68.64 cm^3). Thirty-two microliters of pure essential oil (corresponding to 0.466 $\mu\text{L}/\text{cm}^3$) was used to soak a cotton bud of 2 cm in length glued onto the glass vial cap. Twenty unsexed insects were introduced in the vial which was then tightly screwed. The number of dead insects was recorded each 3 h for 15 h in three separate experiments for each *Thymus* oil and control (without oil). LT_{50} and LT_{90} which represent the times requisite to kill 50% and 90% of exposed insects of the same glass vial, respectively, were determined by probit analysis. Data of the contact and fumigant toxicity tests were corrected by the Abbott formula [16]:

$$M_c(\%) = 100[(M - M_t)/(100 - M_t)]$$

where M_c is the corrected insect mortality; M , the insect mortality in the treated insect population; and M_t , the insect mortality in the control.

Antimicrobial Activity. Seven different bacteria constituted by two Gram-positive (*Bacillus subtilis* ATCC 9372, *Staphylococcus aureus* ATCC 6538), three Gram-negative (*Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739), and two yeast (*Candida albicans* ATCC 24433, *Saccharomyces cerevisiae* ATCC 2601) were used. The strains were provided by the Research and Development Center, SAIDAL Algiers. Sensitivity of the microorganisms to the essential oils was investigated by using the disk diffusion method [17]. Sterile disks (9 mm in diameter) impregnated with the essential oil (10 μL) were placed on the center surfaces of the inoculated Mueller-Hinton agar (for bacteria) and Sabouraud agar (for yeast). After the plates were incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for yeast, the inhibition diameters (mm) were measured. The results are the means of three replicates. The antibiotics ciprofloxacin, piperacillin, and amoxicillin were acted as controls.

Antioxidant Activity

Thiobarbituric Acid Reactive Substances. The ability of samples to inhibit malondialdehyde formation, and therefore lipid peroxidation, was determined by using a modified thiobarbituric acid reactive substances (TBARSs) assay as previously described elsewhere [18, 19]. Briefly, egg yolk homogenates were used as a lipid-rich medium. An aliquot of yolk material was made up to a concentration of 10% (w/v) in KCl (1.15%, w/v) and homogenized. Five hundred microliters of homogenate and 100 μL of sample (essential oil in ethanol or the positive control) were added to the test tube and completed to 1 mL with distilled water, followed by addition of 1.5 mL 20% acetic acid (pH = 3.5) and 1.5 mL 0.8% (w/v) thiobarbituric acid (TBA) in 1.1% sodium dodecyl sulfate. The mixture was vortexed and heated at 95 °C for 1 h. After left to cool at room temperature, 5 mL of *n*-butanol was added to each tube, vortexed, and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. The percentage inhibition was calculated by the formula % Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the fully oxidized control (containing ethanol instead of essential oil) and A_1 the absorbance of the tested sample. Sample concentration able to prevent 50% lipid oxidation (IC_{50}) was determined from the graph plotting inhibition percentage against oil concentration. Butyl hydroxytoluene (BHT) was used as positive control.

ABTS^{•+} Free Radical Scavenging Activity. ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] assay was based on the method of Re et al. [20]. ABTS radical cation (ABTS^{•+}) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS^{•+} solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 25 μ L of sample solution to 1.0 mL of ABTS^{•+} solution, decreasing of absorbance was measured after 7 min at 734 nm. Tests were carried out in triplicate. ABTS^{•+} solution was used as blank sample, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and BHT were used as positive probes. Inhibition (I) of radical ABTS^{•+} as well as IC₅₀ value were determined as reported above.

Free Radical Scavenging Activity (DPPH). About 1950 μ L of 60 μ M ethanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) were incubated in the dark for 30 min with 50 μ L of each sample at different concentrations [19]. Absorbance measurements were made at 517 nm against a blank. IC₅₀ values were determined as reported above. Tests were carried out in triplicate, and BHT was acted as positive control.

Reducing Power Assay. The reducing antioxidant power was assayed by the method of Oyaizu [21]. About 125 μ L of ethanol solutions at the different concentrations of tested samples was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆; 2.5 mL, 1 %]. The mixture was then incubated at 50 °C for 20 min. Afterwards, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1 % w/v), and the absorbance was measured at 700 nm. The assay was fulfilled in three independent experiments, and BHT was acted as positive control. IC₅₀ value corresponding to the absorbance of 0.5 was calculated from the graph plotting absorbance against oil concentration.

Statistical Analysis. Data of bioassays were subjected to one-way analysis of variance (ANOVA) using the SPSS 18.0 software (SPSS Inc.) followed by Tukey's test. The level of significance was set at $P < 0.05$.

Results and Discussion

First of all, it is important to note that the use of the leaves in this work is a strategic choice. Indeed, in a previous study [22], we have shown that oils derived separately from leaves and inflorescences are the same qualitative point of view but they have significant quantitative differences in some compounds and oil yields. The non-homogeneity of the samples due to the unsteadiness of the quantities in leaves and flowers can lead to differences in yields, compositions, and biological activities of oils which could be wrongly interpreted as due to irradiation. Thus, the choice of a single organ (leaves only) is dictated by the desire to eliminate at the outside the influence of the non-homogeneity of the samples.

The percent yield of volatile oil from control and irradiated samples (Table 1) ranged from 1.7% to 1.8% (v/w), showing no major difference with the level of irradiation. Chromatographic analysis of the oils presented in Table 1 showed no qualitative change between control and irradiated samples. The oils were characterized by high content of carvacrol (81.8–85.7%) followed by linalool (3.7–5.0%). This composition pattern is not in good agreement with previously published data on the same species [3, 19, 22, 23] where components like *p*-cymene and γ -terpinene were found to be most important than those of this study (6.2–17.4% vs. 0.2–1.1% and 6.9–14.2% vs. 0.4–0.7%, respectively). Agro-climatic origin is known to influence the oil content tissues a great deal. Except for sample irradiated at 20 kGy, GC analysis

showed that the essential oil compounds of the samples did not considerably change after irradiation. According to our findings, the most changes were recorded after irradiation of 20 kGy for β -myrcene (+0.9%), 3-octanol (−0.8%), *p*-cymene (+0.9%), carvacrol (−2.8%), and β -caryophyllene (+0.7%). The increase of *p*-cymene content up to 20% was noted by other authors in irradiated nutmeg (10.0 kGy) [24]. The decrease of carvacrol amount at 20 kGy is not in accordance with the increase of this compound reported at this dose for carvacrol-rich *Thymus vulgaris* [25]. For all the other samples, the changes, when they exist, ranged from ± 0.2 to $\pm 0.7\%$ with remarkable decrease in linalool for irradiated samples at 5 and 10 kGy (−1.0% and −1.1%, respectively). This result is in good agreement with the literature data which indicate that linalool showed a great sensitivity to γ -radiation [26]. The mechanism by which radiation induces changes in the volatile oil composition is not yet well understood, but the variations in content of the constituents upon gamma irradiation observed in the present study could presumably be due to the radiation sensitivity of compounds with the dose employed. Thus, linalool content which change for 5 and 10 kGy became stable for 20 and 30 kGy. The contents of functional groups in non-irradiated and irradiated samples were equivalent, except for sample irradiated at 20 kGy which records a slight deficit in oxygenated monoterpenes (88.7%) offset by slight increase in monoterpene and sesquiterpene hydrocarbons particularly due to the increase previously pointed out of β -myrcene, *p*-cymene, and β -caryophyllene. The effect of γ -irradiation on volatile compounds of some spices and herbs, reviewed by P. Thongphasuk and J. Thongphasuk [27], highlights that specific effects could be observed for different essential oils.

The effects of irradiation on the antioxidant activities of *T. pallescens* oils were investigated by inhibition of lipid peroxidation (TBARS), ferric reducing power, and scavenging of radicals DPPH and ABTS assays. The data expressed as IC₅₀ values in Table 2 indicated that for whole tests the oils irradiated at 20 and/or 30 kGy were the most active, but remain in general less than the positive controls. Our results show that gamma irradiation for the doses of 20 and 30 kGy affects positively the antioxidant activity of *T. pallescens*. For the other doses (5 and 10 kGy), the antioxidant activity decreases or increases according to each used test. However, the changes recorded between these two doses are in general not significant. Literature data regarding the influence of γ -ray irradiation on the antioxidant activities of herbs or spices is mainly reported for non-volatile extracts while that on essential oils is rather scarce [25, 28, 29] and when it is available it is mainly measured by only one or two tests. Our results are in accordance with those reported for essential oil of *Rosmarinus officinalis* for which the antioxidant activity measured using DPPH and bleaching of β -carotene tests increased with irradiation dose (10–15 kGy) [29]. For the same tests, no significant change in antioxidant activity was noted for *Mentha piperita* essential oil irradiated at 10 and 20 kGy [29]. *T. vulgaris* carvacrol-rich essential oils from Morocco showed a marked increase in DPPH scavenging activity which stabilizes between 20 and 30 kGy [25, 30]. Horváthová et al. reported various trends of antioxidant activity of oregano methanol–water extracts characterized using DPPH, TBARS, ferric reducing power (FRP) and total content of phenolic compounds (TPC) assays [30]. They found that the influence of irradiation of oregano samples at doses of 5–30 kGy on the DPPH radical-scavenging ability and FRP was negligible. On the other hand, the irradiation caused a considerable increase (+18%) of TBARS values of oregano extract prepared from the sample irradiated at 30 kGy.

There is a few data, if any available in the literature on the effect of ionizing radiation on the antibacterial activity of essential

Table 1. Composition (%) of essential oils of *T. pallescens* irradiated for 0, 5, 10, 20, and 30 kGy

N ^o	Components	ERI ^a	γ -Irradiated <i>T. pallescens</i>					Identification ^b
			0 kGy	5 kGy	10 kGy	20 kGy	30 kGy	
1	α -Pinene	937	0.3	0.3	0.3	0.4	0.3	RI, MS, Co-GC
2	β -Pinene	976	0.1	0.1	0.1	0.1	0.1	RI, MS, Co-GC
3	β -Myrcene	990	0.4	0.8	0.9	1.3	0.1	RI, MS, Co-GC
4	3-Octanol	991	1.2	1.0	0.9	0.4	0.7	RI, MS
5	<i>p</i> -Cymene	1022	0.5	1.1	0.7	1.4	0.2	RI, MS, Co-GC
6	γ -Terpinene	1059	0.7	0.5	0.6	0.6	0.4	RI, MS, Co-GC
7	1-Nonen-3-ol	1085	0.1	0.1	0.1	0.1	0.1	RI, MS
8	Terpinolene	1087	0.1	0.1	0.1	0.1	0.1	RI, MS, Co-GC
9	Linalool	1096	4.8	3.7	3.8	5.0	4.5	RI, MS, Co-GC
10	Borneol	1164	0.8	1.4	1.0	0.6	0.1	RI, MS, Co-GC
11	4-Terpineol	1175	0.8	0.7	0.8	0.8	0.9	RI, MS, Co-GC
12	α -Terpineol	1189	0.1	0.1	0.4	0.1	0.1	RI, MS, Co-GC
13	Carvacrol methyl ether	1243	0.1	0.1	0.1	0.1	0.7	RI, MS
14	Thymol	1291	0.6	0.9	0.6	0.3	0.5	RI, MS, Co-GC
15	Carvacrol	1299	84.6	84.5	85.5	81.8	85.7	RI, MS, Co-GC
16	α -Gurjunene	1409	0.3	0.3	0.2	0.4	0.5	RI, MS
17	β -Caryophyllene	1418	0.7	0.7	0.6	1.3	0.9	RI, MS, Co-GC
18	Aromadendrene	1439	0.7	0.6	0.1	0.8	0.4	RI, MS
19	α -Humulene	1453	0.1	0.1	0.1	0.1	0.1	RI, MS
20	Alloaromadendrene	1460	0.1	0.1	0.1	0.1	0.1	RI, MS
21	γ -Murolene	1477	0.1	0.1	0.1	0.1	0.1	RI, MS
22	Ledene	1486	0.1	0.1	0.1	0.1	0.1	RI, MS
23	α -Murolene	1498	0.1	0.3	0.3	0.2	0.2	RI, MS
24	β -Bisabolene	1506	0.1	0.1	0.1	0.1	0.1	RI, MS
25	γ -Cadinene	1513	0.2	0.2	0.2	0.3	0.2	RI, MS
26	δ -Cadinene	1523	0.3	0.3	0.3	0.3	0.2	RI, MS
27	α -Bisabolene	1537	0.5	0.3	0.3	0.5	0.2	RI, MS
28	Caryophyllene oxide	1581	0.4	0.2	0.3	0.5	0.4	RI, MS, Co-GC
Total identification (%)			98.9	98.8	98.7	97.9	98.0	
Monoterpene hydrocarbons			2.1	2.9	2.7	3.9	1.2	
Oxygenated monoterpenes			91.8	91.4	92.2	88.7	92.5	
Sesquiterpene hydrocarbons			3.3	3.2	2.5	4.3	3.1	
Oxygenated sesquiterpenes			0.4	0.2	0.3	0.4	0.4	
Others			1.3	1.1	1.0	0.5	0.8	
Oil yield % (v/w)			1.7	1.7	1.75	1.8	1.8	

^aERI, experimental retention indices relative to C₈–C₁₆ *n*-alkanes on the HP 5MS column.^bIdentification: RI, comparison of retention index with bibliography, MS, comparison of mass spectra with MS libraries, co-GC, comparison with authentic compounds.**Table 2.** Antioxidant activity expressed in IC₅₀ (mg/L) of essential oils of *T. pallescens* irradiated with different doses (0, 5, 10, 20, and 30 kGy), Trolox, and BHT

Plant samples/controls	DPPH	ABTS	TBARS	Reducing power
0 kGy	574 ± 3.6 ^c	21.1 ± 3.9 ^c	387 ± 7 ^c	255 ± 1.9 ^{cd}
5 kGy	631.6 ± 1.4 ^{cd}	18.2 ± 0.1 ^d	378.7 ± 10.8 ^c	249.6 ± 4.6 ^d
10 kGy	627 ± 8.6 ^{cd}	18.3 ± 3.3 ^d	375.1 ± 6.7 ^c	330.2 ± 3.4 ^c
20 kGy	286.7 ± 5.9 ^b	17.9 ± 4.9 ^{cd}	104.5 ± 1.8 ^b	240.4 ± 3.1 ^c
30 kGy	609 ± 3.3 ^c	17.3 ± 0.04 ^c	20.3 ± 0.6 ^a	204.1 ± 3.1 ^b
BHT	28.0 ± 0.7 ^a	5.3 ± 0.01 ^b	98.4 ± 1.7 ^b	64.8 ± 0.7 ^a
Trolox	Nt	2.0 ± 0.1 ^a	Nt	Nt

In each line, values with different letters mean significant differences between values of this line by the Tukey's multiple range test ($p < 0.05$); Nt: not tested.oils. Table 3 shows the effect of gamma irradiation at 5, 10, 20, and 30 kGy on antimicrobial activity of *T. pallescens*. According

to these results, except for *P. aeruginosa*, *T. pallescens* essential oils showed a strong activity on all tested bacterial strains based on the inhibition diameters obtained between 27.0 and 52.8 mm. Highest sensitivities were observed against yeast strains (*C. albicans* and *S. cerevisiae*). Overall, antimicrobial activity of gram-negative and gram-positive bacteria studied was not significantly affected by γ -irradiation dose. Our results are in agreement with those reported for *Zataria multiflora* Boiss. essential oil for which γ -irradiation at 10 and 25 kGy has not affected antimicrobial activity of both gram-positive and gram-negative bacteria [31]. However, a partly disagreement is noted with data reported for *T. vulgaris* essential oil for which γ -irradiation significantly affected the antimicrobial activity against two gram-negative bacteria (*E. coli* and *Salmonella senftenberg*), while gram-positive bacteria (*Listeria monocytogenes* and *S. aureus*) were not affected [25]. On the other hand, our results disagree with those reported by Abdeldaiem et al., for irradiated rosemary (5, 10, and 15 kGy)

Table 3. Diameter of microbial inhibition zone (mm) of antibiotics and *T. pallescens* essential oils extracted from non-irradiated and irradiated plant against a selection of yeast, gram-positive and gram-negative bacteria; disk diameter 9.0 mm

Plant samples/controls	Microorganisms; Inhibition zone (mm)						
	<i>E. coli</i>	<i>K. Pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
0 kGy	27.0 ± 0.2 ^b	32.1 ± 0.5 ^c	13.1 ± 0.2 ^b	42.0 ± 0.4 ^c	40.1 ± 1.0 ^d	52.8 ± 0.2 ^c	45.9 ± 0.3 ^a
5 kGy	27.7 ± 0.8 ^b	32.1 ± 0.2 ^c	13.2 ± 0.2 ^b	42.1 ± 0.2 ^c	41.5 ± 0.2 ^c	52.3 ± 0.3 ^c	46.0 ± 0.2 ^a
10 kGy	27.0 ± 0.4 ^b	32.4 ± 0.3 ^c	12.1 ± 0.2 ^a	42.3 ± 0.2 ^{ef}	40.0 ± 0.5 ^d	51.1 ± 0.2 ^b	46.0 ± 0.3 ^a
20 kGy	28.0 ± 0.3 ^b	32.1 ± 0.4 ^c	12.2 ± 0.2 ^a	40.2 ± 0.5 ^d	42.0 ± 0.5 ^c	50.5 ± 0.2 ^b	47.0 ± 0.3 ^a
30 kGy	33.1 ± 0.3 ^c	34.9 ± 0.3 ^d	12.3 ± 0.5 ^a	43.2 ± 0.1 ^f	41.0 ± 0.1 ^{de}	46.8 ± 0.2 ^a	46.8.0 ± 0.2 ^a
Ciprofloxacin	38.1 ± 0.2 ^d	24.4 ± 0.2 ^b	43.2 ± 0.2 ^d	27.3 ± 0.4 ^b	30.0 ± 0.3 ^c	Nd	Nd
Piperacillin	27.0 ± 0.4 ^b	19.3 ± 0.2 ^a	23.1 ± 0.1 ^c	26.1 ± 0.1 ^a	22.2 ± 0.3 ^b	Nd	Nd
Amoxicillin	24.2 ± 0.3 ^a	19.2 ± 0.2 ^a	Nd	35.0 ± 0.1 ^c	12.1 ± 0.11 ^a	Nd	Nd

In each column, means of three independent experiments ± standard deviations with the same superscript letter are not significantly different ($p < 0.05$). Inhibition zone includes the diameter of the disk (9 mm); Nd: not determined.

essential oil for which the antibacterial activity increases proportionally with irradiation doses [28]. As reported by many researches, the antibacterial potency of the essential oils would be related to the main components including especially the phenolic monoterpenes carvacrol and/or thymol which have been found to exhibit antimicrobial activity against a variety of bacteria, including foodborne pathogens [32–34]. Electron micrographs showed that these compounds, which are lipophilic in nature, act on the cell membrane and cause substantial morphological damage, resulting in a change in permeability and the release of cellular contents [35]. Moreover, some studies showed that their combination with other common compounds led to a synergistic activity that resulted in destabilization of the microbial membrane [31, 36].

Plant essential oils are considered to be an alternative means of controlling many insect pests [37]. In integrated stored product protection, phytochemicals may be used for pest prevention, early pest detection, or pest control [38]. Studies on the mode of action of the natural insecticide have shown that treatments of the insects with natural compounds such as essential oils or pure compounds

may cause symptoms that indicate neurotoxic activity including hyperactivity, seizures, and tremors followed by paralysis (knock down), which are very similar to those produced by the insecticides pyrethroids [39]. It has been recognized that essential oils are potent neurotoxins and could affect through acetylcholinesterase enzyme inhibition in the central nervous system [40].

In fumigant toxicity test, probit analysis showed that the flour beetle *T. confusum* was more susceptible to the oils of *T. pallescens* irradiated at 10 and 20 kGy (Figure 1) with LT_{50} (3.67 and 3.65 h, respectively) and LT_{90} (7.26 and 6.77 h, respectively). For the other samples, no significant differences can be noted in their LT_{90} .

In contact toxicity, probit analysis showed that the oils from samples irradiated at 10 and 20 kGy were also the more toxic against *T. confusum* (LD_{50} = 8.81 μ L/mL corresponding to 0.055 μ L/cm² and LD_{90} = 38.31 μ L/mL corresponding to 0.30 μ L/cm² for 10 kGy vs. LD_{50} = 8.74 μ L/mL corresponding to 0.054 μ L/cm² and LD_{90} = 26.45 μ L/mL corresponding to 0.165 μ L/cm² for 20 kGy) than the other samples (LD_{50} $\geq$ 10.56 μ L/mL or 0.066 μ L/cm² and LD_{90} $\geq$ 64.61 μ L/mL or

Figure 1. Lethal time (h) causing the death of 50% (LT_{50}) and 90% (LT_{90}) of individuals of *Tribolium confusum*. Values in the same column with the same capital letter are not significantly different by the Tukey's multiple range tests ($p < 0.05$)

Figure 2. Lethal concentrations (μ L/mL) causing the death of 50% (LD_{50}) and 90% (LD_{90}) of insect population in contact toxicity test after 4 days. Values in the same column with different capital letter are significantly different by the Tukey's multiple range test ($p < 0.05$)

0.4 $\mu\text{L}/\text{cm}^2$) (Figure 2). The efficiency of the samples increases from control (0 kGy) to reach its maximum at the dose of 20 kGy and then decreased at 30 kGy. The most significant differences are obtained for LD₉₀ values. This result indicates that whole oils are efficient at the beginning of the treatment, but after long exposure time, only irradiated oil at 20 kGy remains more efficient. Therefore, the oil irradiated at this dose could be considered for *T. confusum* control of stored products. The toxicity herein observed might be explained by the presence of carvacrol and *p*-cymene. In fact, it has been previously shown that essential oils rich in these compounds possess acute toxic effects against various storage insect pests [41, 42].

Conclusion

The results of this study allowed us to notice that gamma-irradiation of *T. pallescens* leads either to the retention or to the improvement of the studied biological activities (antioxidant, antimicrobial, and insecticidal activities) in the irradiation range of 10–30 kGy, particularly for the dose of 20 kGy. Comparing our results with those of the literature, it seems to exist for each specific composition of each plant an optimal radiation dose value that can improve or maintain biological activities of the extracts of the plant. To conclude, it can be said that through a preliminary study for each plant, gamma irradiation of spices and herbs could be not only beneficial decontamination perspective but also as an improvement factor of some of their properties.

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