



ISSN 1110-0451



(E S N S A)

Metabolomics Profiling of Chilled (*Coriandrum sativum* L.) Primed by Silicate, Humic acid, and Gamma Radiation

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ARTICLE INFO

Article history:

Received: 20th Nov. 2023.

Accepted: 24th Dec. 2023.

Available online: 7th Mar. 2024

Keywords:

priming;

Chilling stress;

Coriander;

Growth biostimulators;

HPLC;

Metabolic activity.

ABSTRACT

Chilling escape strategy using safe growth bio stimulators viz., is the aim of this work. Dry coriander seeds were primed in humic acid (50 mg. l⁻¹), potassium silicates (80 mM) or irradiated by γ -rays (50 Gy) before priming in tap water, then all previous treatments were incubated for 16 h at 6°C \pm 0.5. The results revealed that priming techniques had enhancement effects on morphological characteristics and metabolomics. Nitrogenous constituents, polyamines, and elements increased nutritional values in chilled or non-chilled coriander plants. While saturated fatty acids decreased in chilled coriander, the unsaturated fatty acids and unsat/sat ratios increased in chilled and primed plants. Also, the data obtained showed fluctuations within quantities of 43 volatile compounds. volatile oil separated by using a GC mass spectrometer either increased, decreased, disappeared, or newly appeared under chilling. Thus, the recommendation is priming coriander seeds before planting is a powerful technique that renders fortification and tolerance to repair physiological damages under chilling.

INTRODUCTION

Chilling causes drastic effects particularly on Mediterranean plant species like maize, pepper, rice, and tomato as well as on tropical or subtropical trees [1; 2]. Previous findings on chilling injury occurs at low temperatures range 1–10°C leads to physiological disturbances of water transport, mineral nutrition, photosynthesis, respiration disturbance, inactivation of metabolic functions of lipids, delay of cell division and death of plants [3-5]. Chilling also affected water loss in sensitive plants, ending with sever wilting because of two main factors: rapid decline of roots ability to absorb and transport water to the shoots [6] and reduced stomatal closure due to water deficit [7, 8]. Subsequently, the interruption of mineral nutrition of plants via interrupting roots absorption of ions, minerals movement in the aboveground parts of plants, and disruption of nutrient distribution between plant organs, which led to a decrease in plant nutrients [9]. Coriander (*Coriandrum sativum* (C. sativum) L.) is a herbal plant belonging to the family *Apiaceae* characterized by abundance of essential oils utilized in food industries as common

spices for seasoning purpose and is regarded as an important ingredient in cosmetics, pharmaceutical, medicinal, and curry powder industries. Humic acid (HA) that originates from soil organic matter (microbes, plants, carbohydrates, proteins, and lignin) is the main constituent of humic substance based on potassium humates. HA was discovered to improve soil fertility and facilitate root uptake under normal or abiotic stress such as drought [10, 11]. Plants absorbed soluble Si from soil solution in the form of monosilicate compound, Si (OH)₄. Though Si was deemed a second abundant element in the earth's crust, it was not attracting scientists' concerns for its invisible deficiency or toxicity symptoms on plants [12]. Si pose roles in assisting plants development, production and health under abiotic and biotic stresses [13]. γ -rays belong to the electromagnetic radiations and are highly penetrating because of low linear energy transfer, it improves the quality of foods, herbal material and reduces the losses due to contamination and insect damage [14]. It has a considerable effect on physiological and biochemical processes in plants [15, 16].

Moreover, (50 Gy) γ -rays found optimal for medicinal plants acclimation to stress achieved useful improvements at germination level, growth parameters, yield and enhancing active ingredients [17-19]. The objective was ameliorating the chilling stress ($6^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) in coriander (*C. sativum* L.) plant originating from chilled seeds using potassium silicate (80 mM), humic acid (50 mg.l⁻¹) and gamma rays (50 Gy) as seeds priming agents on fortification of growth and metabolic activity.

MATERIALS AND METHODS

Coriander (*C. sativum* L.) seeds were purchased from national seeds suppliers from a local retailer. Potassium silicate (99% degree of purity) was purchased from Sigma-Aldrich Company (Cat. No. 792640). Humic acid is registered under the name of "HUMO" with No. 7050 from the Ministry of Agriculture. Coriander seeds were irradiated at NCRRT using Cesium-137 with a dose rate of 0.758 rad/sec. The experiments were repeated in two seasons,

1. Physiological treatments

A pilot experiment was performed with different and gradual concentrations of potassium silicate or humic acid to choose the best treatment. Various ascending concentrations of HA were applied; 5, 10, 25, 50, 75, and 100 mg. l⁻¹. While in potassium silicate; 10, 20, 40, 80, and 160 mM concentrations were applied; the best treatment was 50 mg.l⁻¹ in HA and 80 mM in potassium silicate depending on the highest records of growth parameters and yield components.

1.2 Priming

Seeds were divided into two sets four groups/set (chilling set and control set for comparison).

The first is a chilling set-in which seeds are soaked in an incubator ($6^{\circ}\text{C} \pm 0.5$) for 16 h in: a) Water; (chilling).

b) Potassium silicate (80 mM) solution (chilling+ silicate).

c) Humic acid (50 mg. l⁻¹) (Chilling+ HA).

d) Dry seeds irradiated by (50 Gy) γ -rays then incubated in tap water; (chilling+ γ -rays).

The second: control set -in which seeds are soaked at room temperature at ($20^{\circ}\text{C} \pm 0.5$):

a) Tap water; (control). b) Potassium silicate (80 mM); (silicate). c) Humic acid (50 mg.l⁻¹); (HA).

d) Dry seeds irradiated by (50 Gy) γ -rays then incubated in tap water (γ -rays).

1.3 Experimental design

Before sowing soaked coriander seeds were washed thoroughly with distilled water before planting in (40 cm) plastic pots filled with sandy loam soil. Ten seeds/pot with ten replicates for each treatment and irrigated with tap water regularly till reaching water holding capacity. The experiment was completely randomized. Design.

1.4 Samples

Plants of the vegetative stage are harvested after 75 days from the sowing date. Samples of ten plants from each treatment were taken to measure growth parameters: plant height, root length, number of branches/plants, number of leaves/plant, area of leaves/plant, fresh and dry weights of shoot and root/plant. The experiment was repeated in the next season, and the mean values of growth parameters were recorded. Chemical analysis on coriander leaves at the flowering stage (105 days) was carried out. N-compounds, polyamines, amino acids, fatty acids, and ion composition were determined.

2. Chemical analysis

2.1. Extraction of nitrogenous constituents

The method used was adopted by Yemm and Willis [20] and described by El-Sawy [21]. The dried samples (*C. sativum* L.) were ground, and a known weight was extracted in distilled water by stirring for 30 minutes at room temperature, in a glass mortar. The mixture was then transferred to a test tube quantitatively, and heated in a water bath at 80°C for 5 min. The insoluble residue was filtered and used for total nitrogen determination. The filtrate was made up to a known volume and used for estimation of total soluble nitrogen fraction.

2.2. Estimation of total N and total soluble-N

Total nitrogen (TN) and total soluble nitrogen (TSN) are determined by the conventional semi micro modification of the Kjeldahl method [22, [23]. Put an aliquot of the extract in a digestion flask then heat for 8 hrs.; or weigh 0.02-0.03 g of dry powder heated for at least 2 hrs.; (with 0.5g catalyst K₂SO₄, 80g; CuSO₄.5H₂O, 20g; SeO₂, 0.3g), two milliliters concentrated H₂SO₄ and one ml of distilled water. The digest was quantitatively transferred into the micro-Kjeldahl apparatus with the least amount of bidistilled water and, added 15 ml NaOH (40 %). Ammonia was distilled into 10 ml of 2% boric acid and then titrated against exactly N/70 HCl using bromocresol green-methyl red indicator till a faint red end point. After

correction for the reagent blank, the titration figures were converted into mg nitrogen: 1 ml N/70 HCl = 0.2 mg N.

2.3. Estimation of total amino acids

Amino acids analysis was executed in the amino acids' laboratory, NCRRT [24] after preparing the sample by weighing 100 mg dry powdered sample in a screw-capped glass tube containing 10 ml of 6.0 N HCl. The tubes were kept in an oven at 110°C for 24 hours for complete digestion. The sample was filtered, and the volume was completed to 100 ml with bidistilled water. Five ml of the solution was evaporated to dryness in a rotary evaporator. A suitable volume of sodium citrate buffer (pH 2.2) was added to the dried film of each hydrolyzed sample. After all materials were completely dissolved, the samples were then filtered through a 0.4 µm membrane filter. The extraction procedure of amino acid composition for every treatment (control, stress, etc.) took place from the leaves of three biological and three technical replicates. Aliquots of pooled samples were stored at -80°C after a short snap for 15 seconds in Liquid Nitrogen. In the end, extracted amino acids from the three technical replicates were combined in one sample. Later a combined sample of each physiological status (treatment) was prepared for injection [25]. The amino acids profile was analyzed using Biochroma 20 (Pharmacia Biotech Autosampler) high-performance amino acid analyzer at NCRRT. The data revealed by the chromatogram was analyzed as recommended by the manufacturer user's guide (Ver 6.7).

2.4. Measurement of endogenous polyamines by high-performance liquid chromatography HPLC

2.4.1. Extraction

The dry leaves (0.3 g) were ground in a mortar in the presence of 5% cold HClO₄, centrifuged at 48,000 g for 20 minutes and the supernatant, containing the free polyamine was stable for long-term storage under these conditions [26]. Standard and plant extracts were benzoylated [27]. One ml of 2 N NaOH was mixed with 500µl of HClO₄ extract. The mixture was shaken briefly using a vortex mixer for 10 seconds and incubated for 20 minutes at room temperature, saturated NaCl solution (2 ml) was added. Benzoyl-polyamines were extracted in 2ml diethyl ether (anhydrous). After centrifugation at 1500 g for 5 minutes, the upper organic phase was removed and evaporated to dryness. The residue was dissolved in 100 ml methanol (HPLC grade). Standards were treated similarly, with up to 50 µl of each polyamine in the reaction mixture. The benzoylated

samples were stored at -20 °C. Under these conditions, samples are stable for several months Aliquots of 5-10 µl were taken for injection in the HPLC.

2.4.2. HPLC analysis

For the measurement of different polyamines (putrescine, spermidine, and spermine), the procedure described [28] was applied with some modifications as follows: HPLC was performed on a system consisting of two solvent metering pumps (Shimadzu) programmed with a microprocessor controller, and the solvents were mixed under pressure by automixer (Shimadzu). The benzoylated samples were injected through an autosampler (Knauer) into a fixed 25 µl loop syringe loading injector for loading onto the reserve phase C18 column (RP-C18 µl Bonda Pak, waters) the column used included octadecylsilane (ODS) ultra-sphere particle (5-µm). Samples were eluted at room temperature through a 250× 4.6 mm column with a programmed aqueous 0.2% acetic acid: methanol (v/v) solvent gradient changing 60 to 95 % in 25 minutes at a flow rate of 1 ml /min Elution was completed by 45 minutes. The column was washed with 100% methanol and preequilibrated with 60% methanol. The next sample was injected. The samples were detected at 254 nm using standard a spectrophotometer (Shimadzu). The proper concentrations of endogenous polyamines (putrescine, spermidine and spermine) were obtained by comparing their respective peak areas in the plant extract with their corresponding areas obtained with the authentic samples. The peak area and polyamines concentrations were measured automatically by the software attached to HPLC.

2.5. Estimation of minerals

Plant materials were dried at 80 °C till obtaining constant weight. The dried matter was digested [29] with certain modification. One gram of dried plant material was weighed into 250 ml digestion flask. Ten ml of mixture of concentrated nitric acid and perchloric acid (70%) at the ratio 4: 1 (v/v) were added. The samples were digested on an electric heater until dense fumes appeared and the solution became clear. The samples were then left to cool and diluted with distilled water and quantitatively transferred into a 50 ml volumetric flask. The volume was made up to a known volume. Potassium and sodium were estimated by the flame emission technique [30]. While magnesium, silicon, and calcium were determined simultaneously by ICP Spectroscopy [31].

2.6. Extraction and estimation of fatty acids

The total oil content was extracted from dried powdered leaves using Soxhlet apparatus and hexane as a solvent for 16 h [32]. Hexane was evaporated on the rotavapor apparatus. Fatty acid methyl esters were separated from oil according to protocol 31 of the extraction of lipids and derivative formation [33]. The analysis was carried out at NCRRT using a Hewlett Packard chromatograph with a flame ionization detector (FID), equipped with a 30-m HP 6,890 Innowax-cross linked polyethylene glycol column with 0.32-mm I.D. and 0.5-m film thickness. The oven temperature was programmed from 150 to 450°C for 5 m. The injector and detector were kept at 260 and 275°C, respectively. Nitrogen was used as a carrier gas at 1.5/min flow rate.

2.7. Extraction and analysis of volatile oil

50 g sample from the flowering stage boiled with water in a volatile oil apparatus for 3 hrs. The main essential oil components in *C. sativum* plant are separated using Gas Chromatography-mass spectrometry (GC-MS). GC, Varian 3400 equipped with a DB-5 fused silica capillary column (30 m X 0.25 mm i.d. 0.25 μ m film thickness). The multistep temperature program was increased from 60 °C - 260 °C (held for 10 min) with a rate of 5°C min⁻¹. The carrier gas was helium at a flow rate of 1 ml.min⁻¹, and the sample size was 1 μ l (Injector temperature was 250°C). The mass spectrometer was a Varian- Finnigan SSQ7000 operating in ionizing potential 70 ev and the spectra were scanned in the range of 35-400 amu analysis. The separated components from plants originated from different

treatments at room or chilled temperatures compared with those separated from the untreated control sample. The GC-MS experiment was carried out by injecting a mixed sample of volatile oil separated from three replicates for each treatment.

Statistical analysis

The experimental design of every treatment (control, stress, etc.) was performed using three biological replicates and three technical replicates. Every technical replicate demonstrated the mean of one biological replicate. Every biological replicate represented a combination of plant tissue or leaves of ten plants. The latter procedure was executed in all performed experiments [34], except for the estimation of total amino acids content, volatile oil composition, and estimation of fatty acids in combining and pooling the three extracted technical replicates in one sample to be analyzed.

RESULTS

Priming and γ -rays' effects on morphological characteristics

Chilling stress (6 °C \pm 0.5) caused significant retardation in all growth parameters (shoot and root lengths, fresh and dry weights of shoot and root, number of leaves/plant, number of branches/plant, and leaf areas/ plant). Priming seeds in the pot. silicate, HA, or exposure to γ -rays before planting stimulated growth parameters at all stages of growth as compared with control and chilling-stressed coriander plants (fig1). The most effective treatments were humic acid (HA) in control and chilling-stressed plants.

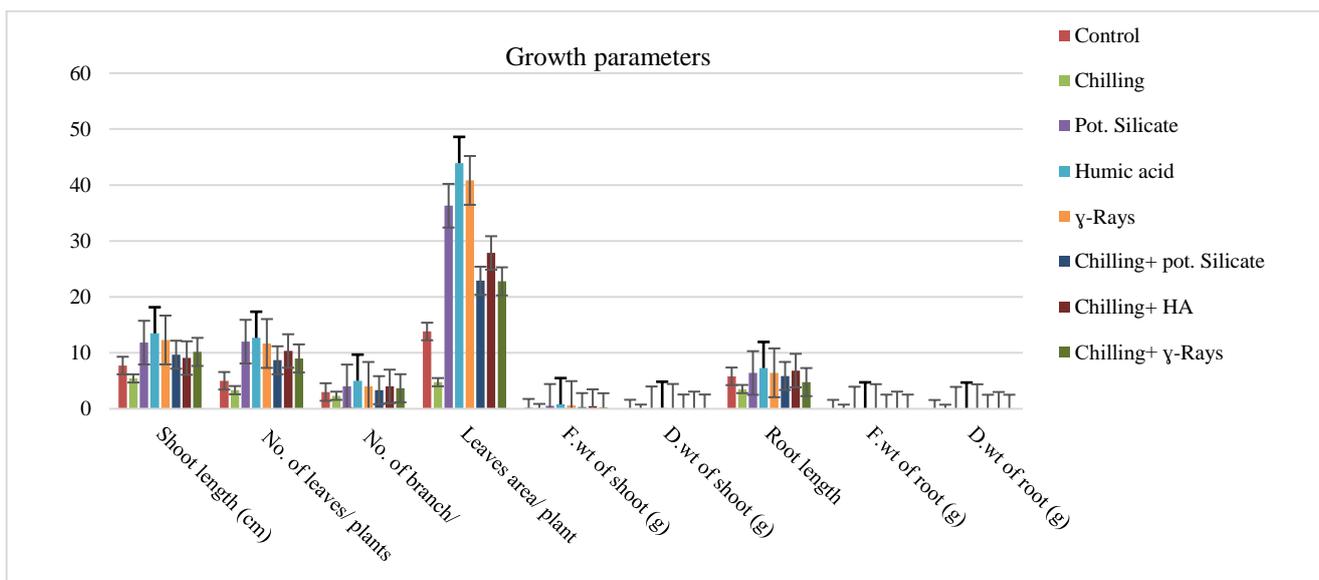


Fig. (1): Effect of chilling stress on (*C. sativum* L) seeds pre-soaked in 80mM pot. silicate, 50 mg. l⁻¹ humic acid or soaked in water after exposure to γ -rays (50 Gy) and the interaction of the alleviation treatments and chilling stress (6 °C \pm 0.5) on growth parameters. The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded. Bar error represents \pm SE.

Priming and γ -rays' effects on nitrogenous constituents

Values of total soluble nitrogen (TSN), protein nitrogen (protein N), and total nitrogen (TN) of coriander plant in response to seeds priming in pot. silicate, HA, or γ -rays were placed in (fig 2). TSN under chilling stress decreased by 34.55%, and increased by 175% and 108%, with pot. silicate treatments of normal and chilled coriander, respectively. Under chilling, protein-N accumulated significantly in plant by 24.86% over untreated control. HA-priming and irradiation techniques were also significant and influential on coriander nitrogenous contents.

Priming and γ -rays' effects on amino acids

Amino acids (AA) composition was listed in (fig 3). Chilling stress increased AA by 51.9% as compared with control plant. It is noteworthy that appearance of

cysteine was detected only in primed chilled seeds with HA or under γ -rays. Generally, coriander plants were rich in essential AA viz., valine, isoleucine, leucine, histidine, and lysine. On the other hand, non-essential AA such as aspartic acid, arginine, alanine, serine, tyrosine, glutamic, and glycine have either increased with the treatments or were not changed by chilling. However, glutamic acid generally decreased except with Pot. silicate treatment which increased by 18.18% over non-chilled plants (fig 3). In this connection, Arginine, aspartic and tyrosine decreased in pre-exposed chilled coriander seeds to γ -rays below the non-primed plants. Glycine and phenyl alanine have similar results to non-chilled coriander plants.

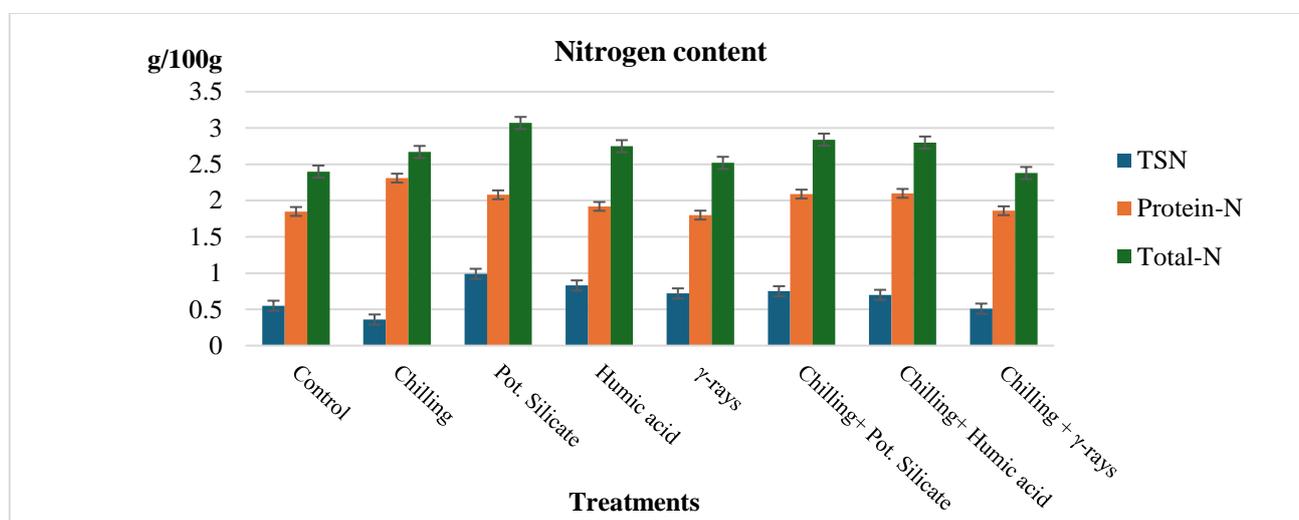


Fig. (2): Impact of alleviation elements on endogenous nitrogenous content (g/100 D. wt.) of (*C. sativum*). Seeds were subjected to pre-soaking treatments in Pot. silicate (80 mM), HA (50 mg l⁻¹) or soaked in water after exposed to γ -Rays (50 Gy) at (6 °C \pm 0.5). The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded. Bar error represents \pm SE.

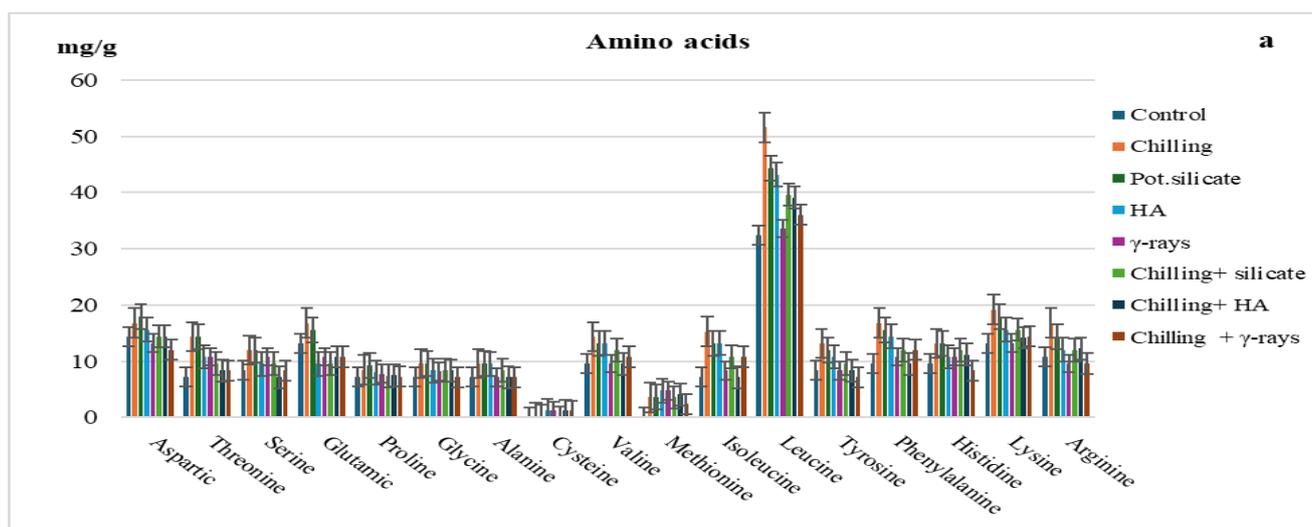


Fig. (3 a): Effect of chilling stress on (*C. sativum* L) seeds pre-soaked in 80mM pot. silicate, 50 mg l⁻¹ HA or soaked in water after exposure to γ -rays (50 Gy) and the interaction of the alleviation treatments and chilling stress (6 °C \pm 0.5) on amino acids as mg/g. The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded. Bar error represents \pm SE.

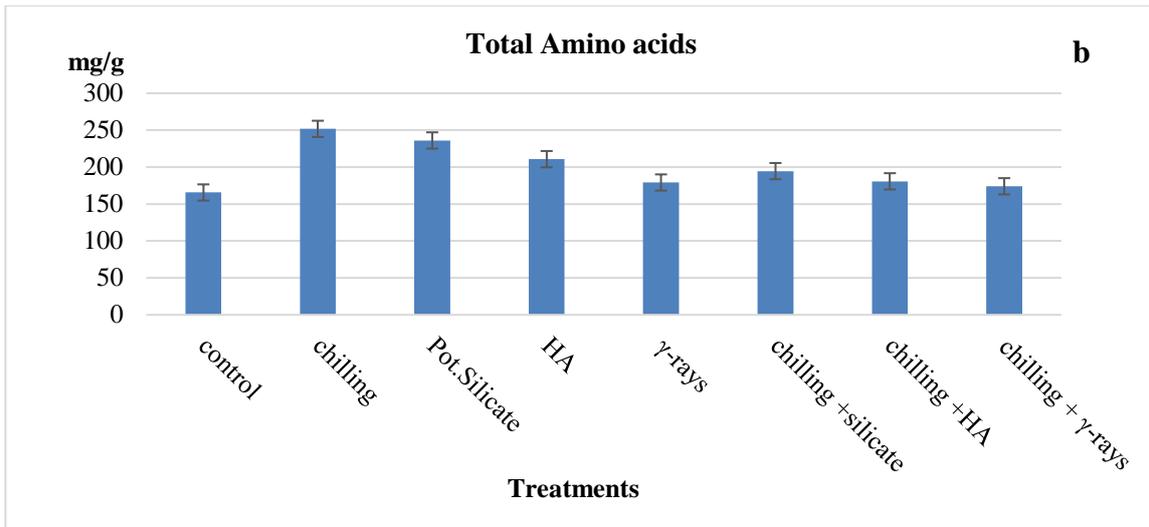


Fig. (3 b): Effect of chilling stress on coriander (*C. sativum* L) seeds pre-soaked in 80mM pot. silicate, 50 mg^l⁻¹ HA or soaked in water after exposure to γ-rays (50 Gy) and the interaction of the alleviation treatments and chilling stress (6 °C ±0.5) on Total amino acids.

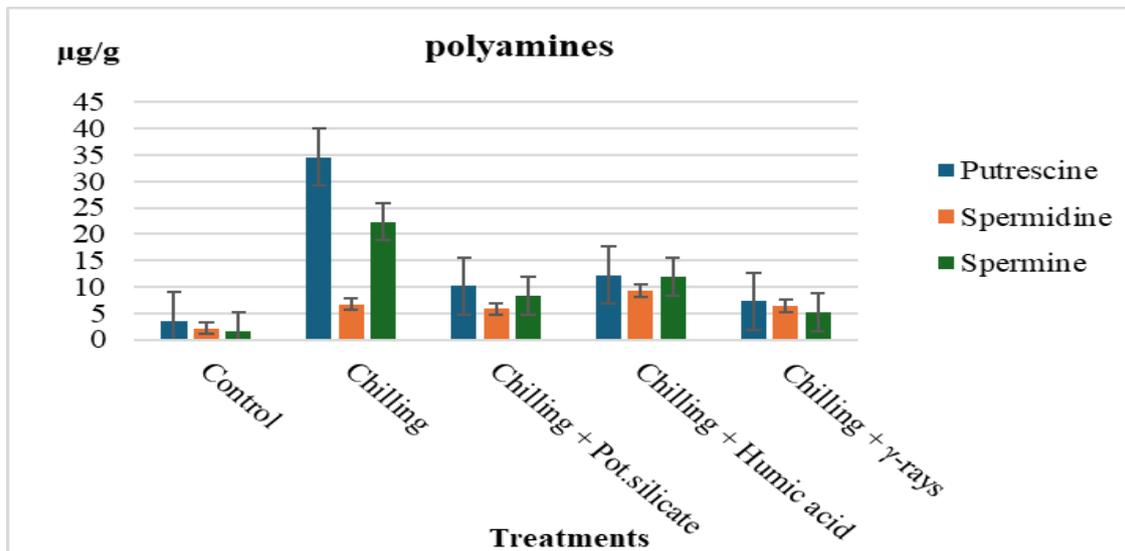


Fig. (4): Effect of chilling stress on (*C. sativum* L) seeds pre-soaked in 80mM pot. silicate, 50 mg^l⁻¹ HA or soaked in water after exposure to γ-rays (50Gy) and the interaction of the alleviation treatments and chilling stress (6 °C ±0.5) on polyamines as µg/g. The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded. Bar error represents ±SE.

Priming and γ-rays' effects on polyamines

Data investigated in (fig 4) revealed chilling impact on increasing levels of putrescine, spermine and spermidine by 874.1%, 1400% and 213.6% over control plant, respectively. As such, total endogenous polyamines viewed eight folds increase compared to control. However, in chilled plants, putrescine accumulation decreased upon priming and irradiation by 70.61%, 64.60%, and 78.90%, with pot. silicate, HA and γ- rays, respectively. In contrast, putrescine in chilled-treated plants evolved by 186.20%, 244.78% and 105.35% compared to control coriander plant,

respectively. In comparison to chilled plants, spermine content decreased with pot. silicate, HA, and γ-rays by 62.77%, 46.44% and 76.82%, respectively, and increased by 458.38%, 703.36% and 247.65% compared to control plants, respectively. Spermidine contents in primed chilled seeds using pot. silicate and γ-radiation decreased by 13.26% and 4.47% compared to chilled plants. However, HA-treated chilled plants increased spermidine up to 38.30% over chilled plants. Total polyamine contents increased in this descending order: chilling > chilling+ HA > chilling+ pot. silicate acid > chilling + γ-rays > control.

Priming and γ -rays' effects on ion content

Changes in nutrition elements (Ca, Mg, K, Na, and Si) in coriander plants were reported in (fig 5). Chilling stress led to a significant decrease in Ca, Mg, and K amounts in coriander leaf by 11.97%, 19.26%, and 14.41%, respectively. Chilling increased Si content significantly (25%) and decreased Na non-significantly (4.166%) as compared to control plants. However, most treatments induced Ca accumulation except Pot. silicate and gamma radiation of chilled plants as both treatments recorded a decrease of 14.40% and 4.80%, respectively. Mg and K ions decreased under chilling stress but increased with all applied methods. Pot. silicate increased Mg and K under normal conditions by 22.96% and 25.23%, respectively, and by 52.29% and 46.32% in chilled experiments, respectively. Chilling stress and all applied treatments caused non-significant changes in Na content. A slight decrease in Na was detected with humic acid primed in the non-chilled experiment and in Pot. silicate treatment with chilling experiment. Si decreased to half in chilled plants treated by γ -rays and increased significantly in response to the other priming methods.

Priming and γ -rays' effects on fatty acids

The fatty acids (FAs) composition as well as the unsaturated/saturated FAs ratio were detected in coriander plants and presented in (fig 6) Oils quality associated with FAs composition is controlled by the presence of oleic, linoleic (omega 6), and linolenic acids (omega 3). Oil of coriander plant grown under normal conditions contains palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C 20:0), in addition to myristic acid (C14:0) as additional FA appeared solely in chilled coriander plant. Moreover, stearic acid has disappeared with gamma radiation. Unsaturated FAs were

higher in percentage in this order: linolenic acid (31.86%), linoleic acids (30.67%) and oleic acids (2.02%) in control coriander, differed in content and accumulation according to chilling stress and priming methods. On the other hand, saturated palmitic (27.45%) was found in high amounts, followed by arachidic acid (5.03%) and stearic acid (1.82%) in control plants (fig 5). Pot. Silicate, HA, γ -rays and their combinations to chilling affected saturated fatty acids, causing palmitic acid decrease compared with control. Stearic acid increased by pot. Silicate and HA disappeared by γ -rays compared to the control. Also, arachidic acid increased over both control and chilled plants with pot. silicate although was never detected with HA or γ -rays. Unsaturated FAs like linolenic, linoleic and oleic acids increased in chilled plants compared to non-chilled plants. Except for linoleic acids with HA, and γ -rays' treatments, the rest of priming treatments led to further unsaturated FAs increase. Total unsaturated FAs exhibited a 10.84% increase in chilled compared to non-chilled coriander, added to 22.10% and 10.16% increase in control and chilled coriander by γ -rays, respectively (fig 6). HA increased total unsaturated FAs by 16.95% and 5.51% in control and chilled plants, respectively. Chilled coriander plants enhanced unsat/sat FAs, as the ratio was further provoked by HA and γ -rays. Concerning unsaturated fatty acid (UFA), chilled plants results showed an increase in linolenic acid, linoleic acids and oleic acids as compared to non-chilled plants. Also, Pot.silicate, HA, γ -rays and their interaction caused additional increase of linolenic acid, linoleic acids, and oleic acids in most of the treatments except linoleic acids in HA and γ -rays plus chilling which were resemble to non-chilled plants. In general, values have even increased over the non-chilled plant. Gamma rays and HA in chilled were the most effective in increasing linolenic acid, linoleic acids, and oleic acids.

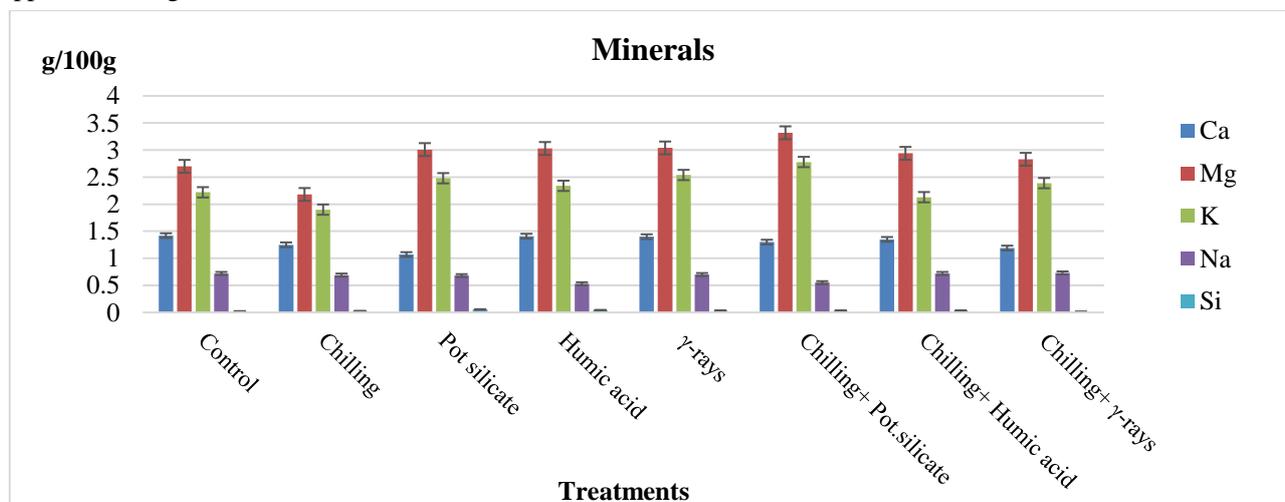


Fig. (5): Effect of chilling stress on (*C. sativum* L) seeds pre-soaked in 80mM pot. silicate, 50 mg⁻¹ HA or soaked in water after exposure to γ -rays (50Gy) and the interaction of the alleviation treatments and chilling stress on minerals composition as g/100g. The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates was recorded. Bar error represents \pm SE.

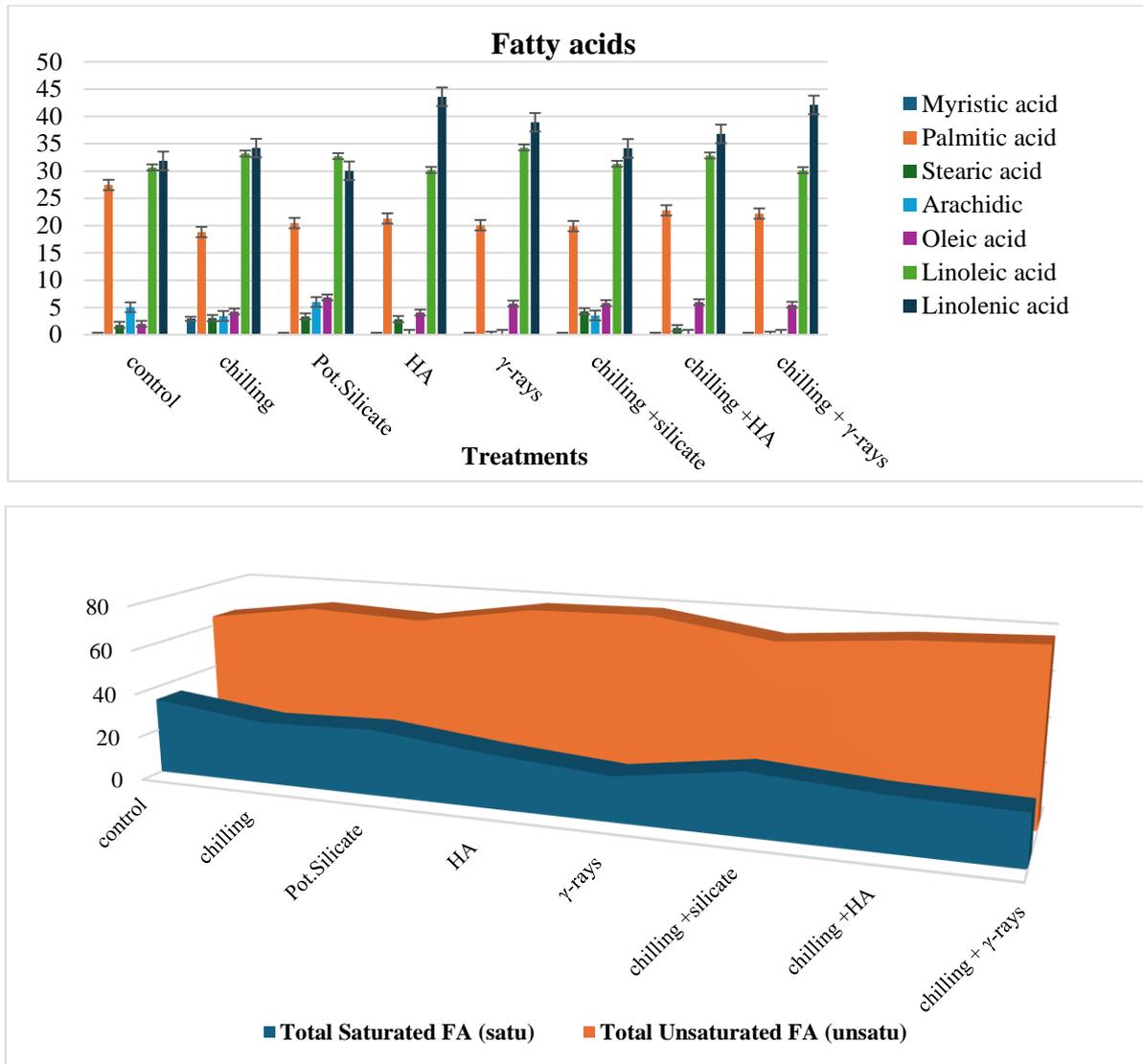


Fig. (6): Effect of chilling stress on (*C. sativum* L) seeds pre-soaked in 80mM pot. silicate, 50 mg l⁻¹ HA or soaked in water after exposure to γ -rays (50Gy) and the interaction of the alleviation treatments and chilling stress (6 °C \pm 0.5) on fatty acid composition as a percentage of leaves oil. The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded. Bar error represents \pm SE.

Priming and γ -rays' effects on volatile oil

The number of detected components in control coriander was 43 compounds (fig 7). The main dominant was decanoic acid (19.5%), Trans-2-decenoic acid (21.37%), and dodecanoic acid (20.73%). While application of chilling, and/or silicate, HA and γ -radiation increased the number of detected components in volatile oil. The maximum number recorded in plants from chilling-stressed seeds with HA, followed by chilling seeds in water and HA at room temperature (30, 29, and 29). The treatments applied were shown to be effective in increasing the health benefits of the coriander plant. After data analysis of GC-MS, the main dominant components of non-

chilled plants were detected as follows; decanoic acid (19.5%), Trans-2-decenoic acid=2 E-2-Dodecnoic acid (21.37%), and dodecanoic acid=Lauric =vulvic acid (20.73%). Whereas the main components of chilled coriander were Trans-2-decenoic acid=2 E-2-Dodecnoic acid (30.08%), Palmitoleic acid=cis-hexadecenoic acid (16.72%), and Myristalchole= tetradecylalchole=tetradecanol (9.39 %). In addition, some components were solely found in untreated control plants like nonane, octanoic acid decyl ester, and nonanoic acid undecyl ester. Also, 2-nonenal and dimethyl 6,7 dichloro2h-1-benzopyrane, 4dicarboxylate were appeared only in coriander plant treated with HA alone and not detected in the other treatments. Moreover, some compounds were disappeared in control under

chilling stress in related to control plant such as heptanal, octanoic acid, 1-hexadecanol, myristilmyristate, eurcic acid, and 1, docosane. Some compounds were newly appeared like 1-decanol, 2-undecanal, cyclododecane, dodecanal, 2-dodecanol, palmitoleic acid, palmitic acid, 2-tridecenoic acid, myristilalcohol, stearic acid, pentadecane, cyclodeceane, 1-octadecanol, and nonacosane. It was worthy to note that palmitic acid was only detected in chilling and Pot. silicate plus chilling samples. On the other side, 1-decanol, 2-dodecanol, palmitoleic acid, 2-tridecenoic acid, and nonacosane appeared in all priming conditions, while the rest were flocculated. The compound heptanal appeared in all priming cases of non-chilled plants and

disappeared under chilling stress condition. However, octanoic acid appeared in all primed-chilled plants. On the same context, 1-hexadecanal was detected in all applied treatments under investigation except in chilled-primed plants with silicate or irradiated with gamma. Myristic acid appeared with all primed non-chilled plants except HA and all primed -chilled. In all priming conditions, except silicate and radiation of chilled-plants, 1-docosane was appeared. The proportion of the following components: nonanoic acid (pelargonic acid), trans-2-undecenoic acid, trans-2-decenoic acid=2 E-2-dodecenoic acid, oleic (9-octadecenoic acid), and 1-nonadecane have increased under chilling condition alone and/or with the priming.

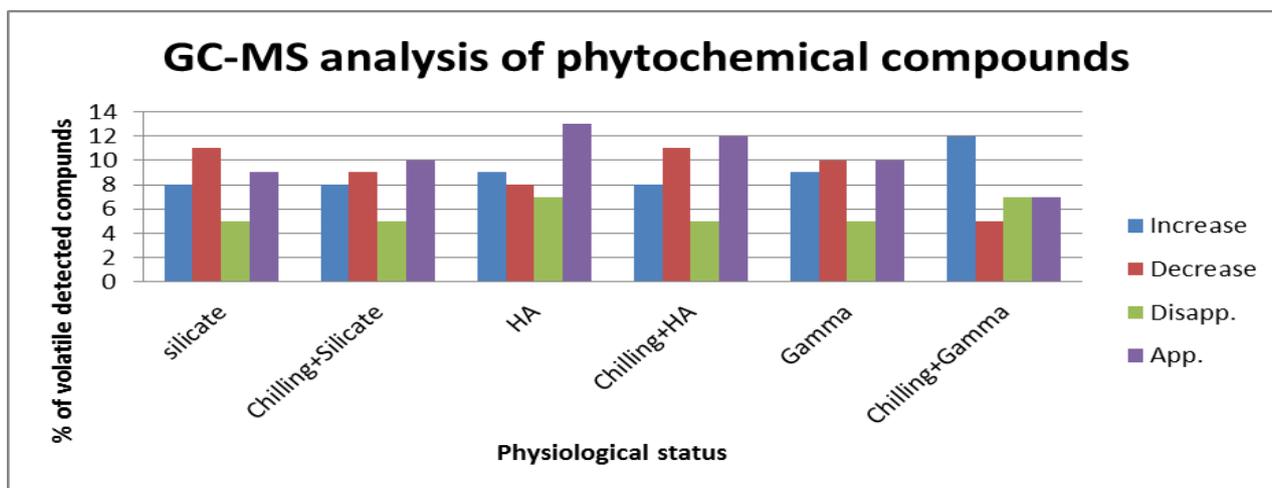


Fig. (7): Percentage of increased, decreased, disappeared, or newly appeared phytochemical compounds to total compounds present in the extracted volatile oil at flowering stage (105-days). Prior planting, Coriander control seeds and chilling-stressed ones ($6\text{ }^{\circ}\text{C} \pm 0.5$) were subjected to pre-soaking in Pot. silicate (80 mM), HA (50 mg l^{-1}) or soaked in water after exposed to γ -Rays (50 Gy). The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded.

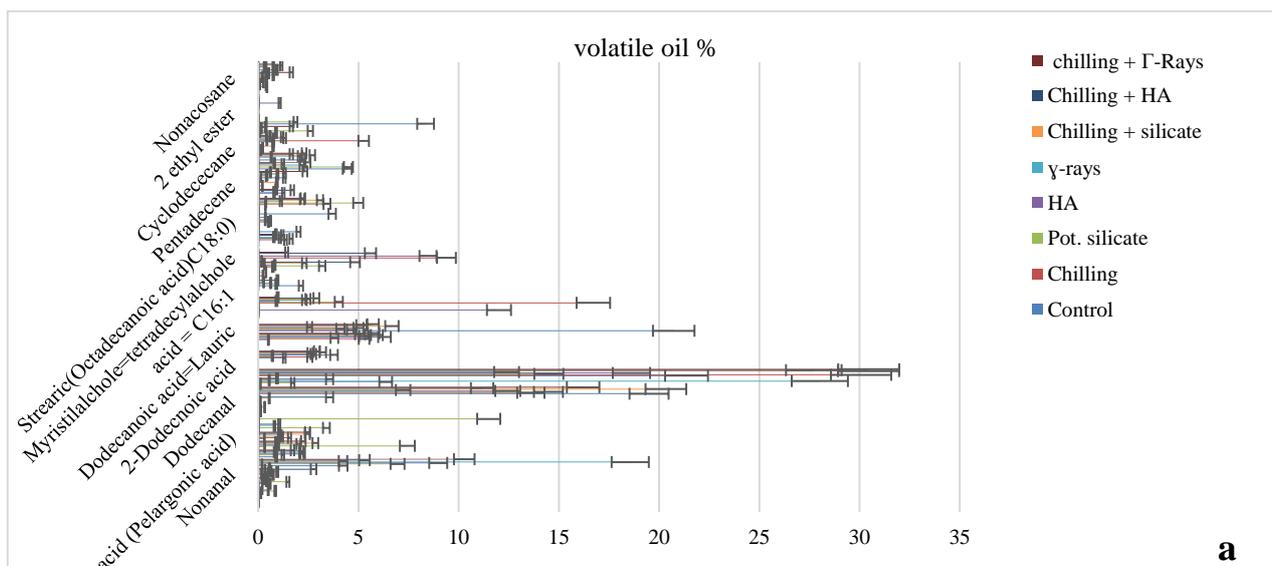


Fig. (7a): GC-MS analysis of phytochemical compounds in the volatile oil extracted from (*C. sativum* L.) leaves and inflorescence by steam distillation at the flowering stage. Prior to planting, Coriander control seeds and chilling-stressed ones ($6\text{ }^{\circ}\text{C} \pm 0.5$) were subjected to pre-soaking in Pot. silicate (80 mM), HA (50 mg l^{-1}) or soaked in water after exposure to γ -Rays (50 Gy). The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded. Bar error represents \pm SE.

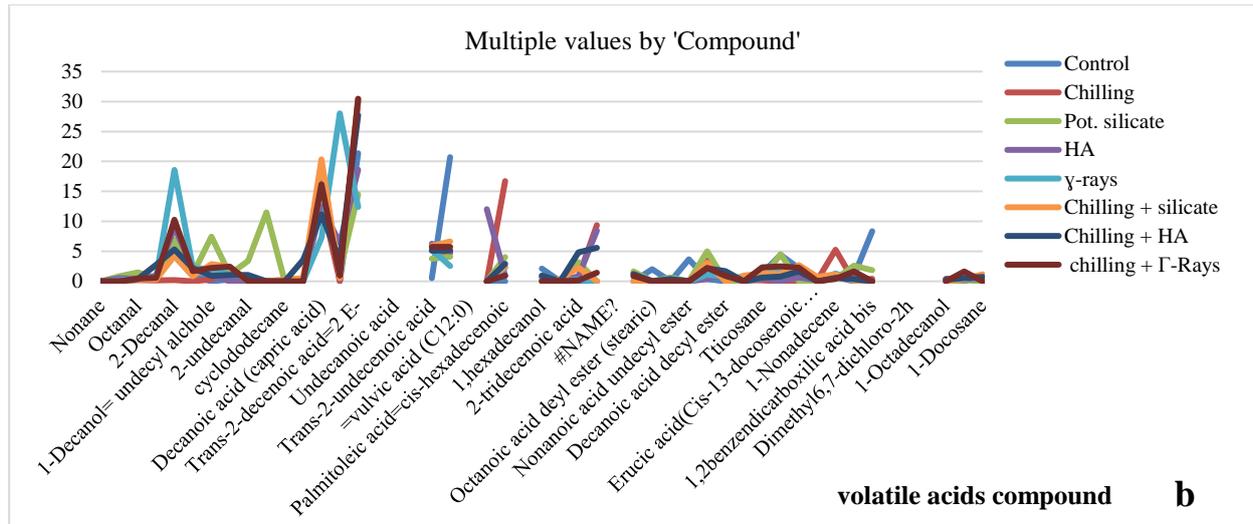


Fig. (7b): The multiple value by compound in volatile oil extracted from (*C. sativum* L.) leaves and inflorescence by steam distillation at the flowering stage. Prior planting, Coriander control seeds and chilling-stressed ones ($6^{\circ}\text{C} \pm 0.5$) were subjected to pre-soaking in Pot. silicate (80 mM), HA (50 mg l^{-1}) or soaked in water after exposed to γ -Rays (50 Gy). The mean value of each biological replicate represents the value of one technical replicate. The reading of the three technical replicates were recorded.

DISCUSSION

In the present study, chilling stress has inhibitory effect on growth parameters in plant. It was observed, as compared with their corresponding control, a decrease in shoot length, number of branches and area of leaves/plant, root length, fresh weight and dry weight of shoot and root of coriander throughout the experiment similar finding has been reported [3, 35, 36]. Reduction in stressed plant growth could be attributed to the decrease in water absorption, cell elongation and alteration of cell division which affect leaf size, weight and minimize their ability to close stomata in response to water deficit similar findings obtained by [7-8]. Also, insufficient water supplies provoked a rapid drop in water loss of leaves during the first hours of cooling [37]. So, the photosynthesis rate decline and affect adversely on CO_2 assimilation which in turn influence growth by lowering the rate of division and elongation. The improvement of growth parameters was triggered by soaking in silicate, HA, and γ -rays to alleviate chilling stress. The most effective treatment was HA in both control and chilling-stressed samples. Humic application has promoted plant growth by acting as a growth regulator. Current results show the effects of priming and gamma radiation in fostering the growth of chilled *C. sativum* till the flowering stage. HA was found crucial for its physiological and metabolic effects on growth and mineral nutrition in plants under stress. Foliar and soil applications using humic substances modulated the chemistry of the soil by changing the pH, increasing the useful microbial flora, and increasing nitrogen and

phosphorus content. Concomitantly, Data showed the potential and superior role of HA in supplying and increasing total nitrogenous compounds, total amino acids, total polyamines, FAs, and minerals in chilled plants till flowering stages by enhancing membrane permeability and root uptake of essential nutrients, alternatively, preventing toxic material uptake. Decreased TSN with HA is ascribed to the shift of amino acid to synthesize polyamines and/or phenolic compounds, also absorption and translocation of nitrate from root to shoots, and/or increasing the protein synthesis. In addition, researchers reported that HA enhanced nutrition with growth-triggered nitrogenous compounds [38,39]. Despite this, the HA effect was even higher than pot. silicate and γ -rays together improved physiological traits, particularly mineral content and polyamines in coriander, pot. silicate was better in restoring N- compounds and γ -rays effect was the best regarding FAs metabolism. Notably total amino acids content increased due to chilling compared to control plant.

In this respect, all applied treatments increased total amino acids content as compared with control plant. High amino acids values were recorded in silicate and HA individually or in combination with chilling stress. Similarly, frequent increments of free amino acid (particularly proline) concentration in plant tissues exposed to many biotic and abiotic stresses were reported [40, 41] as well as alanine which was suggested to play a similar role during osmotic stress [42]. Also, the result was in harmony with Habibi [36] on grapes who found

that silicon supplement to chilled grapes induced higher amino acid concentration as compared with chilled plants which might be attributed to free amino acids accumulation that function as osmolytes to maintain cell turgor and protect membrane from stress damage [43]. These results are in concomitant with those stated that aspartic and glutamic acids were the least to be influenced by the stress, while the contents basic amino acids increased sharply [44]. However, levels of free amino acids, except serine, were enhanced by chilling stress and the applied alleviation treatments. In addition, gamma radiation in exposed coriander to different doses increased most of amino acids concentration in coriander plant similarly to what had been reported [45]. The quantity increases of total amino acids due to either silicon or HA applications can be attributed to increased rate of incorporation of free amino acid(s) into protein. These findings were in consistency with the current results concerning significant increase of protein nitrogen (protein N) constituent, specifically of what concerned effect of silicon or HA applications on chilling alleviation.

Polyamines (PAs) are a group of compatible osmolytes having a crucial role in counteracting the effect of osmotic stress arising from chilling stress. PAs content undergoes a prompt increase in coriander under chilling. Nevertheless, applied treatments particularly γ -rays, followed by pot. silicates exhibited a differential effect although bringing chilled coriander close to normal control condition. Out of total PAs, putrescine (Put, diamine), spermidine (Spd, tri-amine), and spermine (Spm, tetra-amine) were involved with various biochemical and physiological process such as replication, transcription and translation, stabilization of membranes, and modulation of enzyme activities in addition to stress tolerance related to plant growth and development [46]. PAs functioned as antioxidants, having polycationic properties for binding to negatively charged molecules like phospholipids to maintain plasma membrane integrity and protect plant cells from chilling-induced cell damage [47]. Furthermore, PAs bind to radicals of reactive oxygen species to stabilize plasma membrane under stress [48]. The unsaturated fatty acids C18 is the aliphatic ingredients of membrane glycerolipids, involved by themselves or by their triacylglycerols in various defense mechanism against stress, particularly membrane fluidity which been seriously affected during cold thereby, the degree of phosphatidylglycerol unsaturation became an indicator of cold tolerance [49]. However, during cold, membrane rigidity led to membrane malfunction, electrolyte leakage and proteins inactivation [50]. Among the three most

abundant FAs in coriander: linoleic, linolenic, and palmitic, increased linolenic acid and decreased palmitic acids were in alignment with chilling-tolerance in tolerant genotypes [51,52]. Controversy, an opposite behavior was detected in sensitive genotypes pointing to role of lipid unsaturation in restoring membrane stability during chilling [53]. Thus, unsat/sat ratio was enhanced in chilled coriander in comparison to control by humic acid and γ -rays in control- and chilled plants, suggesting HA and γ -rays' intrinsic roles in enhancing lipid unsaturation. This study hypothesized that the ionizing effect of γ -rays that targeted FAs building blocks of plasma membrane, induced membrane overwhelmed and imbalanced FAs profile and distribution in coriander, just to sustain tolerance strategy in coriander as observed with all γ -rays' treatments. For example, the saturated-C14- myristic acid disappeared in control and upon the treatments and appeared at low concentration under chilling disclosing its involvement with chilling escape mechanism. Nevertheless, the simultaneous increase of unsaturated and decrease of saturated FAs is a tolerance mechanism that occurred under water limited conditions [54]. Here, γ -irradiation contributed the most to support coriander tolerance via genotypic modifications, followed by HA. Stearic acid behavior was different and reported to accumulate sharply under salinity and water stress [55, 56]. Stearic acid also increased here under chilling, though was never detected with γ - rays, accounting for the detrimental effect of excess stearic acid as a saturated FA under stress conditions.

In chilled environment, growth inhibition is due to suppression of mineral uptake and mineral allocation between plants parts [57]. However, a non-significant change of particularly, K, Ca, and Na in coriander plants under chilling was reported [58]. Controversially, an increase in Mg and Ca under chilling was recorded [59]. Our data reveal a decline of all nutritional elements either significantly or non-significantly with chilling. Ca diminishes under chilling contradicting previous studies which proposed that cold sensitive- and tolerant plants increased their cytosolic Ca when exposed to chilling stress by Ca influx from intercellular spaces [60]. The HA role of triggering ion transfer at the root level, activating the oxidation-reduction state of the medium, and increasing the absorption of nutrients studied [61]. In addition, HA capability to promote hormonal activity as well as promoted antioxidant production in plants which, in turn, reduces free radicals, and increases root vitality to improve nutrient uptake [62]. Limited information was

reported about the essential oil composition of inflorescence associated with leaves in coriander plants.

In the present work, GC–MS revealed that the essential oil extracted from the coriander inflorescence is marked by a significant percentage of aliphatic aldehydes, alkenals, alkanals and alcohols, saturated and unsaturated fatty acid, among which heptanal, nonanal, 1-decanol, 2-dodecanol and 2-decenal. Major changes of detected phytochemicals were shown and denoted downwards. HA followed by γ -radiation were the most effective alleviation elements that affected the phytochemical profile of coriander plants. Coriander essential oil was considered as a source of bioactive compounds with many health benefits. Chilling stress has increased Trans-2-decenoic acid by 30.08 % over control plant and triggered the appearance of new compounds, especially monounsaturated fatty acid palmitoleic (omega-7; 16.72%) and oleic acid (omega-9) in chilling and all applied treatments. Later Fatty acids (omega-7 and omega-9) are regarded as an alternative source of natural antioxidants [63]. So, coriander has protective effects as an antioxidant against oxidative stress-induced diseases. Volatile oil encloses multiple antioxidative stress compounds that participate in defense mechanisms under stress conditions.

CONCLUSIONS

Applications of humic acid, potassium silicate, and γ -radiation individually or in association with chilling stress are safe methods for improving and stimulating bioactive and healthy components in coriander plants through their protective effects as antioxidants. Humic acid was the best effective priming agent shown in this study followed by potassium silicate.

Acknowledgments All gratitude to NCRRT for support and providing crucial research grant without which this work would not have appear to light. Also, the authors appreciate the collaboration of postgraduate studies and research affairs, Faculty of Science, Ain Shams University, Cairo, Egypt.

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ABBREVIATIONS:

Coriandrum sativum (*C. sativum*); Humic acids (HA); Potassium silicate (Pot. Silicate); Gamma rays (γ -rays); Fatty acid (FA); Polyamines (PAs); putrescine (Put); spermidine (Spd); spermine (Spm); Amino acids (AA); saturated fatty acids (SFA); unsaturated fatty acid (UFA).