Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: March 2022 **2022, 19(5): 966-975** **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2022.6161

Evaluation of Atmospheric Cold Plasma Technique Activity on Phenylpropanoids Gene Expression and Essential Oil Contents and Different Traits of *Ocimum basilicum* L.

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Received 26/3/2021, Accepted 1/9/2021, Published Online First 20/3/2022

Abstract:

The current study was conducted for studying the impact of cold plasma on the expression level of three genes that participate in the biosynthesis of the phenylpropanoid pathway in *Ocimum basilicum.* These studied genes were cinnamate 4-hydroxylase (c4h), 4-coumarate CoA ligase (4cl), and eugenol O-methyl transferase (eomt). Also, the cold plasma impact was studied on the essential oil components and their relation with the gene expression level .The results demonstrated that cold plasma seeds germination of the treated groups 2 (initially for 3 minutes and 3 minutes after 7 days) ,and group 3(initially for 5 minutes and 3 minutes after 7 days) were faster than the control group. Also, the height average of the mature plants of groups 2and 3 was between (50 to73), (50 to 100) cm, respectively compared to the control group which was (40 to 70cm). Moreover, the results indicated significant differences (P-value ≤0.01) in the level of gene expression, which increased for the c4h, 4cl, and eomt genes in group 2 about (5.63 ±0.39), (3.42 ±040) ,and (5.41±0.23) folds respectively compared with untreated control. Additionally, the level of gene expression increased for the c4h, 4cl, and eomt genes in group 3 about (42.34 ±049), (4.13 ±0.38), and (6.29 ±0.71) folds compared with untreated control(1.00 ±0.00) folds.Concerning the contents of essential oil for the control group, group 2, and group 3 were 0.434%, 0.713, and 0.792% (v/w) respectively. Moreover, the general composition of the essential oil in the examined sweet basil, phenylpropanoids was the eugenol compound and its derivatives for the control group, group 2, and group 3 which were 2.76%, 8%, and 11% (v/w) respectively. We concluded that the atmospheric cold plasma has shown an effect on gene expression and essential oil content of phenylpropanoid compounds in *Ocimum basilicum* L. cultivated in Iraq, as the essential oil contents have important therapeutic properties.

Keywords: Cinnamate 4-hydroxylase (c4h), Cold plasma, 4-coumarate coA ligase (4cl), Eugenol O-methyl transferase (eomt), *Ocimum basilicum* **.**

Introduction:

Ocimum basilicum, belonging to Lamiaceae family, is the genera famous for its medical features and aromatic oils which are important economically [1],and is mostly cultivated to produce essential oils .It also which has a high component of phenylpropanoid derivatives, like eugenol, methyleugenol, chavicol ,metheylchavicol, linalool, a monoterpene, and sesquiterpenes. The phenylpropanoids are phenolic small molecules, which are an important component in many herbs such as basil, cinnamon, cloves, and tarragon [2] .These components are synthesized and stored in peltate glands which are found on the surface of leaves, stems, and flowers [3] . *Ocimum* genus is known in high phenolic compounds and also has therapeutic potentials [4]. *O. basilicum* has analgesic, anti-inflammatory, antimicrobial, and cardiac stimulating [5]. The essential aroma components in the basil volatile extracts displayed anti-oxidative activity, inhibit oxidative damage which is related

to cancer, atherosclerosis, premature aging, and diabetes [6].Besides, the essential oils of plants included major constituents such as general terpenoids and phenylpropanoid, which are substantial sources of aromatic and food flavoring, industrial, and the products of pharmaceutical [7]. It has known little about the biosynthesis and organizing of the compounds responsible for the flavor quality of the sweet basil, although the importance of its oils and is used widely [8].The biosynthesis of phenylpropanoid is produced from the pathway of shikimate and organized by many groups of the reactions enzymatic via metabolic channels[9]. The phenylpropanoid derivatives are obtained from cinnamic acid, which is created from phenylalanine via the deamination action of phenylalanine ammonia-lyase (PAL). PAL is an important enzyme which has a role in the organization and the production of phenylpropanoid in plants [10].Cinnamate4-hydroxylase is a major enzyme in the pathway of phenylpropanoid [11]. The c4h has a role in catalyzing the hydroxylation of trans-cinnamic acid to p-coumaric acid [12] ,which is considered the second enzyme in the biosynthetic pathway of phenylpropanoid [13]. 4-coumarate CoA ligase (4cl) is considered the third enzyme for the phenylpropanoid pathway, which has a focal role in organizing the total flow of the hydroxycinnamic acids into pathways of subsequent biosynthetic [14] . Concerning the final biosynthetic step, it includes the formation of methyl eugenol catalyzed via eugenol O-methyltransferase enzyme (eomt) [15]. It represents the predominant compound in the essential oil and eugenol possesses anticancer activity against various types of cancers and reported to have excellent inhibitory effect against number of cancer cell lines [16] .Eugenol compound and its derivatives have medical and pharmaceutical properties[17].

So there are three genes responsible for organizing the gene expression of these enzymes, these are c4h, 4cl ,and eomt [2] . The sequence availability for these genes especially assists in the recognition of the conditions under which expression levels are promoted [18].The cold plasmas have developed especially and purposefully relying on their non-equilibrium properties and their ability to cause physical and chemical reactions with the gas at relatively low temperatures[19, 20]. Atmospheric non-thermal plasma dielectric barrier discharge (DBD) is a plasma type that has a non-uniform distribution of energy [21]. It gains reactivity from the high-energy electrons, while the ions and neutral species remain cold [20] . Also, DBD is considered typical to generate a large volume of non-equilibrium atmospheric pressure diffuse plasma. The utilization of cold plasma technology in many various fields of work includes the effect of cold plasma on seed germination [22]. Furthermore, the promising properties for cold atmospheric pressure plasma (CAP) appeared, because it has a dual feature that it eliminates pathogenic microorganisms from the surface of seeds and it assists the germination of seed [23]. Also, the atmospheric non-thermal plasma is important for the treatment of biological systems in its application at physiological temperatures. Moreover, the occurrence and constancy of plasma constituents (reactive oxygen , UV, nitrogen species, visible light, and electric magnetic fields) are restricted in time and space and do not drop out residues of synthetic chemical on the target [24] . Therefore, this study aims to the utilization of cold plasma feature in assisting the germination of *Ocimum* seeds and increasing the organization of gene expression of these enzymes which are c4h, 4cl, and eomt that have a role in the biosynthesis of phenylpropanoid derivatives production, considered the major compounds in the essential oil of *Ocimum* and has therapeutic potentials.

Material and Methods:

Plant material and germination conditions

Ocimum basilicum seeds (sweet basil) 5 Kg were cultivated in Iraq and have been obtained from AL- Diaa Office for Agricultural and Veterinary Consultation plant in Baghdad belonging to the family Lamiaceae which is classified by the herbarium of Department Biology/College of Science/ Baghdad University .The seeds were divided into three groups and all these groups have been planted and grown in the greenhouse of the Botanical Garden/College of the Science / University of Baghdad.

Experimental Apparatus (DBD)

The experiment was carried out in cold plasma technique Laboratory, Department of Physics, College of Science for women, University of Baghdad. An atmospheric dielectric barrier discharge (DBD) system was used to generate non-thermal plasma or cold plasma, the device composed of two parallel electrodes made of copper material with a diameter of (90mm), surrounded by an insulating material of Teflon with a thickness of (10mm), a high power, as well as the exposure to the plasma which is placed on it. The upper electrode is connected to a vertically moving base to change the distance between the two electrodes and this electrode is connected to the end of the high voltage outlet of the high power supply and the two electrodes are separated from each other with an insulating material of coloration or glass. It is

placed above the lower pole of the system, and the dielectric system is fed with a variable high voltage by connecting it to a transformer whose output voltage is (12) kv, type (Ac) with specifications (220 volt input, frequency 50 Hz, and 15 Kvolt output voltage).

Seeds treatment via atmospheric pressure plasma system

The plant seeds were divided into three groups as follows:

Group 1. Control group (1 Kg), seeds without Non-thermal Plasma Dielectric Barrier Discharge (DBD) treatment.

Group 2. Seeds group (1 Kg), treated with Non-thermal plasma Dielectric Barrier Discharge (DBD) for 3 minutes and repeat the treatment for 3 minutes after 7days.

Group 3. Seeds group (1 Kg), treated with plasma for 5 minutes and repeat the treatment for 3 minutes after 7 days.

Sequentially all seed groups were subjected to direct treatment which is directly placed between the electrodes or placed under the plasma system and the treatment worked by being placed inside the glass of the Petri dish. The seeds uniformly distributed at the bottom dielectric plate were treated for regular time intervals in a DBD system operated at atmospheric pressure in the atmosphere dielectric barrier discharge system [25, 26].

Conditions of growth and harvesting of *Ocimum basilicum*L. plant

The groups of seeds from sweet basil (*Ocimum basilicum*), have been planted and grown between August and October in 2020. The plants were watered every day and maintained at day/night temperatures of 37-35^{o} C and 30-25 ºC, respectively .The first harvesting phase of the control group and treatment group 2 and treatment group 3 sequentially, took place in the seedling stage. Once the seeds sprouted and there was visible evidence of seedlings, in the stage of two pairs of leaves, (two weeks), and all these samples were immediately frozen and preserved at -20 °C for genetic analysis. The other of the seedlings from all groups were left to grow and all plant height was monitored up to the time of second harvesting. Once a critical mass was reached, plants were harvested for the next step [25, 27].

RNA extraction and cDNA synthesis

Total RNA extraction from the plant was done according to the protocol of [28] by using TRIzol reagent (Thermo Scientific, USA). Quantus Fluorometer (Promega, USA) was used to detect the concentration of extracted RNA or cDNA to evaluate the goodness of samples by using Quantifluor RNA System .199µl of diluted QuantiFluor Dye was mixed with 1 µl of cDNA or RNA, after 5 minutes of incubation at room temperature, RNA concentration was measured.

PCR primer and the analysis of Real-Time PCR

Syber Green quantitative PCR was done according to the manufacturer's instruction of Go Taq one-Step RT- qPCR (Promega ,USA).The used primers (Macrogen, Korea) for quantitative real-time polymerase chain reaction are chosen according to [29] for (c4h) forward 5`GGATCATTCTTGCCTTGCCTATACT3`and reverse 5`ATA ACAATGGTGGAGTGCTTCA AAA-3`. While according to [2] the primer of 4cl was chosen the forward 5`-TCGCAAAA CAGCCACTA CCGAC-3`and the reverse 5`-AGGTG CAGCAAGTTT GGC TCTC-3`. Whereas according to(30) the forward of (eomt) was 5`-TGTCGACAGAGCAACTTCTT-3`and the reverse 5`-GGATAAGCCTCTATGAGA GACC-3`While tubulin chosen according to [30] the forward 5`-CTCCTTGAGCTA GTCGTCGC-3`and the reverse 5-AACAAGG CAAAA ACATTCCG 3`.

Ocimum basilicum c4h, 4cl, and eomt genes were amplified from the synthesized cDNA with primers. Moreover, tubulin (Tub) was established as a housekeeping gene to normalize the dissimilar RNA concentrations during RNA extraction. The reverse transcriptase enzyme activation was performed in duplicate at 37 ˚C for 15 min in 1 cycle, to convert RNA to cDNA, and initial denaturation began at 95˚C for 5 min, followed by 40 cycles for the last three-step: denaturation at 95˚C for 30 seconds, annealing at 60˚C for 30 sec and extension at 72˚C for 30 seconds.

Plant Extraction by Supercritical Fluid Extraction Technique (SFE)

After the second harvest of the plants from each group (control group, group 2 and group 3), the collected plant material (aerial parts of a plant) were dried and stored at room temperature .The samples of each group were ground .An essential oil (phenylpropanoids) compounds were extracted from aerial parts of the plants, the experimental runs were conducted in the SFT unit, containing extraction cell of approximate length of 30 cm and inside diameter of 5 cm) and 200 bar pressure, the fixed bed was formed inside a nylon basket . Commercial carbon dioxide was used for Supercritical fluid SFT (99.99) purity. A total of 500 g of *Ocimum basilicum* L. of each group was added to the extraction basket; the temperature was 50^{o}C . After reaching thermal equilibrium, the system was slowly pressurized by opening the valve at the extractor's inlet and allowed CO_2 to flow through the extraction basket at 200 bar pressure, when the

system reached the operating pressure and stabilized, the valve from the extractor's outlet was opened and the extraction process began.

Qualitative and quantitative identification of active compounds (Phenylpropanoids)in *Ocimum basilicum* extracts groups by using GC- MASS technique.

A combination of plasma-treated groups (2, 3) and non-treated group oil extracts that were extracted from *Ocimum basilicum* L.(Sweet basil) groups grown in a greenhouse that was supercritical fluid extraction from the aerial parts of the plant were analyzed performed using GC-MS (model Shimadzu Qp 2010, Germany) equipped with a VF-5 fused silica capillary column (30 m ^{x} 0.25 i. d. mm film thickness 0.25µm, Germany). For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml. Injection temperature was 280 $^{\circ}$C. Injector and mass transfer line temperature set at 250 $^{\circ}$C to 280 $^{\circ}$C. The oven temperature was 80 $^{\circ}$C. Diluted samples (which were prepared in methanol) of 0.3 µl were manually injected in the splitless mode .Identification of Compounds of the samples was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with the standard. The start time was 2.98 min and the end time was 33.00 min.

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means in this study [31].

Results:

Effect of treated plasma on seed germination and growth

The groups of *Ocimum basilicum* L. plant seeds showed morphological changes in the external surface when exposed to non-thermal plasma by atmospheric dielectric barrier discharge DBD technique. The seed surface of groups 2 and 3 became post-treated more hydrophilic than the control group. Also, the germination of treated groups 2 and 3 was faster than the control group where the germination process of 2 and 3 treated groups sprouted after 5 days after planted.The height average of the mature plants of groups 2and 3 was between (50 to73),(50 to 100) cm, respectively, whereas the height average of the control group was (40 to 70cm).

The effect of atmospheric non-thermal plasma Dielectric barrier discharge (DBD) on gene expression

The plasma treatment for 3 minutes and 3 minutes after 7days by dielectric barrier discharge up-regulated the transcription of the c4h, 4cl, and eomt genes in group 2 about (5.63 ±0.39), (3.42 ±040) and (5.41±0.23) folds respectively compared with untreated control (1.00 ±0.00). Moreover, The plasma treatment for 3 minutes and 5 minutes after 7days up-regulated the transcription of the c4h, 4cl, and eomt genes in group 3 by about (42.34 ±049) , (4.13 ±0.38), and(6.29 ±0.71) folds compared with untreated control(1.00 ±0.00). So, the results indicated significant differences with a p-value $\leq$ 0.01 as shown in Table(1).Strikingly that plasma treatment by dielectric barrier discharge demonstrated to alter the expression for genes at the transcriptional level. Additionally, the exposure times caused the raising of the induced expression of c4h, 4cl, and eomt genes. The effects of cold plasma on gene expression for c4h, 4cl, and eomt genes fig.1 (A, B, and C).

Moreover, the effects treatment via cold plasma appeared in seedlings of group 3 which had a maximum stimulatory effect than in seedlings of group 2.

Table 1. Show the comparison between different groups in fold change of c4h, 4cl, and eomt genes in *Ocimum basilicum* L.

Groups	Groups Mean±SE			LSD (P-value)
	Group1	Group2	Group3	
c4h	1.00 ±0.00 **c**	5.63 ±0.39 b	42.34 ±049 a	1.26** (0.0001)
4cl	1.00 ±0.00 **b**	3.42 ±040 a	4.13 ±0.38 a	1.112** (0.0012)
eomt	1.00 ±0.00 **b**	5.41 ±0.23 a	6.29 ±0.71 a	1.50** (0.0003)

This means having the different letters in the same row differed significantly. ** (P≤0.01).

Group1: Control without treated cold plasma; Group2: treated 3 minutes and after 7day treated with 3 minutes. Group3: treated 5 minutes and after 7day treated with 3 minutes.

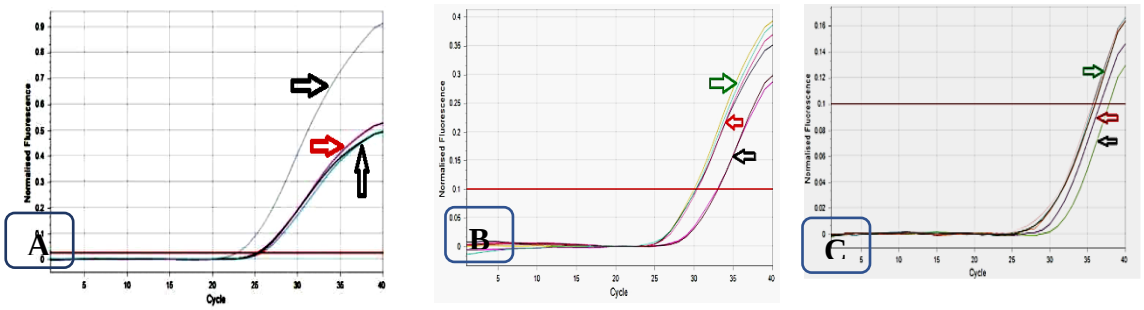

Figure 1. Quantitative real -time PCR (gene expression) curves for genes of *Ocimum basilicum* L. (A) c4h, (B) 4cl and (C) eomt in the three groups :group 1 control without treated(⇨), group 2 (⇨) treated 3 minutes and after 7day treated for 3 minutes and group 3(⇨) treated for 5 minutes and after 7day treated for 3 minutes. A high variance in the effectiveness of cold plasma appeared through the basil plant phenylpropanoid compounds genes expression curves, and showed high significant effect compared to the control (group 1) and the variation in expression between basil plant genes with the group (2), which indicates the efficiency of cold plasma technique in improving the quality of plant and quantity of foods essential oils. Cold plasma involves high different chemical reactive species and high numbers of reactions, which means understanding their interactions with plant components is very complex. However, the reactive species of plasma is the main factor for all changes in the chemical quality characteristics of the treated plants.

Qualitative and Quantitative Characterization of eugenol and its derivatives in *Ocimum basilicum* L. Plant Extracts using GC-MASS

The contents of essential oil ,which were extracted using supercritical fluid extraction technique (SFT) for the control group, group 2, and group 3 were 0.434%, 0.713, and0.792 % (v/w) respectively. The samples were characterized as qualitative and quantitative by chromatographic method GC-MS . Moreover, the results revealed that the general component of the essential oil in the examined sweet basil, phenylpropanoids was the eugenol compound and its derivatives for the control group, group 2, and group 3 which were 2.67%,8%, and 11% (v/w) respectively as shown in figures(2,3,and 4).

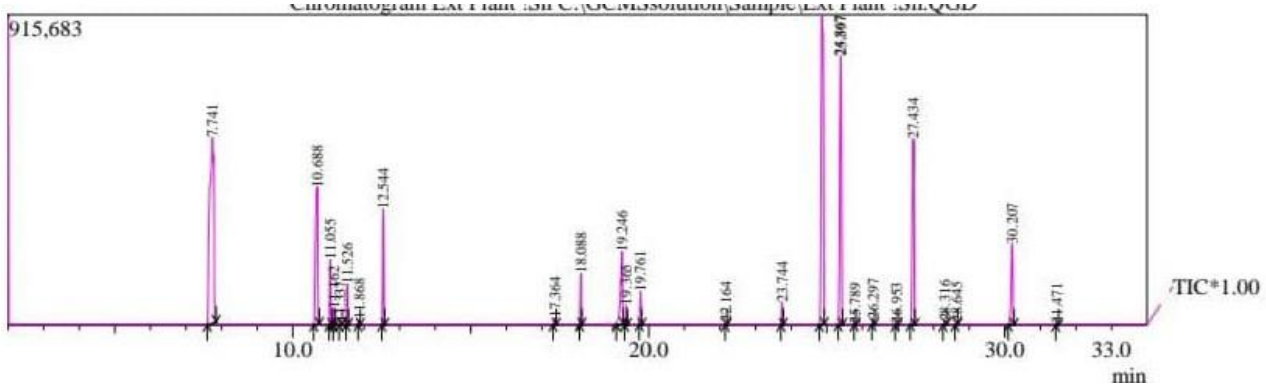

Figure 2. Gas chromatography-mass spectrometry chromatogram of *Ocimum basilicum* L. essential oil extracts for the control group.

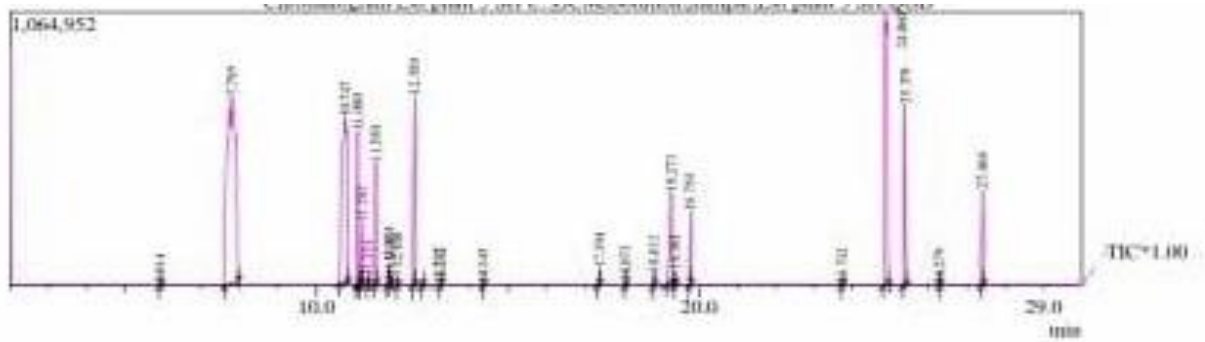

Figure 3. Gas chromatography-mass spectrometry chromatogram of *Ocimum basilicum* L. essential oil extracts for group 2.

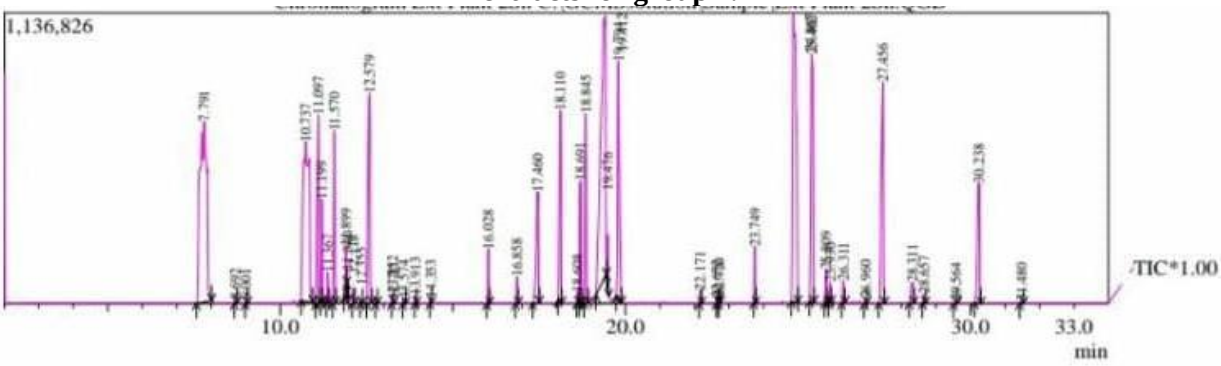

Figure 4. Gas chromatography-mass spectrometry chromatogram of *Ocimum basilicum* L. essential oil extracts for group 3.

Discussion:

Through the results of our study, it has been observed that there is agreement with another study that used an atmospheric pressure discharge and showed that dielectric barrier discharges (DBD) increase gene expression which is responsible for growth and development. Additionally, other researchers who applied cold plasma technology have confirmed which it is superior in the treatment of particulates to other technologies [32]. Besides, the exposure time of cold plasma affects the germination of *Moringa oleifera* seeds by using the different exposure times (1, 5, 10, and 15 minutes) compared to untreated seeds [33].The processing of cold plasma is a novel technique that increases the yield of plants that are known to be key producers of essential oils specially phenylpropanoids compounds [19, 34]. Other studies showed an increase in activities and gene expression of c4h and 4cl when the plant treated with atmospheric non thermal plasma (DBD) compared with control group [35].

Other studies observed an increase in the expression of genes in roots and shoots of NTP-treated wheat seedlings post-treatment by non-thermal plasma also the induction of expression being more faster in aerial parts and roots [36, 37]. Furthermore, one of the studies proved that treatment by cold plasma improves the rice seed germination which was affected via heat stress through affecting the epigenetic regulation [38] . In this current study, the effects of treatment via

atmospheric non thermal plasma dielectric barrier discharge (DBD) appeared in seedlings of group 3 which had a maximum stimulatory effect than in seedlings of group 2. Plasma chemistry is a complex science involving numerous species in a myriad of chemical reactions occurring in different time scales [39.] For example, air plasma involves over 75 different chemical species in almost 500 chemical reactions, making it more complex to understand their interaction with food components. However, plasma reactive species are considered to be the major factor for all the observed changes in the chemical quality attributes of the treated products, which are discussed in the following sections. It is worth noting that plasma reactive species are largely dependent on the gas used for plasma generation, making this one of the most critical factors for chemical changes [40].

Strikingly, there is a relationship between atmospheric non-thermal plasma dielectric barrier discharges (DBD), gene expression, enzyme activity, secondary products, and essential oil yield. As explained in the results, the extracts obtained using the supercritical fluid extraction technique had major compounds identified and detected in essential oils (phenylpropanoids) in the aerial parts of *Ocimum basilicum* L. plant at 50 ˚C and 200 bar of pressure. Supercritical fluid extraction (SFE) is interesting for processing natural products because it produces extracts without organic residues, also the temperature of the process can be reduced, therefore it can preserve thermosensitive compounds [41]. Total essential oil contents in this study according to GC-MASS results indicated that the effect time exposure of atmospheric non-thermal dielectric barrier discharge (DBD) in group 3 influenced strongly on the expression of essential oil phenylpropanoids compounds in group 3 and more effective than control and group 2.

According to the results of this study, the maximum quantity of essential oils was eugenol and in its derivatives for the aerial parts of the plant during the vegetative growth it was observed the maximum value in group 3.

It has been concluded that the c4h, 4cl, eomt representing the regulatory enzymes in the phenylpropanoid pathway play a key role in the regulation of eugenol and its derivatives level in the *Ocimum basilicum* L. plant where the level of gene transcription and activity for c4h, 4cl, and eomt increased during the vegetative growth. The results in this study were in accordance with other researcher's observations, the gene expression of several genes for seeds of diverse plant species was affected by cold plasma [42] . Additionally atmospheric non thermal plasma dielectric barrier discharge (DBD) caused increase in gene expression through the methylation of RNA which represent Chemical modification of RNAs are invaluable ways to increase the regulation in genes [35,43] .

Also, atmospheric non-thermal plasma dielectric barrier discharge (DBD) influenced the priming of sunflower as it was related to transcriptional responses and variation in phytohormones [44]. Taking together, the results along with these reports underlining the hypothesis that atmospheric non-thermal plasma dielectric barrier discharge (DBD) can modify transcription of genes, that way improve plant growth and metabolism. Consequently, more genetic studies are required to elucidate the potentially involved mechanisms in plant responses by using atmospheric non- thermal plasma Dielectric barrier discharge (DBD).

Conclusion:

This study is considered the first of its type as it has tackled the evaluated of atmospheric non-thermal plasma dielectric barrier discharge (DBD) on gene expression and the essential oil content. Which represent an advanced step in molecular biology to increase the production of secondary compounds of medical importance and increase the agricultural production of plants with high-importance properties and without damage to the environment.

Acknowledgments

We are thankful to Prof. Dr. Hamed Hafidh Murbut in Laboratory plasma /Department of Physics/College of Science for women / University of Baghdad., also grated to Environment and Water Directorate /Ministry of Science and Technology/Iraq.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors' contributions statement:

Shaimaa Fakhri Jasim , Labeeb Ahmed Al-zubaidi and Nemat J.Abdulbaqi conceived of the presented idea. Shaimaa Fakhri Jasim and Labeeb Ahmed Al-

zubaidi developed the theory and performed the computations .
Shaimaa Fakhri Jasim and Abdulrazaq Dawood Jasim verified the analytical methods. .
Labeeb Ahmed Al-zubaidi and Nemat J. Abdulbaqi supervised the findings of this work. All authors discussed the results and contributed to the final manuscript

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Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: March 2022 **2022, 19(5): 966-975** **E-ISSN: 2411-7986**

تقييم نشاط تقنية البلازما الباردة في الغلاف الجوي على التعبير الجيني للفينيل بروبانويد ومحتويات الزيت العطري والسمات المختلفة لنبات الريحان *Ocimum basilicum* L.

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الخلاصة:

اجريت الدراسة الحالية لدراسة تأثير بلازما الباردة على مستوى التعبير لثلاثة جينات تشارك في التخليق الحيوي لمسار فينيل بروبانويد في نبات الريحان. كانت هذه الجينات المدروسة (c4h) cinnamate 4-hydroxylase، (4cl) 4-coumarate CoA ligase ، و (eomt) eugenol O-methyl transferase. كما تم دراسة تأثير البلازما الباردة على مكونات الزيت العطري وعلاقتها بمستوى التعبير الجيني. أظهرت النتائج أن البلازما الباردة زادت من انبات البذور للمجموعة الثانية (تمت معاملتها بالبلازما لمدة 3 دقائق و 3 دقائق بعد 7 أيام) ، والمجموعة الثالثة (تمت معاملتها بالبلازما لمدة 5 دقائق و 3 دقائق بعد 7 أيام) كانت أسرع من مجموعة السيطرة الغيرمعاملة. كما تراوح متوسط ارتفاع النباتات الناضجة للمجموعتين الثانية والثالثة بين (50-73) و (50-100) سم على التوالي مقارنة بمجموعة السيطرة (40-70) سم.. كما أشارت النتائج إلى وجود فروق ذات دلالة إحصائية في مستوى التعبير الجيني عند مستوى احتمالية ≥ 0.01 التي زادت بالنسبة لجينات c4h و 4cl و eomt في المجموعة الثانية حوالي (5.63 ± 0.39) و (3.43 ± 0.40) و (5.41 ± 0.23) على التوالي مقارنةً بمجموعة السيطرة . بالإضافة إلى ذلك ، ارتفع مستوى التعبير الجيني لجينات c4h و 4cl و eomt في المجموعة الثالثة حوالي (42.34± 0.49) و (4.13 ± 0.38) و (6.29± 0.71) مقارنةً بالسيطرة (1.00± 0.00). فيما يتعلق بمحتويات الزيت العطري لمجموعة السيطرة والمجموعة الثانية والمجموعة الثالثة كانت 0.434٪ و 0.713 و 0.792٪ (حجم / وزن) على التوالي. علاوة على ذلك ، كان المكون العام للزيت العطري في نبات الريحان الحلو هو مركب يوجينول ومشتقاته لمجموعة االسيطرة والمجموعة الثانية والمجموعة الثالثة والتي كانت 2.76٪ و 8٪ و 11٪ (حجم / وزن) على التوالي. تم الاستنتاج إلى أن البلازما الباردة في الغلاف الجوي قد أظهرت تأثيرًا على التعبير الجيني ومحتوى الزيت الأساسي لمركبات فينيل بروبانويد في نبات الريحان *Ocimum basilicum* . المزروع في العراق ، اذ أن محتويات الزيت العطري لها خصائص علاجية مهمة.

الكلمات المفتاحية: سينااميت 4-هيدروكسيلاز ، البلازما الباردة، 4- كوماريت-coA- لايكيز، ، يوجينول O- ميثيل ترانسفيراز، نبات الريحان.