MARSH BULLETIN
EISSN 2957-9848

The effect of different concentrations of Cd on the active chemical compounds of the *Nigella sativa* L. plant

Wajehaa Musa Issa and Widad M. Taher Al-Asadi
Dept. of Horticulture and Gardens - College of Agriculture - University of Basrah
Dept. of Ecology - College of Science - University of Basrah
Widad.taher@uobasrah.edu.iq

Abstract
The impact of different cadmium concentrations (0, 10, 25, and 50 mg/L) on the bioactive chemical components of the Nigella sativa L. extract was examined in this study. The amount and kind of chemical components found in the plant after exposure to cadmium were found to differ significantly, according to the results. Nearly forty-three chemicals were found at a concentration of 50 mg/L. Compared to the control sample, which contained 32 of the 60 compounds found, this number dropped to 25 and 27 compounds for 10 and 25 mg/L, respectively. The content of some of the chemicals significantly decreased when exposed to cadmium, which was an intriguing finding. At a concentration of 50 mg/L, the 5-hydroxymethylfurfural level dropped from 1.1114% to 0.5652%. Conversely, certain compounds exhibited increases in concentrations of cadmium. Using 9,12,15-octadecatrienoic acid (Z,Z,Z) as an example, the concentration rose from 2.4546% in the control sample to 18.551% at 50 mg/L. On the other hand, n-hexadecanoic acid increased remarkably from 4.9558% in the control sample to 7.5414% at 50 mg/L. According to the study, these are the active molecules that vanished at different cadmium concentrations: 35 at 10 mg/L, 33 at 25 mg/L, and 17 at 50 mg/L. In comparison, 28 of the 60 detected chemicals were lost in the control sample. These results highlight the complex relationship that exists between Nigella sativa's chemical profiles and cadmium exposure.
Keywords: active chemical compounds, *Nigella sativa* L., Cd, plants
Received:18/10/2023 Accepted: 23/3/2024

Introduction

Nigella sativa L. is a medicinal plant widely used throughout the world. Its seeds and oil have a long history of popular use as medicines and foods. Its seeds have been widely used in the treatment of various diseases. It is considered one of the greatest types of therapeutic medicine. It has been widely used as an antihypertensive, liver tonic, diuretic, antidiarrheal, appetite stimulant, analgesic, antibacterial and other diseases. Extensive studies have been conducted on *N. sativa* by many researchers and a wide range of its effects have been explored. Pharmacological properties, which may include anti-diabetic, anti-cancer, immunomodulatory, analgesic, anti-microbial, anti-inflammatory, spasmolytic, bronchodilator, liver and kidney protectant, and has stomach-protective and antioxidant properties, most of the therapeutic properties of this plant are due to the presence of thymoquinone, which is the component the main bioactive substance in essential oil (Aftab *et al*., 2013).

Heavy metals are important environmental pollutants, and their toxicity greatly affects environmental, evolutionary and food systems. The sources of pollution of medicinal plants that contain heavy metals are areas irrigated with long-term wastewater, whether treated or untreated, as well as cultivated lands that are close to traffic. Vehicles passing or near sanitary landfill or waste areas, oil areas or other types of waste deposits that may contain toxic substances such as heavy metals, including Cd, copper, zinc, nickel, cobalt, chromium, lead and nitrogen (Yadav *et al*., 2010; Pavel *et al*., 2010). Increasing concentrations of Cd in soil represent a threat to plants due to its movement and toxicity. It is a non-essential element that inhibits plant growth and development, and causes its death even at very low concentrations (Wahid *et al*., 2010).

Asiminicesei *et al* (2020) showed heavy metal pollution of medicinal plants and the potential effects on human health resulting from many different industrial activities, and explained that plants can easily absorb organic and inorganic substances from all elements of the environment (water, soil, air) and accumulate in their various parts. (root, stem, leaves, fruits and seeds), which can enter and be transmitted in the food chain through ingestion by humans or animals, especially after medicinal plants have been used in health care because they do not have harmful effects on public health.

The study by Alexandra *et al*. (2015) Heavy metal content in selected medicinal plants, components commonly used in herbal formulations, and their study aimed to evaluate the content of heavy metals and metalloids in selected medicinal plants, including *Foeniculum vulgare* Mill., *Matricaria chamomilla* L., *Melissa officinalis* L., *Mentha piperita* L., which is traditionally used in alternative medicine. The results obtained showed that the contents of potentially toxic elements. The medicinal plant species studied above were within the recommended limits for containing toxic elements.

El-Ghamery and Mousa (2018) used salicylic acid to stimulate the roots of the *N. sativa* plant to adapt against the cytotoxicity of Cd, as they showed that this element is a toxic heavy metal that pollutes the environment, which prevents plant growth and development and causes plant death even at very low concentrations. The researchers treated the root tip cells of the *N. sativa* plant with different concentrations (5, 10, 25, 50)ppm of Cd for (3, 6, 12, 24)hours. All concentrations of Cd used showed a decrease in the level of cell division in the tips of the treated roots, and the concentration of 50 ppm for 24 hours was the most effective, which caused distortions in root growth. As for the roots to which concentrations of (0.01, 0.1, 0.2) mm of salicylic acid were added, it led to an increase in growth. The roots were not distorted, and thus the researchers demonstrated that the acid is effective in reducing the toxic effects of heavy metals.

Confirmed El-Said *et al*. (2009) in their study on the removal of arsenic by adsorption method in its As(III) and As(V) forms from wastewater using *N. sativa* seeds. The study focused on using plant seeds as an alternative absorbent material for removing As(III) and As(V). From wastewater, preliminary experiments showed that alkaline solutions (pH>9) without *N. sativa* cause homogeneous oxidation of As(III) to As(V), so the adsorption process was examined at pH 2-8. It was found that the absorption of As ion by *N. sativa* at a low pH value (3.0) is higher than at other values, and that plant seeds can be a good absorbent material for removing cationic metals coming from wastewater.

Abdelhamid *et al*. (2018) Biomass of *N. sativa* seeds as an absorbent for lead from aqueous solutions and wastewater. The adsorption capacity of *N. sativa* seeds towards the element lead was determined, as the concentration of lead absorbed on the surfaces of the seeds of the studied plant was found to be as high as 90.31mg.g^{-1}. The experiment Removal of lead from activated sludge waste and its effluent confirms the efficiency of this biomass, which reaches 97% and 64%. *N. sativa* seed waste biomass appears as an environmentally friendly and low-cost adsorbent for absorbing lead from wastewater.

Materials and Methods

The experiment was conducted in the fabric canopy (covered with saran cloth) of the College of Agriculture/University of Basra, Karma Ali site, for the agricultural season 2021-2022.

It included planting *N. sativa* L. seeds. The seeds were planted on 10/15/2021 in cork dishes with 205 cells filled with a growing medium consisting of German peat moss only, produced by Kekila, at a concentration of 4%. After the seeds germinated, that is, three days after planting them, and after two to three pairs of leaves had formed, they were separated into pots with a diameter of 10 cm after filling them with agricultural medium prepared from a mixture of agricultural seeds, which was sterilized with formaldehyde at a concentration of 4%, according to the method of Al-Saeed and Al-Douri (1982). And peat moss at a ratio of 1:2 (Hassan, 2009), respectively, with one plant per pot.

Experiment design

The experiment was carried out according to a Randomized Complete Block Design (R.C.B.D) with a factorial experiment, which included: preparing standard solutions using a standard solution (Stock solution) with a concentration of 1000 mg.L^{-1} of dissolving Cd nitrate salts Cd(NO3)2 based on the equation described (Muhammed *et al*., 2023).

$$\textbf{Weight (g)} = \frac{\text{mg.L}^{-1}}{1000} \times \frac{\text{Molecular weight of the compound}}{\text{Atomic weight of the element} \times \text{Number of its atoms}} \times \frac{\text{Required volume (ml)}}{1000}$$

To determine the concentrations at which plants grow.

Plastic pots prepared for cultivation were used, with 3 replicates for each concentration, so the number of experimental units was 12. The experiment continued for 40 days in canopy conditions.

Experimental transactions

The experiment included studying the effect of prepared concentrations of Cd

First: Watering plants with Cd in three selected concentrations:

0 -1Change the treatment (comparison).

10 -2mg. L^{-1}

25 -3mg. L^{-1}

50 -4mg. L^{-1}

Then the plants were watered using a hand sprinkler at a rate of three waterings during the growing season, as follows:

1- The first watering ten days after rotation.

2- The second watering 15 days after the first watering.

3- The third watering 15 days after the second watering.

Service operations

All agricultural operations used in *N. sativa* cultivation were carried out completely for all experimental units whenever necessary, including irrigation, hoeing, and weeding.

Experimental measurements

After the end of the experiment and 40 days of treatment, the plants were harvested in pots and collected. They were brought to the Medicinal and Aromatic Plants Unit at the College of Agriculture, and the samples were air-dried by spreading them on newspaper, and turning them every day to avoid damage to the samples. After they dried completely, the weight was stabilized. The samples were ground and the powder was placed in plastic containers, after which the information for each sample was written on it until the extraction process was carried out.

Estimation of active chemicals in plants

The active chemicals in the studied plants were estimated according to the source. (Haarborn, 1984) After drying and grinding the plants, take 1 gm of the dried and ground plant and place it in a 100 ml glass beaker, then add 10 ml of distilled water to it, shake well and place the mixture on the magnetic stirrer device. For three hours, after that, the extract was left for 24 hours and then filtered using filter papers. The filtrate was collected, placed in a test tube, and sent to the Nahran Omar laboratories of the Basrah Oil Company in Basrah Governorate to conduct chemical analysis using a GC-MS device.

Results and Discussion

Table (1) and Figure (1) show the number of active chemical compounds that appeared in the *Nigella sativa* plant exposed to the highest concentration of 50 mg.L^{-1} of the element Cd, which was 43 compounds, and it decreased at the concentrations of 10 and 25 mg.L^{-1} to 25 and 27 compounds. Respectively, compared to the control sample that recorded 32 compounds out of a total of 60 compounds. The reason for the increase in the number of compounds at high concentrations of Cd may be due to the stress resulting from adding heavy metals to the *N. sativa* plant, or to the fact that these compounds act as antioxidants to protect against pollution (Chatterjce *et al*, 2009).

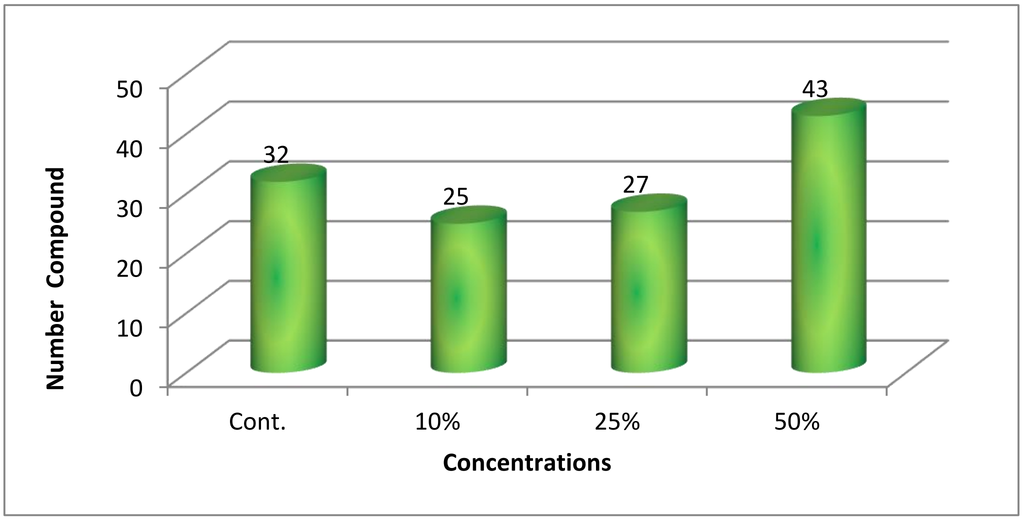

Figure (1): The effect of different concentrations of Cd on the active compounds in *N. sativa* extract

Also, Table (1) and Figures (3 and 4) show the active compounds that were recorded in the extract of the *N. sativa* plant exposed to different concentrations of the element Cd, as it was noted that some compounds decreased in value significantly when exposed to different concentrations, such as the compound 5-Hydroxymethylfurfural, whose concentration was 1.1114%. When treated with the control, it decreased to 0.5652% at the concentration of 50mg.L^{-1}, as well as the compound DL-Glucitol, which reached a percentage of 50.844% in the control sample, to become at the end of the experiment at the concentration of 50 mg.L^{-1} about 40.7328%, and compounds whose percentages increased at the end of the experiment, including the compound - 9,12,15 (Octadecatrienoic acid, (Z,Z,Z), which recorded a percentage of 2.4546% and increased at the last concentration to become 18.551%, and the compound n-Hexadecanoic acid, which recorded its highest percentage of about 7.5414% at the highest concentration compared to the control treatment, which was Its rate is 4.9558%.

Also, many of the active compounds disappeared for each concentration studied from a total of 60 compounds that appeared in the GC-MS analysis of the *N. sativa* plant extract (Table 1 and Figure 2), as it was found in the studied plant extract and at the concentrations of 10 and 25 mg.L^{-1} that it was There are large differences in the number of compounds that did not appear in the analysis, the loss was about 35 and 33 compounds, respectively at the concentration of 50 mg.L^{-1}, the number of losses was less, with about 17 compounds compared to the control sample in which the loss of compounds was about 28 compounds out of 60 a compound, the reason may be because when the plant is exposed to high toxicity from the element Cd, and to reduce the toxic stress on it, it secretes or forms a large number of phenolic compounds and other compounds as protection for it. The reason may also be due to a decrease or decrease in the percentage of these effective compounds in the plants due to the occurrence of changes in the enzymatic activity of antioxidants inside the plant, as pollutants, especially heavy metals, cause an increase in oxygen O2, thus producing toxic oxygen compounds such as H2O2, OH- and O, which in turn interact with different types of unsaturated acids inside the plant cell, leading to an oxidation process, for fats in the cytoplasmic membrane or cell bacilli (mitochondrion), which leads to leaching of cell contents, dehydration, and death.

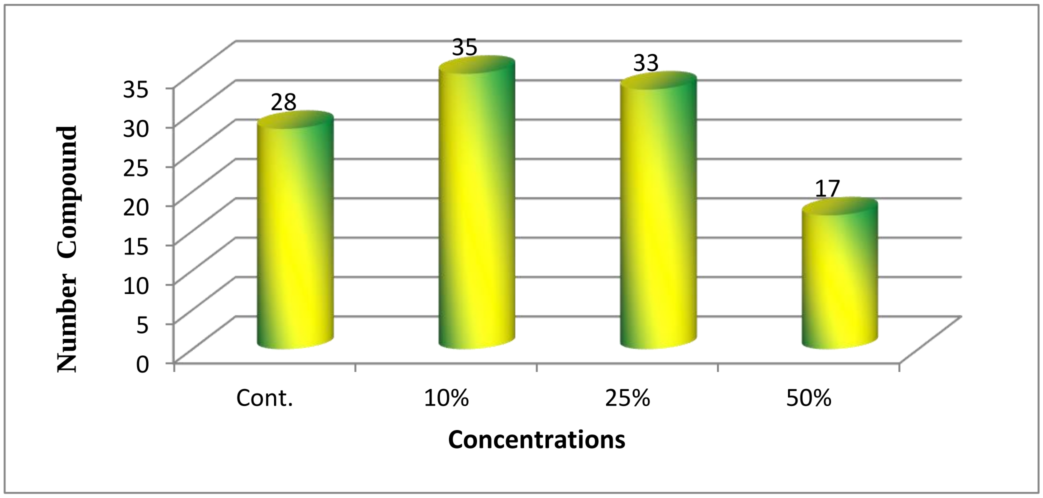

Figure (2): The number of active compounds missing in the *N. sativa* plant extract for each concentration of Cd.

Table (1): The effect of different concentrations of Cd on the active compounds in *N. sativa* extract

Compound	Cont.	10%	25%	50%
(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-methanol	0.5989	0.5721	0.1742	
.alpha.-D-Mannopyranoside, methyl 3,6-anhydro-	0.5171			
1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	0.7039	0.9553	0.7143	
1,1-Dimethylethylamine, N-methoxycarbonyloxy			0.3691	0.6561
1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-			0.7612	1.0019
1,2-Bis(trimethylsilyl)benzene				0.8071
1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester				2.0155
1,3-Dioxane, 2,4-dimethyl-	1.3007	1.0552		
1,4-Bis(trimethylsilyl)benzene	0.6714	0.9351	0.9601	1.0455
1,4-Butanediamine			0.1883	0.4947
1,6-Octadiene, 5,7-dimethyl-, (R)-				1.3721
1-Heptadecanamine				0.4697
1-Hexanamine	0.263	0.2774	0.4381	0.4678
1-Octanamine			0.0551	0.197
2,2-Dimethyl-1,3-butanediol				0.2765
2'-Hydroxy-5'-methylacetophenone, TMS derivative				1.2661
2-Methoxy-4-vinylphenol	0.7045	0.3056		
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester				0.5577
3,4-Furandiol, tetrahydro-, cis-				0.9142
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2.238	1.9547	0.7553	
5-Hydroxymethylfurfural	1.1114	1.0774	0.8362	0.5652
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	2.4546	2.0741	7.9921	18.551

Compound	Cont.	10%	25%	50%
Arsenous acid, tris(trimethylsilyl) ester	0.8089	0.9033	0.7551	0.2905
Benzaldehyde				0.321
Benzeneacetaldehyde	0.8054			
Benzo[h]quinoline, 2,4-dimethyl-	1.4667	1.6062	1.8443	1.9799
Bis(2-ethylhexyl) phthalate	1.2086	0.7922		
Butane, 1,1-diethoxy-3-methyl-	0.6892	0.9261	1.0053	
Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	0.652			
Cyclononasiloxane, octadecamethyl-	0.83	0.8661		1.7664
Cyclooctanone			0.2884	0.3941
Cyclopentadecane	0.9726	0.3995		
Dichloroacetic acid, heptadecyl ester				0.8057
Diglycolamine				0.29
DL-Glucitol	50.844	33.7443	27.9013	40.7328
Eicosane	0.5538	0.0732	0.3859	0.5334
Ethanol, 2-(2-ethoxyethoxy)-	0.6573	0.3922		
Ethyl hydrogen succinate			0.9225	1.1494
Heptadecyl heptafluorobutyrate	1.2878			
Hexacosane	2.0812	2.0184		1.4597
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.0334	1.1882	1.2743	1.3989
Hexasiloxane, tetradecamethyl-	2.6613			2.0255
Methyl-.alpha.-d-ribofuranoside	1.0967	0.9551	0.6591	
N,N-Dimethylsuccinamic acid				0.7911
N-[3-[N-Aziridyl]propylidene]-3-dimethylaminopropylamine			0.4339	0.3604
Neophytadiene				3.0325
n-Hexadecanoic acid	4.9558	5.1773		7.5414
Nonane			0.2733	0.5443
Octadecanoic acid	2.7182	1.8441		
Octane, 2-methyl-			0.4517	0.6796
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-				1.3168
Pentanoic acid, 1-methylethyl ester			1.0551	1.1057
Pentanoic acid, 4-methyl-	5.1579	3.8821		
Phenol, 2,5-bis(1,1-dimethylethyl)-				1.2275
Ritalinic acid, TMS derivative	2.9024			
Stigmasterol			4.8812	3.2106
Styrene				5.1938
Sulfurous acid, pentadecyl 2-propyl ester	0.7202		0.4551	0.5131
Tridecane	0.5639	0.7493		1.5288
Urethane			0.4331	0.2345

The *N. sativa* plant also exhibited some behaviors in its resistance against Cd pollution. Some important compounds that are considered antioxidants increased their percentages in the plant as defensive factors for it, such as the compound n-Hexadecanoic acid, whose percentage increased to 7.5414% at high concentration compared to the two control plants in which it was recorded. Its value is 4.9558%. It is one of the most important saturated acids, as it is found in many plants such as palm trees. It is a major compound of palm oil and is an important antioxidant and vitamin (French *et al*., 2002).

As for the compound octadecanoic acid, which is one of the fatty acids that belong to the omega-9 compounds, and it is an important compound found in many plants and is used to lower blood pressure, it appeared in the control treatment and concentration 10 mg.L^{-1} at rates of 2.7182 and 1.8441%, respectively, and disappeared at the concentrations of 25 and 50mg.L^{-1}.

Many active compounds appeared that were not recorded in the control sample for the treated concentrations, most of them at the highest concentration. The compound Neophytadiene, which is a volatile oil in plants and an antioxidant for bacteria (Gakuubi *et al*., 2016), recorded a percentage of about 3.0325%, and the compound 1,4-Butanediamine. Which is one of the rapidly oxidizing and toxic compounds, which is formed at a rate of about 0.1883 and 0.4947% at concentrations of 25 and 50 mg.L^{-1} and other compounds.

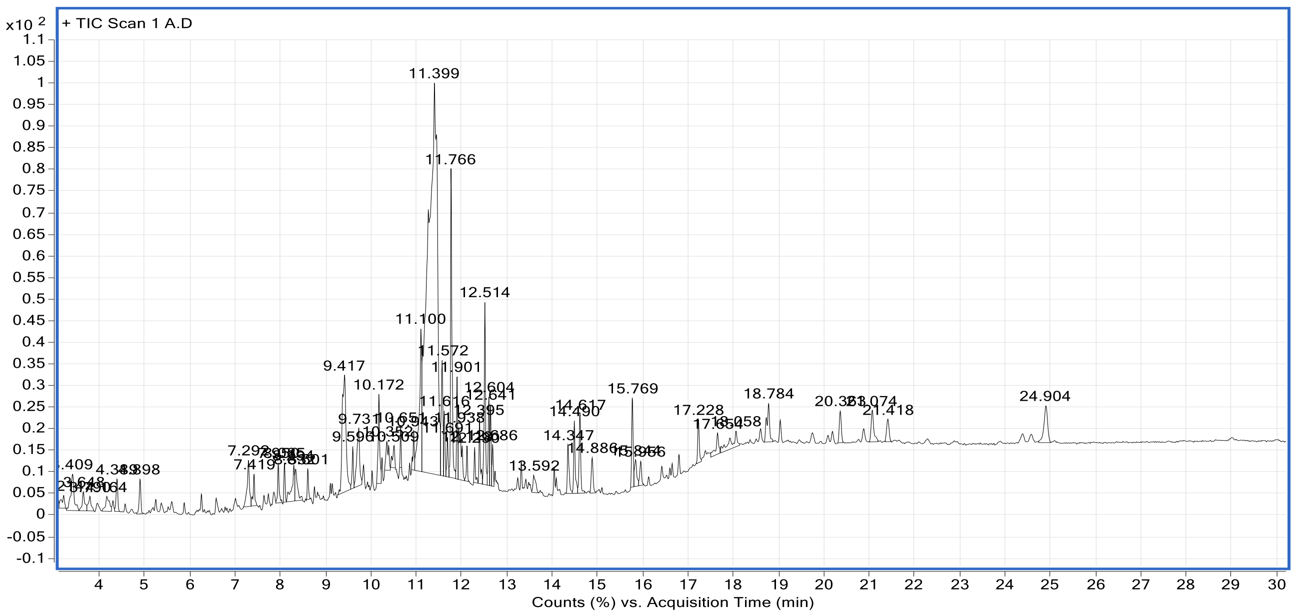

Figure (3): A diagram of the active compounds in the *N. sativa* plant for the control sample

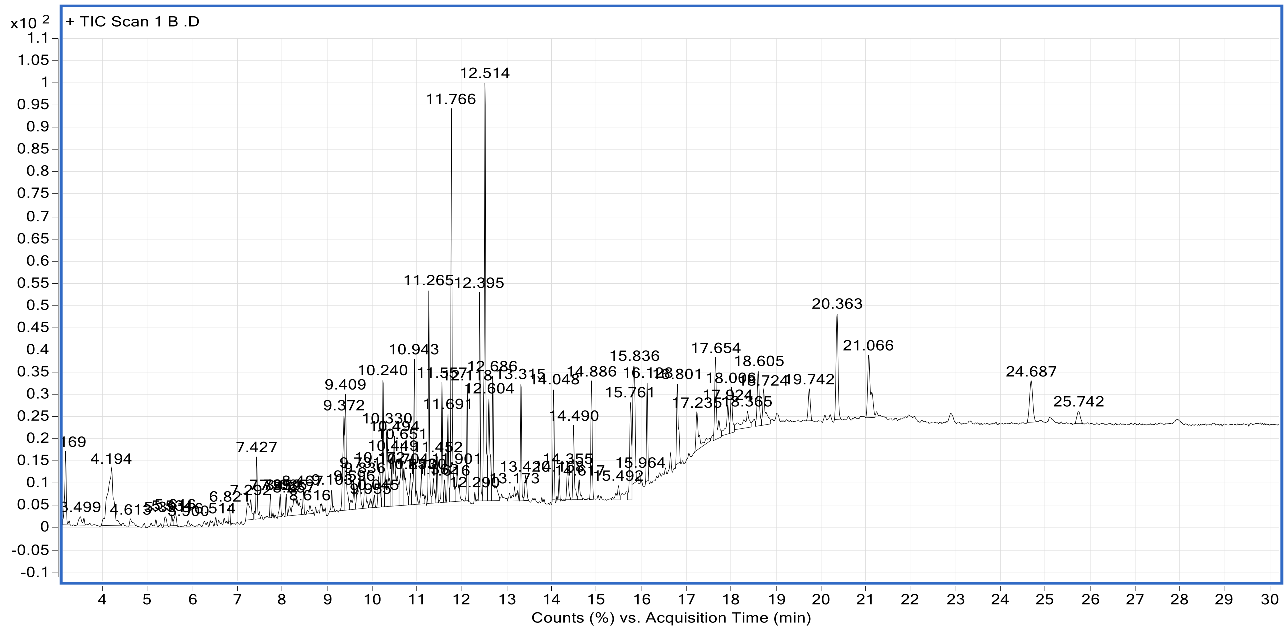

Figure (4): A diagram of the active compounds in the *N. sativa* plant at a concentration of 50

Conclusions

We conclude from this study that plants of medical or nutritional importance, when exposed to different concentrations of heavy elements, lead to the production of active chemical compounds that may be toxic to humans, as well as the disappearance of compounds of medical importance and an increase or decrease in the concentrations of some compounds.

Acknowledgements

We thank the College of Agriculture and the Medicinal and Aromatic Plants Unit for facilitating the process of growing plants used in research in their plant canopy. We also thank the laboratories of the Basrah Oil Company, Nahran Omar, for conducting chemical tests with the GC-MS device.

Referances

Abdelhamid, A.; Noureddine, B. and Maria, E. (2018). Oriental Journal Of Chemistry, 34(2): 638-647

Aftab, A.; Asif, H.; Mohd, M.; Shah, A.K.; Abul, K.N.; Nasir, A.S.; Zoheir, A.D. and Firoz, A. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed 2013; 3(5): 337-352.

Aleksandra, S.T.; Anojkov, I.C.; Seb, I.C. and Anojkov, I.C. (2015). Heavy Metals Content in Selected Medicinal Plants Commonly Used as Components for Herbal Formulations, Tarım Bilimleri Dergisi Journal of Agricultural Sciences, 21: 317-325.

Asiminicesei, D.M.; Vasilachi, I.C. and Gavrilescu, M. (2020). Heavy Metal Contamination of Medicinal Plants and Potential Implications on Human Health, Rev. Chim., 71 (7): 16-36.

El-Ghamery, A.A. and Mousa, M.A. (2018). Salicylic Acid Triggers Adaptation Cd Cytogenetic Toxicity in Roots of *Nigella sativa* L. Egypt. J. Bot., 58(2): 297-310.

El-Said,S.M.; Alamri, M.B.S.; El-Barak, A.B.S. and Alsogair, O. (2009). Adsorptive Removal of Arsenate as (III) and Arsenate as (V) Heavy Metals from Waste Water using *Nigella sativa* L. Asian Journal of Scientific Research, 2: 96-104.

French, M.A.; Sundram, K. and Clandinin, M.T. (2002). "Cholesterolaemic effect of palmitic acid in relation to other dietary fatty acids". Asia Pacific journal of clinical nutrition. 11(7): 401-7.

Gakuubi, M.M.; Wagacha, J.M.; Saifuddin,F.D. and Wycliffe, W. (2016). Chemical composition and antifungal activity of essential oils of *Tagetes minuta* (Asteraceae) against selected phytopathogenic fungi. American Journal of Essential Oils and Natural Products 2016; 4(3): 16-26.

Haarborn, J.B. (1984).Phytochemical methods. A guid to modern ed. Chapman and Hall, London, New York, 282p.

Khan, A. and Khan, A.A. (2015).Effect of Heavy Metal Stress on The Performance of *Nigella sativa* L.

Muhammed, A.H.; Taher, W.M. and Abduljalee, S.A. (2023). Toxic effect of lead and Cd on morphological and chemicalcharacteristics of *Trigonella foenum-graecum*, MARSH BULLETIN, (1): 52-70.

Pavel, V.L.; Bulgariu, D.; Bulgariu, L.; Hlihor, R.M.; Gavrilescu, M. (2010). Analysis of factors determining the behaviour of chromium in some Romanian soils, Environ Eng. Manag. J., 9: 89-94.

Yadav, S.K. (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants, S. Afr. J. Bot., 76: 167-179.

تأثير التراكيز المختلفة لعنصر الكادميوم على المركبات الكيميائية الفعالة لنبات حبة البركة

وجيهة موسى عيسى* و وداد مزبان طاهر الاسدي
*قسم البستنة والحدائق-كلية الزراعة- جامعة البصرة
قسم علم البيئة-كلية العلوم-جامعة البصرة

المستخلص

أظهرت الدراسة الحالية تأثير التراكيز (0، 10، 25، 50) ملجم.لتر$^{-1}$ من عنصر الكادميوم على المواد الكيميائية الفعالة في مستخلص نبات الحبة السوداء، حيث لوحظ أن هذه التراكيز أثرت على عدد المركبات التي ظهرت في النبات المدروس، اذ سجل حوالي 43 مركب عند تركيز 50 ملغم.لتر-1 من العنصر، وانخفض عند تركيز 10 و25 ملغم.لتر-1 إلى 25 و27 مركب على التوالي، مقارنة بعينة السيطرة التي سجلت 32 مركب من إجمالي 60 مركب. كما لوحظ أن قيمة بعض المركبات انخفضت بشكل ملحوظ عند تعرضها لتركيزات مختلفة مثل مركب 5-هيدروكسي ميثيل فورفورال الذي بلغ تركيزه 1.1114% في معاملة السيطرة وانخفض إلى 0.5652% عند تعرضه لتركيز 50 ملغم.لتر$^{-1}$، ومركبات زادت نسبتها عند نهاية التجربة ومنها مركب -9,12,15 حمض أوكتاديكاترينويك (Z,Z,Z) الذي سجل نسبة 2.4546% وزاد عند آخر تركيز ليصبح 18.551%، ومركب ن-حمض هيكساديكانويك الذي سجل أعلى نسبة له بلغت حوالي 7.5414% عند أعلى تركيز مقارنة بمعاملة السيطرة وكانت النسبة 4.9558%. كما اختفت العديد من المركبات الفعالة لكل تركيز مدروس فعند تركيز 10 و 25 ملغم.لتر-1 كان أن الفقد في المركبات بلغ 35 و 33 مركب على التوالي وعند تركيز 50 ملغم.لتر$^{-1}$ كان عدد المركبات أقلها بحوالي 17 مركب مقارنة بعينة السيطرة التي بلغ الفقد فيها 28 مركب من أصل 60.