Effect of abiotic stresses on the level of alkaloids in *peganum Harmala* L plant

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Abstract:

This study was conducted in the tissue culture laboratory, Al Mussaib Technical College , Al-Furat Al-Awsat Technical University, and the other part in the laboratories of the Ministry of Science and Technology for the period from 15/8/2020 to 28/3/2021.To know the effect of abiotic stresses represented by salt stress, sodium chloride (Nacl) and dehydration stress polyethylene glycol (PEG 6000) on callus growth and the presence of some active compounds in it. The secondary compounds were diagnosed using the GC Chromatography-Mass device. The results showed the following:

The results of the GC analysis of β-carboline alkaloids showed that the highest concentration of β-carboline was the concentration of al-Haramin, which reached 48.32 µg for the 200 mM (NaCl) treatment, and the lowest concentration of HARMINE when the control treatment was (0,35.23) µg, while the highest production of Harmalol appeared at the 200 µg concentration. 4.91 mcg, Harmalin alkaloid wire was the same as the active substances (Al-Harameen and Hermalon), where the higher the salt concentrations (NaCl stress), the higher the concentration of the active substance. The study also included the effect of dehydration stress with concentrations (0.1%,2%,3%), respectively, where the concentration of 3% gave the highest concentration of Harameen alkaloid reaching 34.15 µg, while the control treatment without any stress gave the lowest concentration of alkaloid which was 29.18 µg, while The highest concentration of Hermalon alkaloid was 3.92 µg compared to the control treatment, which gave the lowest concentration of 2.73 µg. Also, drought stress led to an increase in the concentration of the Harmaline alkaloid, where it reached 3% when treated, the highest concentration was (21.33) µg.

Introduction :

peganum Harmala L. plant is a medicinal plant belonging to the Nitrariaceae family that is distributed mainly in dry soils and deserts in the central and southern regions of Iraq [27].A wide range of organic compounds called secondary compounds is produced by plants that do not contribute to the normal growth, reproduction, or development of the plant and are not essential for survival but support the plant in protection and competition [25] and [28]. Many of the structural modifications of synthetic and semi-synthetic drugs are made from alkaloids, and they are used as psychoactive drugs such as cathinone, or as relaxants such as harmine and harmaline that stimulate the central nervous system [19]. These active compounds can be augmented by a plant tissue culture technique that is widely used to grow or maintain plant cells in a method known as micropropagation by producing undifferentiated cells called callus[1].Since the production of the secondary compound increases when the plant is exposed to various environmental stresses [30]. Callus cells were exposed to several biological stresses through tissue culture technology to produce secondary or active materials (secondary metabolism) in all seasons and to provide these materials throughout the year due to the increased demand for them to produce secondary compounds as well as their increased, and it has largely succeeded in providing them in tissue culture laboratories in large quantities and good concentrations [11]. Many researchers have studied the overall changes that occur under the influence of salinity on plants, such as changes in the growth and production of chemical components such as alkaloids, protein, free amino acids, and some nutrients [16], [3] and [2].

Materials and Methods

1. Preparation and culture of saline medium

To prepare 1 M/L of sodium chloride salt solution, 58.44 g of NaCl is dissolved in one liter of distilled water to make the solution 1M and then diluted to dilute concentrations of (200, 150, 100, 50) mM L^{-1}. The growing callus was taken in the callus induction experiment (in which the hormones BA and 2,4-D mg/L each were added to the nutrient medium, which gave the best results in callus induction. The callus masses with a diameter of 5 mm per mass were distributed to homogenize the cultured masses as much as possible to a new medium with the same concentrations of hormones with the addition of Nacl salt at the above concentrations. The experiment was conducted with ten replicates for each concentration of salt and the cultures were incubated in the dark.

2. Preparation and culture in drought medium:

Polyethylene glycol (PEG) was used in concentrations (0,2,1,3)% per 100 ml of medium, in the callus formation medium.The growing callus was taken in the callus induction experiment (in which the hormones 2,4-D and BA were used mg/L added to the nutrient medium, which gave the best results in the induction of callus at an average of 50 mg of callus to a new medium with the same hormone concentrations with the addition of (PEG6000) at the above concentrations. The experiment was conducted with ten replicates for each concentration of drought.The medium was distributed on ampoules (withstand a temperature of 120º), followed by the sterilization process, we leave it in the refrigerator until use .Callus mass with a diameter of 5 mm per block was distributed and the cultured masses were homogenized as much as possible into each bottle and incubated under the same conditions (25 ± 2 °C in the dark). Fresh weight was measured after callus growth. Callus was weighed with bottles of different PEG concentrations, all cultures used, 10 replicates per cultivar,With only a middle tube placed to control the weight loss resulting from evaporation, after two weeks it was weighed with packages and repeated this process for 45 days, after 45 days of growth,Callus samples were taken from each treatment and dried in an electric oven at 70° C until the weight was stabilized using a sensitive electronic balance.

Prepare callus extract:

The seed callus of *Peganum harmala* and grinding callus with two concentrations separately, and the extraction process was conducted using pure methyl alcohol for each 5 mg of callus powder (ratio 1:3 volume: weight), and placed in a shaker for 30 minutes with light shaking, then the shaking process was repeated, but with rapid shaking for two minutes, Then the mixture was placed in the tubes of a centrifuge for 10 minutes at a speed of 10,000 revolutions. 1 minute After that, the sewage was filtered with a filter of diameter 0. m) 22) micrometer and kept in special bottles at a temperature of 4 °C until use.

GC gas chromatography:

The chemical compounds include alkaloids diagnosed using gas chromatography (GC) SHIMADZU 2010 model. DB5 type separator column with dimensions of 30 mm x 0.25 mm x 0.25 mm.The temperature started at 90°C to reach 220°C and gradually increased by 10°/min.While the temperature of the injection area and the detector area were 280 and 340 °C, respectively. In the detector area, the indicator type was a flammable ionizing reagent to

indicate the active compound. While nitrogen N was used as the carrier gas.

Preparation of standard solutions of Hermalin, Harmine and Hermalol alkaloids:

1. Preparation of Standard Solutions:

0.01 g of high-purity standardized harmalin and harmine was taken to dissolve in 2 ml of high-purity methanol,

The volume is then completed to 100 mL methanol to obtain a concentration of 100 ppm

1 mL of the standard compounds was injected into the GC device for the diagnosis of secondary compounds depending on the RT (Retention Time) that corresponds to the RT of the standard substance. The concentration calculation was based on retention time and sample area according to the formula below [14]. Sample concentration = standard concentration * sample area / standard area * dilution factor / sample weight

2. Identification of secondary compounds:

Table (1) Working conditions of the GC-MS device for secondary compounds analysis

Device type	GC-Mass Shimadzu 2010Qp 2010Plus
The type of injection used	Split 1:20
Separator column type	HP-5MS capillary column(30m*250mm
injection temperature	°280
column temperature	It starts at 40 °C and stabilizes for 2 minutes and then increases by 4 °C every minute until it reaches a temperature of 280 °C and stays that way for 5 minutes and then cools down.
used gas	Helium He Purity 99.99
mass spectrometry	Electron Impact Ionization (EI);recorded inintervals from 40 to 600m/z
Sample volume at injection	2 µl
mass spectrometer temperature	Inter face

Results and discussion :

Effect of different concentrations of sodium chloride added to MS medium with growth regulators 2,4-D and AB (0.25 mL^{-1} x 0.50 mL^{1}) on the concentration of alkaloids (μg^{-1}) in the calcium produced from the lower embryonic leaf of the seeds of the rue plant.The standard sample of Haramin, Harmalin and Harmalol has been clarified in the form of the standard sample used as a sample for calculating the concentration of Harmaline, Harmalin and Harmalol in the callus sample through the formula shown in the materials and methods of work. The results in Table (1) show the effect of salinity on the concentration of (Haramin, Harmalin and Harmalol) after GC analysis of callus.The high concentration of Haramin was recorded, as it gave the highest significant difference, which amounted to 48.32 mg^{-1} on a medium of 200 mm, where the concentration (0) of the control treatment gave the lowest significant difference in concentration (from Haramin substance) which amounted to 35.23 μg^{-1}. While the medium 200 mm gave the highest significant difference, where the concentration of Harmalol was 4.91 μg^{-1}, and treatment (0) gave the control treatment the least significant difference, which amounted to 3.73 μg^{-1}.While the active substance (Hermalein) behaved the same as the rest of the concentration, where the greater the salt stress, the higher the concentration of the active substance, giving the highest concentration of Dehralene on a medium of 200 mm, amounting to 24.12 μg^{-1} Whereas, treatment (0) gave the control treatment the lowest concentration of Hermalin, which was 22.13 μg^{-1}. The concentration of Haramin, Haramin, and Harmalol was increased when the salinity increased, which led to the improvement of salinity extraction on the plant cells to increase the production of active secondary substances .This is consistent with [13] in their work on radish sprouts (Raphanus sativus L.) when the nutrient medium was prepared with 100 mM NaCl, They observed an increase in the total glucosinolates in 5- and 7-day-old sprouts by 50% and 127%, respectively, and the phenol contents in 3- and 5-day-age sprouts by 20% and 140%, respectively.While these contents were reduced by a low and medium level of salt stress (10-50 mm of NaCl) in the action of [23] on P.harmala plant,They aimed to regenerate the plant in vitro and in vivo and found that harmaline increased with abiotic stresses but decreased significantly in all treatments exposed to dark callus (1 ABA $mg.mL^{-1}$,ABA $mg.mL^{-1}$, BAP 1 $mg.mL^{-1}$) As stress factors on the other hand [17] The alkaloids harmalin and hermaline were identified in the callus of the plant after incubation on different combinations of NAA and BAP revealed [22] through their research

that calcium and magnesium ions are necessary for the sensitive production of the alkaloid content of beta-carboline in the callus of the plant due to changes in the composition of the media.[20] indicated that salt stress led to the improvement of some chemical and physical properties related to the quality of tomato fruits, such as an increase in the fruit's content of soluble solids. Salt stress affects the lycopene content of the tomato plant in a twofold increase in the Marmara tomato cross.[4] indicates that the innovators can stimulate different classes of secondary substances and be affected by the concentration of these compounds in different media, but depending on the genetic types and plant varieties instead of the elite nature.

Table (1) Effect of different concentrations of sodium chloride added to (MS) medium with growth regulators 2,4-D&AB (0.25 mL^{-1}x 0.50 mL^{-1}) on the concentration of alkaloids (μg^{-1}) in the callus produced from the seeds of the rue plant(lower Hypocotyl).

Haramain Concentration μg^{-1}	Harmaline concentration $1\mu g^{-1}$	formalin concentration 1 μg^{-1}	NaCl salinity concentration μg^{-1}
35.23	3.73	22.13	00
39.32	3.76	22.86	50
42.67	3.93	23.23	100
43.45	4.62	23.88	150
48.32	4.91	24.12	200
4.23	0.54	1.02	L.S.D0.05

Effect of different concentrations of (6000PEG) added to (MS) medium with growth regulators (-D&BA 0.25mL^{-1}), 4-2 0.50 ml^{-1}) on the concentration of alkaloids (µg $^{-1}$) in the calcium produced from the seeds of the plant stalk (stalk). lower embryonic). The results in Table (2) show the effect of (PEG6000) on the concentration of Harameen, Harmalin and Harmalol. After the GC analysis of callus, the high concentration of Harameen was recorded, reaching 34.15 µg-1 on a medium of 3%.Whereas, the concentration (0) gave the control treatment the lowest concentration of Harameen substance, which amounted to 29.18 mg^{-1}, while the highest concentration of Harmalol was 3.92 µg^{-1}, compared with the control treatment, which amounted to 2.73 µg^{-1}, on a medium of 3%.While the active substance Hermaline behaved the same as the rest of the concentrations, where the higher the stress concentrations (drought stress), the higher the concentration of the active substance. Whereas, treatment (0) gave the lowest concentration of Harmalin, which amounted to 19.3 µg^{-1}. Compounds play an important role as they act as anti-inflammatory, anti-microbial and anti-cancer [29] It was concluded that the manufacture of these compounds depends on the combination of the nutrient medium, growth regulators and stimuli to encourage callus to manufacture the active compounds in different proportions [15].The results of the study conducted by [10] and [8] also revealed that this plant contains β-Carboline alkaloids, which are represented in Harmaline, Harmine, and Harmalol, which are the most types of alkaloids found in it. The reason for the different types of alkaloid compounds from one plant to another is due to the factors that the plant is exposed to, whether outside or by adding stimulants in the agricultural food circles .Which controls the production of active substances in addition to the quality of the solvent used in the chromatographic analysis.These results are verified by a number of researchers who noticed an increase in the content of some alkaloid and phenolic compounds that stimulate the effect of abiotic stresses, salt stress [24], [21] and [7].

Table (2) Effect of different concentrations of (PEG) added to (MS) medium with growth regulators ((-12.4-D&BA 0.25ml 0.50 ml^{-1}) on the concentration of alkaloids (μg^{-1}) in the calcium produced from the seeds of the plant (Hypocotyl).

Haramain Concentration	Hermalon concentration	Harmalin concentration	PEG dehydration concentration%
29.18	2.73	19.31	00%
29.32	3.01	20.12	1%
32.24	3.53	21.22	2%
34.15	3.92	21.33	3%
2.21	0.86	0.91	L.S.D0.05%

Conclusions

1. Alkaloids increase with increasing stress, as shown by the results of the analysis of secondary compounds with the GC-MS device for long-acting callus (AB 0.25 ml^{-1} and 2,4-D0.50ml^{-1}) and sodium chloride salt of NaCl in different concentrations (0,50,100,150,200)Mm were added to the medium, which gave the highest concentration of salt the highest concentration of the active substances (Harameen, Harmelol, Al-Harmin) and for the same combinations of the dry medium with polyethylene glycol added. With different concentrations (0, 1%, 2%, 3%) the highest concentration of 3% gave the highest concentration of the active substances (Harmine, Harmalin, Harmalol).

2. Possibility of obtaining alkaloids from callus Harmal instead of seeds.

References

1-Ahmadian, E., Lolaei, A., Mobasheri, S. and Bemana, R. (2013). Investigation of importance parameters of plant tissue (review). International journal of agriculture and crop sciences. 5(8), pp. 900-905.

2-Al-Hatami, Karim Taleb (2001) Response of four hybrids of tomato to endurance of salt stress using the technique of culturing plant tissues, Master's thesis, College of Science, University of Kufa.

3-Ali.R.M.(1991). Changes in Chemei Cal Compostion of fruits of salinzied Datura Stramonium.Jornal of Islamic .Cairo univ.Fayoum Egypt. 4:4,289-292.

4-Baenas,N.;G.Viguera and D. A.,Moreno (2014).Elicitation :Atool for enriching the bioactive Composition of foods. Molecules,19:13541-13563.

5-Benzekria, R., Bouslamaa L., Papettib, A., Hammamia, M., Smaouic, A., and Limam, F. (2018). Anti HSV-2 activity of Peganum harmala L. and isolation of the active compound, Microbial Pathogenesis 114. Pp.291–298.

6-Berrougui H, Martín-Cordero C, Khalil A, Hmamouchi M, Ettaib A, Marhuenda E, et al. (2006). Vasorelaxant effects of harmine and harmaline extracted from Peganum harmala L. seeds in isolated rat aorta. Pharmacol Res. (54) 150–7.

7-Borgognone ,D.;M.,Cardare lli;E.,Rea;L.,Lucini and G.,Colla (2014).Salinity source –induced changes in yield ,mineral composition ,phenolic acids and flavonoids in leaves of artichoke and cardoon grown in floating system .J.Sci. Food Agric .,94(6):1231-1237.

8-BOUABEDELLI F., MISSOUN F., BENHAMIMED E., DJEBLI N., 2016-Phytochemical and antimicrobial study of the seeds and leaves of peganum harmala L. against urinary tract infection pathogens . Asian pacific journal of tropical disease.p : 932.

9-Bremner P, Rivera D, Calzado MA, Obón C, Inocencio C, Beckwith C, et al. (2009). Assessing medicinal plants from

South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-Kappa B and other pro-inflammatory mediators. J Ethnopharmacol. (124) 295–305.

10-ELGUBBI S.H., GHAITH S.G., ALNAAS M., BALTO M., 2016- Cytotoxicity studies of harmine isolated and purified from peganum harmal L. seeds.The third symposium and applications of basic and biosciences.p: 32.

11-Gonçalves, Sandra and Romano, Anabela (2018). Production of Plant Secondary Metabolites by Using Biotechnological Tools. IntechOpen, Chapter 5. http://dx.doi.org/10.5772/intechopen.76414 .

12-Guergour, H., Rima A.R., and Abdelouahab B. A. (2018) Phytochemical Screening, Antioxidant Activity of the Various Extracts from *peganum harmala* Der Pharma Chemica, , 10(3): 146-150 Algeria.

13-Guo,R.,Yuan,G.,and Wang,Q.(2011). Sucrose enhances the accumulation of anthocyanins and glucosinolates in broccoli sprouts .Food Chem .,129,1080-1087.

14-Hinshaw, J. V. 2001. Basic GC Measurements and Calculations. Chrom Source, Franklin, Tennessee, USA. pp. 1-5.

15-Irawati ,I.;A.,Rahmawati and R.R.,Esyanti(2014). Analysis of secondary metabolite production in somatic embryo of pasak bumi (Eurycoma longifolia Jack .Proced .Chem .,13:112-118.

16-Jeschke ,W.o;Wolf,O.(1988). Effect of Nacl Salinity on growth develpment ion distribution and ion translocation Castor bean (*Ricinus communis L*.).J.plant physiol.,132:45-53.

17-Juma,G.M.(2005) Peganum harmala Production .European Journal of Scientific Research .1-11.

18-Lamchouri, F., Zemzami, M., Jossang, A., Abdellatif, A., Israili, Z.H., and Lyoussi, B. (2013). Cytotoxicity of alkaloids isolated from Peganum harmala seeds. Pak J Pharm Sci. 26(4):699-706.

19-Manfred, H. (2002). Alkaloids: Nature's Curse or Blessing? Wiley-VCH. ISBN 978-3-906390-24-6 p 309.

20-Najla,s.(2013). Effect of salt stress on some fruit qualities of two tomato hybrids

.Damascus Univ.journal of agriculture science (DjAS). 29(2). Pp. 57-66.

21-Navarro,J.M.;P.,Flores;c., Garrido and v.Martinez (2016).changes in the contents of antioxidant compounds in pepper fruits at ripening stages as affected by salinity .Food chem.,96:66-73.

22-Nettlesship ,L.and Slaytor,M.(1974) Ad-aptation of peganum harmala Callus to alkaloid production .Journal of Experimental Botany .25(6),PP.1114-1124.

23-Onlu,S.,Bingol,O.,Tuker,M., and Ozdmir ,F.A.(2018).B-carboline production from in vitro regenerated peganum harmala L. plant Fresenius Environment al Bulletin. Volume 27(12):pp.8447-8451.

24-Parida, A.K. and A.B. Das. 2005. Salt tolerance and salinity effects on plants: a review. Ecotox. Eviron. Safety, 60: 324–349.

25-Pichersky, E. & Gang, D.R. (2000). Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends in plant science, 5, 439-445.

26-Rezaeia, M., Nasrib, S., Roughanic M., Niknamib Z. and Ziai S. A. (2016). Peganum harmala L. extract reduces oxidative stress and improves symptoms in 6-hydroxydopamine-induced Parkinson's disease in rats. Iranian Journal of Pharmaceutical Research. 15 (1): pp. 275-281.

27-Ridda, Th. J. and W.H. Daood. 1982. Geographical distribution of wild vascular plants of Iraq. National Herbarium of Iraq. (Unpublished) pp. 140.

28-Sheela, J. A. H. (2011). Secondary metabolites of the plants clematis gouriana. International journal of mathematics trends and technology, 2, (2): 1-4.

29-Sheoran ,N.;A.V., Nadakkakakatha ;V.,Munjal; A.; kundu; K.,subaharan ;V.,Venugopal; s., Rajamma;S. J., Eapen and A., kumar (2015). Genetic analysis of plant entophytic

pseudomonas putida BP25and chemo-profiling of its antimicrobial volatile organic compounds. Microbiologic .Res.,173:66-70.

30-Wink, M. and O., Schimmer (1999). Modes of Action of Defensive Secondary Metabolites In: Wink, M. Ed. Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology. Sheffield Academic Press. Pp. 17 -133.