Iraqi Journal of Market Research and Consumer Protection
(2025) 17(1): 193-203
DOI:
Jasim & *Habeeb*

المجلة العراقية لبحوث السوق
وحماية المستهلك

CALLUS INDICATION OF (*Salvadora Persica. L)* AND ECONAMICAL IMPORTANCE

Hadaia Y. Jasim [1] , Hadeel M. Habeeb [2]
[1]Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq. Hedaia.jassim1602@csw.uobaghdad.edu.iq
[2]Assistant Professor PhD, Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq. Hadeel.makkki@csw.uobaghdad.edu.iq

Received 10/ 9/ 2023, Accepted 21/ 11/ 2023, Published 31/ 3/ 2025

This work is licensed under a CCBY 4.0 https://creativecommons.org/licenses/by/4.0

ABSTRACT

The aim of the research is to use *in vitro* plant tissue culture technique to determine the effect of growth regulators on the amount of callus produced. Callus was induced from the *Salvadora persica* or Miswak (Arak) plant by using a combination of Auxin (NAA) and Cytokinin (KIN). In the experiment, the plant parts (seeds, nodes, and leaves) were sterilized with mercury chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) for two periods of time (5 and 10 minutes). The results showed the best material for sterilizing plant parts is ($HgCl_2$). Different plant parts (leaf, internode, node, and root) were cultivated on MS medium accompanied by a combination of hormones of (NAA) and (KIN)with different concentrations, and after four weeks of cultivation, significant differences were recorded. The best combinations of plant growth regulators (PGEs) for callus induction rates (2.2%) from leaves at a combination of 1 KIN and 1 NAA mg/L. While internodes at 0 mg/L KIN, and 1 mg/L NAA gave 1.33%. The heights percentage rate (2.25%) of callus induction was obtained from nodes at (1 mg/L KIN, 2 mg/L NAA). The results showed the highest fresh weight of the callus that was obtained from the leaves was 0.4823 g. While the internodes gave 0.3195 g. whereas the height fresh weight of callus produced from nodes was 0.6791 g. As for the roots, there is no response for callus induction.

Keywords: plant tissue culture, *Salvadora persica* (Miswak), KIN, NAA

استحثاث كالس من المسواك *(Salvadora Persica. L)* واهميتة الاقتصادية

هدايه يعقوب جاسم [1] , هديل مكي حبيب [2]
[1] قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.Hedaia.jassim1602@csw.uobaghdad.edu.iq
[2] استاذ مساعد دكتور، قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق. Hadeel.makkki@csw.uobaghdad.edu.iq

الخلاصة

الهدف من البحث هو استخدام تقنية زراعة الأنسجة النباتية في المختبر لمعرفة تأثير منظمات النمو على كمية الكالس المنتجة.تم استحثاث الكالس من نبات *Salvadora persica* باستخدام توليفة من الأوكسين (NAA) والسيتوكينين (KIN) . في التجربة، تم تعقيم أجزاء النبات (البذور والعقد والاوراق) باستخدام ($HgCl_2$) و (NaOCl) لفترتين زمنيتين (5 و 10 دقائق). ظهرت النتائج أن أفضل مادة لتعقيم أجزاء النبات هي ($HgCl_2$) . تمت زراعة أجزاء النبات المختلفة (الورقة ,السلامية ,العقدة والجذر) على وسط MS مصحوبة بمزيج من الهرمونات وتراكيز مختلفة من (NAA)و (KIN). وبعد أربعة أسابيع من الزراعة سجلت اختلافات معنوية. أفضل توليفات لمنظمات نمو النبات (PGEs)لمعدلات تحفيز الكالس من الأوراق عند 1ملغم/لتر KIN و1 ملغم/لترNAA كانت 2.2%. في حين كانت أفضل توليفة للسلاميات هي 0 ملغم/لتر KIN و 1 ملغم/لتر NAA بنسبة 1.33 .%تم الحصول على معدل استحثاث الكالس من العقد عند (1 ملغم/لترKIN، 2 ملغم/لتر NAA) التي أعطت 2.25%.أظهرت النتائج أن أعلى وزن طري للكالس تم الحصول عليه من الأوراق كان 0.4823 غرام .بينما أعطت السلامية 0.3195غم وزن طري. بينما كان اعلى وزن الطري للكالس الناتج من العقد 0.6791 غرام. أما بالنسبة للجذور فلا يوجد استجابة لتحريض الكالس.

الكلمات المفتاحية: زراعة الأنسجة النباتية، السلفادورا بيرسيكا (المسواك)، (KIN)، (NAA) .

INTRODUCTION

As one of the most well-known methods, plant tissue culture has been used since 1902 to create healthy, disease-free plants with the right quality at a faster rate of production throughout the year, regardless of the season. Every plant has a unique shape and set of dietary needs. Furthermore, the tissues in each section of the plant have different nutritional needs. **(Sudheer *et al.*, 2022).** The technique of tissue culture has made it possible to multiply and genetically enhance economically useful plants. Under controlled conditions, tissue culture has been utilized to research aspects of plant physiology, development, metabolism, reproduction, and nutritional requirements. With little plant tissue, the plantlets are created in a relatively short period of time. The growth of many plants in the absence of seeds or necessary pollinators for the formation of seeds **(Baan *et al.*, 2020).** The Salvadoracea family includes *Salvadora persica SP*, often known as the Miswak or Arak tree, which grows in dry areas of Africa, Asia, and Arab nations **(Mohamed *et al.*, 2021**). Around the world, it is known by a variety of names, including Arak, Miswak, Siwak (Arabic), Merge, pilau (India), Caday (Somalia), Omungambu (Southern Africa), and Toothbrush Tree (English) **(Ronse & Wanntorp, 2009)**. The Spanish pharmacist Juan Salvadory Bosca was honored with the genus' name by French botanist Laurent Garcinin in 1749. The species name persica comes from the fact that the type specimen was found in Persia **(Ahmad & Rajagopal, 2014)**. It can be said that S. persica originated in a variety of locations around the world, including Saudi Arabia, Yemen, Iran, Iraq, Egypt, India, Pakistan, Malaysia, Sudan, Ethiopia, and Mauritania, as well as nations in Central Africa, Southwestern Africa, and South America **(Waseem *et al.*, 2019)** The halophyte S. persica is perennial and evergreen. According to **(Muhammad *et al.*, 2018)**, it may thrive in extremely harsh settings, including extremely dry soils. The *Salvadora persica* tree can grow to a height of 3 m and bears thick, succulent, tiny leaves **(Jamal *et al.*, 2011)** Young stem branches are green to greyish in color, while older stem branches are dark brown **(Ehsan *et al.*, 2012)** *Salvadora persica* 's root, seed, flower, fruit, leaf, bark, stem, and twigs have all been thoroughly screened. Determining the existence of flavonoids, terpenes, sterols, alkaloids, and glycosides required extensive phytochemical investigation. Ascorbic acid, fluoride, calcium, phosphorus, silica, organic sulfur compounds, and elemental sulfur are also present in trace amounts **(Muhammad *et al.*, 2017)**. The plant has short stems, green leaves, and fruits that are used in cooking and in traditional herbal therapy to treat rheumatic illnesses such as rheumatoid arthritis and asthma. *Salvadora persica* has been shown to have beneficial effects on cytotoxicity, antiulcer, antibacterial, antimitotic, mycosis, antifertility, and antioxidants **(Mohamed *et al.*, 2021);(Gamal *et al.*, 2017)**. The significant effect of *Salvadora persica* as an anti-cancer has recently increased after it was shown that the alcoholic extracts of the stem and fruits had an impact on a variety of cancer cells **(Bayan *et al.*, 2018; Mohammed *et al.*,2020; Disha *et al.*,2020).**

Pramod (2019) and others, in their research, aimed to shed light on the method of callus induction of *Salvadora persica L*. by taking healthy explants from the healthy mother plant and planting it on MS medium, which contains different hormonal combinations.

MATERIALS AND METHODS

The experiments of this study were obtained in the plant tissue culture laboratory in the biology department of the College of Science for Women at the University of Baghdad.

Explant collection and sterilization

The plant and the seeds were collected and then classified from local plantations from Hilla in Babylon province. The seeds, leaf, internode root, and nods from the intact plant were washed under running tap water in the laboratory for 30 minutes to remove any adherent particles, the washed seeds were surface sterilization by sodium hypochlorite with sterilized Distilled Water (DW) 1:1, 1:2, and 1:3 concentrations and 0.1% Mercuric chloride ($HgCl_2$) for (5 and 10) minutes then rinsed with sterilized (DW) for 5 minutes three time then culture on MS medium aseptically.

Culture media

A- Murashige and Skoog media (MS) (1962) was used for the induction of plantlets of *Salvadora persica L.* The pH was adjusted from 5.5 to 5.8. and autoclaved at 15 psi and 121°C. For callus induction, another MS media was prepared and supplemented with a combination of PGRs (KIN: NAA) with different concentrations (0,1,2,3) mg/L. two types of media were sterilized in an autoclave under 15 psi and 121°C **(Ali & Bushra, 2023)** and incubated for 3 days before starting culturing.

B- The sterilized seeds were cultured in vials on free MS medium under sterile conditions inside a Laminar air cabinet and then incubated at a temperature of 25± 2°C after three weeks of seed germination, the plantlets were produced. The seed planting with ten replicates

C- The leaf, internode, root, and nods from the plantlets and intact plant were separated and cultured in MS media supplemented with a combination of (PGRs) and incubated under at a temperature 25± 2°C under (8:16) (dark: light) for 1 month. The explants planting with ten replicates.

D- The weight of the callus produced was measured using a sensitive balance Samples were taken randomly of different concentrations and determine the morphological appearance of callus.

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study. **(SAS, 2018)**

RESULTS AND DISCUSSION

Sterilization experiment

The results of the sterilization experiment of using two types of sterilization material mercury chloride ($HgCl_2$) and three concentrations of sodium hypochlorite (NaOCl) for five and ten minutes for three types of explants were studied. Using $HgCl_2$ at a 0.1% concentration as sterilizing agent for seeds, nodes, and leaves, complete sterilization was achieved within 5 minutes, resulting in sterilization rates of 100%. Simultaneously, the survival rates for these explants were (80,80, and 85) % respectively. When the same 0.1% concentration of $HgCl_2$ was applied for 10 minutes, the sterilization rates were (100,85, and 100) % for seeds, nodes,

and leaves, respectively, while the survival rates were (50,75, and 80) for the same explants, respectively. Sodium hypochlorite (NaOCl) where used in three concentrations. The most notable sterilization efficiency was achieved at a 1:1 concentration ratio, particularly after a 10-minute treatment. During this duration, seeds, nodes, and leaves displayed sterilization rates of (100, 85, and 100) %, respectively. Additionally, the corresponding survival rates for these explants were (100, 85, and 850 %, respectively, demonstrating that they maintained high levels of viability despite the sterilization process. While the lowest sterilization efficiency was observed when using a 1:3 concentration ratio at both 5 and 10-minute time intervals. It's important to note that the results varied depending on the type of explants being studied. Briefly, after 5 minutes of treatment, the sterilization rates for seeds, nodes, and leaves were (20, 42, and 66) %, respectively, while the corresponding survival rates were (33, 71%, and 70) %, respectively. In contrast, when the sterilization duration was extended to 10 minutes, the same explants exhibited sterilization rates of (30, 50, and 50) %, respectively, while the survival rates for these explants were (50, 66, and 50) %, respectively. This result agrees with **(Ali & Mundher, 2017)** of using NaOCl as a sterilizer to get rid of microbes.

The results in (Table 1) illustrate that sterilization with (HgCl2) at 0.1% for 5 minutes was the best process for all explants than (NaOCl). Both HgCl2 and NaOCl are toxic to the plant tissues, thus, proper concentrations of these sterilizing agents should be carefully selected. Here it was observed that some nodal segments did not survive, it was probably due to damages that occurred during the ring sterilization procedure and/or due to damaged explants during excision. NaOCl, as a weak/or mild sterilizing agent, should be used in a higher percentage to control contamination. However, HgCl2, which mainly has a bactericidal action, was more effective and showed better decontamination percentages **(Hail *et al*., 2012)** The results of the study agree with **(Pramod *et al*.,2019)** in leaf sterilization and disagree in nods and internodes sterilization and similar kind of results agree with **(Ravindra *et al*., 2017)** by using HgCl2 0.1%. And disagree with **Rasha & Ansam (2020)** they found the optimum results were obtained using NaOCl for 10 minutes for all examined parameters.

Table (1): Effect of $HgCl_2$ and Sodium hypochlorite on sterilization and survival rates of different explants following three weeks of culture at different time intervals.

Plant Part	Time (min.)	Sterilization rate %					Survival rate %				
		$HgCl_2$	Sodium hypochlorite (NaOCl)			LSD value	$HgCl_2$	Sodium hypochlorite (NaOCl)			LSD value
			1:1	1:2	1:3			1:1	1:2	1:3	
Seed	5	100	50	30	20	8.71 *	80	80	40	33	8.46 *
	10	100	80	40	30	9.02 *	50	100	66	50	9.51 *
Nod	5	100	71	57	42	9.57 *	80	75	71	71	6.85 *
	10	85	80	75	50	8.33 *	75	85	100	66	8.36 *
Leaf	5	100	85	80	66	7.94 *	85	85	100	70	8.93 *
	10	100	100	85	50	9.15 *	80	85	85	50	8.14 *
LSD value	---	8.02 *	11.37 *	9.62 *	9.55 *	---	7.59 *	10.42 *	10.07 *	9.83 *	---
* ($P \leq 0.05$).											

Callus induction

In this stage, the nod, internode, leaf, and root were used from seedlings and intact plants as explants for callus induction. the explants were used and cultivated on MS media containing a combination of cytokine (KIN) and auxin (NAA) at various concentrations (0,1,2 and 3) 3mg/L after 4 weeks of cultures. The results are shown in (Table 2,3,4) callus induction rate of the leaf, internode, and node. These explants (leaf, internode, node, and root) were taken from an intact plant and were relied upon for callus initiation due to the small amount of callus produced from the seedling which is not a sufficient amount (Figure 1). The MS medium supplemented with 1 KIN and 1 NAA mg/L concentration, has the best callus initiation rate was 2.2% from leaf segments as seen in (Table 2). Callus-induced rates from internode sections of *S. persica* was 1.33% under the influence of 0 KIN, 1 NAA mg/L concentration is represented in (Table 3). Evidently, based on the data in (Table 4) the node was the best explant for the callus initiation rate of 2.25% at 1KIN and 2NAA mg/L concentration. the roots didn't give a response for callus induction either from seedlings or plantlets. In addition, the callus induction rate was 0 in the control media because there were no PGRs present thus phytohormones are certain to have a considerable impact on the callus-stimulating process in plant tissue culture so the addition of PGRs to MS media is necessary to induction callus. In the current study. The various explants displayed variations in callus induction as measured by the percentage and weight of the callus (Table 5) showing the greatest amount of fresh weight callus was 2 KIN 1 NAA from nods and their weight was 0.6791g Figure 2 and their total amount of fresh weight was 4.2774 g. The texture of callus in all explant types was friable and

the color ranged from white to yellowish (Table 5) which aligned with the result of **(Sara, 2016)** which illustrated that the two concentrations (1.5 and 3.0 mg/L NAA) giving the maximum percentage of callus induction reaching (97%) for each of the two concentrations, which were noticeably better than the NAA-free medium. It is well-recognized that auxins are important for the development of calluses, and different forms of auxins have diverse effects **(Baskaran *et al.*, 2006)**. Additionally, the cytokinins improved the efficiency of auxin's callus-induction impact **(Rao *et al.*, 2006)** The cultured explants' varied responses to callus induction may be explained by the fact that they are made up of distinct tissues, including meristematic, parenchymatous, and cells with less differentiation. Thus, the nature of the callus may depend on the cells' exposure to growth regulators. However, distinct subsets of the callus' cells are also triggered differentially by the same PGRs **(Aftab *et al.*, 2010)**, Similar kinds of results agree with **(Shamil, 2018)** which demonstrates that the types of explants that were employed as sources for callus production differ significantly from one another, and that the production rate was significantly affected by the different PEG concentrations. Also, the amount of callus depends on the presence of hormones inside the cells that agree with **(Ibrahim & Ranin, 2017)** has the same opinion of Callus induction, which generally depends on the internal level of growth regulators and the amount of their absorption to reach an appropriate balance for the formation and growth of callus. The effect of the hormone differs in different cells due to the effect of the genotype, which was suggested by **(Filomena *et al.*, 2010)** that explained the effect of the genotype on the cellular response of cells, also the experiments **(Khansaa, 2012)** have shown that it is possible to induce shoot differentiation and complete plantlet development from nodal explants of *Arachis hypogea L* .by using hormone combination, BA (auxin) with Kin(cytokine). In addition to that, adding growth regulators in different concentrations leads to differences in the weight and quantity of callus produced, and this is confirmed by **(Ali & Jassim, 2022)** their experiments show the difference in the combination and concentration of growth regulators cause differences in plant weight.

A B

Figure (1): The amount of callus indication from different explants of seedlings with various concentration of hormones A) internode (2 mg/L KIN and 1 mg/L NAA) B) leaf (1 mg/L KIN and 1 mg/L NAA).

Table (2): Effect of KIN (mg/L), NAA (mg/L), and their concentration on the percentage callus induction from the leaf of intact plants after 30 days of culture on MS medium.

KIN (mg/L)	NAA (mg/L)				
	0	1	2	3	Mean
0	0.00%	1.66%	0.625%	0.25%	0.633
1	0.00%	%2.2	0.4%	1.6%	1.050
2	0.33%	0.00%	0.00%	2%	0.582
3	0.8%	1%	0.833%	0.00%	0.658
Mean	0.282	1.21	0.464	0.962	---
LSD value: KIN: 0.407 *, NAA: 0.407 * , KIN x NAA: 0.772 * . * (P≤0.05).					

Table (3): Effect of (mg/L), NAA (mg/L), and their concentrations on the percentage callus induction from internodes of intact plants after 30 days of culture on MS medium.

KIN (mg/L)	NAA (mg/L)				
	0	1	2	3	Mean
0	0.00%	1.33%	0.00%	0.125%	0.363
1	1%	0.66%	1.2%	0.00%	0.715
2	0.2%	0.00%	0.00%	0.00%	0.050
3	0.00%	0.00%	1.16%	0.83%	0.497
Mean	0.30	0.497	0.590	0.238	---
LSD value: KIN: 0.291 * , NAA: 0.291 * , KIN x NAA: 0.452 * . * (P≤0.05).					

Table (4): Effect of (mg/L), NAA (mg/L), and their concentrations on the percentage callus induction from nodes of intact plants after 30 days of culture on MS medium.

KIN (mg/L)	NAA (mg/L)				
	0	1	2	3	Mean
0	0.00%	1.66%	0.33%	2%	0.997
1	2%	0.00%	2.25%	0.5%	1.19
2	1.6%	0.00%	0.00%	2%	0.90
3	1.75%	0.66%	0.00%	1.33%	0.935
Mean	1.34	0.58	0.645	1.46	---
LSD value: KIN: 0.437 *, NAA: 0.437 * , KIN x NAA: 0.791 * . * (P≤0.05).					

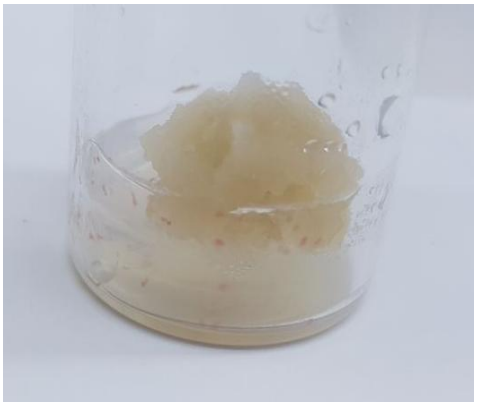

Figure (2): the highest amount of callus induction from node of intact plants when culture it on MS media with 1 mg/L KIN and 2 mg/L NAA.

Table (5): Effect of KIN and NAA on fresh weights of *Salvadora persica* callus and their morphological by using parts of intact plants as explants.

PGRs concentration (mg/l)		Part of plant	% of callus induction	Mean fresh weight of callus (g/explant)	Color of callus	Texture of callus
KIN	NAA					
0	3	Leaf	40	0.2903	Yellowish	Friable
1	3	Leaf	66.6	0.4823	Yellowish	Friable
1	1	Leaf	100	0.3766	White	Friable
3	1	Leaf	80	0.1122	Wight	Friable
LSD value			**8.71 ***	**0.219 ***	---	---
0	1	Internodes	100	0.3195	Yellowish	Friable
0	3	Internodes	33.33	0.0546	Yellowish	Friable
1	1	Internodes	80	0.1302	Yellowish	Friable
3	3	Internodes	66.66	0.2764	Yellowish	Friable
LSD value			**8.97 ***	**0.155 ***	---	---
0	3	Nodes	100	0.5276	White	Friable
1	0	Nodes	100	0.3213	White	Friable
1	3	Nodes	66.6	0.1749	Yellowish	Friable
1	2	Nodes	100	0.6791	Yellowish	Friable
LSD value			**8.57 ***	**0.225 ***	---	---
LSD / Total			**14.72 ***	**0.296 ***	---	---
* ($P \leq 0.05$).						

CONCLUSION

The study concluded that growth regulators KIN and NAA have a beneficial effect on the amount and fresh weight of *Salvadora Persica L.* callus. nodes were the best plant part for induction callus, and the best source for callus initiation was the intact plant.

REFERENCES

1. Aftab, F., Akram, S. & Iqbal, J. (2010). Estimation of fixed oils from various explants and in vitro callus cultures of Jojoba (*Simmondsia chinensis*). *Pakistan Journal of Botany*, 40 (4), 1467-1471.
2. *Ahmad, H., & Rajagopal, K. (2014). Salvadora persica L. (Meswak) in dental hygiene. The Saudi Journal for Dental Research, 5, 130-134.*
3. Ali, A. A., & Mundher, Kh. J. (2017). In Vitro Detection TaSOS1gene in four Iraq genotypes of Bread Wheat under different salt stress levels. *Iraqi Journal of Biotechnology*, 16 (4),69-78.
4. Ali, N. A., & Bushra, S. A., A. (2023). Efficacy of pseudomonas fluorescent and iron chelated FE-EDDHA against *fusarium oxysporumf sp.* the causal agent of root rot and wilt disease on pepper. *Iraqi Journal of Market Research and Consumer Protection*, 15(1): 81-91.
5. Ali, W. R. A., & Jassim M. A. A. (2022). Study of some growth criteria yield of soybean crop with the effect of boron and some growth regulators. *Iraqi Journal of Market Research and Consumer Protection*, 14(1): 137-145.
6. Baan, M. T., Zena, H. J., & Nazmul, M. H. (2020). Trends in the Use of Tissue Culture, Applications and Future Aspects. *International journal of plant biology*, ***11***(1): 8385
7. Baskaran, P., Raja, R. B., & Jayabalan, N. (2006). Development of an in vitro regeneration system in sorghum (*Sorghum bicolor L. Moench*) using root transverse thin cell layers (tTCLs). *Turkish Journal of Botany*, 30 (1), 1-9.
8. Bayan, A., Ismail, A. E., Chandraprabha, M., Ahmed, A., & Amr, A.(2018). *Salvadora persica* (Miswak): antioxidant and promising antiangiogenic insights. *American Journal of Plant Sciences*, 9 (6), 1228-1244
9. Disha, V., Chou, M. C., Aisha, A., Kok, S. L., & Swee, H. E. L. (2020). Middle Eastern plant extracts: an alternative to modern medicine problems. *Molecules*, ***25***(5), 1126.
10. Ehsan, M., Mohammad, R. Sh., Saeed, A., Mitra, M., Mohammad, M. H., & Saeed, E. (2012) In Vitro Antimicrobial Activity of Salvadora persica Extract on Helicobacter pylori Strains Isolated from Duodenal Ulcer Biopsies. *Microbiology Research, 3*(1), 44-49.
11. Filomena, G., Mafalda, S., Maria, L. L., & Jorge M. C. (2010). Effect of plant growth regulators and genotype on the micropropagation of adult trees *of Arbutus unedo L.* (strawberry tree). *New biotechnology journal*,27(6):882-892
12. Gamal, A. S., Majid, A. G., Hassan, N. A., Faisal, F. A., Mohammed, A. S., & Maged, S. A. (2017). Extract of *Salvadora persica* roots attenuates lead acetate-induced testicular oxidative stress in rats. *Journal of Pharmacy and Pharmacognosy Research*, 5 (4), 238-250.
13. Hail, Z. R., Mohammed, A., Fadil, A., & Michael, P. F. (2012). The effect of using PPM (plant preservative mixture) on the development of cauliflower microshoots and the quality of artificial seed produced. *Scientia Horticulturae*,141(4) :47-52.

14. Hilal, A. & Rajagopal, K. (2013). Biological Activities of *Salvadora persica L*. (Meswak). *Medicinal and Aromatic Plants*, 2(4):1
15. Ibrahim, A. H., & Ranin, J. A. (2017). Roll of plant growth regulators in callus initiation and plantlets reproducing of tow Alfalfa cultivars. *The Iraqi Journal of Agricultural Sciences*, 48 (3):765-772
16. Jamal, A., Khalid, M. S., Salma, B., & Mohd, M. (2011).A review on phytochemical and pharmacological investigations of Miswak (*Salvadora persica* Linn.). *Journal of Pharmacy and Bioallied Science*, 3(1): 113–117.
17. Khansaa, R. A. (2012). In Vitro Propagation of Groundnut (*Arachis hypogeaL.*). *Ibn Al-Haitham Journal for Pure and Applied Science*, 25(3).
18. Mohamed, M., Mathias, G., Manoj, K., Radha , Yasmine, H., & Christof, D. (2021). *Salvadora persica*: Nature's Gift for Periodontal Health. *Multidisciplinary Digital Publishing Institute journals*, 10(5):712.
19. Mohammed, A., Hafiz, A. M., Hassan A. A., Sohier, M. S., Ashraf, N. A., Husham, E. H., Shahnaz, S., Waquar, A., & Asaad, K. (2020). Phytochemical, cytotoxic, and antimicrobial evaluation of the fruits of miswak plant, *Salvadora persica L. Journal of Chemistry*, 1 (3):1-11
20. Muhammad, Z. A., Gokhan, Z., & Mohamad, F. M. (2018). A review of the traditional and modern uses of ***Salvadora persica*** L. (Miswak): Toothbrush tree of Prophet Muhammad. *Journal of Ethnopharmacology*, 213 (1): 409-444.
21. Muhammad, Z. A., Gokhan, Z., & Mohamad, F. M. (2017).A review of the traditional and modern uses of *Salvadora persica L.* (Miswak): Toothbrush tree of Prophet Muhammad. *Journal of Ethnopharmacology*, 1(213) :409-444.
22. Pramod, F., Sangeeta, A., & Narayan, B. P. (2019). In Vitro Callus Induction studies in *Salvadora persica* L. *International Journal of Science and Research*,10(3):976-978.
23. Rao, A. Q., Hussain, S. S., Shahzad, M. S., Bokhari, S. Y. A., Raza, M. H., Rakha, A., Majeed, A., Shahid, A. A., Saleem, Z., Husnain, T., & Riazuddin, S. (2006). Somatic embryogenesis in wild relatives of cotton (*Gossypium* Spp.).*Journal of Zhejiang University SCIENCE*, 7(4): 291–298.
24. Rasha, K. M. Al., & Ansam, G.A. (2020). Callus Induction and Shoot Formation for Mexican Red Bean *(Phaseolus vulgaris L.)* Pinto Cultivar in Vitro. *Iraqi Journal of Science*, 61(8): 1887-1893.
25. Ravindra, K. K., Kanwar, P. S., Raju, D.V.S., Sapna, P., Reeta, B., Pradeep, J., & Vinod. (2017). Standardization of rapid multiplication protocol in petaloid male sterilelines of African marigold (*Tagetes erecta*) through in vitro culture. *Indian Journal of Agricultural Sciences*, 87 :(10): 1295–1302.
26. Ronse, D. C. L., & Wanntorp, L. (2009). Floral development and anatomy of Salvadoraceae. *Annals of Botany*, 104(5): 913–923.
27. Sara.R.KH.Al. (2016). Influnce of growth regulators on callus induction of *citrus Volkameriana* in vitro. *The Iraqi Journal of Agricultural Sciences*, 47(3): 723-731.
28. SAS. 2018. *Statistical Analysis System*, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
29. Shamil S. N. (2018). Invitro production of some terpenoids compounds from Nigella sativa with differnt explants type and PEG concentrations. *Iraqi Journal of Agricultural Sciences*, 49(4):534-540.

30. Sher, H., & Alyemeni, M. (2011). Pharmaceutically important plants used in traditional system of Arab medicine for the treatment of livestock ailments in the kingdom of Saudi Arabia. *African Journal of Biotechnology* ,10(45) :9153–9159.
31. Sudheer, W. N., Praveen, N., Al-Khayri, J. M., & Jain, S. M. (2022). *Advances in Plant Tissue Culture*. 1st., Academic Press is an imprint of Elsevier, United Kingdom, p.51-83.
32. Waseem, M. A., Furkhan, A. M., &. Syed, S. R. (2019). Plant and Human Health. 1st., Springer, Nature Switzerland, p.353-