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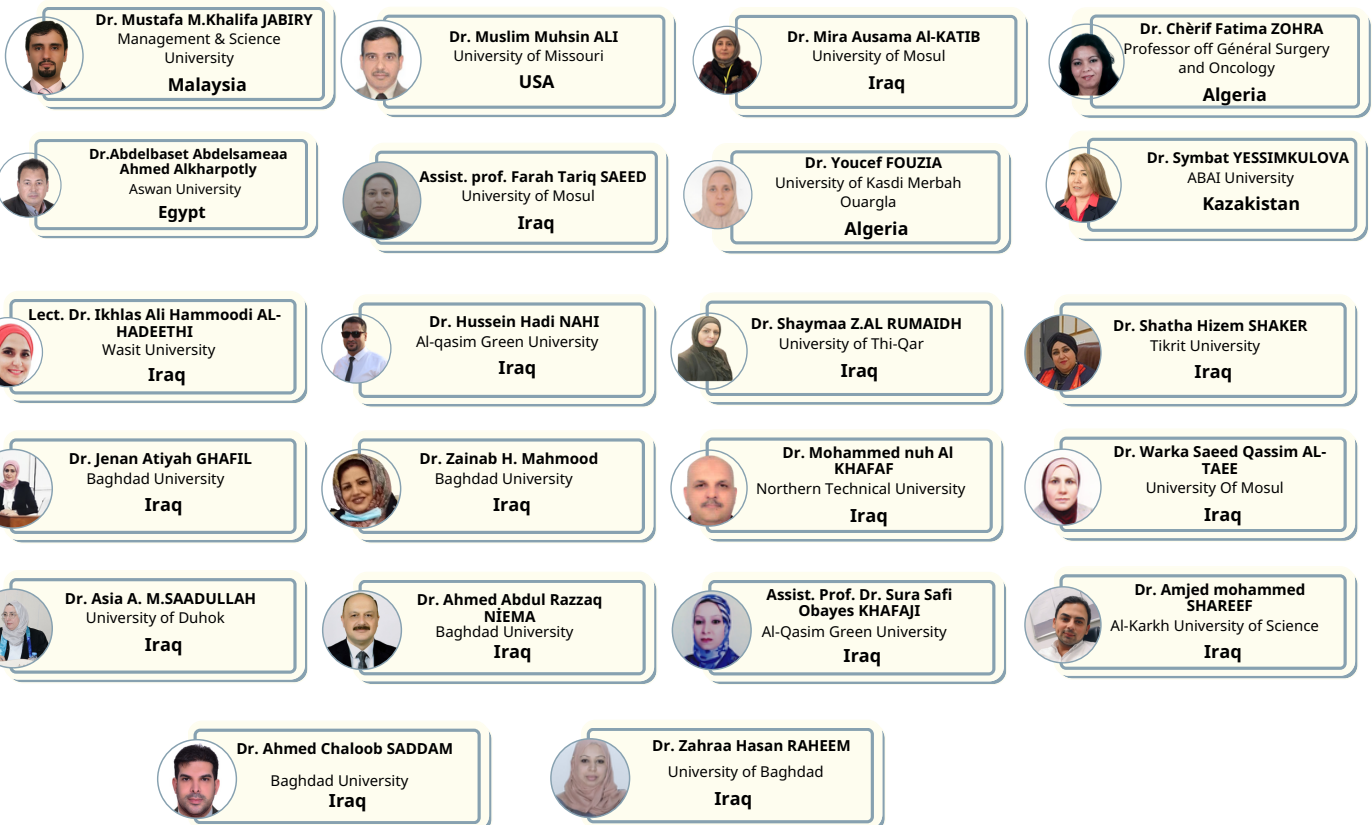
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# Minar Congress

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# ÖNSÖZ

Rimar Academy ile İstanbul Gedik Üniversitesi arasında 14/15/16/02-2023 Şubat tarihlerinde yapılan “İstanbul Gedik Üniversitesi’nin paydaş olduğu 8. Uluslararası Fen, Uygulamalı ve Teknolojik Araştırmalar Kongresi (MINAR KONGRESİ) kurum ve kuruluşların kararlı işbirliğinin, çalışmalarının, maddi ve manevi katkılarının sonucunda gerçekleştirilmiştir. Rimar Academy Türk dünyasının farklı akademik çalışmalarını ortak bir zeminde buluşturmaktadır. Bilim dünyasının değerli insanlarını farklı çevrelere ilişkin; eğitim, edebi, kültürel, sosyal, siyasi, ekonomi ve diğer konulardaki gelişme düzeyinin artırılmasına, ikili ya da bölgesel sorunların çözümüne dair alternatiflerin sunulmasına yönelik bilimsel çalışmaları paylaşmak kongrenin asıl amaçlarından biridir. Rimar Academy Kongre Bildiri Kitabı, bilimsel üretimin geleceğe birikim ve katkı olarak aktarılması hedefiyle hazırlanmıştır. Bu kongreye yurtdışı ve yurtiçinde olmak üzere toplamda 89 kişi başvurmuştur. 63 kişi bilim kurulu tarafından kabul edildiği; kabul edilen bildirilerin 2’si Türkiye’den, 61’i Türkiye dışından 4 ülkeden katılım sağlanmıştır. 39 katılımcı yüz yüze, 24 katılımcı online olarak kongreye katılmıştır. Kongreye 24’ü tam metin bildiri, kalan diğer makaleler Minar Journal dergisinde yayınlanmaya bilim kurulunca uygun görülmüştür. Kongremize değerli katkılarından dolayı tüm bilim insanlarına, teşekkür ediyor ve saygılarımı sunuyorum.

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**ANOVA ANALYSIS FOR MONITORING PERFORMANCE OF CONTACT TIME-DEPENDENT  
MULTIWALL CARBON NANOTUBES (MWCNTs) FOR REMOVAL ELEMENTS OF  
INDUSTRIAL REFINERY WASTEWATER**

**H.I.ABDULGAFOUR** <sup>1</sup>

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**Abstract:**

In this article, multiwall carbon nanotube (MWCNT) was used to remove contaminants from wastewater of the Al-Dora refinery in Baghdad\ Iraq and all data were studied and analyzed by the ANOVA method. A total suspended solids (TSS), nitrate oxides (NO<sub>3</sub>, NO<sub>2</sub>), chemical oxygen demand (COD), and trihalomethanes (THMs) have been detected at a range time limit between 10 to 80 mins with an increasing 10 mins for each step in experiments. A significant difference in the value of organic contaminants has been observed by using MWCNTs as an anti-pollutant, with the best value after 70 mins. Statistical calculations were applied to analyze all the results with the function of time such as models, coded coefficients, regression equations in un-coded units, and the effects of Pareto, by using Minitab software. These results show models of the treatment and absorption stages according to the sizes and lengths of the hydrocarbon bonds.

**Key Words:** Multiwall Carbon Nanotubes, Hydrocarbons, Wastewater Treatment, Al-Dora Refinery, ANOVA Analysis.



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## Introduction:

Water pollution is one of the most important problems in the world and is a direct effect on human health and the environment also it has an impact on social costs and the economy[1, 2]. Water pollution is caused by waste from industries such as petroleum refineries, petrochemical, textile, leather, and others which lead to an increase in the heavy metals in water were the main source of concern for various years.[3] Generally, wastewater was containing a mixture of organic materials, poisonous pulp mills, heavy metals, dyes, and oil.[4] Many different methods of treatment and removal of impurities from wastewater were studied by researchers and developed for removing metal ions and other impurities over the years such as reverse osmosis, chemical precipitation, ion exchange, electrolytic recovery, and adsorption. Unfortunately, these methods have several disadvantages due to their produce large quantities of sludge, chemical requirement, low efficiency and need high energy.[5-10] Of these techniques, nanotechnology is one of the finest and most advanced ways for wastewater treatment and has a unique ability to treat water from contaminants due to its versatility for nanoparticles, highly absorbed, and reacting capabilities with impurities.[11-14]

MWCNTs have significant interest in wastewater treatment due to their flexible surface area, distinct structure, large specific surface area, and lower mass loss in reactive. Generally, MWCNTs have been used for the treatment of wastewater from organic and inorganic materials such as dyes and elements respectively, [15-17] and were reported by several scientists and researchers, for instance, Zeng WJ et al. synthesized graphene oxide/CNTs composite membranes by intercalation MWCNTs-COOH into the graphene oxide and used as a highly efficient of dyes removal from wastewater[18], although Carmen R. et al. reported the effect of walls in MWCNT membranes in a case of nanocomposite (CNT) in RO membrane. By non-equilibrium molecular dynamics simulations, The two types of vertical MWCNT membranes (6,6) and (8,8) were analyzed theoretically to study the effect of wall numbers on RO simulation. The results were shown that the MWCNT (6,6) has a high ion ( $\text{Na}^+$  and  $\text{Cl}^-$ ) rejection while for MWCNT (8,8), the percentage of ion rejection was decreased [19]. Furthermore, Anku W. et al. synthesized MWCNTs and Nd modified ZnO nanocomposite and characterized them by XRD. TEM and FTIR. The analysis showed that the MWCNTs and Nd (0.6%) decreased the energy gap of ZnO to 2.7 eV which enhanced the light absorption and photodegradation efficiency. The modified compound degraded the organic compound (dyes) 99% by visible light irradiation in 4 hours [20]. Generally, Many mechanisms have been signified for removing the contaminates from wastewater by using MWCNTs such as hydrogen bonding, hydrophobic interaction, Lewis acid-base interaction, electrostatic interaction, and  $\pi$ - $\pi$  interaction [21-23] and all these mechanisms gave the efficient absorption of contaminates. This paper describes using MWCNTs for treating the samples of wastewater from the Al- Dora refinery and discussed the effect of temperature at

40 °C and times (10, 20, 30, 40, 50, 60, 70, and 80 mins) for removal of heavy metals and hydrocarbons with other materials such as NO<sub>3</sub>, Oil, THM, NO<sub>2</sub>, COD, and TSS.

### **Materials and methods**

MWCNTs Us Research Nanomaterials Inc., USA, purity above 95%, outside and inside diameter are (5-15 nm) and (3-5 nm) respectively. Other chemicals were purchased from Alfa Aesar and Sigma Aldrich. The wastewater was supplied from the Al-Dora refinery, Baghdad, Iraq. To analyze all data theoretically, researchers Thomas A. Ryan, Jr., Barbara F. Ryan, and Bryan L. Joiner utilized the ANOVA approach with Minitab software. Also, Minitab can be defined as one of the statics packages created at Pennsylvania State University. Gas chromatography–mass spectrometry (GC-MS) were performed by Perkin-Elmer Clarsus 680 system (Perkin-Elmer; Inc., Shelton, USA). Atomic Absorption Spectrometry were recorded by AAS, Nova 400; Analytic Jena, Germany, and UV/visible spectrophotometer were performed by Cecil/ UK, and .

The method was described in previous work [24], MWCNTs (1gm) was added to 25 mL of 8M HNO<sub>3</sub> for 12 hrs, and vigorously stirred at room temperature. The solution was filtered and washed with (50 mL) of deionized water 4 times and dried at 100 C for 2 hrs.

MWCNTs (50 mg) was added to 10 mL of wastewater from the Al-Dora refinery and sonicated at 40 °C for variable times (10, 20, 30, 40, 50, 60, and 70 mins). then the mixture was filtered and the difference between the initial and final concentration of wastewater was measured by atomic absorption spectrophotometer. Other content concentrations such as oil, NO<sub>2</sub>, NO<sub>3</sub>, THM, COD, and TSS were tested by UV/visible spectrophotometer, and gas chromatography.

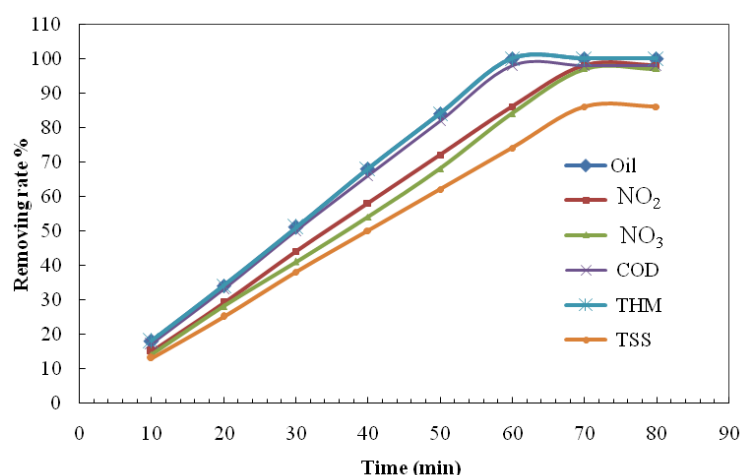
### **RESULTS AND DISCUSSIONS**

The functionalized of MWCNTs by chemical treatment related to oxidation, represented a high-removal approach for a variety of metal ions. Commonly, oxidizing a carbon surface might result in a more hydrophilic surface structure and increase the number of oxygen-containing functional groups, hence increasing the carbon material's ion-exchange capabilities. Nitric acid treatment was a redaction of some groups while also forming a new acidic group dominating the CNT's surface charge. Our tests were conducted solely under acidic conditions because grown MWCNTs have a lower adsorption capacity than MWCNTs that were activated *via* concentrated nitric acid[25]. Activated MWCNTs were used for characterizing and treating the samples of wastewater from the Al- Dora refinery. Our previous study explains the effect of temperature on the heavy elements in wastewater, and we found that the best result has been obtained at 40°C [24], then we attempted to study the effect of time of treatment on these elements at that temperature; therefore, our results show removal of hydrocarbons and some other compounds and materials such as NO<sub>3</sub>, Oil, THM, NO<sub>2</sub>, COD, and TSS from waste-water of Al- Dora refinery at a temperature of 40 °C at

variable times and The maximum percentage of adsorption was pointed at 70 and 80 mins as shown in Table 1, and Figure 1

**Table 1. The removal ratio of different elements by MWCNTs from wastewater of Al-Dora refinery.**

t (min)	Oil%	NO <sub>2</sub> %	NO <sub>3</sub> %	COD%	THM%	TSS%
10	18	15	14	17	18	13
20	34	29	28	33	34	25
30	51	44	41	50	51	38
40	68	58	54	66	68	50
50	84	72	68	82	84	62
60	100	86	84	98	100	74
70	100	98	97	98	100	86
80	100	98	97	98	100	86



**Figure 1. The removal rate of the elements at different times.**

Table 2A shows the GC-MS spectrometry of the hydrocarbon compounds in the wastewater of the Al-Dora refinery before being treated by activated MWCNTs, which gave an important indicator of such types of organic compounds. Table 2B shows the compounds that have been removed from the Al-Dora refinery's wastewater by treating the activated MWCNTs at 40 °C for 70 mins; these results explain how the MWCNTs can be effective on some kinds of hydrocarbon series from wastewater of Al-Dora refinery.



**Table 2: A) the GC-MS data of organic compounds in wastewater of the Al-Dora refinery before treatment by MWCNTs, B) the removed compounds in wastewater after treatment by MWCNTs.**

<b>(A) the wastewater before treatment</b>			
<b>Peak No.</b>	<b>R. Time</b>	<b>Area</b>	<b>Name</b>
1	2.126	3773858	Toluene, Benzene, Methyl
2	2.320	383117	Octane, n-Octane
3	2.921	314835	Benzene, (3,3-dimethylecyl)
4	3.008	334920	p-Xylene, Benzene, 1,4-dimethyl
5	3.318	878776	Nonane, n-Nonane
6	3.771	381753	Octanol, 2-methyl
7	4.182	629903	Octadecane, 6-methyl
8	4.699	2066018	Undecane, n-Undecane
9	5.054	340059	Heptane,5-ethyl--methyl
10	6.259	1365039	Benzamido-4-methylbenzanilide
11	7.835	603195	Dodecane, n-Dodecane
12	9.350	303661	Tridecane, n-Tridencane
13	10.783	1144635	Tetradecane,n-Tetradecane
14	12.134	756203	Pentadecane, n-Pentadecane
15	13.409	672419	Eicosane, 10-methyl
16	14.616	521647	Dodecane, 2- methyl-6-propyl
17	15.762	595594	Methoxyacetic acid, 4-tridecyl ester
18	16.849	298770	Eicosane, 10-methyl
19	17.449	1776838	n-Hexadecanoic acid
20	19.243	2139301	9-Hexadecanoic acid
21	19.886	2163496	1- Heptadecanol acetate
22	20.310	487321	7,10-Hexadecadienoic acid, methyl ester
23	20.795	374656	Decanoic acid, 5-ethyl-3,5,9- trimethyl, methyl ester
24	21.310	4860631	9- Octadecenamide,-(Z)
25	21.593	644167	Hexanedioic acid, bis(2-ethylhexyl) ester
26	21.658	674339	1-Heptadecanol, acetate
27	21.878	691153	Phynol, 2,2 -methylienebis
28	22.478	540098	1-Ethanone
29	22.879	14339055	1,2-Benzenedicarboxlic acid
30	24.866	1097539	Octadecanoic acid
31	27.529	4651968	9-Octadecenoic acid
32	27.741	6562824	1-Tridecanethiol
33	28.270	2143694	Phosphine oxide, heptyldiphenyl

<b>(B) The removed organic compounds in wastewater after treatment by MWCNTs</b>			
<b>peak No.</b>	<b>R. Time</b>	<b>Area</b>	<b>Name</b>
1	2.320	383117	Octane, n-Octane
2	2.921	314835	Benzene, (3,3-dimethylecyl)
3	3.008	334920	p-Xylene, Benzene, 1,4-dimethyl
4	3.318	878776	Nonane, n-Nonane
5	3.771	381753	Octanol, 2-methyl
6	5.054	340059	Heptane,5-ethyl--methyl
7	6.259	136503 9	Benzamido-4-methylbenzanilide
8	9.350	303661	Tridecane, n-Tridencane
9	15.762	595594	Methoxyacetic acid, 4-tridecyl ester
10	20.795	374656	Decanoic acid, 5-ethyl-3,5,9-trimethyl, methyl ester
11	21.310	486063 1	9- Octadecenamide, -(Z)
12	21.593	644167	Hexanedioic acid, bis(2-Ethylhexyl) ester
13	21.658	674339	1-Heptadecanol, acetate
14	21.878	691153	Phynol, 2,2 - methylenebis
15	27.741	656282 4	1-Tridecanethiol
16	28.270	214369 4	Phosphine oxide, heptyldiphenyl

### The kinetics of the contact time and adsorption

From 0 to 80 mins at constant temperature 40 °C, the effect of time on adsorption of the wastewater (100 mL) from refinery with MWCNT nano-composite (2 mg /mL) has been detected. The GC-MS shows the adsorption approach for various organic materials during the contact period between the adsorbate and adsorbent. In about 60-80 minutes, the adsorption rose progressively indicate that the equilibrium of the output results. There are a numerous active binding parts in the surface of MWCNTs which binding with the active parts of the organic compounds and other materials such as NO<sub>2</sub>, NO<sub>3</sub>, THM, COD. The slow diffusion of organic compounds and materials to inner pores and/or steady depletion of adsorption binding sites on adsorbent could explain the adsorption rate identified every 10 minutes of contact time. The physisorption process is followed by pseudo-first-order kinetics, with diffusion as the rate-determining step. The functional groups such as -C=O, -OH, and -COOH can be purposefully placed onto MWCNT surfaces *via* air or acid oxidation. MWCNTs are more hydrophilic as a result of their functional groups, making them suited for the adsorption of low molecular weight and polar contaminants. MWCNTs might be utilized as good scaffolds for the macro-molecules or metal oxides with intrinsic adsorption capabilities and functioning as direct adsorbents. MWCNTs are a useful basis for composite adsorbents due to their customizable surface chemistry and variable pore size. Furthermore, MWCNTs' distinctive electrical characteristics which can be used to improve adsorption with electrochemical assist [26].

Contaminates concentration were decrease gradually through the time but after 60 minutes, time had no impact since all contaminants had been eliminated from the wastewater as a result of ultrasonic irradiation with molecular concentrations of C-NPs over a long period of time. Furthermore, the results of Tukey's test revealed that additional NO<sub>2</sub> settings, such as 10, (20, 30), (30, 40), (40, 50) and (50, 60). This might be because the surface chemistry regarding the MWCNTs has an impact on their adsorption behavior. Also, the value of linear saturated hydrocarbons (n-alkanes) in wastewater and the effectiveness of elimination for pollutant wastewater was evaluated and explained using the Gas chromatography-mass detection technology (GC-MS) as an approach of hydrocarbon (alkenes, alkanes, dienes). The GC-MS method is a strong analytical tool for identifying organic pollutants in the environmental samples. The mass spectra regarding main peaks in the gas chromatograms of refinery wastewater at specific retention time and have been put to comparison with NIST library showing the existence of some aliphatic and aromatic organic compounds such as long-chain alkanes (nonadecane, hexadecane, undecaneetc), acids (Cis-10- onadecenoic Acid, Acetyl benzoic acid, Methoxyacetic acid, and Cis-13-Eicosenoic acid), esters and phthalate. The aromatic and aliphatic petroleum hydro-carbons were represented with methyl-tetrabutyl ether, naphthalene, phenol, 2,3,5,6-tetramethyl-phenol, tetradecane, xylene, 3-tert-butylphenol and 4-chloro-3-methylphenol.[27, 28]

The concentrations for these removal materials such as oil, NO<sub>3</sub>, NO<sub>2</sub>, THM, COD, and TSS have been obtained by HORIBA, UV/vis. spectrophotometer, gas chromatography, and Weighted method. Analysis of variance (ANOVA) allows the testing quality of refinery water after treatment of using carbon nanotubes; the results showed a significant effect for time on the selected materials. Furthermore, a mathematical model (ANOVA) was used to analyze the effect of time on oil removal as results elucidate the benefits of statistical methods for assessing and interpreting data to get better information about the effects of temperature and the design of the monitoring network. Time is considered as an influential factor on the selected responses oil, NO<sub>2</sub>, NO<sub>3</sub>, COD, THM, and TSS. Eight different setting of time (10, 20, 30, 40, 50, 60, 70, 80 mins) was selected to investigate the effect of time on six selected responses. The results of 16 experiments were analyzed using ANOVA. The results of ANOVA are presented in Table 1 for all selected responses showed that time is an influential variable on all responses, which indicates that changing the time will give different results of responses (P-value < 0.0001). Significant effect of time could be achieved due to the treatment combination of CNTs and ultrasonic irradiations, this combination will produce a low molecular concentration of C- nanoparticle within a short period and high within a longer period; thus, the concentration of removing the contaminant from wastewater is during a short period and a higher percentage of contaminant removal during a longer period of time.[29-31] The following tables from 4 to 14, explains the analysis of variances a function of time, the models, coded coefficients, regression equation in uncoded units, and the effects Pareto for (% Oil, % NO<sub>2</sub>, % NO<sub>3</sub>, % COD, % THM, and % TSS), furthermore the

error value, regression equation in uncoded units, fits, and diagnostics for unusual observations were obtained to analyze the treatment quality.

The source of variation is considered significant if it satisfies the condition in equation (1):

$$F \Rightarrow F_t (B_2, V_n, V_e) \dots \dots \dots (1)$$

Where: F is calculated F-ratio of given source,  $F_t$  is the tabulated F-ratio,  $B_2$  is the level of significance used in the test ( $B_2 = 0.05$ ),  $V_n$  is the degree of freedom of given sources,  $V_e$  is the degree of freedom error.

The tabulated  $F_t$  ratios for all sources that based on 5% level of significance and the degree of freedom for tables 4,5,6,7,8 and 9 are :-

$$F_t (0.05, 1, 5) = 6.6080 \dots \dots \dots \text{For (t and } t^2) \text{ sources}$$

$$F_t (0.05, 2, 5) = 5.7861 \dots \dots \dots \text{For (model) sources}$$

While, The tabulated  $F_t$  ratios for all sources that based on 5% level of significance and the degree of freedom for table 10 is :-

$$F_t (0.05, 1, 1) = 161.469 \text{ for (oil \%, NO}_2 \text{ \%, NO}_3 \text{ \%, COD \%, TSS \%)}.$$

For this reason and based on ANOVA (table, 4, 5, 7, and 8), the (model, t, and  $t^*t$  ( $t^2$ ) sources are considered significant parameters as their P-values < 0.05 and there F-values are higher than tabulated ( $F_t$ ) values.

Based on ANOVA analysis, table 6, and 9 (model, and t) sources are significant because P-Values < 0.05 and F-Values >  $F_t$  values. However, the square source ( $t^*t$ ) are statistically considered in significant as their P and F values do not satisfy the conditions mentioned before.

Table 10 has been observed that all sources of variations are insignificant as their P and F values and that don't satisfy the required statistical conditions (  $F < F_t$ ,  $P > 0.05$ )

The regression model is considered reliable as  $R^2$  is closed to 1 (98.81%)

**Table 4. Effects Pareto for %Oil) Response Surface Regression: % Oil versus time, Model Summary, Coded Coefficients and Regression Equation in Uncoded Units**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	2	7157.7	3578.86	178.68	0.000
Linear	1	6776.7	6776.72	338.33	0.000
t	1	6776.7	6776.72	338.33	0.000
Square	1	381.0	381.01	19.02	0.007
t*t	1	381.0	381.01	19.02	0.007
Error	5	100.1	20.03		
Total	7	7257.9			

$$\% \text{ Oil} = -10.37 + 2.626 t - 0.01506 t*t$$

**Table 5. Effects Pareto for %NO<sub>2</sub> Response Surface Regression: % NO<sub>2</sub> versus time, Model Summary, Coded Coefficients, and Regression Equation in Uncoded Units**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	2	6852.62	3426.31	333.42	0.000
Linear	1	6764.02	6764.02	658.22	0.000
t	1	6764.02	6764.02	658.22	0.000
Square	1	88.60	88.60	8.62	0.032
t*t	1	88.60	88.60	8.62	0.032
Error	5	51.38	10.28		
Total	7	6904.00			

$$\% \text{ NO}_2 = -5.50 + 1.923 t - 0.00726 t*t$$

**Table 6. Effects Pareto for % NO<sub>3</sub>)Response Surface Regression: % NO<sub>3</sub> versus time, Model Summary ,Coded Coefficients, Regression Equation in Uncoded Units and Fits and Diagnostics for Unusual Observations.**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	2	6843.15	3421.58	241.91	0.000
Linear	1	6802.15	6802.15	480.92	0.000
t	1	6802.15	6802.15	480.92	0.000
Square	1	41.01	41.01	2.90	0.149
t*t	1	41.01	41.01	2.90	0.149
Error	5	70.72	14.14		

Total	7	6913.88
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**Table 7. Effects Pareto for % COD) Response Surface Regression: % COD versus time, Model Summary, Coded Coefficients and Regression Equation in Uncoded Units.**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	2	6953.62	3476.81	181.31	0.000
Linear	1	6587.52	6587.52	343.53	0.000
t	1	6587.52	6587.52	343.53	0.000
Square	1	366.10	366.10	19.09	0.007
t*t	1	366.10	366.10	19.09	0.007
Error	5	95.88	19.18		
Total	7	7049.50			

$$\% \text{NO}_3 = -4.30 + 1.717t - 0.00494t*t$$

$$\% \text{ COD} = -10.75 + 2.581t - 0.01476 t^2$$

**Table 8. Effects Pareto for % THM|Response Surface Regression: % THM versus time, Model summary, Coded Coefficients, Regression Equation in Uncoded Units**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	1	10.37	10.37	0.05483	0.829
Error	19	371.607	19.5583		
Total	20	381.977			
Corrected Total	19	371.607			

S = 4.42167  
 R-Sq = 2.73%  
 R-Sq(Adj) = 0.00%  
 T-Value of F = 1.60767, P = 0.05483  
 T-Value of F = 1.60767, P = 0.05483

$$\% \text{THM} = -10.37 + 2.626T - 0.01506 t * t$$

**%TSS**  $\pm$  -4.116<sup>+</sup>71.607<sup>+</sup>6.720.0054833<sup>+</sup>t



**Table 9. Effects Pareto for % TSS)Response Surface Regression: % TSS versus time, Model Summary, Coded Coefficients, Regression Equation in Uncoded Units, Fits and Diagnostics for Unusual Observations**

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	2	5265.24	2632.62	326.94	0.000
Linear	1	5214.86	5214.86	647.62	0.000
t	1	5214.86	5214.86	647.62	0.000
Square	1	50.38	50.38	6.26	0.054
t*t	1	50.38	50.38	6.26	0.054
Error	5	40.26	8.05		
Total	7	5305.50			

**Table 10. Response Surface Regressions: t versus % Oil; % NO<sub>2</sub>; %THM; and % TSS**

**Model Summary**

Source	D F	Adj SS	Adj MS	F- Value	P- Value	S	R-sq	R- sq(adj)	R- sq(pred)
Model	6	4150.0 0	691.66 7	13.83	0.203	7.0710 7	98.81 %	91.67%	*
Linear	5	3411.0 2	682.20 5	13.64	0.203				
% Oil	1	0.00	0.000	0.00	1.000				
% NO <sub>2</sub>	1	0.06	0.057	0.00	0.978				
% NO <sub>3</sub>	1	0.00	0.000	0.00	1.000				
% COD	1	0.22	0.221	0.00	0.958				
% TSS	1	0.04	0.043	0.00	0.981				
Square	1	0.00	0.000	0.00	1.000				
% Oil*% Oil	1	0.00	0.000	0.00	1.000				
Error	1	50.00	50.000						
Total	7	4200.0 0							

**Table 11. Coded Coefficients and Regression Equation in Uncoded Units**

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	42.5	13.7	3.11	0.198	
% oil	-0	921	-0.00	1.000	73234.69
% NO <sub>2</sub>	311	9210	0.03	0.978	6800826.32
% NO <sub>3</sub>	-0	423	-0.00	1.000	14397.45
% COD	-51	761	-0.07	0.958	49769.23
% TSS	-228	7761	-0.03	0.981	4796993.85
% oil*% oil	0.0	14.9	0.00	1.000	6.02

**Regression Equation in Uncoded Units**

$$t = -0.0 + 0.0 \% \text{ Oil} + 7 \% \text{ NO}_2 + 0.0 \% \text{ NO}_3 - 1.2 \% \text{ COD} - 6 \% \text{ TSS} - 0.00000 \% \text{ Oil} * \% \text{ Oil}$$

**Table 12. Effects Pareto for time Design Summary**

Factors: 6 Replicates: 1  
 Base runs: 13 Total runs: 13  
 Base blocks: 1 Total blocks: 1  
 Center points: 1

StdOrder	RunOrder	PtType	Blocks	A	B	C	D	E	F
6	1	2	1	-1	-1	0	-1	1	1
12	2	2	1	-1	-1	1	1	-1	0
8	3	2	1	-1	1	-1	0	-1	1
9	4	2	1	1	-1	-1	1	0	1
2	5	2	1	0	-1	-1	-1	-1	-1
1	6	2	1	0	1	1	1	1	1
7	7	2	1	1	-1	1	0	1	-1
10	8	2	1	-1	1	1	-1	0	-1
13	9	0	1	0	0	0	0	0	0
11	10	2	1	1	1	-1	-1	1	0
5	11	2	1	1	1	0	1	-1	-1
3	12	2	1	1	0	1	-1	-1	1

4	13	2	1	-1	0	-1	1	1	-1
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## CONCLUSION

MWCNTs show a significant effect on the wastewater of Al-Dora refinery for the removal materials content; total suspended solids (TSS), nitrate oxides ( $\text{NO}_3$ ,  $\text{NO}_2$ ), chemical oxygen demand (COD), and trihalomethanes (THMs) at time range (10- 80 mins). The experimental results show that the (MWCNTs) has the maximum value of removing elements at 70 min for anti-pollutant from wastewater. The mathematical model description the effect of time on oil removal had been obtained. These results show efficient treatment and absorption stages according to the sizes and lengths of the hydrocarbon bonds.

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## **DETERMINING AN APPROPRIATE INITIAL VALUE OF ECCENTRICITY FOR LOW EARTH SATELLITES USING EULER METHOD**

**Rasha H. IBRAHIM**<sup>1</sup>

### **Abstract:**

The major goal of this research was to use the Euler method to determine the best starting value for eccentricity. Various heights were chosen for satellites that were affected by atmospheric drag. It was explained how to turn the position and velocity components into orbital elements. Also, Euler integration method was explained. The results indicated that the drag is deviated the satellite trajectory from a keplerian orbit. As a result, the Keplerian orbital elements alter throughout time. Additionally, the current analysis showed that Euler method could only be used for low Earth orbits between (100 and 500) km and very small eccentricity ( $e = 0.001$ ).

**Key Words:** Orbit, Orbital Elements, Satellite Perturbation, Euler Method, State Vectors.



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## 1. Introduction:

A precise evaluation of the real orbit is necessary for perturbation to be evaluated precisely. A satellite's true orbit and keplerian orbit are separated by the perturbation [1, 2]. The atmospheric drag changes the size and shape of the orbit. The main non-conservative force that affects a satellite at low heights is one that causes friction on it, which drains energy from the orbit. Additionally, the energy reduction results in a satellite orbit degrading until it re-enters the atmosphere [3]. There are many numerical integration methods, which are used in orbit integration. In general, they are classified into two types: single step methods and multi-step methods. Both of those methods can be used as fixed step or variable step. In fixed step, the step size is constant and the integration method is carried out using this step size. On the other hand, in variable step, the step size depends on the precision of integration [4, 5]. The satellite equation is solved numerically using Euler method, but this differential equation must be transformed to a first order differential equation. By integrating the perturbed acceleration, the satellite position, velocity and orbital elements at any moments of time are computed [6].

## 2. Previous Works

The integration methods for the satellite's equation attracted the attention of numerous scholars; Roberto and Sergio studied two conventional perturbation methods for integrating satellite orbits numerically in the formulation utilizing Cartesian coordinates. They compared specifically with Encke and the variation-of-parameters (VOP) techniques [7]. Balázs and Lóránt estimated the orbit of the most modern LEO spacecraft; conventional numerical integration methods were tested. In general, the space between two discrete observed epochs is often filled using numerical integration techniques for orbit determination including Runge-Kutta, Bulirsch-Stoer, and Adams-Moulton [8]. Jeffrey *et al.* proposed an unique variable-step Gauss-Legendre implicit-Runge-Kutta (VGL-IRK) method for orbit and the spread of instability [9]. Thomas and Dimitrios focused on the accuracy evaluation of numerical integrator in the single-step and multistep techniques categories [10]. Mishra *et al.* determined the satellite's orbit includes a number of methods for measuring the motion of the satellite in terms of its position and speed [11]. Thangavel *et al.* evaluated the  $J_2$  effect and aerodynamic forces. Keeping satellites moves in relation to one another in the presence of  $J_2$  disruption to track error bounds [12]. Saneliso and Wei-Hsi created a numerical model in two dimension of a lunar-influenced Earth satellite orbit. The orbits of the satellites around the Earth were employed in the first portion of the numerical simulation [13]. Ahmed *et al.* examined the accuracy and effectiveness of numerical integration methods for solving the two-body gravitationally perturbed equation [14]. Rasha and Abdul-Rahman provided a suitable eccentricity value for satellites in Low Earth Orbit (LEO) using Runge-Kutta method. It was explained how to transform the orbital components into state vectors [15]. In the next year, Rasha and Abdul-Rahman used the fourth order Adams-Bashforth method to solve the perturbed equation of motion, it was employed in this



research to analyze the modification of orbital elements under the impact of drag [16]. Geul *et al.* calculated mathematically the satellite position and velocity over time by verified the interval orbit propagation [17].

### 3. Methodology

#### 3.1. The Satellite's State Vector

The satellite is moving in the equatorial plane with the following position and velocity [18]:

$$\left. \begin{aligned} X &= P_x x_w + Q_x y_w + W_x z_w \\ Y &= P_y x_w + Q_y y_w + W_y z_w \\ Z &= P_z x_w + Q_z y_w + W_z z_w \end{aligned} \right\} \quad (1)$$

$$\left. \begin{aligned} \dot{X} &= P_x \dot{x}_w + Q_x \dot{y}_w + W_x \dot{z}_w \\ \dot{Y} &= P_y \dot{x}_w + Q_y \dot{y}_w + W_y \dot{z}_w \\ \dot{Z} &= P_z \dot{x}_w + Q_z \dot{y}_w + W_z \dot{z}_w \end{aligned} \right\} \quad (2)$$

Where,  $P_x, P_y, P_z, Q_x, Q_y, Q_z, W_x, W_y, W_z$  are Euler angles.

$x_w, y_w, z_w, \dot{x}_w, \dot{y}_w, \dot{z}_w$  are the satellite position and velocity in the plane of the orbit can be written as the following [3]:

$$\left. \begin{aligned} x_w &= a(\cos Ec - e) \\ y_w &= a\sqrt{1-e^2} \sin Ec \\ z_w &= 0 \end{aligned} \right\} \quad (3)$$

$$\left. \begin{aligned} \dot{x}_w &= -\frac{\sqrt{\mu a}}{r} \sin Ec \\ \dot{y}_w &= \frac{\sqrt{\mu a (1-e^2)}}{r} \cos Ec \\ \dot{z}_w &= 0 \end{aligned} \right\} \quad (4)$$

Where:

Ec: is eccentric anomaly.

a: is semi-major axis.

e: is eccentricity.

$\mu$  : is the Earth's gravitational constant equal to  $398600 \text{ km}^3/\text{sec}^2$ .

$\theta$ : is true anomaly.

r: is the distance.

The calculation of the parameters above will explain later in orbital elements section.

### 3.2. The Satellite's Orbital Elements

The satellite has six orbital elements, which are:

- Semi-major axis is given by [19]:

$$a = \left( \frac{h^2}{\mu} \times \frac{1}{1 - e^2} \right) \quad (5)$$

Here,  $h$  is the angular momentum.

- Eccentricity can be calculated by [20]:

$$\mathbf{e} = \frac{1}{\mu} \left[ \left( |\mathbf{v}|^2 - \frac{\mu}{r} \right) \cdot \mathbf{r} - (\mathbf{r} \cdot \mathbf{v}) \cdot \mathbf{v} \right] \quad (6)$$

Where:

$\mathbf{r}$ : is the vector of the position.

$\mathbf{v}$ : is the vector of the velocity.

$r$ : is the distance.

- Inclination is defined as follows [19]:

$$\cos i = h_z / h \quad (7)$$

Where:

$h_z = x_w \dot{y}_w - y_w \dot{x}_w$ , which is the angular momentum in Z-direction.

$h$ : is the angular momentum magnitude.

- Longitude of ascending node is [19]:

$$\cos \Omega = N_x / N \quad (8)$$

Here,  $N_x$  is the line of node vector in X-direction, equal to  $\mathbf{k}_x \times \mathbf{h}_x$ .

$N$ : is the magnitude of the line of node.

- Argument of perigee is applied as [19]:

$$\cos \omega = \mathbf{N} \cdot \mathbf{e} / N \cdot e \quad (9)$$

Where:

$\mathbf{e}$ : is eccentricity vector.

- True anomaly are calculated by [19]:

$$\cos \theta = \mathbf{e} \cdot \mathbf{r} / e \cdot r \quad (10)$$

### 3.3. The Perturbed Acceleration

This type of perturbation is represented as below [3]:

$$\ddot{\mathbf{r}}_{\text{Drag}} = -\frac{1}{2} C_D \frac{A}{M} \rho \frac{\mathbf{v}_r}{v_r} \quad (11)$$

Where:

$C_D$ : is drag's parameter.

$A/M$ : is the ratio of the satellite's area to its mass.

$\mathbf{v}_r$ : is the satellite's relative velocity vector.

$v_r$ : is the satellite's relative velocity magnitude.

$\rho$ : is the density of the atmosphere, this density is computed by NRLMSISE-00.

The equation of motion without perturbation is [3]:

$$\ddot{\mathbf{r}} = -\frac{\mu}{r^3} \mathbf{r} \quad (12)$$

Adding equation (11) to (12), the final representation of the equation of motion (under the influence of atmospheric drag) is [20]:

$$\ddot{\mathbf{r}}_P = -\frac{\mu}{r^3} \mathbf{r} + \ddot{\mathbf{r}}_{\text{Drag}} \quad (13)$$

**Table (1): Give a representation of the parameter's initial values utilized in the program [3, 4, 15, 16, 21].**

Parameter	Case (1)	Case (2)	Case (3)	Case (4)
$h_p$ (km)	250	250	500	900
$a$ (km)	6625	6685	6885	7385.53
$e$	0.001	0.01	0.001	0.001
$i$ (deg)	70	70	70	70
$\Omega$ , (deg)	50	50	50	50
$\omega$ (deg)	100	100	100	100
$M_e$ (deg)	0	0	0	0
$E_c$ (deg)	0	0	0	0
$\theta$ (deg)	0	0	0	0
$C_R$	2.2	2.2	2.2	2.2
$C_D$	1.3	1.3	1.3	1.3
$A / M$ (m <sup>2</sup> /kg)	0.0052	0.0052	0.0052	0.0052
$h$	5.4393	5.4393	5.6855	6.1886

### 3.4. Euler Method

The new satellite's position and velocity components are computed as [3, 6]:

$$X_f = X_i + \frac{h}{6}(A_X)$$

$$\left. \begin{aligned} Y_f &= Y_i + \frac{h}{6}(A_Y) \\ Z_f &= Z_i + \frac{h}{6}(A_Z) \end{aligned} \right\} \quad (14)$$

$$\dot{X}_f = \dot{X}_i + \frac{h}{6}(B_X)$$

$$\left. \begin{aligned} \dot{Y}_f &= \dot{Y}_i + \frac{h}{6}(B_Y) \\ \dot{Z}_f &= \dot{Z}_i + \frac{h}{6}(B_Z) \end{aligned} \right\} \quad (15)$$

Where:

$X_i, Y_i, Z_i$ : is the initial position in X, Y, and Z.

$X_f, Y_f, Z_f$ : is the final position in X, Y, and Z.

$\dot{X}_i, \dot{Y}_i, \dot{Z}_i$ : is the initial velocity in X, Y, and Z.

$\dot{X}_f, \dot{Y}_f, \dot{Z}_f$ : is the final velocity in X, Y, and Z.

The six parameters  $A_X, A_Y, A_Z, B_X, B_Y, B_Z$  are computed as following:

$$A_X = \dot{X}_i, A_Y = \dot{Y}_i, A_Z = \dot{Z}_i$$

$$B_X = \ddot{r}_{X0}, B_Y = \ddot{r}_{Y0}, B_Z = \ddot{r}_{Z0}$$

Where:

$\ddot{r}_{X0}, \ddot{r}_{Y0}, \ddot{r}_{Z0}$  is initial perturbed acceleration in X, Y, and Z direction.

h: is the integration step size.

### 4. Results and Discussion

The evaluation for six orbital elements was utilized to explain the satellite's orbit under the influence of atmospheric drag. This analysis was made by using different cases, as presented in table (1). Figure (1) demonstrated how the drag affected the semi-major axis. It was observed that the value of semi-major axis for case (1) is monotonically dropping with time. In case 2, this element has a fast decreasing with respect to the number of days, also after the fourth day, it has a constant appearance. In case 3, semi-major axis was linearly decreasing with time, but in case 4, it was linearly increasing, so it was observed that the small value of eccentricity is more affected by drag than other heights.

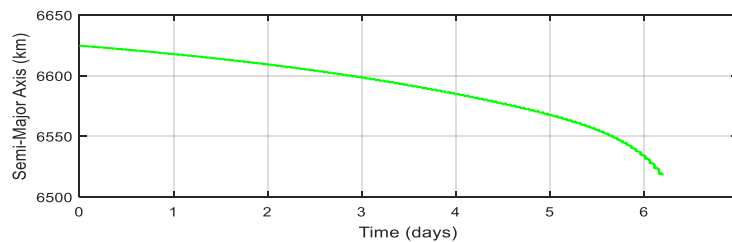
In ideal conditions, the appearance of eccentricity was constant with time, but in figure (2), case 1, it reduced gradually from (0.0001 to 0.00098). The performance of eccentricity in case (2) was also decreasing then be constant with time. In case (3), it observed that the value of eccentricity is dropping with time like semi-major axis. This element in case (4), has a slow growing relationship with the number of days.

Figure (3) depicted the influence of eccentricity and the height on inclination angle. In case 1, this element decreases a little as the time passes and then decreases very fast until reaches the value  $69.973^\circ$ . In case 2, it has a sharp dropping after the second day, this element drops very fast until reaches to a constant value  $69.99652^\circ$ . In case (3) and (4), inclination was linearly reducing with time.

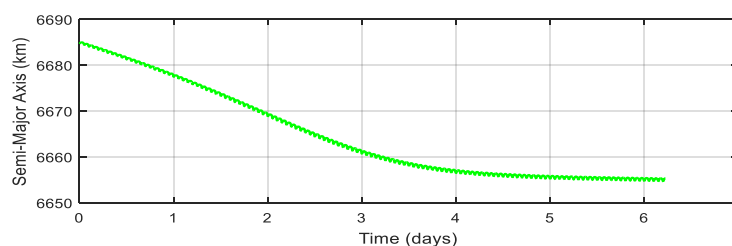
Figure (4) illustrated right ascension of ascending node, which has a value between  $(50-50.007)^\circ$  in case 1. Therefore, the drag moved away this element from its desired value. In case (2), right ascension of ascending node has a hump, then decreasing with time. This element in case (3) and (4), was linearly increasing as the time passes. The ideal value of this element was  $50^\circ$ , but with the assumption of atmospheric drag, it reaches to  $50.0001^\circ$  on the sixth day.

The influence of atmospheric drag on argument of perigee for all cases was linearly growing with time. Also, this perturbation makes this element deflects from its ideal value, as in figure (5).

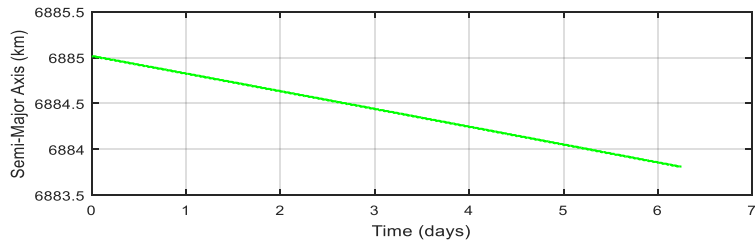
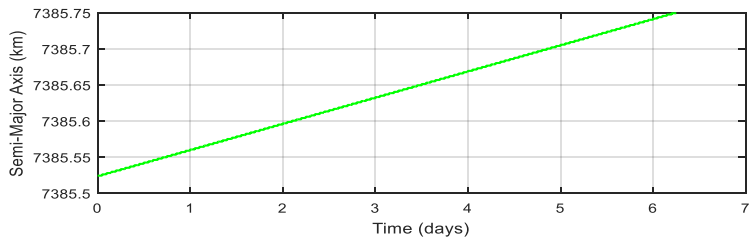
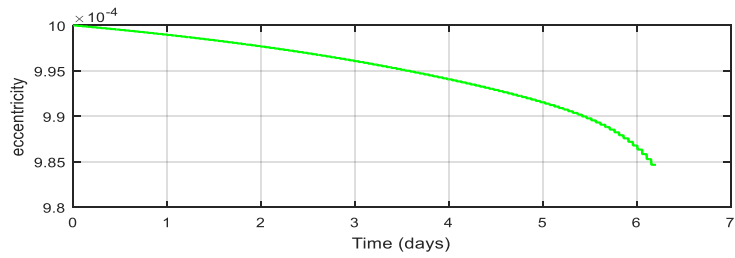
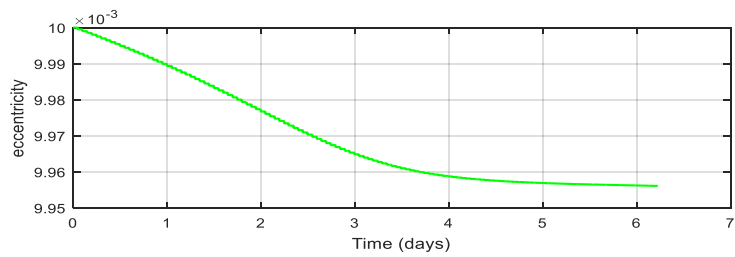
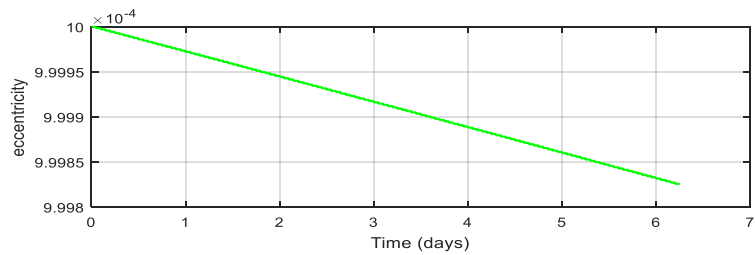
Finally, true anomaly increases from 0 to  $360^\circ$  for one period. It has a toothed appearance in every case and for seven days, as shown in figure (6).

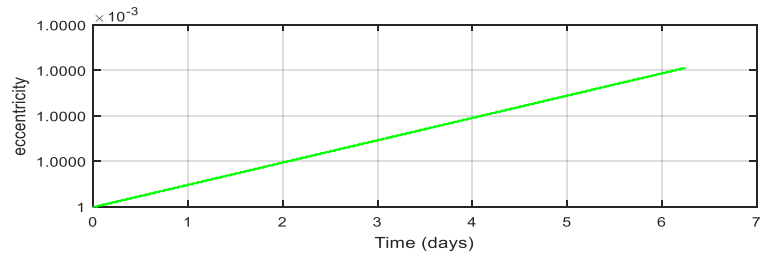


**Case (1)**



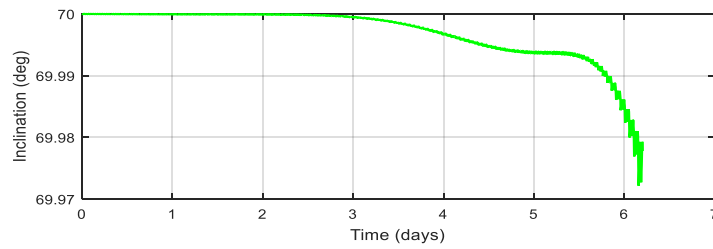
**Case (2)**

**Case (3)****Case (4)****Figure (1): Semi-major axis with different cases.****Case (1)****Case (2)****Case (3)**

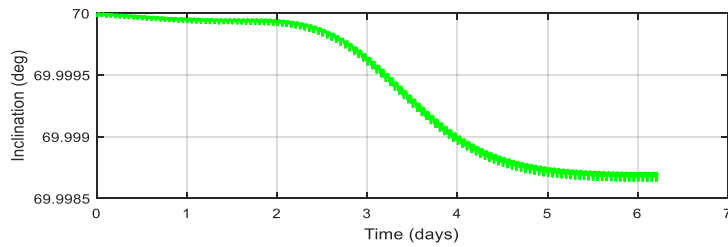


Case (4)

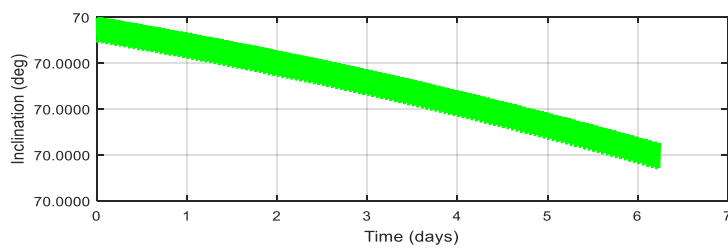
Figure (2): Eccentricity with various cases.



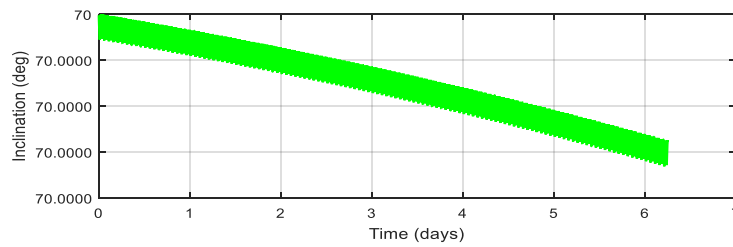
Case (1)



Case (2)

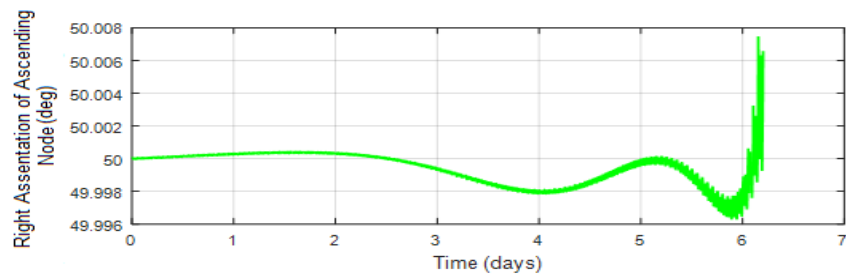
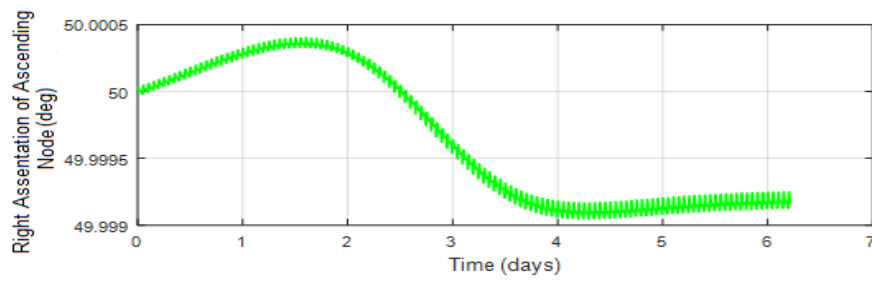
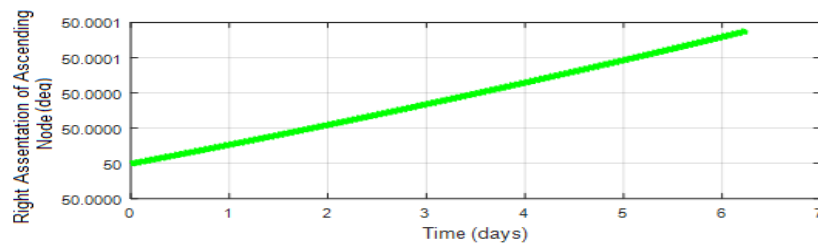
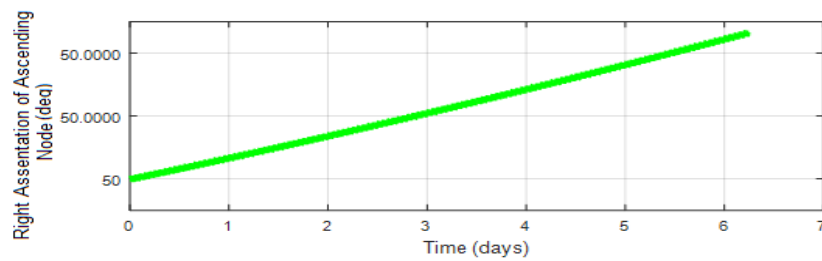


Case (3)

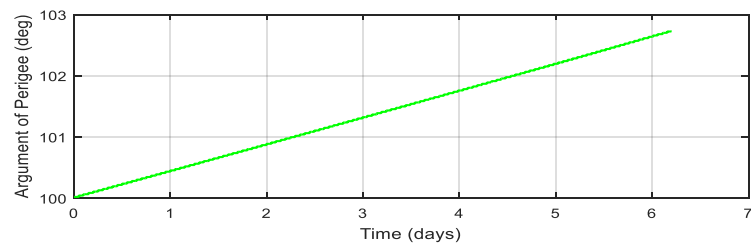
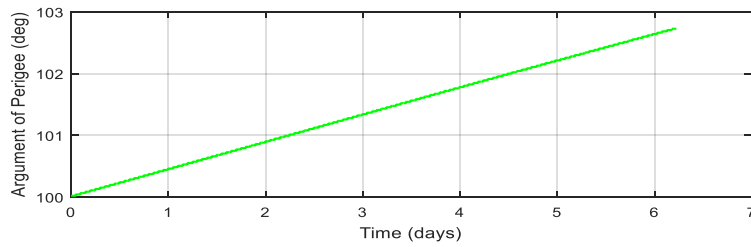
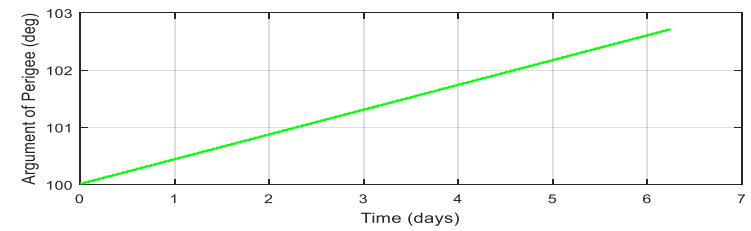
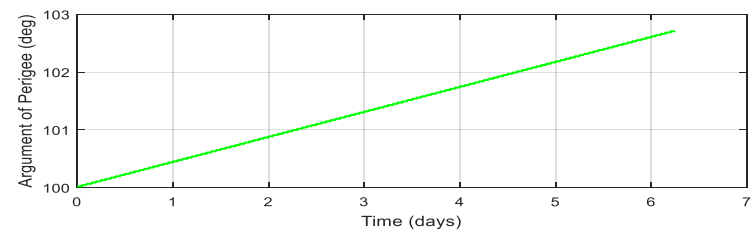


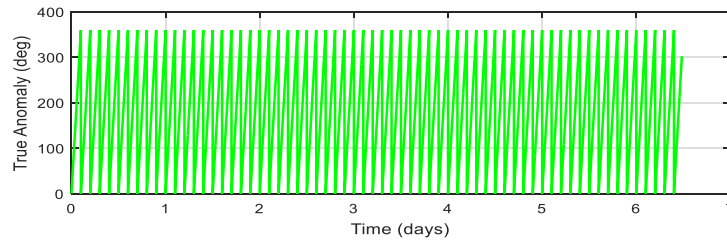
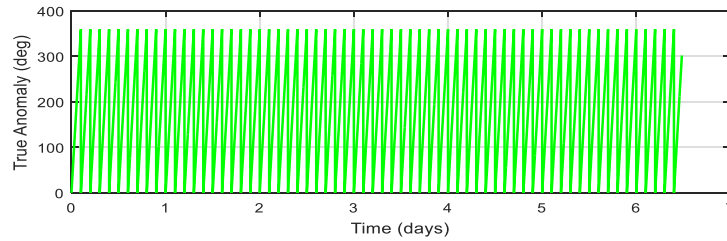
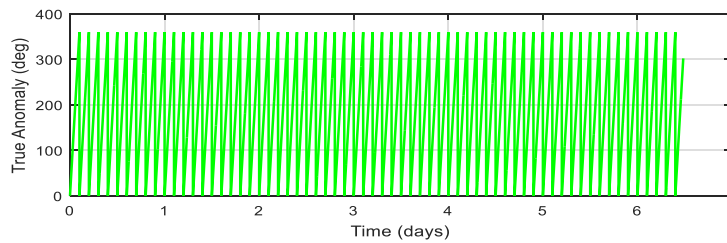
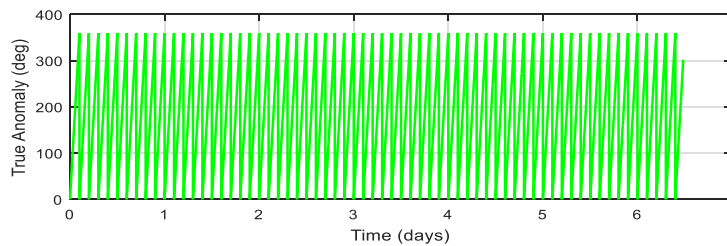
Case (4)

Figure (3): Inclination with diverse cases.

**Case (1)****Case (2)****Case (3)****Case (4)****Figure (4): Right assentation of ascending node with different cases.**



**Case (1)****Case (2)****Case (3)****Case (4)****Figure (5): Argument of perigee with various cases.**

**Case (1)****Case (2)****Case (3)****Case (4)****Figure (6): True anomaly with different cases.**

## 5. Conclusions

1. From the appearance of the semi-major axis and eccentricity, Euler method was applicable for very small values of eccentricity, which was equal to or smaller than 0.0001 and very low heights started from (100-500) km.
2. The directional elements, inclination, longitude of the ascending node, argument of perigee, and true anomaly at heights greater than 500 km, are unaffected by eccentricity regardless of its value.
3. Eccentricity and semi-major axis were rapidly decreased, particularly in recent days.

4. The results demonstrated a good agreement with a number of previously published researches that used the Runge-Kutta method of the fourth order [15, 16, 22, 23] and Adams-Bashforth Method of the fourth order [24].

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**THE KNOWLEDGE NEEDS OF VEGETABLE FARMERS IN HAMAM AL-ALIL DISTRICT  
/ NINEVEH GOVERNORATE IN THE FIELD OF GRYLLOTALPA GRYLLOTALPA  
CONTROLLING**

**Mohammed Ahmed MAHAL <sup>1</sup>**

**Abstract:**

The aim of the research is to identify some of the personal and communicative characteristics of vegetable farmers in Hammam Al-Alil district of Nineveh Governorate / Iraq, in addition to their knowledge needs in the field of Gryllotalpa gryllotalpa control. The research tool included two parts, the first part of which was devoted to the independent variables (age, educational level, number of years of work in vegetable cultivation, level of pest infestation in the field in the last 5 years, level of exposure to sources of information on control of Gryllotalpa gryllotalpa, while the second part included three fields that consisted of 20 items and were distributed respectively (chemical control and it included 6 items, mechanical control and it consisted of 6 items, the insect life cycle included 8 items) the results showed that 88% of the respondents had a medium cognitive need and tended to be high, and with regard to the independent variables, it was found that the age variables, the level of pest infestation in the field in the last 5 years, and the level of exposure to information sources have a correlation with the dependent variable, while it was not found that there is a correlation between the variables of educational level and number of years of work in vegetable cultivation on the one hand, and the level of knowledge needs of vegetable farmers in the field of Gryllotalpa gryllotalpa control on the other hand.

**Key Words:** Knowledge Needs, Vegetable Farmers, Gryllotalpa Gryllotalpa.



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## Introduction:

Gryllotalpa Gryllotalpa is a pest that infects crops in many agricultural areas around the world, causing economic losses due to seedling, root, and tuber damage (Javadzadeh et al., 2017) . It is an important economic insect classified as Orthoptera and its family is Gryllotalpidae (Al-Mallah, 2006). This family contains four races of the insect (Scapteriscus, Neocurtilla, Gryllotalpa, Triamescaptor) (peggy et al, 2002) . There are 34 species found in various countries around the world, and the insect is frequently found around marshes and wetlands. (Danlik & Kristin , 2022). It has developed over time to living under the surface of the soil (De Graaf , 2005) . It makes tunnels near the soil surface and damages plants by cutting roots when digging under the soil (kazemi et al , 2013 ). It often disappear by day under stones, leaves or below trees, and at night they start to come out of their hideouts (Al-saffar & augul , 2018 ) . In Iraq, the insect can be found in light areas, orchards, near water sources, and in household gardens (Ismail & dabdoub , 2009) . The insect in Iraq caused a lot of economic losses to agricultural crops, as the infestation in potatoes was 21.43%, cowpea 22.5%, sunflower 20.5, okra 18%, watermelon 17.7%, and cucumber 15.34% (Al-Jassany & Al-Joboory , 2016 ) .

Based on the communication of the director of the agricultural branch in the study area with the researcher and informing him of the presence of infections in the agricultural fields of Gryllotalpa gryllotalpa despite the farmers making many attempts to control, he decided to investigate the knowledge needs of farmers in the field of insect control to identify weaknesses in this type of agricultural operations.

## Research Objectives

- 1- Determining the personal characteristics of the respondents
- 2- Determining the knowledge needs of farmers in the field of Gryllotalpa gryllotalpa control of the respondents
- 3- Identifying the correlation of the dependent variable with the following independent factors (age, educational level, number of years of vegetable cultivation, pest infestation level of the field in the last 5 years, exposure level to information sources on insect control).

## Materials and Methods

The research questionnaire was divided into two components. The first section contained information about personal respondents as well as agricultural work in their fields (age, educational level, number of years working in vegetable cultivation , The level of field infestation with pests in the last 5 years, the level of exposure to information sources on pest control) . As for the second part, it consisted of three fields as follows (chemical control 6 items, mechanical control 6 items, life cycle 8 items) and the alternatives allocated to each paragraph were (I need a large degree, I need a medium degree, I need a little degree,

I don't need), and then the tool was presented to a number of specialists in agricultural extension and plant protection in order to ascertain the apparent validity and the extent to which the items covered the aspects of the subject, and then the data was collected in July 2022

### **Research and Sample Society**

The search area was located south of Mosul. The area is close to the Tigris River. The largest local inhabitants of its villages grow vegetables. The search community included all 600 vegetable farmers in Hamam Al-Alil district. A random sample of 60 farmers was taken (10%).

### **survey sample**

The questionnaires were distributed to 30 researchers, all of whom were excluded from the final research sample. Thereafter, The reliability factor was found through the alpha-Cronbach equation and the reliability coefficient was (0.89). This value is considered good according to many scientific references related to social statistics.

### **Independent variables measurement.**

- 1- Age was measured by asking the respondents about their age at the time of data collection
- 2- Educational level: The following choices (Illiterate , read and write, primary, secondary , , institute, diploma , bachelor's degree and above) were used to assess this variable, and the following numerical values were assigned to them, correspondingly (1, 2, 3, 4, 5, 6 , 7)
- 3- Number of years of work in vegetable cultivation: This variable was assessed by asking the respondent how many years he had worked in agriculture from the first year of his career to the time of data collection.
- 4- The level of field infestation with pests in the last 5 years: this variable has been measured by the following alternatives (Many injuries, average injuries, few injuries) and numerical values (3, 2, 1) were allocated respectively
- 5- Level of exposure to pest control information sources: it was measured using 10 information sources questions and the options assigned to responding them (always, sometimes, very few, I do not acquire information from this source)and the numerical values were ( 4 , 3 , 2 , 1 ) correspondingly.



### Measurement of the dependent variable

The dependent variable consisted of three fields, the first of which contained six items, the second of which contained six items, and the third of which contained eight items, and the following alternatives were placed for them, respectively (need to a large degree, need to a moderate degree, need to a low degree, did not need) and the following numbers were assigned, respectively, to each alternative (4, 3, 2, 1), and the theoretical range for the first and second fields was between (6 – 24), whereas the range of the third area was (8 - 32) and to extract the actual value of the cognitive need, the actual scores of the three fields obtained by each respondent were collected separately .

### Results

#### 1- First Objective : Identifying the personal characteristics of the respondents

**Age :** The highest digital value of the age of the participants in the questionnaire was found to be 57 years and the lowest is 25 years. The calculation average of the age variable was found to be 42.6 and a standard deviation (8.06). The age groups were extracted by finding the range between the highest value and the lowest value, as shown in table 1.

**Table No. 1 shows the age distribution of respondents**

Categories	Number	Percentage %
Low Category (25-35)	9	15
Intermediate category (36-46)	37	62
High category (47-57)	14	23
Sum	60	100

It is clear from the above table that the middle age group (36-46) reached (62%) is the most numerous, and the group that came next is the high age group (47-57) and its rate was 23%, while the low age group (25-35) it accounted for (23%)

**educational attainment :****Table No. 2 shows the educational attainment distribution of respondents**

Categories	Number	%
Illiterate	7	12
read and write	12	20
primary	9	15
secondary	9	15
institute	15	25
diploma	6	10
bachelor's degree and above	2	3
<b>sum</b>	<b>60</b>	<b>100</b>

According to Table No. 2, the majority of respondents finished preparatory school, with a percentage of (25%), while those who read and write had a percentage of (20%), and the number of those who completed primary and intermediate education had the same percentage (15%). This was followed by the illiterate, diploma, and those with a bachelor's degree and above, with respective percentages of (12%), (10%), and (3%).

**Number of years working in vegetable cultivation :** the lowest number of years of work in agriculture for researchers was found to be (13) and the highest number was (42) with an average of (32.65) and a standard deviation (7.84). The respondents were then divided into three categories based on the range between the highest and the lowest value.

**Table No. (3) Distribution of respondents according to the number of years of their working in vegetable cultivation**

Categories	Numb er	%
Low category ( 13 – 22 )	12	%20
Intermediate category ( 23 - 32 )	28	47%
High category ( 33 – 42)	20	33%

According to Table 3, the Intermediate group (23–32) has the largest percentage of respondents, at 47%, followed by the high category (33–42), at 33%, and the low category (13–22), at 20%.

**The level of pest infestation in the field in the last (5) years :****Table No. (4) Distribution of respondents according to the level of pest infestation in their fields in the last five years**

Categories	Nu mber	%
Weak infestation	7	%12
Intermediate infestation	21	35%
High infestation	32	%53

Through the results, it was found that the majority of the respondents are those who face an increase in the level of injuries to their fields with pests during the last five years and their number was (32) respondents by 53%, followed by the respondents who have the level of injuries in their fields of medium number by 35%, and the last category was the number of respondents whose number was weak in their fields by (7) individuals and their percentage were 12%

**Level of exposure to pest control information sources:**

The lowest numerical value of exposure to information sources was (13) while the highest value was (36) with an average calculation of (22.46) and a standard deviation of (6.81) and then the respondents were divided into three categories based on their exposure to sources of information about insect control under study depending on the range between the highest value and the lowest value

**Table No. (5) Distribution of Respondents According to Exposure to Information Sources**

Categories	Nu mber	%
Weak exposure ( 13 – 20 )	30	50
Intermediate exposure ( 21 - 28 )	16	27
High exposure ( 29 – 36)	14	23

According to Table No. (5), half of the respondents who responded to the questionnaire had a low exposure to knowledge sources about insect control, while the Intermediate group (21-28) had medium exposure to information sources of 27%, and the respondents who were heavily exposed to information sources (29-36) had a 23% exposure.

## 2- Second Objective : Determining the level of knowledge needs of farmers in the field of Gryllotalpa gryllotalpa control

The results showed that the highest value for vegetable farmers' cognitive needs in the control of *Gryllotalpa gryllotalpa* was (77) and the lowest value was (27) with mean that was (53.3) and a standard deviation (11.6), and respondents were divided into three categories after calculation the range between the highest value and the lowest value and dividing the output by the number of categories shown in table (6).

**Table (6) depicts the distribution of respondents into knowledge needs categories.**

Category	(Number)	(%)
Low need (27 - 43)	7	12
Medium need (44 - 60)	38	63
High need (61 - 77)	15	25

From the table above, 88% of respondents showed that their cognitive needs in combating the insect are medium and tend to rise. this indicates that researchers have high needs in their farms and need to see practical control procedures to reduce the amount of losses caused by this insect

## 3- Third Objective: Identifying the correlation between the knowledge needs of the respondents in the field of controlling *Gryllotalpa gryllotalpa* and the following independent variables ( age , educational attainment, number of years working in vegetable cultivation , the level of pest infestation in the field in the last (5) years, level of exposure to pest control information sources )

**Table (7) shows the correlation between independent variables and cognitive needs**

independent variables	Pearson correlation coefficient	Spearman correlation coefficient
age,	**0.865	
education level		0.037
Number of years of work in vegetable cultivation	0.294	
The level of pest infestation in the field in the last (5) years	0.648**	
exposure level to information sources on insect control	0.745**	

**age :** The value of the Pearson correlation coefficient (0.865)\*\* which is significant at the probability level (0.01) This indicates that the respondents who are older need to improve their cognitive levels in the field of identifying the techniques necessary to control of *Gryllotalpa gryllotalpa* and the reason for this cognitive deficiency may be because the elderly respondents are preoccupied with other responsibilities, and this result differs with what reached (Al-Shadaideh and Badour, 2014) and (Al-Hafez, 2011)

**educational attainment :** It turns out that there is no correlation between the educational level and the cognitive needs of vegetable farmers in their control of this insect, and this may be because the information received by the respondents who had educational years and certificates of study is not of such scientific value that makes it influential on their cognitive needs related to the control of *Gryllotalpa gryllotalpa* and this result differs with what reached (Al-Shadaideh and Badour, 2014) and (Al-Hafez, 2011).

**number of years of work in vegetable cultivation :** There was no correlation between vegetable growers' cognitive needs in the field of *Gryllotalpa gryllotalpa* control and the number of years worked in vegetable cultivation where the value of the Pearson Simple Binding Coefficient (0.249) This may indicate that the prevalence of the insect in the study areas is still recent, although it causes significant losses at the level of quantitative production. This result differs with what reached (Al-Shadaideh and Badour, 2014).

**The level of pest infestation in the field in the last (5) years :** There has been a positive correlation between farmers' cognitive needs in the field of *Gryllotalpa gryllotalpa* control and the level of pest infestation in the field in the last (5) years, where the value of the Pearson correlation coefficient (0.648 \* \*) which is significant at the level (0.01). This may be explained by the fact that the damage to the farmers' field spaces has made them feel how little they know about this insect, and thus by expressing their relatively high cognitive needs for the control of this insect.

**Level of exposure to information sources for insect control :** Through the results, it was found that there is a significant correlation between the knowledge needs of vegetable farmers in the field of *Gryllotalpa gryllotalpa* control and the level of exposure of farmers to information sources, where the value of the Pearson correlation coefficient was (0.745\*\*), which is significant at the level (0.01) and this may be due to the fact that the sources of information on this insect has provided farmers with information about the danger of this insect and the extent of the economic losses it causes without providing the necessary detailed information about controlling it. thus, this was the reason for the positive correlation between the dependent variable and the independent variable, and this result differs with what was reached (Al-Hafiz, 2011)

**Conclusions**

- 1- The majority of respondents need training courses in order to improve their levels of knowledge that help them in Gryllotalpa gryllotalpa control
- 2- In addition to the infection of the fields in the study area with Gryllotalpa gryllotalpa, there are also other pests that settle agricultural fields
- 3- This insect infects the fields of elderly farmers with more economic losses than other farmers of the rest of the age groups

**Recommendations**

- 1- The necessity for the agricultural extension system in the research area to benefit from local or foreign parties' expertise in the field of carob insect management and work to direct them toward local farmers and supply them in a thorough and simplified way appropriate with their educational levels.
- 2- Intensifying the reconnaissance activities of the extension system towards the community of farmers in order to see other independent variables that may affect the level of their knowledge needs related to the control of Gryllotalpa gryllotalpa
- 3- Follow up the fields where the insect did not appear and make sure that farmers could control it in the future if it appears in their farms

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# ASSESSMENT OF THE LEVEL OF INTERLEUKIN- 10 AND INTERFERON -GAMMA IN CHILDREN INFECTED WITH AEROMONAS HEMOPHILIA ISOLATED FROM DIARRHEA

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## Abstract:

Background: Aeromonas hydrophila causes bacterial diseases that lead to intestinal and extra-intestinal infections. Objective: The study aimed to isolate Aeromonas hydrophila from patients suffering diarrhea and evaluate the level of some interleukins in patients and compare them with control and resistance of bacteria to antibiotics. Materials and methods collect 150 stool samples of patients with diarrhea. Six isolates of A. hemophila were isolated from children suffering diarrhea from some Baghdad hospitals. Results, all bacterial isolates were identified by the biochemical, cultural and microbial characteristics and confirmed by VITK2 System. The study showed the resistance of A. hemophila to many antibiotics, the highest resistance to Tetracycline and Oxacillin and the rate of resistance was 100%, while the bacteria had the lowest resistance 16.7%, to Erythromycin. The present results exhibited Interleukin-10 level was elevated in the patients and it was controlled by a significant level. The present results besides exhibited that the concentration of Interferon- gamma (IFN- $\gamma$ ) of patients and control group with non-significant difference.

**Key Words:** Aeromonas Hydrophila, Interferon-  $\Gamma$ , Interleukin-10.



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**Introduction:**

*Aeromonas hydrophila* causes bacterial diseases that cause intestinal and extra-intestinal infections. *Aeromonas* species are pathogens that infect both humans and animals. It is connected with numerous food-borne epidemics and is separate from diarrhoeal travelers [1]. The zoonotic agent *A. hydrophila* causes infections. The host tissue may receive local pathology from it. The host's reaction could result in tissue proliferating, deteriorating, and inflaming [2]. *A. hydrophilas* pathogenicity is multifactorial, involving adhesions (such as pili), the S-layer & lipopolysaccharide. Exotoxins & exoenzymes such as enterotoxins, hemolysins, lipases, gelatinases, caseinases, lecithinases, deoxyribonucleases, and proteases are examples of extracellular factors [3]. These virulence factors cause them to invade a host and cause diseases as from non-life-threatening gastroenteritis to soft tissue infections that cause cellulitis or life-threatening conditions like necrotizing fasciitis and septicemia [4]. *A. hydrophila* also causing various diseases, such as meningitis, infections of the heart muscles, blood infections at the digestive tract such as diarrhea, abdominal pain, nausea, headaches and symptoms associated with fever and watery diarrhea [5]. *Aeromonads* have chromosomally mediated microbial resistance. Though, lactamases can be encoded via plasmids or integrons [6]. Different  $\beta$ -lactamases produced by *Aeromonas* spp. discuss resistance to a extensive variety of  $\beta$ -lactam drugs. show that the widespread usage of antibiotics for prevention and treatment in people and fish undoubtedly contributes to the rise in the amount of resistant *A. hydrophila* strains. [7]. An crucial cytokine for both innate and adaptive immunity against protozoan, bacterial, and viral infections is type II interferon, also known as IFN- $\gamma$ . Important macrophage activators and inducers of class II molecules from the major histocompatibility complex include IFN- $\gamma$  [8].

**The aim of the study** Diagnosis of bacteria isolated from children's diarrhea and finding the level of interleukin-10 and interferon- $\gamma$  in patients' sera and comparing it with control

**Materials and methods****Samples collection**

Collect 150 stool samples with an aseptic technique in sterile cups Co. (USA) of patients with diarrhea. These samples were collected from Teaching Laboratory in Medical City Hospitals, Imam Ali and Al-Sader general hospital in Baghdad. Initial identification of bacteria was based on colony characteristics of the confirmed bacteria; at that point various biochemical tests and even VITEK2 system (BioMerieux, France) [9].

**The sensitivity test** was isolates against (6) different antibiotics, including the most important used tablets (Bio analyse/Turke). The test was carried out using Mueller-Hinton agar, and the standard diameters were adopted according to what was stated in CLSI (10)

**Interleukin- 10(IL-10) & Interferon -Gamma (IFN- $\gamma$ )**, were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) Human Cytokine Kit (Cusabio/China) with two sets of each parameter.

### Analysis of Statistical

The Analysis of Statistical system- SAS (2012) software was utilized to examine how the factors of study differed [11].

### Discuss the results

Isolation and identification of bacteria a whole of 150 clinical samples of stool were collected from patients suffering from diarrhea attending different hospitals in Baghdad City. The results of biochemical tests, table (1) were compared with the characteristics of *Aeromonas* spp. [9], and one species of *Aeromonas* was identified which was *Aeromonas hydrophila*, all (6) isolates of *A. hydrophila* were non-lactose fermenters with the presence of oxidase and catalase, while IMVC tests gave different results.

**Table 1: *A. hydrophila* Isolated from a Stool Sample Underwent Biochemical Testing**

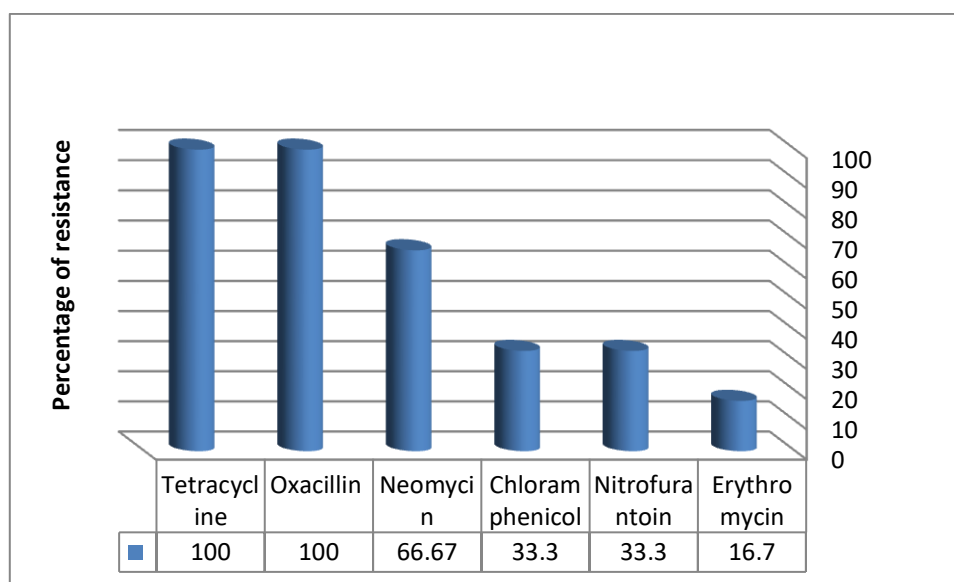
Test	Catalase	Oxidase	Indole	Methyl Red	Voges proskauer	Citrate utilization	Lactose fermentation
Isolate							
<i>A. hydrophila</i>	+	+	+	±	+	±	-
( + ): positive result; ( - ): negative result; (±):Variable							

### Susceptibility to Antibiotics

The results of the susceptibility tests to eight different antibiotics are shown in figure (1) and table (2). Chloramphenicol, Nitrofurantoin, and Erythromycin were the most effective antibiotics, with resistance percentages of 33.3%, 33.3%, and 16.7%, respectively. Neomycin was ineffective, with resistance percentages of 66.68% and 100% for Tetracycline and Oxacillin, respectively.

In contrast to being resistant to penicillin and ampicillin, multi-resistant *A. hydrophila* has been reported to be susceptible to chloramphenicol, quinolones, trimethoprim, sulfamethoxazole, tetracycline, aminoglycosides and second- and third-generation cephalosporins [12], [13]. Since the growth of *A. hydrophila* antibiotic resistance is a community health difficult, a concerted effort should be made to track the incidence of this opportunistic infection globally [14].

In developing nations where gastroenteritis is common and many drugs are commonly misused, antibiotic resistance amongst enteric bacteria is a severe issue. Antibiotic resistance is mainly important in pathogenic *Aeromonas* strain, where multiple resistance has frequently been discovered in addition to the traditional resistance to -lactamic drugs [15]. These microbes can acquire and give other Gram negative microbes antibiotic resistance genes (16).



**Figure 1: Percentage of antibiotic resistance *A. hydrophila* isolates**

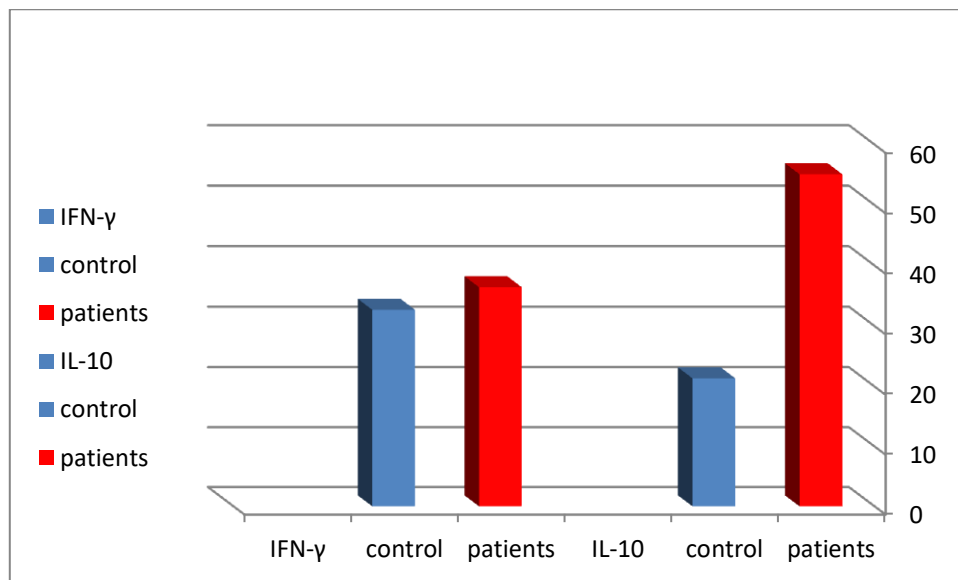
### Immunological markers

The results showed, as shown in the table-2, that the level of interferon-gamma in patients was ( $36.42 \pm 24.31$ ), while the level in healthy subjects was ( $32.68 \pm 22.56$ ), and there was no significant difference between them. While the mean for interleukin-10 was in the patients ( $55.09 \pm 52.18$ ) compared with the control ( $21.36 \pm 19.23$ ), with a significant difference between the two cases.

**Table 2 : Comparison of immunological markers(IFN-  $\gamma$  and IL-10) between patients and healthy**

Parameters (pg /ml)	cases NO.	control NO.	P-value
	(Mean $\pm$ S.D)		
IFN- $\gamma$	36.42 $\pm$ 24.31	32.68 $\pm$ 22.56	P>0.05 no Significant
IL-10	55.09 $\pm$ 52.18	21.36 $\pm$ 19.23	P<0.05 Significant

T-helper cells not only serve crucial pathogenic functions in many human autoimmune disorders, but they also play essential roles in host defense against certain extracellular bacteria and fungi [17].

**Figure 2: IFN- $\gamma$  and IL-10 serum levels in patients and controls**

T cells, B cells, and monocytes/macrophages are only a few of the diverse kinds of cells that secrete the anti-inflammatory cytokine IL-10, when the immune system is activated in various ways. IL-10, a cytokine of the Th2-type, has been demonstrated to suppress a wide spectrum of inflammatory replies & is recognized to show a significant role in preserving the homeostasis of all immune responses. As a result, IL-10-based new

therapeutics have been developed for a number of human diseases, including autoimmune disorders and allergic reactions[18].

Interferon was once assumed to be an antiviral substance that stopped virus reproduction in mammalian cells. They are secreted by infected cells, where they increase cytokine synthesis in the innate immune system as well as the activity of natural killer cells and antigen presentation[19]. A distinguishing feature of both natural and adaptive immunity is the creation of interferon-gamma (IFN-  $\gamma$ ) in reaction to infection. In addition to playing a crucial function in host defense, IFN-  $\gamma$  has also been linked to the pathophysiology of autoimmune and chronic inflammatory diseases [20]. In reality, IFN-  $\gamma$  acting a critical part in mediating a number of pathogenic processes connected to chronic immunological activation, as shown by knockout models. The idea that IFN-  $\gamma$  plays a dual character in inflammation, on the other hand, has gained support in recent years.

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## IMMUNOLOGICAL DETECTION OF RUBELLA VIRUS IN ABORTED WOMEN OF AL-MUTHANNA PROVINCE

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### Abstract:

**Objectives:** Maternal infection with rubella virus during gestation should be considered a risk factor for adverse pregnancy outcomes in human, so, the study aimed to detect Rubella virus in aborted women using serological technique. **Methodology:** The samples were collected at Al-Muthanna Teaching Hospital. Forty nine women were selected for this study, who had a single or repeated miscarriages , and with a physician's referral, they had the TORCH (Toxoplasma, Rubella, CMV, and Herpes) test to establish the exact cause of their loss. Indirect enzyme-linked immunosorbent assay IgG was done to diagnose the Rubella virus infection. **Results:** The results showed that 9 (18.3%) were positive for Rubella indirect ELISA IgG.

**Conclusions :** High prevalence of rubella virus among Iraqi aborted women in Al-muthanna Province. Early diagnosis will help in proper management.

**Key Words:** Rubella Miscarriage – Rubella Igg– TORCH Test.



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**Introduction:**

Miscarriage has been ascribed to various factors involved in reproduction of human . Hereditary and uterine anomalies, endocrine-disorder and immunity dysfunctions, infectious microorganism, ecological-pollution, endometriosis and psycho genetic factors are most essential motives <sup>1, 2</sup> of spontaneous abortion. Some maternal infections, especially at some stages in the beginning of gestation, can lead to malformations or fetal loss due to the fact that the capability of the fetus to oppose the irresistible microorganisms is constrained and the fetus immunity doesn't able to escape the scattering of infectious agents to different tissues<sup>3</sup>.

TORCH {toxoplasmosis, rubella virus, cytomegalo virus (CMV) and herpes simplex virus}, which can cause pregnant women to become ill and can also lead to infant birth defects. This entire infectious microorganism causes a change in immune response from T-helper 2 to T-helper 1 and clinically detectable apoptosis throughout pregnancy as miscarriage<sup>4</sup>. Rubella is associated with a large range of fetal defects and also infects the placenta, raising the likelihood of stillbirth. However, congenital rubella infection in developing nations, due to widespread vaccination program, is extraordinarily uncommon <sup>7</sup> . Rubella is related with a 80%riskof typically more than one congenital abnormalities if acquired in the first three months of pregnancy <sup>8</sup> , particularly the first (2-2.5) months, and result in problems with fetal growth or stillbirth <sup>9</sup> . It is distributed by airborne respiratory droplets. <sup>10</sup> . Replication of the virus originally occur in the lymph nodes and mucosa of nasopharynx, the virus infects the placenta and the developing fetus during pregnancy. Infants with congenital rubella could even continue to shed the virus for 12 months or longer, through urine and pharynx secretions. The incidence and severity of fetal injury decreases dramatically if maternal infection is present after the first3 months of pregnancy.<sup>10</sup> Ophthalmic defects include a number of congenital rubella syndrome (CRS) disorders (e.g. cataracts, glaucoma, pigmentary retinopathy), Cardiac (peripheral stenosis of the pulmonary artery, ductus arteriosus or defects of ventricular septal), auditory (e.g. visual deafness), craniofacial (microcephalus), microcephalus and developmental delay <sup>9</sup> and damage to the lungs, liver and spleen <sup>11</sup> .

## **Materials and methods**

### **Subjects:**

The samples were collected at Al-Muthanna Teaching Hospital from October to March 2021 . Forty nine women were selected for this study aged ( $\geq 20$ - 45)years and had a single or repeated miscarriages with a physician's referral, they had the TORCH (Toxoplasma, Rubella, CMV, and Herpes) test to establish the precise cause of their miscarriages. Blood was withdrawn using sterile medical syringes, then the serum was transferred to sterile tubes and keep into a deep freeze at a degree of (-20) until use.

### **ELISA test:**

Indirect enzyme-linked immunosorbent assay IgG . (Human Gesellschaft Biochemica / Germany) was done According to manufacture instructions the required number of microtiter strips were selected, running duplicates of controls, then controls and specimens were pipetting carefully on the bottom of microwells, after washing, substrate was added and incubated for a period in the dark, substrate initiated a kinetic reaction, which is terminated by the stop solution.

### **Results and discussion:**

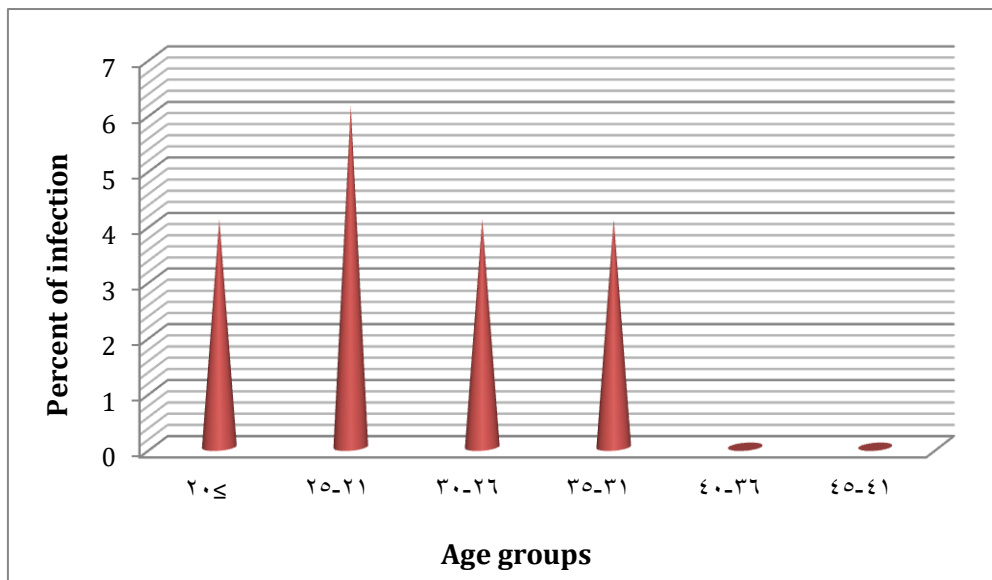
The scientific explanation for using this test is that blood test scans for rubella to see if a woman has rubella virus antibodies. Antibodies are proteins formed by the immune system to help combat infections and to prevent us from deadly diseases. They are directed at various pathogens, bacteria, and other threats. IgG persists in the body for life, indicating that a woman in the past has either the disease or the vaccine and is still immune to the virus <sup>13</sup>. Out of 49 miscarriaged women, only 9 (18.3%) women were infected with Rubella virus. Table (1) shows the demographic characteristics of the (9) Rubella miscarriaged women divided according to age, region, recurrence of miscarriages and finally the trimester of miscarriage.

**Table (1): Demographic characteristics distribution**

	Item	Frequency	Total
<b>Age groups</b>	≥20	2(4.08%)	9 (18.3%) P. value = 0.89
	21-25	3(6.12%)	
	26-30	2(4.08%)	
	31-35	2(4.08%)	
	36-40	0 %	
	41-45	0 %	
<b>Region</b>	Rural	5(10.2%)	9 (18.3%) P. value = 0.66
	Urban	4(8.16%)	
<b>Recurrence</b>	Single	6(12.2%)	9 (18.3%) P. value = 0.67
	Recurrent	3(6.12%)	
<b>Trimester of miscarriage</b>	1 <sup>st</sup>	8(16.23%)	9 (18.3%) P. value = 0.3
	2 <sup>nd</sup>	1(2.04%)	
	3 <sup>rd</sup>	0%	

**P ≤ 0.05 is significant**

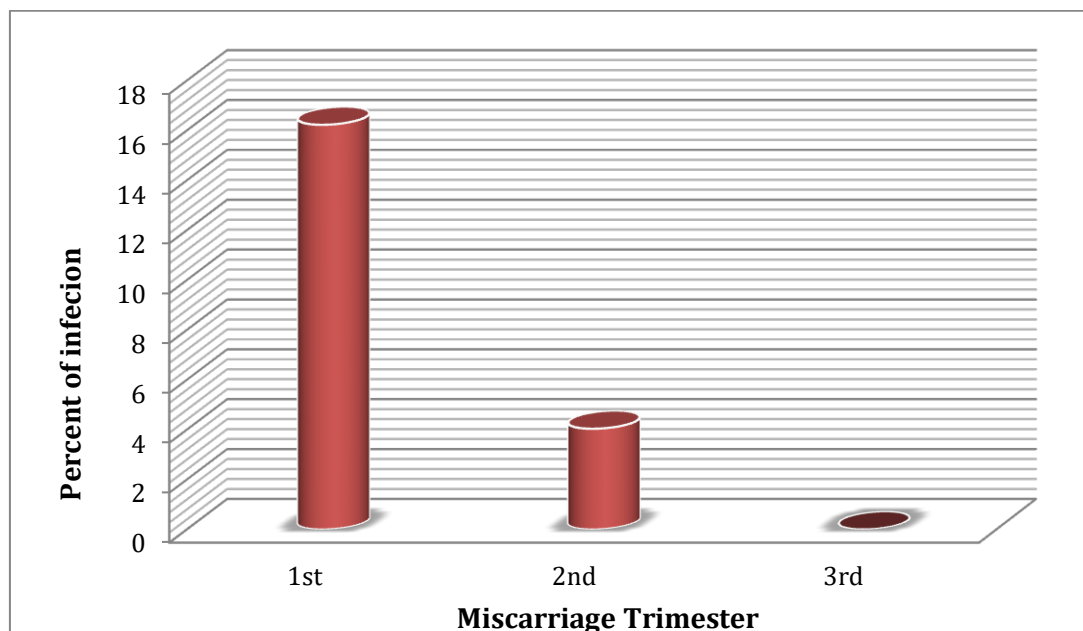
The age group of (21-25) years were the most group showed infection with rubella (6.12%) , In younger women, infection rates are highest, in part because their immature and childish cervical cells are more fragile to infection <sup>14</sup> (Fig. 1). A research by (Mohammed, 2015) indicate that the incidence of IgG seropositivity reduced with growing age, probably increase in the frequency contact to rubella virus among the younger individuals, with seropositivity reducing with age 15. These findings were almost consistent with prior studies which observed that IgG positive cases decreased as the age increased.<sup>16</sup> Another research that confirms also with current study which showed a higher prevalence of IgM antibodies among women between the ages of 25-34 years .<sup>17</sup>.



**Fig 1: Distribution of infection according to Age groups**

The percentage of rural area infections was 5(10.2%) . This is in agreement with (Khudhair and Ahmed, 2015), who reported the number of positive cases between rural areas were 18(51.4%) and urban area were 17(48.6%) and also (Mohammed and Kozaz, 2019) they figured out that urban residences in urban communities have such a relatively high IgG positivity rate as they're more capable of living in cramped conditions and become vulnerable to the widespread risks of disease. <sup>19</sup>.MR vaccine has been prescribed in developing countries in the world as an universal public early childhood vaccine just because rubella causes significant infections, including congenital rubella syndrome, pneumonia and widely disseminated disease, in infants and young children <sup>20</sup> . Most of the cases 6(12.2%) were single miscarriage, this agree with <sup>18</sup> , who reported the IgG seroprevalence was the highest in pregnant women who had one miscarriage in comparison with multiple miscarriages which means that IgG antibodies had a very important role in decreasing the rate of abortion in pregnant women <sup>18</sup>.

(Hammod *et al.*, 2012) previously reported that the seropositivity levels for anti-rubella IgG was substantially greater in pregnant women without prior miscarriage relative to expectant mothers with prior abortion (96.1% vs 76%)<sup>21</sup> , yet this is contrary to (Mohammed, 2015) noting that the relatively high antibody levels, the higher the chance of abortion, meaning that those with a stronger abortion rate had an elevated antibody production, he found that the frequency of rubella increased dramatically with the number of abortions given the association between the abortion rate and the occurrence of rubella<sup>15</sup>.

**Fig(2): Distribution of infection according to Miscarriage trimester**

8(16.23%) of infected women were miscarried in the 1<sup>st</sup> trimester of gestation, while the other were in 2<sup>nd</sup> trimester, this compatible with (Mohammed and Kozaz, 2019), who recorded (59.34%) of infected women were at 1<sup>st</sup> trimester and (28.57%) in 2<sup>nd</sup> trimester<sup>19</sup>. To interpret that some maternal diseases, particularly in the early pregnancy, can bring about fetal miscarriage or distortions on the grounds that the capacity of the fetus to oppose irresistible microorganisms is restricted and the fetal immunity can't forestall the scattering of irresistible microorganisms to different tissues<sup>22, 23</sup>. Within the first trimester of pregnancy, maternal infection may cause congenital distortion of the fetus, a known medically as "Congenital-Rubella-Syndrome".<sup>24</sup> (Fig 2). On the other hand some studies implies that rubella infections are not major causes of abortion (Hussein and Balatay, 2019) referred that the prevalence of acute rubella is low in women with abortion. It is recommended that patients should be screened for those infections within an acceptable timeframe. There is no antiviral treatment for CMV and rubella after abortion as the infection is self-limiting<sup>25</sup>.

**4. Conclusions:** High prevalence of rubella virus among Iraqi aborted women in Almutahanna Province. Early diagnosis will help in proper management.

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## ARTICLE REVIEW: ENHANCED APPROACHES FOR HIGH LEVEL OF SECURITY USING BASICALLY RAIL FENCE ALGORITHM

**Mays M. HOOBI <sup>1</sup>**

### **Abstract:**

Due to the security issues, online information sharing has currently become a serious challenge. Therefore, new methods are needed for safeguarding and protecting shared data over an unsecured channel. The art of obtaining security through encoding messages and rendering them unintelligible is known as cryptography, which is the science of securing data. This science aid to encrypt data such that it could be decrypted independently of the sender. A variety of algorithms are available for decrypting and encrypting data, including (Caesar, Rail Fence, Hill cipher, Vigenere, and others). They are frequently utilized in conjunction with other ciphers or other methods to make a product cipher, which increases the complexity of encryption. This study reviews different effective methods from a number of earlier works. The findings demonstrate that each work utilized a particular enhancement regarding Rail Fence algorithm with various data types (image, text, and others) in order to achieve a high level of security.

**Key Words:** Cipher Text, Cryptography, Decryption, Encryption, Plain Text, Rail Fence, Security.



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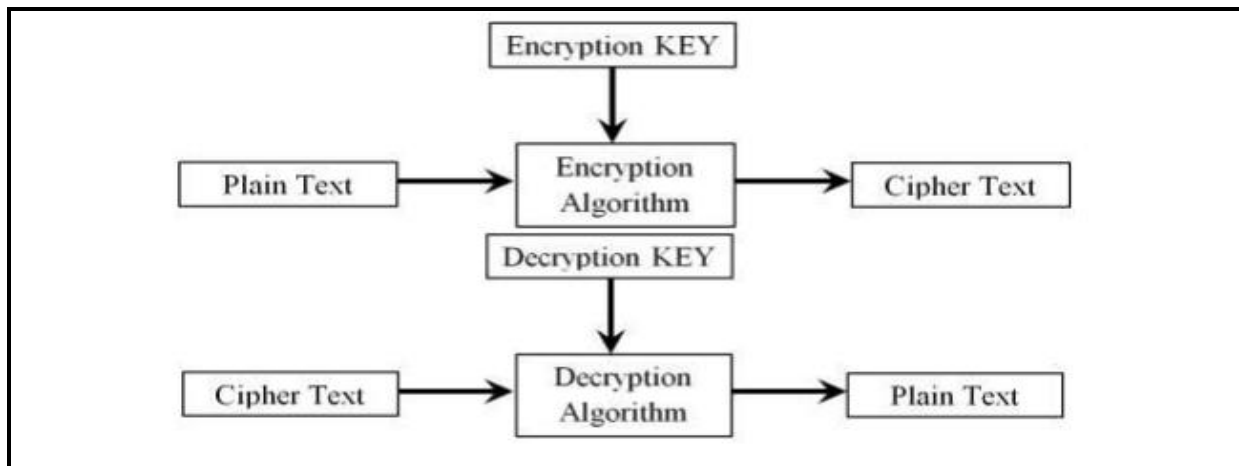


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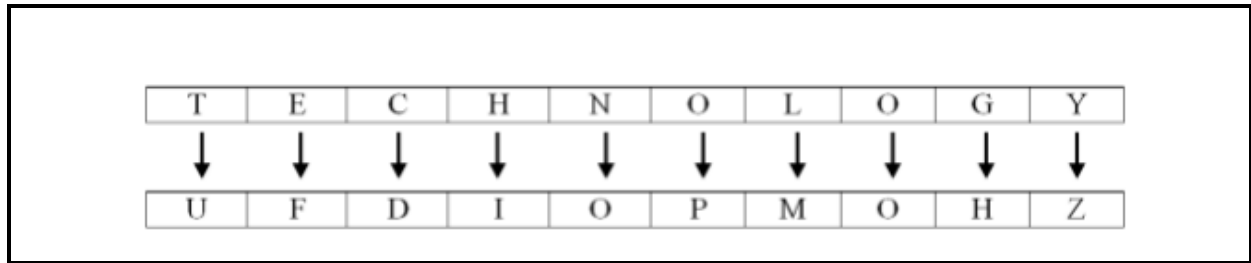
## 1. Introduction:

As technology and science have advanced significantly, this has caused numerous changes in how people live their daily lives, including alterations to their behavior and habits. Men used telegrams, letters, and radio to communicate in the past. Currently, telegrams and letters were supplanted by digital communications that use internet technology, online chat, mobile phones and other tools. Cryptography is utilized for hiding user data while transferring it across an unstable channel. [1][2]. Using specialized algorithms, cryptography creates ciphertext that renders data unintelligible unless it is decrypted using algorithms that have been predefined via the sender. The following three aims (integrity, confidentiality, and authenticity) can be maintained via cryptography [3]. The original message is labeled as "plain text" and encrypted message is labeled as "cipher text" in cryptography. The encryption is the opposed to decryption, encryption is the transformation of plain text into cipher text, as shown in figure(1) [4].

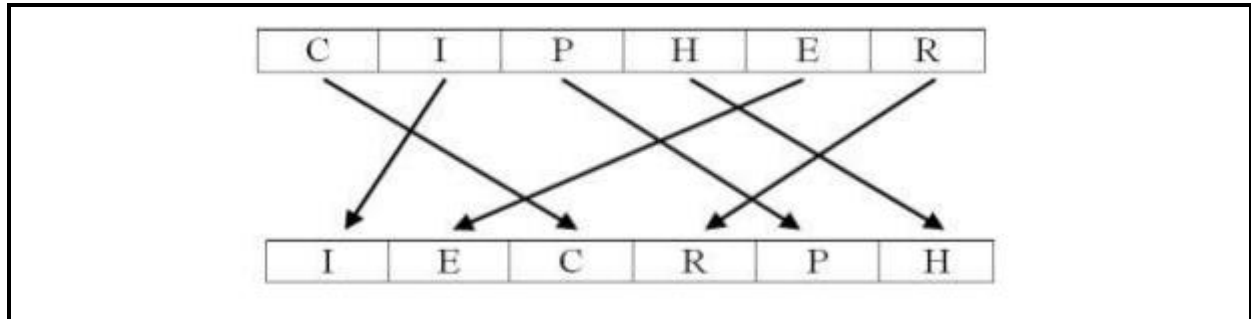


**Figure-1: Encryption and Decryption.**

The process of trying to decode is referred to as cryptanalysis and the outsider who attempts it as a cryptanalyst. Any cryptographic algorithm needs a cryptographic mechanism and a key [5]. Substitution cipher and transposition cipher are the two types of cryptographic methods. In the substitution cipher, several other symbols or characters are used to replace each character in the plain text. The transposition cipher conducts combinations and permutations, as shown in figures (2 - 3) [4].



**Figure-2: Encryption by Substitution Cipher**



**Figure-3: Encryption by Transposition Cipher**

The keys could be divided into two groups based on how decryption and encryption are performed: keys that are asymmetric and symmetric. The latter is one in which the key applied for encrypting plain text and decrypting cipher text is the same key. Because the key in such system should be kept secret or private, it is also known as a private key system. The former is one in which the key utilized for decryption and encryption differs. Public key system is another name for this system [6]. The next sections regarding this study examined the most effective improved cryptography algorithms that had been employed in various prior data encryption works. The basic cryptography algorithm employed in such works was the Rail Fence algorithm.

## 2. Cryptography Algorithms

### 2.1 Rail Fence Algorithm:

A symmetric key cryptography scheme called Rail Fence uses the transposition cipher technique. Additionally, the stream cipher known as Rail Fence carries out the encryption bit by bit [7]. The cipher text is produced by permuting the characters in plaintext. A fairly basic pattern is used to generate the permutation in rail fence cipher. A positive integer serves as the encryption key for rail fence cipher [8]. This method calls for writing the plain text as a chain of diagonals, row after row, it will be read to form the final cipher text [5]. For example, the arrangement of the text "COMPUTER SCIENCE DEPARTMENT" should match that in figure (4) in the case when we want to encrypt the message with five rails. The key to this approach is the decision of how many rails to utilize. Both decryption and encryption employ the same number of rails.

Row 1	C				-				-					E	
Row 2		O			R	S			E	D				R	N
Row 3			H		E			C		C		E		T	
Row 4			P		T			I	N			P		R	
Row 5				U				E					A		

**Figure (4): Rail Fence Encryption.**

To obtain the cipher text following the arrangement, the characters are read line by line from left to right. Figure (4) makes it clear that "C—EORSEDMNMECCETTPTINPRUEA" is the encrypted message that was created [3].

## 2.2 Caesar Algorithm:

Caesar cipher, which uses a substitution cipher where the matching alphabet is substituted by its subsequent third letter, is one of the earliest ciphering methods. For further information, see table (1) [9].

### Table-1: Caesar Cipher Table

Alphabet	Position
A	D
B	E
C	F
D	G
E	H
F	I
G	J
H	K
I	L
J	M
K	N
L	O
M	P
N	Q
O	R
P	S
Q	T
R	U
S	V
T	W
U	X
V	Y
W	Z
X	A
Y	B
Z	C

This method's encryption algorithm can be written as  $F(x) = (x + n) \bmod 26$  (where  $n=3$ ). For instance, Plaintext LUSICT, Ciphertext: XVLFW, this method becomes antiquated quickly, makes it simple for attackers to break the cipher, and doesn't allow alphanumeric characters [2].

### 2.3 Vigenere Algorithm:

A more sophisticated Caesar cipher known as the Vigenere Cipher encrypts alphabetic text by employing a number of different Caesar ciphers that depend on the letters of a keyword. Vigenere square or table is utilized in this cipher. The 26 potential Caesar ciphers are represented by alphabets that are written out 26 times in various rows, with each alphabet shifting cyclically to the previous alphabet. Regular keyword changes could be beneficial since hackers might never successfully predict the keyword. Additionally, this approach's table only contains the english alphabets. Yet, the level of security will be increased by incorporating numbers and symbols into this table. Even in the case when such numbers and symbols are inserted arbitrarily in between alphabets, the level of security will still be raised [10].

### 2.4 RSA Algorithm:

The cryptographic algorithm known as RSA, which stands for Rivest-Shamir-Adleman, is utilized by contemporary computers for encrypting and decrypting messages. This cryptographic algorithm is asymmetric [10]. While the private key is just maintained by the user or the authenticate recipient, the public key is published to all other users, making it known to all users. For more information and an illustration, see figure (5)[11][12].

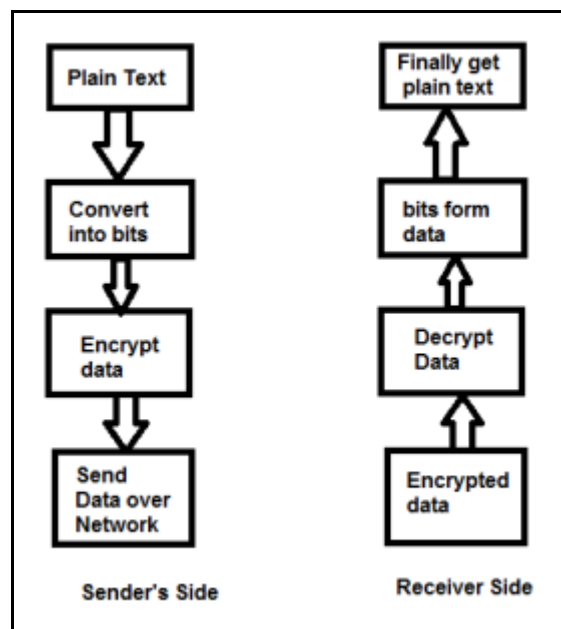


Figure-5: RSA Algorithm Work.

RSA is regarded as the most secure approach for encryption and decryption. This approach requires certain mathematical calculations that require a moderate amount of processing power. The following are the procedures to be followed with regard to RSA key generation:

- Choose two distinct prime numbers  $p$  and  $q$
- Compute  $n = p \cdot q$
- Compute  $\phi(n) = \phi(p) \cdot \phi(q) = (p - 1)(q - 1)$ , where  $\phi$  is Euler's function.
- Choose an integer  $e$  such that  $1 < e < \phi(n)$  and  $\gcd(e, \phi(n)) = 1$ ; i.e.,  $e$  and  $\phi(n)$  are co-prime.
- Determine  $d$  as  $d = e^{-1} \pmod{\phi(n)}$ ; i.e.,  $d$  is the modular multiplicative inverse of  $e \pmod{\phi(n)}$  [10]

## 2.5 DES Algorithm:

DES can be defined as a block cipher that works on 64-bit plain text and use the same 64-bit key for encrypting and decrypting data blocks (symmetric key encryption scheme). The identical procedure is followed in reverse order during DES decryption and encryption. With regard to DES, plaintext undergoes a challenging set of procedures to create cipher text (of the same length). The 8-bits that make up each byte are formed by all blocks, which are numbered from left to right. DES runs 16-rounds to generate 64-bit output data. Figure (6) provides a description of DES [13].

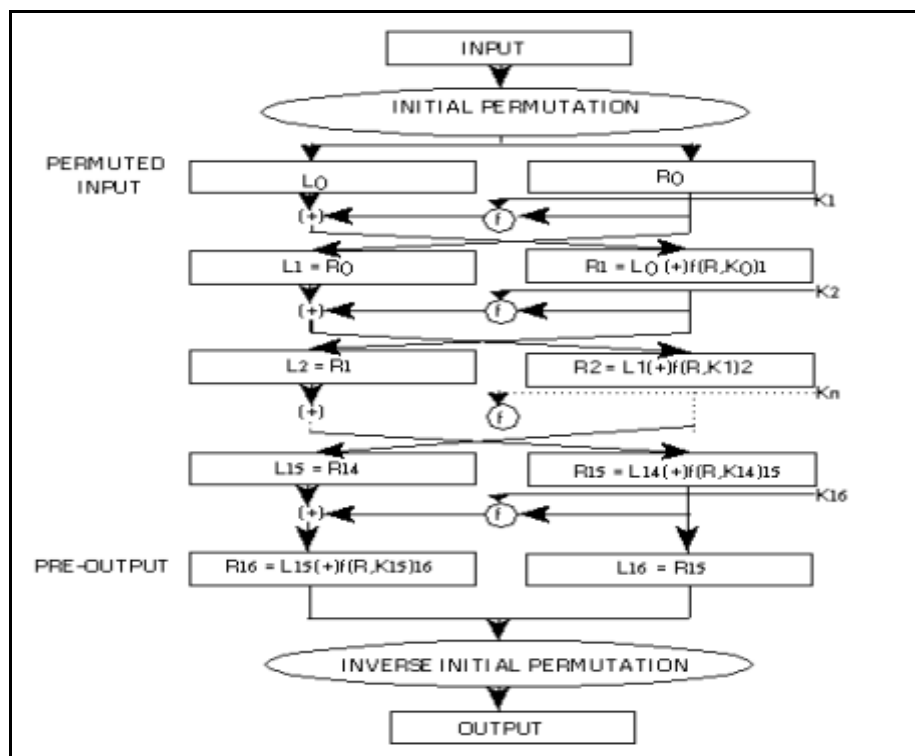


Figure-6: DES Encryption(64-bit).

## 2.6 Hill Cipher Algorithm:

A cryptosystem which enciphers blocks is called Hill cipher. Any block size can be chosen, however finding good keys to decrypt huge blocks could be challenging. A block cipher is a type of cipher that enciphers blocks of letters that are of the same length. Hill cipher requires that each one of the characters in the alphabet of coded letters be given a number before it can be used to encrypt a message. As shown in figure (7)[8], A=0, B=1, C=1, and so on.

Letter	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Number	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

**Figure-7: Scheme of Letters of Alphabet**

Each block of  $n$  letters, which is thought of as  $n$ -component vector, is multiplied by an invertible  $n \times n$  matrix against modulus 26 for encrypting a message. Each one of the blocks is multiplied by the inverse of encryption matrix for decrypting the message. The cipher key is the matrix utilized for encryption, and it must be randomly selected from the collection of invertible  $n \times n$  matrices (modulo 26). The cipher may be modified to work with any number of letters in the alphabet; all arithmetic simply needs to be performed modulo that number of letters rather than modulo 26 [8].

## 2.7 Vernam Cipher Algorithm:

A stream cipher which employs bit-by-bit encryption is the Vernam cipher. It is an encryption algorithm that transforms plain text into cipher text using a transposition mechanism. Vernam applies the provided key's bits to the plain text's bits with the use of Boolean "exclusive or" (XOR) operation. The symbol for the XOR function is  $\oplus$ . The key is utilized for producing the cipher text as  $\text{Plaintext} \oplus \text{Key} = \text{Ciphertext}$ . Additionally, the plaintext is reproduced as  $\text{Ciphertext} \oplus \text{Key} = \text{Plaintext}$ , as shown in figure (8) [7].

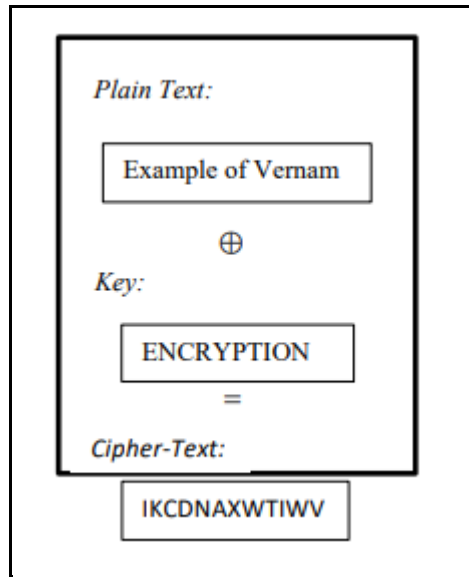


Figure-8: Vernam Cipher

### 3. Literature Review

Due to the fact that communication has always been a topic of attention. Massive data information is disseminating every day as a result of the internet's rapid expansion. Therefore, while data is being transmitted over the internet, data security is a highly significant topic. It becomes open to unauthorized hacker or malicious user attacks. Given that it is possible to break the cipher text using one of the many cryptographic techniques, it was attempted to suggest a system. A large number of effective research were viewed in this part; such investigations used the Rail Fence as its basic algorithm. First, have a look at table (2), which shows how certain earlier studies utilized Rail Fence to provide a high level of security.

Table-2: Literature Review studies

Study No.	Study Title	Author(s)	Publication Year	Methods
1	Modified Ceaser Cipher and Rail fence Technique to Enhance Security	Baljit Saini	2015	Caesar +Rail Fence
2	Efficient Watermarking Technique using DWT, SVD, Rail fence and 10's complement applied on Digital Images	1-Kulakarni Shylesh 2-Chirag Sharma	2015	Rail Fence with image Water marking
3	Maintaining User-Level Security in Short Message Service	1-Arudchelvam. T 2-Fernando.N	2016	Caesar + Vigenere + Rail Fence +RSA
4	Rail Fence Cryptography in Securing Information	Andysah Putera Utama Siahaan	2016	Rail Fence
5	Enhancing the performance of Data Encryption Standard algorithm by using Rail Fencing	1-Ritwik Goyal 2- Binod Kumar Mishra 3-Prashant Lakkadwala	2017	Rail Fence + DES
6	A new cryptography method based on Hill and Rail Fence	1-Ashty M. 2-Ann Z.	2017	Rail Fence +Hill Cipher

	Algorithm			
7	An Improved Algorithmic Implementation of Rail Fence Cipher	1-Samarth Godara 2-Shakti Kundu 3- Ravi Kaler	2018	Rail Fence
8	Mercurial Cipher: A new cipher technique and comparative analysis with classical cipher techniques	1-Karandeep Singh 2-Rahul Johari 3-Kulbeer Singh 4-imanshu Tyagi	2019	Key management with Classical Cipher
9	Combination of Rail Fence Cipher Algorithm and Least Significant Bit Technique to Secure the Image File	1-Dian Rachmawati 2-Mohammad Budiman 3-Akhmad Yusuf	2020	Rail Fence +Steganography
10	Improved Approach of Rail Fence for Enhancing Security	1-Khairun Nahar 2-Partha Chakraborty	2020	Enhanced Rail Fence
11	Merging Vernam Cipher stream and Rail Fence Algorithms and How Effective They are on IoT Devices	1-Adnan Adel Bitar 2- Dr. V. Sujatha	2021	Rail Fence + Vernam
12	International Research Journal of Engineering and Technology (IRJET)	1-A. Ashok Kumar 2- S. Kiran2 3-R. Pradeep Kumar Reddy2, 3-ndeepp Reddy Devara	2021	Enhanced Rail Fence

Author of study no. 1 under title " **Modified Ceaser Cipher and Rail Fence Technique to Enhance Security**", as in [9]. This work employed both transposition and substitution techniques for encrypting data. The key size is fixed at two in the modified Caesar cipher procedure. Additionally, the alphabet index is checked, added to the key, and its modulus is calculated. The message was then encrypted once more using the Rail Fence on index value. This work demonstrates an experiment using the well-known encryption methods Rail Fence and Ceaser cipher to give them some further strength. The suggested approach shown that it is superior in terms of enhancing text message security. The improved method could securely encrypt data, and the cipher text produced by the suggested algorithm will be challenging for an attacker to decipher.

In study No. 2 as [14] authors presented "**Efficient watermarking technique using DWT, SVD, rail fence and 10's complement applied on digital images**". The watermarking approach presented in this research works well on both digital color and greyscale images and is based on Singular Value Decomposition (SVD), Discrete Wavelet Transformation (DWT), and Rail Fence techniques. When the first 10's complement and Rail-Fence approach are applied to the watermark image, the watermark image is transformed. The suggested methodology has made it possible to develop a reliable watermarking security, secure the watermark image, and enable safe digital data



transmission. Lastly, even after applying some different image processing attacks, this work still provided good NC and PSNR values.

Authors in study no. 3 as [10] presented " **Maintaining User-Level Security in Short Message Service** ". In the apps for mobile phones, this work offers a technique for keeping user security up to par. Along with the Vigenere cipher and RSA algorithms, enhancements and implementations are made to the Rail Fence and Caesar cipher algorithms. Because the user could choose the encryption technique and the key in this study, the user might assure the security of messages through periodically switching the key and the encryption approach. In addition to being able to send and receive messages safely, users might also save their information on their own mobile phone to protect it from unauthorized parties. Additionally, users might encrypt any message with the use of any of the application's cipher methods. The key and cipher approach must be covertly sent to the message in the case when the message is encrypted before being sent. Additionally, both the cipher and the key technique could be changed on a regular addition. As a result, the likelihood of hacking might be decreased. After receiving that, in the case when a message is encrypted, the phone owner must keep the cipher technique and key in mind in case they want to retain that message for later use. This app helps data exchange messages and save secret data and messages by the user. Even if the phone or mobile device is lost, the data that was stored in this way is safe from unauthorized access.

The study number 4 as [15] title is "**Rail Fence Cryptography in Securing Information**" This work demonstrated the decryption and encryption of the Rail Fence. In addition, Rail Fence is a straightforward cryptography algorithm, and the number of rows used to implement it is the key. A brute-force approach could be used to guess it, and the decryption procedure might be swiftly accomplished by hand as well. Furthermore, a computer can solve it more quickly. Random transposition positions may strengthen security as a means of raising it. This work showed that Rail Fence should be developed to be more protective and should be used in conjunction with another technology or modified by altering the trajectory of the table.

According to study no. 5 as stated by [16], "**Enhancing the performance of Data Encryption Standard algorithm by using Rail Fencing**". DES encryption method utilized in this work, is still in use today despite its simplicity and lack of significant flaws. The author proposed a method that combines the usage of the traditional symmetric key encryption algorithm "DES" and the cryptographic transposition method Rail Fencing in order to tackle such problems. A transposition method is introduced before the DES algorithm for the encryption process to make it more complex and effective. This increases the DES algorithm's performance with regard to processing time without sacrificing security. Over the already available DES and a select few other ways, this newly suggested solution offers unquestionably a little increase in security. According to evaluation results, the suggested algorithm processes information much more quickly compared to symmetric key methods.

According to study no. 6 as [8], titled “**A New Cryptography Method Based on Hill and Rail Fence Algorithms**” In order to create a new secure approach that is challenging to break, this work offered a novel approach of ciphering that combines the transposition (Rail Fence) and substitution (Hill Cipher) techniques. This mixing served as a link between traditional and contemporary ciphers. Hill cipher and Rail Fence appear to require the generation of random Matrix for the modified Hill cipher Decryption and Encryption, which is effectively the security of RailHill. As far as we are aware, the matrix's inverse is needed for Hill cipher decryption. As a result, there is an issue with decryption because the inverse of matrix does not often exist. Consequently, encrypted text cannot be deciphered in the case when the matrix is not invertible. Yet the modified Hill cipher algorithm totally gets rid of this flaw. The inverse of numerous square matrices must also be found, which is a difficult computing time, in order to use this approach. This mixed system uses a challenging cipher that a cracker will find tough to break. After measuring the time regarding such algorithm and the time at which it runs, it appears to be more secure compared to the transposition and substitution techniques.

Authors in study no.7 as [4] titled “**An Improved Algorithmic Implementation of Rail Fence Cipher**”. This work suggested an algorithm for implementing rail fence transposition cipher with an  $O(n)$  time and space complexity. Additionally, the author indicated the optimized rail number that provides the best randomness in the cipher. A new approach was suggested with the use regarding the rail fence cipher for increasing the cipher's complexity without increasing the time complexity related to the encryption algorithm. As a result, the block rail fence cipher was presented as a way to use rail fence ciphers to raise their complexity without raising the time complexity regarding the encryption algorithm. The suggested algorithm was also created to provide an easy method for creating programs for decryption and encryption by utilizing a variety of rails. The suggested algorithm's time and space complexity is linear, or  $O(n)$ . The arrangement of cipher characters in cipher text will be close to their neighboring plain text characters in the case when the number of rails is minimal; nevertheless, if the number of rails is more than the number of characters in the message, the cipher text will be identical to the provided message.

Authors In study no.8 as [2] presented “**Mercurial Cipher: A new cipher technique and comparative analysis with classical cipher techniques**”. This study suggested a novel ciphering method that addresses the drawbacks of the current conventional ciphering methods and compared it with regard to reliability, performance, and efficiency. A novel ciphering method called Mercurial Cipher was developed by studying the weaknesses, defects, and vulnerabilities of current (XOR cipher) and traditional ciphering methods (Vigenere, Caesar, Rail Fence). The major goal is to find a solution to the key management issue because the majority of ciphering techniques rely on the key, which is the most vulnerable component of every encrypted message and which, if discovered by an attacker, can be used to decrypt any message. The objective of achieving security is wholly defeated.

As a result, the sender's machine information was included into this encryption approach so that the intended message can understand the original message and understand where it came from. To improve the reliability and security regarding the data to be communicated, new or improved ciphering methods are thus necessary.

According to study no.9 as [17], presented **“Combination of Rail Fence Cipher Algorithm and Least Significant Bit Technique to Secure the Image File”**. The secret message cannot be seen in plain view thanks to the combination of Rail and steganography used in this investigation. Steganography is used to conceal secret communications in other messages, making the existence of the secret very difficult to discover. The LSB is one of many Steganographic techniques. The LSB principle operates by changing the RGB image's final bit, when the message is first translated into bits. The four primary menus in sycg implementation are extraction LSB, embedding LSB, encryption Rail Fence, and decryption Rail Fence. Based on the results of this work, it could be said that the process of running time of decryption and encryption rail fence algorithm is the amount of time required as image resolution increases. Along with using the rail fence algorithm for cryptography and the LSB for steganography, both techniques could be used together.

Authors in study no.10 as [5] presented **“Improved Approach of Rail Fence for Enhancing Security”**. This study redesigned the current Rail Fence by applying the three basic phases:

Phase 1: Substitution: Represent the Plain text by the special symbol.

Phase 2: Transposition: Circular Shift.

Phase3: Transposition: Rail Fence.

The suggested method makes sure that the final cipher text is a combination of different symbols that are all determined by a chart. Through transforming plain text unexpected and unreadable, the suggested algorithm removes the previous algorithm's drawback and considerably enhances performance.

Authors In study no.11 as [7] presented **“Merging Vernam Cipher stream and Rail Fence Algorithms and How Effective They are on IoT Devices”**. This work combined the simple standard algorithms "Rail-Fence stream" and "Vernam Cipher" to create the more powerful algorithm "Railve,". The two algorithms are used on a laptop and a mobile phone, respectively. The work's findings suggest that the suggested algorithm "Railve," which performs well and uses little RAM, will take a lot of time and effort to break.

Authors in study no.12 as [3] presented **“A New Variant of Rail Fence Cipher using Hybrid Block-Swap Method”**. The author in this study modified the conventional Rail Fence cipher and fixed the key size as two. The proposed Hybrid Block-Swap Rail Fence Algorithm is an extension of the Rail Fence Algorithm. In this method, the Rail Fence algorithm along with Exclusive-OR (XOR) operation is performed with the key and internal swapping in blocks. If the length of plain text is not an integral multiple of 32-bits, then padding for plain text is required. A hybrid algorithm is proposed to overcome the said

limitations of the existing rail fence algorithm. Every character of the ciphertext generated using this proposed algorithm will be in the range of 0 – 127 ASCII values. The key generated in the proposed algorithm is a Random 64bit, this is additional security, in addition, the hybrid block-swap Rail Fence algorithm gives the best randomness in the cipher.

#### **4. Conclusions:**

The most efficient way to meet the requirements of privacy and security is through encryption. One of the most crucial factors regarding any encryption algorithm is performance. As mentioned in previous sections It is clear that, Rail Fence algorithm can provide a good level of security. Several previous studies in cryptography field used Rail Fence cipher with different algorithms like(Vernam, Vigenere, Caesar,...etc.) and different techniques like(Water marking, Steganography,...etc.). Rail Fence can be implemented with different data type like text, image, and others, also Rail Fence improved by applying different SIW and HIW that help to less amount of cipher text size (reduce memory space) and encryption time, which it presents a better security.

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**STUDYING THE ANTIMICROBIAL EFFECT OF PHENOLIC COMPOUNDS EXTRACTED  
FROM CAULIFLOWER BRASSICA OLERACEA VAR. BOTRYTIS AGAINST SOME  
SPECIES OF PATHOGENIC CANDIDA**

**Alaa M. HASAN<sup>1</sup>**

**Abstract:**

Increasing microbial and fungal resistance to antibiotics is one of the biggest challenges facing the scientific community, therefore, finding alternative treatments is one of the ideal options to overcome this problem. The cruciferous family is one of the richest plants worldwide because it contains an important secondary metabolite .Phenolics compound which are known for their antimicrobial properties. The sScop of this work is to estimate the antimicrobial influence of phenolic compound against four Candida spp. (C. albicans, C. tropicales, C. kyfer and C. parasilopses). The activity of five concentrations (200, 400, 600, 800, and 1000 µg/ml) prepared from crud alcoholic phenol extract were investigated. Data showed that the highest antifungal effect of phenol was against C. albicans and C. parasilopses respectively at the concentration 1000 µg/ml, while the lowest inhibitory effect was against C. albicans at the concentration 200 µg/ml, C. kyfer at the concentration 200 µg/ml, and C. tropicales at the concentration 400 µg/ml. In general, it has been noticed that the inhibitory effect for the rest concentrations concerning Candida spp. was concentration-dependent manner.

**Key Words:** Phenolic Compounds, Brassica Oleracea Var. Botrytis, Candida Species, Human Pathogens, Soxhlet, Ethanol.



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## Introduction:

The cruciferous vegetables are one of the most important good sources of natural compounds that have been studied worldwide, due to its possession of many therapeutic properties, the most important of which are antioxidants, anti-inflammatory and regenerative properties, as well as its wide spread all over the world. **Mattosinhos et al., 2022.**

The Brassicaceae family is the most widespread and well-known among all the plants of the plant kingdom, as its most important areas are located in the northern hemisphere, specifically in its temperate regions, extending from the Mediterranean to Central Asia **Warwick et al., 2006.** These plants have many benefits, especially in the field of medicine and drugs, due to their chemical compositions, in particular flavonoids, minerals, amino acids, folic acid and vitamins **Gerszberg et al., 2019** This aforementioned importance is due to the fact that members of this family contain many secondary metabolite compounds that have an active role in the therapeutic and anti-inflammatory characteristics. **Kohli et al., 2020** .In addition to this efficacy expected to be obtained when compared with the original industrial medicines, obtaining them is less expensive, which makes obtaining these new treatments easier and available to the population. The edible plant parts of all cruciferous plants contain many biologically active components that contain health promoters such as vitamins, glucosinolates and polyphenols. **Koss-Mikołajczyk et al., 2019.**

Treatments for diseases of fungal origin are not sufficiently available, in addition to their lack of effectiveness and lack of development in a manner commensurate with the abundance and complexity of these diseases. In addition, these treatments have begun to face increasing resistance, which has led to doubts about their efficacy in the first place **Gintjee et al., 2020.** On the other hand, the pharmaceutical industry has to some extent ignored the focus and investment on making new drugs with high efficacy against fungi effectively, and for the purpose of largely eliminating fungal diseases, it is necessary to focus on developing these antibiotics by resorting to alternative treatments, such as the use of plant extracts, which have recently drawn attention due to its multiple benefits in treating fungal and microbial diseases **Carvalho et al., 2019.** To this day, it has been concluded that few plant extracts or compounds are able to be highly effective against fungi that cause many diseases that affect humans and animals **Panzella et al., 2020; Veiga et al., 2020.** Phenols, which are one of the widely distributed natural plant compounds, have many biological properties, the most important of which is their anti-fungal activity **Simonetti et al., 2020.** In current work, the crude phenol compound was extracted by one of the traditional and well-known extraction methods using the soxhlet device (fig,1). This method relies on the use of large volumes of solvent for the extraction process, in addition to being a manual and sequential method for solid and liquid extraction (SLE). (**Alara et al., 2018 a,b).**



The aim of current research is studying the inhibitory effectiveness of a crude phenolic compound extracted from cauliflower against human pathogenic *Candida* species, and the possibility of adopting these natural compounds as alternative pharmaceutical treatments.

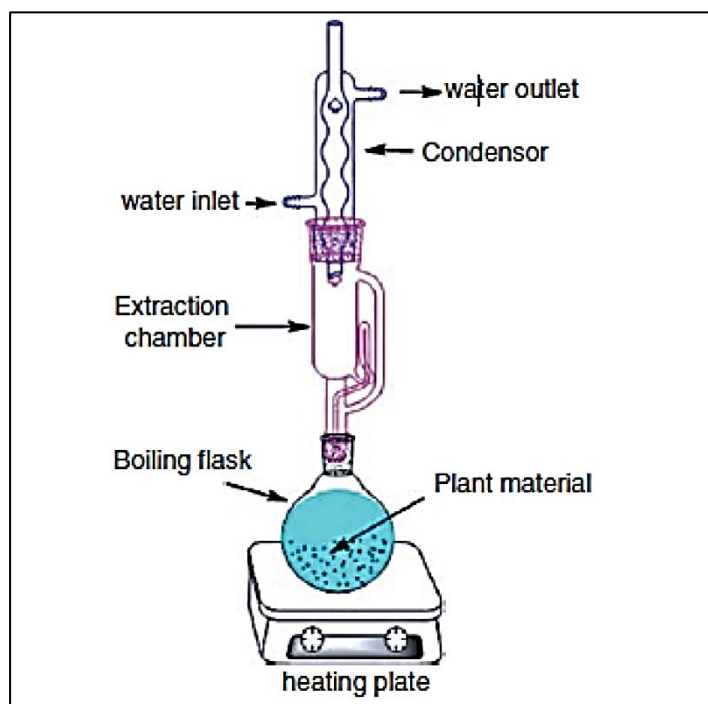


Figure 1, Soxhlet extraction

(Alara *et al.*, 2021)

### Material and methods

This study was performed at plant laboratories of Biology department/ collage of Science/ University of Baghdad/ Baghdad/ Iraq, from February 2022 to October 2022.

#### **Plant material collection:**

The cauliflower plant was obtained from the local markets, the edible parts were cut into small parts and rinsed well with water several times to get rid of dirt and dust. Then it was left to dry well, later the collected parts were placed on a tray and placed in the oven at a moderate temperature of 40 °C for a period of 4 to 6 hr. After making sure that it had dried completely, the obtained amount was ground by means of a food processor.

#### **Preparation plant extract using the soxhlet apparatus**

One hundred gm of dried plant material were put in filter paper and the filter paper were placed in thimble flask. Two hundred ml of solvent (ethanol) were poured in to the solvent flask, after that the flask was placed on heating source (electric heater) which was adjusted according to ethanol boiling point. When the temperature reaches the boiling point, the solvent started to evaporate, this vapor goes up to the condenser which located on the

thimble flask and its responsible for cooling the solvent's vapor. After that, the solvent's vapor started to condense in to thimble flask, later, when the solvent reaches the siphon level the first cycle is completed and the solid plant material is washed with solvent. This cycle was repeated for overnight **Şengün, 2018**.

### **Antimicrobial assay:**

#### **1- Diffusion method**

Antimicrobial effect of different concentrations of phenol compound were evaluated by measuring their inhibition clear zone against four pathogenic yeast isolates on sabouraud's Dextrose Agar (SDA) medium dishes.

#### **2- Microdilution method**

Minimum inhibition concentration (MIC) of phenol compound was determined according the technique outlined by **Andrews, 2001** the double serial dilution of extract was started from 200 to 1000 µg/ml as follows (200, 400, 600, 800, and 1000 µg/ml) which all set from stock solution (1450 µg/ml) using Sabouraud's Dextrose broth (SDB) as a diluent. Absolute ethanol was used as control. Each concentration was tested in triplicate. Then, the dishes were incubated under aerobic conditions for 24 hr. at 37 C ° [**Andrews, 2001**].

### **Preparation of Candida isolates:**

Previously diagnosed yeast isolates from previous work in the botany laboratory at the Department of Biology, College of Science, University of Baghdad [ **Hasan, 2017**] were used for the purpose of measuring the activity of the phenolic compound at different concentrations, which was obtained from the alcoholic extract of cauliflower. The following isolates were used (*C. albicans*, *C. tropicales*, *C. kyfer* and *C. parasilopses*).

### **Preparation of culture media**

A loopful from each candida isolates were taken and cultured in tubes with (SDB) medium, then it was placed in the incubator at 37 C° degrees for one night. later, one drop of 0.1 ml of the inoculum was taken and spread on the (previously pored dishes with cork borer) petri dish with SDA medium and put in incubator for 2- 3 days at 37 C°.

### **Statistical analysis**

Statistical analysis and graphs were done by using Origin 8 software. The data were expressed as means ± SE. The chi-square was used A value of P<0.05 was considered to be statistically significant.

## Results

### Antimicrobial (inhibitory effect) of phenols

The antifungal effect of alcohol extraction of phenols was estimated in current research against four *Candida* species. The antifungal effect of five concentrations of ethanol-phenol extract against the previously mentioned spp. of candida is illustrated in (Table 1).

Data showed that the highest antifungal effect of phenolic compound was against *C. albicans* and *C. parasilopses* respectively at the concentration 1000 µg/ml, while the lowest inhibitory effect was against *C. albicans* at the concentration 200 µg/ml, *C. kyfer* at the concentration 200 µg/ml, and *C. tropicales* at the concentration 400 µg/ml. In general, it has been noticed that the inhibitory effect for the rest concentrations concerning *Candida* spp. was concentration-dependent manner.

**Table 1: Antimicrobial activity of different concentrations of ethanol-phenol extract expressed as diameter of inhibition zones in millimeters (mm) against 4 Candida species.**

Candida spp.	control	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
<i>C. albicans</i>	NS	11±1.4	13±1.2	14±2.0	16±1.4	19±1.5
<i>C. tropicales</i>	NS	NS	12±1.1	14±1.6	14±1.7	16±1.3
<i>C. kyfer</i>	NS	12±1.2	13±1.4	15±1.5	16±1.8	16±1.9
<i>C. parasilopses</i>	NS	NS	12±1.5	14±2.0	15±1.7	18±1.6

\* Each value represents the mean of triplicate ± SE.

\*\*The test (Chi-square) was used under probability value of P<0.05.

\*\*\* NS: non-significant inhibition zone (less than 10 mm).

## Discussion

Fungal infections have a major role in increasing the death rate and disease incidence all over the world **Bongomin et al., 2017**. With regard to pathogenic yeasts, the species *C. albicans* is the most common cause of many diseases, according to their locations in the human body **Paramythiotou et al., 2017**. Therefore, there is an increasing need to find safe and effective new treatments against these highly prevalent pathogens that are resistant to many drugs.

In current study, results in (table,1) showed significant differences that concerning the antimicrobial effect using ethanol-phenol extract in concentration-dependent manner against pathogenic *Candida* spp.

Studies have shown that the Brassicaceae family has a positive effect in terms of making the wound healing process faster, in addition to controlling skin infections and reducing the severity of edema **Mattosinhos et al., 2022**.

Phenol compounds are found naturally in many legume crops, in addition to vegetables, fruits, and tea, where they are responsible for the sensory properties of plant foods. Among the types of phenols present in plants are tannin, phenolic acids, flavonoids, and lignans. **Alara et al., 2021**. One of the most important properties that attracted and continues to attract the interest of researchers in the field of drugs and treatments is the distinctive property of polyphenols (anti-oxidants) because of their protective role in many cases related to oxidative stress. **(Alara et al, 2018 a,b)**

The use of solvents for the purpose of extracting plant extracts is one of the most common methods, in addition to the development of other auxiliary methods for the same purpose, such as the use of ultrasound and the use of a microwave device according to a special method **(Selvamuthukumar and Shi, 2017)**. The chemical composition of phenolic compounds is distinguished by its structure containing several hydroxyl groups, and this is the reason for the toxicity of these compounds towards the microorganisms that are dealt with **Alara et al., 2021**. The quantity and type of phenolic compounds extracted from plants varies according to several factors, such as the extraction method used, the storage conditions of the plant or the extract, the chemical nature of the extracts, the particle size of the material to be extracted, and the presence of interference with other compounds **(Suwal and Marciniak, 2018)**.

**Przybylska-Balcerek et al., 2022** had conformed in their study the antifungal activity of phenolic acids against fungi from the genus *Fusarium*. **Simonetti et al., 2019** had evaluated the antifungal efficiency of raw phenolic compounds extracted from premature grapes in MIC ranged from 53.58 to 214.31 µg/ml. On the same path, **Peixoto et al., 2018** had tested the activity of fluid extract from Brazilian Merlot grapes using MIC value ranged from 500- 1000 µg/ml. on three *Candida* spp. (*C. albicans*, *C. krusei* and *C. parapsilosis*). While the aqueous extract of the leaves recorded stronger activity versus the same species, as it gave 20 mm diameter of inhibition zone at 1250 µg/ml.

Furthermore, **Yigit and collaborators ,2009** clarified the antifungal efficiency of both methanolic and water extracts of leaves, fruits and seeds of *V. vinifera* L. cv. Karaerik against 90 *Candida* spp. such as (*C. albicans*, *C. glabrata* (*Torulopsis glabrata*), *C. guilliermondii*, *C. kefyr*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis* (*C. kefyr*), *C. tropicalis* and *Geotrichum candidum*). **Kumar and Vijayalakshmi, 2013** used ethanol extract from *V. vinifera* seed at MIC value of 1000 µg/mL showed inhibitory effect versus *C. albicans* while at 500 µg/mL showed inhibitory effect versus *C. tropicalis*.

**Cheng et al., 2012** in their study on crud phenolics in wine residue they used both acetone/water, and methanol/water extracts at MIC value of 780 µg/mL which were effective versus *C. albicans* ATCC 10261 strain. As for Oliveira et al., 2013 they tested the activity of Gallic acid and the results were positive at MIC value of 500 µg/mL versus *C. albicans* ATCC 14053 and *C. krusei* ATCC 6258 strains as well as MIC value of 1000 µg/mL versus *C. parapsilosis* strain ATCC 22019.

The reason behind using the soxhlet device in the process of extracting phenols is that the extraction process is continuous, it's an advantageous method because it is convenience and needs less time and solvent in comparison to percolation and maceration methods (**Azwanida, 2015**). Furthermore, the solvents are variable, it is possible to use one of these solvents such as methanol, water, chloroform, n-hexane, ethanol, propanol, ethyl acetate, and acetone according to their polarity as a result of the different effect of each solvent in the process of extracting chemical compounds of plant origin during the extraction process (**Zhang, 2018**).

## **Conclusion**

Phenolic compounds have many benefits and are abundant in many plants. One of these plants that has been focused on in this research is cauliflower, a member of the cruciferous family that is rich in a wide range of antioxidants and antimicrobial substances. Through the results obtained, it was found that phenolic compounds have a promising future in the treatment of many types of candidiasis in the event that it is manufactured safely, it can be used as an *In Vivo* treatment.

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## RESPONSE OF TWO WHEAT CULTIVARS TO THE ALLELOPATHIC EFFECT OF ONION PEELS

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### **Abstract:**

An Experiment was carried out in greenhouse to research the impact of the powder of dry onion peels added to the soil at a ratio (1,2%) w/w. on seed germination and growth of two wheat cultivars, Results showed an inhibition in percent of seed germination and growth represented by shoot, root lengths, dry weight of plants grown in soil containing onion peels powder compared to the control treatment (without addition). This effect was reflected on some characteristics of the yield, as there was a reduction in (shoot height, dry weight, root growth, as well as the in length of spike, number of grains in the spike, dry weight of grains in the spike, the highest reduction ratios (38.8, 81.6, 85.9%) in cultivar Iba-99 at the ratio 2% of onion peels in the growth characteristics of the aforementioned spike are as follows compared to the control. The cultivars are differing in their response to the effect, as the cultivar Ibaa-99 showed sensitivity to the treatment, while cultivar Abu Ghraib-3 showed more resistance.

**Key Words:** Allelopathy, Wheat, Peel, Onion, Cultivars.



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## Introduction

Many plants have ability to produce chemical compounds released to the environment in various ways affecting neighboring receptor plants called allelochemicals which a selective effect decreases the development of some species at a certain level while accelerating the growth of other species. (Zhou et al. 2011). such as flavonoids, terpenoids, alkaloids, glucosinolates, phenolic compounds, and amino acids (Fahey et al. 2001; Velasco et al. 2008). causing changes its nature or changes some of the characteristics and nature of the soil, Messiha et al., 2018; El-Dabaa et al. 2019).

In no-tillage situations, plant wastes may include allelochemicals that can hinder the growth and establishment of other vegetable species (Campiglio et al., 2012,). These substances are broken down by soil microbes from plant organic matter and/or secondary metabolism. (Zhang et al., 2011).

Nath (2016) Allelopathy is the word for this type of plant interaction where distinct secondary metabolites produced by different plants can either inhibit or encourage the growth and development of other plants existing in its vicinity. In some countries found that cover crop residues showed an allelopathic effect on seed germination and growth of subsequent plants (followed by cultivation) (El-Masry et al., 2019). Because of the chemical compounds released during the decomposition of their plant residues, which are released directly into the soil. As the effect may appear during the life cycle of the crop or on subsequent crops, different types of compounds affecting the growth and development of plants and organisms present in the soil were separated and identified (Weir et al., 2004). Studies and research have indicated many crops with allelopathic activity (inhibitory or encouraging), and their effects on other crops that accompany or follow them in agriculture. Which includes sunflower, yellow corn, wheat, barley, rice and others. (Saleh et al., 2019).

Effetores (2012) The growth of *Euphorbia heterophylla* L. and *Ipomoea grandifolia* seedlings was found to be both quantitatively and qualitatively inhibited by the peels of freshly discarded oranges when they came into contact with the oil vapor.

Onions: *Allium cepa* L. It is an annual herbaceous plant. The onion is oval in shape, covered with a brown scaly cover. It grows under the sand 16 cm deep. The leaves of the onions are bright green, their number is between 3-6 and they are striped leaves, and there is one stem bearing an inflorescence of purple or pink flowers. Onions contain volatile oils along with sulfur substances such as allicin, flavonoids, sterols, phenolic acids and gels.

## Materials and method

**1-Collect onion peels:** Dry onion peels (red) were collected and crushed using a Blender device, and kept in plastic bags until use. obtained: Two cultivars of bread wheat (*Triticum aestivum* L.) were selected from a specialized seed certification center/ Nineveh, their viability tested and recorded for Abu Ghraib-3 (95%) and Ibaa-99 (96%) The experiment was conducted in the greenhouse of biology Department /College of Science/Mosul University at 2021 -2022.

**2- Prepare the soil used in the experiment:** placed in a plastic pot (diameter 25 cm, height 20 cm). Onion peels powder was mixed with air-dried soil with addition ratio 1 and 2% w: w, soil without addition was used for control, then soil incubated for 3 weeks by adding 250 ml of water to each pot and covered with perforated plastic sheets, left in the greenhouse until the end of the incubation period (three weeks). Then 10 healthy grains of the two wheat cultivars, approximately equal distances from each other With 4 replicates for each treatment. It was watered with equal quantities of water, as needed, until the end of the experiment.

**Seed germination percentage:** After the stability of germination, the percentage of germination was calculated according to the following equation:

$$\% \text{ germination} = (\text{the apparent normal seedlings}) / (\text{the number of sown seeds}) \times 100$$
  
(ISTA, 1976) seedlings was reduced to five in each pot,

3- After three months of planting. wheat plants were harvested, shoot was separated from the root, the length of each of them was measured, then putting in oven at a temperature of 70 °C for 48 hours, and their dry weights were recorded.

4- **characteristics studies:** Five months after planting, wheat plants were harvested and separated shoots from roots. The length of each was measured and dried in an electric oven at a temperature of 70 °C for 48 hours, and the dry weights were recorded. It was measured: spike length (cm). Number of grains/spike, grains weights

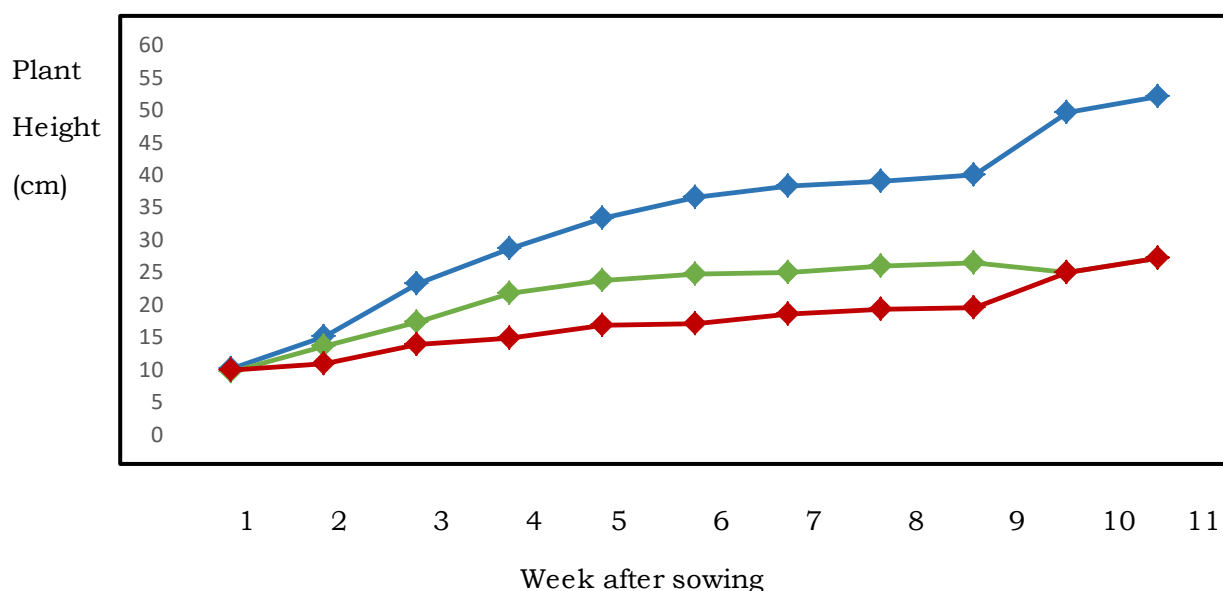
#### **Statistical analysis:**

Factorial experiments using a completely randomized design (C. R. D.) were used for the experiment. means the averages were compared using Duncan's test at a probability of 5%. The percentage of inhibition from the control was calculated according to the following equation:

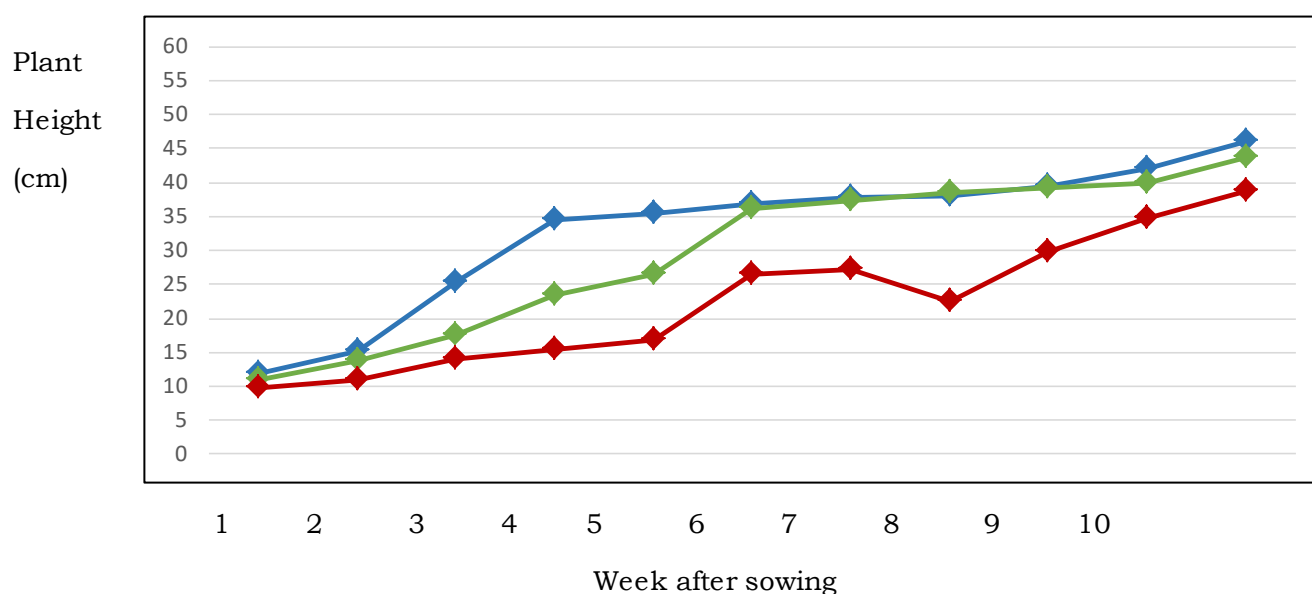
$$\text{Percentage of inhibition} = (\text{treatment-control}) / \text{control} \times 100 \text{ (Chung et al., 2001).}$$

## Results

A significant difference was observed between the two cultivars in root length and dry weight of the shoot. And from the interaction between the cultivars and the addition ratio, the lowest length of the total and its dry weight was 27.2 cm and 3.21 g in the plants of the variety Iba-99 when 2% of onion peels were added, while the lowest length of the root group and its dry weight was 27.1 cm and 1.14 gm in plants of the variety Abu Ghraib-3 by the effect of 2% addition of onion peels.



**Figure 1** Effect of onion peels on the growth of wheat cultivar after weeks of sowing (A. Ibaa-99)



**Figure 2** Effect of onion peels on the growth of wheat cultivar after weeks of sowing (B. Abo-Graib-3)

Results in Table (1) indicate a significant difference in the growth of wheat plants and some traits of yield in the two cultivars of wheat grown in soil containing onion peels added at ratio of 1 and 2% compared with the control treatment. showing a significant decrease in plant height of these plants as mentioned 59.1 cm to 36.3 cm, also the shoot dry weight reduction from 5.25 in control to 4.15 by the effect of a was accompanied by a reduction in the growth of the root system, as it recorded 40.8 cm in control and 35.9 cm at addition ratio 2% of onion peels. And its dry weight was reduced from (3.4 - 2.04) gm. The inhibitory effect was accompanied by some characteristics of the yield, represented by the length of the spike, the number of grains \ spike, and the dry weight of the grains spike. The two cultivars showed a significant difference between them in some traits, as from the interaction between the cultivars and the addition ratio, the lowest shoot length was 34.7 cm, and its dry weight of the vegetative group was 4.1 gm, in Abu Ghraib plants growing in the soil with addition 2% of onion peels, while the rest of the traits recorded the lowest dry weight of the root total 1.99 gm, the spike length 7.4 cm, no. of grains \ spike 2.5 and the weight of the grains.

The spike was 0.065 gm in Ibaa 99 cultivar at the addition ratio 2% of onion peel, while the highest height was 62.2 cm and the shoot length was 42.1 and the maximum dry weight of the shoot was 5.5 gm in Ibaa when in control plants treatment (without addition) adding peels at 2%.

**Table (1): Effect of adding onion peels to the soil on growth and some yield traits of two wheat cultivars (Abu Ghraib-3 and Ibaa-99)**

cultivars	Treatm Addition ratio %	Shoot length cm	Root Length(cm)	Shoot dry weight	root dry weight	Spike Length cm	Number of grains spike	Grains weight   n \ spike
Abo Graib-3	control	56.1* a	39.6 a	5.0 a	3.8 a	12.2 a	13.4 a	<b>0.397 a</b>
	1%	43.5 b	36.5 b	4.2 b	2.9 b	10.5 b	7.8 b	0.198 b
	2%	35.3 c	34.7 c	4.1 c	2.1 b	7.6 c	3.8 c	0.077 c
<b>Mean of cultivars</b>		<b>44.93 b</b>	<b>36.6 b</b>	<b>4.43 a</b>	<b>2.93 a</b>	<b>10.1 a</b>	<b>8.5 a</b>	<b>0.224 b</b>
Ibaa 99	control	62.2 a	42.1 a	5.5 a	3.12 a	12.1 a	13.6 a	0.464 a
	1%	46.5 b	39.4 b	4.4 b	2.32 b	8.8 b	6.4 b	0.177 b
	2%	37.4 c	37.2 b	4.2 c	1.99 c	7.4 c	2.5 c	0.065 a
Mean of cultivars		48.7 a	39.5 a	4.7 a	2.21 a	9.43 a	7.45 b	0.242 a
Mean of ratio	control	59.1 a	40.8 a	a 5.25	a 3.46	a 12.15	13.5 a	0.43 a
	1%	44.9 b	37.9 b	4.3 b	2.61 b	9.65 b	7.1 b	0.187 b
	2%	36.3c	35.9 b	4.15 b	2.04 b	7.5 c	3.15 c	0.081 c

**Similar letters indicate that there are no significant differences at the 5% level, according to Duncan's multiple range.**

### Discussion:

The Inhibition occurred in the germination of seeds and the growth of wheat plants grown in the soil to which onion peels were added by 1 and 2%, may be due to the chemical compounds (allelopathic) present in those peels, which were released into the soil either by washing (water-soluble) or as a result of Biological processes in the presence of microorganisms in the soil (Rice, 1984), The findings suggested that citrus peel essential oils had substantial potential for use in allelopathic processes by significantly inhibiting the growth of the species identified.. El Sawia (2019). which are secondary metabolites produced in different plant parts, which vary according to their chemical nature as well as their

concentrations. Research has indicated the possibility of liberating these compounds from plant parts, whether they are alive or dead, through washing and the art of volatilization (volatile compounds) or from the secretions of roots, and the decomposition of plant residues in the soil by the action of microorganisms in it, the conditions are available for decomposition, and the period of decomposition varies depending on

The nature of the residues, effectiveness of microorganisms, the compounds are released into the soil and affect the plant grown in it. The effect caused by these compounds may be direct, as they are absorbed by the seeds. Researchers attributed the reason to the effect through the effect on the process of absorption as well as the effect on the process of cell division. The assay results demonstrated that the most inhibiting essential oil was that taken from *C. reticulata* peels, followed by essential oils from *C. aurantium* and *C. sinensis* peels. In all concentrations, the essential oils of *C. reticulata* and *C. aurantium* peels totally prevented *H. annuus* seed germination and seedling growth. The essential oil of *Citrus aurantium* (2%), fully prevented the germination and growth of *Portulaca oleracea* seeds.. (El Sawia, 2019)

As for the shoot and root, the highest percentage of inhibition reached (45.9, 18.5) %, respectively, Ibaa-99 cultivars with the effect of onion peels added at ratio 2%. The effect was reflected in the dry weight of both the shoot and root system, as the highest inhibition rate was (27, 50) %, respectively, in plants (Abu Ghraib-3) cultivars at ratio 2%. Thus, it can be concluded that the growth of the shoot system was affected and inhibited more than the root system (variation in response in the plant part and the allelopathic influence). (Masood et al., 2020) Onions (*Allium cepa*) are a rich source of flavonoids, especially the peels, including quercetin, Kaemeferol, luteolin, and quercetin derivatives, which are inhibitory factors for the growth of fungi and bacteria (Sagar and Pareek, 2020). The inhibitory effect on the growth of wheat plants may be due in our study to those compounds which effect is determined by the effective concentration. The reduction in the growth of wheat plants was reflected in some characteristics of the yield, and the effect continued until the period of spike formation,

The inhibition that occurred in the germination of seeds and the growth of wheat plants grown in the soil to which onion peels were added by 1 and 2%, may be due to the chemical compounds (allelopathic) present in those peels, which were released into the soil either by washing (water-soluble) or as a result of Biological processes in the presence of microorganisms in the soil (Rice, 1984),

(Abdul Majeed 2012, examined the allelopathic effects of aqueous extracts of sugarcane (*Saccharum officinale* L.) cultivar root, stem peels, and leaves at concentrations 0, 2.5, 5.0, 7.5, and 10.0 g/l on germination indices and seedling biomass of wheat (*Triticum aestivum* L.). The results showed that higher concentrations (10.0 g/l) of extracts of roots, stem peels, and leaves decreased mean germination time (MGT) significantly while increasing shoot and seminal root growth and seedling dry weight. However, % was not impacted by extract concentration or the plant parts used in the study. Up to 7.5 g/l of

extract concentration had no impact on the wheat parameters under investigation. Our findings imply that wheat might be grown in sequential rotation under field settings and that sugarcane allelopathy exhibits beneficial impacts on wheat growth. (Pirsabak-2005)



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## JET PLASMA EFFECTIVENESS IN PH, MITOTIC AND BLASTOGENIC INDEX ASSAYS

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**Sura Allawi OBAID <sup>2</sup>**

### **Abstract:**

The aim of this work is determine the effect of jet plasma on biological parameters of blood serum or Lymphocytes (Blast and Mitotic index assays) for both genders of mice. The blood samples are took direct from mice before and after jet plasma exposure. So the rate of cell division was measured for all animals.

all animals are treated by jet plasma with a voltage of (175 volts) and (2 L/min as flow of gas), the animals are exposed with jet plasma for (five and ten Mints) along (1 Month). The results of blood serum as mitosis or number of dividing cells per thousand cells (MI) showed that the increased of MI is almost non- existent at five mints plasma exposure, like for the results of blast cells(blast cells per thousand cells BI) as shown in tables(1,2 and 5) and figures (1 and 2). At ten Mints of plasma exposure the results showed the jet plasma affected on male mice, so MI result recorded (1.812, 2.17) for control and treated respectively as shown in tables(3,4 and 5) and figures (3 and 4), its weakly effect on male mice. BI results showed that the plasma effected on number of blast cells for both genders by the same way at this time of exposure. About the results of PH of blood serum the results showed that clearly increased for both genders at both time of plasma exposure but greater ratio at ten Mints, so the mean of serum PH results recorded (6.6,6.8), (7.1,7.28) and its recorded (6.65,7.8) and(7.19,7.4) for control and treated group respectively, and the blood serum has become base.

**Key Words:** Jet Plasma, Mitotic Index Assay (MI), Blastogenic (BI), Blood.



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**Introduction:**

Atmospheric-pressure plasmas are widely used in biomedical applications (Sch et al. 1999). The direct application of plasma in medicine is to understand the biological, physical, chemical mechanisms of direct jet plasma interaction with living cells (Jabur, Hammed, and Khalaf 2021) (Mazhir and Ali 2019). Engineering works has resulted in many advances in health care. Ionizing radiation and laser are examples of technological breakthroughs that have created diagnosis and new treatments for the disease. Jet plasma medical techniques also have important therapeutic effects and lead to new medical diagnostic tools, Some of these factors include understanding stopping bleeding, treated damage tissues and pathogens without damaging surrounded healthy tissues, promoting wound healing and treating cancer (Muryoush and Ali 2019). The measurement of PH in the blood serum is one of the most important measurements for estimating the variables that occur in the pH of the blood because it is an important factor for regulating the physiology of the work of cells in the body (Ali et al. 2017) (Muryoush 2022). As these changes in the level of acidity has an impact on the cell works, organ and whole body, The balance between the ratio of bases and acids in the blood serum is vital for the normal functioning of the organs. The increase or decrease in the degree of blood PH that indicate the presence of disease or defect in the cells work (Kellum 2000). Cell-based test that determined the growth of cells and quantitate the grist of cells in certain stages of the cell cycle are more and more important in drug checking environments (Miller et al. n.d.). The mitotic index assays (MI), or the ratio of a cell population that is undergoing mitosis, is an examination used usually to mark the health of cells in a population. The mitotic index assay is oftentimes increase in cancerous cell populations due to increased cell generation (Label free mitotic index | Application Note n.d.). Blood cells make up the vast majority of our body. These cells may have to double to allow our organism to keep normal homeostasis. Development occurs through a very tightly regulated operation called the cell cycle. During this process, a cell finally divides into two similar cells within 24 or 48 a hours. Deregulation of the cell cycle may result in cell killing or in uncontrolled cell duplication leading to cancer [2]. In addition, exposure to toxic compounds may also effect in change to cell growth or cell death (Pognonec et al. 2021) (Noori 2017). Mitotic index (MI) is the standard cytotoxic parameter for determining which test concentrations will be evaluated for chromosome aberrations. Assessment of the MI is performed microscopically by determining the frequency of mitotic cells in a population of 1000 cell, blasogenic index assay (BI) is a relation between the blast cells in interphase to 1000 cell.

### Experimental Work:

The male and female mice were exposed with direct jet plasma. The jet plasma was used with voltage (175v) for (5 and 10 mints /day) along 1 month. Blood samples were collected from the animals (male and female), and cultured inside laminar airflow to prevent the contamination. Using test tube with (4.5 ml) culture medium (RPMI – 1640) used for this work, 0.5 ml of whole blood it's separated in these tubes. (0.2 ml) of PHA was added to each tube. The tubes are tightly closed by a screw cap for avoid contamination and incubated in incubator at 37°C for 72 hours. Colchicine (0.1 ml) was added in the last hour of the incubation process. Lymphocytes cells were harvesting using hypotonic solution (kcl), so the wight blood cells are settled at the bottom of the test tube and the supernatant was clear and using pastor pipette, the supernatant was gently removed and the pellet was left in the bottom of tube with small amount of culture medium. The cells treated with Methanol: Acetic Acid by ratio (3:1) for three times. The cells separated on the slid by dropping and stained using Geimsa stain. The slides tested using optical microscope. Blood samples put in centrifuge device (1500 rpm / 10 min) for separated blood serum. PH test was measured before and after plasma exposure.

For calculations of the mitotic (MI) and blastogenic index assay (BI) using eq.(1)(2) [9].

$$MI = (\text{No .of dividing cells} / 1000 \text{ cell}) \times 100\% \quad (1).$$

$$BI = (\text{No .of non-dividing cells} / 1000 \text{ cell}) \times 100\% \quad (2).$$

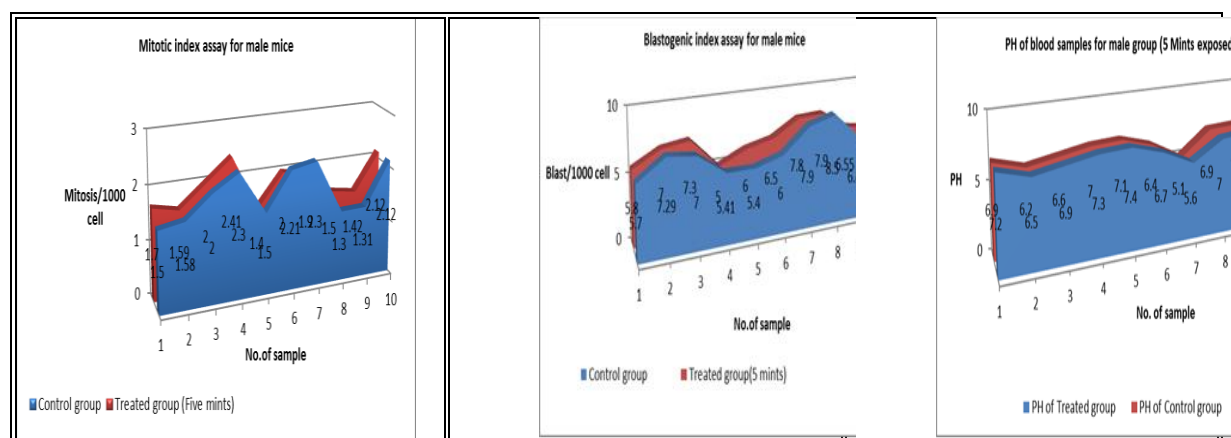
MI was measured by number of cell which undergone mitotic divisions divided by all cells in a microscopic view into 100.

### Results and Discussion:

In this work, blood samples used to staminate the effectiveness jet plasma on blood cells (cell cycle) by determine mitotic index (MI) or mitotic (M-Phase), in which the nuclei disappear their membrane, nucleoli disappear and chromosomes are physically sorted to be equally split among two identical two daughter cells and blast index assay(BI), as well as measuring the PH of blood.

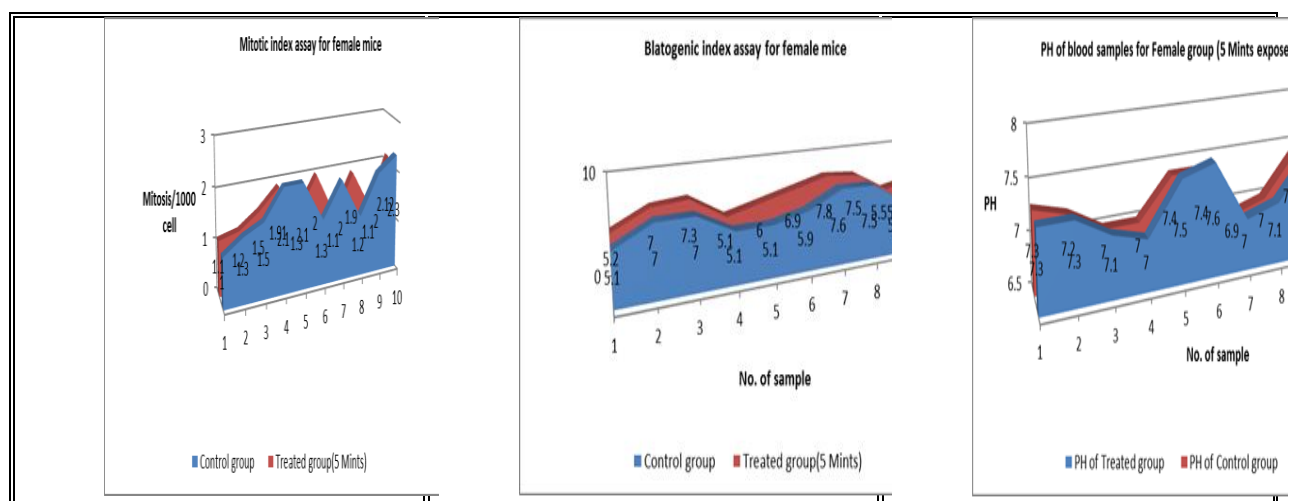
**Table1- Mitotic and Blastogenic index Assays, PH of blood samples for Male group.**

No. of Sample	Time of Plasma exposure	Mitosis / 1000cell (control group)	Mitosis / 1000cell (treated group)	Blast cell/ 1000cell (control group)	Blast cell/ 1000cell (treated group)	PH (Contr ol group)	PH (Treate d group)
1.	<u>Five mints</u>	1.5	1.7	5.7	5.8	6.9	7.2
2.		1.58	1.59	7.29	7.00	6.2	6.5
3.		2.0	2.0	7.0	7.3	6.6	6.9
4.		2.3	2.41	5.41	5.00	7.0	7.3
5.		1.5	1.4	5.4	6.00	7.1	7.4
6.		2.21	2.00	6.00	6.5	6.4	6.7
7.		2.30	1.90	7.90	7.8	5.1	5.6
8.		1.3	1.5	8.5	7.9	6.9	7.0
9.		1.31	1.42	6.42	6.55	7.0	7.2
10.		2.12	2.12	6.12	6.22	6.3	7.0
Mean		1.812	1.804	6.574	6.607	6.65	6.85

**Figure1- Mitotic and Blastogenic index Assays, PH of blood samples for Male group (5 Mints) time of plasma exposure.**

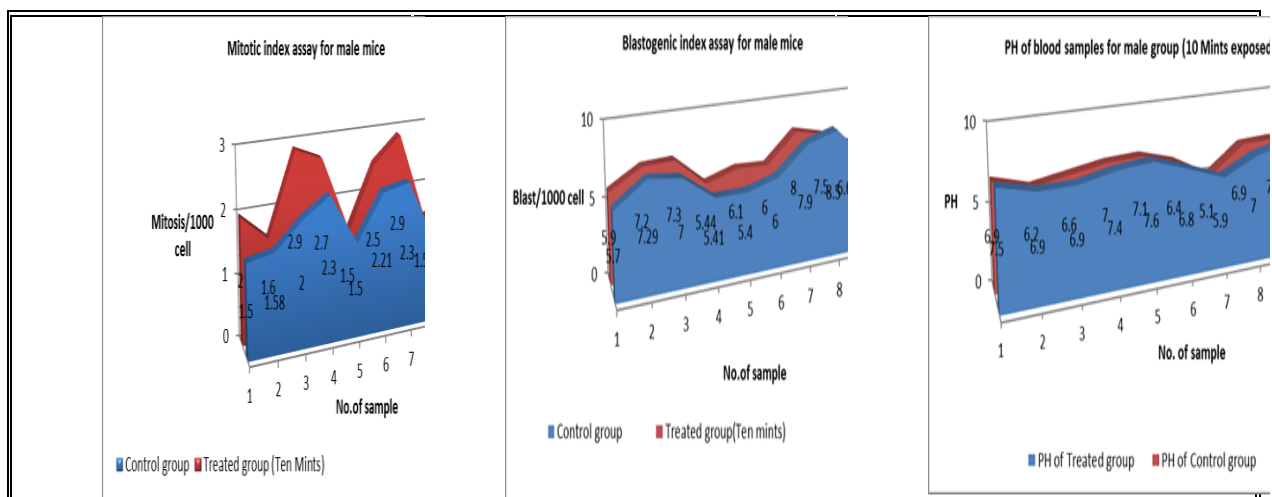
**Table 2- Mitotic and Blastogenic index Assays, PH of blood samples for Female group.**

No. of Sample	Time of Plasma exposure	Mitosis /1000cell (control group)	Mitosis /1000cell (treated group)	Blast cell/1000cell (control group)	Blast cell/1000cell (treated group)	PH (Control group)	PH (Treated group)
1.	<u>Five mints</u>	1.00	1.1	5.1	5.2	7.3	7.3
2.		1.3	1.2	7.00	7.00	7.2	7.3
3.		1.5	1.5	7.0	7.3	7	7.1
4.		2.1	1.91	5.1	5.1	7	7.0
5.		1.3	1.3	5.1	6.00	7.4	7.5
6.		2.00	2.00	5.90	6.9	7.4	7.6
7.		1.2	1.10	7.60	7.8	6.9	7.0
8.		2.00	1.9	7.5	7.5	7	7.1
9.		1.00	1.1	5.42	5.55	7.4	7.5
10.		2.30	2.12	6.00	6.22	7.3	7.4
Mean		1.57	1.52	6.457	6.452	7.19	7.28

**Figure2- Mitotic and Blastogenic index Assays, PH of blood samples for Female group (5 Mints) time of plasma exposure.**

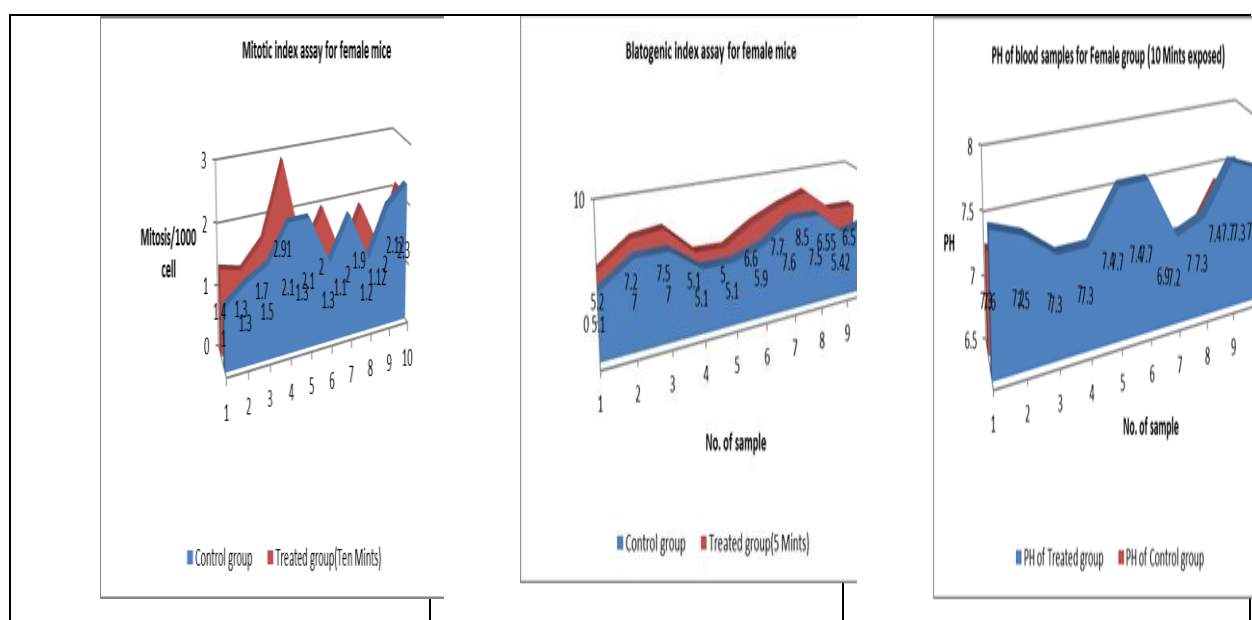
**Table3- Mitotic and Blastogenic index Assays, PH of blood samples for Male Mice Group.**

No. of Sample	Time of Plasma exposure	Mitosis / 1000cell (control group)	Mitosis / 1000cell (treated group)	Blast cell/ 1000cell (control group)	Blast cell/ 1000cell (treated group)	PH (Control group)	PH (Treated group)
1.	<u>Ten mints</u>	1.5	2	5.7	5.9	6.9	7.5
2.		1.58	1.6	7.29	7.20	6.2	6.9
3.		2.0	2.9	7.0	7.2	6.6	6.9
4.		2.3	2.7	5.41	5.44	7.0	7.4
5.		1.5	1.5	5.4	6.10	7.1	7.6
6.		2.21	2.5	6.00	6.00	6.4	6.8
7.		2.30	2.90	7.90	8.0	5.1	5.9
8.		1.3	1.5	8.5	7.5	6.9	7.0
9.		1.31	1.62	6.42	6.65	7.0	7.5
10.		2.12	2.5	6.12	6.32	6.3	7.3
Mean		1.812	2.17	6.574	6.641	6.65	7.8

**Figure3- Mitotic and Blastogenic index Assays, PH of blood samples for Male Mice (10 Mints) time of plasma exposure.**

**Table4- Mitotic and Blastogenic index Assays, PH of blood samples for Female Mice group.**

No. of Sample	Time of Plasma exposure	Mitosis /1000 cell (control group)	Mitosis /1000 cell (treated group)	Blast cell/1000 cell (control group)	Blast cell/1000 cell (treated group)	PH (Control group)	PH (Treated group)
1.	<u>ten mints</u>	1.00	1.4	5.1	5.2	7.3	7.6
2.		1.3	1.3	7.00	7.20	7.2	7.5
3.		1.5	1.7	7.0	7.5	7	7.3
4.		2.1	2.91	5.1	5.1	7	7.3
5.		1.3	1.3	5.1	5.00	7.4	7.7
6.		2.00	2.00	5.90	6.6	7.4	7.7
7.		1.2	1.10	7.60	7.7	6.9	7.2
8.		2.00	1.9	7.5	8.5	7	7.3
9.		1.00	1.10	5.42	6.55	7.4	7.7
10.		2.30	2.12	6.00	6.5	7.3	7.6
Mean		1.57	1.6	6.457	6.55	7.19	7.49

**Figure4- Mitotic and Blastogenic index Assays, PH of blood samples for Female Mice (10 Mints) time of plasma exposure.**



**Table5- Mean of Mitotic and Blastogenic index Assays, PH of blood samples for all groups.**

Mean	Male group				Female group			
	Control	Treated (5 Mints)	Control	Treated (10 Mints)	Control	Treated (5 Mints)	Control	Treated (10 Mints)
MI	1.812	1.804	1.812	2.17	1.57	1.52	1.57	1.6
BI	6.574	6.607	6.574	6.641	6.457	6.452	6.457	6.55
PH	6.65	6.8	6.65	7.8	7.19	7.28	7.19	7.49

**Discussion:**

The results as shown in the tables and Figures above. The means of MI, BI and PH. The results showed that the effect of jet plasma on blood cells division at time exposure (5 Mints) on male mice, MI was imperceptible effect for both gender. BI results recorded very slight increase at (5 Mints) exposure for male mice only, its recorded (6.57, 6.607) for control and treated group respectively, and imperceptible effect for female mice. At (10 Mints) time of the plasma exposure the results showed the jet plasma affected on male mice, so MI result recorded (1.812, 2.17) for control and treated respectively, its weakly effect on female mice. BI result showed that plasma effected on number of blast cells for both genders by the same way at (10 Mints) time of exposure, these small differences in the results of MI and BI due to the physical nature of both genders. The blood serum PH results showed that clearly increased for both genders at both time exposure but greater ratio at (10 mints), so the mean of serum PH results recorded (6.6, 6.8), (7.1, 7.28) and its recorded (6.65, 7.8) and (7.19, 7.4) for control and treated group respectively. That means the blood serum of male was more acidic before plasma exposure. after treatment with plasma, the acidity of serum decreased and the serum became more alkaline for both genders.

**Conclusions:**

For illustrating the safety application of jet plasma with living cells and extermination the number of dividing and blast cells as so as blood PH. The results showed that the main non-toxic properties of jet plasma for (5, 10 Mints) time of plasma exposure, are due to low energy plasma. So no real changes in the numbers of dividing cells. Jet plasma has a direct effect on PH blood serum especially at (10 Mint) time of plasma exposure, the acidity of blood serum decreased and became more alkaline. That indicated the jet plasma hasn't effects on the lymphocytes behavior and its cells division.

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## SUBCLINICAL HYPOTHYROIDISM AND THEIR RELATION WITH DYSLIPIDEMIA IN IRAQI WOMEN

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### Abstract:

Subclinical hypothyroidism could be developed into serious health issues that affect the quality of life of the affected individuals. This study examines the potential association between subclinical hypothyroidism and dyslipidemia.

**Subjects and Methods:** Forty women were recruited in this study and divided into two groups consisted of 20 women with subclinical hypothyroidism and 20 apparently healthy women designated as a control group. The participants' age ranged between 20 to 42 years. Peripheral venous blood samples were collected, post 12hrs post fasting, for the measurement of serum thyroid parameters and lipid profile.

**Results:** The current study findings demonstrated significant differences ( $P > 0.05$ ) between the occurrence of subclinical hypothyroidism and disturbances of lipid profile including elevated level of cholesterol, triglyceride (TG), LDL-C, and VLDL-C in subclinical hypothyroidism cases than that of their healthy counterparts. Whereas HDL-C level dropped in the subclinical hypothyroidism group compare to that of the healthy controls, but these differences did not reach the significant levels.

**Conclusion:** Findings of the present study propose that subjects with subclinical hypothyroidism are at increased risk of dyslipidaemia. This is quite interesting as the co-occurrence of dyslipidaemia and subclinical hypothyroidism is closely connected to cardiovascular disease development.

**Key Words:** SCH, TSH, T4, T3, TG, LDL-C, HDL-C.



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## Introduction

Subclinical hypothyroidism (SCH) is manifested by combination of raised serum levels of thyroid-stimulating hormone (TSH) that is accompanied with normal levels of thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>). Such situation can be either mild (TSH <10mIU/L) or severe (TSH ≥10mIU/L) (1). SCH's frequency account for 3% to 8% of the general population and its occurrence seems to be women predominant, with an age-dependent prevalence that is increased with advanced age. The vast majority of SCH patients (80%) exhibited TSH serum levels lower than 10mIU/L. This confer high predisposition for the development of clinical hypothyroidism status. However, debate continues about considering SCH as a good risk factor for cardiovascular complications (2). Accordingly, quantifying of serum TSH can be a key examination for the detection of mild thyroid failure in the case of subjects exhibiting normal peripheral thyroid hormone ranges (3). It is known that approximately 1.4 to 13% of hyperlipidaemia patients have the symptoms of hypothyroidism. This highlights the notion of the commonality of thyroid failure that could be left undetected in those patients (4).

In patients with declined thyroid functions, serum is marked by elevated lipid levels. Several lines of evidence have shown significant excess of total cholesterol and low-density lipoprotein-cholesterol (LDL-C) mean levels in patients having TSH values ranged between 5.1 and 10mIU/L (5). Based on that, it is thought that subclinical hypothyroidism could be essential reason behind hyperlipidaemia and consequently implicated to the increasing risk of several serious complications including atherosclerosis, and coronary heart disease "CHD" (6).

Cholesterol has essential biological functions in building cellular membranes, many hormones, and vitamin D) which is catalysed with the help of liver known as "enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)" and circulated via lipoproteins. As a potential predictor of atherogenicity, much of the current literature on CHD pays particular attention to the LDL-C (7). Whereas, cardiovascular protective effects exert by HDL-C, as it is mediated of cholesterol reverse transport to the liver via the circulation. The aforementioned biological events is believed to be highly influenced by thyroid hormone activity which is lipid metabolism's key regulator promoting liver's *de novo* fatty acid synthesis, lipids mobilization and degradation (8). In the cases of hypothyroidism, evidence showed that hypercholesterolemia is largely as a result of lowering LDL receptor's activity of. Such effect is typically attended with concomitant diminishing control by triiodothyronine (T<sub>3</sub>), where the later modifies cholesterol biosynthesis via controlling rate-limit-degrading HMG-CoA enzyme's activity (9). Thyroid hormone impact on bile acids has recently appeared as a recognizable hypocholesterolemic effect. In this framework, increased bile acids flow causes the reduction of the hepatic cholesterol reservoir tracked by the mean of increasing liver's cholesterol synthesis and the hepatic uptake of cholesterol from the circulation. Interestingly, high thyroid-stimulating hormone (TSH) levels are reported to be related with a non-favourable lipid profile (10).

Furthermore, meta-analysis of cross-sectional studies has concluded that metabolic syndrome and its cluster of conditions (including hypertension, increased triglycerides level, weight gain, and insulin resistance, along with high serum cholesterol level) are strictly connected to subclinical hypothyroidism (11). These conditions are connected with a collection of lipid and lipoprotein aberrations, and such aberrations have been denoted to as the “atherogenic dyslipidaemia” (12). This atherogenic dyslipidaemia status is categorized by the occurrence of the subsequent collection of anomalies: hypertriglyceridemia (as a sign of elevated triglyceride), low HDL-C levels, high LDL levels, and high of the rest of lipoproteins levels (13). Of interest, thyroid function is considered as an integral part of a number of factors that have impact in determining population body weight. Indeed, it is reported that even slight increase serum TSH levels are linked to rise in the incidence of obesity (14). The present study was set to examine the potential association between lipid profile and subclinical hypothyroidism.

## **Materials and Methods**

### **Subjects:**

A cohort consisted of 40 participants (their age ranged between 20 to 42 years) were recruited in the study. Participants were subgrouped into two groups: the first one including twenty women with subclinical hypothyroidism was donated as a patients group; while the second group of twenty age-matched apparently healthy women was included as a control group. The patient's inclusion criteria were based on physical examination by specialist doctors. While the exclusion criteria included the excluded of subjects on medication for thyroid diseases. Additionally subjects taking medications that can influence hypothalamus-pituitary-thyroid gland function (such as amiodarone and glucocorticoids) were not recruited.

For the purpose of assessing of serum thyroid parameters and lipid profile in serum, peripheral blood samples were collected from all of participants 12hrs post requested fasting.

### **Laboratory evaluation**

Cholesterol, triglyceride, LDL-C and HDL-C levels were estimated by enzyme-based assays. According to the provide reference ranges, normal TSH levels lie between 0.27 to 4.2 mIU/L. While the expected readings for euthyroid subjects were within 66–181 nmol/L of T<sub>4</sub>, and 1.3–3.1 nmol/L of T<sub>3</sub>. Serum concentrations of TSH, T<sub>4</sub>, and T<sub>3</sub> were specified utilizing electro-chemiluminescence immunoassay (ECLIA, Roche-Diagnostics, Germany).

## Results:

The mean age of subclinical hypothyroidism group was  $37.40 \pm 12.8$  years which is comparable to that of the control group ( $36.62 \pm 10.12$  years). As shown in table 1, cholesterol levels elevated significantly ( $P < 0.05$ ) in subclinical hypothyroidism group in comparison to their healthy counterparts. Similarly, significant increase ( $P < 0.05$ ) was observed in the levels of the other investigated lipid profile component, (TG, LDL-C, and VLDL-C) in subclinical hypothyroidism group compared to that of the healthy control group. Whereas HDL-C level was slightly decreased in subclinical hypothyroidism subjects when it is compared to that of the healthy counterparts, however, such differences did not reach significant level ( $P > 0.05$ ).

**Table 1: Evaluation of lipid profile value in subclinical hypothyroidism and control groups**

Groups	Lipid profile (mmol/l)				
	Cholesterol	TG	HDL-C	LDL-C	VLDL-C
<b>Subclinical hypothyroidism</b>	$6.42 \pm 0.16^*$	$3.34 \pm 0.25^*$	$1.08 \pm 0.10$	$4.91 \pm 0.62^*$	$1.10 \pm 0.12^*$
<b>Healthy controls</b>	$6.11 \pm 0.10$	$2.60 \pm 0.43$	$1.12 \pm 0.11$	$3.92 \pm 0.35$	$0.82 \pm 0.11$

**-Results are expressed as mean  $\pm$  SD.**

**-\*denoted for statistically significant differences ( $P < 0.05$ ).**

Interestingly, our study finding showed highly significant ( $P < 0.001$ ) elevation in the TSH level in subclinical hypothyroidism ( $5.45 \pm 1.64$  mIU/ml) as compared with healthy controls ( $1.78 \pm 0.91$  mIU/ml). This three folds excess in the TSH levels in the subclinical hypothyroidism in comparison to the healthy controls suggests a potential impact of this hormone in subclinical hypothyroidism pathogenicity. However, no significant differences in mean levels of T4 and T3 were observed between subclinical hypothyroidism group and the control group ( $88.28 \pm 0.20$  nmol/L,  $1.40 \pm 0.51$  nmol/L vs.  $86.88 \pm 0.19$  nmol/L,  $1.38 \pm 0.48$  nmol/L, respectively, Table 2).

**Table 2: Thyroid gland parameters in subclinical hypothyroidism subjects and their healthy counterparts**

Parameters	Subclinical hypothyroidism	Control	P value
<b>TSH (mIU/ml)</b>	5.45±1.64*	1.78±0.91	<0.001*
<b>T4 (nmol/L)</b>	88.28±0.20	86.88±0.19	0.631
<b>T3 (nmol/L)</b>	1.40±0.51	1.38±0.48	0.377

**-Results are expressed as mean±SD.**

**-\*denoted for statistically significant differences (P <0.001).**

### **Discussion:**

Efforts concentrated to understand the underlying disease-associated biological changes are of great interest as they contribute to development of potential biomarkers for patient's stratification and disease management. In the same vein, the current study results revealed that subclinical hypothyroidism is associated with dyslipidemia and thus have the potential to confer coronary heart disease. Our study results are consistent with the findings of Tagami and colleagues (4) who stated that hypothyroidism greater frequency was higher among the subjects with dyslipidemia. Similarly, Canaris and co-researcher (15) have observed increased lipid levels in patients with declined thyroid function. While Duntas (16) highlighted that subclinical hypothyroidism is marked with disrupted lipid profiles characterized by normal to slightly raised total cholesterol, increased LDL, and lower HDL levels. Abnormal shift in cholesterol and lipoprotein metabolism in SCH has been reported when serum TSH is exceeding 10 mIU/L (17). Several lines of evidence have observed lipid profile component abnormalities potential accelerated risk for CVD. In this regard, it is believed that the impact of SCH on lipids is comparative to the TSH elevation and retains more substantial significance in the conversion of SCH to an overt disease. Sigalet *et al.* (18) demonstrated that TG and phospholipids transfer to HDL and were significantly lower in SCH subjects than healthy controls. Additionally, large TG-rich very low-density lipoprotein (VLDL) particles secretion from liver resultant in higher plasma VLDL-TG concentration was distinguished in SCH cases. Moreover, Kanaya and colleagues (19) reported that lower thyroid function can contribute to poorer lipid profile in old patients. In a cross-sectional study based on data collected from 2,799 participants aged 70–79 years, high TSH (> 5.5 mIU/L) was linked to higher cholesterol level. Likewise, serum levels of both LDL-C and LDL/HDL-C values significantly increased in patients older than 65 years (especially in those having TSH

between 3.6 and 10mIU/L).Also in patients havingTSH> 10mIU/L, TC levels seemed to increase too. Collectively, these studies concludedthat SCH could worsen the increase of lipids with age (20).

In postmenopausal women, evidence has shownthat SCH is associated with atherogenic lipid profiles even in those with a mild riseof serum TSH.Such effect seemed to be independent of the influence thyroid hormones (21).Lee *et al.* (22) demonstrated an association between metabolic syndrome cluster of conditions(especially hypertension and high serum triglycerides) andsubclinical hypothyroidism.Other study conducted byNader and colleagues (11) revealed that euthyroid children with no history of hypo/hyperthyroidism, increasing levels of TSH and decreasing levels of free T4 exhibited higher triglyceride levels,weight gain, elevated insulin resistancesigns, and perhaps high serum cholesterol level.

Furthermore, evidencebased on clinical and mouse model studies suggest that obesity reads to fat gathering in the thyroid gland and this may disrupt thyroid hormone creation and driving hypothyroidism (23).

### **Conclusions:**

Overall, the present study findings suggest an involvement of dyslipidaemia in subclinical hypothyroidism pathogenicity (SCH). As such association could lead to cardiovascular disease development,biochemical screening for thyroid dysfunction is recommended in all patients with dyslipidemia.

In addition, it is recommended to conduct furtherlarge scale studies are needed to assess the impact of SCH on cardiovascular diseases, such as coronary artery disease and hypertension.



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## EFFECT OF POTASSIUM SOURCE, HUMIC ACID AND IRRIGATION WATER SALINITY ON SOME PHYSIOLOGICAL TRAITS, GROWTH AND YIELD OF BROCCOLI

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### Abstract:

A field experiment was carried out in the season 2020-2021 in a field site in Al-Dora/Baghdad area in a clay mixture under the Type Torrifluent group, on broccoli plant. Potassium sulfate was formed in three concentrations (0, 60, 120) Kg K ha<sup>-1</sup> and irrigated with two types of irrigation water (river and well) under the drip irrigation system. The results showed a significant increase of potassium sources on the leaf area. The treatment of fertilization with potassium sulfate was superior at the second level. (HS2) in the values of leaf area (40.66) cm<sup>2</sup>. The interaction between potassium sources and salinity of irrigation water was also recorded, and the highest value was recorded when treatment (S1HS2) was (66.86) cm<sup>2</sup>. A significant decrease in leaf area was recorded with the increase in salinity of irrigation water (S2) (24.08). ) cm<sup>2</sup>, and the results of the research indicated a significant increase of potassium sources in the percentage of chlorophyll in the leaves. The treatment of fertilization with potassium sulfate at the second level (HS1) was superior to the values of chlorophyll (1.23) mg liter<sup>-1</sup>. The interaction between potassium sources and salinity of irrigation water was also recorded. The highest value was observed when treatment (S2HS1) was (1.06) mg liter<sup>-1</sup>, and there was a significant increase in biological yield and economic yield due to potassium sources. The treatment of fertilizing with potassium sulfate at the level (HS) was (1530.5) gm plant<sup>-1</sup> and (20.2) ton ha<sup>-1</sup> for both biological yield and economic yield, respectively. Also, the interaction between potassium sources and irrigation water salinity recorded the highest value when treatment (S1HS1) and it was (2033) gm plant<sup>-1</sup> and (23.8) ton ha<sup>-1</sup> for both biological yield and economic yield Respectively and there was a significant increase in the potassium content in the plant due to potassium sources.

**Key words:** Total Chlorophyll, Leaf Area, Biological Yield, Soil Potassium, Plant Potassium.



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## Introduction:

Broccoli belongs to the cruciferous family, and it is an important vegetable that is grown for its leaves as fodder for animals and its flowers for human consumption, which are eaten either fresh or for the purpose of making salads, soups, or the rest of the dishes. In regulating the nutritional status of plants and as a result of the increase in global pollution rates, as indicated by reports and studies, and for the success of agriculture under the conditions of salty irrigation water, it is necessary to focus on strategies to improve the physical and chemical properties of the soil (30). And the use of various practical methods such as choosing appropriate irrigation systems and choosing tolerant crops. salinity and an effective puncture system (26).

There is an urgent need to use alternatives to chemical fertilization such as organic fertilizers (humic acid), which can improve soil properties and plant metabolism processes (29), as it urges the plant to withstand various environmental stresses as well as its content of nutrients for the plant and can be obtained as a commercial product that contributes to Reducing the negative effects of salinity and maintaining sustainable agriculture (28).

Potassium is an essential nutrient for the plant that enables it to withstand non-vital factors such as (frost, drought, and salinity) and vital factors such as disease, and contributes to photosynthesis of the plant, cell division, and the transfer of materials from the source to other parts of the plant, and raising the plant's efficiency in absorbing nutrients, especially Nitrogen and phosphorous, and this is an improvement in the nutritional balance, which leads to improved plant growth and an increase in production and quality (25). When using salty irrigation water, the production may be subject to damage due to the absorption of salt by the plant, resulting in osmotic pressures and nutritional imbalance (27), so we decided to use humic acid and potassium in multiple concentrations and under two types of irrigation water (6).

## Materials and methods:

A field experiment was carried out in the season 2020-2021 in a field site in the Dora / Baghdad area in a clay mixture under the Type Torrfluvent group, the characteristics of which are shown in (Table 1), where the broccoli was planted in a tray and after the completion of the fourth leaf, it was transferred to the permanent field, which was plowed with a disc plow Then the irrigation system was extended and the plants were planted at a distance of 40 cm, and the soil treatment included three levels of humic acid, Speedy growth, an addition of ground and a concentration of (0,1,2) gm liter<sup>-1</sup>, which symbolized by H0, H1 and H2, respectively, and potassium was added in the form of potassium sulfate in three concentrations. (0, 60, 120) Kg K ha<sup>-1</sup>, symbolized by H0, HS1 and HS2, and nitrogen was added at the chemical recommendation at a rate (96 kg N ha<sup>-1</sup>) in the form of urea (46% N) and phosphorous at a rate of (184 kg P ha<sup>-1</sup>) in the form of superphosphate (46%P) (3,4)

and was irrigated with two types of irrigation water (river, well), each of which has a symbol S1 and S2 for each of the two types, respectively, under the drip irrigation system and by designing random sectors and table (3) ) shows some characteristics of water (9,10).

The electrical conductivity (EC) of a soil:water extract (1:1) was estimated using an EC-meter, and the degree of soil reaction (pH) was estimated in a soil:water extract (1:1) using a pH-meter (5).

**Table (1): Some chemical and physical properties of the soil of the experimental field**

properties		value
pH		7.70
EC ( dS.m <sup>-1</sup> )		3.52
Available nitrogen	Elements (mg.kg <sup>-1</sup> )	35.00
Available potassium		297
Available phosphorous		0.012
Ca <sup>++</sup>	Cations Cmol L <sup>-1</sup>	1.10
K <sup>+</sup>		0.12
Na <sup>+</sup>		1.00
Mg <sup>++</sup>		0.40
SO <sub>4</sub> <sup>=</sup>	Anions Cmol L <sup>-1</sup>	1.23
Cl <sup>-</sup>		0.80
HCO <sub>3</sub> <sup>-</sup>		0.60
CO <sub>3</sub> <sup>=</sup>		Non
Sand	aggregate soil gm Kg <sup>-1</sup>	147
Silt		432
Clay		430

- The positive ions (Ca<sup>+2</sup>, Mg<sup>+2</sup>, and Na<sup>+1</sup>) were estimated in a 1:1 extract, Ca<sup>+2</sup> and Mg<sup>+2</sup> were measured by scaling method using EDTA-Na<sub>2</sub> and the dissolved Na<sup>+1</sup> was estimated by a flame apparatus (5).
- The carbonates and bicarbonates were determined by slaking with H<sub>2</sub>SO<sub>4</sub> (0.01 N) acid.(5)
- The chlorine was measured by grinding with silver nitrate (N0.005) (5).
- Sulfate by turbidity using barium chloride (5).
- The determination of the prepared nitrogen was carried out using the microkjeldahl apparatus, according to the method used by Bremner (5).
- The available phosphorous was measured according to the (Olsen) method (5).

- The available potassium was extracted by means of ammonium acetate and measured using a flame photometer (5).
- Estimate the volume distribution of soil particles by density method (5).
- Humic acid was determined according to the method described in (8,9) by adding 0.1 N sodium hydroxide and precipitating humic acid using 2 N hydrochloric acid, then separated by centrifuge and dried at a temperature of 40 C °.

The following analyzes were performed on humic acid:

-Samples of humic acid were analyzed using sulfuric and pyrochloric acid (10) and the elements were estimated:

Nitrogen by Kjeldahl device according to (5).

-Potassium by means of a flimphotometer, according to (5).

-Phosphorous with a spectrophotometer according to the method mentioned in (5).

- Chlorophyll was analyzed according to (2).

The result was collected at the end of the experiment and the fourth leaf was taken from the developing top of a plant for the purpose of laboratory analysis. The statistical analysis was done according to the complete random sectors (1).

**Table (2): Some chemical properties of humic acid**

property	pH	phosphate	potassium	nitrogen	humic acid
measuring unit		%	%	%	mg.kg <sup>-1</sup> dry matter
value	6.20	0.90	6.00	3.20	128000

**Table (3) Some characteristics of irrigation water**

			dissolved ions Cmol L <sup>-1</sup>						
	pH	EC ds m <sup>-1</sup>	SO <sub>4</sub>	HCO <sub>3</sub>	Cl	K	Na	Mg	Ca
<b>S1</b>	7.78	0.72	0.06	0.38	0.34	0.02	0.33	0.11	0.11
<b>S2</b>	7.67	3.56	0.58	0.36	1.00	0.16	1.62	0.28	0.20

**Results and discussion:****1- Leaf area and total chlorophyll content in leaves.****Table (4): Effect of potassium source and irrigation water salinity on leaf area (cm<sup>2</sup>) of broccoli plant.**

Water Quality S	Sources of Potassium - H					water quality S
	H0	H1	H2	HS1	HS2	
S1	21.93	24.00	30.30	44.33	66.86	37.48
S2	20.65	20.88	27.40	24.82	26.63	24.08
Potassium source - H	24.19			40.66		H*S
LSD 0.05	Potassium source H			Salinity-S		
	2.23			2.82		

The results of the research and from Table 4 indicate that there is a significant increase of potassium sources on the leaf area. The treatment of fertilizing with potassium sulfate at the second level (HS2) was superior in the leaf area values (40.66) cm<sup>2</sup>. The interaction between potassium sources and salinity of irrigation water was also recorded, and the highest value was recorded in the treatment (S1HS2). ) and was (66.86) cm<sup>2</sup>.

A significant decrease was recorded in the leaf area with an increase in the salinity of irrigation water (S2) (24.08) cm<sup>2</sup>, as the results of the research indicated in Table 5 that there was a significant increase of potassium sources in the percentage of chlorophyll in the leaves. The treatment of fertilization with potassium sulfate at the second level (HS1) in the values of Chlorophyll (1.23) mg L<sup>-1</sup> also recorded the interaction between potassium sources and irrigation water salinity, and the highest value was recorded when treatment (S2HS1) was (1.06) mg L<sup>-1</sup>, and no significant difference was recorded in chlorophyll with increasing salinity of irrigation water (6,14).

**Table (5) Effect of potassium source and irrigation water salinity on total chlorophyll (mg L<sup>-1</sup>) of broccoli**

Water Quality S	Sources of Potassium - H					water quality S
	H0	H1	H2	HS1	HS2	
S1	0.43	0.77	0.87	1.23	1.17	0.89
S2	0.39	0.62	1.13	0.68	1.18	0.80
Potassium source - H	0.70			1.06		H*S
LSD 0.05	Potassium source H			Salinity-S		
	0.18			0.22		0.23

The presence of a significant effect of humic acid and potassium sulfate on the construction of pigments can be explained by the fact that the formation of chlorophyll of types A and B and carotenoids depends on three factors: genetic factor, light, availability of nutrients such as magnesium, calcium, sulfur and nitrogen, and the addition and presence of an increase in humic acid in the soil increases the readiness and raises the concentrations of magnesium Calcium and the rest of the nutrients, thus increasing the efficiency of building chlorophyll and carotene pigments. (7,19) He explained the importance of chloroplasts being the center of photosynthesis in plants, in which chlorophyll molecules and other auxiliary pigments that participate in the photosynthesis process are organized.

The humic acids, including humic, increase the leaves' content of chlorophyll and carotene, as they are a necessary food source for building structural and protective pigments, and thus increase the availability of energy for building processes (4,7,20), as well as their role in delaying the aging of leaves with its content of organic compounds.

## 2- Biological yield and total yield

It is evident from the results of the research and from Tables 6 and 7 that there is a significant increase in the biological yield and the economic yield due to potassium sources. The treatment of fertilization with potassium sulfate at the (HS) level excelled, and it was (1530.5) gm plant<sup>-1</sup> and (20.2) tons ha<sup>-1</sup> for both biological yield and economic yield. Also, the interaction between potassium sources and salinity of irrigation water recorded the highest value when treatment (S1HS1) and it was (2033) gm plant<sup>-1</sup> and (23.8) tons ha<sup>-1</sup> for biological yield and economic yield, respectively.



**Table (6): Effect of potassium source and irrigation water salinity on the biological yield (gm plant<sup>-1</sup>) of broccoli**

Water Quality  S	Sources of Potassium - H					water quality  S
	H0	H1	H2	HS1	HS2	
S1	892	1273	1800	1800	2033	1559.6
S2	886	1000	1076	986	1303	1050.2
Potassium source - H	1154.5			1530.5		H*S
LSD 0.05	Potassium source H			Salinity -S		
	236.1			238.3		

**Table (7) Effect of potassium source and irrigation water salinity on the economic yield Mg.ha<sup>-1</sup> of broccoli plant**

Water Quality S	Sources of Potassium - H					water quality S
	H0	H1	H2	HS1	HS2	
S1	5.2	15.5	17.4	23.4	23.8	17.1
S2	4.6	13.3	16.5	12.9	20.8	13.6
Potassium source - H	12.0			20.2		H*S
LSD 0.05	Potassium source H			Salinity -S		
	1.3			1.6		

The increase in yield when adding humic acid is due to its content of nitrogen, potassium and phosphorous elements (Table 2), and humic acid contains effective groups that participate in the reactions of humic acids with cations of the elements to form organic-metallic complexes, which are It is of great importance in the readiness of the elements, and that all of these groups behave like electrons donor, so they have the ability to bind with cations of positively charged nutrients, including potassium and micronutrients, which behave as electrons acceptor (25). Humic contributes to decreasing the degree of reaction of the soil solution and thus increasing the availability of nutrients in the soil solution and their uptake by plant roots (15,17,18,32,33,34) .

### 3- Potassium content of plants and soil

It is evident from the results of the research and from tables 8 and 9 that there is a significant increase in the potassium content in the plant due to potassium sources. The treatment of fertilization with potassium sulfate at the level (HS) was (1.45) %, while no significant differences were recorded in the soil potassium content, as well as the interaction between the sources Potassium and the salinity of irrigation water had the highest value when treatment (S1HS2) and it was (1.68%).

**Table (8): Effect of potassium source and irrigation water salinity on potassium content in plants(%)**

Water Quality S	Sources of Potassium - H					water quality S
	H0	H1	H2	HS1	HS2	
S1	0.69	1.30	1.62	1.45	1.68	1.35
S2	0.65	1.13	1.23	1.30	1.38	1.14
Potassium source - H	1.10			1.45		H*S
LSD 0.05	Potassium source H			Salinity -S		
	0.16			0.18		

No significant differences were recorded in the soil potassium content in plants and soil when the salinity of the irrigation water changed, as shown in Tables 8 and 9. The interaction between potassium sources and irrigation water salinity recorded the highest value when treatment (S1HS2) and it was (314 mg Kg soil<sup>-1</sup>). (14,21,22,23,31).

**Table (9): Effect of potassium source and irrigation water salinity on soil potassium content (mg Kg soil<sup>-1</sup>)**

Water Quality  S	Sources of Potassium - H					water quality  S
	H0	H1	H2	HS1	HS2	
S1	266	308	313	299	314	300
S2	264	275	297	300	303	289
Potassium source - H	287			304		H*S
LSD 0.05	Potassium source H			Salinity -S		
	42			36		65

The difference in the quality of irrigation water generates significant differences in the values of the remaining potassium in the soil due to the difference in the quality and proportion of clay minerals in the soil (17), the greater the potassium depletion, the greater the stabilizing effort of potassium in that soil (2,11), the exchange capacity of soil for clay soils High (16) and this encourages the washing of elements, including potassium, which explains the low content in the soil. The reason for the decrease in the amount of potassium in the plant with the increase in the salinity of the irrigation water can be attributed to the osmotic effect of sodium and the competitive effect between potassium and sodium, which leads to a decrease in potassium absorption. (16,13).

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## REDUCING HEAT STRESS ON MAIZE DURING SPRING SEASON BY USING SELENIUM AND ITS REFLECTION ON POLLEN VITALITY AND GRAIN YIELD

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### Abstract:

This study was aimed to investigate the effect of seed soaking and spraying with selenium treatments on reducing the effect of heat stress at the pollination and fertilization stage of maize planted in the spring season in Iraq. Field experiment was conducted in the experimental farm, Agricultural Engineering Sciences, University of Baghdad-Iraq, during the season 2021. The experiment was laid out in Randomized Complete Blocks Design within split plot arrangement with three replicates. Main plots included two cultivars of maize (Baghdad 3 and cultivar 5018), while the sub plots included four levels of selenium: (soaking seed with selenium at concentrations 2 and 5 mgL<sup>-1</sup> and spraying plants with selenium at concentrations 10 and 20 mg L<sup>-1</sup> in addition to the two control treatments which are soaking seeds with distilled water and dry seeds. The results showed that the cultivar 5018 was superior in pollen vitality (89.92%), fertility percentage (94.56%) and grain yield (11.47 Mg ha<sup>-1</sup>). The two treatments of selenium spraying at concentrations of 10 and 20 mg L<sup>-1</sup> were superior without significant differences between them and gave the highest means in pollen viability (93.31% and 93.39%), fertility percentage (98.49% and 98.31%) and grain yield (13.07 and 13.55 Mg ha<sup>-1</sup>). The interaction was significant in the most of studied traits.

**Key words:** Maize, Selenium, Grain Yield, Cultivars, Heat Stress.



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### Introduction:

Maize (*Zea mays* L.) is an important cereal crop for its various nutritional and industrial uses. It is grown in Iraq for two seasons: the autumn, which is prevalent to suit the climatic conditions, and the spring, which is still not prevalent and planted in very few areas due to its low yield compared to the yield of the autumn season due to the inappropriate climatic conditions for it, also high temperatures and the increase in photoperiod at the stage of pollination and fertilization, and then increase the duration of exposure to high temperatures with low relative humidity, which affects the vitality of pollen and the success of the pollination and fertilization process. The stage of pollination and fertilization is one of the most critical stages in the life of the crop, so the exposure during the stage of pollen dispersal and the silking to high temperatures, can cause a loss in grain yield more than any other stage of growth. An increasing the temperature up 30°C during the flowering leads to a delay in the silking and acceleration the release of pollen, and then there is a lack of Asynchrony between male and female flowering which negatively affects the percentage of fertility through its effect on the growth and development of the anthers. The decrease in pollen vitality, the drying of the stigmas, the lack of opportunities for fixing the pollen on them, and the speed of the growth of the pollen tube to deliver the pollen to the ovules in the ovary caused decreased in the rate of pollination and fertilization, which negatively affects the grain yield (Hussain *et al.*, 2006 and Elsahookie *et al.*, 2006). Studies indicated that high temperatures during flowering stage negatively affect the growth of the male and female reproductive parts, the speed of silk growth and the spread of pollen, which led to an increase in the time interval between the appearance of the male inflorescence and the emergence of the silk, and then incompatibility and failure of the fertilization and pollination process, as well as reducing the vitality of pollen and reducing the production of maize by 73.6%. (Hatfield *et al.*, 2011, Alam *et al.* 2017, Wang *et al.*, 2019 and 2020).

The difference between cultivars is due to their different response to temperature and their variation in the tolerance to high temperatures in the stages of flowering, fertilization and dry matter accumulation

during the grain filling phase, as well as their different efficiency in transferring the products of the photosynthesis process from the source (AL-

shahadeh *et al.*, 2014 and Hadi and Hassan, 2021). Also, grain yield is greatly influenced by the combinations of genes possessed by the genotype (variety), as well as by the interactions of the genotype with the growth factors available in that environment (Lee and Tollenaar, 2007, Kazem and Hassan, 2020). It is possible to reduce the harmful effects of high temperatures in the pollination and fertilization phase of maize during spring season by using some antioxidants such as selenium as nutrient and antioxidant which has the ability to combine with proteins to form mineral proteins that help the plant withstand environmental stresses. Selenium can inhibits the formation of free radicals and



contributes with most of the antioxidant enzyme systems due to its antioxidant effect, also selenium has positive effects on the growth and development of plants, especially those that grow under stress conditions (Seppänen *et al.*, 2003 and Perveen, *et al.*, 2015).

Due to the economic importance of the maize crop especially its yield in the spring season, because of the easy and speed of drying compared to the autumn season, this study aimed to raise the productivity of two varieties of maize in spring season by using selenium to reduce the negative impact of heat stress on pollen vitality and the process of pollination and fertilization.

## MATERIALS AND METHODS

An experiment was carried at the Department of Field Crops - College of Agricultural Engineering Sciences - University of Baghdad (Al-Jadriya) Iraq, located at latitude of 33° 32' N and longitude of 44° 23' E. during 2021 to study the effect of selenium in reducing heat stress to which maize is exposed during the stage of pollination and fertilization in the spring season, and its reflection on the grain yield of maize. Randomized Complete Blocks Design within split plot arrangement, with three replicates was used, where the main plots included two cultivars of maize (Baghdad 3 and cultivar 5018), while the sub plots included six levels of selenium: (soaking the seeds with selenium at concentrations 2 and 5 mg L<sup>-1</sup> and spraying with selenium at concentrations 10 and 20 mg L<sup>-1</sup> in addition to the two control treatments which are soaking seeds with distilled water and dry seeds, the number of experimental units is 12 for each replicate. Selenium sprayed in three stages: the first stage when the 6 leaves appear, the second at 12 leaves appear, and the third at the male flowering stage (with the same concentration).

The research field was prepared and divided with standard production practices units, and the area of each experimental unit was 7.5 m<sup>2</sup> consisted of four lines of 2.5 m length and 75 cm apart, and the distance between plants 20 cm, so that the plant density becomes 66,666 thousand plants ha<sup>-1</sup>. The nitrogen fertilizer in the form of DAP fertilizer (46% P<sub>2</sub>O<sub>5</sub> and 18%N) was applied at a rate of 240 kg ha<sup>-1</sup> and potassium sulfate fertilizer (52% K<sub>2</sub>O) at a rate of 200 kg ha<sup>-1</sup> at planting (16-3-2021), and urea fertilizer (46% N) at a rate of 360 kg ha<sup>-1</sup> in two splits; the first one month after the date of planting and the second at the male flowering stage (Saleh and Salman, 2005).

Hydrogen sodium selenite solution (NaHSeO<sub>3</sub>) is a standard high solubility in water was prepared by dissolving 1.91 g in a liter of distilled water to obtain a concentration of 1000 mg L<sup>-1</sup> as a standard solution and using the dilution equation, the concentrations required for soaking 2 and 5 mg L<sup>-1</sup> and spraying concentrations of 10 and 20 mg L<sup>-1</sup> were prepared, after which the seeds were placed in solutions for 10 hours at room temperature 25°C.

**studied traits**

- 1- No. of days from planting until 100% tasseling (day)
- 2- No. of days from planting until 100% silking (day)
- 3- No. of days between male and female flowering (Anthesis -Silking interval) (day).
- 4- Vitality of pollen: after collecting pollen from the spikelets of male inflorescences of the studied genotypes at the age of 2-4 days in the early morning, from five plants and placed in plastic bags to prevent moisture loss. It was sent to the laboratory to be dyed using the dye (safranin - glycerin), and it was examined using a light microscope at a magnification of 40X as the live pollen are pink in color and are full and spherical in shape, while the dead pollen are not colored and wrinkled in shape, the vitality ratio was extracted by dividing the number of live pollen by the total number of pollen examined in the field of view of the slide multiplied by \* 100 (Al-Dubaisi, 2008).
- 5- Fertility percentage (%); It was calculated by dividing the number of ear divided by the number of total ovaries in the ear (seeded and seedless sites) multiplied by 100. (Gardner *et al.*, 2017).
- 6- Grain yield ( $Mg\ h^{-1}$ ); at full maturity, five plants of each experimental unit were harvested to calculate the mean yield of plant ( $gm\ plant^{-1}$ ) then multiply by the plant density and the data was converted to  $Mg\ ha^{-1}$  based on moisture 15.5% according to the following equation:-

$$\text{Dry weight} = \frac{100\% \text{moist}}{100 - 15.5\% \text{moist}} \times \text{total fresh weight} \quad (\text{AL-Sahoki, 1990}).$$

**Results and discussion****No. of days from planting until 100% tasseling (day)**

The effect of cultivars, selenium treatments and the interaction in the number of days from planting until 100% tasseling (day) were significant (Table 1). The cultivar Baghdad 3 gave the least period to complete male flowering (57.62 days), due to the different combination of genes that make up each variety, as well as the number and action of the genes that govern this trait (Hamdan and Bektash, 2010). This is in agreement with others (Al-Khazaali *et al.*, 2013, Al-Hilfy and Al-Tamimi, 2017 and Al-Tamimi and Al-Hilfy, 2021).

All treatments of soaking and spraying of selenium had a significant effect in reducing the number of days compared to the control treatment. The treatment of selenium 10  $mg\ L^{-1}$  was superior, as it gave the lowest mean of 55.22 days, with a decrease rate 7.16% compared to the control treatment. This may be due to the role of selenium in shortening the period from planting until the flowering of 100% of plants, as it had a role in increasing

the leaf area and its evidence (unpublished data) which leads to the preservation of the origins of flowers by achieving a state of continuous food supply with nutrients, which promoted the acceleration of physiological processes within the plant, (Hell and Mendel, 2010). Regarding the interaction the two cultivars showed a different response to the treatments of soaking and spraying selenium than the control treatment, The combination of spraying selenium  $10 \text{ mg L}^{-1}$  for Baghdad 3 cultivar was superior, with the lowest mean reaching 55.47 days, the combination of the control treatment (witho ut soaking or spraying) of cultivar 5018 gave the highest mean days(60.27)

**Table 1. Effect of cultivars and selenium on the number of days from planting until 100% tasseling (day) of spring maize for the growing season 2021**

Treatments	Cultivars		Mean
	Baghdad 3	Cultivar5018	
Control	59.27	60.27	59.77
seeds Soaking in distilled water	59.07	58.93	59.00
seeds Soaking with selenium2 mg L <sup>-1</sup>	57.30	57.80	57.55
seeds Soaking with selenium 5 mg L <sup>-1</sup>	57.47	56.87	57.17
Spray Selenium 10 mg L <sup>-1</sup>	55.47	56.47	55.97
Spray Selenium 20 mg L <sup>-1</sup>	57.13	57.43	57.28
L.S.D. 5%	0.487		0.487
Mean	57.62	57.96	
L.S.D. 5%	0.213		

#### **No. of days from planting until 100% of Silking (day).**

Results in Table 2 showed a significant effect of cultivars , selenium treatments and the interaction in the number of days from planting until 100% Silking .The cultivar Baghdad 3 gave the least period to complete flowering (60.86 days), this may be due to this characteristic determined by the genetic composition,

All soaking and selenium spraying treatments had a significant effect in reducing the number of days compared to the control treatment. Selenium spray at a concentration of  $10 \text{ mg L}^{-1}$  was superior, it gave the lowest mean of 58.98 days, with a decrease rate of 7.79% compared to the control treatments, due to the role of selenium in increasing gibberellic acid, which has a role in encouraging flowering and cellular elongation, as well as a role in improving the absorption of water and nutrients from the soil, which led to an increase in

flower growth, especially phosphorous (El-Ramady *et al.*, 2015). As for the interaction, the two cultivars responded differently to the treatments of soaking and spraying selenium. It is noted that the number of days decreased with the application of treatments of soaking and spraying with selenium, but it increased when the concentration of selenium increased, as the amount of increase in this treatment was more in the Baghdad3 than in the 5018 cultivar. The combination of spraying selenium 10 mg L<sup>-1</sup> with Baghdad 3, was superior with the lowest mean of 58.97 days for this trait, while the combination of the control treatment for 5018 cultivar gave the highest mean ( 64.60 days).

**Table 2. Effect of cultivars and selenium on the number of days from planting until 100% of Silking (day) of spring maize for the growing season 2021**

Treatments	Cultivars		Mean
	Baghdad 3	Cultivar 5018	
Control	63.33	64.60	63.97
seeds Soaking in distilled water	62.47	63.10	62.78
seeds Soaking with selenium2 mg L-1	60.53	61.40	60.97
seeds Soaking with selenium 5 mg L-1	59.83	60.83	60.33
Spray Selenium 10 mg L-1	58.97	59.00	58.98
Spray Selenium 20 mg L-1	60.00	59.63	59.82
L.S.D .5%	0.297		0.210
Mean	60.86	61.43	
L.S.D.5%	0.133		

**No. of days between male and female flowering (Anthesis -Silking interval) (day)**

It is obvious from Table 3 a significant effect of selenium treatments in Anthesis - Silking interval and the interaction. Baghdad 3 gave the lowest mean ( 3.24 days) this may be due to the different response of the varieties to the environmental conditions

All soaking and spraying of selenium had reduce the time between male and female flowering compared to the control treatment. Selenium sprayed at 20 mg L<sup>-1</sup> was superior in the lowest mean of 2.53 days with a decrease rate of 39.69% compared to control treatment( 4.20 days). this is for earlier in reaching 100% male and female flowering (Tables 1 and 2), which was reflected in the interval between male and female flowering, as

the increase in the Anthesis-Silking interval has a significant impact on the process of pollination and fertilization, as it is one of the factors that cause an increase in the number of unfertilized grains (Shrestha, 2013).

As for the interaction, the interval decreased with the increase of selenium concentration in soaking and spraying at Baghdad 3 cultivar. The combination of spraying selenium at 20 mg L<sup>-1</sup> with cultivar 5018 gave the lowest mean of 2.20 days, while the control treatment with 5018 gave the longest interval (4.33 days).

**Table 3. Effect of cultivars and selenium on Anthesis -Silking interval (day) of spring maize for the growing season 2021**

Treatments	Cultivars		Mean
	Baghdad 3	Cultivar 5018	
Control	4.07	4.33	4.20
seeds Soaking in distilled water	3.40	4.17	3.78
seeds Soaking with selenium 2 mg L <sup>-1</sup>	3.23	3.60	3.42
seeds Soaking with selenium 5 mg L <sup>-1</sup>	2.37	3.97	3.17
Spray Selenium 10 mg L <sup>-1</sup>	3.50	2.53	3.02
Spray Selenium 20 mg L <sup>-1</sup>	2.87	2.20	2.53
L.S.D. 5%	0.503		0.356
Mean	3.24	3.47	
L.S.D. 5%	n.s		

### **Pollen Vitality (%)**

There was a significant effect of cultivars and selenium treatments on pollen viability (%) in this trait (Table 4). The cultivar 5018 was superior in giving the highest mean (89.92%) compared to the cultivar Baghdad (89.52%) due to the different response to the environmental conditions surrounding the growth.

Soaking and spraying selenium increase pollen viability significantly compared to the control treatment. Selenium at 20 mg L<sup>-1</sup> was superior in highest mean (93.39%), with no significant differences from the treatment of spraying selenium at a concentration of 10 mg L<sup>-1</sup> (93.31%), and with an increase of percentages that amounted to 13.77% and 13.67%

compared to the control treatment for the two treatments sequentially .There is a significant increase in pollen vitality was obtained when the seeds were soaked with distilled water reached (83.72% ) compared to the control treatment which gave less mean reached 82.09% .The reason for the

decrease in pollen vitality at control treatment may be attributed to the exposure of plants in the pollination and fertilization stage to high temperatures, as heat stress reduces the percentage of effective pollen and the duration of pollen survival time, as high temperatures severely affect the basic metabolic pathways and thus generates pollen with poor vitality (Also, high temperatures in the pollen dissemination stage lead to an increase in the accumulation of reactive oxygen species (ROS) in pollen, which leads to damage the pollen membrane and deterioration and drying of pollen, as both pollen and pistil accumulate increased levels of ROS under heat stress, This leads to damage to membranes and other organelles (Djanaguiraman *et al.*, 2018). While the selenium led to a significant increase in the viability of the active pollen due to its role in suppression of ROS produced under heat stress condition which leads to an increase in pollen viability (Djanaguiraman *et al.*, 2010).

**Table 4. Effect of cultivars and selenium on pollen vitality (%) of spring maize for the growing season 2021**

Treatments	Cultivars		Mean
	Baghdad 3	Cultivar 5018	
Control	81.49	82.68	82.09
seeds Soaking in distilled water	83.50	83.93	83.72
seeds Soaking with selenium2 mg L <sup>-1</sup>	93.35	92.73	93.04
seeds Soaking with selenium 5 mg L <sup>-1</sup>	92.48	93.03	92.76
Spray Selenium 10 mg L <sup>-1</sup>	92.94	93.67	93.31
Spray Selenium 20 mg L <sup>-1</sup>	93.34	93.44	93.39
L.S.D. 5%	ns		0.574
Mean	89.52	89.92	
L.S.D. 5%	0.043		

### **Fertility percentage (%)**

It is clear from Table 5 that the effect of the cultivars, selenium treatments and their interaction on the fertility percentage was significant. Cultivar 5018 gave the highest percentage (94.56%) compared to Baghdad 3 which gave 94.43%. due to the different response of cultivars to temperatures and their variation in tolerance to high temperatures in the stages of flowering, fertilization and accumulation of dry matter during the period of grain filling, and this result in agreement with Siddiq and Muhammad (2012), who obtained significant differences in the fertility percentage between the synthetic cultivars of maize.

All soaking and selenium spraying treatments had a significant effect on increasing the fertility percentage compared to the control treatment. The treatment of selenium spraying at 10 mg L<sup>-1</sup> was superior in giving the highest mean (98.46%) with an increase of 13.98% compared to the control treatment, due to the role of selenium in reducing the interval between the male and female flowering dates (Table 3), which reduce the number of pollen grains reaching the female inflorescence which ultimately leads to decrease the number of grains in the ear)

Abdul Hamid and Adra, 2011). Another role for selenium is in maintaining the antioxidant enzyme systems (unpublished data) which enhances the flow of sugar and starch towards the developing ovaries in the stage of pollination and fertilization when applied before the stage of tasseling, which significantly reduced the rate of abortion of maize ovaries under heat stress conditions (Yun-Ying *et al.*, 2008 and Del Pino *et al.*, 2019). It was noticed that a temperature exceeded more than 40°C during the stage of pollination and fertilization causing a decrease in the number of fertilized grains due to a decrease in pollen viability and an increase in the rate of ovarian abortion, also because of lack of the products of photosynthesis process, which leads to the abortion of grains (Shen and *et al.*, 2020), in addition to that the yield decrease due to the low content of soluble sugar and starch in grains under heat leads to ovarian abortion (Gao *et al.* 2020). As for the interaction the combination of spraying with a concentration of 20 mg L<sup>-1</sup> for 5018, was superior with the highest mean (98.56%). Whereas, the combination of the control treatment for Baghdad 3 gave the lowest mean (86.12%).

**Table 5. Effect of cultivars and selenium on the fertility percentage (%) of spring maize for the growing season 2021.**

Treatments	Cultivars		Mean
	Baghdad 3	Cultivar 5018	
Control	86.12	86.70	86.41
seeds Soaking in distilled water	87.47	87.99	87.73
seeds Soaking with selenium2 mg L <sup>-1</sup>	98.04	97.71	97.87
seeds Soaking with selenium 5 mg L <sup>-1</sup>	98.40	97.89	98.14
Spray Selenium 10 mg L <sup>-1</sup>	98.46	98.53	98.49
Spray Selenium 20 mg L <sup>-1</sup>	98.07	98.56	98.31
LSD 5%	0.584		0.413
Mean	94.43	94.56	
L.S.D. 5%	0.117		

**grains yield (Mg ha<sup>-1</sup>)**

Data in Table 6 showed a significant effect of the cultivars, selenium treatments and their interaction in the grains yield. Cultivar 5018 excelled in giving the highest mean ( 11.47 Mg ha<sup>-1</sup> ) due to the different combinations of genes and the physiological processes that regulate the evolutionary stages of the cultivar (Khan *et al.*, 2002 and Shankar *et al.*, 2009). These results are in agreement with those obtained by Ali (2022). Selenium at 20 mg L<sup>-1</sup>

was superior in highest mean of this trait ( 13.55 Mg ha<sup>-1</sup> ), due to the roles of Selenium in increased the main and secondary yield components, which was reflected in an increase in the grain yield. As for the interaction the combination of spraying selenium at a concentration of 20 mg L<sup>-1</sup> for 5018 had the highest mean, ( 13.94 Mg ha<sup>-1</sup> ) while the combination of the control treatment for Baghdad 3 gave the lowest value (7.38 Mg ha<sup>-1</sup>).



**Table 6. Effect of cultivars and selenium on grain yield ( $Mg\ ha^{-1}$ ) of spring maize for the growing season 2021**

Treatments	Cultivars		Mean
	Baghdad 3	Cultivar 5018	
Control	7.38	8.04	7.71
seeds Soaking in distilled water	8.76	9.09	8.93
seeds Soaking with selenium2 mg L <sup>-1</sup>	11.57	12.01	11.79
seeds Soaking with selenium 5 mg L <sup>-1</sup>	12.54	12.66	12.60
Spray Selenium 10 mg L <sup>-1</sup>	13.03	13.11	13.07
Spray Selenium 20 mg L <sup>-1</sup>	13.15	13.94	13.55
L.S.D. 5%	0.358		0.253
Mean	11.07	11.47	
L.S.D. 5%	0.120		

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## EFFECT OF NITROGEN FERTILIZER AND ASCORBIC ACID ON SOME CHEMICAL AND ANATOMICAL CHARACTERISTICS LEAVES OF PLANT PAULOWNIA TOMENTOSA

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### Abstract:

This study was carried out at the Agricultural Research Station of the University of Basra's College of Agriculture in a canopy covered with saran cloth on paulownia tree seedlings. All experimental plants were fertilized with compound fertilizer (20-20-20) NPK monthly throughout the study period at a rate of 1 g per pot as a constant factor. Two factors were examined in the study: the first is nitrogen fertilization with urea fertilizer (46% nitrogen) using three levels (0, 1.6, 3.2 g plant<sup>-1</sup>), and the second factor is spraying with three concentrations of ascorbic acid (0, 30, 60 mg l<sup>-1</sup>). The anatomical characteristics of paulownia leaves were studied after six months of treatment, as the anatomical sections were prepared using paraffin technology. According to the study's findings, the comparison treatment of nitrogen fertilizer compared to the other concentrations, was noticeably superior and the highest value for the thickness of the cuticle layer was 17.33 micrometers, while the treatment recorded 2.3 g. Plant<sup>-1</sup> had the lowest value of 10.33 micrometers. The comparison treatment of nitrogen fertilizer gave the lowest values for leaves content of total chlorophyll and carbohydrates, thickness of the epidermis layer, length of columnar cells, a diameter of spongy cells, and diameter of small and large vascular bundles (83, 86.80, 8.72, 166.67, 90, 146.67, 686.67), respectively, while the treatment recorded 2.3 g Plant<sup>-1</sup> has the highest rates for the same traits. The treatment with ascorbic acid 60 mg l<sup>-1</sup> compared to the other concentrations, was noticeably superior were recorded for the chemical and anatomical characteristics, except for the thickness of the cuticle layer. As for the interaction between nitrogen fertilizer and ascorbic acid, the treatment of the interaction between nitrogen fertilizer 3.2 g plant<sup>-1</sup>, ascorbic acid 60 mg l<sup>-1</sup> recorded the highest rate for all chemical and anatomical characteristics and significantly different from the other interactions, which has a good impact on the paulownia plant's growth properties.

**Key words:** Fertilizer; Nitrogenous; Ascorbic Acid; Chemical Anatomical Characteristics; Plant; Paulownia.



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## Introduction:

The paulownia is a princess tree or the royal tree Royal tree, and the reason for naming it paulownia is after a Russian princess (Anna Paulonia), the Russian Tsar Paul I's daughter. Due to the shape of the spring tree, it was given the name royal covered with flowers with a sweet smell that smells like vanilla. The paulownia tree is what it is known as in environmental studies (the empress). For its advantages to the environment the Japanese made it a state medal and changed the name of the state medal from the Bright Land to the paulownia flower, and in 2003 it was awarded in the name of the Japanese Emperor (Clatterbuck 2004).

The paulownia tree, native to China, is a well-known environmentally friendly tree that follows the family Paulownaceae, the order of the Lamiaceae. Nine species belong to the genus Paulownia, are fast-growing plants with hardwood (Bergmann, 2003). The tree has many benefits as it helps germinate other trees around it because It has abundant nitrogen in its leaves and blooms and useful as a natural fertilizer. It is one of the elegant and beautiful-looking ornamental trees with benefits, and It has multiple uses and can withstand various weather conditions, such as extreme heat and cold, below zero. It is planted as ornamental trees in public parks (Anglove, 2010).

Nitrogen feeding controls a large degree of plant growth rate by regulating the action of plant hormones (auxins and cytokinins), which increases the number of meristematic cell divisions. The necessary nutrients from the soil and its representation, especially phosphorus and potassium, increase water consumption efficiency and resistance to external stresses, prolonging the growth period and delaying aging (Woods, 2008). Spraying with ascorbic acid on the shoots of the plant was found to increase cellulose, and this is a result of the increase in chlorophyll building, which affects the increase in the metabolic materials produced as a result of photosynthesis, which leads to the accumulation of soluble sugars in plant tissues, and this is evidence that ascorbic acid is part of Plant defence system. (Liu et al., 2008). Since the nitrogen concentration of a plant ranges from 2 to 5 percent of its dry weight, it is one of the most crucial components required for the plant. It plays a crucial function in the nutrition and physiology of the plant because it creates the amino acids needed to make proteins, enzymes, and cell membranes. It also contributes to the production of chlorophyll, nucleic acids, and vitamins (Wang et al.; 2014). Also, nitrogen causes an increase in plant biomass as a result of increasing the efficiency of photosynthesis, which causes an increase in the plant content of carbohydrates (Gilman et al. 2006), since nitrogen is the primary nutrient and what determines how fertilization works.

The anatomical characteristics of the plant have been used as a tool for classification for more than a hundred years, and its results are useful in the field of classification; in addition to that it is considered one of the changes that occur due to the environmental conditions that the plant passes through, and it is considered a vital indicator for the response of plants to various treatments (Athbi et al., 2010).

## Materials and Methods

The investigation was carried out on paulownia tree seedlings at the Agricultural Research Station of the College of Agriculture, University of Basrah, under a saran cloth-covered canopy. After being turned into 28 cm-diameter pots, they were thoroughly cleansed with water, formalin-sterilized, and then filled with sterilized growth media, one plant per pot. All experimental plants were fertilized with compound fertilizer (20-20-20) NPK monthly throughout the study period at a rate of 1 g per pot as a constant factor with all treatments.

### The study included the following treatments:

1- Nitrogen fertilization with urea fertilizer (46% nitrogen) using three levels (0, 1.6, 3.2 g plant<sup>-1</sup>).

2- Spraying by three concentrations of ascorbic acid (0, 30, 60 mg l<sup>-1</sup>).

The treatments were sprayed on the leaves until complete wetness with a 5-liter hand sprayer after adding the Tween 20 dispersant.

### Leaves content of total chlorophyll (mg100g<sup>-1</sup>)

According to the method, the chlorophyll pigment in the green leaves was estimated by taking the third leaf from the growing apex of each treatment (Al-Sahhaf, 1989).

### Carbohydrate content of leaves (mg. 100g<sup>-1</sup>)

Modification of Phenol-Sulphric acid Colorimetric Method prepared by (1956) Dobois et al.

### Anatomical features

The anatomical characteristics of paulownia leaves were studied after six months of treatment, as the anatomical sections were prepared using paraffin technology according to the method mentioned in Khafaji (2001). Leaf samples were collected, and the fixation was performed on them in F.A.A solution for 48 hours; then, the cut parts were passed with increasing concentrations of Ethyl alcohol. The samples were buried with paraffin wax at a temperature of 58 °C, after that the samples were cut by a Rotary Microtome with a thickness of 10 micrometers, loaded on slides, stained with Safranin dye, and then placed in Fast green dye, then loaded with drops of DPX and covered with a slide cover, after that it was Studying the anatomical characteristics of the leaves. Measurements were taken in micrometres  $\mu\text{m}$  using an ocular micrometre in an Olympus light microscope equipped with a camera attached to a calculator.

## Statistical analysis

The study's factorial experimental design followed the layout of the fully randomized sectors. To ensure that there were significant differences among the traits examined using the GenStat statistical program, the findings were analyzed using analysis of variance. Moreover, means were examined, and the modified least significant difference test (L.S.D) was used to determine significance at the probability level of 50.0. (Bashir, 2003).

## Results and discussion

### Leaves content of total chlorophyll (mg100g<sup>-1</sup>)

The treatment with nitrogen fertilizer 3.2 g plant<sup>-1</sup> was noticeably superior to the other concentrations and recorded the highest value of 12.25 mg100 g<sup>-1</sup> in the data reported in Table (1). This resulted in a total chlorophyll content of paulownia plant leaves of 12.25 mg100 g<sup>-1</sup>. The lowest value, 8.72 mg, was observed for the comparative therapy. 100 g<sup>-1</sup>. When compared to the other concentrations, the treatment using 60 mg l<sup>-1</sup> of ascorbic acid recorded the highest value of 12.22 mg100 g<sup>-1</sup>, while the comparison treatment recorded the lowest rate of 8.08 mg100 g<sup>-1</sup>.

As for the interaction between nitrogen fertilizer and ascorbic acid, the treatment of the interaction between nitrogen fertilizer 3.2 gm plant<sup>-1</sup> and ascorbic acid 60 mg l<sup>-1</sup> recorded a highest leaf chlorophyll content rate of 13.42 mg100 g<sup>-1</sup>. At the same time, the comparison treatment for both recorded the lowest value of 6.02 mg100 g<sup>-1</sup>, as compared to the other interactions, with a significant difference.

Table (1) Effect of nitrogen fertilizer, and ascorbic acid on leaves content of total chlorophyll (mg 100 g<sup>-1</sup>)

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	6.02	9.23	10.92	8.72
1.6	7.33	11.22	12.33	10.29
3.2	10.88	12.44	13.42	12.25
Average of ascorbic effect	8.08	10.96	12.22	
L.S.D.	Urea=0.68	Ascorbic0.68 =	Interaction= 1.01	



**Carbohydrate content of leaves (mg 100g<sup>-1</sup>)**

The results are shown in Table 1. The treatment with nitrogen fertilizer at 3.2 g plant<sup>-1</sup> was significantly better than the other concentrations and recorded the highest value of 108.47 mg100 g<sup>-1</sup>, while the comparison treatment recorded the lowest value of 86.8 mg100 g<sup>-1</sup>. This study examined the effects of nitrogen fertilizer and ascorbic acid on the total chlorophyll content of paulownia leaves. In comparison to the other concentrations, the treatment using 60 mg l<sup>-1</sup> of ascorbic acid recorded the highest value, 109.07 mg100 g<sup>-1</sup>, while the comparison treatment recorded the lowest rate, 79.30 mg100 g<sup>-1</sup>.

As for the interaction between nitrogen fertilizer and ascorbic acid, the treatment of the interaction between nitrogen fertilizer 3.2 g plant<sup>-1</sup>, and ascorbic acid 60 mg l<sup>-1</sup> recorded a highest leaf chlorophyll content rate, 120.50 mg 100 g<sup>-1</sup>. The comparison treatment for both recorded the lowest value of 69.70 mg100 g<sup>-1</sup>, significantly different from the others of interactions.

Table (2) Effect of nitrogen fertilizer, and ascorbic acid on Carbohydrate content of leaves (mg 100g<sup>-1</sup>)

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	69.70	93.10	97.60	86.80
1.6	82.30	107.20	109.10	99.53
3.2	85.90	119	120.50	108.47
Average of ascorbic effect	79.30	106.43	109.07	
L.S.D.	Urea= 1.21	Ascorbic = 1.21	Interaction= 2.16	

**The thickness of the cuticle layer (μm)**

Ascorbic acid and nitrogen fertilizer both had an impact on the anatomical features of paulownia plant leaves, as shown by the results in Table (3) and Figure (1). The nitrogen fertilizer comparison treatment significantly outperformed the other concentrations and recorded the highest value of 17.33 m, while the treatment recorded 2.3 g. plant<sup>-1</sup> 10.33 m was the value at the bottom. The ascorbic acid comparison treatment greatly outperformed the other concentrations, recording the greatest value of 16 m, while the ascorbic acid treatment at 60 mg l<sup>-1</sup> recorded the lowest value of 10.67 m.

The comparison treatment for both substances recorded the highest value of 20 m for the interaction between nitrogen fertilizer and ascorbic acid, which is significantly different

from the other interactions. Whereas ascorbic acid 60 mg/l and nitrogen fertilizer 20 g plant<sup>-1</sup> had the lowest interaction rate (8 m), respectively.

Table (3) Effect of nitrogen fertilizer, and ascorbic acid on the thickness of the cuticle layer ( $\mu\text{m}$ )

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	20	17	15	17.33
1.6	15	12	9	12
3.2	13	10	8	10.33
Average of ascorbic effect	16	13	10.67	
L.S.D.	Urea= 1.23	Ascorbic = 1.23	Interaction= 2.11	

### The thickness of the epidermal layer

Ascorbic acid and nitrogen fertilizer had an impact on the anatomical features of paulownia plant leaves, as shown by the results in Table (4) and Figure (1). The nitrogen fertilizer treatment at 3.2 g plant<sup>-1</sup> was significantly better than the other concentrations, and the highest value of the epidermal layer thickness was measured at 108.33 m while on this treatment. The lowest value was 83 m, which was obtained with the comparison treatment. The greatest value was 108.33 m and the lowest value was 85.33 m for the comparator treatment, indicating that the therapy using 60 mg l<sup>-1</sup> of ascorbic acid was much better than the other concentrations.

In terms of the interaction between ascorbic acid and nitrogen fertilizer, the interaction treatment between ascorbic acid 60 mg l<sup>-1</sup> and nitrogen fertilizer 3.2 g plant<sup>-1</sup> recorded the greatest rate of an epidermal thickness of 120 m. The lowest value for both, 75 m, was obtained by the comparison treatment, which differed significantly from the rest of the interferences.

Table (4) Effect of nitrogen fertilizer, and ascorbic acid on the thickness of the epidermal layer ( $\mu\text{m}$ )

Urea concentrations $\text{g plant}^{-1}$	Ascorbic concentration $\text{mg l}^{-1}$			Average of urea effect
	0	30	60	
0	75	84	90	83
1.6	86	105	115	102
3.2	95	110	120	108.33
Average of ascorbic effect	85.33	99.67	108.33	
L.S.D.	Urea= 5.44	Ascorbic = 5.44	Interaction= 8.11	

### Palisade cells length

The palisade cells are one of the most important components of the mesophyll in plant leaves because these parenchyma cells have a vital role in the process of photosynthesis, as they are characterized by the abundance of chloroplasts in them.

Ascorbic acid and nitrogen fertilizer had an impact on the anatomical features of paulownia plant leaves, as shown by the results in Table (5) and Figure (1). The nitrogen fertilizer treatment at  $3.2 \text{ g plant}^{-1}$  was significantly better than the other concentrations, and the highest value of the length of the palisade cells was recorded at 218.33 m while on this treatment. The lowest value, 166.67 m, was reported by the comparison treatment. The maximum value, 223.33 m, was recorded by the treatment using  $60 \text{ mg l}^{-1}$  ascorbic acid, while the lowest value, 155 m, was obtained by the comparison treatment. This therapy was clearly superior to the other concentrations.

Regarding the interaction of ascorbic acid and nitrogen fertilizer, the treatment of the interaction between ascorbic acid  $60 \text{ mg l}^{-1}$  and nitrogen fertilizer  $3.2 \text{ g plant}^{-1}$  recorded the highest rate of palisade cell length, reaching 250 m. The lowest value for both, 155 m, was obtained by the comparison treatment, which was significantly different from the rest of the interactions.

Table (5) Effect of nitrogen fertilizer, and ascorbic acid on palisade cells length ( $\mu\text{m}$ )

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	130	170	200	166.67
1.6	160	190	220	190
3.2	175	230	250	218.33
Average of ascorbic effect	155	196.67	223.33	
L.S.D.	Urea= 12.28	Ascorbic = 12.28	Interaction= 19.56	

### Spongy cells diameter

The spongy cells are one of the most important components of the mesophyll in plant leaves because of the vital role of these parenchyma cells in the storage of nutrients and their role in the process of photosynthesis.

The paulownia plant leaves' anatomical characteristics were affected by nitrogen fertilizer and ascorbic acid, as shown by the results in Table (6) and Figure (1). The nitrogen fertilizer treatment at 3.2 g plant<sup>-1</sup> was significantly better than the other concentrations, and the highest value for the diameter of the spongy cells was recorded at 123 m while using this fertilizer. 90 m was the lowest value measured in the comparator treatment. The ascorbic acid treatment at 60 mg l<sup>-1</sup> was noticeably better than the other concentrations, recording the highest value of 120 m, while the comparison treatment recorded the lowest rate of 90 m. When it comes to the interaction between ascorbic acid and nitrogen fertilizer, the treatment including 3.2 g plant<sup>-1</sup> of nitrogen fertilizer and 60 mg l<sup>-1</sup> of ascorbic acid had the largest rate of sponge cell diameter, reaching 140 m. When compared to the remainder of the interferences, the comparative treatment for both reported the lowest value of 80 m.

Table (6) Effect of nitrogen fertilizer, and ascorbic acid on spongy cells diameter ( $\mu\text{m}$ )

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	80	90	100	90
1.6	90	115	120	108.33
3.2	100	129	140	123
Average of ascorbic effect	90	111.33	120	
L.S.D.	Urea = 6.23	Ascorbic = 6.23	Interaction = 9.35	

### Small vascular bundles diameter

The small vascular bundles are one of the branches of the large vascular bundles, and they are one of the most important components of the vascular tissue in the plant because these bundles have a vital role in transporting water, salts, and nutrients within the plant.

Ascorbic acid and nitrogen fertilizer had an impact on the anatomical features of paulownia plant leaves, as shown by the results in Table (7) and Figure (2). The nitrogen fertilizer treatment, which used 3.2 g plant<sup>-1</sup>, was significantly better than the other concentrations, and the diameter of the small vascular bundles was recorded at its highest value—256.67  $\mu\text{m}$ —while the comparison treatment recorded the lowest value—146.67  $\mu\text{m}$ . The greatest value was 256.67  $\mu\text{m}$  for the ascorbic acid treatment, which was considerably superior than the other concentrations, while the lowest value was 126.67  $\mu\text{m}$  for the comparison treatment.

Regarding the interaction of ascorbic acid and nitrogen fertilizer, the treatment of the interaction between ascorbic acid 60 mg l<sup>-1</sup> and nitrogen fertilizer 3.2 g plant<sup>-1</sup> recorded the maximum average width of tiny vascular bundles reaching 300  $\mu\text{m}$ . The lowest value of 80  $\mu\text{m}$  was obtained by the comparison treatment for both, which was significantly different from the rest of the interferences.

Table (7) Effect of nitrogen fertilizer, and ascorbic acid on small vascular bundles diameter ( $\mu\text{m}$ )

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	80	170	190	146.67
1.6	100	230	280	203.33
3.2	200	270	300	256.67
Average of ascorbic effect	126.67	223.33	256.67	
L.S.D.	Urea= 12.64	Ascorbic = 12.64	Interaction= 18.23	

### Vascular bundles diameter

The vascular bundles are one of the most important components of the vascular tissue in the plant because of the vital role of these bundles in transporting water, salts and nutrients inside the plant.

The diameter of the large vascular bundles reached 1050 m in the nitrogen fertilizer treatment, which was significantly higher than the other concentrations, while the comparison treatment recorded the lowest value of 686.67 m. The results are shown in Table (8) and Figure (2), which demonstrate the effects of nitrogen fertilizer and ascorbic acid on the anatomical characteristics of paulownia plant leaves. In comparison to the other concentrations, the treatment using 60 mg l<sup>-1</sup> ascorbic acid recorded the highest value, 1030 m, while the comparison treatment recorded the lowest rate, 641.67 m.

The treatment of the interaction between 3.2 g plant<sup>-1</sup> of nitrogen fertilizer and 60 mg/l of ascorbic acid resulted in the largest average diameter of the major vascular bundles, measuring 1300 m. The lowest value for both treatments, 550 m, came from the comparison treatment, which differed significantly from the other interactions.

Table (8) Effect of nitrogen fertilizer, and ascorbic acid on vascular bundles diameter  
( $\mu\text{m}$ )

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	550	700	810	686.67
1.6	625	850	980	818.33
3.2	750	1100	1300	1050
Average of ascorbic effect	641.67	883.33	1030	
L.S.D.	Urea= 25.25	Ascorbic = 25.25	Interaction= 30.88	

#### Vascular bundle sheath diameter

The vascular bundle sheath is one of the most important components of the vascular tissue in plants because these bundles have a vital role in photosynthesis.

The results are shown in Table (9), which demonstrates the impact of nitrogen fertilizer and ascorbic acid on the anatomical features of paulownia plant leaves. The nitrogen fertilizer treatment, which used 3.2 g plant<sup>-1</sup>, was significantly better than the other concentrations, and the diameter of the bundle envelope cells was measured at 158.33  $\mu\text{m}$ , while the comparison treatment only measured 158.03  $\mu\text{m}$ . A value of 113.33  $\mu\text{m}$  was the lowest. The ascorbic acid treatment, which used 60 mg l<sup>-1</sup>, outperformed the other concentrations significantly. It achieved the maximum value, 165  $\mu\text{m}$ , while the comparator treatment only managed 110  $\mu\text{m}$ .

In regard to the relationship between ascorbic acid and nitrogen fertilizer, the treatment of the interaction between ascorbic acid 60 mg l<sup>-1</sup> and nitrogen fertilizer 3.2 g plant<sup>-1</sup> recorded the maximum average diameter of the cells of the bundle envelope reaching 200  $\mu\text{m}$ . The lowest value of 100  $\mu\text{m}$  was obtained by the comparison treatment for both, which was significantly different from the rest of the interferences.

Table (9) Effect of nitrogen fertilizer, and ascorbic acid on vascular bundle sheath diameter ( $\mu\text{m}$ )

Urea concentrations g plant <sup>-1</sup>	Ascorbic concentration mg l <sup>-1</sup>			Average of urea effect
	0	30	60	
0	100	115	125	113.33
1.6	110	130	170	136.67
3.2	120	155	200	158.33
Average of ascorbic effect	110	133.33	165	
L.S.D.	Urea= 8.44	Ascorbic = 8.44	Interaction= 10.21	

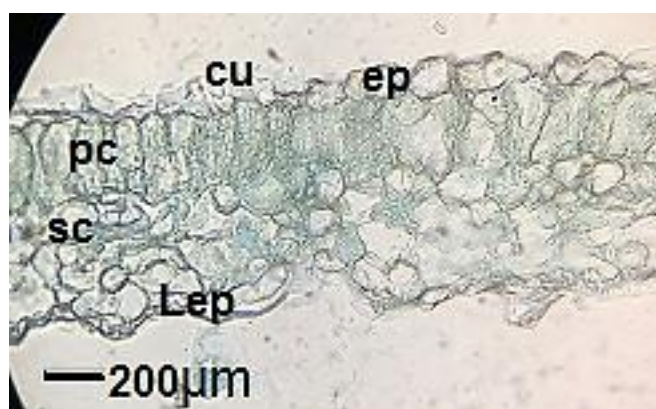
The study's results showed that the treatment with nitrogen fertilizer and ascorbic acid caused a significant decrease in the thickness of the cuticle layer in the leaves of the paulownia plant. The cuticle layer's thickness is what causes it to expand. The results of the study showed that the treatment with nitrogen fertilizer and ascorbic acid improved the anatomical characteristics of paulownia plant leaves, the creation of amino acids, particularly the amino acid tryptophan, which is a beginning molecule for the formation of auxins and, as a result, an increase in cell division and the expansion of their size, may have a significant role in boosting the level of plant hormone production in plants which was positively reflected on the tissue of the leaves (Farahat et al. 2014). The cause could be the quick solubility of nitrogen, which is evidence of its availability and readiness for absorption by the plant. Nitrogen is also a component of the chlorophyll molecule and other vital substances like proteins, nucleic acids, enzymes, and energy compounds that help to increase the size and number of cells, which has a positive impact on vegetative growth and plant size (Abdul Jabbar, 2012).

through nitrogen's function in constructing protein and boosting meristematic efficiency. Furthermore, it can be found in amine derivatives like choline. With the aid of light energy, nitrogen also plays a role in the transformation of carbon dioxide and water into sugars. Since nitrogen efficiently absorbs nutrients, the morphological properties of leaf tissues are enhanced (Blumenthal and Sauder, 2002).

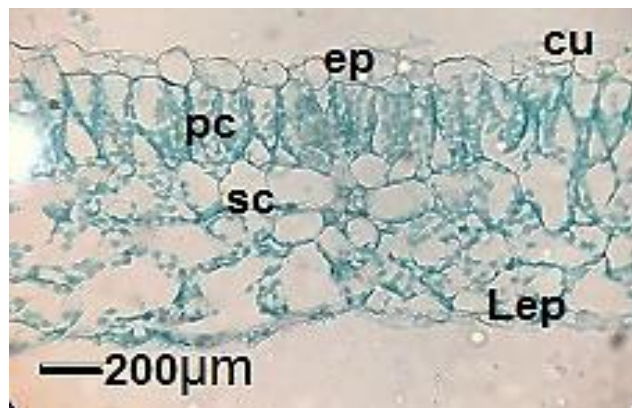
Ascorbic acid's impact on the process of photosynthesis may be the cause which stimulates the growth and elongation of leaf tissue cells as a result of its products' use in the photosynthesis processes growth (Muhammad et al., 2016). Contributes to many biological activities in the plant as a donor or gainer in the transfer of electrons, which



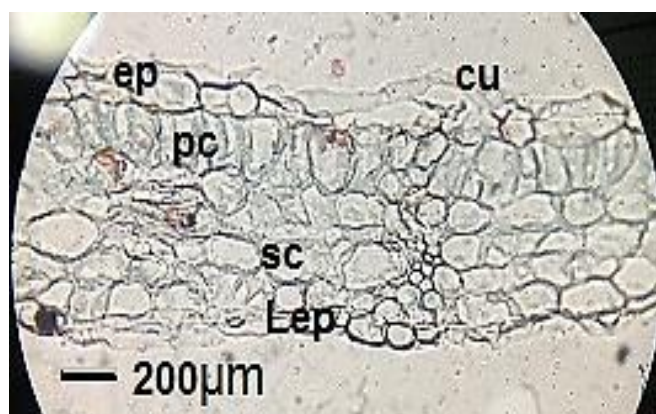
ultimately leads to the expansion and elongation of cells, which increases plant tissue volume (Conklin, 2001).



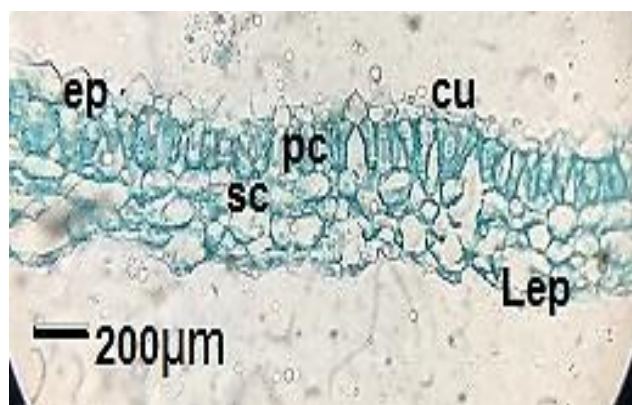
nitrogen fertilizer (3.2 g plant<sup>-1</sup>)



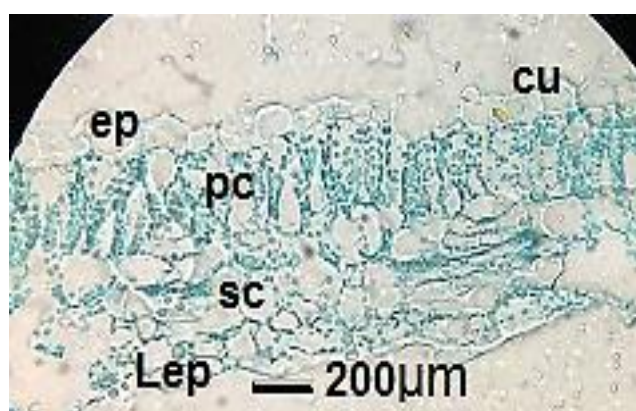
Nitrogen fertilizer (comparison)



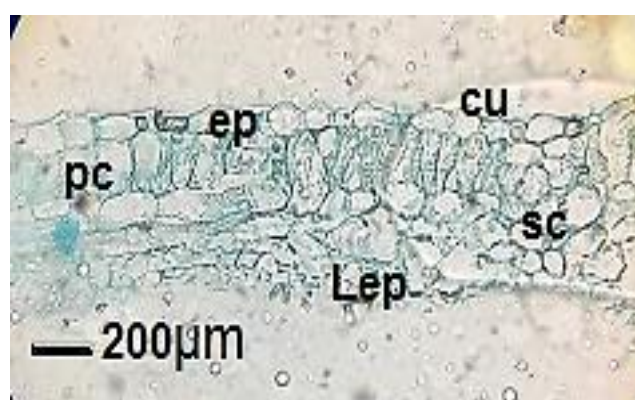
Ascorbic (60 mg l<sup>-1</sup>)



Ascorbic (0 mg l<sup>-1</sup>)



Nitrogen fertilizer 3.2 x 60 mg l<sup>-1</sup> Ascorbic

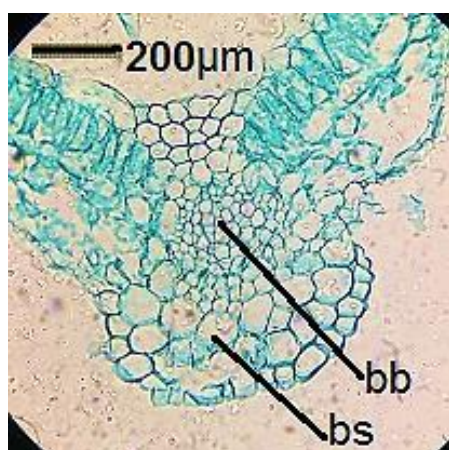
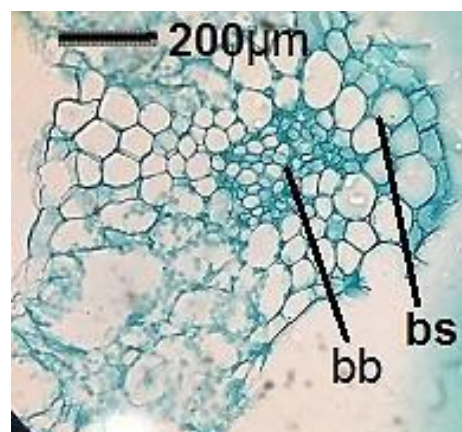


Nitrogen fertilizer 0x0 ascorbic

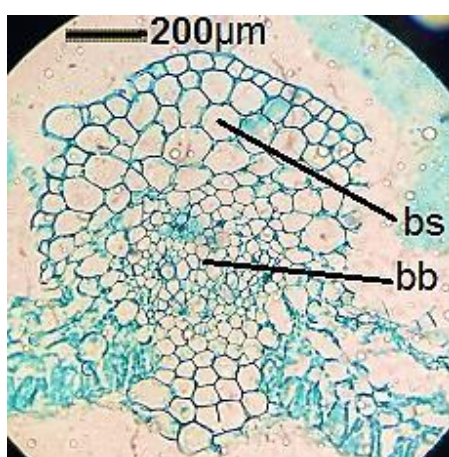
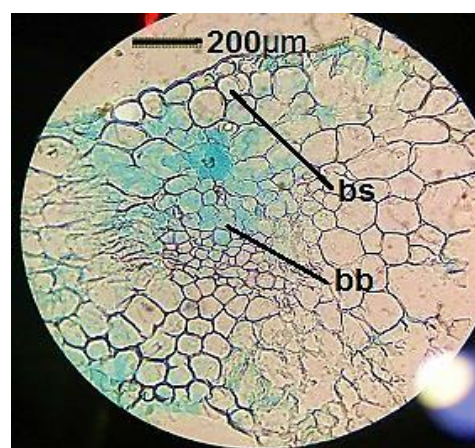
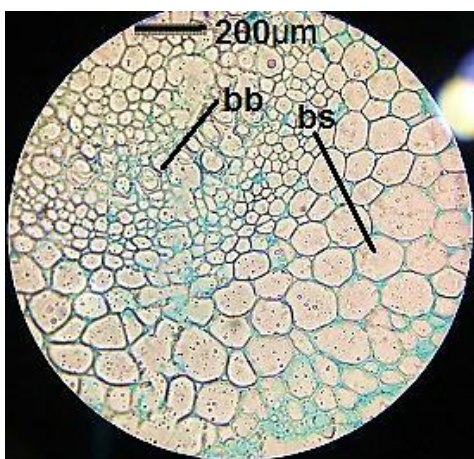
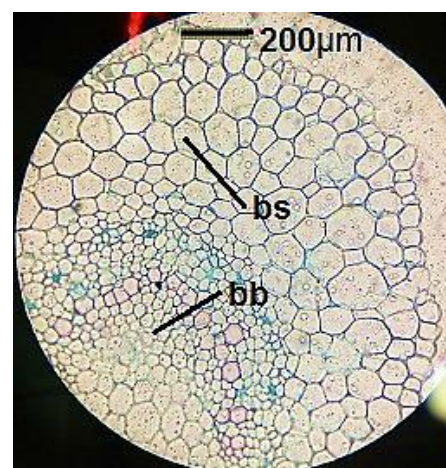
**Figure (1) Effect of nitrogen fertilizer, and ascorbic acid on the anatomical characteristics of paulownia leaves (μm)**

Cu=cuticle ep=epidermis pc=palisid cell sc=sponge cell



nitrogen fertilizer (3.2 g plant<sup>-1</sup>)

Nitrogen fertilizer (comparison)

Ascorbic (60 mg l<sup>-1</sup>)Ascorbic (0 mg l<sup>-1</sup>)Nitrogen fertilizer 3.2 x 60 mg l<sup>-1</sup> Ascorbic

Nitrogen fertilizer 0x0 ascorbic

**Figure (2) Effect of treatment with nitrogen fertilizer and ascorbic acid on the characteristics of the vascular bundles of paulownia leaves (μm)**

bb= big bundle bs=bundle sheath

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**SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF  $\alpha$ -MnO<sub>2</sub> NANORODS BEFORE AND AFTER DOPING WITH SOME TRANSITION METAL IONS****Zahraa Hasan RAHEEM<sup>1</sup>****Mayson Thafir HADI<sup>2</sup>****Saba jaafar AJEENA<sup>3</sup>****Abstract:**

The over use of some antibiotics can lead to the appearance of some resistant bacteria that can cause some serious illnesses. Hence, many intentions to prepare new materials that can act as antibacterial agents have been made over the last few years. Some nanomaterials are known to have good antibacterial activity. The aim of this paper is to describe the synthesis of manganese dioxide  $\alpha$ -MnO<sub>2</sub> nanorods before and after doping with (5% wt.) of the following transition metal ions (Fe<sup>3+</sup>, Co<sup>3+</sup>, Ni<sup>2+</sup> and Cu<sup>2+</sup>) using a simple hydrothermal method and to compare the antibacterial activity of these materials. After the synthesis process, the materials were characterized using XRD, EDX, and FE-SEM to confirm the structure, elemental components, and morphology of the samples. The morphologies of pure samples were nanorods of uniformed size with diameters around (40-60 nm) and lengths around (1-2  $\mu$ m). The doped samples were slightly wider in diameter due to the differences between the ionic radii of Mn<sup>4+</sup> ions and the dopant ions. Afterward, the antibacterial activity of the prepared  $\alpha$ -MnO<sub>2</sub> nanorods (pure and doped) was measured against Escherichia coli using the disk diffusion method to compare the antibacterial activity of the doped and undoped  $\alpha$ -MnO<sub>2</sub> nanorods. The Cu doped sample showed the highest antibacterial activity with an inhibition zone diameter of 24 mm at the concentration of (600  $\mu$ g/ml).

**Key words:** Antibacterial, Doping With Metal Ions, Hydrothermal Synthesis, MnO<sub>2</sub>.



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## Introduction:

Manganese dioxide has been used in many applications for so many years due to its unique chemical and physical properties: it has high electrochemical activity, natural abundance, a low cost, and is environmentally safe <sup>(1, 2)</sup>. It has been used in energy storage devices such as lithium-ion batteries <sup>(3,4,5)</sup>, alkaline batteries and capacitors <sup>(6,7)</sup>, in medicine <sup>(8, 9, 10)</sup>, as antibacterial <sup>(11,12,13,14,15,16)</sup> and antifungal agents <sup>(17)</sup>, as a catalyst <sup>(18, 19)</sup>, gas a sensor <sup>(20,21,22,23,24)</sup>, solar cells <sup>(25, 26)</sup>, and many other applications. Manganese dioxide has variable crystal forms or polymorphs ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\lambda$  and  $\epsilon$ ) <sup>(27)</sup>, which are formed from different connecting ways of the crystal units of  $[\text{MnO}_6]$  octahedral which either are by sharing corners or edges <sup>(28, 29)</sup>. By sharing edges to form a tunnel structure in the form of  $(2 \times 2)$  and  $(1 \times 1)$ , double chains with octahedral units of  $[\text{MnO}_6]$  form a polymorphic  $\alpha$ - $\text{MnO}_2$ . These tunnels have inner spaces of  $(4.6 \times 4.6 \text{ \AA})$  and  $(1.89 \times 1.89 \text{ \AA})$  respectively <sup>(30)</sup>. By using cations (such as  $\text{Ba}^{2+}$ ,  $\text{Ca}^{2+}$  or  $\text{K}^+$ ) or water molecules, large tunnels of the type  $(2 \times 2)$  can be occupied <sup>(31,32)</sup>. For the great importance of manganese dioxide in different industrial fields, the synthesis of these metal oxides has been accomplished in the nano the scale through different synthesis systems, such as co-precipitation <sup>(33,34)</sup>, sol-gel <sup>(35, 36)</sup>, and the hydrothermal method <sup>(37,38,39)</sup>. Doping metal oxides with other metals is a strategy used to enhance their properties <sup>(40, 41)</sup> such as thermal and electrical conductivity, optical, magnetic, and antimicrobial properties <sup>(42, 43)</sup>. Hence, in this paper the synthesis procedure of pure  $\alpha$ - $\text{MnO}_2$  nanorod shapes and  $\alpha$ - $\text{MnO}_2$  nanorod shapes doped with some transition metal ions ( $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  $\text{Ni}^{+2}$  and  $\text{Cu}^{+2}$ ), and the antibacterial activity of  $\alpha$ - $\text{MnO}_2$  nanorods pure and doped with some metal ions are discussed.

## Materials and methods

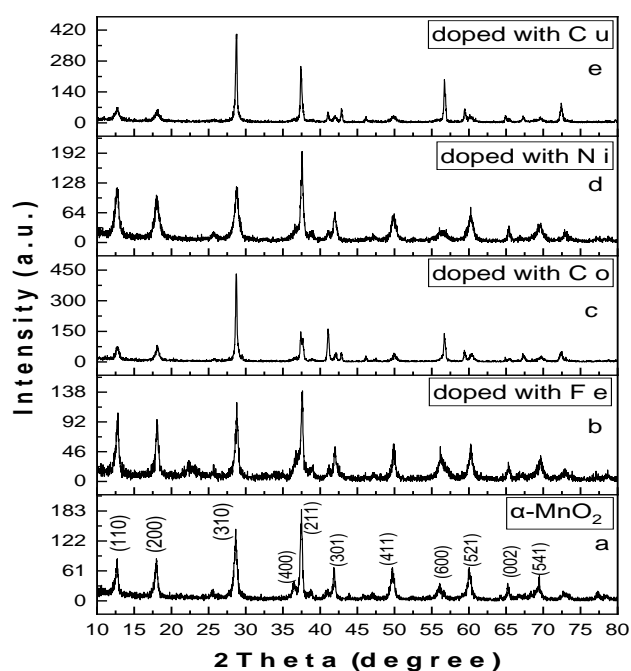
All chemicals and reagents were of high purity and were supplied from Sigma-Aldrich/India. The synthesis procedures of pure  $\alpha$ - $\text{MnO}_2$  nanorods and doped  $\alpha$ - $\text{MnO}_2$  nanorods with the transition metal ions ( $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  $\text{Ni}^{+2}$  and  $\text{Cu}^{+2}$ ) were performed according to the following procedure:

For the synthesis of pure  $\alpha$ - $\text{MnO}_2$  nanorods a redox reaction between  $\text{KMnO}_4$  and  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  with (1:1) mole ratio was used <sup>(44)</sup>, the materials were dissolved in distilled water using a magnetic stirrer and, then the solution was transferred to a (100 ml) Teflon liner filled to 70% of its capacity, then hydrothermally heated at  $160^\circ\text{C}$  for 10 hours, and then the reactor was hydrothermally heated at  $140^\circ\text{C}$  for 12 hours. After that the reactor was left to cool down to room temperature, and the dark brown  $\alpha$ - $\text{MnO}_2$  precipitate was collected, rinsed several times with distilled water followed by drying at  $70^\circ\text{C}$  for 6 hours. For the purpose of synthesizing  $\alpha$ - $\text{MnO}_2$  nanorods doped with metal ions ( $\text{Fe}^{+3}$ ,  $\text{Co}^{+3}$ ,  $\text{Ni}^{+2}$  and  $\text{Cu}^{+2}$ ), the pure  $\alpha$ - $\text{MnO}_2$  nanorod synthesis procedure was applied with some modification where 5% of the weight of these metal ions as nitrate salts ( $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  and  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ) were added separately into the reaction solution followed

by the same steps mentioned earlier. Afterwards, XRD (SHIMADZU/ XRD 6000/Japan) coupled with Cu K $\alpha$  radiation ( $\lambda=1.54180\text{\AA}$ ) was used for characterization, and sample morphology was assessed using FE-SEM (Hitachi /S-4160 /Japan). Finally, (Bruker X Flash /6110) was employed for EDX analysis.

## Results and discussion

Figure (1a) shows the X-ray diffraction patterns for pure and doped  $\alpha\text{-MnO}_2$  nanorods. The diffraction peaks of  $2\theta$  recorded values ( $12.7^\circ$ ,  $18.1^\circ$ ,  $28.8^\circ$ ,  $37.5^\circ$ ,  $41.9^\circ$ ,  $49.8^\circ$ ,  $60.2^\circ$ , and  $69.7^\circ$ ) are indexed to (110, 200, 310, 211, 301, 411, 521, and 541) <sup>(44)</sup> respectively. These values indicate the tetragonal phase of  $\alpha\text{-MnO}_2$  (JCPDS 44-0141) with crystal parameters ( $a=b=9.7847\text{\AA}$ ,  $c=2.8630\text{\AA}$ ) <sup>(45, 46)</sup>. No other additional impurity peaks have been observed for  $\text{MnO}_2$ . Sharp peaks indicate the highly crystallized nature of  $\alpha\text{-MnO}_2$  nanorods. Figures (1 b, c, d and e) shows the XRD patterns  $\alpha\text{-MnO}_2$  nanorods doped with 5% wt. of (Fe, Co Ni and Cu) respectively. The XRD patterns of the doped  $\alpha\text{-MnO}_2$  nanorods had small differences in some peak's intensities, some peaks are enhanced in their intensity due to the doping metals that have become part of the  $\alpha\text{-MnO}_2$  nanorods crystal lattice and some Mn ions are replaced by them, and some other peaks are slightly quenched which may be due to the slight difference in the ionic size diameters of the doping metal ions than of  $\text{Mn}^{+4}$  which may cause some differences in the interplanar space of the crystals <sup>(47, 48)</sup>. Table (1) displays differences in the lattice constant and crystal volume for the doped and pure  $\alpha\text{-MnO}_2$  nanorods. Differences in the values of lattice constants and unit cell volumes are due to the difference between the ionic radii of ( $\text{Mn}^{+4} = 67\text{pm}$ ) and the ionic radii of the dopant metal ions ( $\text{Fe}^{+3} = 78\text{pm}$ ,  $\text{Co}^{+3} = 75\text{pm}$ ,  $\text{Ni}^{+2} = 83\text{pm}$  and  $\text{Cu}^{+2} = 87\text{pm}$ ) <sup>(49)</sup>, the values of lattice constants and unit cell volume in this table increased with the increase in the ionic radii of these dopant metal ions .



**Figure (1):** XRD patterns of (a) pure synthesized  $\alpha$ - $\text{MnO}_2$  nanorod shapes (b) Fe doped  $\alpha$ - $\text{MnO}_2$  nano rods (c) Co doped  $\alpha$ - $\text{MnO}_2$  nano rods (d) Ni doped  $\alpha$ - $\text{MnO}_2$  nano rods (e) Cu doped  $\alpha$ - $\text{MnO}_2$  nano rods.

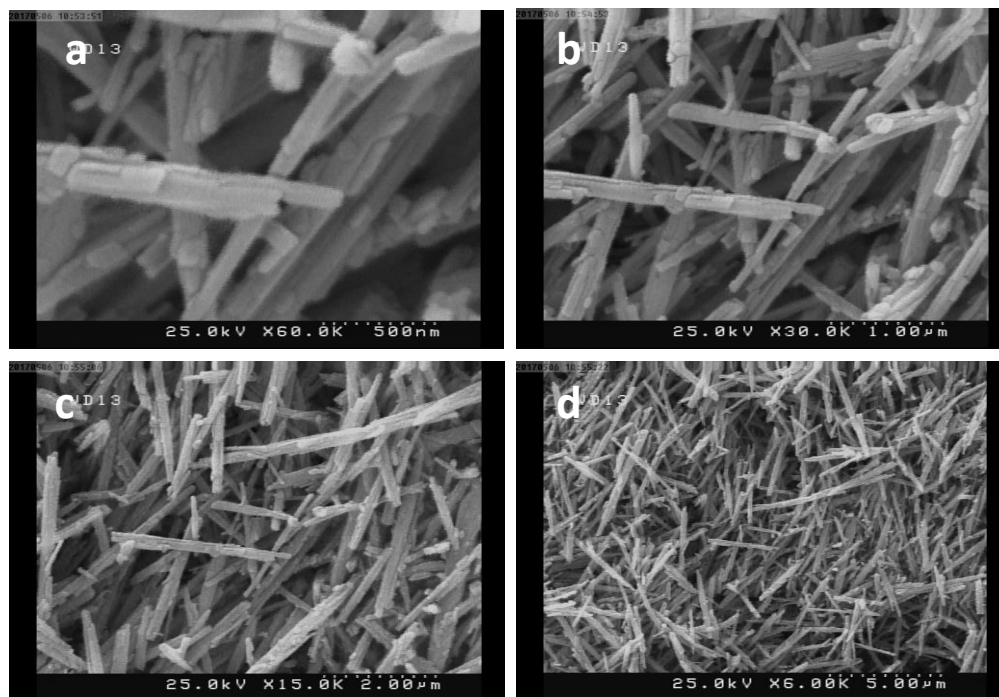
**Table (1):** Calculated lattice constant and crystal volume for the pure and doped  $\alpha$ - $\text{MnO}_2$  nanorods.

sample	Lattice constant $a=b$ (Å)	Lattice constant $c$ (Å)	Unit cell volume (Å <sup>3</sup> )
Pure $\alpha$ - $\text{MnO}_2$ nanorods	9.7373	2.8518	270.394 5
Fe doped $\alpha$ - $\text{MnO}_2$ nanorods	9.7788	2.8560	273.111 6
Co doped $\alpha$ - $\text{MnO}_2$ nanorods	9.7455	2.8525	270.924 5
Ni doped $\alpha$ - $\text{MnO}_2$ nanorods	9.7939	2.8543	273.790 2
Cu doped $\alpha$ - $\text{MnO}_2$ nanorods	9.8114	2.8699	276.270 6

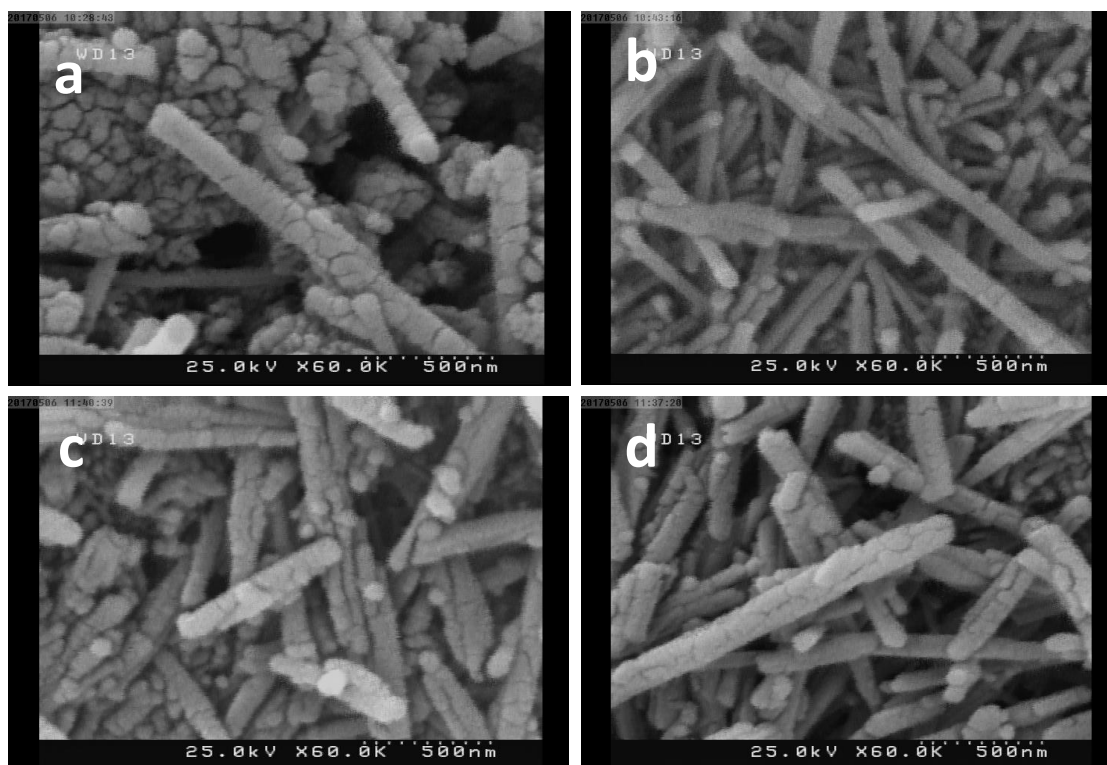
Figure (2) shows the FE-SEM images of pure  $\alpha$ - $\text{MnO}_2$  nano rods in different magnifications, it showed that  $\alpha$ - $\text{MnO}_2$  nanorods had diameters around (40-60 nm) and length about (1-2  $\mu\text{m}$ ) .



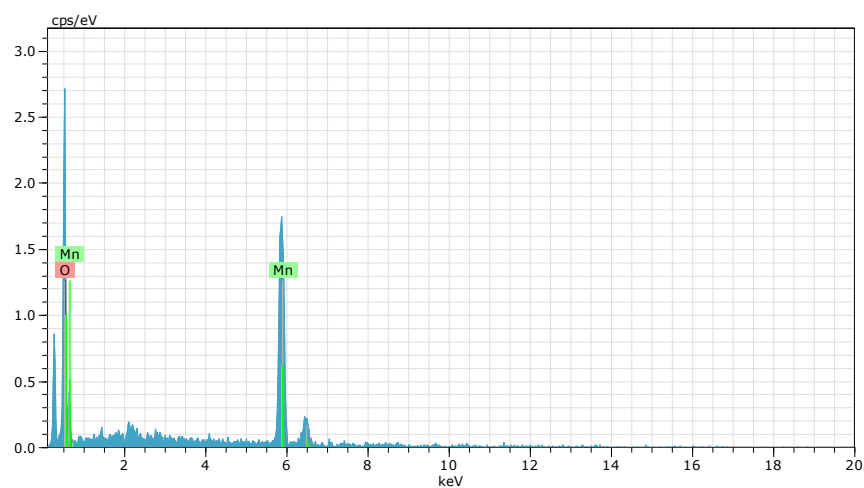
Figure (3) shows the FE-SEM images of doped  $\alpha$ -MnO<sub>2</sub> nanorods, and it is clear that the morphologies of these nanostructures are still nanorods with some increase in the diameters and the surface of the doped nanorods has some roughness compared with the pure  $\alpha$ -MnO<sub>2</sub> nanorods. Figure (4) shows the EDX analysis of pure  $\alpha$ -MnO<sub>2</sub> nanorods and it had 62.52 wt. % manganese and 37.48 wt. % oxygen. Figure (5) shows the EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with iron which display a weight percent of 63.74 % manganese , 26.26% oxygen, 5.80% potassium and 3.50% iron. Figure (6) shows the EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with cobalt and it is displaying a weight percent of 60.99 % manganese , 32.87 % oxygen, 5.98 % of potassium and 3.16% cobalt. Figure (7) shows the EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with Ni, and it displays a weight percent of 63.89 % manganese , 28. 48 % oxygen, 5.22 % potassium and 2.41 % Ni. Figure (8) shows the EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with Cu, and it is showing a weight percent of 61.07 % manganese, 31. 03 % oxygen, 2.67 % potassium and 3.96 % copper.



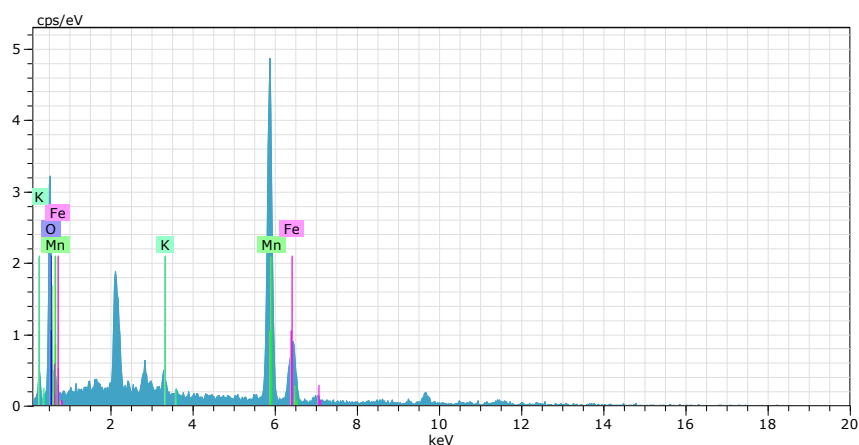
**Figure (2):** FE-SEM images of  $\alpha$ -MnO<sub>2</sub> nanorods in different magnifications.



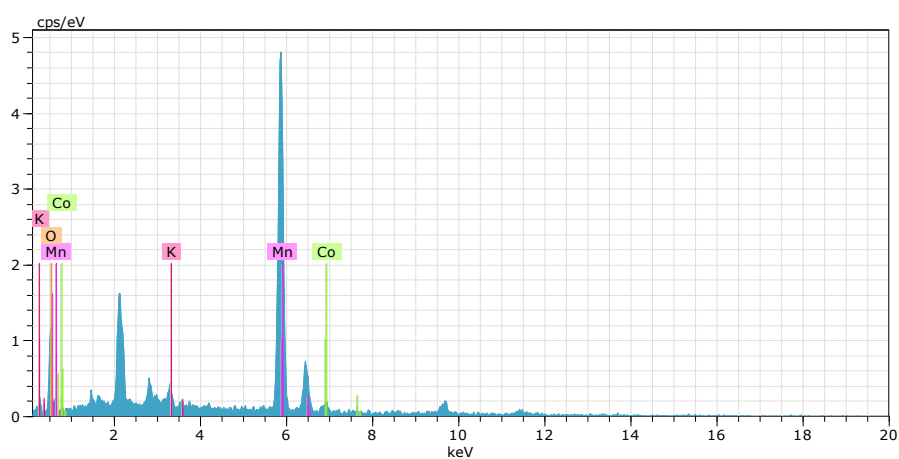
**Figure (3):** FE-SEM images of doped  $\alpha$ -MnO<sub>2</sub> nanorods (a) with Fe (b) with Co (c) with Ni and (d) with Cu.



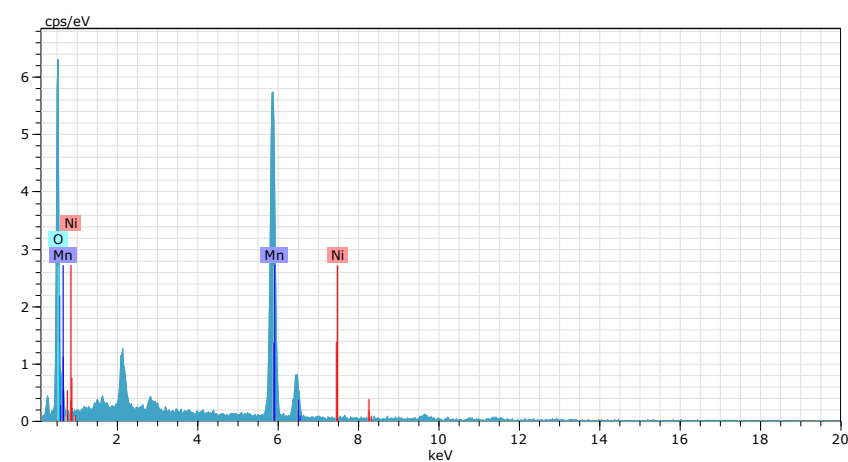
**Figure (4):** EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods.



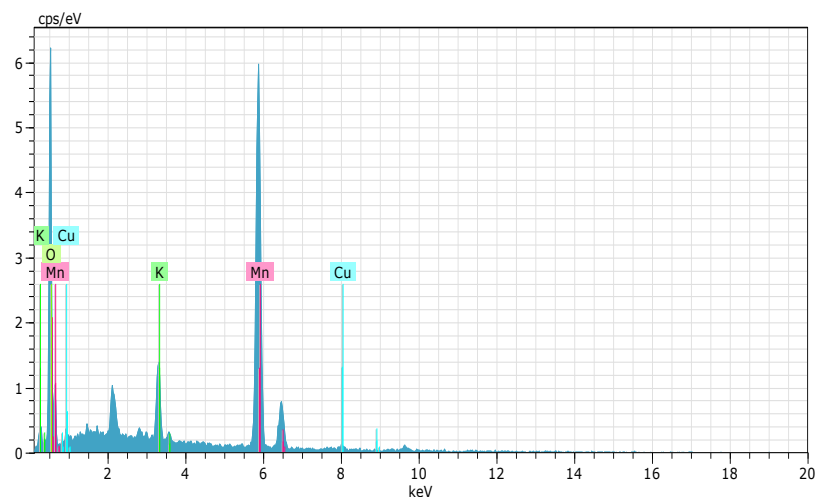
**Figure (5):** EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with (5% wt. Fe).



**Figure (6):** EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with (5% wt. Co).



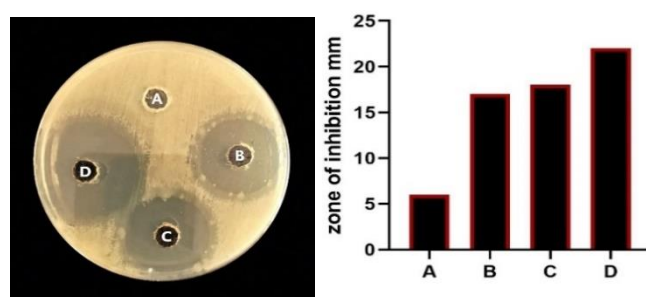
**Figure (7):** EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with (5% wt. Ni)



**Figure (8):** EDX analysis of  $\alpha$ -MnO<sub>2</sub> nanorods doped with (5% wt. Cu)

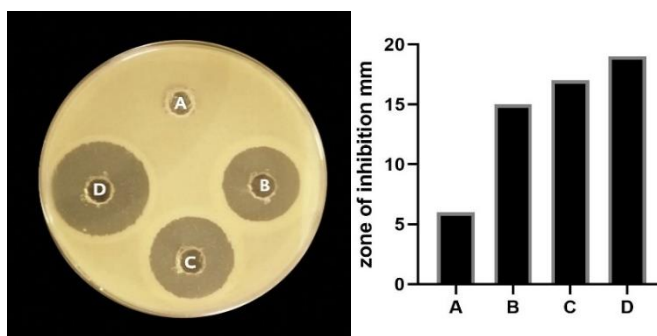
### Antibacterial analysis

In the current study, the ability of pure synthesized samples from  $\alpha$ -MnO<sub>2</sub> nanorods and  $\alpha$ -MnO<sub>2</sub> nanorods doped with (Fe, Co, Ni and Cu) elements to act against (*Escherichia coli*) gram-negative bacteria was investigated using an agar well diffusion assay. Figures (9), (10) (11), (12) and (13) demonstrate results of the antibacterial activity assay. About 20 ml of sterilized Muller-Hinton (MH) agar was poured into sterile Petri dishes. Bacterial samples were prepared from bacterial stock cultures. After culturing, a sterile tip was used to prepare 6 mm-diameter wells in the agar plates. (200, 400 and 600  $\mu$ g/ml) concentrations of each sample were prepared and put into agar wells individually. The cultured plates were incubated overnight at 37°C and the average diameter of growth inhibition zones were measured and recorded. Data from the three experiments were represented as mean  $\pm$  SD. Significant difference was statistically indicated at  $p < 0.05$  (50, 51, 52).

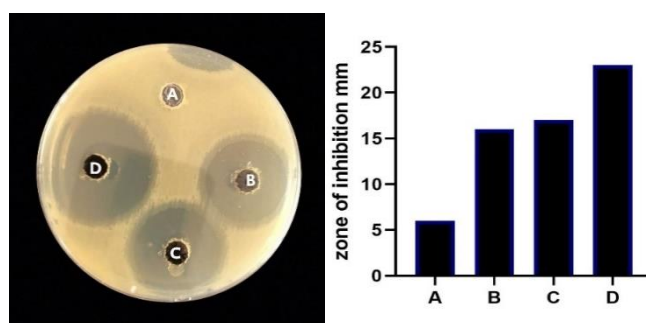


**Figure (9):** On the left the antibacterial activity of synthesized pure

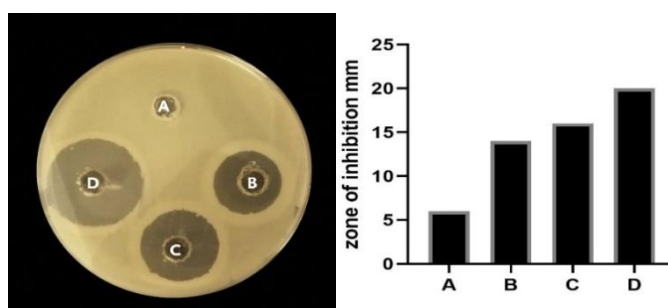
$\alpha$ -MnO<sub>2</sub> nanorods against Gram-negative (*Escherichia coli*) using three concentrations: (A) control (B) 200  $\mu$ g/ml (C) 400  $\mu$ g/ml (D) 600  $\mu$ g/ml. On the right the diagram of the inhibition zone diameters in mm with concentrations.



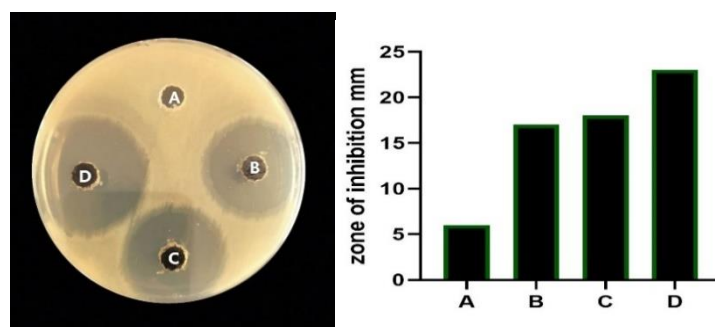
**Figure(10):** On the left the antibacterial activity of  $\alpha$ - $\text{MnO}_2$  nanorods doped with Fe against Gram-negative (*Escherichia coli*) concentrations: (A)control (B)200  $\mu\text{g/ml}$  (C)400  $\mu\text{g/ml}$  (D)600  $\mu\text{g/ml}$  . On the right the diagram of the inhibition zone diameters in mm with concentrations.



**Figure (11):** On the left the antibacterial activity of  $\alpha$ - $\text{MnO}_2$  nanorods doped with Co against Gram-negative (*Escherichia coli*) using concentrations : (A) control(B)200  $\mu\text{g/ml}$  (C)400  $\mu\text{g/ml}$  (D)600  $\mu\text{g/ml}$  . On the right the diagram of the inhibition zone diameters in mm with concentrations.



**Figure (12):** On the left the antibacterial activity of  $\alpha$ - $\text{MnO}_2$  nanorods doped with Ni against Gram-negative (*Escherichia coli*) using concentrations: (A) control(B) 200 $\mu\text{g/ml}$  (C) 400 $\mu\text{g/ml}$  (D) 600 $\mu\text{g/ml}$ . On the right the diagram of the inhibition zone diameters in mm with concentrations



**Figure (13):** On the left the antibacterial activity of  $\alpha$ -MnO<sub>2</sub> nanorods doped with Cu

against Gram-negative (*Escherichia coli*) using three different concentrations (A) control (B) 200  $\mu$ g/ml (C) 400  $\mu$ g/ml (D) 600  $\mu$ g/ml. On the right the diagram of the inhibition zone diameters in mm with concentrations

The diameters of the inhibition zones of pure  $\alpha$ -MnO<sub>2</sub> nanorods and  $\alpha$ -MnO<sub>2</sub> nanorods doped with Co and Cu are listed in table(2).

**Table (2):** The diameters of inhibition zone of pure  $\alpha$ -MnO<sub>2</sub> nanorods and doped with Co and Cu.

Sample	Inhibition zone diameters			
		B	C	D
pure $\alpha$ -MnO <sub>2</sub> nanorods		16	17	22
$\alpha$ -MnO <sub>2</sub> nanorods doped with Fe		15	17	19
$\alpha$ -MnO <sub>2</sub> nanorods doped with Co		17	18	23
$\alpha$ -MnO <sub>2</sub> nanorods doped with Ni		14	16	20
$\alpha$ -MnO <sub>2</sub> nanorods doped with Cu		17	18	24

Both pure and doped samples showed very good inhibition against *Escherichia coli*. The doped samples of  $\alpha$ -MnO<sub>2</sub> nanorods with Cu showed the highest inhibition against *Escherichia coli* at concentration (600  $\mu$ g/ml) with an inhibition zone of 24 mm. The other doped samples also showed improvement in the inhibition against *Escherichia coli*, though the inhibition of all samples was not very high compared to the pure  $\alpha$ -MnO<sub>2</sub> nanorods, which may be due to the low doping percent (5%).

## Conclusions

Synthesis of pure phase of  $\alpha$ -MnO<sub>2</sub> nanorods and doping  $\alpha$ -MnO<sub>2</sub> with some metal ions could be achieved by hydrothermal method. The pure alpha phase MnO<sub>2</sub> was confirmed using XRD, the morphology and dimensions of all the synthesized samples, according to FE-SEM, showed nanorod like shapes, and the elemental composition of the samples were confirmed by the EDX analysis. The antibacterial activity of pure  $\alpha$ -MnO<sub>2</sub> nano rods and  $\alpha$ -MnO<sub>2</sub> nano rods doped with (5%wt) Fe, Co, Ni and Cu was achieved, all samples showed good antibacterial activity against *Escherichia coli*, the  $\alpha$ -MnO<sub>2</sub> nanorods doped with Cu<sup>+2</sup> showed the highest inhibition zone, but not very much higher than the pure  $\alpha$ -MnO<sub>2</sub> nanorods.



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**SECTION OF VETERINARY MEDICINE: MICROBIOLOGY, IMMUNITY AND VIROLOGY.  
THE BACTERIAL CONTAMINATION WITH PROTEUS AND E. COLI IN CERVIX AND  
UTERINE OF COWS DURING THE DIFFERENT ESTRUS PHASES**

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**Abstract:**

The herein research was carried out in order to identified the presence of bacteria in cervix and uterine lumen in Iraqi cattle during the different estrus phase with focusing on Protus and E coli. Estrus phases were determined by the structures which found on ovary (follicular growth for pro-estrus, mature growing follicle for estrus, hemorrhagic corpus luteam for meta-estrus and active corpus luteam for di-eatrus). Forty cervical swabs (ten for each estrus phase) and forty uterine swabs (ten for each estrus phase) were taken from macroscopically healthy reproductive animals after slaughtering and cultivated on nutrient agar and blood agar, the bacterial isolation were identified with biochemical teats. The present study found that (65%) of cervical swabs were bacterial positive and the bacterial isolates were higher in the pro-estrus and meta-estrus phases 70% than estrus and diestrus 60%, the Protus spp. Could not been isolated from cervix or uterine during estrus phases, while E coli isolated during three first phases and disappear during diestrus phase, and appear as 10 single and 10 mixed isolated during follicular phase and metaestrus phase in cervical swabs. A total of five different microorganisms were isolated from cervical swabs (Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus hominies and Staphylococcus epidermidis) with twelve single isolation and fourteen mixed isolation. The present study found that (47.5%) of uterine swabs were bacterial positive and the bacterial isolates were higher in the pro-estrus, estrus and meta-estrus phases 50% than estrus and diestrus 40%, E coli isolated during estrus and diestrus phases only, and appear as 7 single and 2 mixed isolated during those two phases in uterine swabs. A total of five different microorganisms were isolated from uterine swabs (Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus hominies and Staphylococcus epidermidis) with fourteen single isolation and five mixed isolation.



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This research was conclude that the possibility of isolation different bacterial species during different estrus phases in cervical and uterine lumen from slaughtered cows, in addition significantly cervical had more isolates than uterine, and estrus and diestrus phase show lower bacterial isolation than other phases.

**Key words:** Bacterial Flora, Cervical Cavity, Uterine Cavity, Slaughtered Cows, Estrus Phases.

### Introduction:

The genital tract infection with bacteria will reduce reproductive efficiency (Ahmed *et al.*, 2017). The venereal disease that came from bacterial microorganism due to decrease immunity of genital system in addition to the coast of using antibiotics to treat these infections will multiplied the problem (Martins *et al.*, 2009). Several types had been isolated from genital system of ruminant like: *Staphylococcus* spp., *Streptococcus* spp. and *Proteus* spp. (Al-Sariy, 2014). Al-Zubaidi *et al.* (2013) also isolate same microbes' types in addition with *E. coli*, *Klebsiella* and *Pseudomonas*. All these bacterial agents cause reproductive reduce due to genital infection which involve clinical vaginal discharge (Shallali *et al.*, 2001, El-Arabi *et al.*, 2013 and Yaseen *et al.*, 2019). *E. coli* was an opportunistic invader caused genital infections in ruminants animals and isolated all the time from these species of animals (Ababneh and Degefa, 2006; Sargison *et al.*, 2007 and Manes *et al.*, 2010). Those coliform species play a role in pathogens in reproductive cavity (Yaseen *et al.*, 2019), when the animals exposed to stress condition that decrease immunity of reproductive organs then those microbes caused reproductive infection that lead to infertility (Mshelia *et al.*, 2014). The infection of vaginal cavity (vaginitis) often caused from secondary infection mainly *E. coli* (Manes *et al.*, 2010). Some workers found that the bacteria may be found inside uterus after complicated parturition, like *E. coli*, *Staphylococcus* spp. and *Streptococcus* spp. (Mavrogianni *et al.*, 2007). All these bacterial types considered as normal flora exists in vaginal cavity (El-Arabi *et al.*, 2013). The using of progesterone therapy as intravaginal sponge or implant may be lead to vaginitis due to *E. coli* spreading (Vasconcelos *et al.*, 2016). *Staphylococcus aureus* occupy normally the vulva and vagina and caused vaginitis under stress condition (Donders *et al.*, 2002 and Braganca *et al.*, 2017). *Bacillus* spp. and *Corynebacterium* spp. also isolated from ruminant vagina (Manes *et al.*, 2010).

### Materials and methods:

**Sample collection:** bacterial isolation samples were about 80, 40 of it from vaginal cavity and the 40 remaining samples were taken from uterine cavity, from abattoir genital samples after slaughtering from Baghdad province. The estrus phases were determined according to the structures that found on ovaries as: follicular growth to detect pro-estrus phase, mature Graffian follicle to insure estrus phase, hemorrhagic corpus luteum for determine meta-estrus phase and active corpus luteum to insure diestrus phase. Forty cervical and uterine swabs were taken as: ten for each estrus phase from macroscopically

healthy cattle females after slaughter. Samples were taken with transported media (Amies), whole of the genital tracts were cleaned with normal saline washing then transported in thermal container to the laboratory within two hours after slaughtering. The out surfaces were disinfecting with alcohol 70% then swabs was taken from vaginal cavity and uterine cavity after surgical incision.

**Samples culturing:** different culture media were used to bacterial isolation and identifying including blood agar and nutrient agar and incubated under aerobic condition at 37°C for 24 to 48 hrs., the characteristic feature of cultured colonies (shape, size, consistently, color and pigment production) and gram staining under microscope examination. This was done according to (Quinn *et al.*, 2004 and Forbes *et al.*, 2007).

**Bacterial identification:** many biochemical tests were used in order to identifying isolated bacteria include using of coagulase, catalase, oxidase, urease, gelatin liquefaction, hemolysis on blood agar, carbohydrate utilization and motility tests, IMVIC and TSI.

**Statistical analysis:** Statistical test was using Q square test to detect the variation between the percentage between groups at ( $P < 0.01$  and  $P < 0.05$ ). This was done using system of SAS (2012).

## Results:

The table 1 show that the positive isolation and positive percentage of cervical samples during different estrus phases from slaughtering cattle. From ten samples of pro-estrus seven of them were positive isolation which represents 70% of percentage (Table 1). During estrus phase show decrease to 6 (60%) positive isolation, while during meta-estrus phase it was increased to reach 7 (70%) positive isolation, the last phase (diestrus) showed 6 (60%) positive bacterial detection (Table 1). Table 2 shows that five (50%) positive isolation during follicular phase and meta-estrus and then decreased into four (40%) positive isolation during diestrus from uterine swabs. The total isolation from cervical cavity was 65% 26 positive isolation from total 40 swabs, and this percentage decreased from uterine samples to reach 47.5% as 19 positive isolation from total 40 samples (Table 1 and 2). *E. coli* was isolated during the three estrus phases (pro-estrus, estrus and meta-estrus), with 10 single isolation and 10 mixed isolation from cervical samples (Table 3). *Protus* spp. could not isolate from all samples (Table 3 and 4). Five microbes were isolated from cervix cavity as: *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus hominies* and *Staphylococcus epidermidis*, and 12 single isolate and 14 mixed isolate (Table 3). Uterine isolation samples showed that *E coli* isolated during estrus and diestrus phases only, and appear as 7 single and 2 mixed isolated during those two phases in uterine swabs (Table 4). Also five different bacteria were isolated from uterine cavity as: *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus hominies* and *Staphylococcus epidermidis* with 14 single and 5 mixed isolation (Table 4). There was a significant



differences  $P < 0.01$  between cervical swabs and uterine swabs in positive isolation of bacteria.

**Table 1: The number of cervical samples, positive isolation and positive percentage during different estrus phases of slaughtering cows.**

Estrus phase	Samples number	Positive isolation	Percentage %	Significance
Pro-estrus	10	7	70%	nil
Estrus	10	6	60%	nil
Meta-estrus	10	7	70%	nil
Di-estrus	10	6	60%	nil
Total	40	26	65%	nil

• Different small letters represent significant differences at  $P < 0.01$ .

**Table 2: The number of uterine samples, positive isolation and positive percentage during different estrus phases of slaughtering cows.**

Estrus phase	Samples number	Positive isolation	Percentage %	Significance
Pro-estrus	10	5	50%	nil
Estrus	10	5	50%	nil
Meta-estrus	10	5	50%	nil
Di-estrus	10	4	40%	nil
Total	40	19	47.5%	nil

• Different small letters represent significant differences at  $P < 0.01$ .

**Table 3: Types of isolated bacteria from cervical samples during different estrus phases of slaughtering cows.**

Estrus phase	Bacterial isolated types	Single or mixed isolation
Pro-estrus	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	4 single 3 mixed
Estrus	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	3 single 3 mixed
Meta-estrus	<i>Staphylococcus hominies</i> <i>Escherichia coli</i>	3 single 4 mixed
Di-estrus	<i>Streptococcus faecalis</i> <i>Staphylococcus epidermidis</i>	2 single 4 mixed
Total	<i>Escherichia coli</i> <i>Streptococcus faecalis</i> <i>Staphylococcus aureus</i> <i>Staphylococcus hominies</i> <i>Staphylococcus epidermidis</i>	12 single 14 mixed

**Table 4: Types of isolated bacteria from uterine samples during different estrus phases of slaughtering cows.**

<b>Estrus phase</b>	<b>Bacterial isolated types</b>	<b>Single or mixed isolation</b>
<b>Pro-estrus</b>	<i>Streptococcus faecalis</i> <i>Staphylococcus aureus</i>	4 single 1 mixed
<b>Estrus</b>	<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	3 single 2 mixed
<b>Meta-estrus</b>	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i>	3 single 2 mixed
<b>Di-estrus</b>	<i>Staphylococcus hominies</i> <i>Escherichia coli</i>	4 single
<b>Total</b>	<i>Escherichia coli</i> <i>Streptococcus faecalis</i> <i>Staphylococcus aureus</i> <i>Staphylococcus hominies</i> <i>Staphylococcus epidermidis</i>	14 single 5 mixed

### Discussion:

The herein study revealed that there were several isolation were identifying several bacterial species from cervix and uterine. The predominant bacteria was *E. coli* with other four positive bacteria from genital system of cows during different estrus phases form both cervix and uterine cavities. This result was fit with (Bukar-Kolo *et al.*, 2007; Manes *et al.*, 2010; Al-Zubaidi *et al.*, 2013; Mashelia *et al.*, 2014 and Ahmed *et al.*, 2017) who found that there were 6 different types of bacteria inside ruminant genital system frequently isolated, (Bukar-Kolo *et al.*, 2007) stated that the *E. coli* was 44% of isolation. Also the (Al-Hilli and Ajeel, 2015) said that dominate bacteria isolation from genital tract was *E. coli*. These types of isolated bacteria was disagree with (Al-Delemi, 2005) who said that *actinomyces*, *Klebsiella* and *Staphylococcus* were most isolated than other types in small ruminant. The source of *E. coli*, *Staphylococcus aureus* and *Streptococcus* spp. from fecal contamination. This was agreeing with result of (Shallali *et al.*, 2001) in their study on small ruminants. The localization of bacteria inside of vagina and uterine cause infertility in ruminants. This was fact with (Ahmed *et al.*, 2017). It was very important to identify these bacterial types in view to decrease fertility. This was became fit the recommendation of (Ahmed *et al.*, 2017). There were several positive isolation from cervix and uterine cavity could be done, this indicate that the presence of normal flora inside reproductive organs without causing any infection. This agree with several studies (Aziz *et al.*, 2000; Al-Hilali and Al-Delemi, 2001; Al-Delimi, 2002, Al-Delemi, 2005 and Ahmed *et al.*, 2017). Otherwise Zaid (2009) determine about 12 positive isolation from reproductive tract of ruminant and the probability to isolate more than one type in the same swab sample. Martins *et al.* (2009) stated that changing of pH of vagina during estrus could change microflora.



**Conclusion:**

this research indicated the possibility to isolate various bacterial types during estrus phases in cattle from cervix and uterine cavities, as total 5 different types of bacteria were isolated and the predominant one was the *E. coli*. The cervical isolation was significantly more than uterine swabs, and the estrus and diestrus phases were the lower percentage of isolation than other estrus phases.

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## THE EFFECT OF ADDING DATE PALM RESIDUES, IRRIGATION WATER, AND HUMIC ACIDS ON ONION YIELD AND WATER CONSUMPTION (*ALLIUM CEPA* L.)

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### Abstract:

To investigate the influence of adding date palm residues and humic acids, as well as the level of irrigation water quantity, on onion yield, water consumption, and water usage efficiency an experiment was implemented in 2018 2019 season. Date palm residues were applying at two concentration (0 and 5% of the soil volume), and humic acids were added with irrigation water at three concentration (0, 15, and 30) kg.ha<sup>-1</sup>, with irrigation water added at 100 and 50% of the net irrigation depth. Within the Randomized Complete Block design with three replicates, the study treatments were dispersed using the split-split plots approach. On the 22nd of December 2018, onion bulblets (local variety) were planted, and irrigation was planned based on the American evaporation basin, type A. The results show that adding date palm residues 5% increased total bulbs yield and water use efficiency significantly; additionally, adding humic acids with irrigation water at 15 kg.ha<sup>-1</sup> increased total bulbs yield and water use efficiency significantly; and the amount of irrigation water at 100% increased total bulbs yield but had no effect on water use efficiency. The interaction treatments between the study factors also have a an effect on total bulbs yield and water use efficiency.

**Key words:** Irrigation, Date Palm, Water Consumption, Onion, Bulb.



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## Introduction:

Onion (*Allium cepa* L.), a member of Alliaceae family, is one of Iraq's and world's most significant strategic crops. This is significant since it is an antioxidant and anticancer agent (Patil, Bhimanagouda S and Pike, Leonard M and Yoo 1995). The applying of soil conditioners improves soil qualities such as moisture retention, water conductivity, and bulk density decrease, also gases diffusion such as O and CO<sub>2</sub> (Ati *et al.* 2007). (Kahlel2013) Using poultry manure (Italpollina) on three rats (20, 30, 40 kg. 100 m<sup>-2</sup>) and discovered a rate of 40 kg. Increase considerably the (vegetative growth parameters, the average bulb weight, the average bulb diameter, the plant, and the total bulb production).

(Almamori *et al.*, 2020) discovered that incorporating vermicompost into potato plants greatly enhances the number of tubers per plant, plant production, total yield, nitrogen, and phosphorus availability in the soil. It has been discovered that using Monocarps leftovers as soil conditioners in potato plants is beneficial. (Al-Dulaimi, 2020). (Sajidet al., 2012) used Humic at four different levels and discovered that the level 2 kg. ha<sup>-1</sup> had a significant effect on some vegetative growth, and bulb weight, and total yield compared to the other levels. Humic acid contributes significantly to soil pH improvement, which increases the availability of elements for absorption by plant roots and, as a result, improves plant development and production (Marschner 2012).

Doklega 2017) discovered that applying humic acid at rates of 5, 10, and 15 kg.fed<sup>-1</sup> resulted in significant increases in plant dry weight, plant height, chlorophyll content, crude protein percent, total carbs percent, TSS percent, sulfur volatile oil percent, and total bulb yield. At 15 kg. fed.<sup>-1</sup>, (Al-Fraihat1 *et al.*, 2018) found that foliar application of onion plants with Humic acid significantly increased all vegetative growth characters than control.

To reduce water usage, numerous organic and inorganic components were added to the soil (Evans 2004).. Iraq suffers from water shortages due to a lack of yearly water flows from the Tigris and Euphrates rivers (AL-Shahrabali 2009). Many strategies have been implemented to reduce water needs and through creative scientific methods, such as, adding small amount of water that are less than the real water requirement (Ati *et al.*,2010). The use of current irrigation technologies is a progression of our understanding of water transfer from soil to plants, which leads to optimal water use, and that the process of water transfer in the root zone is influenced by soil and plant features as well as climatic elements (Shankar *et al.*, 2013). As a result, determining water demands became vital, particularly in arid and semi-arid countries where water is a major determinant of production (Al-Janabi 2005) The seasonal water consumption of the onion crop was 353 and 392 mm for conventional drip and strip drip irrigation, respectively, while the efficiency of water utilization when employing different irrigation periods of 2 days and 3 days under the drip irrigation system was 0.94 and 1.02 tons (Bagali, A., H. Patil 2012).

The study sought to determine the influence of date palm and humic acid in yield, water consumption, and water use efficiency in onion crops.

## Materials and Methods

The experiment was carried out at Anbar on a soil with the parameters listed in Table 1. (Olsen *et al.*, 1982).

**TABLE 1.** Pre Cultivation Properties of Farm Soil

Soil properties	Value	Method
	Sandy Clay Loam	
Soil texture	Clay      silt      sand	Method of mechanical analysis (hydrometer)
	g.kg <sup>-1</sup>	
	240      232      115	
Organic matter g.kg <sup>-1</sup>	8.3	Potassium dichromate oxidation method
CaSO <sub>4</sub> g.kg <sup>-1</sup>	31	Hydrochloric acid extraction
CaCO <sub>3</sub> g.kg <sup>-1</sup>	119	Calcimeter method
PH	7.5	PH- Meter device
Electrical      Conductivity	1.6	EC-Meter device
dS.m <sup>-1</sup>		
Nitrogen	53	Kildahle method
Phosphorus      g.kg <sup>-1</sup>	27	Spectrophotometer
Potassium	144	Flame photometer
Field capacity %	30	Pressure membrane device
Bulk density Mgm.m <sup>-1</sup>	1.28	Paraffin wax method

In this study, the split-plots system inside the RCBD (randomized complete blocks) design was employed, with three replications and three factors as follows:

- 1- Date palm residues: Date trash was added to the soil at two concentrations (0 and 5% of total soil weight) in main plots.
- 2- Humic acids: Humic and fulvic acids were applied to the soil at three concentrations (0, 15, and 30 kg.ha<sup>-1</sup>) and placed in secondary plots.
- 3-Irrigation Depth: Irrigation water was put in the subplots at two levels (100 and 50 percent of the net depth of irrigation).

Before cultivation, the land was ploughed and leveled, and fertilizer was applied, with DAB (Di-Ammonium Phosphate) fertilizer and potassium sulfate applied at rates of 260 kg in P<sub>2</sub>O<sub>5</sub>/ha and 200 kg K<sub>2</sub>O/ha, respectively, and urea fertilizer applied in two batches, the first after two months and the second after three months (Sebah et al.1991).

On the 22<sup>nd</sup> of December 2018, onion bulbs of a local variety were planted 20 cm apart in a single line in the center of the furrow . Each experimental unit has three 40 cm broad ridges totalling 4.8 m<sup>2</sup> in size. In the experimental unit, a total of 60 plants were employed. Irrigation was then planned using the evaporated water from the American

evaporation pan class A by adding 100% and 50% of the net irrigation depth. Where the following equations were used to measure the net irrigation depth:

1- Calculation of evaporation - reference transpiration: (Al-Hadithy 2010).

$$ET_o = K_p * E_{pan} \quad (1)$$

Where:

ET<sub>o</sub>: evapotranspiration reference (mm. Day<sup>-1</sup>)

E<sub>pan</sub>: - measured evaporation from the basin (mm. Day<sup>-1</sup>)

K<sub>p</sub>: - a coefficient specific to the evaporation basin and varies according to the type of the basin, the vegetation cover surrounding the basin, and the nature of the soil surface, as mentioned in (Al-Hadithy, (2010).

2-Calculation of evaporation: the actual yield, which is equivalent to the actual water consumption of a potato crop, watered using surface irrigation or sprinkler methods , as calculated by the following equation:

$$ET_a = K_c * ET_o \quad (2)$$

As

ET<sub>a</sub>: - actual evaporation - transpiration (mm.day<sup>-1</sup>)

K<sub>c</sub>: - yield factor

The values (0.70 - 0.80, 0.95 - 1.1, 0.85 - 0.90 and 0.75 - 0.85) mentioned in (FAO 1998).

With drips that discharge at a rate of 4 liters per hour<sup>-1</sup>. Tape was utilized to create a drip irrigation system. Irrigation water was applied in two batches separated by six hours using the double addition technique. The irrigation time was calculated using the equation in (Al-Hadithi, 2010).

$$q \times t = a \times d \quad (3)$$

Whereas

q: drainage gave to the sidelines (m<sup>3</sup>. hour<sup>-1</sup>), t: irrigation time (hour), a: planted area (m<sup>2</sup>), and d: added water depth (m).

Study indicators

At the end of the experiment, the bulbs for all plants in the experimental equipment were manually gathered, and the measurements listed below were taken:

1- The overall bulb yield, derived from the experimental unit's production relative to the hectare area.

2- Water Use Efficiency (WUE) or Water Productivity: Using the equation given in (Hillel 2008), the water use efficiency (WUE) or water productivity was calculated by dividing the total yield (kg.ha<sup>-1</sup>) by the volume of water added (m<sup>3</sup>.ha<sup>-1</sup> season) (Doorenbos 1977).

$$WUE (kg.m^{-3}) = \frac{\text{Total Yied (kg.ha}^{-1})}{\text{Added water quantity (m}^3\text{.ha}^{-1})} \quad (4)$$

The data were analyzed using the utilized design at a probability threshold of 0.05, and the means were verified using the L.S.D. test (Al-Rawy 2000).

## RESULTS and DISCUSSION

### Water consumption of the onion crop

Table 2 displays the values of water consumption of onion plants based on growth phases, as the depth of the added water reached 22.8 mm during the germination stage, then rose with the advancement of the growth stage as the plants' need for water increased, in addition to raising the temperature as the growth season progresses. Also, the table shows that the most water depth was added during the bulb formation stage, reaching 84.68 mm at 100 percent irrigation depth treatment and then decreasing to 48.68 mm for the same treatment during the full maturity stage, which could be due to a decrease in the plants' need for water at this stage when the plant tissues mature and dry. While it reached the greatest depth of rainwater at the period of bulb formation, as it reached 87 mm, it had a positive impact on limiting the onion crop's water use. As a result of this, supplementary irrigation using rainwater, particularly in assured or semi-guaranteed rain locations, can be used to irrigate the onion crop while restricting water use for this crop.

Table 1 depicts the change in the number of irrigations at each stage of plant growth, with a total water consumption of 248.28 mm. Season<sup>-1</sup> and the greatest number of irrigations occurred during the vegetative development stage. This outcome might be attributed to the length of time for this stage (53 days), which corresponds to (Salem 2006), in addition to the total rainfall depths throughout the growth season, which was 170.75 mm. Table 1 shows that the overall water demands for onion crop during the growth season reached 3.977 m<sup>3</sup>.ha<sup>-1</sup>, with the germination stage requiring the least amount of water (208.5 m<sup>3</sup>.ha<sup>-1</sup>), accounting for 5.24 percent of the total water needs.

Table 1 also shows that water demands rise as the growing season progresses, with the greatest value during the bulb formation stage reaching 1605.15 m<sup>3</sup>.ha<sup>-1</sup> and accounting for 40.36 percent of total water needs. (Abubaker et al 2006).

**TABLE 2.** Depth of adding water and rainfall depth during growth season

Growth Stages	Times Interval	Irrig. Events	water Depth (mm.) 50%	Addition 100%	Rainfall Depth (mm.)	Water Uses (m <sup>3</sup> .ha <sup>-1</sup> )
Germination	22 /12/2018- 4/1/2019	2	22.80	22.80	5	208.5
Vegetative Growth	5/1- 26/2/2019	6	20.12	43.04	67.75	981.825
Flowering	37/2- 20/3/2019	3	24.50	49.00	9.75	624.375
Yield content	21/3- 20/4/2019	5	43.34	84.68	87	1605.15
Maturity	21/4- 1/5/2019	1	24.38	48.76	1.25	557.925
Total		17	131.34	248.28	170.75	3.977.775



Table 3 demonstrates that adding 5% date palm residue to the soil resulted in a significant increase in overall bulb production, with (18.770 ton.ha<sup>-1</sup>) than zero applying treatment. Furthermore, irrigation at 100% resulted in significant improvement in over-irrigation at 50%. The best humic acid concentration was (15 kg ha<sup>-1</sup>) .

The combination of applying Date palm and irrigation at (100%), was significantly superior than other treatments(20.073 ton. ha<sup>-1</sup> ) in the other hand the lowest bulb yield was (9.100 ton. ha<sup>-1</sup>)

Combination treatment of applying date palm 5% with humic 15 Kg. ha<sup>-1</sup> gave the maximum bulb production ,while the combination treatment of adding the date palm 5 % and humic 15 Kg. ha<sup>-1</sup>, gave the lowest bulb yield (2.500 ton. ha<sup>-1</sup>) In the relationship between irrigation and humic, the combination treatment of irrigation 100% and the applying of humic 30 kg.ha<sup>-1</sup> produced higher total bulb yield (24.110 ton.ha<sup>-1</sup>), while the combination treatment of irrigation 50% and the applying humic 15 kg.ha<sup>-1</sup> produced the lowest bulb yield (3.550 ton.ha<sup>-1</sup>).

The greatest bulb production from the triple interaction was from combination treatment of 5% date palm residues, 100% irrigation, and humic, 15 kg.ha<sup>-1</sup>, while the lowest bulb yield was from the combination of zero date palm, 50% irrigation, and humic, 15 kg.ha<sup>-1</sup>.

**TABLE 3.** Effect of date palm residues, humic acid, and the level of irrigation in total yield of bulbs (ton.ha<sup>-1</sup>).

Date residues(D)	Irrigation Depth (I)	Humic acids kg. ha <sup>-1</sup> (H)			(D)*(I)	(D) means	(I) means
		0	15	30			
0%	50%	2.000 k	15.000 f	10.300 h	9.100 d	9.966 b	13.283 b
	100%	3.000 k	17.400 e	12.100 g	10.833 C		15.453 a
	50%	5.1000 j	27.300 b	20.000 d	17.466 b		18.770 a
5%	100%	7.200 i	30.820 a	22.200 c	20.073 a		
(D)*(H)	0%	2.500 f	16.200 c	11.200 b			
	5%	6.150e	29.060 a	21.100 b			
	50%	5.100e	3.550 f	24.110 a			
(I)*(H)	100%	21.150 b	17.150 c	15.150d			
	(H) means	4.325 c	22.630 a	16.150 b			

\*According to the Duncan multiple range test, means followed by the same letter are not substantially different at the P=0.05 level of probability.

The applying of date palm 5% increased water use efficiency and gave (7.00 Kg. m<sup>3</sup>) than the not additional treatment that . While irrigation treatments show no differences. In

the other hand humic acid ( $15 \text{ kg. ha}^{-1}$ ) inras significantly the water use efficiency as mentioned in (table4)

Considering the binary combination interaction between date palm and irrigation, it was found that the highest water use efficiency was ( $7.66 \text{ Kg. m}^3$ ) from combination treatment of applying date palm and irrigation at 50%, while the lowest water use efficiency was ( $3.40 \text{ Kg. m}^3$ ) from the combination between zero date palm and irrigation at 100%.

The maximum water usage efficiency was discovered for the binary interaction of date palm 5% and humic  $15 \text{ Kg. ha}^{-1}$  which was ( $10.90 \text{ Kg. m}^3$ ), whereas the lowest water use efficiency was ( $0.85 \text{ Kg. m}^3$ ) from the combination treatment of zero date palm and zero humic

In the bilateral combination of irrigation and humic, it was found that the highest water use efficiency ( $9.30 \text{ Kg. m}^3$ ) was from the combination treatment of irrigation 100% and the applying of humic  $15 \text{ kg.ha}^{-1}$ , while the lowest water use efficiency was ( $1.50 \text{ Kg. m}^3$ ) from the combination of irrigation 100 % and the applying of humic  $0 \text{ kg.ha}^{-1}$

.From the triple combination the highest water use efficiency was ( $12.00 \text{ Kg. m}^3$ ) from the combination of date palm 5%, irrigation 50% and humic,  $15 \text{ kg.ha}^{-1}$ , , while the lowest water use efficiency ( $0.80 \text{ Kg. m}^3$ ) was from the combination of zero date palm , 100% irrigation, and zero humic.

**TABLE 4.** Effect of date residues, humic acid, and the level of irrigation in water use efficiency (WUE) ( $\text{Kg. m}^3$ ).

Date residues(D)	Irrigation Depth (I)	Humic acids $\text{kg / ha}^{-1}$ (H)			(D)*(I)	(D) means	(I) means
		0	15	30			
0%	50%	0.80 f	6.60 bcd	4.50 def	3.96 b	3.68 b	5.81 a
	100%	0.90 f	5.50 cde	3.80 def	3.40 b		4.86 a
5%	50%	2.20 ef	12.00 a	8.80 abc	7.66 a	7.00 a	
	100%	2.20 ef	9.80 ab	7.00 bcd	6.33 a		
(D)*(H)	0%	0.85 e	6.05 bc	4.15 cd			
	5%	2.2 de	10.90 a	7.90 b			
(I)*(H)	50%	1.50 c	9.30 a	6.65 ab			
	100%	1.50 c	7.65 ab	5.40 b			
(H) means		1.50 c	8.47 a	6.02 b			

\*According to the Duncan multiple range test, means followed by the same letter are not substantially different at the  $P=0.05$  level of probability.

According to the findings, the examined parameters had a substantial impact on bulb yield and water usage efficiency. The applying of dates palm at 5 %, humic acid at 15 % and irrigation at a 100% level led to the highest value of bulbs yield while the highest values of water use efficiency were from the applying of dates palm at 5 %, humic acid at 15

% and irrigation at 50% level . This results is in harmony with that found by ( Ghehsareh 2013).

The applying of soil conditioners improves soil characteristics such as water conductivity, moisture retention, and bulk density decrease, also enhancing gases diffusion such as O and CO<sub>2</sub>. (Hassan 1994 ; *Ati, et al.*, 2007).

(El-Dardiry 2007) indicate that reducing the effect of water stress by applying date palm which improving the distribution of soil pores.

Increasing absorption capacity of nutrients in the soil by applying humic acid which chelating the elements or reducing the loss of these nutrients (Tan 2003 ;Pettit 2003). Sajid et al., 2012 revealed that humic acid increasing the fertility of the soil, and increase the availability of essential nutrients which increase in the yield of bulbs . Gerjes (2013) and Bettoni (2016)revealed that spraying humic acid on onion plants increased growth, bulb yield, and chemical composition.

(Calvo, 2014) revealed that Humic substances promoting plant growth through enhancing root growth and nutrient uptake so it considered as plant bio stimulants. (Baldotto et al., 2009; Oliveira et al., 2009;Gulser, 2010; Daur 2013) and increasing photosynthesis. (Baldottoet al., 2009 ;Ertani et al., 2011), , cell respiration and protein synthesis, enzyme activities (Allison 2006), and leaf water retention (Bettoni et al., 2014).

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## ANTIOXIDANT EFFECT OF AQUEOUS EXTRACT OF (DIANTHUS CARYOPHYLLUS L.) ON MICE EXPOSED TO OXIDATIVE STRESS

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### Abstract:

This study aims to investigate the antioxidant effect of the aqueous extract of cloves (*Dianthus caryophyllus* L.) at a concentration of, 150 mg / kg of body weight. on stress oxidative induced by hydrogen peroxide H<sub>2</sub>O<sub>2</sub> at a concentration of 1% on male mice Swiss, as some biochemical variables were measured in blood serum, such as vitamin C and vitamin E, The level of glutathione was also measured GSH and malondialdehyde MDA, and the superoxide enzyme dysmutasis SOD, where the results showed that the treatment with H<sub>2</sub>O<sub>2</sub> led to a significant decrease compared with the control group, treatment with an aqueous extract of cloves at a concentration of. 150 mg / kg, a significant increase ( $P \leq 0.05$ ) in the level of vitamin E, C, SOD, and GSH, so a decrease significantly ( $P \leq 0.05$ ) in the MDA in the groups that were treated with it compared with the control group.

We conclude that the aqueous extract of cloves has an antioxidant effect on exposed mice for oxidative stress.

**Key words:** Antioxidant, *Dianthus Caryophyllus* L, Oxidative Stress.



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## التأثير المضاد للأكسدة للمستخلص المائي للقرنفل (*Dianthus caryophyllus* L.)

### على الفئران المعرضة للإجهاد التأكسدي

سلوان وعبدالله يوسف

خنساء عزيز يونس

#### ملخص:

يهدف هذا البحث إلى دراسة التأثير المضاد للأكسدة للمستخلص المائي لنبات القرنفل (*Dianthus caryophyllus* L.) بتركيز 150 ملغم / كغم من وزن الجسم على الإجهاد التأكسدي المستحدث بواسطة بيروكسيد الهيدروجين  $H_2O_2$  بتركيز 1% على ذكور الفئران السويسرية إذ تم قياس بعض المتغيرات الكيمو حيوية في مصل الدم مثل فيتامين C وفيتامين E كذلك تم قياس مستوى الكلوتاثيون GSH والمالوندايديهايد MDA وانزيم السوبراوكسيد دسميوتيز SOD حيث أظهرت النتائج ان المعاملة ب  $H_2O_2$  ادى إلى انخفاضاً معنوي  $P \leq 0.05$  في مستوى فيتامين C، و GSH، SOD وارتفاعاً معنوياً  $P \leq 0.05$  في MDA مقارنة مع مجموعة السيطرة، وقد أظهرت المجاميع المعاملة بالمستخلص المائي لنبات القرنفل بتركيز 150 ملغم / كغم ارتفاعاً معنوياً  $P \leq 0.05$  في مستوى فيتامين C، و SOD، GSH وانخفاضاً معنوياً  $P \leq 0.05$  في MDA في المجاميع التي عوملت به مقارنة مع مجموعة السيطرة، نستنتج ان المستخلص المائي لنبات القرنفل له تأثير مضاد للأكسدة على الفئران المعرضة للإجهاد التأكسدي.

**الكلمات المفتاحية:** المضاد للأكسدة، *Dianthus caryophyllus* L، الإجهاد التأكسدي.

#### المقدمة

مضادات الأكسدة Antioxidant هي جزيئات قادرة على أبطاء أو منع تأكسد الجزيئات الشاردة الجذور الحرة Free radical وهو تفاعل يقوم بتحويل الإلكترونات من مادة معينة إلى عامل مؤكسد الذي يعمل على اتلاف الخلايا حيث تقوم مضادات الأكسدة بالمحافظة على الخلايا من التلف الذي تسببه الجذور الحرة (1)

والجذور الحرة هي نتاج طبيعي من عمليات الايض كما ان الجهاز المناعي يستخدمها من اجل القضاء على البكتريا وبالتالي حاجتها إلى مضادات الأكسدة امر لافهم منه (2)

والطبيعه غنية بالعديد من من النباتات والمركبات الموجودة في معظم الخضراوات والفواكه والأعشاب الحاوية على مضادات الأكسدة لتحمي النباتات نفسها من الشوارد الحرة ويمكن للانسان ان يستفاد من خصائصها عندما يتناولها وتشمل الكاروتينات Carotenoid والفلافونويدات Flavonoids وعديد الفينولات polyphenoles (3)

تعمل مضادات الأكسدة الموجودة في النباتات كمضادات للالتهابات حيث انها تمنع أكسدة الدهون وايضا تعمل على زيادة المناعة حيث تحمي من بعض أنواع السرطانات والتأثيرات الجانبية للأدوية المضادة للسرطان وتعمل على مكافحة الشيخوخة (4، 5)



ويعد نبات القرنفل Clove نوع من نباتات الفصيلة الاسبية وهو من اقدم واشهر التوابل، وبذورها تشبه المسامير وهي أكثر الأجزاء استعمالاً وتسمى ايضاً بعود النوار ويكون لونها أحمر ويتحول إلى البني عندما تصبح يابسة (6)

يوصف القرنفل بأنه طارد للحمى، ومطهر ومعقم للمعدة، يشفي من القروح ويحمي من الأوبئة ويساعد على الهضم ومسكن لآلام الاسنان ويقلل الالتهابات والحساسية وينبه القلب والمعدة ويستخرج من بذور القرنفل زيت طيار يسمى الاوجينول Eugenol ذات خصائص علاجية واسعة، وخاصة لآلام الاسنان (7)

### المواد وطرق العمل

حيوانات الدراسة: استخدمت ذكور الفئران البيض السويسرية Mus musculus بأعمار تتراوح بين 3-4 اشهر واوزان 25-30 غم مع اعطائها العليقه القياسيه وظروف اضاءة وتهوية مناسبة .

تحضير المستخلص المائي لنبات القرنفل: تم شراء بذور نبات القرنفل من الاسواق المحلية لمدينة الموصل نقيت من الشوائب وغسلت جيداً ثم تركت لتجف بعدها تم تحضير المستخلص المائي لبذور نبات القرنفل حسب طريقة (8) حيث نقيت البذور من الشوائب وغسلت جيداً ثم تركت لتجف بعدها تم سحق البذور باستخدام الهاون الخزفي وأخذ 150 غرام من المسحوق وضيف اليه الماء المقطر واكمل الحجم النهائي إلى 1 لتر وترك لمدة 30 دقيقة في جهاز الهزاز الافقي بعدها وضع الراشح في جهاز الطرد المركزي وبسرعة 3500 دورة /دقيقة لمدة 15 دقيقة بعد ذلك تم تبخير الماء من المستخلص باستخدام جهاز Vacuum evaporator ثم تم حفظ المستخلص لحين الاستخدام في التلاجة.

- تحديد الجرعة الفعالة: تم استخدام 15 فاراً ذكراً وقسمت إلى ثلاثة مجاميع واعطيت تراكيز مختلفة من المستخلص المائي لنبات القرنفل لمعرفة الجرعة الفعالة وهي 150 ملغم /كغم من وزن الجسم.
- تصميم التجارب : قسمت الحيوانات إلى اربع مجاميع وكل مجموعة احتوت على خمسة حيوانات.
- المجموعة الاولى:مجموعة السيطرة وقد اعطيت ماء الشرب الاعتيادي مع تجريعها لمدة 30 يوم بماء مقطر.
- المجموعة الثانية: تم اعطاؤها 1٪ بيروكسيد الهيدروجين مع ماء الشرب لمدة 30 يوم .
- المجموعة الثالثة: تم تجريعها بالمستخلص المائي لنبات القرنفل بتركيز 150 ملغم/كغم من وزن الجسم مع اخذ 1٪ بيروكسيد الهيدروجين مع ماء الشرب لمدة 30 يوم .
- المجموعة الرابعة: تم تجريعها بالمستخلص المائي لنبات القرنفل بتركيز 150 ملغم/كغم من وزن الجسم لمدة 30 يوم .

جمع نماذج الدم: جمعت عينات الدم من الحيوانات بعد انتهاء فترة التجريع وهي 30 يوم من وريد العين بواسطة انبوبة شعرية تحتوي على الهيبارين وتم الحصول على مصل الدم لاجراء الاختبارات .

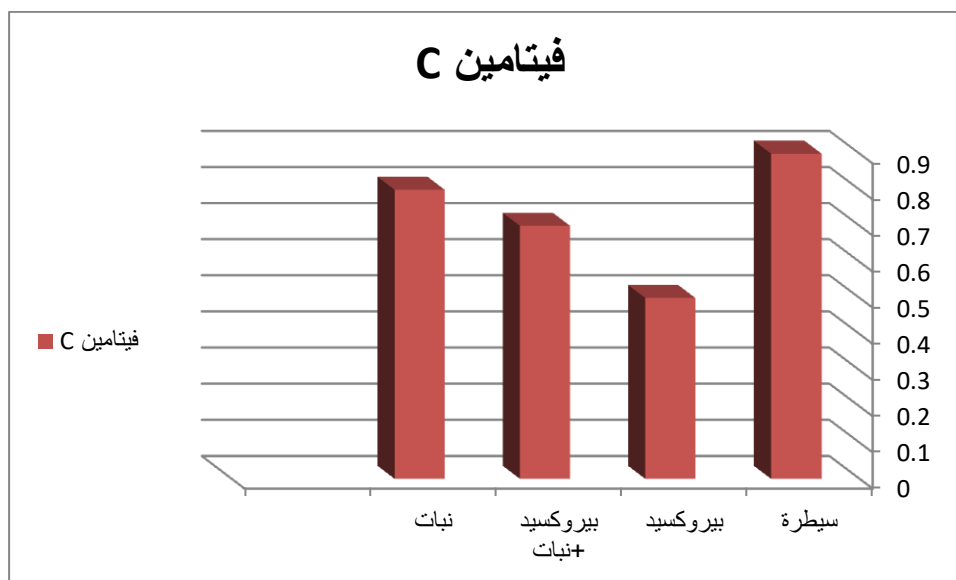
## الاختبارات الكيموحيوية:

تم تقدير فيتامين C حسب الطريقة المتبعة من قبل (9) والتي تضمنت التفاعل الذي يحدث بين حامض الاسكوربيك المؤكسد مع مركب 2، 4 ثنائي نايثرو فينيل هايدرازان ليعطي ناتج يمتص عند طول موجي (520) نانوميتر ، كذلك قدر فيتامين E من خلال الطريقة (10) والتي تضمنت اختزال ايون الحديدك  $Fe^{+3}$  بواسطة التوكوفيرول وتفاعله مع محلول a,a -dipyridil ليكون معقدا يمتص عند طول موجي 520 نانوميتر كذلك تم تقدير مستوى الكلوتاثيون GSH من خلال استخدام الطريقة المتبعة من قبل (11) والتي تم فيها استخدام محلول المان Elmans reagent وقياس الامتصاصية عند طول موجي 420 نانوميتر وتم تقدير تركيز مستوى المالوندايالديهيد MDA وفق طريقة (12) عن طريق تفاعله مع حامض الثايوباربيوتيك TBA ويكون ناتج يقاس الامتصاصية له عند طول موجي (532) نانوميتر كما تم تقدير فعالية انزيم السوبر اوكسيد دسميوتيز SOD باتباع الطريقة الواردة في (13) بواسطة استخدام سيانيد الصوديوم حيث يقوم بتثبيط انزيم البيروكسيداز من خلال تغيير الكثافة الضوئية للفورمازين المتكون وأخيرا تم تقدير جذر البيروكسي نيتريت باستخدام طريقة (14) والتي تتضمن نيترة nitration الفينول من قبل جذر بيروكسي نيتريت وتكوين نايثروفينول ويقاس عند الطول الموجي (412).

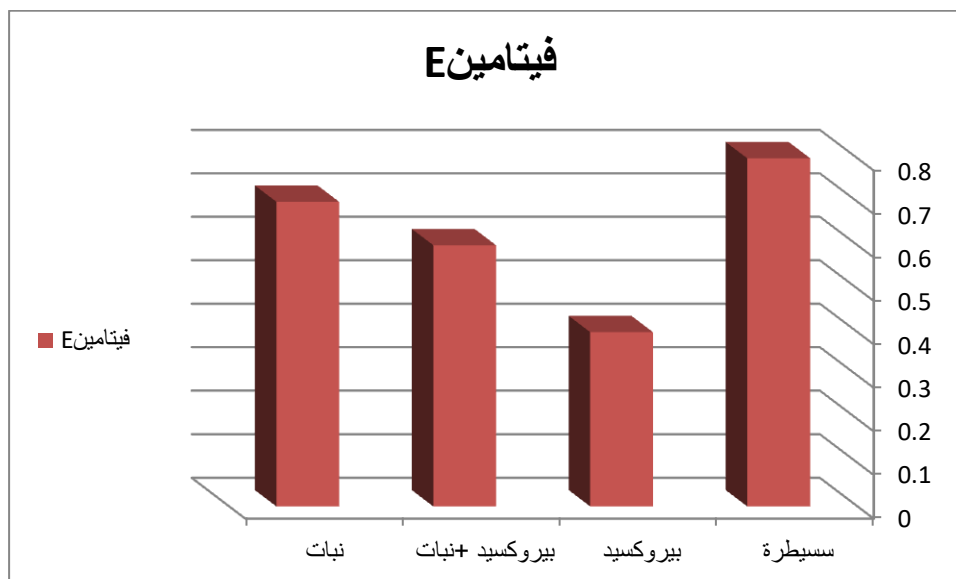
## النتائج والمناقشة

### 1- تأثير المستخلص المائي لنبات القرنفل بتركيز 150 ملغم / كغم على مستوى فيتامين C وفيتامين E .

بينت النتائج الموضحة في الشكل 1 و 2 ان استخدام بيروكسيد الهيدروجين بتركيز 1% في ماء الشرب لمدة 30 يوم ادت إلى ظهور انخفاضاً معنوياً عند مستوى احتمالية  $P \leq 0.05$  في مستوى فيتامين C و E عند المجموعة التي عوملت به مقارنة مع مجموعة السيطرة حيث تعتبر مادة بيروكسيد الهيدروجين من احد العوامل المؤكسدة القوية والتي تؤدي إلى ظهور حالة الاجهاد التأكسدي oxidative stress حيث تقوم بزيادة الجذور الحرة وتؤدي إلى نقص النظام الدفاعي حيث تؤدي إلى ظهور العديد من الامراض والحالة غير الطبيعية في جسم الانسان والحيوان (15) وادت المجاميع المعاملة بالمستخلص المائي لنبات القرنفل بتركيز 150 ملغم / كغم من وزن الجسم إلى رفع مستوى فيتامين C و E عند مستوى احتمالية  $P \leq 0.05$  مقارنة مع نظيرتها المستخدم فيها البيروكسيد فقط حيث تمكن مستخلص القرنفل في رفع في رفع مستوى فيتامين C و E اذ يعتبر القرنفل من اقوى النباتات المضادة للأكسدة وذلك لاحتوائه على العديد من المركبات الفعالة وخاصة الزيوت الطيارة مثل زيت الاوجينول Eugenol (16) الذي له خصائص كاسحة للجذور الحرة وفعالية مضادة للأكسدة وهو من المركبات الفينولية الفعالة التي تقلل من الاجهاد التأكسدي وتمنع بيروكسدة الدهون (17).



شكل (1): تأثير بيروكسيد الهيدروجين 1% ومستخلص نبات القرنفل بتركيز 150 ملغم / ملغم مستوى فيتامين C في الفئران .



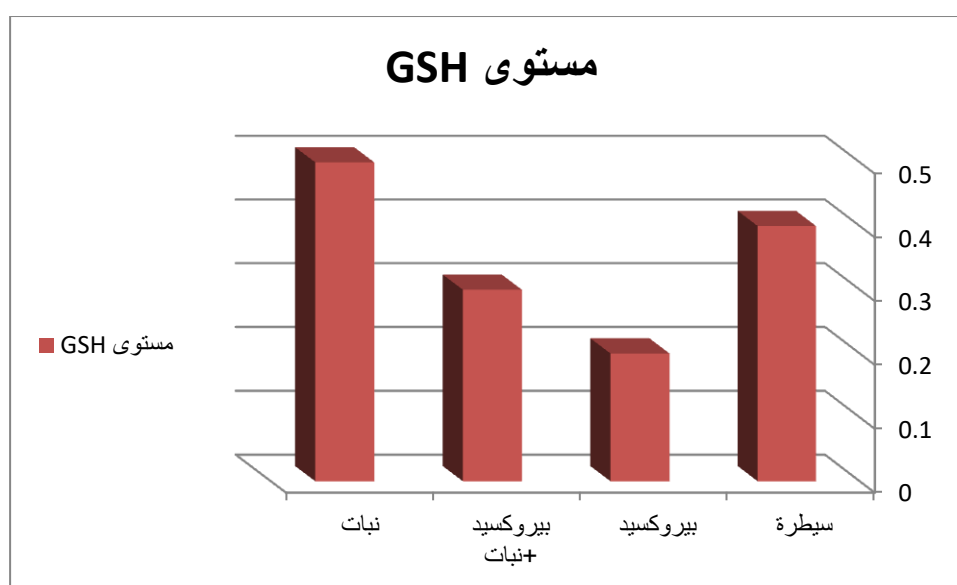
شكل (2): تأثير بيروكسيد الهيدروجين 1% ومستخلص نبات القرنفل بتركيز 150 ملغم / ملغم مستوى فيتامين E في الفئران .

## 2- تأثير المستخلص المائي لنبات القرنفل بتركيز 150 ملغم/كغم من وزن الجسم على مستوى الكلوتاثيون GSH وانزيم السوبر اوكسيد دسميوتيز SOD .

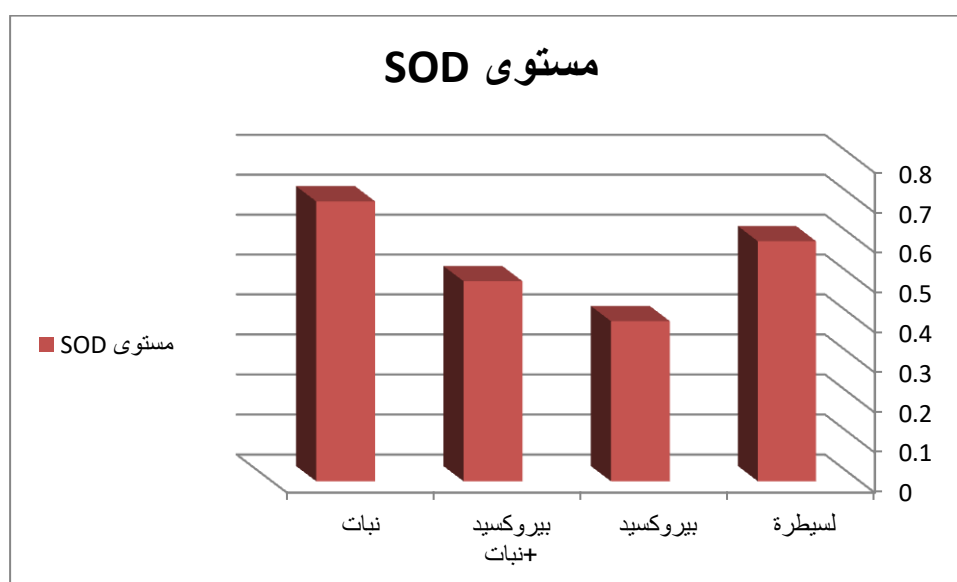
أظهرت النتائج الموضحة في الأشكال 3 و 4 حدوث انخفاض معنوي في مستوى GSH و SOD عند مستوى احتمالية  $P \leq 0.05$  في مجموعة الحيوانات التي عوملت ببيروكسيد الهيدروجين بتركيز 1% مقارنة مع مجموعة السيطرة. في حين حدث ارتفاع معنوي عند مستوى احتمالية  $P \leq 0.05$  على مستوى GSH ، SOD في المجاميع المعاملة بالمستخلص المائي لنبات القرنفل مقارنة مع المجموعة التي عوملت بالبيروكسيد لوحده. وهذا يدل على دور GSH الحيوي في التفاعلات الحيوية وخاصة تفاعلات الأكسدة والاختزال حيث ان اعطاء بيروكسيد الهيدروجين في ماء الشرب يعمل على افراز GSH في الانسجة والدم (18) حيث ان حالة الاجهاد التأكسدي تؤدي إلى حدوث ارتفاعا في

أكسدة GSH في شكل ثنائي الكبريت GSSG عن طريق تثبيطه وتحويله إلى مسار السكر الخماسي Pentose phosphate مما يؤدي إلى الحد من إنتاج NADPH الضروري لفعالية انزيم GSH رديكتيز لإعادة تخليقه إلى الشكل المؤكسد (19) كما ان انزيم SOD يعمل على استعادة حيوية الخلايا وتقليل سرعة تدميرها، ويقوم بمعادلة بعض أنواع الجذور الحرة، كما يساعد في عمله على الاستفادة من عنصري الزنك والنحاس اذ يقوم بإزالة جذر الاوكسجين O<sub>2</sub> عن طريق تسريع معدل تحوله إلى H<sub>2</sub>O<sub>2</sub> بمساعدة عدد من المعادن مثل السيلينيوم، النحاس (20).

وقد ادت المعاملة بالمستخلص المائي لنبات القرنفل إلى رفع مستوى GSH و SOD في جميع فترات المعاملة مقارنة مع المجاميع التي عوملت بيروكسيد الهيدروجين وهذا يوضح قدرة نبات القرنفل على خفض الجذور الحرة وكبح الاجهاد التأكسدي حيث يعمل على استقرار الاغشية الخلوية كونه اقوى مضادات الأكسدة الفينولية الكاسحة للجذور الحرة ومنع بيروكسدة الدهن lipid peroxidation (21).



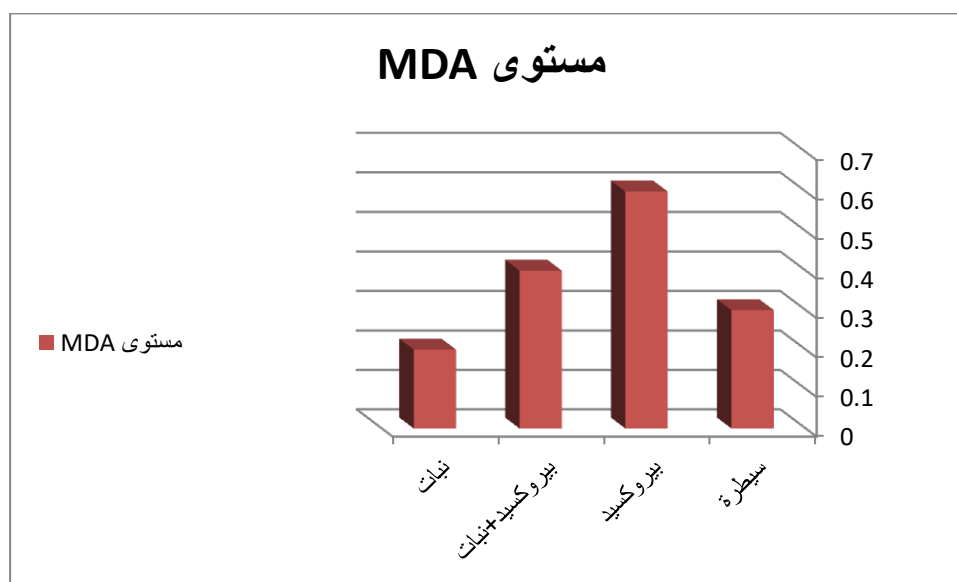
شكل (3): تأثير بيروكسيد الهيدروجين 1% ومستخلص نبات القرنفل بتركيز 150 ملغم /ملغم مستوى GSH في الفئران .



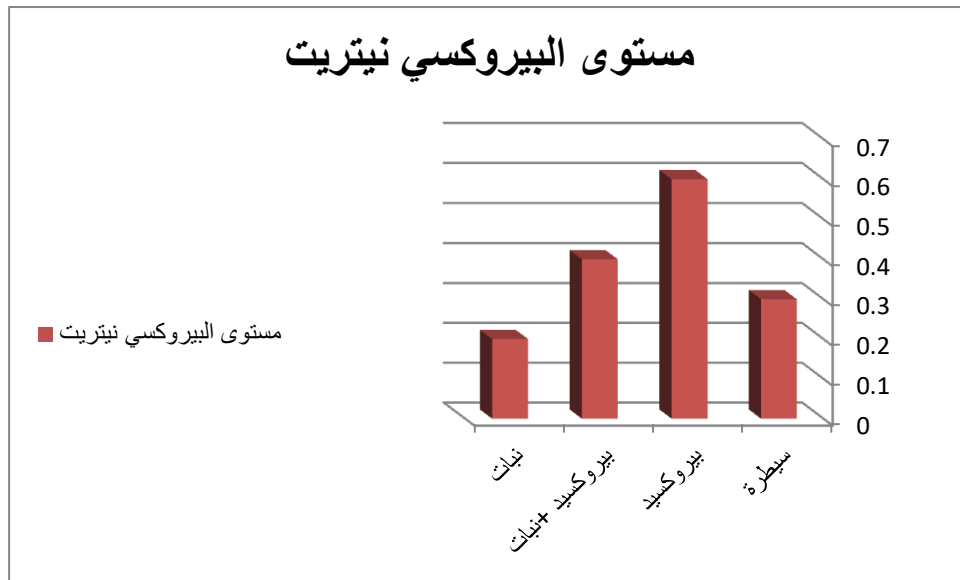
شكل (4): تأثير بيروكسيد الهيدروجين 1% ومستخلص نبات القرنفل بتركيز 150 ملغم /ملغم مستوى SOD في الفئران

3- تأثير المستخلص المائي لنبات القرنفل بتركيز 150 ملغم /كغم من وزن الجسم على مستوى المألوندا بالديهايد MDA وجذر البيروكسي نيتريت .

أظهرت النتائج الموضحة في الأشكال 5 و 6 حدوث ارتفاع معنوي عند مستوى احتمالية  $P \leq 0.05$  في مستوى MDA وجذر البيروكسي نيتريت في المجاميع المعاملة ببيروكسيد الهيدروجين بتركيز 1% مقارنة مع مجموعة السيطرة في حين بينت النتائج انخفاضاً معنوياً  $P \leq 0.05$  في مستوى MDA وجذر البيروكسي نيتريت عند المجاميع المعاملة بالمستخلص المائي لنبات القرنفل. بتركيز 150 ملغم /كغم من وزن الجسم، في المجاميع التي عوملت به مقارنة مع المجاميع التي عوملت ببيروكسيد الهيدروجين وهذا يدل على ان استحداث حالة الجهاد التأكسدي بواسطة بيروكسيد الهيدروجين يؤدي إلى حدوث اضراراً تأكسدية تعمل على بيروكسدة الدهون غير المشبعة للدهون المفسفرة في اغشية الخلايا الحية حيث يؤدي إلى انتاج مركبات سامة للخلايا منها MDA وجذر البيروكسي نيتريت (22، 23) كما ان المعاملة بالمستخلص المائي لنبات القرنفل ادى إلى التقليل من هذا الضرر وارجاعها إلى المستويات الطبيعية بسبب خصائصه المضادة للأكسدة التي لها القدرة على اكتساح الجور الحرة والتقليل من مستويات ROS و RNS (24، 25) .



شكل (5): تأثير بيروكسيد الهيدروجين 1% ومستخلص نبات القرنفل بتركيز 150 ملغم /ملغم مستوى MDA في الفئران



شكل (6): تأثير بيروكسيد الهيدروجين 1% ومستخلص نبات القرنفل بتركيز 150 ملغم /ملغم مستوى جذر البيروكسي نيتريت في الفئران .

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# EFFECT OF SPRAYING DIFFERENT CONCENTRATIONS OF FOLIAR FERTILIZER AND BALANCED NUTRIENT SOLUTION ON SOME VEGETATIVE AND ROOT GROWTH CHARACTERISTICS OF BRASSICA CAMPESTRIS L

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## Abstract:

The study was conducted in Harmoush farms on the Najaf-Karbala road during the agricultural season 2021-2022. To study the effect of spraying different concentrations of foliar fertilizer and balanced nutrient solution on some vegetative and root growth characteristics of *Brassica campestris* L.. A factorial experiment ( $3 \times 3$ ) was carried out like the first factor, with three concentrations of foliar fertilizer (0, 3, and 6) ml. L-1 and the second three concentrations of the nutrient solution (0, 2, 4) ml. L-1 according to the completely random block design (R.C.B.D). The results showed that spraying foliar fertilizer at a concentration of 6 ml.L-1 led to an improvement in the vegetative and root growth indicators, as it increased (plant height, number of leaves, shoot fresh weight, shoot dry weight, total chlorophyll content, root diameter, root circumference, and the dry weight of the roots) while a treatment of 3 ml. L-1 was given the highest rate of root system weight compared to plants that were not sprayed, which gave the lowest values. Table (4) and (5) show that spraying with a nutrient solution at a concentration of 4 ml. L-1 had a significant effect on vegetative and root growth indicators (plant height, number of leaves, shoot fresh weight, shoot dry weight, leaves content of total chlorophyll, root diameter and root dry weight) while a 2 ml L-1 treatment was given highest rate in the root circumference compared to plants that were not sprayed, which gave the lowest values. The results showed that the spraying with the two fertilizers and the interaction between them had a significant effect on the vegetative and root growth characteristics, as it gave the highest rate when the interaction between (6 ml.L-1) of foliar fertilizer and (4 ml.L-1) of the nutrient solution in (plant height, number Leaves, fresh and dry weight of shoot, leaves content of total chlorophyll, root diameter, fresh weight of total root and dry weight of total root) while (6 ml.L-1) and (2 ml.L-1) treatment gave the highest rate of Root circumference compared to the control treatment that gave the lowest values for these traits.

**Key words:** *Brassica Campestris* L, Effect of spraying, Foliar Fertilizer.



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## تأثير رش تراكيز مختلفة من السماد الورقي والمحلول المغذي المتوازن في بعض صفات النمو الخضري والجذري لنبات اللفت *Brassica campestris L*.

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### ملخص:

أجريت الدراسة في مزارع الهرموش على طريق النجف - كربلاء خلال الموسم الزراعي 2021-2022. لدراسة تأثير رش تراكيز مختلفة من السماد الورقي والمحلول المغذي المتوازن في بعض صفات النمو الخضري والجذري لنبات اللفت *Brassica campestris L*. نفذت تجربة عاملية (3 × 3) مثل العامل الأول ثلاثة تراكيز من السماد الورقي (0، 3، 6) مل. لتر-1 والثاني ثلاثة تراكيز من المحلول المغذي (0، 2، 4) مل. لتر-1 وفق تصميم القطاعات العشوائية الكاملة (R.C.B.D). اظهرت النتائج ان رش السماد الورقي بتركيز 6 مل. لتر-1 ادى إلى تحسين مؤشرات النمو الخضري والجذري اذ ازداد (ارتفاع النبات، عدد الأوراق، الوزن الرطب للمجموع الخضري، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي، قطر الجذر، محيط الجذر، والوزن الجاف للجذور) بينما أعطت معاملة 3 مل. لتر-1 أعلى معدل في وزن المجموع الجذري مقارنة بالنباتات غير المرشوشة والتي أعطت أقل القيم. بين جدول (4) و (5) ان الرش بالمحلول المغذي بتركيز 4 مل. لتر-1 أثر معنوياً في مؤشرات النمو الخضري والجذري (ارتفاع النبات، عدد الأوراق، الوزن الرطب للمجموع الخضري، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي، قطر الجذر والوزن الجاف للجذور) بينما أعطت معاملة 2 مل. لتر-1 أعلى معدل في محيط الجذر مقارنة بالنباتات غير المرشوشة والتي أعطت أقل القيم. اظهرت النتائج ان الرش بالسمادين والتداخل بينهما اثر معنوياً في صفات النمو الخضري والجذري اذ اعطى أعلى معدل عند التداخل بين (6 مل. لتر-1) من السماد الورقي و (4 مل. لتر-1) من المحلول المغذي في (ارتفاع النبات، عدد الأوراق، الوزن الرطب، الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي، قطر الجذر، الوزن الرطب للمجموع الجذري والوزن الجاف للمجموع الجذري) بينما أعطت معاملة (6 مل، لتر-1) و (2 مل. لتر-1) أعلى معدل في محيط الجذور مقارنة بمعاملة المقارنة التي أعطت أقل القيم لهذه الصفات.

**الكلمات المفتاحية:** السماد الورقي، نبات اللفت، الرش.

## المقدمة

ينتمي نبات لفت المائدة. *Brassica campestris* L الى العائلة الصليبية Cruciferae نبات عشبي حولي تستخدم جذوره في التخليل او بشكل طازج (بوراس وآخرون، 2006)، يعد لفت المائدة احد الخضر الجذرية الهامة و من اقدم المحاصيل الخضرية في العالم، موطنه الأصلي المنطقة المعتدلة من أوروبا إلى جميع أنحاء العالم، وان مركز نشأته الأولى كانت في منطقة البحر الأبيض المتوسط التي تطورت منها الطرز المستعملة حالياً في الزراعة في اوربا وافغانستان (مصطفى، 2010)، يتكون المجموع الجذري من جذر وتدي يتعمق إلى 100 سم أو أكثر ويتكون الجزء المتضخم، والمستعمل في التغذية تحت سطح الأرض أما من تضخم السويقة الجذنية السفلى او من تضخم الجزء العلوي من الجزء العلوي من الجذر الوتدي مع السويقة الجذنية السفلى، الساق قصيرة جدا تحمل برعما طرفيا تحيط به مجموعة من الأوراق، الأوراق تخرج على الساق القصيرة متزاحمة على شكل حزمة قائمة كبيرة الحجم عنقها طويل وسميكة عند القاعدة، النصل بيضاوي حافته مفصصة، الازهار تحمل في نورات عنقودية والزهرة خنثى (بوراس وآخرون، 2005)، وتعتبر جذور اللفت غنية في محتواها من العناصر الغذائية سواء الأساسية أو الفيتامينات والمعادن والالياف أما الأوراق فأنها تعتبر أغنى من الجذور في محتواها من العناصر المعدنية والفيتامينات والبروتينات والكربوهيدرات وذات فوائد طبية عديدة منها فاتح للشهية جداً، زيادة العصارة المعدنية، منشط لوظائف المعدة، وتستخدم بذور اللفت في تفتيت حصا الكلية ويستخدم في عمليات التجميل بإزالة الكلف والنمش من البشرة ويعالج حالات الاسهال ونزلات البرد (مصطفى، 2010). ومن الوسائل المتبعة لتنظيم نمو النبات للحصول على نباتات ذات مجموع خضري وجذري جيد وذات مواصفات جيدة استخدام التسميد الورقي ان إضافة الأسمدة عن طريق الرش الورقي يزيد من امتصاص العناصر الغذائية وهي طريقة حديثة في التسميد الا انها ليست بديلا عن التسميد الارضي وانما مكملة له، وتحتوي الأسمدة العضوية على العناصر المغذية الكبرى النتروجين والفسفور والبوتاسيوم اذ يعتبر عنصر النتروجين من العناصر التي تدخل في بناء العديد من المركبات الضرورية في نمو النبات، إضافة ان عنصر الفسفور يدخل في تركيب الأحماض النووية والأمينية وتكوين مركبات الطاقة الضرورية إلى نمو النبات، اما عنصر البوتاسيوم يعتبر هذا العنصر عامل مساعد في تكوين الكربوهيدرات وتكوين الأحماض الأمينية والبروتينات إضافة إلى أهميته في انقسام الخلايا (Jones، 1991)، ويعد عنصر البورون احد العناصر الغذائية المهمة في النبات، اذ يلعب دور مهم في الايض الغذائي للنبات، ويدخل في عملية نقل السكريات و التفاعلات الانزيمية إضافة إلى تمثيل البروتين و بناء الهرمونات النباتية وانقسام الخلايا (ابو ضاحي، 1989)، يلعب الزنك دور مهم في عمليتي الأكسدة والاختزال التي تلعب دور في عملية التمثيل الغذائي ويدخل في تكوين الحامض الأميني التربتوفان الذي يعد البادئ لتكوين اندول حامض الخليك (IAA) المهم في انقسام الخلايا (النعمي، 1999)، كذلك يلعب المنغنيز دور في العديد من العمليات الحيوية داخل النبات التي تؤدي إلى زيادة الإنتاج وتحسين جودة الحاصل وهو يلعب دور في عمليات الأكسدة والاختزال (Dris و El-Shazly، 2004)، يعد عنصر الحديد قليل الحركة في النبات وله دور مهم في العديد من الانظمة الانزيمية التي تدخل في عمليتي الأكسدة والاختزال ويساعد في بناء الكلوروفيل (صقر، 2010)، اما عنصر النحاس ضروري في عملية البناء الضوئي ويشترك في ايض البروتينات والكربوهيدرات وتثبيت النتروجين (الريس، 1987). فقد وجد Gehan وآخرون (2013) ان رش البورون على هيئة حامض البوريك بتركيز 2 غم. لتر<sup>-1</sup> على نبات بنجر المائدة ادى إلى تحسين مؤشرات النمو الخضري والحاصل ونوعية الجذور. هذا ما أكدته التجربة التي أجريت على نبات الألبازة التي تمت معاملتها بمستخلص السماد

العضوي وكبريتات البوتاسيوم وكبريتات الزنك حيث أعطت أعلى معدلات ارتفاع النبات والوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي (اليساري، 2019).

وعلى الرغم من أهمية هذا البحث واستعمالاته الكثيرة فلا تزال الأبحاث المجراة حوله قليلة لذا أجريت هذه التجربة لبيان أهمية رش تراكيز مختلفة من السماد الورقي والمحلل المغذي المتوازن في بعض صفات النمو الخضري والجذري لنبات اللفت .

### المواد وطريقة العمل

نفذت التجربة في مزارع الهرموش طريق النجف - كربلاء في الموسم الزراعي 2021- 2022 على نبات اللفت لدراسة تأثير رش تراكيز مختلفة من السماد الورقي والمحلل المغذي المتوازن في بعض صفات النمو الخضري والجذري لنبات اللفت *Brassica campestris* L.. نفذت تجربة عاملية (3×3) شمل العامل الأول ثلاثة تراكيز من السماد الورقي (6,3,0) مل. لتر<sup>-1</sup> والعامل الثاني ثلاثة تراكيز من المحلول المغذي (4,2,0) مل. لتر<sup>-1</sup> وفق تصميم القطاعات العشوائية الكاملة (R.C.B.D) بثلاث مكررات، احتوى كل مكرر على تسعة معاملات، زرعت البذور غوطه الشام المنشأ سوري على شكل دايات بتاريخ 2021/10/4، نقلت الشتلات الجاهزة للشتل بتاريخ 2021/11/26 بعد ظهور اربعة أوراق حقيقية إلى جور على مساطب طول 1 م والعرض 85 سم والمسافة بين نبات وآخر 20 سم بواقع خمس نباتات لكل وحدة تجريبية مع اجراء كافة عمليات الخدمة كلما دعت الحاجة. تم رش السماد الورقي بتاريخ 2021/12/20 بواقع رشتين بينهما 10 ايام، وأجريت عملية الرش بالمحلل المغذي بتاريخ 2021/12/24 بواقع رشتين بينهما عشرة ايام، وحضرت تراكيز السماد الورقي وذلك بأخذ 3 مل من السماد الورقي واكمل الحجم إلى 1 لتر بالماء المقطر لغرض الحصول على تراكيز 3 مل. لتر<sup>-1</sup> وهكذا لبقية التراكيز مع إضافة مادة الزاهي لغرض كسر الشد السطحي وتمت عملية الرش في الصباح الباكر بواقع رشتين. حللت النتائج حسب تحليل التباين (ANOVA)، وقورنت المتوسطات حسب اختبار أقل فرق معنوي L.S.D وعلى مستوى احتمال 0.05 (الراوي، 2000).

جدول (1) بعض الصفات الفيزيائية والكيميائية لتربة حقل التجربة

نسجة	رمل	غرين	طين	Ca	K	P	N	O.M	Ec	PH
رملية	889	50	48 غم.ك	3.5 ملغم	1.55 ملغم	0.57 ملغم	15.6 ملغم	3.52%	3.27 ديسيميتر	7.34
	غم.كغم <sup>1</sup>	غم.كغم <sup>1</sup>								

جدول (2) مكونات السماد الورقي

النسبة	العناصر
2.1 %	نتروجين على هيئة يوريا
17.9 %	نتروجين على هيئة امونيا
20 %	فسفور على هيئة خامس اوكسيد الفسفور
20 %	بوتاسيوم على هيئة كبريتات البوتاسيوم
0.1 %	حديد مخلبي
0.1 %	خارصين مخلبي
0.05 %	نحاس مخلبي
0.05 %	منغنيز مخلبي
0.05 %	بورون مخلبي
0.02 %	مولبيدنيوم مخلبي
0.005 %	فيتامين (B)

جدول (3) يبين مكونات المحلول المغذي المتوازن

النسبة	العناصر
15.0% W/V	Total Nitrogen (N) ureic
15.0% W/V	Phosphoric Anggdride ( $P_2O_5$ )
15.0% W/V	Potassium Oxide ( $K_2O$ )
0.026 % W/V	Boron (B)
0.066 % W/V	Copper (Co)
0.066 % W/V	Iron (Fe)
0.066 % W/V	Manganese (Mn)
0.026 % W/V	Molybdenum (Mo)
0.013 % W/V	Zinc(Zn)

وفي نهاية التجربة تم حساب الصفات التالية التي اخذت عشوائيا من كل وحدة تجريبية هي :-

### مؤشرات النمو الخضري:

1-1- ارتفاع النبات (سم): تم اخذ ثلاث نباتات من كل وحدة تجريبية واخذت قياس اطوالها ومن ثم اخذ متوسط الطول لكل نبات.

1-2- عدد الأوراق (ورقة. نبات-<sup>1</sup>): تم حساب عدد الأوراق لكل نبات ومن ثم استخراج المعدل

1-3- الوزن الرطب للمجموع الخضري (غم. نبات -<sup>1</sup>): تم اخذ الوزن الرطب للمجموع الخضري بواسطة ميزان حساس واستخرج المعدل.

1-4- الوزن الجاف للمجموع الخضري (غم. نبات-<sup>1</sup>) تم حساب الوزن الجاف للمجموع الخضري لكل نبات واستخرج المعدل

1-5- محتوى الأوراق من الكلوروفيل الكلي (ملغم. 100 غم<sup>-1</sup> وزن طري):- تم تقدير الكلوروفيل الكلي حسب طريقة (Goodwin , 1976)

مؤشرات النمو الجذري:-

2-1- قطر الجذر (سم):- تم اخذ قياس قطر الجذر بواسطة القدمة Vernier بين ابعدين نقطتين.

2-2- محيط الجذر (سم. نبات -<sup>1</sup>): تم قياس محيط الجذور عن طريق لف خيط حول اعرض مكان بالجذر، وبعدها اخذ قياس طول الخيط بواسطة المسطرة لكل وحدة تجريبية واستخرج المعدل لخمسة جذور.

2-3- الوزن الطري للجذور (غم):- فصل المجموع الجذري عن المجموع الخضري واخذ القياس الاوزان بواسطة الميزان الحساس ولكل نبات واستخرج المعدل.

2-4- الوزن الجاف للجذور (غم):- اخذ الوزن الجاف للجذور بواسطة الميزان الحساس ولكل نبات واستخرج المعدل.

### النتائج والمناقشة

#### أولاً: تأثير رش السماد الورقي في صفات النمو لنبات اللفت المائدة

أظهرت نتائج جدول (4) ان رش السماد الورقي بالمستوى 6 مل.لتر<sup>-1</sup> اثر معنوياً على صفات النمو التالية (ارتفاع النبات، عدد الأوراق، الوزن الرطب للمجموع الخضري، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي) اذ بلغ ( 19.69 سم، 34.70 ورقة.نبات<sup>-1</sup> ، 121.1 غم، 9.28 غم، 76.2 ملغم. 100 غم<sup>-1</sup> وزن طري) مقارنة بالنباتات غير المسمدة والتي أعطت أقل معدل بلغ (7.51 سم، 16.05 ورقة.نبات<sup>-1</sup>، 51.3 غم، 5.62 غم، 61.4 ملغم. 100 غم<sup>-1</sup> وزن طري) وعلى التتابع ويرجع السبب إلى دور السماد الورقي الحاوي على العديد من العناصر الغذائية الكبرى والصغرى ومنها النتروجين الذي يلعب دوراً في بناء أنسجة النبات لأنه الأساس في تركيب الكلوروفيل وضروري لتكوين الانزيمات والفيتامينات مما يزيد من حجم الأوراق والساق اما الفسفور يعتبر احد العناصر الضرورية لتغذية النبات وله دور رئيسي في عملية التمثيل الضوئي والبروتوبلازم وعملية التنفس اما عنصر البوتاسيوم يلعب دور في زيادة الطاقة ويؤثر ايجابيا في زيادة الاستفادة من النتروجين والفسفور يسيطر على الفعاليات الحيوية

المرتبطة بنمو النبات وتنشيط الانزيمات وله دور في انتقال السكريات من الأوراق إلى اجزاء النبات الاخرى وزيادة تركيز الكربوهيدرات (العابدي، 2010) وهذا يتفق مع ما اشار اليه (زيدان واخرون، 2016) في دراسة على نبات البطاطا (صنف سبونت) أجريت في جامعة تشرين في سوريا لدراسة تأثير الأسمدة العضوية اذ اثر معنويا في ارتفاع النبات قياسا بمعاملة المقارنة والتي أعطت أقل القيم وبين (عداي وحمد، 2017) في تجربة أجريت في جامعة بغداد كلية الزراعة (ابوغريب) لدراسة تأثير الأسمدة العضوية حامض الهيومك والحديد في بعض صفات نمو وحاصل صنفين من البطاطا رشا على الأوراق، قد ادت إلى زيادة مؤشرات النمو الخضري. وهذا يتفق مع ما ذكره Saadoun و Al-juthery (2018) في دراسة تأثير بعض أسمدة المغذيات الصغرى في نمو وحاصل الالمازة، تفوق معاملة رش رباعي (Mn+Fe+Zn+Cu) معنويا في حاصل الوزن الجاف للمجموع الخضري. ويتضح من الجدول (5) ان الرش بالسماذ الورقي عند المستوى 6 مل. لتر<sup>-1</sup> في صفات النمو الجذري (قطر الجذر، محيط الجذر و الوزن الجاف للمجموع الجذري) اذ بلغ (7.244 سم، 23.49 سم. نبات<sup>-1</sup> و 14.84 غم) مقارنة بمعاملة عدم التسميد والتي أعطت أقل القيم بلغ (6.367 سم، 20.62 سم. نبات<sup>-1</sup> و 11.15 غم) بينما أعطت معاملة 3 مل. لتر<sup>-1</sup> أعلى معدل في الوزن الرطب للمجموع الجذري بلغ 154.1 غم مقارنة بمعاملة المقارنة بلغت 137.8 غم وقد يعزى سبب هذه الزيادة إلى دور العناصر الداخلة في تركيبة السماذ الورقي لاسيما عنصر النتروجين ، اذ يسهم في جميع الخطوات المرتبطة بتفاعلات البروتوبلازم وعمليات البناء الضوئي وبالتالي زيادة نمو النبات، فيما يعطي الفسفور قوة في النمو ويعمل على زيادة وتقوية المجموع الجذري، ويعمل البوتاسيوم على تشجيع نمو الانسجة المرستيمية ومن ثم تكوين نمو خضري وجذري جيدين مما يزيد من كفاءة امتصاص الماء والمغذيات الجاهزة من التربة (عواد، 1987)، وهذا يتفق مع ما اشار اليه El-Sayed واخرون (2014) تأثير حامض الهيومك ومصدر الفسفور على نمو ومحتوى الفجل وقد وجد ان تركيز 0.1 % من حامض الهيومك الغني بالعناصر الغذائية ادى إلى زيادة صفات النمو الجذري قطر الجذر والوزن الرطب للجذور والوزن الجاف للجذور. وبين حماد (2015) في دراسة تأثير مستخلص هيويمكس (أي ان تي) على نمو وإنتاجية نبات الفجل بينت النتائج ان إضافة مستخلص الهيويمكس الغني بالعناصر بتركيز 2، 4 سم<sup>3</sup>. لتر زاد من صفات النمو الجذري قطر الجذر والوزن الجاف للجذور.

#### ثانياً: تأثير رش المحلول المغذي في صفات النمو لنبات اللفت المائدة

يتضح من الجدول (4) ان رش المحلول المغذي بالمستوى 4 مل. لتر<sup>-1</sup> اثر معنويا على صفات النمو التالية (ارتفاع النبات، عدد الأوراق، الوزن الرطب للمجموع الخضري، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي) اذ بلغ (15.60 سم، 35.10 ورقة. نبات<sup>-1</sup>، 116.9 غم، 10.31 غم، 80.6 ملغم. 100 غم<sup>-1</sup> وزن طري) مقارنة بالنباتات غير المسمدة والتي أعطت أقل معدل بلغ (12.85 سم، 13.48 ورقة. نبات<sup>-1</sup>، 52.0 غم، 5.06 غم، 51.2 ملغم. 100 غم<sup>-1</sup> وزن طري) ويعزى سبب زيادة في هذه الصفات إلى ما يحتويه هذا المحلول من عناصر غذائية كافية لما يحتاجه النبات في عمليتي انقسام الخلايا واستطالتها، لاسيما النتروجين الذي يؤثر في زيادة نشاط القمم المرستيمية التي تعمل على زيادة انقسام الخلايا واستطالتها نتيجة زيادة تركيز الاوكسجين او لجاهزية المواد الأساسية التي يحتاجها النبات في عمليات البناء بالحوامض الأمينية وبعض المرافقات الانزيمية NAD و NADP التي يدخل النتروجين في تركيبها (الريس، 1987)، فضلاً عن دور الفسفور في تكوين المركبات الغنية بالطاقة ATP و UTP و GTP الضرورية لتكوين الفوسفوليبيدات والمرافقات الانزيمية وال NADP التي تسهم في السيطرة على العديد من الفعاليات الحيوية



للنبات مما يؤدي إلى زيادة في النمو الخضري بشكل عام (Jayapaul و Devasgayam، 1997)، اما التأثير الايجابي للبوتاسيوم فربما يعود إلى كونه منشط لتمثيل البروتين والانزيمات التي تصاحب تمثيل الكربوهيدرات فضلا عن كونه منظما ازموزيا ايونيا يشترك في عمليتي فتح وغلق الثغور وما يتبع ذلك من تأثير في امتصاص الماء والمغذيات التي تعمل على زيادة النمو الخضري فضلا عن تأثير البوتاسيوم الذي يعمل على تنشيط الانزيمات وتراكم المواد الكربوهيدراتية نتيجة لدخوله في عمليات البناء البروتوبلازمي (ابوضاحي، 1988)، هذا ما أكدته التجربة التي أجريت على نبات الألامازة التي تمت معاملتها بمستخلص السماد العضوي وكبريتات البوتاسيوم وكبريتات الزنك حيث أعطت أعلى معدلات ارتفاع النبات والوزن الجاف للمجموع الخضري ومحتوى الأوراق من الكلوروفيل الكلي (اليساري، 2019).

ويتضح من الجدول (5) ان الرش بالمحلول المغذي عند المستوى 4 مل.لتر<sup>-1</sup> في صفات النمو الجذري (قطر الجذر، الوزن الرطب للمجموع الجذري و الوزن الجاف للمجموع الجذري) اذ بلغ (7.844 سم، 171.4 غم و 18.56 غم) وعلى التوالي مقارنة بمعاملة المقارنة والتي أعطت أقل القيم اذ بلغ (4.967 سم، 113.3 غم و 5.72 غم) بينما أعطت معاملة 2 مل. لتر<sup>-1</sup> أعلى معدل بلغ 24.19 سم مقارنة بمعاملة المقارنة التي أعطت أقل القيم. ويرجع السبب إلى دور البورون في العمليات الحيوية والفلسجية في تكوين وانقسام وتطور الخلية النباتية وبالتالي يؤدي إلى استتالة نهايات الجذور، وان البورون يلعب دورا كبيرا في استهلاك النتروجين وتحفيز العمليات الحيوية ال RNA وبالتالي يزيد من نمو الجذور، ويلعب دور في تمثيل البروتين والأحماض النووية ويسير انتقال السكريات في النبات يعتمد على صفة حامض البوريك الذي يكون مركبات معقدة مع ال polyhydroxyl من خلال ما ذكره (النعمي، 2000). اثر البوتاسيوم معنويا في صفات النمو الجذري ويعزى السبب إلى ان البوتاسيوم يساعد النبات في امتصاص كمية اكبر من النتروجين يمتص النتروجين على صورة نترات او امونيوم وتختزل إلى الشكل العضوي المهم في بناء الخلية النباتية وتتحد مع المواد الكربوهيدراتية لتكوين البروتينات ويدخل في تركيب الأحماض النووية RNA وجزئ الكوروفيل والانزيمات التي تشترك في عملية التركيب الضوئي (الصحاف، 1989)، ودور البوتاسيوم في تكوين مجموع جذري قوي يساعد على زيادة امتصاص الماء والعناصر المغذية مما يؤدي إلى زيادة النمو الخضري وبالتالي زيادة وحدة المساحة (النعمي، 1999). ويلعب دور في انقسام الخلايا وتشجيع نمو الانسجة النباتية مما شجع تكوين جذور قوية (العابدي، 2010).

### ثالثا: تأثير التداخل بين السماد الورقي و المحلول المغذي في صفات النمو لنبات اللفت المائدة

يلاحظ من الجدول (4) ان المستوى (6 مل.لتر<sup>-1</sup> السماد الورقي) و (4 مل.لتر<sup>-1</sup> المحلول المغذي) اثر معنويا في صفات النمو الخضري (ارتفاع النبات، عدد الأوراق، الوزن الرطب للمجموع الخضري، الوزن الجاف للمجموع الخضري، محتوى الأوراق من الكلوروفيل الكلي) مقارنة بمعاملة عدم التسميد والتي أعطت أقل القيم، اما التداخل بين السماد الورقي والمحلول المغذي في جدول (5) يبين ان المستوى (6 مل.لتر<sup>-1</sup> السماد الورقي) و (4 مل.لتر<sup>-1</sup> المحلول المغذي) اثر معنويا في صفات النمو الجذري (قطر الجذر، الوزن الرطب للمجموع الجذري، الوزن الجاف للمجموع الجذري) مقارنة بمعاملة المقارنة، بينما أعطت معاملة (6 مل.لتر<sup>-1</sup>) و (2 مل.لتر<sup>-1</sup>) أعلى معدل في محيط الجذور مقارنة بمعاملة المقارنة التي أعطت أقل القيم لهذه الصفات.



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**INVESTIGATION THE MOST EFFICIENT EXTRACTION METHOD FOR OLEANOLIC ACID FROM OLIVE PLANT AND ITS CHARACTERIZATION****Nidhal. M.S. AL-JANABI <sup>1</sup>****Sarah Abd-ALK. M <sup>2</sup>****Abstract:**

The effect of five solvents was tested, including water, ethyl alcohol 80%, methyl 80%, ethyl acetate and butanol, in order to reach the most efficient solvent for extraction oleanolic acid (Triterpen), depending on the extraction yield, which reached the in leaves 0.9, 1, 0.2, 0.2, and 0.5 g /100 g, respectively, less than in fruits and then Kernels. Five extraction methods were tested .Maceration in hot water, ethyl alcohol (80%), Soxhlet method, Reflux extraction, Microwave, and Defatting with petroleum ether (DPE) method. The latter method distinguished by the amount of yield by 4g/100g, the yield of other extraction methods ranged from 0.1-0.7g/100g. The results of the qualitative chemical detection of the active compounds from the three parts of the olive plant (leaves, fruits and Kernels) showed that the leaves were distinguished by containing a number of active compounds with the appearance of a precipitate, color or foam, as the contained terpenes. total terpenes another indicator to determine the plant part with the highest quantitative content. The results of the experiment indicated the superiority of olive leaves in their content of total terpenes for the five solvents with rates ranging from 40-90% in the leaves and then 39-70% in the fruits and the Kernels are lowest 20-50. Crystals of Oleanolic acid were appeared yellow and needle-shaped under microscope melting point was 299-205 C 0 .

**Key Words:** Olea Europaea, Extraction, Crystallization, Melting Point, Triterpenes.



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## Introduction:

The type of olive *Olea europaea* is the most widespread species around the world (9), the medicinal and important part of this tree is the fruits, leaves and oil (18), which are used in food and in the prevention of many diseases, the leaves contain many Effective compounds, but they are rich in terpenes, especially Oleanolic acid (3, 5). Terpenes are a huge group of natural products with a variety of carbon structures, starting from simple linear chains to multi-ring and they include a large number of important plant substances the most important of which are volatile oils, carotenoids, rubber and some hormones (32), which are food preservatives, stimulate appetite, facilitate digestion and analgesic, and are extracted by a number of solvents such as water, ethyl and methyl alcohol, chloroform and ether (2). Triterpenes the most common type of terpenesis in nature are White solid compounds, most of them molten at a high temperature, are optically effective in nature, freely or bound together (16), while the active groups found in building their structures are mostly groups that are Hydroxyl, carboxylic and aldehyde, or these groups may be multiple in one structure (21), terpenes are produced from wide types of plants, animals and microorganisms and have several functional roles in plants such as chlorophyll dye and carry electrons through the electron transport chain in mitochondria and act as antioxidants. Membranes protect against free radicals (11). Oleanolic acid is a three-tricyclic five-cyclic terpene found in nature free or bound within saponified terpenes (17). It is widely found in fruits, leaves and stem bark of various types of edible and medicinal plants (28), in the olive plant, which produces a high yield from it, it is the commercial source for it (25). Olive leaves contain high levels of terpenes, on top of which is Oleanolic acid, with higher concentrations than the rest of the terpenes in the range of 3.0-3.5%. It is found in fruits, specifically in the peel, by 30 times less than its presence in the leaf (10). This study was aimed to investigate the best solvent and extraction method to get oleanolic acid biologically active compound.

## MATERIALS AND METHODS

Preparation of plant samples: Olive plant samples were collected from the gardens of the University of Baghdad / Al-Jadryia in the ninth month of 2020, the plant was identified by the National Herbarium - General Authority for Seed Examination and Certification - Ministry of Agriculture, *Olea europea* L. This was followed by the washing and drying process by brushing it in the shade with continuous stirring, and then the samples were crushed when conducting the tests.

### Extraction

#### Maceration by hot water

According to the method described by Rafiee *et al.*, (20), So 10 gm of olive sample powder was extracted with 150 ml of distilled water at boiling point and left for an hour on a

magnetic stirrer at room temperature, then the extract was filtered. Then concentrated in a rotary evaporator at 60 ° C, then poured the concentrated extract into a petri dish and placed in the electric oven at a temperature of 40 ° C for 24 ho. The dried powder was scraped and collected in dry bottles .

#### **Maceration by ethyl alcohol(80%)**

According to the method described by Peragon *et al.*, **(19)**, 10 gm of olive sample powder was extracted with 150 ml of ethyl alcohol (80%) and left for an hour on a magnetic stirrer at room temperature, then the extract was filtered ,concentrated in a rotary evaporator at 40 °C, then poured the concentrated extract into a petri dish and placed it ( oven 40 °C / 24 ho). The dried powder was scraped and kept in dry bottles in the refrigerator until use.

#### **Soxhlet Method**

It was done with water and methyl alcohol according to the method described by Fernandez - Hernandez *et al.*, **(8)** with some modifications using the aforementioned solvents instead of hexane, 10 gm of sample powder with 150 ml of the solvent, for 6-8 hours after that a rotary evaporator 40 C<sup>0</sup>, drying the extract in the air oven for 24 hours, then scraping it and keeping it in approved bottles in the refrigerator until use.

#### **Reflux Method**

The method described by Singh and Das, **(27)** was using water and methyl alcohol separately, by extracting 10 gm of plant sample powder in a reflux device flask with 150 ml of the ementioned solvents, heating for an hour, then filtering the extract Whatman. No.1 and then remove the solvent using a rotary evaporator at a temperature of 40 C<sup>0</sup>. The extract is dried in the electric oven, the extract is scraped and kept in the refrigerator until use.

#### **Assist extraction by Microwave**

Extraction was carried out according to the method described by Rafiee *et al.*, **(20)**, 10 gm of olive sample powder was extracted with 150 ml of water and methyl alcohol separately in a microwave device, and for 10 min with no boiling of the extract after that. The extract was filtered and concentrated in a rotary evaporator at a temperature of 40 °C, the concentrated was poured in to a Petri dish and dried for 24 hours, then scraped and kept in until use.

### Defatting with Petroleum ether and extraction by ethyl alcohol (DRE)

It was carried out according to the method described by Vyas *et al.*, (33) and Verma *et al.*, (31), 10 gm of olive sample powder was maceration in petroleum ether for 3 days and extracted with ethyl alcohol 80%. For four days at room temperature, the extract was filtered under vacuum pressure by a rotary evaporator at a temperature of 40 °C for an hour, dried and kept until use.

### Qualitative chemical detection of the active compounds in the olive plant (fruits, leaves and kernels).

We followed the methods described by Shihata (26), which appeared in (1) to estimate the pH and detect tannins, glycosides, flavonoids, saponins and resins, while the method mentioned in Fahmy (6) for alkaloids and Geisman's method was adopted. 1962 for the coumarin that was mentioned in (1) and the qualitative and quantitative detection of terpenes was carried out according to the method mentioned in, (14) and the method mentioned in (29) was adopted for phenols.

### Quantification of terpenes

Add 100 mg of dried extract of the plant sample (Wi) to 9 ml of ethyl alcohol and leave the mixture for 24 hr, then filter and mix the product with 10 ml of petroleum ether and leave in a separating funnel, the petroleum layer is separated and dried By placing it in a plate with a known weight and by taking the difference between the weight of the empty plate and the weight of the plate with the sample after drying, we get (Wf), (14) and by applying the following equation to find out the percentage of total :

$$\text{Amount of total terpenes \%} = \frac{W_f - W_i}{W_i} \times 100$$

Wf = extract Dryer weight.

Wi = weight of the extract after separation.

### Characterization of oleanolic acid crystals

Oleanolic acid was extracted by DPE method for the extract after concentration in a rotary evaporator (40°C), and the extract was placed with chloroform in the refrigerator for 24 hr for the purpose of precipitation.

#### 1- Description of oleanolic acid crystals

The crystals are described morphologically in terms of color with the naked eye, and the shape of the crystals is determined by the optical microscope, by taking part of the crystals and placing them on a glass slide then covering them with a piece of the cover slide.



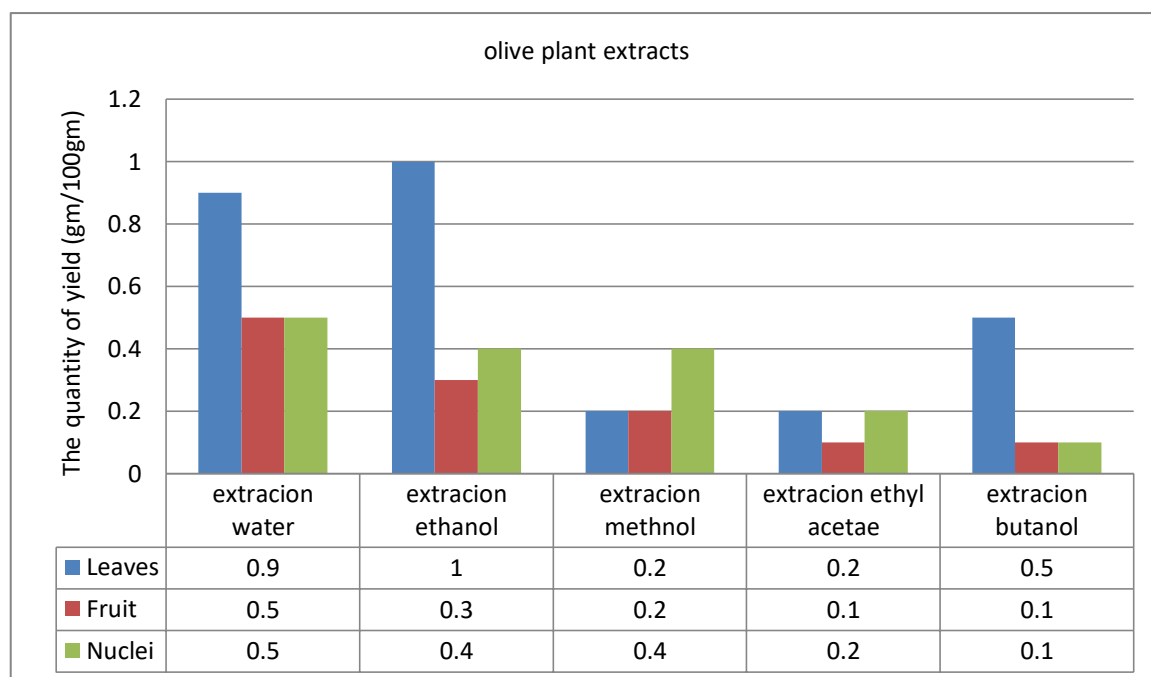
## 2- Determination of the melting point

The melting point of Oleanolic acid was determined in the Food Safety Laboratories / Department of Environment and Water / Ministry of Science and Technology, by placing an amount of it in a capillary tube clogged at one end and then placed in a melting Point measuring device and recording the beginning and end of the melting point.

## RESULTS AND DISCUSSION

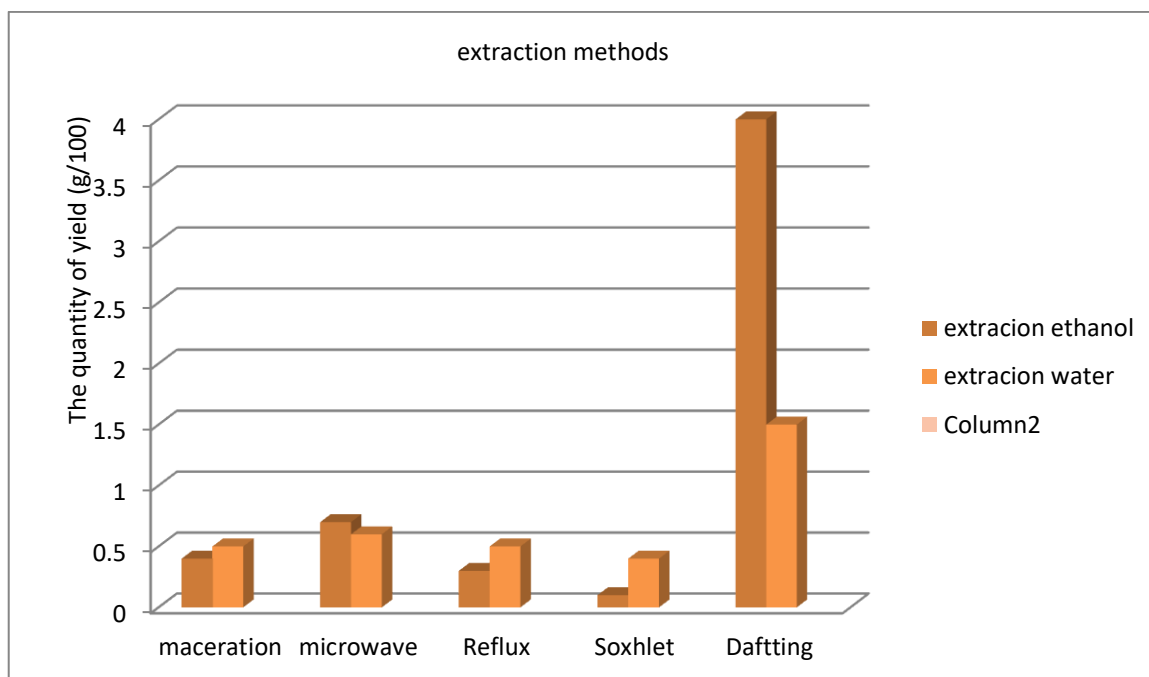
After the three plant parts of the olive plant (leaves, fruit and kernels) were extracted by maceration with five types of solvents (water, ethanol, methanol, ethyl acetate and butanol) the yield of each type of solvent was calculated (Figure 1). The step of selecting the extraction solvent for plant models is critical because it will determine the quantity and type of extracted compounds **(12)**, as the extraction efficiency depends on the type of solvent **(35)**, a number of different polar solvents were chosen. It included water, ethanol, methanol, butanol, and ethyl acetate in order to study their effect on the amount of extraction and thus the content of extracts from terpenes. ethanolic extract gave the highest yield, which may be due to ethanol concentration in the efficiency of terpenes extraction in general. This agrees with Fang *et al.*, **(7)** found that the low concentration of ethanol is suitable for extracting the most beneficial active compounds the percentage of yield was with this concentration 1.85% for a period of one hour extraction.

The use of free organic solvents with low solubility of some plant compounds, which is the result of strengthening the hydrogen bonds between the compounds capable of forming hydrogen bonds and proteins in those solutions **(15)**. Depending on the quantity of the yield or the solvent test, water and ethanol 80%, identification plant part that contained the highest yield which is leaves of olive plant .



**Figure.1 Yield for olive plant extracts for its three parts (leaves, fruit and kernel) for five tested solvents by maceration method**

In terms of extraction methods, the Defatting Petroleum ether and methnol (DPE) method, then extraction with ethanol, with a concentration of ( 80%) DPE method was distinguishrd from other tested extraction methods, with a yield of 4 g / 100 g of the sample using DPEM method, and this yieldt was more than what was obtained. By Vyas *et al.* (33), which was 0.9 g / 100 g of the sample, , the amount of yield for other extraction methods ranged between 0.7-0.1 g / 100 g of the plant sample as shown in Figure (2) based on what was obtained from the extraction results with different solvents and various methods. We concluded that the extraction with (DPE) method and the plant part (leaves) was characterized by the highest extraction yield.



**Figure. 2 Yield of the five extraction methods for olive leaves**

#### **Qualitative chemical detection of the active compounds of the olive plant (leaves, fruit and kernels)**

After choosing the most efficient solvent and the most efficient extraction method, a qualitative chemical detection of the active compounds of the three parts of the olive plant (leaves, fruit and kernel) was adopted as a second indicator in addition to the first indicator and the extraction result for choosing the plant part with the highest content of active compounds, the table(1) shows results of tests to reveal the nature and quality of the active compounds present in the olive plant, as it shows the presence of flavonoids, glycosides, terpenes, saponins, resins and coumarins, and the absence of tannins in the leaves and kernels, and this is consistent with what was indicated by Llias *et al.*, (13) and Salih (24) found that the olive plant contains these compounds especially the leaves. The results of the current experiment are in agreement with what Guinda *et al.*, (10) inferred that the olive plant is rich in terpenes, especially the tested leaves with their content of oleanolic acid.

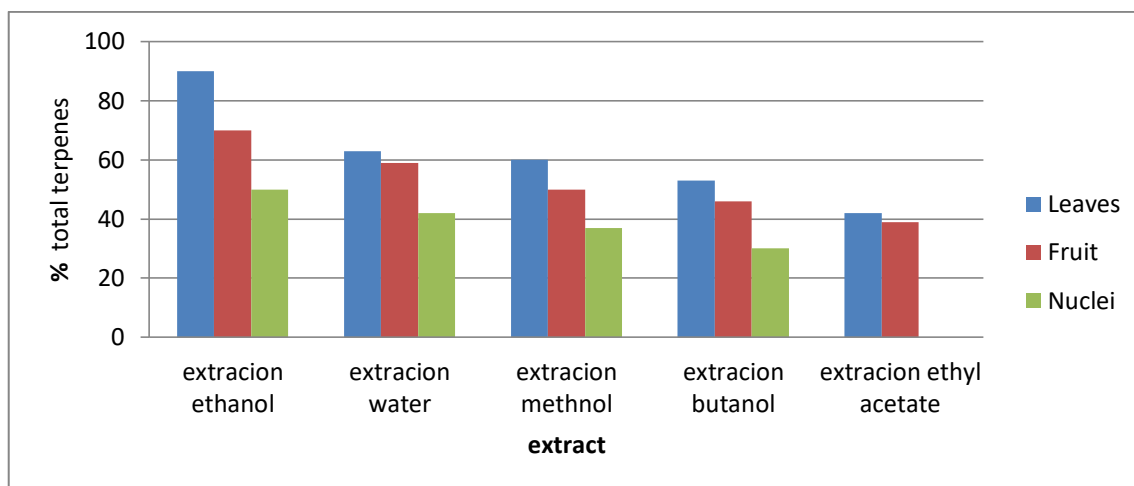
**Table.1 Qualitative chemical detection of the active compounds in olive plant extracts**

kernel	fruit	leaves	indicator Detection	Test detection	Active compound
±	+	++	reddishbrown precipitate	Ethanol 99% + petroleum ether + sulfuric acid	Terpenes
±	+	++	reddish precipitate	A-Fehlink reagent b- Fehlink reagent	Glycosides
±	+	++	yellow color	Ethyl alcohol 95%+ potassium hydroxide 50%	Flavonoids
–	+	–	White jelly precipitate	A - lead acetate 1%	Tannins
–	+	–	Greenish blue color	B-ferric chloride 1%	
–	–	++	Greenish blue color	Moisturizing Filter Paper With Dilute NaOH Solution UV	Coumarin
–	+	++	lees	Ethyl alcohol 95% boiling and distilled water	Resins
–	+	++	foam	Shake the aqueous extract	Saponins
+	+	+	white precipitate	1- Mayer's reagent	Alkaloids
-	+	+	yellow precipitate	2- Picric acid	

It indicates a positive detection. (+)

Indicates negative detection. It (-)

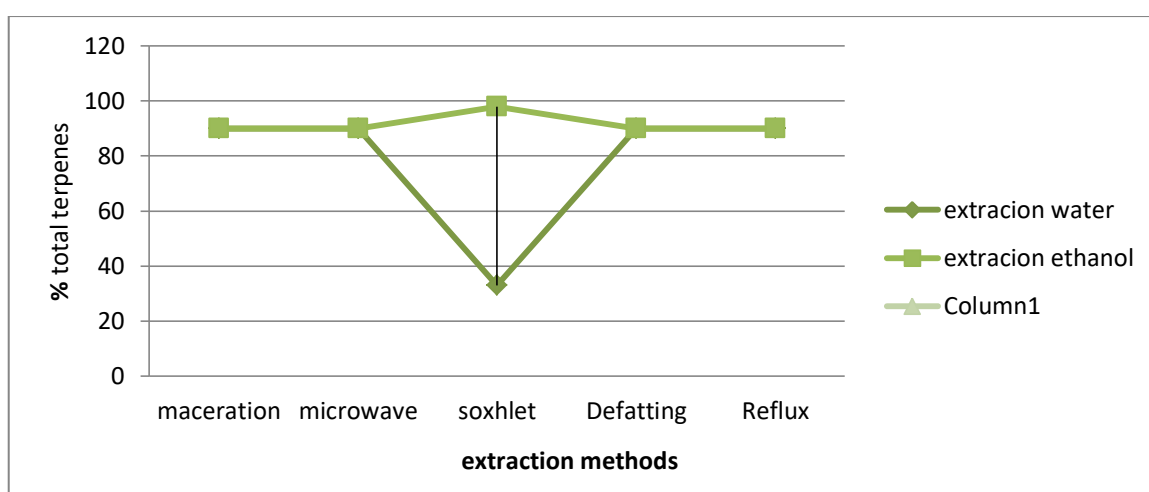
It is inferred from the above table about the presence of alkaloid compounds, flavonoids, saponins, terpenes, coumarins and resins, with the variation of their presence in the three parts of the olive plant.



**Figure. 3 Total terpenes of the three parts of the olive plant (leaves, fruits and kernel) with five tested solvents**

### Quantification of total terpenes

The quantitative estimation of the total terpenes was adopted in order to reach and determine the plant part with the highest quantitative content of them. Figure (4) shows the content of the total terpenes in the three parts of the olive plant tested. It was found that the olive leaves had the highest percentage of the total terpenes, followed by the fruit and then the kernels. While the ethanol extract for olive leaves, fruit and kernels was distinguished as the best extraction solvent based on the extracted terpenes ratios, the results ranged between 40-90% for leaves and 39-70% less for the fruit, and the lowest percentage in the kernels was 20-50%, starting with the ethanolic extract and then the aqueous extract, methanol and butanol, the lowest of them to extract the total terpenes represented by ethyl acetate.

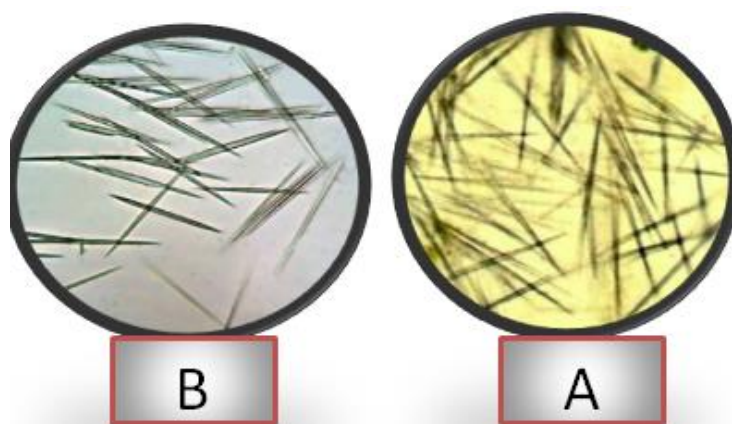


**Figure. 4 Total terpenes by extraction methods with aqueous and ethanolic extract.**

As for the extraction methods applied to the aqueous and ethanolic extract, the highest percentage of total terpenes was concerned. It was noted from Figure (4) that the percentage of the total terpenes was approximately when using ethanol 80% for the maceration, microwave, reflux, and DEP method, the Soxhlet method was distinguished in the total terpenes content by a rate of 98% with ethanol, while it was 90% for the other four tested methods, but the total terpenes content has decreased to 33% when the same extraction methods were applied with water.

### Characterization of oleanolic acid crystals

The physical and chemical properties of oleanolic acid produced from olive leaves were studied, as the crystals obtained from DPE extract were subjected to some tests to infer their purity and conformity to the findings of previous studies. Figure (5) shows the crystals of oleanolic acid produced in our current study under the microscope in the form of crystals A yellow needle, and this is identical to what was described by Yang *et al.*, (34) and Tong *et al.*, (30).



**Figure. 5 Oleanolic acid crystals (A: the acid produced from olive leaves B: the standard).**

Jose *et al.*, (16) found solid crystals that appear polymorphous when crystallized with chloroform showing amorphous shapes and fine that crystallize from plant extracts of olive leaves.

### Melting Point of Oleanolic Acid

Each chemical compound has a certain melting point that differs from other compounds, and this physical property can be used to detect the purity of compounds, and accordingly this examination is a method for detecting the purity of crystals of oleanolic acid that approach 299-305°C agree with what Reyes-Zuri *et al.*, (22), Ribeiro *et al.*, (23) and Banarase *et al.*, (4) obtained that the melting point of oleanolic acid is 306-308°C.

### **Conclusion**

The olive plant has nutritional and therapeutic benefits as it contains different active compounds especially triterpenes including oleanolic acid which has various and important benefits that direct us to work to obtain it in a vailable and cheap sources,we thank the ministry of agriculture-national herbarium and ministry of sciences and technology-food safety laborates.

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## ANALYTICAL STUDIES AND THE EFFECT OF TRYPAN BLUE IN HUMAN ESOPHAGUS CANCER CELLS

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Omar Naji ALI<sup>3</sup>

### Abstract:

Analytical studies of Trypan Blue (TB) diazo dye; showed, highest solubility in water without deviation in the linearity between a dipole moment constants and maximum wavelength. Because of the absorption spectrum of TB affected by using a range of different solvents via solvation and dielectric constant (D). The linear relationship between  $\lambda_{\max}$  and each function  $F(D)$ ,  $\phi(D)$  and  $(D-1)/(D+1)$ , Shown that the D is the only operator that controls shift in the peak. Results also showed, that the deviation from the linear relationship of DMSO is explained on the basis of the formation of strong hydrogen bonding between the solvent and the solute molecules. Though, pH effect, also studied in some variety buffer solutions. The protonation (pKb) and ionization (pKa) constants were intended, owing to suggest the ionization scheme in acidic and basic media. TB was non-toxic and it isn't shown any hemolysis effect in the normal human cells. Further, the MTT assay using human esophagus cancer cells (SK-GT-4) was applied using three different concentrations of TB, the inhibition rate of the cell growth was seeming to be higher than (50%) using 250  $\mu\text{g/ml}$  concentration in contrast with the control. But, there is no effect of TB using 500  $\mu\text{g/ml}$  concentration. Furthermore, the results were indicated that the TB was generating a negative response using high concentration (1000  $\mu\text{g/ml}$ ). In this study TB recommended to use as anti- human esophagus cancer cells (SK-GT-4) .

**Key Words:** Trypan Blue, Analytical studies, Ionization constants, Protonation constant, Esophagus and Cancer Cells.



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## Introduction:

In scientific research azo compounds have receiving high attention,<sup>1,2,3,4</sup> because their significance in the analytical chemistry.<sup>5,6,7</sup> A powerfully colored compounds were status as DNA and RNA inhibitors, antidiabetic, antineoplastic, antibacterial,<sup>5</sup> and can use in the synthesis of protein.<sup>8</sup> These compounds also used against human breast MDA-MB231 cancer cells,<sup>9,10</sup> using cytotoxicity assay to receive an appropriate results within a short time in vitro, which is used earlier than in vivo testing. Because of, in vitro cultures can be refined the results under a controlled environment, due to minimize experimental errors.<sup>10</sup> Therefore, in this study, MTT assay applied in esophageal cancer (EC) which is related to high death worldwide,<sup>11</sup> using diazo day TB as model.

## 1. Materials and Methods

Absorption spectrum was determined on a spectrophotometer. Human cell line was gained from Iraq Biotech Cell Bank/ Basrah, and preserved in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/ ml and 100 µg/ml of penicillin and streptomycin respectively. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37 °C and 5% CO<sub>2</sub>.

### 2.1 pH effect

Each buffer solution, ( $1 \times 10^{-4}$  M) ranging (2-12).

### 2.2 The effect of solvents with different polarity

Individually, TB solution in: water (TB1), ethanol (TB2), methanol (TB3), chloroform (TB4), ethylene chloride (TB5), triethylamine (TB6) and dimethylsulphoxide (DMSO) (TB7), was prepared, ( $1 \times 10^{-4}$  M).

### 2.3 Cellular poisonousness

Toxity of different concentrations of TB, (50,100, 250, 500 and 1000 µg/ml) was measured.<sup>12</sup> Then, the results were recorded after the incubation for three hrs.

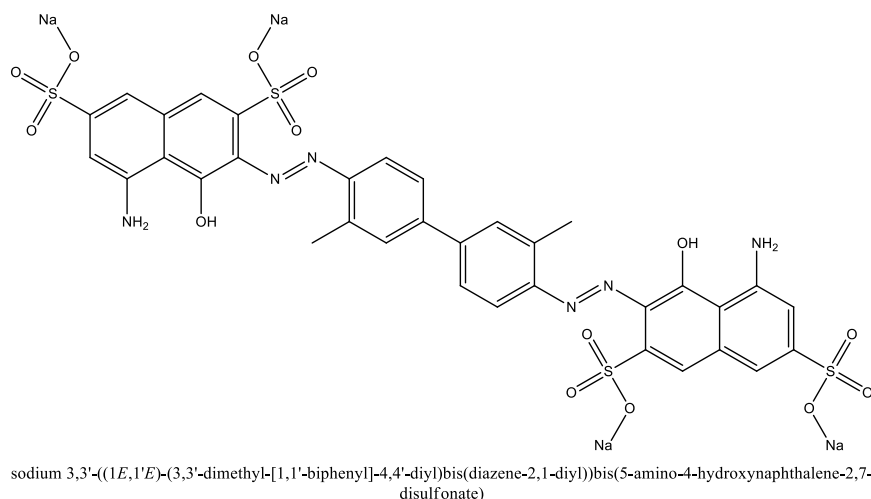
### 2.4 MTT Assay

Human esophagus cell viability assay was directed on 96-well plates, ( $1 \times 10^4$  cells/well). After day. cells were treated with TB, but the cell viability was measured after three days. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 100 µL of DMSO followed by 37 °C incubation for 15 min with shaking. The absorbance was intended by using microplate reader at 620 nm; the test was repeated thee times. The inhibition rate was calculated as the following

equation: Proliferation rate as (PR)=  $B/A \times 100$  where A is the mean optical density of untreated wells and B is the optical density of treated wells and IR= 100- PR.

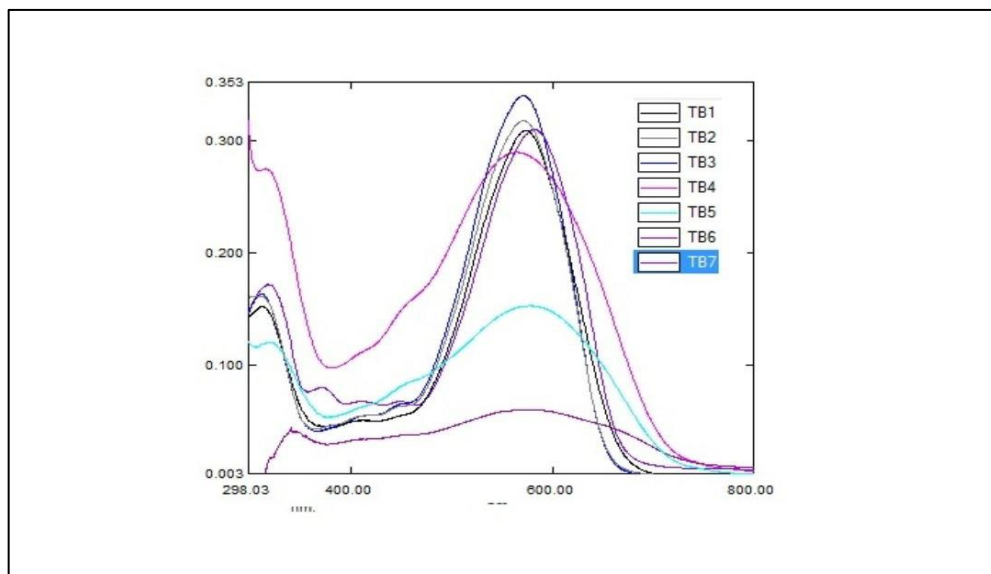
### 3. Results and Discussion

Analytical studies were focused on Trypan Blue, <sup>13</sup> (TB, Figure 1).



**Figure 1. The structure of TB.**

The solvents effects in TB was studied using a set of solvents, water (TB1), ethanol (TB2), methanol (TB3), chloroform (TB4), ethylene chloride (TB5), triethylamine (TB6) and DMSO (TB7), (Figure 2).



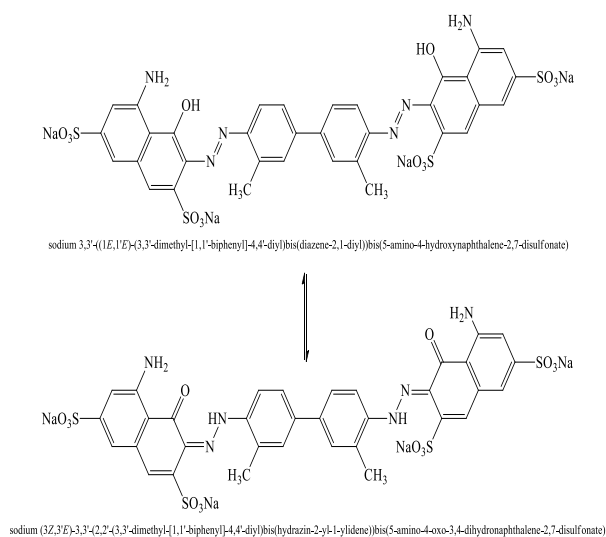
**Figure 2. The solvents effects.**

Figure (2) above shows different values of  $\lambda_{\max}$  and the best solubility of TB was in water, (Table 1).

**Table 1. The solvents effects in TB**

Solvent	(TB)	
	$\lambda_{\max}$ (nm)	$\epsilon_{\max} (\times 10^{-4})$
TB1	315	0.153
TB2	310	0.162
TB3	310	0.164
TB4	315	0.275
TB5	320	0.121
TB6	340	0.044
TB7	370	0.081

The results were revealed that the peaks in the ranges (310-375) were associated with  $\pi-\pi^*$  transitions of N=N due to indicate, that the presence of additional  $\lambda_{\max}$ , (550-580) nm is related to the hydrazine form, which is in equilibrium with azo form with respect the symmetry in the structure of TB, (Figure 3).

**Figure 3. The equilibrium of di azo form with di hydrazine form.**

Designated absorption spectrum of TB affected by using a range of different solvents by meaning of solvation and D, which can be stated,<sup>14</sup> (equation1).

$$\Delta\tilde{\nu} = [(a-b)(n^2-1 / 2n^2+1)] + b(D-1 / D+1) \dots\dots (1)$$

The results from equation (1) are giving an indication that the shift in the peak can occur in response to the change in the solvation energy or in related to the exact dielectric constant. In more precisely the functions F(D) and  $\phi(D)$  lead to linear relation, when the D is the only operator that controls the shift.

$$F(D) = \frac{2(D-1)}{2D+1}$$

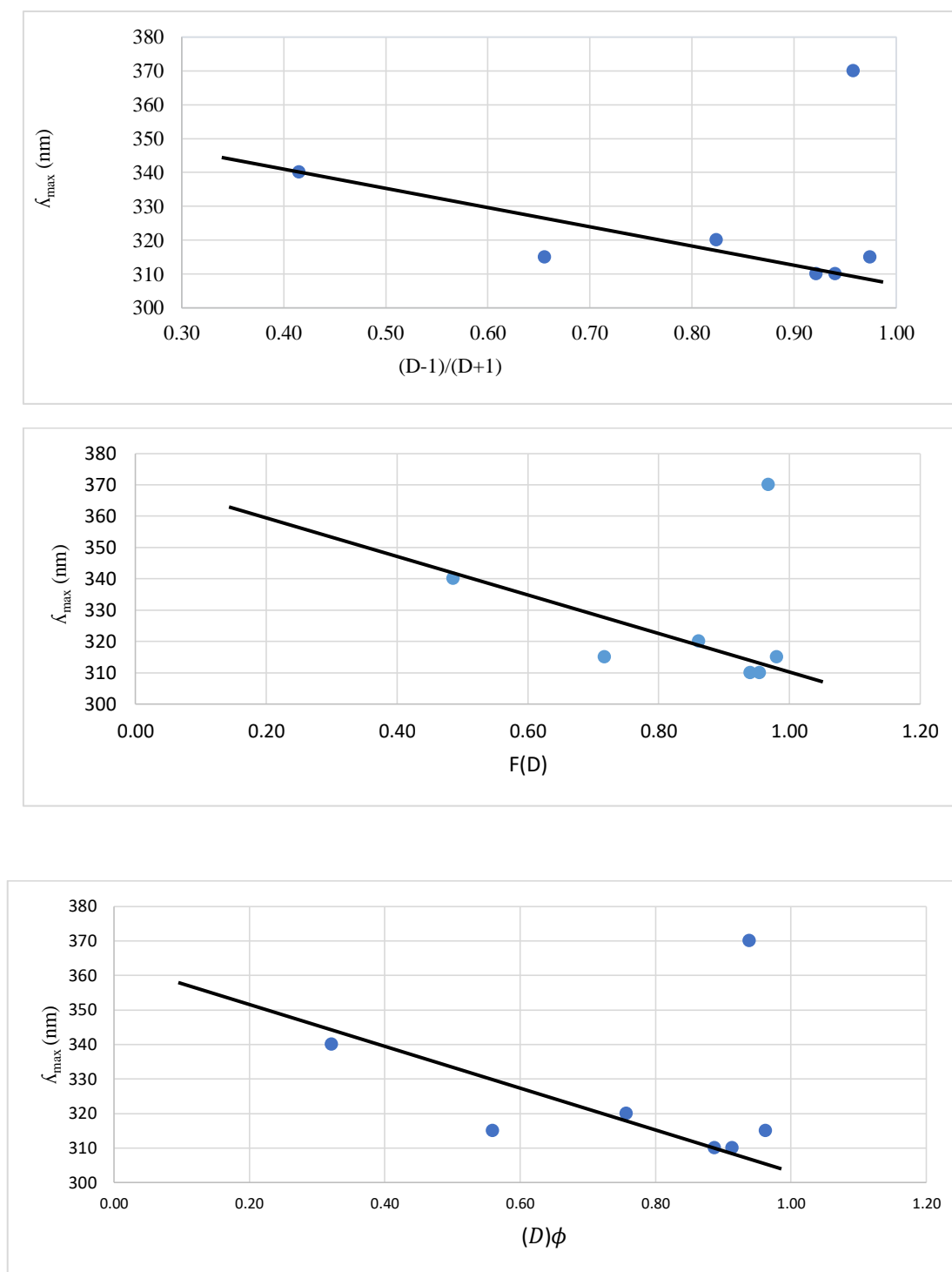
$$\phi(D) = \frac{D-1}{D+2}$$

Therefore, F(D),  $\phi(D)$  and (D-1)/(D+1) were presented in Table (2) below.

**Table 2. Dielectric constants of selected solvents and some functions**

Solvent		(TB)			
		D	(D-1)/(D+1)	F(D)	$\phi(D)$
TB1	Water	78.30	0.97	0.98	0.96
TB2	Ethanol	24.55	0.92	0.94	0.89
TB3	Methanol	32.70	0.94	0.95	0.91
TB4	Chloroform	4.810	0.66	0.72	0.56
TB5	Ethylene chloride	10.36	0.82	0.86	0.76
TB6	Triethylamine	2.420	0.42	0.49	0.32
TB7	DMSO	46.68	0.96	0.97	0.94

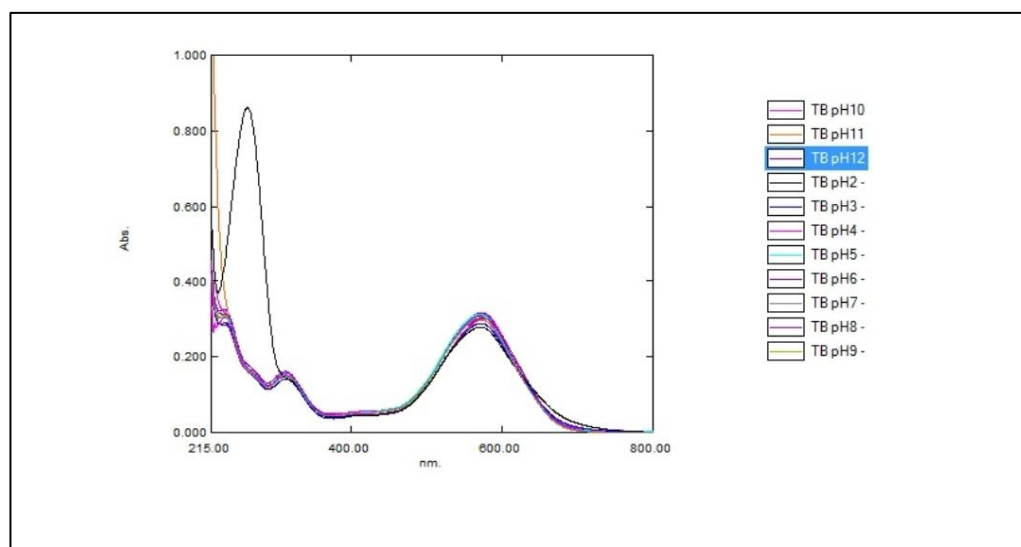
Therefore, the linear relationship or close it of selected solvents were designated in Figure (4) below.



**Figure 4. The linear relationship between  $\lambda_{\max}$  and some functions of different solvents.**

The results were showed that the deviation from the linear relationship of DMSO is explained on the basis of the formation of strong hydrogen bonding between the solvent and the solute molecules. Absorption of each solution of TB, (pH 2-12) was also attended at  $\lambda$  (200-800) nm as seen in Figure (5) below.





**Figure 5. Absorption of TB solutions, (pH=2-12).**

Figure (5) above shows two isopiestic points in the spectrum at (540 and 630 nm) and the suitable pH value was in the pH10. Add to which, the half- height method was used to calculate the pK<sub>b</sub> and pK<sub>a</sub> of nitrogen atom and hydroxyl group respectively via equations (2) and (3) underneath, (Table 3).

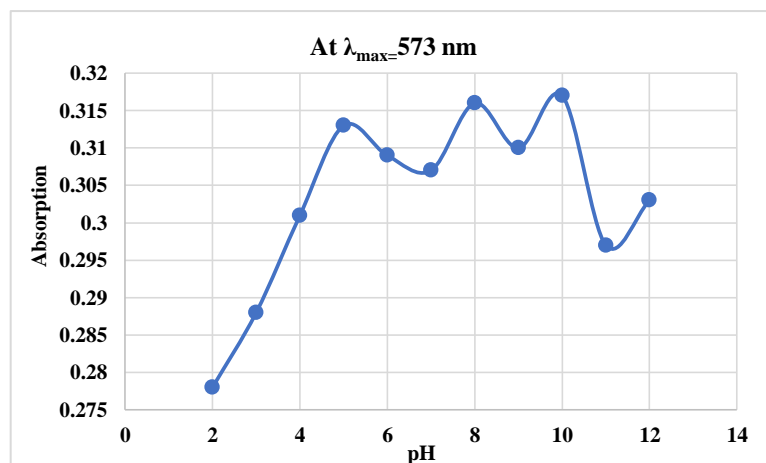
$$\text{pK}_a = \text{pH (at } A_{1/2}) \dots\dots\dots (2)$$

$$A_{1/2} = \frac{A_l + A_{min}}{2} \dots\dots\dots (3)$$

**Table 3. The values of pK**

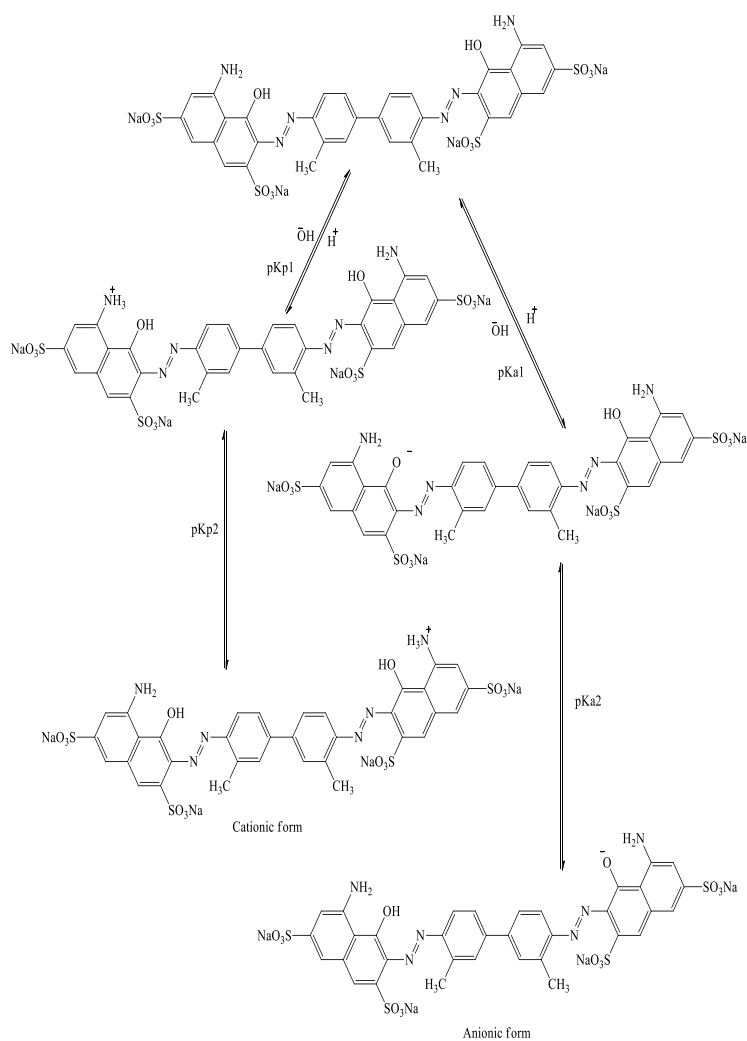
Id	$\lambda_{\text{max}}$	$A_{1/2p1}$	pK <sub>P1</sub>	$A_{1/2p2}$	pK <sub>p2</sub>	$A_{1/2a1}$	pK <sub>a1</sub>	$A_{1/2a2}$	pK <sub>a2</sub>
TB	573 nm	0.295	3.5	0.313	7.6	0.315	9.5	0.300	11.5

The pK at ( $A_{1/2}$ ) of (TB) in acidic and basic media was envisioned since the pH - absorbance curve identified, (Figure 6).



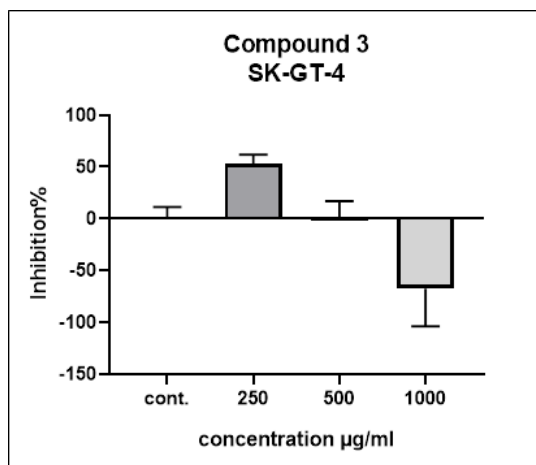
**Figure 6. Curve of pH versus absorbance of TB.**

The results of TB in acidic and basic media were displayed the suggesting ionization scheme, (1).



**Scheme 1. Suggested ionization scheme of TB.**

TB toxicity using hemolytic red blood cells in vitro was also studied and the results were indicated the non-toxic effects of different concentrations of TB, that did not display hemolysis effect. MTT cell viability assay was used, (Figure 7). TB was diminishing the growth of esophagus (SK-GT-4) cancer cells.



**Figure 7. Ability of TB in destroying esophagus cancer cells.**

Cytotoxic effect of TB achieved using three different concentrations, inhibition rate of the cell growth seeming to be higher than (50%) using 250  $\mu\text{g/ml}$  in contrast with the control. But, there is no effect of TB using 500  $\mu\text{g/mL}$  concentration on the cancer cells. Further, the results were giving negative response using 1000  $\mu\text{g/ ml}$  concentration in contrast with the control.

#### 4. Conclusion

TB was gained good color, high soluble in water, have non-toxic effects and non-hemolysis effect in the human cells. TB also had variable results using different buffer solution, besides their ability to bind esophagus cancer (SK-GT-4) cells and affect their viability. The inhibition rate of the cell growth was more than 50% and start a negative response at concentration higher than 500  $\mu\text{g/ml}$ . Owing to indorse the diazo dye as anti-human esophagus cancer cells (SK-GT-4).

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FUZZY 2-MAXIMAL  $R$  -MODULESMohammed D. SALMAN <sup>1</sup>Aliaa Aqeel MAJEED <sup>2</sup>**Abstract:**

Suppose  $R$  is a commutative ring with unity and an  $R$ -module  $M$  is referred to be a 2 - maximal  $R$  -module iff  $\sqrt{\text{ann}_R M}$  is a 2 -maximal ideal of  $R$ . The essential goal of this work is to fuzzy this concept, analyze its fundamental aspects, and analyze the sufficient conditions of the F- 2 - maximum  $R$ -module.

**Key Words:** F- 2 - maximal  $R$ -module,  $F$  - 2 -maximal , F-2 -maximal idal.



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## Introduction:

L.A.in [1] developed the notion of fuzzy sets in 1965, and W.J. Liu [2] proposed the notion of fuzzy modules and fuzzy submodules in 1993, and numerous articles have been published since subsequently in a wide range of mathematics both in theory and in applications. Meanwhile, the concept of the *m*aximal ideal was originally introduced by D.M. Burton in [3], Burton defined the *m*aximal idels of  $\mathcal{R}$ , by an ideal  $\mathfrak{p}$  of  $\mathcal{R}$  is said to maximal idel if it provided that  $\mathfrak{p} \neq \mathcal{R}$  and whenever  $\mathcal{I}$  is an idal of  $\mathcal{R}$  with  $\mathfrak{p} \subset \mathcal{I} \subseteq \mathcal{R}$ , then  $\mathcal{I} = \mathcal{R}$ . Later Abdul-AL-kalik A.J. in [4] introduce a concept of *M*ax- module as follows:  $\mathcal{M}$  is called a *M*axmodule if  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{N}}$  is a maximal idal of  $\mathcal{R}$ , for each non- zero submodule  $\mathcal{N}$  of  $\mathcal{M}$  where  $\text{ann}_{\mathcal{R}} \mathcal{N} = \{r: r \in \mathcal{R} \text{ and } r \mathcal{N} = 0\}$

Numerous researchers have attempted to bridge the gap between the two notions and established new theories, such as J.N. Mordeson and D.S. Malik in [5], who proposed the concept of fuzzy maximal modules in 1998, where specific properties and theorems of FMR were introduced. In Mohammed D.S in [6] introduced the concept of 2-MI of  $\mathcal{R}$  where An ideal  $\mathcal{I}$  of a ring  $\mathcal{R}$  is 2-MI iff  $\mathcal{R}/\mathcal{I}$  is 2-Regular ring as generalization of *m*aximal ideal, and by presenting the idea of a fuzzy *P*rimel idel. If  $\mathcal{R}$  is a ring in which each *m*aximal ideal is *p*rimel, then each *m*aximal fuzzy idal is fuzzy *p*rimel, according to SWAMY in [7]. Therefore, in this study, we aim to expand the work in maximal  $\mathcal{R}$ -module to fuzzy 2-maximal  $\mathcal{R}$ -module.

Finally, (shortly fuzzy 2\_ maximal  $\mathcal{R}$ \_module, fuzzy 2\_ maximal idal, 2-maximal idal, cyclic  $\mathcal{R}$ \_module in to F-2MRM and F-2MI, 2MI, CRM).

**Definition (1.1):**

A non-zero  $R$ - $M$  is called F-2MRM if and only if  $\sqrt{\text{ann}_R M}$  is F-2MI of  $R$ .

**Remarks and Examples (1.2):**

1) A F-2MRM is not necessarily a  $\mathfrak{m}$ ax-module. For example,  $4\mathbb{Z}$  is the F-2MI in a ring  $\mathbb{Z}$ , therefore  $4\mathbb{Z}$  is the F-2MZM, and  $\mathbb{Z}_6$  as a  $\mathbb{Z}$ -module is the F-2MZM but not  $\mathfrak{m}$ ax  $\mathbb{Z}$ -module, since  $\sqrt{\text{ann}_{\mathbb{Z}} \mathbb{Z}_6} = \sqrt{6\mathbb{Z}} = 6\mathbb{Z}$  is the F-2MI of  $\mathbb{Z}$  but not the  $\mathfrak{m}$ aximal of  $\mathbb{Z}$ .

(2)  $\mathbb{Z}_9$  as a  $\mathbb{Z}$ -module is F-2MZM. Therefore its  $\mathfrak{f}$ uzzy, Since  $\sqrt{\text{ann}_{\mathbb{Z}} \mathbb{Z}_9} = \sqrt{9\mathbb{Z}} = 3\mathbb{Z}$  is F-2MI of  $\mathbb{Z}$ , Since  $\frac{\mathbb{Z}}{3\mathbb{Z}} \cong \mathbb{Z}_3$  is 2-regular ring while  $\mathbb{Z}_3$  is a field.

(3)  $\mathbb{Z}_8$  as a  $\mathbb{Z}$ -module is not F-2MZM.

(4) The F-2MRM includes all sub-modules.

Proof (4):

Allow  $M$  to be a 2MRM so that its  $\mathfrak{f}$ uzzy, and allow  $0 \neq \mathcal{A} \subseteq M$ . To illustrate that  $\sqrt{\text{ann}_R \mathcal{A}}$  is F-2MI of  $R$ , because  $M$  is F-2MRM then  $\sqrt{\text{ann}_R M}$  is F-2MI of  $R$ . Since  $\mathcal{N} \subseteq M$  then  $\text{ann}_R \mathcal{M} \subseteq \text{ann}_R \mathcal{N}$ , then  $\sqrt{\text{ann}_R \mathcal{M}} \subseteq \sqrt{\text{ann}_R \mathcal{N}}$ . Thus by [8]  $\sqrt{\text{ann}_R \mathcal{N}}$  is  $\mathfrak{f}$ uzzy 2- $\mathfrak{m}$ aximal idal of  $R$ , Hence  $\mathcal{N}$  is F-2MRM.

**Theorem (1.3):**

Let  $M$  be CRM. Then the following statements are equivalents :-

(1)  $M$  is F-2MRM.

(2)  $[\sqrt{\text{ann}_R M} : J]$  is F-2MI of  $R$ ,  $\forall$  ideal  $J$  of  $R$  with  $J \not\subseteq \sqrt{\text{ann}_R M}$

(3)  $[\sqrt{\text{ann}_R M} : x]$  is F-2MI of  $R$ ,  $\forall$  element  $x \in R$  with  $x \notin \sqrt{\text{ann}_R M}$ .

(4)  $\sqrt{\text{ann}_R (m)}$  is  $\mathfrak{f}$ uzzy 2- $\mathfrak{m}$ aximal idal of  $R$ , for  $0 \neq m \in M$ .

**Proof (4) :**

Let  $M$  be 2MRM so its  $\mathfrak{f}$ uzzy, and allow  $\mathcal{A} \subseteq M$   $0 \neq$  sub module of  $M$ . To show that  $\sqrt{\text{ann}_R \mathcal{A}}$  is F-2MI of  $R$ . Since  $M$  is 2MRM then  $\sqrt{\text{ann}_R M}$  is  $\mathfrak{f}$ uzzy 2MI of  $R$ . And

since  $\mathcal{M} \subseteq \mathcal{N}$  then  $\text{ann}_{\mathcal{R}} \mathcal{M} \subseteq \text{ann}_{\mathcal{R}} \mathcal{N}$ , then  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} \subseteq \sqrt{\text{ann}_{\mathcal{R}} \mathcal{N}}$ . Thus by [8],  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{N}}$  is F-2MI of  $\mathcal{R}$ , Hence  $\mathcal{N}$  is F-2MRM.

**Theorem (1.4) :**

Let  $\mathcal{M}$  be a cyclic  $\mathcal{R}$ -module. Then the following statements are equivalents:-

- (1)  $\mathcal{M}$  is F-2MRM.
- (2)  $[\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} : \mathcal{J}]$  is F-2MI of  $\mathcal{R}$ ,  $\forall$  ideal  $\mathcal{J}$  of  $\mathcal{R}$  with  $\mathcal{J} \not\subseteq \sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$
- (3)  $[\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} : x]$  is F-2MI of  $\mathcal{R}$ ,  $\forall$  element  $x \in \mathcal{R}$  with  $x \notin \sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$ .
- (4)  $\sqrt{\text{ann}_{\mathcal{R}} m}$  is F-2MI of  $\mathcal{R}$ ,  $\forall 0 \neq m \in \mathcal{M}$ .

**Proof:**

(1)  $\Rightarrow$  (2) Since  $\mathcal{M}$  is F-2MRM, then  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  is F-2MI of  $\mathcal{R}$ . Let  $\mathcal{J}$  be an FI of a ring  $\mathcal{R}$  with  $\mathcal{J} \not\subseteq \sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$ . But  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} \subseteq \sqrt{[\text{ann}_{\mathcal{R}} \mathcal{M} : \mathcal{J}]}$ , then by proposition in [8] we have  $\sqrt{[\text{ann}_{\mathcal{R}} \mathcal{M} : \mathcal{J}]}$  is 2MI of  $\mathcal{R}$  so that its fuzzy.

(2)  $\Rightarrow$  (3) Taking  $\mathcal{J} = \mathcal{R}$ , then  $\sqrt{[\text{ann}_{\mathcal{R}} \mathcal{M} : \mathcal{J}]} = \sqrt{[\text{ann}_{\mathcal{R}} \mathcal{M} : \mathcal{R}]} = \sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} \subseteq [\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} : t]$  for every element  $t$  in  $\mathcal{R}$ ,  $t \notin \sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$ . Thus  $[\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} : t]$  is 2MI of  $\mathcal{R}$  so that its F-2MI.

(3)  $\Rightarrow$  (4) Let  $m$  be a non-zero element in  $\mathcal{M}$ . Since  $1 \notin \sqrt{\text{ann}_{\mathcal{R}}(m)}$ , then by assumption  $[\sqrt{\text{ann}_{\mathcal{R}}(m)} : \mathcal{R}] = \sqrt{\text{ann}_{\mathcal{R}}(m)}$  is F-2MI of  $\mathcal{R}$ .

(4)  $\Rightarrow$  (1) Since  $\mathcal{M}$  is cyclic  $\mathcal{R}$ -module then  $\mathcal{M} \cong \mathcal{R}/\text{ann}_{\mathcal{R}}(m)$ . That is  $\text{ann}_{\mathcal{R}} \mathcal{M} = \text{ann}_{\mathcal{R}} \left( \frac{\mathcal{R}}{\text{ann}_{\mathcal{R}}(m)} \right) = [\text{ann}_{\mathcal{R}}(m) : \mathcal{R}] = \text{ann}_{\mathcal{R}}(m)$  which implies that  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}} = \sqrt{\text{ann}_{\mathcal{R}}(m)}$ . Hence  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  is F-2MI of  $\mathcal{R}$ . Therefore  $\mathcal{M}$  is F-2MRM.



**Proposition (1.5)**

Every  $\mathcal{R}$ -module over Boolean ring is F-2MRM .

**Proof:**

By Remarks and Examples (2.1.2) (2)[6] every ideal of  $\mathcal{R}$  is 2MI. Tus  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  is 2MI .  $\mathcal{M}$  is F-2MRM .

**Proposition (1.6)**

Every  $\mathcal{R}\mathcal{M}$  over a semi- simple ring is F-2MRM .

**proof:**

Also remarks and Examples (2.1.2)(7)[6] every ideal of  $\mathcal{R}$  is 2MI. Tus  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  is F-2MIof  $\mathcal{R}$ .  $\mathcal{M}$  is F-2MRM .

**Proposition (1.7)**

Let  $\mathcal{M}$  be a cyclic semi-simple  $\mathcal{R}$ -module , and  $\text{ann}_{\mathcal{R}}(x)$  is a semiprime ideal of  $\mathcal{R} \forall 0 \neq x \in \mathcal{M}$  . Then  $\mathcal{M}$  is F-2MRM .

**Proof:**

Since  $\mathcal{M}$  is a cyclic , then  $\mathcal{M} = \mathcal{R}x$  for non – zero  $x$  in  $\mathcal{M}$  . Then  $\mathcal{M} \cong \mathcal{R} / \text{ann}_{\mathcal{R}}(x)$  . Since  $\mathcal{M}$  is a semisimple , Then by Corollary (1.2.4)[6]  $\mathcal{M}$  is 2-Regular  $\mathcal{R}$ -module . Hence  $\mathcal{R} / \text{ann}_{\mathcal{R}}(x)$  is 2-Re  $\mathcal{R}$ -module . Thus  $\text{ann}_{\mathcal{R}}(x)$  is 2MIof  $\mathcal{R}$  so is F-2MI. But  $\text{ann}_{\mathcal{R}}(x)$  is a semiprime then  $\sqrt{\text{ann}_{\mathcal{R}}(x)} = \text{ann}_{\mathcal{R}}(x)$  , then  $\sqrt{\text{ann}_{\mathcal{R}}(x)}$  is F-2MIof  $\mathcal{R}$  . Hence by Theorem (1.4)  $\mathcal{M}$  is F-2MRM .

**Proposition (1.8):**

Let  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  is 2MIof  $\mathcal{R}$  , Then  $\mathcal{M}$  is F-2MRM .

**Proof:**

Since  $\text{ann}_{\mathcal{R}} \mathcal{M} \subseteq \sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  , and  $\text{ann}_{\mathcal{R}} \mathcal{M}$  is MI of  $\mathcal{R}$  then by (2.1.11)(2)[6]  $\sqrt{\text{ann}_{\mathcal{R}} \mathcal{M}}$  F-2MIof  $\mathcal{R}$  Thus  $\mathcal{M}$  is F-2MRM .

**Proposition (1.9):**

Every F.G.  $R$  module  $M$  is F-2MRM .

**Proof:**

Since  $M$  is finitely generated regular  $R$  -module then by Proposition (2.1.28)[6]  $\text{ann}_R M$  is 2MI of  $R$  . Then by Proposition (1.8)  $M$  is F-2MRM .

**Proposition (1.9)**

Every F.G co-semisimple  $R$  module  $M$  is F-2MRM .

**Proof:**

Since  $M$  is F.G co-semisimple  $R$  -module then by Proposition (2.1.26)[6]  $\text{ann}_R M$  is 2MI of  $R$  . then by Proposition (1.8)  $M$  is F-2MRM

Keep in mind an  $R$  -module  $M$  is called a prime if  $\text{ann}_R M = \text{ann}_R N$  for every non-zero submodule  $N$  of  $M$  [12] .

**Proposition (1.10) :**

**Suppose**  $M$  be a prime 2-  $R$  egular  $R$  -module over PIR  $R$ . Then  $M$  is F-2MRM

**Proof:**

Since  $M$  is 2-  $R$  egular , then by Proposition (1.2.15) in [6]  $R/\text{ann}_R(x)$  is 2-Regular  $R$  -module for each non-zero  $x$  in  $M$ . Then  $\text{ann}_R(x)$  2MI of  $R$  so its fuzzy. But  $M$  is a prime , then  $\text{ann}_R M = \text{ann}_R(x)$  for each non-zero  $x$  in  $M$  . Thus  $\text{ann}_R M$  is F-2MI of  $R$  . then by Proposition (1.7)  $M$  is F-2MRM . Recall that an  $R$  -module  $M$  is co-prime if  $\text{ann}_R M = \text{ann}_{R_R}(\frac{M}{N})$  for every non-zero sub-module  $N$  of  $M$  [9].

**Proposition (1.11):**

Suppose  $M$  be a co-prime  $R$  -module over 2-  $R$  egular ring  $R$  , Then  $M/N$  is F-2MRM , for every non-zero submodule  $N$  of  $M$  .

**Proof:**

Since  $R$  is 2-  $R$  egular ring , then every ideal of  $R$  is 2MI. Thus  $\text{ann}_R M$  is 2MI so its F-2MI. But  $M$  is co-prime  $R$ -module , Then  $\text{ann}_R M = \text{ann}_R(\frac{M}{N})$  for every non-zero submodule  $N$  of  $M$ . Hence  $\text{ann}_R(\frac{M}{N})$  is F-2MI of  $R$  . But  $\text{ann}_R(\frac{M}{N}) \subseteq \sqrt{\text{ann}_R(\frac{M}{N})}$  ,

Then by proposition (2.1.11)(2)[6]  $\sqrt{\text{ann}_{\mathcal{R}}(\frac{\mathcal{M}}{\mathcal{N}})}$  is F-2MI of  $\mathcal{R}$ . Therefore  $\frac{\mathcal{M}}{\mathcal{N}}$  is  $\mathcal{M}$  is F-2MRM.

Keep in mind an  $\mathcal{R}$ -module  $\mathcal{M}$  is a primary if  $(0)$  is a primary submodule of  $\mathcal{M}$ , where a submodule  $\mathcal{N}$  of  $\mathcal{M}$  is called a primary  $\mathcal{N} \neq \mathcal{M}$  and wherever  $rx \in \mathcal{N}$  for  $r \in \mathcal{R}, x \in \mathcal{M}$ , we have either  $x \in \mathcal{N}$  or  $r^n \in [\mathcal{N} : \mathcal{M}]_{[10]}$ .

**Proposition (1.12)**

Let  $\mathcal{M}$  be an  $\mathcal{R}$ -module with  $(0)$  is a primary submodule of  $\mathcal{M}$ , and every proper submodule of  $\mathcal{M}$  is 2MRM. Then  $\mathcal{M}$  is F-2MRM.

**Proof:**

Since  $(0)$  primary submodule of  $\mathcal{M}$ , then by Proposition (1.1.57)[6] we have  $\sqrt{\text{an}_{\mathcal{R}} \mathcal{M}} = \sqrt{\text{an}_{\mathcal{R}} \mathcal{N}}$  for every proper submodule  $\mathcal{N}$  of  $\mathcal{M}$ , But  $\mathcal{N}$  is 2MRM Then  $\sqrt{\text{an}_{\mathcal{R}} \mathcal{N}}$  is 2MI of  $\mathcal{R}$ , Hence  $\sqrt{\text{an}_{\mathcal{R}} \mathcal{N}}$  is F-2MI of  $\mathcal{R}$ . Therefore  $\mathcal{M}$  is F-2MRM

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## EVALUATION THE SURFACE ROUGHNESS OF POLYMETHYLMETHACRYLATE AFTER REINFORCEMENT WITH MAGNESIUM OXIDE

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Sara Abdulbasit TURKI <sup>2</sup>

### Abstract:

Nanotechnology has recently been used in the prosthodontics field for material enhancement purposes. The major goal of this research was to evaluate the effect of inclusion Magnesium oxides nanoparticles on the surface roughness of heat cured poly-methyl methacrylate. Forty (40) disc shape of PMMA specimens with a diameter of (12±0.1) mm and a thickness of (2±0.1) mm were prepared and subdivided into 4 groups according to nanoparticles addition. (0,1,3,5 %) of magnesium oxide powder were added to heat cured PMMA. Then, they cured, finished and polished. Finally, they were subjected to roughness test. Statistical analysis represent the highest mean value was in group without any addition of NPs (1.4520). while, (3%) group of NPs was the lowest mean value (0.0465).

Conclusion: Nps can improve the properties of poly methylmethacrylate when added in limited concentrations.

**Key Words:** Magnesium Oxides Particles, Surface Roughness, Heat-Cured Acrylic Resins.



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## Introduction:

Over decades, denture have been fabricated by using many of materials, in 1930, Polymethyl methacrylate (PMMA) and for more than eighty five years it has been the material of choice for denture base construction (1). Till now, over 95% of removable dentures are consist of PMMA for many reasons like: esthetics, ease of manipulation, stability in the oral condition, biocompatibility, and good oral hygiene (2). On the other hand, it has some disadvantages such as: poor strength, low resistance to abrasion, and shrinkage on polymerization (3). Thus, Many attempts have been used to overcoming these limitations and optimization of their physical properties such as: incorporation of some rubber phase, changes in their chemistry, addition of multifunctional crosslinking factor such as polyethylene glycoldimethacrylate, metal oxides, metal wires or fiber oxides of (Al, Mg, Zr)(4). The inclusion of magnesium oxide in different dental materials was investigated and shown to be as an important inorganic material, non-toxic and antibacterial agent. The roughness of acrylic resin surfaces is un desirable property due to irregularities of the surface increase the likelihood of microorganisms remaining when the prosthesis is cleaned (4,5). In this study we investigate the effects of magnesium oxide with different concentrations on the surface roughness of heat-cured acrylic resins.

## 2. MATERIALS AND METHODS:

Forty (40) heat-cured samples of acrylic resins have been prepared by conventional methods. Then, they are sub grouped according to concentrations of magnesium oxide particles have been added. Firstly, silicon mold with a  $(12 \pm 0.1)$  mm of diameter and a  $2(\pm 0.1)$  mm of thickness was used for waxing of specimens which have been flaked Fig.(1), (2). Then, metal flasks technique is used by coating them with separating medium (cold mold seal). The lower half is filled with dental stone then the disc shape wax is fixed on it, after the stone sets, separation medium was applied and left to dry. Following that, the upper half of flask was filled with stone and vibrated to prevent air entrapped. Then, wax elimination is accomplished according to instructions, the two halves of flasks is opened and coated with the separation medium to be packed later. The mixing proportion of acrylic resins was 3/1 by volume (P/L), while the manipulation and mixing were based on the guidelines of the manufacturer. The mixing was performed in a dry clean jar, then it wrapped till the dough stage have been reached. Three different concentrations of magnesium oxide were selected in this study (1% ,3% and 5%). For each group, the electronic balance was used to weigh the required amount of the polymer and Mgo powders as shown in table (1). the premeasured amount of nanoparticles were incorporated to the monomer and they are completely distributed by using of probe sonication device (120 W,100 KHz) for about 3 min. as shown in Fig.(3), after that the monomer and nanoparticle powder were mixed directly with the polymer's powder for lowering the particle collection possibilities & phase separations. After that, the mixture is stand until the dough have been reached. Thepacking stage was done after resin's reaching to dough phase. The acrylic was removed from glass container and

packed in a flask which was coated with a separation medium and left to dry. Then, nylon sheets was used to aid in separation the two halves of the flask and remove or added another amount of resin. The flask's two halves are closed tightly and put at a hydraulic press. (85-90 bar for 1minute). The pressure was applied in a slow rate on flask and the flow of the dough was evenly done via the space of the flask. Then, the pressure is released and opened, then the excessive material is removed via using a sharp knife. Then, the closure for another time was done and a nylon sheet is removed. Finally, the flask's two halves were closed tightly and clamped to transfer to the water bath for curing in accordance with the guidelines of the manufacturer. Finally, disc samples are removed from the flask and hand finished by using acrylic bur to remove the excess flash of acrylic resin. The samples were stored in sterilized water in 37C° for forty-eight hours before they were examined in relation to American Dental Association specification NO.12,1999)(6) All the samples subjected to roughness test by using the profilometer device to inspect the influence of Nano filler reinforcement on microstructure of the tested surfaces. The apparatus is comes with analyzing of surface (sharp stylus) composed from diamond. The profilometer records all of the peaks and recesses which characterized the test surface by its scale. The acrylic samples were placed on its stable stage and the location of the test area was chosen then the analyzer passed in the correct direction along the surface of the samples as shown in Fig. (4).

### 3.RESULTS:

**Table (2): Descriptive statistics for surface roughness test group**

Groups	Mean	Std. Deviation	N
<b>PMMA(control)</b>	1.4520	0.35398	10
<b>PMMA+1%MgO</b>	1.1204	0.33846	10
<b>PMMA+3%Mgo</b>	0.0465	0.00178	10
<b>PMMA+5%Mgo</b>	.07720	0.00460	10

Table (2) results showed, the highest mean of roughness value was for PMMA without any treatment (control) followed by PMMA treated with 1% of MgO nanoparticles group (1.4520) (1.1204), while the lowest roughness value was for PMMA with 3% of MgO group which is (0.0465).

**Table (3): LSD test for roughness test groups.**

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PMMA	PMMA+1%Mgo	.3414	.15487	.160	-.0863	.6793
	PMMA+3% Mgo	1.4154*	.11184	.000	1.0560	1.6648
	PMMA+5% Mgo	1.3847*	.11185	.000	1.0252	1.6342
PMMA+1% Mgo	PMMA+3%Mgo	1.0739*	.10703	.000	.6398	1.3080
	PMMA+5%Mgo	1.0432*	.10704	.000	.6091	1.1773
PMMA+3% Mgo	PMMA+5%Mgo	-.0307*	.00146	.000	-.0253	-.0161

Table (3) represent the least significant difference which have been used to determine the source of variance among the groups of roughness test ,their results showed there is high significant difference in the roughness value among treated groups with magnesium oxides nanoparticles except for control and PMMA with 1% of Mgo there is non-significant difference between them.

#### 4.DISCUSSION:

Polymethylmethacrylate which is a material selected for denture base construction due to its appropriate properties for working, easy of manipulation, precise fits, stable in the oral environments, low cost with superior esthetic. Nevertheless, it isn't ideal due to its relative reduced mechanical power that may lead to denture's fracture, thus, there are many reinforcement substances like metal oxides, clay mineral as well as fibers of Carbon graphite's (7).These particles' shapes, sizes and distributions play essential roles on the mechanical characteristics of the polymer's (8).In the current study, magnesium oxide powder is used due to its non-toxic and inorganic material in addition to their white color doesn't alter their esthetic (9) .Thus, different concentrations 1%, 3% and 5% of Magnesium oxide nanoparticles have been used to evaluate surface roughness of acrylic resin denture base.

##### 4.1. Roughness test of PMMA + 1%, 3% and 5% of MgO nanoparticles:

The surface irregularities or roughness on denture base materials are necessary and important property due to which it affects the oral tissues when it comes into direct contact with dentures. Thus, the materials have been used as a dental prosthesis should be inspected before their use in the mouth ,since rough surfaces can cause discoloration of the prosthesis , be an irritant to patients and it may also contribute to colonization of microbial and biofilm formation(10)(11).In the current study, profilometer device was used to estimate the effect of



incorporation magnesium oxide particles on surface geometry of the specimens because this device appear to be excellent device to evaluate surface roughness by giving quantitative measurement that can be evaluated and compared statistically. From the results of the roughness test as Shown in tables: (2),(3) .The highest mean value was for Control group which untreated with nanoparticles (1.4520) followed by PMMA with 1% of Mgo nanoparticles which is (1.1204) this may be due to environmental reasons, w:p ratio, mixing time or method of addition the nanoparticles to mixture(12). On the other hand, 3% of Mgo nanoparticle (0.0465) was the lowest mean value. This can have explained by the interaction between the reinforcement material and the polymer matrix by Cross-link formation or supra-molecular bonding that coats nanoparticles and avoids the crack's and roughness propagation (13)(14). The low percentage may be acted as impurities within the PMMA which lead to decrease the roughness value than high percentage (15)(16). This coincide with (2) who found that addition of Nano filler materials in low percentage causes reduction in surface roughness.

#### **CONCLUSIONS:**

According to the result obtained in this study we found that:

Adding limited concentration of magnesium oxide (3%) have positive impacts on the roughness of polymethylmethacrylate and any increased of magnesium oxide concentration will affect adversely on the roughness of acrylic resins.

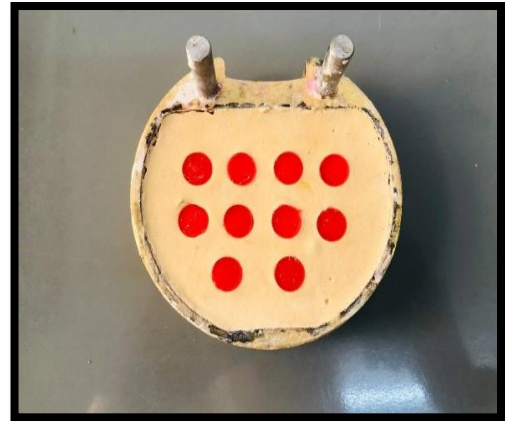
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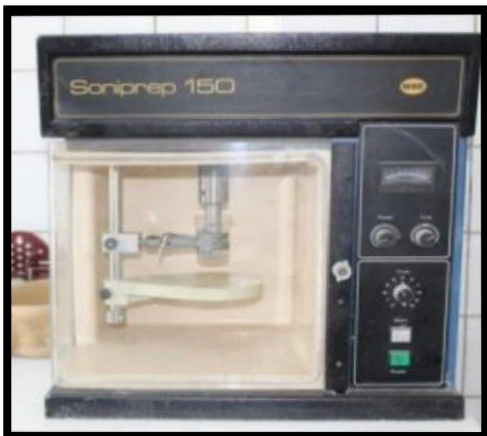
**Figures:**



**Figure (1) Silicon mould for waxing**



**Figure (2) disc shaped wax specimens**



**Figure (3): probe sonication**



**Figure (4): profilometer device**

Tables:

**Table (1): Mixing ratio of acrylic resin with different nanoparticles concentration of MgO**

<b>Percentage</b>	<b>Amount of reinforcement (addition)</b>	<b>Amount of polymer</b>	<b>Amount of monomer</b>
<b>untreated (0%)</b>	<b>0</b>	<b>100g</b>	<b>40ml</b>
<b>MgO (1%)</b>	<b>1g</b>	<b>99 g</b>	<b>40ml</b>
<b>MgO (3%)</b>	<b>3 g</b>	<b>97 g</b>	<b>40ml</b>
<b>MgO (5%)</b>	<b>5 g</b>	<b>95 g</b>	<b>40ml</b>

## USING LIGHT FIDELITY (Li-Fi) TO TRANSMIT DATA

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### Abstract:

Li-Fi (Light Fidelity) is a revolutionary technology for optical wireless communication systems that using LEDs as a source of transmission. Different of radio signals, data is transferred via light signals. The Li-Fi technology are employed for internal wireless optical communication by using a white LED as the source and a solar cell for detection. We transmit an image between two computers at a link distance of 100 cm with the concept of line of sight (LOS). To demonstrate the effect of temperature on the receiver image, we vary the hot air parameter from different angles. We use measures such as the average square error (MSE) and signal-to-noise ratio (SNR) to evaluate image quality.

**Key Words:** Optical wireless communication, Indoor Channel, Li-Fi, Light-Fidelity.



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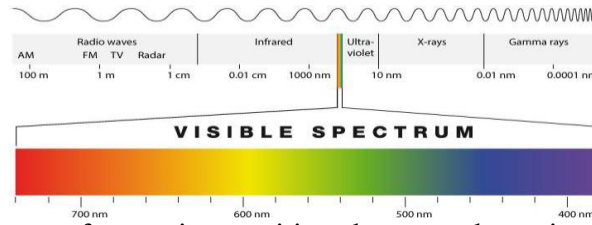


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## Introduction:

Li-Fi (Light Fidelity) is a wireless connection that travels very quickly using visible light, and then named visible light communication. Data is transmitted through various LEDs according to their intensity[1]. These high data bandwidths and high data transmission confidentiality can be available in next-generation communications called LIGHT FIDILITY or Li-Fi. This technique uses a visible wavelength of about 300nm-700nm as shown in Figure 1, using this spectrum set allows us to use It

Light is used between 400 THz (780 nm) and 800 THZ (375 nm). The data in this wave cannot be penetrate the walls (because they are light waves)[2].



It makes them important for use in sensitive places such as aircraft and hospital Hata! Başvuru kaynağı bulunamadı.. Hata! Başvuru kaynağı bulunamadı..

Fig.(1) photo of the spectrum

This technology uses white LEDs as transmitters [4]. These devices are usually used for lighting only by applying constant current. With the

Fast and accurate variations of the current, the optical output can be changed at very high speeds. When the LED turns on, we're sending one digit. If it is stopped, this means sending zero (0). (LED) at high speed which gives good opportunities for data transmission at high speed.

So, all that is required is some LEDs and a controller that allows the coding of the data required through the LED.

Some improvements can also be made to this method by using LEDs to transfer data. This can be done by using a combination of green, red, and blue LEDs to change the frequency of light. Each frequency is coded for a different data channel.

These developments are expected in the future at speeds of up to 10 Gp/s. This means we can download the full-resolution film in about half a minute (30s).

## Historical review:

In 2011, Harald Hass discussed the potential of Li-Fi technology and how it could potentially replace Wi-Fi. Wi-Fi is useful for general wireless coverage within buildings, while Li-Fi is ideal for high-density wireless data coverage in areas with no obstacles, using light-emitting diodes (LEDs) for data transmission[3]. In 2016, D. Samudika and his team implemented two prototype stereo and audio streaming methods using Visible Light

Communication (VLC), including a Pulse Code Modulation Streaming (PCM) prototype and MP3 streaming prototype. They observed that the PCM prototype had lower system complexity compared to MP3, and at higher data rates, the audio quality reached an optimal level. However, as the channel length increased, the audio quality decreased to a minimum level[4]. In 2013, K.P. Pujapanda discussed Power-line Communications (PLC) for data transmission over power lines. The use of lines as a medium of communication is common. An integrated system with PLC and VLC offering low-cost and efficient broadband access for home networking. For receiving higher data rates in MHz, the PLC channel was simulated using the DMT-QAM modulation scheme[5].

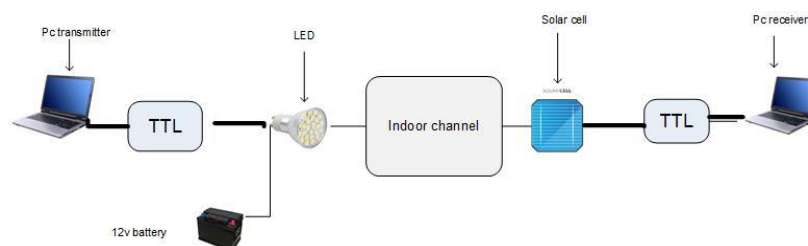
In this study, sensors were able to harvest energy from LED light and communicate with each other using Li-Fi, a technology that has the potential to bring significant benefits and enable effective features in high-performance buildings. In 2016, H. Saini and his team presented a literature review of Li-Fi technology, which described its workings, advantages, and various future aspects[6].

### **The theoretical part:-**

#### **Light-Fidelity or (Li-Fi) Concept:**

VLC technology has proposed providing high-speed internet connection from ceiling lamps, also known as Light-Fidelity or "optical Wi-Fi." VLC technology offers a massive free bandwidth, making it ideal for high-speed internet connections in small spaces, such as offices with distances of only a few meters between the ceiling and the office. VLCs can provide multi-gigabit connections in such spaces, making Li-Fi the preferred option for this application. In this system, data, image, and video transmissions are evaluated for indoor LED (Li-Fi) channels. The system comprises two main components: a Li-Fi router that transforms the incoming data from the internet source into a light signal and light sources that switch on and off at frequencies negligible to humans. The receiver then transforms the light signal into numerical data,

which is provided to the mobile station. For uploading, infrared links are used



**.Fig. (2): Diagram of (Li-Fi) system**

The fig. (2) represented the diagram of (Li-Fi) system.

### **Line of Sight (LoS), Propagation Models:-**

Indoor optical wireless communication (OWC) systems use LED as a source and large-area photodetectors. The behavior of the optical band is illustrated in equation (1). Attenuation due to absorption and scattering is minimal because the link length for indoor OWC is relatively short. Assuming an OWC link with a Lambertian source and a receiver with an optical band-pass filter of transmission  $T_s(\psi)$  and a nonimaging concentrator of gain  $g(\psi)$ , the DC gain for a receiver located at a distance of  $d$  and angle  $\phi$  with respect to the transmitter can be expressed using Lambert's mode number, which approximates the directivity of the source beam [7].

$$H_{\text{lon}}(0) = \begin{cases} \frac{A_1(m_1+1)}{2\pi f} \cos^m(\phi) T(\psi) g(\psi) \cos\psi & 0 \leq \psi \leq \Psi \\ 0 & \text{otherwise} \end{cases} \quad \text{.....(1)}$$

### **The Signal to Noise Ratio (SNR).**

The proportion of signal power to noise power can be defined as the signal to noise ratio [9]. The term (SNR) in experimental measurements is often an estimate of a ratio of currents, voltages, or power. The SNR is easy to determine by using the following equations [10]:

The signal intensity at the photodiode when the LED is emitting light (signal + noise) is referred to as High, while the noise present when the LED is turned off is referred to as Low. The Mean Square Error (MSE) is a measure of the difference between the estimated values and the true values, which is calculated by taking the average squared difference between the input and output image pixels as shown in equation (3) [11].

$$MSE = \frac{1}{NM} \sum_{m=1}^{M-1} \sum_{n=1}^{N-1} e(m, N)^2 \quad \text{.....}$$

(3)

Where (m, n) is the error difference between the original and the distorted images. The M and N are the numbers of rows and columns in the input images

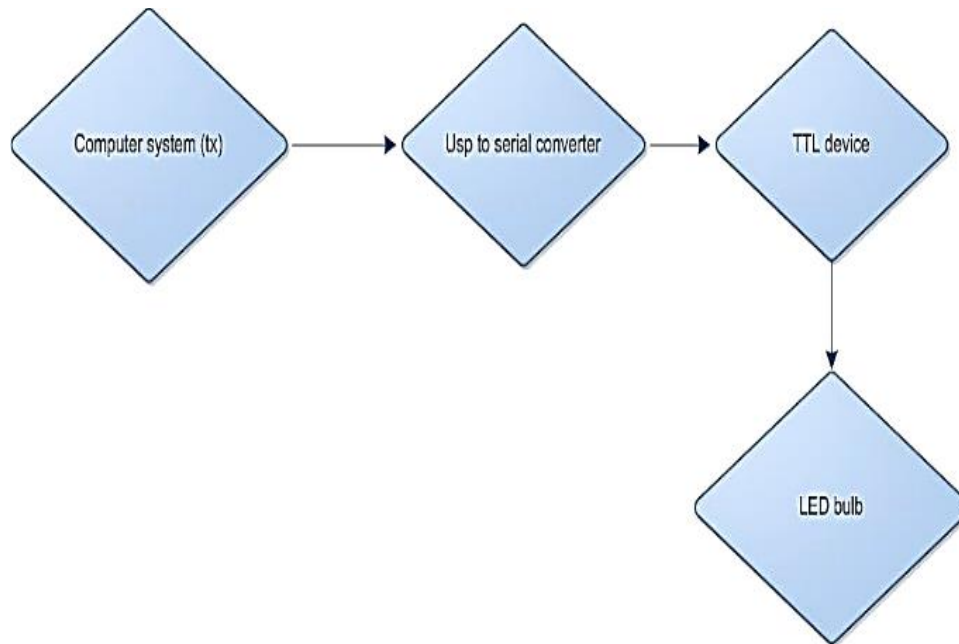
### **Experimental part**

Indoor Li-Fi systems can be divided into two main parts. The first component is the sender which represents the transmission performed by sending data in an indoor channel, and the second part in the Li-Fi system is the receiver which represents the reception performed. Transmission and receiving can be clarified in two block diagrams as follows: -



**Block diagram for the transmitter: -**

The block diagram for the transmitter model to the indoor Li-Fi channel is shown in Figure (3).



**Figure 3: Data transmitter diagram[12]**

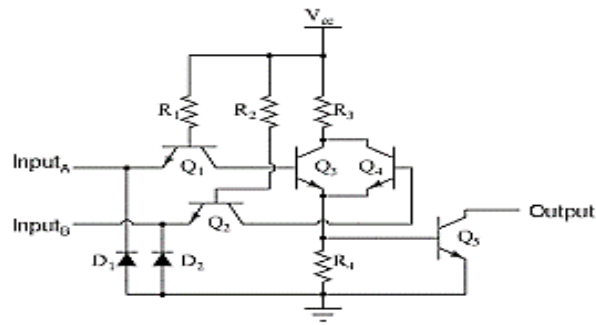
The transmitter module described in the paper includes several components and processes. The first component is

A. a computer system that performs image processing and sends the image signal or information to the transmitter module. The image signal is sent in a serial manner using a USB-to-Serial converter. The signal is then received by a TTL receiver, which converts it into a Pulse Width Modulation (PWM) signal. The PWM signal is transmitted to the LED driver for amplification. The TTL also generates a PWM signal that varies the brightness of a LED connected to it, demonstrating PWM. An LCD is connected to the TTL to display the data transmission status. Overall, the transmitter module is responsible for receiving image signals, converting them into PWM signals, and amplifying them for transmission through LED lights.

B. A USB adapter converter is a type of protocol used for converting USB data signals to and from other communications standards. USB adapters convert USB data to standard serial port data. The USB connector plugs into the computer's USB port. The serial device transmits data directly to the USB port.

**C. The TTL:**

It is a logic family structure made from bipolar junction transistors. Its name signifies that transistors include both the logic function (first transistor) and the amplifying function (second transistor); it is the same naming convention used in diode-transistor logic (DTL) and resistor-transistor logic (RTL). Figure (4) illustrates the circuit of this logic family.

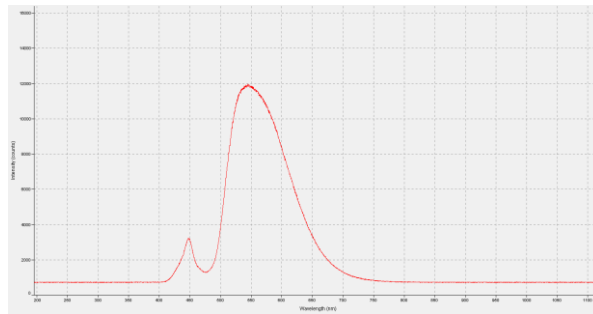


**Figure4: TTL circuit.[13]**

D. LED source:

That is correct. LED (Light Emitting Diode) is a semiconductor device that emits light when a current flows through it. In optical communication, LED is used as a source of light for transmitting data. The LED can be modulated to produce an optical signal by turning it on and off at a very high speed. This modulation produces a series of light pulses that can be detected and decoded by a receiver to retrieve the transmitted data. The LED has several advantages as a light source for communication, such as low cost, high reliability, high efficiency, and compatibility with electronic devices.

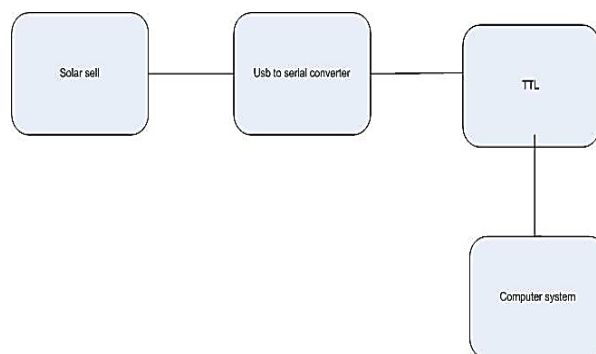
The bulb spectrum was shown in Figure (5).



**Figure 5: Spectrum of the LED used in transmission part.**

#### Receiver module: -

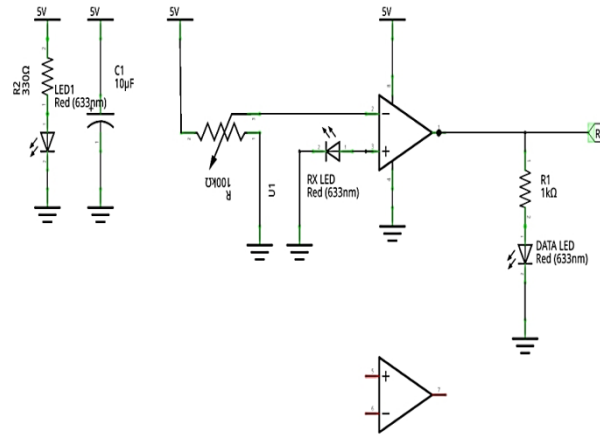
The block diagram for the receiver module to the Li-Fi channel system is shown in Figure (6).



**Figure 6: Data receiver model.**

a- Solar cell: - We used a high-efficiency solar cell panel as a receiver, due to its ease of use and connection to the laptop, and the large surface area of the solar cell compared to the photodetected one. with electrical specifications and dimensions as follows Dimension is 6cm \* 11cm and is electrical specifications 6v, 1w

b- Tuning circuit: - We used a small electronic circuit with the solar cell to synthesize the best signal and electronic circuit as shown in Figure 7.



**Figure 7: Receiver circuit.**

c- TTL: - Which is the same as the transmitter, has been used and as explained previously with the computer to receive the transmitted image signal

Working: -

The bandwidth was 4.8 kbps used for transmission data. The USB serial port was be directed for both the recipient and sender on the program so as not to conflict in the transmission of data. A file is created in the receiving computer to save the sent image from the sending computer. The important point is that the generated image file must be extended within the known image extensions and match the extension of the sent file.

As for the transmit part, when the send button is opened in order to send the image file to be sent. The transmitter will be in stages: -

A. Solar cells. The solar cell was used as a receiver, due to its ease of use and connection to the laptop. The large surface area of the solar cell compared to the photo detected it being slow to respond.

At the receiver side, the solar cell will register a binary (0) when the light is off and (1) when the light is on.

B. TTL receiver.

At the receive, the TTL conducted the binary conversation with the solar cell input data.

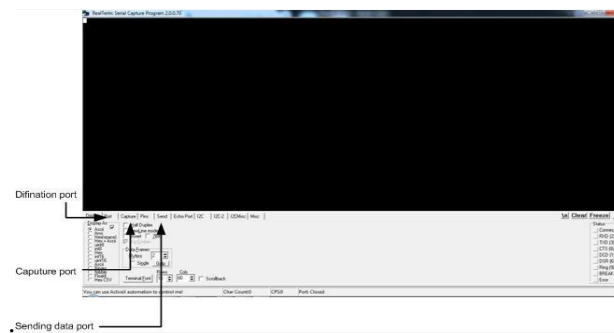
C. Computer system (receiving computer)

This paragraph is a technical description of how data is received by the receiver side in Li-Fi technology. It explains that the receiver side is connected to the TTL via a serial port and

uses an application called "real term" to process the data and regenerate the sent image. It also mentions that the data is sent using Li-Fi technology.

#### **Applications (Software) used: -**

The Realterm application, which is used for serial communication between the transmitter and receiver computers in the context of the image transmission system being described. The application is noted to have various uses, including engineering, debugging, data logging, and testing, and it can be used by other programs as a serial component. The paragraph also mentions that the application has an extensive command line interface and provides a figure (8) to clarify the program interface with some buttons.



**Fig. (8) real term application interface**

2. Matlab application: used to compare the sent and received images.

The Matlab software used with different codes to study quality of the received images based on the parameters such as (MSE, SNR). The code in the appendix is used to show the values of the parameters in terms of image quality.

This code calculates the MSE and SNR for the transmitted image.

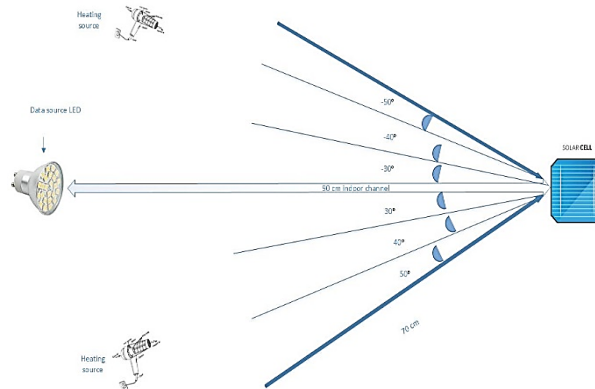
#### **Hardware parts:-**

1- TTL: - transistor logic is one of the most reliable devices used in transferring data between two computers. In this system, two TTL pieces are used, one in the receiver and another in the transmitter.

2- Solar cell: The solar cell is an electrical device that converts the energy of light directly to an electric signal or analog signal by the photovoltaic effect, which is a physical phenomenon[14].

**Methodology and experimental setup: -**

An electric circuit biased by 12V is used to operate the LED and to connect the transmitter computer via a TTL circuit. On the other side, the solar cell separated from the LED at a distance of 90 cm. The hot air source was 30 degrees from the LED and the solar cell) and the distance was 70 cm far from the solar cell as shown in Fig. (9).



**Figure 9: Transmitter and Receiver diagram.**

The data is sent from the transmitter PC to the receiver PC in this case.

Then increase the angle of hot air to 40° and 50° and repeat the steps. A file is created for each image sent case.

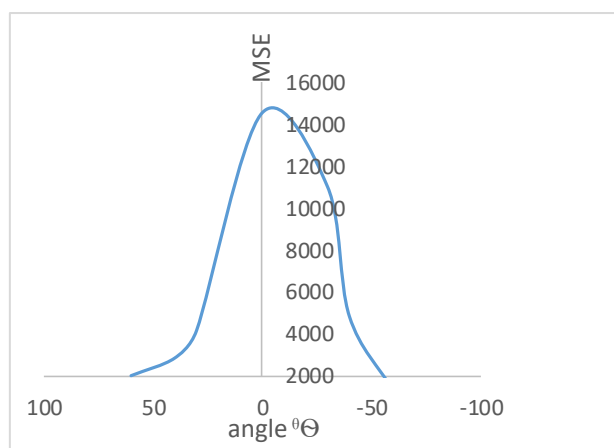
The steps are repeated on the other side. The data of the image transmitted is also saved in the same file to compare it by the Matlab application to determine the mean square error (MSE) for every case and compare it with the original image that is transferred in line of sight (LOS) without distortion.

Table (1) explains how (MSE) behaves when the angle is changed

.Table (1).MSE vs. received angle  $\Theta^0$

Angle $\Theta^0$	MSE
50	2011
40	2851
30	4160
0	14538
-30	11025
-40	4850
-50	1314

As we moved further from this line, the MSE values decreased. This result makes sense in accordance with reality. This is because MSE is computed by averaging the squared intensity of the original (input) image and the resultant (output) image pixels outlined in Fig. (10).

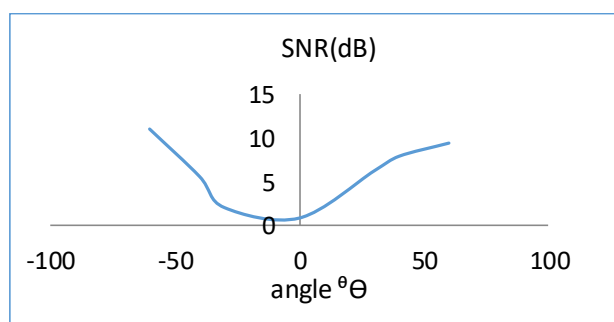


**Fig. (10) MSE vs angle ( $\Theta^\circ$ ) of hot air source**

Fig. (10) show that the relation between mean square error (MSE) and degree of hot air source. When the

angle between the (LOS) and the link from hot air source to the receiver increase, the (MSE) is decreasing, also observe the two side is not symmetry because of the alignment and effect of out lighting.

The fig. (11) illustrate the relation between the signal noise ratio (SNR) and the angle between hot air source and line of side (LOS). The (SNR) is increase when the angle is increasing also.



**Fig. (11) SNR vs angle ( $\Theta^\circ$ ) with hot air source**

## Conclusion

In this paper, an image was sent using Li-Fi technology. The distance between the transmitter of data (LED) and the reception (photodetector) was 100 cm, since this distance is a suitable for some tests in indoor localization. From the results of the parameters used (MSE and SNR), we can conclude that the best image when the heat source is far from the (LOS) (the straight line between the transmitter and the reception). Conversely, the more distorted appear in the image when the thermal source is closer to the LOS where the temperature have an important effect on the quality of the data transmitted by using the Li-Fi technology especially in indoor localization.

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## REGULARITY VIA PRE- GENERALIZED OPEN SETS

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### Abstract:

By use the notions pre-g-closedness and pre-g-openness we have generalized a class of separation axioms in topological spaces. In particular, we presented in this paper new types of regularities, which we named  $\rho g$ -regularity and Spg-regularity. Many results and properties of both types have been investigated and have illustrated by examples.

**Key Words:** Pre-Open Set, Pre-Closed Set, Pre-Function, Pre-Continuous-Function.



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## Introduction:

The nature of this work is to explore and extract a specific kind of axioms of generalized separation in topological spaces (or spaces for short). Before we know about this new genre, the generalized axioms of separation has been fully constructed by several authors ( cf. [ 1, 2, 3] ). A *pre-open* was studied in 1982 by Abd El-Monsef, Mashhour and El-Deeb [4]. A subset  $B$  of a space  $X$  is *pre-open* if  $B \subseteq \text{int}(\text{cl}(B))$ , the collection of all *pre-closed* (resp. *pre-open*) is symbolized by  $\text{PC}(X)$  (resp.  $\text{PO}(X)$ ), where the  $\text{cl}(B)$  and the  $\text{int}(B)$  are the closure and the interior operators of  $B$  respectively, the complement of a *pre-open* set is called *pre-closed*. If  $B$  is a subset of a space  $X$ , we denote the *pre-closure* of  $B$  and the *pre-interior* of  $B$  by  $\rho\text{-cl}(B)$ ,  $\rho\text{-int}(B)$  respectively, where the *pre-interior*( $B$ ) is the union of all *pre-open* sets in  $X$  which are subsets of  $B$  and the *pre-clouser*( $B$ ) is the intersection of all *pre-closed* sets containing  $B$ . Recall that [5, 6] a subset  $B$  of a space  $X$  is namely *pre -g-closed* set, denoted by  $\rho g\text{-closed}$ , if  $\rho\text{-cl}(B) \subseteq \mathcal{U}$  whenever  $B \subseteq \mathcal{U}$  and  $\mathcal{U}$  is a *pre-open* set in  $X$ . the complement of  $B$  is called  $\rho g\text{-open}$  and the collection of all  $\rho g\text{-closed}$  (resp.  $\rho g\text{-open}$ ) is symbolized by  $\rho g\text{-C}(X)$  (resp.  $\rho g\text{-O}(X)$ ). A map  $f$  from a space  $X$  to a space  $Y$  is called *pre-irresolute* [7] if  $f^{-1}(\mathcal{V}) \in \text{PO}(X)$  for each  $\mathcal{V} \in \text{PO}(Y)$  and it is called *M-pre-closed* [4] if the image of each *pre-closed* set in  $X$  is *pre-closed* set in  $Y$ . Also a map  $f: X \rightarrow Y$  is known  $\rho g\text{-irresolute}$  if  $f^{-1}(\mathcal{V}) \in \rho g\text{-O}(X)$  for each  $\mathcal{V} \in \rho g\text{-O}(Y)$  and it is known  $\rho g\text{-open}$  (resp.  $\rho g\text{-closed}$ ) if the image of each  $\rho g\text{-open}$  (resp.  $\rho g\text{-closed}$ ) set in  $X$  is  $\rho g\text{-open}$  (resp.  $\rho g\text{-closed}$ ) set in  $Y$  [for instance see 4, 8].

## 2. pre -generalized regular space

In the beginning of this section we will give a definition of *g-regular spaces*.

**Definition 2.1.** a space  $X$  is called *pre-generalized regular space* and shortly  $\rho g\text{-R-space}$  if for each  $\mathfrak{c} \in X$  and for every closed set  $\hat{F}$  in  $X$  such that  $\mathfrak{c} \notin \hat{F}$ , there are two disjoint  $\rho g\text{-open}$  sets  $\mathcal{U}$  and  $\mathcal{V}$  where  $\mathfrak{c} \in \mathcal{U}$  and  $\hat{F} \subset \mathcal{V}$ .

It is easy to see that every *regular space* is  $\rho g\text{-regular}$  but the convers is not necessarily true, for example take  $X = \{\mathfrak{c}_1, \mathfrak{c}_2, \mathfrak{c}_3\}$ ,  $\mathcal{C} = \{X, \emptyset, \{\mathfrak{c}_1, \mathfrak{c}_2\}\}$ , hence  $\mathcal{C}^c = \{X, \emptyset, \{\mathfrak{c}_3\}\}$ . The collection of  $\rho g\text{-open}$  as follows:  $\rho g\text{-O}(X) = \{X, \emptyset, \{\mathfrak{c}_1\}, \{\mathfrak{c}_2\}, \{\mathfrak{c}_1, \mathfrak{c}_2\}, \{\mathfrak{c}_1, \mathfrak{c}_3\}, \{\mathfrak{c}_2, \mathfrak{c}_3\}\}$ . It is clear that  $(X, \mathcal{C})$  is  $\rho g\text{-R-space}$  however it's not *regular space*.

**Theorem 2.2.** A space  $X$  is  $\rho g\text{-R-space}$  if for every  $\mathfrak{c} \in X$  and for every open set  $\mathcal{U}$  containing  $\mathfrak{c}$ , there exist a *pre-open* set  $\mathcal{V}$  such that  $\mathfrak{c} \in \mathcal{V} \subset \rho\text{-cl}(\mathcal{V}) \subset \mathcal{U}$ .

**Proof:** Let  $E$  be a closed and  $\mathfrak{c} \notin E$ , so  $\mathfrak{c} \in E^c$  and  $E^c$  is open. By assumption we have  $\mathcal{V} \in \text{PO}(Y)$  such that  $\mathfrak{c} \in \mathcal{V} \subset \rho\text{-cl}(\mathcal{V}) \subset E^c$ , hence  $E \subset (\rho\text{-cl}(\mathcal{V}))^c$ . But  $\rho\text{-cl}(\mathcal{V})$  is *pre-closed* [4], also each of  $\mathcal{V}$  and  $(\rho\text{-cl}(\mathcal{V}))^c$  are  $\rho g\text{-open}$  [9]. It is clear that  $\mathcal{V}$  and  $(\rho\text{-cl}(\mathcal{V}))^c$  are disjoint and containing  $\mathfrak{c}$  and  $E$  respectively, so we have done.

**Remark 2.3.** A space  $X$  is  $\rho g$ - $\mathcal{R}$ -space if for every  $\epsilon \in X$  and every open set  $\mathcal{V}$  containing  $\epsilon$ , there exists a  $\rho g$ -open set  $\mathcal{W}$  such that  $\epsilon \in \mathcal{V}$  and  $\rho\text{-cl}(\mathcal{W}) \subset \mathcal{V}$ .

**Proof:** since  $\epsilon \notin \mathcal{V}^c$  and  $\mathcal{V}^c$  is closed, there exist disjoint  $\mathcal{W}_1, \mathcal{W}_2 \in \rho g\text{-}\mathcal{O}(X)$  such that  $\epsilon \in \mathcal{W}_1$  and  $\mathcal{V}^c \subset \mathcal{W}_2$  implies  $\mathcal{W}_1 \subset (\mathcal{W}_2)^c \subset \mathcal{V}$ . Further  $(\mathcal{W}_2)^c$  is  $\rho g$ -closed and  $\mathcal{V}$  is pre-open [9], then  $\rho\text{-cl}(\mathcal{W}_1) \subset \rho\text{-cl}(\mathcal{W}_2)^c \subset \mathcal{V}$ , so  $\mathcal{W}_1$  will be the  $\rho g$ -open set we need.

**Proposition 2.4.** If  $X$  is  $\rho g$ - $\mathcal{R}$ -space then each neighbourhood of  $\epsilon$  contain a  $\rho g$ -closed subset consist of  $\epsilon$ .

**Proof:** Let  $\epsilon \in X$  and a neighbourhood  $\mathcal{M}$  of  $\epsilon$ , then there is an open set  $\mathcal{H} \subset X$  such that  $\epsilon \in \mathcal{H} \subset \mathcal{M}$  implies  $\epsilon \notin X - \mathcal{H}$ . Since  $X$  is  $\rho g$ - $\mathcal{R}$ -space, so we have  $\mathcal{W}_1, \mathcal{W}_2 \in \rho g\text{-}\mathcal{O}(X)$  such that  $\epsilon \in \mathcal{W}_1$ ,  $(X - \mathcal{H}) \subset \mathcal{W}_2$  and  $\mathcal{W}_2 \cap \mathcal{W}_1 = \emptyset$ . Hence  $\epsilon \in \mathcal{W}_1 \subset X - \mathcal{W}_2 \subset \mathcal{H} \subset \mathcal{M}$ , therefore  $X - \mathcal{W}_2$  will be the required  $\rho g$ -closed set.

The following example shows that the union of each  $\rho g$ -closed subsets is  $\rho g$ -closed, nevertheless this fact is not true in general.

**Example 2.5.** let  $X = \{\epsilon_1, \epsilon_2, \epsilon_3\}$ ,  $\mathcal{C} = \{X, \emptyset, \{\epsilon_1\}, \{\epsilon_1, \epsilon_2\}\}$ , So  $\text{PO}(X) = \{X, \emptyset, \{\epsilon_1\}, \{\epsilon_1, \epsilon_2\}, \{\epsilon_1, \epsilon_3\}\}$ , hence  $\rho g\text{-}\mathcal{C}(X) = \{X, \emptyset, \{\epsilon_2\}, \{\epsilon_3\}, \{\epsilon_2, \epsilon_3\}\}$ .

We mean by a  $\rho g$ -open subset  $B$  has a property  $\Gamma^*$  if  $B \cap \mathcal{H}$  is  $\rho g$ -open for each  $\rho g$ -open  $\mathcal{H}$  in a  $(X, \mathcal{C})$ .

**Theorem 2.6.** The  $\rho g$ -regularity is hereditary property restricted on subspaces have property  $\Gamma^*$ .

**Proof:** Let  $Y$  be a subspace of a space  $(X, \mathcal{C})$  has property  $\Gamma^*$  and let  $F$  be a closed subset of  $Y$  such that  $\epsilon \notin F$  for some  $\epsilon \in Y$ . Now  $F$  is closed, so there is a closed subset  $E$  in  $X$  such that  $F = E \cap Y$  [10]. It is clear  $\epsilon \notin E$  and since  $X$  is  $\rho g$ - $\mathcal{R}$ -space, hence there are two disjoint  $\rho g$ -open sets in  $X$  say  $\mathcal{M}_1$  and  $\mathcal{M}_2$  such that  $\epsilon \in \mathcal{M}_1$ ,  $F \in \mathcal{M}_2$ . By the  $\Gamma^*$  property of  $Y$ , we have  $Y \cap \mathcal{M}_1$  and  $Y \cap \mathcal{M}_2$  are  $\rho g$ -open subsets in  $Y$  containing  $\epsilon$  and  $F$  respectively and since  $(Y \cap \mathcal{M}_1) \cap (Y \cap \mathcal{M}_2) = \emptyset$  [11], so we have done.

**Definition 2.7.** A space  $X$  is called pre-generalized Hausdorff space (or shortly  $g$ -Hausdorff space)  $\Leftrightarrow$  for any elements  $\epsilon_1 \neq \epsilon_2$ , there are  $\rho g$ -open sets  $\mathcal{V}$  and  $\mathcal{U}$  satisfy  $\epsilon_1 \in \mathcal{U}$ ,  $\epsilon_2 \in \mathcal{V}$  and  $\mathcal{V} \cap \mathcal{U} = \emptyset$ .

The following example shows that the quotient topology of  $\rho g$ -regular space is  $\rho g$ -Hausdorff.

**Example 2.8.** Let  $X$  be  $\rho g$ -regular space and  $F$  be any closed subset of  $X$ . Define a relation  $R$  on  $X$  as follows:  $\epsilon R v \Leftrightarrow \epsilon$  and  $v$  belong to  $F$  then  $\epsilon = v$ . It can be seen that  $R$  is an equivalence relation on  $X$ . to prove that  $X/R$  with the quotient topology is  $\rho g$ -Hausdorff space take  $[\epsilon], [v] \in X/R$  such that  $[\epsilon] \neq [v]$  hence either  $\epsilon$  or  $v$  belong to  $F$  and the other may not so let  $\epsilon \in F$  and  $v \notin F$  then there exist  $\rho g$ -open sets  $U$  and  $V$  subsets of  $X$  such that  $[\epsilon] \in F \subset U$  and  $[v] \in V$ , implies that the the quotient topology is  $\rho g$ -Hausdorff space.

**Theorem 2.9.** Let  $f: X \rightarrow Y$  be a closed,  $\rho g$ -irresolute and injective function and  $Y$  is  $\rho g$ -regular spaces, then  $X$  is  $\rho g$ -regular space.

**Proof:** let  $c \in X$  and  $B$  be any closed subset of  $X$  such that  $c \notin B$ , then  $f(B)$  is closed subset of a space  $Y$  such that  $f(c) \notin f(B)$  and since  $Y$  is  $\rho g$ -regular so there exist disjoint  $\rho g$ -open sets  $\mathcal{V}, \mathcal{M}$  such that  $f(B) \subset \mathcal{V}$  and  $f(c) \in \mathcal{M}$ . clear that  $B \subset f^{-1}(f(B)) \subset f^{-1}(\mathcal{V})$  and  $c \in f^{-1}(\mathcal{M})$  also  $f^{-1}(\mathcal{V}) \cap f^{-1}(\mathcal{M}) = \emptyset$  see [11], and since  $f$  is  $\rho g$ -irresolute implies  $f^{-1}(\mathcal{V}), f^{-1}(\mathcal{M})$  are  $\rho g$ -open subsets of  $X$ , which complete the proof.

**Theorem 2.10.** Let  $f: X \rightarrow Y$  be a continuous,  $\rho g$ -open and bijective function and  $X$  is  $g$ -regular space, then  $Y$  is  $\rho g$ -regular space.

**Proof.** Let  $E$  be closed set in  $Y$  and  $v \notin Y$ , then  $f^{-1}(E) \in X$  and  $f^{-1}(v) \notin f^{-1}(E)$ . Since  $f$  is continuous then  $f^{-1}(E)$  is closed in  $X$ , and by  $\rho g$ -regularity of  $X$  there exist disjoint  $\rho g$ -open sets  $\mathcal{U}$  and  $\mathcal{M}$  in  $X$  such that  $f^{-1}(E) \subset \mathcal{U}$  and  $f^{-1}(v) \subset \mathcal{M}$ . Now  $f$  is  $\rho g$ -open hence  $f(\mathcal{U})$  and  $f(\mathcal{M})$  are disjoint  $\rho g$ -open sets in  $Y$  containing  $E$  and  $y$  respectively.

### 3. Strongly pre-generalized regular spaces

In the beginning of this section we give the definition of *Spg-regular space*.

**Definition 3.1.** a space  $X$  is called strongly pre-generalized regular space and shortly *Spg-R-space* if for each  $c \in X$  and for every  $\rho g$ -closed set  $E$  in  $X$  such that  $c \notin E$ , there are two disjoint open sets  $\mathcal{U}$  and  $\mathcal{V}$  where  $c \in \mathcal{U}$  and  $E \subset \mathcal{V}$ .

It is easy to see that every *Spg-regular space* is  $\rho g$ -regular but the convers is not necessarily true, for example take  $X = \{c_1, c_2, c_3\}$ ,  $\tau = \{X, \emptyset, \{c_1, c_2\}\}$  Then  $\rho gO(X) = \{X, \emptyset, \{c_1\}, \{c_2\}, \{c_1, c_2\}, \{c_1, c_3\}, \{c_2, c_3\}\}$  and  $\rho gC(X) = \{X, \emptyset, \{c_1\}, \{c_2\}, \{c_3\}, \{c_1, c_3\}, \{c_2, c_3\}\}$ .

**Theorem 3.2.** A space  $X$  is *Spg-R-space*  $\Leftrightarrow$  for every  $c \in X$  and for every  $\rho g$ -open set  $\mathcal{U}$  containing  $c$ , there exist an open set  $\mathcal{V}$  such that  $c \in \mathcal{V} \subset cl(\mathcal{V}) \subset \mathcal{U}$ .

**Proof:**

Necessity; Since  $c \notin \mathcal{U}^c$  and  $\mathcal{U}^c$  is  $\rho g$ -closed, so by *Spg-regularity* of  $X$  there exist disjoint open sets  $\mathcal{V}$  and  $\mathcal{M}$  where  $c \in \mathcal{V}$  and  $\mathcal{U}^c \subset \mathcal{M}$ , but  $\mathcal{M}^c$  is closed and  $\mathcal{V} \subset \mathcal{M}^c$ , this mean that  $cl(\mathcal{M}^c) = \mathcal{M}^c$  and  $cl(\mathcal{V}) \subset cl(\mathcal{M}^c)$ , hence  $\mathcal{V} \subset cl(\mathcal{V}) \subset \mathcal{M}^c \subset \mathcal{U}$ , therefore  $c \in \mathcal{V} \subset cl(\mathcal{V}) \subset \mathcal{U}$ .

Sufficient; Let  $E$  be a  $\rho g$ -closed and  $c \notin E$ , so  $c \in E$  and  $E^c$  is  $\rho g$ -open implies that there exist open set  $\mathcal{V}$  such that  $c \in \mathcal{V} \subset cl(\mathcal{V}) \subset E^c$ , but  $E \subset (cl(\mathcal{V}))^c$ , this mean that  $cl(\mathcal{V})$  is closed and  $\mathcal{V} \cap (cl(\mathcal{V}))^c = \emptyset$ , therefor  $c \in \mathcal{V}$  and  $E \subset (cl(\mathcal{V}))^c$ , so  $X$  is *Spg-R-space*.

The following theorem gives a characterization of a topological space to be strongly pre-generalized regular space.

**Theorem 3.3.** A space  $X$  is *Spg-R-space* if for each  $c \in X$ , then any  $\rho g$ -open containing  $c$  consists of a pre-closed neighbourhood of  $c$ .

**Proof:** Let  $c \in X$  and a  $\rho g$ -closed set  $E \subset X - \{c\}$ . thus  $X - E$  is  $\rho g$ -open and containd  $c$ , so there is a pre-closed neighborhood  $\mathcal{V}$  of  $c$  such that  $\mathcal{V} \subset X - E$ . Now let  $\mathcal{W}_1 = X - \mathcal{V}$ , then  $\mathcal{W}_1$

is *pre-open* and  $E \subset \mathcal{W}_1$ . On the other hand  $\mathcal{V}$  is a neighbourhood of  $\mathfrak{c}$ , hence there is an *open* set  $\mathcal{W}_2$  such that  $\mathfrak{c} \in \mathcal{W}_2 \subset \mathcal{V}$ . Thus  $\mathcal{W}_1 \cap \mathcal{W}_2 \subset \mathcal{V} \cap (X - \mathcal{V}) = \emptyset$  and since  $\mathcal{W}_2$  is  $\rho g$ -open[9] the proof is complete.

**Remark 3.4.** A space  $X$  is  $\rho g$ - $\mathcal{R}$ -space if for each  $\mathfrak{c} \in X$ , then any  $\rho g$ -open containing  $\mathfrak{c}$  consists of a neighbourhood of  $\mathfrak{c}$ .

**Proof:** The proof is similar to theorem (3.3).

**Theorem 3.5.** Let  $f: X \rightarrow Y$  be a  $\rho g$ -closed, continuous, injective function and  $Y$  is *Spg-regular spaces*, then  $X$  is *Spg-regular space*.

**Proof:** let  $\mathfrak{c} \in X$  and  $\mathcal{B}$  be any  $\rho g$ -closed subset of  $X$  such that  $\mathfrak{c} \notin \mathcal{B}$ , then  $f(\mathcal{B})$  is  $\rho g$ -closed subset of a space  $Y$  such that  $f(\mathfrak{c}) \notin f(\mathcal{B})$  and since  $Y$  is *Spg-regular* so there exist disjoint open sets  $\mathcal{V}, \mathcal{M}$  such that  $f(\mathcal{B}) \subset \mathcal{V}$  and  $f(\mathfrak{c}) \in \mathcal{M}$ . Clear that  $\mathcal{B} \subset f^{-1}(f(\mathcal{B})) \subset f^{-1}(\mathcal{V})$  and  $\mathfrak{c} \in f^{-1}(\mathcal{M})$  also  $f^{-1}(\mathcal{V}) \cap f^{-1}(\mathcal{M}) = \emptyset$ , and since  $f$  is continuous then  $f^{-1}(\mathcal{V}), f^{-1}(\mathcal{M})$  are open subsets of  $X$ , which complete the proof.

Finally we will define a weak form of *g-Hausdorff spaces*.

**Definition 3.6.** A space  $X$  is called weakly  $\rho g$ -Hausdorff space  $\Leftrightarrow$  for each distinct points  $\mathfrak{c}, \mathfrak{v}$  in  $X$  such that  $\mathfrak{c} \notin P\text{-cl}(\mathcal{V}_{\mathfrak{v}})$ , where  $\mathcal{V}_{\mathfrak{v}}$  be any  $\rho g$ -open set containing  $\mathfrak{v}$ , then there exist disjoint  $\rho g$ -open sets  $\mathcal{V}, \mathcal{W}$  containing  $\mathfrak{c}$  and  $\mathfrak{v}$  respectively

**Proposition 3.7.** Every  $\text{Spg-}\mathcal{R}$ -space is weakly  $\rho g$ -Hausdorff space.

**Proof:** Let  $X$  be a  $\text{Spg-}\mathcal{R}$ -space and  $\mathfrak{c}, \mathfrak{v} \in X$  such that  $\mathfrak{c} \neq \mathfrak{v}$  and let  $\mathfrak{c} \notin P\text{-cl}(\mathcal{V}_{\mathfrak{v}})$ , where  $\mathcal{V}_{\mathfrak{v}}$  be any  $\rho g$ -open set containing  $\mathfrak{v}$  but  $P\text{-cl}(\mathcal{V}_{\mathfrak{v}})$  is *pre-closed* [1], hence it is  $\rho g$ -closed [4] so by the *Spg-regularity* of  $X$  there exist disjoint open sets  $\mathcal{U}_{\mathfrak{c}}$  and  $\mathcal{U}_{\mathfrak{v}}$  such that  $\mathfrak{c} \in \mathcal{U}_{\mathfrak{c}}$  and  $\mathfrak{v} \in P\text{-cl}(\mathcal{V}_{\mathfrak{v}}) \subset \mathcal{U}_{\mathfrak{v}}$  implies  $X$  is weakly  $\rho g$ -Hausdorff.

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