مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد السابع والعشرون - المجلد الثاني - علوم الحياة - آذار 2026 م
doi.org/10.52866/esj.2026.02.27.14

Evaluation of the Efficacy of Biosynthesised Copper Oxide Nanoparticles Towards Some Bacterial Species Isolated from the Housefly (*Muscadomestica* L.)

Sondos Abdalrahman Hussien , Harith Ahmed Mustafa
Department of Biology, College of Education, University of Samarra, Samarra, Iraq
Corresponding author email: sondosabdalrahman4@gmail.com.

Abstract:

This study was conducted to evaluate the effectiveness of copper oxide nanoparticles prepared biologcally from *Ficuscarica* leaf extract towards pathogens isolated from Housefly(*Muscadomestica*). 150 houseflies were collected from different places (vegetable market, grocery stores and some houses). The results of isolation and diagnosis of bacterial species showed that 118 samples of houseflies carried bacteria with a percentage of 78.66%, while the number of samples in which no bacterial growth occurred was 32 samples (21.33%). As for the number of bacterial isolates, 266 bacterial isolates were obtained, and the results showed the predominance of bacterial isolates negative for Cram's stain (86.46%) over those positive for Cram's stain (13.53%). The bacterial isolates were diagnosed as *Klebsiellaoxytoca.Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and *Enterobacter cloacae*,The first ranking was topped by*K. oxytoca* with 36.84% while *E. coli came* second with 28.94%. The study proved that the aqueous extract of *Ficuscarica* leaves can be used as a biological agent in the preparation of copper oxide nanoparticles as it had a surface topography size of 41.45 nm when examined by atomic force microscopy and a nanosize of 24.06 nm with a spherical shape when examined by field emission scanning electron microscopy. Two absorption peaks appeared at wavelengths of 240 and 350 nm, according to the UV/Vis spectrophotometer results, and the evaluation of CuO NPs' ability to inhibit bacterial species revealed that the concentrations of CuO NPs varied significantly. The concentrations that were employed (300, 400, and 500 micrograms/ml) varied significantly, and the inhibitory diameters increased in direct proportion to the nanoconcentration. Additionally, the results demonstrated that all bacterial isolates were resistant to the concentrations of CuO NPs (100 and 200 micrograms/ml). The highest inhibition diameter was 17.67± 0.577 mm with E. coli at 500 µg/ml, while the lowest inhibition diameter was 10.67 ± 0.577 mm with P. mirabilis at 300 µg/ml.

Key word: Houseflies, Pathogenic bacteria, CuO NPs, *Ficuscarica.*

تقييم فاعلية اوكسيد النحاس النانوي المحضر حيويًا تجاه بعض الأنواع البكتيرية المعزولة من حشرة الذبابة المنزلية (*Muscadomestica* L.)

سندس عبد الرحمن حسين ، حارث أحمد مصطفى
قسم علوم الحياة، كلية التربية، جامعة سامراء، سامراء، العراق

مستخلص:

اجريت هذه الدراسة من اجل تقييم فعالية اوكسيد النحاس النانوي والمحضر بطريقة حيوية من مستخلص اوراق التين *Ficuscarica* اتجاه المسببات المرضية المعزولة من الذباب المنزلي (*Muscadomestica*) Housefly، إذ جمعت 150 حشرة من الذباب المنزلي من اماكن مختلفة (سوق الخضروات والقصابة وبعض المنازل)، إذ أوضحت نتائج تشخيص الانواع البكتيرية أن 118 عينة من الذباب المنزلي تحمل البكتيريا وبنسبة 78.66٪ في حين بلغ عدد العينات التي لم يحصل فيها أي نمو بكتيري 32 عينة وبنسبة 21.33٪ وبعدد عزلات بكتيرية كانت266 عزلة، كما واوضحت النتائج تفوق العزلات البكتيرية السالبة لصبغة كرام وبنسبة 86.46٪ في حين كانت نسبة البكتيريا الموجبة لصبغة كرام 13.53٪، وشُخصت العزلات البكتيرية المعزولة إلى *Klebsiellaoxytoca* و *Escherichia coli* و *Staphylococcus aureus* و *Proteus mirabilis* و *Enterobacter cloacae* وقد تصدرت المرتبة الاولى النوع البكتيري *K.oxytoca* بنسبة 36.84٪ في حين جاءت بالمرتبة الثانية النوع *E. coli* بنسبة 28.94٪، وأثبتت الدراسة إمكانية استعمال المستخلص المائي لأوراق التين *Ficuscarica* كعامل حيوي في تحضير جسيمات أوكسيد النحاس النانوي إذ كان حجم تضاريس السطح لها 41.45 نانوميتر عند فحصها بمجهر القوة الذرية وبحجم نانوي 24.06 نانوميتر وذات شكل كروي عند فحصها في المجهر الالكتروني الماسح للانبعاثات الميدانية كما ظهرت قمتاامتصاص عند أطوال موجية 240 و350 نانومتر،وفقًالنتائج مطياف الأشعة فوق البنفسجية/ المرئية،وكشفت نتائج تقييم قدرة جسيمات أكسيدالنحاس النانوية على تثبيط الأنواع ا لبكتيرية وجود فروق معنوية بين التراكيز المستعملة (300 و400 و500 ميكروغرام/ مل) وبشكل كبير، حيث ازدادت أقطارالتثبيط بشكل طردي معا لتركيز النانوي. بالإضافة إلى ذلك،أظهرت النتائج أن جميع العزلات البكتيرية كانت مقاومة لتركيزات جسيمات أكسيد النحاس النانوية (100 و200 ميكروغرام/ مل). كما كان أعلى قطر تثبيط 17.67 ± 0.577 mm مع الإشريكية القولونية عند 500 ميكروغرام/ مل،بينما كان أقل قطر تثبيط10.67 ± 0.577 mm مع *P. mirabilis* عند 300 ميكروغرام/ مل.

الكلمات المفتاحية: الذباب المنزلي، البكتيريا المرضية ، *Ficuscarica*، CuO NPs.

Introductions:

Insects represent approximately 67% of known living organisms and are one of the most widespread and present organisms of the animal kingdom on the surface of the globe, which includes at least one million species of insects and is a large and integral part of ecosystems [1,2],So scientific studies have focused on insects for many reasons, including their large numbers and the seriousness of their impact on human life and the organisms that live with them, their transmission of many pathogens and the damage of products and materials used by humans [3]. Housefly(*Muscadomestica*) is one of the largest health pests in the world, and it is a global pest that poses great concerns as it has a close association and relationship with humans as well as with the animals and livestock that they raise and thus transmit many pathogens to humans and animals[4]. house flies transmit many dangerous diseases and epidemics including Typhoid, Cholera, Paratyphoid, Diarrhoea, Dysentery, Smallpox and Anthrax [5].Studies have revealed that flies play a major role in the transmission of antibiotic-resistant bacteria and the spread of antibiotic-resistant strains from the hospital environment to other environments, as well as their role in spreading epidemics, especially intestinal epidemics such as diarrhoea and other diseases [6].

However, certain current medications are no longer effective against multidrug-resistant bacteria, which have proliferated. Antibiotic dosages have increased as a result, and new medications must be created. In order to solve this issue, researchers must identify alternatives to medications, which presents a significant obstacle. The characteristics of nanomaterials, such as their size, shape, appearance, charge, and surface area, have made them a viable new solution in recent years. Because of this, they are now the subject of studies on the problem of antibiotic resistance. [7,8], Furthermore, the use of plant extracts and parts to create nanomaterials has become very popular recently because it is a safe, cost-effective, environmentally friendly, and quick process, as well as because plants contain efficient

metabolic compounds that contribute to the reduction of nanomaterials. [9]. Among the plants that have been used in the preparation of nanomaterials is *Ficuscarica*, as it is rich in natural metabolic compounds such as phenols, alkaloids, flavonoids, terpenes, tannins and other minor ones [10].The aim of this study was to find a safe, environmentally friendly and inexpensive way to synthesise copper oxide particles from fig leaf (*Ficuscarica*) extract and use it to inhibit bacterial species isolated from the housefly.

Methods

Preparation of culture media

The culture media required in the experiment were nutrient broth, nutrient agar, brain-heart infusion broth, Mueller-Hinton agar, MacConkey agar, mannitol salt agar, brilliant green agar, eosin methylene blue agar, and blood agar. Prepared according to the manufacturer's instructions with pH regulation 7. Sterilized under known sterilization conditions in an autoclave, and then placed at 37°C in the incubator for 24 hours to ensure the absence of any contamination, and then stored at 4°C in the refrigerator until use. Biochemical tests were performed according to De la Maza*et al.* [11] to diagnose bacterial isolates. The biochemical tests were the oxidase test, catalase test, indole test, methyl red test, citrate utilization test, and urease test.

Isolation and diagnosis of bacteria from house flies

A total of 150 samples of houseflies were collected from different places (vegetable market, grocery market, and some houses). The insects were anesthetized and frozen. 2 ml of normal saline was added to the sterile tubes, then the solution was shaken using Vortex. A sample was taken using Loop and sown on pre-prepared culture media, and bacterial species were diagnosed according to their phenotypic characteristics, microscopic examination, and some biochemical tests in addition to diagnosis using the Vitek Compact system. [12,13].

Preparation of hot aqueous extract of *Ficuscarica*

Collected *F. carica* leaves, washed with well-distilled water and cut

into small pieces, 5 g of them were weighed, then 125 ml of ionic water was added to them and the plant mixture was placed in a sterile glass flask and the mouth of the flask was closed to prevent evaporation of the mixture and the mixture was placed in a water bath shaker at 60°C for one hour, then the mixture was filtered using medical gauze to remove plant parts and then Whatman No. 1 filter paper was used. The mixture was placed in a sterile glass bottle and kept in the refrigerator until use [14].

Preparation of copper sulfate solution and synthesis of CuO NPs

Copper sulfate solution was prepared at a concentration of 0.1 mol/L by weighing 24.97 g of CuSO4.5H2O in 800 mL of deionized water. The color of this solution was light blue, and this solution was placed on an electric heater with a magnetic stirrer at a temperature of 60-70°C. Fig leaf extract was added (10 ml), and the color changed to brown. Then it was washed by centrifugation. [15].

In order to confirm the synthesis of CuONP nanoparticles by the green manufacturing method.Several tests were conducted at Ames Research Laboratory in Baghdad.Including Field Emission Scanning Electron Microscopy, Atomic Force Microscopy and UV/Vis Spectrophotometer [16,17].

Evaluation of the efficacy of CuO NPs against bacterial species isolated from house flies

To evaluate the efficacy of CuO NPs against bacterial species isolated from houseflies. The concentrations (100, 200, 300, 400, and 500 µg/ml) of CuO NPs were determined. The etch diffusion method was used to detect the activity of the samples. The isolates were grown in nutrient broth medium and incubated at 37°C. 10 µL of the growth was added to 5 mL of saline solution per tube, and the tubes were shaken. A portion of the bacterial suspension (1.5 x 10^8 CFU/mL) was transferred and spread on the prepared Mueller-Hinton agar medium. After 10 min, a cork drill was used to make 5 holes in the medium, and the nanomaterial was added according to the concentrations, and the diameters of inhibition were measured. [17].

Statistical analysis

The results were statistically analyzed using the ANOVA test, completely randomized design (CRD), and the arithmetic means were compared using Duncan's multiple range test at a probability level of 0.05% .[18].

Results

According to the results of the isolation of bacterial species from 150 samples of houseflies (*Muscadomestica*).Collected from different locations (vegetable markets, grocery stores, and some homes). The diagnostic results showed that 118 samples of houseflies were positive for bacterial growth at a rate of 78.66%. The number of samples in which no bacterial growth was obtained was 32 samples, or 21.33%. The number of bacterial isolates obtained amounted to 266 isolates, as shown in Table 1.

Table 1: Bacteriological examination of housefly samples

Bacteriological test for houseflies	Positive samples	%Percentage	*P value*
Positive samples	118	78.66	$P \leq 0.001$
Negative samples	32	21.33	
Total number	150	100	

The results of staining the 266 bacterial isolates isolated from *M. domestica* samples with Grams stain showed that 230 isolates were negative for Grams stain 86.46%, while 36 bacterial isolates were positive for Grams stain 13.53% as shown in Figure 1.

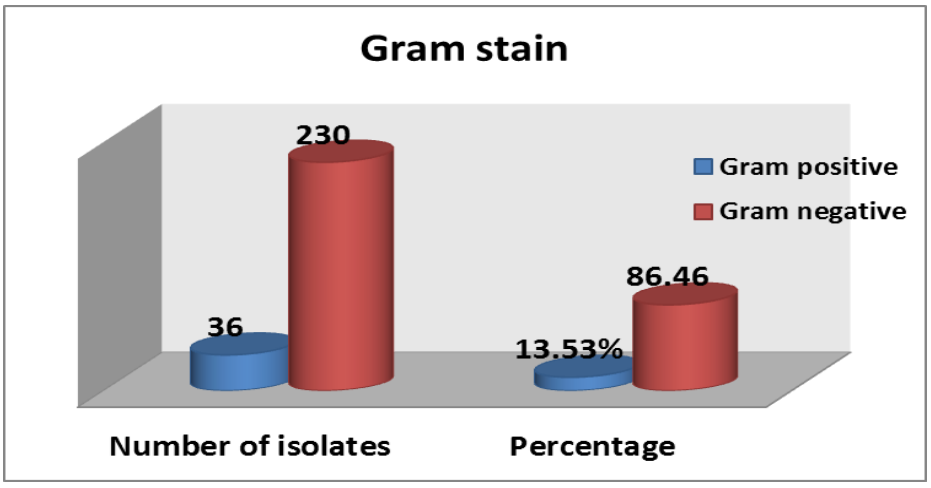


Figure 1: Positive and negative isolates of Cram's stain isolated from houseflies.

The results of the diagnosis of the 266 bacterial isolates of *M. domestica*, based on culture and microscopic characteristics, biochemical tests and Vitek Compact System tests, showed that the following bacterial species: *Klebsiellaoxytoca*, *Escherichia coli, Staphylococcus aureus*, *Proteus mirabilis* and *Enterobacter cloacae*.*K. oxytoca* came first with 98 isolates and a percentage of 36.84%.*E. coli* came second with 77 isolates and a percentage of 28.94%.*S. aureus* came third with 36 isolates and a percentage of 13.53%.*P. mirabilis* and *E. cloacae* had 30 and 25 isolates and a percentage of 11.27% and 9.39%, respectively, The results also showed that no bacterial species was obtained within the natural flora of the insect except for *E. coli* as shown inFigure 2and 3.

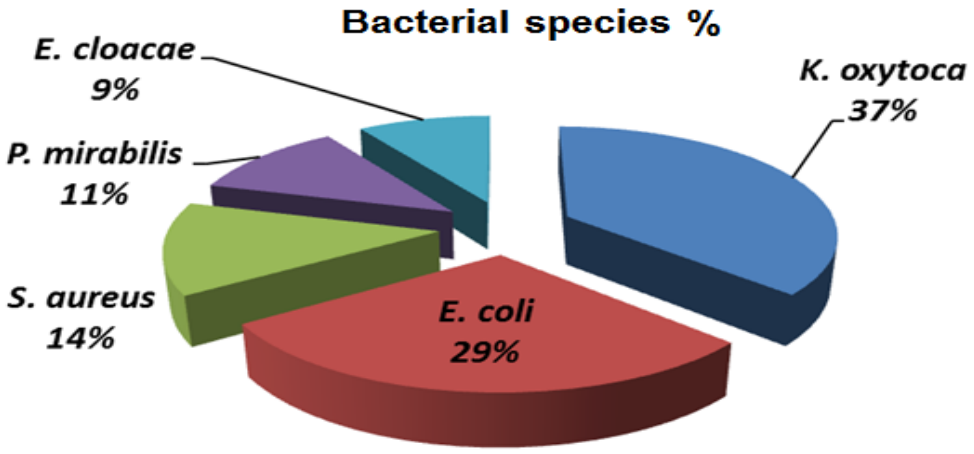


Figure 2: Bacterial species isolated from house flies with their percentages

Regarding the culture characteristics of the diagnosed bacterial isolates., the bacteria grew as orange to yellow colored colonies as in Figure 3.

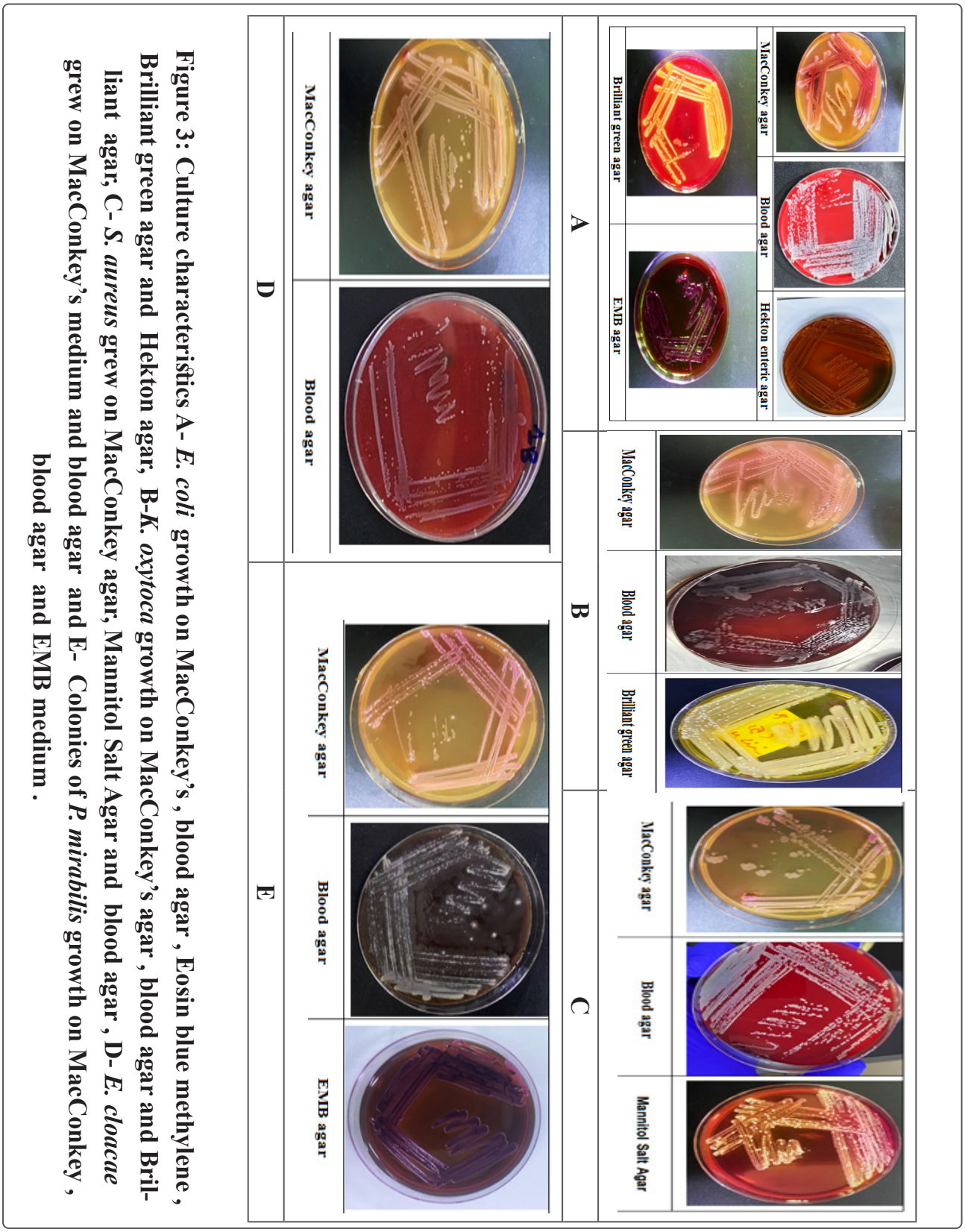


Figure 3: Culture characteristics A- *E. coli* growth on MacConkey's , blood agar , Eosin blue methylene , Brilliant green agar and Hekton agar, B-*K. oxytoca* growth on MacConkey's agar , blood agar and Brilliant agar, C- *S. aureus* grew on MacConkey agar, Mannitol Salt Agar and blood agar , D- *E. cloacae* grew on MacConkey's medium and blood agar and E- Colonies of *P. mirabilis* growth on MacConkey , blood agar and EMB medium .

The results revealed the synthesis of CuO NPs by green synthesis method using *Ficuscarica* leaf extract as a reducing agent for copper sulfate CuSO4. 5H2O to CuONPs .The results showed that the nanoparticles could be prepared and through the color change. The solution changed to dark brown color indicating the reduction of copper sulfate to CuO NPsas shown in Figure 4.

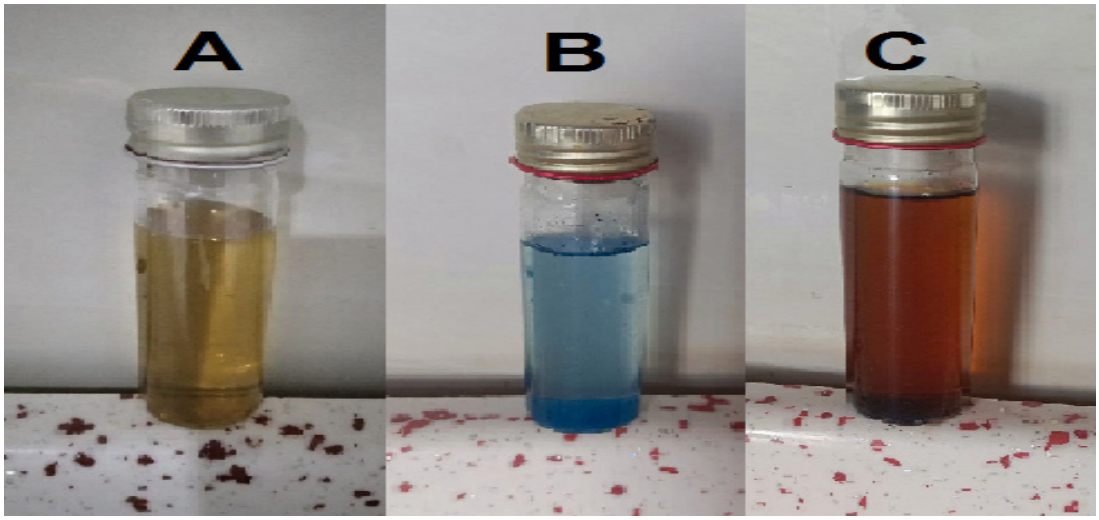


Figure 4:Color change A- plant extract B- copper sulfate C-CuO NPs

The results of the confirmatory tests for the formation of CuO NPs as the UV/Vis spectrophotometer showed two absorption peaks at wavelengths of 240 nm and 350 nm as shown in Figure 5.

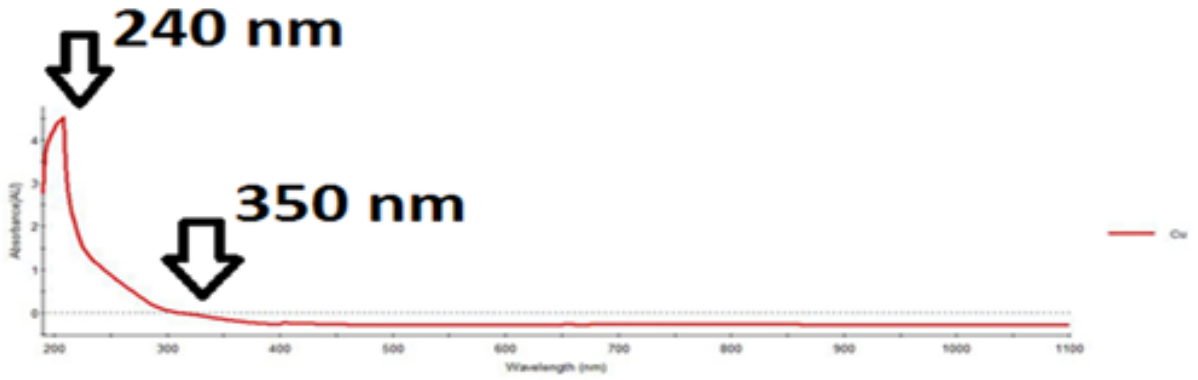


Figure 5: UV/Vis spectrophotometer of CuONPs .

Atomic force microscopy (AFM) results showed that there is a variation in the height and depth of the surfaces. This variation reflects the surface roughness of the nanoparticle.The sample as shown in 2D and 3Dduring AFM scanning as in figures6 and 7.

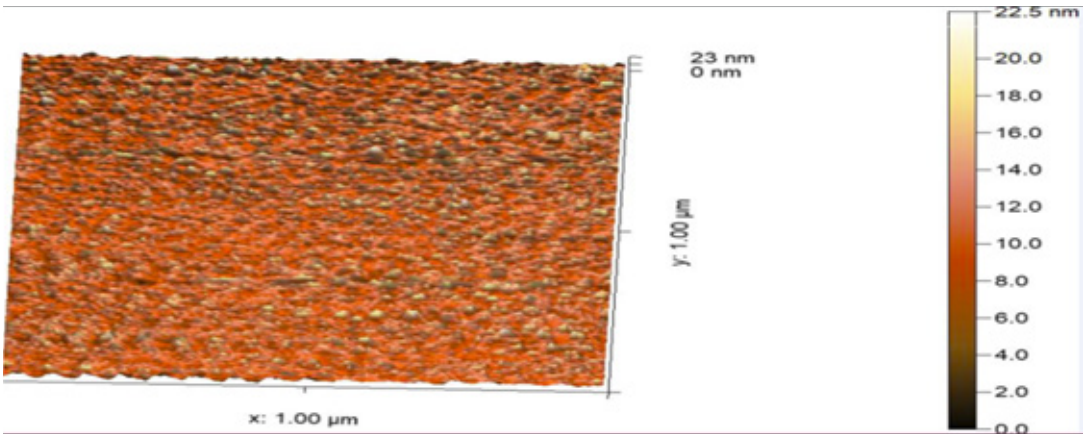


Figure 6:Two-dimensionalduring AFM scanning

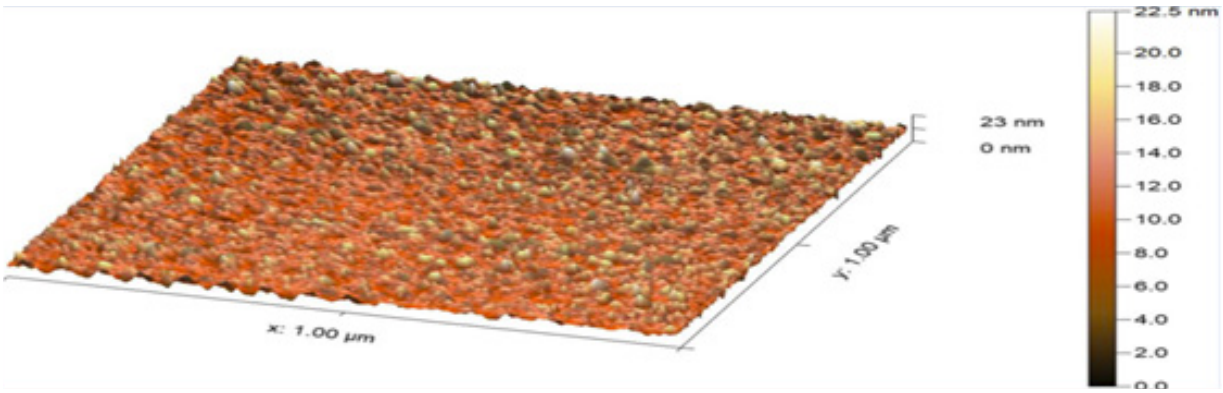


Figure 7:Three-dimensionalduring AFM scanning

The results also showed the distribution of CuO NPs a surface topography sizewith nanoscale sizes ranging from 0 - 66 nm.An average nanoscale size for CuO NPs of 41.45 nm as shown in Figure 8.

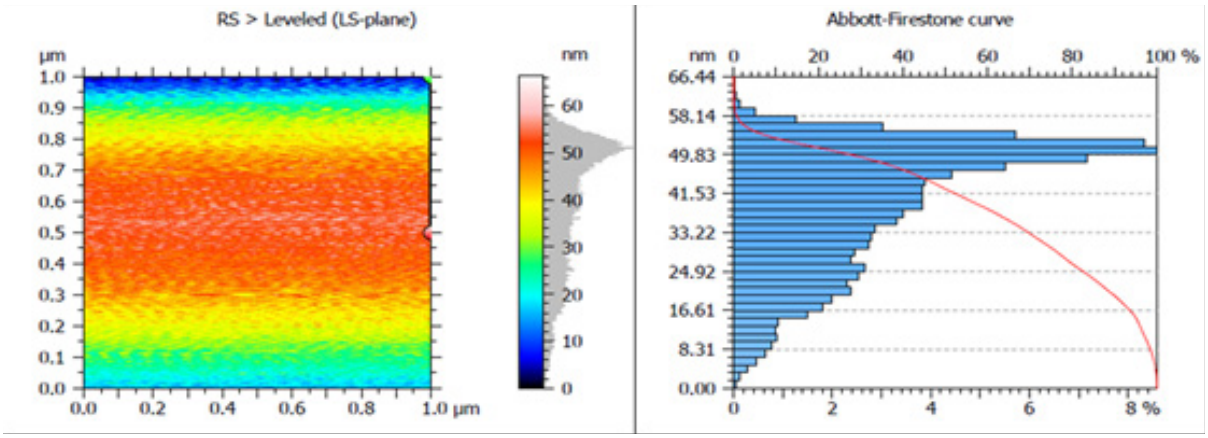


Figure 8:Surface topography size of CuO NPs during AFM scanning

The results of the bio-prepared CuO NPs when examined by Field-emission Scanning Electron Microscope showed that CuO NPs have a spherical to oval shape.The results showed some agglomerates and clusters of nanoparticles. Nanoparticles of CuO NPsas shown in Fig.9 under 200 nm scanning power of FE-SEM.

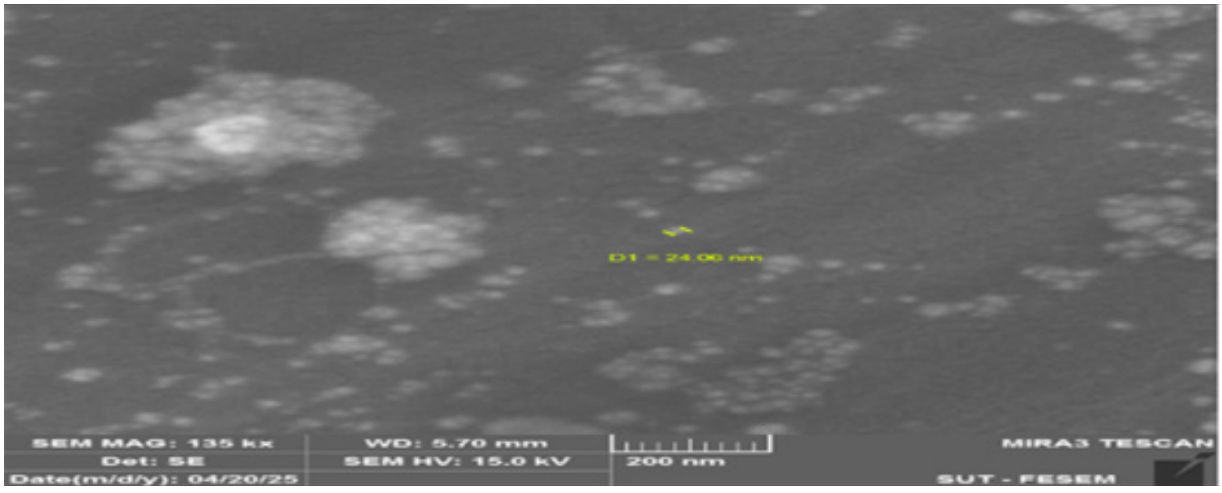


Fig.9:CuO NPs under 200 nm scanning power of FE-SEM.

The copper oxide nanoparticles examined in the FE-SEM microscope under 1µm magnification are shown in Figure 10.

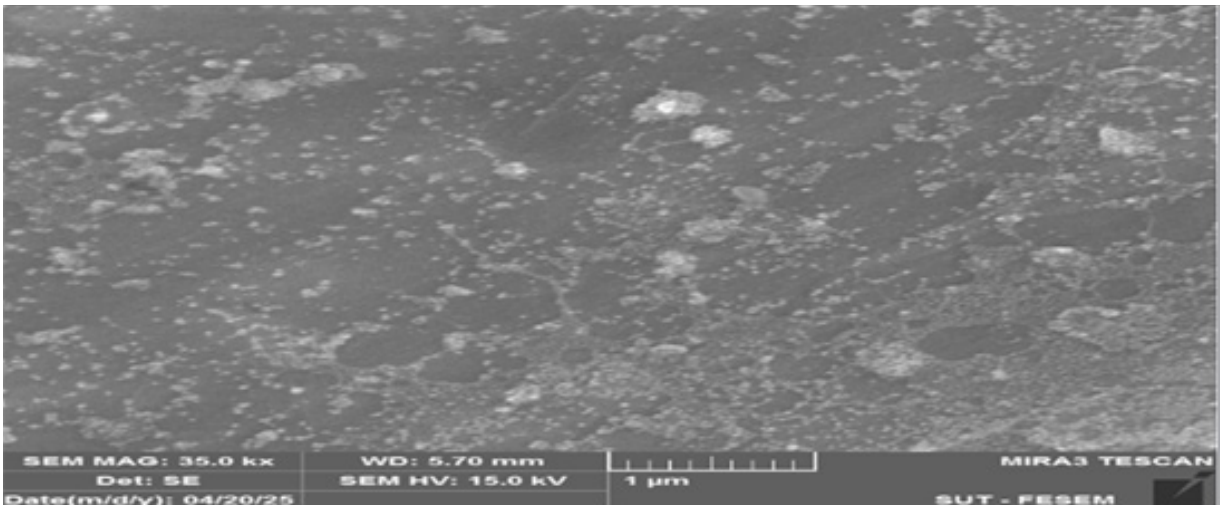


Fig.10: CuO NPs under 1µm scanning power of FE-SEM.

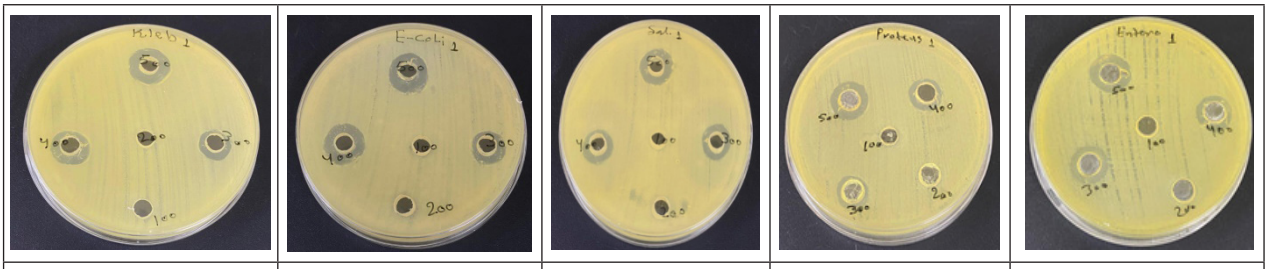
An experiment evaluating the efficacy of bio-prepared copper oxide nanoparticles against bacterial species isolated from house flies, including five species (*K. oxytoca, E. coli, S. aureus, P. mirabilis, E. cloacae*). The results indicated that there were significant differences between the concentrations used (300, 400 and 500 µg/ml) as there was a direct proportionality of increasing inhibition diameters with increasing concentration of copper oxide particles for all bacterial isolates. All bacterial isolates were resistant or desensitised to both 100 and 200 µg/ml concentrations of CuO NPs as no inhibition occurred for all bacterial isolates under study at these concentrations, and the results showed that the highest diameter of inhibition was17.67 ± 0. 577 mm and17.00 ± 1.00 mm with *E. coli* and *E. cloacae*, respectively, at 500 µg/ml of CuONPs.The lowest inhibition diameter was10.67 ± 0.577 mm with *P. mirabilis*at 300 µg/ml.

The results also showed that the highest inhibition diameter of CuONPs with *K. oxytoca* was 15.33 ± 0.577mm at a concentration of 500 µg/ml of CuONPs.The highest inhibition diameter with *S. aureus*and *P. mirabilis*was 15.67 ± 0.577 mm at a concentration of 500 µg/ml of CuO NPs as shown in Table 3 and Figure 11.

Table 3: Evaluation of the efficacy of CuO NPs towards bacterial isolated from Houseflies

Bacterial species	Concentrations µg/ml	Mean ± standard deviation	*P value*
K. oxytoca	100	0.00d ± 0.00	*P ≤ 0.05*
	200	0.00d ± 0.00	
	300	11.33c ± 0.577	
	400	13.33b ± 0.577	
	500	15.33a ± 0.577	
E.coli	100	0.00d ± 0.00	
	200	0.00d ± 0.00	
	300	13.33c ± 0.577	
	400	15.67b ± 0.577	
	500	17.67a ± 0.577	
S. aureus	100	0.00d ± 0.00	
	200	0.00d ± 0.00	
	300	11.67c ± 0.577	
	400	13.67b ± 0.577	
	500	15.67a ± 0.577	
E. cloacae	100	0.00d ± 0.00	
	200	0.00d ± 0.00	
	300	11.33c ± 0.577	
	400	12.67b ± 0.577	
	500	17.00a ± 1.00	
P. mirabilis	100	0.00d ± 0.00	
	200	0.00d ± 0.00	
	300	10.67c ± 0.577	
	400	13.67b ± 0.577	
	500	15.67a ± 0.577	

*** Similar letters mean that there are no significant differences .Different letters between coefficients mean that there are significant differences below the probability level *P ≤ 0.05*.**



A	B	C	D	E

Figure 7: Evaluation of the efficacy of CuO NPs towards bacterial isolated from Houseflies

K. oxytoca*, (B) *E. coli* , (C) *S. aureus* , (D) *P. mirabilis* and (E) *E. cloacae.

Discussion:

This study was conducted to isolate and characterise bacterial species from the surface of house flies. In addition, the efficacy of bio-prepared copper oxide nanoparticles from fig leaf extract in killing and inhibiting these bacterial isolates was evaluated. The study showed that CuO nanoparticles provide an important aspect in killing bacterial species that possess antibiotic resistance. The results of our study showed that CuO NPs are more effective towards Gram negative bacteria (G-ve bacteria) than Gram-positive. Due to the difference in cell wall composition. The wall of Gram-negative bacteria is characterised by a peptidoglycan substance that the nanoparticles can penetrate due to its small size. The wall of Gram-positive bacteria is thick and forms a barrier, reducing the effect of these nanoparticles and minimising their penetration into the cell. Regarding the results of isolation and diagnostic results of bacterial isolates from houseflies, they are consistent with the findings of Saleem *et al.*,[19] and Sudagidan *et al.,*[20] who showed that the surface of houseflies is a good habitat for different types of pathogenic and non-pathogenic bacteria. The reason for the presence of bacteria on the surface of flies is due to their movement and movement from one place to another between food waste, waste, and blood in cutting shops, so bacteria can attach to the surface of the flies. [21].

The results of our study showed a high percentage of Gram negative bacteria (G-ve bacteria). These results

were consistent with the study of Nguyen *et al.,* [22] and Adhim *et al*.,[12] who showed that Gram-negative bacterial species are the most present on the surface of domestic flies in homes, slaughterhouses, markets and others. The reasons are due to the availability of suitable and ideal conditions for the growth of bacteria due to accumulated organic matter, raw meat in its raw state and the spread of blood and may be due to watering fruits and vegetables with water contaminated with bacteria. Population density in urban areas and markets also plays a role in the spread of bacteria. On the other hand, the results of our study did not agree with the study of Shehata*et al*.[23] who found a 77% increase in Gram-positive bacteria isolated from houseflies. This may be due to the bacteria's thick wall, which makes it more resistant to dry conditions, as well as its spore formation and virulence factors that enable it to adhere to the surface of houseflies.

As for the results of CuO NPs biosynthesis from fig leaves, they agreed with Taha*et al*.,[24] and Atiya*et al.,[*15] who showed the possibility of using *Ficuscarica* extracts as a biological agent in the reduction of copper sulfate CuSO4. 5H2O to copper oxide nanoparticles and showed that it is a safe, environmentally friendly and economical method for the synthesis of nanoparticles and because of the plant's effective and active chemical substances such as phenols, flavonoids, acids, carbohydrates, proteins and others, these substances can be Plants are easy to prepare and do not require additional processes such as isolation, cultivation and media preparation as in the case of fungal and bacterial extracts [25]. The colour change can also be due to the Surface Plasmon phenomenon,one of the optical properties of nanoparticles, is the collective movement and vibration of electrons as they are exposed to light[26],The UV/Vis Spectrophotometer results were in agreement with Jabeen *et al*.,[27] who showed that the absorbance values of CuO at 248 nm, and on the other hand did not agree with Maurya*et al*.,[28] who showed that the absorbance value of CuO bio-prepared from *Ficusracemosa* cluster fig extract is between the wavelengths of 500 - 590 nm. 590 nm, the reason for this difference may be

due to the concentration of the solution at the time of examination and the method of titration and dilution, as well as the incubation period that has an effect on the absorbance, as well as the difference in the plant type of figs used to prepare the plant extract.

The microscopy results were in agreement with Bhavyasree and Xavier [29] who showed that CuO nanoparticles prepared from fig plant extract had sizes ranging from 15-45 nm. The reason for the agglomerates and clusters that appeared in the FE- SEM could be due to the increased surface area of the nanomaterials. And the strong attraction of these materials due to the Vander Waals force. It can also due to the reduced kinetic energy exhibited by the nanoparticles or due to the agglomeration of organic matter present in the extracts.[30].The smaller nanoparticle size has a large surface area and therefore contacts the bacterial cells and can reach the cytoplasm more than large nanoparticles as it leads to membrane damage which can lead to leakage of cellular contents and death of the bacteria [31].

The results of the evaluation of the effectiveness of copper oxide nanoparticles in inhibiting and killing bacterial species isolated from flies came with Zhang *et al.,*[32] and Bai *et al.,[*17] who showed that high concentrations of copper oxide nanoparticles 500 µg/ml inhibited bacterial species such *as E.coli, S. aureus* and *Klebsiella*. The reason for this is that copper oxide nanoparticles cause oxidative stress that causes irreparable DNA damage. As well as damage to bacterial proteins. The reason for the inhibition is mechanistically due to the negative charges of the bacterial surface attracting the positive charges of the created nanoparticles. Degrading the cell wall at first and then reaching the extracellular lipids, sugars and other granular parts. Finally, copper oxide nanoparticles reach deep into the bacterium and its bacterial nuclear material and affect its functions and death.[33].

Conclusions:

The study showed that house flies are carriers of many bacteria. The most common bacterial species found on the surface of the flies were *Klebsiella oxytoca* and *Escherichia coli*. Through the

study, the bioengineered copper oxide nanoparticles proved to be effective in killing pathogenic and antibiotic-resistant bacterial species. This opens future prospects in introducing nanoparticles in the pharmaceutical industry or making nanomaterials that can carry active substances of drugs, especially with bacterial species that possess multiple antimicrobial resistance.

References:

1. Mohammed, W. J., Mohammed, B. M., & Hassan, H. F. (2016). Screening for Pathogenic Parasites in the Muscadomestica L.(Diptera: Muscidae) in some areas of Kirkuk Provinces. Tikrit Journal of Pure Science, 21(4), 60-66.
2. Redak, R. (2023). Introduction to and Importance of Insects. In Forest Entomology and Pathology: Volume 1: Entomology (pp. 1-17). Cham: Springer International Publishing.
3. Belluco, S., Bertola, M., Montarsi, F., Di Martino, G., Granato, A., Stella, R., Mutinelli, F. (2023). Insects and public health: an overview. Insects, 14(3), 240.
4. Geden, C. J., Nayduch, D., Scott, J. G., Burgess IV, E. R., Gerry, A. C., Kaufman, P. E., &Machtinger, E. T. (2021). House fly (Diptera: Muscidae): biology, pest status, current management prospects, and research needs. Journal of Integrated Pest Management, 12(1), 39.
5. Olagunju, E. A. (2022). Housefly: Common zoonotic diseases trans-

mitted and control. Journal of Zoonotic Diseases, 6(1), 1-10.

6. Monyama, M. C., Onyiche, E. T., Taioe, M. O., Nkhebenyane, J. S., &Thekisoe, O. M. (2022). Bacterial pathogens identified from houseflies in different human and animal settings: a systematic review and meta-analysis. Veterinary Medicine and Science, 8(2), 827-844.
7. Mubeen, B., Ansar, A. N., Rasool, R., Ullah, I., Imam, S. S., Alshehri, S., ...&Kazmi, I. (2021). Nanotechnology as a novel approach in combating microbes providing an alternative to antibiotics. Antibiotics, 10(12), 1473.
8. Omar, S. T., Ali, W. K., &Karim, A. S. (2023). The Effects of Zinc Oxide nanoparticles synthesized from Eucalyptus plant extracts against mealworm stages Tenebriomolitor L., 1758 (Tenebrionidae: Coleopetera). Tikrit Journal of Pure Science, 28(6), 11-24.
9. Ying, S., Guan, Z., Ofoegbu, P. C., Clubb, P., Rico, C., He, F., & Hong, J. (2022). Green synthesis of nanoparticles: Current developments and limitations. Environmental Technology & Innovation, 26, 102336.
10. Üstün, E., Önbaş, S. C., Çelik, S. K., Ayvaz, M. Ç., &Şahin, N. (2022). Green synthesis of iron oxide nanoparticles by using *Ficuscarica* leaf extract and its antioxidant activity. Biointerface Res. Appl. Chem, 12(2), 2108-2116.
11. De la Maza, L. M., Pezzlo, M. T., Bittencourt, C. E., and Peterson, E. M.(2020). Color atlas of medical bacteriology. John Wiley and Sons.270p.
12. Adhim, R., Oleiwi, A. H., &khassafAtya, A. (2023). Pathogenic Bacteria Isolated from House Flies and Compared with Pathogenic Bacteria Isolated from Children with Diarrhea. University of Thi-Qar Journal of Science, 10(2), 108-114.
13. Váradi, L., Luo, J. L., Hibbs, D. E., Perry, J. D., Anderson, R. J., Orenga, S., and Groundwater, P. W.(2017). Methods for the detection and identification of pathogenic bacteria: past, present, and future. Chemical Society Reviews , 46(16), 4818-4832.
14. Al-MaSoudi, S. T. O. (2020). The

antibacterial effect of extracts of the banyan branch and grape vine on Some types of bacteria isolated from different clinical Samples, MaSter ́s thesis, College of Education, UniverSity of Diyala.128.

15. Atiya, M. A., Hassan, A. K., &Luaibi, I. M. (2022). Green synthesis of bimetallic iron/copper nanoparticles using ficus leaves extract for removing orange G (OG) dye from aqueous medium. Nat. Environ. Pollut. Technol, 21(1), 355-365.
16. Saleh, R. A. M., & Mustafa, H. A. (2024). Analysis of the Synergistic Effect of Antibiotics with Copper Oxide Nanoparticles Against Pseudomonas aeruginosa Isolated from Different Clinical Cases. Tikrit Journal of Pure Science, 29(4), 41-51.
17. Bai, B., Saranya, S., Dheepaasri, V., Muniasamy, S., Alharbi, N. S., Selvaraj, B., ...&Gnanamangai, B. M. (2022). Biosynthesized copper oxide nanoparticles (CuO NPs) enhances the anti-biofilm efficacy against *K. pneumoniae* and *S. aureus*. Journal of King Saud University-Science, 34(6), 102120.
18. SAS, J. (2012). Statistical Analysis System, v. 10.0. 2. Journal of Cary, North Carolina. USA, 45(5): 1977-1983.
19. Saleem, A., Khan, M., & Ahmed, S. (2021). High Bacterial Load on Houseflies in Karachi Markets . PLOS ONE , 16(7), 1–14.
20. Sudagidan, M., Ozalp, V. C., Can, Ö.,Eligül, H., Yurt, M. N. Z., Tasbasi, B. B., ... &Koçak, O. (2022). Surface microbiota and associated staphylococci of houseflies (*Muscadomestica*) collected from different environmental sources. Microbial pathogenesis, 164, 105439.
21. Yap, K. L., Kalpana, M., & Lee, H. L. (2008). Wings of the common house fly (*Muscadomestica* L.): importance in mechanical transmission of Vibrio cholerae. Trop Biomed, 25(1), 1-8.
22. Nguyen, T., Moee, H., & Tran, Q. (2023). Urban Flies as Vectors of Pathogens in Vietnam . Environmental Microbiology Reports , 15(2), 234–245.
23. Shehata, A., El-Waseif, A., Waheeb, H., Emam, D., El-Tabakh, M., &Elghwas, D. (2024). Abundance and

Molecular Identification of House Fly *Muscadomestica* (Diptera: Muscidae) With Study to Associated Pathogenic Bacteria. Journal of microbiology, biotechnology and food sciences, 13(5), e10580-e10580.

24. Taha, J. H., Abbas, N. K., & Al-Attraqchi, A. A. (2020). Green synthesis and evaluation of copper oxide nanoparticles using fig leaves and their antifungal and antibacterial activities. Int. J. Drug. Deliv. Technol, 10, 378-382.
25. Bachheti, R.K. and Bachheti, A. (2023). Secondary Metabolites from Medicinal Plants Nanoparticles Synthesis and their Applications. Book. CRC Press. Taylor & Francis Group .London-New York .p:119.
26. Narayana, A., Bhat, S. A., Fathima, A., Lokesh, S. V., Surya, S. G., &Yelamaggad, C. V. (2020). Green and low-cost synthesis of zinc oxide nanoparticles and their application in transistor-based carbon monoxide sensing. RSC advances, 10(23), 13532-13542.
27. Jabeen, S., Siddiqui, V. U., Bala, S., Mishra, N., Mishra, A., Lawrence, R., ...& Khan, T. (2024). Biogenic synthesis of copper oxide nanoparticles from aloe vera: antibacterial activity, molecular docking, and photocatalytic dye degradation. ACS omega, 9(28), 30190-30204.
28. Maurya, A. K., Varshney, S., Upadhyay, S., Gupta, A., Jeyakumar, E., Lawrence, R., & Mishra, N. (2024). Green synthesis of copper oxide nanoparticles using *Ficusracemosa* leaf extract and investigation of its antibacterial properties. Advances in Materials and Processing Technologies, 1-21.
29. Bhavyasree, P. G., & Xavier, T. S. (2021). A critical green biosynthesis of novel CuO/C porous nanocomposite via the aqueous leaf extract of *Ficusreligiosa* and their antimicrobial, antioxidant, and adsorption properties. Chemical Engineering Journal Advances, 8, 100152.
30. Inam, H., Fatima, U., Shahid, S., Mansoor, S., Yasin, A., Javed, M., ...&Elkaeed, E. B. (2023). Nanotheranostic fabrication of iron oxide for rapid photocatalytic degradation of organic dyes and antifungal

potential. Journal of Saudi Chemical Society, 27(5), 101689.

31. Abdelsattar, A. S., Nofal, R., Makky, S., Safwat, A., Taha, A., and El-Shibiny, A. (2021). The synergistic effect of biosynthesized silver nanoparticles and phage zcse2 as a novel approach to combat multidrug-resistant Salmonella enterica. Antibiotics, 10(6), 678.
32. Zhang, Xiaoli; Wang, Yiming; Chen, Zhijun (2023) 'High-dose CuO NPs completely inhibit *E. coli* growth'. Frontiers in Microbiology, 14, p. 1188765.
33. Fernandez, A. C., &Archana, K. M. (2020). Green synthesis, characterization, catalytic and antibacterial studies of copper iodide nanoparticles synthesized using *Brassica oleracea* var. capitata f. rubra extract. Chemical Data Collections, 29, 100538.