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Original Research Article

Resistance Efficiency of Some Bacterial Isolates Against Malathion Pesticide

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ABSTRACT

Chemical pesticides have an impact on other living organisms in addition to their intended target organisms. Any chemical pesticide is therefore made safe for use by examining its biological characteristics and side effects. The present study was aimed at determining the resistance efficiency of six bacterial isolates obtained from malathion-contaminated soils. Bacteria were isolated from soil samples collected in Adhamiya, Baghdad, Iraq. Biochemical tests and VITEK 2 compact equipment were used to identify the bacterial isolates. Primary and secondary screening tests were conducted on the bacterial isolates for resistance against malathion pesticides. The optimal bacterial growth conditions were determined in malathion-contaminated media. The results demonstrated that the bacterial isolates 1, 3, 4, and 8 grew best on malathion-contaminated (100 mgL^{-1}) mineral salt medium (MSM). Isolates 1 and 2 had a MIC of 500 mgL^{-1}, where they continued to grow until the seventh day of incubation. *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Aeromonas hydrophilia*, and *Escherichia coli* were the identified bacterial isolates. These isolates showed optimal bacterial growth in the test conditions (temperature, incubation period, and pH), indicating their efficiency and ability to degrade malathion. The highest average growth of *P. aeruginosa* at 35 °C was 70.08 mm, while that of *P. putida* was 40.10 mm during the 7-day incubation period. Also, the highest values of average growth were observed in the same bacteria at pH 7, with a value of 26.98 mm. The findings of this study reveal that *Pseudomonas aeruginosa* and *Pseudomonas putida* were the best bacterial isolates for biodegrading malathion.

Keywords: Bacterial isolate, Bioremediation, Incubation period, Minimum inhibitory concentration, Pesticide, pH, Temperature .

Introduction

The effect of any chemical pesticide is not only on the target organisms but also extends to other living organisms.[1] Therefore, evaluating the biological properties of any chemical pesticide, as well as investigating its side effects, makes it safe for use. Malathion is a pesticide that has a short lifespan in the environment.[2] It is extremely toxic to both humans and animals, and such toxicity rises when the pesticide breaks down into other compounds.[3] However, it also has an impact on biological processes by inhibiting vital enzymes such as the ATPase enzyme.[4] Tetra ethyl pyrophosphate (TEPP) was one of the first pesticides to be produced after the 1928 discovery of organophosphate insecticides.[3] The German scientist, Schradan and his team identified its action on insects in 1947, when a wide field opened for the generation of a huge number of phosphorous compounds, including gases utilized in World War II.[5] As it is not stored in fatty tissues and has the potential to dissolve in water, this group of pesticides is considered to have one of the strongest effects on insects compared to chlorinated hydrocarbons.[1] This makes it easier for the organism to get rid of it by diuresis.[5] The widespread use of TEPP is due to several factors. Firstly, its compounds are very efficient against insects and also have a high starting effect because they contain phosphorous, which has several properties, including having several valences (pentavalent); its derivative, phosphoric acid, is one of the most important elements in biological processes; helps to regulate the process of energy production; and participates in the synthesis of phosphoryl lipids, which are present in the cell membrane, nucleic acids, and animal fats in the nucleus.[6,7] Secondly, this group has a variety of specifications, including fast-degrading pesticides (like nucose, malathion, and TEPP), slow-degrading pesticides (like diazinon), specialized pesticides (like Schradan), and non-specialized pesticides (such as parathion). Thirdly, it is characterized by low chronic toxicity as a result of its decomposition into non-toxic products in humans and animals. Lastly, it is also characterized by low fish toxicity.[8]

One of the disadvantages of pesticides is that they are very harmful to humans and animals. This is because these pesticides are aliphatic chemicals, straight, without rings, like malathion, which was used and is still used in Iraq to control sucking insects like aphids, white flies, dubas, and guantlets (LD_{50} = 1000 mg/kg).[5] Several studies indicated the possibility of isolation and characterization of various bacteria such as *Pseudomonas putida*, *Aeromonas media*, *Sphingobacterium spiritivorum*, *Flavobacterium breve*, *Serratia liquefaciens*, and *Klebsiella oxytoca* that are capable of producing various types of hydrocarbon and phosphorous compounds. The majority of these bacteria are gram-negative.[9] Some researchers were able to isolate many aerobic and anaerobic bacteria in wells as well as soil contaminated with pesticides and oil.[8] In a different investigation, some researchers discovered that certain species of *Bacillus* were capable of decomposing pesticides and oil pollutants, as well as separating various varieties of them from contaminated deep sea water, sediments, and soil.[2]

The present study was, therefore, conducted to determine the malathion pesticide resistance efficiency of some bacterial isolates.

Materials and Methods

Soil sample collection

Soil samples were collected at depths ranging from 5 to 10 cm by the Uker device from different sites throughout Baghdad, Iraq, notably from many nurseries in Adhamiya city. Twenty soil samples were

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obtained from six sites and were collected from September 2020 to March 2021. The soil of these nurseries was contaminated with many pesticides, including malathion insecticides. One hundred grams (100 g) of the soil samples were collected from each site and placed in a sterile nylon bag with the following information recorded: sample number, soil type, location, and date of sample collection. The soil samples were taken to the laboratory.[10,11]

Isolation and purification of bacterial isolates

Serial dilutions of the soil samples were made, and 0.1 ml (10^{-2} diluent) was spread on the surface of nutrient agar. The cultures were incubated for 24 to 48 hours at temperatures between 15 and 45°C. The purified bacterial isolate was stored in a solid nutrient medium at 4°C until needed for identification.[12]

Identification of bacterial isolates

Biochemical tests were conducted to identify the species and genera of the bacterial isolates. Moreover, the VITEK 2 compact equipment was utilized for the confirmation of the identity of the isolates.[13]

Primary screening of bacterial isolates for resistance against malathion pesticide

After serial dilutions, 500 µl of the supernatant was spread on an agricultural medium containing solid mineral salt medium (MSM) to screen for pesticide resistance.[14] Malathion was used as a carbon source in the MSM to evaluate the test bacteria's ability to decompose it. The isolates that showed higher biodegradation capabilities of pesticides were selected for further investigations.

Secondary screening of bacterial isolates in solid media

In the present study, the bacterial isolates that showed high degradability of malathion in the primary screening test were sub-cultured into solid MSM containing different concentrations (ranging from 10-500 mgL^{-1}) of the pesticide as the only carbon source. Also, the minimum inhibitory concentration (MIC) was determined as the lowest concentration that inhibited bacterial growth. The MSM medium was used to select the pesticide concentrations, which ranged from 50-500 mgL^{-1} and the cultures were incubated at 28°C for 7 days. The growth of bacterial isolates was evaluated based on the formation of clear zones around bacterial colonies.[15,16]

Determination of optimal bacterial growth conditions in malathion pesticide-contaminated medium

Some factors, such as temperature, incubation period, and pH, that affect the growth of bacterial isolates were evaluated.[17]

Statistical analysis

The Statistical Analysis System (SAS) program,[18] was used to determine the effect of different factors on the research parameters. The least significant difference (LSD) test and analysis of variance (ANOVA) were used for mean comparison.

Results and Discussion

Three of the nine distinct bacterial isolates were gram-negative, and six were gram-positive. To obtain single and pure bacterial isolates, the bacterial isolates were purified on a solid nutrient medium. They were then grown on a mineral salt medium and exposed to 100 mgL^{-1} of the pesticide malathion to determine which bacterial isolates were able to use the pesticide as a carbon source for nutrition. Table 1 illustrates the growth of the bacterial isolates in the MSM.

In the MSM, where growth typically began on the second and third day and lasted until the seventh day for most samples, enhanced growth was observed in the bacterial isolates 1, 2, 3, and 8. The bacteria also utilized the pesticide as a carbon source for nutrition. The remaining bacterial isolates (5, 6, 7, and 9) did not demonstrate good growth in the MSM. Statistical analysis revealed significant differences between days 4, 5, 6, and 7 of bacterial growth. Meanwhile, the remaining days did not reveal any significant differences (Table 1). The growth of bacterial isolates increased after the second and third days of incubation in the media containing the pesticide. This observation was consistent with the previous findings.[19,20] The reason for the slow growth of bacteria may be attributed to the fact that they do not have the metabolic ability for these bacterial pesticides. The bacterial isolates that do not survive in agricultural media contaminated with malathion pesticides may not have a specialized enzymatic system. Also, the low solubility of chemicals (pesticides) in the agricultural medium may be the cause of their slow growth because there is not enough food for bacterial isolates to grow.[21,14]

The bacterial isolates that showed good growth in the primary screening test with MSM contaminated with malathion pesticide at 100 mgL^{-1} were selected for the second round of screening. The bacterial isolates (1, 2, 3, 4, and 8) were sub-cultured onto MSM contaminated with different concentrations (ranging from 10 to 500 mgL^{-1}) of malathion pesticide as the only source of carbon. There was a growth of the isolates until the seventh day of incubation. However, not all of the isolates recorded growth at the same concentration as shown in Table 2. All of the isolates continued to grow in all concentrations except for the second isolate, and they all ceased their growth at a concentration of 150 mgL^{-1}. The reason for the growth of bacterial isolates in different concentrations of the pesticide could be that these isolates evolved mechanisms that allowed them to grow there, as earlier reported.[22,23]

In total, four bacterial isolates with optimum growth were selected in the secondary screening assay to determine the MIC. They were sub-cultured into different concentrations (ranging from 50 to 500 mgL^{-1}) of the MSM contaminated with malathion pesticide and incubated at 28°C for 7 days. The results indicated that the isolates (1 and 23) had optimal growth in all media concentrations contaminated with malathion. The first and second isolates had the highest growth measured at O.D 600 nm, which was 490 and 488 in the 50 mgL^{-1} concentration, respectively. The MIC was determined to be 500 mgL^{-1} for the fourth isolate in the malathion-containing medium. There was no growth recorded in the 500 mgL^{-1} concentration. The statistical analysis revealed that there were significant ($p \leq 0.05$) differences between pesticide concentrations, as depicted in Table 3. The growth rate of the bacteria decreased with an increase in pesticide concentrations. This observation could be due to the stress caused by the bacteria's adaptation to growth and the use of the medium as a food source.[24] Another cause of a decrease in bacterial growth could be attributed to the fact that the pesticide that the bacteria use as food has been depleted or has turned into metabolic products that are poisonous to the microorganisms.[25]

Optimal conditions for bacterial growth

Based on morphological characteristics and biochemical tests, as well as confirmation of the isolates using the VITEK 2 compact device, the four isolates in this study were identified and demonstrated to be effective at growing in an agricultural medium treated with malathion. The results of the tests revealed that the first isolate was *Pseudomonas aeruginosa*.

Table 1: Growth of bacterial isolates in MSM contaminated with malathion pesticide at a concentration of 100 mg L^{-1}.

Bacterial Isolate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	-	+	+	++	++	++	++
2	-	-	-	-	-	+	+
3	-	+	+	+	++	++	++
4	-	-	+	+	++	++	++
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	+	+
8	-	-	-	+	+	++	++
9	-	-	-	+	+	-	-
LSD value	NS	NS	NS	0.05*	0.05*	0.05*	0.05*

-: No growth; +: Good growth; ++: Perfect growth; MSM: Mineral salt medium; *: $p \leq 0.05$

The second, third, and fourth isolates were *Pseudomonas putida*, *Aeromonas hydrophilia*, and *Escherichia coli*, respectively.[26] The optimal temperature, incubation period, and pH for bacterial growth were determined in MSM liquid medium contaminated with malathion pesticide at 100 mgL^{-1}. The results showed that *P. aeruginosa* and *A. hydrophilia* had the highest average growth, recording values of between 66.78 and 70.08, respectively, at 35°C. Meanwhile, *E. coli* had the lowest average growth, with a value of 11.88 mm at 50°C. The statistical analysis revealed significant ($p \leq 0.05$) differences between the bacterial types as well as the temperatures, as shown by the LSD values in Table 4. There are significant differences among growth averages.

It was observed that bacterial systems can biodegrade pesticides at high temperatures ranging from 15 to 35°C. These results are in agreement with studies that have shown that bacteria can grow rapidly and biodegrade organic cellulosic materials at standard laboratory temperatures,[27] and that temperature also affects the enzymatic activity of bacteria. The decomposition of compounds increases with temperature, providing the necessary nutrients for bacterial growth and activity.[6] For the incubation periods, it was observed that *P. aeruginosa* and *P. putida* had the highest average growth on the seventh day of incubation, with values of 39.85 and 40.10 mm, respectively. Meanwhile, *E. coli* had the lowest average growth on the seventh day of incubation with a value of 22.54 mm. According to the statistical analysis, there were no significant differences between incubation days 0, 1, 2, 3, and 4, but there were significant differences between incubation days 5, 6, and 7, as displayed in Table 5. According to the statistical analysis, there were significant ($p \leq 0.05$) differences in the bacterial growth averages. Many organisms can secrete the enzymes required to break down pesticides, and they can transform pesticides from hazardous materials into less toxic ones. Considering it as a food source in previous studies,[28,29] an increase in the number of bacteria was observed with an increasing incubation period. This finding agrees with the results of the current study.

A pH range of 5 to 10 was chosen to determine the optimal pH for bacterial growth at 100% pesticide concentration. This pH range was chosen because *P. aeruginosa*, *Aeromonas hydrophilia*, and *P. putida* all showed comparable average growth (25.80, 26.98, and 25.88 mm, respectively) at pH 7. *Pseudomonas putida* had the highest average growth (26.98 mm), while *E. coli* had the lowest average growth (18.73 mm). According to statistical analysis, there were significant ($p \leq 0.05$) differences between bacterial average growth (Table 6). The results of the present study support the previous finding,[30] which showed that bacterial growth increases with increasing acidity and that the optimal pH range for bacterial growth is 7-8. At pH 7, microorganisms have high physiological activity because the enzymes that dissolve pesticides and transfer them to simpler materials, such as carbonic food sources, are more effective.[31] Microorganisms, such as bacteria, viruses, and parasites, can be identified from their specific hosts using molecular techniques like a polymerase chain reaction. *Clostridium perfringens*,[32] *Brucella melitensis*,[33] *Proteus vulgaris*,[34,35] *Staphylococcus aureus*,[36] *Pseudomonas aeruginosa*,[37] and *Toxoplasma* sp.,[38,39] SARS-Cov-2,[40] are among these bacteria.

Table 2: Second screening and MIC of bacterial isolates grown in MSM contaminated with malathion pesticide.

Incubation period (days)	Malathion MIC (mg/l)	Bacterial isolate
7	500	1
3-7	150	2
7	450	3
7	450	4
7	300	8
1.751*	62.073*	**Value of LSD**

MIC: Minimum inhibitory concentration; MSM: Mineral salt medium; *: $p \leq 0.05$

Table 3: MIC of bacterial isolates in a medium contaminated with malathion pesticide.

Bacterial isolates	Malathion concentration										LSD
	500%	450%	400%	350%	300%	250%	200%	150%	100%	50%	
1	50.00	100.00	101.30	200.5	300.9	300.10	340.55	390.60	480.50	490.50	42.57*
2	30.00	97.00	112.3	197.3	270.9	300.12	340.50	370.60	480.50	488.30	51.74
3	3.00	42.00	100.00	130.00	197.00	200.7	310.35	350.50	440.30	370.30	58.97*
4	00.00	30.00	100.00	121.00	150.00	200.2	290.10	310.30	400.30	310.00	48.55*
LSD value	17.93*	26.58*	15.43 NS	25.04*	28.47*	37.64*	37.51*	41.85*	34.66*	39.02*	------

MIC: Minimum inhibitory concentration; *: $p \leq 0.05$

Table 4: Average growth of bacterial isolates in agricultural medium contaminated with malathion pesticide (100% concentration) incubated at different temperatures for 7 days.

Temperature (°C)	*E. coli*	*A. hydrophilia*	*P. putida*	*P. aeruginosa*	LSD value
25	12.30	20.21	20.51	20.76	4.59 *
30	15.50	30.70	35.90	37.90	7.04 *
35	50.10	66.78	63.80	70.08	7.16 *
40	41.66	55.15	63.97	66.01	6.52 *
45	13.79	26.11	30.13	33.00	5.38 *
50	11.88	12.08	16.91	20.60	4.87 *
LSD value	6.48 *	7.32 *	6.91 *	5.84 *	----------

Measurement was recorded at 600 nm; *: $p \leq 0.05$.

Table 5: Average growth of bacterial isolates in agricultural medium contaminated with malathion pesticide (100% concentration) incubated at different periods.

Days of incubation	*E. coli*	*A. hydrophilia*	*P. putida*	*P. aeruginosa*	LSD value
1	5.20	7.93	9.13	7.81	4.62 NS
2	6.03	7.97	9.33	7.81	4.08 NS
3	6.36	8.99	9.96	9.32	3.92 NS
4	7.18	9.00	9.96	9.68	2.87 NS
5	8.20	11.24	12.45	11.91	4.37 NS
6	12.77	20.37	23.50	21.98	6.31*
7	20.33	30.65	36.31	35.70	7.05*
LSD value	22.54	36.40	40.10	39.85	6.84*
---	6.41*	7.26*	8.07*	7.63*	---

Measurement was recorded at 600 nm; *: p≤0.05

Table 6: Average growth of bacterial isolates in agricultural medium contaminated with malathion pesticide (100% concentration) at different media pH incubated for 7 days.

Media pH	*E. coli*	*A. hydrophilia*	*P. putida*	*P. aeruginosa*	LSD value
5	5.33	7.97	8.30	8.63	3.09 NS
6	16.36	18.87	18.90	19.77	3.57 NS
7	18.73	25.80	26.98	25.88	4.61 *
8	20.58	26.11	26.80	25.97	4.97 *
9	16.18	21.13	24.45	22.33	5.07 *
10	15.31	18.85	19.53	18.99	3.69 NS
LSD value	5.28 *	5.97 *	6.68 *	5.07 *	------

Measurement was recorded at 600 nm; *: p≤0.05

Conclusion

The research work established that *Pseudomonas aeruginosa* and *Pseudomonas putida* are easier to cultivate on sandy-loamy soil. Two bacterial isolates were able to grow in the highest concentration of malathion, which may be due to the already evolved mechanisms that allowed them to thrive in such conditions. With the right environment, both bacteria thrived to produce the greatest possible outcome.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Cresswell JE, Robert FX, Florance H, Smirnoff N. Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*). Pest Manag Sci. 2014; 70(2):332-337.
2. Rahman KS, Rahman T, Lakshmanaperumalsamy P, Banat IM. Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. J Basic Microbiol. 2002; 42(4):284-291.
3. Čolović M, Krstić D, Petrović S, Leskovac A, Joksić G, Savić J, Franko M, Trebše P, Vasić V. Toxic effects of diazinon and its photodegradation products. Toxicol Lett. 2010; 193(1):9-18.
4. Vasić V, Momić T, Petković M, Krstić D. Na+, K+-ATPase as the target enzyme for organic and inorganic compounds. Sensors. 2008; 8(12):8321-8360.
5. Cutler GC, Purdy J, Giesy JP, Solomon KR. Risk to pollinators from the use of chlorpyrifos in the United States. Ecological risk assessment for chlorpyrifos in terrestrial and aquatic systems in the United States. Rev Environ Contam Toxicol. 2014; 219-265.
6. Hardstone MC, Scott JG. Is *Apis mellifera* more sensitive to insecticides than other insects? Pest Manag Sci. 2010; 66(11):1171-1180.
7. Feitkenhauer H, Müller R. Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 6070 C by *Thermus* and *Bacillus* spp. Biodegradation. 2012; 14(6):367-372.
8. Mas Gordi J, Vigués Frantzen N, Sanchez Martinez O. Presence of opportunistic oil-degrading microorganisms operating at the initial steps of oil extraction and handling. Int Microbiol. 2006; 9(2):119-124.
9. Boboye B, Olukunle OF, Adetuyi FC. Degradative activity of bacteria isolated from hydrocarbon-polluted site in Ilaje, Ondo State, Nigeria. Afr J Microbiol Res. 2010; 4(23):2484-2491.
10. Kanekar PP, Bhadbhade BJ, Deshpande NM, Sarnaik SS. Biodegradation of organophosphorus pesticides. Proc Indian National Sci Acad. 2004; 70(1):57-70.
11. Al Fatlawy YFK, Mahdii BA, Kadhim F. Study the Tigris river in the South of Baghdad City and may use it as irrigation water. Int J Agric Stat Sci. 2021; 17:1167–1172.

12. Yoo IY, Han J, Ha SI, Cha YJ, Pil SD, Park YJ. Clinical performance of ASTA SepsiPrep kit in direct bacterial identification and antimicrobial susceptibility test using MicroIDSys Elite and VITEK-2 system. J Clin Lab Anal 2021;35(6):e23744. doi: 10.1002/jcla.23744.
13. MacFaddin JF. Biochemical tests for identification of medical bacteria. Williams and Wilkins. Philadelphia, PA. 2000; 113p.
14. Goda SK, Elsayed IE, Khodair TA, El-Sayed W, Mohamed ME. Screening for and isolation and identification of malathion-degrading bacteria: cloning and sequencing a gene that potentially encodes the malathion-degrading enzyme, carboxylestrase in soil bacteria. Biodegradation. 2010; 21(6):903-913.
15. Zhongli C, Shunpeng L, Guoping F. Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. Appl Environ Microbiol. 2001; 67(10):4922-4925.
16. Fang H, Xiang YQ, Hao YJ, Chu XQ, Pan XD, Yu JQ, Yu YL. Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi. Int Biodeterior Biodegr. 2008; 61(4):294-303.
17. Karunya SK, Reetha D. Efficiency of bacterial isolates in the degradation of malathion and parathion. Int J Pharm Biolog Arch. 2012; 3:659-665.
18. Cary N. Statistical analysis system, User's guide. Statistical. Version 9. SAS. Inst. Inc. USA. 2012.
19. Abboud HY, Jaber MI and Salman FM. Effect of soil salinity and glyphosate pesticide on the preparation of nitrification bacteria for soils of different textures. Al-Furat J Agric Sci. 2016; 1:181-176.
20. Emran FK. Biodegradation of Malathion and Dursban by Mono and Mixed Bacterial Cultures. Ph.D. Thesis. University of Baghdad, Baghdad, Iraq. 2015.
21. Mansour EA, Hammadi A. Study the effect of pesticides commonly used locally on the biomass and activity in the soil. AUBER. 2015; 18 (1):43-54.
22. Eman A, Abdel-Megeed A, Suliman A, Sadik M, Sholkamy EN. Biodegradation of glyphosate by fungal strains isolated from herbicides polluted-soils in Riyadh area. Br J Environ Sci. 2013; 1:7-29.
23. Okoh AI. Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. Afr J Biotechnol. 2003; 2(5):104-108.
24. Nielsen LN, Roager HM, Casas ME, Frandsen HL, Gosewinkel U, Bester K, Licht TR, Hendriksen NB, Bahl MI. Glyphosate has limited short-term effects on commensal bacterial community composition in the gut environment due to sufficient aromatic amino acid levels. Environ Pollut. 2018; 233:364-376.
25. Blanco P, Hernando-Amado S, Reales-Calderon JA, Corona F, Lira F, Alcalde-Rico M, Bernardini A, Sanchez MB, Martinez JL. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms. 2016; 4(1):14; doi:10.3390/microorganisms4010014
26. Alnazzal A, Aghared A, Yassamin LK. Isolation and identification of pathogenic bacteria from drinking water in Salahdeen province by using membrane filter method. Anbar J. Pure Sci 2009; 3(3):1-7.
27. Deka D, Das SP, Sahoo N, Das D, Jawed M, Goyal D, Goyal A. Enhanced cellulase production from *Bacillus subtilis* by optimizing physical parameters for bioethanol production. ISRN Biotechnol. 2013; 2013:965310. doi: 10.5402/2013/965310.
28. Atiya SA, Labeeb AK, Sanaa KM, Hassena WM, Shahlaa KF. Study of some optimum conditions of *Bacillus subtilis* for the biological activity of cellulose degradation, Iraqi J Sci Technol. 2019;10 (2):1-7.
29. Milošević NA, Govedarica MM. Effect of herbicides on microbiological properties of soil. Zb Matice Srp Prir Nauke. 2002; (102):5-21.
30. Goyal V, Mittal A, Bhuwal AK, Singh G, Yadav A, Aggarwal NK. Parametric optimization of cultural conditions for carboxymethyl cellulase production using pretreated rice straw by *Bacillus* sp. 313SI under stationary and shaking conditions. Biotechnol Res Int. 2014 (1):1-7 .https://doi.org/10.1155/2014/651839.
31. Ray AK, Bairagi A, Ghosh KS, Sen SK. Optimization of fermentation conditions for cellulase production by *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 isolated from fish gut. Acta Ichthyol Piscat. 2007; 37(1):47-53.
32. Hashim ST, Fakhry SS, Rasoul LM, Saleh TH, Alrubaii BAL. Genotyping toxins of *Clostridium perfringens* strains of rabbit and other animal origins. Trop J Nat Prod Res. 2021; 5(4):613–616.
33. Abdulkaliq AH, Hammed ZN, Hamzah SS, Saleh TH, AL-Rubaii BAL. Molecular identification of intracellular survival related *Brucella melitensis* virulence factors. Biomedicine. (India) 2022; 42(4):761–765.
34. Abdul-Gani MN, Laftaah BA. Purification and characterization of chondroitinase ABC from *Proteus vulgaris*, an Iraqi clinically isolate. Curr Sci 2017; 113(11):2134-2140.
35. Kadhim AL-Imam MJ, AL-Rubaii BAL. The influence of some amino acids, vitamins and anti-inflammatory drugs on activity of chondroitinase produced by *Proteus vulgaris* caused urinary tract infection. Iraqi J Sci. 2016; 57(4A):2412-2421.
36. Sabah FS, Noori HZ, Abdul-elah BW, ALRubaii BAL. Identification of methicillin-resistant strains of *Staphylococcus aureus* isolated from humans and food sources by use mecA 1 and mecA 2 genes in Pulsed-field gel electrophoresis technique. Bionatura. 2022; 7(2):44. http://dx.doi.org/10.21931/RB/2022.07.02.44.
37. Shehab ZH, AL-Rubaii BAL. Effect of D-mannose on gene expression of neuraminidase produced from different clinical isolates of *Pseudomonas aeruginosa*. Baghdad Sci J. 2019;16(2):291–298.
38. Abdulla L, Ismael MK, Salih TA, Malik SN, Al-Rubaii BAL. Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV-2 infections. Ann Parasitol. 2022; 68(2):385–390.
39. Jiad AL, Ismael MK, Muhsin SS, Al-Rubaii BAL.ND2 Gene Sequencing of Sub fertile Patients Recovered from COVID-19 in Association with Toxoplasmosis. Bionatura. 2022; 7(3):45. http://dx.doi.org/10.21931/RB/2022.07.03.45.
40. Rasoul LM, Nsaif MM, Al-Tameemi MT, Al-Rubaii BA. Estimation of primer efficiency in multiplex PCR for detecting SARS-Cov-2 variants. Bionatura. 2022; 7(3):48. http://dx.doi.org/10.21931/RB/2022.07.03.49.