Iraqi Journal of Market Research and Consumer Protection
(2024) 16(2): 136-148
DOI: http://dx.doi.org/10.28936/jmracpc16.2.2024.(12)
Ali & et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

DETERMINATION OF OPTIMUM CONDITIONS FOR BIOREMEDIATION OF IMIDACLOPRID BY *RHIZOBIUM PUSENSE* STRAIN IHB 1(OP218458.1) AND *PSYCHROBACTER CELER* STRAIN IHB2(OP672320.1)

Ihab Hammood Ali[1*], Aswan Hamdullah Abbood Bayar[2], Mokhtar Abd-Alsatar Arif [3]
[1]Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq. ehabhomoud@gmail.com
[2]Professor PhD., Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, Iraq. aswan@coagri.uobaghdad.edu.iq
[3]Co-advisor The National Center for Pesticides Control, Ministry of Agriculture, Baghdad, Iraq. mokhtar.a.arif@gmail.com

Received 5/ 6/ 2023, Accepted 2/ 8/ 2023, Published 31/ 12/ 2024

ABSTRACT

Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine), is a recent systemic and contact insecticide with high activity against a wide range of pests. Continuous dispersion of this pesticide in the environment and its stability in soil results in environmental pollution which demands remediation. Used in this research *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1). which were previously isolated from botanical fields soil of greenhouses which has been using imidacloprid pesticides for many years to determine optimum condition and degradation ability for imidacloprid by tested in minimal salt medium (MSM) for a duration of 21 days. The temperature, pH number, and concentration of the pesticide were determined for the growth of bacteria. The best growth of *Psychrobacter celer* strain IHB2(OP672320.1) was at 28°C, pH 6, and pesticide concentration 50ppm, while Rhizobium pusense strain IHB1 (OP218458.1) had the best growth at 24°C, pH 7, and pesticide concentration 75ppm.Levels of imidacloprid in MSM medium were analyzed by high-performance liquid chromatography (HPLC). *Rhizobium pusense* strain IHB 1 (OP218458.1) was able to degrade 50.2% and *Psychrobacter celer* strain IHB2(OP672320.1) was able to degrade 59.01% of the initial amount of imidacloprid at the concentration of 25 mg /L in MSM media. All bacteria introduced in this study were among the first reports of imidacloprid degrading isolates in MSM-limited media from greenhouse soil. Therefore, the results of this study demonstrate the effectiveness of using soil bacteria for microbial degradation of imidacloprid. These findings suggest that these strains may be promising candidates for bioremediation of imidacloprid-contaminated soils.

Keywords: MSM, *Psychrobacter*, *Rhizobium*, HPLC.

[*]**The article is taken from the doctoral thesis of the first researcher.**

المجلة العراقية لبحوث السوق وحماية المستهلك

Iraqi Journal of Market Research and Consumer Protection

Ali & et al.,

(2024) 16(2): 136-148

تحديد الظروف المثلى للمعالجة الحيوية للإيميداكلوبريد بواسطة *سلالة* ***Rhizobium pusense*** **IHB 1 (OP218458.1) وسلالة *Psychrobacter celer* IHB2(OP672320.1)**

ايهاب حمود علي[1] ,إسوان حمد الله بيار[2] , مختار عبد الستار عارف[3]

[1] باحث, قسم علوم الاغذية, كلية علوم الهندسة الزراعية, جامعة بغداد, بغداد, العراق. ehabhomoud@gmail.com

[2] أستاذ دكتوره، قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق. aswan@coagri.uobaghdad.edu.iq

[3] مشرف مساعد، المركز الوطني للسيطرة على المبيدات، وزارة الزراعة، بغداد، العراق.mokhtar.a.arif@gmail.com

الخلاصة

يعرف Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl] -N-nitro-2- imidazolidinimine بانه مبيد حشري جهازي ويعمل بالملامسة أيضا وذو فعالية عالية ضد مجموعة واسعة من الآفات. ويؤدي التشتت المستمر لهذا المبيد في البيئة واستقراره في التربة إلى تلوث بيئي يتطلب معالجة. استخدمت في هذا البحث بكتيريا *Psychrobacter celer* strain IHB2 (OP672320.1) و *Rhizobium pusense* strain IHB 1 (OP218458.1) التي تم عزلها سابقًا من ترب البيوت البلاستيكية، والتي تستخدم مبيدات الآفات الاميداكلوبريد لسنوات عديدة وذلك لتحديد الظروف المثلى لها وقدرتها على تحليل الاميداكلوبريد عن طريق اختبارها في الوسط الملحي (MSM)لمدة ٢١ يومًا. جرى تحديد كل من درجة الحرارة والرقم الهيدروجيني وتركيز المبيد في نمو البكتريا. كان أفضل نمو لبكتريا *Psychrobacter celer* strain IHB2 (OP672320.1) في درجة ٢٨ مئوية و pH٦ وتركيز المبيد ppm٥٠، اما بكتريا *Rhizobium pusense* strain IHB 1 (OP218458.1) فكان أفضل نمو في درجة ٢٤مئوية و pH٧ وتركيز المبيد ppm.٧٥ تم تحليل مستويات إيميداكلوبريد في الوسط الملحي MSM بواسطة تحليل الكروماتوجرافي السائل عالي الأداء (HPLC)، واستطاعت سلالة*(OP218458.1) *Rhizobium pusense* IHB 1 أن تحلل بنسبة ٥٠,٢٪ وسلالة *Psychrobacter celer* IHB2 (OP672320.1) أن تحلل بنسبة ٥٩,٠١٪ من الكمية الأولية من إيميداكلوبريد بتركيز ٢٥ ملغم/ لتر في الوسط الملحي MSM. كانت أنواع البكتريا التي تم إدخالها في هذه الدراسة من بين التقارير الأولى للعزلات المحطمة للإيميداكلوبريد في الوسط الملحي MSM المعزولة من ترب البيوت البلاستيكية، لذلك أظهرت نتائج هذه الدراسة فاعلية استخدام بكتيريا التربة في التحلل الحيوي لمادة الاميداكلوبريد. تشير هذه النتائج إلى أن هذه السلالات قد تكون مرشحة واعدة للمعالجة الحيوية للتربة الملوثة بالاميداكلوبريد.

الكلمات المفتاحية: وسط ملحي MSM، *Psychrobacter*، *Rhizobium* ، كروماتوجرافي سائل عالي الأداء.

INTRODUCTION

Pesticides are a significant environmental risk where three million metric tons of pesticides are used globally each year because of their enduring effects on the environment. The organic decomposition of many of these pesticides in the soil is incomplete and enter the food chain through bioaccumulation and biomagnification, having an effect on both the target and non-target organisms, including people. Since farmers who spray pesticides in greenhouse fields are also affected by them, pesticide residues are viewed as a significant risk factor in society **(Awad *et al.*, 2018; Othman & Kakey, 2018)**. One million of the three million individuals who are fatally poisoned by pesticides each year do not even know it, according to the World Health Organization (WHO) **(Miccoli *et al.*, 2016)**. Neonicotinoid insecticides are widely used because insect pests are a widespread issue. accounting neonicotinoid around 25% of the global pesticide market **(Zhang *et al.*, 2019)**. These compounds are becoming more and more well-liked as an alternative to pyrethroid and organophosphate due to their unique mechanism of action **(Abdel-Ghany *et al.*, 2016)**. More and more academics have been focusing on the effects on organisms, the environment and activities using these substances through Studies on aquatic creatures **(Chen *et al.*, 2019)**, birds **(Humann-Guilleminot *et al.*, 2019)**, bees **(Zhu *et al.*, 2019; Strobl *et al.*, 2021)** and mammals (including humans) have shown that exposure to neonicotinoid pesticides may cause acute or

chronic toxicity **(Ali *et al*., 2018; Saqer *et al*., 2018).** As of late. The residual duration after pollution has been removed is an important indication. The amount of time that neonicotinoids stay in the soil varies greatly, from a few hundred to several thousand days **(Schaafsma *et al*., 2016)**. Imidacloprid, the first half-life of one neonicotinoid insecticide is 28–1250 days, while the half-life of clothianidin is 148–6931 days. There hasn't been much investigation into the precise reasons behind the significant inter-annual variation in neonicotinoid residual durations in the environment. The most popular insecticide is imidacloprid, also known as (1-[(6-chloro-3-pyridinyl) methyl] The first neonicotinoid pesticide on the market was N-nitro-2-imidazolidinimine. It is used in more than 100 countries and more than 140 different types of crops **(Jeschke & Nauen, 2018)**. More than two-thirds of the world's imidacloprid is produced in China, with 12,000 tons produced in 2012. 2019 China exported 31,595 tons of imidacloprid-related goods in 2017 **(Chen *et al*., 2019).** IMI has been used heavily and widely, but this has led to several problems, including environmental contamination, insect resistance, and the extinction of natural adversaries **(Liu *et al*., 2013)**. Because of the increased attention being paid to environmental issues, IMI's microbial degradation has been studied to eliminate IMI residues in ecosystems. It was discovered that IMI can remain in the soil for up to 156 days before it starts to lose its effectiveness **(Jeschke & Nauen, 2018)**. The most common method for converting synthetic chemicals into inorganic compounds is biodegradation, and both biotic and abiotic components of soil, such as elements, sunlight, and microbes, aid in this process. Soil microorganisms have been shown to degrade Heavy Metals and imidacloprid in several studies **(Al-Soufi *et al*., 2015; Garg *et al*.,2021)**, also found the ability of pseudomonas sp. To biodegradation of imidacloprid, hydrocarbons and Phenol in soil **(Hatit *et al*., 2013; Nafal & Abdulhay, 2020; Kridi *et al*., 2021; Dhari & Hetite, 2019)** and bioremediation of imidacloprid using Azospirillium biofertilizer and Rhizobium biofertilizer **(Kulkarni *et al*., 2022)**. This research aimed to ability of *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1) the best degrading of imidacloprid in communities' soil samples for greenhouses and identify the best.

MATERIAL AND METHODS

Chemicals and organisms

By the National Center for Pesticide Control in Baghdad provided imidacloprid (purity >97%). All solvents were bought from Merck KGaA in Germany (99.9% purity) and were of HPLC grade. England company Romil developed HPLC-grade water. A nylon filter (0.45) µm was used when applying HPLC-grade H_2O and C_2H_3N. Using a criterion solution, analytical criterion for HPLC, standardization in the 1–50 mg/L range was produced. *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2 (OP672320.1) provided from the Department of Biofertilizers by the Plant Protection Director in Baghdad.

Culture of microbe growth

According to the manufacturer's recommendations, distilled water was used to create MSM (Mineral Salt Medium) media and was autoclaved for 15 minutes at 121ºC to guarantee that the solutions was sterilized (Sigma-Aldrich, USA). Imidacloprid (25 ppm) was administered using a syringe filter. According to **(Zhao *et al*.,2018)** the mineral salt medium (MSM) was employed for biodegradation testing and contains (g/L) ammonium sulfate (2.0), potassium hydrogen phosphate (0.625), sodium dihydrogen phosphate (0.6), magnesium sulfate (0.2), and calcium chloride. Antarctictite (hexahydrate) (0.15) (pH 7.0). The medium was cleaned by autoclaving it at 121C degrees for 15 minutes.

Instruments

An HPLC (LC-2010 A HT Shimadzu Japan model) with a DAD was used for the analysis of imidacloprid. ChemStation software was used for all data collection and processing. Each sample was separated using a 250 4.6 mm Orbit C18 reversed-phase column. At 1 ml/min at a flow rate and an oven temperature of 40 °C, the mobile phase's binary composition was 60% water and 40% acetonitrile. The detector's wavelength was adjusted at 270 nm. Each time a sample was inspected, the calibration curve was verified. was developed with the use of external standards and a quantitative technique known as linear regression analysis. In order to identify imidacloprid, the retention time was employed. For measuring the optical density was employed., a spectrophotometer (Cecil/ CE 7200) **(Sabourmoghaddam *et al.*, 2015)**.

Temperature Variation Assay

Temperature Tolerance for *psychrobacter* sp. and *Rhizobium* sp. were investigated by incubating bacterial cultures in MSM medium with 25 ppm imidacloprid at 20 ºC, 24 ºC, 28 ºC, 32 ºC, and 37 ºC and after that measured optical density after 1 and 3 days.

PH Variation Assay

The ability of *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2 (OP672320.1) isolates to grow at different pH was tested separately in MSM broth with 25 ppm imidacloprid by adjusting the pH to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 with NaOH and HCl. Growth of 1×10^7cfu/ml at different pH in optimum temperature was determined by measuring O.D. at 550 nm after 1-4-7 days. Each experiment was performed in triplicates.

Imidacloprid Concentration Variation Assay

The ability of cultures *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1) to grow in different imidacloprid concentrations was tested by adding them concentrations 1×10^7 separately in MSM medium containing 25, 50, 75, 100, 150, and 200 ppm of imidacloprid with incubation at optimum temperature and pH.

Biodegradation of Pesticides in Minimal Salt Medium10^7

Sterilized 50 ml falcon tubes were used in all biodegradation experiments. Each tube received 30 ml of MSM containing 25 mg/L imidacloprid. This MSM functioned as the only source of carbon and nitrogen in a carbon-limited medium. Also used control tubes containing medium and a pesticide (without any bacteria) to prevent environmental effects such as photodegradation. for imidacloprid. 10^7 bacterial cells per ml were cultivated in the falcon tubes for three weeks at optimum temperature in the dark on a rotating shaker set to 120 rpm. For analyses of pesticide residue from imidacloprid taken subsamples from each treatment were taken after 1, 7, 15, and 21 days. A one-half milliliter of each subsample was combined with a one-half milliliter of acetonitrile in two milliliters Eppendorf tubes, and the resulting mixture was centrifuged at 12,000 rpm for five minutes to measure the concentration of each treatment. To store the supernatant used Pasteur pipettes were used to transfer it to the amber HPLC vials and kept in a refrigerator. Each sample was injected into the HPLC at a volume of 50 microliters. And also measure the growth bacteria in each falcon tubes at the same time on 550nm optical density (OD) was recorded. **(Sabourmoghaddam *et al*, 2015)**.

Statistical Analysis

The standard error of mean results for each experiment was calculated after each experiment was carried out in triplicate. It was done using one-way analysis of variance (ANOVA). based on HPLC data indicating variations in the concentration of imidacloprid in treatments. The LSD test ($p< 0.05$) in SPSS 19 was used to assess mean differences. Controls were only utilized to monitor the growth of the isolates; they were not employed in the data analysis. **(Cary., 2012)**.

RESULTS AND DISCUSSION

HPLC calibration

Imidacloprid retention time were 4.934 minutes under experiment conditions (Fig. 1). Working standard solutions of imidacloprid were produced at different dilutions (1, 5, 25, and 50 ppm) and used to calibrate the instrument before any sample analyses were injected in order to determine the sensitivity of the HPLC. The correlation coefficient for imidacloprid was 0.999, indicating a linear relationship between the amount of standard solution injected and the resulting peak area.

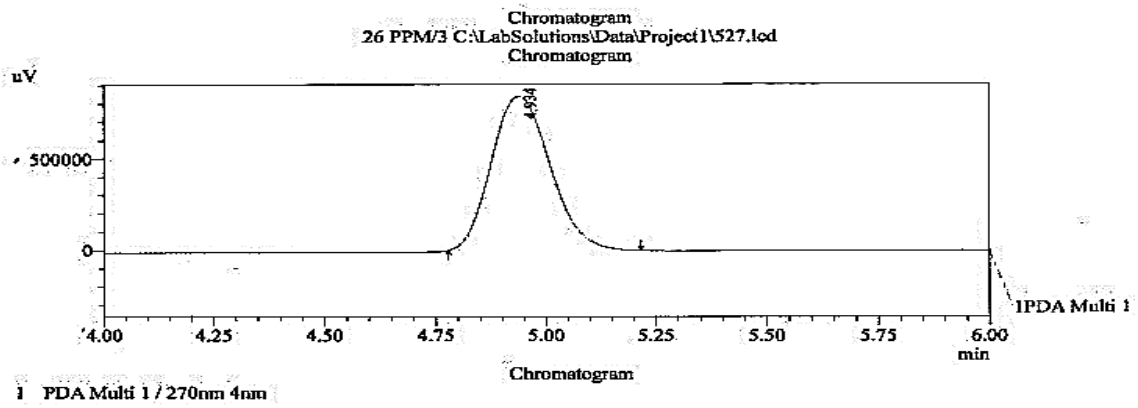

Figure (1): Separation of the imidacloprid (4.934 min) 1 ml per minute flow rate on a C18 column.

Optimum condition for Growth isolates:

Temperature effects

The optimal temperature for growth *Psychrobacter celer* strain IHB2(OP672320.1) was 28°C (Fig.2), while temperatures higher than 32°C reached 37°C reduced growth markedly. The effect of temperature on growth rate was determined for several selected temperatures (Fig. 3), and the temperature for maximal growth rate was also 28°C. while the optimal temperature for growth and maximal growth of *Rhizobium pusense* strain IHB 1 (OP218458.1) was 24°C and reduced markedly growth was determined towards 37°C which according with **(Bipte *et al.*, 2012)** when used optimum temperature for *Rhizobium* sp. to degraded imidacloprid and fipronil.

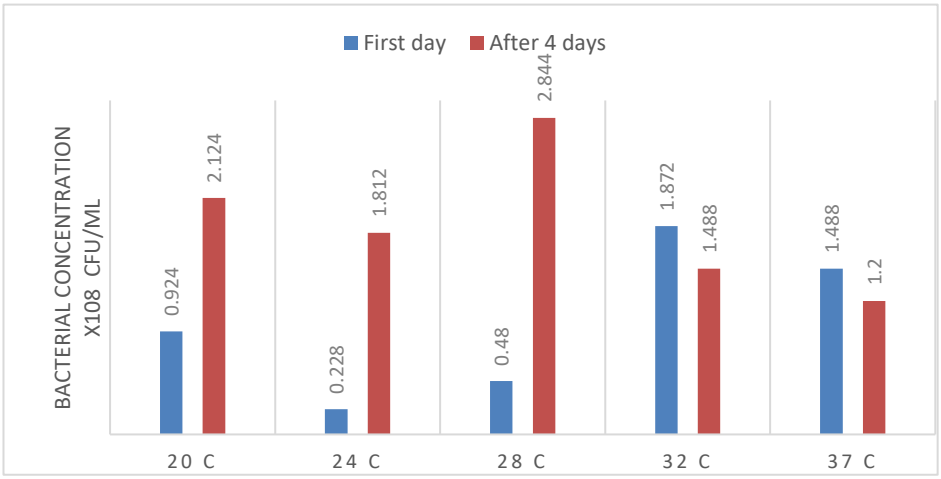

Figure (2): Tolerance of *Psychrobacter celer* strain IHB2(OP672320.1) to Temp.

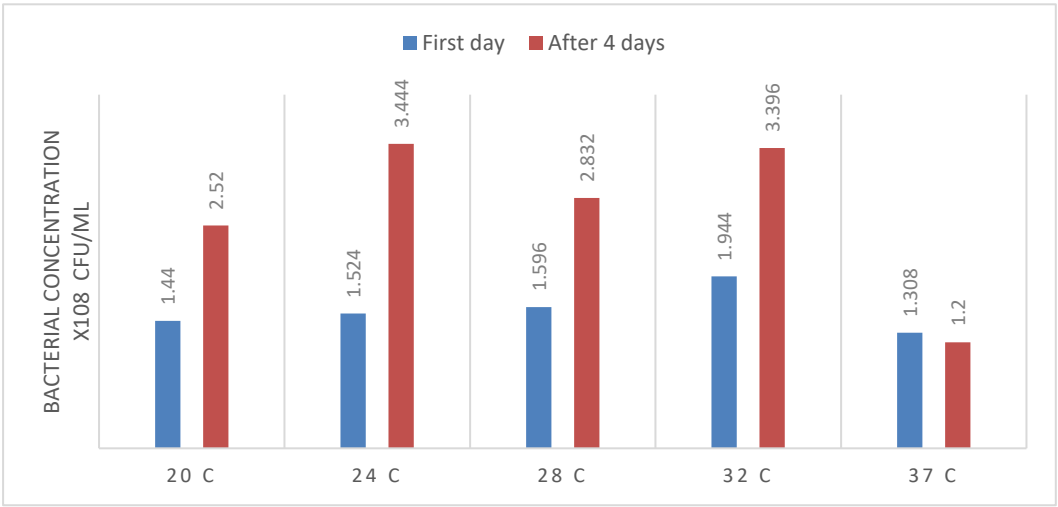

Figure (3): Tolerance of *Rhizobium pusense strain* IHB 1 (OP218458.1) to Temp.

Effect of pH

The optimum pH for *Psychrobacter celer* strain IHB2(OP672320.1) growth was 6 within the range of 4.0-9.0 with limited growth toward acid (pH 4) and normal growth toward neutral and base (pH 7 to pH9) after 7 days (Fig.4). While the optimum pH for *Rhizobium pusense* strain IHB1 (OP218458.1) growth was 7 within the same range of 4.0-9.0 after 7 days of incubation which according with **(Madariaga-Navarrete *et al.*, 2017)** when used optimum pH for bioremediation of Atrazine pesticide (Fig.5).

Figure (4): Tolerance of *Psychrobacter celer* strain IHB2(OP672320.1) to pH.

Figure (5): Tolerance of *Rhizobium pusense strain* IHB 1 (OP218458.1) to pH.

Effects of Imidacloprid Concentration

The optimal concentration of Imidacloprid for degradation by *Psychrobacter celer* strain IHB2(OP672320.1) within the range used of 25-200 ppm was (25 and 50 ppm) in optimum temperature and pH at 21 days of incubation and the degradation becomes lower toward high concentration of imidacloprid (Fig.6), while the result when used the same range concentration of imidacloprid with *Rhizobium pusense* strain IHB 1 (OP218458.1) was 75 ppm within optimal conditions from temperature and pH for it, with continues fewer growth markedly toward high concentration of imidacloprid which indicates tolerance to high concentrations of pesticides, which is compatible with **(Madariaga-Navarrete *et al.*, 2017)** when *Rhizobium* sp exposed. to 10,000 mg L^{-1} from Atrazine and it continues growth lowest which suggests that the bacteria are resistant to the Atrazine herbicide in high concentration (Fig.7).

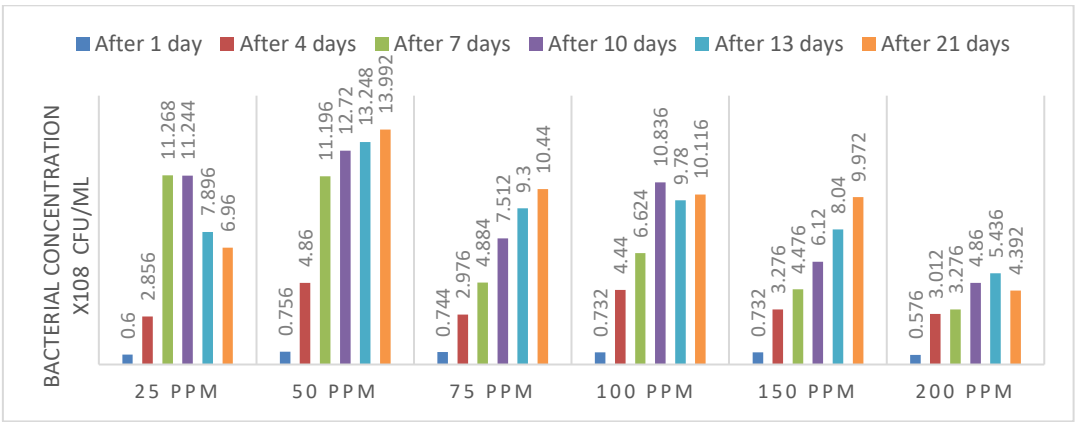

Figure (6): Tolerance of *Psychrobacter celer* strain IHB2(OP672320.1) to Imidacloprid concentrations.

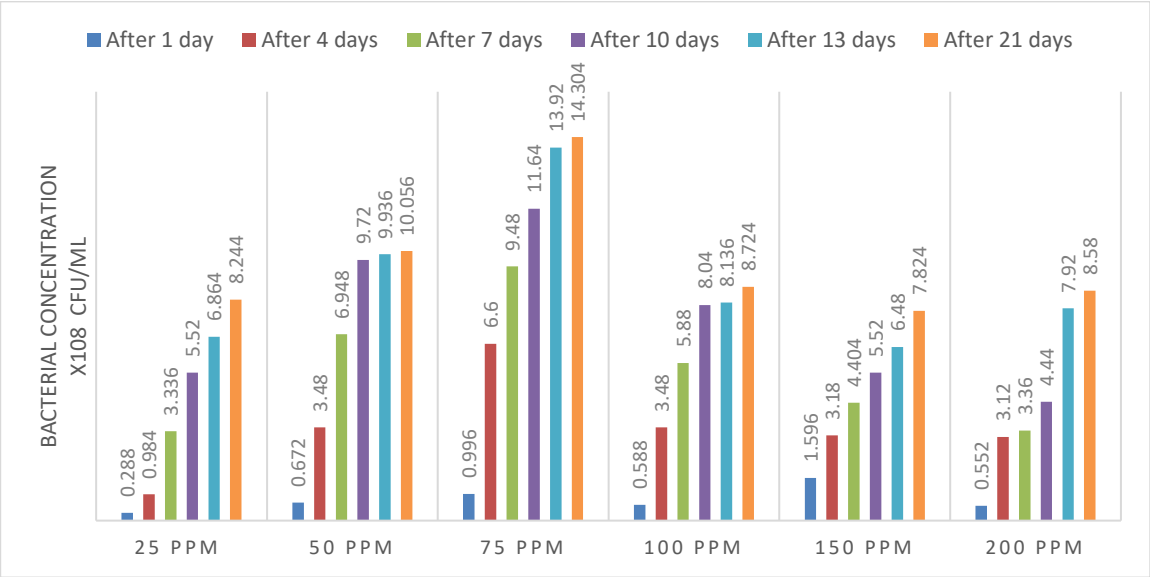

Figure (7): Tolerance of *Rhizobium pusense strain* IHB 1 (OP218458.1) to Imidacloprid concentrations.

Measured Imidacloprid Residues by Degrading Bacteria

Rhizobium pusense strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB 2(OP672320.1) bacterial strains were used to test imidacloprid biodegradation in optimum condition for 21 days and measured residual. A one-way ANOVA was employed to examine variations between the isolates taken from the MSM medium. The One-way analysis of variance (ANOVA) was used to see whether there were any statistically significant differences between the two groups. The daily concentration means (7, 15, and 21) days were shown to be substantially different from one another at a 95% confidence interval. was significant for all groups after days 7, 15, and 21 were also significant for differences between groups. (Table 1). Imidacloprid biodegradation by two isolates was between 39.52% to 50.23% after seven days and 45.07 % to 53.50% on day 15 of incubation, although it increased afterward to 59.01% and 50.20% of the spiking quantity by day 21 of incubation. Statistically, the analysis found a significant relationship between the growth of isolate and days, and, a significant correlation between bacterial population size and degradation ability which according to with study **(Sabourmoghaddam *et al.*, 2015)**.

Table (1): A one-way ANOVA test of the Significance for Days (1,7,15,21).

Days.	Groups	T	Df	Mean Square	F	Sig.
After 1 day	Between Groups	1.046	2	0.523	0.557	0.600
	Within Groups	5.637	6	0.939		
	Total	6.683	8			
After 7 days	Between Groups	137.197	2	68.599	24.864	0.001
	Within Groups	16.554	6	2.759		
	Total	153.751	8			
After 15 days	Between Groups	174.736	2	87.368	51.522	0.000
	Within Groups	10.175	6	1.696		
	Total	184.911	8			
After 21 days	Between Groups	152.716	2	76.358	33.465	0.001
	Within Groups	13.690	6	2.282		
	Total	166.407	8			

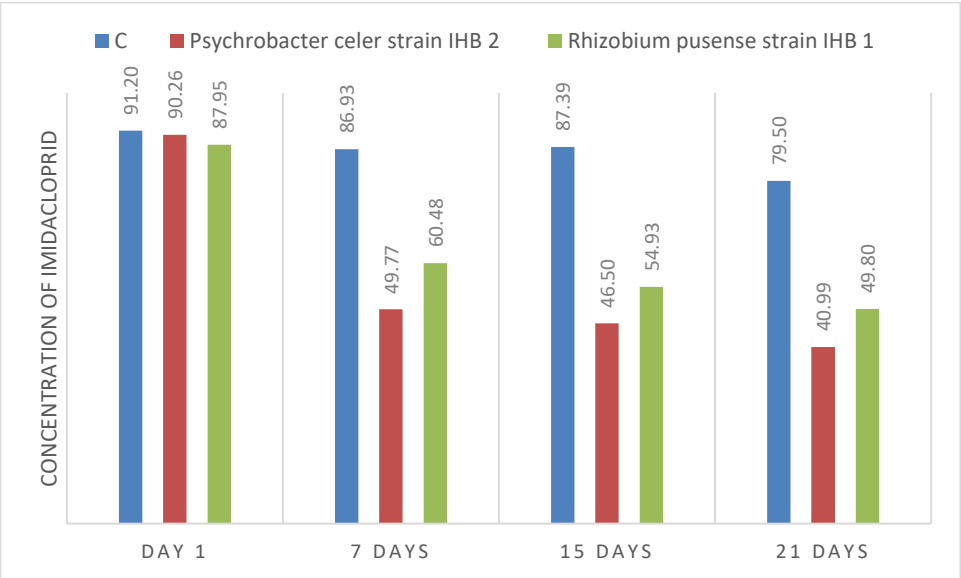

Figure (8): Degradation of Imidacloprid by isolates in 21 days.

CONCLUSIONS

The results of this research demonstrated that imidacloprid might be degraded by *Rhizobium pusense* strain IHB 1 (OP218458.1) and *Psychrobacter celer* strain IHB2(OP672320.1) in optimum conditions. The chosen bacterial isolates from soil were able to degrade between 50.2 and 59.01% of imidacloprid by 21 days. However, isolates bacteria (*Rhizobium and psychrobacter*) introduced in this study were the first locally isolated from a greenhouse in Iraq about the degradation of imidacloprid in MSM media. These bacterial conversions of imidacloprid open up new avenues for its chemical degradation in soil.

REFERENCES

1- Abdel-Ghany, M. F., Hussein, L. A., El Azab, N. F., El-Khatib, A. H., & Linscheid, M. W. (2016). Simultaneous determination of eight neonicotinoid insecticide residues and two primary metabolites in cucumbers and soil by liquid chromatography–tandem mass spectrometry coupled with QuEChERS. *Journal of Chromatography* B, 1031, 15-28.

2- Ali, I. H., Musa, T. N. and Ali, A. J. (2018). Effect of acetamiprid residues in tomato fruits on some blood profile parameters of male mice. *The Iraqi Journal of Agricultural Science*, 49(6),1110-1117.

3- Al-Soufi, M. A., Muhyaddin, M. O., & Al-Musawi, A. T. (2015). Determination of some Optimum Conditions for Bioremediation of Some Heavy Metals by Saccharomyces cerevisiae. *Iraq journal of market research and consumer protection*, *7*(2),271-290.

4- Awad, B. M., Musa, T. N., & Ali, A. J. (2018). Determination of deltamethrin residues in mandarin orange and studying its physiological effects on male mice blood profile. *The Iraqi Journal of Agricultural Science*, 49(6), 1160-1167.

5- Bipte, S. R., Parmar, P. S., & Musaddiq, M. (2012). Isolation and identification of pesticide resistant bacteria from agricultural soil. *Nature, Environment and Pollution Technology*, *11*(1), 139-140.

6- Cary, N. (2012). *Statistical analysis system, User's guide. Statistical.* Version 9. SAS. Inst. Inc. USA.

7- Chen, Y., Zang, L., Liu, M., Zhang, C., Shen, G., Du, W., ... & Zhao, M. (2019). Ecological risk assessment of the increasing use of the neonicotinoid insecticides along the east coast of China. *Environment international*, 127, 550-557.

8- Dhari, S. F., & Hetite, W. A. (2019). Expression of Dampen in *Pseudomonas putida* for degradation of phenol in soil. *Iraqi Journal of Agricultural Sciences*, 50,54-63.

9- Garg, N., Bhattacherjee, A. K., Shukla, P. K., & Singh, B. (2021). Influence of imidacloprid on bacterial community diversity of mango orchard soil assessed through 16S rRNA sequencing-based metagenomic analysis. *Environmental Monitoring and Assessment*, 193, 1-10.

10- Hatit, W. A., Ghaima, K. K., Ali, S. A., & Mohammed, M. M. (2013). Study the Growth Kinetics of *Pseudomonas aeruginosa* Degrading Some Pesticides Which Isolated from Cultivated Soll. *Iraq journal of market research and consumer protection*, 5(1),157-167.

11- Humann-Guilleminot, S., Clément, S., Desprat, J., Binkowski, Ł. J., Glauser, G., & Helfenstein, F. (2019). A large-scale survey of house sparrows feathers reveals ubiquitous presence of neonicotinoids in farmlands. *Science of the Total Environment*, 660, 1091-1097.

12- Jeschke, P. & Nauen, R. (2005) *Neonicotinoid insecticides. In: Gilbert L, Iatrou K, Gill SS (eds)Comprehensive molecular insect science*, vol 5. Elsevier Pergamon, Oxford, 52–105.

13- Kridi, N., Al-Shater, M. S., & Al Zoubi, M. M. (2021). Isolation and identification of some bacterial isolates from soil contaminated with crude oil and Testing Their Effectiveness. *Baghdad Science Journal*, 18(4), 1476-1484.

14- Kulkarni, K., Chawan, A., Kulkarni, A., & Gharat, S. (2022). Bioremediation of imidacloprid using Azospirillium biofertilizer and Rhizobium biofertilizer. *Korean Journal of Chemical Engineering*, 39(10), 2702-2712.

15- Liu, Z., Dai, Y., Huan, Y., Liu, Z., Sun, L., Zhou, Q., ... & Yuan, S. (2013). Different utilizable substrates have different effects on cometabolic fate of imidacloprid in Stenotrophomonas maltophilia. *Applied microbiology and biotechnology*, 97, 6537-6547.

16- Madariaga-Navarrete, A., Rodríguez-Pastrana, B. R., Villagómez-Ibarra, J. R., Acevedo-Sandoval, O. A., Perry, G., & Islas-Pelcastre, M. (2017). Bioremediation model for atrazine contaminated agricultural soils using phytoremediation (using Phaseolus vulgaris L.) and a locally adapted microbial consortium. *Journal of Environmental Science and Health, Part B*, *52*(6), 367-375.

17- Miccoli, S., Finucci, F., & Murro, R. (2016). Feeding the cities through urban agriculture the community esteem value. *Agriculture and Agricultural Science Procedia*, 8, 128-134.

18- Nafal, D. H., & Abdulhay, H. S. (2020). Bioremediation of petroleum polluted soils using consortium bacteria. *Iraqi Journal of Science*, 61(5), 961-969.

19- Othman, B. A., & Kakey, E. S. (2021). Pesticides bioaccumulation and their soil pollutant effect. *Iraqi Journal of Agricultural Sciences*, 52(1), 36-47.

20- Sabourmoghaddam, N., Zakaria, M. P., & Omar, D. (2015). Evidence for the microbial degradation of imidacloprid in soils of Cameron Highlands. *Journal of the Saudi Society of Agricultural Sciences*, 14(2), 182-188.

21- Saqer, B. T., Al-Aubadi, I. M. & Ali, A. J. (2018). Determination of imidacloprid pesticide residues in cucumber and effect of some food preparation methods in reducing residues. *Int. J. Agricult. Stat. Sci*. 14(1), 311-318.

22- Schaafsma, A., Limay-Rios, V., Xue, Y., Smith, J., & Baute, T. (2016). Field-scale examination of neonicotinoid insecticide persistence in soil as a result of seed treatment use in commercial maize (corn) fields in southwestern Ontario. *Environmental Toxicology and Chemistry*, 35(2), 295-302.

23- Strobl, V., Albrecht, M., Villamar-Bouza, L., Tosi, S., Neumann, P., & Straub, L. (2021). The neonicotinoid thiamethoxam impairs male fertility in solitary bees, Osmia cornuta. *Environmental pollution*, 284, 117106

24- Zhang, Q., Lu, Z., Chang, C. H., Yu, C., Wang, X., & Lu, C. (2019). Dietary risk of neonicotinoid insecticides through fruit and vegetable consumption in school-age children. *Environment international*, 126, 672-681.

25- Zhao, H., Zhu, J., Liu, S., & Zhou, X. (2018). Kinetics study of nicosulfuron degradation by a *Pseudomonas nitroreducens* strain NSA02. *Biodegradation*, 29, 271-283.

26- Zhu, Y. C., Yao, J., & Adamczyk, J. (2019). Long-term risk assessment on noneffective and effective toxic doses of imidacloprid to honeybee workers. *Journal of Applied Entomology*, 143(1-2), 118-128.