https://doi.org/10.48047/AFJBS.6.Si2.2024.5286- 5301

Optimization Of Isolated Strain *Pseudomonas Stutzeri* (HR-115) Using Response Surface Methodology For Monocrotophos Degradation.

Rohit, Sweeta soni, Shruti Dudeja, Hemlata Badgujar, Mohit Kumar, Swati Jaast, Anil Kumar

Department of Bio and Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar-125001, Haryana, India.

Article History
Volume6,IssueSi2,2024
Received:18Apr2024
Accepted:20Jun2024
doi:10.48047/AFJBS.6.Si2.2024.5286-5301

Abstract

The indiscriminate use of various pesticides causes contamination of different biological systems and accumulates in food chains and crops, thereby resulting in several health hazards for humans. Monocrotophos is a hazardous pesticide used globally, and thus its biodegradation is needed at this time. In this study, HR-115 (bacterial strain) was evaluated for its monocrotophos degradation potential using high-pressure liquid chromatography (HPLC). Five parameters inoculum size, pH, rpm, time, and temperature were optimized using response surface methodology to enhance the strain's ability to degrade the pesticide in the minimum incubation period. The model was found to be fit with an R^2 of 0.97, showing non-significant lack of fit and P-values less than 0.0500. The potent degrader HR-115 was further employed for Sanger sequencing and identified as *Pseudomonas stutzeri* (MRCJ-1020). HPLC results revealed a pesticide degradation potential of 64.89% after 72 hours of incubation.

Keywords : Organophosphate, Monocrotophos, MCP, RSM, HPLC.

Introduction

India is an agrarian economy where the majority of the population relies on agriculture for their livelihood. Agriculture contributes significantly to India's GDP and is an essential sector for the country's economic growth. India has a total land area of 328.7 million hectares, out of which 158.4 million hectares (48.2%) are agricultural land (Ministry of Agriculture and Farmers Welfare, 2021). Several factors, such as weeds, pests, rodents, and diseases, significantly impact agricultural produce. To prevent significant losses, the extensive use of

pesticides becomes unavoidable. Pesticides protect crop from reduction of product quality, potential yield losses, and to minimize infestations by pests, therefore broadly used in many areas of crop production. Due to excessive concern and a lack of precise knowledge, the indiscriminate use of pesticides has resulted in both short- and long-term health hazards. These pesticides interact with the biological systems in the environment due to their widespread use over the years; hence give rise to numerous problems related to environment pollution (Despotovic *et al*., 2019).

Current pesticide consumption patterns with various groups of pesticides (organophosphorous, organochlorine, carbamate, pyrethroids, pyrethrins, and neonicotinoids) can be seen all over the world out of which organophosphorous group accounts 36%. Organophosphates used as warfare agents for protecting crops, livestock, human health during last 60 years and are divided into around thirteen categories on the basis of structural characteristics, including phosphonates, phosphorothioates phosphates, etc. (Zhang *et al*., 2011). The adverse short-term effects of organophosphates exposure have been studied mostly in the nervous system (Gupta *et al*., 2006). In India, malathion, qinalphos, monocrotophos, chlorpyrifos, methyl parathion, and parathion are used, among these, monocrotophos is widely used but less studied. Monocrotophos is produced throughout the world and has been registered for use on many crops. Monocrotophos is relatively immobile and adsorbs to the soil and has been detected in food, sediments, surface water, aquatic organisms, rainwater, and atmospheric samples. The heavy pesticide usage would have an enhanced risk of exposure to huge number of peoples living within or nearby areas through skin contact with contaminated plants.

Monocrotophos is a compound that appears colourless when in its pure form, but takes on a reddish-brown hue in its technical grade form. The Environmental Protection Agency has classified it as highly toxic, with a median lethal dose of 18-20 mg for mammals and 0.9-6.5 mg for birds. Although it is easily soluble in water and has 100% solubility, its hydrophilic nature means that it is poorly absorbed by soil granules, making it a potential threat to groundwater contamination via leaching. Its half-life can vary from 17-96 days depending on the pH and temperature (Kaur and Goyal., 2019).

Pesticide concentration in soil, water and food chains has been increased due to its continuous application. Synthetic non-degradable pesticides even present in minute quantities, leave residues for prolonged periods in various living systems and show adverse effect on ecological systems (Sivaperumal and Sankar 2008). Pesticides cause risks to human health as well as the environment, as they target systems or enzymes in different pests which

usually similar to enzymes in human beings (Children's Health and the Environment, 2008). The level of environmental risk for the inhabitants of nearby villages and occupationally exposed population has been increased as a result of dispersion mechanism (Kalipci *et. al.*, 2010). Because of bioaccumulation in non-target organisms and rapid fat solubility pesticides cause serious health risks to living beings (Xue *et al.*, 1988).

For the removal of organophosphorous pesticides in soil, there are different possible mechanisms for example, chemical treatment, volatilization and incineration. Besides their feasibility volatilization and chemical treatment, are risky as huge volume of alkalis and acids are generated, which need to be disposed off. Incineration, although, a reliable physico-chemical method for degradation of these compounds, cannot be used because of its potential harmful emissions, serious public opposition, and its high financial burden (Kearney, 1998; Zhang and Quiao, 2002). As result of current environmental concerns associated with the aggregation of pesticides in food products and water supplies, there is a great demand to develop economically feasible, safe and convenient methods to obtain energy, carbon, or nitrogen from the pesticide molecules (Zhang and Quiao, 2002). Biodegradation is a cost-effective, environment-friendly and minimally hazardous (Finley *et al.,* 2010).

Material and Methods

Chemicals and Media

Analytical grade monocrotophos (99% purity), was purchased from Sigma Aldrich. The commercial formulation was dissolved in sterile distilled water to achieve the desired concentrations for addition to soils and media. Himedia- HPLC grade Acetonitrile, Methanol and water was used. All reagents used in this study were of high purity and analytical grade. Minimal salt media containing g/l of 1.5g KH_2PO_4, 0.5g K_2HPO_4, and 1g $(NH4)_2SO_4$, 0.2 $MgSO_4$, 0.02g $FeSO_4$ and 0.5g NaCl with pH 7.2 amended with monocrotophos analytical grade as a carbon source. Nutrient agar and nutrient broth are used for bacteria culturing.

Enrichment and Isolation of Monocrotophos degrading strains

Soil samples were collected from agricultural fields in Haryana, India, where there has been prolonged exposure to monocrotophos. A 20 g portion of soil sample was thoroughly mixed with monocrotophos and maintained at 37 ± 2°C for 5-7 days. Following the incubation period, the soil samples were serially diluted, and these soil samples were spread onto MSM agar plates containing monocrotophos. Afterward, the plates were placed in an incubator at 37°C for 5 to 7 days. Subsequently, unique colonies were identified, isolated, and conserved on identical plates and MSM agar slants for further examination and use.

Degradation of Monocrotophos

All bacterial strains obtained from soil samples underwent screening for their tolerance to monocrotophos using a plate assay method. MSM agar plates with varying concentrations (ranging from 100 to 500 ppm) of monocrotophos were employed for this purpose. Among the bacterial strains tested, designated as HR-115 was demonstrating the highest tolerance to monocrotophos were selected for further experimentation as individual pure bacterial inoculants. The investigation into pesticide degradation was conducted using sterile minimal salt broth containing: 0.5 g KH_2PO_4, 0.2 g $MgSO_4$, 1.5 g NH_4NO_3, 0.5 g NaCl, 1.5 g K_2HPO_4, per litre and having pH 7.0 (Srinivasulu *et al*., 2017). The MSM broth was supplemented with filter-sterilized monocrotophos as a carbon source, added at a concentration of 500 ppm.

Analytical instrumentation and monitoring of percentage degradation

High performance liquid chromatography was carried out for confirmation of monocrotophos degradation. To prepare samples, the MSM cultures were centrifuged at 9000 rpm for 10 minutes at 10 °C using a refrigerated centrifuge. The resulting cell-free supernatant was utilized for estimating residual monocrotophos content, which was filtered using 0.2 µm pore size syringe filter for subsequent analysis. HPLC analysis utilized a Reverse phase C18 column with dimensions of 4.6×250 mm and a particle size of 5 µm. The mobile phase, operated in isocratic mode, comprised a blend of acetonitrile and high-performance liquid chromatography (HPLC) grade water in an 80:20 ratio. The sample was injected at a flow rate of 1 ml/min; with a volume of 20 µl. Monitoring degradation was achieved using a UV detector scanning within the range of 214 nm (Abraham *et al*., 2014).

Molecular identification

Bacterial strains with the ability to degrade monocrotophos were identified through a process involving morphological characteristics, followed by molecular identification using 16s rRNA gene sequencing conducted by Macrogen Korea. The resulting sequences were then submitted to the NCBI gene bank.

Response surface methodology for optimization

Optimization using response surface methodology (RSM) which comprises regression analysis and factorial design, aids in measuring the significant parameters, in order to study the relations among the factors as well as choosing the optimal conditions of factors or appropriate responses (Ibrahim et al., 2015). In this study, optimization of monocrotophos degrading strain were conducted by significant variables obtain were optimized further using Box Behnken Design(BBD) model with 45 experiments(Table1)indicate the estimation of

monocrotophos degradation percentage termed as response(R).Independent variables X1 (pH 5-9) X2 (Time 24-72 hrs.), X3 (30˚- 45˚) X4 (RPM 60-120) and X5 (1-3 ml per ml contained 3×10^8).The percentage of monocrotophos degradation was taken as the response (Rajendran *et al.,* 2014).Analysis of variance(ANOVA)plot of residuals will describe the success of designed model and 3D colour contour plots of fitted surfaces indicate the effect of variables in combined and individual form (Beniwal V*et al.,* 2015).

Table 1: Different sets of Experimental designs with their Response.

		Factor 1	Factor2	Factor 3	Factor 4	Factor 5	Response 1
Std	Run	pH	Time	Temperature	RPM	Inoculum Size	Degradation
							%
30	1	7	48	45	90	1	46.22
5	2	7	48	30	60	2	44.93
24	3	7	72	45	90	2	59.22
45	4	7	48	37.5	90	2	52.82
21	5	7	24	30	90	2	21.22
35	6	5	48	37.5	90	3	43.69
29	7	7	48	30	90	1	46.99
31	8	7	48	30	90	3	50.26
16	9	9	48	45	90	2	47.04
14	10	9	48	30	90	2	47.84
36	11	9	48	37.5	90	3	53.27
38	12	7	72	37.5	60	2	62.99
25	13	5	48	37.5	60	2	40.77
6	14	7	48	45	60	2	46.01
37	15	7	24	37.5	60	2	26.91
43	16	7	48	37.5	90	2	51.91
34	17	9	48	37.5	90	1	48.4
41	18	7	48	37.5	90	2	50.99
15	19	5	48	45	90	2	44.46

42	20	7	48	37.5	90	2	53.32
4	21	9	72	37.5	90	2	61.07
28	22	9	48	37.5	120	2	49.06
39	23	7	24	37.5	120	2	28.38
44	24	7	48	37.5	90	2	51.01
33	25	5	48	37.5	90	1	41.09
11	26	7	24	37.5	90	3	30.44
22	27	7	72	30	90	2	58.99
19	28	7	48	37.5	60	3	47.29
7	29	7	48	30	120	2	48.83
23	30	7	24	45	90	2	23.39
17	31	7	48	37.5	60	1	45.62
10	32	7	72	37.5	90	1	63.91
2	33	9	24	37.5	90	2	29.14
20	34	7	48	37.5	120	3	54.84
8	35	7	48	45	120	2	48.85
18	36	7	48	37.5	120	1	49.48
27	37	5	48	37.5	120	2	44.29
3	38	5	72	37.5	90	2	57.2
26	39	9	48	37.5	60	2	46.7
40	40	7	72	37.5	120	2	62.28
9	41	7	24	37.5	90	1	29.82
32	42	7	48	45	90	3	49.81
12	43	7	72	37.5	90	3	64.89
13	44	5	48	30	90	2	43.99
1	45	5	24	37.5	90	2	22.27

Results

Enrichment and Isolation of Monocrotophos Degrading Soil Bacteria

A total of 15bacterial strains were obtained after isolated by enrichment of soil samples utilizing monocrotophos as energy and carbon source. The most potent bacterial isolate namely HR-115 was found to be capable of utilizing monocrotophos up to the concentration of 500 ppm. After 72 h of incubation at culture condition of 37.5˚ C temperature, pH 7 and 90 RPM, up to 64.89%, degradation was achieved by this bacterial isolate.

Identification of Isolates

Molecular identification of isolated culture was done using 16s rRNA gene sequencing from Macrogen Korea. Sequencing results revealed that the culture labelled HR-115 identified as *Pseudomonas stutzeri*, and was submitted at the Microbial Resource Center, Jammu with accession no (MRCJ1020). The sequences of the 16s rRNA gene for bacterial strains are depicted in Figure 1. given below. *Pseudomonas* is a genus of Gram-negative, aerobic gamma proteo bacteria in the family Pseudomonadaceae, comprising 191 validly described species. Members of this genus exhibit significant metabolic diversity, allowing them to colonize a wide range of environments. *Pseudomonas stutzeri*, a Gram-negative bacterium, is particularly noted for its versatile metabolism. Individual *P. stutzeri* cells are rod-shaped, possess a single polar flagellum and opportunistic pathogenic.

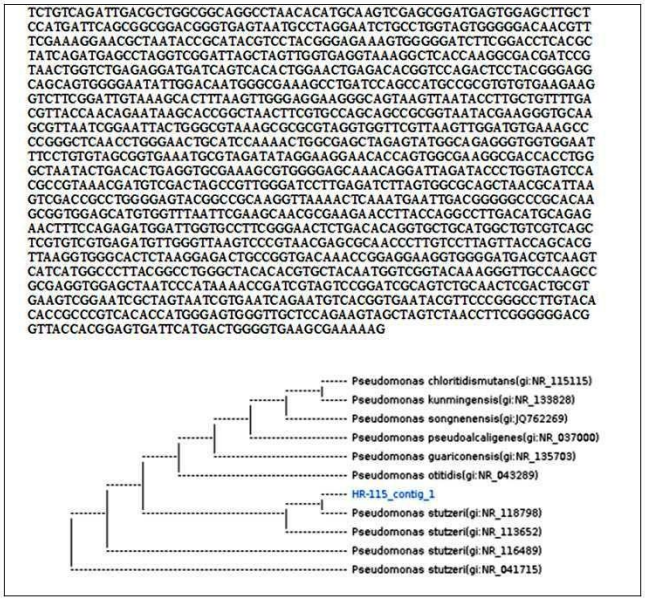

Figure 1: Sequences of the 16s rRNA gene for bacterial strain HR-115.

Degradation of Monocrotophos analysed using HPLC

HPLC was employed to validate the degradation study of monocrotophos. Six different concentrations of analytical-grade Sigma-Aldrich were analyzed using HPLC with a DAD detector to check the response. The standard calibration curve for the analyzed monocrotophos pesticide showed a strong regression within the explored concentration range of 100 ppm to 500 ppm (Figure 2a). The analytical peak was very clear, with a retention time fixed at around 4.5 minutes (Figure 2b). *Pseudomonas stutzeri* (HR-115) found able to degrade monocrotophos maximum up to 64.89 % at end of 72h, degrade maximum 54.84% and minimum 40.77% in 48 h, and degrade maximum 30.44% and minimum 21.22 % in 24 h (Table 1). Degradation shown by strain *Pseudomonas stutzeri* (HR-115) at optimizes the physical parameters X1 (pH), X2(Time), X3(Temperature), X4(RPM) and X5(Inoculum size) using BBD model of design expert software.

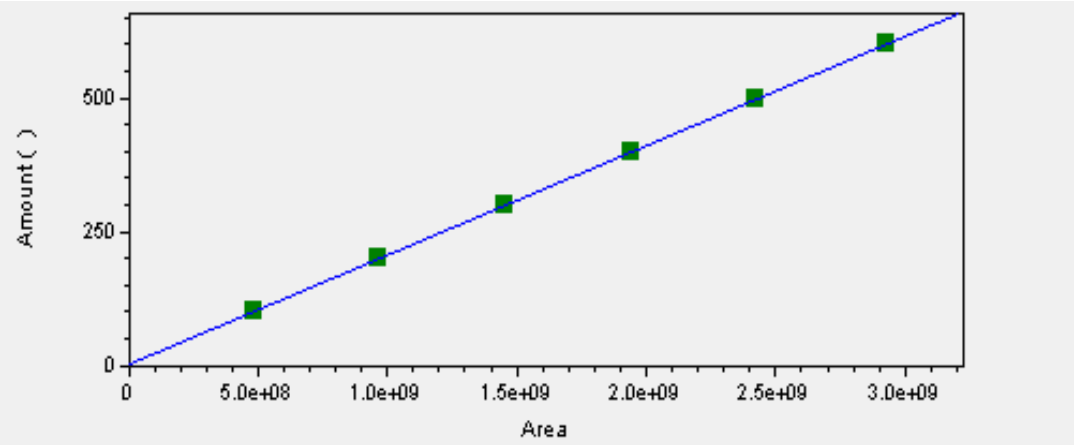

Figure 2 (a): Standard calibration curve at concentrations 100-600ppm.

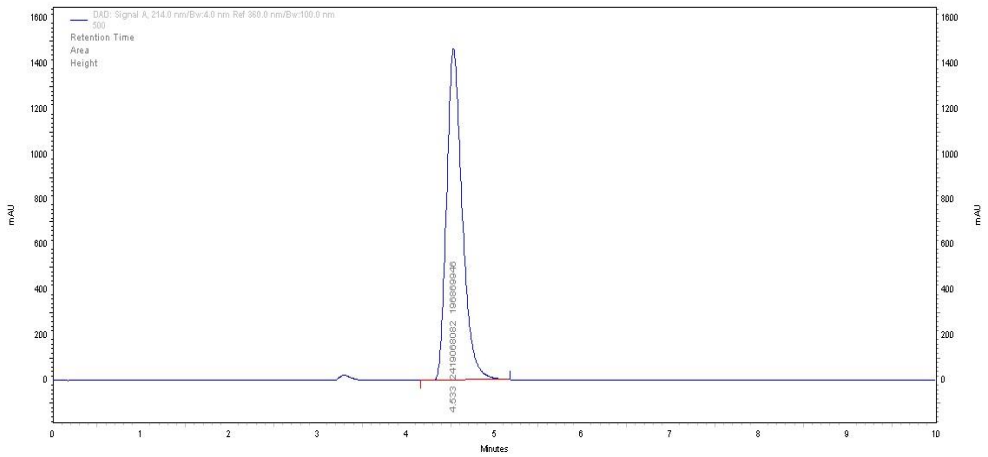

Figure 2 (b): Represents chromatogram of standard monocrotophos at 500ppm concentration.

Response surface methodology

Box Behnken Design is aquadratic rotatable model its function gives estimation of percentage degradation 3D coloured plots of fitted surfaces, test of lack of fit ANOVA and plots of residuals. Effective degradation could be achieved by providing optimum condition to cultures. The Model F-value of 72.91 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, D, E, A^2, B^2, C^2, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 3.85 implies there is a 9.96% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad we want the model to fit. This relatively low probability (<10%) is troubling (shown in Table 2).Maximum degradation achieved under the condition of (RPM 90), (pH 7), (temperature 37.5˚C) and (inoculum size of 3 ml per ml contained 3×10^8) around 64.89%within 72 hours of incubation period (Table1). Pino *et al.*, 2011 described adaptation and acclimatization with environmental conditions explains its effective degradation.

Table 2: ANOVA result sheet for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5445.62	20	272.28	72.91	< 0.0001	significant
A-pH	125.22	1	125.22	33.53	< 0.0001	
B-Time	4864.37	1	4864.37	1302.60	< 0.0001	
C-Temperature	0.2377	1	0.2377	0.0636	0.8030	
D-RPM	38.41	1	38.41	10.29	0.0038	
E-Inoculum Size	32.95	1	32.95	8.82	0.0067	
AB	2.25	1	2.25	0.6025	0.4452	
AC	0.4032	1	0.4032	0.1080	0.7453	
AD	0.3364	1	0.3364	0.0901	0.7667	
AE	1.29	1	1.29	0.3450	0.5625	
BC	0.9409	1	0.9409	0.2520	0.6203	
BD	1.19	1	1.19	0.3182	0.5780	
BE	0.0324	1	0.0324	0.0087	0.9266	

CD	0.2809	1	0.2809	0.0752	0.7862	
CE	0.0256	1	0.0256	0.0069	0.9347	
DE	3.40	1	3.40	0.9115	0.3492	
A^2	139.79	1	139.79	37.43	< 0.0001	
B^2	260.49	1	260.49	69.76	< 0.0001	
C^2	99.53	1	99.53	26.65	< 0.0001	
D^2	29.35	1	29.35	7.86	0.0098	
E^2	0.9051	1	0.9051	0.2424	0.6270	
Residual	89.62	24	3.73			
Lack of Fit	85.20	20	4.26	3.85	0.0996	not significant
Pure Error	4.42	4	1.11			
Cor Total	5535.25	44				

The Predicted R^2 of 0.9372 is in reasonable agreement with the Adjusted R^2 of 0.9703; i.e. the difference is less than 0.2. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 33.146 indicates an adequate signal. This model can be used to navigate the design space. coloured graph Figure3 (a,b,c,d,e,f,g,h,i,j) each 3D graph and 2D contour plot represented two independent variables against R (percentage of degradation).

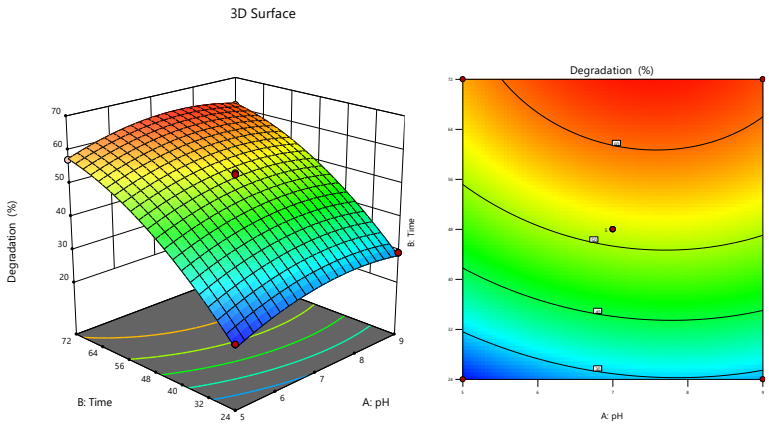

Figure3 (a): Model plot displaying 3D and 2D contour plotting for the effect of pH and time on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

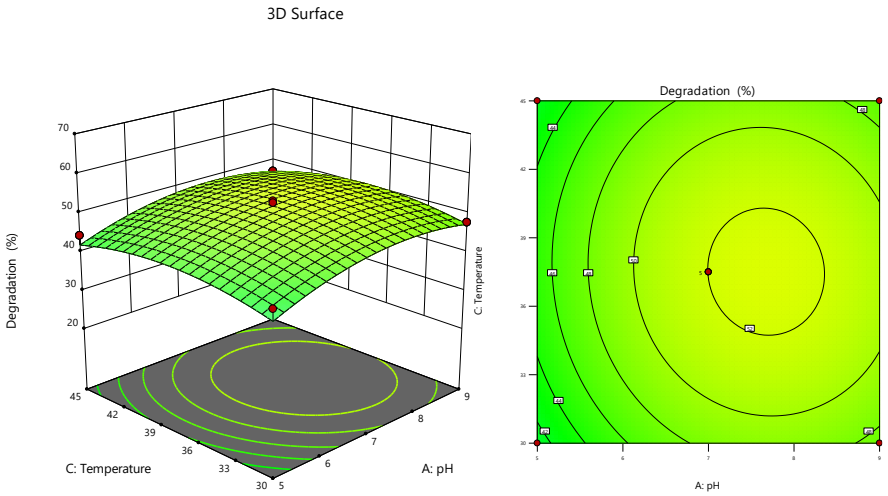

Figure 3 (b): Model plot displaying 3D and 2D contour plotting for the effect of pH and temperature on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

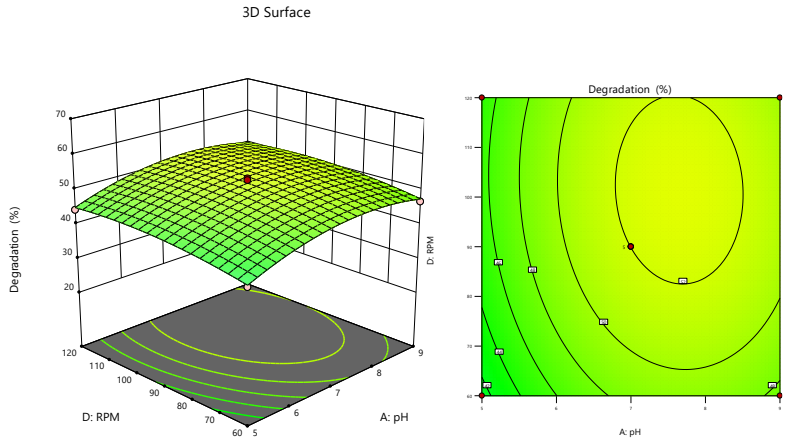

Figure 3 (c): Model plot displaying 3D and 2D contour plotting for the effect of pH and RPM on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115)

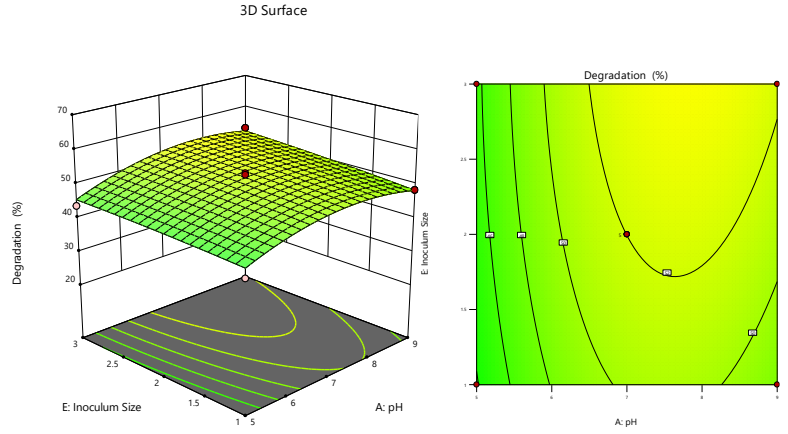

Figure 3(d): Model plot displaying 3D and 2D contour plotting for the effect of pH and Inoculum size on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

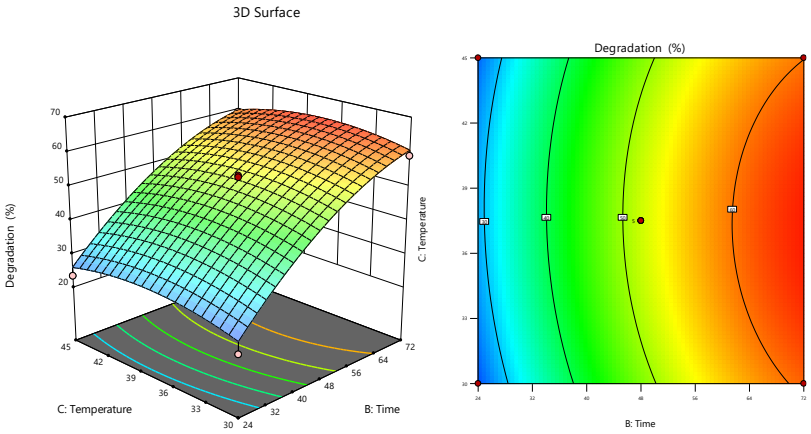

Figure 3(e): Model plot displaying 3D and 2D contour plotting for the effect of time and temperature on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

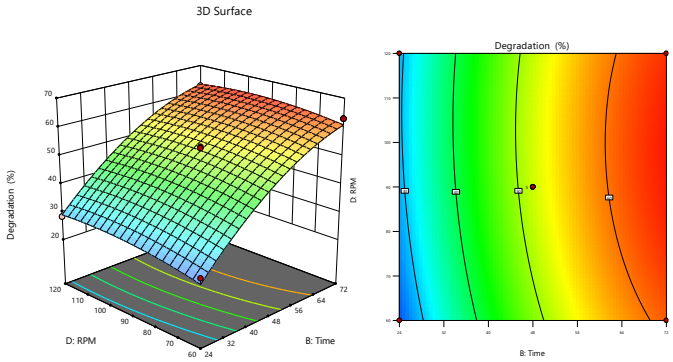

Figure 3(f): Model plot displaying 3D and 2D contour plotting for the effect of time and RPM on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

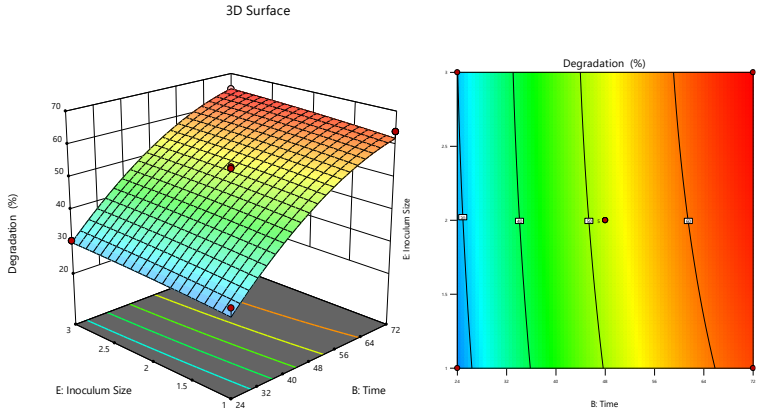

Figure 3(g): Model plot displaying 3D and 2D contour plotting for the effect of time and inoculum size on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

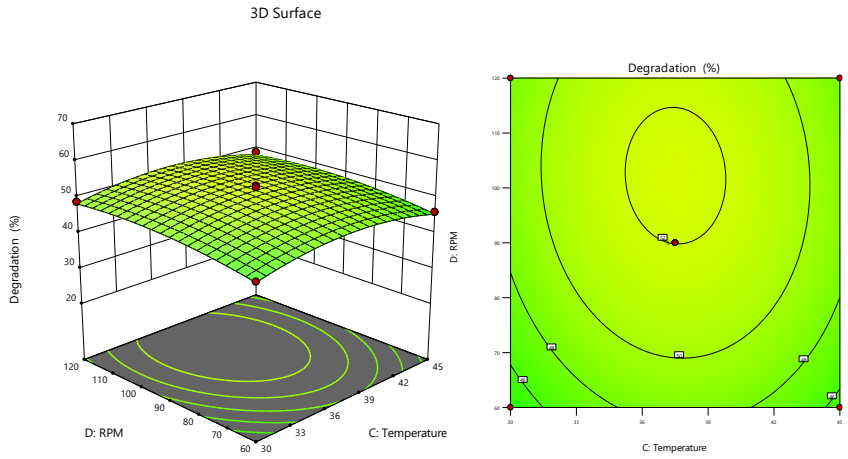

Figure 3(h): Model plot displaying 3D and 2D contour plotting for the effect of temperature and RPM on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

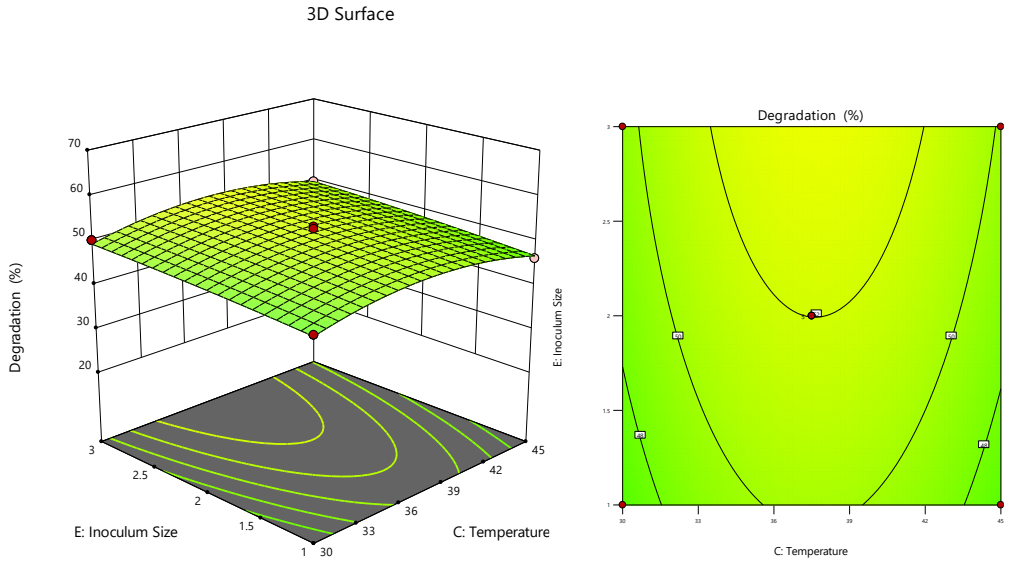

Figure 3(i): Model plot displaying 3D and 2D contour plotting for the effect of temperature and inoculum size on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

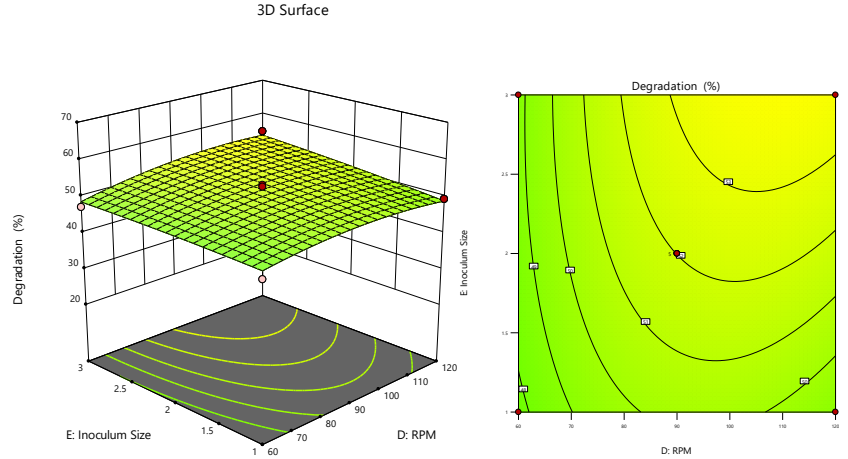

Figure 3(j): Model plot displaying 3D and 2D contour plotting for the effect of RPM andinoculum size on the degradation of monocrotophos by *Pseudomonas stutzeri* (HR-115).

Conclusion

In current study, a highly efficient monocrotophos-degrading *P. stutzeri* sp. strain (MRCJ-1020) was isolated from agricultural soil using an enrichment method. The response surface methodology, a powerful optimization method was applied to assess the effects of five variables: pH, temperature, RPM, time, and inoculum size, as well as their interactions on monocrotophos degradation. *P. stutzeri* (HR-115) was found capable of degrading monocrotophos up to a maximum of 64.89% after 72 hours and a minimum of 21.11% after 24 hours at a concentration of 500 ppm, within a temperature range of 30-45°C, and pH range of 5-9. The similarity between predicted and experimental results confirms the applicability and validity of the RSM-BBD model in the optimization process. The results indicate that statistical optimization methods are effective in predicting the biodegradation activity of pesticides. The highest degradation, approximately 64.89%, was achieved under the following conditions: 90 RPM, pH 7, temperature of 37.5˚C, and an inoculum size of 3 ml per ml containing 3× 10^8 cells/ml, during a 72-hour incubation period. The isolated *P. stutzeri* sp. strain (MRCJ-1020) holds potential for substantial applications in monocrotophos bioremediation and in the remediation of other pesticide-polluted soils.

References

- Abraham, J., Silambarasan, S., &Logeswari, P. (2014). Simultaneous degradation of organophosphorus and organochlorine pesticides by bacterial consortium. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(5), 2590-2596.
- Beniwal, V., Sharma, A., Marwah, S., & Goel, G. (2015). Use of chickpea (Cicer arietinum L.) milling agrowaste for the production of tannase using co-cultures of Aspergillus awamori MTCC 9299 and Aspergillus heteromorphus MTCC 8818. *Annals of microbiology*, *65*, 1277-1286.
- Das, S. DK (2003) Utilization of monocrotophos as phosphorus source by Pseudomonas aeruginosa F10B and Clavibactermichiganense subsp. insidiosum SBL 11. *Can J Microbiol*, *49*(2), 101-109.

- Despotović, J., Rodić, V., & Caracciolo, F. (2019). Factors affecting farmers' adoption of integrated pest management in Serbia: An application of the theory of planned behavior. *Journal of Cleaner Production*, *228*, 1196-1205.
- Finley, S.D.; Broadbelt, L.J. &Hatzimanikatis, V. (2010). In Silico Feasibility of Novel Biodegradation Pathways for 1,2,4-Trichlorobenzene, BMC Systems Biology, Vol.4, No.7, (February 2010), pp.4-14, ISSN 1752-0509.
- Gupta, V. K., Ali, I., &Saini, V. K. (2006). Adsorption of 2, 4-D and carbofuran pesticides using fertilizer and steel industry wastes. *Journal of Colloid and Interface Science*, *299*(2), 556-563.
- Ibrahim, S., Shukor, M. Y., Khalil, K. A., Halmi, M. I. E., Syed, M. A., & Ahmad, S. A. (2015). Application of response surface methodology for optimising caffeine-degrading parameters by sp. strain SIU Leifsonia. *Journal of environmental biology*, *36*, 1215-1221.
- Kalipci Erkan, ÖzdemirCelalettin, ÖztasFulya and SahinkayaSerkan, 2010. Ecotoxicological effects of Methyl parathion on living things and environment. African Journal of Agricultural Research Vol. 5(8), pp. 712-718,
- Kearney, M. (1998). Transnationalism in California and Mexico at the end of empire. *Border Identities: Nation and state at international frontiers*, 117-141.
- Kaur, R., &Goyal, D. (2019). Toxicity and degradation of the insecticide monocrotophos. *Environmental Chemistry Letters*, *17*, 1299-1324.
- Lappalainen, R., Kearney, J., &Gibney, M. (1998). A pan EU survey of consumer attitudes to food, nutrition and health: an overview. *Food quality and Preference*, *9*(6), 467-478.
- Ministry of Agriculture and Farmers Welfare, Government of India (2021). Agricultural Statistics at a Glance 2021. Retrieved from https://eands.dacnet.nic.in/PDF/Agricultural_Statistics_At_Glance-2021.pdf
- Pino, N. J., Dominguez, M. C., & Penuela, G. A. (2011). Isolation of a selected microbial consortium capable of degrading methyl parathion and p-nitrophenol from a contaminated soil site. *Journal of Environmental Science and Health, Part B*, *46*(2), 173-180.
- Rajendran, D., Venkatachalam, P., & Ramakrishnan, J. (2014). Response surface methodology: optimisation of antifungal bioemulsifier from novel Bacillus thuringiensis. *The Scientific World Journal*, *2014*(1), 423289.

- Sivaperumal, P., & Sankar, T. V. (2008). *The influence of organophosphorus pesticide-methylparathion on protein, lipid metabolism and detoxifying enzymes in rohu (Labeorohita)* (Doctoral dissertation, Cochin University of Science and Technology).
- Srinivasulu, M., Nilanjan, P. C., Chakravarthi, B. V. S. K., Jayabaskaran, C., Jaffer, M. G., Naga, R. M., ... &Rangaswamy, V. (2017). Biodegradation of monocrotophos by bacteria isolated from soil. *African Journal of Biotechnology*, *16*(9), 408-417.
- Sousa Fragoeiro, S. I. (2005). *Use of fungi in bioremediation of pesticides* (Doctoral dissertation, Cranfield University).
- Xue, Y., Zhao, L., Liu, H. W., & Sherman, D. H. (1998). A gene cluster for macrolide antibiotic biosynthesis in Streptomyces venezuelae: architecture of metabolic diversity. *Proceedings of the National Academy of Sciences*, *95*(21), 12111-12116.
- Zhang, H. X., &Sultatos, L. G. (1991). Biotransformation of the organophosphorus insecticides parathion and methyl parathion in male and female rat livers perfused in situ. *Drug metabolism and disposition*, *19*(2), 473-477.
- Zhang, J. L., &Qiao, C. L. (2002). Novel approaches for remediation of pesticide pollutants. *International journal of environment and pollution*, *18*(5), 423-433.
- Zhang, W., Jiang, F., &Ou, J. (2011). Global pesticide consumption and pollution: with China as a focus. *Proceedings of the international academy of ecology and environmental sciences*, *1*(2), 125.