Journal of Advanced Zoology

ISSN: 0253-7214

Volume ***44*** *Issue S-****05 Year 2023*** *Page* ***2435:2440***

Comparative Optimization Studies Of Various Parameters Towards Chlorantraniliprole Degradation By Gram Positive Pdb

Gade Pavan Kumar[1], K.Bramarambica[2], P.Chandrasekhar[3], Y. Vijay Kumar[4], Swapna M, Rajender Vadluri*

[1]Department of Biotechnology, Chaitanya Deemed to be University, Hanumakonda -506001.
[2]Department of Biotechnology, JJTU, Jhunjhunu, Rajasthan -333013.
[3]Department of Botany, SGA Government Degree College, Elamanchilli, Andhra Pradesh -531005.
[4]Department of Botany, SCIM Govt. College, Tanuku, Andhara Pradesh -534211.

***Corresponding Author:** Rajender Vadluri*
Email: rajenderbio@gmail.com.

Article History

Received: 22/10/2023
Revised: 22/12/2023
Accepted: 25/12/2023

Abstract

Biodegradation of pesticides using various microbes such as PDBs is the best alternative for eco-friendly as well as economical way to mitigate bioaccumulation as well as biomagnification of these chemicals into ecological pyramids. The microbes have the property of degrading pesticides also known as PDB (pesticide degrading bacteria) in their vicinity using their metabolic pathways which was explored in the present study to biologically degrade organophosphorus based pesticide Chlorantraniliprole using a gram positive bacteria isolated from agriculture soils. The results of this study has improved the scientific way of exploring *Staphylococcus hemolyticus* for its potency towards bioremediation property also in biodegrading chemical based pesticide. A comparative study has revealed the influence of various optimized parameters towards the pesticide degradation activity of isolates from three study sites which has opened the unexplored zones of scientific understanding of a microbial behaviour towards specific activity.

Keywords: PDB, Chlorantraniliprole, bioremediation, biodegradation, gram positive bacteria.

Introduction:

Chlorantraniliprole is an insecticide which also shows pesticidal activity is widely used to kill pests that attack the Tomato plants. This pesticide is been categorised under organophosphorus class of pesticide which show the mode of action on the pest's by inhibiting its neurotransmitters such as Acetylcholinesterase (AChE) (Rathnayake et al., 2016). When such pesticides are been bioaccumulated and enter into human systems, they are showing worse effects. This pesticide is so toxic that use of it even in lesser quantities can kill the pests effectively which is saving farmer's money and duration to deal with pests and hence preferred more for economic reasons. But the adverse effects of this pesticide was known later when biomagnification of it through food chain and food webs.

In order to degrade this pesticide, biological way is proven to be the best and economical than the conventional methods where chemicals are used. Microbes are the best biological agents that can degrade

these organophosphorus based pesticides through their metabolic pathways (Soulas and Lagacherie, 2001). These microbes release certain enzymes which specifically degrade the organophosphorus based pesticides and for the gene responsible for these enzymatic release is 'oph gene' also known as organophosphorus hydrolase gene. The enzyme produced by this gene is OPH (Organophosphorus hydrolase). This enzyme not only shows hydrolytic activity in the vicinity of the substrate organophosphorus pesticides but also through co-metabolism can effectively convert these toxic xenobiotic compounds into simple organic compounds (Singh and Walker, 2006).

A wide range of bacteria which are of gram negative nature are reported to degrade these organophosphorus based pesticides in their habitat such as *Pseudomonas*, *Flavobacterium*, *Bacillus* and*Enterobacterium* but very few research is done on gram positive organisms. The present research has revealed that gram positive *Staphylococci* are also very good biodegraders of these pesticides when optimal conditions are provided as in case of substrate , when glucose was provided to *Pseudomonas*, it could degrade Chloropyrifos at maximum (Ambreen and Yasmin, 2020). When pH 7 was provided for gram negative bacteria *Bacillus* it showed maximum degradation (Awad et al., 2011). A comparative study on their potentiality has been discussed in the present research article.

Materials and Methodology:

Samples of soil were isolated from three study areas of Telangana region from Tomato field and were serial diluted upto 10^{-6} so that isolated colonies can be obtained. Then this concentration was spread onto prepared nutrient agar plates and incubated for 24 hours. After incubation plates were observed for microbial growth.

Then loopful of isolated colonies were taken and mixed with minimal salt media flask and incubated in shaker incubator at room temperature for 24 hours at 120 rpm. After incubation the flask was removed and observed for bacterial enumeration. A loopful of colonies were taken and steaked onto minimal salt media for further morphological as well as biochemical studies using standard protocols. For molecular studies of the isolates from three study sites, DNA was isolated and sequenced using standard protocols which gives species confirmation.

Optimization studies were done taking parameters such as temperature, pH, pesticide concentration, and substrate as mentioned in (GadePavan Kumar et al., 2023).

Results and discussion:

Figure 1 A: Isolate from SS1 **Figure 1 B: Isolate from SS2**

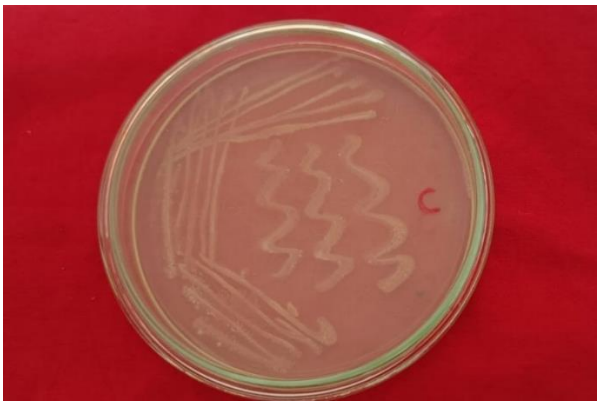

Figure 1 C: Isolate from SS3

Table 1: Comparative temperature optimization studies for three study sites SS1,SS2 and SS3

Parameter	Optimization	Study stations	Pesticide degradation percentage				
			1sthr	2nd hr	3rd hr	4th hr	5th hr
Temperature	37°C	SS1	27%	47%	51%	75%	83%
Temperature	37°C	SS2	29%	48%	55%	76%	93%
Temperature	37°C	SS3	32%	51%	59%	86%	97%

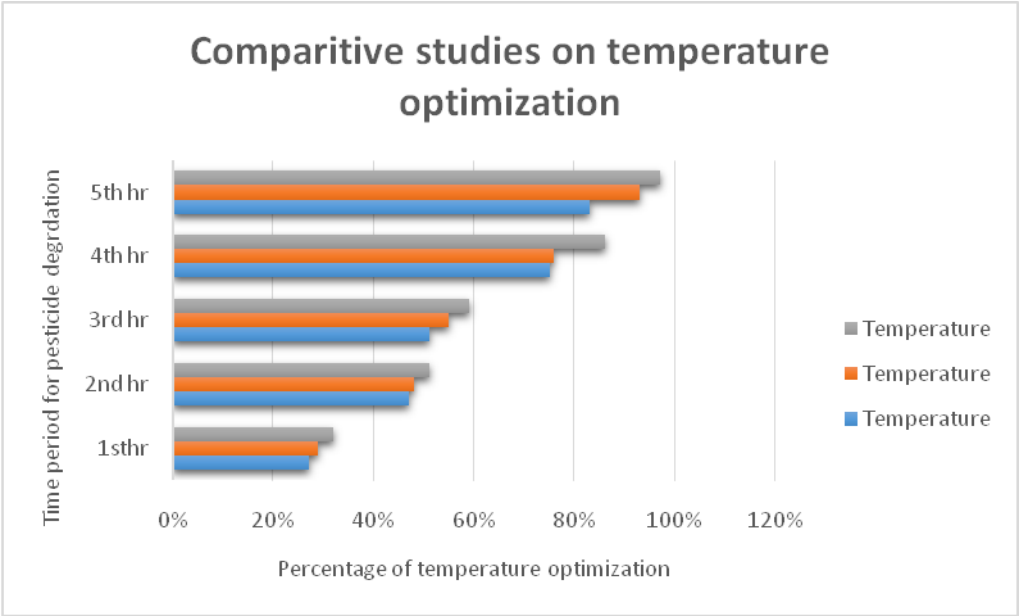

Figure 2: Comparative optimization of temperature for study sites SS1, SS2 and SS3

Table 2: Comparative pH optimization studies for the three study sites SS1, SS2, and SS3

Parameter	Optimization	Study stations	Pesticide degradation percentage				
			1sthr	2nd hr	3rd hr	4th hr	5th hr
pH	7	SS1	36%	52%	89%	97%	97%
pH	7	SS2	38%	56%	87%	97%	99%
pH	7	SS3	39%	59%	89%	96%	98%

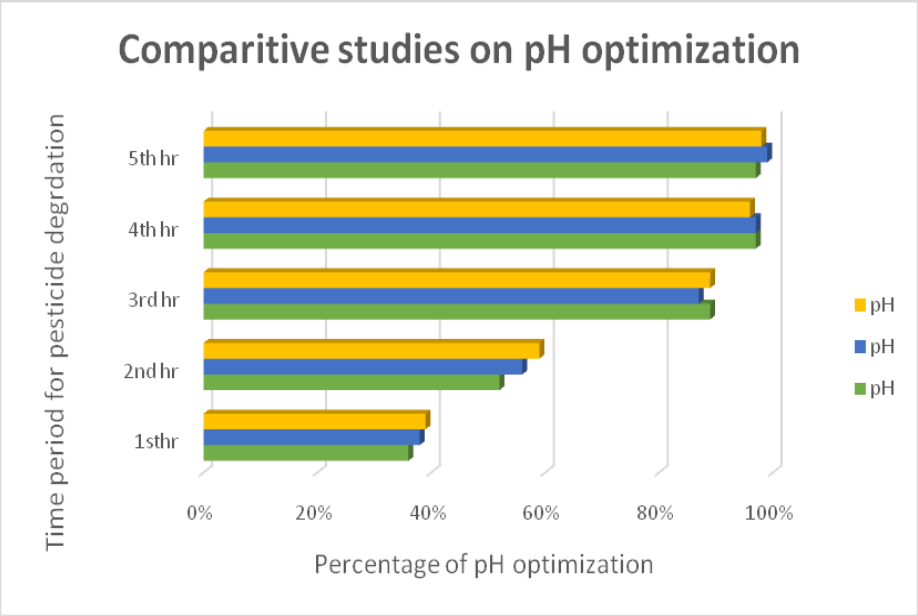

Figure 3: Comparative optimization of pH for three study sites SS1, SS2 and SS3

Table 3: Comparative pesticide concentration optimization studies for the three study sites SS1, SS2 and SS3

Parameter	Optimization	Study stations	Pesticide degradation percentage				
			1sthr	2nd hr	3rd hr	4th hr	5th hr
Pesticide conc.	10 mg	SS1	92%	98%	98%	98%	98%
Pesticide conc.	10 mg	SS2	90%	96%	97%	98%	98%
Pesticide conc.	10 mg	SS3	91%	93%	95%	97%	98%

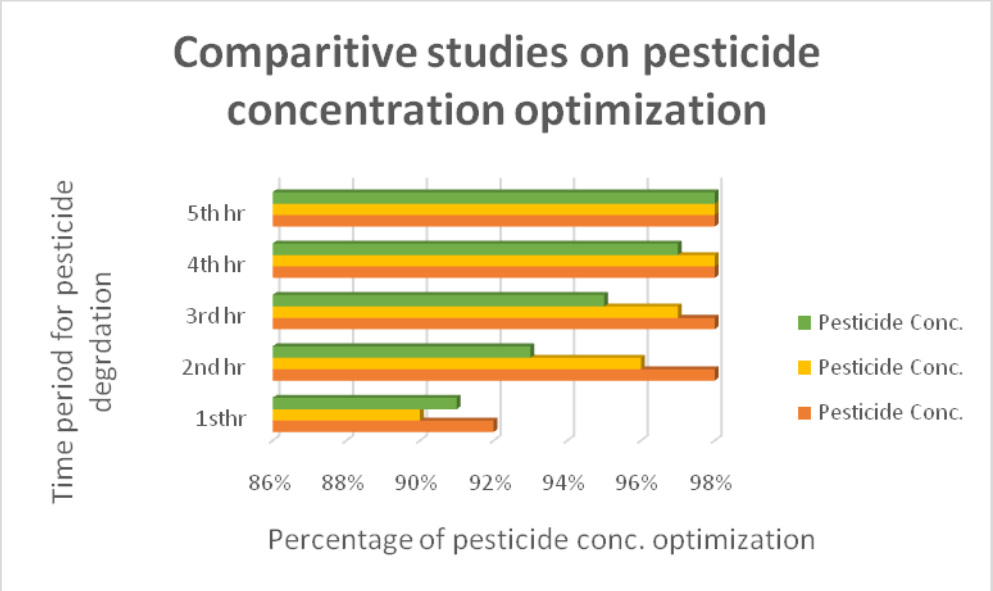

Figure 4: Pesticide concentration optimization studies comparison for study sites SS1, SS2 and SS3

Table 4: Comparative substrate optimization studies of three study sites SS1,SS2 and SS3

Parameter	Optimization	Study stations	Pesticide degradation percentage				
			1sthr	2nd hr	3rd hr	4th hr	5th hr
Substrate	Fructose	SS1	93%	94%	98%	98%	98%
Substrate	Fructose	SS2	94%	95%	96%	97%	99%
Substrate	Fructose	SS3	95%	96%	97%	98%	98%

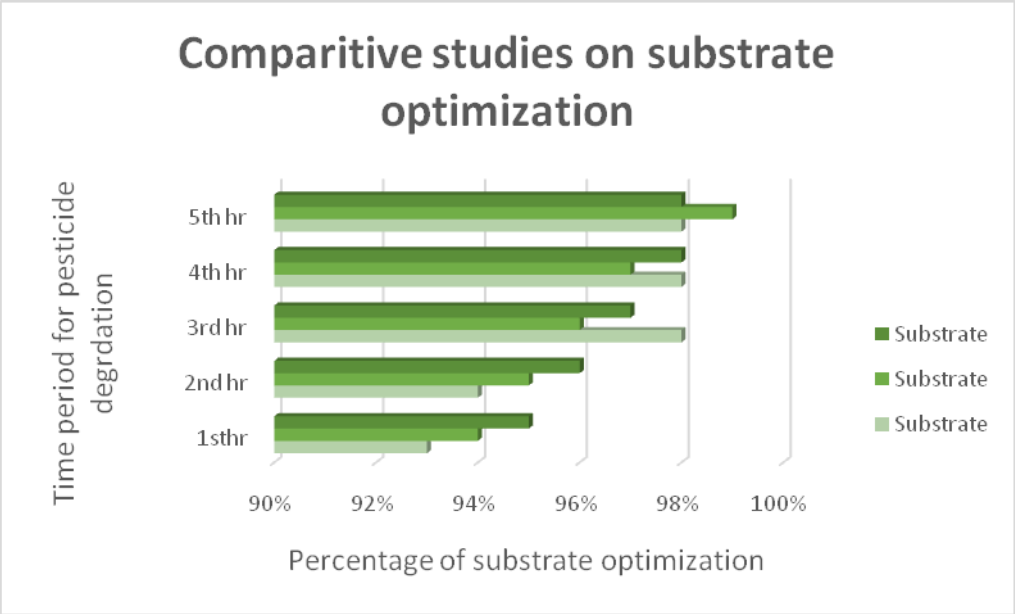

Figure 5: Comparative substrate optimization studies from study sites SS1, SS2 and SS3

Discussion:

Agriculture sector has been the backbone for many countries in contributing major share of food to people. The reforms in this sector to meet the demand has led the 'Green Revolution' which was achieved by use of pesticides and fertilizers of chemical origin. This criteria has no doubt solved the demand of food for the population but also caused great affects towards agriculture soil, and accumulation of these chemicals in food chains as well as food webs thereby resulting in bioaccumulated biomagnification. The very need for controlled use of these chemical based pesticides was understood as the adverse effects were directly related to neurological disorders in humans and various fauna. Pesticides belonging to various class have various targetdisorders, in the present study the pesticide belonging to organophosphorus class Chlorantraniliprole shows its activity by inhibiting the neurotransmitter acetylcholine.

Biodegradation of such pesticides is a challenging work as it needs considerations of various factors associated with it. Microbial degradation is one of the best approach as they degrade these pesticides through their metabolism in useful way. For this to be achieved, optimization of parameters such as temperature at 37^0C, pH at 7, fructose as substrate and 10 mg pesticide concentration was done which was compared among the three bacterial samples as shown in figure 1A, 1 B and 1 C isolated from three study sites SS1, SS2 and SS3.They revealed that maximum pesticide degradation activity of isolate from SS2 was shown for optimized substrate and pH as shown in tables 2 and 4 and figures 5 and 3, the isolate from SS3 has shown greater pesticide degradation activity than the other two isolates at optimized temperature as shown in table1 and figure 2 and all the three isolates has shown maximum pesticide degradation activity at optimized pesticide concentration as shown in table 3 and figure 4.

These efficacy differences among the isolates from various sampling areas may be due to the effect of parameters on the activity of organism in terms of co-metabolism (Gao et al., 2019& Gupta et al., 2015). Much literature was reported for gram negative bacteria in degradation of organophosphorus based pesticides but in the present study a gram positive bacteria has shown much efficient degradation activity. The bacterial isolate form three study sites was *Staphylococcus hemolyticus* which was biochemically as well as through molecular characterization was confirmed.

It is understood through the present study that even though the organism is same from three study sites, the effectiveness towards degradation of Chlorantraniliprole was different when studied under same optimized conditions. The microbes isolated from various soil samples show various efficacy due to long time exposure to diversifying abiotic as well as biotic factors (Bucibo et al.,2010). It is reported that *Staphylococcus* mixed with other pesticide degrading bacteria has shown good degradation activity (Kadhim et al., 2015). But the present study revealed that monoculture of *Staphylococcus hemolyticus* has much greater biodegradation of organophosphorus pesticides when optimum conditions were provided. And a comparative study among the three isolates of this microbe has given a clear picture of their diverse nature towards pesticide degradation activity even though the optimization parameters were same.

Conclusion:

Exploring the biodegradation activity of gram positive bacteria *Staphylococcus hemolyticus* towards Chlorantraniliprole was done in which optimised conditions were maintained. A comparison between the isolates from three study sites for this degradation activity was studied to reveal their potency. This study helped for understanding the influence of sample source of isolated samples towards biodegradation activity when other parameters were constant. The seasonal impact on collected samples that is from December to February which is winter in Tropical countries like India has also shown influence for better co-metabolic way of pesticide degradation by the isolates.

Further research has to be done in molecular level to reveal the potency of these bioremediating gram positive *Staphylococcus hemolyticus* to understand the variants of mechanism behind their efficiency towards biodegradation of pesticides.

Reference:

1. Ambreen, S., & Yasmin, A. (2020). Isolation, characterization and identification of organophosphate pesticide degrading bacterial isolates and optimization of their potential to degrade chlorpyrifos. *International Journal of Agriculture and Biology*, *24*(4), 699-706.
2. Awad NS, HH Sabit, EM Salah, SEM Abo-Aba, RA Bayoumi (2011). Isolation, characterization and fingerprinting of some chlorpyrifos-degrading bacterial strains isolated from Egyptian pesticides-polluted soils. *Afr J Microbiol Res* 5:2855–2862.
3. Bucibo M, Nontutu, N, Gadihele G, (2010) Identification and quantification of selected pesticides in surface water in Southern gauteng region, Magister Technologiae (Chemistry), the Department of Chemistry.
4. GadePavan Kumar, K. Bramarambica, Swapna Mittapalli, Dhiddi Bindu Sri, M. Akshay Kumar, V. Rajender (2023). Optimization studies in terms of substrate, temperature, pesticide concentration and pH on pesticide degrading bacteria from agricultural soil, *Journal of Composition theory*, Volume 16, Issue 9, 21-32.
5. Gao, W., Li, D., & You, H. (2019). Functional characterization and genomic analysis of the chlorantraniliprole-degrading strain Pseudomonas sp. GW13. *Bioengineering*, *6*(4), 106.
6. Gupta, M.; Mathur, S.; Sharma, T.; Rana, M.; Gairola, A.; Navani, N.; Pathania, R (2015). A study on metabolic prowess of Pseudomonas sp. RPT 52 to degrade imidacloprid, endosulfan and coragen. J. Hazard. Mater,301, 250–258.
7. Kadhim, F., Rabee, A. M., &Abdalraheem, E. (2015). Biodegradation of malathion by selected bacterial isolates. *Journal of International Environmental Application and Science*, *10*(3), 361-366.
8. Rathnayake, L.K.; Northrup, S.H (2016). Structure and mode of action of organophosphate pesticides: A computational study. Comput. Theor. Chem. 1088, 9–23.
9. Singh, B. K., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS microbiology reviews*, *30*(3), 428-471.
10. Soulas, G.; Lagacherie, B (2001). Modelling of microbial degradation of pesticides in soils. Biol. Fertil. Soils, 33, 551–557.