JournalofAdvancedZoology
*ISSN:0253-7214*
*Volume**44**Issue**S-5Year**2023**Page2400:2404*

# OrganophosphorusBasedPesticideDegradingBacterialScreeningFrom Agriculture Soils Of Telangana Region.

**GadePavanKumar[1],K.Bramarambica[2],Y.VijayaKumar[3],P.ChandraSekhar[4],Swapna Mittapalli[1],Rajender Vadluri[1*]**

*[1*]DepartmentofBiotechnology,ChaitanyaDeemedtobeUniversity,Hanumakonda-506001.*
*[2]AssociateProfessor,DepartmentofBiotechnology,JJTU,Jhunjhunu,Rajasthan-333013.*
*[3]LecturerinBotany,SCIMGovt.College,Tanuku,AndharaPradesh-534211.*
*[4]Principal,SGAGovernmentDegreeCollege,Elamanchilli,AndhraPradesh-531005.*

***Corresponding Author:***
*RajenderVadluri*
*Email:rajenderv@chaitanya.edu.in.*

***ArticleHistory***

Received:
Revised:
Accepted:

***Abstract***

Organophosphorus based pesticides are chemical pesticides which are widely used in India for controlling the pests, insects and plant pathogens. The mode of action of pesticides belonging to this class is on neurotransmitter inhibition as well as muscle suppression by which the target organisms are killed. But since the use of these pesticides is in an uncontrolled and indiscriminate manner in agriculture sector, there is a need to degrade them to protect their harmful effects on environment. Biological degradation of these pesticides areconsidered as better alternatives as this method is both economical as well as eco-friendly.

Bioremediation of organophosphorus pesticides inthevicinity of soil has gained much attention recently because the microbes involved in this process are naturally having the potency to degrade the pesticides in their habitat which is called principle of infallibility. This property was explored for the biological degradation of pesticides. The microorganisms have a special gene known as organophosphate hydrolase or 'oph' whose enzyme product when come into contact with the organophosphorus compounds readily degrades them. This organophosphate hydrolase enzyme is well known for its broad spectrum of substrates hydrolysis activity. This activity was taken as a tool for screening of potent bacteria from agriculture soils towards organophosphorus degradation in the present study.

Serial dilutions of the soil samples collected from three different regions of Telanganastate were donetodecline the microbial loadand thenthrough spread plate and streaking techniques, isolated bacterial colonies were obtained. Screening of these colonies for the presence of OPH enzyme was done by mixing the OPDs onto OP medium.

After screening for the potent isolates showing OPH enzyme, it is revealed that the potent isolates which were successful in degradation of organophosphorus pesticideChlorantraniliproleindicatedbybacterialgrowtheveninthepresence of pesticide and also due to the presence of 'oph' gene which was produced OPH enzyme.



**Keywords:**Organophosphoruspesticides,Chlorantraniliprole,OPH,PDBs

**Introduction:**

Organophosphorus pesticides are xenobiotic compounds that act as inhibitors of neuro transmitters such as acetylcholinesterase leading to neurological disorders and cancer related diseases when accumulated into food chain and food web. Soils have the capability of dissoluting them but it takes longer periods (Satish et al., 2017). Annually millions of deaths and chronic poison in there were reported due to the residual deposition of these pesticides in soil, water as well as air (Frazer, 2000). The pesticides such as Chloropyrifos, parathion, methylparathion, glyphosate and Chlorantraniliprole are dangerous to mammals as their half-life in degradation in the soil takes much time (Singh and Walker, 2006) so, without disturbing the balance of ecological systems and one such alternative is using of microorganisms based degradation methods.

Microorganismssuch as bacteriaareabletometabolize thesexenobioticcompounds into organiccompounds through various degrading pathways. The enzymes produced by these organisms help in dissolution of the harmful active ingredients of pesticides such as Coumaphos degraded by *Falavobacterium*and *Achrobacterium*enzymes degrades Carbofuran (Mustapha et al., 2019). Organophosphorus pesticides are degraded by bacteria because those bacteria produce OPH enzyme which helps in hydrolysing of these pesticide class. In the present study an attempt was done to screen the bacteria that produce OPH enzyme which were of agriculture soil origin.

**Experimental:**

**Soilsamplecollectionandculturingof organisms:**

Soil samples were collected from three study areas of Telangana region of Khammam district from tomato fields where agriculture practice wasdonefrom a decade so that microorganismsdiversity can befound. The collected soil samples were serially diluted to reduce the microbial load in order to get isolated colonies.Then the last dilution factor that is $10^{-6}$ was taken and spread onto pre sterilized and prepared nutrient agar plates in sterile conditions and incubated at $37^0$C for 24 hours to get the microbial growth. After incubation, the growth was observed and loopful of colonies were inoculated streaking of the isolated colonies was done onto fresh nutrient agar plates which were sterile for sub-culturing.

**DetailsofChlorantraniliprole(GadePavanet.al.,2023).**

**Common name** : Chlorantraniliprole
**Molecularweight** :483.15g.mol$^{-1}$
**Colour** :Whiteorcolourlesscrystallinesolid
**IUPACFormula** :5-bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-2-(3-chloropyridin-2-yl)pyrazole-3-carboxamide
**ChemicalFormula** :$C_{18}H_{14}BrCl_2N_5O_2$
**Stability** :StableinairandisnotsensitivetoUVradiation,itisstable to neutral and weakly acidic solutions, but is hydrolysed by strongbases.

**Structureof Chlorantraniliprole:**

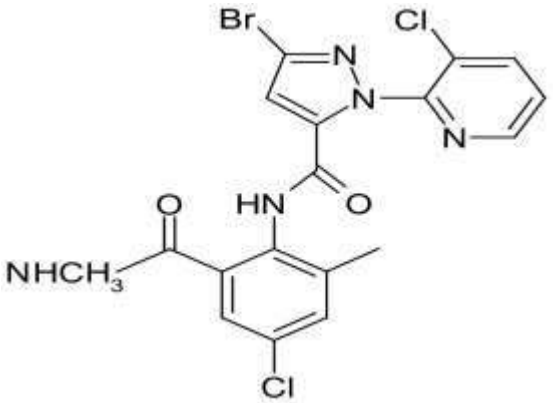


**Figure1:Chlorantraniliprolestructure(Aghrisetal.,2022).**

**OptimizationconditionsfollowedforPDBisolation:**
The accelerated degradation of pesticide Chlorantraniliprole whose structure is shown in figure 1, was found in minimal salt media at $37^0$C, pH7 in 5hours 30minutes when sucrose was added as substrate and pesticide was10mg/L, and the methodology was mentioned in (Gade Pavan et.al., 2023).

**Microbialgrowthonselectivemedia:**
The morphology of the colonies were observed under microscope such as size, shape, colour, sporeformation and gram's stain, then selective media was prepared by using sucrose as one of the ingredient in nutrient agar medium composition as shown in table 1. Then the colonies were spread onto the selective mediaplatesandpesticideof10mgconcentration wasusedtofurther screenthecoloniesandthe plates were incubated at $37^0$C for 24 hours.

**Table1:Compositionofselectivemedia(MinimalSaltmedia)**

| S.No | Minimalsaltmediaingredients | Composition |
|---|---|---|
| 1. | $K_2HPO_4$ | 0.6g/L |
| 2. | $KH_2PO_4$ | 0.2g/L |
| 3. | $MgSO_4.7H_2O$ | 0.08g/L |
| 4. | NaCl | 0.2g/L |
| 5. | $NH_4NO_3$ | 0.6g/L |
| 6. | Sucrose | 2.0 g/L |
| 7. | Distilledwater | 1000mL |
| 8. | pH | 7.0 |
| 9. | Agar | 4grams |

**Screeningofcoloniesshowingpesticidedegradation:**
After incubation for 24 hours on selective media with pesticide Chlorantraniliprole, the zone of clearance were observed which were measured using a ruler.

**Resultsand discussion:**

The soil samples collected from Khammam district from the Tomato fields wereserially diluted and the serial dilution concentration of $10^{-6}$ was spread onto sterile nutrient agar media plate and incubated. Then from the colonies formed on the plate a loopful of colonies were streaked onto fresh nutrient agar media to get isolated colonies as shown in figure 2.

**Figure2:Isolatedbacterialcolonies**

**Morphological studies:**
The obtained isolated colonies were observed for morphological features which revealed that the organism was motile, rod shaped gram negative bacteria when observed under microscope which are the feature of *Pseudomonas* genus.

**Optimizationstudies:**
Optimization studies were done to get a bacterial strain that is capable of degrading organophosphorus pesticide with the optimized parameters such as temperature, pH, substrate, time period as well as pesticide concentration which were published in (Gade

Pavanet.al.,2023).Suchpotentcoloniesweregrowninbrothandincubatedforfurtherscreeningstudies.

**ScreeningstudiesofPDB:**

Screening was done using sucrose as substrate in nutrient agar media and fresh colonies from the broth were spread and Chlorantraniliprole pesticide was also added of 10mg concentration and after incubating for 24 hours no zones were observed which indicated the organophosphate hydrolase enzyme activity of the bacterial isolates towards the pesticide degradation which were shown in figure 3.

**Figure3:Noclearzonesareseenduetopesticidedegradationbybacterialhydrolase activity**

Pesticide degrading bacteria are regarded as the better alternative for degrading pesticides which were accumulated in soil of agriculture fields. The bioremediation properties of microorganisms in particular bacteria is influenced by factors such as application of pesticide, their frequency of application, soil pH, stability of other flora, gap between the applications and half–life of the pesticide in the soil (Singh and Walker, 2006).

So to understand the metabolism of the PDBs for their activity, they need to beisolated and screened fortheir potency which was attempted in the present work through a method targeting 'oph' gene which produces organophosphate hydrolase enzyme which is produced when the bacteria degrades pesticides that belongs tothe class organophosphorus which wasevident by nozones and luxurious growth of bacteria even in the presence of pesticide as shown in figure 3, this luxurious growth is due to chemiolithotrophic nutrition of PDBs.

It is reported that *Klebsiella aerogenes* degrade pesticides belonging to class methyl as well as dimethyl phosphates due to the presence of phosphomonoesterase and diesterase enzymes (Wolfenden and Spence, 1967). Even *Enterobacter* species were reported to use organophosphorus compounds and are able to metabolize them to simple compounds (Singh et.al., 2003 and Singh et al., 2004). This OPH enzyme wasfirstobservedin*Falvobacterium*strainATCC27551aswellas*Pseudomonasdiminuta*andtheircodinggenes on extrachromosomal plasmid were similar (Qiongetal., 2010).

OPH enzyme isolated form *Pseudomonas* species have shown more thermos stability which helped the scientists to explore these organisms for understanding the enzyme activity toward pesticide degradation (Su et al., 2011). This very property has increased the interest in screening for the microbes related to *Pseudomonas* species which was shown in present work.

**Conclusion:**

Organophosphate hydrolase enzyme is the xenobiotic compound breaking down enzyme which specifically targets on organophosphorus compounds of pesticides such as Chlorantraniliprole which was used in the present study. This enzyme is naturally doing this activity in the vicinity of soil in agricultural fields where these class of pesticides are extensively used. Biological degradation of these compounds is necessary asthey are bioaccumulating and biomagnifying themselves and taking much time to degrade in soil.

*Pseudomonas* species are one of the best organophosphorus compounds degraders as the OPH enzyme in them is naturally a thermostable one. This is evident that the obtaining of *Pseudomonas* in the present study as potent isolate for degradation of Chlorantraniliprole in optimized conditions.

Future studies are focused on testing the efficacy of OPH enzyme from*Pseudomonas* in degrading a widerange of class of pesticides by understanding the enzyme activity at molecular level.

**References:**


1. Aghris, S.; Alaoui, O.T.; Laghrib, F.; Farahi, A.; Bakasse, M.; Saqrane, S.; Lahrich, S.; Mhammedi, E (2022).Extractionanddeterminationofflubendiamideinsecticideinfoodsamples:Areview.Curr.Res. Food Sci. 5, 401–413.
2. FrazerC(2000).The Bioremediation and Phytoremediation of Pesticide-contaminated sites. Prepared for U.sE.P.A, WashingtonD.C.49.
3. Gade Pavan Kumar, K.Bramarambica, Swapna Mittapalli, DhiddiBindu Sri, M. AkshayKumar, V. Rajender (2023). Optimization studies in terms of substrate, temperature,pesticide concentration andpH on pesticide degrading bacteria from agricultural soil, *Journal of Composition theory*, Volume16, Issue9, 21-32.
4. Mustapha, M. U., Halimoon, N., Johar, W. L. W., &AbdShukor, M. Y. (2019). An overview on biodegradation of carbamate pesticides by soil bacteria. *Pertanika Journal of Science &Technology*, *27*(2).
5. Qiong, L., Qing,T., Xudong, X ., Hong, G (2010). Expression of organophosphorus-degradation gene (opd) in aggregating and non-aggregating filamentous nitrogen-fixing cyanobacteria .*Chin JOceanolLimnol*, vol .28,pp. 1248-1253.
6. Satish,G.,Ashokrao,D.M.,&Arun,S.K.(2017).Microbialdegradationofpesticide:areview. *Africanjournalofmicrobiologyresearch*,*11*(24),992-1012.
7. SinghBK, WalkerA, MorganJAW&WrightDJ (2003c).Effect of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. ApplEnviron Microbiol69:5198–5206.
8. Singh BK, Walker A, Morgan JAW & Wright DJ (2004). Biodegradation of Chlorpyrifos by Enterobacter strain B-14 and its use in the bioremediation of contaminated soils.ApplEnvironMicrobiol70:4855–4863.
9. Singh,B.K.,&Walker,A.(2006).Microbial degradation of organophosphorus compounds. *FEMS microbiology reviews*, *30*(3), 428-471.
10. Su,Y.,Tian,J.,Wang,P.,Chu,X.,Liu,G.,Wu,N.,Fan,Y(2011).Improving the Thermo stability of a Methyl ParathionHydrolasebyAdding theIonicBond onProteinSurface.*ApplBiochemBiotechnol*,vol.165, pp. 989–999.
11. Wolfenden R&Spence G (1967).Depression of phosphomonoesterase and phosphodiesterase activitiesin *Aerobacter aerogenes*. BiochemBiophysActa 146:296–298.