International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 1970-1974

Received: 11-01-2018
Accepted: 12-02-2018

Uma
Department of Agril. Microbiology, Indira Gandhi Krishi Vishvavidyalaya, Krishak Nagar, Raipur, India.

Tapas Chowdhury
Department of Agril. Microbiology, Indira Gandhi Krishi Vishvavidyalaya, Krishak Nagar, Raipur, India.

SB Gupta
Department of Agril. Microbiology, Indira Gandhi Krishi Vishvavidyalaya, Krishak Nagar, Raipur, India.

Dhanni Devi
Department of Agril. Microbiology, Indira Gandhi Krishi Vishvavidyalaya, Krishak Nagar, Raipur, India.

Ashok Kumar Koshariya
Department of Plant Pathology, Indira Gandhi Krishi Vishvavidyalaya, Krishak Nagar, Raipur, India.

Correspondence
Uma
Department of Agril. Microbiology, Indira Gandhi Krishi Vishvavidyalaya, Krishak Nagar, Raipur, India.

Isolation and characterization of eco-friendly microflora for degradation of herbicide and promoting the soil health

Uma, Tapas Chowdhury, SB Gupta, Dhanni Devi and Ashok Kumar Koshariya

Abstract
The investigation was carried out in an Inceptisol with *Rabi* season chickpea to evaluate the isolation of eco-friendly microflora for degradation of herbicide and promoting the soil health. Fifteen soil samples were collected from different plots of a long term herbicidal trail where different herbicides were applied in *kharif* and *rabi* season continuously for last five years in a rice-chickpea cropping system. From these soil samples 11 crop beneficial herbicide tolerant microbial isolates were collected. These isolates were further characterized with respect to their cultural characteristics, Dehydrogenase activity and these selected isolates were further tested for their potentiality of pendimethalin degradation. Three rhizobial and four phosphobacterial isolates were taken in a study for their herbicide degradation potential under *in-vitro* conditions. All isolates had shown good growth in their respective culture media containing 5000 ppm pendimethalin. Twenty treatments were taken under this study comprising single and dual inoculation of above symbiotic N-fixing and P-solubilizing microorganisms, including control. All the treatments were compared with uninoculated control. Treatment No. 19 dual inoculation of rhizobial isolate *Rhizobium*-3 and phosphobacterial isolate PSB-4 was found to be best for rapid degradation of herbicide pendimethalin, followed by single inoculation of phosphobacterial isolate PSB-4. These native isolates had soon highest Dehydrogenase activity over control at 50 DAS. The combined application of *Rhizobium*-3 and PSB-4 was also found supreme to increase the chickpea yield at highest level followed by isolate PSB-4 by mobilizing more nitrogen and phosphorus in crop rhizosphere.

Keywords: Chickpea, Isolation, Characterization, Biochemical, Degradability

1. Introduction
Chemical herbicides are all probably the most important component of weed management system for most of the major crops. The ultimate destination of herbicidal chemicals is the soil where they come in contact with different microflora which are responsible for different biochemical transformations related to mineral nutrition to plants. Different reports envisaged that herbicidal application has adverse effects on bacterial, fungal (Shukla. 1997) [6] and actinomycetes population. Herbicides are often applied to crops to provide season-long weed control. However, herbicidal activity is beneficial only for the time it is needed. Longer herbicide activity can cause injury to subsequent crops. The length of time a herbicide remains active in the soil is called soil residual life or soil persistence of a herbicide. Several herbicides commonly persist in the soil long enough to injure subsequent crops. In addition, misapplication, crop failures, late applications, and adverse weather conditions can result in carryover (residual) of herbicides that normally decompose slowly. Microbial decomposition is one of the most important methods by which herbicides are decomposed in soil. Microorganisms consume the herbicide molecules and utilize them as a source of energy and nutrients for growth and reproduction. In agriculture many crop beneficial microorganisms are used as bio-fertilizer to increase the nutrient availability and enhance crop productivity. Many of them may degrade the herbicides at a faster rate. So by exploiting them as a microbial agents we can sustain the crop productivity and conserve environment simultaneously from the toxicity of herbicide chemicals.

2. Methodology
The experiment was conducted during *Kharif* 2014 at green house conditions, IGKV, Raipur in an Inceptisol with *Rabi*

season chickpea to evaluate the identification of Suitable Crop Beneficial Microbe(s) for Rapid Degradation of Applied Herbicides. Fifteen soil samples were collected from different plots of a long term herbicide trail where different herbicides were applied in *kharif* and *rabi* season continuously for last five years in a rice-chickpea cropping system. From these soil samples three rhizobial, four phosphobacterial, two *Azotobactor* and two *Azospirillum* isolates were collected. The experiment included 20 treatments *viz.*T1: *Rhizobium*-1, 1. T2: *Rhizobium*-2, T3: *Rhizobium*-3, T4: PSB-1, T5: PSB-2, T6: PSB-3, T7: PSB-4, T8: *Rhizobium*-1+PSB-1, T9: *Rhizobium*-1+PSB-2, T10: *Rhizobium*-1+PSB-3, T11: *Rhizobium*-1+PSB-4, T12: *Rhizobium*-2+PSB-1, T13: *Rhizobium*-2+PSB-2, T14: *Rhizobium*-2+PSB-3, T15: *Rhizobium*-2+PSB-4, T16: *Rhizobium*-3+PSB-1, T17: *Rhizobium*-3+PSB-2, T18: *Rhizobium*-3+PSB-3, T19: *Rhizobium*-3+PSB-4, T20: Uninoculated control. The experiment was conducted on *Rabi* chickpea (*Cicer arietinum*) with test variety JG-130. The pendimethalin herbicide was applied at pre emergence stage of crop i.e. 1 DAS of the crop. The soil was Inceptisol (pH: 11, EC: 0.35 dSm^{-1}, available N: 223.57 kg ha^{-1}, available P: 8.96 kg ha^{-1}, and available K: 388.86 kg ha^{-1}, available Zn: 1.91 ppm, available Cu: 0.66 ppm, available Fe: 2.32 ppm, available Mn: 6.22 ppm). The treatments were replicated thrice under completely randomized design. Rhizosphere soil was collected at a depth of 4.5-7.0 cm at different stages of crop growth for the purpose of analysis. Soil sampling was done at 10, 20, 30, 40, and 50 days after sowing of crop. The soil samples were subjected to analysis for degradation potential of herbicide and Dehydrogenase activity.

3. Results & Discussion

In this experiment different microbial isolates were tested as individual or in combination to evaluate their degradation potential of pendimethalin herbicide with respect to microbial properties of rhizosphere soil of chickpea. The results obtained from these studies are as follows:

3.1. Collection

Fifteen soil samples were collected from different plots of a long term herbicidal trial where different herbicides were applied in *kharif* and *rabi* seasons continuously for last 5 years in a rice-chickpea cropping system. From these soil samples eleven isolates of different crop beneficial microorganisms were collected which belong to *Rhizobium*, *Pseudomonas*, *Bacillus*, *Azotobactor* and *Azospirillum* genus.

3.2. Characterization

These isolates were further characterized with respect to their cultural characteristics and gram reaction. Confirmation of *Rhizobium*, and PSB isolates was done by plant infection and phosphate solubilizing capacity, respectively. The *Rhizobium* colonies were found white in colour, circular, entire and convex. Likewise *PSB* isolates were grown on pikovskaya's plates. The colonies of *PSB* were confirmed by the hollow zone formed around the colonies, light yellow in colour, circular, undulated and raised. The colonies of *Azotobacter* on Jensen's agar media was found small, white in colour and elevated. The margin of colonies was observed entire. The isolation of *Azospirillum* was done by using Okon's medium. Earlier the medium was green in colour but after growth of above bacterium the colour of the medium turned dark blue which was the indication of growth of above bacterium. The colonies of *Azospirillum* were round in shape, elevated and their margin was found entire. Other crop beneficial microorganisms were isolated by culturing them on their respective media (Table-1).

3.3. Degradation potential of pendimethalin herbicide

In the above isolation study three *Rhizobium* and four phosphobacterial (PSB) isolates were obtained. These isolates were further tested for their ability to grow in a medium containing different concentration of pendimethalin herbicide which commonly used in chickpea to control weed. The isolates of *Rhizobium* and PSB were grown in their respective nutrient liquid culture medium containing pendimethalin at different concentrations (4000, 5000 and 6000 ppm). These isolates were subjected to continuous incubation at 28±2° C with 150 rpm for 15 days. After incubation the survibility of different isolates was tested by growing them on their respective media. At 6000 ppm pendimethalin concentration four isolates had shown poor growth while three did not show any growth. At 4000 and 5000 ppm concentrations all of the isolates had shown growth but at varying level. Phosphobacterial isolate PSB-4 had shown highest population followed by PSB-1 in 4000 ppm concentration and PSB-2 in 5000 ppm concentration (Table-2). The results are in agreement with previous finding reported by Elsayed *et al*. (2013) [2] who reported that different microbial types produce different enzymes have different degradation preferences. In our study the isolate shown maximum growth in pendimethalin contaminated media was having the characteristics of *Pseudomonas*.

Table 1: Characterization of isolated microorganisms collected from herbicide treated plots

S. N.	Herbicide used	Isolated microorganisms		Characteristics				
			Colour	Forms	Gram reaction	Plant infection (Chickpea)	Margins	Elevation
1.	*Kharif* : Oxadiargyl and	PSB-3*	White	Irregular	+	-	Undulate	Raised
	Bispyribac	PSB-1*	Light yellow	Circular	-	-	Undulate	Raised
		Rhizobium-2**	White	Circular	-	+	Entire	Convex
		Azotobactor-1***	White	Circular	-	-	Entire	Convex
		Azospirillum-1****	Dark blue	Circular	-	-	Entire	Convex
	Rabi : Pendimethalin							
2.	*Kharif* : Pyrazosulfuron	*Rhizobium*-3**	White	Circular	-	+	Entire	Convex
		Azotobactor-2***	White	Circular	-	-	Entire	Convex
		Azospirillum-2****	Dark blue	Circular	-	-	Entire	Convex
	Rabi : Pendimethalin							
3.	*Kharif* : Fenoxaprop+	*Rhizobium*-1**	White	Circular	-	+	Entire	Convex
	Chloromuron ethyle+	*Azotobacto*r-3***	White	Circular	-	-	Entire	Convex
	Metsulfuron methye	PSB-2*	White	Irregular	+	-	Undulate	Raised
		PSB-4*	Light yellow	Circular	-	-	Undulate	Raised
	Rabi : Pendimethalin							

*Grown on Pikovskaya's medium **Grown on YEMA medium *** Grown on Jensen's agar media **** Grown on Okon's agar media

Table 4.2: Growth ability of the bacterial isolates in liquid culture media containing Pendimethalin

S.N.	Isolates	Growth ability		
		Pendimethalin concentration		
		4000	5000	6000
		ppm	ppm	ppm
		($x10^5$)	($x10^3$)	($x10^2$)
1.	*Rhizobium*-1	1.74	9.7	3.65
2.	*Rhizobium*-2	1.58	8.0	-
3.	*Rhizobium*-3	1.62	8.3	-
4.	PSB-1	1.85	11.0	5.1
5.	PSB-2	1.88	11.5	5.3
6.	PSB-3	1.53	7.6	-
7.	PSB-4	1.98	12.8	6.2

Media Used: (i) For *Rhizobium*: YEMA broth
(ii) For PSB: Pikovskaya's broth

Chemical composition of media

1. YEMA for *Rhizobium*

Mannital	10.0gm
K2HPO4	0.5gm
MgSO4. 7H2O	0.2gm
NaCl	0.1gm
Yeast extract	1.0gm
Agar	20.0gm
Distilled water	1000ml
Congo red soln (1%)	2.5ml
pH	7.0

2. Pikovskaya's medium for PSB

Mannital	10.0gm
K2HPO4	0.5gm
MgSO4. 7H2O	0.2gm
NaCl	0.1gm
Yeast extract	1.0gm
Agar	20.0gm
Distilled water	1000ml
Congo red soln (1%)	2.5ml
pH	7.0

3. Jensen's agar medium for *Azotobacter*

Sucrose	20.0gm
K_2HPO_4	1gm
$MgSO_4$	0.5gm
NaCl	0.5gm
$FeSo_4$	0.1gm
Na2MoO4	0.001gm
$CaCo_3$	2gm
Agar	15. 0gm
Distilled Water	1000ml
pH	7.0-7.2

4. Okon's medium for *Azospirillum*

Malic acid	5.0gm
KOH	4. 0gm
K_2HPO_4	0.5gm
$MgSO_4$	0.1gm
NaCl	0. 02gm
$FeSo_4$	0. 05gm
Na_2MoO_4	0.002gm
$CaCl_2$	0. 01gm
$MnSo_4$	0. 01gm
NH_4Cl	1.5gm
0.5%BTB	2.0gm
Agar	18. 0gm
Distilled Water	1000ml
pH	6.6-7. 0

4-Dehydrogenase activity

Application of pendimethalin significantly inhibited the DHA after its application *i.e*. 1 DAS of chickpea crop, except isolate PSB-4, *Rhizobium*-2, PSB-2 and *Rhizobium*-3+PSB-4. The dehydrogenase activity in soil inoculated with different isolates significantly inhibited by applied pendimethalin up to 20 DAS. However, from 30 DAS onwards a gradual increase in DHA was noticed in all treatments which indicated the initiation of dissipation of pendimethalin. At 50 DAS highest DHA was estimated in soil in all the treatments in comparison to other crop growth stages. At this stage all the treatments had shown significantly superior DHA value over control which indicated the complete dissipation of applied herbicide. At this stage maximum dehydrogenase activity in soil (79.2 µg TPF) was recorded due to dual inoculation of *Rhizobium*-3 & PSB- 4 and minimum DHA in soil (63.6 µg TPF) was observed due to dual application of *Rhizobium*-1 & PSB-3 (Table-3). Above observations were in close agreement with Nowark (1996) and Wyszkowska and Kucharski (2004) [8] who claimed that dehydrogenase are an objective reflection of the biological state of soil. They also reported that highest rate of triflurotox depressed the dehydrogenase activity by 28.7 % before sowing and 35.6 to 72.6 % after harvest of crop, in comparison to the control. The inhibitory effect of herbicides on the activity of dehydrogenase has been also reported by other researchers: Strzelec (1986) and Pietr and Zablonska (1987) [4]. From 30 DAS onwards the dissipation of pendimethalin was observed in different treatments. At 40 DAS the DHA activity increased significantly due to application of some treatments viz. T_1, T_7, T_8, T_{14}, T_{17} and T_{19}.

At 50 DAS the dehydrogenase activity was increased in some treatments due to application of *Rhizobium* and phosphobacterial isolates alone and in combination. The treatments which had shown significant increment in dehydrogenase activity over control were T_1-T_5, T_7- T_9, T_{11}-T_{18} and T_{19}. It is apparent from the study that above treatments were significant to degrade pendimethalin at a faster rate. Belal *et al*. (2008) [1] also stated that microorganisms can use a variety of xenobiotic compounds including pesticides for their growth, mineralize and detoxify them. The highest dehydrogenase activity was observed due to dual inoculation of treatment *Rhizobium*-3 and PSB-4 (79.2) and lowest in treatment comprising *Rhizobium*-1+PSB-3 isolate.

Table 3: Effect of pendimethalin (Herbicide) on Dehydrogenase activity (µg TPF /h/g) in rhizosphere soil at different growth stages of chick pea

Tr. No.	Treatment	Days after sowing				
		10	20	30	40	50
T1	*Rhizobium*-1	24.5	22.2	27.2	40.7	71.1
T2	*Rhizobium*-2	21.5	18.4	23.3	37.7	65.1
T3	*Rhizobium*-3	21.8	18.6	23.5	36.0	65.6
T4	PSB-1	24.9	22.3	27.5	41.0	72.3
T5	PSB-2	24.7	22.6	27.3	41.1	72.5
T6	PSB-3	21.5	18.2	23.1	35.4	64.7
T7	PSB-4	27.4	24.2	29.5	45.3	77.7
T8	*Rhizobium*-1+ PSB-1	23.1	20.7	26.3	39.3	69.8
T9	*Rhizobium*-1+ PSB-2	22.9	20.3	25.8	38.8	69.3
T10	*Rhizobium*-1+ PSB-3	21.2	17.7	22.3	34.6	63.6
T11	*Rhizobium*-1+ PSB-4	24.1	21.3	26.8	40.3	71.2
T12	*Rhizobium*-2+ PSB-1	23.9	21.7	26.8	42.6	74.8
T13	*Rhizobium*-2+ PSB-2	24.2	22.4	27.3	42.0	73.7
T14	*Rhizobium*-2+ PSB-3	23.4	21.2	26.5	39.7	70.4
T15	*Rhizobium*-2+ PSB-4	22.5	20.1	25.3	38.1	68.3
T16	*Rhizobium*-3+ PSB-1	22.3	19.2	25.0	37.7	67.8
T17	*Rhizobium*-3+ PSB-2	23.5	21.0	26.7	40.0	70.9
T18	*Rhizobium*-3+ PSB-3	21.1	19.0	24.6	37.1	66.8
T19	*Rhizobium*-3+ PSB-4	25.6	23.1	28.9	46.6	79.2
T20	Control	20.2	17.2	22.0	34.0	63.0
	SEm(±)	1.52	1.646	1.689	0.661	0.578
	C.D. (0.05)	NS	NS	4.23	1.896	1.659

Initial DHA value: 37.2 µg TPF /h/g

Conclusion

From these soil samples three rhizobial, four phosphobacterial, two *Azotobactor* and two *Azospirillum* isolates were collected. These isolates were further characterized with respect to their cultural characteristics and behavior towards gram reaction. At 4000 and 5000 ppm concentrations all of the isolates had shown growth but at varying level. Phophobacterial isolate PSB-4 had shown highest population followed by PSB-1 in 4000 ppm concentration and PSB-2 in 5000 ppm concentration. At 50 DAS highest DHA was estimated in soil in all the treatments in comparison to other crop growth stages. At this stage all

the treatments had shown significantly superior DHA value over control which indicated the complete dissipation of applied herbicide. At this stage maximum dehydrogenase activity in DHA in soil (63.6 µg TPF) was observed due to dual application of *Rhizobium*-1 & PSB-3.soil (79.2 µg TPF) was recorded due to dual inoculation of *Rhizobium*-3 & PSB-4 and minimum DHA in soil (63.6 µg TPF) was observed due to dual application of *Rhizobium*-1 & PSB-3.

Acknowledgment

I am grateful to the Department of Agril. Microbiology, IGKV. Raipur (C.G.), for providing facilities for this research.

References

1. Belal EB, Zidan NA, Mahmoud HA, Eissa FI. Bioremediation of pesticides-contaminated soils. J. Agric. Res. Kafrelssheikh Univ. 2008; 34:588-608.
2. Elsayed BB, Mohamed F, Nady E. Bioremediation of pendimethalin-contaminated soil. African Journal of Microbiology Research. 2013; **7**(21):2574-2588.
3. Nowak J. Interactions between biodegradation tetrachlorwin-fosu and chlorfenwin-fosu but in different conditions amount of alive biomass mikroor-ganizmow tempearatury and humidity of soil. Zesz. Sciences. Stettin AR. 1996; 173(63):191.
4. Pietr S, zablonska B. The effect of action of herbicides on some chemical parameters and the enzymatic activity of soils. Polish J. Soil Sci. 1987; 20(2):17-25.
5. Rajendran K, Lourdaraj AC. Residual effect of herbicides in Rice ecosystem a review. Agril, Rev, 1999; 20:48-52.
6. Shukla AK. Effect of herbicides butachlor, fluchloralin, 2,4-D and oxyfluorfen on microbial population and enzyme activities of rice field soil. Indian Journal of Ecology, 1997; 24:189-192.
7. Strzelec A. Influence of specificity of soil on their reactions of on herbicides treatment. Rocz. Soils, 1986; 37(1):129.
8. Wyszkowska J, Kucharski J. Biochemical and Physicochemical Properties of Soil Contaminated with Herbicide Triflurotox 250 Ec. Polish Journal of Environmental Studies, 2004; 13(2): 223-231.