Eco. Env. & Cons. 29 (May Suppl. Issue) : 2023; pp. (S206-S210)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i03s.039

Studies on Pesticide Resistant Phosphate Solubilizing Bacteria from Grape Yard Soil

***Jadhav A. V.[1], Shaikh N. R.[2] and Pathade G. R.[1]**

[1]Krishna Institute of Allied Sciences,
Krishna Institute of Medical Sciences, "Deemed to be University", Karad, M.S., India
[2]Yashwantrao Chavan College of science, Karad, M.S., India

(Received 10 November, 2022; Accepted 20 January, 2023)

ABSTRACT

Dimethoate, a widely used organophosphate insecticide and acaricide to grape plants, is readily absorbed and distributed throughout plant tissues. The present work was based on isolation and studies on pesticide resistant phosphate solubilizing bacteria from grape yard soil. The Minimum inhibitory concentration [MIC] of normal garden microflora was observed to be 0.8% of dimethoate. The total of 6 pesticide resistant phosphate solubilizing bacteria [PP1, PP2, PP3, PP4, PP5, and PP6] were isolated from grape yard soil on Katznelson's and Bose agar. Colony characteristics, morphological characteristics, motility, sporulation and capsulation of isolates were studied. Biochemical characteristics including carbohydrate utilization, H_2S production and enzyme activities were studied. Agricultural importance of the isolates with respect to indole acetic acid production, nitrogen fixation, cellulose degradation, antagonistic effects on *Azotobacter spp.* and *Rhizobium spp.* were also studied. The pesticide tolerance was checked and seed germination test were performed. The effect of each isolate and mixed cultures on plant growth of moth bean seeds was studied. From the results, the isolate PP3 was found to be most important agriculturally producing indole acetic acid. The mixed culture also improved shoot and root growth. Further studies in this context are in process.

Key words: *Pesticide resistant phosphate solubilizing bacteria, Minimum inhibitory concentration, Agricultural importance, Grape yard soil.*

Introduction

India being an agricultural country has been a dumping ground for all sorts of pesticides, killing pests at all cost to increase agricultural production. Pesticides are chemical substances designed to control a specific pest of economic crops. Organochlorine, organophosphates, carbamates and pyrethroid are pesticides used to control various plant diseases and insect pests of grape yard. Dimethoate, an organophosphate, is an acetylcholinesterase inhibitor which disables cholinesterase, an enzyme essential for central nervous system function. The half-life of dimethoate in soil is 4-6 days, or as high as 122 days (Howard, 1989). A serious problem of agricultural community is the accumulation of pesticide residues causing damage to the ecosystem. They may also adversely influence microbial processes involving carbon, nitrogen, phosphorous and sulphur cycles. Many pesticides can bemicrobially transformed in soil. Plants require nutrients for their growth. Phosphorous is second next to nitrogen as a mineral nutrient. Plants get phosphate from the soil only in free available form phosphate anion (Alexander, 1997). Since 95- 99% soil phosphorous is insoluble and unavailable, some soil microorganisms are able to con-

vert unavailable phosphorous to available form in a cheap, safe and an ecofriendly mode. Hence use of phosphate solubilizing microorganisms is best option to avail phosphorous to plants (Jones, 1987). Many bacteria like *Bacillus, Pseudomonas,* fungi like *Penicillium, Cephalosporium,* cyanobacteria like *Tolpothrix, Nostocsp*, algae like *Chlorella sp, Anabaena moveculais* and actinomycetes like *Streptomyces* are potential solubilizers of bound phosphates in soil.

In the present work, pesticide resistant phosphate solubilizing bacteria are isolated and studied for their agricultural importance.

Materials and Methods

Soil from rhizospheric area of grape plant about 15cm below from grape yard farm from village Valva, Dist. Sangli, Maharashtra, India was collected and brought to laboratory in sterile container and maintained at 28 ^{0}C. Garden soil suspension without pesticide was streaked on sterile nutrient agar plates containing varying concentration of dimethoate- 0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.2% and incubated at 28 ^{o}C for 2 days for determination of Minimum inhibitory concentration. Grape yard soil suspension was streak inoculated on sterile Katznelson and Bose medium containing 0.4%, 0.6%, 0.8% and 1.0% of dimethoate and incubated at 28 ^{o}C for 2 days. After incubation colonies with different morphological features were selected from each plate containing 0.8% dimethoate and studied for their gram nature, motility, spore and capsule production. All isolates were coded, maintained and refrigerated on 0.8% dimethoate containing nutrient agar. Further study for different carbohydrate fermentation, enzymatic activities with respect to production of catalase, oxidase, urease, caseinase, gelatinase, amylase, nitrate reductase and H_2S production and hydrolysis of tributyrin was done. Oxidative and/or fermentative nature was studied by Hugh and Leifson's test. Agricultural importance of the isolates was studied by detecting their ability to degrade cellulose, indole acetic acid production, effect on *Azotobacter* spp. and *Rhizobium* spp., pesticide tolerance, phosphate solubilization and nitrogen fixation. The pot culture study was carried out after performing seed germination test by treating moth bean seeds with varying concentrations of dimethoate solution and observing the number of seeds germinated.

Results and Discussion

The Minimum inhibitory concentration of normal garden microflora was 0.8% of dimethoate. Total of 6 pesticide resistant phosphate solubilizing bacteria were isolated from grape yard soil on Katznelson'sand Bose agar. The isolates obtained from 0.8% concentration were coded as PP1, PP2, PP3, PP4, PP5 and PP6.

It is evident from the Table 1 that all the isolates showed round and moist colonies. All were white

Table 1. Colony characters of isolates on pesticide containing Katznelson's and Bose agar.

Sr. No.	Code of isolate	Colony characters						
		Size	Shape	Colour	Elevation	Margin	Opacity	Consistency
1	PP1	3mm	Round	White	Flat	Wavy	Translucent	Moist
2	PP2	1mm	Round	White	Flat	Entire	Transparent	Moist
3	PP3	2mm	Round	White	Raised	Entire	Translucent	Moist
4	PP4	3mm	Round	White	Raised	Entire	Translucent	Moist
5	PP5	2mm	Round	Colourless	Raised	Entire	Transparent	Moist
6	PP6	3mm	Round	Colourless	Raised	Wavy	Transparent	Moist

Table 2. Gram nature, morphological characteristics, motility, sporulation and capsulation of isolates.

Sr. No.	Isolate code	Gram staining	Morphology	Motility	Spore staining	Capsule staining
1	**PP1**	Gram positive	Cocci in pair	Motile	Non sporing	Non capsulated
2	**PP2**	Gram positive	Single cocci	Motile	Non sporing	Non capsulated
3	**PP3**	Gram positive	Cocci in pair	Actively motile	Non sporing	Non capsulated
4	**PP4**	Gram positive	Cocci in Clusters	Actively motile	Non sporing	Non capsulated
5	**PP5**	Gram positive	Cocci in chain	Actively motile	Non sporing	Non capsulated
6	**PP6**	Gram positive	Cocci in pair	Non motile	Non sporing	Non capsulated

Table 3. Carbohydrate fermentation by the isolates:

Sr. No.	Carbohydrate	Bacterial isolates					
		PP1	PP2	PP3	PP4	PP5	PP6
1	Glucose	-	-	+	+	+	-
2	Lactose	+	-	-	-	+	-
3	Mannose	+	+	+	+	+	-
4	Sucrose	+	-	+	+	+	-
5	Maltose	+	-	+	+	+	+
6	Arabinose	+	+	+	+	+	+
7	Mannitol	-	-	+	+	[+]	[+]
8	Raffinose	-	-	+	-	[+]	[+]
9	Ribose	+	+	-	+	[+]	[+]
10	Galactose	+	+	+	+	[+]	[+]
11	Xylose	+	+	+	-	[+]	[+]
12	Fructose	-	-	+	+	[+]	[+]

+ = Acid production [+] = Acid and gas production - = No acid and gas production
From the Table 3, it was found that most of the carbohydrates were utilized by the isolates.

Table 4. H_2S Production, Enzymatic activities and Oxidative and fermentative natureof the isolates.

Isolates	PP1	PP2	PP3	PP4	PP5	PP6
H_2S production	-	+	+	-	-	+
Oxidase production	+	+	+	+	+	+
Catalase production	+	+	+	+	+	+
Urease production	+	-	++	-	+	+
Amylase production	-	+	++	-	+	+
Gelatinase production	+	+	-	-	-	-
Protease production	-	-	++	-	-	++
Lipase production	-	-	++	-	-	+
Nitrate reductase production	+	+	++	++	+	++
Oxidative and/or fermentative nature						
Aerobic tube	+	+	+	+	+	+
Anaerobic tube	+	+	+	+	+	+

+ = Positive test, ++ = strongly positive test, - = Negative test
From the Table 4, isolates PP2, PP3 and PP6 are H_2S producing. Most of the isolates are producing above enzymes. All are facultative in nature.

Table 5. Phosphate solubilization, IAA production, Nitrogen fixation and Cellulose degradation abilities.

Test	Bacterial Isolates					
	PP1	PP2	PP3	PP4	PP5	PP6
Phosphate solubilization	+	+	+	+	+	+
IAA production	++	+	+	++	++	++
Nitrogen fixation	+	+	+	+	+	+
Cellulose degradation (with pesticide)	+	+	++	+	-	-
Cellulose degradation (without pesticide)	-	-	++	-	+	-
Effect of association of isolates on soil microorganisms						
Azotobacter spp.	-	-	-	-	-	-
Rhizobium spp.	-	-	-	-	-	-

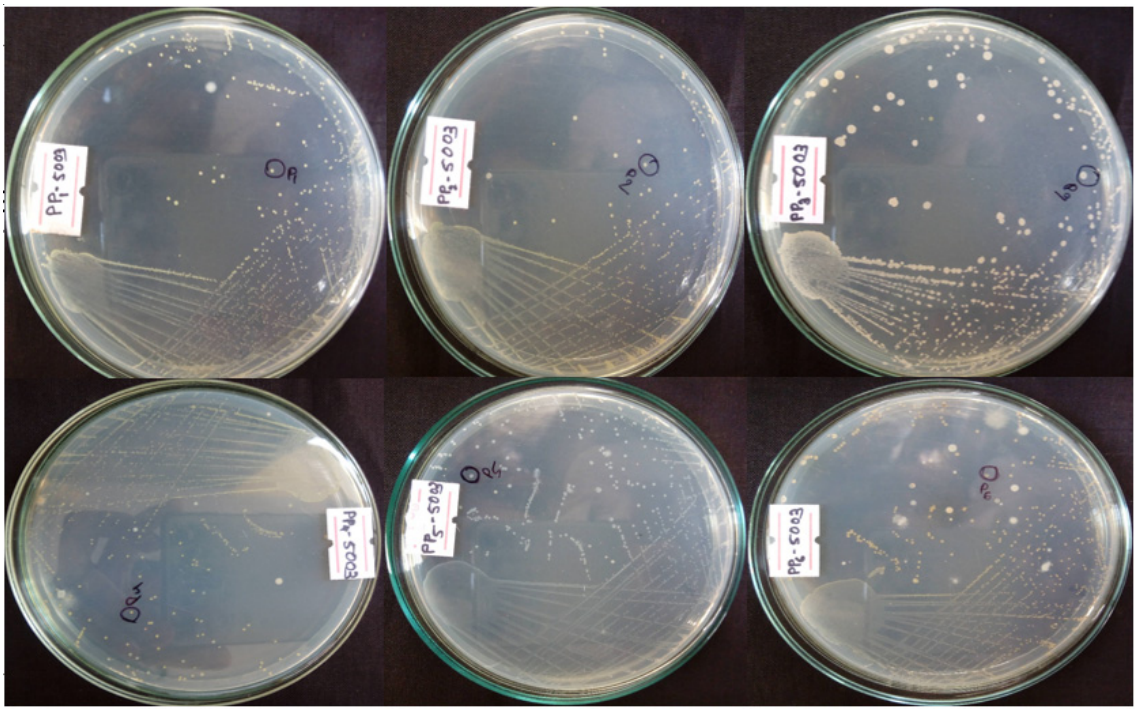
+ = Positive test ++ = strongly positive test - = Negative test.

coloured and PP5 and PP6 were colourless. PP1 and PP2 were flat but rests were raised colonies. PP1 and PP6 were wavy and remaining were entire colonies. PP1, PP3 and PP4 were translucent colonies but PP2, PP5 and PP6 were transparent colonies.

From 2, it is clear that all the isolates were Gram positive cocci, non-spore formers and non-capsulated. PP1 and PP2 were motile, PP3, PP4 and PP5 were actively motile and PP6 was non-motile.

It can be seen from Table 5 that all the isolates are Phosphate solubilizing, IAA producing, nitrogen fixing and had no effect on *Azotobacter* spp. and *Rhizobium* spp. Isolate PP3 degrades cellulose in presence and absence of dimethoate. PP1, PP2 and PP4 degraded cellulose in presence of dimethoate. PP5 degraded cellulose in absence of dimethoate.

From the Table 6 it was clear that higher concentration of dimethoate showed effect on the isolates. All the isolates could tolerate 0.6% dimethoate. 0.7% dimethoate could be tolerated by the isolates PP1



Photograph 1: Pesticide resistant phosphate solubilizing bacterial isolates PP1, PP2, PP3, PP4, PP5 and PP6 on Katznelson's and Bose agar after 48hrs of incubation at 28°C.

Table 6. Results of Pesticide tolerance of the isolates.

MIC of Dimethoate (%)	(average of triplicates)			Bacterial isolates		
	PP1	PP2	PP3	PP4	PP5	PP6
0.5	+	+	+	+	+	+
0.6	+	+	+	+	+	+
0.7	+	-	+	-	-	-
0.8	-	-	+	-	-	-

+ = Growth - = No growth

Table 7. Results of seed germination test. [Average of triplicates]

Concentration of dimethoate used	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%	0.7%	0.8%	0.9%	1.0%
Seeds germinated	22	6	10	22	6	6	4	5	2	0
% germinated	88	24	40	88	24	24	16	20	8	0

Table 8. Effects of isolates on plant growth promotion.

Characters	Bacterial isolates [Measurements without pesticide]							
	PP1	PP2	PP3	PP4	PP5	PP6	Control	Mixed
Height of shoot (cm)	10.8	10.1	12.3	9.5	10.4	11.6	13.8	13
Height of root (cm)	2.6	2.4	1.8	1.8	2.3	3.3	2.6	2.6
No. of leaves	2	2	3	2	2	2	2	2
Extent of branching at root	+	+	++	++	+	++	+	++
Dry weight (g) of plant	0.01	0.005	0.006	0.006	0.01	0.01	0.01	0.01
Measurements in presence of pesticide								
Height of shoot (cm)	12	13	12.5	11.8	12.3	10.7	11.6	12.5
Height of root (cm)	3.1	2.5	3.1	3.7	2.7	2.6	3.1	2.7
No. of leaves	2	2	3	2	2	2	2	3
Extent of branching at root	+++	++	+	+++	++	++	+	++
Dry weight (g) of plant	0.01	0.007	0.01	0.015	0.01	0.008	0.008	0.013

and PP3. But 0.8% of dimethoate could be tolerated by the only isolate PP3.

From the Table 7 it was clear that 0.9% of dimethoate was the highest concentration at which the moth bean showed growth.

From the Table 8, isolate PP6, mixed culture and control shows efficient growth in absence of pesticide. In presence of pesticide, all the isolates except PP5, shows good result.

Conflict of Research

No conflict of research.

Conclusion

During the course study of this project, the soil sample from region near Valva, Sangli were used for isolation of pesticide resistant phosphate solubilizing bacteria. Six Pesticide resistant phosphate solubilizingbacterial isolates were obtained namely PP1, PP2, PP3, PP4, PP5 and PP6. The minimum inhibitory concentration was determined to be 0.4%. All the bacterial isolates were Gram positive cocci, non-spore formers and non-capsulated. All were facultative and showed fermentation of various sugars. All produced catalase and oxidase, fixed nitrogen and producedindole. All showed dimethoate tolerance up to 0.6%, but the isolate PP1 tolerated 0.7% and PP3 tolerated 0.8% dimethoate. Isolate PP3 could degrade cellulose both in presence and absence of dimethoate. None of them showed any effect on *Azotobacter* spp. and *Rhizobium* spp. Isolates PP3 and PP6 showed all the enzyme production except urease. Isolate PP3 showed most of the tests positive. All of them were agriculturally important.

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