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Evaluation of compatibility of native plant growth promoting rhizobacterial isolates against chemical pesticides

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ABSTRACT

Conventional agriculture relies heavily on chemical fertilizers, pesticides and weedicides to control plant diseases, pests and weeds to improve production. Consequently, the soil receives the bulk of complex agrochemical compounds many of which are poisonous to the activity of non-target beneficial soil micro-organisms. There is a need to check the tolerance of biocontrol agents like Plant Growth Promoting Rhizobacterial (PGPR) to various pesticides before their practical application. Taking this into account, the investigation was conducted for the screening of the PGPR isolates against chemical pesticide degradation potentialities in Plant Bacteriological Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during 2021-22. All the native rhizobacterial, *Bacillus* and *Fluorescent pseudomonad* isolates tested had good potentiality to tolerate Carbendazim and Glyphosate. Among native *Bacillus* isolates, BRB89, BRB42, PR 19, PR 20, BRB 52, BRB 56, BRB 74, and SM 9 showed moderate tolerance, whereas among all the native *Fluorescent pseudomonads*, all except GP2 showed high tolerance against Paraquat. All native *Bacillus* isolates except PR16 were highly sensitive against Chorpyriphos. However, all the native fluorescent pseudomonads showed tolerance against Chorpyriphos even at 2000 ppm concentration.

Key words: *Chemical pesticides, compatibility, Plant growth-promoting rhizobacteria*

Introduction

Plant growth-promoting rhizobacteria (PGPR)are the important biocontrol agents (Abdallah *et al.,* 2018) and effective in reducing both abiotic and biotic stresses (Mishra *et al.,* 2017). They competitively colonize the roots of plant and can enhance plant growth (Ramadan *et al.,* 2016). PGPRs, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Pantoea*, *Bacillus*, *Serratia* and *Rhizobium,* have shown an ability to improve plant growth (Verma *et al.,* 2019). Among these, species of *Bacillus* and *Pseudomonas* are predominant because of their distinctive plant growth promoting characteristics (Karnwal, 2017). Biofertilization, phytostimulation and biological characteristics can be exploited to develop formulations for management of several phytopathogens, enhancement of yield and food production by using fewer resources and less reliance on the chemical fertilizers and pesticides (Bhattacharyya and Jha, 2012). Because of the broad-host range of pest and

pathogens, changing climates, high prices of agrochemicals and ecological crises, devising multi-purpose bio-formulations will be a more practical strategy for integrated pest and nutrient management. They also reduce the application of chemical fertilizers which would be economically feasible and ecofriendly for lower production cost as well as recognize the best management practices of soil and crop to achieve more sustainable agriculture as well as fertility of soil. Variations in the PGPR are bound to occur in different agro-climatic regions. Some native rhizobacterial isolates may not be compatible with different pesticides that adversely affects their effectiveness. There is a need to check the compatibility of different rhizobacteria with native strains before their practical application. Moreover, there is no information available on compatibility of different native rhizobacteria with the commonly used pesticides particularly in this agroclimatic zone. Keeping this in mind, the present investigation was undertaken to evaluate the compatibility of native plant growth promoting rhizobacterial isolates against chemical pesticides.

Materials and Methods

The investigation was carried out *in-vitro* in Plant Bacteriological Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal during 2021-22. The preparation of media, sterilization, isolation and maintenance of microbial cultures, etc., were done following the method developed by Dhingra and Sinclair (1995) and Aneja (2003) with slight modification.

Collection of native plant growth promoting rhizobacteria: Thirteen isolates of fluorescent pseudomonads and thirteen isolates of *Bacillus* sp., were obtained from the Plant Bacteriological Laboratory, Department of Plant Pathology, BCKV and these strains were maintained by frequent sub-culturing after 30 days interval and stored at 4 °C in the test tube slants of Nutrient Agar (NA) media. The isolates with their identified species are listed in Table 1.

Test of compatibility of native rhizobacterial isolates against commonly used chemical pesticides: Compatibility of bacterial test isolates with commonly used chemical pesticides like fungicides (Carbendazim 50% WP), herbicides (Paraquat dichloride 24% SL, and Glyphosate 71% SG) and insecticides (Chlorpyriphos 20% EC) were tested at different concentrations *viz*., 0 ppm (control), 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. The pesticides were added to the Potato Dextrose Agar (PDA) media at above concentrations and all the rhizobacteria were streaked over the solidified plates. The plates were maintained at 27±1°C for 2 days and growth on different concentrations were compared with control plates (no pesticides). The growth of the isolates was measured under different concentrations of different chemical pesticides in qualitative manner (++: Profuse growth; +: medium growth to poor growth; -: no growth). After incubation of 48 hrs at 27 ± 1°C, growth of colonies was visualized and marked as (++/ + /-).

Table 1. List of native rhizobacterial isolates

Native *Bacillus* isolates	Name of Rhizobacteria	Native Fluorescent Pseudomonads	Name of Rhizobacteria
BRB 88	*Bacillus subtilis* subsp.*subtilis*	BCLP4	*Pseudomonas fluorescens*
BRB 89	*Bacillus pumilus*	CK2LPP	——–
BRB 35	*Bacillus altitudinis*	CK2LP8	——–
BRB 42	*Bacillus rugosus*	CK2LP12	——–
BRB 52	*Bacillus pumilus*	GP2	*Pseudomonas aeruginosa*
BRB 56	*Bacillusamyloliquefaciens*	GP8	*Pseudomonas aeruginosa*
BRB 74	*Bacillus subtilis*	G11SP37	*Pseudomonas geniculata*
PR 16	*Bacillus australimaris*	G15SP38	*Pseudomonas putida*
PR 18	——–	K11SP4	*Pseudomonas baetica*
PR 19	*Bacillus subtilis* subsp. *subtilis*	K22SP8	*Pseudomonas fluorescens*
PR 20	*Bacillus subtilis*	SS2PP	——–
SM 9	——–	SS2LP	——–
SM 14	——–	S21SP14	*Pseudomonas putida*

Results and Discussion

Compatibility with fungicides: All the tested native rhizobacterial isolates were resistant to Carbendazim 50% WP (Table 2) and profuse growth (++) was recorded for all the native rhizobacterial isolates at 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm concentrations. Thus, the present findings indicated that the native rhizobacterial isolates exhibited enhanced tolerance against Carbendazim 50% WP even at 2000 ppm concentration. This is in agreement with Mishra *et al.,* (2013) who reported that the plant growth-promoting rhizobacteria were compatible with fungicides. Madhavi (2006) stated that Carbendazim was found to be compatible at their recommended and half the recommended dosages with *Pseudomonas fluorescens* strains *in-vitro.*

Compatibility with herbicides: The native rhizobacterial isolates were tested with the two commonly used herbicides, Paraquat dichloride 24% SL and Glyphosate 71% SG. The growth of all the *Bacillus* isolates were inhibited (-) by Paraquat even at 500 ppm concentration and some of the *Bacillus* isolates *viz*., BRB 89 (500-1500 ppm); BRB 42 (500-2000 ppm); PR 19, PR 20, BRB 52, BRB 56, BRB 74, and SM 9 (500 ppm), showed moderate growth (+) (Table 3). But fluorescent pseudomonad isolates (except GP2) were found tolerant and exhibited profuse growth (++) even at 2000 ppm concentration. The native rhizobacterial isolates were found resistant (++) to

Table 2. Effect of different concentrations of Carbendazim on native rhizobacterial isolates

Native *Bacillus* sp.	Concentration (ppm)					Native Fluorescent Pseudomonads	Concentration (ppm)				
	0	500	1000	1500	2000		0	500	1000	1500	2000
BRB 88	++	++	++	++	++	BCLP4	++	++	++	++	++
BRB 89	++	++	++	++	++	CK2LPP	++	++	++	++	++
BRB 35	++	++	++	++	++	CK2LP8	++	++	++	++	++
BRB 42	++	++	++	++	++	CK2LP12	++	++	++	++	++
BRB 52	++	++	++	++	++	GP2	++	++	++	++	++
BRB 56	++	++	++	++	++	GP8	++	++	++	++	++
BRB 74	++	++	++	++	++	G11SP37	++	++	++	++	++
PR 16	++	++	++	++	++	G15SP38	++	++	++	++	++
PR 18	++	++	++	++	++	K11SP4	++	++	++	++	++
PR 19	++	++	++	++	++	K22SP8	++	++	++	++	++
PR 20	++	++	++	++	++	SS2PP	++	++	++	++	++
SM 9	++	++	++	++	++	SS2LP	++	++	++	++	++
SM 14	++	++	++	++	++	S21SP14	++	++	++	++	++

Table 3. Effect of different concentrations of Paraquat on native rhizobacterial isolates

Native *Bacillus* sp.	Concentration (ppm)					Native Fluorescent Pseudomonads	Concentration (ppm)				
	0	500	1000	1500	2000		0	500	1000	1500	2000
BRB 88	++	-	-	-	-	BCLP4	++	++	++	++	++
BRB 89	++	+	+	+	-	CK2LPP	++	++	++	++	++
BRB 35	++	-	-	-	-	CK2LP8	++	++	++	++	++
BRB 42	++	+	+	+	+	CK2LP12	++	++	++	++	++
BRB 52	++	+	-	-	-	GP2	++	-	-	-	-
BRB 56	++	+	-	-	-	GP8	++	++	++	++	++
BRB 74	++	+	-	-	-	G11SP37	++	++	++	++	++
PR 16	++	-	-	-	-	G15SP38	++	++	++	++	++
PR 18	++	-	-	-	-	K11SP4	++	++	++	++	++
PR 19	++	+	-	-	-	K22SP8	++	++	++	++	++
PR 20	++	+	-	-	-	SS2PP	++	++	++	++	++
SM 9	++	+	-	-	-	SS2LP	++	++	++	++	++
SM 14	++	-	-	-	-	S21SP14	++	++	++	++	++

Glyphosate at 500-2000 ppm concentrations (Table 4). The deleterious effect of Paraquat has been also reported by Adomako and Akyempong (2016) as its treatment resulted in reduction in the beneficial bacterial population in soil. Busse *et al.* (2001) reported that Glyphosate had no effects on PGPR. *Bacillus* sp. and fluorescent pseudomonads are the most efficient organisms in degrading the organic compounds to fulfill their nutritional requirements (Ermakova *et al.*, 2010; Abdel-Hadi *et al.*, 2012; Kryuchkova *et al.*, 2014).

Compatibility with insecticides: All the native rhizobacterial isolates fluorescent pseudomonads (13) and *Bacillus* sp. (13) was screened for their compatibility with insecticide, Chlorpyriphos 20% EC. Results revealed that the growth (++) of PGPR fluorescent pseudomonad isolates were not inhibited by Chlorpyriphos at all the concentrations indicating their compatibility with the native fluorescent pseudomonads used in the present study (Table 5). Hence, any of these isolates can be used in combination with any of the above insecticides for development of integrated pest management package. However, Chlorpyriphos drastically inhibited the growth (-) of all native *Bacillus* isolates except PR 16 (++) isolate (Table 5). So, the native *Bacillus* isolates should be used in IPM package along with insecticides for pest and disease management. Enhanced degradation of Chlorpyriphos by *Enterobacter* strain followed by *Pseudomonas fluorescens* and *Bacillus subtilis* was

Table 4. Effect of different concentrations of Glyphosate on native rhizobacterial isolates

Native *Bacillus* sp.	Concentration (ppm)					Native Fluorescent Pseudomonads	Concentration (ppm)				
	0	500	1000	1500	2000		0	500	1000	1500	2000
BRB 88	++	++	++	++	++	BCLP4	++	++	++	++	++
BRB 89	++	++	++	++	++	CK2LPP	++	++	++	++	++
BRB 35	++	++	++	++	++	CK2LP8	++	++	++	++	++
BRB 42	++	++	++	++	++	CK2LP12	++	++	++	++	++
BRB 52	++	++	++	++	++	GP2	++	++	++	++	++
BRB 56	++	++	++	++	++	GP8	++	++	++	++	++
BRB 74	++	++	++	++	++	G11SP37	++	++	++	++	++
PR 16	++	++	++	++	++	G15SP38	++	++	++	++	++
PR 18	++	++	++	++	++	K11SP4	++	++	++	++	++
PR 19	++	++	++	++	++	K22SP8	++	++	++	++	++
PR 20	++	++	++	++	++	SS2PP	++	++	++	++	++
SM 9	++	++	++	++	++	SS2LP	++	++	++	++	++
SM 14	++	++	++	++	++	S21SP14	++	++	++	++	++

Table 5. Effect of different concentrations of Chlorpyriphos on native rhizobacterial isolates

Native *Bacillus* sp.	Concentration (ppm)					Native Fluorescent Pseudomonads	Concentration (ppm)				
	0	500	1000	1500	2000		0	500	1000	1500	2000
BRB 88	++	-	-	-	-	BCLP4	++	++	++	++	++
BRB 89	++	-	-	-	-	CK2LPP	++	++	++	++	++
BRB 35	++	-	-	-	-	CK2LP8	++	++	++	++	++
BRB 42	++	+	-	-	-	CK2LP12	++	++	++	++	++
BRB 52	++	-	-	-	-	GP2	++	++	++	++	++
BRB 56	++	+	-	-	-	GP8	++	++	++	++	++
BRB 74	++	-	-	-	-	G11SP37	++	++	++	++	++
PR 16	++	++	++	++	++	G15SP38	++	++	++	++	++
PR 18	++	-	-	-	-	K11SP4	++	++	++	++	++
PR 19	++	-	-	-	-	K22SP8	++	++	++	++	++
PR 20	++	-	-	-	-	SS2PP	++	++	++	++	++
SM 9	++	-	-	-	-	SS2LP	++	++	++	++	++
SM 14	++	-	-	-	-	S21SP14	++	++	++	++	++

reported by Singh *et al.,*(2003). *Pseudomonas* sp. (ChlD), isolated from agricultural soil by enrichment culture technique, was capable of producing biosurfactant (rhamnolipids) and degrading Chlorpyriphos (Singh *et al.,* 2009). *Bacillus* sp., including *Bacillus pumilus* (Anwar *et al.,* 2009), and *Bacillus subtilis* strain Y242 (El-Helow *et al.,* 2013) were also demonstrated to show almost complete degradation in soil contaminated with Chlorpyriphos

Conclusion

All the native rhizobacterial isolates had good potentiality to tolerate Carbendazim and Glyphosate even at high concentration (2000 ppm). *Bacillus* isolates, BRB 89, BRB 42, PR 19, PR 20, BRB 52, BRB 56, BRB 74, and SM 9, showed moderate tolerance and all the native fluorescent pseudomonads except GP2, showed high tolerance against Paraquat. All native *Bacillus* isolates except PR 16, were highly sensitive against Chlorpyriphos. However, all the native fluorescent pseudomonads showed tolerance against Chlorpyriphos even at 2000 ppm concentration. Integration of pesticide tolerant PGPR in integrated disease management package is necessary for management of different diseases of crops. Nanoencapsulation technology might be utilized as a multipurpose tool to protect PGPR, enhancing their shelf life, dispersion, controlled release and stress alleviating activities.

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Conflict of Interest: There is no competing interest exists.

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