RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Effect of Pesticides on the Phosphate Solubilization Capacity of Microbial Isolate

KEYWORDS: Phosphorus, Phosphate solubilizer, pesticides, *Citrobacter freundii*

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ABSTRACT *Phosphorus (P) is one of the essential elements for all biological entities and an adequate supply of P in the early stages of plant growth is important for the proper growth and development of plants. Many microbes having phosphate solubilizing ability have been used as the soil inoculants to improve the uptake of phosphorus by plants. The use of pesticides is becoming very common in agriculture as plant protection measures. A potential inoculant organism must be tested thoroughly in lab under the conditions that will be experienced by the farmers. The major concern regarding the use of pesticides is that their use may adversely affect non - target soil microflora and fauna. Phosphate solubilizing micro-organisms which are useful soil inhabiting microflora and of importance to soil fertility may be affected by some insecticides and herbicides. The isolate, Citrobacter freundii, used in present study is an efficient phosphate solubilizer and its effect on plants in presence as well as in absence of select pesticides was studied.*

Introduction

Phosphate solubilizing microorganisms include bacteria and fungi which convert insoluble inorganic phosphatic compounds into soluble form. The use of pesticides is becoming very common in agriculture as plant protection measures and during storage of food grains. The use of organophosphorus and carbamate insecticides has been increasing whereas the use of chlorinated insecticides has decreased as these are very stable and persist in the environment for a long time (Vimal Ramani and H.H.Patel,2011). A potential inoculant organism must be tested under conditions that will be experienced by the farmers (Leggett *et al.,* 1998). Although scientists continue to identify less persistent agrochemicals, microbiologists are concerned regarding their use, because some of these chemicals may adversely affect non-target soil microflora and fauna. Phosphate solubilizing micro-organisms which are useful soil inhabiting microflora and of importance to soil fertility may be affected by some insecticides and herbicides (Gaur, 1990). Raghu and Mac Rae (1967) found an increase in the population of anaerobic phosphorus solubilizers after treatment of soil with lindane. Effect of pesticides on microbial solubilization of rock phosphate in Pikovskaya medium was studied by Gaur (1990). *Pseudomonas striata* significantly augmented rock phosphate solubilization. Maximum phosphorus solubilization by the bacteria was obtained in the presence of carbofuran followed by oxyfluorfen at normal dosage. In a similar study the plant growth promoting traits of the strain *Pseudomonas aeruginosa* decreased consistently as the concentrations of each pesticide was increased from the recommended dose to the higher ones (Ahemad M,Khan MS,2011). Several studies have indicated augmented phosphate solubilization in presence of few pesticides and herbicides, while many other workers have reported significant decrease in phosphate solubi lization efficiency.

In the present study attempt has been made to study the effect of various pesticides, which are used commonly by the farmers of this region of Madhya Pradesh on phosphate solubilizaton by *C. freundii.*

Materials & Methods

The culture *Citrobacter freundii* was maintained on Pikovskaya's agar pH 7.2 at 4°C and sub cultured every month.

Inoculum and Incubation

Overnight grown culture of *Citrobacter freundii* in nutrient broth was harvested by centrifugation and resuspended in broth to have 1 O.D. mL-1at 660nm.Inoculum thus prepared was transferred under aseptic conditions to 100 mL Pikovskaya's broth in 250 mL Erlenmeyer conical flasks. In one experimental set up the broth contained TCP 50 mg% equivalent to 114.5 mg P_2O_5 100 mL-1. To study the phosphate solubilization from URP (27.6 mg% P_2O_5), it was added equivalent to 50 mg P_2O_5 100 mL-1. The flasks were incubated at 28°C ± 2°C under static conditions with intermittent shaking at 12hr interval up to 7 and 15 days for TCP and URP respectively.

Phosphate estimation

Soluble phosphorus from the broth was estimated using chlorostannous reduced molybdophosphoric acid blue method as it is reported to be highly sensitive and satisfactory method. (Jackson M L,1973).

Culture media

Pikovskaya's broth containing 50 mg % TCP and Modified Pikovskaya's broth medium where TCP in Pikovskaya's broth medium was replaced by URP equivalent to 50 mg % P_2O_5 was used.

The required numbers of flasks with 100 ml of media mentioned above were sterilized. To each of these flasks the pesticides (malathion, chloropyriphos, dimethoate, monocrotophos, dicofol and phosphomidon) in the quantity of "recommended dose" as mentioned on the commercial packet and double the quantity of "recommended dose" (double dose) were added aseptically to separate flasks. The flasks containing Pikovskaya's broth and modified pikovskaya's broth without added pesticides were kept as control.

Results and Discussions

The effect of various pesticides on TCP solubilization by *C. freundii* is shown in Table 1. The results show significant decrease in phosphate solubilizing activity in presence of most of the pesticides under study. Maximum phosphate solubilizing activity was affected by both the single as well as double dose of the "recommended dose" of pesticides. However, in presence of pesticide phosphomidon, the organism shows

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

better solubilization of TCP as compared to control.

Table 1
Effect of different pesticides on TCP solubilization

Treatments/ Dose	P solubilized as mg % P_2O_5		
	Days of incubation		
	5	10	15
C.freundii (C)	59.21±0.67	30.36±0.34	22.50± 0.48
C + Mal X	8.69±0.52	9.66±0.27	17.58±0.86
C + Mal 2X	7.53±0.55	8.32±0.94	15.41±0.86
C + Chl X	8.67±0.53	16.53±1.07	17.58±0.86
C + Chi 2X	6.39±0.77	14.32±0.54	17.68±0.86
C + Dmt X	11.11±0.24	7.66±0.37	17.92±0.13
C + Dmt 2X	0.00	7.80±0.29	22.19±0.25
C + Mono X	13.73±0.56	9.59±0.22	26.72±0.42
C + Mono2X	8.90±0.16 10.13±0.29	12.35±0.98	18.16±0.41
C + Dico X	9.32±0.76	14.68±0.63	17.41±0.59
C + Dico 2X	59.81±0.79	12.86±0.24	16.98±0.22
C + Pho X	60.41±0.38	47.51±0.65	43.21±0.86
C + Pho 2X		51.32±0.20	53.32 ±2.08

Mal= malathion, Mono = monocrotophos Chl= chlorpyriphos, Dico=dicofol, Dmt= dimethoate Pho = phosphomidon

X = recommended dose

2X = double dose

The maximum TCP solubilization occurred in the presence of single and double dose of malathion, chloropyriphos, dimethoate, monocrotophos and dicofol on 15th day while for phosphomidon it occurred on 5th day.

For all pesticides under study only phosphomidon did not affect phosphate solubilization adversely, rather it showed better activity as compared to controls. Malathion showed maximum solubilization up to 17.58 mg % with single dose on 15th day which was very low as compared to control where maximum solubilization is 59.21 mg % on 5th day. Similarly, chloropyriphos produces maximum solubilization with single dose (17.58 mg %) and double dose (17.68 mg %) on 15th day. Dimethoate also adversely affects TCP solubilization. Maximum activity is obtained on 15th day at double recommended dose (22.19 mg %). Monocrotophos showed maximum activity at single dose (26.72 mg %). Dicofol also showed maximum solubilization of TCP at single dose (17.41 mg %). Maximum TCP solubilization activity occurred in presence of double the recommended dose of phosphomidon (60.41 mg %) which was more than control. Thus this was the only pesticide which did not adversely affect phosphate solubilization.

TCP solubilization in the presence of different pesticides (recommended dose) can be arranged in following decreasing order:

Phosphomidon > monocrotophos > dimethoate > chloropyriphos > malathion > dicofol.

Effect of pesticides on URP solubilization:
Table 2 presents URP solubilization by *Citrobacter freundii* in the presence of various pesticides in a single and double dose. URP solubilization is also adversely affected by the presence of pesticides except that phosphomidon and monocrotophos showed good solubilization. In presence of phosphomidon maximum URP solubilization (11.11 mg %) was obtained on 5th day. Maximum URP solubilization (13.15 mg %) in presence of monocrotophos occurred on 15th day. Maximum solubilization (7.31 mg %) of URP in presence of malathion occurred on 15th day at single dose. In presence of chloropyriphos maximum activity (6.96 mg%) was observed at double dose on 5th day. Dimethoate showed maximum solubilization up to 4.82 mg % at single dose on 10th day. Monocrotophos showed maximum solubilization (13.15mg %) at single dose on 15th day. In presence of dicofol very less URP was solubilized up to 3.93 mg % on 10th day at single dose. At double dose of dicofol no solubilization was obtained on 15th day. In presence of phosphomidon maximum solubilization (11.11 mg %) of URP was obtained on 5th day at single dose.

URP solubilization in presence of different pesticides (recommended dose) can be arranged in following decreasing order:

Monocrotophos > phosphomidon > malathion > chlorpyiphos > dimethoate > dicofol.

TABLE 2
Effect of different pesticides on URP solubilization

Treatments/ Dose	P solubilized as mg % P_2O_5		
	Days of incubation		
	5	10	15
C. freundii (C)	15.91±0.22	10.12±0.33	9.62±1.16
C + Mal X	4.32±0.78	5.62±0.35	7.31±0.56
C + Mal 2X	2.59±0.35	5.31±0.29	2.44±0.35
C + Chl X	5.42±0.36	4.01±0.62	3.62±0.52
C + Chi 2X	6.96±0.47	3.98±0.59	2.32±0.28
C + Dmt X	2.32±0.56	4.82±0.53	3.63±0.53
C + Dmt 2X	0.00	3.88±0.48	2.10±0.43
C + Mono X	10.18±1.19	9.45±0.82	13.15±0.26
C + Mono 2X	9.86±0.29	11.00±0.27	6.34±0.59
C + Dico X	2.36±0.25	3.93±0.54	1.81±0.19
C + Dico 2X	1.08±0.4	0.14±0.00	0.00
C + Pho X	11.11±0.24	10.36±0.61	6.72±0.88
C + Pho 2X	10.81±0.52	9.63±0.15	4.44±0.46

CONCLUSIONS
The studies on phosphate solubilization in presence of pesticides by *Citrobacter freundii* revealed that most of the pesticides used by the farmers in the field exert negative effect on solubilization although the isolate in the present study could survive the double recommended dose of pesticides but its phosphate solubilizing activity was drastically and adversely affected by the presence of the pesticides. The results are in contrast to the studies performed on Pseudomonas species by Gaur and his co-workers.

Acknowledgments
The author is highly indebted to the support provided by Dr.H.H.Patel (Bhavnagar) and Dr.K.C.Patel (Vallabh Vidyanagar).

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

REFERENCE [1]Ahemad, M.,Khan, M.S. (2011), "Toxicological assessment of selective pesticides towards plant growth promoting activities of phosphate solubilizing Pseudomonas aeruginosa." Acta Microbiol Immunol Hung., 58(3):169-87. | [2]Gaur, A. C. (1990), "Phosphate solubilizing microorganisms as biofertilizer." Omega scientific publishers, New Delhi. | [3] Jackson, M. L. (1973), In: "Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd." New Delhi, pp 134-182. | [4] Leggett, M., Gleddie, S. and Holloway, G. (1998), "Phosphate solubilizing microorganisms and their use". Summary, Workshop on New concepts in plant nutrient acquisition held at Ibavaki, Japan. | [5]Raghu, K. and Mac Rae, I. C. (1967), "The effect of gamma isomer of BHC upon the microflora of submerged rice soils. II. Effect on nitrogen mineralization and fixation of selected bacteria." Can. J. Microbiol. 13: 621. | [6]Vimal Ramani and H.H.Patel (2011), "Phosphate solubilization by Bacillus sphaericus and Burkholderia cepacia in presence of pesticides." Journal of Agricultural Technology, 7(5): 1331-1337.