Pesticide Biochemistry and Physiology 100 (2011) 51–56

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Effects of insecticides on plant-growth-promoting activities of phosphate solubilizing rhizobacterium *Klebsiella* sp. strain PS19

Munees Ahemad, Mohammad Saghir Khan *

Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202 002, UP, India

ARTICLE INFO

Article history:
Received 4 December 2010
Accepted 20 February 2011
Available online 23 February 2011

Keywords:
Klebsiella
Phosphate solubilization
Insecticide
Rhizosphere
Toxicity

ABSTRACT

In this study, four technical grade insecticides, fipronil, pyriproxyfen, imidacloprid and thiamethoxam were applied at the recommended and the higher doses to investigate their effects on plant growth-promoting activities of phosphate-solubilizing *Klebsiella* sp. strain PS19, isolated from mustard rhizosphere. All tested insecticides displayed a concentration-dependent inhibition in plant growth promoting traits, like, inorganic phosphate solubilization, biosynthesis of phytohormones and siderophores, of rhizobacterial strain PS19. For example, the phosphate-solubilizing activity of *Klebsiella* sp. PS 19 was reduced maximally by 95%, at 3900 $\mu g\, l^{-1}$ pyriproxyfen over control. At the recommended rate, the magnitude of toxicity of insecticides to plant growth promoting traits was less severe compared to the higher doses. The sequence of insecticide-toxicity expressed as percent decrease, determined at highest dose rate of each insecticide, over control was: pyriproxyfen (95) = imidacloprid (95) > thiamethoxam (94) > fipronil (85), for phosphate-solubilizing activity while for salicylic acid (SA) it was: thiamethoxam > pyriproxyfen = imidacloprid > fipronil. The impact of the highest dose rate of insecticides on 2,3-dihydroxybenzoic acid (DHBA) was almost equal to those observed for SA. Thiamethoxam decreased the indole acetic acid (IAA) synthesis maximally by 86% whereas fipronil had least toxicity and reduced it by 67% relative to the control. Among the experimental insecticides, pyriproxyfen at 3900 $\mu g\, l^{-1}$ in general, had the greatest toxic effects for plant growth promoting activities of the test strain. The study inferred that insecticides affect the plant beneficial activities of rhizobacteria adversely. These findings are likely to add a new insight into the pest management practices.

1. Introduction

Soil is a complex ecosystem established and stabilized by several diverse interactions involving biotic and abiotic constituents. Among the biotic components, the heterogeneous microbial flora and their associated functional activities have a crucial impact on soil fertility mediated by metabolic processes leading to improved plant growth [1–2]. Among microbes, a group of bacteria often termed PGPR (plant-growth-promoting rhizobacteria), facilitate the plant growth through N_2 fixation, solubilization of insoluble phosphate (P), production of siderophores, synthesizing phytohormones, lowering of ethylene concentration, production of antibiotics and antifungal metabolites and induced systemic resistance [3–5]. However, accumulation of variable pesticides into soils beyond certain threshold levels due to its frequent application in fields to overcome different noxious and resistant pests is detrimental to both rhizosphere microorganisms and their activities [6–7].

Although *Klebsiella* has been used as a versatile model to study a range of N_2-fixation processes and other mechanisms, the plant growth promoting traits of *Klebsiella* species of rhizosphere-niche have however, not been comprehensively explored. In addition, studies on the effect of various pesticides have largely been focused on assessing/identifying changes in populations of soil microflora including PGPR. However, the reports on *in vitro* plant growth promoting activities of PGPR specifically *Klebsiella* sp. in the presence of pesticides are rare. Considering these scientific gaps, the present study was, therefore, designed to evaluate the effects of four insecticides, fipronil, pyriproxyfen, imidacloprid, thiamethoxam, at recommended (1×), double (2×) and three times (3×) the recommended rates on the survival and *in vitro* plant growth promoting (PGP) activities of *Klebsiella* sp. as a model PGPR.

2. Materials and methods

2.1. Isolation and screening of phosphate solubilizing bacteria

Three rhizosphere soil samples (10 g each) of mustard (*Brassica campestris*) cultivated in experimental fields (alluvial sandy clay loam, sand 667 $g\, kg^{-1}$, silt 190 $g\, kg^{-1}$, clay 143 $g\, kg^{-1}$, organic

* Corresponding author. Fax: +91 571 2703516.
E-mail address: khanms17@rediffmail.com (M.S. Khan).

0048-3575/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2011.02.004

matter 6.2 g kg^{-1}, Kjeldahl N 0.75 g kg^{-1}, Olsen P 16 mg kg^{-1}, pH 7.2 and water holding capacity 0.44 ml g^{-1}, cation exchange capacity 11.7 and 5.1 cmol kg^{-1} anion exchange capacity) of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh (27°29′ latitude and 72°29′ longitude), Uttar Pradesh, India, were collected in sterile polythene bags (15 × 12 cm^2) and thoroughly mixed. In order to isolate the phosphate solubilizing bacteria, a serial dilution assay was carried out in 0.9% NaCl solution and 10 μl of diluted suspension was spread plated on Pikovskaya agar medium (g/l: glucose 10; $Ca_3(PO_4)_2$ 5; $(NH_4)_2SO_4$ 0.5; NaCl 0.2; $MgSO_4 7H_2O$ 0.1; KCl 0.1; yeast extract 0.5; $MnSO_4$ and $FeSO_4$ trace; agar 15; pH 7). Plates were incubated at 28 ± 2 °C for 7 days. The isolates showing clear halo within 7 days around bacterial colonies were considered as P-solubilizers. A total of 50 P-solubilizing isolates with maximum halo sizes and different pigmentations and morphological parameters were selected.

2.2. Assessment of bacterial strains for pesticide tolerance

The bacterial strains were tested further for their sensitivity/resistance to four insecticides, fipronil, pyriproxyfen, imidacloprid and thiamethoxam (Table 1) by agar plate dilution method using minimal salt agar medium (g/l: KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $MgSO_4{\cdot}7H_2O$ 0.2; $CaCl_2{\cdot}2H_2O$ 0.02; $FeSO_4{\cdot}7H_2O$ 0.01; agar 15; pH 6.5). The freshly prepared agar plates amended separately with increasing concentration (0–3200 μg ml^{-1}; at a two fold dilution interval) of insecticides were spot inoculated with 10 μl of 10^8 cells ml^{-1} of bacterial strains. Plates were incubated at 28 ± 2 °C for 3 days and the highest concentration of insecticides supporting bacterial growth was defined as the maximum tolerance level (MTL). Out of 50, a total of 18 bacterial isolates showing higher MTL values (>600 μg ml^{-1}) against most of the insecticides and greater halo size (>4 mm) were selected and maintained on solid Pikovskaya agar medium for further use.

2.3. Bacterial characterization

Among 18 bacterial strains, the strain PS19 showing higher MTL values and phosphate solubilization was further selected. The strain PS19 was identified by the morphological, physiological and biochemical tests which included Gram reaction, citrate utilization test, indole production test, methyl red test, nitrate reduction, Voges Proskaeur, catalase test, carbohydrates (dextrose, mannitol and sucrose) utilization test, starch hydrolysis, and gelatin liquefaction test. These tests were performed following the standard methods outlined in Bergey's Manual of Determinative Bacteriology [8].

2.4. 16S rDNA based identification

The sequencing of 16S rDNA of the strain PS19 was done commercially by DNA Sequencing Service, Macrogen, Inc., Seoul, South Korea using universal primers, 518F (5′CCAGCAGCCGCGGTAATACG3′) and 800R (5′TACCAGGGTATCTAATCC3′). Later, nucleotide sequence data was deposited in the Gen-Bank sequence database.

The online program BLAST was used to find out the related sequences with known taxonomic information in the databank at NCBI website (http://www.ncbi.nlm.nih.gov/BLAST) to accurately identify the strain PS19.

2.5. Bioassays of plant growth promoting activities under insecticide-stress

The PGP activities, P-solubilization, indole acetic acid, siderophore, exo-polysaccharides, hydrogen cyanide and ammonia production, of P-solubilizing bacteria were assayed both in the presence and absence of the selected insecticides under *in vitro* conditions.

2.5.1. Phosphate solubilization by bacterial strains

The bacterial strains showing P-solubilizing activity in the screening process were inoculated into Pikovskaya agar medium supplemented with 0, 1×, 2× and 3× of the recommended rate of each insecticide and incubated at 28 ± 2 °C for 7 days and observed for halo formation. The halo formed around the bacterial colony was measured. The bacterial strains were further used to determine the extent of P-solubilization in Pikovskaya broth by the chlorostannous-reduced molybdophosphoric acid blue method [9–10]. Briefly, 100 ml Pikovskaya broth treated with 0, 1×, 2× and 3× of each insecticide was inoculated with 1 ml of 10^8 cells ml^{-1} of each culture. The flasks were incubated for 7 days with shaking (120*g*) at 28 ± 2 °C. A 20 ml culture broth from each flask was removed and centrifuged (9000*g*) for 30 min and the amount of soluble P released into the supernatant was determined. To 10 ml of supernatant, 10 ml chloromolybdic acid and five drops of chlorostannous acid was added and volume was adjusted to 50 ml with distilled water. The absorbance of blue color developed was read at 600 nm. The amount of P solubilized was calculated using the calibration curve of KH_2PO_4. The change in pH following tri-calcium phosphate (TCP) solubilization was also recorded.

2.5.2. Bioassay for indole-3-acetic acid production

The indole-3-acetic acid (IAA) was quantitatively analyzed by the method of Gordon and Weber [11], later modified by Brick et al. [12]. Briefly, the phosphate solubilizing bacterial strains were grown in Luria Bertani (LB) broth (g l^{-1}: tryptone 10; yeast extract 5; NaCl 10 and pH 7.5). A-100 ml LB broth with 100 μg tryptophan ml^{-1} supplemented with 0, 1×, 2× and 3× of each insecticide was inoculated with 1 ml culture containing 10^8 cells ml^{-1} bacterial isolate and was incubated for 24 h at 28 ± 2 °C with shaking at 125 rpm. After 24 h, 5 ml of each culture was centrifuged at 9000*g* for 15 min and 2 ml of Salkowsky reagent prepared in 2% 0.5 M $FeCl_3$ in 35% perchloric acid, was added to 2 ml of supernatant and incubated at 28 °C in darkness for 1 h. The IAA concentration in the supernatant was determined using a spectrophotometer (λ 540 nm) against a standard curve.

Table 1
Insecticides used in the present study.

Common name	Grade (purity)	Chemical name	Chemical family	Recommended dose (μg l^{-1})
Fipronil	Technical (98%w/w)	5-Amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile	Phenylpyrazole	200
Pyriproxyfen	Technical (98%w/w)	4-Phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether	Juvenile hormone mimics	1300
Imidacloprid	Technical (100% EC)	(*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine	Pyridyl methylamine	100
Thiamethoxam	Technical (100%w/w)	(*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine	Thiazole	25