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Clothianidin Tolerant Plant Growth Promoting Bacteria Isolated From The Soil

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Abstract

Use of pesticides is incrementing that ensures food security at various levels for the growing population, during production, harvesting and storage. Along with the use of pesticides, the retention of pesticide residues in the environment possesses potential health issues and negatively influences the indigenous micro-organisms in the soil. In the present study two types of soil samples i.e. soil without exposure of pesticide and soil exposed to pesticide (clothianidin) were selected for the isolation of bacteria. A total of 40 bacterial strains (10 isolates from non exposed soil and 30 isolates from exposed soil) were isolated which were subjected to pesticide (Clothianidin) tolerance test. Out of 40 isolates, 12 isolates (2 from non-exposed and 10 from exposed soil) showed tolerance to pesticide up to 100 mM concentration. Further, these 12 isolates were grown on Jensen's media and Pikovskayas media to detect the nitrogen fixing and phosphate solubilisation acitivity. Out of 12 isolates, 10 isolates were able to grow on Jensen's media whereas only two isolates (C1 and P7) showed phosphate solubilisation on Pikovskayas media. The isolate P7 was found to be a most efficient isolate and based on biochemical characterization it was identified as *Bacillus* sp. The results of the present showed that *Bacillus* isolate (P7) is a potential candidate for the development of biofertilizer formulation.

Keywords: Pesticide tolerant, clothianidin, nitrogen fixer, phosphate solubilizer.

INTRODUCTION

Soil is an amalgam of various macro and micro nutrients that supports diverse form of life. It acts as a habitat for numerous animals ranging from small to enormous size and plants as well [1]. All these forms interact with each other in a specific manner with respect to different parameters of soil. The soil parameters are responsible for the productivity of soil that supports plant growth, these plants can act as a source of food for the growing population [2]. Soil is an eminent habitat that inhabitant both producers and decomposers thus regulating the nutrient cycle [3]. With increase in population demand of food increases day by day showing need of food security that includes improving the quality of food grains, crop protection from pests to enhance agricultural development. Indian economy has a major cornerstone in terms of agriculture, to ensure the high yield of crops and food facility for the increasing population protection from pests is necessary [4,5]. The problem of food loss due to these infestations can be controlled efficiently with the help of pesticides use either in treatment of seeds or directly as spray in fields [6].

Pesticides are chemical formulations that are premediated extensively to kill any pest either by acting specifically or on a broad scale action. Almost 98% of the pesticides are contributing as a pollutant in various resources as they have the capability of high persistence in the soil, leaching out in the soil leads to ground water contamination and non-specific effect on the beneficial organisms [4,7]. There are various pesticides that have high persistence in the soil thus do not degrade easily and effect the beneficial microflora of soil leading to deterioration of soil quality by their accumulation [4,8]. Now a day neonicotinoid (clothianidin) is extensively used new generation insecticides all over the world. They are nicotine derivatives and can potentially cause

death of insecticide at low concentration by targeting their central nervous system [9]. These are primarily used in various developed countries for a huge diversity of crops such as canola, corn, soybean, cotton, rice, sorghum, sugar beets, sweet corn, and wheat [10]. Degradation or leaching in soil water is the most probable causes of neonicotinoids loss from agricultural soil [11]. These have a potential half-life of about 3000 days thus do not degrade easily and can undergo accumulation in the soil that deteriorates the soil quality and affect soil flora [12].

To overcome the problem of non-target effect of pesticides, bioremediation is the potential approach that includes use of live forms specifically the microorganisms for degradation of pesticides. The microorganisms break the complex forms into simpler forms and utilize them further in their metabolic activities. It is highly cost-effective approach that results in cleaning of major pollutants with increasing efficiency [13]. The degradation rate of these agro organic pollutants in the soil depends on numerous factors including the temperature, moisture content, pH of the soil and type of indigenous microflora. The bacteria and fungi are the commonly found to play an important role in the pesticide's biodegradation efficiently [14]. There are number of bacterial species that are involved in bioremediation belonging to the genera *Bacillus sp., Micrococcus sp., Acidomonas sp., Streptomyces sp., Achromobacter sp., Pseudomas sp.,* etc., that possess the enzymes involved in degradation of pesticides [5,15]. Current study was focused on the isolation of Clothianidin tolerant/degrading microorganisms, which help in enhancing plant growth and productivity.

MATERIAL AND METHODS

Collection of soil sample

Soil sample was collected from two different states, one having no exposure of pesticides from last 3 years from village Manpul, District Hamirpur, Himachal Pradesh (India) in a sterile vial (altitude- 508m, latitude-31.77805˚N and 76.3445˚E longitude). The second sample was collected from Village Chak Fateh Singh Bhal, Bathinda district of Punjab (India) that was continuously in exposure with pesticides (altitude- 210m, latitude-30.2110˚ N, Longitude-74.9455˚ E), 50 gm of soil sample was collected in a sterile container and then were transported to the laboratory for the isolation of pesticide tolerant bacterial species.

Isolation of bacteria

Soil sample was used to isolate the bacteria by standard serial dilution techniques and bacteria were isolated on nutrient agar plates supplemented with recommended dose of filter sterilized pesticide clothianidin (0.48 mM). The plates were incubated at 30˚C for 24 hours and after incubation bacterial colonies were selected on morphotyping for further microbiological analysis.

Screening of isolated bacteria for the tolerance to pesticide

The isolated bacterial isolates were subjected to pesticide (Clothianidin) tolerant test for which isolates were subjected to different concentrations of the pesticide, ranging from 1mM to 150 mM in nutrient broth. The pure isolates were inoculated separately for each tested concentration of pesticide. The inoculated tubes were incubated at 30˚C for 24 hrs and after incubation the optical density was recorded at 600 nm.

Nitrogen fixation and phosphate solubilization test

The isolates were grown on Jensen's media and Pikoskvayas media to detect their ability to act as nitrogen fixers and phosphate solubilizers respectively [16]. The nitrogen fixers will be able to grow on Jensen's media and phosphate solubilizers will form clear zone around the colonies on Pikoskvayas media.

Biochemical Identification of pesticide tolerant bacterial isolate

The most efficient pesticide tolerant isolate was identified based on biochemical tests as described by Bergey et al. [17].

RESULTS AND DISCUSSION

To protect the crops from pest infestation, use of pesticides is incrementing that ensures food security at various levels, during production, harvesting and storage [18]. Along with the use of pesticides, the retention of pesticide residues in the environment possesses potential health issues and negatively influences the indigenous micro-organisms. In the present study to check the effect of pesticides residue on indigenous micro-organisms two different soil samples were taken, one with no exposure of pesticides (control soil) and another with repeated exposure of Clothianidin (exposed soil) from last 1.5 years.

The isolates from both Clothianidin exposed soil and control soil were morphologically identified on the basis of their colony characteristics as shown in Table 1 and Table 2. Based on morpho-typing, a total of 10 and 30 bacterial isolates were obtained from control and exposed soil respectively. The most of the morphotypes were smooth/ sticky colonies with entire or umbonate margins, pin-pointers to large in size, white or yellowish colonies. Earlier many research workers have used morphotyping of isolates for the grouping purpose of the isolates [19,20].

Table 1. Morphological characteristics of isolated bacterial colonies from control soil.

Isolates	Colour	Margin	Size	Shape	Elevation	Texture
C1	Pale yellow	entire	punctiform	Circular	Raised	Smooth
C2	Yellow	entire	small	Circular	Raised	Smooth
C3	White	entire	punctiform	Circular	Pin-pointed	Smooth
C4	Pale yellow	entire	small	Irregular	Flat	Smooth
C5	White	undulate	large	Irregular	Flat	Smooth
C6	White	entire	small	Circular	Raised	Smooth
C7	Yellow	entire	punctiform	Circular	Flat	Smooth
C8	Pale yellow	entire	moderate	Irregular	Flat	Smooth
C9	White	undulate	large	Irregular	Raised	Rough
C10	White	entire	small	Circular	Pin-pointed	Smooth

Table 2. Morphological characteristics of isolated bacterial colonies from pesticide exposed soil.

Isolates	Colour	Shape	Size	Margin	Elevation	Texture
P1	White	circular	punctiform	Entire	raised	Smooth
P2	White	circular	Small	Entire	raised	Smooth
P3	Pale yellow	punctiform	Small	Entire	Pin-pointed	Smooth
P4	White	circular	Small	Entire	raised	Rough
P5	White	irregular	Large	Irregular	flat	Smooth
P6	White	circular	Small	Entire	raised	Smooth
P7	Yellow	irregular	Large	Irregular	flat	Smooth
P8	Yellow	circular	Small	entire	raised	Smooth
P9	Yellow	circular	Small	Entire	flat	Smooth
P10	Pale yellow	circular	Moderate	Entire	raised	Smooth
P11	White	circular	Large	umbonate	flat	Smooth
P12	White	circular	Moderate	Irregular	flat	Rough
P13	Yellow	irregular	Small	entire	flat	Smooth

P14	White	irregular	Small	Entire	raised	Smooth
P15	White	circular	Large	Irregular	flat	Smooth
P16	White	circular	Small	Entire	raised	Smooth
P17	Yellow	circular	punctiform	Entire	Pin-pointed	Rough
P18	Pale yellow	irregular	Small	Entire	raised	Smooth
P19	Yellow	circular	Small	Entire	flat	Smooth
P20	White	circular	punctiform	Entire	raised	Smooth
P21	White	circular	small	Entire	raised	Smooth
P22	White	circular	small	Entire	flat	
P23	White	circular	large	Irregular	raised	Smooth
P24	White	circular	small	Entire	raised	Smooth
P25	Yellow	circular	punctiform	Entire	Pin-pointed	Rough
P26	Pale yellow	circular	small	Entire	flat	Smooth
P27	Pale yellow	irregular	punctiform	Entire	flat	Smooth
P28	White	circular	small	Entire	flat	Smooth
P29	White	circular	small	Entire	raised	Smooth
P30	Yellow	irregular	large	Irregular	flat	Smooth

Pesticide tolerance test for isolates

The different bacterial strains isolated from control soil and clothianidin exposed soil were subjected to tolerance check against different concentrations of clothianidin ranging from 10mM to 150mM and the minimum inhibitory concentration per isolate was observed. Out of the total 40 isolates, four isolates (C1, C4, C5 and C7) from control soil and ninteen isolates from pesticides exposed soil showed growth at a concentration of 30mM (Table 3 and Table 4).

The smaller number of isolates from control soil showed tolerance to the tested pesticide whereas higher number of isolates from exposed soil showed resistance because of repeated and inappropriate use of Clothianidin which leads to the development of resistance in the microbes. Also, it is reported in the literature that pesticide resistant isolates may use pesticide as their food [21,22,23].

Table 3. Pesticide (Clothianidin) tolerance test for the bacterial strains isolated from control soil (in the table OD values are given).

Isolates	10mM	20mM	30mM	50mM	70mM	100mM	150mM
C1	0.231	0.162	0.148	0.052	-	-	-
C2	0.132	0.031	-	-	-	-	-
C3	0.109	0.071	-	-	-	-	-
C4	0.134	0.096	0.075	-	-	-	-
C5	0.297	0.156	0.132	-	-	-	-
C6	0.196	0.072	-	-	-	-	-
C7	0.393	0.234	0.156	0.096	-	-	-
C8	0134	-	-	-	-	-	-
C9	0.108	0.073	-	-	-	-	-
C10	0.084	-	-	-	-	-	-

Out of the 10 isolates from control soil, only two isolates (C1, and C7) showed growth at a concentration of 50mM, whereas out of thirty isolates from pesticides exposed soil 10 isolates showed growth at a concentration 50mM, reflecting the development of tolerance or resistance in

isolates that are in continuous exposure with Clothianidin. Bacterial isolates P7 and P11 from pesticide exposed soil were able to tolerate clothianidin concentration up to 100 Mm.

Table 4. Pesticide (Clothianidin) tolerance test for the bacterial strains isolated from clothianidin exposed soil (in the table OD values are given).

isolates	10mM	20mM	30mM	50mM	70mM	100mM	150Mm
P1	0.582	0.438	0.301	0.193	0.072	-	-
P2	0.433	0.297	0.123	0.083	-	-	-
P3	0.265	0.145	0.063	-	-	-	-
P4	0.132	-	-	-	-	-	-
P5	0.342	0.213	0.102	0.062	-	-	-
P6	0.234	0.108	0.073	-	-	-	-
P7	0.986	0.902	0.889	0.566	0.498	0.236	-
P8	0.186	-	-	-	-	-	-
P9	0.632	0.452	0.312	0.156	0.097	-	-
P10	0.329	0.196	0.074	-	-	-	-
P11	0.834	0.731	0.543	0.398	0.213	0.102	-
P12	0.234	0.101	-	-	-	-	-
P13	0.455	0.312	0.123	-	-	-	-
P14	0.321	0.112	-	-	-	-	-
P15	0.564	0.401	0.312	0.156	0.076	-	-
P16	0.323	0.189	0.113	-	-	-	-
P17	0.276	0.096	-	-	-	-	-
P18	0.116	-	-	-	-	-	-
P19	0.564	0.329	0.152	-	-	-	-
P20	0.387	0.257	0.095	-	-	-	-
P21	0.667	0.506	0.385	0.195	-	-	-
P22	0.456	0.397	0.213	0.152	-	-	-
P23	0.121	-	-	-	-	-	-
P24	0.564	0.317	0.125	-	-	-	-
P25	0.456	0.249	0.212	0.173	-	-	-
P26	0.431	0.352	0.149	-	-	-	-
P27	0.345	0.137	-	-	-	-	-
P28	0.251	0.105	-	-	-	-	-
P29	0.323	0.179	-	-	-	-	-
P30	0.176	-	-	-	-	-	-

Nitrogen fixation and phosphate solubilization

Nitrogen and phosphate are two very essential nutrients for the plant growth [24,25,26]. Many microorganisms isolated from soil showed nitrogen fixing and phosphate solubilising activities which are very beneficial not only to the microbes but also to the plants [26,27]. Therefore, in the

present study isolates which showed growth at higher concentrations (50 mM) of clothianidin were checked for their ability to grow on nitrogen free medium and P-solubilisation activity on Pikovskaya's media (Table 5). Out of 12 isolates, 10 showed growth on nitrogen free Jensen's media and 2 isolates showed zone of solubilisation on Pikoskvayas media, reflecting their nitrogen fixation and phosphate solubilization activity respectively. Out of the 12 isolates, two were from control soil and ten from clothianidin exposed soil. Two isolates from control soil and 8 from pesticides exposed soil showed growth on Jensen's media. For phosphate solubilization, one isolate (C1) from control soil and one isolate (P7) from pesticides exposed soil showed clear zones around the colonies on Pikoskvayas media.

Table 5. Nitrogen and Phosphate solubilising activity of screened pesticide tolerant isolates.

S.No.	isolates	Nitozen fixation	Phosphate solubilization
1	C1	+ve	+ve
2	C7	+ve	-ve
3	P1	-ve	-ve
4	P2	+ve	-ve
5	P5	+ve	-ve
6	P7	+ve	+ve
7	P9	-ve	-ve
8	P11	+ve	-ve
9	P15	+ve	-ve
10	P21	+ve	-ve
11	P22	+ve	-ve
12	P25	+ve	-ve

Biochemical Identification

The most efficient pesticide tolerant isolate (P7) which also showed growth on nitrogen free media and phosphate solubilisation activity on Pikovskaya's media was identified by performing various biochemical tests. The isolate P7 was a gram positives rods, catalase and oxidase positive, indole and urease negative, MR and VP positive, able to ferment glucose, sucrose and fructose. Based on these tests the isolate P7 was identified as *Bacillus* sp.

CONCLUSIONS

Use of pesticides is incrementing that ensures food security at various levels for the growing population, during production, harvesting and storage. Along with the use of pesticides, the retention of pesticide residues in the environment possesses potential health issues and negatively influence the indigenous micro-organisms in the soil. In the present study two types of soil samples i.e. soil without exposure of pesticide and soil exposed to pesticide (clothianidin) were selected for the isolation of bacteria. Out of 40 bacteria strains, (10 isolates from non exposed soil and 30 isolates from exposed soil) isolate P7 found the most efficient isolate which showed tolerance to the tested pesticide at 100 mM concentration and also it showed growth on nitrogen free media as well as showed phosphate solubilisation activity. Based on biochemical characterization isolate P7 was identified as *Bacillus* sp. Since, the P7 isolate showed tolerance to high concentration of pesticide as well showed two important plant growth promoting activity (nitrogen fixation and phosphate solubilization) so this isolate could be a potential strain for the development of biofertilizer inoculants.

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