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Profex Induced Changes on the Growth and Indole-3-Acetic Acid and Siderophore Production by Tomato Crop Field Soil Bacteria

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A B S T R A C T

Profex is an organophosphorus pesticide used against insect pests. Its widely applied in vegetable and cotton crop fields. Extensive and repeated use of Profex pesticide in agriculture fields could adversely affect the non-target organisms. In this context, the present study was designed to evaluate the impact of Profex pesticide on the bacterial growth and its plant growth promoting traits. Bacteria prevalent in Profex applied crop fields were isolated and tested for their ability to degrade Profex. In this study, the growth of dominant bacteria, *Bacillus licheniforms, Bacillus cereus* and *Paenibacillus polymyca* in minimal salt medium augmented with Profex was assessed and its impact on the production of indole-3-acetic acid (IAA) and siderophore were assayed. The results obtained reveal significant reduction in the growth of bacteria and consequent reduction in IAA and siderophore production. Thus, the soil biological processes could be altered, which could affect the soil quality and crop yield.

***Key words*:** Profex, *Bacillus sp. Pseudomonas polymyca,* Indole-3-acetic acid (IAA), Siderophore

The application of pesticides to enhance crop production also causes adverse effect on microbial activity of microbes persisting in agricultural field soil. Some of these microbes are capable of using these pesticides as a sole source of carbon and are able to mineralize it. On the other hand, the microbial activity like siderophore production, indole acetic acid production, phosphate solubilization etc., are hindered. The production of indole-3- acetic acid varies between species and are influenced by the culture conditions. IAA serve as signal molecules [1]. Soil microbes play an important role in sustaining soil fertility through decomposition of organic matter and nutrient cycling [2-3]. Siderophore exhibit high affinity for chelating agents secreted by microbes. Several pesticides are known to influence the growth of microbes and microbial activity [4]. Keeping this in view, the present experiment was designed to determine the effect of Profex pesticide on bacterial growth and its plant growth promoting traits such as IAA and siderophore production.

MATERIALS AND METHODS

Determination of growth curve of bacteria in minimal salt medium under Profex pesticide stress

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100 µl (21 × 10^{-9} cfu / ml) of 48 hours of *Bacillus licheniforms* nutrient broth culture was inoculated into conical flask containing 100 ml of sterilised minimal salt medium and Profex pesticide was added (22 ppm and 44 ppm) separately and kept in the orbital shaker at 150 rpm at 30°C for 96 hours in triplicates. Simultaneously, control was maintained without addition of pesticide in minimal salt medium containing bacteria. 3 ml of sample was withdrawn and the optical density at initial, 4^{th}, 8^{th}, 16^{th}, 24^{th}, 48^{th}, 72^{nd} and 96 hours OD was read at 620 nm using colorimeter. Simultaneously, colony forming units on nutrient agar plates of each test and bacterial control were recorded from 100 µl of 10^{-9} dilution factor. Same procedure was followed for *Bacillus cereus* and *Paenibacillus polymyca*.

Impact Profex pesticide on IAA production by Bacillus licheniforms, Bacillus cereus and Paenibacillus polymyca

Indole-3-acetic acid synthesized by bacterial strains was quantitatively evaluated by the method of Gordon and Weber [5] and later modified by Brick *et al.* [6]. Selected bacteria strains were grown in Luria Bertani (LB) broth. Luria Bertani broth (100 ml) having fixed concentration of tryptophan (100 mg / ml) and supplemented with 22 ppm and 44 ppm of recommended rate of pesticide was inoculated with 1 ml culture of *Bacillus licheniforms* bacterial isolates (cfu / ml) and was incubated for seven days at 28 ± 2°C with shaking at 120 rpm. Simultaneously, control was maintained without addition of pesticide in MSM containing bacteria. After seven days, 5 ml of culture of each treatment was centrifuged (9,000 rpm) for 15 minutes and an aliquot of 2 ml supernatant was mixed 100 µl of orthophosphoric acid and 4 ml of salkowsky reagent (2% 0.5 M

$FeCl_3$ in 35% per-chloric acid) and incubated at 28 ± 2°C in darkness for 1 hour. The absorbance of developed pink colour was read at 530 nm. IAA concentration in the supernatant was determined using a calibration curve of pure IAA as a standard. Cfu also was determined simultaneously. Same procedure was followed for *Bacillus cereus* and *Paenibacillus polymyca*.

Impact of Profex pesticide on siderophore production by Bacillus icheniforms, Bacillus cereus and Paenibacillus polymyca [7]

Siderophore production was studied by inoculating *Bacillus licheniforms* in conical flask containing 1200 ml succinate medium (K_2HPO_4 6.0, KH_2PO_4 3.0, $MgSO_4$ 0.2, $(NH_4)_2SO_4$ 1.0 and succinic acid 4.0 gm L^{-1}, pH 7.0) and supplemented with 22 ppm and 44 ppm of Profex pesticide separately. *Bacillus licheniformis* was inoculated and incubated in succinate medium for 24 – 72 hours at 28°C with constant shaking at 120 rpm on rotary shaker. Simultaneously, control was maintained without addition of pesticide. After incubation, the fermented broth were centrifuged at 10, 000 rpm in cooling centrifuge at 4°C for 10 minutes and cell free supernatant was then mixed with 0.5 ml CAS (Chrome Azurol Solution) solution and 10 µl shuttling solution (sulfosalicyclic acid). The optical density was read in the spectrophotometer at absorbance 630 nm. The percentage of siderophore units was estimated as the proportion of CAS color shifted using the formula:

$$[(Ar-As) / Ar] \times 100$$

Where, Ar is the A630 nm of reference sample (medium + CAS assay solution + shuttle solution) and As is the A630 nm of the sample (supernatant + CAS assay solution + shuttle solution). Colony forming units (cfu) also was determined simultaneously. Same procedure was followed for *Bacillus cereus* and *Paenibacillus polymyca*.

Statistical analysis

The data obtained were subjected to One way and Two-way ANOVA using version SPSS 16.0.

Table 1 Variation in the growth (OD) of bacteria on exposure to Profex pesticide in minimal salt medium

Treatment	Optical density		
	Bacillus licheniforms	*Bacillus cereus*	*Paenibacillus polymyca*
Control	0.3150[a]	0.3438[a]	0.3917[a]
22 ppm	0.2496[b]	0.3138[b]	0.3412[b]
44 ppm	0.2250[c]	0.2667[c]	0.3062[c]
F value	3.399E3***	2.899E3***	2.124E3***
P	0.001	0.001	0.001

n = 3, Values are mean, ***Significant at P < 0.001, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

RESULTS AND DISCUSSION

In general, a dose-dependent decline in the growth of bacteria exposure to profex (22 ppm and 44 ppm) was evinced in terms of optical density compared to the control (0.3150). Significant (F = 3.399E3; P < 0.001) decrease in the growth of *Bacillus licheniforms* was evinced at 22 ppm (0.2496) and 44 ppm (0.2250) (Table 1). Significant (F = 2.899E3; P < 0.001) decrease in the growth of *Bacillus cereus* was evinced on exposure to Profex pesticide (22 ppm: 0.3138; 44 ppm: 0.2667) when compared to the control (0.3438). The growth of *Paenibacillus polymyca* significantly (F = 2.124E3; P < 0.001) declined on exposure to Profex pesticide (22 ppm: 0.3412; 44 ppm: 0.3062) when compared to the control (0.3917) (Table 1).

From the (Table 2), its observed that optimal growth of bacteria was evinced at 72 hours, irrespective of the bacterial species.

Table 2 Growth of bacteria (OD) on exposure to Profex pesticide in minimal salt medium

Time	Optical density		
	Bacillus licheniforms	*Bacillus cereus*	*Paenibacillus polymyca*
0 hours	0.0100[h]	0.0100[h]	0.0100[h]
4 hours	0.0467[g]	0.0533[g]	0.0633[g]
8 hours	0.0833[f]	0.1067[f]	0.1222[f]
16 hours	0.1167[e]	0.1300[e]	0.1511[e]
24 hours	0.4767[c]	0.5533[c]	0.6267[c]
48 hours	0.5578[b]	0.6611[b]	0.7356[b]
72 hours	0.6189[a]	0.7411[a]	0.8167[a]
96 hours	0.1956[d]	0.2089[d]	0.2456[d]
F value	3.604E4***	6.255E4***	4.585E4***
P	0.001	0.001	0.001

n = 3, Values are mean, ***Significant at P < 0.001, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

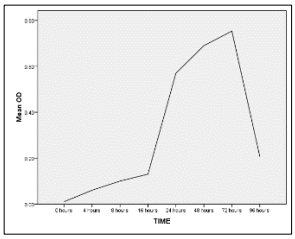

Fig 1 Growth Curve of (OD) *Bacillus licheniforms* in Minimal Salt Medium (Control)

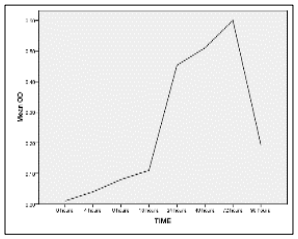

Fig 2 Growth Curve (OD) of *Bacillus licheniforms* in minimal salt medium containing 22 ppm of Profex pesticide

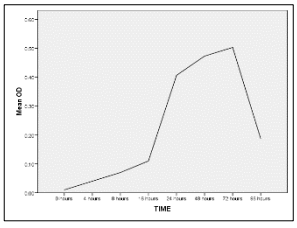

Fig 3 Growth Curve (OD) of *Bacillus licheniforms* in minimal salt medium containing 44 ppm of Profex pesticide

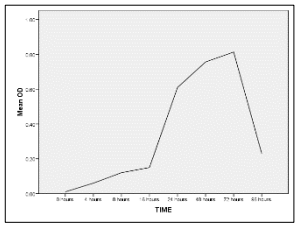

Fig 4 Growth Curve (OD) of *Bacillus cereus* in minimal salt medium (Control)

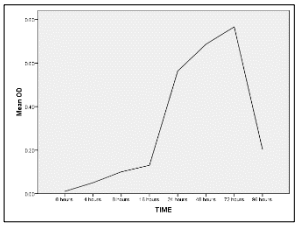

Fig 5 Growth Curve (OD) of *Bacillus cereus* in minimal salt medium containing 22 ppm of Profex pesticide

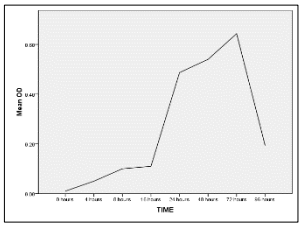

Fig 6 Growth Curve (OD) of *Bacillus cereus* in minimal salt medium containing 44 ppm of Profex pesticide

The growth curve of *Bacillus licheniforms* in terms of optical density is depicted in (Fig 1). Lag phase lasted till 16 hours, log phase till 56 hours, followed by decline phase. On exposure to Profex (22 ppm and 44 ppm), similar trend was evinced (Fig 2-3). The growth curve of *Bacillus cereus* in terms of OD displayed in (Fig 4) reveals lag period of 16 hours, followed by log period 56 hours followed by decline phase. Similar growth curve pattern was evinced on exposure to Profex

pesticide (22 ppm and 44 ppm) (Fig 5-6). From (Fig 7), it is evident that, the growth curve of *Paenibacillus polymyca* reveals lag phase of 16 hours, followed by log phase of 56 hours and then the decline phase. Similar response with respect to growth curve was elicited by *Paenibacillus polymyca* as exposure to Profex pesticide (Fig 8-9).

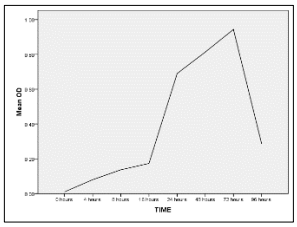

Fig 7 Growth Curve (OD) of *Paenibacillus polymyca* in minimal salt medium (Control)

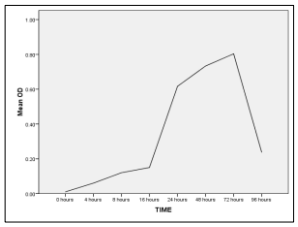

Fig 8 Growth Curve (OD) of *Paenibacillus polymyca* in minimal salt medium containing 22 ppm of Profex pesticide

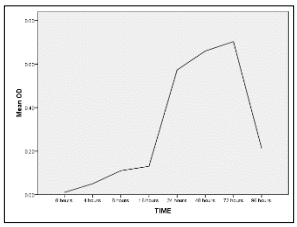

Fig 9 Growth Curve (OD) of *Paenibacillus polymyca* in minimal salt medium containing 44 ppm of Profex pesticide

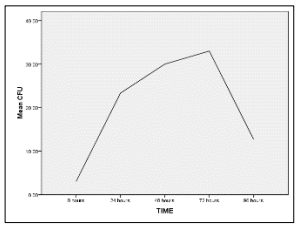

Fig 10 Growth Curve of *Bacillus licheniforms* in minimal salt medium (Control) (X 10^{-9} cfu/ml)

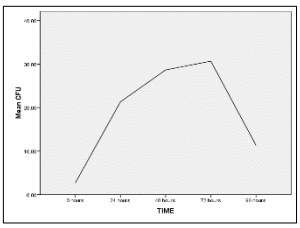

Fig 11 Growth Curve of *Bacillus licheniforms* in minimal salt medium containing 22 ppm Profex pesticide (X 10^{-9} cfu/ml)

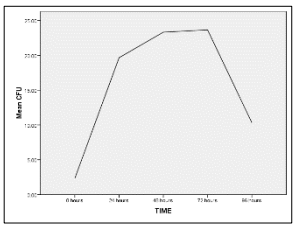

Fig 12 Growth Curve of *Bacillus licheniforms* in minimal salt medium containing 44ppm Profex pesticide (X 10^{-9} cfu/ml)

Table 3 Variation in the growth (cfu) of bacteria on exposure to Profex pesticide in minimal salt medium

Treatment ($X10^9$ cfu/ml)	*Bacillus licheniforms* ($X10^9$ cfu/ml)	*Bacillus cereus* ($X10^9$ cfu/ml)	*Paenibacillus polymyca* ($X10^9$ cfu/ml)
Control	20.4000^a	22.9333^a	25.0000^a
22 ppm	18.9333^b	20.6667^b	22.8000^b
44 ppm	15.8667^c	18.1333^c	20.0000^c
F value	301.000^{***}	486.500^{***}	471.000^{***}
P value	0.001	0.001	0.001

cfu- Colony forming units, ***Significant at P < 0.001, n = 3, Values are expressed as mean, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

The data displayed in (Table 3), indicate that Profex pesticide significantly (F = 301.000; P < 0.001) declined the population of *Bacillus licheniforms* (22 ppm: 18.9333 × 10^9 cfu / ml; 44 ppm: 15.8667 × 10^9 cfu / ml) when compared to the control (20.4000 × 10^9 cfu / ml). *Bacillus cereus* also elicited a similar response. On exposure to Profex pesticide, the population of *Bacillus cereus* declined significantly (F= 486.500; P < 0.001) in a dose-dependent manner (22 ppm: 20.6667 × 10^9 cfu / ml; 44 ppm: 18.1333 × 10^9 cfu / ml). On the other hand, control registered *Bacillus cereus* population of 22.9333 × 10^9 cfu / ml. *Paenibacillus polymyca* also elicited in a similar response on exposure to Profex pesticide. *Paenibacillus polymyca* population significantly (F = 471.000; P < 0.001) declined in a dose-dependent manner (22 ppm: 22.8000 × 10^9 cfu / ml; 44 ppm: 20.0000 × 10^9 cfu / ml) when compared to the control (25.0000 × 10^9 cfu / ml). From (Table 4), the optimal growth of bacteria was evinced at 72 hours, irrespective of the bacterial species.

Table 4 Variation in the population of bacteria exposure to Profex pesticide in minimal salt medium

Time	*Bacillus licheniforms* ($X10^9$ cfu/ml)	*Bacillus cereus* ($X10^9$ cfu/ml)	*Paenibacillus polymyca* ($X10^9$ cfu/ml)
0 hours	2.6667^e	3.7778^e	3.5556^e
24 hours	21.4444^c	24.1111^c	27.1111^c
48 hours	27.3333^b	30.7778^b	33.0000^b
72 hours	29.1111^a	33.3333^a	36.6667^a
96 hours	11.4444^d	10.8889^d	12.6667^d
F value	$4.216E3^{***}$	$8.294E3^{***}$	$8.862E3^{***}$
P	0.001	0.001	0.001

cfu- Colony forming units, ***Significant at P < 0.001, n = 3, Values are expressed as mean, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

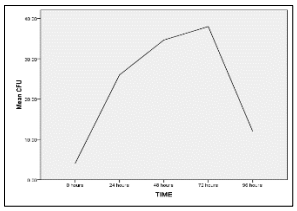

Fig 13 Growth Curve of *Bacillus cereus* in minimal salt medium (Control) (X 10^{-9} cfu/ml)

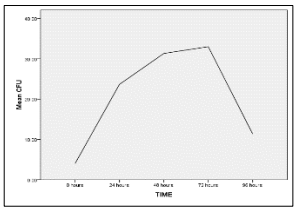

Fig 14 Growth Curve of *Bacillus cereus* in minimal salt medium containing 22 ppm Profex pesticide (X 10^{-9} cfu/ml)

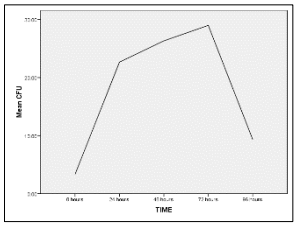

Fig 15 Growth Curve of *Bacillus cereus* in minimal salt medium containing 44ppm Profex pesticide (X 10^{-9} cfu/ml)

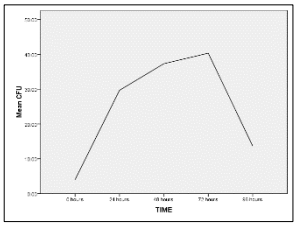

Fig 16 Growth Curve of *Paenibacillus polymyca* in Minimal Salt Medium (Control) (X 10^{-9} cfu/ml)

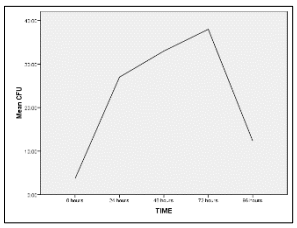

Fig 17 Growth Curve of *Paenibacillus polymyca* in Minimal Salt Medium containing 22ppm Profex pesticide (X 10^{-9} cfu/ml)

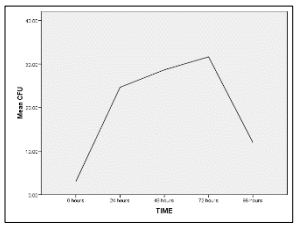

Fig 18 Growth Curve of *Paenibacillus polymyca* in Minimal Salt Medium containing 44 ppm Profex pesticide (X 10^{-9} cfu/ml)

Growth curve of *Bacillus licheniforms* in terms of colony forming units is expressed in (Fig 10). The log phase lasted till 24 hours followed by log phase till 72 hours, and then the decline phase. Similar response was elicited by *Bacillus*

licheniforms on exposure to Profex pesticide (Fig 11-12). The growth curve of *Bacillus cereus* is displayed in (Fig 13), the lag phase lasted till 24 hours followed by log phase of 48 hours and then the decline phase. On exposure to Profex pesticide, similar pattern of growth curve of *Bacillus cereus* was observed in terms of colony forming units (cfu) (Fig 14-15). (Fig 16), exhibits the growth curve of *Paenibacillus polymyca* in minimal salt medium in terms of colony forming units. The log phase lasted till 24 hours, followed by log phase till 72 hours and thereafter, decline phase. Similar growth curve pattern was exhibited by *Paenibacillus polymyca* on exposure to Profex pesticide (Fig 17-18).

Table 5 Impact of Profex pesticide on IAA production by *Bacillus licheniforms*, *Bacillus cereus* and *Paenibacillus polymyca*

Treatment	Indole-3-Acetic Acid (µl/ml)		
	Bacillus licheniforms	*Bacillus cereus*	*Paenibacillus polymyca*
Control	0.6867 ± 0.00333^{a}	0.7033 ± 0.00333^{a}	0.7167 ± 0.00333^{a}
22 ppm	0.3867 ± 0.00333^{b}	0.4167 ± 0.00333^{b}	0.3767 ± 0.00667^{b}
44 ppm	0.2733 ± 0.00667^{c}	0.3233 ± 0.00333^{c}	0.2367 ± 0.00333^{c}
F value	2.053E3***	3.529E3***	2.742E3***
P	0.001	0.001	0.001

n = 3, Values are mean, ***Significant at P < 0.001, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Table 6 Variation in *Bacillus licheniforms*, *Bacillus cereus* and *Paenibacillus polymyca* population due to Profex pesticide exposure during IAA production for a period of 7 days

Treatment (X10^{9} cfu/ml)	*Bacillus licheniforms* (X10^{9} cfu/ml)	*Bacillus cereus* (X10^{9} cfu/ml)	*Paenibacillus polymyca* (X10^{9} cfu/ml)
Control	16.6667 ± 0.33333^{a}	18.3333 ± 0.33333^{a}	17.3333 ± 0.66667^{a}
22 ppm	12.0000 ± 0.00001^{b}	13.3333 ± 0.33333^{b}	10.3333 ± 0.33333^{b}
44 ppm	8.6667 ± 0.33333^{c}	10.6667 ± 0.33333^{c}	7.3333 ± 0.33333^{c}
F value	218.000***	136.333***	118.500***
P value	0.001	0.001	0.001

cfu- Colony forming units, ***Significant at P < 0.001, n = 3, Values are expressed as mean, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Indole-acetic acid (IAA) production by *Bacillus licheniforms* declined significantly (F = 2.053E3; P < 0.001) in a dose-dependent manner (22 ppm: 0.3867 ± 0.00333 µl / ml; 44 ppm: 0.2733 ± 0.00667 µl / ml) when compared to the control (0.6867 ± 0.00333 µl / ml). Similar response was elicited by *Bacillus cereus*. Profex pesticide significantly (F = 3.529E3; P < 0. 001) reduced the production of IAA by *Bacillus cereus* (control: 0.7033 ± 0.00333 µl / ml; 22 ppm: 0.4167 ± 0.00333 µl / ml; 44 ppm: 0.3233 ± 0.00333 µl / ml). Dose-dependent decline in IAA production by *Paenibacillus polymyca* was evinced (control: 0.7167 ± 0.00333 µl / ml; 22 ppm: 0.3767 ± 0.00667 µl / ml; 44 ppm: 0.2367 ± 0.00333 µl / ml; F = 2.742E3; P < 0.001) (Table 5). In general, as the dose of Profex pesticide increased, the growth of bacteria (both in terms of optical density and colony forming units) significantly declined (Table 6). Control registered population of (16.6667 ± 0.33333 ×10^{9} cfu / ml). On the other hand, Profex exposure, significantly declined (F = 218.000; P < 0.001) the population of *Bacillus licheniforms* (22 ppm: 12.0000 ± 0.00001 × 10^{9} cfu / ml; 44 ppm: 8.6667 ± 0.33333 × 10^{9} cfu / ml). Similarly, significant reduction (F = 136.000; P < 0.001) in *Bacillus cereus* population was evinced (control: 18.3333 ± 0.33333 × 10^{9} cfu / ml; 22 ppm: 13.3333 ± 0.33333 × 10^{9} cfu / ml; 44 ppm: 10.6667 ± 0.33333 × 10^{9} cfu / ml) on exposure to Profex. The population of *Paenibacillus polymyca* was significantly reduced (F = 118.500; P < 0.001) on exposure to Profex pesticide (control: 17.3333 ± 0.66667 × 10^{9} cfu / ml; 22 ppm: 10.3333 ± 0.33333 × 10^{9} cfu / ml; 44 ppm: 7.3333 ± 0.33333 × 10^{9} cfu / ml).

Table 7 Impact of Profex pesticide on siderophore production by *Bacillus licheniforms*, *Bacillus cereus* and *Paenibacillus polymyca*

Treatment	Siderophore production (%)		
	Bacillus licheniforms	*Bacillus cereus*	*Paenibacillus polymyca*
Control	0.6867 ± 0.00333^{a}	0.7767 ± 0.00333^{a}	0.7367 ± 0.00333^{a}
22 ppm	0.4067 ± 0.00333^{b}	0.4833 ± 0.00667^{b}	0.3767 ± 0.00333^{b}
44 ppm	0.3333 ± 0.00333^{c}	0.4133 ± 0.00667^{c}	0.2967 ± 0.00333^{c}
F value	3.129E3***	1.115E3***	4.944E3***
P	0.001	0.001	0.001

n = 3, Values are mean, ***Significant at P < 0.001, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Table 8 Variation in *Bacillus licheniforms*, *Bacillus cereus* and *Paenibacillus polymyca* population Profex pesticide exposure during Siderophore production for a period of 7 days

Treatment (X10^{9} cfu/ml)	*Bacillus licheniforms* (X10^{9} cfu/ml)	*Bacillus cereus* (X10^{9} cfu/ml)	*Paenibacillus polymyca* (X10^{9} cfu/ml)
Control	10.6667 ± 0.33333^{a}	12.0000 ± 0.57735^{a}	9.6667 ± 0.33333^{a}
22 ppm	8.3333 ± 0.66667^{b}	10.3333 ± 0.33333^{b}	7.3333 ± 0.33333^{b}
44 ppm	7.6667 ± 0.33333^{c}	9.6667 ± 0.33333^{c}	5.3333 ± 0.33333^{c}
F value	11.167**	7.800*	42.333 ***
P value	0.009	0.021	0.001

cfu- Colony forming units, ***Significant at P < 0.001, n = 3, Values are expressed as mean, In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

The production of siderophore by *Bacillus licheniforms* (0.6867 ± 0.00333%), *Bacillus cereus* (0.7767 ± 0.00333) and *Paenibacillus polymyca* (0.7367 ± 0.00333%) is displayed in (Table 7). Significant decline in the production of siderophore by these bacteria on exposure to profex was observed. As the concentration of Profex increased, the production of siderophore by these bacteria decreased (*Bacillus licheniforms*: control: 0.6867 ± 0.00333%; 22 ppm: 0.4067 ± 0.00333%; 44 ppm: 0.3333 ± 0.00333% F = 3.129E3; P < 0.001, *Bacillus cereus*: control: 0.7767 ± 0.00333%; 22 ppm: 0.4833 ± 0.00667%; 44 ppm: 0.4133 ± 0.00667%; F = 1.115E3; P < 0.001, *Paenibacillus polymyca*: control: 0.7367 ± 0.00333%; 22 ppm: 0.3767 ± 0.00333%; 44 ppm: 0.2967 ± 0.00333%; F =

4.944E3; P < 0.001). The bacterial population also declined during in the siderophore production on Profex exposure (*Bacillus licheniforms*: control: 10.6667 ± 0.33333 × 10^9 cfu / ml; 22 ppm: 8.3333 ± 0.66667 × 10^9 cfu / ml; 44 ppm: 7.6667 ± 0.33333 X10^9 cfu / ml; F = 11.167; P < 0.01; *Bacillus cereus*: control: 12.0000 ± 0.57735 × 10^9 cfu / ml; 22 ppm: 10.3333 ± 0.33333 × 10^9 cfu / ml; 44 ppm: 9.6667 ± 0.33333 × 10^9 cfu / ml; F = 7.800; P < 0.05; *Paenibacillus polymyca*: control: 9.6667 ± 0.33333 × 10^9 cfu / ml; 22 ppm: 7.3333 ± 0.33333 × 10^9 cfu / ml; 44 ppm: 5.3333 ± 0.33333 × 10^9 cfu / ml; F = 42.333; P < 0.001) (Table 8). The decline in population by these bacteria on exposure to Profex pesticide could be a possible cause for significant reduction in siderophore and IAA production.

The present findings are in good accord with the observations of Kumar *et al.* [8] who have also have evinced the ability of *Pseudomonas putida* and *Bacillus amyloliquefaciens* to utilize Carbendazim (0.2-1%), Glyphosate (2-10%), Imidacloprid (2-10%) as a sole source of carbon. According to Vijay Kumar *et al.* [1] acephate, glyphosate, monocrotophos and photate (0.25 – 250 mg / L) significantly reduced the siderophore production by *Pseudomonas fluorescens*, *Azotobacter vinelandii*, *Bacillus brevis*, *Rhizobium leguminosarum* and *Salmonella typhimurium*. These observations are in line with the present findings. In the opinion of Lo [9] mixed form of pesticides cause adverse effect on the soil microorganisms through inhibition of plant growth promoting traits. Findings of this study is also in accordance with Madhaiyan *et al.* [10] who have evinced that Monocrotophos, Lindane and Dichlorvos reduced the production of Indole acetic acid and gibberellic acid by *Gluconacetobacter diazotrophicus* in minimal salt medium isolated from sugarcane roots.

Mundi *et al.* [11] have reported that higher doses of chlorpyrifos (8 ppm) and isoproturon (495 ppm) than the recommended dose elicited adverse impact on the soil bacterial activity. Further, they have also observed that Indole acetic acid production by *Azotobacter* was significantly reduced due to exposure to higher doses (above use recommended rates of chlorpyrifos and isoproturon). The observations are in consistent with the present findings. Similar to the reduction in siderophore production observed in this study, Mundi *et al.* [11] have also observed reduction in siderophore production by *Azotobacter* on exposure to higher doses of chlorpyrifos (8 ppm) and isoproturon (495 ppm). In coincidence with the present findings, Supreeth *et al.* [12] have reported that chlorpyrifos exhibited deleterious effect on the soil bacteria. Researchers have claimed that this might have occurred due to inability of these bacteria to use the profex as a nutrient source.

The findings reported are in accordance with the study conducted by Ahemad and Khan [13] who have also reported that Imidacloprid at 300 µg / L, Thiamethoxam at 50 µg / L and 75 µg / L, Hexaconazole at 80 and 120 µg / L and Metalaxyl at 4,500 µg / L reduced the siderophore production by *Bradyrhizobium* strains MRM6. Further, they also have evinced that Metribuzin (1,700 and 2,550 µg / L), Glyphosate (2,888 µg / L, 4,332 µg / L) Imidacloprid (200 and 300 µg / L), Thiamethoxam (50 and 75 µg / L), Hexaconazole (80 and 120 µg / L), Metalaxyl (1,500 and 3,000 µg / L) and Kitazin (192 and 288 µg / L) significantly reduced the production of IAA by *Bradyrhizobium sp.* when compared to their recommended dose (850 µg / kg^{-1}, 1,444 µg / kg^{-1}, 100 µg / L^{-1}, 25 µl / L^{-1}, 40 µl / kg^{-1} and 96 µl / kg^{-1}, respectively). The results find support with the observation made by Ahemad and Khan [14], where the researchers have observed that fungicides like Tebuconazole, Hexaconazole, Metalaxyl and Kitazin caused minor effect on the production of Indole acetic acid and siderophore by *Pseudomonas putida* at recommended dose and the effect intensified at higher doses. Our results are also in agreement with those obtained by Ahemad and Khan [15] who have reported that Indole acetic acid and siderophore production by *Klebsiella sp.* PS19 decreased on exposure to Fipronil, Pyriproxyfen, Imidacloprid and Thiamethoxam in a dose-dependent manner. The dose-dependent decline in IAA and siderophore production by bacteria on exposure to Profex pesticide observed in this study gains support form the findings of Silambarasan and Vangnai [16] who have also observed dose-dependent decline in IAA production by *Acinetobacter sp.* on exposure to 4-nitroaniline at 25 mg / L^{-1} and 50 mg / L^{-1}.

Shahid *et al.* [17] have demonstrated that increase in the concentration of pesticides hexaconazole (600 µg / mL^{-1}, 1200 µg / mL^{-1}, 1800 µg / mL^{-1}), kitazin (800 µg / mL^{-1}, 1600 µg / mL^{-1}, 2400 µg / mL^{-1}), imidacloprid (900 µg / mL^{-1}, 1800 µg / mL^{-1}, 2700 µg / mL^{-1}) glyphosate (300 µg / mL^{-1}, 600 µg / mL^{-1}, 900 µg / mL^{-1}), fipronil (600 µg / mL^{-1}, 1200 µg / mL^{-1}, 1800 µg / mL^{-1}), monocrotophos (700 µg / mL^{-1}, 1400 µg / mL^{-1}, 2100 µg / mL^{-1}), quizalofop (400 µg / mL^{-1}, 800 µg / mL^{-1}, 1200 µg / mL^{-1}), atrazine (300 µg / mL^{-1}, 600 µg / mL^{-1}, 900 µg / mL^{-1}) and metalaxyl (500 µg / mL^{-1}, 100 µg / mL^{-1}0, 1500 µg / mL^{-1}) significantly decreased the production of IAA and siderophore by *Azotobacter vinelandii*.

In consistent with the present findings, Ahemad and Khan [18] have also observed significant reduction in IAA and siderophore production by *Pseudomonas aeruginosa* strain on Tebuconazole exposure (100, 200 and 300 µg / mL^{-1}) several bacteria like *Pseudomonas fluorescens*, *Azospirillum lipoferum*, *Bacillus coagulans*, *Brevibacillus brevis*, *Aeromonas sp.*, *Bacillus sp.*, *Azospirillum brasilens*, *Enterobacter sp.*, *Pseudomonas striata*, *Stenotrophomonas*, *Azotobacter sp.*, *Alcaligen sp.* are known to produce siderophore and IAA [19-22]. The siderophore are low molecular weight (500-1000 daltons) ion chelating compounds. Our results are in accordance with Subramanium and Sundaram [5] who have also proved that *Pseudomonas aeruginosa* PSA01 and *Pseudomonas fluorescences* PSF02 produced siderophore.

CONCLUSION

The impact of Profex on the soil bacteria has been assessed in this study. Profex at 22 ppm and 44 ppm elicited significant decline in the growth of *Bacillus licheniforms*, *Bacillus cereus* and *Paenibacillus polymyca*. This has in turn resulted in significant decline in production of Indole-3-Acetic Acid (IAA) and siderophore by these bacteria in a dose-dependent manner. Thus, ecofriendly approach to control the pest has to be devised.

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