

Original Research Article

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Rhizosphere Microbial Population of Pea Genotypes under Different Level of Aluminium

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ABSTRACT

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The investigation was conducted to examine the effects of aluminium on the soil micro-organism in the rhizosphere of pea genotypes with varying Al treatment. The experiment was layout in a factorial completely randomized design with the first factor comprised of 4 pea genotypes (2 tolerant and 2 susceptible each) and the second factor with 3 levels of Al treatment (control, 12 ppm and 24 ppm Al). The classical serial dilution technique was used for isolation of total bacterial and fungal population from the soil sample by spread plate technique on appropriate media. It was observed that aluminium caused marked influence on the bacterial and fungal population in the rhizosphere of pea genotypes. The fungal population showed a marked reduction at 24 ppm level of aluminium treatment in all genotypes except intolerant genotype (Kashi Samridhi) where an increase in the fungal population was observed. The bacterial population showed a positive relation with aluminium treatment. Low level of Al treatment showed an increase in the bacterial population in the rhizosphere of susceptible genotype but at a higher level of Al the rhizosphere of tolerant genotype had a higher bacterial population.

Introduction

Micro-organism plays a vital role in soil fertility and crop growth. High rainfall, use of acidic fertilizer and release of industrial effluents in the soil has acidified it, rendering unsuitable for cultivation of the crop. One of the consequences of soil acidification is the increase in bioavailable toxic metals in soil especially aluminium (Al), iron and manganese. Microorganisms continuously interact with varied inorganic ions, some of which are essential for biological functions

whereas others exert inhibitory effects that limit normal development. Microbial communities in the soil can react to both natural and anthropogenic influences related to soil chemistry (Schallmach *et al.*, 2000), the presence of different plants (Marschner *et al.*, 2001) changes in soil moisture (Wilkinson *et al.*, 2002) and input of toxic pollutants (Joner *et al.*, 2001) Aluminium is a metal lacking biological functions and in this respect pertains to the non-essential class of chemical elements. The presence of toxic ions in the environment may select for the

appearance of tolerant microbial variants possessing genetic determinants which confer resistance to the poisonous compounds (Slawson *et al.*, 1992). Although the toxic effects of high Al concentrations on plants have been known for a long time back (Hartwell and Pember, 1918) and a large number of investigations into the effects of high Al concentrations on cultivated plants, very few studies concerning the influence of Al concentration on free-living soil micro-organisms has been conducted. The few microbiological works on this topic were mostly carried out in marginal fields. This neglect is surprising, given the importance of free-living soil bacteria and fungi. It was therefore the aim of this investigation to examine effects of aluminium on the soil micro-organism of pea genotypes with varying Al treatment.

Materials and Methods

The experiment was layout in a factorial completely randomized design with the first factor comprised of 4 pea genotypes *viz.* Two tolerant genotypes (Kashi Samrath and Kashi Samridhi) were collected from ICAR-IIVR, Varanasi and two susceptible genotypes Azad Pea-3 (CSAUA&T, Kanpur) and Matar Ageta-7 (PAU, Ludhiana) and the second factor comprised of 3 levels of Al treatment (control, 12 ppm and 24 ppm). The Al tolerance of pea genotypes was determined based on morphological parameters using sand culture experiments (data not reported here).

The genotypes were grown in soil culture in the pot under polyhouse condition at College of Horticulture and Forestry in the year 2018-2019. Plastic pots (30 × 25 cm) were filled with 7 kg well-mixed sandy loam soil and treatment was given using AlCl₃.7H₂O. The soil has a pre-cropping value of organic carbon (1.5%) (Walkley and Black, 1932),

available nitrogen (100.8 mg/kg) (Subbiah and Asija, 1956) available phosphorus (19 mg/kg) (Bray and Kurtz, 1945), available potassium (120 mg/kg) (Hanway and Heidel, 1952) Exchangeable calcium and magnesium (8 mg/kg and 2.4 mg/kg, respectively) (Tucker and Kurtz, 1961) and Exchangeable Al (KCl) (0.2 mg/kg) (Barnhisel and Bertsch, 1983) before sowing. The pea crop was grown for three months the representative soil samples were collected, sieved through a 2 mm stainless steel sieve and kept in a plastic container and analyzed for the microbial population at Basic science laboratory, College of Horticulture and Forestry, Pasighat.

Isolation and identification

The classical serial dilution technique was used for isolation of total bacterial and fungal population from the soil sample by spread plate technique on appropriate media. Initially, the soil sample of 1 gram each was suspended in 9 ml of sterile distilled water (water blank) and from this original suspension, further dilutions were made aseptically. For isolation of bacteria from soil, 10⁻⁵ dilution was finally selected. With the help of a sterile pipette, 0.1 ml of the soil suspension was transferred from the final dilution to the sterilized Petri plate containing Nutrient agar (HIMEDIA) and the composition of media is given in Table 1.

Fungi from the soil were also isolated in a similar manner. But here, 0.1 ml of the soil suspension was transferred from 10⁻³ dilution to a sterilized Petri plate containing Rose Bengal Agar (HIMEDIA) and the composition of media is given in Table 2. Then, the plates were incubated at 20-30°C for about 45 hours and the microbial populations were estimated as colony forming unit (cfu) g⁻¹ soil on a dry weight basis.

Composition of the media

28 g of this was suspended in 1000 ml distilled water and then boiled to dissolve the media completely. It was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The solution was mixed well before pouring.

31.55 g of this was suspended in 1000 ml distilled water and then warmed gently to dissolve the media completely. It was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The solution was mixed well before pouring into Petri plates.

Results and Discussion

Bacterial Population (cfu/g soil)

Averaged across Al treatment Matar Ageta-7 had the highest bacterial population (20.12×10^5 cfu/g soil) which was at par with AP-3 (18.03×10^5 cfu/g soil) (Table 3).

The bacterial population was found low in tolerant genotype *viz.* Kashi Samrath (13.22×10^5 cfu/g soil) and Kashi Samridhi (13.00×10^5 cfu/g soil). Due to Al treatment, the bacterial population increased from 10.72×10^5 cfu/g soil in control to 20.72×10^5 cfu/g soil at 12 ppm Al level. However, at 24 ppm Al level the bacterial population was

16.89×10^5 cfu/g soil which was significantly lower than 12 ppm Al level but significantly higher than control (Table 3).

Interaction between genotypes and Al was found to be significant for the bacterial population (Fig. 1). In the soil of susceptible variety *viz.* Matar Ageta-7 and AP-3 significant increase in bacterial population was observed at 12 ppm Al level with respect to control.

However, there was no significant difference at 24 ppm Al level with respect to control. In the soil of tolerant genotype *viz.* Kashi Samrath and Kashi Samridhi there was no significant difference in the bacterial population at 12 ppm Al level with respect to control. However, at 24 ppm Al level significant increase in bacterial population was observed with respect to control.

Fungal Population (cfu/g soil)

The fungal population of soil was found highest in Kashi Samridhi (3.43×10^3 cfu/g soil) followed by Kashi Samrath (2.58×10^3 cfu/g soil) (Table 3). Application of 12 ppm Al in the soil doesn't have a significant difference in fungal population compared to control. However, at 24 ppm Al level fungal population significantly decreased with respect to control.

Table.1 Nutrient agar for bacteria

Ingredients	Amount (g lit ⁻¹)
Peptic digest of animal tissue	5.00 g
Beef extract	1.5 g
Sodium chloride	5.00 g
Yeast	1.5 g
Distilled water	1000ml
Agar	15.00 g
pH	6.8-7.0 (at 25°C)

Table.2 Rose Bengal agar with chlortetracycline for fungi

Ingredients	Amount (g lit ⁻¹)
Peptone	5.0
Dextrose	30.0
Sodium nitrate	2.0
Monopotassium phosphate	1.0
Magnesium sulphate	0.5
Rose Bengal	0.05
Agar	15.0
Distilled water	1000ml
Final pH (at 25°C)	7.3±0.2

Table.3 Influence of Al on soil microbes

Genotypes	Bacterial Population (10 ⁵ cfu/g soil)	Fungal Population (10 ³ cfu/g soil)
Matar Ageta-7	20.12	1.24
AP-3	18.03	2.32
Kashi Samrath	13.22	2.58
Kashi Samridhi	13.00	3.43
C.D (0.05)	3.16	0.326
Al treatment		
Control	10.72	2.725
12 PPM Al	20.68	2.625
24 PPM Al	16.89	1.829
C.D. (0.05)	2.73	0.282

Fig.1 Effect of Al on bacterial population (10⁵ cfu/g) of soil, * indicates a significant difference with control [LSD (0.05) -5.47]

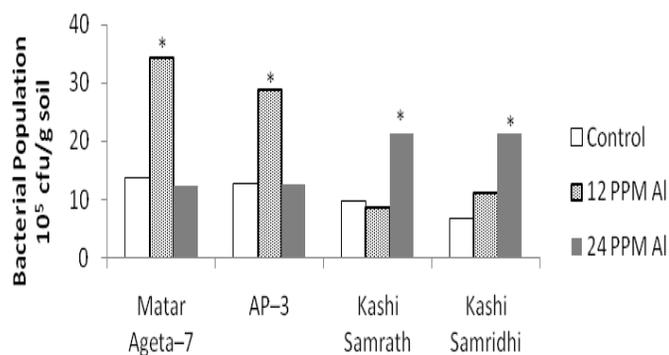
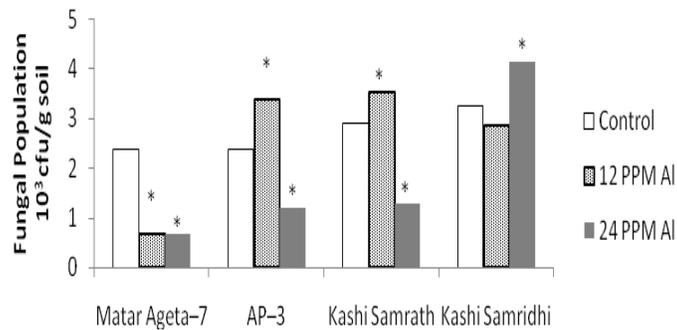


Fig.2 Effect of Al on the fungal population (10^3 cfu/g) of soil, * indicates a significant difference with control [LSD (0.05)-0.565]



Interaction between genotypes and Al had a significant effect on the fungal population of soil. In control and 24 ppm, Al level fungal population was highest in the soil of Kashi Samridhi (3.27×10^3 cfu/g soil) and (4.15×10^3 cfu/g soil), respectively (Fig. 2). However, at 12 ppm Al level fungal population was found highest in Kashi Samrath (3.53×10^3 cfu/g soil) (Fig. 2).

The bacterial population showed a positive relation with aluminium treatment. Low level of Al treatment showed an increase in the bacterial population in soil grown with susceptible genotype but at a higher level of Al the soil of tolerant genotype had a higher bacterial population. Joner *et al.*, (2005) reported an increase in biomass of Gram-positive bacteria and actinomycetes in the organic horizon of Al treated plots in forest soil. Guida *et al.*, (1992) when studying aluminium toxicity towards *Escherichia coli*, found that growth inhibition was markedly dependent on pH, recording sensitivity to 0.9 and 2.25 mM Al at pH 5.4 and 6.6, respectively; aluminium toxicity increased when iron was omitted from the medium, which suggests that aluminium uptake involves iron transport systems, as previously reported (Davis *et al.*, 1971). Accordingly, Gascoyne *et al.*, (1991) found that some siderophore-producing alkalophilic bacteria were able to accumulate aluminium, as well

as iron and gallium, from culture media. It has generally been easier to detect changes in soil chemistry and adverse effects of Al on aerial plant parts than to demonstrate Al induced stress symptoms on roots (Janhunnen *et al.*, 1995). A negative impact on microorganisms is frequently detected in vitro but is far more difficult to detect when working with indigenous microbial communities in soil (Illmer *et al.*, 1995; Raubuch *et al.*, 1999).

The fungal population reduced at a higher level of aluminium treatment. However, there was an increase in the fungal population of tolerant genotype Kashi Samridhi. Illmer *et al.*, (1995) reported that the microbial biomass decreased with increasing aluminium concentration independent of the pH. Not only plants are damaged by high concentrations of available Al (as is well known) but free living soil micro-organisms are inhibited too. The increase in fungal population in the rhizosphere of the tolerant genotype Kashi Samridhi may be due to the influence of excreted organic acid which neutralizes the Al in soil

From the present study, it was concluded that aluminium caused marked influence on the bacterial and fungal population in the soils grown with pea genotypes. The fungal population reduced at a higher level of aluminium treatment. However, there was an

increase in the fungal population of tolerant genotype Kashi Samridhi. The bacterial population showed a positive relation with aluminium treatment. Low level of Al treatment showed an increase in the bacterial population in soil grown with susceptible genotype but at a higher level of Al the soil of tolerant genotype had a higher bacterial population. Further study is required to see the effect on different fungal and bacterial strain to find out the tolerant microbial strain. The exudation of organic acid is an external defense mechanism of legumes to neutralize toxic Al (Matsumoto and Motoda, 2012).

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