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Study on Phosphate Solubilization of Salt Tolerant Soil Yeast Isolates and Effects on Maize Germination and Growth

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ABSTRACT: Among 12 isolated soil yeasts, four isolates were selected according to their salt tolerance and these four isolates tolerated 14% NaCl. Moreover, they can tolerant to KCl, $MgCl_2$ and $CaCl_2$. Phosphate solubilization of selected soil yeast isolates were detected in Pikovskaya's broth supplemented with various NaCl concentrations (ranging from 0% to 10%). They can solubilize insoluble phosphate at all NaCl concentrations. But with increasing NaCl concentration, phosphate solubilization was decreased and the best solubilization was occurred at 6 days incubation period. Above 10% NaCl concentration, these isolates cannot solubilize insoluble phosphate, $Ca_3(PO_4)_2$. On maize germination under NaCl stressed conditions, it was found that soil yeast isolates enhanced maize germination when compared with uninoculated treatment. Above 0.5% NaCl concentration, germination percentage of maize was obviously different between inoculated and uninoculated treatments. Like in phosphate solubilization, germination percentage was decreased with increasing NaCl concentration. At 2% NaCl concentration, germination was not found. So these isolates tolerated to some degree of NaCl, there is limited range for their functioning. After treating salt affected soils with soil yeast isolates for four weeks, salinity of treated soils was slightly decreased but total nitrogen content, K^+, and available nutrients (P and K_2O) were slightly increased when compared with those of untreated soil.

KEYWORDS: NaCl, Phosphate solubilization, Pikovskaya's broth, Soil yeasts, Maize germination.

1 INTRODUCTION

Nearly 40% of world's surface has salinity problems [1]. Salinization of soil is a serious problem and is increasing gradually in many parts of the world, particularly in arid and semiarid areas. The phosphorus deficiency frequently compounds the problems of saline soils of the tropics [2]. High salinity affects plant growth through, (i) the osmotic effects; (ii) toxicity of salt ions; and (iii) the changes in the physical and chemical properties of soil [3]. It also suppresses the phosphorus uptake by plant roots and reduces the available phosphorus by sorption processes and low solubility of Ca-P minerals [4]. Since phosphorus is a critical nutrient limiting plant growth [5].

A wide variety of heterotrophic fungi and bacteria have been shown to be capable of solubilizing insoluble phosphate ([6], [7]). In fact, the ability of fungi and heterotrophic bacteria to solubilize insoluble phosphate is well documented ([6], [7], [8], [9], [10]). Although soils are known to contain yeasts, little is known about their ecology and the role which they play in mineral cycling. This lack of interest probably reflects the low population density and relatively small biomass of yeasts in most soils ([11], [12]).

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Yeast are ubiquitous unicellular microorganisms in the natural environments (including aquatic systems) as well as industrial effluents where they are exposed to a variety of conditions with respect to nutrient availability, temperature, pH, osmotic pressure, access to oxygen and water activity, all of which induce stress responses [13]. They offer the advantages of having cell wall materials which show excellent metal binding properties [14].

Data on phosphate solubilization *in vitro* by soil yeasts are scarce or lacking. Reference [15] isolated soils yeasts and studied phosphate solubilizing of thes soil yeasts. It was reported that isolated soil yeasts solubilized insoluble phosphate *in vitro* leading to the formation of large amounts of soluble phosphate. Moreover, Reference [16] studied phosphate solubilization of a species of Candida isolated from soil. It has been found that the soil yeast *Williopsis califurnica* is able to oxidize ammonium sulphate to nitrate via nitrite and it could also solubilize insoluble phosphate [17].

The present investigation was undertaken to study the role of selected soil yeasts, isolated from Myanma Agricultural Soils, on salt tolerance, the solubilization of insoluble phosphate, $Ca_3(PO_4)_2$, effectss on germination and growth of maize.

Application of yeast produced the highest spike number irrespective of salinity level and cultivar type [18].

An improved salinity and tolerance of crop plants by using bio-fertilizers was investigated in several studies. Reference [19] studied the effect of of biofertilization of yeast (*Rhodotorula glutinis*) on four Egyptian maize varities (Giza 2, One Way Cross 10, One Way Cross 129 and Three Way Cross 352) grown under different salinity levels. From their study, they reported that bio-fertilization alleviated adverse effects of high levels of salinity and plants accumulated more polyamines than those, which received no bio-fertilizer, especially at high salinity levels.

2 MATERIALS AND METHODS

2.1 SOIL SAMPLING AND ISOLATION OF SOIL YEAST

Soil samples were collected from around Yangon Region, Kyaukse Twonship, Patheingyi Township in Mandalay Region. Soil yeasts were isolated on PYG media (peptone - 2 %, yeast extract - 1%, and glucose - 2%) containing 0.05 % Chloramphenicol. The culture plates were incubated at 37ºC for overnight. After overnight incubation, growth of yeast colonies on PYG media was observed and pure strains were obtained by subculturing.

2.2 SCREENING OF SALT TOLERANCE OF SOIL YEAST ISOLATES

The cultures obtained on PYG media were further screened for their salt-tolerance. For this purpose, PYG broth supplemented with various concentrations of NaCl (ranging from 1-14%) was used for inoculation of soil yeast isolates and PYG broth without NaCl was used as a control and incubation was carried at 37ºC. Total viable count of all cultures was determined on PYG media. The isolates showing high salt-tolerance were selected for further study.

Salt tolerance of soil yeast isolates were also screened on other salts ($MgCl_2$, $CaCl_2$, and KCl) using PYG media.

2.3 CONVENTIONAL IDENTIFICATION OF SOIL YEAST ISOLATES

Isolates soil yeasts were identified through colonial and microscopic morphology, sugar assimilation and fermentation abilities, some biochemical characteristics.

2.4 PHOSPHATE SOLUBILIZATION OF SOIL YEAST ISOLATES UNDER SALT-STRESS CONDITIONS

Phosphate solubilization of soil yeast isolates under salt stressed conditions was determined in Pikovskaya' broth [20] by Spectrophotometric method [21]. Pikovskaya's broth with $Ca_3(PO_4)_2$ was used and Pikovskaya's broth (30 ml) with different NaCl concentrations (0%, 2%, 4%, 6%, 8%, and 10%) was prepared for salt induced phosphate solubilization. Sample (10 ml) from each flask was withdrawn aseptically at 3 days, 6 days and 9 days. Phosphate solubilization in Pikovaskaia's broth media was quantified in a flask (10 ml) and incubated in water batch shaker at 37ºC for five days. Uninoculated medium served as control. After incubation, the culture broth was passed through the cation exchange resin and $(PO_4)^{3-}$ solution was reacted with color forming reagent. After color development, phosphate solubilizing activity was measured by UV-vis spectrophotometric method at 830 nm.

2.5 Effects of Soil yeast Isolates on Maize Germination under Salt Stressed Conditions

Maize seeds were surface disinfected by immersion in 70% ethanol for 1 min followed by 10 min in 2% sodium hypochlorite. They were then washed three times with sterile distilled water. An inoculum of soil yeast isolates was prepared in PYG broth. The disinfected seeds were immersed in either the inoculums or in PYG broth without any yeast isolates for one hour and were used as inoculated and uninoculated treatments. Germination was assayed according to International Seed Testing Association [22]. Four replicates of 25 seeds were germinated in Sterile Petri dishes containing two sheets of filter papers moistened initially with 4 ml of sterilized tricalcium phosphate solution supplemented with NaCl at 0%, 0.5%, 1%, 1.5%, and 2%. The Petri dishes were then placed in room temperature and germination was assayed daily and recorded.

2.6 Treatment of Salt Affected Soils with Isolated Soil Yeasts

Salt affected soils were obtained from Myitthar Township, Mandalay Region Myanmar. Agricultural land of this area was affected by salinity and so this has been left without cultivation. Growth of plants in this soil was tested (Figure 1). 10 kg of soil samples were separately placed. An inoculum was prepared by preculturing the yeast isolates in PYG broth at 37ºC. 50 ml of PYG medium was inoculated with exponentially growing yeast isolates of inoculums (5 ml). After two days incubation at 37ºC, 50 ml of PYG broth culture solution of each isolate was poured to soil samples and mixed well. Moisture of soil samples was maintained enough to grow the soil yeast isolates. Yeast broth culture solution was poured weekly. In order to compare the soil salinity and some mineral contents of treated and untreated soils, untreated soil was used as control. After four week treatment, treated soil samples were analysed for electricial conductivity (EC), and total nitrogen by the Kjeldahl method [23], available phosphorus (P) by the Olsen method [24] and the potassium by flame photometry mineral contents.

2.7 Growth of Maize in Treated Salt Affected Soils

After four weeks treatment of salt affected soils with soil yeast isolates, maize seeds were sown in pot trial and growth of maize were studied. Pot experiment was carried out by sowing five seeds of maize per pot containing 1 kg of treated soil sample. After sowing, yeast broth culture of each isolate (OD – 0.5) was poured to each pot at every weekend and pots were also watered. After four weeks, growth of maize was studied and the height of maize was measured.

3 Results and Discussion

A total of 12 soil yeasts were isolated on PYG medium from collected soil samples. After purifying isolated soil yeasts on PYG media, salt tolerance of soil yeasts were screened on PYG media supplemented with various NaCl concentrations (ranging 1-14%). Among 12 isolates, four isolates (I1, I2, I3 and I4) tolerated NaCl to 14% (Table 1) and selected after plate screening. TAKAKUW [25] selected yeast strains that tolerated at 10% NaCl for study of Glucosylcramide. Besides NaCl tolerance, tolerance of selected four soil yeast isolates were screened on other salts (KCl, $CaCl_2$ and $MgCl_2$). Among three types of salts, they can tolerant to these salts, but growth rates of soil yeast isolates on PYG media supplemented with $MgCl_2$ were less when compared with the growth on other media.

3.1 Sugar Assimilation and Fermentation Abilities and Biochemical Characteristics

Sugar assimilation and fermentation abilities of selected soil yeast isolates were shown in Table (2). Some biochemical characteristics were shown in Table (3).

3.2 Phosphate Solubilization of Soil Yeast Isolates under Salt Stressed Conditions

Phosphate solubilization of soil yeast isolates were evaluated in Pikovskaya's broth supplemented with different NaCl concentrations. This study revealed that all four yeast isolates solubilized insoluble $Ca_3(PO_4)_2$ under NaCl stressed conditions. Therefore, it seemed that they had been well adapted to the salt stressed conditions and they have genetic potential to solubilize the insoluble phosphate at high salt concentration. Stress induced phosphate solubilization has been studied by several researchers ([26], [27], [28]). The insoluble phosphate solubilizing of isolated soil yeasts was increased at 6 days incubation period and soluble phosphate concentrations were almost the same between 0% and 2% NaCl concentration (Figure 2). With increasing NaCl concentration, insoluble phosphate solubilizing activity was drastically decreased. These results could be due to influence of NaCl on their growth. It was also said that halotolerant microorganisms have limited degree of salt tolerance. It was also reported that solubilization in presence of 10% sodium chloride but there is a general trend of decrease in solubilization activity with the increase of sodium chloride concentration. This might have two reasons either two stresses at the same time may harm cell growth and proliferation which result in less efficiency of solubilization or it might be possible that too much chloride ions may chelate or neutralize proton ions or acid produced in the medium ([29], [30], [31], [32]).

3.3 EFFECTS OF SOIL YEAST ISOLATES ON MAIZE GERMINATION

To study the effects of isolated soil yeasts on maize germination under saline conditions, the suspension of soil yeast isolates was used to inoculate maize seeds prior to germinate under saline conditions supplemented with 0%, 0.5%, 1%, 1.5% and 2% NaCl concentrations. The germination percentage of inoculated with soil yeast isolates and uninoculated seeds were compared (Figure 3). Uninoculated treatments were conducted at all NaCl concentrations along with the inoculated treatments. This study was found that germination percentage of inoculated and uninoculated treatments was decreased with increasing NaCl concentrations. But germination percentage of inoculated treatments was higher than uninoculated treatments. Germination percentage of inoculated and uninoculated treatments was not significantly different between 0% and 0.5% NaCl concentration, but above 0.5% NaCl, germination percentage was obviously different between inoculated and uninoculated treatments. Salinity affect on germination by interfering with the uptake of essential nutrients and the direct toxicity effects of salt ions and prevention of weed water uptake in the first phase of germination ([33], [34]). In this study, it was also seen that decreasing of germination percentage of uninoculated treatment was due to salinity effects and might be due to the lack of soluble phosphorus supply from microbial activity. Maize germination inoculated with soil yeasts was obviously higher than those of uninoculated treatment. This might be a result of inoculation of maize seeds with salt tolerant soil yeast isolates and increasing available phosphorus from activity of yeasts. Seed or soil inoculations with phosphate solubilizing microbes (PSM) have largely been used to improve crop growth and production by solubilizing of fixed and applied phosphates [27].The inoculation of bean seeds with yeast under saline conditions increased the percentage of germination compared to non inoculated seeds [19].

3.4 TREATMENT OF SALT AFFECTED SOILS BY SOIL YEAST ISOLATES

Collected salt affected soils were treated with soil yeast in order to study the tolerance of soils yeasts and to promote soil fertility. After four weeks treatment, salinity was measured. According to this study, salinity of treated soils was slightly decreased but not obvious, and some mineral contents (total N_2, available P and K, P_2O_5) were slightly increased (Table 4). Before treatment of salt affected soils (initial soil), total nitrogen content, K^+, available nutrients (P and K_2O) were lower than those of treated soils with salt tolerant soil yeasts. But increasing of some mineral contents was not obvious. It may be due to treatment duration in this study. Nitrogen fixing and potassium decomposing activities of soil yeast were not detected, so it was not known whether these isolates have the above activities. But increasing of total nitrogen content and potassium in soils might be due to the supply of soil yeasts to the growth of other beneficial microorganisms in soils. But from this study, it is believed that isolated salt tolerant yeasts can contribute positive effects when they are applied in salt affected soils. At high salinity level, it was found that treatments supplied by biofertilization with yeast decreased the adverse effect of salinity. Halophilic microorganisms are already in use for some biotechnological processes, such as commercial production of carotene, polymers, enzymes, compatible solutes [1]. Reference [19] also said that bio-fertilization of four Egyptian maize varieties grown in saline conditions with *Rhoditorula glutinis* influenced the plant K-content.

After four weeks treatment of salt affected soils with soil yeast isolates, maize were grown in these treated and untreated soils. At first, germination of maize was first seen from treated soils. In untreated soil, germination took more time than those in treated soils. The growth of maize from all treatments was measured in height. After four weeks, height of maize from treated soils was higher than those of untreated soil (Table 5). An enhancement of growth of maize might be a result of increasing available phosphorus from microbial activity and promotion of soil yeasts to growth of other beneficial microbes in soils. Similar finding was also proposed in [35]. The use of micro-organisms to increase the salt tolerance of maize was also studied in [36]. A growing number of studies indicate that plant root growth may be directly or indirectly enhanced by yeasts in the rhizosphere ([37], [38], [39]). A wide diversity of soil yeasts have been researched for their potential as bio-fertilizers ([40], [41]).

4 Tables and Figures

4.1 Tables

Table 1. NaCl Tolerance of Isolated Yeast Strains on PYG Media Supplemented with Different NaCl Concentrations

Isolates	*NaCl concentrations*					
	0%	3%	6%	9%	12%	14%
I1	+++	+++	+++	++++	++	+
I2	+++	+++	+++	+++	++	+
I3	+++	+++	+++	+++	++	+
I4	+++	+++	+++	+++	++	+
I5	+	+	-	-	-	-
I6	+	+	+	-	-	-
I7	+	+	-	-	-	-
I8	+	+	-	-	-	-
I9	+	+	+	-	-	-
I10	+	+	-	-	-	-
I11	+	+	+	-	-	-
I12	+	+	-	-	-	-

Table 2. Sugar Assimilation and Fermentation Abilities of Selected Yeast Isolates

Sugars	I1		I2		I3		I4	
	SA	SF	SA	SF	SA	SF	SA	SF
Fructose	+	+	+	+	+	+	+	+
Lactose	+ (WG)	+	-	-	-	-	+(WG)	+
Mannitol	+	+	+	+	+	-	+	-
Maltose	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Xylose	+	-	+	-	+	-	+	-
Sucrose	+	+	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+	+	+

SA – Sugar Assimilation, SF – Sugar Fermentation, WG – Weak Growth

Table 3. Some biochemical characteristics of soil yeast isolates

Isolates	Urease	Utilizing of citrate	Utilizing of methanol	Utilizing of ethanol	Utilizing of glycerol
I1	+	+	-	+	+
I2	+	+	-	+	+
I3	-	+	-	+	+
I4	-	+	-	+	+

Table 4. Salinity (EC ms/cm), Total N_2 (%), K^+, and available nutrients (P and K_2O) of treated and untreated soils after four weeks treatment

Samples	EC ms/cm	Total N2 %	K^+ meg/100gm	Available Nutrients	
				P(ppm)	K_2O mg/100
T1 (I1)	0.95	0.183	1.56	16.42	73.27
T2 (I2)	1.06	0.183	1.64	18.94	77.35
T3 (I3)	0.96	0.202	1.40	16.57	66.10
T4 (I4)	1.07	0.183	1.44	16.84	67.78
T5 (Initial soil)	1.89	0.181	1.38	15.66	65.08

Table 5. Growth of maize biofertilized with salt tolerant soil yeasts after four weeks

Treatment	Plant heights(cm)
T1 (I1)	32
T2 (I2)	25.33
T3 (I3)	34.44
T4 (4)	26.44
T5 (Initial soil)	24.11

4.2 Figures

(i) (ii) (iii)

Fig. 1. Growth of mustard and maize in collected salt affected soils and normal cultivated agricultural soils;(i) growth of mustard, (ii) growth of maize in normal cultivated agricultural soil, and (iii) growth of maize in collected salt affected soil

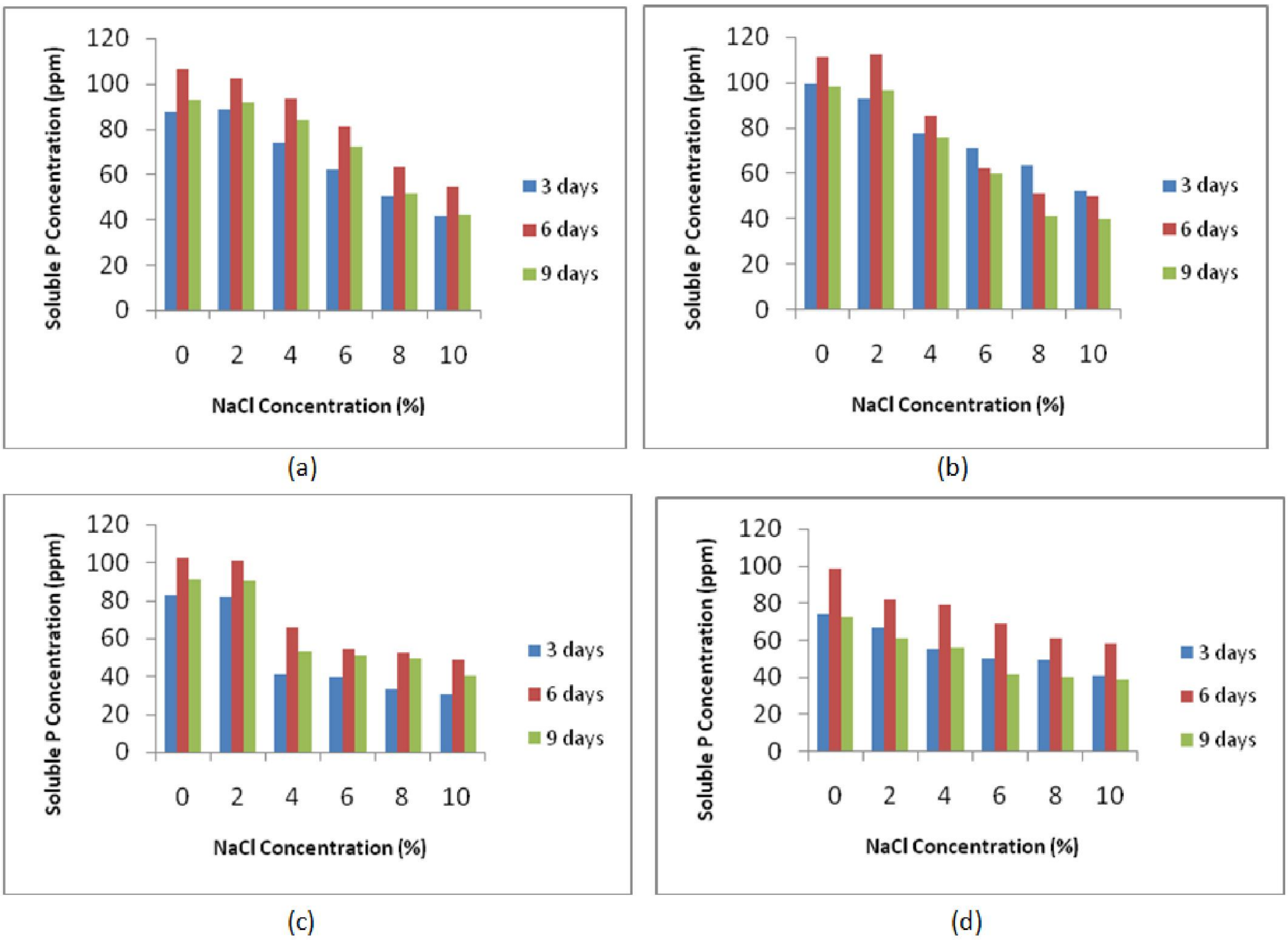

Fig. 2.   Soluble phosphate (ppm) solubilized by four soil yeast isolates in Pikovskaya's broth containing tricalcium phosphate at various NaCl concentration: (a) I1 isolate, (b) I2 isolate, (c) I3 isolate and (d) I4 isolate

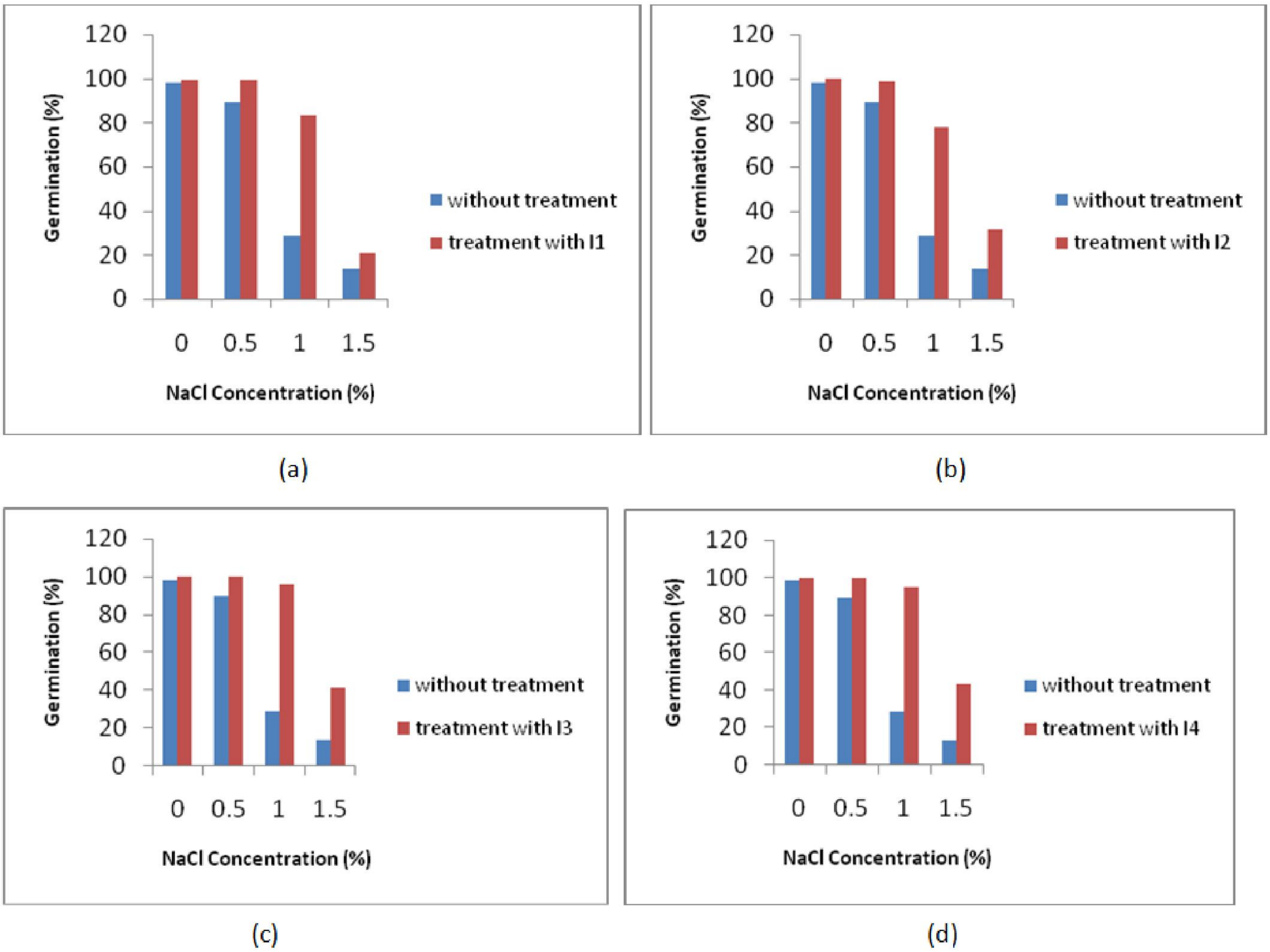

Fig. 3. Effects of soil yeast isolates on maize germination under NaCl stressed conditions; (a) I1, (b) I2, (c) I3 and (d) I4

5 CONCLUSION

Four soil yeast isolates were obtained. These four yeast isolates tolerated to NaCl at 14% and to $MgCl_2$, KCl and $CaCl_2$. Phosphate solubilization of soils yeast isolates was decreased with increasing NaCl concentration. Above 10% NaCl concentration, soluble phosphorus solubilized by soil yeasts was not detected. Although they can solubilize insoluble phosphate at 10% NaCl concentration, germination of maize grown in 2% NaCl concentration was not enhanced. So these isolates limited range of NaCl tolerance for their function. Treatment of salt affected soils by these isolates, some mineral contents in soils were slightly increased but not obvious. Growths of maize in treated soils were slightly higher than those of untreated soils.

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