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Effect of nanoprosil-1 (lus-1) in combination with vermicompost for production of pseudomonas fluorescens inoculants on the growth and yields of maize

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The purpose of this study was to determine capability of bacteria Pseudomonas fluorescence survival on nanoprosil-1, vermicompost, rock phosphate, bentonite and three formulations of them and also, their effect on yield, growth indices and phosphorus uptake in maize. After inoculation of examined carriers with bacteria Pseudomonas fluorescence, inoculants were maintained in 28 ̊C for 15 days and then till the end of 180 days in the refrigerator. Bacteria populations were measured at time 180 day through CFU method. Then inoculants were used for greenhouse culture of maize. The experiment was performed through complete randomized block design with five inoculants and two fertilizers treatments in four replicates. After 60 days, some plant growth indices and phosphorous in soil and shoot were determined. The results showed that use of bacteria Pseudomonas fluorescens inoculants based on vermicompost and also the combination of vermicompost and nanoprosile-1 promoted growth indices, soil and plant phosphorus in comparison with control plant significantly ($P<0.05$). Based on the results, triple phosphate treatment was relatively the best treatment regarding to phosphorus, but this difference was not significant with vermicompost and combination of vermicompost and nanoprosil-1 treatments ($p>0.05$).

1. Introduction

Growing population and consequent increase of indiscriminate use of chemical fertilizers to increase agricultural and horticultural crops along with industrial development and mass production of fertilizers, the world faces the threat of environmental pollution (Adesemoye et al, 2009; Cordell, 2009). All these imply that healthy food production should be much more efficient in the near future than past .It is believed that nano-fertilizers application is the most effective and simplest way to reduce nutrient losses and increase the efficiency of chemical fertilizers. In this regard, the use of nano-fertilizers can be an effective step towards achieving environmentally sustainable agriculture through precise control of nutrient release (Cui, 2006). As general, advantages of nanofertilizers compared with conventional chemical fertilizers are as following: (1) increase efficiency due to no fertilizer loss by leaching and complete uptake of fertilizer nutrients by plants because of suitable release speed of nutrients from fertilizer (2) reducing soil compaction (3) Reduce plant toxicity and stress induced by high local concentration of salt in the soil (4) Increasing crop yields due to favorable nutritional status (5) improve the handling and storage characteristics and fertilizer transportation facilitation (Cui, 2006). Due to the release of nutrients from nanofertilizers in accordance with the plant requirements, only once application of nanofertilizers can be sufficient for nutrient need of plants throughout the growth season. So using them as compared to conventional chemical fertilizers, which require multiple applications during the growing season have resulted in reducing costs of the application of fertilizers in the farm (Shaviv, 2005). According to an estimate made in Canadian funds by taking nano-fertilizers can save $ 2,000 million because the low efficiency of conventional fertilizer nutrients by crops can be prevented (Monreal, 2010). Liu et al. (2006) state that conventional chemical fertilizers covered by nanomembranes can release nutrients slowly and steadily. Few literatures have been published on the role of nanoparticles as bacteria carriers. Thus, due to usefulness and high potential of nanomaterials in agriculture and biotechnology, and positive effect of silica on corn, the aim of this study was to investigate the potential of nanoparticle nanoprosil-1 (lus-1) in the production process of nano-inoculant and its role in promoting growth of maize.

2. Materials and methods

In this experiment, four materials including nanoprosil-1, vermicompost, rock phosphate, bentonite and different combinations of them were used as carrier. Porous silicate nanoparticles (lus-1) were prepared from department of inorganic chemistry of Tehran University and its hydrophilic form was used in this experiment. It was patented in Tehran University and Laval University in Canada in 2001 (Benoit, 2001). The materials were grounded and passed through a 250 µm sieve. Then, they were oven-dried. Some physical and chemical properties of these materials were determined (Table 1) (Sparks, 1996; Carter and Gregorich, 2008). For inoculants production, first carriers were sterilized and then Pseudomonas fluorescens suspension was prepared and added to the carriers. Subsequently, inoculants were kept for 15 days in incubator at 28̊C and then till the end of 180 days in the refrigerator. Inoculants population was determined through CFU method at the end of 6-month preservation period (Table 2) (Albareda et al., 2011). For greenhouse tests, surface soil (0-30 cm depth) was sampled from Kordan in Karaj city (Alborz Province) and transferred to the laboratory, air-dried, grinded, passed through 4mm sieve and added to the pots. Physicochemical properties of soil were measured after passing it through 2mm sieve (Table 3). SC 260 cultivar of corn seeds were obtained from the Seed and Plant Improvement Institute Gene Bank. For inoculation, the surface of seeds were sterilized and then seeds were planted at a depth of approximately 4 cm in each pot and according to type of carrier treatment, 0.1 g of carriers were added to the soil around the seed (Bashan, 1998). Prior to planting, based on the results of soil test, urea (equivalent to 300 kg N per hectare) and potassium sulfate (equivalent to 270mg per kg) were added to the pots. Also, triple and single superphosphate were added to the pots 179.9 and 420 kg ha-1, respectively as phosphorus fertilizer treatments. The Hoagland solution was added to plant twice in order to supply trace elements. The experiment condition was as the following: growth period, 60 days; light intensity, 15000 Lux and light period at the first and the end of the growth period, 12 and 14 hours, respectively. Average greenhouse temperature was 26 ̊C and temperature range was between 17 and 25 ̊C. Irrigation was performed at 80% field capacity once a day. After the plant growth period (60 days), soil phosphorus, and shoot length were measured. Total leaf area was measured by leaf area meter model CI-202 as cm2. Also, stem diameter was measured by digital caliper with resolution of 0.05 mm. After harvest, root volume was determined by cylinder method. Dry weight of shoot and root (resolution 0.01 g) were measured after drying in 75 ̊C oven for 72 h and weighting. Then, the dried shoots were grinded and extracted (Cottenie, 1980) and their shoot phosphorus was measured (Ryan et al., 2001). The experiment was performed in a randomized complete block design (RCBD) with five inoculants and two fertilizer (triple and single superphosphate) treatments and control in four replicates. Data were analyzed using SAS statistical software. The mean comparison of data was performed by Duncan,s test at 5 percent confidence limit. Excel 2007 software was used for plotting the curve.

3. Results and discussion

As Table 1, the materials were used as carrier had different characteristics; so vermicompost had the highest amount of organic matter and nutrients among them, but because of the differences in materials, such as electrical conductivity, pH and organic carbon, etc, three formulations were prepared by mixing of these materials.

Table 1
Some physical and chemical characteristics of carrier materials.

Parameter	POlsen (mgkg-1)	N (%)	pH	EC(dSm^{-1})	bulk density (g/cm^{-3})	Organic carbon (%)
Nanoprosil-1	0	0	9.3	3.95	0.16	0
Vermicompost	1375	7.4	7.34	1.72	0.7	17.7
Rock phosphate	2	0	7.64	0.558	1.65	0
Bentonite	0	0	8.02	0.96	0.97	0

After 6 months of inoculants storage, their population was measured through CFU method during inoculation to corn. The treatments 5 (Nanoprosil-1 + bentonite + vermicompost), 4 (Nanoprosil-1 + bentonite + vermicompost + rock phosphate) and 1 (vermicompost) had the largest population with 6.44×107, 4.18×107 and 2.41× 107 bacteria per gram of carrier, respectively. Since nanoprosil-1 and nanoprosil-1+ bentonite + rock phosphate inoculants could not maintain optimum bacterial population at the end of 6 months storage, these two treatments were not used for maize culture.

Table 2
Cell count of inoculants during inoculation.

Treatment	Carrier	CFU/gr
1	Vermicompost (V)	2.41×107
2	Bentonite (B)	8.31×105
3	Rock phosphate (R)	5.48×105
4	Nanoprosil-1+ Bentonite+ Vermicompost+ Rock phosphate (N+B+V+R)	4.18×107
5	Nanoprosil-1+ Bentonite+ Vermicompost (N+B+V)	6.44×107

According to the results of soil physical and chemical properties has appropriate for this study in terms of high pH, low phosphorus and organic matter, so that loamy texture of this soil is one of favorable properties for maize cultivation.

Table 3
The physicochemical characteristics of studied Soil.

Parameter	Texture	EC)dS/m(	pH	OC (%)	Nt (%)	Pa (mgkg-1)	Pt (mgkg-1)	Ka (mgkg-1)	)(CFU/gr
Result	loam	0.7	8.20	0.09	0.053	4.1	1200	190	9.2×106

Analysis of variance (Table 4) showed that the effect of treatments on shoot dry weight, root dry weight, root volume, chlorophyll, leaf area, stem diameter, shoot phosphorous and soil phosphorous at 1% and the plant height and root dry weight at 5% level were significant.

Table 4
Analysis of variance of treatments effect on growth parameters of maize.

S.O.V	DF	Shoot dry weight (g)	Root dry weight (g)	Root volume	Leaf erea	Plant height	Steam diameter	P shoot (%)	P soil (mg/kg)
Block	3	105.6 ns	0.47ns	309.4ns	459551**	500.8*	0.038*	0.0001ns	3.6ns
Treatment	7	159.1**	2.3*	747.7**	505121**	315.5*	0.069**	0.0009**	45.6**
Error	21	30.5	0.78	72.5	83389	108.9	0.010	0.0002	6.2
CV	-	11.7	10.5	13.2	8.7	8.91	7.38	15.7	16.3

The results of mean comparisons (Table 5) showed that although triple and single super phosphate had the highest shoot and root dry weight, there was not significant differences with inoculants no.1 (vermicompost) and no.5 (nanoprosil-1 + bentonite + vermicompost compost) showed. Mehnaz et al (2009) stated Pseudomonas fluorescens bacteria increase dry matter and yield of the plant by producing Indole-3-acetic acid (IAA) and phosphate solubilizing acids. Poonguzhali et al (2005) examined 10 strains of Pseudomonas which were capable of producing ACC-deaminase and dissolving phosphate and found that those strains increased root growth and dry weight. Also, Piromyou et al (2011) showed that maize seed inoculation with phosphate solubilizing bacteria Pseudomonas sp. and Brevibacillus with compost increased root weight. Shaharoona et al (2006) studied the effect of different strains of Pseudomonas in different conditions of fertilizer on the growth of maize and showed that different strains of the bacteria can increase dry weight of corn according to nitrogen fertilizer between 15.2-19.7 percent compared with the control. Yang et al (2008) stated that addition of optimum value of silicon in phosphorus deficiency conditions increased the shoot dry weight and also uptake and accumulation of phosphorus and silicon in the shoot of plant significantly that is an explanation for the effect of silicon on dry weight increase of maize which observed in inoculant no.5. Since the inoculant (1) (vermicompost) had a positive effect on shoot dry weight and vermicompost existed in other inoculants (ie.1 and 5) which resulted in high dry weight, some of this increase can be attributed to the presence of vermicompost in them. The favorable effect of vermicompost is probably due to the relatively higher levels of nutrients and hence macro and micro nutrients availability (Jat and Ahlavat, 2008).

Table 5
Mean comparison among experimental treatments.

Treatment	Shoot dry weight (g)	Root dry weight (g)	Root volume	Leaf erea	plant height	Steam diameter	P soil (mg/kg)
1- Vermicompost (V)	51.42^{ac}	7.95^{ab}	70^{b}	3541^{ab}	124.3^{ab}	1.51^{ab}	16.46^{ab}
2- Rock phosphate (R)	40.10^{c}	6.55^{c}	51.20^{c}	2917^{c}	102.3^{c}	1.30^{c}	9.69^{d}
3- Bentonite (B)	41.70^{c}	6.52^{c}	56.75^{c}	2970^{c}	112.7^{ac}	1.36^{bc}	11.76^{cd}
4- N+B+V+R	46.35^{b}	7.22b^{c}	53.26^{c}	3271^{bc}	116.0^{bc}	1.40^{bc}	13.10^{bc}
5- N+ B+ V	48.02^{ac}	7.60^{ab}	71.25^{b}	3265^{bc}	117.2^{bc}	1.38^{bc}	14.01^{bc}
SSP	54.32^{ab}	8.05^{ab}	80.07^{ab}	3509^{ab}	128.2^{a}	1.64^{a}	15.50^{bc}
TSP	55.91^{a}	8.40^{a}	85.00^{a}	3895^{a}	125.8^{a}	1.60^{a}	19.45^{a}
Blank	39.92^{c}	6.27^{c}	50.03^{c}	2985^{c}	108.0^{c}	1.30^{c}	9.32^{d}

According to Table 5, triple super phosphate had the highest leaf area, but no significant difference between this fertilizer treatment and inoculant no.1 (vermicompost) was observed. Studies have shown that PGPR bacteria increase the leaf area. Gholami et al (2009) expressed elevated levels of leaf area in bacteria treatments can be due to plant hormones production and increase of nutrients availability (phosphate dissolution). It has been observed that in different plants the use of rhizobacteria stimulating plant growth resulted in increase in leaf area and this has been attributed to the ability of bacteria to produce IAA.

Another reason for the increase in these parameters can be expressed due to vermicompost. Asciutto et al (2006) observed an increase in the amount of vermicompost substrates increases root dry weight and leaf area. Golchin et al (2006) reported leaf area index of pistachio leaves in treatments of vermicompost was higher than control. But the results showed that the single and triple super phosphate had the highest root volume and significant difference with inoculants. Correlation coefficient between root dry weight and volume (r2 = 0.70) indicates that there is a direct relationship between root volume and dry weight. Also, the results show that in the treatments the greater root dry weight, the higher root volume.

The result of mean comparisons of the treatment effects on plant height and stem diameter at 5% level showed the highest amount was related to single superphosphate, then followed by triple super phosphate and vermicompost that there was not significant differences between these two treatments with single superphosphate. Rodriguez and Fraga (1999) stated some strains of Pseudomonas putida and Pseudomonas fluorescens caused the stem elongation of canola, lettuce and tomatoes. Jarak et al (2012) showed that hybrid inoculation of maize with Pseudomonas fluorescens, Bacillus and Azotobacter significantly increased plant dry weight and also, the maximum height plant was obtained from the combined treatment of Pseudomonas fluorescens + Bacillus. Ekin (2010) describes the use of insoluble phosphate solubilizing bacteria with different amounts of phosphate fertilizer increased sunflower stem diameter. Mean comparisons of the treatments on soil phosphorus (Table 5) showed that inoculant Pseudomonas fluorescens based on vermicompost caused significant increase of soil soluble phosphorus in comparison with control at 5% level. Although triple superphosphate fertilizer treatment had the highest soil soluble phosphorus, vermicompost inoculant did not show significant difference with this treatment. Many researchers stated that the seed or soil inoculation with phosphate solubilizing bacteria can dissolve stabilized soil phosphorus and change it to available phosphorus, thus increase crop yield (Puente et al, 2004; Canbolat et al, 2006). Another reason for vermicompost priority is considered the higher amount of soluble phosphorus in vermicompost (1375 mg kg-1) even than total phosphorus in studied soil (1200 mg kg-1).The researchers stated that vermicompost increased phosphatase activity and changed phosphorus to available form (Pramanik et al, 2007; Saha et al, 2008). The result of mean comparisons among treatments on shoot phosphorus at 5% level showed (Figure 1) that the highest shoot phosphorus content was related to triple super phosphate treatment, followed by vermicompost inoculant and single super phosphate treatments which this difference was not significant.

Hamda et al (2008) showed that the use of phosphate solubilizing bacteria Pseudomonas fluorescens and Serratia marcescens with rock phosphate in the greenhouse conditions increased plant phosphorus 42 and 47 percent, respectively. Also, the increase of phosphorus uptake and total phosphorus potential of many legume plants by the use of phosphate solubilizing bacteria has been reported by many researchers. Also, about vermicompost treatment, Prabha et al (2007) showed that vermicompost as an organic source increased available phosphorous, potassium and iron. Studies suggest that an increase in organic matter, while improving its ability to uptake phosphorus, increases application efficiency. About the effect of silica on plant phosphorous, hydroponic experiments by Ma and Takashi (2002) showed that when phosphorus was low, the use of silica increased phosphorus availability and its uptake. Results of Yang et al (2008) showed that adding the optimum amount of silicon in phosphorus deficiency conditions increased stem, root and leaf dry weight, leaf chlorophyll, uptake and accumulation of phosphorous and silicon in stem and leaves significantly. These results indicate that application of silicon in phosphorus stress conditions improved the situations significantly.

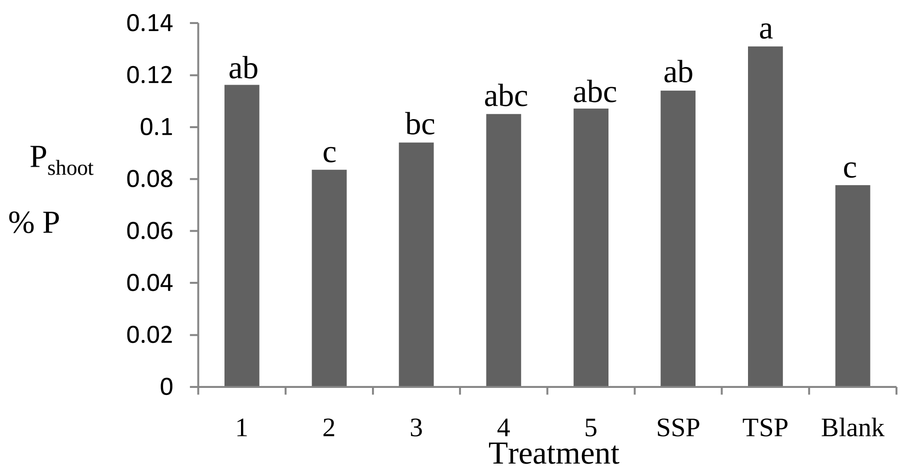

Fig. 1. Mean comparison of phosphorus in shoot.

4. Conclusions

According to the results it can be expressed using this nanoparticle as a carrier alone is not suitable, but it can be a appropriate formulation for nanobiologic inoculants when combined with vermicompost. Findings showed that using Pseudomonas fluorescens inoculants increased growth indices and shoot phosphorus in maize significantly. Pseudomonas fluorescens inoculants based on vermicompost increased dry weight of shoot, soil and leaf phosphorus at 28, 77 and 51 percent, respectively. Therefore it can be concluded that the use of Pseudomonas fluorescens enhances maize yield and reduces fertilizer consumption.

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