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Research article

Consolidated effect of bacterial strains as bio-inoculants on growth of maize: a comparative approach

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HIGHLIGHTS

- Use of bio-inoculants can reduce the cost of chemical fertilizer with increase in production
- Bio-inoculants can be helpful to achieve increase in production

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ABSTRACT

Maize is third important crop grown in Pakistan contributing 2.2 percent to the quality included farming and 0.5 percent to GDP. Chemical fertilizers are frequently used in Pakistan which increases cost of production and give less profit to the farmer. In this study two main bio-inoculants namely PGPR & PSB were used in-combination with chemical fertilizer to compare the effect of both as single/co inoculants on maize crop. A pot experiment was conducted where 9 treatments of bio-inoculants and chemical fertilizer combined/ separately connected at the sowing time. Information was treated by ANOVA Least Significant Difference (LSD) test at probability level 0.05 was used to seclude the techniques when the ANOVA F-test showed a vital effect of treatments. The co-inoculation of PGPR + PSB + K + ¾ N + ¾ P shows significant results among nine treatments of all parameters that were observed. So use of bio-inoculants can reduce the cost of chemical fertilizer with increase in production.

Key words: Maize, Bio-inoculants, Rhizobacteria, P-solubilizing bacteria

1. Introduction

Maize (*Zea mays. L),* being the most imperative yielding among grains on the earth is of critical significance for countries like Pakistan, where rapidly extending people has formally out stripped the open support supplies. Plant development advancing PGPR are class of microscopic organisms that effectively colonize plant roots and improves its development and yield (Wu *et al.,* 2005).Various techniques are used by which plant growth is promoted such as enhanced plant hormone concentration, a symbiotic N_2 fixation, antagonistic effect on phytopathogenic microorganism and solubilization of different soil nutrients (Burd *et al.,* 2000; Zahir *et al.,* 2003). Inoculation of microbial cultures can be significant to increase plant growth, seed germination, seedling emergence, responses to external harmful factors, disease protection and root growth pattern (Lugtenberg *et al.,* 2002). Maize inoculation with PGPR *(Azospirillum)* dominantly impacts plant dry weight, height; fertility and grain yield (Biari *et al.,* 2008). Seed inoculation of maize with isolated PGPR strains does not only increase crop yield per unit area but also improved yield stability for better plant fitness (Hegedus *et al.,* 2003). Phosphate solubilizing microorganisms (PSB) are used as bio fertilizerfor last 50 years (Kudashev, 1956). They discharge distinctive sorts of organic acids e.g. carboxylic acid (Deubel & Merbach, 2005) thus decrease the pH in the rhizosphere and subsequently separate the bound forms of phosphate like $Ca_3(PO_4)_2$ in calcareous soils. Synthetic Phosphorus is exceptionally costly and its utilization effectiveness by products may extend from 10 - 25%. Solubilization of fixed soil P through the use of micro-organisms is available option to make phosphorus available in easily assailable form by the crops. A compelling increase was noticed in grain yield ranged from 27-50% over the control because of inoculation of PSB (Khokhar *et al.,* 2006)**.**Inoculations with beneficial bacteria has been recommended to ensure crop inoculation response in field to cope with variability in soil conditions. Seed inoculation decrease of 50% in mineral fertilizer application rates for maize (Shatokhina and Khristenko, 1996). Co-inoculation of PGPR and phosphate solubilizing bacteria was more proficient than single inoculation for providing an effective and balanced nutrition to plants (Belimov *et al.,* 1995). This study was conducted with specific objectives of (i) to study out the vegetative parameters of maize using synthetic fertilizer and co-inoculation of PGPR and PSB; (ii) to study the suitable level of chemical fertilizers and plant uptake of nutrients; (iii) to explore the result of phosphorus rate with and without phosphate-soluble bacteria and (iv) to minimize the expenses on chemical fertilizers.

2. Materials and methods

2.1. Study area

The National Agricultural Research Centre (NARC), Islamabad (latitude 33º. 43′ N, longitude 73º 04′ E, with altitude 490 Msl) was selected as a study area (Figure 1). The site contains Nabi pur soil series, was classified as *Typic Camborthid* (*Calcaric Fluvisols*). It is developed from a mixed, calcareous alluvium, and is weakly differentiated, they are generally medium textured to clay-loam with native organic matter as low as <2.5g kg-1. The study range has a regular atmosphere adaptation of humid subtropical, a claim to fame of covering the all major five seasons. The average low temperature is 2 °C (35.6 °F) in January, while the normal high temperature is 38.1 °C (100.6 °F) in June.

2.2. Isolation

Isolation was carried out from maize, *ipil ipil* and white clover. Serial dilution method was adopted for the isolation. Seven screw cap tubes was used with 9ml sterilized distilled water. One gram rhizosphere soil in case of maize and white clover was taken in first tube shaken vigorously for 1-2 minutes on reciprocating shaker. 1gm crushed nodules were taken from *ipil ipil* roots and same process was carried out. 1ml of suspension was transferred through micro pipette into test tube no 1. The procedure was repeated till up to tube no 7.For the growth of bacterial colonies we use dilution 5,6,7 and 0.1ml was taken from each dilution and spread on the media plate with the help of spreader, mark the plate. The procedure was repeated for other dilutions as well. Then inverted plates were placed in an incubator at 28 ^{o}C for 24 hrs. Purification of strains was done by streaking method. Bacterial colony was taken by sterilized loop and streak on the media plates three ways streaking was done. The cultures were incubated at 28 ^{o}C for 24 hrs to obtain pure cultures of bacterial isolates. Gram staining was done to characterize the isolated and purified bacterial strains; microscopic slides were prepared and examined for cell morphology (Loynachan, 1988).

2.3. Phosphate solubilization

Phosphate solubilizing bacteria was characterized by the formation of transparent hollow zone, zone diameter measurement, solubilization index (SI), pH change of media. Quantification of Phosphate solubilized by Phosphate solubilizing bacteria using, phospho-molybdate method through spectrophotometer at 882 nm (Murphy and Riley, 1962).

2.4. Quantification of indole acetic acid

Indole Acetic Acid (IAA) was characterized by using fully grown cultures centrifuged at 3000 rpm for 30min. The supernatant (2ml) was mixed with two drops of orthophosphoric acid and 4ml of the Salkowski reagent (50ml, 35% of per-chloric acid, 1ml 0.5M $FeCl_3$ solution).The optical density (O.D) was resulted as 530 nm using spectrophotometer. The level of IAA produced was estimated by a standard IAA graph (Ahmad *et al*., 2005).

2.5. Experimental design

An experiment was conducted on maize with nine treatments following Complete Randomized Design (CRD), with four replications. Fertilizer nitrogen @ 120kg N ha^{-1}, 80kg P_2O_5 ha^{-1} and K_2O $ha^{-1}$was applied to un-inoculated treatments as basal/ starter dose. PGPR strain (MZ-100) isolated from maize, phosphate solubilizing bacteria PSB (WPS-9) isolated from white clover and *Rhizobium* strain isolated from root nodule of *LeaucenealeucocephalaI pilIpil* (II) was used. Bacterial strains were utilized as maize seed coatings. Seed surface sterilization was done with 35% hydrogen peroxide for 120 seconds and bathe thoroughly in sterile distilled water. For inoculation seeds were coated by using carrier black soil putting bacterial broth cultures in the packets of sterilized carrier soil (85 ml culture/500 g soil). The formation of coating material and seed coating was carried out in laminar flow to avoid any contamination. Plastic pots were used with capacity of 6 kg soil. All treatments (inoculated + un-inoculated) were arranged in 36 pots i.e. 9 treatments with 4 replications for each treatment. Low chemical dose were applied in the experiment except three treatments, one with no inoculation, other with PGPR and PSB, third one with II and PSB. Growth parameters of maize was analyzed including plant height, chlorophyll content, no. of leaves, tasseling of plants and no. of corns. Data was factually treated by ANOVA minimum huge distinction (LSD) test at likelihood level 0.05 was utilized to isolate the methods when the ANOVA F-test demonstrated a noteworthy impact of treatments.

3. Results and discussion

Inoculation purpose 3 bacterial strains were isolated and purified from roots collected from different fields. These strains were identified and on the basis of their Gram reaction, colony color, size, shape, and cell morphology.

3.1. Colony and cell morphology

Out of three isolates two were Gram negative (i.e.) II, & W.C and third was Gram positive. The shape of all 3 isolates was regular. The cell shape short rod (II), cocci (MZ-100), and rod (WPS-9) were observed (Table 1)

3.2. Solubilization and quantification of P

Only WPS-9 shows P-Solubilization by the formation of hollow zone and co-relation with decrease in pH of culture media after incubation of 7 days. The solubilization of P computed by spectrophotometer and was found within the range from 0.2-0.8 %. Results of 3 isolates conclude 1 as PSB and two were PGPR (Figure 3).

3.3. IAA production

All the isolates showed significant production of indole acetic acid (IAA). The IAA production from these isolates ranged 0.14–0.20 µg/ml in culture medium. The isolates MZ-100 and II have highest concentration of IAA (Figure 4).

Table 1: Morphological characteristics of different bacterial isolates

Bacterial isolates	Gram Staining	Shape/form	Elevation	Margin	Opacity	Cell Shape
Ipillpil	-ve	Regular	Raised	Entire	Translucent	Short Rod
Maize	+ve	Regular	Flat	Undulate	Translucent	Cocci
White Clover	-ve	Regular	Flat	Entire	Opaque	Rod
Sunflower	+ve	Irregular	Raised	Undulate	Opaque	Rod/ Circular

3.4. Plant growth

The results of pot experiment shown that, In case of plant height the maximum height was observed (88.7cm) in treatment where PGPR + PSB + K + ¾N + ¾P was applied**.** The results of experiment conducted by (Nezarat and Gholami, 2009) showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants in non-sterile than sterile soil. Seed treatment with bio-inoculants enhanced the grain yield of field-grown maize by 85% compared to the un-inoculated (Hameeda *et al.*, 2008).The integration of PGPR and PSB shows convincing increase on plant height at 5% probability over control. Bacterial treatments has 30% higher plant growth with respective to fertilizer application. The treatment of *ipil ipil* phosphate solubilizing bacteria with fertilizer shows 21% significant plant height over un-inoculated control (Figure 5)

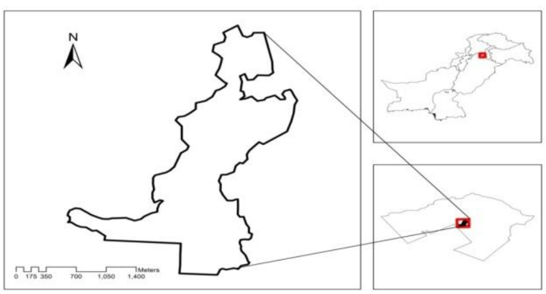

Figure 1: Study area NARC Islamabad

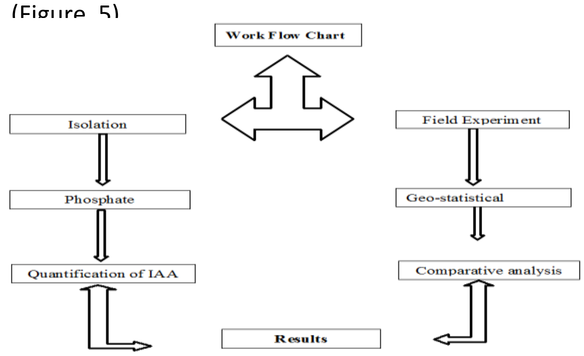

Figure 2: Work flow chart

Table 2: Pre experiment Physio-chemical analysis of soil

Parameters	Amounts/class
Organic matter	0.9%
NO_3^-N	1.13 µg g^{-1}
Available P	0.017ppm
Available K (extractable)	13.6 ppm
Electrical Conductivity (EC e)	0.5 dS m^{-1}
pH	8.73
Particle size analysis (textural class)	Loamy sand
Saturation percentage	47%
Zinc	2.443
Manganese	2.563
Iron	4.573
Copper	1.39ppm
Boron	0.88ppm

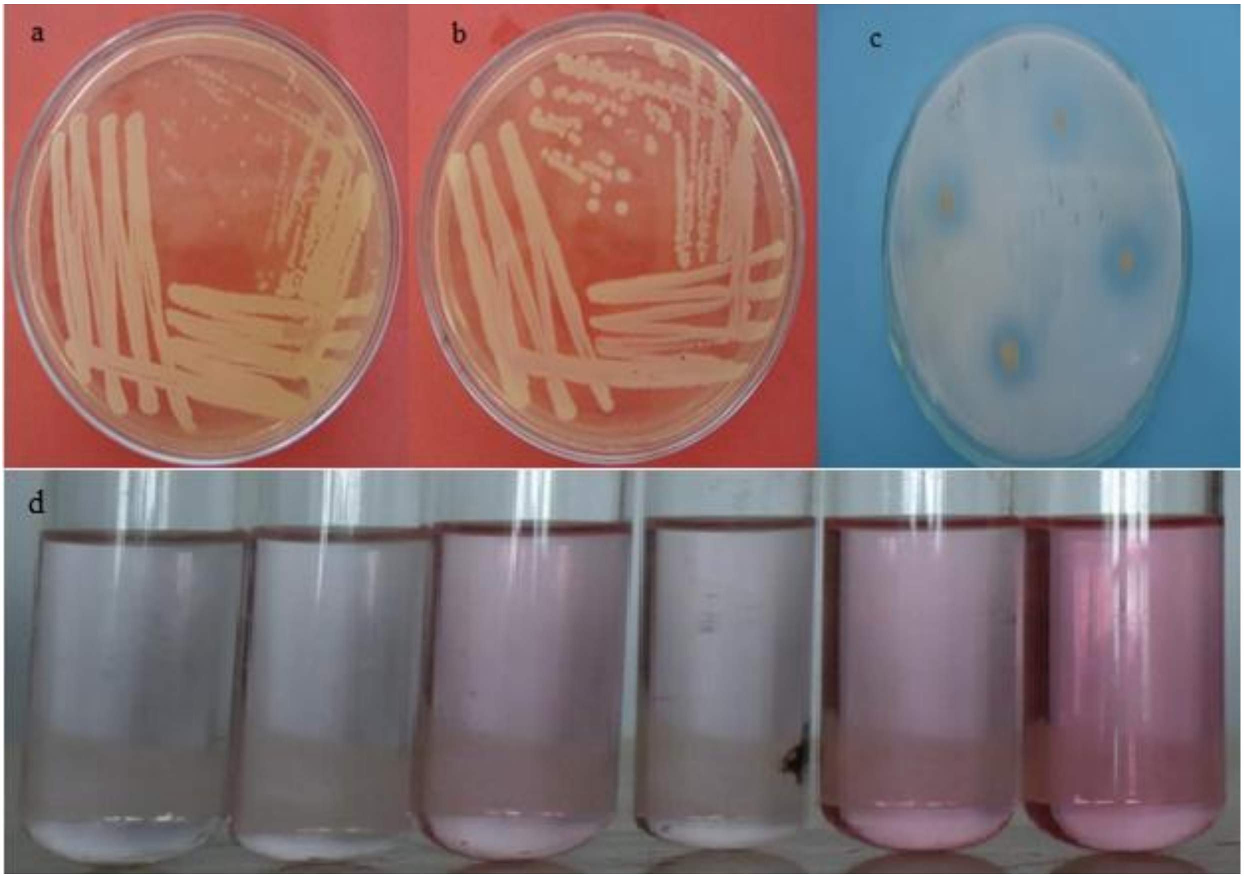

Figure 3: Bacterial isolates (a,b) and phosphate solubilization (c) and quantification of IAA (d)

3.5. Chlorophyll content

Maximum chlorophyll content was observed (50.03%) in treatment where PGPR + PSB + K + ¾N + ¾P. Treatment with PGPR(s) significantly increased plant height, shoot and seed dry weight, ear dry weight and length and number of seeds per row. Plants nutrient uptake of N, P, K, Fe, Zn, Mn and Cu were also significantly influenced by application of PGPR(s) (Biari *et al*., 2008).The integration of PGPR and PSB with K+ ¾ P+ ¾ N shows significant response on chlorophyll content of maize at 5% probability over control. Again it has 25% higher chlorophyll as compare to fertilizer application. The integration of *ipil ipil* phosphate solubilizing bacteria with NPK results 10% higher plant growth over NPK (Figure 5).

3.6. Number of leaves

The maximum no. of leaves/plant (11) was obtained in treatment where PGPR + PSB + K + ¾N + ¾P were applied. The integration of PGPR and PSB with K+¾ N+ ¾ P statistically shows significant density on leaves of maize per plant at 5% probability over control. The Stimulatory effects of bio-organic treatment on maize growth, yield, soil microbes and mineral content in grain and leaves were recorded. Bio-organic treatments greatly enhanced growth parameters of maize plant. Also, mineral content indicated high response reflected on grains yield (El-Gawad, 2008).

The combine effect has 27% more leaves as compare to control. The integration of *ipil ipil* phosphate solubilizing bacteria with NPK results 14% higher leaves in count over NPK (Figure 5).

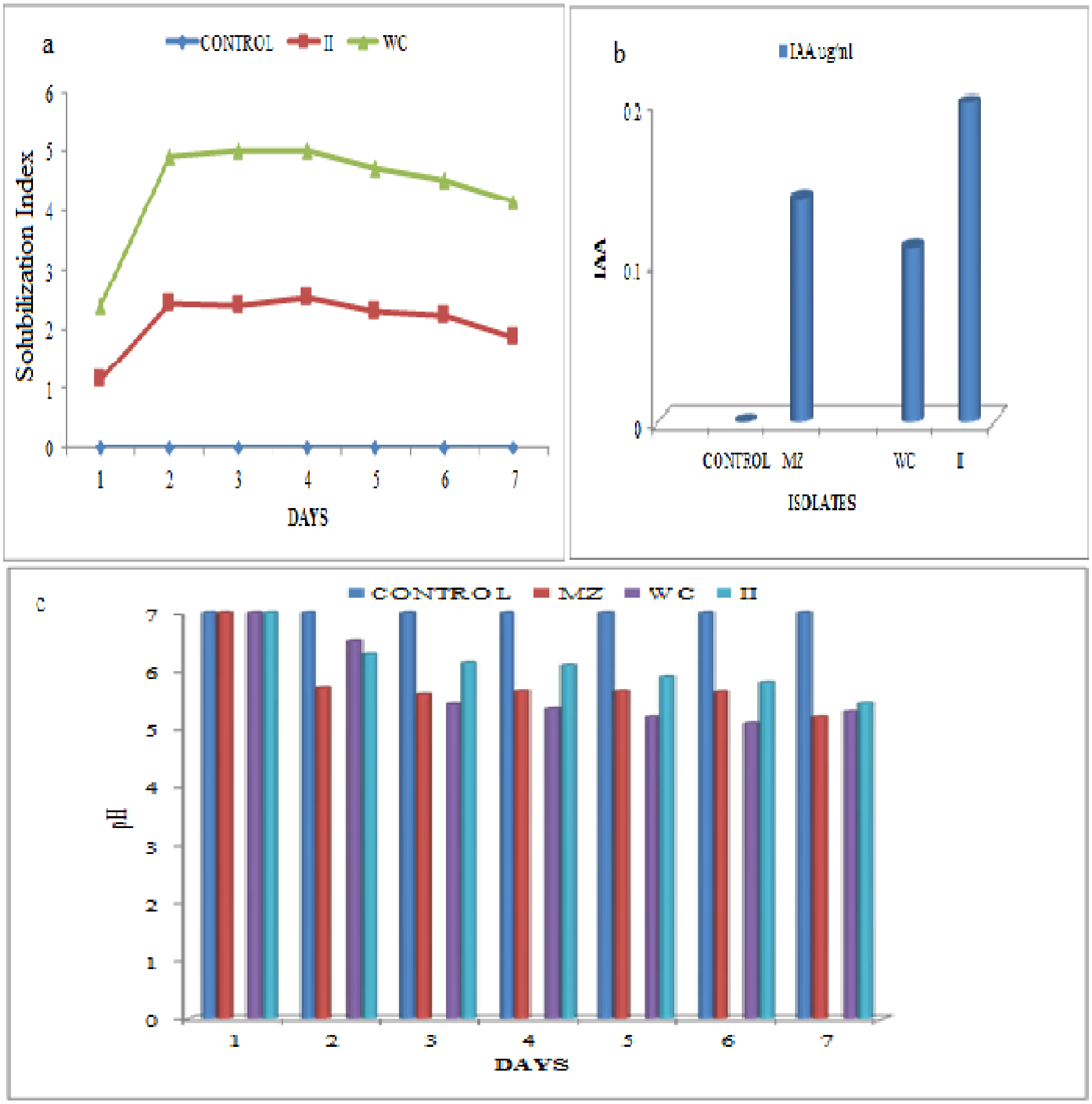

Figure 4: Solubilization Index, IAA and pH value among isolates

3.7. Number of fruits

The maximum no. of fruits/plant (8) was obtained in treatment where PGPR + PSB + K + ¾ N + ¾ P were applied. Seed inoculation with selected PGPR strains does not only increase crop yield per unit area but also improved yield stability for better plant fitness (Hegedus *et al*., 2003). The integration of PGPR and PSB with K+ ¾ N + ¾ P statistically shows significant density on leaves of crop at 5% probability over control. Bio-fertilizer application also dominantly enhanced uptake of N, P and K. The fluoresces biotype G (N3) containing bio-fertilizer was found best to increase grain yield and nutrient uptake both in the absence or presence of 88 kg N ha^{-1} (Naveed *et al.*, 2008).

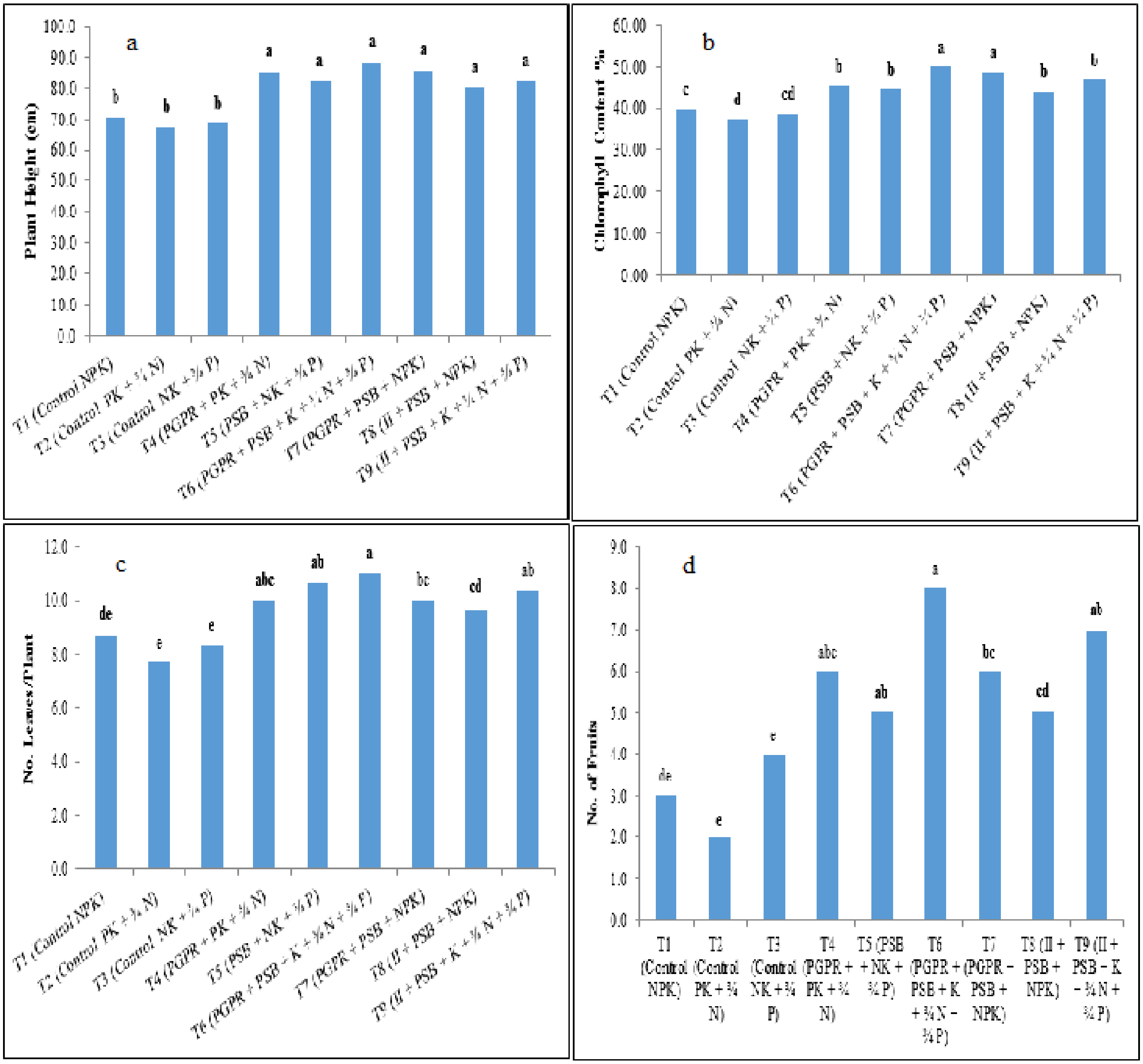

Figure 5: Integrated Effect of PGPR, PSB and chemical fertilizer on plant height (a), chlorophyll content (b), no. of leaves/plant (c) and no. of fruits (d) of maize

The combine effect has 25% more leaves with respective to chemical control NPK. The combine effect of *ipil ipil* phosphate solubilizing bacteria with NPK shows 12% higher no. of leaves over NPK (Figure 5).

3.8. Geo-statistical analysis using arc-GIS

Further statistical verifications was done by using Arc GIS**.** The quantile-quantile (q-q) plot is a graphical technique by which we can determine the two data sets come from populations with a common distribution. It is a plot of the quantiles of the first data set against the quantiles of the second data set. The quantile here means the fraction (or percent) of points below the given value. The distribution of all the parameters was observed and it was revealed that all the results were normally distributed as shown in (Figure 6).

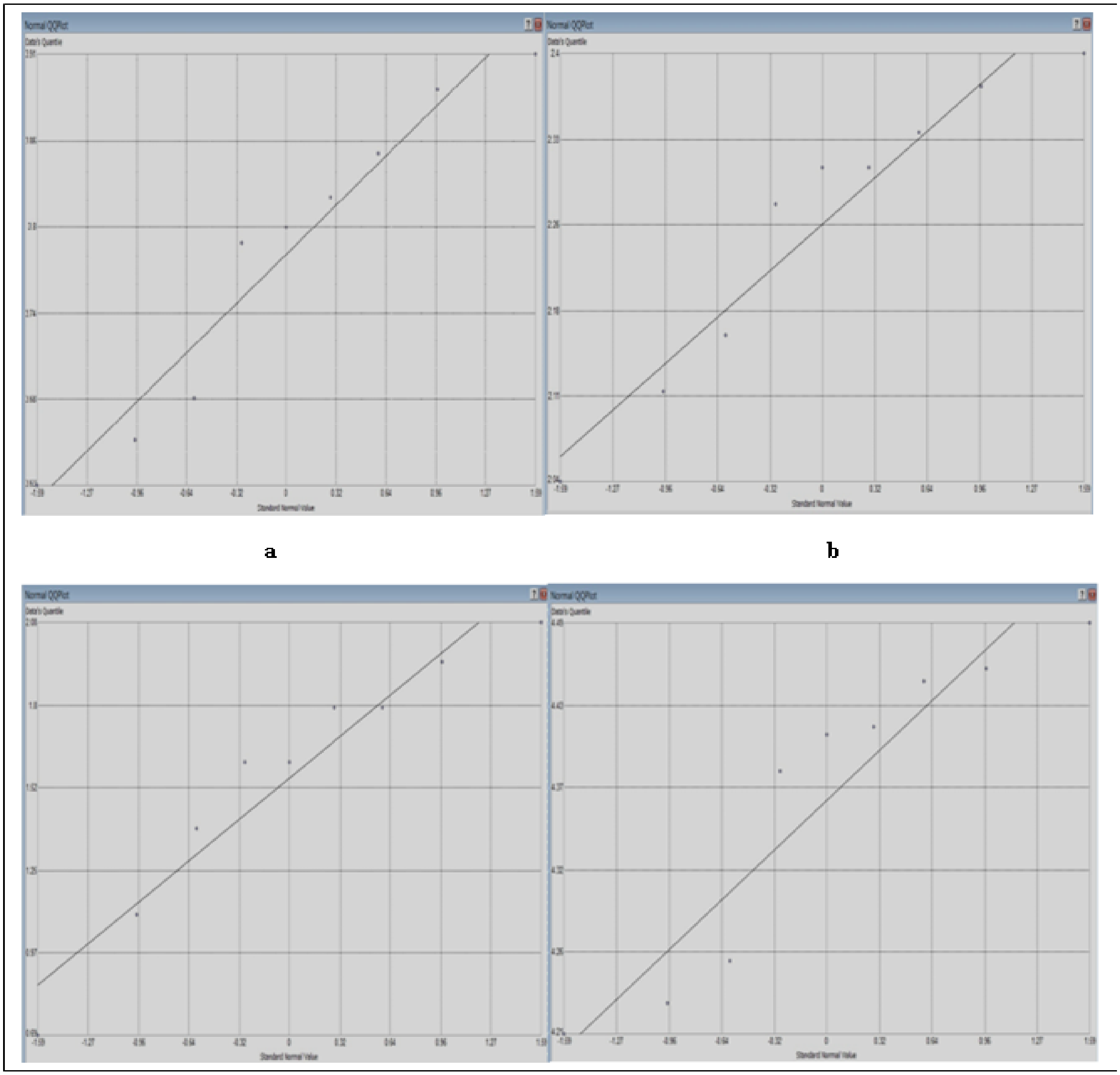

Figure 6: Normal Q-Q plot using Arc-GIS of, (a) chlorophyll content (b), no. of leaves/plant (c), no. of fruits (d)

4. Conclusions

The results of this study strongly supports the argument that the bio-inoculants work efficiently in fertilizer uptake and reduce the cost of chemical fertilizer. The treatments which contain bio-inoculants have significant growth in terms of vegetative and reproductive parameters. Bio-inoculants also create resistance against pests attack. The use of bio-inoculants will enhance soil properties and produce healthy/organic food. It is simple in application as well as financially cheap. For future recommendation bio-inoculants should be used on large scale field experiments.

References

Ahmad, F., Ahmad, I. and KHAN, M.S. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk. J. Biol., 29(1): 29-34.

Belimov, A.A., Kojemiakov, A.P. and Chuvarliyeva, C.N., 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant and Soil, 173(1):29-37.

Berg, R.K., Loynachan, T.E., Zablotowicz, R.M. and Lieberman, M.T. 1988. Nodule occupancy by introduced *Bradyrhizobium japonicum* in Iowa soils. Agron j., 80(6): 876-881.

Biari, A., Gholami, A. and Rahmani, H.A. 2008. Growth promotion and enhanced nutrient uptake of maize (Zea mays L.) by application of plant growth promoting rhizobacteria in arid region of Iran. J. Biol. Sci, 8(6): 1015-1020.

Burd, G.I., Dixon, D.G. and Glick, B.R. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol., 46(3): 237-245.

Deubel, A. and Merbach, W. 2005. Influence of microorganisms on phosphorus bioavailability in soils. In Microorganisms in soils: roles in genesis and functions. Springer Berlin Heidelberg, 177-191.

El-Gawad, A. 2008. Employment of bio-organic agriculture technology for Zea mays cultivation in some desert soils. Res. J. Agri. Biol. Sci. 4(5): 553-565.

Hameeda, B., Harini, G., Rupela, O.P., Wani, S.P. and Reddy, G., 2008. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol. Res., 163(2): 234-242.

Hegedus, A., Oldal, B., Jevcsak, I., Bayoumi, H.E.A.F. and Kecskes, M. 2003. Effect of seed treatment with microorganisms on the crop yield and quality of maize hybrids. Agrokemia es Talajtan Hungary.

Khokhar, S.N., Khan, M.A., Afzal, A. and Ahmed, R., 2006. Interaction of diazotrophs with phosphorus-solubilizing bacteria: their effect on seed germination, growth and grain-yield of maize, under rainfed conditions. Int. J. Biol Biotechnol., 3: 773-77.

Kudashev, I.S. 1956. The effect of phosphobacterin on the yield and protein content in grains of Autumm wheat, maize and soybean. Doki. Akad. Skh. Nauk, 8: 20-23.

Lugtenberg, B.J., Chin-A-Woeng, T.F. and Bloemberg, G.V., 2002. Microbe–plant interactions:principles and mechanisms. Antonie Van Leeuwenhoek 81(1-4): 373-383.

Murphy and, J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analyt. Chimica acta 27: 31-36.

Naveed, M., M. Khalid. D.L. Jones. R. Ahmad and Z.A. Zahir. 2008. Relative efficacy of Pseudomonas spp., containing ACC-deaminase for improving growth and yield of maize (Zea mays L.) in the presence of organic fertilizer. Pak. J. Bot, 40(3): 1243-1251.

Nezarat, S. and A. Gholami. 2009. Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize.Pak. J. Biol. Sci, 12(1):26-32.

Shatokhina, S.F. and Khristenko, S.I. 1996. Effect of Different Fertilizer Systems and Seed Inoculation on the Microflora, Biochemical and Agrochemical Characteristics of Typical Chernozem, and Corn Yield and Quality of Silage. Eurasian Soil Sci., 29(8): 956-962.

Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C. and Wong, M.H. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma, 125(1): 155-166.

Zahir, Z.A., Arshad, M. and Frankenberger, W.T. 2003. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv. Agron., 81: 97-168.