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ORIGINAL ARTICLE

Effect of nanozeolite and plant growth promoting rhizobacteria on maize

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Abstract

Plant growth promoting rhizobacteria are key to soil and plant health maintenance. In the present study, two PGPR strains which were identified as *Bacillus* spp. (accession number KX650178 and KX650179) with nanozeolite (50 ppm) were applied to the seeds in different combinations and tested on growth profile of maize crop. Various growth related parameters, including plant height, leaf area, number of leaves chlorophyll and total protein were positively increased up to twofold by the nanocompound treatment. GC–MS results reveal increase in total phenolic and acid ester compounds after the treatment of nanozeolite and PGPR, which are responsible for stress tolerance mechanism. Soil physicochemical parameters (organic carbon, phosphorous, potassium, ammoniacal nitrogen and nitrate nitrogen) were assessed qualitatively and a shift towards higher amount was observed. Various biochemical parameters of soil health like dehydrogenase, fluorescein diacetate hydrolysis and alkaline phosphatase activity were significantly enhanced up to threefold with the application of different treatments. The results, for the first time, demonstrate successful use of nanozeolite in enhancing growth of *Zea mays*, under controlled conditions and present a viable alternative to GM crop for ensuring food security.

Keywords PGPR · Rhizosphere · Bioinoculant · Nanozeolite · *Zea mays*

Abbreviations

AC	Absolute control
SS	Sterilized soil
FDA	Fluorescein diacetate
OOC	Oxidizable organic carbon
APH	Available phosphorous
NN	Nitrate nitrogen
USS	Unsterilized soil
AN	Ammoniacal nitrogen
NZ	Nanozeolite
APT	Available potassium

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Introduction

Maize is one of the most important cereal crop of world and third most important crop after rice and wheat in India. It requires good agronomic practices for improvement in yield as it is very sensitive to nutrient status of soil (Suriyaprabha et al. 2014). Nanoparticles are the particles with one or more dimension in nanosize (0.2–100 nm). Nanotechnology in agriculture have several applications but the major concern of the present study is the application of nanocompounds for the crop improvement.

Several studied regarding impact of nanoparticles on plant growth have already been done (Khan and Bano 2016). The majority of reported studies point to positive impact with a few isolated studies pertaining to negative effect. Some studies demonstrate that TiO_2 nanoparticles enhance photosynthesis and nitrogen metabolism and thus improve growth of spinach at a very low concentration, i.e., 20 mg/L

(Yang et al. 2006). Lin and Xing (2008) investigated toxicity of nanoparticles (carbon nanotubes, aluminium, zinc, zinc oxide) on root growth and seed germination of six plant species (radish, rape, rye grass, lettuce, corn and cucumber). Nanotechnology has begun to make efficient use of different nanoparticles but on the other hand increasing quantities in which nanoparticles are produced and applied, as well as unique characteristics resulting from nanoscale size, has led to the concern over toxicological implication of exposure for nontarget.

Zeolites are complicated silicate minerals with pores and channels within its crystal structure. It has unique higher Cation exchange capacity (CEC) due to which it has high affinity towards cations like Na^+, K^+, Ca^{2+} (Navrotsky et al. 1995). Zeolites are responsible for selective retention of NH^{4+} and K^+ ions in soil system (Petrovic 1993). Very few studies have investigated the effect of nanozeolite on maize growth. Nanozeolite are the least worked out nanoparticles on plant growth promotion and soil health, but can give extensive advantage with least side effect due to its biodegradability. Aminiyan et al. (2015) studied the effect of some natural products, zeolite and nanozeolite on aggregation stability and organic carbon status of soil and observed that the nanozeolite gave best results by enhancing the organic carbon of the soil and stabilizing the micro and macroaggregates followed by zeolite.

Plant growth promontory rhizobacteria are already known to support plant growth promotion. (Kloepper et al. 1980). They are also among the most complex and important assemblages in the biosphere found in the vicinity of plant rhizosphere (Khan 2005). PGPR like *P. aeruginosa, P. putida, P. fluorescens, B. subtilis* and soil nitrogen cycle bacteria (nitrifying bacteria and denitrifying bacteria) have shown varying degree of inhibition when exposed to nanoparticles in pure culture or in aqueous suspensions (Mishra and Kumar 2009). Iron and copper-based nanoparticles are presumed to react with peroxides present in the environment and generate free radicals that are highly toxic to microorganisms like *P. aeruginosa*. A sublethal dose of CuO nanoparticle impaired pyoverdine (PVD) function in a Gram-negative bacterium. Although most of the reports point out negative effect of nanoparticles on PGPRs but the effect of nanozeolite on PGPRs was never worked out before and nanoparticles like nanozeolite are least toxic and biodegradable, hence are supposed to support the growth of PGPR due to enhanced nutrient use efficiency.

The present study was planned to evaluate the tremendous advantage of nanozeolite in combination of PGPR isolates on maize growth promotion and to look after the possible side effects in parallel. The plant growth parameters (plant height, leave area, number of leaves, chlorophyll content and total protein content) were investigated to observe the direct effect of nanozeolite on plant growth viability but on the other side soil health parameters (pH, Organic carbon, nitrogen, available phosphorous, potassium, FDA hydrolysis activity and enzyme assays) were also investigated to see the effect of nanoparticles on soil health.

Materials and methods

Chemicals used

The nanozeolite was purchased from Intelligent material Pvt. Ltd India and physicochemical properties as supplied are given in Supplementary material 1. Other chemical were purchased from SRL and Hi media Laboratories Pvt. Ltd. India.

Soil collection

The soil was taken from the previous field experiment where the nanoparticles were applied by spray method at a rate of 0.03 g each in 1.5 L. The experimental site was at Norman E. Borlogue Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Dist.Udham Singh Nagar (Uttarakhand), India. This center is situated at an altitude of 243.84 above mean sea level, 29°N latitude and 79.3°E longitudes. The soil without nanoparticle was considered as control.

Soil characteristics

The soil of the experimental plot was classified under order—Mollisol, sub order—udoll, great group—Hapludoll (Deshpande et al. 1971). The composite soil sample was prepared by mixing the soils from different locations in the field. Soil was dug up to a depth of 15 cm and analyzed for different physicochemical properties. The soil was silt clay loam in texture, medium in organic carbon, low in available nitrogen and medium in available phosphorus, and potassium, and near neutral in reaction (Khati et al. 2017b).

Seed variety

Pant sankarmakka 1, a high yielding variety of maize was obtained from Crop Research Centre, Pantnagar. The duration of the crop is 90–120 days (Khati et al. 2017a).

Culture conditions

The bacterial isolates (PS2 and PS10) used for the experiment were Gram-positive rod-shaped PGPRs, and characterized as *Bacillus* spp. according to 16SrDNA sequencing (Khati et al. 2017a).

Inoculation procedure

The seeds were bacterized in 1 mL of broth (population in broth was maintained up to 10^6) amended with carboxymethyl cellulose, which act as binding agent.

Seed germination assay

Different treatments were made according to different combinations of nanozeolite and bacterial isolates. The pot trial was conducted with sterilized and unsterilized soil to see the effect of nanozeolite in controlled and natural conditions, respectively (Table 1). Plants were thinned to 10 plants per pot to maintain equal number for further observations.

Analysis of plant sample

The plant materials were sampled at 30 day after sowing.

Physical parameters

Shoot length, root length, leaf area, and number of leaves were measured and mean values were considered for further analysis (Yoshida et al. 1972).

Plant biochemical analysis

Chlorophyll content Fresh leaves (500 mg) were ground in 10 mL of 80% acetone. The homogenate was centrifuged at 8000 rpm for 10 min and supernatant is used for chlorophyll estimation by taking the absorbance at 645 and 663 nm (Arnon 1949). The total amount of chlorophyll a and chlorophyll b was calculated on the basis of formula:

$$\text{Chlorophyll a } \left(\text{mg g}^{-1}\,\text{FW}\right) = 0.0127 \times \text{OD}_{663} - 0.00269 \times \text{OD}_{645}$$

$$\text{Chlorophyll b } \left(\text{mg g}^{-1}\,\text{FW}\right) = 0.229 \times \text{OD}_{645} - 0.00269 \times \text{OD}_{663}$$

$$\text{Total chlorophyll } \left(\text{mg g}^{-1}\,\text{FW}\right) = 0.0202 \times \text{OD}_{645} + 0.00802 \times \text{OD}_{663}$$

Total protein extraction and estimation The total protein content determination according to Bradford method using bovine serum albumin (BSA) as the standard (Bradford 1976). One gram of fresh leaves were ground with 0.2 M Tris (pH 8) in pestle and mortar and the slurry was centrifuged at 10,000 rpm at 4 °C for 20 min. the protein concentration was determined according to the formula:

$$\text{Protein concentration } \left(\mu g\,\mu L^{-1}\right) = \frac{\text{Absorbance} \times \text{dilution factor}}{\text{Slope } \left(\mu g^{-1}\right) \times \text{volume of diluted protein used for assay } \left(\mu L^{-1}\right)}$$

Gas chromatography–mass spectroscopy

Shade-dried maize leaves (20 days old) were extracted by methanolic extraction and analyzed by gas chromatography–mass spectroscopy (Thermo GC-Trace Ultra Ver. 5.0; Thermo Scientific MS DSQ II). The GC silica column dimension was 30 m 90.25 mm at a flow rate of 1 mL min^{-1} at 8 °C and then, the temperature was raised to 24 °C. The volatile compounds present in the control, nano silica and bulk silica treated samples were identified by comparing with the standards or the mass spectrum matched with the inbuilt library (Wiley 9).

Physicochemical analysis of soil

The test soil sample was collected after 30 day of treatment. Different physicochemical tests (pH, Organic carbon, available potassium, available phosphorous, nitrate nitrogen and ammoniacal nitrogen) were performed qualitatively with the HiMedia kit.

Table 1 Different treatments for pot trial

Serial no.	Treatments	Abbreviations used
1	Nanozeolite treatment with PS2 in sterilized soil	NZ + PS2 + SS
2	Nanozeolite treatment with PS2 in unsterilized soil	NZ + PS2 + USS
3	Nanozeolite treatment with PS10 in sterilized soil	NZ + PS10 + SS
4	Nanozeolite treatment with PS10 in unsterilized soil	NZ + PS10 + USS
5	Nanozeolite treatment in sterilized soil	NZ + SS
6	Nanozeolite treatment in unsterilized soil	NZ + USS
7	PS2 in sterilized soil	PS2 + SS
8	PS2 in unsterilized soil	PS2 + USS
9	PS10 in sterilized soil	PS10 + SS
10	PS10 in unsterilized soil	PS10 + USS
11	Absolute control in sterilized soil	AC + SS
12	Absolute control in unsterilized soil	AC + USS

Soil enzymes assays

Fluorescein diacetate hydrolysis (FDA)

FDA hydrolysis assay was done according to Schurer and Rosswall (1982) method. One g of soil sample was incubated with 50 mL of 60 mM Sodium phosphate buffer pH 7.6 and 0.50 mL of FDA solution (5 mg in 10 mL acetone). The suspension is incubated at 24 °C. Aliquots were withdrawn at regular interval of 1, 2 and 3 h and FDA hydrolysis was stopped by adding 1 mL acetone to a 6 mL aliquot. Slurry was centrifuged (8000 rpm for 5 min) and filtered. Absorbance was recorded at 490 nm.

Dehydrogenase activity

Modified method of Casida et al. (1964) was followed for the dehydrogenase assay of soil samples. To 5 g soil sample 5 mL of 2,3,5-triphenyltetrazolium chloride (TTC) solution (2 g in 100 mL 0.1 M tris buffer pH 7.4) was added. After incubation (37 °C, 120 rpm for 8 h) 25 mL acetone or methanol was added to extract Triphenylformazan (TPF). Centrifugation (4500 rpm for 10 min at 4 °C) to obtained supernatant was done and absorbance recorded at 485 nm.

Alkaline phosphatase activity

One g of each dry soil was mixed with 0.25 mL toluene, 4 mL of Modified universal buffer (MUB) (100 mM, pH 11) and 1 mL p-nitrophenyl phosphatase (pNPP) (25 mM) were added. After incubation at 37 °C for 1 h. 1 mL $CaCl_2$ (0.5 M) and 4 mL of tris buffer (0.1 M, pH 12) were added to stop the reaction. Intensity of color was determined using spectrophotometer at 400 nm (Tabatabai and Bremner 1969).

Statistical analysis

The pot trial was performed on the basis of CRD with three replicate per treatment. The data were statistically treated using general linear model procedure (SPSS, Ver 16.0) to reveal significant effect of nanozeolite treatment with PGPR isolates on maize crop. The results were analyzed using one way analysis of variance (ANOVA).

Results

Seed germination assay

Percentage germination of *Zea mays* seeds were positively affected by nanozeolite treatment at 50 mg/L concentration (Fig. 1). Briefly, seed germination was highest (94.44%) in NZ + PS2 + SS (nanozeolite with PS2 and sterilized soil), NZ + PS2 + USS (nanozeolite with PS2 and unsterilized soil), NZ + PS10 + USS (nanozeolite with PS10 and unsterilized soil), PS10 + USS (PS10 and unsterilized soil), PS2 + USS (PS2 and unsterilized soil). In case of NZ + PS10 + SS (nanozeolite with PS10 in sterilized soil), NZ + SS (nanozeolite in sterilized soil), PS10 + SS (PS10 in sterilized soil) the percent seed germination was about 88.89, followed by NZ + USS (nanozeolite in unsterilized soil) with 83.33% and PS2 + USS (PS2 in unsterilized soil) with 72.22%. Least germination was found in AC + SS (absolute control) with 44.44 and AC + USS (absolute control with unsterilized soil) with 33.33%. A statistically significant increase in percent germination was recorded at 50 mg/L NZ (nanozeolite) treatment. This indicates that application of nanozeolite together with PGPR isolates enhance the seed germination maximally, but when nanozeolite or PGPR applied individually the seed germination was slightly slower but still better than the control.

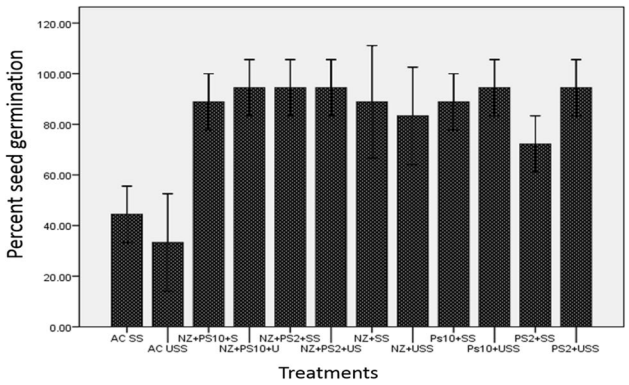

Fig. 1 Percent seed germination of different treatments. The bars shows the mean value ± SD

Effect of nanozeolite treatment on growth profile of treated seedling

The plant height was significantly increased with nanozeolite treatment in comparison to control. A maximum increase of 1.5 fold was observed in seedling treated with nanozeolite with PS2 in sterilized soil (Figs. 2 and 3). However, no significant change in case of number of leaves was observed and even slight increase in leaf area with a maximum value (30.49 cm^2) was observed in case of PS2 treated sterilized soil in comparison to control (24.52 cm^2). Total chlorophyll content was also increased by 1.6-folds in comparison to control (Fig. 4). Protein content of plants was also enhanced 1.5–1.8-folds in the treatment of nanozeolite with PS2 in sterilized soil.

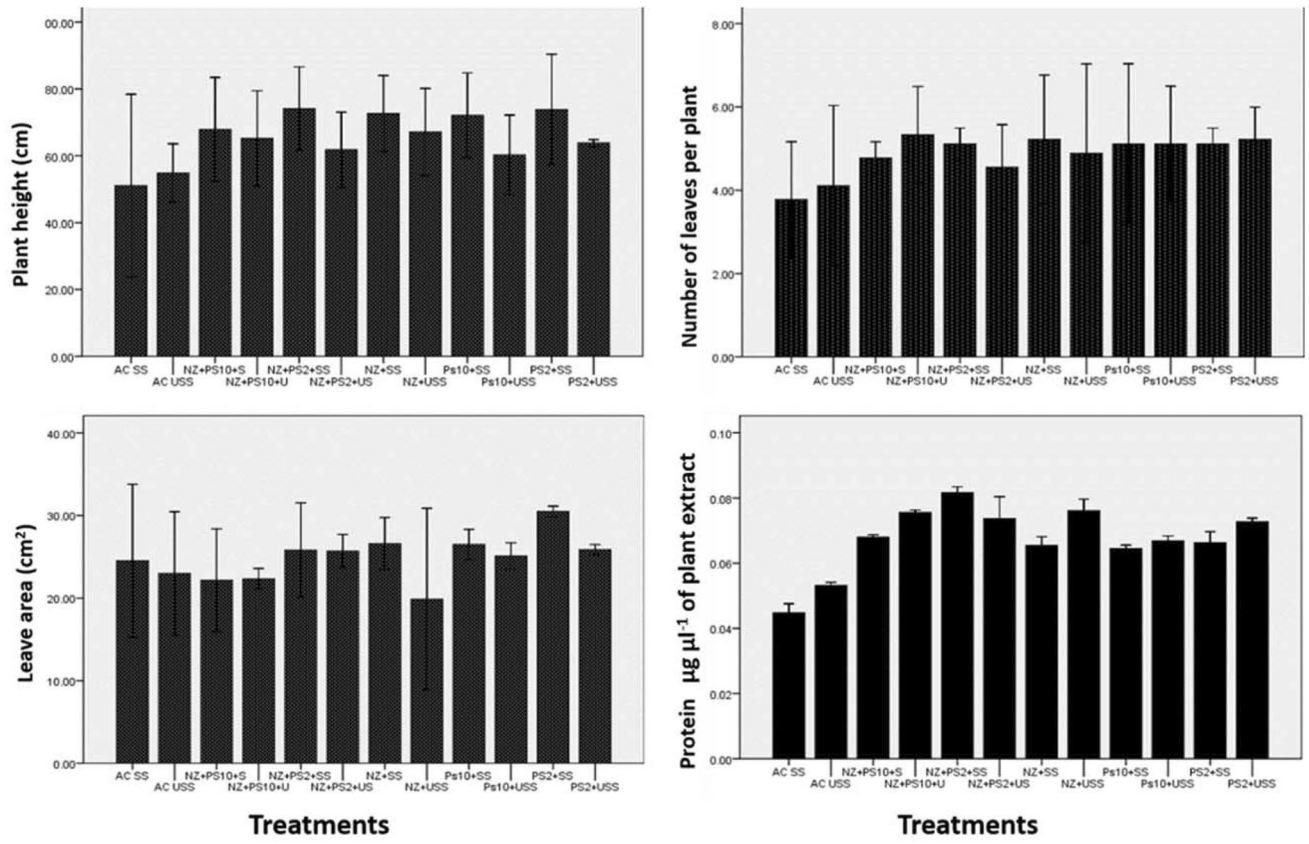

Fig. 2 Effect of treatments on growth parameters in maize

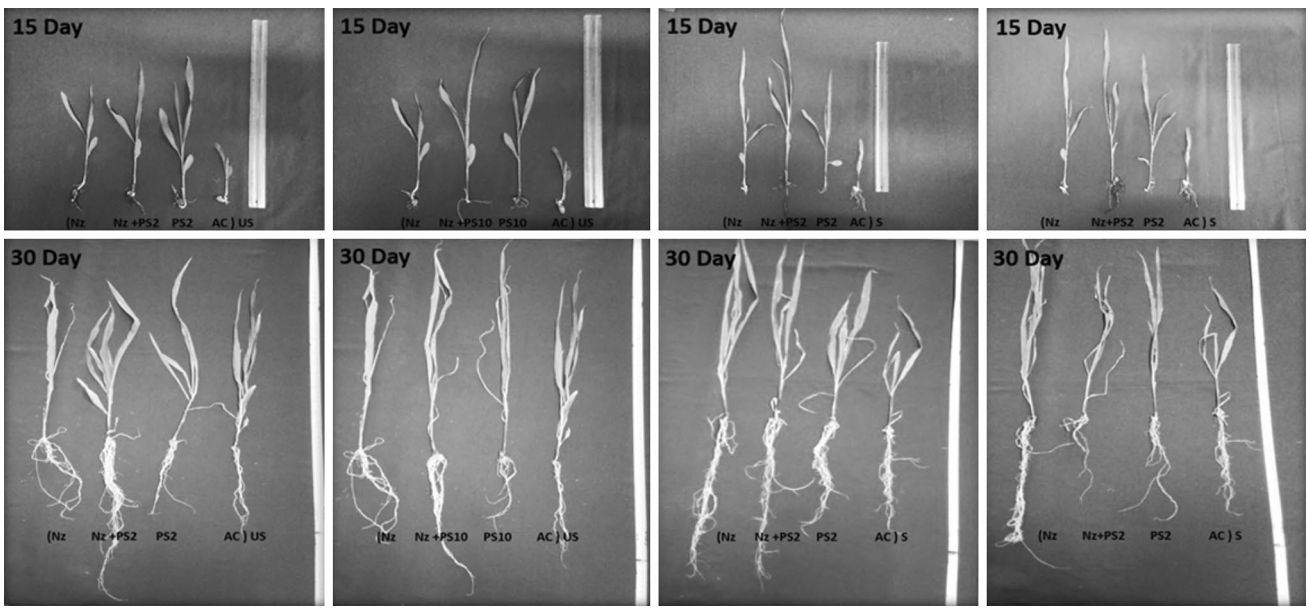

Fig. 3 Plant height measurement after 15th and 30th day

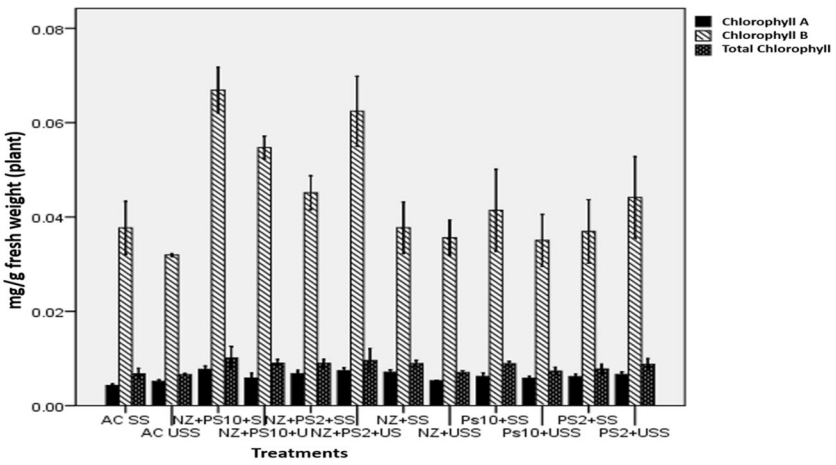

Fig. 4 Variations in chlorophyll content in maize leave samples amended with Nanozeolite and bacterial isolates in different combinations

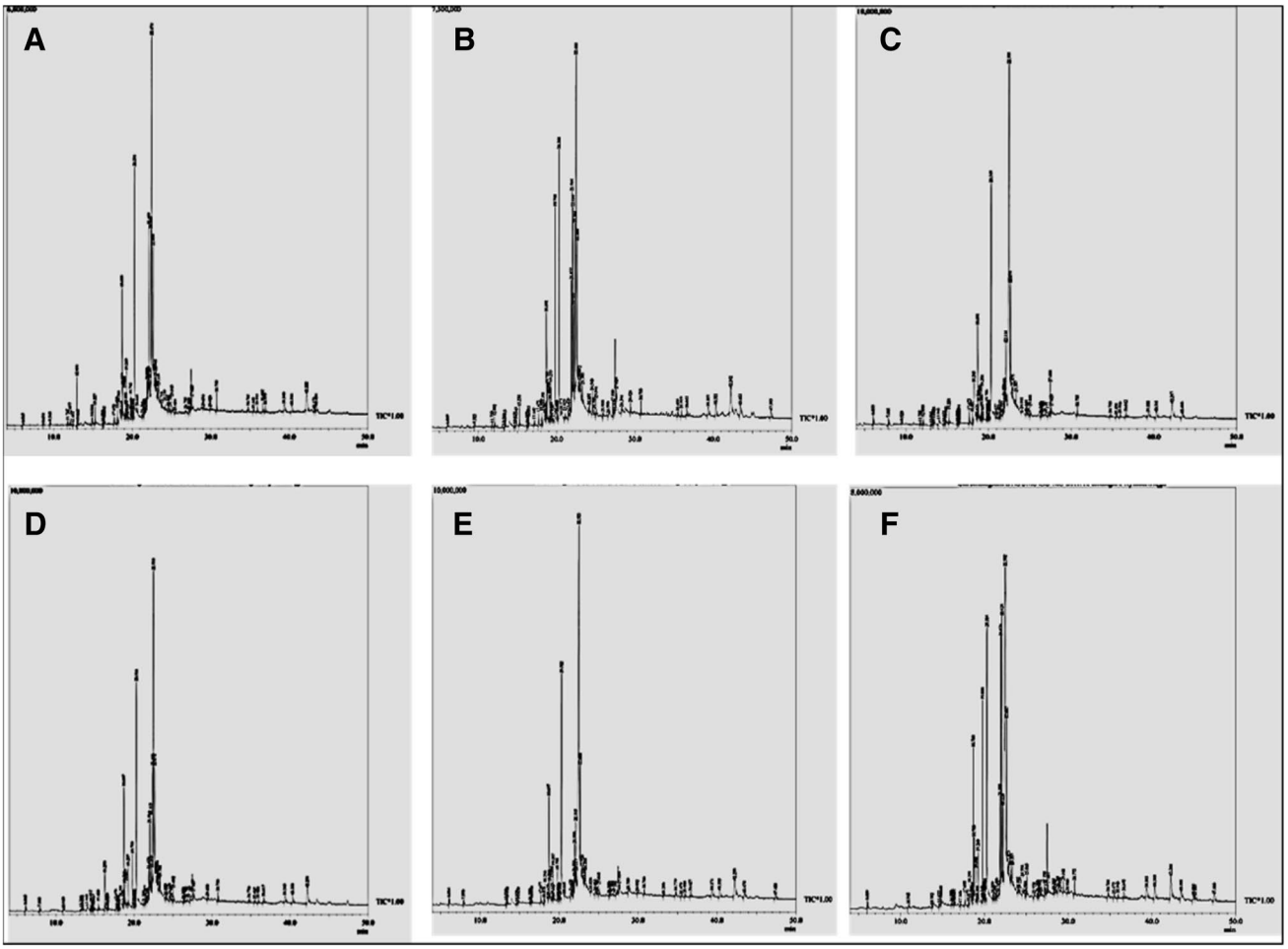

Fig. 5 GC–MS chromatogram of maize leaf extract with respect to different treatments: **a** NZ + PS2, **b** NZ + PS10, **c** NZ, **d** PS2, **e** PS10 and **f** absolute control

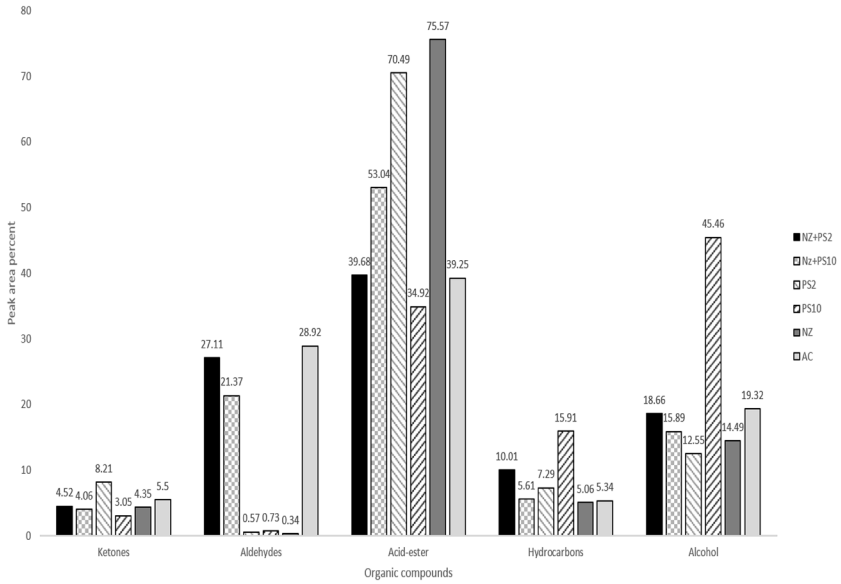

Fig. 6 Total area percentage of organic compounds in maize leaf extract of different treatments

GC–MS

The modulations in total organic compounds according to the treatments of nanozeolite and bacterial isolates are elucidated from GC–MS results. A relative abundance of volatile compounds in maize leaf extract with respect to the retention time is presented in chromatograph (Fig. 5). The GC–MS results revealed increased acid esters and alcohols in treated plant samples which are responsible for stress tolerance (Fig. 6). This can be correlated to the better stress adaptation by the plant in the presence of nanocompound alone or in combination with PGPR.

Soil physicochemical properties

Soil physicochemical properties are summarized in Table 2. The pH of soil was alkaline with some variation in all the treatments. The results indicate increase in % OOC (percent oxidizable organic carbon) from M (medium: 0.5–0.75 Kg ha^{-1}) to MH (medium high: 0.75–1 Kg ha^{-1}) nanozeolite treatment with PS2, Nanozeolite alone and PS10 alone in unsterilized soil, but no increase was recorded when nanozeolite treated with PS10, PS10 and PS2 alone in sterilized soil. This indicates that nanozeolite play significant role in increasing OC (organic carbon) content of soil.

Two type of nitrogen estimation was done to target ammoniacal nitrogen and the nitrate nitrogen. Ammoniacal nitrogen was found to be very low in the control soil (without any treatment). Slight increment was observed when NZ treated with PS2 and PS10 (SS) from low to medium. Similarly in PS2 and PS10 alone medium level of AN was found. It means nanozeolite alone do not have significant effect on AN, rather the PGPR isolates alone or in combination with NZ are positively effecting AN status of soil in case of Nitrate nitrogen (NN), soil without any treatment have very low (04 Kg ha^{-1}) NN which was slightly enhanced in NZ treated soil, PS2 treated soil and PS10 (USS), NZ with PS2 (SS) and NZ with PS10 (SS) with low level of NN (about 10 Kg ha^{-1}). Medium level of NN was found when NZ treated with PS2 and PS10 in unsterilized soil.

It is clearly evident from the results that nanozeolite when treated either with PS2 or PS10 gave best result (MH: medium high (56–73 Kg ha^{-1}) as compared to control (medium: 22–56 Kg ha^{-1}), but when applied alone (NZ, PS2 and PS10) good results were only observed in sterilized soil, which indicate that competition with the indigenous microflora suppressed the beneficial traits.

The K content of the soil was found to be low (> 112 Kg ha^{-1}) in most of the cases, but slight increment in the level (from low to medium) was observed in case of nanozeolite when treated with PS2 in sterilized soil, nanozeolite with PS10 in unsterilized soil and PS2 alone in unsterilized soil. The observation shows NZ and PS2 are more beneficial for available potassium status.

Soil enzymes

Significant increase in dehydrogenase activity was observed in all treatments in comparison to untreated soil, with maximum activity in case of PS2 treatment in unsterilized soil (0.050473 U) (Fig. 7). Highest activity of FDA hydrolysis was obtained in nanozeolite treated with PS2 in unsterilized soil (0.912 U). Least enzyme activity was observed in absolute controlled unsterilized (0.338431 U) and absolute control sterilized (0.21817 U), slight increase in the activity was observed in PS2 alone in sterilized soil (0.3307 U) and PS10 alone in sterilized

Table 2 Soil physicochemical properties after 30 day (as per "K054 soil testing Kit; Himedia Laboratories Pvt Ltd India" Black boxes shoe respective test results and are a mean of three replicates)

Treatments scale	Soil pH	OC[a]					APH[b]					APT[c]				AN[d]			NN[e]			
		L	ML	M	MH	H	B	L	M	MH	H	L	M	H	VH	L	M	H	VL	L	M	H
NZ+PS2+SS	8.616667				■					■			■				■			■		
NZ+PS2+USS	8.57				■					■		■					■				■	
NZ+PS10+SS	8.186667			■						■		■					■			■		
NZ+PS10+USS	8.423333			■						■			■			■					■	
NZ+USS	8.13				■				■			■				■				■		
NZ+SS	7.85				■					■		■				■				■		
PS2+USS	8.26			■					■				■			■				■		
PS2+SS	8.45			■						■		■					■			■		
Ps10+USS	8.246667				■				■			■					■			■		
Ps10+SS	8.41			■						■		■					■		■			
AC USS	8.15			■					■			■				■			■			
AC SS	8.363333			■					■			■				■			■			

[a]*OC* organic carbon (oxidizable organic carbon)

L low (0.1–0.3), *ML* medium low (0.300–0.500), *M* medium (0.500–0.750), *MH* medium high (0.750–1.00), *H* high (1.00–1.50)

[b]*APH* available phosphate as P_2O_5 ($Kgha^{-1}$)

B Blank, *L* low (< 22), *M* medium (22–56), *MH* medium high (56–73), *H* high (< 73)

[c]*APT* Available potassium as K_2O ($Kgha^{-1}$)

L Low (> 112), *M* medium (112–280), *H* high (280–392), *VH* very high (< 393)

[d]*AN* Ammonical nitrogen ($Kgha^{-1}$)

L Low (about 15), *M* medium (about 73), *H* high (about 202)

[e]*NN* Nitrate nitrogen ($Kgha^{-1}$)

VL Very low (about 04), *L* low (about 10), *M* medium (about 20), *H* high (about 50)

soil (0.3593 U) and a significant increase in activity in case of all the nanozeolite treated soil with or without PGPR isolates. The results suggest the fact that enzyme activity was enhanced by nanozeolite in soil but when combined with PGPR isolates the synergistic effect was two–threefold enhancement in FDA hydrolysis activity.

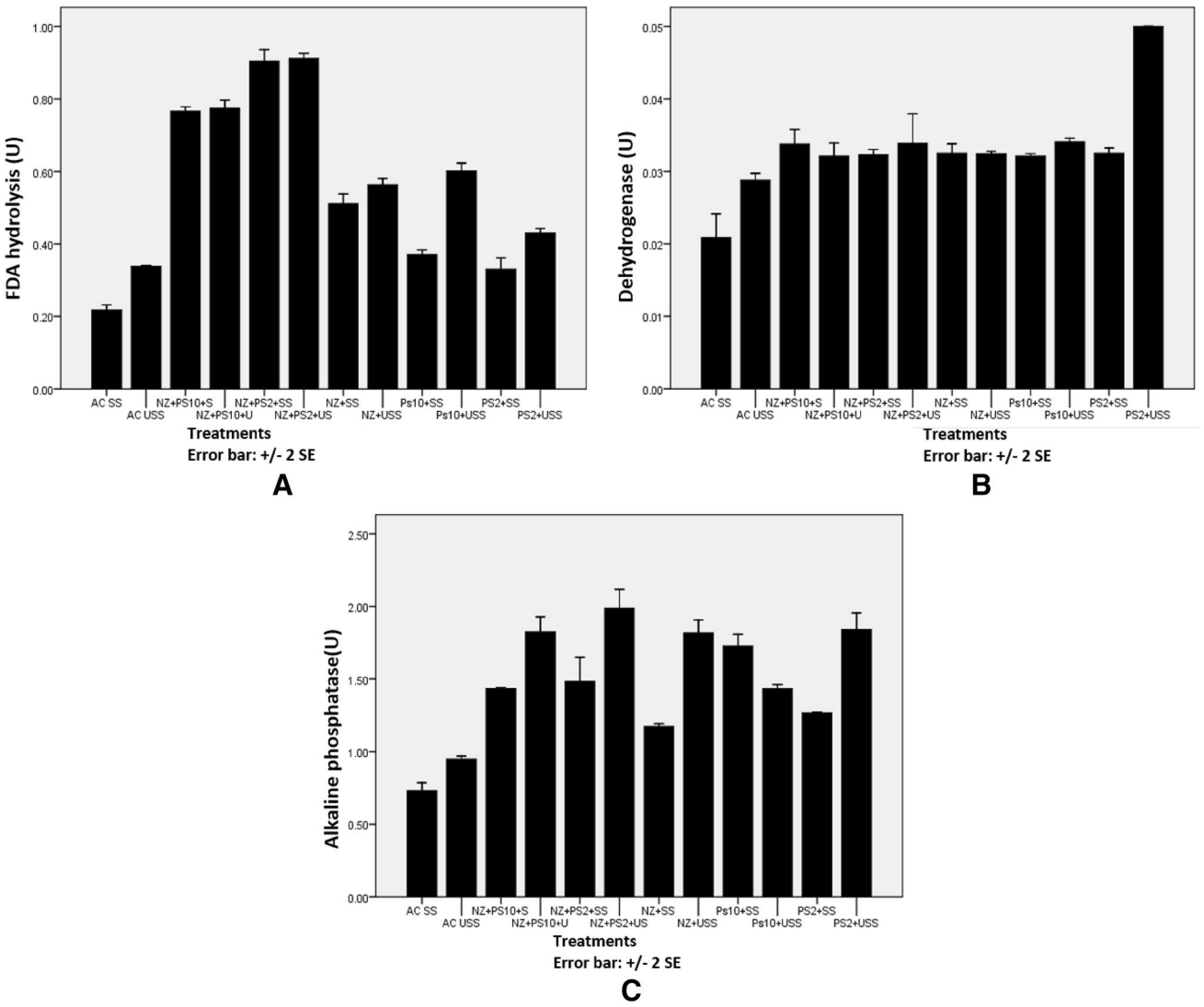

Fig. 7 Soil enzyme assay **a** FDA hydrolysis, **b** Dehydrogenase and **c** Alkaline phosphatase

Increase in enzyme activity from 0.9477 U in case of absolute control in unsterilized soil and 0.731 U in case of absolute control in sterilized soil to a maximum of 1.989 U in case of nanozeolite treatment with PS2 in unsterilized soil. While in other treatments also significant increment (one to two folds) in the alkaline phosphatase activity was observed. This shows enzyme activity was significantly enhanced in nanozeolite treated soil and a synergistic effect was observed in nanozeolite with PS2 and also in nanozeolite with PS10 for smaller extent.

Discussion

Seed germination percent was significantly enhanced in different treatments. Similarly Khodakovskaya et al. (2009) also exposed MWCHTs (Multi walled carbon nanotubes) to tomato seeds and observed enhanced seed germination. MWCNT also enhanced efficiency of water uptake as well as Ca and Fe nutrients uptake which are responsible for enhanced seed germination (Villagarcia et al. 2012). Wheat seed germination was found to be enhanced by ZnO NP (Ramesh et al. 2010; Prasad et al. 2012). RuO_2 nanoparticles were found to increase seed germination in *Brassica spp*. (Singh et al. 2015).

So far the proposed mechanisms could be due to the increased permeability of seed capsule, facilitating the admission of water and di-oxygen into the cells, which accelerates the metabolism and germination process (Zheng et al. 2005). Increase in nitrate reductase activity, higher utilization of fertilizers and enhanced antioxidant system could be another possible mechanism for enhanced seed germination (Lu et al. 2002).

The mechanism of plant height enhancement may be attributed to increased level of gibberellic acid (GA), which is key hormone responsible for shoot elongation (Stepanova et al. 2007). Seif et al. (2011) also reported stem elongation in *Borago* after silver nanoparticle treatment. Increase in total chlorophyll means increase in photosynthates (Urbonaviciute et al. 2006). Tarafdar (2013) reported that ZnONPs induced a significant improvement in *Cyamopsis tetragonoloba* plant biomass, shoot and root growth, root area, chlorophyll and protein synthesis, rhizospheric microbial population, acid phosphatase, alkaline phosphatase and phytase activity in cluster bean rhizosphere. Total organic compounds, such as phenols, aldehydes, ketones, etc., are found to be enhanced in maize leaves treated with nanozeolite in combination with PGPR. The other organic compounds are not varied considerably. These results are correlated with the study on the protective mechanism of silicon in rice through phenols (Goto et al. 2003).

Though the physicochemical properties are vulnerably influenced by the change of redox conditions in soils (Lamparter et al. 2009). Therefore, it is necessary to determine the underlying impacts induced by nanocompouds on soil physicochemical properties to optimize their use. Organic carbon is the most important element and a key attribute in assessing soil health, generally correlating positively with crop yield (Bennett et al. 2010). The soil organic carbon also attributes to other important functional process in soil such as nutrient storage, mainly Nitrogen, water holding capacity and also stability of aggregates (Silva and SáMendonça 2007). Above all soil organic carbon supports soil microbial activity which is a key step of enhancing soil health. Nitrogen (N) is the most requires plant nutrients, which is found in several chemical forms in soil (Cantarella 2007) resulting in very dynamic behavior.

Phosphorous (P) in soil is a key nutrient that limit agricultural yields and is present as orthophosphate, but microbial P and organic P are also acts as stocks that can rapidly become available. According to Doran et al. (1999) phosphorous is one of the major indicators of productivity and environment quality of soil. Potassium (K) is also another indicator of soil health and productivity. The results indicate that the nanozeolite along with PGPR isolates enhanced the different minerals nutrient levels which are indicators of soil physicochemical properties.

Similar investigation was also done by Zhou et al. (2012) who studied the dynamic influence of three iron based NPs (Fe^0, Fe_3O_4, and Fe_2O_3) on soil physicochemical properties such as pH, dissolved organic carbon (DOC), available ammoniacal nitrogen (AAN) available phosphorous and enzymatic activities. They found that the addition of Fe^0 NP increased DOC and AAN but significantly reduced AP, while Fe_3O_4, and Fe_2O_3NP significantly reduced AAN.

Enzymes are vital activators in life processes, likewise in the soil they are known to play a substantial role in maintaining soil health and its environment. Evaluation of soil health, therefore, requires estimation of these indicators of the same. The soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil (Sinsabaugh et al. 1991). According to Kiss et al. (1978) various substrates are catalyzed by soil enzymes and act as nutrients for microorganisms. These enzymes include amylase, dehydrogenase, phosphatase, β-glucosidase and arylesterase.

Dehydrogenases are the most important group of enzymes used as indicator of biological activity in soils (Burns 1978). Dehydrogenases are the major indicators of microbiological redox system and possible measure of microbial oxidative activity (Tabatabai 1982; Trevors 1984).

FDA [3′6′-diacetylfluorescein] hydrolysis can also be used as a measure of microbial activity in soils (Schnurer and Rosswall 1982). FDA is hydrolyzed by a number of different enzymes, such as protease, lipase and esterase.

Phosphatase are the group of enzymes believed to play pivotal role in phosphorous (P) cycling (Speir and Ross 1978; Sylvia et al. 2005). Apart from being good indicator of soil health these enzymes plays key role in soil system (Eivazi and Tabatabai 1977; Dick et al. 2000) by enhancing the P solubilization when deficiency is recorded. Raliya et al. (2015) studied effect of TiO_2 nanoparticles on Mung bean and observed the increase in activity of acid phosphatase (67.3%), alkaline phosphatase (72%), phytase (64%) and dehydrogenase (108.7%). Similarly a significant improvement in acid phosphatase (73.5%), alkaline phosphatase (48.7%), and phytase (72.4%) activity in cluster bean rhizosphere was observed over control in 6-week-old plants due to application of nanoZnO (Raliya and Tarafdar 2013).

The results suggest the application of nanocompound is not only beneficial for the plant health parameters but also for the maintenance of soil health which is key to sustainable agriculture system.

Conclusion

The results obtained suggest the application of nanozeolite in combination with PGPR is beneficial to enhance plant health parameters and thus crop productivity. The different enzymes selected for the soil health assessment acted as fingerprints and revealed the status of test soil with respect to different treatments. The present findings are novel and may help in standardization of alternate technology for ensuring world food security and also ensure soil health maintenance.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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