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CONTENTS

Performance of plant growth promotory rhizobacteria on maize and soil characteristics under the influence of TiO_2 nanoparticles

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ABSTRACT: In the present study effect of TiO_2 nanoparticles was tested on six plant growth promotory rhizobacteria (HS2, HS10. HS12, HS11, HM4 and HR11) recovered from soyabean and maize rhizosphere. HM4 showed highest IAA (56.31 µg/ml) production and HR11 showed highest siderophore (56%) production. All the isolates showed maximum growth at 50 ppm TiO_2 in nutrient broth, HS10 and HS12 showed best growth. Effect of TiO_2 nanoparticles was evaluated on plant vigour of maize treated with selected plant growth promotory bacteria. Bacterial treatment showed enhanced shoot germination, plant height and leaf area over control in the presence of TiO_2 nanoparticles. Average total chlorophyll in maize plant was also maximum (5.04 µg/g) in the presence of TiO_2 nanoparticle. Performance of HS12 was best among all treatments. After 45 days of pot experiment, fluorescein diacetate hydrolysis (57.91µg/ml), dehydrogenase (38.10µg/ml) and alkaline phosphatase (207.16 µg/ml) was reported in the presence of nanoparticles and bacterial cultures. HM4 and HS12 treatment in the presence of 10 ppm TiO_2 enhanced enzyme activities.

Key words: Maize, PGPR, TiO_2 nanoparticles

Soil quality is the capacity of a soil to function within natural or managed ecosystem boundaries to enhance plant and animal productivity, retain water and air quality and human health. Plant growth promoting rhizobacteria (PGPR) promote plant growth either by colonizing around the root zone and helping plants to get nutrient through various mechanisms. Population of soil microorganisms is greatly influenced by natural and anthropogenic manipulations in the soil. Hence, protection of microbial biomass and diversity of soil is one of the major challenges for sustainable resource use because higher level of microbial biomass and diversity lead to more nutrient turnover (Torsvik and Ovreas, 2002). Nanoparticles are particles with a size range of 1 to 100 nanometers. They have small diameter but have large volume-surface area and thus find subsidiary in various fields (Darroudi *et al*., 2012). Nanoparticles pose a direct effect on soil biota and sometimes through change in availability of toxins or nutrient by interacting with them (Simonet and Valcarcel, 2009).

Iron and copper nanoparticles react with peroxides present in the environment and show highly toxic behaviour towards microorganisms like *P. aeruginosa* (Saliba *et al*., 2006). Presence of SiO_2, ZrO_2 and Al_2O_3 microparticles triggers the growth of bacterial population in soil which is responsible for enhancement of soil nutrient value. Nanosilica promotes 100% seed germination in maize therefore, it shows auspicious effect on bacterial population and nutrient value of soil (Karunakaran *et al*., 2013). Titanium dioxide (TiO_2) nanoparticles are white solid inorganic substance that is thermostable, non-flammable and poorly soluble in water. TiO_2 nanoparticles saliently promote aged seeds vigor and chlorophyll content and hence increases photosynthesis, there by indirectly promoting plant growth and development (Yang *et al*., 2006). These particles were found to enhance seed germination and promote radicle and plumule growth of canola seedlings by Mahmoodzadeh *et al*. (2013). According to Jaberzadeh *et al*. (2013) TiO_2 nanoparticles augmented wheat plant growth and yielded components under deficit stress condition. Appropriate use of these nanoparticles is beneficial otherwise higher dose astringently eradicate indigenous microbial population. There is an immense need to study and evaluate the effect of the nanoparticles on microbial diversity and soil health.

MATERIALS AND METHODS

Soil sample

Soil samples from soyabean and maize rhizosphere were collected from Crop Research Centre of the University in the month of November, 2015 and preserved at -20^{0}C till use.

Bacterial isolation

Isolation of bacterial isolates from rhizospheric soil was done on nutrient agar, King's B medium and Tryptic soya agar using serial dilution.

Screening of potent bacterial isolate

A sum of 25 bacterial isolates, recovered from

rhizospheric soil of soyabean and maize plants were tested for their plant growth promotory properties like Siderophore production (Schwyn and Neilands, 1987), P-solubilization (Wahyudi *et al.,* 2011), Indole acetic acid (IAA) (Patten and Glick, 2002), Ammonia production (Cappuccino and Sherman, 1992) and HCN production (Donate-Correa *et al*., 2005).

Morphological and biochemical characterization

Morphological and Biochemical characterization (lipolytic, gelatinase, amylolytic, protease, cellulase, pectinolytic, catalase, urease activity and citrate utilization) of selected six bacterial isolates were performed according to Madigan *et al*. (2012). Bacterial isolates were tested for antibiotic sensitivity against Co-trimoxazole (25mcg/disc), Chloramphenicol (30 mcg/disc), Ampicillin (10mcg/disc), Tetracyclin (30 mcg/disc), Kanamycin (30mcg/disc) and Methilicin (25mcg/disc).

Effect of TiO_2 nanoparticle on the growth of bacteria

One ml of each bacterial isolate was inoculated in nutrient broth, supplemented with different concentrations of TiO_2 nanoparticle (10, 30, 50 and 100 ppm). Inoculated flasks were incubated on a rotatory shaker at 28$\pm$2°C. Aliquots of 3 ml were withdrawn at the interval of 12 h upto 4 days. Absorbance was taken at 600 nm.

Experimental details for pot trial

A pot experiment was conducted, using Complete Randomize Design (CRD) in winter season (January 2016) to study the impact of TiO_2 nanoparticles (10ppm) on the soil health and plant health with 5 seeds per pot in unsterilized soil. Harvesting was done after 45 days.

Preparation of bacterial inoculums

Out of the 25 bacterial isolates recovered in the same experiments, six bacterial cultures (HS2, HS10, HS11, HS12, HM4 and HR11) which were tested best for plant growth promotory activities were selected for pot trial (Table 1). The bacterial isolates were grown in 50 ml of nutrient broth, at 30^0C and adjusted to 10^5 to 10^6 CFUml^{-1}.

Seed sterilization and bacterization

Maize seeds were treated with acidified (0.1%) $HgCl_2$ for 3 min and washed thoroughly before coating with inoculum. Surface sterilized seeds were dried and treated with fresh bacterial cultures, individually to obtain 10^8 cfu/ml. One per cent carboxy methyl cellulose (CMC) was also added for proper adherence.

Treatments

The treatments used for pot experiments were as follows:

1. Soil conditions: Sterilized soil
2. Bacterial cultures: Six (HS2, HS10, HS11, HS12, HM4 and HR11) (Table 1)

The experiment was conducted in a replication of three in the month of February, 2015.

Observation

Plant Height was measured and mean values were presented in centimeter. Germination percentage (%) and Leaf Area of all plants were also measured (Yoshida *et al*., 1972).

Biochemical Analysis of Plant and Soil

Estimation of chlorophyll

Chlorophyll was estimated according to Hiscox and Israelstam (1979). Fifty mg fresh leaves were added in 7 ml of DMSO. After incubation at 65°C for 3 h absorbance of chlorophyll extract (obtained by centrifugation at 2000 rpm for 10 min) at 663nm, 645nm and 470nm was recorded using visible spectrophotometer (Varion).

Estimation of Enzyme Activities of soil used in pot experiment

Fluorescein Diacetate (FDA) hydrolysis: FDA activity was determined according to Schnurer and Rosswall, (1982). One gram soil was mixed with 50ml sodium phosphate buffer (pH 7.6). Provided FDA solution (0.5ml) was added and incubated for 2 hr at 24°C . Two ml acetone was added to terminate the FDA hydrolysis. Soil suspension was centrifuged (8000 rpm for 5 min) and filtered through Whatmann No. 2 filter paper. Absorbance of the filtrate was measured at 490nm. FDA hydrolysis activity was expressed as µg flurorescein g^{-1} dry soil h^{-1} with fluorescein as standard

Dehydrogenase Activity: Dehydrogenase activity was determined by the method of Casida *et al*. (1964). Five gram soil was mixed in 5ml of 2, 3, 5- triphenyltetrazolium chloride (TTC) solution and incubated for 16 h at 37°C. Now 25 ml acetone was added to the mixture and centrifuged at 4500rpm for 10min at 4°C. Obtained supernatant was filtered through Whatmann No.2. Absorbance of the filtrate was taken on spectrophotometer at 485nm. The activity of dehydrogenase was expressed as µg TPF 5g^{-1} dry soil 16hr^{-1}.Triphenyl

Table 1: Layout plan for the pot experiment

Treatment I	Absolute control	Treatment VIII	HS11 + TiO_2
Treatment II	TiO_2 control	Treatment IX	HS12
Treatment III	HS2	Treatment X	HS12 + TiO_2
Treatment IV	HS2 + TiO_2	Treatment XI	HM4
Treatment V	HS10	Treatment XII	HM4 + TiO_2
Treatment VI	HS10 + TiO_2	Treatment XIII	HR11
Treatment VII	HS11	Treatment XIV	HR11 + TiO_2

Parameters taken

i. Absolute control (no culture, no TiO_2 nanoparticles)
ii. Only bacterial culture(s)
iii. Bacterial culture + TiO_2 nanoparticles
iv. Only TiO_2 nanoparticles

formazan (TPF) (100µg/ml) was used as standard.

Alkaline Phosphatase Activity: Alkaline Phosphatase activity was determined by the method of Tabatabai and Bremner (1969) method. To 1 g soil sample, 0.25ml toluene, 4ml Modified Universal Buffer(MUB) buffer and 1ml p-nitrophenyl phosphatase (pNPP) (25mM) were added. Tubes were incubated at 37°C for 2h. After incubation, 1ml $CaCl_2$ and 4ml Tris buffer (0.1M, pH 12) were added to stop the reaction. The sample was incubated for 2h. Intensity of the colour was determined at 400nm. pNP was used to draw standard curve.

Statistical analysis of data

The experimental data was analyzed using procedure for factorial CRD according to Cochran and Cox (1957). The significance of treatment means was tested using F- test. Critical difference (C.D.) value at 5% of significance was calculated for comparison of difference among treatment means, if the F-test was significant. Standard error of mean (SEm+) and coefficient of variance (CV 5%) was also calculated in each case.

Molecular characterization of the bacterial isolates

Extraction of genomic DNA of two bacterial isolates (HS12 and HS10) was according to Bazzicalupo and Fani (1995). Amplification of DNA was done according to Lane *et al*. (1985) using 16SrDNA primers (1492R and 27F) Amplified products were sequenced and identity was matched through basic local alignment sequence tool (BLAST).

RESULTS AND DISCUSSION

Morphological and Biochemical characterization of selected bacterial isolates

All the recovered bacterial isolates cultures were Gram negative except, HS10 which was Gram positive and had long rods. Six rhizospheric bacterial isolates were tested for the production of extracellular enzymes. Biochemical characteristics of bacterial isolates are given in Table 2.

Screening of potent bacterial cultures

Twenty five bacterial isolates recovered from rhizospheric soil were tested for their plant growth promotory properties. Eight bacterial isolates (HS2, HS10, HS11, HS12, HM4, HM6, HM7 and HR11) produced siderophore on Chrome Azurol S-Agar (CAS) and this property was confirmed by the presence of orange zones around the bacterial colonies on blue black background. On quantification, highest value of siderophore was observed in HR11 (56%) followed by HS12 (53%), HM4 (49.56%), HS2 (48.26%), HS11 (39.32) and HS10 (38.19%) (Table 3). Eleven isolates (HS2, HS10, HS11, HS12, HS14, HM1, HM4, HM5, HM8, HM9 and HR11**)** had the ability to solubilize inorganic phosphorous. Six bacterial isolates produced IAA significantly. Highest IAA production was reported in HM4 (56.31 µg/ml) after 72 h of incubation in dark followed by HS2 (53.43 µg/ml), HS12 (47.12 µg/ml), HS11 (34.32 µg/ml), HR11 (32.88 µg/ml) and HS10 (26.71 µg/ml) (Fig 1). Nine bacterial isolates (HS1, HS2, HS3, HS5, HS7, HS11, HM3, HM4 and HM7) produced HCN and twenty three bacterial isolates were good ammonia producer.

Effect of TiO_2 nanoparticle on bacterial growth

All the bacterial isolates showed better growth in the presence of different concentrations of TiO_2 (10, 30, 50 and 100ppm) than control, HS10 and HS12 showed best growth in the presence of 50ppm TiO_2. There was continuous increase in bacterial growth (as evidenced by O.D.) up to 50 ppm TiO_2 nanoparticles but a slight decrease in population was observed at 100 ppm TiO_2(Fig. 2). HS12 and HS10 showed better result. Chatterjee *et al*. (2011) have reported no significant difference in the

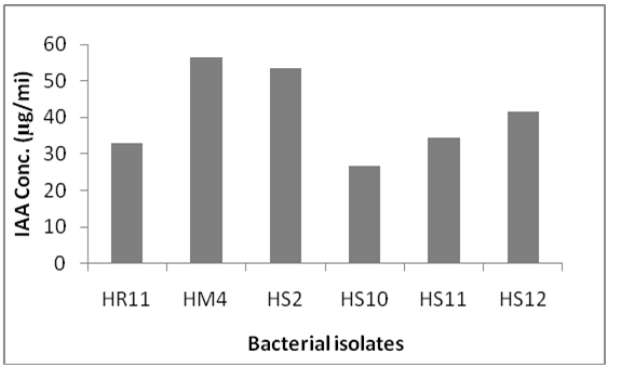

Fig 1: Quantitative estimation of IAA production by bacterial isolates

Table 2: Cell morphology and Colony characteristics of selected bacterial isolates

S. No.	Bacterial isolates	Gram's Reaction	Cell morphology	Arrangement	Colony characteristics				
					Shape	Edge	Elevation	Surface	Chromogenesis
1	HR11	-ve	Short rod	Scattered	Circular	Entire	Convex	Glistering	Fade yellow
2	HM4	-ve	Very short rod	Scattered	Circular	Entire	Convex	Smooth	White
3	HS2	-ve	Very short rod	Scattered	Circular	Undulate	Raised	Smooth	White creamy
4	HS10	+ve	Long rod	Scattered	Circular	Undulate	Umbonate	Wrinkled	Off white
5	HS11	-ve	Short rod	Scattered	Circular	Exntire	Convex	Smooth	Golden
6	HS12	-ve	Short rod	scattered	Circular	Entire	Convex	Smooth	Light yellow

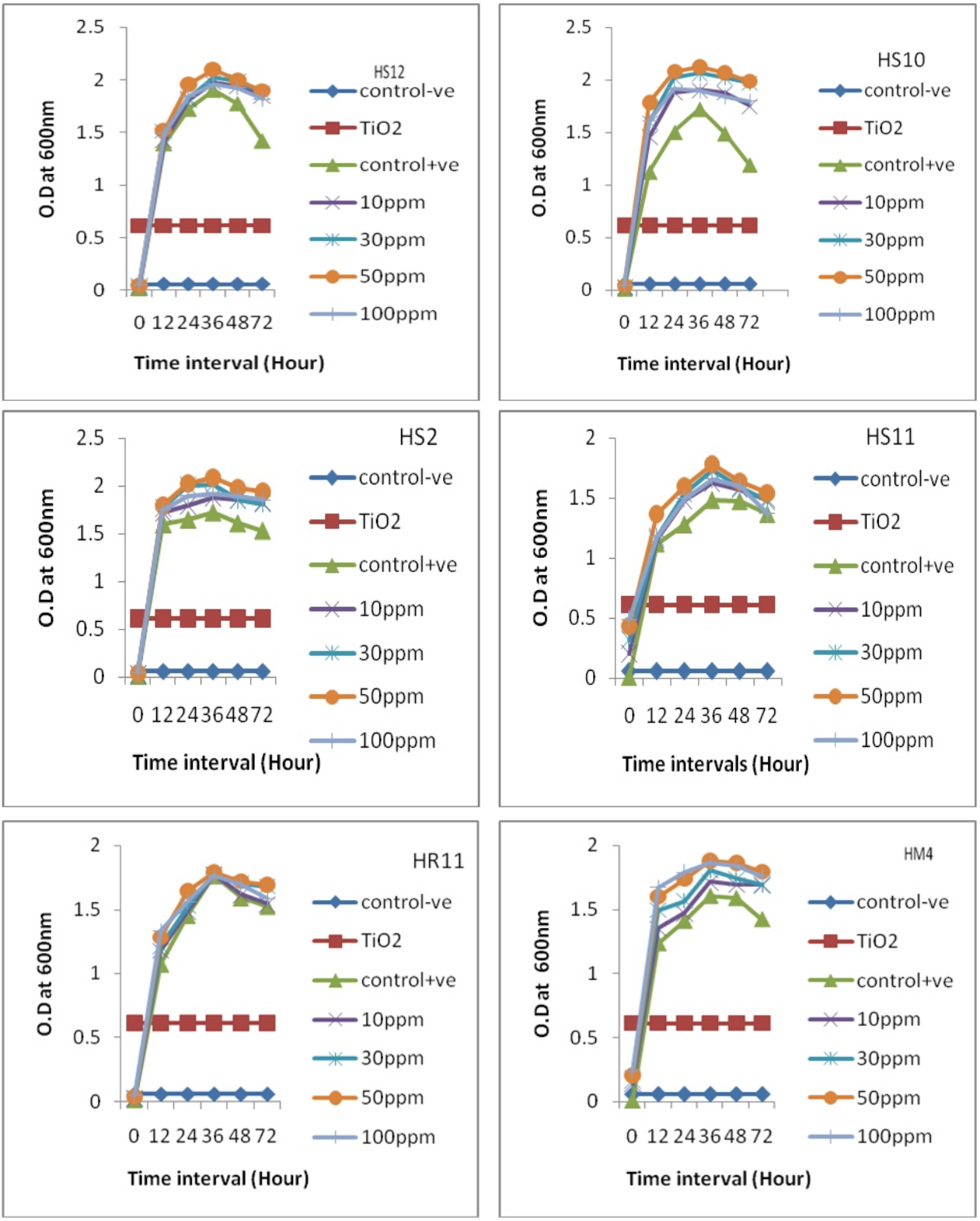

Fig.2: Graphical representation of growth pattern of plant growth promotory rhizobacteria through their absorbance at 600nm due to different concentration of TiO_2 Nanoparticles.

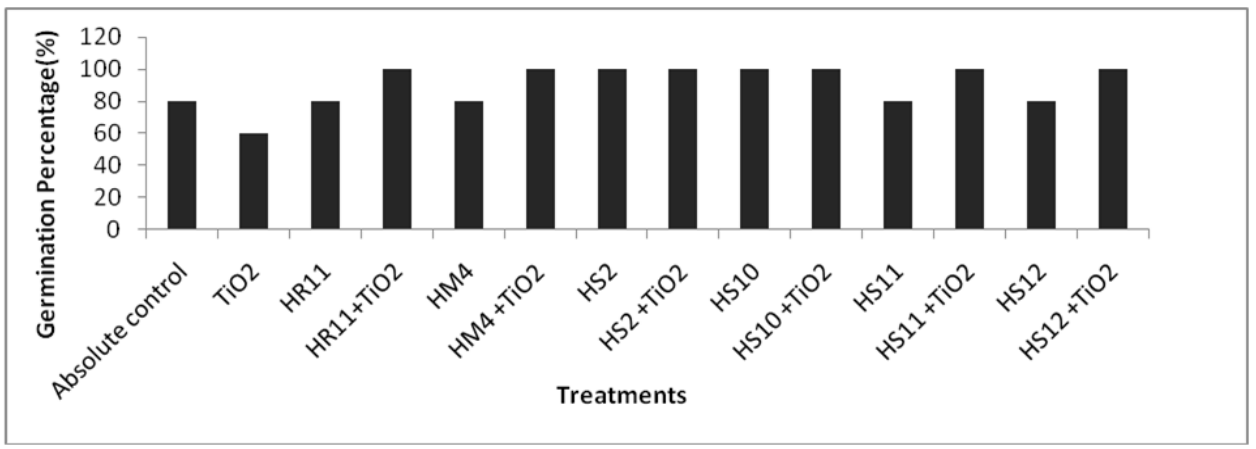

Fig. 3.1: Bar diagram to show variation in percent seed germination (Y axis) under TiO_2 nanoparticles and bacterial treatments (X axis)

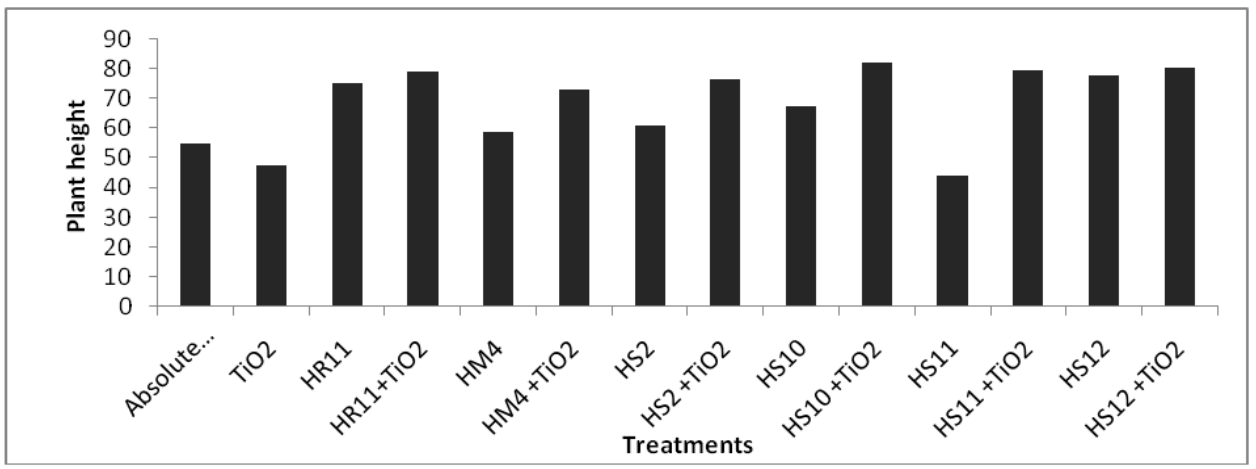

Fig.3.2: Bar diagram to represent effect of different treatments (X axis) on plant height (Y axis)

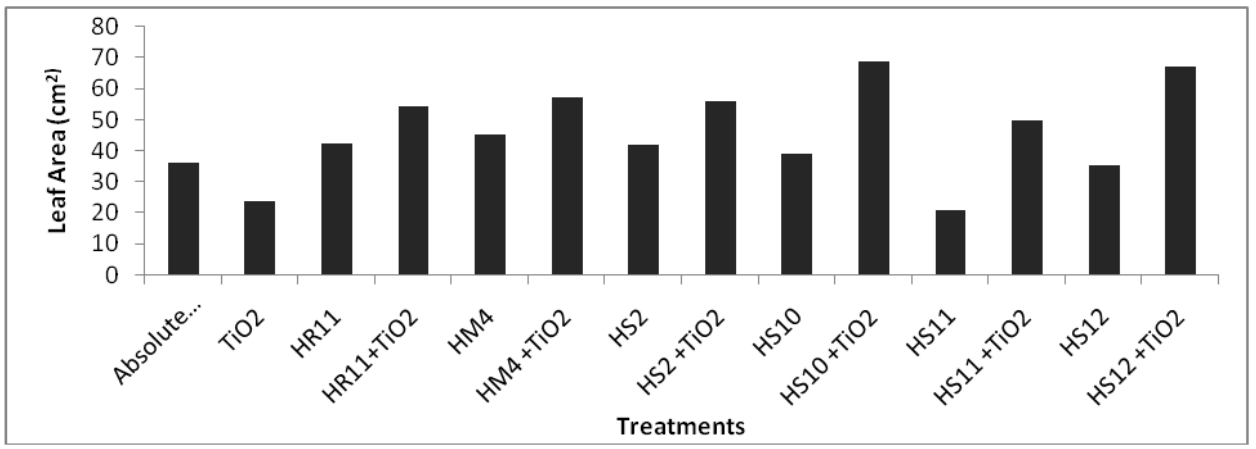

Fig. 3.3: Bar diagram to represent effect of different treatments (X axis) on total leaf area

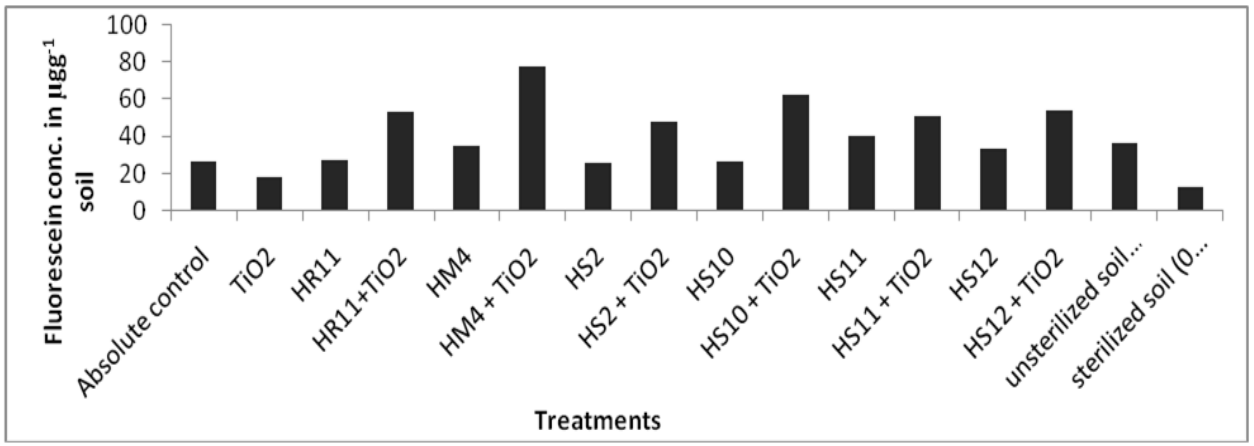

Fig.4.1: Fluorescein Hydrolysis pattern in soil under the influence of different treatments

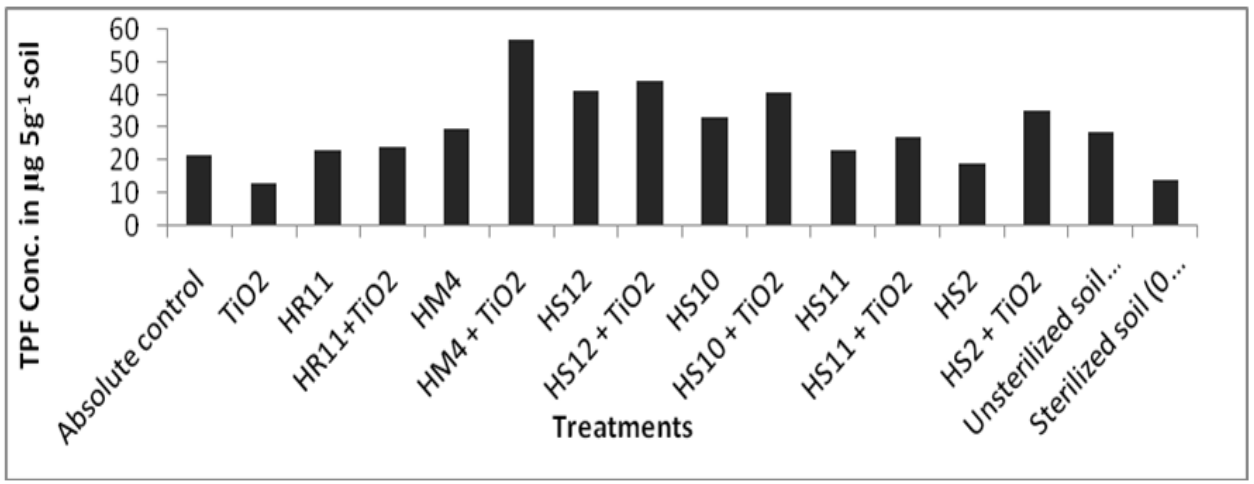

Fig. 4.2: Representation of dehydrogenase activity

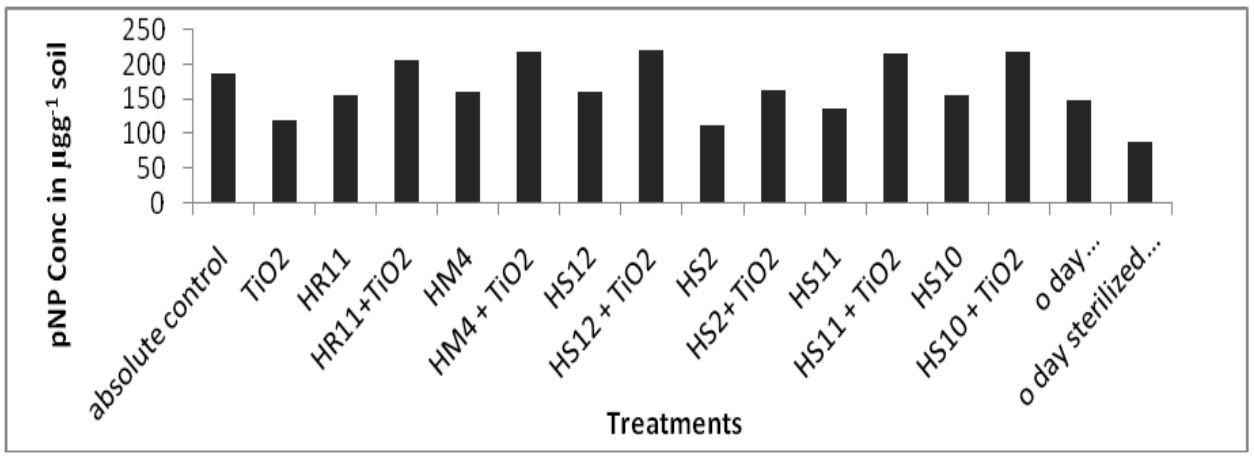

Fig 4.3: Release of pNP during alkaline phosphatase activity of soil sample under different treatments through the release of TPF in soil samples under different treatments

growth and cfu counts when *E. coli* was treated with various concentrations of gold nanoparticle. TiO_2 treatment affected survival rate of *E. coli*, *B. subtilis*, and *S. cerevisiae,* where *S. cerevisiae* showed highest survival rate (71%) and *E. coli* showed the lowest (36%) (Park *et al.,* 2012). Nanogypsum enhanced the growth of *Pseudomonas taiwanensis* and *Pantoea agglomerans* reported by Chaudhary and Sharma, 2019. Khati *et al.* (2019) found that nanozeolite improved the protein content of Bacillus sp. and enhanced their growth pattern.

Pot experiment: Plant height, Germination percentage and Leaf area

Growth of TiO_2 nanoparticle treated maize plants was rapid as compared to only bacterial treatments. Plants grown with TiO_2 + bacterial culture were healthy and green than the plants treated only with bacterial culture. HS10 + TiO_2treatment showed highest (82cm) plant height (Fig 3.1, Table 4). Germination percentage of maize seeds was high in TiO_2+ culture treatments than the treatment, only with culture (Fig 3.2). Among all the treatments, maximum leaf area was observed in HS10 + TiO_2 treatment (68.99 cm^2). Enhanced total leaf area was reported in the presence of nanoparticles (Fig 3.3, Table 5). Maximum plant height was observed in HS10 +TiO2 treated maize, however all the combination of bacteria +TiO2 supported plant height as compare to only bacterial treatment. A significant increase in plant height was observed in HM4, HS2, HS10 and HS11 in the presence of TiO_2.

Enhancement in per cent seed germination of maize seed was observed in bacteria +nanoparticle treatment. Four combinations of bacterial inoculum (HR11, HM4, HS11 and HS12) and nanoparticles supported germination significantly. Although in isolation TiO_2showed minimum percent germination. Five treatments of bacterial inoculum (HR11. HM4, HS2, HS10, HS11 and HS12) with TiO_2 supported maximum leave area. Highest leave area was observed in HS10 + TiO_2

Biochemical Analysis of Plant and Soil

Estimation of chlorophyll

All the treatments consisted of bacterial inoculum and nanoparticles (TiO_2) supported the chlorophyll in maize plants. In general, chlorophyll content was highest in plant samples treated with bacterial cultures + TiO_2. Total Chlorophyll content was highest in HS12 treatment with TiO_2, and was in the range of 7.783 µg g^{-1} fresh weight of plant sample. Level of chlorophyll *a*, chlorophyll *b* and total chlorophyll is given in Table 6. Application of TiO_2 alone enhanced chlorophyll by two fold times than the absolute control

Soil Enzyme Activity

Fluorescein diacetate (FDA) hydrolysis activity: All

Table 3: Plant growth promotory characteristics of the isolated bacterial cultures

Bacterial isolates	Siderophore production	Phosphate solubilization	IAA production	Ammonia production	HCN production
HS1	-	-	-	+ve	+ve
HS2	+ve	+ve	+ve	+ve	+ve
HS3	-	-	-	+ve	+ve
HS4	-	-	-	+ve	-
HS5	-	-	-	-	+ve
HS6	-	-	-	+ve	-
HS7	-	-	-	+ve	+ve
HS8	-	-	-	-	-
HS9	-	-	-	+ve	-
HS10	+ve	+ve	+ve	+ve	-
HS11	+ve	+ve	+ve	+ve	+ve
HS12	+ve	+ve	+ve	+ve	-
HS13	-		-	+ve	-
HS14	-	+ve	-	+ve	-
HM1	-	+ve	-	+ve	-
HM2	-	-	-	+ve	-
HM3	-	-	-	+ve	+ve
HM4	+ve	+ve	+ve	+ve	+ve
HM5	-	+ve	-	+ve	-
HM6	+ve	-	-	+ve	-
HM7	+ve	-	-	+ve	+ve
HM8	-	+ve	-	+ve	-
HM9	-	+ve	-	+ve	-
HM10	-	-	-	+ve	-
HR11	+ve	+ve	+ve	+ve	-

the bacterial treatment with TiO_2 significantly enhanced FDA hydrolysis activity (Fig 3.6). An average of 31.54µg and 57.91µg fluorescein production per g soil was observed under bacterial treatments and culture and TiO_2 treatment respectively from the hydrolysis of FDA in 2h (Fig 4.1 and Table 7). Soil sample from HM4 + TiO_2 treatment showed highest FDA activity (78.15µg/ml). At 0 day, FDA activity was 36.78 µg/ml in unsterilized soil and 12.75 µg/ml in sterilized soil.

Dehydrogenase activity: An average of 28.30µg and 38.10µg TPF (triphenyl formazan) was observed from five gram soil sample treated with culture and culture + TiO_2 by the action of dehydrogenase activity in 16 h of incubation (Fig 4.2 and Table 7). Soil sample from HM4 + TiO_2 treatment showed maximum dehydrogenase activity whereas unsterilized soil showed 28.5 µg and sterilized soil 14.33 µg dehydrogenase activity at 0 day. TPF release was significant with respect to incubation. None of the bacterial treatment along with TiO_2 depressed dehydrogenase activity.

Alkaline Phosphatase activity: An average of 146.9µg and 207.16µg pNP were released per gram of soil sample treated with culture and culture + TiO_2 respectively (Table 7 and Fig 4.3). Soil sample from HS12+ TiO_2 treatment showed highest alkaline phosphatase activity and release of pNP/ml was 225µg/ml. Release of pNP was significant with respect to incubation time. At 0 day, alkaline phosphatase activity was 147.5 µg/ml in unsterilized soil and 87.5 µg/ml in sterilized soil.

PGPR are plant growth promotory bacteria with numerous characteristic mechanisms like siderophore production, N_2 fixation, antibiotic production, IAA production and solubilization of P and Zn. The bacteria were applied as seed coating or inoculum which enhances plant growth (Kloepper *et al*., 1978). In the present study out of 25 bacterial isolates recovered from maize and soyabean rhizosphere only six were selected on the basis of PGPR properties. All the test isolates were good siderophore producers as maximum unit of % siderophore was in HS12. Siderophore production help in Fe_2+ sequestration from the soil and may act as biocontrol agent or directly provide Fe_2+ to host plant (Schwyn and Neilands, 1987). Chandra *et al.* (2007) reported production of 24 µg/ml of IAA by *Mesorhizobium loti* after 48 h of incubation which is in correlation of our results. Bacterial isolates from rhizospheric region of rice, mangrove, chick pea and effluent contaminated soil showed large amount of HCN production (Joseph *et al.,* 2007; Samuel and Muthukkaruppan, 2011). Production of HCN along with siderophore has been reported as the major factor in biocontrol activity for the protection of Black pepper and ginger against *Phytophthora capsici* (Diby, 2004).

Out of six bacterial isolates four showed positive test for HCN production. Ammonia was also invariably observed in all the bacterial isolates. Accumulation of ammonia in soil may lead to increased pH which may suppress fungal growth. Mishra *et al.(*2010) reported that *B subtilis* strain MA-2 and *P fluorescence* MA-4 were good ammonia producers and enhanced biomass of *Geranim amedicin* and aromatic plants.

All the selected isolates solubilized insoluble phosphate in pikovaskya agar plate. Glick (1995) reported that seven bacterial and fungal strains had phosphate solubilization capabilities and promote plant growth by providing them soluble form of phosphorous. These bacteria dissolve soil P through the production of low molecular weight organic acids, mainly gluconic acid and ketogluconic acid (Deubel, 2000).

Based on PGPR properties six bacterial isolates were selected for pot experiment on maize to observe the effect of TiO_2 on plant vigour of maize.

Effect of TiO_2 on plant growth pattern of selected bacterial isolates revealed that HS10 and HS12 showed best growth in the presence of 50ppm TiO_2. There was continuous increase in bacterial growth (as evidenced by O.D.) up to 50 ppm TiO_2 nanoparticles but a slight decrease in population was observed at 100 ppm TiO_2. Chatterjee *et al*. (2011) observed no significant difference in the growth and CFU counts when *E. coli* was treated with various concentrations of gold nanoparticle. Park *et al*. (2012) reported that when *E. coli*, *B. subtilis*, and *S. cerevisiae* were treated with TiO_2, *S. cerevisiae* showed highest survival rate (71%) and *E. coli* showed the lowest (36%). Growth of *E. coli* got suppressed when treated with 50 µg ml^{-1} Cu nanoparticles indicating that Cu could inhibit the growth and reproduction of bacterial cells (Jamshidi and Jahangirirad, 2014). Study conducted by Vani *et al*. (2011) showed antibacterial activity of ZnO nanoparticles against *S. aureus*.

Effect of bacterial inoculation on maize plant under TiO2 nanoparticles treatment revealed that growth of maize plant was rapid with TiO_2 treatment as compare to only bacterial treatment. Plant growth with TiO_2 + bacterial cultures was healthy and green than those treated with bacterial cultures only. Germination of all treatments (TiO_2 + bacterial cultures) was 100%. Hojjat (2015) reported highest germination percentage (78%) in Fenugreek seeds when treated with 20µg mL^{-1} of silver nanoparticles. Nanochitosan and *Bacillus* spp. enhanced the growth of maize health parameters (Khati *et al*., 2017).

Plants may serve as a potential pathway for NP transport and bioaccumulation into the food chain. NPs could pass through the plant epidermis and cortex via the apoplast

Table 4: Shoot, root and plant height under the influence of different treatments

Treatments	Shoot Height (cm)	Root Height (cm)	Plant Height (cm)
Absoiute control	28.00	26.00	55.00
HR11	43.00	32.33	75.33
HR11 + TiO_2	44.50	34.66	79.16
HM4	34.66	24.00	58.66
HM4 + TiO_2	46.66	24.66	73.33
HS2	38.66	22.33	61.00
HS2 + TiO_2	50.16	32.33	76.66
HS10	35.33	29.00	67.33
HS10 + TiO_2	51.33	32.00	82.33
HS11	24.66	19.33	44.00
HS11 + TiO_2	51.00	28.83	79.83
HS12	43.33	28.66	78.00
HS12 + TiO_2	52.00	38.00	80.66
	Treatment × Day	Treatment × Day	Treatment × Day
Sem+	0.655	0.320	2.919
Cd at 5 %	1.898	0.929	8.457

Table 5: Total leaf area of Maize plants

Treatments	Leaf Length (cm)	Leaf Breadth (cm)	Kemp's constant (K)	Leaf Area (cm^2)
Absolute control	24.19	2.26	0.66	36.09
TiO_2	23.16	1.56	0.66	23.63
HR11	32.33	1.96	0.66	42.13
HR11 + TiO_2	34	2.1	0.66	54.23
HM4	34.16	2.13	0.66	45.34
HM4 + TiO_2	35.83	2.26	0.66	57.23
HS2	28	2.26	0.66	41.81
HS2 + TiO_2	33	2.56	0.66	56.16
HS10	28.26	2.1	0.66	39.13
HS10 + TiO_2	37	2.83	0.66	68.99
HS11	18	1.73	0.66	20.65
HS11 + TiO_2	36	3.1	0.66	49.63
HS12	27.66	2.46	0.66	35.35
HS12 + TiO_2	35	2.93	0.66	67.33
	Treatment× Day	Treatment× Day	-	Treatment× Day
Sem+	0.504	0.0426	-	3.392
Cd at 5 %	1.461	0.1234	-	9.828

Table 6: Chlorophyll content of Maize plant

	Chlorophyll in µg g^{-1} fresh weight		
Treatments	**Chlorophyll *a***	**Chlorophyll *b***	**Total chlorophyll**
Absolute control	1.55	8.14	1.82
TiO_2	1.95	10.19	3.45
HR11	2.41	12.06	2.77
HR11+TiO_2	2.95	18.24	3.73
HM4	2.99	18.45	3.77
HM4 + TiO_2	3.04	18.99	3.93
HS2	1.62	10.31	2.07
HS2 + TiO_2	3.39	23.77	4.55
HS10	2.64	14.48	3.16
HS10 + TiO_2	4.18	28.02	5.48
HS12	2.24	11.90	2.64
HS12 + TiO_2	4.55	54.66	7.77
HS11	1.32	8.60	1.71
HS11 + TiO_2	2.99	29.39	4.82
	Treatment × Day	Treatment × Day	Treatment × Day
Sem+	0.315	0.315	0.059
Cd at 5%	0.912	0.912	0.173

Table: 7: Enzyme activities of soil under the influence of different treatments

Treatments	FDA (µg/ml)	Dehydrogenase (µg/ml)	Alkaline phosphatase (µg/ml)
Absolute control	26.93	21.83	187
TiO_2	18.38	13.33	118
HR11	27.28	23.33	155.5
HR11 + TiO_2	53.07	24	205.5
HM4	34.78	29.5	161
HM4 + TiO_2	78.15	57	218.5
HS12	33.85	41.56	160.5
HS12 + TiO_2	54.36	44.166	221.5
HS2	26.23	19	112.5
HS2+ TiO_2	47.79	35.5	163
HS11	40.29	23	136
HS11 + TiO_2	51.19	27.16	216
HS10	26.81	33.48	156
HS10 + TiO_2	62.91	40.83	218.5
Unsterilized soil (0 day)	36.78	28.5	147.5
Sterilized soil (0 day)	12.75	14.33	87.5
	Treatment × Day	Treatment × Day	Treatment × Day
Sem±	0.8299	0.3499	2.5502
Cd at 5%	2.4041	1.0136	7.3879

pathway. TiO_2 NPs also agglomerated in soil medium or on the surface of periderm cells. Salama (2012) found that treatment with 60 ppm silver nanoparticles increased chlorophyll *a* and chlorophyll *b* by 49% and 33% as compared to the control in common bean (*Phaseolus vulgaris*). In corn (*Zea mays*) the chlorophyll *a* and *b* increased by 46% and 26% as compared to control respectively. Raliya *et al*. (2015) observed increase in shoot length (17.02%), root length (49.6%), root area (43%), root nodule (67.5%), chlorophyll content (46.4%) and total soluble leaf protein (94%) of mung bean plants on foliar spray of TiO_2nanoparticles. Durairaj *et al.* (2015) reported a gradual increase in *Trigonella foenumgraecum,* plant parameters such as plant height, chlorophyll content, germination rate, seed vigour and protein content when treated with 50µg of TiO_2 nanoparticle. Foliar spray with magnetite nanoparticles (MNPs) on pear saplings resulted in increased biomass parameters i.e. sapling height, stem diameter, leaf area and dry weight along with increased total carbohydrates, total amino acids, nitrogen and iron

content, total chlorophyll and carotenoids content (Nasr *et al*., 2015). ZnO nanoparticles also improved plant (cluster bean) phenology such as stem height, root volume, and biochemical indicators such as leaf protein and chlorophyll contents (Raliya and Tarafdar, 2013). Frazier *et al.* (2014) reported that plantlets exposed to TiO_2 nanoparticles (range 1000–25,000 ppm) for three weeks showed enhanced leaf count, root length and plant biomass and was in correlation with Ti concentrations.

Enzymes are the integral part of nutrient cycles in soil. They act as marker for viable cells, so enzyme activities such as FDA, dehydrogenase, phosphatase and many other can be related with soil microbial activity or soil health (Bandick and Dick, 1999). In a study, foliar spray of TiO_2 nanoparticles at 10 mgL^{-1} concentration on the leaves of 14 days old mung bean plant, enhanced alkaline phosphatase activity by 72% and dehydrogenase activity by 108.7% over control when tested after six weeks (Raliya *et al*., 2015). Application of nanozeolite and *Bacillus* spp. enhanced the seed germination, plant height, chlorophyll, carotenoid and protein content over control in pot and field experiment (Khati *et al.*, 2018; Chaudhary *et al*., 2021a). Raliya and Tarafdar, (2013) reported that Zn acts as a cofactor for P solubilizing enzymes such as phosphatase and hence increase their activity between 84 and 108%. Sunghyum *et al.* (2011) reported an increased in soil enzyme activity and growth of *Cucumis sativus* from 0 days of sowing period to 45 days of harvesting period when treated with Zn or ZnO nanoparticles. Zinc nanoparticles induced FDA, phosphatase (acid and alkaline) and dehydrogenase enzyme activities (Sindhura *et al.,* 2015).

CONCLUSION

On the basis of above results it can be concluded that nanoparticles make the products better in terms of functionality and do not disturb soil health if used in agriculture. Nanotechnology is a promising field for sustainable agriculture. Different nanoparticles possess different properties which make them harmful or harmless. At present nanoparticles are used in medicines, mechanics, and software technologies. They can also be applied in agriculture for enhanced crop production, pest manifestation and for eliminating the natural plant pathogen. Therefore use of nanoparticles under optimum concentrations results in beneficial effect such as improvement in plant-bacterial interaction, promotion of plant growth related parameters, increased microbial diversity and maintenance of soil health. Chaudhary *et al.* (2021b) also reported that nanozeolite and nanochitosan @ 50 mg/L improved the dehydogenase and alkaline phosphatase activity under maize cultivation.

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