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Bacillus subtilis, a bacterial inhabitant in calcareous soil and its ability to demineralize fixed Fe and Zn salt in its habitat

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Abstract

Calcareous soils are unproductive for agriculture. World over 30 percent of soils are calcareous soils limiting crop cultivation and productivity. In calcareous soils, most of the plant nutrients and micronutrients are not freely available to the crop plants as these often lie in fixed or bonded form. Therefore, these nutrients have to be released from their bonded form into freely available form in such soils to make them available for crop plant growth. The application of microbial species native to such calcareous soils and having the ability and capacity to release such bonded nutrients into freely available form is a need of the day to convert these unproductive soils into productive ones.

In the present investigation, we studied the presence of microbial flora particularly the bacterial *species* in the calcareous soil in western Maharashtra, India, and their role in the release of the bonded Fe and Zn micronutrients into a freely available form, from such soil to make these available for the kidney-bean plant growth.

Six bacterial isolates of distinct colony morphology were isolated from the calcareous soil as the calcareous soil-inhabiting bacterial species, on a specialized enriched media. Their ability as Fe and Zn salt-releasing/de-mineralizing bacterial species was assessed. The release of Fe and Zn from their fixed form by these bacterial species was 0.80 and 1.80 µg/g of calcareous soil respectively. The release of Fe and Zn in calcareous soil was found to play a role in the growth and yield-attributing parameters of kidney-bean plants in calcareous soil. These morphologically distinct bacterial cultures were identified as *Bacillus subtilis* and *Bacillus sp* by using the MALDI Bio Typer classification protocol. These calcareous soils inhabiting bacterial species can be applied as bio-inoculants to the calcareous soil regularly to make them crop productive as well as for the reclamation of such calcareous soils.

Keywords: Calcareous soil; Bacterial species; Micronutrient-releaser/de-mineralizer; Plant-growth parameters; Soil reclamation

1. Introduction

Crop production and productivity are affected by soil characteristics. Normal soils favor a better crop growth than the saline/acidic /calcareous soils. World over 30 percent of soils are calcareous soils limiting crop growth and productivity (Marschner, 1995; Samal and Gautam,2020). The reason for this unproductiveness of calcareous soils is that in calcareous soils most of the plant nutrients and micronutrients are not freely available to the crop plants as these often lie in fixed or bonded form (Meshram et.al, 2023). Therefore, these nutrients have to be released from their bonded

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form to freely available form in such soils to make them available for crop plant growth. There is a need to have eco-friendly, cheap technology for the reclamations of such calcareous soils.

Nature has a variety of microbes in all the ecological habitats, so in the calcareous soils too. The microbial flora of calcareous soils is studied by various workers (Maheshwari and Raj, 2020; Basu et.al, 2016a,b), however, their role in the release of fixed micronutrient-salt in these calcareous soils have not yet been reported. These fixed micronutrient salts can be made available to the plants by these microbes by three microbial functions viz. solubilizing of the fixed nutrient/micronutrient-salt so that plant root can absorb them e.g. Phosphorus solubilization by phosphate solubilizing microbes (Liu et.al, 2015), Nutrient mobilizer e.g. Mycorrhiza mobilizing plant nutrients (Wahid et.al, 2020), and a micronutrient salt demineralizing microbe (Reddy, 2016). Special natural habitat has special microbes and they play a specific role in their habitat (Harwood and Buckley, 2008).

In the present investigation, we studied the presence of microbial flora particularly the bacterial *species* in the calcareous soil in western Maharashtra, India, and their role in the release of the bonded Fe and Zn micronutrients from these calcareous soils, and further studied their availability for the kidney-bean plant growth under glass-house condition. These results can pave the way for microbial reclamation of calcareous soils.

2. Material and Methods

2.1. Collection of Calcareous soil sample

The calcareous soil sample was collected from the calcareous soils prevalent in B block of survey number 50 at the post-graduate farm of Mahatma Phule Agriculture University, Rahuri. The soil sample was obtained with a soil auger from a soil depth of 15 cm by standard procedure (Benton, 1999), collected in a polythene bag, and brought to the laboratory for the soil analysis and isolation of bacteria species habituating the calcareous soil.

2.2. Analysis of calcareous soil sample for soil properties

The soil properties like soil pH, EC, organic carbon, available N, P, K, $CaCO_3$, and micronutrients like Fe, Zn, Mn, and Cu were estimated by employing the standard protocol of soil analysis (SPAC, 2000).

2.3. Isolation of bacterial species inhabiting the Calcareous soil

2.3.1. Medium used for isolation of bacterial species from calcareous soil

The medium used for the isolation of bacterial species from calcareous soil samples is given in Table 1.

Table 1 Medium used for isolation of bacterial species dwelling in calcareous soil

Medium numbers	Name of medium	Composition of medium/L
1	Nutrient-Agar (NA)medium	Peptone 5 g, Yeast extract 3 g, sucrose. 20.0 g, Agar. 20.0 g, Distilled water. 1 L, pH 8-9
2	NA- $CaCO_3$ salt medium	NA enriched with 0.2 percent $CaCO_3$ salt
3	NA- $Mg(OH)_2$ salt medium	NA enriched with 0.2 percent Mg $(OH)_2$ salt
4	NA- $CaCO_3$-$Mg(OH)_2$ salt medium	NA enriched with 0.2 per cent $CaCO_3$ and 0.2 per cent $Mg(OH)_2$ salt
5	NA- $CaCO_3$,- $Mg(OH)_2$,- $Ca(OH)_2$ salt medium	NA enriched with 0.2 percent $CaCO_3$, 0.2 percent Mg $(OH)_2$ and 0.2 percent Ca $(OH)_2$ salt
6	NA- $CaCO_3$, -$Mg(OH)_2$, -$Ca(OH)_2$,- $FeSO_4$ -$ZnSO_4$ salt medium	NA enriched with 0. 2 percent $CaCO_3$, 0.2 percent Mg $(OH)_2$, 0.2 percent Ca $(OH)_2$ salt, and 0.01 percent each of $FeSO_4$ and $ZnSO_4$ salt
7	NA- $CaCO_3$, -$Mg(OH)_2$, -$Ca(OH)_2$,- $FeSO_4$ -$ZnSO_4$ –K_2HPO_4 salt medium	NA enriched with 0. 2 percent $CaCO_3$, 0.2 percent Mg $(OH)_2$, 0.2 percent Ca $(OH)_2$ salt, and 0.01 percent each of $FeSO_4$, $ZnSO_4$ salt and K_2HPO_4

2.3.2. Isolation of Bacterial inhabitant of calcareous soil

Bacterial species inhabiting calcareous soils were isolated from collected calcareous soil samples by pour plate method using medium numbers 1 to 7.

A 1 g fine soil sample of calcareous soil was taken into a 100 mL conical flask and 25 mL sterilized water was added to it. It was placed on a rotary shaker for 1 h with shaking (1200 rpm) and allowed to set soil particles. From this 1 mL aliquot suspension was transferred into sterilized petri plates (in 3 replicates) followed by pouring of sterilized respective lukewarm media and rotated to mix the content. These plates were incubated at room temp (30 ^{0}C) for 3 days and the appeared bacterial colonies were selected and maintained on respective media slant for further studies.

2.3.3. Characterization and identification of bacterial isolates appeared on Fe and Zn salt-enriched media

The bacterial isolates that appeared on the Fe and Zn enriched media were studied for their morphological characteristics *viz.* bacterial colony growth, colony form, colony margin, colony elevation, colony color, gram staining reaction, bacterial shape and cell size, etc. The identification of bacterial isolates up to the species level was carried out by employing the MALDI Bio-typer classification protocol (Sogawa et.al, 2011)

2.3.4. In vitro testing efficacy of isolated bacterial species on solubilization of Fe and Zn salt

The ability of bacterial isolates to solubilize Fe and Zn salt was tested on an NA medium containing Zn salt (0.1g), Fe salt (0.1g), and Mg salt (0.1g) respectively. The bacterial cultures were streaked on these respective salt-enriched mediums under aseptic conditions. The bacterial inoculated plates were incubated at 30 ^{0}C for 7 days. The bacterial growth and clear zone around the growth, if any, was recorded as a solubilization ability of the bacterial species of the concern salt.

2.3.5. Testing efficacy of isolated bacterial species on release of fixed Fe and Zn salt from Calcareous soils under in vitro experiment.

The calcareous soil was collected from the source plot. The bacterial cultures were grown separately in respective salt-enriched NA broth for 5 days, added to the calcareous soil, and mixed well. In general, 500 mL of bacterial-growth broth was added to 1 kg calcareous soil, mixed well, filled in a transparent polythene bag, closed its mouth tightly, and incubated for 30 days for the growth of bacteria in calcareous soil. The efficacy of the bacterial cultures in the release of fixed micronutrient Fe and Zn from the calcareous soil was analyzed as per the standard method of DTPA-micronutrient by using an Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978).

2.3.6. Testing efficacy of demineralized/released Fe and Zn salt of Calcareous soil on kidney-bean plant growth (under pot culture experiment).

The bacterial growth enriched calcareous soil as prepared above was filled in the earthen pots. The absolute control with calcareous soil alone and normal soil alone was kept. 5 seeds of the Kidney bean variety Varun were dibbled in each pot at a depth of 3 cm. These pots were kept under environmental control (temp 28 ^{0}C, RH 89 percent) in poly-house facilities. The pot culture experiment was conducted with 3 replications.

Agronomic practices like watering, weeding, *etc* are carried out as per requirements. The plant growth parameters like plant height, no. of plants/pot, no. of leaves, flowers, and pod beans/plants were recorded at regular intervals.

2.3.7. Quantification of the demineralized Fe and Zn micronutrients in Calcareous soil and assimilation in plant samples after crop harvest

The laboratory analysis of the calcareous soil samples after treatment with bacterial inoculants, and plant samples grown in these soils as well as in control treatments were quantified for the available Zn and Fe in the soil as well as in plant samples after crop harvest as per standard method of soil and plant analysis done by DTPA-micronutrient (Fe and Zn) by using Atomic Absorption Spectrophotometer for soil analysis (Lindsay and Norvell, 1978) and plant analysis (Zososki and Burau, 1977).

3. Results

3.1. Analysis of Calcareous soil sample for soil properties

The results of the soil analysis are summarized in Table 2.

Table 2 Properties of calcareous soil used in the experimentation

Sr.no	Soil properties	value
1	pH	8.4
2	Electrical Conductivity	0.46
3	Organic carbon	0.42 per cent
4	Available nitrogen	246.85 kg/ha
5	Available phosphorus	10.23kg/ha
6	Available potash	404.12 kg/ha
7	Calcium carbonate(CaCO3)	19.62 per cent
8	Available Fe	3.67 µg/g
9	Available Zn	0.647 µg/g
10	Available Mn	11.983 µg/g
11	Available Cu	2.353 µg/g

3.2. Isolation of bacterial species inhabiting the calcareous soil and their identification

The bacterial species inhabiting the calcareous soil were isolated on (1). Regular NA media, (2). NA media enriched with $CaCO_3$ salt, (3). NA media enriched with Mg $(OH)_2$ salt; (4).NA media enriched with $CaCO_3$ + Mg $(OH)_2$ salt, (5). NA media enriched with $CaCO_3$ + Mg $(OH)_2$ + Ca $(OH)_2$ salt, (6). NA media enriched with $CaCO_3$ + Mg $(OH)_2$ + Ca $(OH)_2$ + $FeSO_4$ + $ZnSO_4$ salts, and (7). NA media enriched with $CaCO_3$ + Mg $(OH)_2$ + $FeSO_4$ + $ZnSO_4$+ K_2HPO_4 (as these salts are present in the calcareous soils).

The results (table 3) indicated that for isolation of calcareous soil inhabiting bacterial species CaCO3, and Mg $(OH)_2$ salts were important constituents of the NA media. In the absence of these salts in the NA media the bacterial colonies dwelling in calcareous soil did not appear on the media. The presence of Ca $(OH)_2$ salt in the media was detrimental to the growth of bacterial isolates inhabiting calcareous soil.

Table 3 Bacterial isolates appeared on respective media

Medium number	Salts in the medium (as available in Calcareous soil)	Appearance of bacterial colonies	No. of distinct bacterial isolates	Designation of bacterial isolate number
1	Regular NA media	-	0	-
2	NA with $CaCO_3$,	+	2	I,II
3	NA with $Mg(OH)_2$	+	1	III
4	NA with $CaCO_3$ + $Mg(OH)_2$	+	3	IV, V,VI
5	NA with $CaCO_3$ + Mg $(OH)_2$ + Ca $(OH)_2$,	-	0	-
6	NA with $CaCO_3$ + Mg $(OH)_2$ + Ca $(OH)_2$ + $FeSO_4$ + $ZnSO_4$.	-	0	-
7	NA with $CaCO_3$ + $Mg(OH)_2$ + $FeSO_4$ + $ZnSO_4$+ K_2HPO_4	-	6	I, II, III, IV, V, VI

-= no bacterial colony appeared, + = appearance of bacterial colonies

The bacterial colonies that appeared on particular salt-enriched media were designated a different isolate number based on its distinct colony morphology. The bacterial isolates which were obtained on medium number 2 were

designated as isolate number I and II, while the isolates obtained on medium number 3 were designated as isolate number III and the isolates that appeared on media number 4 were designated as isolate numbers IV, V, and VI. On media number 7 all the above 6 isolates were able to grow. The characters of these 6 bacterial isolates are summarized in Table 4.

Table 4 Characteristics of different bacterial isolates inhabiting calcareous soil

Bacterial Isolate number	Bacterial colony growth	Bacterial Colony form	Bacterial Colony margin	Bacterial Colony elevation	Bacterial Colony color	Gram staining reaction	Bacterial shape & cell size(µm)
I	moderate	circular	entire	flat	creamy	+ve	Rod, 2.3x0.6
II	slow	irregular	lobate	raised	creamy	+ve	Rod, 2.2x0.7
III	slow	Irregular	lobate	umbonate	Navy blue	+ve	Rod, 3.2x0.8
IV	moderate	circular	entire	flat	creamy	+ve	Rod 4.2x1.0
V	high	circular	entire	raised	white	+ve	Rod, 2.3x0.9
VI	high	circular	entire	flat	white	+ve	Rod, 2.5x0.7

All the bacterial isolates were gram-positive in gram staining reaction but differed in other bacterial colony characteristics, and bacterial shape, and cell size. The bacterial isolates number I and IV have moderate growth with a circular colony, entire margin, flat elevation, and creamy colony color as common characteristics but differ in their bacterial cell size. Isolate numbers II and III have slow colony growth, with irregular form, lobate margin, raised/umbonate elevation, and creamy/navy-blue colony color. The bacterial isolate numbers V and VI have fast colony growth with circular colony form, entire margin, raised/flat elevation, and white colony color.

3.3. Differentiation of bacterial isolates based on their calcareous soils' salt preference.

The six bacterial isolates were further differentiated based on their calcium carbonate/Magnesium hydroxide/calcium-carbonate + Magnesium hydroxide salt preference (table 5). Bacterial isolate number I and II preferred $CaCO_3$ salt, isolate number III preferred $Mg(OH)_2$ salt while isolate number IV, V, and VI preferred a combination of CaCO3 + $Mg(OH)_2$ salt.

Table 5 Differentiation of bacterial isolates based on calcareous soils' salt preference

Sr no.	Calcareous soil salt preferred for growth	Bacterial isolate number
1	CaCO3	I, II
2	Mg(OH)2	III
3	CaCO3 + Mg(OH)2	IV, V,VI

3.4. Identification of bacterial isolates inhabiting calcareous soil by using the MALDI Bio Typer identification protocol

The 6 bacterial isolates inhabiting the calcareous soils were identified into bacteria genera and species by using the MALDI Bio Typer protocol by Microbial Culture Collection, National Centre for Cell Science, Pune, India (table 6). The bacterial isolate numbers I to IV was identified as *Bacillus subtilis* while isolate numbers V and VI were identified as *Bacillus sp*. based on the best match culture with score value.

Table 6 Identification of bacterial isolates inhabiting calcareous soil by using the MALDI Bio Typer identification protocol

Bacterial isolate number	Bacterial Genus and species	Score value with available culture reference number
I	*Bacillus subtilis*	2.238 (DSM 5552)
II	*Bacillus subtilis*	2.083 (MCC 2511)
III	*Bacillus subtilis*	2.037 (MCC 2511)
IV	*Bacillus subtilis*	2.192 (DSM 5611)
V	*Bacillus sp*	2.444 (MCC 2505)
VI	*Bacillus sp*	2.077 (MCC 2510)

3.5. Assessment of the sustainability of *Bacillus subtilis* and *Bacillus sp* isolates to various salts of calcareous soil

Calcareous soil salts particularly Fe, Zn, and Mg alone and in combination were assessed for their effect on the growth of these 6 bacterial isolates isolated from calcareous soil. The results (table 7) indicated that all the bacterial isolates grew well on the NA media containing Fe, Zn, and Mg salt.

Table 7 Sustainability of *Bacillus subtilis* and *Bacillus sp* to various salts of calcareous soils

Bacterial isolate no/bacterial sp	Growth of bacterial sp on various salts			
	NA with Fe salt (0.1g)	NA with Zn salt (0.1g)	NA with Mg salt (0.1g)	NA with Fe + Zn + Mg salt (0.1g each)
I : Bacillus subtilis	+	+	+	+
II : Bacillus subtilis	+	+	+	+
III : Bacillus subtilis	+	+	+	+
IV : Bacillus subtilis	+	+	+	+
V : Bacillus sp.	+	+	+	+
VI : Bacillus sp.	+	+	+	+

3.6. Effect of *Bacillus* culture inoculant on de-mineralization/release of Fe and Zn salt from calcareous soil

The micronutrients Fe and Zn are fixed in the calcareous soil and therefore are not readily available for the plant's growth and plant metabolic activities in these soils. The *Bacillus* isolates that sustained the calcareous soil condition were assessed for their ability to de-mineralize/release these nutrients (Fe and Zn) salt from their fixed form to freely available form.

The results (table 8) indicated that the *Bacillus* isolates applied as bio-inoculants into the calcareous soil released the fixed Fe and Zn from this soil and the release for fixed Fe and Zn was 0.80 and 1.80 µg/g of soil respectively. This was evident from the table as the initial status of these salts was less than the status after adding the bacterial bio-inoculants into the experimental calcareous soil, indicating that the increase in the amount of respective salt was due to their de-mineralization/release from the fixed form to freely available form.

Table 8 The ability of *Bacillus* bio-inoculant in the release of fixed Fe and Zn salts from calcareous soil

Micronutrient Salt	Initial status in calcareous soil including fixed salts (µg/g of soil)	The amount available after adding *Bacillus* isolates (µg/g of soil)	The amount released due to *Bacillus* inoculants (µg/g of soil)
Fe	3.67	4.47	0.80
Zn	0.647	2.447	1.80

Thus, the calcareous soil inhabiting *Bacillus subtilis* and *Bacillus sp* have the Fe and Zn salt de-mineralization and releasing ability.

3.7. Effect of demineralizing *Bacillus* inoculants on the growth parameters of Kidney bean plant in calcareous soil

It was observed that the inoculation of *Bacillus* bio-inoculants in calcareous soil increased the plant growth parameters of the kidney-bean plant (table 9) as compared to the plant grown in calcareous soil. There was no flowering and bean pod formation in the surviving plants in calcareous soil, while in the salt-releasing *Bacillus* bio-inoculants treated soil there was flowering and bean pod formation. Though normal (non-calcareous) soil has high plant growth parameters, the results of *Bacillus* bio-inoculants-treated soils were better than non-treated calcareous soils. It was evident from these results that the bio-inoculants treatment by salt-releasing *Bacillus* isolates helped to improve these soils for plant growth parameters.

Table 9 Effect of *Bacillus* bio-inoculants on the plant growth parameters of kidney-bean plant in calcareous soil

Treatment	Treatment details	Plant growth parameters (at 55 d) in calcareous soil				
		Plant survival (Average)	Plant height (cm)	Leaves/ plant	Number of flowers/ plant	Number of bean pods/plant
1	Calcareous soil without bacterial bio-inoculants	0.33	9.33	3.67	0.0	0.0
2	Calcareous soil with bacterial bio-inoculants	4.0	17.33	7.0	3.0	1.0
3	Normal (non-calcareous) soil without bacterial bio-inoculants	5.0	53.67	11.0	6.67	3.67

3.8. Assimilation and Availability of Micro-nutrient Fe and Zn in calcareous soil after Crop harvest, due to *Bacillus* bio-inoculants

Table 10 Assimilation of Fe and Zn in kidney-bean plant and/in calcareous soil due to *Bacillus* bio-inoculants

Treatment	Treatment details	Availability in soil sample (µg/g soil)		Availability in kidney-bean plant sample (µg/g plant)	
		Fe	Zn	Fe	Zn
1	Calcareous soil without bacterial bio-inoculants	3.67	0.64	58.3	16.67
2	Calcareous soil with bacterial bio-inoculants	4.34	2.42	155.0	10.0
3	Normal soil without bacterial bio-inoculants	4.17	1.87	109.6	31.67

The results (table 10) indicated that there was more assimilation of Fe and Zn micronutrients in kidney-bean plants grown in *Bacillus* bio-inoculants-treated calcareous soil as compared to untreated soil. Similarly, there was the release of fixed Fe and Zn in the calcareous soil in their available form. The availability of Fe and Zn in the calcareous soil was to the tune of 5.22 and 2.53µg/g of soil respectively after the salt-releasing *Bacillus* bio-inoculants treatment as against 3.67 and 0.64 µg/g of Fe and Zn in the absence of *Bacillus* bio-inoculants treatment, after the kidney-bean plant harvest.

4. Discussion

Calcareous soils of India with high soil pH and nutrient deficiency have always been a problem for crop cultivation. Crops grown in these soils record severe nutrient deficiency and yield loss. Biological remediation by the microorganism is a novel approach for problematic calcareous soils and can play a major role in enhancing crop production.

The beneficial bacterial micro-flora in soil plays an important role like the salt solubilizing agent, salt mobilizing agent, or salt releasing/de-mineralizing agent. The bacterial species working as salt-solubilizing agents always form a solubilization zone around the bacterial colony while salt mobilizing /salt-releasing agents did not form a solubilization zone around the bacterial growth. Our *Bacillus* isolates were the salt-releasing/de-mineralizing agent as these did not form the solubilization zone around the *Bacillus* growth. The release of Fe and Zn from their fixed form was 0.80 and 1.80 µg/g of calcareous soil respectively by the *bacillus* bio-inoculants in the calcareous soil. The release of Fe and Zn in calcareous soil was found to play a role in the growth and yield-attributing parameters of kidney-bean plants in calcareous soil. There was more assimilation of Fe and Zn micronutrients in kidney-bean plants grown in *Bacillus* bio-inoculants-treated calcareous soil as compared to untreated soil. Similarly, there was a release of fixed Fe and Zn in the calcareous soil in their available form. The assimilation and availability of these two micronutrients in bean plants are in the range of reports of other authors like Lamptey et.al (2023) where Fe concentration in the common bean seeds ranged from 60.68 to 101.66 ppm while Zn levels ranged from 25.87 to 34.04 ppm. The Iron, and Zinc content in raw beans reported by Carvalho et.al. (2012) was 53.1-74.7 and 33.5-43.1 mg/kg. According to Freirs (1997) and Costa et.al (2006) the iron and zinc contents of common bean ranges from 18.8 – 82.4 mg of Fe/g and from 32.6 to 70.2 mg of Zn/kg. However, these levels may vary in function depending on various factors viz. species; variety; and processing factors, such as storage time, temperature, and food preparation (Welch and Graham,2002).

5. Conclusion

Six bacterial cultures of distinct colony morphology were isolated from the calcareous soil as the calcareous soil-inhabiting bacterial species, on a specialized enriched media. They did not form the solubilization zone on the Fe and Zn enriched media but can act as Fe and Zn salt-de-mineralizing/releasing microbes. The release of Fe and Zn from their fixed form was 0.80 and 1.80 µg/g of calcareous soil respectively by the bacterial bio-inoculants. These calcareous soil-inhabiting bacteria were identified as *Bacillus subtilis* and *Bacillus sp*. The release of Fe and Zn in calcareous soil by these *Bacillus* bio-inoculants was found to play a role in the growth and yield attributing parameters of kidney-bean plant in calcareous soil and therefore, can be applied as bio-inoculants into the calcareous soil regularly to make them crop productive and for the reclamation of these calcareous soils.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Basu, K., Vimala kumara TG., Kharkwal AC., Varma.A,. Kumar V. 2016a. Alkali-tolerant *Penicillium asturianum* as a plant growth promoter for nutrient-deficient calcareous soil. Innovare J. Agri. Res. 4(2): 8-12.

[2] Basu, K., Vimala kumara TG., Kharkwal AC., Abdin, MZ., Vivek K., and Varma. A. 2016b. Bioremediation potential of soil microbes for nutrient management in calcareous soil. International J. Chem Tech Research. 9(9): 447-455.

[3] Benton J. Jr. 1999. Soil and Plant Analysis. Laboratory Registry for the United States and Canada, Second Edition. Routledge publication (Taylor & Francis group). ISBN:9781574441796.

[4] Carvalho LM, Corrêa MM, Pereira EJ, Nutti MR, Carvalho JL, Ribeiro EM, Freitas SC. 2012. Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. Food Nutr Res. 56. doi: 10.3402/fnr.v 56i0.15618.

[5] Costa GEA, Queiroz-Monici KS, Reis SMPM, Oliveira AC.2006. Chemical composition, dietary fiber, and resistant starch contents of raw and cooked pea, common bean, chickpea, and lentil legumes. *Food Chem.*94:327–30.

[6] Freire WB.1997. Strategies of the Pan American Health Organization/World Health Organization for the control of iron deficiency in Latin America. *Nutr Rev.*55:183–188.

[7] Harwood. C and Buckley. M. 2008. The uncharted microbial world: microbes and their activities in the environment. A Report from the American Academy of Microbiology, Washington. Pages 41. http://www.asm,org

[8] Lamptey. M., H. Adu-Dapaah., F. O. Amoako-Andoh , L. Butare ., K. A. Bediako. , R. A. Amoah ., I. Tawiah , S. Yeboah , J. Y. Asibuo .2023. Genetic studies on iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.) in Ghana. Heliyon. 9 (6): e17303.

[9] Lindsay, WL and Norvell WA.1978. Development of DTPA soil test for Zinc, Iron, Manganese, and Copper. Soil Sci Soc Am. J. 42: 421-428.

[10] Liu. Z., Li. YC., Zhang. S., Fu. Y., Fan. X., Patel. JS., and Zhang, M. 2015. Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. Applied soil Ecology. 96: 217-224.

[11] Maheshwari, P., and Raj. A. 2020. Microbial profile of a calcareous soil of south Tamil Nadu, India. International J. Curr. Microbiol. App. Sci. 9(10):3899-3907.

[12] Marschner, H .1995. Mineral nutrition of higher plants. Academic Press, London

[13] Meshram, NA, Singare, PB and Kausadikar, HK. 2023. Calcareous soils and their management. Agriculture. 3(4):1-6.

[14] Reddy. P. 2016. Microbial status of calcareous soil and the role of microbes in the release of plant micronutrients (Fe and Zn). A M.Sc thesis submitted to Mahatma Phule Agriculture University, Rahuri, India. Pp.72.

[15] Samal, SK, and Gautam, RK. 2020. Nutrient management in calcareous soil. Food and Scientific Reports. 1 (6): 1-3.

[16] SPAC, 2000. Soil Analysis: Handbook of Reference Methods. Taylor & Francis Group publication. ISBN:9780849302053.

[17] Sogawa. K., M. watanabe., F. Nomura. 2011. Use of MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms. Analytical and Bioanalytical Chemistry. 400:1905-1911.

[18] Wahid. F., Fahad. S., Danish. S., Adnan. M., Yue. Z., Saud. S., Siddiqui. MH., Brtnicky. M., Hammerschmiedt. T., and Datta. R. 2020. Sustainable management with Mycorrhizae and Phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. Agriculture. 10(8):334

[19] Welch RM, and Graham RD.2002. Breeding crops for enhanced micronutrient content. *Plant Soil.* 245:205–14.

[20] Zososki, R.L., and Burau, R.G. 1977. A rapid nitric, perchloric acid digestion method for multielement tissue analysis. Soil Sci. Plant Analysis. 8:425-436.