World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

# Fungal *species* inhabiting calcareous soil in western Maharashtra, India, and their role in the release of soil-bonded Fe and Zn micronutrients for crop plant availability in such soils

S. G. Borkar [1,*], Prathyusha Reddy [2] and V. A. Chavan [2]

[1] *Dr. Borkar's Laboratory and Research Centre, Endeavour Scientific Agriculture, 301, Prestige Point Building, In front of Nashik Road police Station, Nashik 422 101, India.*
[2] *Department of Plant Pathology & Agricultural Microbiology, Mahatma Phule Agriculture University, Rahuri 413 722, India.*

World Journal of Advanced Research and Reviews, 2023, 20(03), 1622–1632

Publication history: Received on 12 November 2023; revised on 21 December 2023; accepted on 23 December 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.3.2626

## Abstract

Calcareous soils which are around 30 per cent of the world over are unproductive for agricultural 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 to 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 is a need of the day to convert these unproductive soils into productive ones. The microbial species that work as nutrient/micronutrient-salt solubilizing agents generally form a salt solubilization zone around the microbial colony while salt-mobilizing /salt-releasing microbes do not form such a solubilization zone around the microbial colonies.

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

Six fungal cultures of distinct colony morphology were isolated from the calcareous soil as the calcareous soil- inhabiting fungal species, on a specialized enriched media. They did not form the solubilization zone on the Fe and Zn enriched media and therefore were considered and tested for their efficacy as Fe and Zn salt-releasing/demineralizing microbes. The release for Fe and Zn from their fixed form was 1.68 and 1.91µg/g of calcareous soil respectively by these demineralizing fungal bio-inoculants. 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 fungal cultures were identified as *species* of *Glomus*, *Mucor,* and *Aspergillus.* Therefore, these calcareous soils inhabiting fungal species having demineralization activity can be applied as demineralizing bio-inoculants to the calcareous soil regularly to make them crop productive, and for their reclamation.

**Keywords:** Calcareous Soil; Fungal Species; Micronutrient-Releaser/Demineralizer; Plant-Growth Parameters; Soil Reclamation

* Corresponding author: S. G. Borkar.

## 1. Introduction

Crop production and productivity are affected by soil characteristics. Normal soils favour better crop growth than the saline/acidic /calcareous soils. World over 30 per cent of soils are calcareous soil limiting crop growth and productivity (Marschner, 1995; Samal and Gautam,2020). The reason for this unproductiveness of calcareous soils lies in the fact 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 form to freely available form in such soils to make them available for plant crop 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 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, 2016b), however, their role in the release of fixed micronutrient-salt in calcareous soils have not yet been reported. These fixed micronutrient salts can be made available to the plant by the soil microbes by three microbial functions viz. solubilizing 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 releasing/ de-mineralizing microbes (Reddy, 2016).

Special natural habitat has special microbes and they play a specific role in these habitats (Harwood and Buckley, 2008). In the present investigation, we studied the presence of microbial flora particularly the fungal *species* in the calcareous soil in western Maharashtra, India, and their role in the release of the bonded Fe and Zn micronutrients from the calcareous soil, and further studied their availability for the kidney-bean plant growth in calcareous soil 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 samples

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

*2.3.1. Growth medium used for isolation of fungal micro-flora*

The medium used for the isolation of fungal micro-flora from calcareous soil samples is given in Table 1.

**Table 1** Medium used for isolation of fungal micro-flora

| Medium number | Name of medium | Composition of medium/L |
|---|---|---|
| 1 | Potato-dextrose-agar (PDA)medium | Potato extract. 250 g, dextrose. 20.0 g, Agar. 20.0 g, Distilled water. 1 L, pH 8-9 |
| 2 | PDA- $CaCO_3$ salt medium | PDA enriched with 2 per cent $CaCO_3$ salt |
| 3 | PDA- $Mg(OH)_2$ salt medium | PDA enriched with 1.5 per cent $Mg(OH)_2$ salt |
| 4 | PDA- $CaCO_3$-$Mg(OH)_2$ salt medium | PDA enriched with 2 per cent $CaCO_3$ and 1.5 per cent $Mg(OH)_2$ salt |
| 5 | PDA- $CaCO_3$,- $Mg(OH)_2$ ,- $Ca(OH)_2$ salt medium | PDA enriched with 2 per cent $CaCO_3$ , 1.5 per cent $Mg(OH)_2$ and 2 per cent $Ca(OH)_2$ salt |

| | | |
|---|---|---|
| 6 | PDA- $CaCO_3$, -$Mg(OH)_2$, -$Ca(OH)_2$,-$FeSO_4$ -$ZnSO_4$ salt medium | PDA enriched with 2 per cent $CaCO_3$ , 1.5 per cent $Mg(OH)_2$ , 2 per cent $Ca(OH)_2$ salt, and 0.5 per cent each of $FeSO_4$ and $ZnSO_4$ salt |

*2.3.2. Isolation of Fungi*

Fungal inhabitants of calcareous soil samples and those of Fe and Zn salt demineralizing /releasing fungi were isolated from these soil samples by pour plate method using medium numbers 1 to 6.

A 1 g fine soil sample of calcareous soil was taken into a 100 mL conical flask and 25 mL distilled 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 sterile 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 7 days and the appeared fungal colonies were selected and maintained on respective media slants for further studies.

*2.3.3. Characterization and identification of fungal isolates*

The fungal isolates grown on the Fe and Zn enriched media were studied for their morphological characters *viz.* colony growth, colony colour, septa in hypha, and fungal spores/fruiting bodies for the identification of fungal genus (Dugan, 2006).

*2.3.4. Testing efficacy of isolated fungal species on demineralization/release of fixed Fe and Zn salt of Calcareous soil under in vitro experiment.*

The calcareous soil was collected from the source plot and steamed sterilized for 30 minutes for 3 consecutive days. The test fungal cultures were grown separately in respective salt enriched Potato-dextrose broth for 5 days. In general, 500 mL broth having fungal growth 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 fungi in this calcareous soil, and test their efficacy in the release of fixed micronutrient Fe and Zn from this calcareous soil. The analysis of available Fe and Zn was carried out as per the standard method of DTPA-micronutrient by using an Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978).

*2.3.5. Testing efficacy of demineralized Calcareous soil on plant growth of the kidney-bean plant (under pot culture experiment).*

The fungal 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 plant, variety Varun, were dibbled in each pot at a depth of 3 cm. These pots were kept under environmentally controlled (temp 28 $^0$C, RH 89 per cent) poly-house facilities. The pot culture experiment was conducted with 3 replications.

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

*2.3.6. Quantification and availability of demineralized Fe and Zn in Calcareous soil and harvested kidney-bean plant samples.*

The experimental soil samples as well as plant samples of kidney-bean plants were quantified for the available Zn and Fe after crop harvest. The laboratory analysis was carried out as per the standard method of analysis done by DTPA-micronutrient testing (Fe and Zn) by using an Atomic Absorption Spectrophotometer for soil analysis (Lindsay and Norvell,1978) and plant analysis (Zososki and Burau, 1977).

## 3. Experimental 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 fungal species inhabiting calcareous soil and their identification

The fungal species inhabiting the calcareous soil were subjected to isolation on (1). Regular PDA media, (2). PDA media enriched with $CaCO_3$ salt, (3). PDA media enriched with $Mg(OH)_2$ salt, (4). PDA media enriched with $CaCO_3$ + $Mg(OH)_2$ salt, (5). PDA media enriched with $CaCO_3$ + $Mg(OH)_2$ + $Ca(OH)_2$ salt, and (6). PDA media enriched with $CaCO_3$ + $Mg(OH)_2$ + $Ca(OH)_2$ + $FeSO_4$ + $ZnSO_4$ salts (as these salts are generally present in the calcareous soils).

The results (table 3) indicated that 3 distinct types of fungal colonies developed on the PDA media enriched with $CaCO_3$ + $Mg(OH)_2$ + $Ca(OH)_2$, whereas another 3 distinct types of fungal colonies developed on the PDA medium enriched with $CaCO_3$ + $Mg(OH)_2$ + $Ca(OH)_2$ + $FeSO_4$ + $ZnSO_4$. On regular PDA media or PDA media enriched with CaCO3, $Mg(OH)_2$, or $CaCO_3$+$Mg(OH)_2$ no fungal colonies could be isolated from calcareous soil.

**Table 3** Fungal isolates on respective media

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

- = No fungal colony appeared, + = appearance of fungal colonies

It is apparent from the table that the PDA media that contains $Ca(OH)_2$ salt in combination with $CaCO_3$ favoured the isolation and growth of fungal colonies from calcareous soils.

These fungal colony isolates were designated into different isolate numbers based on their distinct colony characteristics (photo.1). The fungal isolates which were obtained on medium number 5 were distinct from the fungal isolates obtained on medium number 6. The isolates obtained on medium number 5 were designated as isolate numbers I, II, and III, while the isolates obtained on medium number 6 were designated as isolate numbers IV, V and VI. All these

isolates were distinct from each other for mycelia character, fungal colony colour, colony diameter (growth on the 8th day) and fungal spore (table 4)

**Table 4** Characterization of fungal isolates inhabiting calcareous soil

| Isolate number | Fungal Mycelia character | Fungal Colony diameter (at 8 days) | Fungal colony colour | Fungal spores | Fungal genus |
|---|---|---|---|---|---|
| I | septate | 4.10 | Pale yellow Cottony radiating | Chlamydospores | *Glomus* |
| II | coenocytic | 3.60 | Pale yellowish cottony radiating | sporangiospores | *Mucor* |
| III | septate | 3.90 | Pale Yellowish suppressed radiating | sporangiospores | *Aspergillus* |
| IV | coenocytic | 4.00 | Whitish cottony suppressed | Sporangiospores | *Mucor* |
| V | coenocytic | 4.30 | Pale yellowish cottony radiating | sporangiospores | *Mucor* |
| VI | septate | 3.40 | Pale Yellowish suppressed radiating | sporangiospores | *Aspergillus* |

Based on the mycelia characters and the fungal spores forming structures, the fungal colonies growing on medium number 5 were identified as of genus *Glomus, Mucor* and *Aspergills,* while those growing on medium number VI were of *Mucor* and *Aspergillus*. The fungal colonies of *Mucor* and *Aspergillus* on these two different mediums have different growth rates and therefore seem to be different morphotypes.

### 3.3. Assessment of the sustainability of fungal 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 various fungal isolates/fungal genus isolated from calcareous soil. The results (table 5) indicated that all the fungal isolates grew well on the PDA media containing Fe and Mg salt. The isolate numbers I, II, and III were unable to grow on Zn salt-containing media while isolate numbers IV, V, and VI were able to grow on Zn salt-containing medium. The Zn salt sustainability results further confirm that the *Mucor* and *Aspergillus* species grown on medium numbers 5 and 6 are different.

The beneficial fungal micro-flora in soil plays an important role like the salt-solubilizing agent, salt-mobilizing agent or de-mineralizing /salt-releasing agent. The fungal species working as salt-solubilizing agents always form a solubilization zone around the fungal colony while salt-mobilizing /salt-releasing agents did not form a solubilization zone around the fungal growth. Our fungal isolates seem to be de-mineralizing /salt-releasing agents as these did not form the solubilization zone around the fungal growth. Therefore, the fixed salt-releasing ability of these fungal isolates was studied.

**Table 5** Assessment of sustainability of fungal isolates to various salts of calcareous soils.

| **Fungal isolates no/fungal sp** | **Growth of fungal *sp* on various salts** | | | |
|---|---|---|---|---|
| | PDA containing Fe salt (0.1g) | PDA containing Zn salt (0.1g) | PDA containing Mg salt (0.1g) | PDA containing Fe + Zn + Mg salt (0.1g each) |
| I :(*Glomus sp*) | + | - | + | - |
| II :(*Mucor sp*) | + | - | + | - |
| III :(*Aspergillus sp*) | + | - | + | - |
| IV :(*Mucor*) | + | + | + | + |
| V :(*Mucor*) | + | + | + | + |
| VI: (*Aspergillus sp*) | + | + | + | + |

### 3.4. Effect of fungal species as demineralizing bio-inoculants on the 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 plants' growth and plant metabolic activities in these soils. The fungal isolates/species which sustained the calcareous soil condition were assessed for their ability to release these micro-nutrient salts from their fixed status.

The results (table 6) indicated that the fungal isolates applied as demineralizing bio-inoculants into the calcareous soil released the fixed Fe and Zn salt from this soil, and the release of fixed Fe and Zn was 1.68 and 1.91µg/g of soil respectively. It is evident from the table that the initial status of these salts was less than the status after adding the fungal bio-inoculants, indicating that the increase in the amount of respective salt was due to their release from fixed form to freely available form.

**Table 6** The ability of fungal demineralizing bio-inoculant in the release of fixed Fe and Zn salts from calcareous soil

| **Micronutrient Salt** | **Initial salt status in calcareous soil (µg/g of soil)** | **Salt status after adding salt-releasing fungal isolates (µg/g of soil)** | **Amount of fixed salt released due to fungal inoculants (µg/g of soil)** |
|---|---|---|---|
| Fe | 3.67 | 5.35 | 1.68 |
| Zn | 0.647 | 2.557 | 1.91 |

Thus, the fungal isolates of *Mucor, Aspergillus* and *Glomus* isolated from the calcareous soil as its inhabitants are the demineralizer of the fixed Fe and Zn salt/micronutrient salt.

### 3.5. Effect of addition of demineralizing (fixed Fe and Zn salt-releasing) fungal bio-inoculants in calcareous soil on the growth parameters of Kidney bean plant

It was observed that the inoculation of fungal demineralizing bio-inoculants in calcareous soil favours the germination and survival of kidney-bean plant (photo 2) with an increase in the plant growth parameters (table 7) as compared to the plant grown in calcareous soil. There was no flowering and bean pod formation in the surviving plant in calcareous soil, while in the salt demineralizing/releasing bio-inoculants treated soil, there was flowering and bean pod formation. Though normal (non-calcareous) soil has high plant growth parameters, the results of demineralizing bio-inoculants-treated soils were better than non-treated calcareous soils. It is evident from these results that the bio-inoculants treatment by salt-releasing fungal isolates helped to improve these soils for plant growth parameters.

**Table 7** Effect of fungal bio-inoculants on the plant growth parameters of kidney-bean plant

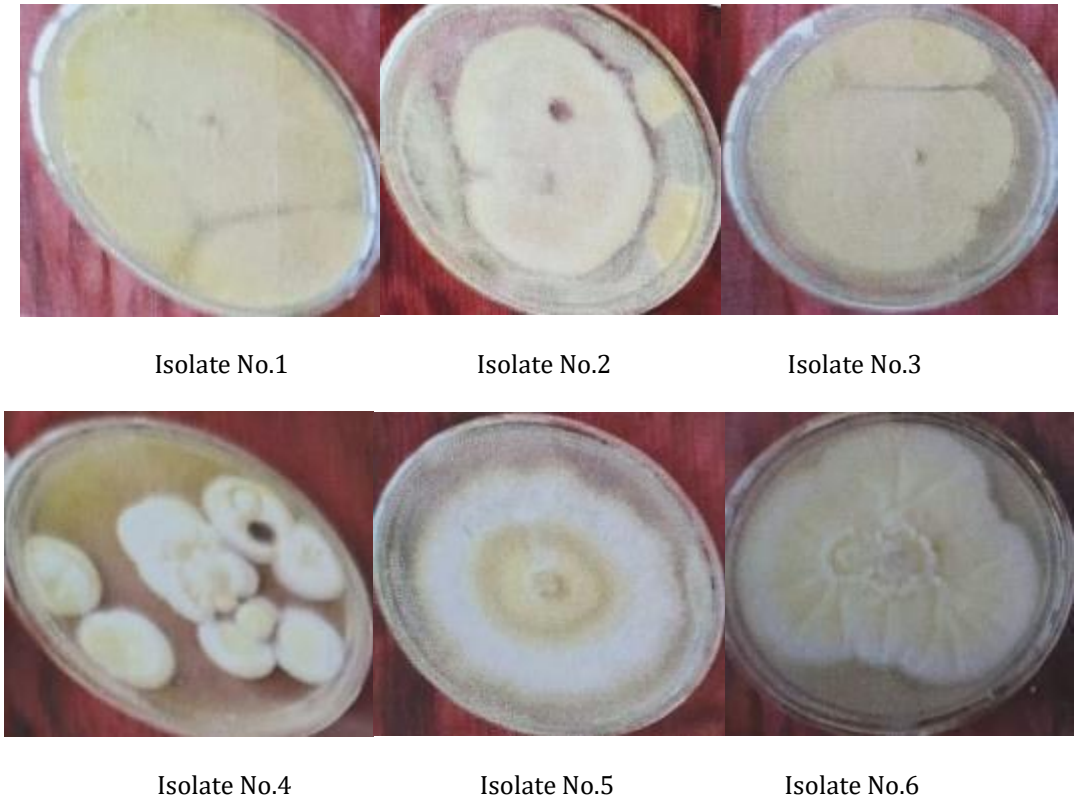
| Treatment | Treatment details | Plant growth parameters (at 55 d) | | | | |
|---|---|---|---|---|---|---|
| | | Plant survival (Average) | Plant height (cm) | Leaves/ plant | Number of flowers/ plant | Number of bean pods/plant |
| 1 | Calcareous soil without fungal inoculants | 0.33 | 9.33 | 3.67 | 0.0 | 0.0 |
| 2 | Calcareous soil with fungal inoculants | 1.33 | 14.33 | 8.0 | 4.33 | 1.67 |
| 3 | Normal (non-calcareous) soil without fungal inoculants | 5 | 53.67 | 11.0 | 6.67 | 3.67 |

### 3.6. Availability status of Fe and Zn in calcareous soil and kidney-bean plant samples after crop harvest, as influenced by the addition of these salt-releasing fungal bio-inoculants

The results (table 8) indicated that there was more assimilation of Fe and Zn micronutrients in kidney-bean plants grown in bio-inoculants (micronutrient releasing) treated calcareous soil as compared to untreated soil. Similarly, there was a release of fixed Fe and Zn in the calcareous soil into 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 bio-inoculants treatment as against 3.67 and 0.64 µg/g of Fe and Zn in the absence of bio-inoculants treatment after the kidney-bean plant harvest. The assimilation of Fe and Zn was more in the kidney-bean plant grown in salt-releasing bio-inoculants-treated calcareous soil as compared to non-treated calcareous soils. The assimilation was nearly double or more than double.

**Table 8** Assimilation of Micro-nutrient Fe and Zn in kidney-bean plant and/in calcareous soil due to fungal bio-inoculant after crop harvest

| Treatment | Treatment details | Availability in a soil sample (µg/g soil) | | Availability in kidney-bean plant sample (µg/g plant sample) | |
|---|---|---|---|---|---|
| | | Fe | Zn | Fe | Zn |
| 1 | Calcareous soil without fungal inoculants | 3.67 | 0.64 | 58.3 | 16.67 |
| 2 | Calcareous soil with fungal inoculants | 5.22 | 2.53 | 111.50 | 43.33 |
| 3 | Normal soil without fungal inoculants | 4.17 | 1.87 | 109.6 | 31.67 |



Isolate No.1 Isolate No.2 Isolate No.3

Isolate No.4 Isolate No.5 Isolate No.6

**Figure 1** Fungal isolates of distinct morphology inhabiting calcareous soil


A. Kidney-bean plant survival in calcareous soil; B. Kidney-bean plant survival in fungal bio-inoculants treated calcareous soil.

**Figure 2** Effect of de-mineralizing fungal isolates on kidney-bean plant growth in calcareous soil in pot experimentation.

## 4. Discussion

Calcareous soils 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. Fungi are ubiquitous inhabitants of soils, rock and mineral surfaces, and can prosper in the most adverse of environments. These are significant geo-active agents capable of numerous transformations of metals and minerals in their habitat, with their activities underpinned by growth form and metabolism (Gadd, 2017). Wherever fungi are found, the transformation of metals and minerals is a key aspect of their activity, with bio-mineralization an important feature. Fungal bio-mineralization is an important facet of geomycology viz. the roles of fungi in geochemical and geophysical processes (Gadd, 2021). Our studies indicated that the fungal species of the genus *Glomus, Aspergillus* and *Mucor* were the inhabitants of the calcareous soil under study and had a functional role as demineralizer of fixed Fe and Zn salt in these soils. The fungal isolates applied as demineralizing bio-inoculants into the calcareous soil released the fixed Fe and Zn salt into the freely available form from this soil, and the release of fixed Fe and Zn was 1.68 and 1.91µg/g of soil respectively. Basu et.al (2016a) isolated alkali-tolerant *Penicillium asturianum* (IPL/KB/F3) from soils with high pH (alkali soils). Their isolate showed a remarkable amount of alkali tolerance and phosphate solubilizing activity along with other insoluble elements. This isolate under *in vitro* study showed 54±1.13 to 92±1.16 µg/ml of phosphate solubilization within 3-5 days, with an ability to tolerate high alkaline pH up to 11.0, grow at a higher concentration of calcium carbonate, and solubilize micronutrients.

Our experimental calcareous soil contained both free-living (*Mucor* and *Aspergillus*), and root-associated Arbuscular mycorrhizae fungi (*Glomos sp*). The plant-root-associated fungi known as Mycorrhizas are symbiotic, and found to colonize the roots of most plant genera, where they improve plant nutrition through solubilization of essential minerals and phosphate from soil. Arbuscular mycorrhizae (AM) are an integral part of most plants in nature (Gianinazzi et al., 1982) and occur in nearly 90% of species of the plant community (Smith & Read, 1997). One of the most dramatic effects of mycorrhizal infection on the host plant is an increase in phosphorus (Koide, 1991) and zinc uptake (Lambert et al., 1979; Kothari et al., 1991; Ortas et al., 2001; Ortas, 2003) mainly due to the capacity of the mycorrhizal fungi to absorb these ions from the soil and transfer them to the host roots (Asimi et al., 1980; George et al., 1995). In addition, mycorrhizal infection results in an increase in the uptake of other macro- and micro-nutrients (Marschner & Dell, 1994). In our studies, we found *Glomos sp* of Mycorrhizae fungi as inhabitants of calcareous soil and with an evident involvement in bio-mineralization of Fe and Zn salt. The content of Fe and Zn in kidney-bean plant samples in our experimentation was 58.3 and 16.67 µg/g of plant sample respectively in the plants grown in calcareous soil while it was 111.50 and 43.33 µg/g of plant samples respectively in plants grown in demineralizing bio-inoculants treated calcareous soil, indicating that there was more assimilation of Fe and Zn in the plant grown in demineralizing bio-inoculants treated calcareous soils as compared to untreated soils. Lamptey et.al, (2023) reported the Fe content of the common bean seeds in the range of 60.68 to 101.66 ppm while zinc content was in the range of 25.87 to 34.04 ppm. Carvalho et.al. (2012) reported the Iron and Zinc content in raw beans in the range of 53.1-74.7 and 33.5-43.1 mg/kg. The common bean (*Phaseolus vulgaris* L.) content of iron and zinc ranges from 18.8 – 82.4 mg of Fe/g and from 32.6 to 70.2 mg of Zn/kg (Freirs, 1997., Costa et. al,2006). Thus, various researchers reported different levels of Fe and Zn in bean plants which may vary depending on various factors like crop species; variety; and processing factors, such as storage time, temperature, and food preparation (Welch and Graham, 2002).

Ortas and Akpinar,(2006) observed that kidney bean plants grown for 8 weeks in two widely distributed calcareous clay soils (Central Anatolian Sultanönü and Konya soil )with low nutrient content with two selected arbuscular mycorrhizal sp (*Glomus mosseae* and *G. etunicatum)* increased the plant growth as well as P and Zn uptake in the Sultanönü soil. The positive effect of mycorrhizal inoculation on plant P content and uptake was found to be higher when higher levels of phosphorus were applied. In our studies, the Fe and Zn concentration of the Kidney-Bean plant is also increased with the application of Zn and Fe-releasing bio-inoculants.

## 5. Conclusion

Six fungal cultures of distinct colony morphology were isolated from the calcareous soil as the calcareous soil-inhabiting fungal species, on a specialized enriched media. They did not form the solubilization zone on the Fe and Zn enriched media and appeared as Fe and Zn salt-releasing microbes. The release for Fe and Zn from their fixed form was 1.68 and 1.91ug/g of calcareous soil respectively by these fungal- bio-inoculants. 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 fungal cultures were identified as *species* of *Glomous*, *Mucor,* and *Aspergillus.* Therefore, these fungal species

inhabiting calcareous soils can be applied as bio-inoculants to the calcareous soil regularly to make them crop productive and for their reclamation.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

## References


[1] Asimi , S. , Gianinazzi-Pearson , V. and Gianinazzi , S. 1980 . Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhizae and *Rhizobium* in soybeans. *Canadian Journal of Botany*, 58: 2200 – 2205.

[2] Basu, K., Vimala kumara T.G., Kharkwal A.C., 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.

[3] Basu, K., Vimala kumara T.G., Kharkwal A.C., Abdin, M.Z., 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.

[4] 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.

[5] Carvalho L.M, Corrêa M.M, Pereira E.J, Nutti M.R, Carvalho J.L, Ribeiro E.M, and Freitas S.C. 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.

[6] Costa G.E.A, Queiroz-Monici K.S, Reis S.M.P.M, Oliveira A.C.2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chem.*94:327–330.

[7] Dugan, F.M. 2006. The Identification of Fungi- An Illustrated Introduction with Keys, Glossary, and Guide to Literature. American Phytopathological Society publication. ISBN: 9780890543368.

[8] 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.

[9] Gadd. GM. 2017. Fungi, Rocks and Minerals. Elements.13(3): 171-176.

[10] Gadd. GM.2021. Fungal biomineralization. Current Biology.31(24): R 1557- 1563.

[11] George, E., Marschner, H. and Jakobsen, I. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, 1: 257 – 270.

[12] Gianinazzi-Pearson, S., Gianzinazzi-Pearson, V. and Trouvelot, A. 1982. *Mycorrhizae, an integral part of plants: biology and perspectives for their use*, INRA-Presse, Paris, France.

[13] 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

[14] Koide, R.T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist*, 117: 365 – 386.

[15] Kothari, S.K., Marschner, H. and Romheld .V. 1991. Contribution of the VA mycorrhizal hyphae in the acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant and Soil*, 131: 177 – 185.

[16] Lambert, D.H., Baker, D.E. and Cole, H. 1979. The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. *Soil Science Society of America Journal*, 43: 976 – 980.

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

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

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

[20] 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.

[21] Marschner, H. and Dell, B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil*, 159: 89 – 102.

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

[23] Meshram, N.A, Singare, P.B and Kausadikar, H.K. 2023. Calcareous soils and their management. Agriculture. 3(4):1-6.

[24] Ortas, I., Kaya, Z., & Çakmak, I. 2001. Influence of VA-mycorrhiza inoculation on growth of maize and green pepper plants in phosphorus and zinc-deficient soils. In *Plant Nutrition- Food Security and Sustainability of Agro-ecosystems*. pp. 632 – 633.

[25] Ortas, I. 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Harran Plain in south Anatolia. *Journal of Plant Nutrition*, 26: 1 – 17.

[26] Ortas, I. and Akpinar. C. 2006. Response of kidney-bean to Arbuscular Mycorrhizal inoculation and mycorrhizal dependency in P and Zn deficient soils. Acta Agriculturae scandinaviea. Section B. Soil and Plant Science. 56(2): 101-109.

[27] 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.

[28] Samal,S.K, and Gautam, R.K. 2020. Nutrient management in calcareous soil. Food and Scientific Reports. 1 (6): 1-3.

[29] Smith, S.E. and Read, D.J. 1997. *Mycorrhizal symbiosis*, 2nd ed, Academic, San Diego.

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

[31] Wahid. F., Fahad. S., Danish. S., Adnan. M., Yue. Z., Saud. S., Siddiqui. M.H., 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

[32] Welch RM, Graham RD.2002. Breeding crops for enhanced micronutrient content. *Plant Soil.* 245:205–214.

[33] 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.