

ISOLATION AND CHARACTERIZATION OF ZINC SOLUBILIZING MICROORGANISMS AND THEIR EFFECTS ON AEROBIC RICE



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**ISOLATION AND CHARACTERIZATION OF
ZINC SOLUBILIZING MICROORGANISMS
AND THEIR EFFECTS ON AEROBIC RICE**

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University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
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MASTER OF SCIENCE (Agriculture)
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By
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UNIVERSITY OF AGRICULTURAL SCIENCES
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**DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
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BANGALORE**

CERTIFICATE

This is to certify that the thesis entitled “ISOLATION AND CHARACTERIZATION OF ZINC SOLUBILISING MICROORGANISMS AND THEIR EFFECTS ON AEROBIC RICE” submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE in AGRICULTURAL MICROBIOLOGY** to the University of Agricultural Sciences, Bangalore is a record of *bona fide* research work carried out by **Ms. GAGANASHREE, A., ID No. PAMB 0170**, during the period of her study in this University under my guidance and supervision. The thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

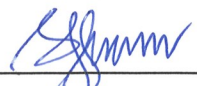
**Place: Bengaluru
April, 2023**


GIRISHA, H.C.
(Major Advisor)

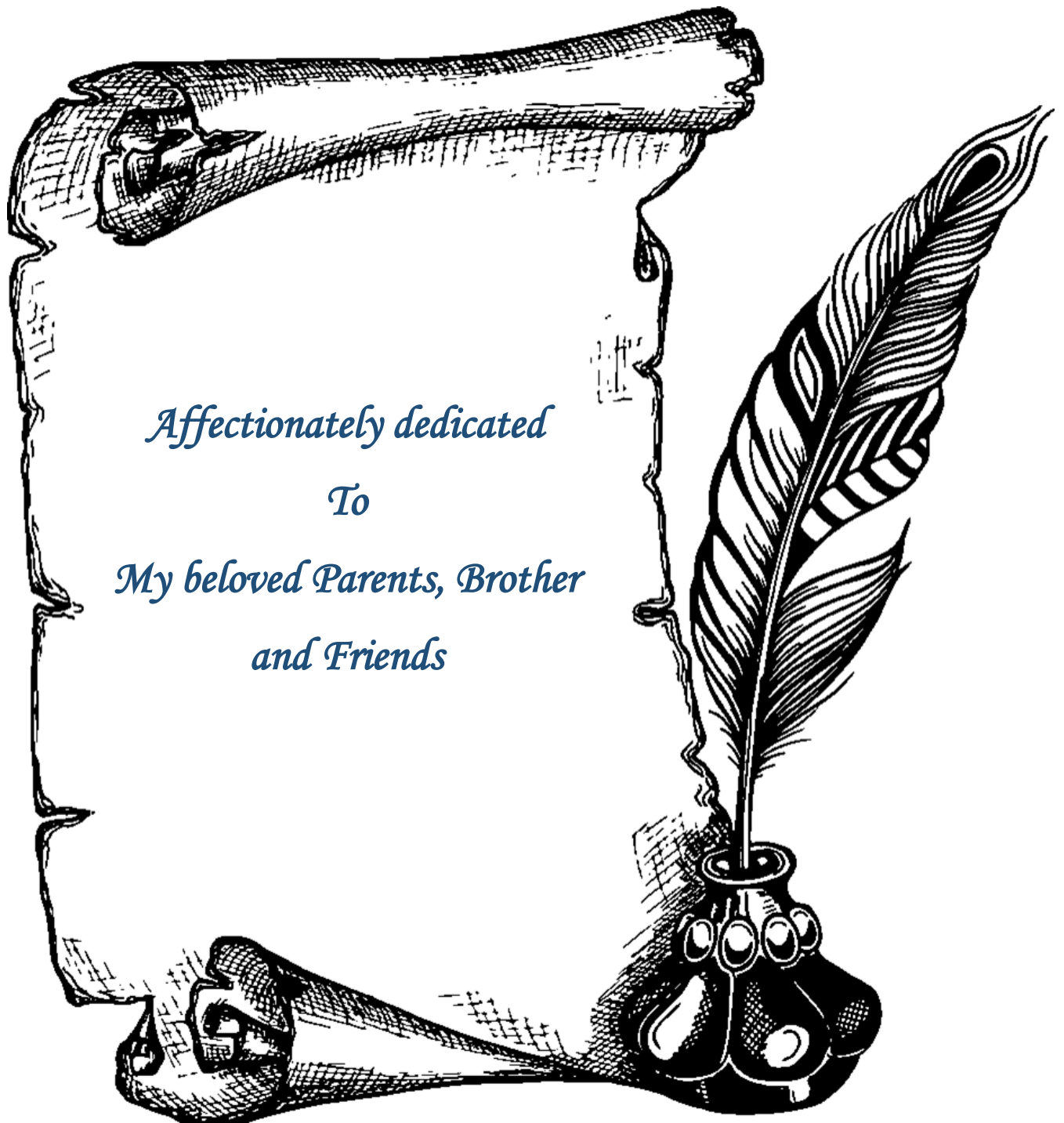
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3. 
(CHANNAKESHA, S.)



Affectionately dedicated
To
My beloved Parents, Brother
and Friends

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Isolation and Characterization of Zinc Solubilizing Microorganisms For Zinc Utilization in Rice

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Introduction

- Zinc is one of the essential elements required for the functioning of more than 300 enzymes and its deficiency disturbs multiple biological functions.
- Zinc deficiency limits crop production, nutritional quality of the produce and human health.
- The zinc deficiency syndrome is considered as an important nutritional problem next to iron anemia in the world.
- Microbial transformation of unavailable forms of soil zinc to plant available zinc is an important approach contributing to plant zinc nutrition and growth promotion.
- Zinc plays a pivotal role in physiological and biochemical functions of the plants. Both quantitative and qualitative yield of the plants are strongly dependent on this micronutrient. Supplementation of zinc in the form of synthetic fertilizer is proved to be inappropriate due to its unavailability to plants. This crisis can be prevented by the identification of rhizospheric micro-organisms which has the potential to transform various unavailable forms of the metal to available forms. (Saravanan *et al.*, 2004)

Objectives

- To isolate and characterize of Zinc solubilizing microorganisms from soils of Western Ghats of Karnataka.
- To screen the isolates for Zinc solubilization under *in-vitro* conditions.

Material and methods

➤ Isolation and characterization of Zinc solubilising microorganisms from soils of Western Ghats.

The zinc solubilizing bacteria and fungal strains isolated from soils of western ghats by using Standard plate count method on Bunt and Rovira medium enriched with different insoluble sources of zinc (ZnO, ZnCO₃ and Zn (PO₃)₄) at 0.1%.

Out of 25, ten fungal and out of 15, ten bacterial isolates were selected based on colony characteristics from the soils of western ghats and they were maintained on potato dextrose medium and nutrient agar medium respectively at 4°C throughout the study.

➤ Screening of isolates for Zinc solubilisation *in-vitro*.

The selected isolates were tested for solubilization efficiency by means of plate assay using modified mineral agar medium containing 0.1% of ZnO, ZnCO₃ and Zn (PO₃)₄ as insoluble source. The plates were incubated at 30°C for seven days. By measuring the diameter of the clear zone and colony growth, Zn solubilization efficiency was estimated (Sunithakumari *et al.*, 2016)

Solubilization = $\frac{\text{Solubilization diameter} - \text{colony diameter}}{\text{diameter of colony growth (mm)}}$ X 100 efficiency

The selected isolates were grown in modified mineral liquid medium with different sources of insoluble zinc viz. ZnO, ZnCO₃ and Zn (PO₃)₄. Broth incubated for 7 days and available zinc in the medium was estimated using atomic absorption spectrophotometer.

Results

Table 1: Effect of inoculation with zinc solubilizing bacterial isolates to broth having insoluble zinc sources on solubilizing efficiency and available zinc

Isolates	Solubilizing Efficiency(%)			Available Zinc (ppm)		
	ZnO	Zn ₃ (PO ₄) ₂	ZnCO ₃	ZnO	Zn ₃ (PO ₄) ₂	ZnCO ₃
ZSBI-1	44.44 ^{cd} (6.71)	85.70 ^d (9.56)	39.97 ^c (6.38)	8.00 ^d (3.00)	26.08 ^a (5.20)	9.36 ^c (3.22)
ZSBI-2	350 ^a (18.73)	90 ^d (9.51)	158.17 ^a (12.62)	29.67 ^a (5.54)	19.32 ^b (4.51)	25.43 ^a (5.14)
ZSBI-3	11.43 ^g (3.51)	41.80 ^{ef} (6.54)	0 ^f (1)	3.53 ^{cf} (2.13)	4.96 ^f (2.44)	0.96 ^{ef} (1.40)
ZSBI-4	27.77 ^{ef} (5.35)	55.50 ^e (7.43)	25 ^d (5.1)	7.73 ^d (2.95)	9.06 ^d (3.17)	7.13 ^d (2.85)
ZSBI-5	17.2 ^g (4.15)	33.30 ^f (5.86)	0 ^f (1)	4.27 ^e (2.29)	7.73 ^a (2.95)	0.79 ^{ef} (1.34)
ZSBI-6	44.43 ^c (6.71)	187.67 ^a (13.74)	44.43 ^f (6.71)	10.6 ^c (3.40)	26.70 ^a (5.26)	13.17 ^b (3.76)
ZSBI-7	44.18 ^{cd} (6.4)	119.87 ^c (10.99)	25.00 ^d (5.1)	2.36 ^f (1.83)	26.38 ^a (5.23)	1.1 ^{cf} (1.45)
ZSBI-8	17.2 ^g (4.15)	11.53 ^g (3.51)	7.41 ^c (2.89)	2.5 ^f (1.87)	2.53 ^g (1.88)	2.13 ^c (1.77)
ZSBI-9	153.69 ^b (12.42)	146.21 ^b (12.13)	65.47 ^b (8.15)	18.83 ^b (4.45)	26.16 ^a (5.21)	13.00 ^b (3.74)
ZSBI-10	31.67 ^{cd} (5.71)	75.77 ^d (8.76)	0 ^f (1)	11.53 ^c (3.54)	12.68 ^c (3.70)	0.23 ^f (1.11)

Table 2: Effect of inoculation with zinc solubilizing fungal isolates to broth having insoluble zinc sources on solubilizing efficiency and available zinc

Isolates	Solubilizing Efficiency(%)			Available zinc (ppm)		
	ZnO	Zn ₃ (PO ₄) ₂	ZnCO ₃	ZnO	Zn ₃ (PO ₄) ₂	ZnCO ₃
ZSFI-1	29.52 ^c (5.52)	6.083 ^a (2.64)	2.04 ^{ef} (1.74)	6.70 ^f (2.77)	4.08 ^f (2.25)	1.05 ^{ef} (1.43)
ZSFI-2	8.40 ^g (3.06)	6.813 ^a (2.79)	4.87 ^c (2.41)	2.02 ^b (1.74)	5.32 ^f (2.51)	1.55 ^e (1.59)
ZSFI-3	132.56 ^c (11.55)	31.67 ^d (5.71)	4.96 ^c (2.43)	15.63 ^d (4.08)	11.66 ^d (3.56)	1.33 ^{ef} (1.53)
ZSFI-4	303.70 ^a (17.46)	466.60 ^a (21.62)	193.33 ^a (13.94)	22.96 ^a (4.89)	27.23 ^a (5.31)	14.27 ^a (3.91)
ZSFI-5	227.38 ^b (15.10)	404.83 ^b (20.14)	113.33 ^b (10.68)	19.65 ^b (4.54)	23.24 ^b (4.92)	8.53 ^c (3.09)
ZSFI-6	72.24 ^d (8.56)	31.20 ^d (5.67)	16.88 ^d (4.22)	8.97 ^c (3.16)	11.55 ^d (3.53)	5.21 ^d (2.49)
ZSFI-7	13.143 ^f (3.75)	17.58 ^e (4.30)	3.17 ^e (2.04)	3.4 ^e (2.10)	7.9 ^e (2.98)	1.16 ^{ef} (1.47)
ZSFI-8	3.02 ^h (1.99)	14.19 ^{ef} (3.85)	0.00 ^f (1.00)	0.91 ^g (1.38)	0.9 ^g (1.38)	1.54 ^e (1.59)
ZSFI-9	4.29 ^h (2.30)	11.11 ^g (3.48)	0.00 ^f (1.00)	0.36 ^e (1.16)	4.42 ^f (2.33)	0.33 ^f (1.15)
ZSFI-10	141.67 ^c (11.93)	308.87 ^c (17.59)	67.49 ^c (8.28)	17.73 ^c (4.33)	20.4 ^c (4.63)	12.11 ^b (3.62)

These tables shows the solubilizing efficiency and solubilized zinc in the broth, inoculated by isolated bacterial and fungal strains

Discussion

Ten zinc solubilizing fungal isolates and bacterial isolates were isolated using bunt and rovara medium enriched with different insoluble sources of zinc [ZnO, ZnCO₃ and Zn₃(PO₄)₂] at 0.1%.

The isolate ZSBI-2 showed significantly high solubilizing efficiency among bacterial isolates in all three different insoluble sources of zinc ranging from 90% to 350%. The fungal isolate ZSFI-4 showed significantly high solubilizing efficiency in ZnO and Zn₃(PO₄)₂ i.e. 303.7% and 466.6% respectively.

All the isolates solubilized insoluble zinc under *in-vitro* condition. Among the bacterial isolates ZSBI-2 solubilized significantly high zinc i.e. 29.92 ppm(ZnO) and among the fungal isolates ZSFI-4 solubilized significantly high zinc i.e. 27.22 ppm[Zn₃(PO₄)₂]

ZnO Zn₃(PO₄)₂ ZnCO₃

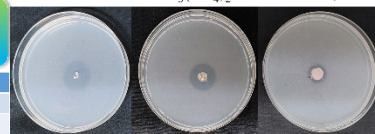


Plate 1: Zinc solubilizing bacterial isolate-2 with different insoluble zinc source on bunt and rovara medium

ZnO Zn₃(PO₄)₂ ZnCO₃

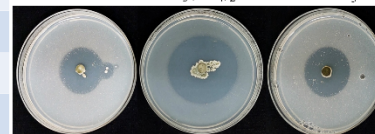


Plate 2: Zinc solubilizing Fungal isolate-4 with different insoluble zinc source on bunt and rovara medium

Summary

Among bacterial isolates, ZSBI-2 selected as efficient zinc solubilizing bacteria based on zone solubilization and solubilized zinc in broth. Among Fungal isolates ZSFI-4 was selected as efficient zinc solubilizing fungal strain based on zone solubilization and solubilized zinc in broth.

These two isolates were selected for further studies on Aerobic rice.

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Advisory committee

Chairperson: Dr. Girisha, H.C.
Members: Dr. S.T. Bhairappanavar
Dr. N. Umashankar
Dr. Channakeshava, S.

ISOLATION AND CHARACTERIZATION OF ZINC SOLUBILISING MICROORGANISMS AND THEIR EFFECTS ON AEROBIC RICE.

GAGANASHREE, A.

ABSTRACT

A study was conducted to isolate Zinc solubilising microorganisms and their effect on Zn uptake in aerobic rice. Forty fungal and twenty bacterial Zn solubilising isolates were isolated from the soils of Western Ghats of Karnataka. Selected isolates were screened for their ability to solubilize zinc in Bunt and Rovira medium supplemented with 0.1 per cent insoluble zinc sources (ZnO , $\text{Zn}_3(\text{PO}_4)_2$, ZnCO_3) in both agar and broth culture medium. Among bacterial isolates, ZSBI-2 showed highest zinc solubilizing efficiency in both agar media (350%, 90% and 158.17% in ZnO , $\text{Zn}_3(\text{PO}_4)_2$ and ZnCO_3 respectively) and broth culture (29.67, 19.32 and 25.43 ppm in ZnO , $\text{Zn}_3(\text{PO}_4)_2$ and ZnCO_3 respectively) after 7 days of inoculation. Among fungal isolates, ZSFI-4 showed highest zinc solubilizing efficiency both in agar (303.70%, 466.6% and 193.33% in ZnO , $\text{Zn}_3(\text{PO}_4)_2$ and ZnCO_3 respectively) and broth culture (22.96 27.23 and 14.27ppm in ZnO , $\text{Zn}_3(\text{PO}_4)_2$ and ZnCO_3 respectively) after 7 days of inoculation. These isolates were identified as *Bacillus subtilis* and *Aspergillus niger*, respectively by molecular characterization. The inoculation of these two identified isolates for Zn uptake in aerobic rice under green house study resulted in higher number of leaves (11.88/plant), number of tillers (7.25/plant), plant height (72.86cm/plant) and biomass (0.75g/plant) in T4 (Inoculated with *Aspergillus niger*) with added soil phosphorus .Among the treatments without phosphorous, “T10” (Inoculated with *Bacillus subtilis* and *Aspergillus niger*) resulted in higher number of leaves (11.50/plant), number of tillers (6.38/plant), plant height (70.22cm/plant) and biomass (0.74g/plant). Inoculation with *A. Niger* strain alone has significantly increased the available Zn content in soil (4.06 and 4.30 mg per kg with and without P respectively) and inoculation with both *B. subtilis* and *A. Niger* strains has improved the Zinc uptake by aerobic rice under pot experiment (45.05 and 48.63 mg per kg with and without P respectively).

April, 2023

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Dr. GIRISHA, H.C.
(Major Advisor)

ಸತು ಕರಗಿಸುವ ಸೂಕ್ಷ್ಮಜೀವಿಗಳ ಪ್ರತ್ಯೇಕತೆ ಮತ್ತು ಗುಣಲಕ್ಷಣಗಳು ಹಾಗು ಏರೋಬಿಕ್
ಭತ್ತದ ಮೇಲೆ ಅವುಗಳ ಪರಿಣಾಮಗಳು

ಗಗನಶ್ರೀ.ಎ.

ಸಾರಾಂಶ

ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಸತು ಕರಗಿಸುವ ಸೂಕ್ಷ್ಮಜೀವಿಗಳನ್ನು ಪ್ರತ್ಯೇಕಿಸಲು ಕರ್ನಾಟಕದ
ಪಶ್ಚಿಮ ಘಟ್ಟದ ಮಣ್ಣಿನಿಂದ ಪ್ರತ್ಯೇಕಿಸಲಾದ 40 ಶಿಲೀಂಧ್ರಗಳು ಮತ್ತು 20 ದುಂಡಾಣುಗಳಲ್ಲಿ
ರೂಪವಿಜ್ಞಾನದ ಗುಣಲಕ್ಷಣಗಳನ್ನು ಆಧರಿಸಿ 10 ಶಿಲೀಂಧ್ರ ಮತ್ತು ದುಂಡಾಣುಗಳ ತಳಿಗಳನ್ನು
ಆಯ್ಕೆ ಮಾಡಲಾಯಿತು. ಆಯ್ಕೆ ಮಾಡಿದ ತಳಿಗಳಲ್ಲಿ ಯಾವುದು ಹೆಚ್ಚು ಸತು ಕರಗಿಸುವಂತಹ
ಶಕ್ತಿಯನ್ನು ಹೊಂದಿದೆಯೆಂದು, ಶೇ. 1 ರಷ್ಟು ಕರಗದ ವಿವಿಧ ಸತುವಿನ ಮೂಲಗಳನ್ನು ಸೇರಿಸಿ
[ZnO, ZnCO₃, Zn₃(PO₄)₂] ಘನರೂಪದ ಮತ್ತು ದ್ರವರೂಪದ ಮಾಧ್ಯಮಗಳಲ್ಲಿ ಪರೀಕ್ಷಿಸಲಾಯಿತು.
ಪರೀಕ್ಷಿಸಿದ 10 ದುಂಡಾಣುಗಳಲ್ಲಿ ZSBI-2 ತಳಿಯು ಹೆಚ್ಚು ಸತು ಕರಗಿಸುವ ಸಾಮರ್ಥ್ಯವನ್ನು
ಹಾಗು ಪರೀಕ್ಷಿಸಿದ 10 ಶಿಲೀಂಧ್ರಗಳಲ್ಲಿ ZSFI-4 ಹೆಚ್ಚು ಸತು ಕರಗಿಸುವ ಸಾಮರ್ಥ್ಯವನ್ನು
ಹೊಂದಿದೆ ಎಂದು ತಿಳಿದುಬಂದಿತು. ಪ್ರತ್ಯೇಕಿಸಲಾದ ಸೂಕ್ಷ್ಮಜೀವಿಗಳಲ್ಲಿ ZSBI-2 ಮತ್ತು ZSBI-4
ಹೆಚ್ಚು ಸತು ಕರಗಿಸುವ ಸಾಮರ್ಥ್ಯವನ್ನು ಹೊಂದಿದೆ ಹಾಗು ಆಣ್ವಿಕ ಪರೀಕ್ಷೆಯ ಮೂಲಕ
ಇವುಗಳನ್ನು ಬ್ಯಾಸಿಲಸ್ ಸಬ್ಬಿಲಿಸ್ ಮತ್ತು ಆಸ್ಪರ್ಜಿಲಸ್ ನೈಜರ್ ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ. ಈ
ಎರಡು ಉತ್ತಮವಾದ ಸತು ಕರಗಿಸುವ ಸೂಕ್ಷ್ಮಜೀವಿಗಳನ್ನು ಏರೋಬಿಕ್ ಭತ್ತದ ಮೇಲೆ ಹಸಿರು
ಮನೆಯ ಪ್ರಯೋಗದಡಿಯಲ್ಲಿ ಉಂಟು ಮಾಡಿದ ಪರಿಣಾಮದಿಂದ ಕಂಡುಬಂದಿದ್ದೇನೆಂದರೆ,
ರಂಜಕದೊಂದಿಗಿನ ಉಪಚಾರಗಳಲ್ಲಿ T₅ (ಬ್ಯಾಸಿಲಸ್ ಸಬ್ಬಿಲಿಸ್ ಮತ್ತು ಆಸ್ಪರ್ಜಿಲಸ್ ನೈಜರ್)
ಗಿಡಗಳಲ್ಲಿ, ಗಿಡದ ಬೆಳವಣಿಗೆಯ ಮೇಲೆ ಅಂದರೆ ಎಲೆಗಳ ಸಂಖ್ಯೆ (11.88/ಗಿಡ), ತೆಂಡೆಗಳ ಸಂಖ್ಯೆ
(7.25/ಗಿಡ), ಎತ್ತರ (72.86 cm/ಗಿಡ) ಮತ್ತು ಒಟ್ಟುಗಿಡದ ತೂಕದಲ್ಲಿ (0.75/ಗಿಡ) ಉತ್ತಮ
ಫಲಿತಾಂಶವನ್ನು ತೋರಿಸಿದೆ. ರಂಜಕವಿಲ್ಲದ ಉಪಚಾರಗಳಲ್ಲಿ T₁₀ (ಬ್ಯಾಸಿಲಸ್
ಸಬ್ಬಿಲಿಸ್+ಆಸ್ಪರ್ಜಿಲಸ್ ನೈಜರ್) ಗಿಡಗಳಲ್ಲಿ ಎಲೆಗಳ ಸಂಖ್ಯೆ (11.50/ಗಿಡ), ತೆಂಡೆಗಳ
ಸಂಖ್ಯೆ(6.38 /ಗಿಡ), ಎತ್ತರ (70.22 cm /ಗಿಡ), ಒಟ್ಟುಗಿಡದ ತೂಕದಲ್ಲಿ (0.74/ಗಿಡ) ಉತ್ತಮ
ಫಲಿತಾಂಶವು ಕಂಡಿದೆ. ರಂಜಕ ಸಹಿತ ಮತ್ತು ರಹಿತ ಉಪಚಾರಗಳಲ್ಲಿ T₄ (ಆಸ್ಪರ್ಜಿಲಸ್ ನೈಜರ್)
ಹಾಗು T₁₀ (ಬ್ಯಾಸಿಲಸ್ ಸಬ್ಬಿಲಿಸ್+ಆಸ್ಪರ್ಜಿಲಸ್ ನೈಜರ್) ಗಣನೀಯವಾಗಿ ಮಣ್ಣಿನ ಸತುವು 4.06
ಮತ್ತು 4.30 ಮಿ.ಗ್ರಾಂ / ಕೆ. ಜಿ. ಹಾಗು ಸಸ್ಯದ ಸತುವು 45.05 ಮತ್ತು 48.63 ಮಿ.ಗ್ರಾಂ / ಕೆ.
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ಏಪ್ರಿಲ್, 2023

ಕೃಷಿ ಸೂಕ್ಷ್ಮಜೀವಿಶಾಸ್ತ್ರ ವಿಭಾಗ

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CONTENTS

Sl. No.	CHAPTER PARTICULARS	PAGE No.
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	4
III	MATERIAL AND METHODS	28
IV	RESULTS AND DISCUSSION	37
V	SUMMARY	57
VI	REFERENCES	61
	APPENDICES	i

LIST OF TABLES

TABLE No.	TITLE	PAGE No.
1	Treatments details	34
2	Initial physico-chemical properties of the soil of pot culture experiment	35
3	Details of Locations for soil sample collection.	38
4	Morphological characteristics of the zinc solubilizing bacterial isolates from soils of western ghats.	39
5	Morphological characteristics of the zinc solubilizing fungi isolates from soils of western ghats	40
6	Solubilizing efficiency and solubilizing index of Zn-solubilizing bacterial isolates for different sources of Zinc	42
7	Solubilizing efficiency and solubilizing index of Zn-solubilizing fungal isolates for different sources of Zinc	43
8	Solubilization of different sources of insoluble Zinc by bacterial isolates in broth culture	44
9	Solubilization of different sources of insoluble Zinc by fungal isolates in broth culture	45
10	Molecular characterization of efficient zinc solubilizing microorganisms	46
11	Effect of zinc solubilizing microorganisms on number of leaves, number of tillers and plant height per plant of aerobic rice under greenhouse condition at 90 DAS.	48
12	Effect of zinc solubilizing microorganisms on biomass of aerobic rice under greenhouse at 90 days after sowing.	50
13	Effect of zinc solubilizing isolates on available Zn (mg kg ⁻¹) in soil 90 DAS of aerobic rice under greenhouse.	53
14	Effect of zinc solubilizing microorganisms on Zn uptake in aerobic rice 90 DAS	54

LIST OF FIGURES

FIG. NO.	TITLE	PAGE NO.
1	Phylogenetic tree zinc solubilizing bacterial strain	47
2	Phylogenetic tree zinc solubilizing fungal strain	47

LIST OF PLATES

PLATE No.	TITLE	BETWEEN PAGES
1	Cell morphology of zinc solubilizing bacterial isolates	40-41
2	Cell morphology of zinc solubilizing fungal isolates	40-41
3	Morphology of efficient zinc solubilizing bacterial and fungal isolate	40-41
4	Zinc solubilization by different fungal isolates	44-45
5	Zinc solubilization by bacterial isolates	44-45
6	Zinc solubilizing bacterial isolate-2 (<i>Bacillus subtilis</i> strain UASB_GHC2) with different insoluble zinc source on bunt and rovira medium	46-47
7	Zinc solubilizing fungal isolate-4 (<i>Aspergillus niger</i> strain UASB_GHC4) with different insoluble zinc source on bunt and rovira medium	46-47
8	Effect of zinc solubilizing microorganisms on growth parameters of aerobic rice under greenhouse condition 90 DAS	48-49
9	Effect of phosphorous on zinc solubilization by zinc solubilizing bacterial and fungal isolates	54-55

LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOLS	ABBREVIATIONS	SYMBOLS	ABBREVIATIONS
%	Per cent	SI	Solubilization index
g	Gram	FAO	Food and Agriculture Organization
mg	Milligram	RDF	Recommended dose of fertilizer
µg	Microgram	NPK	Nitrogen, Phosphorus, Potassium
mL	Millilitre	PGPR	Plant growth promoting rhizobacteria
L	Litre	IAA	Indole-3-acetic acid
cm	Centimeter	HCN	Hydrogen cyanide
m	Meter	H ₂ O ₂	Hydrogen peroxide
°C	Degree celsius	GA	Gibberellic acid
MW	Molecular weight	ABA	Abscisic acid
M	Molar	SA	Salicylic acid
mM	Milli molar	H ₂ S	Hydrogen sulfide
ng g ⁻¹	Nanogram per gram	ISR	Induced systemic resistance
µg mL ⁻¹	Microgram per millilitre	SAR	Systemic acquired resistance
Kg h ⁻¹	Kilogram per hectare	hr.	hour
sp.	Species	min.	minute
spp.	Several species	sec.	second
<i>et al.</i>	Co-workers	DAT	Days after transplanting
<i>i.e.</i>	That is	MC	Microbial consortia
cfu	Colony forming unit	w/ v	Weight per volume
<i>viz.</i>	Namely	CD	Critical difference
Fig.	Figure	SE(m)	Standard error of mean

INTRODUCTION

I INTRODUCTION

Zinc (Zn) is one of the essential micro-nutrients, its primary role in plants is carbohydrate, protein, phosphate metabolism, auxin synthesis, nitrogen assimilation and ribosome production. Zinc deficiency is ranked as the fifth most significant risk factor for illness and mortality in the developing world, according to a report released by the WHO (2002). Regarding micronutrients, Zn deficiency is the top concern in agriculture and is considered a severe threat to global and national food security. Around 40 per cent of soil samples examined in India for available Zn were deficient (Das *et al.*, 2019). According to a survey on the status of accessible micronutrients in India, Zn was found to be insufficient in all 20 states evaluated and in all agroecological zones of the country (Shukla *et al.*, 2021).

Zinc deficiency in humans causes hair and memory loss, skin disorders, muscular weakness, and impaired brain development in the foetus. Zinc deficiency causes plant anomalies such as reduced growth, chlorosis, relatively small leaves, and spikelet sterility. The chemical makeup of the soil's essential ingredients determines how much zinc is present overall in the soil. The value of zinc in the earth's crust is roughly 80 mg per kg, and the value of zinc in soil ranges from 10 to 300 mg per kg on average. In soil, zinc is found in the minerals sphalerite, olivine, hornblende, augite and biotite. The availability of zinc from these sources indeed is influenced by various factors including the metabolic activity of rhizosphere microorganisms which play a significant role in transforming such unavailable forms into available (Bhupinder *et al.*, 2005).

Zinc has been discovered in soils in the following forms: (i) water soluble plus exchangeable (ii) organically bound (iii) amorphous iron oxide bound (iv) crystalline iron oxide bound (v) manganese oxide bound (vi) carbonate and sulphide bound and (vii) residual forms (Shuman, 1985). A trace amount of Zn is present in a water-soluble and exchangeable form. The remaining pools are scarce soluble and so unavailable to the plant. Zn deficit can also be caused by one or more of the following factors: (i) Plant types that are naturally vulnerable to Zn deficiency (*i.e.*, Zn-inefficient cultivars). (ii) High pH and

calcareous soil (iii) anaerobic conditions (iv) Increase the availability of Fe, Ca, Mg, Cu, Mn, and P following flooding (Dobermann and Fairhurst, 2000).

Rice is vulnerable to Zn deficiency, which is the most common micronutrient problem in rice and is typically called "Khaira" disorder. Reduced zinc availability restrict rice plants from transforming amino acids into proteins. Several cases of zinc deficiency in upland rice were recorded by Hogland in 1948. In regions where hybrids or HYV paddy is being grown with recommended or higher NPK doses, zinc deficit is likely to arise. Grain and straw yield in rice can be increased by applying zinc to the soil (Naik and Das, 2010). Various factors, namely soil acidity, available zinc concentration, organic matter, and soil type, affect zinc mobility and uptake in soil. The amount of zinc available for plants decreases due to the high levels of phosphorus in the soil. Some metal cations, like Cu^{2+} and Fe^{2+} , prevent plants from absorbing zinc (Chang *et al.*, 2007). Applying chemical fertilisers and manures to improve soil fertility and crop yields has frequently affected the intricate biogeochemical cycles (Steinshamn *et al.*, 2004). External chemical fertilisers application converts 96-99 percent of available Zn to unavailable Zn reserves by precipitating into carbonates, oxides, phosphates, and some other compounds (Zhang *et al.*, 2017).

Zn deficiency is becoming more common in India for various reasons, including increased use of phosphatic fertilisers, which causes P-induced Zn deficit, low organic matter content and the use of irrigation water of inferior quality with a higher calcium carbonate concentration. Meanwhile, the greater cost of applying Zn fertilisers to rectify Zn deficiency poses a significant burden on poor farmers.

Therefore, the usage of Plant Growth Promoting Rhizobacteria (PGPR) is one of the methods for increasing crop productivity and food quality without inciting environmental problems. These PGPR have the ability to colonise internal plant tissues, the root surface and the rhizosphere. Since zinc is a scarce resource in agriculture, understanding how these bacteria can solubilise zinc and provide it to plants is essential (Saravanan *et al.*, 2003). To enhance zinc availability for crop absorption and meet the

requirement of low-input and sustainable agriculture, it is necessary to utilise beneficial rhizosphere microorganisms as bio-inoculants (He *et al.*, 2010).

A practical option is to use specific soil microbes to convert these fixed forms of zinc into labile zinc forms for enhanced availability and subsequent absorption by plants (Prajapati *et al.*, 2021). Many bacterial genera have been identified as potential Zn solubilizers, which includes *Pseudomonas* sp., *Gluconacetobacter* sp., *Thiobacillus* sp., *Bacillus* sp., *Acinetobacter* sp., etc. *Thiobacillus thiooxidans* and facultative thermophilic iron oxidisers solubilise zinc from sphalerite, as recorded by Hutchins *et al.*, 1986. Zinc solubilising fungi like *Emmericella rugulosa*, *Penicillium citrinum*, *Aspergillus candidus*, *A. terreus*, *A. niger* were isolated from soils of TamilNadu by Anitha *et al.*, 2015.

The current study was conducted to characterise, screen and use zinc-solubilising bacteria to improve the zinc nutrition on aerobic rice. The experiments were carried out at GKVK, Bangalore, under glasshouse conditions during 2021-22 with the following objectives:

1. Isolation and characterisation of Zinc solubilising microorganisms from soils of Western Ghats.
2. Screening of isolates for Zinc solubilisation *in vitro*.
3. Effect of Zinc solubilising microorganisms on aerobic rice under greenhouse conditions.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Zinc (Zn) is one of the essential nutrients for the growth in animals, human beings and plants, which plays a vital in crop nutrition as it is required in various enzymatic reactions, metabolic processes and redox reactions. In addition, Zn is also essential for many enzymes which are needed for nitrogen metabolism, energy transfer and protein synthesis. Zinc deficiency not only retards growth and yield of plants, but it also has affects on human beings. More than 3 billion people worldwide are suffering from Fe and Zn deficiencies and this condition is particularly widespread in areas where population is heavily dependent on an unvaried diet of cereal based foods, in which Fe and Zn are stored almost exclusively in the husk and are reported to be lost during milling and polishing (Cakmak, 2002 and Graham *et al.*, 2001). When the supply of zinc to the plant is inadequate, many important physiological functions are unable to operate normally and plant growth is adversely affected. Most of the soils of the world are too low in available zinc for production of good yields. Certain groups of microorganisms including bacteria, fungi and actinobacteria are known to solubilize zinc minerals into soluble form which can be utilized by the plants. Since the literature pertaining to solubilization of zinc minerals by bacteria is very scanty. The available and relevant literature has been summarised and presented in this chapter.

2.1 Importance of Zn in Humans

Zinc deficiency is common in humans, animals and plants. More than 30 per cent world's population suffers from Zn deficiency (Welch, 2002). According to recent data, zinc deficiency affects 17 per cent of the world's population with the highest estimate in Africa (24%) and Asia (19%) based on dietary supply estimates (Belay *et al.*, 2021). Zinc plays a part in the basic roles of cellular functions in all living organisms and is also involved in improving the human immune system. The optimum dietary intake for human adults is 15 mg of Zn per day. Zinc acts as a catalytic or structural component in various body enzymes.

Unsatisfactory intake and improper absorption of Zn in the body may cause deficiency of Zn. Due to Zn deficiency, the human body will suffer from hair and memory

loss, skin problems and weakness in body muscles. Further insufficient intake of Zn during pregnancy also causes stunted brain development of the foetus. Infertility has also been observed in Zn deficient men. Zinc deficiency may cause congenital diseases like Acrodermatitis enteropathica (Lukaski, 2004 and Morley, 2004). As per recommendations an average male need 11 mg of Zn daily while an average female needs 9 mg of Zn. During pregnancy and lactation, the female needs 13 mg to 14 mg of Zn daily. Infants from 7 months to 3 years need 3 mg, 4 to 8 years need 5 mg and children from 9 to 13 years need 8 mg of Zn daily (Hotz and Brown, 2004).

2.2 Role of Zinc in Plants

Zinc plays vital role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome (Tisdale *et al.*, 1984). Plant enzymes activated by Zn are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation (Marschner, 1995). The regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants are Zn dependent (Cakmak, 2000). Its deficiency results in the development of abnormalities in plants which become visible symptoms such as stunted growth, chlorosis and smaller leaves, spikelet sterility. Zn deficiency can also adversely affect the quality of harvested products; plants susceptibility to injury by high light or temperature intensity and to infection by fungal diseases can also increase (Marschner, 1995 and Cakmak, 2000).

Zinc seems to affect the capacity of water uptake and transport in plants and also reduce the adverse effects of short periods of heat and salt stress (Kasim, 2007, Disante *et al.*, 2010, Peck and McDonald, 2010 and Tavallali *et al.*, 2010). As Zn is required for the synthesis of tryptophan which is a precursor of IAA, it also have an active role in the production of an essential growth hormone auxin (Alloway, 2004 and Brennan, 2005). The Zn is required for integrity of cellular membranes to preserve the structural orientation of macromolecules and ion transport systems. Its interaction with phospholipids and sulfhydryl groups of membrane proteins contributes for the maintenance of membranes

(Cakmak, 2000, Kabata-Pendias, 2001, Alloway, 2004, Dang *et al.*, 2010, Disante *et al.*, 2010).

The Zn-finger transcription factors are involved in the development and function of floral tissues such as anthers, tapetum, pollen and secretory tissues of pistil in many plant species, it is likely one among the transcription factor VvZIP3 may play a key role in both flower and normal fruit development (Kobayashi *et al.*, 1998 and Sharma *et al.*, 1987). The alteration of VvZIP3 expression during flowering and fertilization can modify the distribution and availability of Zn, hence affects normal reproductive development (Kapoor *et al.*, 2002).

2.3 Zinc in soil

Zinc is one among the eight trace elements which are essential for the normal healthy growth and reproduction of crop plants, the other elements are boron, chlorine, copper, iron, manganese, molybdenum and nickel. These elements are referred to as 'essential trace elements' or micronutrients, because they are only required in relatively small concentrations in the plant tissues (5-100 mg kg⁻¹). Some of these elements, namely copper, iron, manganese and zinc, with in addition cobalt, chromium, iodine and selenium, are also essential for animals. Apart from the eight trace elements, nine major elements also essential for plants and these are carbon, hydrogen, oxygen, nitrogen, potassium, calcium, magnesium, phosphorus and sulphur.

All soils contain measurable concentrations of the essential trace elements, as well as other trace elements which are not essential for plants and animals (non-essential elements), but the concentrations can vary considerably, in some cases they can be very low. Trace elements in soils are derived from the geochemical weathering of the rock fragments on which the soil has formed (soil as parent material), together with inputs from atmospheric deposition (as dust and aerosol sized particles in dry deposition or rainfall) and inputs from agricultural activities such as livestock manures, fertilisers and agrichemical sprays. Soils on river flood plains will also have received trace elements from floodwaters and sediments. All of these sources and inputs can vary greatly in magnitude and result in soils having a wide range of total trace element concentrations. The extent to

which the total concentration of a trace element such as zinc in a soil is available for uptake by plants or movement down the soil profile depends on a range of soil properties. The range of total zinc concentrations in soils reported in the literature tends to show an overall mean total concentration of around 55 mg Zn per kg. Alloway (1995) reported a typical range of zinc in soils of 10-300 mg per g with a mean of 50 mg Zn per kg.

2.4 Zinc Deficiency in Soil

Zinc deficiency can be found in every part of the world and almost all crops respond positively to application of Zn (Welch, 2002). Normal soils inherit trace elements which include Zn primarily from the rocks through geochemical and pedochemical weathering processes. Besides mineralogical composition of the parent material, the total amount of Zn present in the soil is also dependent on the type, intensity of weathering, climate and numerous other predominating factors during the process of soil formation (Saeed and Fox, 1977). Meanwhile, high pH and high contents of CaCO_3 , organic matter, clay and phosphate can fix Zn in the soil and give rise to the reduction of available Zn (Imtiaz, 1999). Soils derived from granite and gneiss can be low in total Zn (Krauskopf, 1972). Similarly, total Zn is low in highly leached, acid, sandy soils such as the soil which are found in many coastal areas. Quartz in the soil dilutes Zn from it as the reported concentrations of Zn in quartz are very low, which ranged from 1.0 μg per g to < 5 to 8 μg per g (Helmke *et al.*, 1977 and Brehler and Wedepohl, 1978). According to the Food and Agriculture Organization (FAO), about 30 percento of the cultivable soils of the world contain low levels of plant available Zn (Sillanpaa, 1990). The lowest Zn concentrations were always found in Spodosols (28 $\mu\text{g g}^{-1}$) and luvisols (35 $\mu\text{g g}^{-1}$), while higher levels were found in fluvisols (60 $\mu\text{g g}^{-1}$) and Histosols (58 $\mu\text{g g}^{-1}$) (Kiekens, 1995).

Generally, Zn deficiency is expected in calcareous soils, sandy soils, peat soils, and soils with high phosphorus and silicon (Alloway, 2008 and Alloway, 2004). The submerged soils are well recognized for the lack of Zn availability to the plants, particularly due to the reaction of Zn with free sulphide (Mikkelsen and Shiou, 1977). Flooding and submergence bring decline in available Zn because of the changes in pH and the formation of insoluble Zn compounds. Meanwhile, the insoluble Zn compounds formed are likely to

be with Mn and Fe hydroxides from the breakdown of oxides and adsorption on carbonates, specifically magnesium carbonate. Under the submerged conditions for rice cultivation, Zn is transformed into amorphous sesquioxide precipitates or franklinite; ZnFe_2O_4 (Sajwan and Lindsay, 1988). Zinc deficiency causes multiple symptoms which usually appear 2 to 3 weeks after transplanting of rice seedlings, with leaves developing brown blotches and streaks that may fuse to entirely cover older leaves, and plants remain stunted, whereas in severe cases, the plants may die, while those which recover will show substantial delay in maturity and reduction in yield (Yoshida and Tanaka 1969, Van and Castro 1980, Neue and Lantin 1994).

2.5 Zinc and phosphorous interaction

The interaction of Phosphorous (P) and Zinc has been well researched and documented in the fields of soil chemistry and plant nutrition. Research shows that high rates of P fertilizer without adequate plant available Zn can reduce Zn uptake by the roots, induce Zn deficiency, and decrease plant growth and yield. One of the primary causes of Zn deficiency in soils treated with high amounts of P fertilizer is its reaction with soluble Zn in the soil. This reaction produces insoluble Zn-phosphate complexes which influences the availability of Zn for plant uptake over time (Mortvedt, 1991). A potential outcome is a reduction of Zn availability leading to Zn deficiency and reduced plant growth.

A field study was conducted to determine the effect of applied Zn on wheat (*Triticum aestivum* L.) grown on residual Phosphorous plots and identify the mechanism of P×Zn interaction. This study was conducted by Singh *et al.*, 1986, in a continuous wheat system on a clayey, mixed, frigid 'Typic Hyploboroll' soil that had received 0, 80, and 160 kg P per ha in 1979. Three experiments were carried out with three levels of P as main plots and rates of soil applied Zn-sulphate (0, 5, 10, and 20 kg Zn ha⁻¹) or Zn-chelate (0, 0.5, 1.0, and 2.0 kg Zn ha⁻¹) or foliar applied Zn-chelate (0 and 0.35 kg Zn ha⁻¹) as subplots. A one-time application of 80 or 160 kg P per ha increased wheat yield significantly. In the presence of P, application of Zn resulted in significant increase in grain yield and Zn uptake into grain. Applied P increased soil P levels and tissue P concentration, but resulted in a significant decrease of tissue Zn levels.

The effect of withholding Zinc on the uptake, translocation and accumulation of P was studied in cotton plants (*Gossypium hirsutum* L. cv. Deltapine 15/21) grown in nutrient solutions under controlled environmental conditions by Cakmak and Marschener, 1986. The influence of P on the uptake rate, translocation and distribution of Zn in the plants was also examined. Increasing the P supply resulted in severe Zn deficiency symptoms (interveinal chlorosis) as well as P toxicity symptoms, which were characterized by leaf puckering and greyish-brown marginal necrosis. From the results obtained it is concluded that the P-induced Zn deficiency in cotton, as well as in other species, is primarily caused by enhanced P uptake and translocation and not by inhibition of Zn uptake.

Study conducted for the effects of Phosphorous supply on the root uptake and tissue concentrations of Zn was investigated in wheat (*Triticum aestivum*) grown in different media by Ova *et al.*, 2015. Plants were grown under greenhouse and growth chamber conditions in native soil, autoclaved soil and nutrient solution with different P and Zn supplies. Higher P rates caused substantial decreases in shoot and grain Zn concentrations in native soil but not in autoclaved soil. Treatment of native soil with increasing P significantly reduced mycorrhizal colonization. At low Zn, P applications aggravated Zn deficiency symptoms in both soil and solution culture. In solution culture, root and shoot Zn concentrations were not lowered by higher P rates. Root uptake of Zn from nutrient solution was even depressed at low P.

2.6 Occurrence and isolation of zinc solubilizing microbes (ZSM)

Martino *et al.* (2003) found that Ericoid mycorrhizal fungi enhance the capacity of their host plants to colonize in the soils polluted with toxic heavy metals. *Oidiodendron mains* was identified as the Zinc solubilising fungi from both polluted and unpolluted soils. Strains from polluted land had a very small ability to solubilize both zinc oxide and zinc phosphate. However, strains from unpolluted land showed a greater solubilization potential. They concluded that ericoid mycorrhizal fungi isolated from polluted and unpolluted soils would interact with this metal compounds in different manner.

Saravanan and Raj (2004) isolated zinc solubilizing microbes particularly the bacterial strain from soil and screened both through direct plating and also through the

technique in which medium was enriched with 0.1 percent Zn from an insoluble Zn source. Three bacterial cultures were isolated through direct plating and one through enrichment technique. These bacterial strains were identified as two genera viz. *Bacillus* spp. and *Pseudomonas* spp. Subsequently the Zn solubilization potential was assessed by inoculating them in soybean plant. The result suggested that *Pseudomonas* spp. has a greater capacity to correct Zn deficiency when treated with 1 per cent ZnO.

Madhaiyan *et al.* (2004) isolated zinc solubilizing bacteria from the tissues of surface sterilized roots, stems and leaves of fifty different crop plants. Phenotypic, biochemical tests and species-specific PCR assay permitted identification of four isolates of *Gluconacetobacter diazotrophicus* from root tissues of carrot (*Daucus carota* L.), raddish (*Raphanus sativus* L.), beetroot (*Beta vulgaris* L.) and coffee (*Coffea arabica* L.). All the four isolates were able to effectively solubilise zinc (ZnO).

Intorne *et al.* (2009) isolated *Gluconacetobacter diazotrophicus* from sugar cane rhizosphere. A total of five mutants were identified and these mutants were screened for their ability to solubilise insoluble zinc compounds. All the five mutants were effectively solubilise ZnO both in plate and broth assay.

Silva and Ferreira (2010) isolated 3 bacterial strains from leaves and roots of sugarcane. Subsequently, these isolates were evaluated for their ability to solubilize insoluble nutrients in LGI medium containing calcium tertiary phosphate and zinc oxide. Isolate SCB4789F-1 was the most efficient for the solubilization of phosphorus, with a halo of solubilization diameter on average 0.75 cm. The solubility of zinc isolates SCB4789F-1 and SCB4789F-2 were the most efficient, with a halo whose diameter was on average 1.34 and 1.29 cm, respectively.

Xinxia *et al.* (2011) isolated zinc solubilising bacteria from roots, stems and leaves of Zn/Cd hyperaccumulator *Sedum alfredii*. A total of fourteen bacterial endophytes were isolated and were closely phylogenetically related to *Pseudomonas*, *Bacillus*, *Stenotrophomonas* and *Acinetobacter* by 16S rRNA sequence analysis. Both plate and

broth assay proved that strain VI8L1, VI8L2, II8L4 and VI8R2 were able to effectively solubilise ZnCO_3 and $\text{Zn}_3(\text{PO}_4)_2$.

Mukherjee *et al.* (2011) isolated four bacterial strains from mixed sewage and designated as L4 (1), L4 (2), L4 (3) and L4 (4). Different concentrations of zinc solutions i.e., 100ppm, 500ppm and 1000 ppm were prepared in which each of the four strains were inoculated and incubated at 37°C for 4 days. Uptake at 1000 ppm concentration of zinc was observed by strain L4 (4) (954.7 mg/L) and least by strain L4 (1) (896 mg/L). At 500ppm concentration of zinc, maximum uptake was observed by the strain L4 (3) (464.7 mg/L) and minimum by the strain L4 (4) (442.7 mg/L). L4 (4) was identified to be *Escherichia coli* and L4 (3) was identified with the help of 16S rDNA analysis and was found to be *Pseudomonas pseudoalcaligenes*.

Li *et al.* (2012) isolated several strains of bacteria from the rhizosphere of *S. alfredii*. Among the different strains isolated, *Burkholderia cepacia* showed the highest ability in mobilizing Cd and Zn as well as resisting high concentrations of soluble Zn (500 mg L⁻¹). The soluble Zn concentration in the medium increased from 13 to 72 and 99 per cent ($p < 0.001$) after bacterial inoculation in the medium supplemented with insoluble zinc oxide and zinc carbonate, results implicated that certain bacteria associated with metal hyperaccumulators could contribute significantly in mobilizing heavy metals, which would enhance the phytoextraction process.

Dahaji *et al.* (2012) isolated zinc solubilizing Rhizobium strains from *Phaseolus vulgaris* L. The ability of fourteen superior strains to solubilize phosphorus and zinc was evaluated in the laboratory assays. The highest P and Zn solubility in liquid and solid medium was observed in strains Rb113 and Rb130, respectively.

Desai *et al.* (2012) isolated *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* strains from diverse crop production system and were evaluated for solubilization of zinc and Phosphorous in vitro from insoluble Zinc and Trichloropropane respectively. After 15 days of incubation 15 strains solubilized Zinc and produced more than 50 cm⁻² solubilization zone on solid media.

Zhang *et al.*, (2013) isolated four bacterial strains, *Burkholderia sp.* SaZR4, *Burkholderia sp.* SaMR10, *Sphingomonas sp.* SaMR12 and *Variovorax sp.* SaNR1 from surface-sterilized root of *S. alfredii* which is a Zn and Cd co-hyperaccumulating plant. Laser scanning confocal microscopy (LSCM) revealed that biofilms on roots were formed by gfp-tagged SaZR4, SaMR12, and SaNR1 cells and SaZR4 and SaMR12 cells could invade the root tissues. SaMR10 showed the lowest population and had little effect on plant growth and phytoextraction process. Whereas, SaZR4 influenced only Zn-extraction but not Cd-extraction. SaMR12 and SaNR1 significantly enhanced plant growth in medium supplemented with Zn or Cd. This study revealed that the four native endophytes colonize the host plant root differently and influence metal uptake and growth of plant. SaMR12 and SaNR1 strains were very much promising in association with *S. alfredii* plants for phytoremediation of Zn/Cd-contaminated soil.

Gontia-Mishra *et al.* (2017) isolated and characterized zinc solubilizing bacteria (ZSB) from the rhizosphere of rice and subsequently evaluated these isolates for plant growth promotion of rice. ZSB significantly solubilized both the insoluble zinc compounds however, ZnO was greater solubilized as compared to ZnCO₃. Isolate ZSB 3 showed highest solubilization of Zn in broth assay *i.e.* 62.48 mg per l. The ZSB also had various plant growth promoting (PGP) properties such as 1-aminocyclopropane-l-carboxylic acid (ACC) deaminase synthesis, exopolysaccharide (EPS) production, phosphate and potassium solubilization. The isolates were identified as four genera *i.e.* *Pseudomonas eruginosa*, *Ralstonia picketti*, *Burkholderia cepacia* and *Klebsiella pneumoniae*.

Khanghahi *et al.* (2018) isolated 80 plant growth promoting bacteria (PGPB) strains from rhizosphere of tomato and barley plant. Two strains *viz.* *Agrobacterium tumefaciens* and *Rhizobium sp.* were cultured in Tris-mineral medium enriched with 0.1 per cent ZnO, ZnCO₃ and Zn₃(PO₄)₂ separately. Results showed that these strains had solubilization ability in the range of pH 8-10. However, no solubilisation was noticed at pH 6 and 7.

2.7 Mechanisms of zinc solubilization

Sayer *et al.* (1995) screened *Aspergillus niger* and *Penicillium simplicissimum* for the ability to solubilize insoluble metal compounds. Almost one-third of the natural isolates screened were able to solubilize at least one of ZnO, $\text{Zn}_3(\text{PO}_4)_2$ and $\text{Co}_3(\text{PO}_4)_2$, with five strains (out of 56) being capable of solubilizing all three. Both organisms are able to perform solubilization with production of organic acids.

Sayer and Gadd (1997) studied the zinc and copper solubilizing ability of *Aspergillus niger* by using ZnO, $\text{Zn}_3(\text{PO}_4)_2$ and $\text{Co}_3(\text{PO}_4)_2$. *A. niger* was able to grow on and solubilize all three metal compounds, a crystalline precipitate was observed to form eventually in the clear zone of solubilization under and around the colony. Citric and oxalic acid produced by *A. niger* that helped in solubilization of insoluble metal compounds.

Di simine *et al.* (1998) reported that a strain of *Pseudomonas fluorescense*, able to solubilize zinc phosphate very rapidly. Colonies of the microorganisms produced clear haloes on solid medium incorporating with zinc phosphate only when glucose was provided as a carbon source. Solubilization of zinc phosphate occurred by both an increase H^+ ion concentration of the medium, probably as a consequence of ammonia assimilation, and production of gluconic acid.

Fasim *et al.* (2002) isolated airborne bacteria from a tannery air environment and they were screened for the property of solubilization of insoluble zinc oxide and zinc phosphate. Out of 10 strains tested, a strain of *Pseudomonas aeruginosa* (CMG 823) showed the best solubilization by solubilising both zinc oxide and zinc phosphate. Colonies of the bacterium produced clear haloes on solid medium which contained these insoluble metal compounds, but only when glucose was provided as a carbon source.

Saravanan *et al.* (2003) studied the zinc solubilizing ability of *Bacillus spp.* and *Pseudomonas spp.* by using zinc oxide, zinc sulphide and zinc carbonate in both plate and broth assay. *Bacillus spp.* showed highest solubilization in the presence of zinc sulphide and *Pseudomonas spp.* showed more solubilizing ability in the presence of both ZnO and ZnCO_3 .

Saravanan *et al.* (2006) examined the zinc solubilization potential properties of *Gluconacetobacter diazotrophicus*. PA15 strain effectively solubilized the zinc compounds and 5-ketogluconic acid was identified as the major organic acid aiding the solubilization of zinc oxide.

Saravanan *et al.* (2007) studied the zinc solubilizing potential of *Gluconacetobacter diazotrophicus* under *in-vitro* condition with different Zinc compounds using glucose or sucrose as carbon source. *G. diazotrophicus* solubilize ZnO effectively than ZnCO_3 or $\text{Zn}_3(\text{PO}_4)_2$. Gluconic acid as the major organic acid produced by *G. diazotrophicus* that helped in solubilization of insoluble Zn compounds.

Sarathambal *et al.* (2009) conducted a pot culture experiment to study the zinc solubilization potential of *Gluconacetobacter diazotrophicus*. The treatments were (1) uninoculated control without zinc, (2) ZnCO_3 , (3) $\text{ZnCO}_3 + G. diazotrophicus$, (4) ZnO alone, (5) $\text{ZnO} + G. diazotrophicus$, (6) $\text{Zn}_3(\text{PO}_4)_2$ and (7) $\text{Zn}_3(\text{PO}_4)_2 + G. diazotrophicus$. Maize seedlings developed zinc deficiency after two months of sowing. This deficiency was corrected with the application of insoluble forms of zinc along with the inoculation of *G. diazotrophicus*. The inoculated organism might have released the gluconic acid during its growth, which in turn solubilized the insoluble zinc compounds, thereby making zinc available for plants.

Sarathambal *et al.* (2010) assessed the zinc solubilization ability of *Gluconacetobacter diazotrophicus* in maize rhizosphere using isotope labelled (65) Zinc compounds. (65) ZnCO_3 and (65) ZnO was effectively solubilized by *Gluconacetobacter diazotrophicus* and the uptake of zinc by the plants also more in *G. diazotrophicus* inoculated treatments compared to the uninoculated treatments. Three types of soils (Zn deficient sterile, Zn deficient-unsterile, and Zn sufficient-sterile) were used in experiment. Among the three soils, Zn deficient-unsterile soil registered maximum Zn solubilization compared to other two soils. This may be due to other soil microorganisms in unsterile soil.

Bapiri *et al.* (2012) studied the zinc solubilizing ability of *Pseudomonas fluorescence* using zinc oxide, zinc carbonate and zinc sulphide in both plate and broth

media assays. Forty bacterial strains and 0.1 per cent of each chemical source in six replications were used. Colony and halo diameters were measured after incubating the plates for 48 hour in incubator. Zn solubilizing ability of 40 mentioned strains in three replications was studied with ZnO and ZnCO₃ solutions in broth assay. The soluble zinc and pH were measured after five days. The results showed, only 8 of 40 strains could form clearing zone in plate assay. Halo diameter, ratio of the halo diameter to the colony diameter and area respectively for zinc oxide and zinc carbonate were as following, 0.60-1.32 cm, 1.20-2.64 and 0.95-2.60 cm², 0.13-1.70 cm, 0.27-2.99 and 0.31-4.10 cm² respectively. There were no halos observed in zinc sulphide. The concentration of soluble Zn for ZnO was 28-625 mg per l and pH was shifted from 7.0-7.2 to 3.90-6.50 and for ZnCO₃ was 247- 753 mg per l and pH was shifted from 7.0-7.2 to 3.5-6.3 after 5 days of inoculation in 28°C.

Panneerselvam *et al.* (2012) studied the zinc solubilization potential of the four isolates in zinc phosphate, zinc carbonate and zinc oxide, results indicated that *Pseudomonas putida* solubilized a significantly higher amount of zinc phosphate and zinc oxide than the other three isolates. In general, the rate of zinc carbonate solubilization by all the isolates was low, but *B. subtilis* recorded the highest zinc carbonate solubilization, followed by *P. putida*, *Brevibacillus sp.*, and *P. aeruginosa*. In the present study, the differences in zinc solubilization by *P. putida*, *P. aeruginosa*, *Brevibacillus sp.*, and *B. subtilis* might be due to their inherent genetic variability in organic acid production. Co-inoculation of *G. mosseae* with *P. putida* may be of particular use in alleviating zinc deficiency in guava cultivation.

Panneerselvam *et al.* (2013) isolated and studied the four bacterial isolates for siderophore production, antifungal activity, growth hormone production and zinc solubilization *in vitro*. The *Pseudomonas putida* was positive for siderophore production and antifungal activity against *Fusarium oxysporum*, whereas *Bacillus choshinensis*, *Bacillus parabrevis* and *Providencia rettgeri* showed negative activity. IAA production was significantly higher for *P. putida* than for *B. choshinensis*, *B. parabrevis* and *Pr. rettgeri*. Whereas, for GA₃ production, *B. choshinensis* produced significantly more than the other isolates, which were at par with each other. The zinc solubilization results

revealed that *P. putida* recorded significantly higher zinc phosphate ($206.0 \pm 7.50 \mu\text{g/ml}$) and zinc oxide ($232.4 \pm 8.46 \mu\text{g/ml}$) solubilization, than the other three isolates. In zinc carbonate solubilization, *P. putida* and *B. parabrevis* recorded significantly higher solubilization (17.6 and $14.4 \mu\text{g/ml}$) than the other two isolates. All four isolates were found to solubilize zinc phosphate, zinc carbonate and zinc oxide at varying levels. All four bacterial isolates caused a shift in pH after growth in the broth. After 14 days, the pH of the broth was acidic in all the cultures. The pH shift was from $7.0\text{--}7.1$ to $4.9\text{--}6.6$.

Poonam *et al.* (2014) isolated 48 endophytic bacteria from soybean and summer mungbean rhizosphere and they were screened for zinc solubilizing ability in Tris- minimal medium separately amended with inorganic zinc compounds viz. zinc oxide (ZnO) and zinc phosphate $\text{Zn}_3(\text{PO}_4)_2$ by plate assay method. Further zinc solubilizing isolates were assessed for morphological, biochemical and plant growth promoting (PGP) traits *in vitro*. Out of 48 endophytic bacterial isolates, 3rd and 17th were able to solubilize ZnO whereas 2nd and 12th were able to solubilize $\text{Zn}_3(\text{PO}_4)_2$ on Tris-minimal medium. Endophytes 1J (*Klebsiella spp.*) and 19D (*Pseudomonas spp.*) were found to be promising bacterial isolates as they solubilized both inorganic sources of zinc separately supplemented in Tris-minimal medium along with good PGP traits (P-solubilization & indole acetic acid) and can be exploited as potential biofertilizers.

Ramesh *et al.* (2014) studied *in vitro* zinc solubilization ability of *Bacillus aryabhattai* strains MDSR7, MDSR11 and MDSR14 in medium supplemented with three insoluble zinc salts (zinc oxide, zinc carbonate and zinc phosphate). While testing under *in vitro*, all the three strains possessed IAA, siderophore and ammonia producing traits. The strains MDSR7 and MDSR14 produced substantially higher soluble zinc content with significant decline in pH and increase in total organic acid production in Tris-minimal broth supplemented with insoluble zinc compounds.

2.8 Effect of Zinc solubilizing microorganism on plant growth, yield and quality of plants.

Microbial transformation of unavailable forms of zinc to bio-available zinc positively influence the plant zinc nutrition and growth. Zinc solubilizing microorganism

influences mobilization of zinc from soil to plant and subsequently its concentration increases in edible portion.

Purakayastha and Chhonkar (2001) studied the influence of vesicular-arbuscular mycorrhizal fungi (*Glomus etunicatum* L.) on mobilization of zinc in wetland rice (*Oryza sativa* L.). The experimental design included two rates of inoculation with vesicular-arbuscular mycorrhizal fungi (VAMF), *Glomus etunicatum* (nil and inoculated) and different combinations of organic (farmyard manure, FYM) and inorganic (NPK, ZnSO₄) fertilizers. The results revealed that a high intensity of root colonization in rice inoculated with *G. etunicatum* could be achieved by raising seedlings in phosphorous and zinc-deficient soil in the nursery under aerobic conditions. The application of ZnSO₄ significantly increased the inflow of zinc to rice roots at the panicle-initiation stage (40 days after transplanting) relative to NPK.

Kumar *et al.* (2004) conducted a field experiment using turmeric as test crop to study the effect of Zn enriched organic manures in combination with Zn solubilizers on the yield, curcumin content of turmeric and fertility status of the soil. The treatment FYM + zinc solubilizing bacteria showed greater rhizome yield which was increased by 21.6 per cent than the FYM alone treatment which was higher than control (no manure) by 9.1 per cent. The dry rhizome yield revealed the encouraging result of Zn and Fe supplemented coir pith or FYM, and Zn supplemented coir pith or FYM compared to M1 (no manure) while at M2 (NPK + FYM) and M3 (NPK + FYM + Zn solubilizing bacteria), the foliar spray of Zn + Fe + MOP beaten the remaining treatment.

Saravanan and Raj (2004) took an effort to isolate three zinc solubilizing bacterial (ZSB) strains viz. ZSB-O-1, ZSB-S-2 and ZSB-S-4 from soil and ore (sphalerite) sources both by direct plating and one (ZSB-S-3) by enrichment technique using modified Bunt and Rovira medium supplemented with 0.1 per cent zinc. Among these four strains, ZSB-O-1 and ZSB-S-4 were identified as *Bacillus* sp. and ZSB-S-2 as *Pseudomonas* sp. and they were evaluated for their potential to correct the zinc deficiency in 10 soybean plants which is high Zn requiring crop. The result showed that *Pseudomonas* sp. (ZSB-S-1) was

competent to alleviate the zinc deficiency when it was used along with 1per cent (w/w) zinc oxide.

Subramoniam *et al.* (2006) conducted field experiment with Thompson seedless grapes with treatments involving micronutrients as soil application and foliar spray and application of zinc solubilising bacteria by adopting randomized block design with three replications. Application of recommended doses of N, P and K fertilizers along with foliar sprays of ZnSO_4 (0.2%) + boric acid (0.2%) + FeSO_4 (0.2%) + MnSO_4 (0.2%) + MgSO_4 (0.5%) + CaCl_2 (0.5%) + KNO_3 (0.5%) + urea (1%) and soil application of zinc solubilising bacteria at blooming and 15 days after blooming stages was best. Among all the treatments soil application on ZnSO_4 + Zn solubilising bacteria increases the quality of fruits such as juice content, TSS, titratable acidity, specific gravity, total sugar and TSS, acidity and gives higher yield.

Tariq *et al.* (2007) studied importance of Plant Growth Promoting Rhizobacteria (PGPR) to enhance the native soil zinc (Zn) supply to rice (*Oryza sativa* L.) by conducting a net house micro plot experiment. The application of PGPR alleviated the deficiency symptoms of zinc and simultaneously increased the total biomass (23%), grain yield (65%) and harvest index as well as zinc content in the grain. The inoculated rice plant showed a positive influence on root length (54%), root weight (74%), root volume (62%), root area (75%), shoot weight (23%), panicle emergence index (96%) and exhibited the highest zinc solubilization efficiency as compared with the un-inoculated control. It was well established that, selected PGPR strains can serve as an efficient zinc biofertilizer, permitting farmers to reduce the use of expensive chemical zinc fertilizer in rice crop.

Timothy and Cavagnaro (2008) studied the role of arbuscular mycorrhiza in improving plant zinc nutrition under low soil zinc concentrations. Arbuscular mycorrhizal fungi (AMF) can take up nutrients including zinc and transfer them to the plant thereby enhancing plant nutrition. Under high soil zinc concentrations the formation of AM can also 'protect' against the accumulation of zinc in plant tissues to high concentrations.

Esitken *et al.* (2010) studied the contribution of plant growth promoting bacteria (PGPB) on the fruit yield, growth and nutrient content of strawberry plant. Three PGPB strains viz. *Pseudomonas* (BA-8), *Bacillus* (OSU-142) and *Bacillus* (M-3) were used singly or in combination as a biofertilizer in this experiment. Results from consecutive three years revealed that the use of PGPB markedly increased P and Zn contents in leaves and also enhance the fruit yield and plant growth.

Iqbal *et al.* (2010) studied the effects of five bacterial isolates viz. U, 8M, 36, 102 and 111 on the growth of *Vigna radiata*. Bacterial isolates were employed alone or together with zinc phosphate, $Zn_3(PO_4)_2 \cdot 4H_2O$. The highest increase in all plant growth parameters was observed when seedlings were inoculated with isolate 102. Isolate 36 resulted in maximum increase in seedling length (35.1 cm) amended with 1 mg zinc phosphate as compared to controls. Isolate 111 was the most efficient phosphate solubilizer, releasing 13.29 ppm phosphorous in soil when used in mixture with 1 mg salt, whereas isolate 36 showed highest uptake of phosphorous, leaving only 4.63 ppm in soil.

Janaki and Velu, (2010) studied the effect of different bacterial strains on zinc solubilization in Maize. The result revealed that, among the main plot treatment, sources of zinc i.e. control (No Zn) and application of ZnO at the rate of 8 kg per ha, application of zinc revealed higher grain and Stover yield as well as higher zinc uptake in grain and straw. Among the subplot treatments, T7-T1+Pf-1 $\times$ FP-7 $\times$ *Bacillus* (Consortia) at 2.5 kg per ha registered a higher grain (6135 kg ha^{-1}) as well as stover yield followed by the treatment T8 (5927 kg ha^{-1}), while the control recorded the lowest. And the same treatment recorded 100 grain weight as well as cob length. The zinc uptake was high in grain and straw in the same treatments.

Amalraj *et al.* (2012) studied the *in vivo* and *in vitro* studies of *Bacillus megaterium* var. phosphaticum on nutrient mobilization, antagonism and plant growth promoting traits. They conducted potted plant assay using sunflower, (*Helianthus annuus*) as the test crop which indicated superior plant growth and photosynthetic activities in treatment with *B. megaterium* var. phosphaticum + 75% of recommended chemical fertilizer dose as compared to treatment with 100 per cent chemical fertilizers. It also improved significantly

the uptake of nitrogen (7.97mg/100g dry mass), phosphate (3.41mg/100g dry mass), potash (38.12mg/100g dry mass), zinc (184mg/100g dry mass), iron (743mg/100g dry mass) and manganese (138mg/100g dry mass) as compared to treatments with 100 per cent *B. megaterium* var. phosphaticum, 100 per cent chemical fertilizers and untreated control. The findings of current study suggest reduction of 25 per cent recommended dose of chemical fertilizers in combination with *B. megaterium* var. *phosphaticum* as seed dresser and soil application.

Dehaji *et al.* (2012) studied the effect of *Pseudomonas fluorescence* on improving zinc compounds solubility and zinc uptake by common bean (*Phaseolus vulgaris* L). 40 isolates were evaluated for their ability in solubilization of ZnO, ZnS, and ZnCO₃ compounds. All of the zinc solubilizing isolates could produce auxin and siderophore and they could dissolve phosphate insoluble compounds in both solid and liquid media. The effect of the selected strains on zinc uptake was determined in greenhouse condition. P19 was selected as the most effective strain. It could increase zinc concentration up to 50.1 mg per kg in shoot, at 500 mg per kg concentration of ZnCO₃.

Panneerselvam *et al.* (2012) studied the effect of *Glomus mosseae* associated bacteria and their influence on stimulation of mycorrhizal colonization, sporulation and growth promotion in guava (*Psidium guajava* L.) seedlings. Zinc solubilization potential of the four isolates in zinc phosphate, zinc carbonate and zinc oxide indicated that *P. putida* solubilized a significantly higher amount of zinc phosphate and zinc oxide than the other three isolates. Among all the isolates *P. putida* was selected for growth promotion attributes and ability to stimulate AM fungal colonization and spore proliferation in guava seedlings. Application of *G. mosseae* along with *P. putida* produced significantly higher leaf, stem, shoot and root dry masses, total biomass and total leaf area than other treatments followed by *G. mosseae* and *P. putida* alone. Application of *G. mosseae* with *P. putida* significantly increased the contents of leaf chlorophyll, phenol and total glomalin, acid and alkaline phosphatase activities, AM fungal colonization and spore population in the rhizosphere of guava seedlings compared with either *G. mosseae* or *P. putida* alone. The results obtained show that combined inoculation of *G. mosseae* with its associated bacterium, *P. putida*, significantly improved AM fungal colonization and spore proliferation in addition to

enhancement of the growth promotion in guava seedlings. These findings have a significant impact for the raising of guava seedling root stocks for commercial multiplication.

Joshi *et al.* (2013) conducted pot experiment in green house on wheat (PBW 373). Treatments consist of bacterial isolates in consortium (B1 & B2) with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at 5mM which resulted in dramatic enhancement in plant height, chlorophyll content and grain number per ear over control. Consortium (B1 & B2) treated wheat plants exhibited 31 per cent rise in grain zinc content over control. HPLC analysis of the root extract of inoculated wheat plants showed the presence of several organic acid particularly of oxalic, maleic, ketoglutaric and fumaric acid. There was also presence of higher amount of valine and leucine, sugar, protein and phenolic compounds in the root extracts of consortium treated wheat plants compared to control. Isolate B1 solubilized maximum amount of ZnO (1.348 ppm) after 15 days of incubation.

Goteti *et al.* (2013) screened 10 isolates for zinc solubilisation, among them P29, P33, and B40 formed 22.0 mm clear halo zone on solid medium supplemented with ZnCO_3 . However, P17 and B40 showed 31.0 mm zone in a medium incorporated with ZnO. P29 and B40 released appreciable amount of soluble zinc in broth amended with ZnCO_3 (*i.e.* 17 and 16.8 ppm) and ZnO (*i.e.* 18 and 17 ppm) respectively. The pH of the broth was found to be acidic in all the cases ranging from 3.9 to 6.1 in case of ZnCO_3 and from 4.1 to 6.4 in case of ZnO incorporated medium. Pot culture experiment in green house with maize revealed that seed inoculation with P29 at rate of 10 g per kg significantly increase total dry weight (12.96 g) and uptake of N (2.268 %), K (2.0 %), Mn (60 ppm) and Zn (278.8 ppm).

Panneerselvam *et al.* (2013) studied the effect of mycorrhiza-associated bacteria on enhancing colonization, sporulation of *Glomus mosseae* along with growth promotion studies in sapota seedlings. Four bacterial isolates were chosen because of their growth-promoting attributes where phytohormones, siderophore and hydrogen cyanide production, antifungal, enzyme activity phosphorus and zinc solubilization were evaluated with *G. mosseae* for growth promotion and mycorrhizal development in sapota seedlings. Among these isolates, application of *P. putida* with *G. mosseae* significantly increased

seedling height, fresh and dry shoot and root weight, total biomass, total leaf area and mycorrhizal colonization compared with other bacterial isolates.

Ramesh *et al.* (2014) studied the effect of *Bacillus aryabhattai* strains MDSR7, MDSR11 and MDSR14 on crop growth, soil biological properties, zinc mobilization from native zinc-pool of soil and acquisition by soybean and wheat under microcosm conditions. On inoculation with MDSR7 and MDSR14 rhizosphere soil pH decreased and increased dehydrogenase, glucosidase, auxin production, microbial respiration and microbial biomass in the rhizosphere soils of soybean and wheat. The operation of all these soil processes regulated by *B. aryabhattai* strains resulted in depletion of organically complexed and calcium carbonate bound zinc and an increase in exchangeable and sesquioxide bound zinc in soil. Such enhanced microbial activities and redistribution of different zinc pools in rhizosphere might have paved way for increased plant available zinc resulting in increased growth promotion and zinc assimilation in seeds of soybean and wheat crops.

Nyoki and Ndakidemi (2014) conducted both field and net house experiments to evaluate the effects of *Bacillus japonicum* inoculation using cowpea as a test crop and phosphorus was supplemented to enhance the uptake of some micronutrients. The results indicated a noteworthy improvement in the uptake of micronutrients in the treatments which were inoculated with *B. japonicum* compared to control. Phosphorus enrichment (40 kg/ha) also revealed a substantial increase in the uptake of some micronutrients while declining the uptake of Zn in some plant tissues. There was also a specific interaction between *B. japonicum* inoculation and phosphorus in the uptake of Zn by the plant root in the field experiment.

Vaid *et al.* (2014) reported a comparative study between the isolated native soil borne bacterial strain and chemical Zn fertilizer ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) by conducting a greenhouse experiment by using two rice varieties viz. Zn responsive (NDR 359) and Zn non responsive (PD 16) to evaluate their potential to supplement Zn nutrition. Three bacterial strains viz. BC, AX and AB isolated from a Zn deficient rice-wheat field identified as the genera *Burkholderia* and *Acinetobacter* which were under study for the growth promotion

and Zn uptake by rice plants. The plant growth promoting traits such as Zn solubilization and production of IAA of the isolates were tested. The three isolated strains when treated individually or in combination were found to be very efficient in increasing the productive tillers per plant (15.1 %), number of panicles per plant (13.3 %), number of grains per panicle (12.8 %), mean dry matter weight per pot (12.9 %), straw yield (12.4 %) and grain yield (17.0 %) compared to the control and uninoculated Zn fertilizer treatment, respectively. Bacterial inoculations results in significant increase in the total Zn uptake per pot (52.5%) as well as methionine content of grain (38.8%). Effect of bacterial inoculated treatments on the bioavailability of Zn was evaluated by estimating the concentration of phytic acid in grains. A decline in phytate to zinc ratio in grains by 38.4 per cent was perceived under bacterial inoculated treatments.

Noman *et al.* (2015) showed the significant positive effect of zinc bio-fertilizer on growth and yield attributes (except kernels/pod), pod and haulms yields, shelling percentage and zinc content and uptake. As compared to the control there was an increase in pod yield, haulms yield, net returns, sulphur uptake in kernel and zinc uptake in kernel by 4.7, 6.2, 9.2, 5.2 and 7.9 per cent respectively, as a result application of zinc bio-fertilizer. By averaging two years results, it could be said that response to sulphur and zinc application was found to be quadratic and optimum rate of application was calculated to be 39.0 and 4.5kg/ha, respectively which can improve the productivity and profitability of groundnut in sandy loam soils deficient in sulphur and zinc.

Muhammad *et al.* (2015) isolated 234 microbial strains from the roots of aromatic rice viz. basmati-385 and basmati super rice varieties growing in clay loam and saline soil at different locations of Punjab (Pakistan) respectively. Out of total isolates, 27 had the potential to solubilize zinc phosphate [$\text{Zn}_3(\text{PO}_4)_2$], zinc carbonate (ZnCO_3) and zinc oxide (ZnO). The strain SH-10 had maximum capacity for Zn solubilization forming a halo zone of diameter of 24 mm in a medium supplemented with $\text{Zn}_3(\text{PO}_4)_2$ and strain SH-17 showed maximum potential to solubilize ZnO and ZnCO_3 forming a zone with a diameter of 14-15 mm. These two strains could also solubilize phosphorous (P) and potassium (K) *in vitro* with a solubilisation clearing zone of 38-46 mm and 47-55 mm in diameter respectively. The strains also could inhibit economically important rice pathogens like *Pyricularia*

oryzae and *Fusarium moniliforme* by 22-29 per cent by producing various biocontrol determinants *in vitro*. The strains helped in better translocation of Zn toward grains and subsequently increased the yield of basmati-385 and super basmati rice varieties by 22-49 per cent and 18-47 per cent respectively. These two zinc solubilizing strains were characterized as *Bacillus sp.* and *Bacillus cereus* by 16S rRNA gene analysis.

Naz *et al.* (2016) reported that wheat crop treated with different microbes *viz.* *Pseudomonas* and *Rhizobium* significantly increased zinc concentration in various plant tissues of wheat at different growth stages. These microbes also took part in efficient uptake of other nutrient and also resulted in better crop yield concluding that zinc solubilizers applied in combination with chemical fertilizers could be a better alternative for farmers for sustainable crop production.

Gandhi and Muralidharan (2016) isolated total 143 Zinc solubilizing bacterial strain from rice rhizosphere using Tris-mineral salt medium enriched with insoluble source of zinc such as Zinc Oxide (ZnO) and Zinc Carbonate (ZnCO₃) separately. In the plate assay among these isolates AGM3 had maximum solubilisation potential followed by AGM9, supplemented with both ZnO and ZnCO₃ with the average halo zone diameter of 13.21 mm, 10.71 mm and 11.74 mm, 7.90 mm respectively. Similarly in broth assay, the AGM3 showed greater potential of zinc solubilization than AGM 9 in both ZnO and ZnCO₃ supplemented medium with a value of 36.54 mg per ml Zn, 35.40 mg per ml Zn and 33.14 mg per ml Zn, 32.69 mg per ml Zn respectively. These AGM3 and AGM9 bacterial strains were characterized as *Acinetobacter spp.* through biochemical test and 16S rRNA analysis. IAA production was also highest for AGM3 followed by AGM9 with a value of 45.61 mg per ml and 37.27 mg per ml respectively. In pot experiment, plant growth was maximum when treated with AGM3 and AGM9 with ZnO and ZnCO₃ in combination. Postharvest soil also shows an increase in exchangeable zinc, manganese oxide bound zinc, crystalline and amorphous zinc and a decrease organically bound and carbonate complexed zinc compared to control pot.

Othman *et al.* (2017) conducted two experiments, for the first one, Five-day-old rice plantlets (MR219) were inoculated with bacterial isolates *viz.* *Acinetobacter sp.*

(TM56) and *Serratia* sp. (TM9) in growth chamber under control condition and the roots were cut for analysis using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In the second experiment the treatments consisted two bacterial isolates viz. *Acinetobacter* sp (TM56) and *Serratia* sp. (TM9), two types of zinc sources i.e. ZnSO₄ and ZnO with three levels like 0 mg per L, 0.2 mg per L and 0.4 mg per L. Results showed that *Acinetobacter* sp. and *Serratia* sp. were capable to colonize and even invade in the tissues of rice plant roots. Bacterial populations of *Serratia* sp. were influenced by different levels of zinc. However, rice plant growth was maximum when it was inoculated with *Acinetobacter* sp. (0.2 mg/l) of ZnSO₄.

Khande *et al.* (2017) isolated a total of 115 *Bacilli* spp. from rhizosphere of two soybean varieties viz. JS 93-05 and JS 335. Among these isolates 20 potential zinc solubilizing *Bacilli* spp. were screened. 7 Isolates viz. KMR-5, THB-2, BBU-5, DBU-5, DBU-1, KHKD-1, KKI-6 and BHKD-6 were observed as highly efficient in solubilizing insoluble zinc sources both on solid plate and in broth assay. Twenty *Bacilli* spp. were characterized as *Bacillus cerells*, *B. anthracis*, *B. thuringiensis*, *B. tequilensis* and *B. subtilis* subsp. Inaquosorum through 16S rRNA gene sequencing analysis. When soybean and wheat were inoculated with KMR-5 and KBY-1 strains of *B. cereus* resulted in increased seed yield, but KMR-5, BHKD-6 and DBU-1 enhanced the Zn concentration as well as seed yield in soybean.

Mumtaz *et al.* (2017) isolated several bacterial strains from maize rhizosphere and subsequently screened for their potential to solubilize zinc oxide. All the selected isolates were positive for oxidase activity (except ZM22), HCN production (except ZM27) and citrate utilization. Ammonia, hydrogen cyanide, siderophores, exo-polysaccharides and cellulase were produced by more than 70 per cent of isolates. More than half of isolates proved to be positive for urease activity and lipase production. Chitinase activity was only found by ZM31 and S 10. All these isolates were evaluated for growth promotion of maize under jar trial. Four isolates viz. ZM20, ZM31, ZM63 and S 10 were considered to be best in stimulating the maize yield. These strains were characterized as *Bacillus* sp. (ZM20), *Bacillus aryabhattai* (ZM31 and S 10) and *Bacillus subtilis* (ZM63) through 16S rRNA sequencing.

Dinesh *et al.* (2018) found that among six promising zinc solubilizing bacteria (ZSB), ZSB-2 (*B. megaterium*, KY687496) was found to be the most efficient strain due to its greater Zn solubilisation capacity *in vitro*. Enhanced gluconic acid production resulted in lower pH was responsible for higher rate of Zn release. Amount of gluconic acid produced by ZSB2 was recorded as 1884.7 ± 413.4 µg per mL, which was 35.3-69.7 per cent higher in comparison to other ZSB isolates. On evaluation of ZSB2 alone or in combination with chemical zinc (75 % and 100 % of recommended Zn) was done using turmeric as the test crop in the green house. The results showed that soil available Zn in the treatment with 75 per cent Zn + ZSB2 (12.69 ± 2.96 mg kg⁻¹) was at par with the 100 per cent Zn (12.74 ± 2.63 mg kg⁻¹) at 120 days after planting, while at harvest the treatment with 75 per cent Zn + ZSB2 results 65.0 per cent greater available Zn levels than 100 per cent Zn. The positive influence of ZSB2 was also found on rhizome yield. Besides, the Zn concentration in the rhizome was also higher in the treatment with ZSB2 + 75 per cent Zn (40.5 ± 3.5 mg kg⁻¹), which was at par with 100 per cent Zn. The study revealed that ZSB2 strain has a great potential for enhanced Zn uptake.

Ash *et al.* (2020) conducted study to evaluate the response of rice to different levels of zinc oxide (ZnO) in combination with zinc solubilizing microbes. Zn deficient soil were treated with three different doses of ZnO (0, 2.5 and 5 kg ha⁻¹) along with three types of microbial inoculation, viz. no inoculation (M0), *Enterobacter cloacae* strain ZnPSBJ-6 (M1) and zinc solubilizing fungi (M2). It was found that ZnPSBJ-6 has increased the grain yield by 57.2 per cent and 101.4 per cent while treated with Z1 (2.5 kg ha⁻¹) and Z2 (5 kg ha⁻¹) respectively compared to ZnO. Whereas, in case of zinc solubilizing fungi, grain yield has increased by 96.4 per cent and 127.1 per cent in case of Z1 and Z2 respectively. On the other hand application of Z1 and Z2 have significantly increased the average straw yield by 28.9 per cent and 36.5 per cent respectively over the control.

Among 10 strains isolated by Yasmin *et al.* (2021), *Pseudomonas protegens* (RY2, MF351762) was found to be the most promising strain having zinc-solubilizing potential on 4 different insoluble zinc sources. In quantitative assay, zinc solubilization by RY2 was significantly higher than other strains at different incubation time. Enhanced shoot and root dry weights and lengths were observed in chickpea plants.

Karnwal (2021) isolated and screened zinc solubilizing bacteria from vermicompost for the plant growth promoting properties with okra. 30 bacterial isolates from vermicompost were examined for zinc solubilization with ZnO and ZnCO₃. Two strains VBZ4 and VBZ17, were found most prevalent Zn solubilizing isolates with 32 mm and 43 mm solubilization zone for ZnO supplemented media. VBZ4 displayed significantly higher solubility of ZnO (26.8 ppm) compared with VBZ17 (22.2 ppm). The quantitative results of plant growth promotion characters showed that VBZ4 and VBZ17 solubilize the maximum amount of phosphate with 316.72 µg per ml and 214.0 µg per ml with indole acetic acid production, 10.2l g per ml and 17.1 g per ml respectively. Further, ZSB isolates were introduced with Okra seeds under pot experiment, where treatment P4 (seeds + VBZ4 + VBZ17) found significantly effective on plant growth and maximum zinc content in Okra fruit (2.85 mg/100g) compared to control.

Prajapati *et al.* (2022) conducted test for identifying and characterizing the efficient Zn solubilizing rhizobacteria isolated from chickpea and peas rhizosphere owing distinct plant growth advancing qualities for zinc-inadequate alluvial soil. Out of ten, four strains ZnJ11, (*Klebsiella pneumonia* MT509804), ZnJ10 (*Acinetobacter calcoaceticus*-MT509803), ZnJ15A (*Acinetobacter pittii*- MT509805), and ZnJ2A (*Pantoea agglomerans*- MT509806) were selected using basal medium contained insoluble Zn minerals such as ZnO and ZnPO₄ at concentrations of 2000 mg per mL. Bio-inoculation of *A. calcoaceticus* and *P. agglomerans* was done in rice crop (HUR 105) with different combination of zinc oxide and zinc sulphate in pot experiment. Findings revealed that root growth (31 ± 1.6), straw yield (32.4 ± 1.7), and grain yield (13 ± 2.0) in the treatment of ZnO (Zn-8.0 kg/ha) with Bio-inoculation was found superior over all treatments.

MATERIAL AND METHODS

III MATERIAL AND METHODS

The experiment on isolation and characterization of zinc solubilising microorganisms and their effects on aerobic rice was carried out during the year 2021-22 in Department of Agricultural Microbiology, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bangalore, 560065. The zinc solubilizing bacterial and fungal isolates were isolated from soils of western Ghats of Karnataka. These isolates were characterized and further examined for their ability to solubilize zinc from insoluble zinc sources and for other beneficial traits in aerobic rice (Daksha) under pot culture studies. The materials used, procedures and the techniques adopted are detailed in this chapter.

3.1 General procedure

3.1.1 Sterilization of media and glass wares

All media were autoclaved at 121 °C at 15 PSI for 20 min. Fresh media was prepared every time. The glassware were sterilized in hot air oven at 100 °C for two hours. Laminar air flow chamber was sterilized with UV radiation for 45 min. Heat labile chemicals used in the study were sterilized through membrane filtration. Alcohol was used for sterilizing the hands and all the inoculations were performed in the flame zone of Bunsen burner by using sterilized inoculation loop and micro pipette inside laminar air flow. The laboratory was maintained under utmost hygiene during the research.

3.1.2 Soil sample collection region

Soil samples were collected from soils of Western Ghats (Sakaleshpura) of Karnataka by following standard soil sampling procedure.

3.1.3 Collection of soil sample

Soil samples at a depth of 10 to 15 cm were collected randomly, about 500 g of the soil samples were collected in sterilized polythene containers and transported to the laboratory with proper labelling. A total of 15 soil samples were collected and stored in refrigerator at 4° C.

3.2 Isolation and characterization of zinc solubilizing microorganisms

3.2.1 Isolation and purification of zinc solubilizers

Zinc solubilizers were isolated from soils of Western Ghats by serial dilution plate count method using modified Bunt and Rovira medium (Bunt and Rovira, 1955), containing 0.1 per cent insoluble zinc sources *i.e.*, ZnO, ZnCO₃ and Zn₃(PO₄)₂. The plates were incubated at room temperature (30±1°C) for 4 days and the colonies exhibiting large clear halo zones were selected, purified by streak plate method and were preserved on agar slants and plates at 4°C for further study. Ten gram of soil was added to the 99 ml sterile distilled water and further serially diluted up to 10⁻⁵ dilution and spread plate method was followed for the isolation of bacteria and fungi on Bunt and Rovira medium and plates were incubated at 30°C for 4 days. Totally 40 zinc solubilizing fungi and 20 bacterial isolates were obtained. Among them 10 fungi and bacterial isolates were selected based on morphological characters, stored in potato dextrose agar (PDA) and nutrient agar slants respectively for further screening.

3.2.2 Characterization of the isolates

3.2.2.1 Morphological characterization

All the selected bacterial isolates were examined for the colony morphology, cell shape, and gram reaction as per the standard procedures given by Anonymous (1957), Barthalomew and Mittewer (1950). Colony characteristics viz. shape, size, elevation, surface form, margins, surface texture and colour of isolates were observed in fungal isolates.

3.2.2.2 Molecular characterization of bacterial and fungal isolates

DNA extraction

The genomic DNA of the bacterial isolates was extracted by alkaline lysis method (Felicciello and Chinali, 1993). The bacterial isolates were grown in nutrient broth for 48 hours at 30°C and centrifuged 3 mL of culture at 12,000 rpm for 3 min, bacterial culture was pelleted. The extracted pellet was resuspended in 650 µL of extraction buffer and incubated at 65°C for 30 min. 100 µL of 5 M potassium acetate solution was added to the

extract and allowed for precipitation of protein and carbohydrates by placing it on ice for 15 min. The supernatant was collected by centrifuging at 12,000 rpm for 8 min. The DNA was precipitated by adding an equal volume of ice-cold isopropanol and pelleted at 12,000 rpm for 5 min. The pellet obtained was washed twice with 70 % ethanol, air-dried and dissolved in 1mM TE (10:1). The concentration of the DNA was measured using UV spectrometer at 260 /280 nm wavelength and stored at -20°C. The fungal isolate was grown in potato dextrose broth for 72 hours at 28°C and fungal mats were crushed using 1 mL extraction buffer by rotating it in one direction. Samples were transferred to a 2 mL eppendorf tube having PVP. The extraction buffer (1mL) was added to each tube, mixed well and kept in a water bath for 45 minutes at 65°C. Samples were then transferred to another tube and 1 mL of a mixture of chloroform and isoamyl alcohol (24:1) was added to it and centrifuged at 10,000 rpm for 8 minutes. The supernatant was separated in which 600 µL of chilled isopropanol was added and kept at -20 °C for two hours. The chilled samples were again centrifuged at 10,000 rpm for 10 minutes and the supernatant was discarded. The chilled ethanol (500 µL of 70 %) was added to the pellets formed and again centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and the pellets were air-dried. These pellets were then redissolved in deionized sterile water to which 5µL of RNase enzyme was added and incubated in the water bath at 37°C for 1 hour. The concentration of the DNA was measured using a UV spectrometer at 260 /280 nm wavelength and stored at -20°C.

PCR amplification of the DNA

The two primers (22 bp forward primer 5' GGAGAGTTAGATCTTGGCTCAG 3' and 20 bp reverse primer 5' AAGGAGGGGATCCAGCCGCA 3') already reported for 16S rRNA sequences from the NCBI were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly for PCR reactions for DNA isolated from bacterial isolates. The two primers (forward primer ITS-1 and reverse primer ITS-4) reported for Internal Transcribed Spacer region (ITS) sequencing were diluted for PCR reactions for fungal DNA isolation.

The annealing temperature for primer pair was standardized and PCR was run in 40 μ L reaction mixture containing 1X buffer with MgCl_2 (1.5 mM), dNTPs (200 μ M), forward and reverse primers (0.5 μ M each), Taq DNA polymerase and 2 μ L template DNA (50 ng / μ L). Amplification was carried out with an initial denaturation at 96° C for four minutes followed by 35 amplification cycles consisting of 94° C for one minute, 55° C for 30 seconds and 72° C for one minute and a final extension at 72° C for 12 min. Controls for PCR reactions were carried out with the same primers without providing template DNA (Yurgel *et al.* 2017).

Agarose gel electrophoresis

Agarose gel electrophoresis was performed to resolve the amplified product using 0.8 per cent Agarose in 1X TAE buffer, 0.5 μ g /mL of ethidium bromide and loading buffer (0.25 % bromophenol blue prepared in 40 % sucrose). Five μ L of loading dye was added to 40 μ L of PCR product and loaded to the well. Electrophoresis was carried out at 100 V for two hours. The gel was visualized under a UV transilluminator and documented using a gel documentation unit (Comeau *et al.*, 2016).

Gel elution

The gene JETTM Gel extraction kit (Thermo Scientific) was used for the rapid and efficient separation of DNA fragments from agarose gel. The gel slice containing DNA was excised using a clean razor blade and was placed into a pre-weighed 1.5 mL tube. An equal volume of binding buffer was added to the tube (100 mg gel slice: 100 μ L of binding buffer). The mixture was incubated at 60° C till the gel slice was completely dissolved. The solubilized gel solution was added to the Gene JETTM purification column and centrifuged at 10,000 rpm for 60 sec. The flow-through was discarded and the column was placed back into the same collection tube washed by adding 700 μ L of wash buffer by centrifugation at 10,000 rpm for 60 sec. The tube was again centrifuged for 60 seconds to remove the wash buffer that remained as residue. Then purification column was placed into a clean 1.5 mL microcentrifuge tube and added with 20 μ L of elution buffer and centrifuged at 10,000 rpm for 60 sec. The DNA eluted was checked for its concentration using nanodrop and got

sequenced by Barcode BioSciences, Bengaluru, Karnataka. The sequence data received from the company was analysed for homology.

Sequence analysis and homology search

The sequence analysis and homology search sequence results were analyzed by online software from National Centre for Biotechnology Information (NCBI). The sequence was aligned by CLUSTAL W multiple sequence alignment program and a phylogenetic tree was constructed with bootstrap support based on a neighbor-joining analysis in MEGA X.

3.3 Screening of isolates for zinc solubilisation under *in vitro* condition

3.3.1 Qualitative estimation of zinc solubilization

The Bunt and Rovira medium was prepared by supplementing 0.1 per cent insoluble zinc sources viz., ZnO, ZnCO₃ and Zn₃(PO₄)₂ then autoclaved at 121°C for 20 minutes. Selected bacterial and fungal isolates strain spot inoculated onto the agar and plates were incubated at 30°C. The diameter of clearance halo zone was measured in millimetre successively for 7 days. By measuring the diameter of the clear zone and colony growth, Zn solubilization efficiency and Solubilizing Index was tested (Edi-Premono *et al.*, 1996).

$$\text{Solubilization efficiency} = \frac{\text{Solubilisation diameter-colony diameter}}{\text{Diameter of colony growth}} \times 100$$

$$\text{Solubilization Index} = \frac{\text{Solubilisation diameter} + \text{colony diameter}}{\text{Diameter of colony growth}}$$

3.3.2 Quantitative estimation of zinc solubilization

Bunt and Rovira broth was prepared with 0.1 per cent of insoluble zinc compounds viz., ZnO, ZnCO₃ and Zn₃(PO₄)₂ them autoclaved at 121⁰ C in 15 PSI pressure for 20 minutes. Then the flasks were inoculated with 1 ml suspension of the bacterial culture with a cell load of 10⁹ cells ml⁻¹ and fungal culture with 1 ml fungal spore suspension (10⁹ spore ml⁻¹). Three flasks (uninoculated) were maintained as control for each treatment. Cultures

were incubated at 30⁰C with continuous shaking at 150 Rpm for 7 days, then samples were tested for zinc content seven days after inoculation. Samples were centrifuged at 7000 rpm for 15 minutes to remove the debris and cells. Then the concentration of zinc in the supernatant was estimated in atomic absorption spectrophotometer to determine the available zinc content later expressed in ppm.

3.4 Determination of pH

The changes in the pH of the zinc solubilizing bacterial culture filtrates and the uninoculated samples were determined 7 days after inoculation. The cultures were centrifuged to remove the debris and cells, then the pH was estimated using pH meter.

3.5 Selection of isolates for green house study

Out of ten bacteria and fungi isolates, one bacterial and one fungal isolate selected based on the solubilizing efficiency and solubilizing index in qualitative method and solubilized zinc in quantitative method. Those two isolates were identified through molecular characterization.

3.6 Evaluation of zinc solubilizing isolates on zinc uptake by aerobic rice under greenhouse condition.

The pot culture experiment was conducted using efficient zinc solubilizing bacteria and fungal isolate for its performance on Zn uptake and other growth parameters in aerobic rice.

3.7 Treatments details

The pot culture experiment consisted of ten treatments, each with four replications, were maintained as follows with Completely Randomized Design.

Table 1: Treatments details

Sl. No.	Treatments
T ₁	Absolute control +100% N, P, K
T ₂	Recommended zinc (ZnSO ₄ .7H ₂ O) + 100% N, P, K
T ₃	Inoculated-with bacterial isolate +100% N, P, K
T ₄	Inoculated with fungal isolate +100% N, P, K
T ₅	Inoculated with bacterial and fungal isolate +100% N, P, K
T ₆	Absolute control +100% N, K, 0% P
T ₇	Recommended zinc (ZnSO ₄ .7H ₂ O) + 100% N, K, 0% P
T ₈	Inoculated with bacterial isolate +100% N, K, 0% P
T ₉	Inoculated with fungal isolate +100% N, K, 0% P
T ₁₀	Inoculated with bacterial and fungal isolate +100% N, K, 0% P

3.8 Soil type

The medium red sandy loam soil was collected from fields of GKVK college, University of Agricultural sciences, Bangalore. The soil was sieved to remove weeds, clods, stones and other debris later on filled in to the pots at the rate of 5 kg per pot.

3.9 Soil parameters

The soil was analysed for its available nitrogen content by Kjeldhal method (Subbaiah and Asija, 1956) and organic carbon content by wet oxidation method (Jackson, 1967). The pH of the soil was determined in 1:2.5 soil water suspension using a digital pH meter. Available phosphorus content was determined by Bray's method, the available potassium by flame photometer method (Stanford and English, 1949) and the available zinc and other micro nutrients content was determined by using AAS (Atomic Absorption Spectrophotometer). The chemical and biological properties of soil analysed is presented in Table.

Table 2: Initial physico-chemical properties of the soil of pot culture experiment

Parameter	Value	Status
Physico- chemical properties		
pH	5.83	Acidic
EC (dsm ⁻¹)	0.11	Low
OC (%)	0.48	Low
BD (Mg m ⁻³)	1.31	
Primary nutrients		
Available Nitrogen (kg ha ⁻¹)	221.66	Low
Available Phosphorous (kg ha ⁻¹)	28.73	Medium
Available Potassium (kg ha ⁻¹)	149.34	Medium
Available zinc (ppm)	3.01	Medium

3.10 Seeds

The seeds of *Oryza sativa*, Daksha (KMP-175) used in this study were collected from AICRP on rice, ZARS, V. C. Farm, Mandya.

3.11 Fertilizers

The recommended dose of fertilizer used for aerobic rice (100:50:50 kg NPK per ha and 25 kg zinc per ha) in this experiment.

3.12 Sowing and maintenance

Soil was inoculated with 7 days old culture of fungal isolate, 2 days old culture of bacterial isolate before sowing at the rate of 10 ml per pot. The germinated seeds were sown to the pots containing 5 kg soil. The pots were watered regularly to maintain optimum moisture and other routine care was taken to protect the plants from pests and diseases.

3.13 Plant growth parameters

The observation with respect to the growth parameters including plant height, number of leaves, number of tillers, plant biomass were recorded after 90 days of sowing.

3.14 Estimation of zinc in soil and plant sample

The method developed by Lindsay and Norwell (1978) using DTPA (Diethylene triamine penta acetic acid) extractant was followed for the estimation of zinc. Ten grams soil was shaken with 20 mL of DTPA extractant for 2 hours for the extraction of micronutrient cations. Atomic absorption spectrophotometer was used for measuring the concentration of zinc and presented in ppm.

One gram of the powdered leaf was pre-digested with 10 ml HNO_3 (62 %) for 24 hours, later digested in a digestion chamber at 85°C with the following steps, the pre-digested samples were treated with 10 mL di-acid mixture reagent ($\text{HNO}_3 + \text{HClO}_4$ at 10:4 ratio) and kept in digestion chamber until white precipitate was left at the bottom of the flask. The digested samples were diluted with distilled water to a known volume after filtration. This extract was used in the estimation of zinc by using standard procedures (Lindsay and Norwell, 1978)

3.15 Statistical analysis

The data of the lab and green house experiments were subjected to appropriate statistical analysis. Complete randomized design of statistical analysis was followed for laboratory and green house experiments. The analysis of variance (ANOVA) and interpretation of data were done as per procedures given by (Fisher and Yates, 1963 and Panse and Sukhatme, 1985)

RESULTS AND DISCUSSION

IV RESULTS AND DISCUSSION

This study attempted to isolate zinc solubilizing microorganisms from the Western Ghats soils of Karnataka. The isolates were examined for their ability to solubilize insoluble zinc minerals. The selected isolates were characterized and identified based on morphological and molecular properties. The efficient Zn solubilizing microorganisms were further subjected for their ability to solubilize and release Zn from ZnO, ZnCO₃ and Zn₃(PO₄)₂. Highly efficient Zn solubilizing strains were also tested for their influence on the growth and nutrient uptake of aerobic rice plants with and without phosphorous nutrient application in pot culture experiment under greenhouse conditions. The results obtained in these studies are presented in this chapter.

4.1 Isolation and characterization of zinc solubilizing microorganisms (ZSM) from soils of Western ghats

The soil samples of the Western Ghats were collected and used for the isolation of ZSM. The details of the places from where the soil samples were collected are mentioned in (Table 3). Zinc solubilizing microorganisms were isolated in modified Bunt and Rovira media with three different insoluble zinc sources viz., ZnO, ZnCO₃ and Zn₃(PO₄)₂ by following the spread plate method. Microorganisms that show a halo zone around the colony were selected and these isolates were purified, identified and maintained for further use.

Soil is a dynamic ecosystems inhabited by numerous microorganisms which are closely associated with plants. Microorganisms serve two main functions: pathogens that cause diseases and beneficial activities like biocontrol agents, nutrient mobilizers and solubilizers. Zinc is absorbed in the form of divalent cation (Zn²⁺) by plants from soil solution (Alam *et al.*, 2010 and Alloway, 2008). However, most of the zinc in the soil is present as mineral deposits and is not readily available for plant uptake. Despite the high zinc concentration in the soil, plants cannot utilize it since it is bound to minerals. A number of soil microbes have been reported to solubilize zinc from insoluble mineral sources, resulting in higher availability of zinc to plants (Bunt and Rovira, 1955).

Out of 40 fungal and 20 bacterial isolates, ten fungal and ten bacterial isolates were selected based on morphological characters and colony appearance, they found to produce halo zone (solubilization zone) in media with all three insoluble zinc source, these isolates were selected for further studies. ZnO, ZnCO₃ and Zn₃(PO₄)₂ are the common insoluble forms of zinc found in soil hence these insoluble forms were used on source of zinc in Bunt and Rovira medium.

Table 3: Details of Locations for soil sample collection.

Soil sample	GPS location
Achanahalli 1	13°54'26.8"N 75°24'20.2"E
Achanahalli 2	13°54'32.8"N 75°23'38.6"E
Achanahalli 3	13°54'34.3"N 75°23'24.8"E
Bellanduru	14°00'44.8"N 75°16'00.4"E
Honnnavalli	13°19'29.3"N 76°22'59.6"E
Meega 1	13°27'15.4"N 75°11'45.4"E
Meega 2	13°27'20.1"N 75°12'06.5"E
Meega 3	13°27'16.9"N 75°11'51.4"E
Meega 4	13°27'21.1"N 75°11'52.4"E
Melukoppa 1	13°28'09.3"N 75°14'44.3"E
Melukoppa 2	13°28'11.4"N 75°14'37.0"E
Melukoppa 3	13°27'53.6"N 75°14'52.6"E
Nallur	14°05'13.3"N 75°52'45.5"E
Veera pura 1	13°57'14.6"N 75°39'49.8"E
Veera pura 2	13°48'19.8"N 75°38'44.3"E

4.1.1 Morphological characters of selected zinc solubilizing bacterial isolates

The morphology of selected zinc solubilizing bacterial isolates were studied and is given in Table 4. The zinc solubilizing bacterial isolates colonies were observed as creamy-dense white to yellowish in colour, slimy, mucoid with opaque, circular to irregular, smooth, tiny in texture or dry in texture on the agar medium.

A comprehensive study of the ten bacterial isolates showed that ZSBI-2, ZSBI-5, ZSBI-7, and ZSBI-10 are positive in Gram reactions and endospore production. The other isolates are negative for Gram reaction.

Table 4: Morphological characteristics of the zinc solubilizing bacterial isolates from soils of western ghats.

Isolates	location	Colony characteristics	Gram reaction	Cell shape	Endospore formation
ZSBI-1	Meega 2	Yellowish, slimy, tiny	-	Rod	-
ZSBI-2	Honnavalli	Fuzzy white, rough, opaque with jagged edges	+	Rod	+
ZSBI-3	Achanahalli 3	Creamy white, dense, irregular, tiny	-	Rod	-
ZSBI-4	Melukoppa 2	Dense white, medium, circular	-	Rod	-
ZSBI-5	Meega 4	Creamy white, opaque, dried, medium	+	Rod	+
ZSBI-6	Meega 3	Yellowish, glossy, tiny	-	Rod	-
ZSBI-7	Melukoppa 3	Creamy white, slimy, transparent , tiny	+	Rod	+
ZSBI-8	Veera pura 1	Smooth and transparent milky white	-	Rod	-
ZSBI-9	Achanahalli 1	Creamy white, dense, irregular, tiny	-	Rod	-
ZSBI-10	Nallur	Creamy white, slimy, medium	+	Rod	+

4.1.2 Morphological characters of selected zinc solubilizing fungal isolates

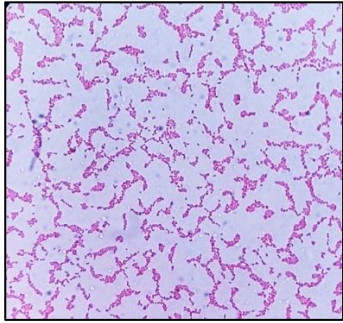
Morphological characteristics of the fungi were observed by growing them on Bunt and Rovira medium and Potato Dextrose Agar. Morphological examination of isolates revealed that all isolates showed different characteristics of growth. The morphological parameters studied by growth on different media, colony colour, colony characteristics and sporulation of all isolated fungi are described in Table 5.

Table 5: Morphological characteristics of the zinc solubilizing fungi isolates from soils of western ghats.

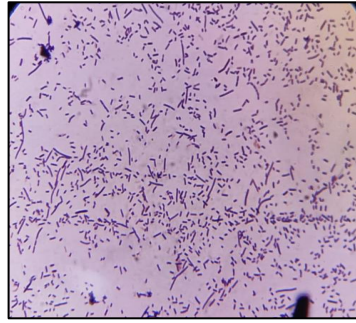
Isolates	location	Colony colour	Colony appearance	Sporulation pattern
ZSFI-1	Melukoppa 1	Green with white margin	Smooth and velvety	Moderate
ZSFI-2	Nallur	Green	Light Velvet	Moderate
ZSFI-3	Honnavalli	Grey with white margin	Powdery	Heavy
ZSFI-4	Melukoppa 2	white to yellow and then turning black	Cottony appearance	Heavy
ZSFI-5	V pura 2	Grey	Powdery	Heavy
ZSFI-6	Meega 3	Greenish yellow	Compact and velvety	heavy
ZSFI-7	Melukoppa 3	Olivaceous green	Powdery	Heavy
ZSFI-8	V pura 1	Off white	Loose, fluffy, cottony white mass	Heavy
ZSFI-9	Bellanduru	Pure white	Tight Cottony white mass	Heavy
ZSFI-10	Aranahalli 3	Cream White	Smooth and velvety	Moderate

The chances of isolating microbes to solubilize insoluble mineral nutrients from the rhizosphere soils are common (Altomare, 1999). Out of 40 fungal isolates, ten were selected based on microscopic observation and colony morphology. Out of 20 bacterial isolates, ten were selected based on microscopic observation. Interestingly, isolates from rhizosphere soils could grow and solubilize the medium containing a fixed or insoluble form of nutrients.

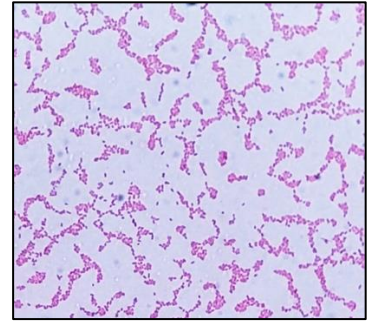
The results are in agreement with the findings of Desai *et al.* (2012) and who also isolated *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* strains from diverse crop production systems as mineral zinc solubilizers. Panneerselvam *et al.* (2012) and Panneerselvam *et al.* (2013) isolated the mycorrhiza associated bacteria which have the zinc solubilization ability viz., *Pseudomonas putida*, *P. aeruginosa*, *Bacillus subtilis* and



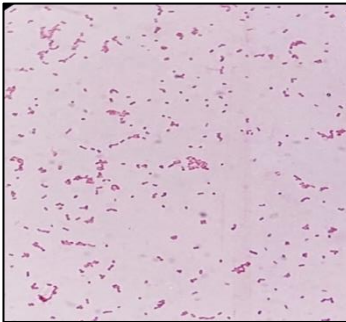
ZSBI-1



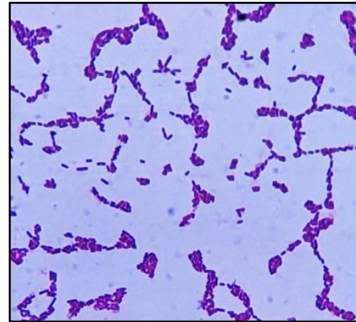
ZSBI-2



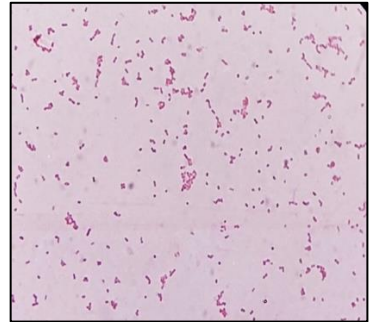
ZSBI-3



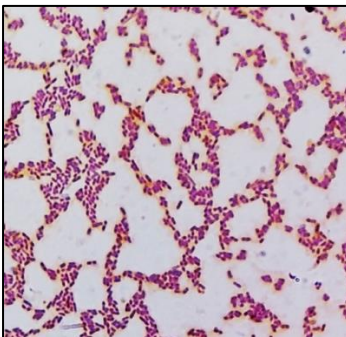
ZSBI-4



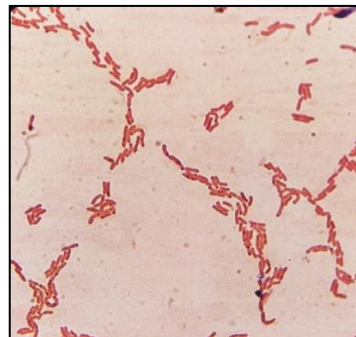
ZSBI-5



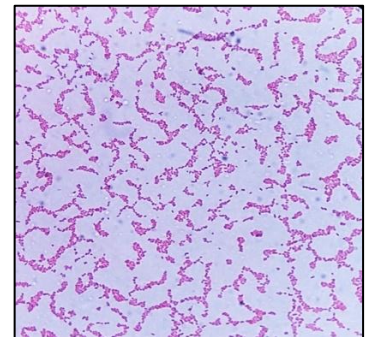
ZSBI-6



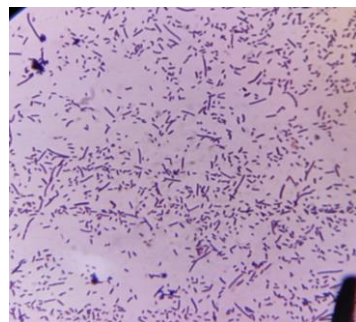
ZSBI-7



ZSBI-8

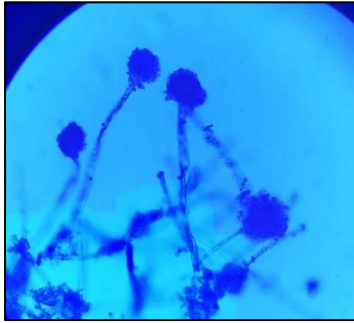


ZSBI-9

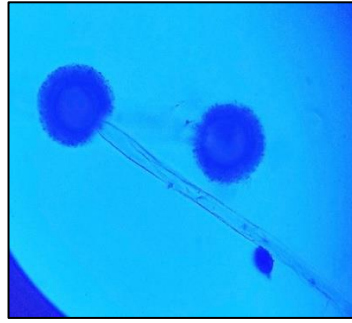


ZSBI-10

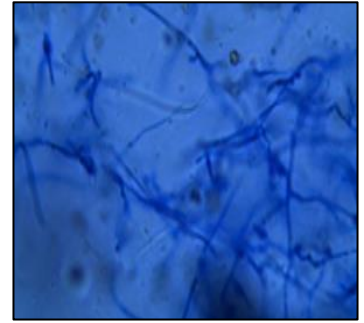
Plate 1: Cell morphology of zinc solubilizing bacterial isolates



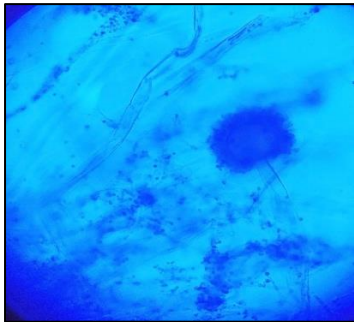
ZSFI-1



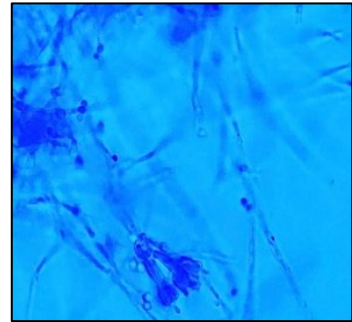
ZSFI-2



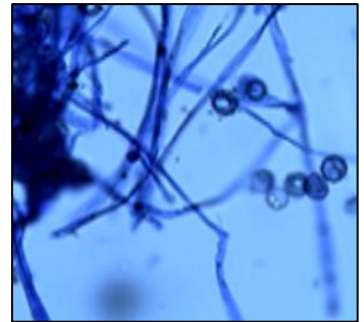
ZSFI-3



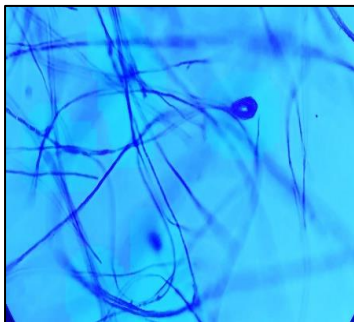
ZSFI-4



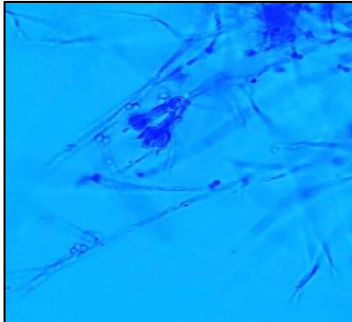
ZSFI-5



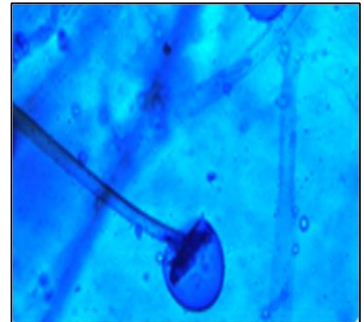
ZSFI-6



ZSFI-7



ZSFI-8



ZSFI-9

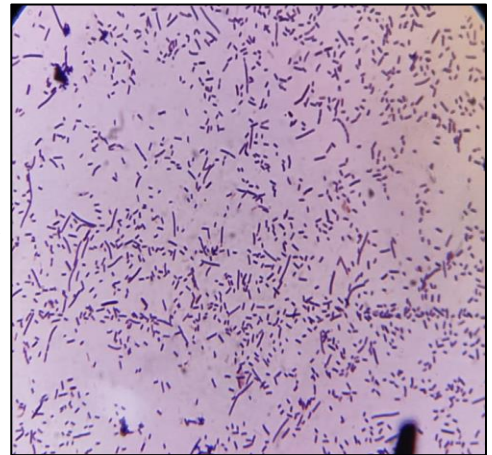


ZSFI-10

Plate 2: Cell morphology of zinc solubilizing fungal isolates



ZSBI-2



ZSFI-4

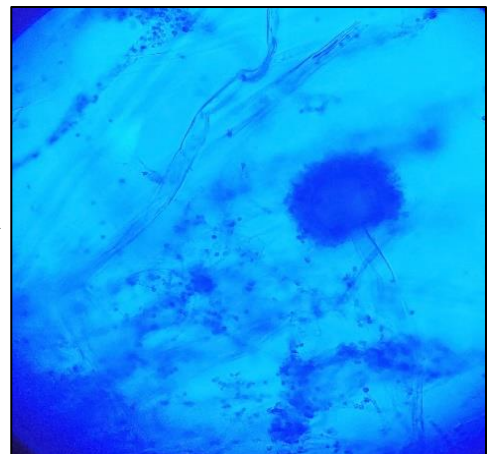


Plate 3: Morphology of efficient zinc solubilizing bacterial and fungal isolate

Brevibacillus sp. from the rhizosphere of guava and *Brevibacillus parabrevis*, *P. putida*, *B. choshinensis* and *Providencia rettgeri* from sapota cropping system. Anitha *et al.*, (2015) isolated *Emericella rugulosa*, *Penicillium citrinum*, *Aspergillus candidus*, *Aspergillus terreus* and *Aspergillus Niger* in the agricultural fields of Tamilnadu. Devi *et al.* (2022) also isolated *Aspergillus* spp. and *Penicillium* sp. from the arid and semi-arid soils of Rajasthan.

In the present study, the results of all the experiments conclude that *Bacillus* sp. and *Aspergillus* sp. were potential to solubilize the zinc mineral. The results are in agreement with the Saravanan and Raj (2004), Xinxian *et al.* (2011), Desai *et al.* (2012) and Mukherjee *et al.* (2011) they isolated *Bacillus* sp. from various sources. Anitha *et al.* (2015), Devi *et al.* (2022) isolated *Aspergillus* sp.

4.2 Screening of isolates for zinc solubilisation under *in vitro* condition

4.2.1 Qualitative estimation of zinc solubilization

Qualitative assay of the selected zinc solubilizing isolates for zinc solubilization abilities was examined on agar medium supplemented with 0.1 per cent of different zinc sources, presented in Table 6, for bacterial isolates and in Table 7, for fungal isolates.

The results indicated that the maximum solubilizing efficiency (350%, 90%, 158.17%) and solubilizing index (5.50, 2.90, 3.58) was observed in the treatments inoculated with ZSBI-2 followed by ZSBI-9 with solubilizing efficiency (153.69%, 146.21%, 65.47%) and solubilizing index (3.53, 3.44, 2.47) and ZSBI-6 with SE (44.43%, 187.67%, 44.43%) and SI (2.43, 3.85, 2.43) with the source of zinc oxide, zinc phosphate and zinc carbonate followed by after seven days of incubation which was significantly different from all the other zinc solubilizing bacterial isolates.

Among the fungal isolates, results indicated that the maximum solubilizing efficiency (303.70%, 466.6%, 193.33%) and solubilizing index (5.04, 6.60, 3.93) was observed in the treatment inoculated with ZSFI-2 followed by ZSFI-5 with solubilizing efficiency (227.38%, 404.83%, 113.33%) and solubilizing index (4.27, 6.02, 3.13) and ZSFI-10 with SE (141.67%, 308.87%, 67.49%) and SI (3.42, 5.06, 2.67) with the source of

of zinc oxide, zinc phosphate and zinc carbonate respectively, after seven days of incubation which was significantly different from all the other zinc solubilizing fungal isolates.

Table 6: Solubilizing efficiency and solubilizing index of Zn-solubilizing bacterial isolates for different sources of zinc *in-vitro*

Isolates	ZnO		Zn ₃ (PO ₄) ₂		ZnCO ₃	
	SE %	SI	SE %	SI	SE %	SI
ZSBI-1	44.44 (6.71)	2.45 (1.86)	85.70 (9.56)	2.87 (1.97)	39.97 (6.38)	2.39 (1.84)
ZSBI-2	350.00 (18.73)	5.50 (2.55)	90.00 (9.51)	2.90 (1.97)	158.17 (12.62)	3.58 (2.14)
ZSBI-3	11.43 (3.51)	2.11 (1.76)	41.80 (6.54)	2.42 (1.85)	0.00 (1.00)	0.00 (1.00)
ZSBI-4	27.77 (5.35)	2.27 (1.81)	55.50 (7.43)	2.50 (1.87)	25.00 (5.10)	1.80 (1.67)
ZSBI-5	17.20 (4.15)	2.17 (1.78)	33.30 (5.86)	2.30 (1.82)	0.00 (1.00)	2.38 (1.84)
ZSBI-6	44.43 (6.71)	2.43 (1.85)	187.67 (13.74)	3.85 (2.20)	44.43 (6.71)	2.43 (1.85)
ZSBI-7	41.80 (6.54)	2.42 (1.85)	119.87 (10.99)	3.20 (2.05)	25.00 (5.10)	1.80 (1.67)
ZSBI-8	17.20 (4.15)	2.17 (1.78)	11.53 (3.51)	2.11 (1.76)	7.41 (2.89)	2.07 (1.75)
ZSBI-9	153.69 (12.42)	3.53 (2.13)	146.21 (12.13)	3.44 (2.11)	65.47 (8.15)	2.47 (1.86)
ZSBI-10	31.67 (5.71)	2.33 (1.82)	75.77 (8.76)	2.75 (1.94)	0.00 (1.00)	0.00 (1.00)
SE(m)±	0.422	0.015	1.015	0.014	0.20	0.013
C.D.	1.253	0.044	0.342	0.042	0.594	0.037

Values in parenthesis are transformed by $\sqrt{(x+1)}$

Table 7: Solubilizing efficiency and solubilizing index of Zn-solubilizing fungal isolates for different sources of Zinc.

Isolates	ZnO		Zn ₃ (PO ₄) ₂		ZnCO ₃	
	SE %	SI	SE %	SI	SE %	SI
ZSFI-1	29.52 (5.52)	2.29 (1.81)	6.08 (2.64)	2.06 (1.75)	2.04 (1.74)	2.04 (1.74)
ZSFI-2	8.40 (3.06)	2.08 (1.75)	6.81 (2.79)	2.06 (1.75)	4.87 (2.41)	2.06 (1.75)
ZSFI-3	132.56 (11.55)	2.33 (1.82)	31.67 (5.71)	3.32 (2.08)	4.96 (2.43)	2.04 (1.74)
ZSFI-4	303.70 (17.46)	5.04 (2.46)	466.60 (21.62)	6.60 (2.76)	193.33 (13.94)	3.93 (2.22)
ZSFI-5	227.38 (15.10)	4.27 (2.30)	404.83 (20.14)	6.02 (2.65)	113.33 (10.68)	3.13 (2.03)
ZSFI-6	72.24 (8.56)	2.72 (1.93)	31.20 (5.67)	2.30 (1.82)	16.88 (4.22)	0.00 (1.00)
ZSFI-7	13.14 (3.75)	2.14 (1.77)	17.58 (4.30)	2.16 (1.78)	3.17 (2.04)	2.03 (1.74)
ZSFI-8	3.02 (1.99)	2.02 (1.74)	14.19 (3.85)	2.15 (1.77)	0.00 (1.00)	0.00 (1.00)
ZSFI-9	4.29 (2.30)	2.04 (1.74)	11.11 (3.48)	2.10 (1.76)	0.00 (1.00)	0.00 (1.00)
ZSFI-10	141.67 (11.93)	3.42 (2.10)	308.87 (17.59)	5.06 (2.46)	67.49 (8.28)	2.67 (1.92)
SE(m)±	0.226	0.013	0.247	0.015	0.169	0.008
C.D.	0.671	0.038	0.735	0.044	0.502	0.023

Values in parenthesis are transformed by $\sqrt{(x+1)}$

4.2.2 Quantitative estimation of zinc solubilization

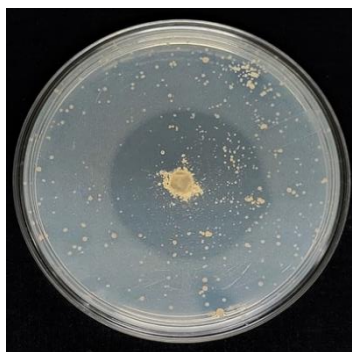
Quantitative estimation of zinc released from zinc oxide, zinc phosphate, zinc carbonate and change in the pH by the bacterial isolates were studied seven days after incubation. The results (Table 8) indicated that the amount of zinc released from zinc sources by all the bacterial isolates increased with decreased pH.

Among the isolates, ZSBI-2 released the maximum (29.67, 19.32 and 25.43 ppm) amount of zinc from zinc oxide, zinc phosphate, zinc carbonate and least pH (3.43, 4.23 and 3.93) followed by ZSBI-9 (18.83, 26.16, 1.10 ppm) with pH (4.93, 4.10, 5.13) and ZSBI-6 (10.60, 26.70, 13.17) with pH (5.07, 4.00, 5.07) which were significantly different from all the other isolates at seven days after incubation. Other zinc-solubilizing bacterial isolates were significantly different from each other with respect to the release of zinc and change in the pH.

Table 8: Solubilization of different sources of insoluble Zinc by bacterial isolates in broth culture

Isolates	ZnO		Zn ₃ (PO ₄) ₂		ZnCO ₃	
	Available zinc (ppm)	pH	Available zinc (ppm)	pH	Available zinc (ppm)	pH
ZSBI-1	8.00	5.13	26.07	3.97	9.36	5.20
ZSBI-2	29.67	3.43	19.32	4.23	25.43	3.93
ZSBI-3	3.53	6.23	4.96	6.03	0.96	6.53
ZSBI-4	7.73	5.47	9.06	5.37	7.13	5.20
ZSBI-5	4.27	5.03	7.73	5.07	0.79	6.43
ZSBI-6	10.60	5.07	26.70	4.00	13.17	5.07
ZSBI-7	2.36	6.40	26.38	4.13	1.10	6.47
ZSBI-8	2.50	6.13	2.53	6.13	2.13	6.03
ZSBI-9	18.83	4.93	26.16	4.10	13.00	5.13
ZSBI-10	11.53	5.20	12.68	5.13	0.23	6.47
SE(m)±	0.53	0.08	0.25	0.05	0.51	0.04
C.D.	1.57	0.26	0.75	0.17	1.53	0.13

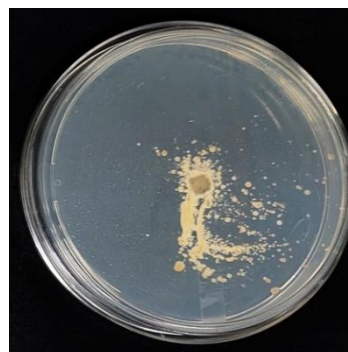
Quantitative estimation of zinc released from zinc oxide, zinc phosphate, zinc carbonate and change in the pH by the fungal isolates were studied seven days after incubation. The results (Table 9) indicated that the amount of zinc released from zinc sources by all the fungal isolates increased with decreased pH.



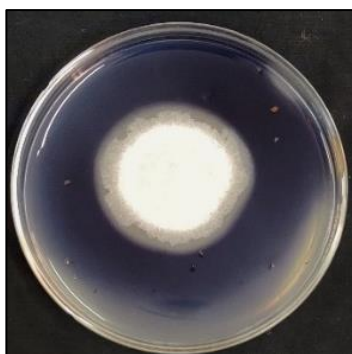
ZnO



$\text{Zn}_3(\text{PO}_4)_2$
ZSFI-3



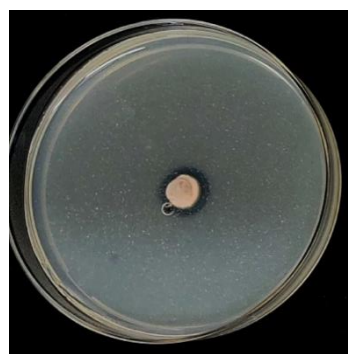
ZnCO_3



ZnO



$\text{Zn}_3(\text{PO}_4)_2$
ZSFI-5



ZnCO_3



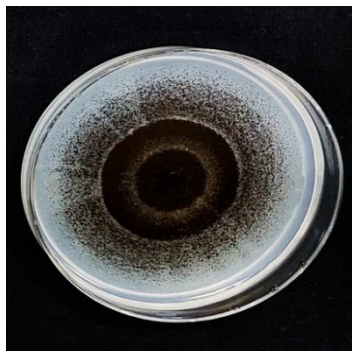
ZnO



$\text{Zn}_3(\text{PO}_4)_2$
ZSFI-7



ZnCO_3



ZnO

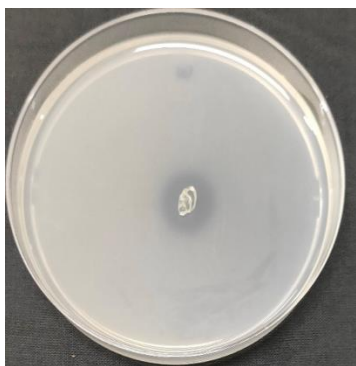


$\text{Zn}_3(\text{PO}_4)_2$
ZSFI-10



ZnCO_3

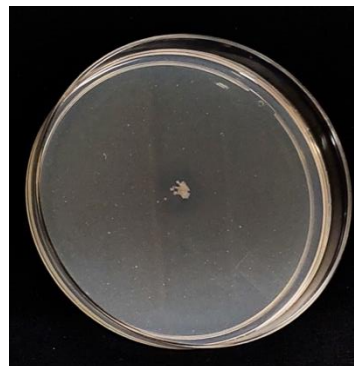
Plate 4: Zinc solubilization by different fungal isolates



ZnO

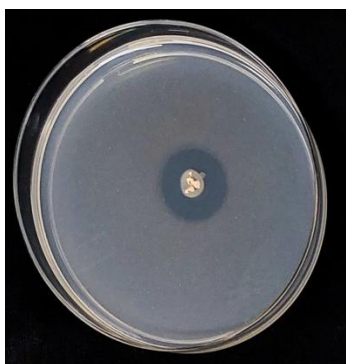


$\text{Zn}_3(\text{PO}_4)_2$

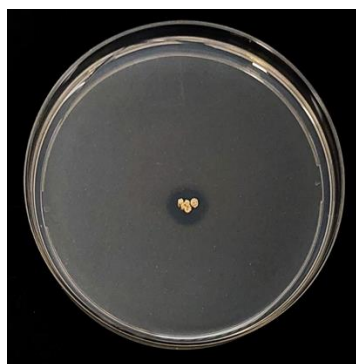


ZnCO_3

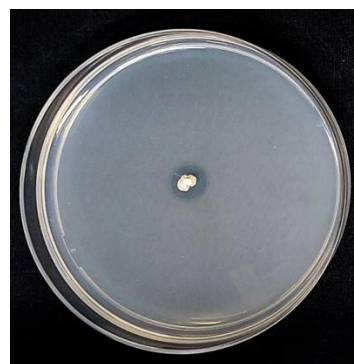
ZSBI-7



ZnO



$\text{Zn}_3(\text{PO}_4)_2$



ZnCO_3

ZSBI-9

Plate 5: Zinc solubilization by bacterial isolates

Among the fungal isolates, ZSFI-4 released the maximum (22.96, 27.23 and 14.27 ppm) amount of zinc from zinc oxide, zinc phosphate, zinc carbonate and least pH (4.23, 4.03 and 4.76) followed by ZSFI-5 (19.65, 23.24, 8.53 ppm) with pH (4.23, 4.03, 4.76) and ZSFI-10 (17.73, 20.40, 12.11) with pH (4.22, 4.03, 4.96) which was significantly different from all the other isolates at seven days after incubation. Other zinc solubilizing fungal isolates were significantly different from each other with respect to the release of zinc and change in the pH.

Table 9: Solubilization of different sources of insoluble Zinc by fungal isolates in broth culture

Isolates	ZnO		Zn ₃ (PO ₄) ₂		ZnCO ₃	
	Available zinc (ppm)	pH	available zinc (ppm)	pH	available zinc (ppm)	pH
ZSFI-1	6.70	6.07	4.08	5.93	1.05	6.30
ZSFI-2	2.02	6.73	5.32	6.13	1.55	6.30
ZSFI-3	15.63	4.92	11.66	4.79	1.33	6.35
ZSFI-4	22.96	4.23	27.23	4.03	14.27	4.76
ZSFI-5	19.65	4.63	23.24	4.13	8.53	5.33
ZSFI-6	8.97	5.93	11.55	5.67	5.21	6.10
ZSFI-7	3.40	6.30	7.90	5.57	1.16	6.40
ZSFI-8	0.91	6.37	0.90	6.60	1.54	6.50
ZSFI-9	0.36	6.43	4.42	5.95	0.33	6.40
ZSFI-10	17.73	4.22	20.40	4.03	12.11	4.96
SE(m)±	0.24	0.07	0.56	0.07	0.34	0.07
C.D.	0.73	0.23	1.68	0.23	1.02	0.22

Qualitative and quantitative estimation of zinc solubilization was done for both bacterial and fungal isolates. Among ten bacterial isolates, ZSBI-2 showed significantly more solubilizing efficiency in agar and broth supplemented with 0.1 per cent of different

insoluble zinc sources viz., ZnO, ZnCO₃ and Zn₃(PO₄)₂. Among Fungal isolates, ZSFI-4 showed significantly high solubilizing efficiency in agar and broth supplemented with 0.1 per cent of different insoluble zinc sources i.e., ZnO, ZnCO₃ and Zn₃(PO₄)₂.

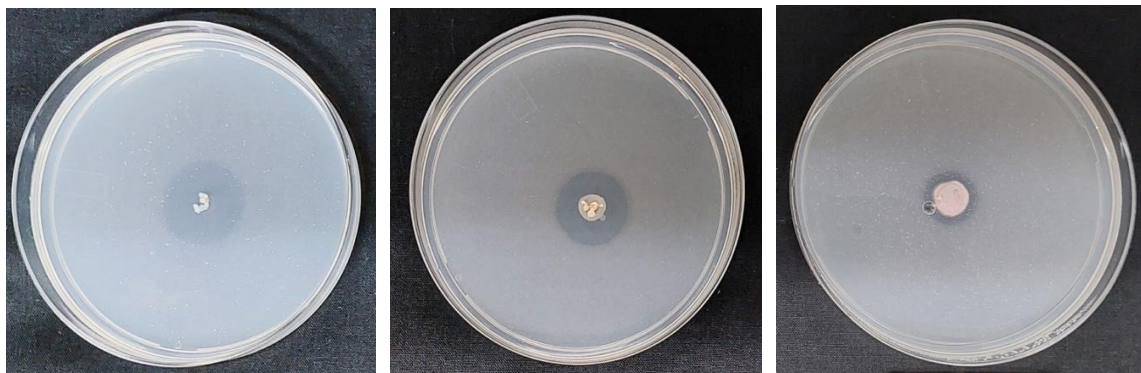
These observations are in agreement with the findings made earlier that among the capable of solubilizing insoluble zinc compounds such as ZnO, ZnCO₃ and Zn₃(PO₄)₂ found to solubilize readily in appreciable amounts (Saravanan *et al.*, 2003, Saravanan *et al.*, 2007, Sarathambal *et al.*, 2010, Panneerselvam *et al.*, 2012, Desai *et al.*, (2012), Panneerselvam *et al.*, 2013 and Ramesh *et al.*, 2014, Anitha *et al.*, 2015). Thus the ability of bacterial and fungal isolates to release Zn largely depends on various parameters like the nature of the zinc compounds, carbon source, temperature, pH and variable concentration of sodium chloride and glucose (Shahab and Ahmed, 2008). The variability among the isolates indicates the importance of the exploration of different zinc solubilizing isolates and understanding their solubilizing mechanisms.

4.3 Molecular characterization of efficient zinc solubilizing microorganisms

After *in-vitro* of all isolates for zinc solubilization, best isolate among bacteria and fungi was selected for molecular characterization and identification. The isolates were identified by 16srRNA and 18s rRNA respectively, which were belongs to *Bacillus subtilis* and *Aspergillus niger*.

Table 10: Molecular characterization of efficient zinc solubilizing microorganisms

Isolate code	Organism identified	Accession number	Closest type strain in the NCBI database	Sequence similarity
ZSBI-2	<i>Bacillus subtilis</i> strain UASB_GHC2	OQ121770	<i>Bacillus subtilis</i> strain DSM 10	99.16%
ZSFI-4	<i>Aspergillus niger</i> strain UASB_GHC4	OQ231494	<i>Aspergillus niger</i> CBS 554.65	99.51%



ZnO

$\text{Zn}_3(\text{PO}_4)_2$

ZnCO_3

Plate 6: Solubilization of zinc by zinc solubilizing bacterial isolate-2 (*Bacillus subtilis* strain UASB_GHC2) in Bunt and Rovira media supplemented with different insoluble zinc source.



ZnO

$\text{Zn}_3(\text{PO}_4)_2$

ZnCO_3

Plate 7: Solubilization of zinc by zinc solubilizing fungal isolate-4(*Aspergillus niger* strain UASB_GHC4) in Bunt and Rovira media supplemented with different insoluble zinc source.

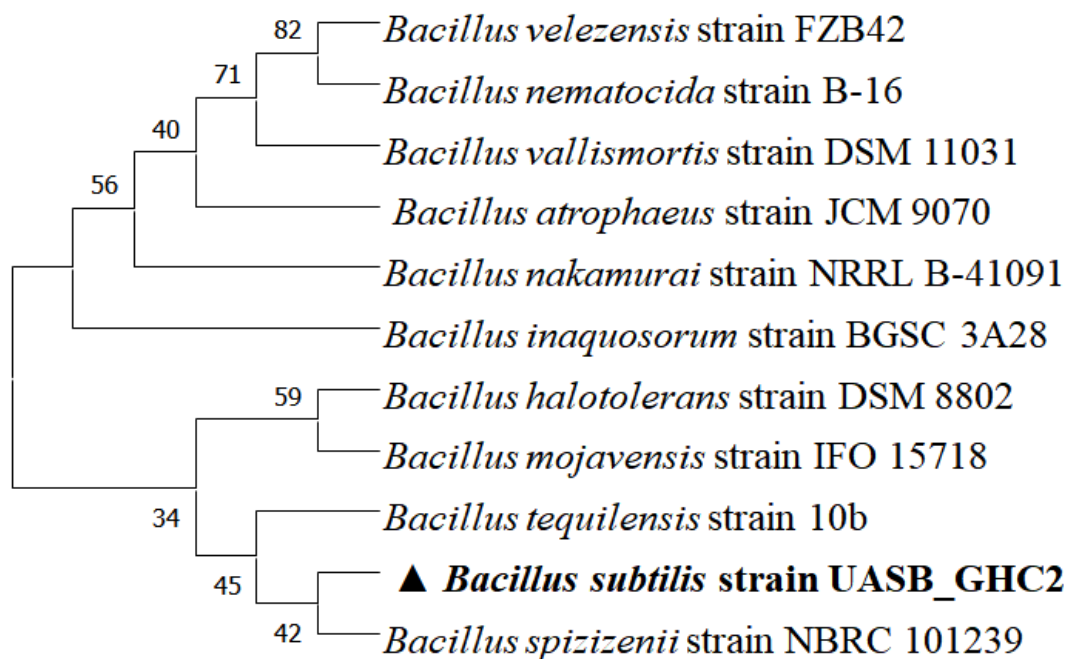


Fig. 1: Phylogenetic tree zinc solubilizing bacterial strain

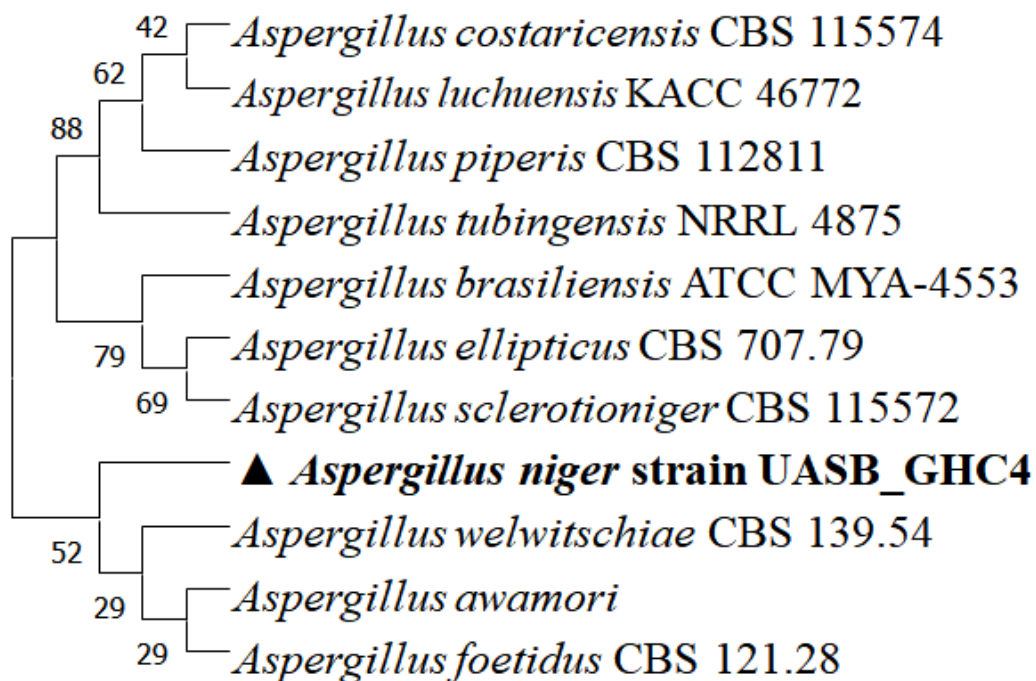


Fig. 2: Phylogenetic tree of zinc solubilizing fungal strain

4.4 Evaluation of efficient zinc solubilizing isolates on growth parameters of aerobic rice under greenhouse condition.

To study the effect of efficient zinc solubilizing isolates on plant growth promotion of aerobic rice (Daksha), a pot culture experiment was conducted and the observation was recorded at 90 days after sowing are presented in Table 11 and Table 12

A critical study of data presented in Table 12 revealed that the height of the aerobic rice increased with the inoculation of zinc solubilizing isolates. The plant height was recorded from 57.2 cm to 72.86 cm. Among these treatments, T5 (72.86cm) showed the highest plant height, followed by T2 (70.70cm), T4 (70.58cm) and T10 (70.22cm). There is no significant difference between treatments T2, T3, T4 and T10. There is a significant difference between the treatments T1 and T6, T2 and T7, T3 and T8, T4 and T9, T5 and T10.

Table 11: Effect of zinc solubilizing microorganisms on number of leaves, number of tillers and plant height per plant of aerobic rice under greenhouse condition at 90 DAS.

Treatments	Plant height (cm)	Number of leaves/plant	Number of tillers/plant
T1	57.20	9.00	4.50
T2	70.70	11.19	6.13
T3	69.54	10.88	5.38
T4	70.58	10.94	5.44
T5	72.86	11.88	7.25
T6	40.16	7.44	2.63
T7	54.51	8.94	5.19
T8	65.83	10.82	5.50
T9	66.51	11.25	5.81
T10	70.22	11.50	6.38
SEm±	0.53	0.61	0.148
CD	1.56	1.76	0.431

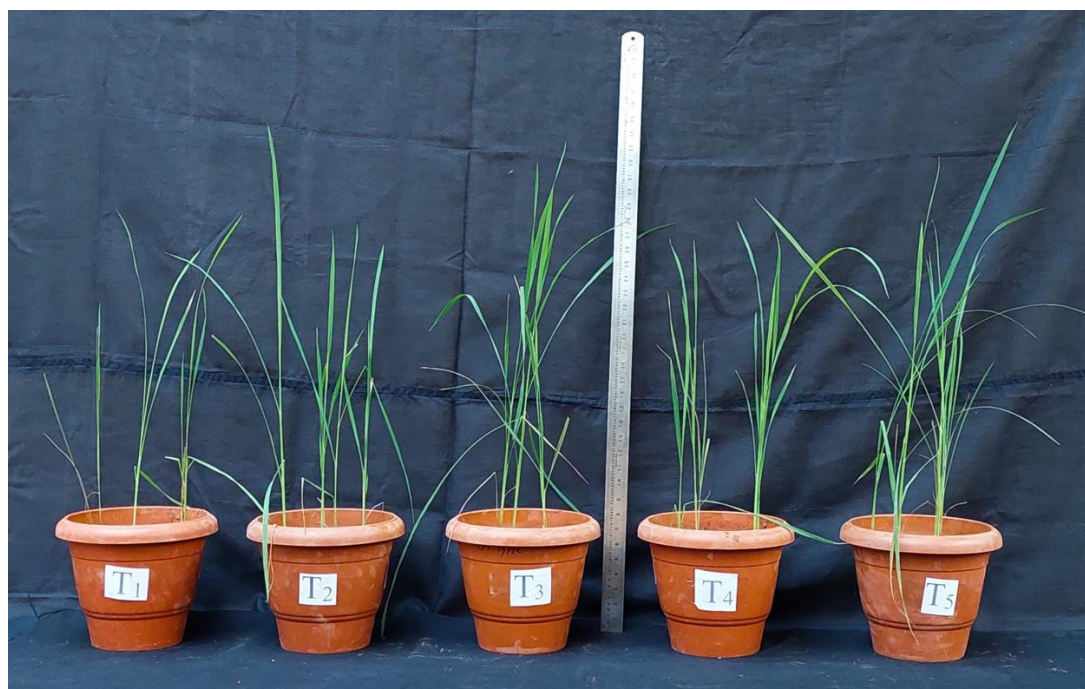


Plate 8: Effect of zinc solubilizing microorganisms on growth parameters of aerobic rice under greenhouse condition 90 DAS

The number of leaves were recorded in the range of 9.00 to 11.88. Among these treatments, T5 (11.88) showed significantly more leaves per plant followed by T10 (11.50), T9 (11.25) and T2 (11.19). These results showed a significant difference from the control T1 (9.00). T1 showed significant difference with T2, T3, T4, T5 and T6 showed a significant difference with T7, T8, T9 and T10. The number of tillers were recorded in the range of 4.50 to 7.25, among these treatments T5 (7.25) followed by T10 (6.38) and T2 (6.13) showed significantly more tillers per plant. T1 to T5 treatments were treated with the recommended dose of Phosphorous and T6 to T10 were not treated with phosphorous. When we compare the same treatments while keeping phosphorous as a variable, Treatments with phosphorous (T1 to T5) showed a significant difference from those without phosphorous (T6 to T10), which shows that phosphorous has an important role in growth parameters.

Data regarding zinc solubilizing microorganisms effect on aerobic rice biomass under greenhouse at 90 days after sowing are presented in Table 12. The result shows that pots with phosphorous biomass of T2, T3, T4 and T5 increased significantly, which were recorded as 0.65, 0.65, 0.67 and 0.75 g per plant, respectively compared to T1 (control) 0.27g per plant. However, T5 (inoculated with both bacterial and fungal strains) showed superior results. Among the pots without phosphorous, the biomass of T7, T8, T9 and T10 increased significantly, which were recorded as 0.27, 0.68, 0.69 and 0.74 g per plant, respectively, compared to control T6 (control) 0.19 g per plant. However, T10 showed superior results.

Treatments inoculated with bacterial isolate (*Bacillus subtilis* strain UASB_GHC2) T3, Fungal isolate (*Aspergillus niger* strain UASB_GHC4) T4 and T2 with the recommended dose of zinc, significantly showed increased values in growth parameters and biomass when compared to T1 (uninoculated with the recommended dose of NPK). However, T5 inoculated with both bacterial and fungal isolates, showed superior results in growth parameters and biomass among the pots treated with phosphorous.

Table 12: Effect of zinc solubilizing microorganisms on biomass of aerobic rice under greenhouse at 90 days after sowing.

Treatments	Dry shoot weight (g)	Dry root weight (g)	Total biomass (g)
T1	0.21	0.06	0.27
T2	0.55	0.10	0.65
T3	0.54	0.11	0.65
T4	0.56	0.11	0.67
T5	0.63	0.13	0.75
T6	0.13	0.05	0.19
T7	0.22	0.05	0.27
T8	0.58	0.10	0.68
T9	0.59	0.11	0.69
T10	0.61	0.13	0.74
SEm±	0.02	0.009	0.027
CD	0.07	0.027	0.079

The phosphorus element plays an important role in transferring energy to plant cells. It encourages root development and early fertilization, strengthens the stem to prevent fall, and increases N uptake at the beginning of growth. The high availability of P in the soil due to the application of P fertilizer can stimulate plant growth and yield (Naomi and Nurmalasari, 2021). Patel and shelek (1999) found that the vegetative growth characteristics (Height of plant, number of secondary and primary branches per plant) of mustard (*B. juncea*) were increased significantly with increasing levels of Phosphorous up to 120 kg per ha.

Among the treatments which were treated without phosphorous, treatments inoculated with bacterial isolate (*Bacillus subtilis* strain UASB_GHC2) T8, Fungal isolate (*Aspergillus niger* strain UASB_GHC4) and T9 with the recommended dose of zinc,

significantly showed increased values in growth parameters when compared to T6 (uninoculated with the recommended dose of NK and 0 % P). However, T10 inoculated with both bacterial and fungal isolates showed superior results in growth parameters.

The greater straw production in fungal and bacterial strain inoculation treatments could be attributed to the solubilization of native inorganic and organic phosphates, as well as the solubilization and uptake of native zinc-bearing minerals by rice. Rice's zinc intake is critical for photosynthesis, nitrogen fixation, root development, flowering, and seed formation. Dubey (1995) also found an improvement in soybean seed and grain production using *Rhizobium* and PSM co-inoculation.

Mumtaza *et al.*, (2017) got positive results in growth promotion of maize by inoculating zinc solubilizing bacterial species *Bacillus* sp. (ZM20), *Bacillus aryabhattai* (ZM31 and S10) and *Bacillus subtilis* (ZM63). Anitha *et al.*, (2015), Devi *et al.*, (2022) isolated *Aspergillus* sp. as zinc solubilizers. Panneerselvam *et al.*, (2012) and Panneerselvam *et al.*, (2013) reported a significant increase in growth promotion, viz., seedling height, total biomass and total leaf area in sapota and leaf, stem, shoot, root, total biomass and total leaf area in guava seedlings co-inoculated with *Glomus mosseae* and zinc solubilizing mycorrhiza associated bacteria. Similarly, Uzma *et al.*, (2010) recorded a maximum increase in all plant growth parameters in *Vigna radiata* when seedlings were inoculated with isolate 102. Isolate 36 with 1 mM zinc phosphate showed the maximum increase in seedling length (35.1 cm) compared to controls. Natheer and Muthukkaruppan (2012) reported that the inoculation of *G. diazotrophicus* isolates with different Zn compounds (ZnO, ZnCO₃, and ZnSO₄) will increase the plant growth of sugarcane.

4.5 Evaluation of efficient zinc solubilizing isolates on zinc uptake by aerobic rice under greenhouse condition.

Data regarding the effect of zinc solubilizing isolates on DTPA (diethylene tri-amine penta- acetic acid) extractable soil zinc content (mg kg⁻¹) in soil presented in the Table 13. Among the treatments, with phosphorous results show that at 90 days after sowing DTPA extractable soil zinc content (mg kg⁻¹) has increased significantly in both treatments T3 (*Bacillus subtilis* strain UASB_GHC2) and T4 (*Aspergillus niger* strain

UASB_GHC4) which are estimated to be 3.83 mg per kg and 4.06 mg per kg when compared to T1(uninoculated). Similarly on DTPA extractable soil, zinc content has significantly increased in the soil treated with T2 having zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. which was estimated as 3.64 mg per kg compared to T1(control) 2.75 mg per kg. DTPA extractable zinc significantly decreased in T5 (*Bacillus subtilis* strain UASB_GHC2+ *Aspergillus niger* strain UASB_GHC4) which was estimated to be 3.84 mg per kg compared to T4.

Among the treatments without phosphorous, results show that at 90 DAS DTPA extractable soil zinc content has increased significantly in treatments T8 (*Bacillus subtilis* strain UASB_GHC2), T9 (*Aspergillus niger* strain UASB_GHC4) and T10 (*Bacillus subtilis* strain UASB_GHC2+ *Aspergillus niger* strain UASB_GHC4) which were estimated as 3.73 mg per kg, 3.90 mg per kg and 4.30 mg per kg as compared to T6 (uninoculated) 2.18 mg per kg. Similarly DTPA extractable soil zinc content has significantly increased in the soil of T7 treated with zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ which was estimated as 3.62 mg per kg compared to T6 (control) 2.18 mg per kg.

When treatments with phosphorous (T1 to T5) compared to without phosphorous treatments (T6 to T10) result shows that DTPA extractable zinc is more in the treatments with phosphorous except in T5. T10 (without phosphorous) treatment showed significantly high zinc content compare to T5 (with phosphorous), it may be due zinc and phosphorous often interact negatively with each other in soil plant systems, and a few mechanisms for such interactions have been proposed by Sanchez-Rodriguez *et al.*, 2017. P induced Zn deficiency is due to negative interaction between Zn and P in the soil. The reason for such increment of available phosphorous in soil is due to the fact that zinc solubilizing microbes are good solubilizers of phosphorous (Dahaji *et al.*, 2012 and Desai *et al.*, 2012). Reddy *et al.*, (2002) isolated three isolates of *Aspergillus tubingensis* and two isolates of *Aspergillus niger* as phosphate solubilizers. *Bacillus subtilis* strain PH was reported as phosphate solubilizer in the study conducted by Mohamed and Azza, 2018, which was in co-ordinance with our conducted study.

Table 13: Effect of zinc solubilizing isolates on available Zn (mg kg⁻¹) in soil 90 DAS under greenhouse.

Treatments	Soil available zinc (mg kg⁻¹)
T1	2.75
T2	3.64
T3	3.83
T4	4.06
T5	3.84
T6	2.18
T7	3.62
T8	3.73
T9	3.90
T10	4.30
SEm±	0.063
CD	0.184

The effect of zinc solubilizing microorganisms on Zn uptake in aerobic rice is presented in Table 14. Among the treatments with phosphorous, results show that at 90 DAS zinc concentration per plant has increased significantly in both treatments T3 (*Bacillus subtilis* strain UASB_GHC2) and T4 (*Aspergillus niger* strain UASB_GHC4) which were estimated as 44.35 mg per kg and 45.05 mg per kg when compared to T1(uninoculated). Similarly zinc concentration has significantly increased in T2, treated with zinc source ZnSO₄.7H₂O, which was estimated as 42.68 mg per kg compared to T1(control) 34.23 mg per kg. Zinc concentration decreased significantly in T5 (*Bacillus subtilis* strain UASB_GHC2 + *Aspergillus niger* strain UASB_GHC4) which was estimated as 31.50 mg per kg compared to T1 (control).

Among treatments without phosphorous, results show that zinc concentration has increased significantly in treatments T8 (*Bacillus subtilis* strain UASB_GHC2), T9 (*Aspergillus niger* strain UASB_GHC4) and T10 (*Bacillus subtilis* strain UASB_GHC2 + *Aspergillus niger* strain UASB_GHC4) which were estimated as 41.73 mg per kg , 43.95 mg per kg and 48.63 mg per kg as compared to T6 (uninoculated) 25.13 mg per kg. Similarly zinc content has significantly increased in T7 treated with zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, which was estimated as 32.18 mg per kg compared to T6 (control) 25.13 mg per kg.

Table 14: Effect of zinc solubilizing microorganisms on Zn uptake in aerobic rice 90 DAS

Treatments	Zinc concentration/plant (mg kg ⁻¹)	Uptake (g/plant)
T1	34.23	0.009
T2	42.68	0.028
T3	44.35	0.029
T4	45.05	0.030
T5	31.50	0.024
T6	25.13	0.005
T7	32.18	0.009
T8	41.73	0.028
T9	43.95	0.031
T10	48.63	0.036
SEm±	0.314	0.001
CD	0.910	0.003

When the treatments with phosphorous (T1 to T5) compared to without phosphorous treatments (T6 to T10), result showed that zinc uptake is more in the

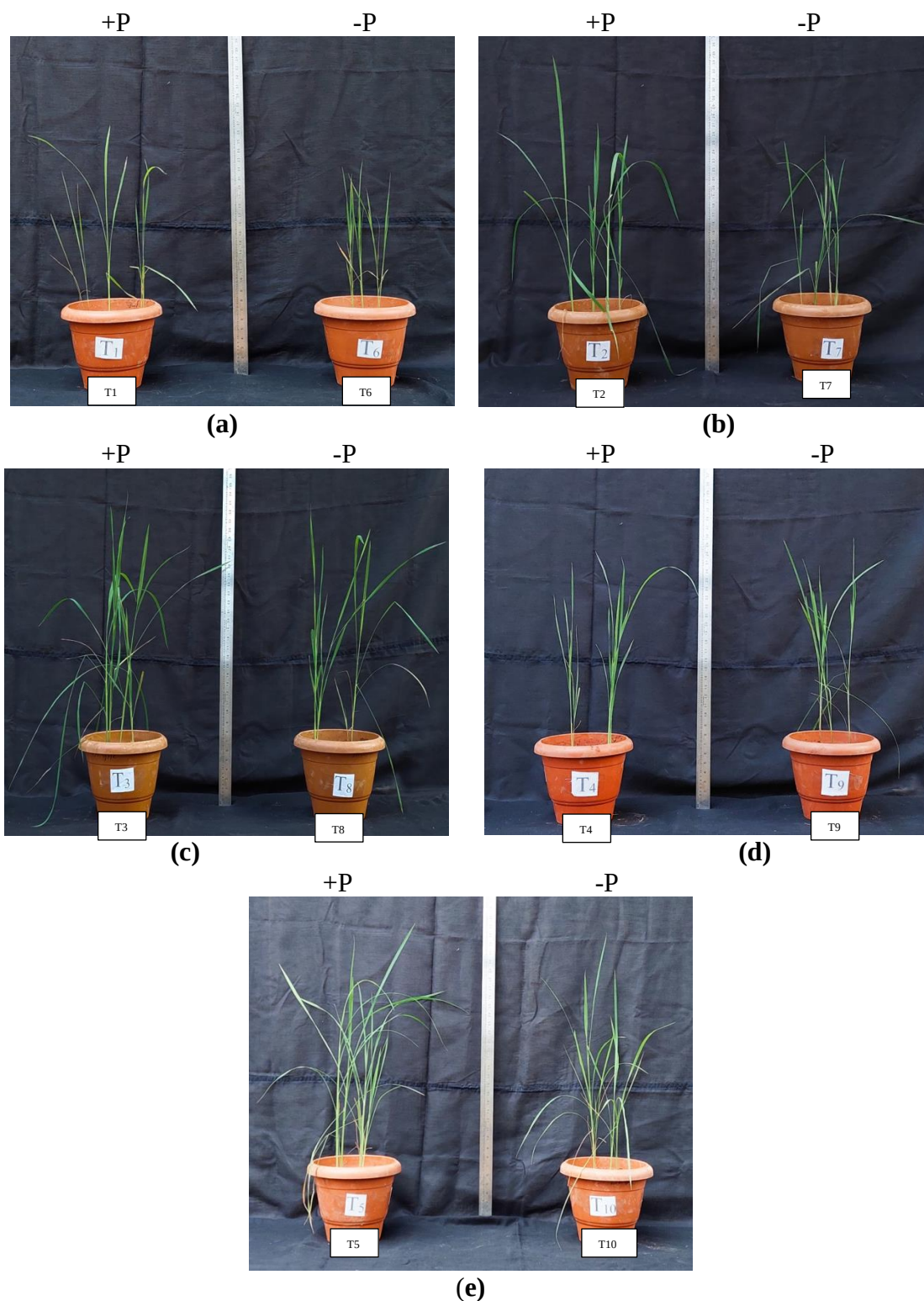


Plate 9: Effect of phosphorus on zinc solubilization by zinc solubilizing bacterial and fungal isolates

+P- With phosphorus, **-P** Without phosphorus, **a-** Control, **b-** Treated with RD of zinc, **c-** Inoculated with bacterial isolate, **d-** Inoculated with fungal isolate and **e-** Inoculated with both bacterial and fungal isolate.

treatments with phosphorous except in T5. T10 (without phosphorous) shows significantly high zinc content compared to T5 (with phosphorous). Zinc solubilizing microbes are also good solubilizers of phosphorous (Dahaji *et al.*, 2012 and Desai *et al.*, 2012). Reddy *et al.*, 2002, isolated three isolates of *Aspergillus tubingensis* and two isolates of *Aspergillus niger* as phosphate solubilizers. The results were similar to study conducted by Mohamed *et al.*, where *Bacillus subtilis* strain PH reported as phosphate solubilizer.

Within plants, phosphorous enhanced growth likely causes 'dilution' of the shoot zinc, loss of tight control over P uptake by genes encoding high-affinity P transporter proteins under Zn deficiency will result in increased P accumulation and further aggravate Zn deficiency (Huang *et al.*, 2000, Sanchez-Rodriguez *et al.*, 2017, Zhu *et al.*, 2001). Zinc and phosphorous often interact negatively with each other in soil plant systems and a few mechanisms for such interactions have been proposed (Sanchez-Rodriguez *et al.*, 2017). Although zinc and phosphorous are retained in soil via different mechanisms in different amounts, by minerals. The mechanisms and amounts vary between different types of soil, high doses of Phosphorous in most cases, affects Zn absorption and reduce plant uptake of Zn (Chen *et al.*, 2019). Excessive application of P results in reduced shoot and grain (Zn) and crop yields (Akhtar *et al.*, 2019, Barben *et al.*, 2011, Zhang *et al.*, 2017). It is yet not clear whether 'P-induced Zn deficiency' is due to the negative interaction between Zn and P in the soil or in the plant. Precipitation of soluble P and Zn (Zn^{2+}) in the soil as $Zn_3(PO_4)_2$ can make both Zn and P unavailable for plants, precipitation of $Zn_3(PO_4)_2$ may also occur in roots and restrict the translocation of Zn from roots to shoots and to the grains (Pongrac *et al.*, 2019 and Zhang *et al.*, 2012).

The growth of paddy, nutrient concentrations and uptake of both Phosphorous and Zinc were all reported to be negatively correlated in Singh and Singh's (1980) study. When P was not added, the weight of the straw and grain increased by 5 and 10 mg of Zn per kilogramme of soil, but only by 5 mg of Zn per kg of soil when P was introduced.

Haldar and Mandal (1981) reported that application of phosphorous and zinc at all the levels significantly increased the dry matter yield of shoots, roots and grains. The increase was highest when both P and Zn were combined with each other at their respective

highest levels, with the mean increase of shoots, roots and grains in the P2Zn2 treatments being 35, 39 and 25 percent respectively over that in the control (P0Zn0). The increase in dry matter yield resulting from combined application of phosphorous and zinc might be due to their beneficial effect on metabolism of plants.

Soltangheisi *et al.*, 2013 conducted study in sweet corn using pot culture experiments containing all combinations of Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) at levels of 0.0, 5.0 and 10.0 mg per kg soil and P(KH_2PO_4) at levels of 0.0, 50.0, 100.0 and 200.0 mg per kg soil, later crop was harvested at 28 days after transplanting. Dry matter yield increased with phosphorous supply, while zinc application did not show any significant effect on this parameter. The zinc and phosphorous uptake by shoots increased with increasing zinc and phosphorous application into the soil. The zinc concentration in shoots decreased with increasing phosphorous supply, but phosphorous concentration and its uptake enhanced indicating phosphorus induced zinc deficiency is due to dilution effect.

Thus, the present study proved that inoculation of zinc solubilizing microorganisms is essential for enhancing growth parameters and Zn concentration in plants with recommended dose of fertilizers. Phosphorous plays a very important role in zinc uptake of crops. The results of this experiment suggest that high phosphorous uptake may inadvertently depress Zn uptake and tissue Zn concentration in crops.

SUMMARY

V SUMMARY

Zinc is a crucial micronutrient for enhancing agricultural yield and reducing human malnutrition, thus ensuring nutritional security. There are several strategies that support raising dietary zinc intake in order to combat malnutrition. An example of such an intervention is the exploration of soil organisms that could mobilize unavailable zinc, accelerate zinc uptake and enhance plant growth and productivity. To boost the availability of native zinc for crop assimilation, beneficial rhizosphere microorganisms can be utilized as bio-inoculants, this not only accomplishes the goal of low input and sustainable agriculture, but it is also a more practical solution to address zinc malnutrition in human society. Therefore, it is feasible that using native zinc mineralizing and solubilizing microorganisms can alleviate the zinc deficit and maximize the zinc availability to crops. Considering all these factors, efforts were undertaken in the current work to identify and characterize efficient isolates that solubilize zinc. Some bacterial and fungal isolates were chosen and evaluated for their ability to raise the zinc content in aerobic rice grown in a greenhouse.

Ten fungal and bacterial isolates were selected based on morphological characters out of 40 fungal and 20 bacterial isolates, which were isolated from the soils of the Western Ghats of Karnataka. Morphological characterization of bacterial and fungal isolates was done. The zinc solubilizing bacterial isolates colonies were observed as creamy-dense white to yellowish in colour, slimy, mucoid with opaque, circular to irregular, smooth, tiny in texture or dry in texture on the agar medium. A comprehensive study of the ten bacterial isolates showed that ZSBI-2, ZSBI-5, ZSBI-7 and ZSBI-10 are positive in Gram reactions and endospore production. The other isolates are negative for Gram reaction. The morphological parameters were studied by growth on different media, colony colour, colony characteristics and sporulation of all isolated fungi.

In Qualitative assay (solubilization zone) of the selected zinc solubilizing bacterial and fungal isolates for zinc solubilization after seven days of incubation, maximum solubilizing efficiency among bacterial isolates was shown by ZSBI-2 with all three sources of zinc (350%, 90% and 158.17% respectively for zinc oxide, zinc phosphate and

zinc carbonate) which was significantly higher than other bacterial isolates. Among fungal isolates, ZSFI-4 showed maximum solubilizing efficiency with all three sources of zinc (303.70%, 466.6% and 193.33% respectively for zinc oxide, zinc phosphate and zinc carbonate).

The quantitative estimation of zinc released from zinc oxide, zinc phosphate, zinc carbonate and change in the pH by the isolates were studied seven days after incubation. Among the bacterial isolates, ZSBI-2 released the maximum (29.67, 19.323 and 25.43 ppm) amount of zinc from zinc oxide, zinc phosphate, zinc carbonate and least pH (3.43, 4.23 and 3.93), which was significantly different from all the other bacterial isolates at seven days after incubation. Among fungal isolates, ZSFI-4 released the maximum (22.96, 27.23 and 14.27 ppm) amount of zinc from zinc oxide, zinc phosphate, zinc carbonate and least pH (4.23, 4.03 and 4.76), which was significantly different from all the other fungal isolates.

Based on qualitative and quantitative analysis, ZSBI-2 and ZSFI-4 were selected as efficient zinc solubilizers among bacterial and fungal isolates, respectively for further studies. Upon subjecting ZSBI-2 and ZSFI-4 to molecular characterization, they were identified as *Bacillus subtilis* and *Aspergillus niger*, respectively.

The effect of zinc solubilizing microorganisms on growth parameters and dry matter among the treatments which were treated with phosphorous T5 showed superior results in the number of leaves (11.88/plant), number of tillers (7.25/plant), plant height per plant (72.86cm/plant) and biomass (0.75g/plant). Among the treatments which were treated without phosphorous, T10 showed superior results in number of leaves (11.50/plant), number of tillers (6.38/plant), plant height per plant (70.22cm/plant) and biomass (0.74g/plant).

The effect of zinc solubilizing isolates on DTPA (diethylene tri-amine penta- acetic acid) extractable soil zinc content (mg kg^{-1}) in soil, among the treatments with phosphorous soil available zinc content (mg kg^{-1}) has increased significantly in both treatments T3 (*Bacillus subtilis* strain UASB_GHC2) and T4 (*Aspergillus niger* strain UASB_GHC4)

which were estimated to be 3.83 mg per kg and 4.06 mg per kg when compared to T1(uninoculated). Similarly, on DTPA extractable soil, zinc content has significantly increased in the soil treated with T2 having zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, which was estimated as 3.64 mg per kg compared to T1(control) 2.75 mg per kg. DTPA extractable zinc significantly decreased in T5 (*Bacillus subtilis* strain UASB_GHC2 + *Aspergillus niger* strain UASB_GHC4), which was estimated to be 3.84 mg per kg compared to T4. Among the treatments without phosphorous, soil zinc content has increased significantly in treatments T8 (*Bacillus subtilis* strain UASB_GHC2), T9 (*Aspergillus niger* strain UASB_GHC4) and T10 (*Bacillus subtilis* strain UASB_GHC2 + *Aspergillus niger* strain UASB_GHC4), which were estimated as 3.73 mg per kg, 3.90 mg per kg and 4.30 mg per kg respectively, as compared to T6 (uninoculated) 2.18 mg per kg. Similarly, soil available zinc content has significantly increased in the soil of T7 treated with zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ which was estimated as 3.62 mg per kg compared to T6 (control) 2.18 mg per kg. T10 showed superior results in soil zinc content compared to all other treatments.

In the study on the effect of zinc solubilizing microorganisms on Zn uptake in aerobic rice 90 DAS among the treatments with phosphorous, zinc concentration per plant has increased significantly in both treatments T3 (*Bacillus subtilis* strain UASB_GHC2) and T4 (*Aspergillus niger* strain UASB_GHC4) which were estimated as 44.35 mg per kg and 45.05 mg per kg when compared to T1(uninoculated). Similarly, zinc concentration has significantly increased in T2, treated with zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, which was estimated as 42.68 mg per kg compared to T1(control) 34.23 mg per kg. Zinc concentration decreased significantly in T5 (*Bacillus subtilis* strain UASB_GHC2 + *Aspergillus niger* strain UASB_GHC4), which was estimated as 31.50 mg per kg compared to T1 (control). Among treatments without phosphorous, results show that zinc concentration has increased significantly in treatments T8 (*Bacillus subtilis* strain UASB_GHC2), T9 (*Aspergillus niger* strain UASB_GHC4) and T10 (*Bacillus subtilis* strain UASB_GHC2 + *Aspergillus niger* strain UASB_GHC4) which were estimated as 41.73 mg per kg, 43.95 mg per kg and 48.63 mg per kg as compared to T6 (uninoculated) 25.13 mg per kg. Similarly, zinc content has significantly increased in T7 treated with zinc source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, which was estimated as 32.18 mg per kg compared to T6 (control) 25.13 mg per kg.

It can be concluded that, when treatments with phosphorous (T1 to T5) were compared to those without phosphorous treatments (T6 to T10), zinc uptake was more in the treatments with phosphorous except in T5. T10 (without phosphorous) shows significantly high zinc content compared to T5 (with phosphorous). The results of this experiment suggest that high phosphorous uptake may inadvertently depress Zn uptake and tissue Zn concentration in plants.

Future line of work

- ❖ Isolation and characterization of potential ZSM from different Agro-ecological zones and cropping system.
- ❖ Molecular characterization of zinc solubilizing isolates.
- ❖ Evaluation of ZSM isolates for relation between zinc and other major and minor nutrients.

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APPENDICES

APPENDIX – I

Composition of stains

1. Gram stain

i) Crystal violet

Solution A

Crystal violet (90 % dye content)	:	2.0 g
Ethyl alcohol (95 %)	:	20 mL

Solution B

Ammonium oxalate	:	0.8 g
Distilled water	:	80 mL

Dissolve crystal violet in ethyl alcohol and the ammonium oxalate in distilled water, mix solution A and B.

ii) Gram's Iodine

Iodine	:	1.0 g
Potassium iodide	:	2.0 g
Distilled water	:	300.0 mL

Dissolve iodine and potassium iodide in distilled water.

iii) Ethyl alcohol (95 %)

Ethyl alcohol (100 %)	:	95.0 mL
Distilled water	:	5.0 mL

iv) Safranin

Safranin (2.5 % solution in 95 %)

Ethyl alcohol (100%)	:	10.0 mL
Distilled water	:	100.0 mL

2. Malachite green

Malachite green	:	05.00 g
Distilled water	:	100 mL

APPENDIX – II

1. Bunt and Rovira medium containing (Bunt and Rovira, 1955)

Glucose	: 10 g
ammonium sulphate	: 1 g
potassium chloride	: 0.2 g
di-potassium hydrogen phosphate	: 0.1 g
magnesium sulphate	: 0.2 g
agar	: 15 g
Zn source	: 0.1%
Distilled water	: 1000 mL
pH	: 7

2. Nutrient Agar

Peptone	: 5.0 g
Beef Extract	: 3.0 g
NaCl	: 5.0 g
Agar	: 18.0 g
Distilled water	: 1000 ml
pH	: 6.8-7.2

3. Potato Dextrose Agar

Potatoes (peeled and diced into small pieces)	: 200.0 g
Dextrose	: 20.0 g
Agar	: 15.0 g
pH	: 5.6±0.2

APPENDIX – III

Sequencing detail of bacterial isolate

1. *Bacillus subtilis* strain UASB_GHC2 (ZSBI-2)

TGCAAGATCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGACGGTGAGTA
ACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCG
GATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGCTACCACTTACAGA
TGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGT
AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGT
GAGTGATGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTC
GAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCG
CAGGCGGTTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGA
AACTGGGGAACTTGAGTGCAGAAGAGGGAGAGTGAAATTCCCCGTGTAGCGGTGAAATG
CGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTCTTGGTCTGTAAC TGACGCT
GAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
CGATGAGTGCTAAGTGTTAGGGGGTTTTCCGCCCCCTTAGTGCTGCAGCTAACGCATTAAG
CACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT
GACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCA
ACCCTTGATTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAA
CCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGGCTACACAC
GTGCTACAATGGACAGAACAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAA
TCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGT
AATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTTACACACCGGCCGTC
ACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTAGGAGCCAGCCGC
CAAGTG

2. *Aspergillus niger* strain UASB_GHC4 (ZSFI-4)

GGGGAAAGCCGGGCTGTTCTAGTATAAGGCACTTTATACTGTGAAACTGCGAATGGCTC
ATTAAATCAGTTATCGTTTATTTGATAGTACCTTACTACATGGATACCTGTGGTAATTC
TAGAGCTAATACATGCTGAAAACCTCGACTTCGGAAGGGGTGTATTTATTAGATAAAAA
ACCAATGCCCTTCGGGGCTCCTTGGTGAATCATAATAACTTAACGAATCGCATGGCCTT
GCGCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGG
CCTACCATGGTGGCAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTG
AGAAACGGCTACCACATCCAAGGAAGGCGCAGGCGCGCAAATTACCCAATCCCGACACG
GGGAGGTAGTGACAATAAATACTGATACGGGGCTCTTTTGGGTCTCGTAATTGGAATGA
GTACAATCTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTT
GAACCTTGGGTCTGGCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTT
TCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGAACCAGGACTTTTACTGT
GAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAG
AATAGGACGTGCGGTTCTATTTTGTGTTTCTAGGACCGCCGTAATGATTAATAGGGA
TAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACT
AACTACTGCGAAAGCATTGCGCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGG
GATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATC
GGACGGTGTTTTCTATTATGACCCGTTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTT
CTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCA
GGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA
AATAAGGATTGACAGATTGAGAGCTCTTCTTGATCTTTTGGATGGTGGTGCATGGCCG
TTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTCGGCCC
TTAAATAGCCCCGGTCCGCATTTGCGGGCCGCTGGCTCTTAGGGGACTATCGCCCAAGCC
CGGAGCCATCC