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ZINC SOLUBILIZING BIOFERTILIZER TECHNOLOGY FOR MAJOR CROPS OF MAHARASHTRA

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CONTENT

Background

Zinc is one of the essential elements required for growth and metabolism of microorganisms and plants. Zinc has an immense role in nutrition of both eukaryotic and prokaryotic organisms as cofactor or metal activator in various enzyme systems. Zinc is critical for several key enzymes in the plant. Zinc binds tightly to Zn-containing essential metabolites in vegetative tissues e.g., Zn activated enzymes such as carbonic anhydrase. Zinc plays a key role in plants with enzymes and proteins involved in carbohydrate metabolism, protein synthesis, gene expression, auxin metabolism, pollen formation, maintenance of biological membranes, protection against photo-oxidative damage and heat stress and resistance to infection by certain pathogens.

Zinc is deficient in Indian soils with an average of about 40 per cent.The water soluble zinc applied as zinc sulphate gets transformed into different forms like Zn (OH) and Zn $(OH)_2$at a pH of 7.7 and 9.1, $ZnCO_3$ in calcium rich alkali soils and Zn $(PO_4)_2$ in near neutral to alkali soils of high phosphorus application and zinc sulphide under reduced condition. About 75 % exogenous application of zinc sources like $ZnSO_4$ get fixed in soil. Fixation of zinc in soils with pH more than 7.0 increases with increasing concentration of carbonates, thus become unavailable and can be reverted back to available form with zinc solubilizers.

Zinc solubilizers have a potential to solubilize zinc from insoluble complexes by different processes, one of which is acidification. Theses zinc solubilizing microbes produce organic acids in soil which sequester the zinc cation and production of organic acids reduces the pH of surrounding soil. Besides, the anions can also chelate zinc and increase the solubility of zinc. Other possible pathways for zinc solubilization involved siderophore production and proton, cell membrane oxidoreductive structures and chelated ligends. Various PGPRs, when inoculated with seed, have shown increased growth and zinc content. PGPRs include strains of *Rhizobium*, strains of *Bacillus* and *Azospirillum. Gluconacetobacter diazotrophicus, Burkholderia cenocepacia, Pseudomonas strita, Pseudomonas fluorescens, Serratia liquefaciens and Bacillus thuringiensis* have been reported to show zinc solubilization on laboratory scale.

Zinc solubilizing biofertilizer technology developed under ICAR - All India Network project on Soil Biodiversity - Biofertilizers (AINP-SSB) at VNMKV, Parbhani is widely used in Maharashtra. The technical bulletin highlights production of zinc solubilizers , quality and field evaluation. The Zinc solubilizing biofertilizers improves crop yield by 4.17% to 56.82%. The products is commercially produced and marketed to farmers. During 2016-2022 revenue generated was Rs. 49.97 lakhs. The technology benefitted to 3260 farmers. The technology has been transferred to two entrepreneurs (M/s Microbial Solutions Pvt. Ltd. Hingoli and Atmanirbhar Baliraja Agri Business Pvt. Ltd., Jintur, Maharashtra) and marketed through 4 KVKs.

Isolation of Zinc Solubilizing Bacteria (*ZSB*)

1. 1 g rhizosphere soils suspended in 9 ml sterile water and mix vigorously.
2. Serial dilution of the sample and dilutions are restricted to 10^{-4} or 10^{-5}
3. Sterilized media is poured in petri plates and allowed for solidification.
4. Using sterile pipettes or micro tip 0.1 ml of each dilution is transferred aseptically to the petri plates containing solid media and spread uniformly with spreader.
5. Petri plates are incubated in BOD incubator at 30 ± 1ºC for 3-5days.

Media for growth of Zinc Solubilizing Bacteria (Modified Pikovskaya's medium)

Composition	Quantity (g/L)
Glucose	10.0
$ZnO/ZnCO_3/Zn_3(PO4)_2$	1.00
$(NH_4)SO_4$	0.50
KCL	0.20
$MgSO_4$	0.10
$FeSO_4$	0.005
$MnSO_4$	0.005
Yeast extract	0.50
pH	7.00
Agar	15-20

Zinc solubilization potential of microorganisms

Colony diameter

Inoculation of *Pseudomonas striata* strain in modified PKV medium shows significantly higher colony diameter in ZnO, $ZnCO_3$ and Zn $(PO)_2$ as insoluble zinc source (1.35 cm, 1.34cm and 1.69 cm respectively).

Clearing zone

PKV medium with inoculation of *Pseudomonas striata* and insoluble zinc source like ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ shows significantly higher size of clearing zone (2.34 cm, 2.43 and 2.11 cm respectively).

Halozone diameter

PKV medium along ZnO, $ZnCO_3$ and $Zn_3(PO_2)_4$and inoculated by *Pseudomonas striata* shows significantly highest halazone diameter (3.55 cm, 3.78cm and 3.80 cm).

Solubilization index and solubilization efficiency

Highest solubilization index and solubilization efficiency in modified PKV medium using ZnO, $ZnCO_3$ and $Zn_3(PO_2)_4$as insoluble zinc sources was found with inoculation of strain *Pseudomonas striata* (3.63 ZNI; 263.20%, 3.82 ZNI; 281.64 % and 3.25 ZNI; 225.38% by using ZnO, $ZnCO_3$ and $Zn_3(PO_2)_4$ sources, respectively).

Zinc solubilization activity of various microorganisms under different insoluble zinc sources on solid media (ZnO)

Microorganisms	ZnO				
	Clearing zone (cm)	Colony diameter (cm)	Halozone diameter (cm)	Solubilization index (ZNI)	Solubilization efficiency (%)
Bacillus *subtilis*	0.63	1.05	1.88	2.79	178.78
Bacillus licheniformis	0.85	1.20	2.25	2.87	187.25
Bacillus megaterium	1.76	1.28	3.34	3.61	261.37
Bacillus thuringiensis	0.56	1.08	1.84	2.70	169.81
Pseudomonas fluorescens	0.83	1.11	2.14	2.94	193.69
Pseudomonas striata	2.34	1.35	3.55	3.63	263.20
Trichoderma viride	1.59	1.25	3.18	3.54	253.51
Trichoderma herzenium	0.55	1.01	1.77	2.75	174.52
Azotobacter chroococcum	0.73	1.10	2.03	2.86	186.31
Azospirillumlipoferum	0.65	1.04	1.81	2.72	171.54

Zinc solubilization activity of various microorganisms under different insoluble zinc sources in solid media ($ZnCO_3$)

Microorganisms	$ZnCO_3$				
	Clearing zone (cm)	Colony diameter (cm)	Halozone diameter (cm)	Solubilization index (ZNI)	Solubilization efficiency (%)
Bacillus *subtilis*	0.84	0.96	1.80	2.87	186.56
Bacillus licheniformis	1.13	1.09	2.22	3.04	203.74
Bacillus megaterium	2.29	1.29	3.57	3.78	277.99
Bacillus thuringiensis	0.71	0.77	1.47	2.92	192.22
Pseudomonas fluorescens	1.00	1.02	2.03	2.98	197.85
Pseudomonas striata	2.43	1.34	3.78	3.82	281.64
Trichoderma viride	2.10	1.25	3.35	3.69	268.54
Trichoderma herzenium	0.67	0.76	1.44	2.88	188.42
Azotobacter chroococcum	0.87	0.92	1.78	2.95	194.93
Azospirillumlipoferum	0.72	0.85	1.57	2.86	185.67

Zinc solubilization activity of various microorganisms under different insoluble zinc sources in solid media ($Zn_3(PO_4)_2$)

Microorganisms	$Zn_3(PO_4)_2$				
	Clearing zone (cm)	Colony diameter (cm)	Halozone diameter (cm)	Solubilization index (ZNI)	Solubilization efficiency (%)
Bacillus *subtilis*	0.84	1.08	1.92	2.78	178.28
Bacillus licheniformis	1.18	1.23	2.41	2.96	196.28
Bacillus megaterium	1.93	1.65	3.58	3.17	217.18
Bacillus thuringiensis	0.75	1.05	1.79	2.72	171.65
Pseudomonas fluorescens	1.02	1.15	2.17	2.89	188.67
Pseudomonas striata	2.11	1.69	3.80	3.25	225.38
Trichoderma viride	1.62	1.50	3.12	3.08	208.48
Trichoderma herzenium	0.69	0.98	1.66	2.70	170.41
Azotobacter chroococcum	0.96	1.07	2.03	2.90	189.87
Azospirillumlipoferum	0.75	0.95	1.70	2.79	178.81

Zinc oxide

Pseudomonas striata

Bacillus megaterium

Trichoderma viride

Azospirillum lipoferum

Zinc carbonate

Pseudomonas striata

Bacillus megaterium

Trichoderma viride

Azospirillum lipoferum

Zinc phosphate

Pseudomonas striata

Bacillus megaterium

Trichoderma viride

Azospirillum lipoferum

Large Scale Production of Liquid *Zinc Solubilizing* Biofertilizers

Liquid zinc solubilizing biofertilizer production technology includes isolation or procurement of efficient bacterial strains for required purpose, selection of suitable effective strain, preparation of mother culture, broth preparation, addition of cell protectants and their mixing, followed by packaging, storage and dispatch. The zinc solubilizing strains most adopted and found suitable in Marathwada region are produced at biofertilizer production unit, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

Preparation of liquid *Zinc Solubilizing* biofertilizers

1. Preparation of mother or starter cultures

Starter cultures of selected strains are obtained after ascertaining their performance in laboratory, pot culture experiments and at field levels. The pure culture of efficient strain of particular microorganism is grown on respective agar medium on slant and maintained in the laboratory. A loopful of inoculum from the slant is transferred in a 250 ml capacity conical flask containing liquid medium. The conical flak was kept on rotary shaker for at least 72 hrs depending whether they are fast growing or slow growing. The content of these flasks usually attains a load of 10^{-14} cells per ml called mother culture or starter culture. This mother culture is further multiplied in larger flasks called as broth preparation.

2. Preparation of broth cultures

Prepare liquid medium for zinc solubilizers. Distribute equal quantity in big conical flasks (1000 ml). Sterilize it in autoclave for half an hour at 15 lbs pressure. After sterilization each flask containing suitable broth is inoculated with the mother culture in 1:5 proportions aseptically under laminar flow. Keep the flaks on rotary shaker for 72 hours or in sterile fermenter until the viable count per ml reaches to 10^{14} cells. The broths become thicker in consistency. This broth culture with population of at least 10^{12} cells per ml should be stored at suitable temperature and condition.

3. Preparation of liquid zinc solubilizing biofertilizer with cell protectant

To prepare the liquid zinc solubilizing biofertilizers from the above prepared broth, all the ingredients required should be mixed thoroughly under laminar flow to avoid the contamination.

Ingredients

Sr.No.	Component
A)	Liquid broth
B)	Cell protectants
C)	Glycerol

* Mix A+B+C as per above and make the volume 100 liters with sterile water

4. Filling and packaging of bottles

After preparation of liquid zinc solubilizing biofertilizer as mentioned above the requisite quantity as per need should be filled in the sterile auto-lock high-density polyethylene (HDPE) plastic bottles under laminar flow to avoid contamination.

Dosage of liquid zinc solubilizing biofertilizer in different crops

Recommended liquid zinc solubilizing biofertilizers, their application methods and quantity to be used for different crops is given follows:

Crop	Recommended Biofertilizer	Application method	Quantity to be used
Sorghum, Pearl millet, Maize, Paddy, Sunflower, Safflower	*Pseudomonas striata*	Seed treatment	100 ml 10 kg^{-1} seed
Cotton	*Pseudomonas striata*	Seed treatment	10 ml kg^{-1} seed
Sugarcane, Potato, Ginger	*Pseudomonas striata*	Planting material	500 ml in 500 lit. of water
Vegetable crops	*Pseudomonas striata*	Seedling deeping	100 ml in 25 lit. of water
Fruit crops	*Pseudomonas striata*	Through drip irrigation	2.5 L. ha^{-1}

Effect of various zinc solubilizing biofertilizers on yield of various crops

Crops	Treatments	Yield (q ha^{-1})		Percent increase in yield
		Uninoculated	Inoculated	
Sorghum	RDF+ Seed treatment with *Pseudomonas striata*	17.46	18.22	4.17
Soybean	RDF+ Seed treatment with *Pseudomonas striata*	19.41	23.45	17.22
Pigeon Pea	RDF+ Seed treatment with *Pseudomonas striata*	14.04	17.68	25.94
Groundnut	RDF+ Seed treatment with *Pseudomonas striata*	23.50	29.10	19.24
Cotton	RDF+ Seed treatment with *Pseudomonas striata*	12.57	9.41	25.13
Ginger	RDF+ Rhizome treatment with *Pseudomonas striata*	98.92 (g pot)	229.12 (g pot)	56.82
Spinach	RDF+ Seed treatment with *Pseudomonas striata*	60.39 (g pot)	188.65 (g pot)	14.98
Guava	RDF+ Application of *Pseudomonas striata* through drip	160.17	210.98	24.08

Evaluation of biofertilizer : Front line demonstration


RDF + *Pseudomona striata*

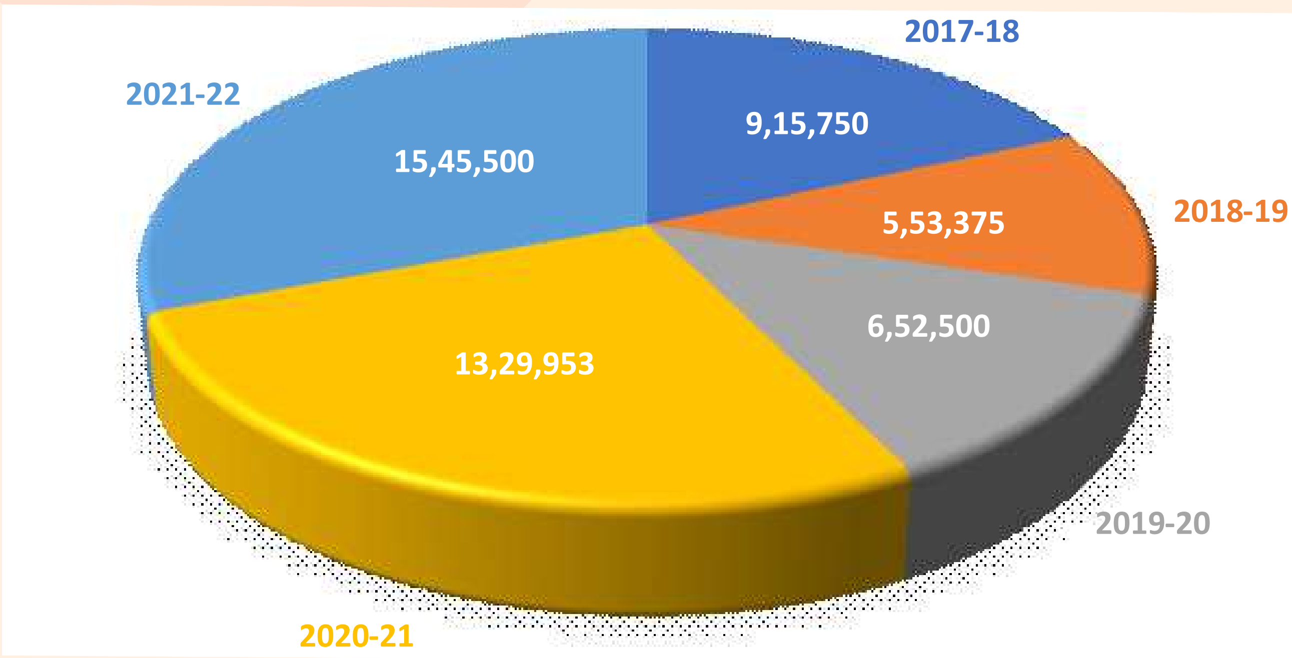

RDF + *Pseudomona striata*


RDF + *Pseudomona striata*


RDF + *Pseudomona striata*

Evaluation of biofertilizer : Front line demonstration

Commercialization and revenue generated through Liquid biofertilizer



Revenue generated (Rs.)

Technology Transferred

Industry	M/s Microbial Solution Pvt. Ltd. Hingoli (Maharashtra)	Atmanirbhar Baliraja Agri Business Pvt. Ltd. Jintur (Maharashtra)

MICROBIAL SOLUTIONS PVT. LTD.

Certificate

This is to certify that we have received Liquid Biofertilizer Technology for (Rhizobium spp., Azotobacter, PSB, Sulphur Oxidizing Bacteria, Zinc Solubilizers) from the ICAR All India Network Project on Soil Biodiversity Biofertilizers, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra on 18/5/2018.The technology is used for commercial production of biofertilizer.

Microbial Solution Pvt. Ltd
Hingoli,Maharashtra

Principal Investigator
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Dated : 25th July 2021

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CIN U2430PN2017PTC173643

Atmanirbhar Baliraja

CERTIFICATE

This is to certify that we have received Liquid Biofertilizer Technology for (Rhizobium Spp., Azotobacter, PSB, Sulphur Oxidizing Bacteria, Zinc Solubilizers) from the ICAR - All India Network Project on Soil Biodiversity Biofertilizers, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani, Maharashtra on 20.12.2021. This technology is used for Commercial Production of Biofertilizer.

Atmanirbhar Baliraja Agri Business Pvt. Ltd.

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Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani

Dated: 20 Dec 2021

Recommnedations

1. Seed treatment of liquid zinc solubilizer *Pseudomonas striata* @ 100 ml / 10 kg seed+ application of $ZnSO_4$ 30 kg ha^{-1} is recommended to soybean along with recommended dose of fertilizers(30:60:30 NPK kg ha^{-1}) for enhancement of soybean seed yield, monetary return and improvement in soil properties,

2. For the enhancement of seed cotton yield, monetary return and improvement in soil properties, drenching of liquid *Trichoderma viride* as zinc solubilizer @ 2.5 liters/ha in 1000 liters of water within 30 days after sowing is recommended to *Bt.* cotton along with recommended dose of fertilizers.

3. For the enhancement of pigeon pea yield, monetary returns and improvement in soil properties, seed treatment of liquid *Pseudomonas striata* as zinc solubilizer @ 100 ml/10 kg seed + application of 30 kg $ZnSO_4$ ha^{-1} is recommended to pigeon pea along with recommended dose of fertilizers.

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Plate : Zinc solubilizing bioferilizer for sale

The information given in the document is based on the experiments carried out at the ICAR - AINP-SBB Centre, Department of Soil Science, VNMKV Parbhani, Maharashtra. For training, demonstration and other enquiries please contact the department.