FINAL REPORT

National Agricultural Innovation Project
(Indian Council of Agricultural Research)

Nano-technology for Enhanced Utilization of Native-Phosphorus by Plants and Higher Moisture Retention in Arid Soils

Central Arid Zone Research Institute, Jodhpur – 342003,
Rajasthan

2014

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Dated : 21 June, 2014

FORWARD

The most exciting scientific developments in recent years are in the area of biochemical expoitation of microorganisms or regulating their behavior. Biological production of nanoparticles is one such approach which unifies science, innovation and opportunity. The world around biological production and use of nanoparticles is constantly changing and evolving. it is now becoming one of the world's fast growing industry having high impact.

At this institute with support from National Agricultural Innovation Project (NAIP) of ICAR; Dr. J.C. Tarafdar and his team along with partners from Birla Institute of Technology & Science (BITS) Pilani; Indian Institute of Technology (IIT) Mumbai; Indian Institute of Soil Science (IISS) Bhopal and Punjab Agricultural University (PAU) Ludhiana; have taken the nanotechnology applications in agriculture from inception to industrial scales, through ever evolving novel approaches. Such a process is not simple. It is plagued by several questions – unknown behavior of nano-materials, ethics of utilization, contamination, environmental consequences and waste disposal. However, the impact of this work has led several firms to commercialize this technology and have signed a Memorandum of Understanding (MoU) with this institute.

I compliment dr. Tarafdar and hsi entire team for such an innovative work for the agricultural sector.

(M.M. Roy) 21/6/14
Director &
Consortium Leader

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Preface

Low utilization of native-P by crops and high fixation of applied P had been a major concern in crop production for over three decades. Different approaches viz., Phosphorus Solubilizing Bacteria (PSB), Phosphatase and Phytase Producing Fungi (PPF), Organic acid producing microorganisms and VAM have been tried in the past to address these problems. But success of these Phosphorus Mobilizing Microorganisms (PMM) had been limited for two reasons: (i) low Soil Carbon (SC) which limit the energy supply of PMM, (ii) high temperature and evaporation from surface soils which adversely affect the survivability of PMM. Thus, proposed project was aimed on increasing the energy supply to PMM by improving moisture retention in soil by increasing production of microbial polysaccharides with the induction of nanoparticles. This cannot be achieved by conventional means viz., incorporation of crop residues and manure due to their limited availability. Further, low SC also limits the availability of C skeleton compounds for the synthesis of exopolysaccharides.

Nanoparticles of Mg, Zn, Fe and P are the structural component of beneficial enzymes (phosphatase, phytase etc.), polysaccharides and chlorophyll. They are known to stabilize the enzyme complexes in plants. We aim on utilizing this property for increasing photosynthesis that would most likely lead to higher exudation which in turn would increase the energy supply and supply of C skeleton compounds to PMM and exopolysaccharide producing microorganisms. The nanoparticles may also trigger the plant enzyme systems to make them more active. This approach would enable in breaking of existing barriers in utilization of native-P and reduce dependence on imported P fertilizers. Nanoparticles also help in increasing the nutrient use efficiency due to more mobilization in the rhizosphere.

This project generated new knowledge of preparation of nanoparticles by biological means and nano-induced polysaccharide powder as well as nano-clay fabrication, which shall address to the current emerging issues of decreasing resource use efficiency with emphasis on nutrients and water. The present results shall help to meet the challenges in technology development and keep pace with the changing scenario of Indian agriculture. The results also facilitate an accelerated and sustained improvement of nutrient and moisture use efficiency through collaborative development and application of newer innovations in partnership with other public/private organizations.

We hope that the result will meet the present depressing scenario with respect to native-P availability and applied P use efficiency.

J. C. Tarafdar

Consortium PI

सारांश

नैनो कण सफलता पूर्वक भौतिक (फॉस्फोरस), रासायनिक (फॉस्फोरस, जस्ता, लौहा), जैविक (फॉस्फोरस, जस्ता, लौहा, मैग्निशियम) और एयरोसोल (मैग्निशियम) तकनीक से विकसित किये गये है। जैविक विधि से तैयार नैनोकण अधिक स्थायी होते है क्योंकि ये मातृ प्रोटीन के द्वारा आवरित रहते है। 58 प्रकार के सूक्ष्म जीव विकसित किये गये है जो कि नैनो कणों के उत्पादन के लिए जिम्मेदार है। धात्विक नैनो कणों के जैव–तकनीक से निर्माण के लिए एक नवाचार विकसित किया गया (पेटेन्ट नं.– 149/DEL/2012)। नैनो खाद के उत्पादन के लिए एक आवरण विधि विकसित की गई जो कि अस्थायी कणों के लिए बहुत उपयोगी सिद्ध हो सकती है। निम्नलिखित तकनीकों के उपयोग के बाद नैनो कणों का निर्धारण किया गया – D.L.S., T.E.M., S.E.M., E.D.S., XRD, जीटा पोटेंश्यिल, AaFM, FTIR और नैनों कणों की अधिकतम सान्द्रता न्यूनतम आकार, आकृति के मानक मूल्य निर्धारित किये गये जिससे कि पादप और सूक्ष्म जीवों पर छिड़काव के समय अधिकतम नैनो कणों का उपयोग हो सकें। मरूस्थलीय फसलों में अधिक तनाव की सहनशक्ति, परतों की क्षति को रोकना और कार्बन के स्त्रःवण को कम करने में नैनो जस्ता और लोहा मदद करता है।

फॉस्फोरस, जस्ता, लौह तत्व और मैग्निशियम नैनो कणों के अनुप्रयोग से अन्न की उत्पादकता 12-54% बढ़ गई और शुष्क द्रव्य भार में 18-39% की वृद्धि देखी गई। 15 प्रकार के सूक्ष्म जीव विकसित किये गये जो कि पॉलीसेकेराइड उत्पन्न करते है। जस्ते और लौहे के नैनोकणों के अनुप्रयोग से पॉलीसेकेराइड के उत्पादन की मात्रा 10-18 गुणा बढ़ गई। पॉलीसेकेराइड चूर्ण के उत्पादन के लिए एक विधि विकसित की गई (पेटेन्ट नं.– 404/DEL/2012)। जिससे उत्पन्न पॉलीसेकेराइड चूर्ण जेन्थेन, कुरडलेन और पोल्यूलेन चिन्हित किये गये। सूक्ष्म जीवों द्वारा उत्पन्न पॉलीसेकेराइड मरूस्थलीय मिट्टी में मृदा के एकत्रिकरण में (33-83%), नमी को रोकने में (10-14%) और कार्बन के निर्माण में (3-5%) बहुत प्रभावी है।

मृदा के एकत्रिकरण के दौरान नैनो पॉलीसेकेराइड के चूर्ण के बंधो के स्थान को नैनो आधारित मिट्टी की बनावट को विकसित किया गया और इसको चिन्हित किया गया। लौहा (30 ppm), जस्ता (10 ppm), मैग्निशियम (20 ppm) फॉस्फोरस (40 ppm) के नैनो कणों की मानक सान्द्रता और मात्रा के अनुप्रयोग से बीजों के अंकुरण, घुलनशील प्रोटीन घटक, मृदा में उपस्थित सूक्ष्मजीवों की संख्या, पादप उत्तक में कुल RNA, चूहों में नैनो–भोजन के उपभोग की दर और उनके शरीर के वजन पर कोई नकारात्मक प्रभाव नहीं पाया गया। नैनो उपचारित पादप उत्तक जैव–सूचना प्रौद्योगिकी के पूर्ण अध्ययन में एन्टो–बी–मेन्नेज नाम एन्जाइम की पहचान की गई। यह एन्जाइम पौधों की वृद्धि और विकास में एक महत्वपूर्ण भूमिका निभाता है। दलहन में नैनो कणों के अनुप्रयोग के परिणामस्वरूप 2488 नया यूनीजीन्स पाया गया हो कि कार्बोहाइड्रेट, लीपीड, न्यूक्लिओटाइड, अमीनो अम्लों के उपापचय में सहायक है। बाजरा और मूंग में निर्धारित मात्रा से 2 से ढ़ाई गुणा अधिक नैनो पोषक पदार्थ और नैनो खाद देने के बावजूद भी कोई नकारात्मक प्रभाव नही आया। नैना उपचारित जाँच पदार्थ देने के बादभी प्राणियों (जन्तुओं) में असामान्य लक्षण और व्यावहारिक क्रिया नहीं पायी गई।

Executive Summary

Nanoparticles were successfully prepared by physical (P), chemical (P, Zn, Fe), biological (P, Zn, Fe, Mg) and aerosol (Mg) technique. Biosynthesis of nanoparticles was found to be more stable due to natural encapsulation by mother protein. Fifty eight microorganisms responsive to nanoparticle production were developed. An eco-friendly low cost protocol for biosynthesis of metal nanoparticles (Patent no: 149/DEL/2012) was unfolded. A coating method has been invented for nano-fertilizer production which may be very useful for unstable particles. A protein which is responsible for nanoparticle production has been identified. The nanoparticles were characterized after using DLS, TEM, SEM, EDS, XRD, Zeta Potential, AFM, FTIR, and stardardized the optimum concentration, size, shape of nanoparticles to be spread to plant and microorganisms for maximum benefit. Nano-Zn and Fe helps in more stress tolerance, prevention of membrane damage and less carbon exudation by arid crops. In general, 12-54% improvement of grain yield and 18-39% dry matter yield of 10 different crops (Cauliflower, Tomato, Capsicum, Maize, Pearl Millet, Castor, Rice, Cluster bean, Mung bean and Moth bean) was observed due to application of P, Zn, Fe and Mg nanoparticles. The Nutrient Use Efficiency (NUE) increased three fold as compared to conventional fertilizers. There was 30% more nutrient mobilization in the rhizosphere compared to control. The beneficial enzyme activities in the rhizosphere increased between 18 and 283%. Fifteen polysaccharide producing microorganisms were developed and their polysaccharide production has been induced from 10-18 times by applying Zn and Fe nanoparticles. A methodology has been developed (Patent no: 404/DEL/2012) for preparation of polysaccharide powder which has been identified as Xantham, Curdlan, Pollulan. The microbial polysaccharide is found to be very effective in soil aggregation (33-83%), moisture retention (10-14%) and carbon build-up (3-5%) in arid soils. Sixty three mineral species has been identified from Udaipur Rock Phosphate (URP) and their characterization has been completed using SEM, EDS, FTIR and XRD. All P minerals were free from heavy metals. The nano based clay fabrications were developed and identified the bonding sites of nano-polysaccharides during soil aggregation. Nanofabrication of phosphorus was done on Kaoline mineral receptacles for its use as advance nano-material including novel fertilizer.

No adverse effect was observed on seed germination, soluble protein content, soil microbial population, total RNA in plant tissue, body weight and consumption rate of nano-food to mice and nanoparticle concentration in seeds with the application of standardized doses of application of Fe (30ppm), Zn (10ppm), Mg (20ppm) and P (40ppm) nanoparticles. A complete bio-informatics on nano treated plant tissues identified an enzyme known as Endo-β-Mannase (EC 3.2.1.78), this enzyme plays a role in plant growth and development. The nanoparticle application of legumes results 2488 new unigenes which help in carbohydrate, lipid, nucleotide, amino-acid metabolism. Pearl millet and mung bean grain with nanonutrients and nanofertilizer did not induce any adverse effects in rats even after feeding more than two and half times limit dose. No abnormal clinical signs, behavioral activity etc. were observed in animals which received nanotreated test materials. Histopathological analysis for estimating toxicological effect showed no adverse effect on liver, kidney and spleen tissues due to intake of nano-foods.

Part-I : General Information of Sub-project

1. **Title of the sub-project :** Nanotechnology for Enhanced Utilization of Native-Phosphorus by Plants and Higher Moisture Retention in Arid Soils
2. **Sub-project code:** 417001-05
3. **Component :** 4
4. **Date of sanction of sub-project :** 18.07.2008
5. **Date of completion :** 31.03.2014
6. **Extension if granted, from :** 1.04.2012 to 31.03.2014
7. **Duration of the sub project :** 5years 8months and 12days
8. **Total sanctioned amount for the sub-project :** 614.7119 lakh
9. **Total expenditure of the sub-project :** 476.60786
10. **Consortium leader :**
    Director, Central Arid Zone Research Institute, Jodhpur - 342003, Rajasthan,
    Phone: 0291- 2786584, Fax: 0291-2788706, E-mail: director@cazri.res.in,
    website: www.cazri.res.in
11. **List of consortium partners :**

	Name of CPI/ CCPI with designation	Name of organization and address, phone & fax, email	Duration (From - To)	Budget (Lakhs)
CPI	Dr. J. C. Tarafdar, Principal Scientist and ICAR National Fellow	Central Arid Zone Research Institute, Jodhpur – 342003, Ph:0291-2785250, +919414118499(mob), Fax: 0291-2788706 Email: jctarafdar@yahoo.in	18.07.2008-31.03.2014	320.1811
CCPI 1	Dr. Jitendra Panwar, Associate Professor	Birla Institute of Technology and Science(BITS) Pilani – 333031, Ph:+91-1596-242126, 09414411654(mob) Fax: +91-1596-244283 Email:jpanwar@bits-pilani.ac.in	18.07.2008-31.03.2014	41.5390
CCPI 2	Dr. A. S. Khanna, Professor	IIT Bombay, Powai, Mumbai -400072, Ph:022-25767891, 09820616372(mob) Fax: 022-25723480 Email: khanna@iitb.ac.in	18.07.2008-31.03.2014	85.6542

CCPI 3	Dr. Siddhartha S. Mukhopadhyay, Professor	Punjab Agricultural Univeristy, Ludhiana – 141004, Ph:+911612401960, +919815993318(mob) Fax: +911612400945 Email: siddhartha_soil@yahoo.co.in	18.07.2008-31.03.2014	63.8760
CCPI 4	Dr. Tapan Adhikari, Principal Scientist	Indian Institute of Soil Science, Nabibagh, Berasia Road Bhopal-462038, MP Ph:0755-2732838, 09303129693(mob) Fax: 0755-2733310 Email: tapan_12000@yahoo.co.uk	18.07.2008-31.03.2014	103.4636

CPI-Consortia Principal Investigator; CCPI-Consortia Co-Principal Investigator

12. Statement of budget released and utilization partner-wise (₹ in Lakhs) :

	CPI/CCPI Name, designation & address	**Total budget sanctioned**	**Fund released (up to closing date)**	**Fund utilized (up to closing date)**
CPI	Dr. J. C. Tarafdar, Principal Scientist and ICAR National Fellow	320.1811	260.38209	271.23453
CCPI 1	Dr. Jitendra Panwar, Associate Professor	41.5390	33.32363	34.96645
CCPI 2	Dr. A. S. Khanna, Professor	85.6542	30.03840	30.03840
CCPI 3	Dr. Siddhartha S. Mukhopadhyay, Professor	63.8760	63.75081	66.25081
CCPI 4	Dr. Tapan Adhikari, Principal Scientist	103.4636	73.72647	74.11767
Total		**614.7119**	**461.22140**	**476.60786**

CPI-Consortia Principal Investigator; CCPI-Consortia Co-Principal Investigator

Part-II : Technical Details

1. Introduction

P is an important nutrient but the concentration of plant available P is generally low (0.72 – 1.2% of total P) in arid soils. The total quantity of P in arid soils is >600kg ha^{-1}, however, the availability of this is restricted thus application of phosphate fertilizer is a must for a good crop. Phosphate fertilizers are mainly synthesized from rock phosphate. Total rock phosphate reserves in India are estimated about 132 m tons which at current rate of exploitation have a life period of 77 years. Thus, it is important to utilize native P both to reduce the cost of production and sustain the mineral reserves for longer period.

Nanoparticles of Mg, Zn, Fe and P are the structural components of enzymes (phosphatases and phytase), polysaccharides and chlorophyll. They are known to stabilize the enzyme complexes in plants. We aim on utilizing this property for increasing photosynthesis that could most likely lead to higher exudation which in turn would increase the energy supply and supply of carbon skeleton compounds to phosphorus mobilizing and exo-polysaccharide producing microorganisms. Thus, this approach would enable in breaking of existing barriers in utilization of native P and reduce dependence on imported P fertilizers.

The proposed project shall generate new knowledge in nanoparticle farming which shall address to the current emerging issue of decreasing resource use efficiency with emphasis on nutrient and water. Likely outcome of the proposal shall be development of newer methods to control and enhance the availability and release of nutrients through basic and strategic research. This shall help to meet the challenges in technology development and keep pace with the changing scenario of Indian agriculture. Thus the project shall facilitate an accelerated and sustained improvement of nutrient and moisture use efficiency through collaborative development and application of newer innovations in partnership with other public/private organizations.

2. Overall Sub-project Objectives

- Enhancing the utilization of native phosphorus by plants using nano-particles of Mg, Zn, and Fe.
- Enhancement of gum production for soil binding and moisture retention by microbes through nano-particle (Mg, Zn, Fe, P) stimulation.
- Synthesis and application of nano-granules of phosphorus from rock phosphate for enhancing its utilization.

3. Sub-project Technical Profile

Objective 1. Enhancing the utilization of native phosphorus by plants using nano-particles of Mg, Zn, and Fe

Work Plan

- Screening, isolation and identification of microorganisms possessing properties of nanoparticle formation.
- Effect of different compounds on yield of nanoparticles from microorganisms.
- Development of pure culture of promising microorganisms and recovery of nanoparticles from bio-nano factories.
- Synthesis/production of nanoparticles of Fe, Zn, Mg and P through physical/chemical approaches.
- Characterization of synthetic and biologically developed nanoparticles.
- Effect of nanoparticles on nutrient use efficiency, plant metabolism and enzyme exudation.

Monitoring indicators

- Development of suitable microorganisms producing nanoparticles.
- Suitable compounds identified and methods developed for production of nanoparticles.
- Suitable methods to be developed for nanoparticle recovery.
- Suitable physical, chemical methods for nanoparticle production.
- Characterization of nanoparticles.
- Plant mechanisms, metabolic changes and enzyme identification and quantification.

Expected output

- Pure culture of microorganisms that produce nanoparticles.
- Compatible microorganisms for maximum production of nanoparticles of Zn, Fe, Mg and P.
- Efficient method for maximum recovery of nanoparticles of Zn, Mg, Fe and P.
- Characterization of nanoparticles of Zn, Fe, Mg and P.
- Metabolic changes and specific enzymatic role quantified.

Expected outcome

- Potential microbe identified and developed.
- Biogenic methods for nanoparticle production standardized.
- Methodology for efficient production of nanoparticle for agricultural use.
- New frontier technology developed.
- Nano nutrients and nano P fertilizer.

Objective 2. Enhancement of gum production for soil binding and moisture retention by microbes through nano-particle (Mg, Zn, Fe, P) stimulation

Work Plan

- Screening of available nanoparticles for increasing gum production from microorganisms.
- Assessment of binding and soil moisture retention efficiency of microbial gum.
- Assessment of soil aggregation, bonding mechanism between different soil particles and exo-polysaccharides.
- Stability of soil aggregates.

Monitoring Indicators

- Screening of nanoparticles on increasing of gum production.
- Microbial polysaccharides for soil binding and moisture retention.
- Understanding the soil binding mechanisms by gum.
- Suitable methods for quantification of soil aggregation by different gums.

Expected output

- Microorganisms and nanoparticles identified for higher gum production.
- Suitable gums identified for soil binding and moisture retention.
- Mechanisms of bonding with different gums.
- Suitable gum for stable aggregates.

Expected outcome

- Developed microorganisms for nano-induced gum production.
- Gum production for soil binding and moisture retention.
- Identified the bonding mechanisms for different gums.
- For stable aggregate, suitable gum may be identified.

Objective 3. Synthesis and application of nano-granules of phosphorus from rock phosphate for enhancing its utilization

Work plan

- Removal of toxic heavy metals from Rock Phosphate.
- Assessing potential of nano-granules for its use as nanofertilizers in selected test plants.
- Effect of nano-material coated fertilizers on plant growth.

Monitoring indicators

- Decontaminated Rock Phosphate.
- Use of P nano-granules and its application doses.
- Response on cereals and legumes for higher uptake and yield.

Expected output

- Pure Rock Phosphate.
- Standardization of dose and application of P nano-granules
- Expected higher yield.

Expected outcome

- Rock Phosphate for preparation of physical nanoparticles.
- Doses for application of P nano-granules.
- Improvement of crop yield and P use efficiency.

4. Baseline Analysis

There was no information available on production on P nano-particles, their purification and interaction with plants. Similarly, information on nano-induced gum production, subsequent soil aggregation and moisture retention is not available. No attempt has been made on development of nano fertilizer, their delivery system and subsequent effect on soil and plant. Finally most of the information related to production of nanoparticles were in chemical means and are not accessible being in patent domain.

Fe, P, Mg and Zn are the structural components of many enzyme systems in plants and in their nano size, can provide stability to the enzyme systems. Higher stability increases the epicacy of the plant processes and may result in higher metabolism, in general, and photosynthesis in particular. Since 25-30% of the photosynthates are exuded from roots, it was logical to hypothesize that increased photosynthesis by nanoparticles would increase root exudates. These root exudates can provide energy to the organisms which in turn would mobilize higher quantity of native P.

5. Research Achievements with Summary

Objective 1. Enhancing the utilization of native phosphorus by plants using nano-particles of Mg, Zn, and Fe

Activity 1: Screening, isolation and identification of microorganisms possessing properties of nanoparticle formation **[CAZRI Jodhpur and BITS Pilani]**

Fifty–seven microorganisms (CAZRI-36; BITS-21) were developed, out of about 1,800 isolated micro-organisms, for preparation of different nanonutrients. The organisms were isolated from the soils collected from different parts of India, and identified through colony morphology, microscopic examination, and biochemical characteristics. The molecular identification of isolated microbial strain was carried out on the basis of 16S rRNA and 18S rRNA gene sequencing using universal primers EUB1, EUB2, ITS1 and ITS2. Nucleotide sequence comparisons were performed using basic local alignment search tool (BLAST) network services of National Center for Biotechnology Information (NCBI) database. A list of identified organisms with the capability of their production of different nanoparticles along with NCBI accession number is given as Table 1.

Table 1: List of Nanoparticle (100%) producing microorganisms developed & published through NCBI

Sr. No.	Name of the organisms	NCBI GenBank Accession No.	Types of Nanoparticles
1.	*Aspergillus terreus CZR-1*	JF681300	Zn, Mg, P, Fe
2.	*Aspergillus flavus CZR-2*	JF681301	Zn, Mg, Ti, Ag, Au
3.	*Aspergillus flavus TFR- 1*	JN194185	Zn, Mg, P, Ti
4.	*Aspergillus terreus TFR-2*	JN194186	Zn, Mg, P, Ag, Au, Fe

5.	*Aspergillus tubingensis TFR- 3*	JN126255	Mg, Fe, P
6.	*Aspergillus fumigatus TFR-8*	JQ675291	Mg, Zn, P
7.	*Aspergillus oryzae TFR-9*	JQ675292	Zn, P, Ag, Au, Fe, Ti
8.	*Aspergillus flavus TFR- 10*	JQ675293	Zn, Ti, Mg
9.	*Aspergillus flavus TFR- 11*	JQ675294	Zn, Ag, Au, Fe
10.	*Aspergillus flavus TFR- 12*	JQ675295	Zn, Mg, P, Ag, Au, Fe, Ti
11.	*Aspergillus niger TFR-4*	JQ675305	Mg, Zn, Ti
12.	*Aspergillus tubingensis TFR-5*	JQ675306	Mg, Ti, Ag, Au, Fe
13.	*Rhizoctonia bataticola TFR-6*	JQ675307	Zn, P
14.	*Aspergillus flavus TFR- 7*	JQ675308	Zn, Mg, P, Fe
15.	*Aspergillus brasiliensis TFR-23*	JX999490	Mg
16.	*Emericella nidulans TFR-14*	KC175549	P, Zn
17.	*Emericella nidulans TFR-15*	KC175550	P
18.	*Emericella variecolor TFR-16*	KC175551	Zn, Fe
19.	*Aspergillus flavus TFR-17*	KC175552	Zn, Ti
20.	*Aspergillus terreus TFR-18*	KC175553	Fe
21.	*Aspergillus flavus TFR-19*	KC175554	Zn
22.	*Emericella nidulans TFR-20*	KC175555	Zn, P
23.	*Aspergillus fumigatus TFR-22*	KC175556	Fe, Zn
24.	*Aspergillus japonicus AJP01*	JF770435	Fe
25.	*Bacillus megaterium JCT 13*	JX442240	P
26.	*Pantoea tarafdar JCT14*	KC806057	N
27.	*Emericella quadrilineata TFR-25*	KC806055	K
28.	*Aspergillus ochraceus TFR-23*	KC806053	K
29.	*Penicillium janthinellum CZF-5*	KC412872	Fe, Zn
30.	*Ascomycota clone CZF-6*	KC412873	Zn
31.	*Aspergillus flavus CZF-3*	KC131548	Fe
32.	*Fusarium solani CZF-4*	KC142125	*
33.	*Penicillium solitum TFR 24*	KC806054	K
34.	*Emericella nidulans TFR-26*	KC806056	P
35.	*Staphylotrichum coccosporum TFR-27*	KF729586	B, S, Mg

36.	*Penicillium limosum TFR-26*	KF729585	S, Mg
37.	*Aspergillus aeneus NJP12*	HM222934	Zn
38.	*Aspergillus flavus NJP08*	HM222933	Zn
39.	*Aspergillus niger NJP09*	HQ710538	*
40.	*Aspergillus ochraceus NJP04*	HQ710534	*
41.	*Aspergillus ochraceus NJP13*	HQ710541	*
42.	*Aspergillus oryzae NJP01*	HQ710532	Zn
43.	*Aspergillus oryzae NJP06*	HQ710536	*
44.	*Aspergillus oryzae NJP10*	HQ710539	*
45.	*Aspergillus oryzae NJP15*	HQ710543	*
46.	*Aspergillus oryzae NJP18*	HQ710546	*
47.	*Aspergillus sp. NJP02*	HM222932	Zn, Ag
48.	*Cladosporium sp. NJP19*	JF298825	*
49.	*Cladosporium sp. NJP05*	HQ710535	*
50.	*Eupenicillium javanicum NJP17*	HQ710545	Fe
51.	*Mortierella sp. NJP14*	HQ710542	*
52.	*Penicillium communeNJP11*	HQ710540	*
53.	*Penicillium commune NJP07*	HQ710537	*
54.	*Penicillium commune NJP03*	HQ710533	*
55.	*Penicillium crustosum NJP16*	HQ710544	*
56.	*Aspergillus flavus AJP02*	JF770436	Fe
57.	*Cladosporium oxysporum AJP03*	KF245937	Au

* Not producing 100% particles in nano form from the respective precursor salts

Activity 2: Effect of different compounds on yield of nano-particles from micro–organisms
[CAZRI Jodhpur]

Different salts were tested for preparation of desired nanoparticles. The concentration for production of different nanoparticles and their optimum pH, temperature were standardized. The proteins responsible for the production of nanoparticles were identified for important particles like Zn, P, Mg and Fe. A list of compounds used for different nanoparticles produced by the action of micro-organisms is listed in Table 2.

Table 2: Effect of different compounds on yield of nano-particles from micro-organisms

Metal	Concentration	Compound
Mg	0.1 mM	MgO, $MgNO_3$, $MgSO_4$
Zn	0.1 mM	ZnO, $ZnCl_2$, $ZnSO_4$, $ZnNO_3$
Fe	0.1 mM	Fe_2O_3,$FeCl_3$, $K_3Fe(CN_6)_6$
P	0.1 mM	$Ca_3(PO_4)_2$, phytin
Ti	0.1 mM	TiO_2
Ag	0.1 mM	$AgNO_3$
Au	0.1 mM	$HAuCl_3$
K	0.1 mM	KNO_3
N	0.1 mM	$NH4(NO_3)_2$
Pt	0.1 mM	$Pt(OH)_2$

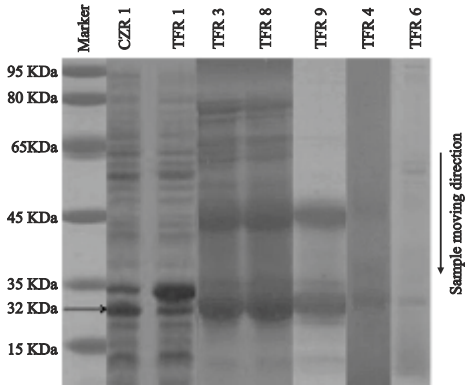


Figure 1: SDS–PAGE profiles of extracellular proteins of different fungal species used for nanoparticle synthesis

The extracellular fungal proteins that were responsible for the synthesis of Zn, Mg, Fe and P nanoparticles from their precursor compounds were subjected to SDS–PAGE analysis to characterize on the basis of molecular weight. In this regards, extracellular proteins isolated from seven different species of 14 fungal isolate were used (Figure 1). As a result of SDS-PAGE various dense and light protein bands were observed after silver staining of poly acryl amide gel. It was very clearly exhibited that a protein band of 32 kDa weight was found in all the seven different fungal species, and band obtained in CZR 1 isolate was highly dense due to more protein contents. Experiments were conducted for optimization of salt concentrations of different nanonutrients. Optimum salt concentration for production of nanoparticles was found to be 0.1mM for Zn, Fe, P and Mg. The optimum conditions for production of nanoparticles from biological sources were standardized and presented as (Figure 2) alongwith optinum doses and mode of application.

BIOSYNTHESIS OF NANOPARTICLES	
Age of fungal culture	: 15 days
Salt concentration	: 0.1 mM
Fungal extract: salt solution	: 1:1
Static/shaker	: Shaking
Reaction period	: 48-72h
pH	: 55 - 7.0
Temperature	: 28^{0}C
Stability	: > 180 days

DOSES OF NANOPARTILES	
Zn	: 10 ppm
Mg	: 20 ppm
Fe	: 30 ppm
P	: 40 ppm

APPLICATION OF NANOPARTICLES	
Spray	: Aerosol
Concentration	: < 5 ppm
Size	: < 20 nm
Shape	: Cube shaped

MODE OF APPLICATION	
Mode application	: Foliar
Plant age	: 2 weeks
Plant parts	: Om leaves
Requirement	: 16-18 L ha^{-1}

Figure 2. Optimum application procedure for bio-synthesis of Fe, Zn, Mg and P nanoparticles

Effect of sonication on nanoparticle size

In order to overcome recurrent problem of agglomeration, the effect of sonication time on the size of biosynthesized nanoparticles were evaluated on the final product. Fifteen minutes of continuous sonication produces least average size of nanoparticles but with further increase in sonication time no significant enhancement in reduction of average particle size was observed.

Activity 3: Development of pure culture of promising micro-organisms and recovery of nano-particles from bio-nano factories **[CAZRI Jodhpur and BITS Pilani]**

After isolation and identification of efficient organisms, the culture of promising organisms was maintained in PDA at -20^{0}C. The subcultures were prepared every month to maintain the culture. The organisms were considered for nanoparticle production only when they produce 100% particles in nano forms in the respective salt solution. A sketch diagram (Figure 3) was presented below for preparation of nanoparticles..

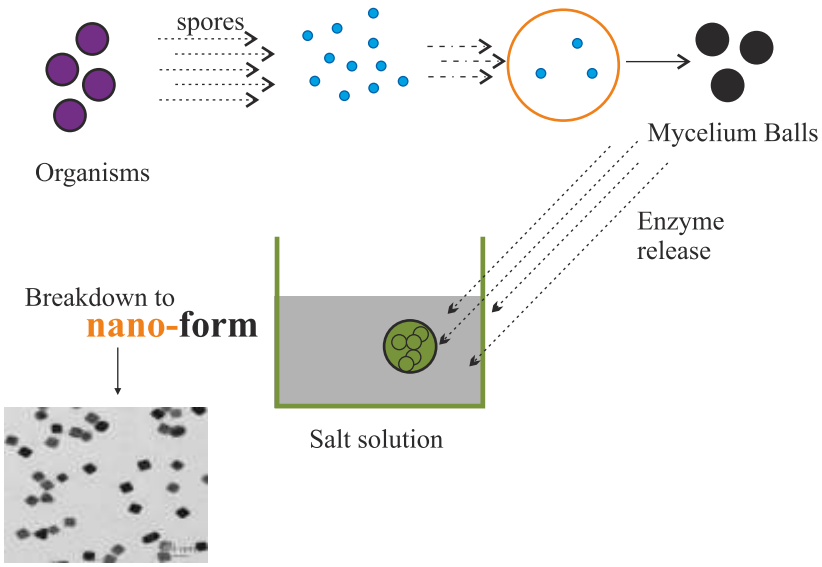


Figure 3 : Breakdown of salts by isolated organisms into nano-form

Effect of centrifugation on the recovery of nanoparticles

The reaction mixture containing nanoparticles was subjected to 6, 20, 40, and 80K rpm centrifugation. The supernatant and the pellet were subjected to particle size analysis. The results indicated higher recovery of nanoparticles in the range of 42.3- 99.7 nm in the pellet fraction when the reaction mixture was centrifuged at 80K for 10 minutes. Centrifugation up to 40K (Table 3) resulted in similar intensity distribution of nanoparticles in supernatant as well as pellet fraction.

Table 3. Effect of different speeds of centrifugation on the recovery of nanoparticles

Fraction	Centrifugation speed (rpm)							
	6000		20,000		40,000		80,000	
	Size (nm)	c.f.	Size (nm)	c.f.	Size (nm)	c.f.	Size (nm)	c.f.
Supernatant	35.2-93.8	7.9	30.0-98.4	7.4	75.1-97.1	6.6	65.7-93.0	3.5%
Pellet	ND	---	ND	---	50.1-99.4	6.6	42.3-99.7	9.3

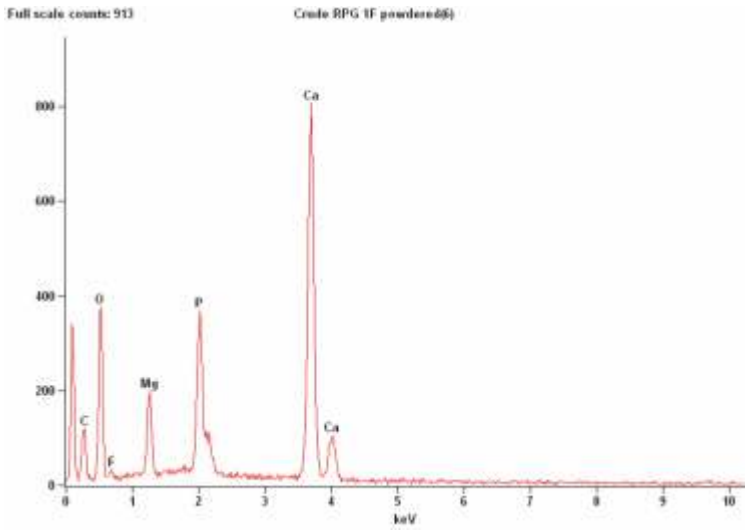
Cf : Intensity distribution

Observations of supernatant and pellet revealed higher distribution of nanoparticles in the supernatant upto 15k rpm while the percent distribution of nanoparticles increased in the pallet after 20k rpm. There was high degree of variability in the results and no fixed conclusions could be drawn from the studies.

Activity 4: Synthesis/production of nano-particles of Fe, Zn, Mg and P through physical/chemical approaches **[PAU Ludhiana; IIT Bombay; CAZRI Jodhpur; IISS Bhopal]**

Physical approach for preparation of nano-phosphorus

Physical synthesis was carried out for phosphorus as the natural mineral deposits was available as rock phosphate. We have collected rock phosphate from Udaipur as well as Masoorie, and tried to purify their minerals to release maximum amount of phosphorus by use of citric, oxalic, formic and acetic acids of various concentration and partitioning processes. A released amount P was estimated by spectrophotometer (blue colour method). Amount of P released from P-rich mineral (RPG 1f) were in the following order: oxalic acid > formic acid > citric acid > acetic acid. It was observed that the reactivity with acids at room temperature was more with powder forms than with grains (0.5 mm-1 mm effective diameter size). This might be due to increase in surface area of P-rich mineral (RPG 1f). The reactions were restricted to room temperature to save on energy consumption. P-rich mineral (RPG 1f) were analyzed by Scanning Electron Microscope (SEM), SEM-EDS (Figure 4) and FT-IR (Figure 5) techniques to estimate amounts of P present in the samples. The EDS showed that P ranged from 3.77 to 6.23% by atom percent.



Quantitative Results RPG 1F

Element	*Weight%*	*Atom%*
C	4.44	8.60
O	36.47	53.10
F	2.01	2.46
Mg	3.31	3.17
P	8.29	6.23
Ca	45.48	26.43
Total	100.00	100.00

Figure 4: SEM-EDS analysis of P minerals

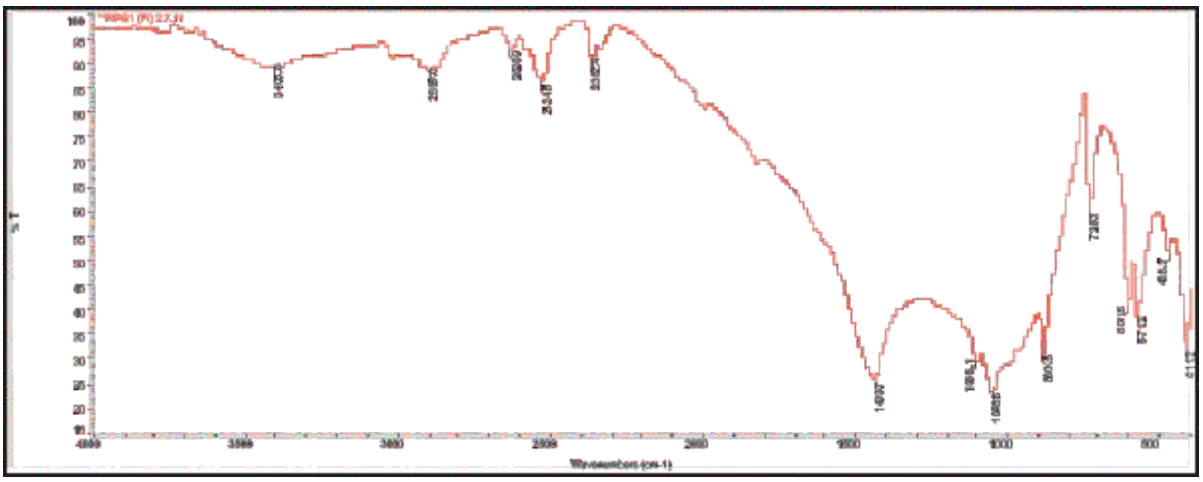
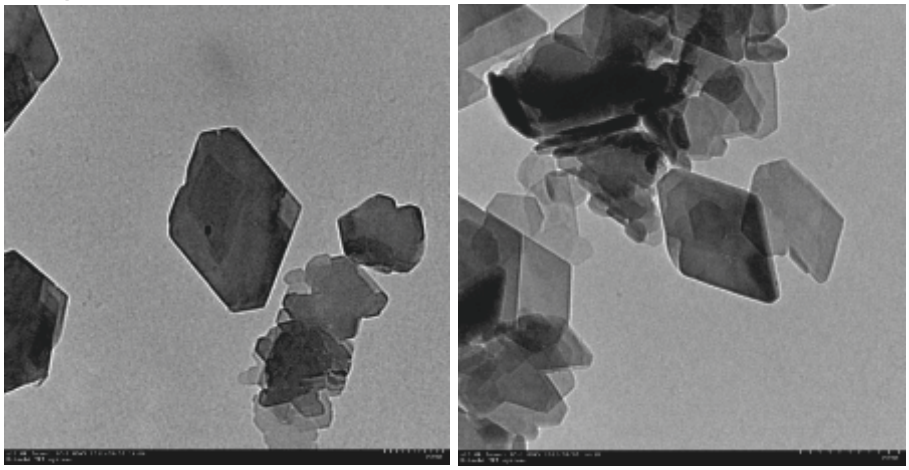


Figure 5: FT-IR spectrum of P-rich mineral (RPG 1f)

The FT-IR peaks at position 3405.6, 1098.1, 1048.6, 601.8, 571.2, and 468.7 indicate the presence of phosphorous (Hydroxyapatite).

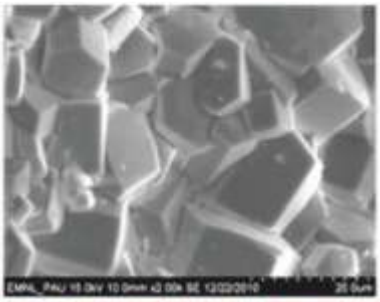
The process of nanosizing of kaolin (by breaking macro and microaggregated to <100 nm effective diameter size without allowing aging to halloysite) for persisting long-time dispersion in aqueous media was achieved by ultrasonication by optimizing cycles, pulses, probe size and time of sonication. The material was then filtered through 0.2 µ nylon filter. The resultant kaolin was in the range of 30-50 nm as determined by the Transmission Electron (Figure 6). The kaolin was also characterized using FT-IR.



6 (a) **6 (b)**

Figure 6: (a) and (b) Shows the TEM Micrograph of nano-kaolin

The FT-IR peaks at position 3695.10, 3620.46, 3620.1, 1112.89, 1010.08, 1032, 913.45, 789.11, 754.32, 696.69, 539.32, 471.01 correspond to the peak of kaolin. After purification of rock phosphate the particles were subject to grind either by high energy ball mill or pot mill to get particles into nano-form, for example, by using high energy ball mill we were getting average P nanoparticle size of 28nm while using pot mill the size was 70nm (Figure 7).



Physical Method (P nano particle)

Method used	Av. size (nm)
High energy ball mill	28.0
High energy pot mill	70.0

Rotor speed mill (Fritsch) & High energy ball mill

Figure 7: Physical synthesis of nanoparticles

Chemical Synthesis of Nanoparticles

Sedimentation technique (Figure 8) was used to prepare Zn nanoparticles after reacting Zinc Acetate in Methanol.

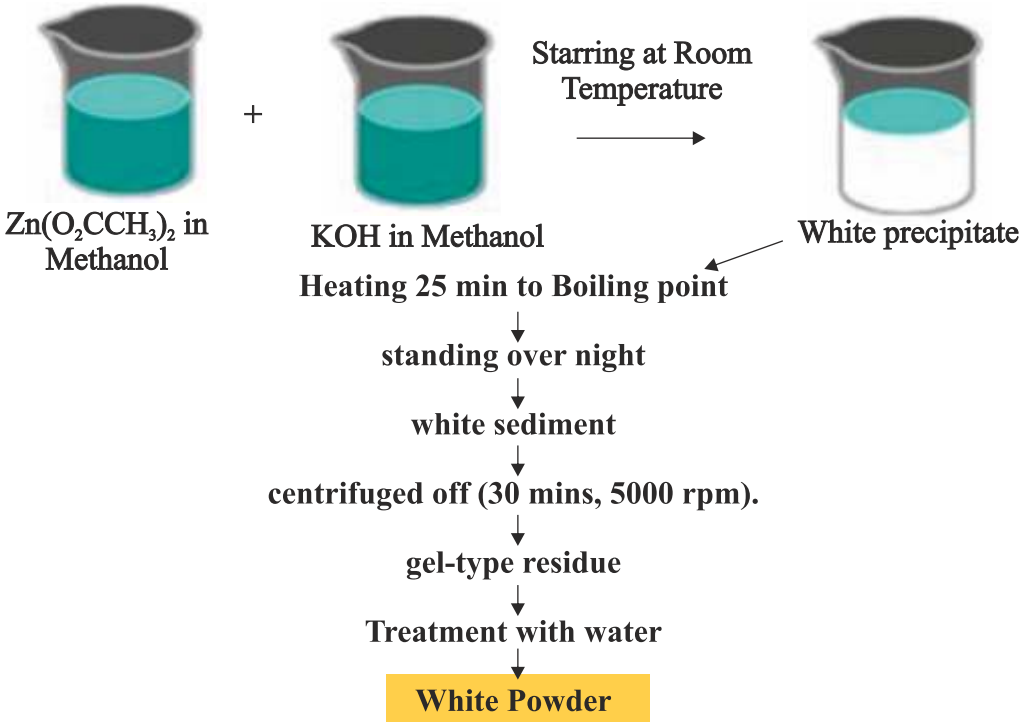
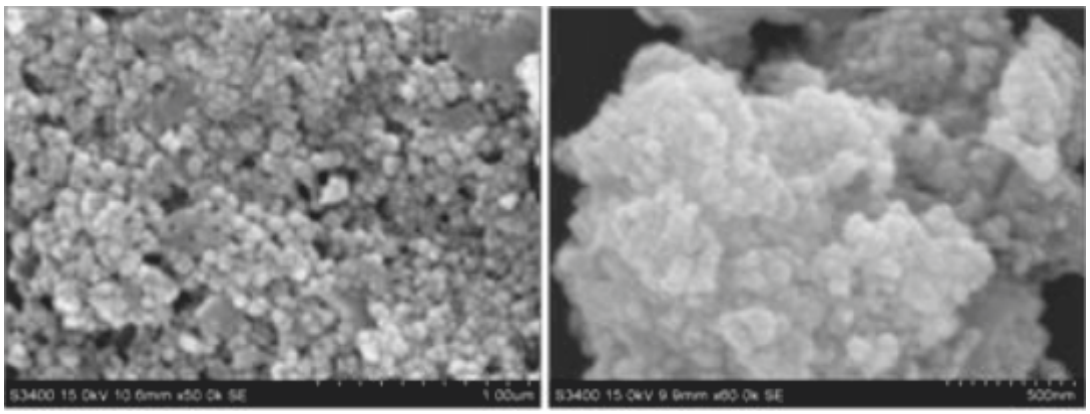


Figure 8: Preparation of nano ZnO by chemical method

The **nano particles of ZnO** were synthesized by mixing the solutions of zinc acetate dehydrate in methanol and potassium hydroxide in methanol at room temperature. The synthesized ZnO was characterized for their size, shape, morphology and structure using TEM, SEM (Figure 9), XRD and FTIR techniques. The hexagonal particles with average size of 20-30 nm were observed in SEM and TEM. A strong band at 500 cm^{-1} corresponding to Zn^{-O} stretching band was observed in FTIR spectrum.



SEM Image of Unmodified ZnO **SEM Image of Oleic acid modified ZnO**

Figure 9. SEM image of modified and unmodified ZnO nanoparticles

Synthesis of Nano-Calcium Phosphate [Hydroxy Apaptite-HA]

HA powder was prepared using a sol–gel method with phosphoric pentoxide (P_2O_5) and calcium nitrate tetrahydrate ($Ca(NO_3)_2$. $4H_2O$). The sol–gel process is a wet chemical method and requires no high pH value and high sintering temperature. This method offers a molecular mixing of the calcium and phosphorus capable of improving the chemical homogeneity. The high reactivity of the sol–gel powders allows a reduction of the processing temperature and of any degradation phenomena during sintering. The reported sol-gel method does not require controlling of pH-value, vigorous stirring and long time for hydrolysis of phosphates. For pilot batch 100mL of 0.5 mol/liter P_2O_5 in ethanol and 100mL of 1.67 mol/liter ($Ca(NO_3)_2$. $4H_2O$) in ethanol were prepared. The two solutions were mixed in a manner to maintain the Ca/P molar ratio as 1.67. The initial precursor solution thus obtained was stirred continuously for 10 min at ambient temperature and then was heated at 60^0C for 1 h to get a white transparent gel. The gel was aged for 4 h at ambient temperature and then dried at 80^0C for 24 h in an air oven. The dried gel was sintered at 700^0C in a furnace for 15 min. The sintered products were crushed using an agate mortar and pestle to obtain resultant powders. The powder obtained as per the above mentioned procedure were characterized using the following techniques Particle size analysis [DLS], TEM analysis, XRD analysis, FTIR analysis.

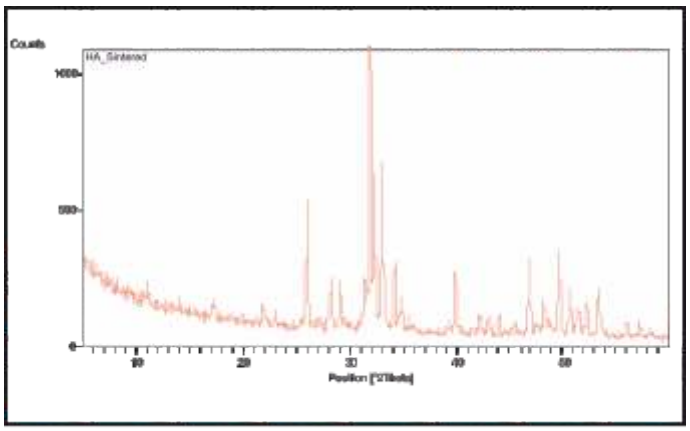
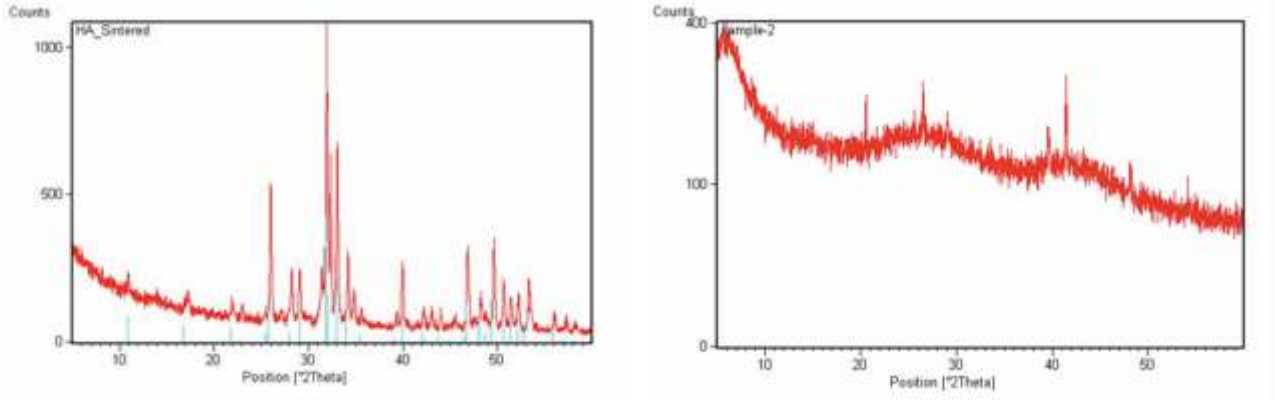


Figure 10. (a) : X-ray diffraction pattern for sintered HA

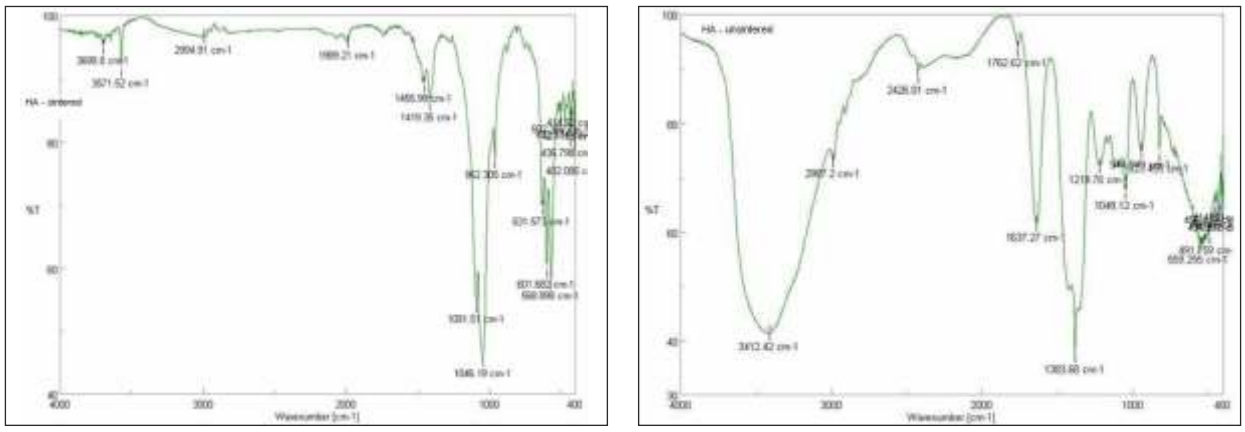


(b) **(c)**

Figure 10. **(b)** XRD pattern of sintered HA with standard reference.
(c) XRD pattern for unsintered HA

Figure 10.a and figure10.b shows the XRD patterns for HA powder aged 4 h un-sintered and sintered at 700^{0}C respectively. The d-spacings were compared with standard reference [Reference code: 01-073-1731, Hydroxyapatite] for sintered HA and a good match was observed with the standard with respect to both intensity and position of lines. XRD pattern of un-sintered HA shows lack of crystallite phase. The XRD patterns thus confirm that sintering at 700^{0}C results in formation of crystallite hydroxyapatite.

The FTIR spectra for HA un-sintered and sintered are shown in Figure 11(a) and Figure 11(b) respectively. The identified peaks for sintered HA correspond to vibration modes of phosphate group [$PO4^{3-}$] viz., at 568, 601, 962 and 1046 cm^{-1} and of carbonate group [$CO3^{2-}$] viz., at 1419 and 1456 cm^{-1} which are characteristics of hydroxyapatite. In un-sintered HA, the vibration band of phosphate group [$PO4^{3-}$] are at 559,944 and 1048 cm^{-1}, but are very weak and not as sharp as in sintered HA. The broad band at 3412 cm^{-1} corresponding to [–OH] stretching for un-sintered HA corresponds to high concentration of alcoholic [-OH] group. For sintered HA, this peak has vanished and a weak peak at 3698 cm^{-1} can be observed indicating very low concentration of alcoholic [-OH] group. The FTIR results thus affirm that the obtained product from sol-gel reaction of calcium nitrate and phosphorus pentoxide is calcium phosphate/ hydroxyapatite.



(a) (b)

Figure.11. (a) FTIR spectra of unsintered HA **(b)** spectra of sintered HA

Synthesis of nano- Fe_2O_3

Iron nanoparticles were synthesized using Iron sulphate hepta-hydrate [$FeSO_{4.7}H_2O$], Sodium acetate [CH_3COONa]. The solutions were mixed in appropriate concentrations at room temperature to get a yellow precipitate. The precipitate formed was washed with distilled water and ethanol and then dried to obtain the nano-iron oxide particles. Figure12 illustrates the synthesis procedure followed for nano-iron oxide. The synthesized Fe_2O_3 was also characterized for their size, shape, morphology and structure using TEM, SEM, XRD and FTIR techniques. In SEM and TEM images, the particles were found to consist of single phase primary particle in rod shape with average length of 180 nm and diameter of about 20 nm.

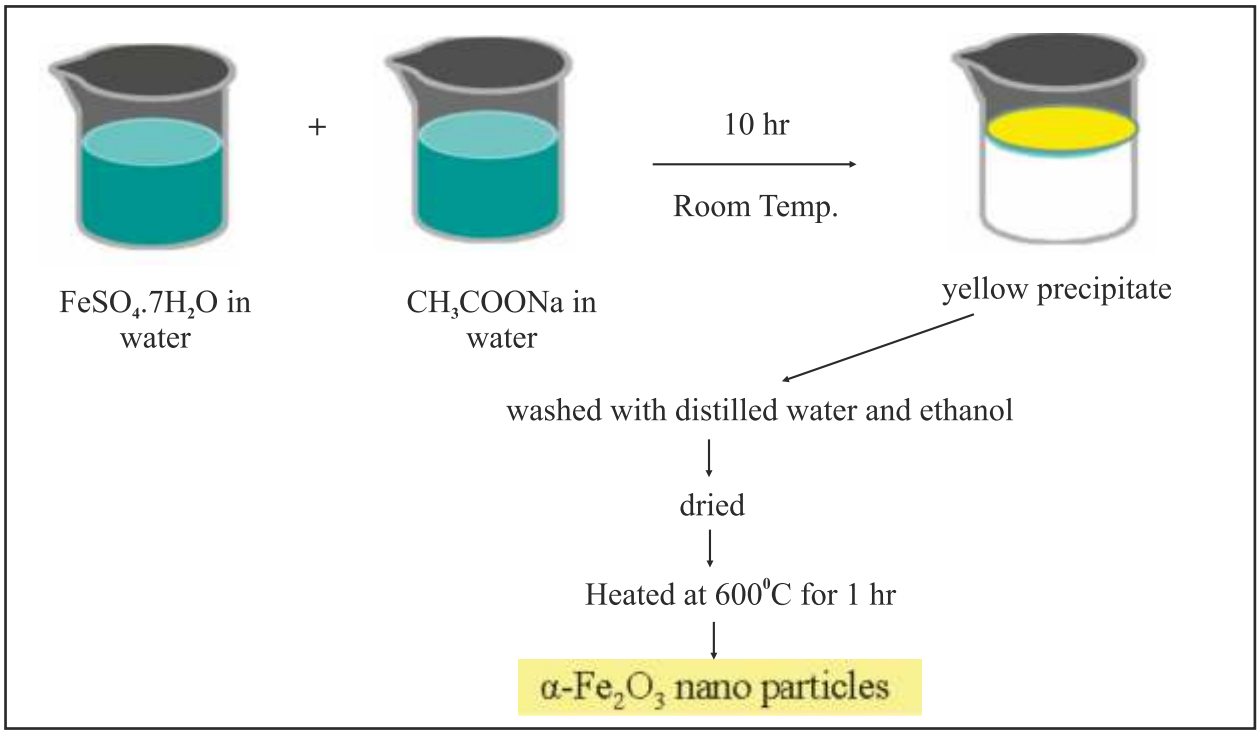


Figure 12: Synthesis procedure for nano-iron oxide

Aerosol Processing of Nanoparticles

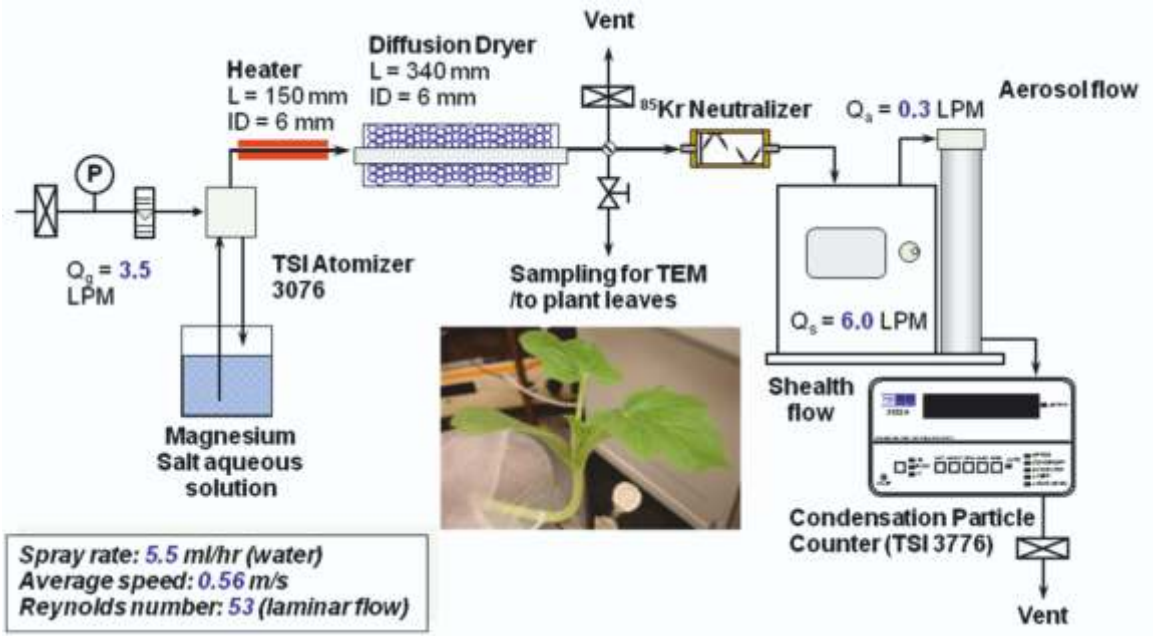


Figure 13. Aerosol synthesis of Mg nanoparticles

Aerosols were generated by the above mentioned TSI atomizer (Figure 13) under flowing air. Before aerosolization, a background measurement was carried out to rule out the interference of solvent. The results show that DI water had very small particle size distribution and extremely low number concentration, implying negligible effect on the aerosol size measurements.

Online size measurements of $Mg(NO_3)_2$ aerosols generated from solutions with different concentrations were first performed. The particle size distributions showed a right shift with precursor concentration. In the meantime, the corresponding total particle number concentration also revealed the same increase tendency. The increase of particle size can be explained following one droplet to one particle (ODOP) principle based on mass conservation. The increase in total number concentration of the aerosol nanoparticles with increasing concentration was caused by a change in the physical properties of suspensions. Previous research showed that the average droplet size decreased with increasing precursor concentration due to the increase in viscosity and density. Since the volume of the precursor sprayed per unit time was found to change little for different concentrations, the number concentration of the droplets, subsequently the aerosol nanoparticles, should increase as precursor concentrations increase. In addition to solutions, a similar precursor concentration effect on aerosol particle size distribution was also found for nanoparticle suspensions. The average particle size estimated from the TEM images also increased with suspension concentration, consistent with the online size measurements. It is clear that the online size measurements (Figure 14) revealed much smaller sizes compared with their corresponding hydrodynamic sizes measured by DLS. This indicates that the aerosolization technique might break up the soft agglomerate structure of nanoparticles in suspensions due to the strong shear force during atomization, resulting in smaller aerosol particles.

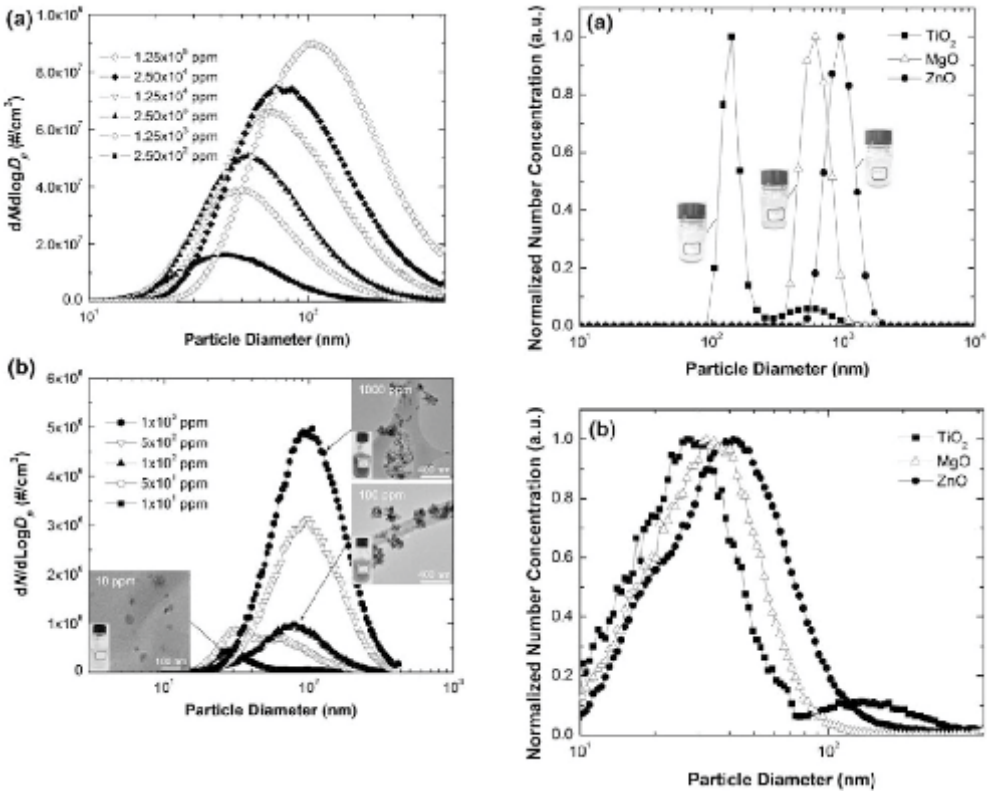


Figure 14. Online measurements of a $Mg(NO_3)_2$ and b Fe_2O_3 aerosols generated from precursors with different concentrations. The insets in b are TEM images of Fe_2O_3 aerosols and corresponding photos of Fe_2O_3 suspensions.

To further compare the offline (DLS) and online aerosol (SMPS) size measurements, TiO_2, MgO, and ZnO suspensions with the same concentration (100 ppm) were used as precursors. The results of DLS measurements were presented in Figure 15, from which the peak diameters of TiO_2, MgO, and ZnO were obtained as 150, 623, and 1,020 nm, respectively. The large sizes were mainly due to the agglomeration of the nanoparticles in their suspensions. While from their corresponding aerosol size measurement results (Figure 15b), much smaller particle sizes were obtained due to the formation of fine droplets, which inhibit the agglomeration of nanoparticles during aerosolization.

Water-soluble magnesium nitrate and sulfate were first tested. The elemental analysis results in terms of Mg were summarized. From the results, minor improvements were observed on the nanoparticle penetration into leaves by the aerosol method over the solution method. For example, the amount of $Mg(NO_3)_2$ NPs (d = 44 nm) in the root was about 5.3 %, of the total recovered amount, slightly larger than that using the solution method (2.2 %). However, the difference is not significant, which may be because of the nature of the materials. Both magnesium nitrate and magnesium sulfate are highly water soluble. Though aerosols of these salts (44 nm for $Mg(NO_3)_2$ and 45 nm for $MgSO_4$ are used, they may eventually dissolve inside the plants and be transported in dissolved form, similar to application of solutions.

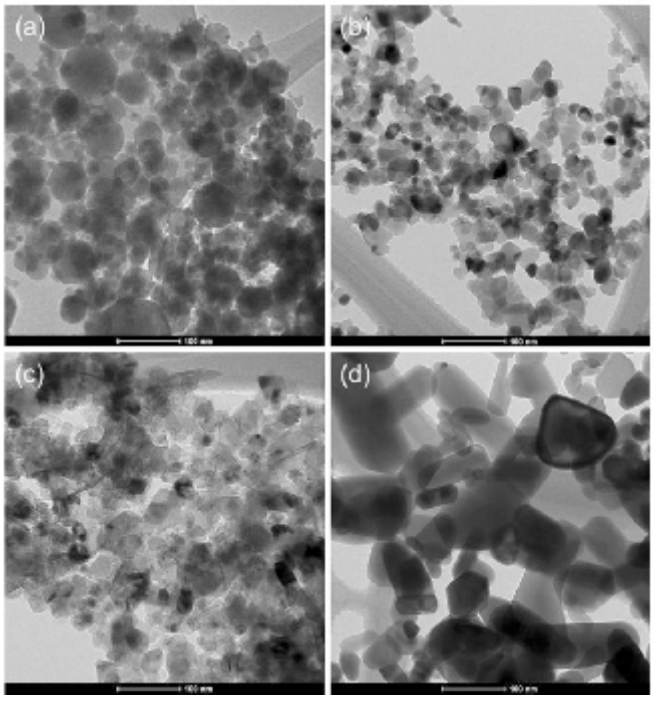


Figure 15. TEM images of nanoparticles. a Fe_2O_3, b TiO_2, c MgO, and d ZnO

***Activity 5: Characterization of synthetic and biologically developed nano-particle*[CAZRI Jodhpur; BITS Pilani; IISS Bhopal; PAU Ludhiana; IIT Bombay]**

Samples of nano product of Zn, Mg, Fe and P nanoparticles were characterized by world-wide accepted nanostructure characterizations techniques like Dynamic Light Scattering (DLS) using particle size analyzer, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Fourier Transform Infrared (FTIR) spectroscopy, X-Ray Diffraction (XRD), and Energy Dispersive Spectroscopy (EDS) techniques.

Characterization of Zn nanoparticles

Biotransformed zinc nanoparticle size was initially measured by DLS technique, which analyses particle size on the basis of intensity, number and volume distribution in the solution. The DLS can also measured zeta potential of the nanoparticles. The graphical representation (Figure 16) clearly exhibit that a portion of particle size ranges between 1 and 8.2 nm and a considerable portion of nanoparticles shows agglomeration stage by forming a cluster. However, the number distribution clearly indicated that most of the particles were within the range of 1 to 8.2 nm (Figure 17). The volume distribution curve further validated that more than 99% of the nanoparticles were measured below 8.2 nm (Figure 18). The detail size distribution of Zn nanoparticles was presented as Table 4. The surface charge of the nanoparticles plays a crucial role during interaction with molecule of other biological system such as plant, which should normally be in the range of -30 to +30 mV. The surface charge of biosynthesized Zn nanoparticle was measured as zeta potential of -5.70 mV using DLS, which was graphically represented as Figure 19.

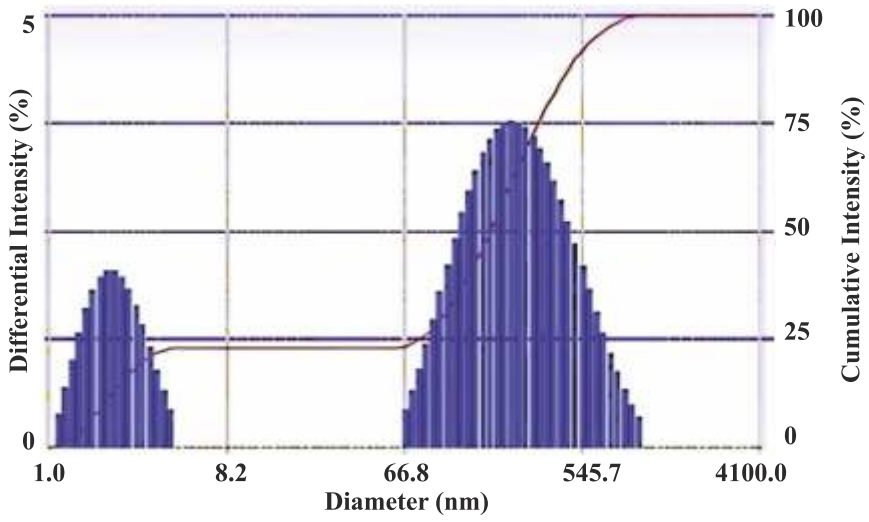


Figure 16. Intensity distribution of biologically synthesized Zn nanoparticles

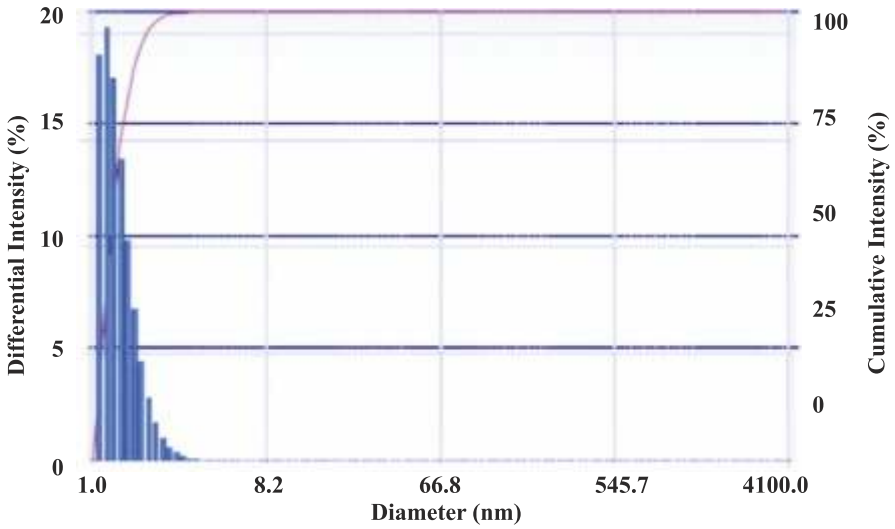


Figure 17. Number distribution of biologically synthesized Zn nanoparticles

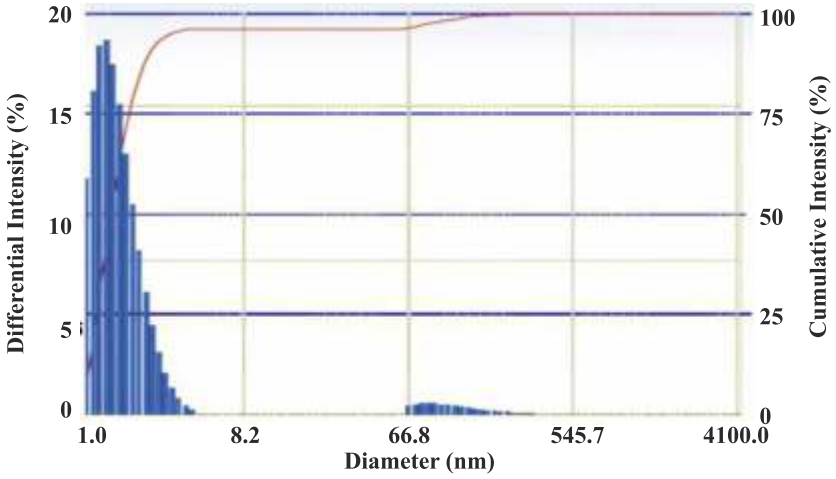


Figure 18. Volume distribution of biologically synthesized Zn nanoparticles

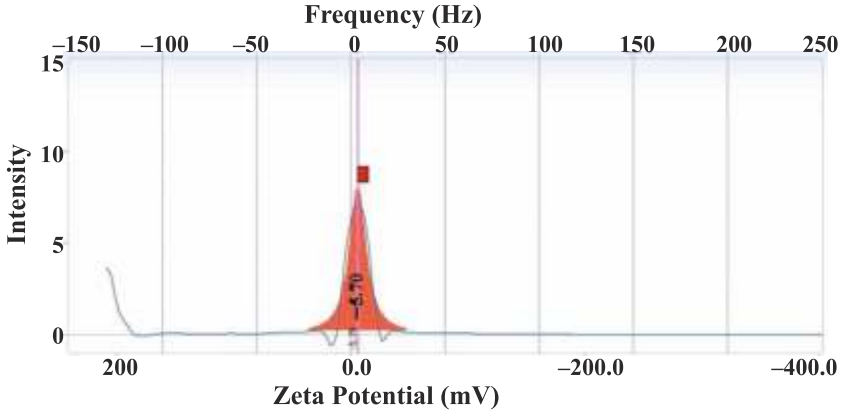


Figure 19. Zeta potential of biologically synthesized Zn nanoparticles

Table 4. Size distribution of biosynthesized Zn nanoparticles

Nanoparticle size	Intensity distribution	Number distribution	Volume distribution
Cumulative per cent nonoparticle			
1.0	5.8	45.3	41.4
2.0	18.6	64.8	48.6
3.0	36.0	74.3	78.8
4.0	42.3	78.5	83.6
5.0	43.4	86.8	87.4
6.0	44.3	93.4	98.2
7.0	44.8	94.2	98.6
8.0	45.3	99.9	99.4
9.0	45.4	100.0	99.8
10.0	45.6	100.0	99.8
20.0	46.8	100.0	99.8
30.0	46.8	100.0	99.8
40.0	46.9	100.0	99.8
50.0	47.2	100.0	99.8
100.0	78.9	100.0	100.0

It can be clearly seen in the low magnification image (Figure 20) that all the nanoparticles were present in mono-disperse stage and a fairly good idea of the size can be seen in the further highly magnified TEM micrograph. An insight of figure 21 shows SAED patterns of a single Zn nanoparticle, where white array of spot shows the crystalline nature of a nanoparticle. The high resolution TEM micrograph of a single Zn nanoparticle shows the arrangements of atoms within the nanoparticle. The presence of lines also depicts the crystallity of Zn nanoparticles which was otherwise absent in nonmetallic organic molecules.

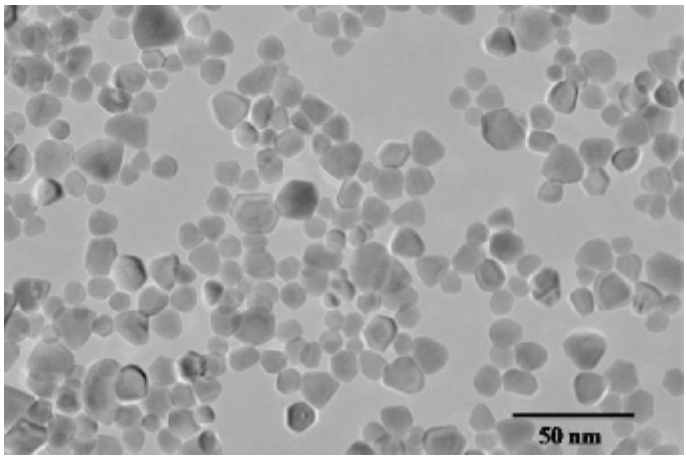


Figure 20. Low magnification TEM micrograph of biologically synthesized Zn nanoparticles at 50 nm scale bar

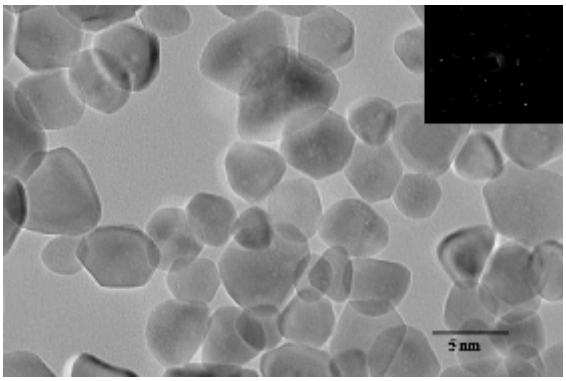


Figure 21. High magnification TEM micrograph of biologically synthesized Zn nanoparticles at 5 nm scale bar, inset showing the SAED pattern of a single Zn nanoparticle

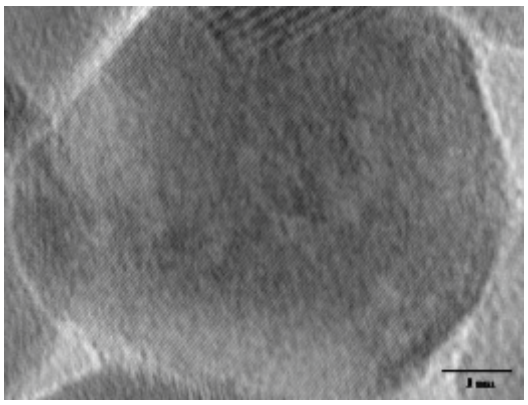


Figure 22. HR–TEM micrograph of biologically synthesized single Zn nanoparticle

The biosynthesized single Zn nanoparticle is shown as figure 22. To ascertain the surface morphology, the nanoparticles were coated with heavy metal gold and subjected to SEM imaging. A micrograph (Figure 23) gives a fairly good idea that the Zn nanoparticles were having rough outer surface and showing a certain degree of agglomeration. To further validate the surface morphology drop coated AFM three dimensional images were taken in non contact mode. One such AFM 3-D micrograph (Figure 24) clearly shows that the rough surface of the biosynthesized Zn nanoparticles.

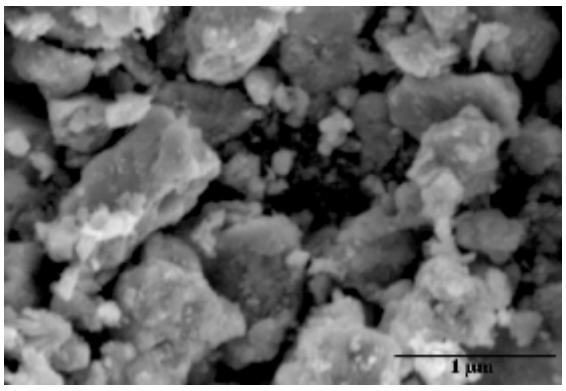


Figure 23. SEM micrograph of biologically synthesized Zn nanoparticles

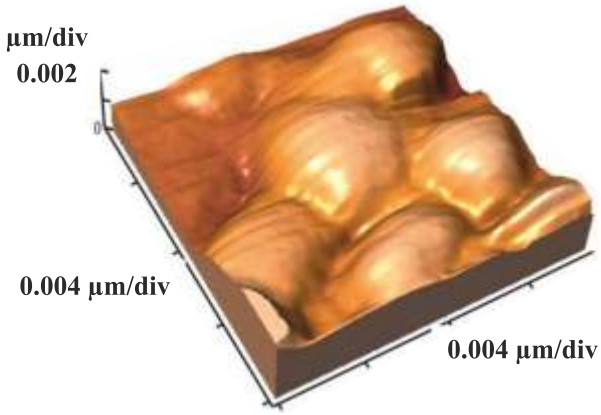


Figure 24. AFM micrograph of biologically synthesized Zn nanoparticles

Mechanically developed nanoparticles need encapsulation by proteins to make them eco-friendly. However, biologically synthesized Zn nanoparticles using fungal extracellular enzymatic system were naturally encapsulated the Zn metal particles. In order to validate the formation of nanoparticles and their encapsulation by the fungal protein, the biosynthesized nanoparticles were subjected to FTIR spectroscopy. The graphical representation shows signature peaks for Zn metal marked with arrow and other peaks are due to fungal protein encapsulating the synthesized nanoparticles (Figure 25).

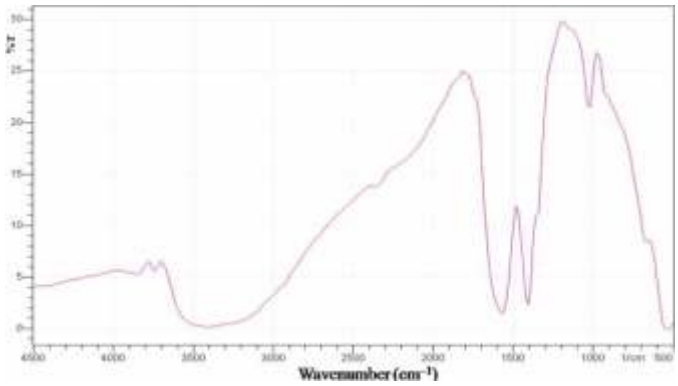


Figure 25. FTIR spectra of biosynthesized Zn nanoparticles

The crystallity of zinc nanoparticles was assessed using XRD at 2 angle of Bragg's equation (Figure 26). The typical seven characteristic peaks of Zn metal finally validated the crystallity of Zn metal only.

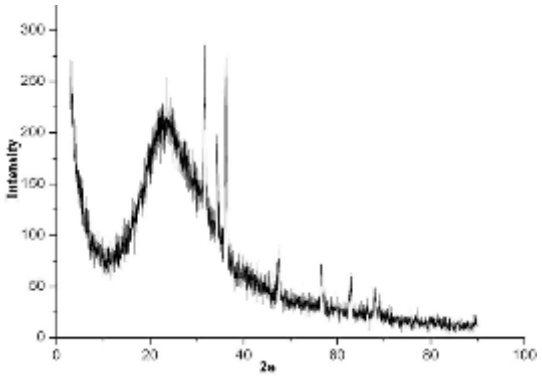


Figure 26. XRD spectra of biosynthesized Zn nanoparticles

The elemental compositions of bio-transformed product containing Zn nanoparticles in the solution were confirmed by TEM equipped with energy dispersive X-ray spectroscopy (EDX). The results of TEM- EDX (Figure 27) clearly shown that the Zn nanoparticles were highly intense and the maximum intensity was found at 1keV. The oxygen intensity was also observed up to 600 due to disassociation of Zn and oxygen molecules from the precursor salt ZnO used for biosynthesis of Zn nanoparticles. Results clearly depicts that sample contains 98% atom of Zn metal only.

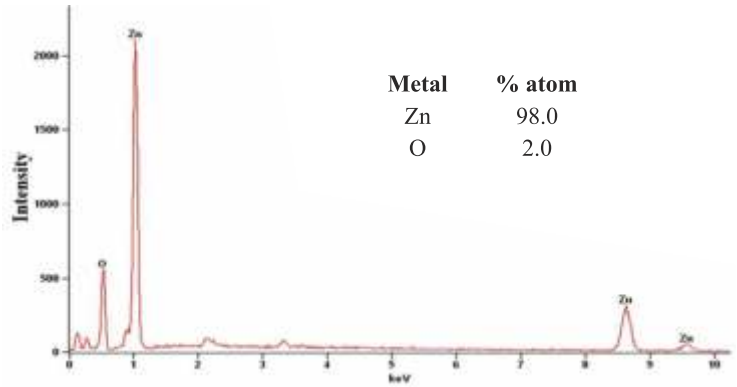


Figure 27. EDX spectrum of biologically synthesized Zn nanoparticle

Characterization of Mg nanoparticles

Fungal extracellular enzyme mediated synthesis of magnesium nanoparticles size was initially calculated by DLS technique, which, analyses particle size on the basis of intensity, number and volume distribution in the solution. The DLS also measured zeta potential of the nanoparticles. The graphical depiction (Figure 28) clearly exhibits that particles between one and 6.4 nm and the number distribution also indicated that most of the particles were within the range of one to 6 nm (Figure 29). The results of intensity and number distribution were further validated by the volume distribution curve, which shows that 100 % of the nanoparticles were measured below 6.4 nm (Figure 30). The detailed size distributions of Mg nanoparticles were presented as Table 5. The surface charge of the nanoparticles plays a crucial role during interaction with molecule more particularly biological system such as plant, which should normally be in the range of –30 to +30 mV. The surface charge of biosynthesized Mg nanoparticle was measured as zeta potential of –6.66 mV using DLS was graphically represented (Figure 31).

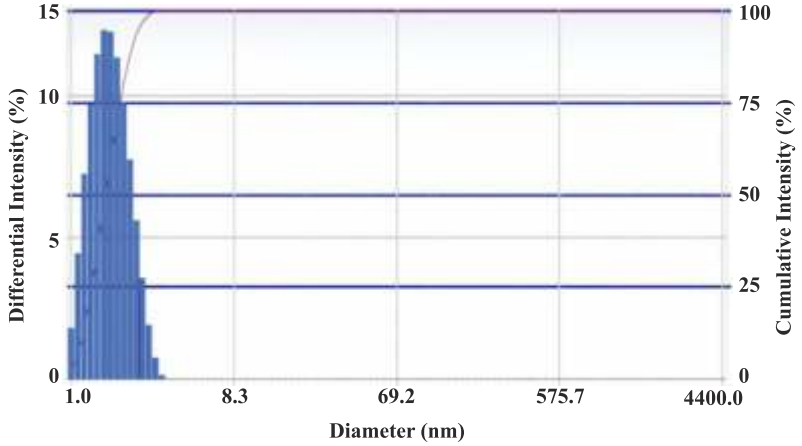


Figure 28. Intensity distribution of biologically synthesized Mg nanoparticles

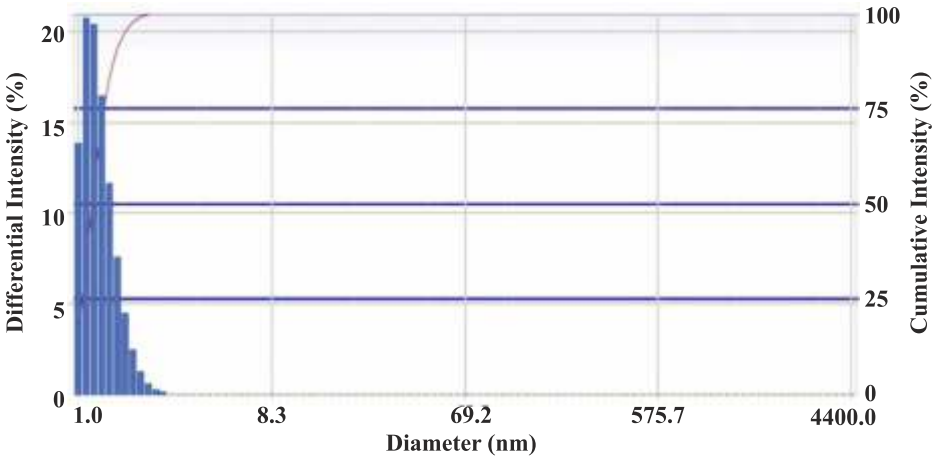


Figure 29. Number distribution of biologically synthesized Mg nanoparticles

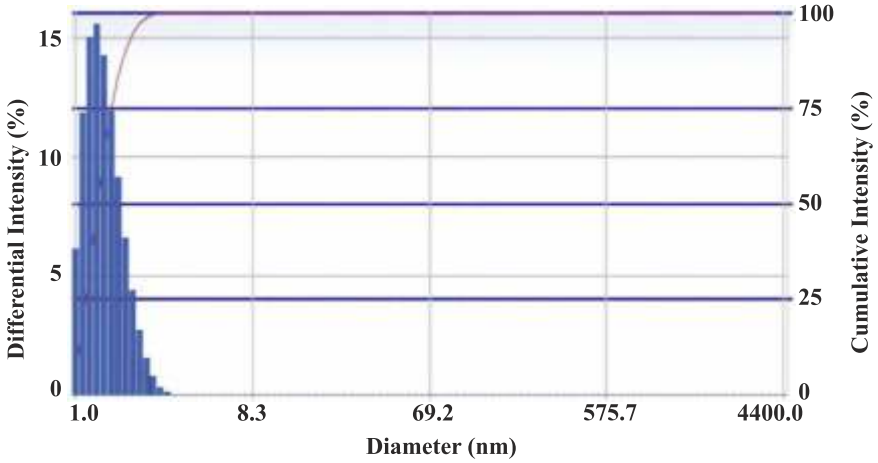


Figure 30. Volume distribution of biologically synthesized Mg nanoparticles

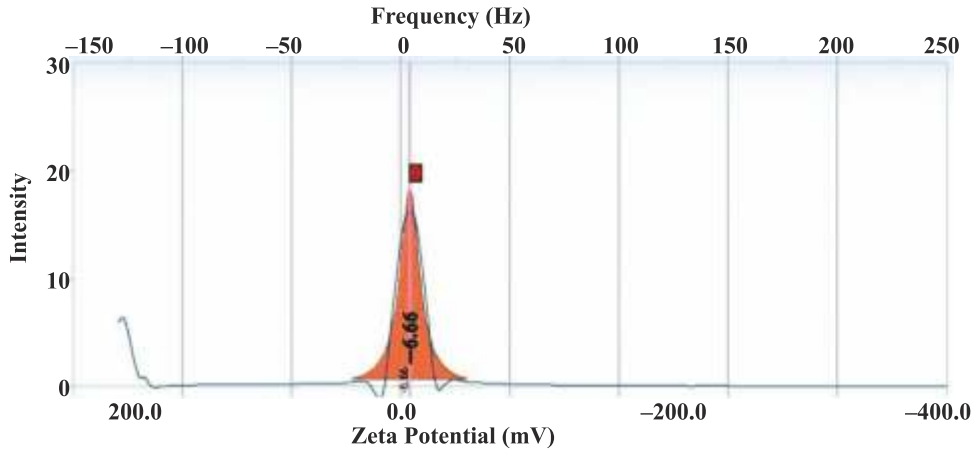


Figure 31. Zeta potential of biologically synthesized Mg nanoparticles

Table 5. Size distribution of biosynthesized Mg nanoparticles

Nanoparticle size	Intensity distribution	Number distribution	Volume distribution
Cumulative per cent nonoparticle			
1.0	58.5	72.5	69.2
2.0	66.2	84.8	75.6
3.0	78.4	87.6	79.8
4.0	89.2	92.4	86.2
5.0	96.4	98.9	92.4
6.0	99.9	100.0	99.3
7.0	100.0	100.0	100.0

Once the size of nanoparticle was confirmed, it was subjected to electron microscopy for detail morphological studies. The transmission electron microscopic images of biosynthesized Mg nanoparticles were shown (Figure 32–34). It can be clearly seen in the low magnification image at 50 nm scale bar (Figure 32) that all the particles were below 10 nm and show mono-dispersity whereas a reasonably good idea about shape and size can be obtained under highly magnified TEM micrograph at 5 nm scale bar (Figure 33).

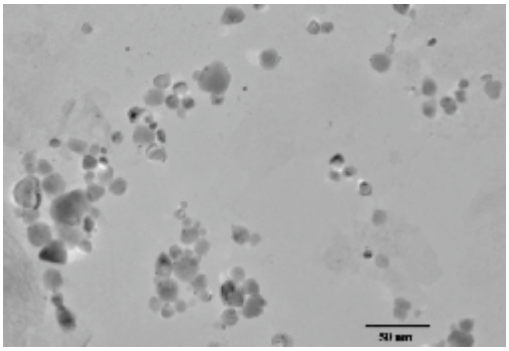


Figure 32. Low magnification TEM micrograph of biologically synthesized Mg nanoparticles at 50 nm scale bar

Insight of Figure 33 shows SAED patterns of a single Mg nanoparticle, where white array of spot supports the crystalline nature of nanoparticle. The high resolution TEM micrograph of a single Mg nanoparticle shows the linear arrangements of atoms within the nanoparticle, and the presence of lines also depicts the crystallity of Mg nanoparticles which was otherwise deficient in nonmetallic organic compounds and molecules. The TEM image of single Mg nanoparticles was also presented as Figure 34.

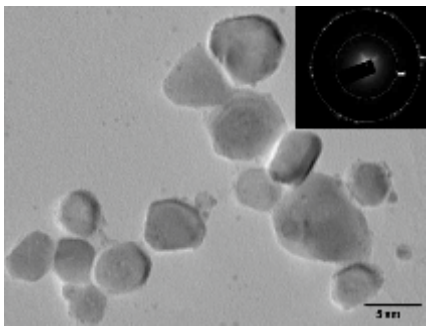


Figure 33. High magnification TEM micrograph of biologically synthesized Mg nanoparticles at 5 nm scale bar, inset showing the SAED pattern of a single Mg nanoparticle

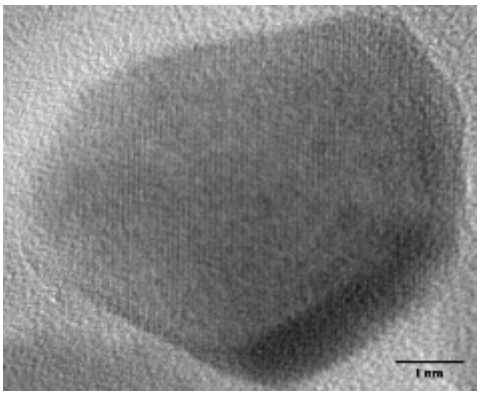


Figure 34. HR–TEM micrograph of biologically synthesized single Mg nanoparticle

The sample was subjected for heavy metal gold coating followed by SEM imaging to determine the surface structure of biosynthesized Mg nanoparticles. A micrograph shown (Figure 35) gives a fairly good idea that the Mg nanoparticles were having rough outer surface and showing well distribution of the particles. To further validate the surface morphology and size, three dimensional images were taken under non contact mode of AFM. One such 3-D micrograph shown (Figure 36) clearly exhibited that the rough surface of the biosynthesized Mg nanoparticles and also confirms the mono-dispersity.

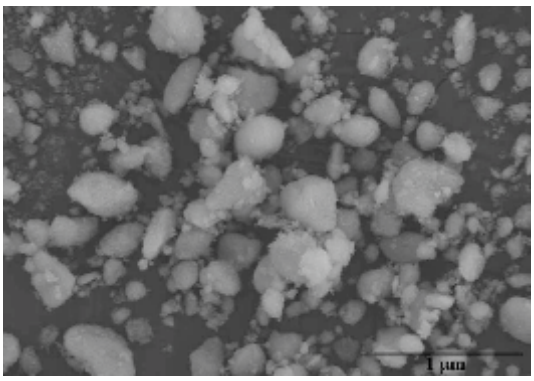


Figure 35. SEM micrograph of biologically synthesized Mg nanoparticles

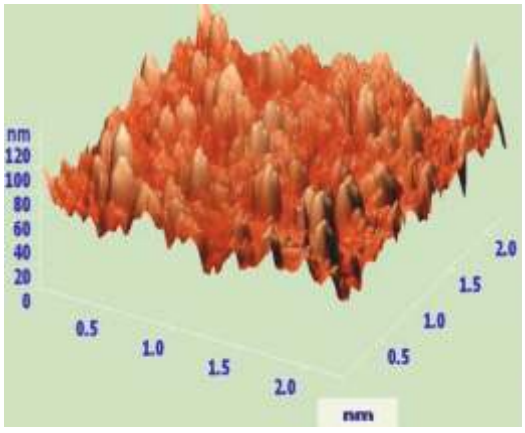


Figure 36. AFM micrograph of biologically synthesized Mg nanoparticles

Instinctively developed nanoparticles need encapsulation by organic molecule such as proteins to make them environmentally benign. The biologically synthesized Mg nanoparticles get naturally encapsulated by the fungal extracellular enzymatic system during the metal protein interaction. In order to confirm the encapsulation of nanoparticles by fungal protein, the biotransformed products were subjected to FTIR spectroscopy. The graphical representation in the Figure 37 shows the signature peak for Mg metal marked with arrow and other peaks are due to fungal extracellular protein encapsulating the synthesized nanoparticles.

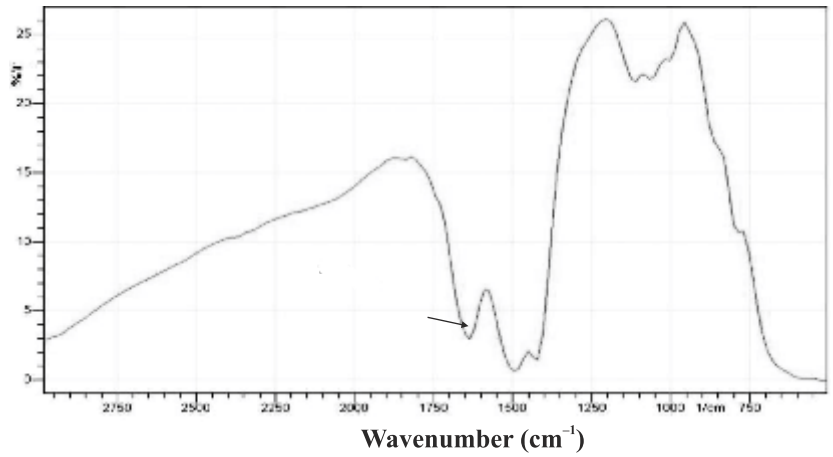


Figure 37. FTIR spectra of biosynthesized Mg nanoparticles

The crystallity of magnesium nanoparticles was further confirmed using XRD at 2 angle of Bragg's equation (Figure 38). The typical three characteristic peaks of Mg metal finally validated the crystallity of Mg metal.

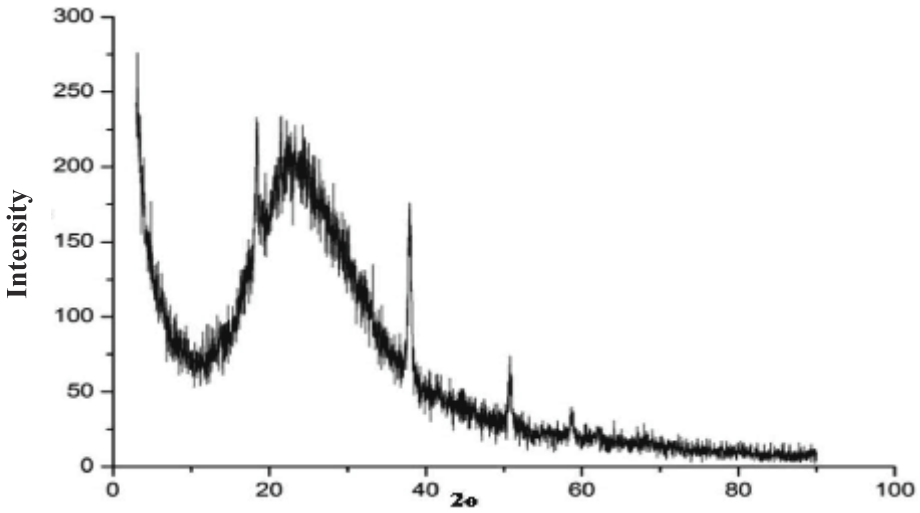
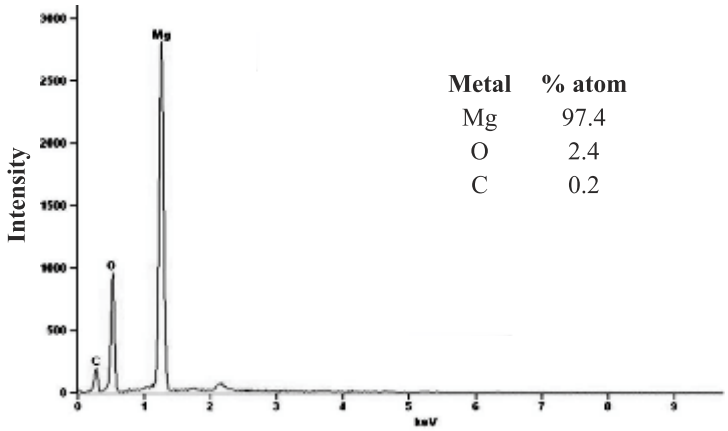


Figure 38. XRD spectra of biosynthesized Mg nanoparticles

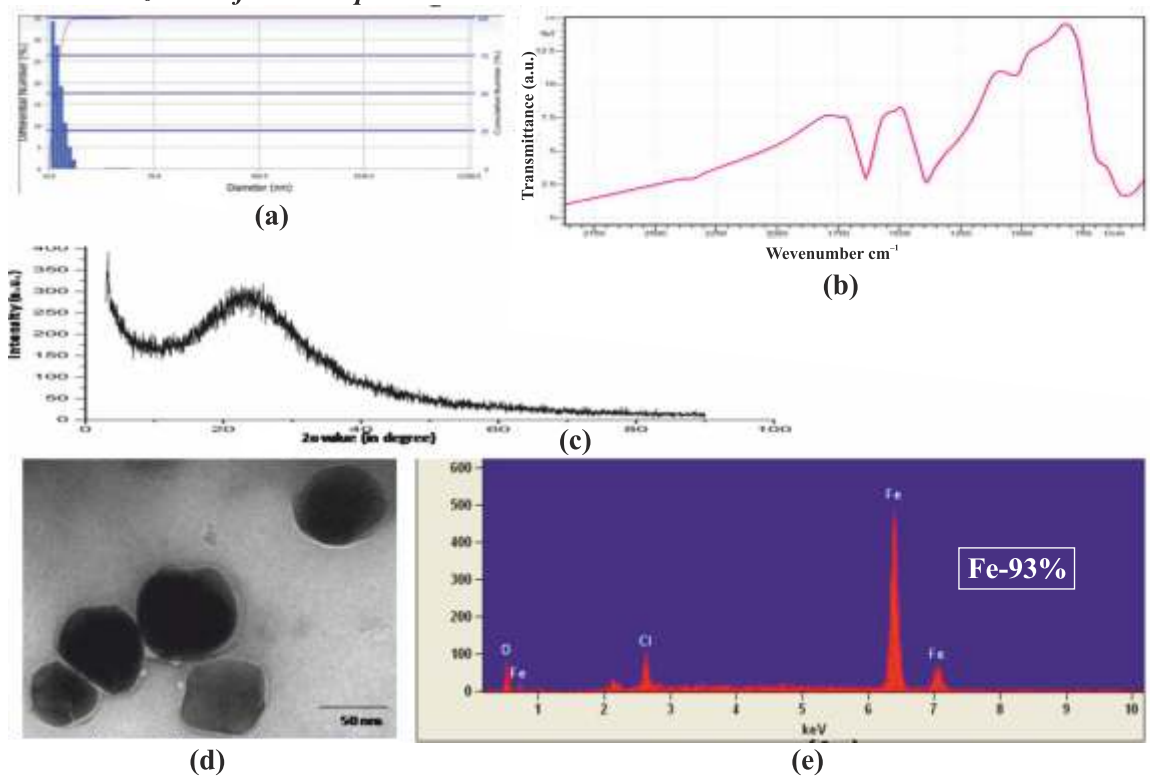
The elemental proportions of biotransformed product containing Mg nanoparticles in the solution were confirmed by TEM equipped with energy dispersive X-ray spectroscopy (EDX). The results of TEM- EDX have clearly shown that the Mg nanoparticles were highly intense and the maximum intensity of 2800 was found at 1.3keV (Figure 39). The peak intensity of 1000 about oxygen was due to disassociation of Mg and oxygen molecules from the precursor salt MgO used for biosynthesis of Mg nanoparticles and another low intensity carbon peak found at 0.2keV was due to organic molecules of fungal protein. Results clearly depicts that sample contains 97.4% atom of Mg metal only.



Metal	% atom
Mg	97.4
O	2.4
C	0.2

Figure 39. EDX spectrum of biologically synthesized Mg nanoparticle

Characterization of Fe nanoparticles



(a) **(b)** **(c)** **(d)** **(e)**

Figure 40. Complete characterization of Fe nanoparticles **(a)** Particle size distribution **(b)** FTIR spectra **(c)** XRD stpectra **(d)** TEM micrograph **(e)** EDX Spectrum

The nanoparticle of Fe ranges between 10 and 70nm with 93% purity (Figure 40). In general, nanoparticles were oval shaped with average diameter 11.7nm and a PDI of 0.471 indicate the particle are monodispersed. The results also indicate 93% atom of Fe metal only.

Characterization of P nanoparticles

The average size of P nanoparticles measured by DLS is 5.8nm (Figure 42) with 67% purity from Calcium phosphate salts. In general, nanoparticles were oval shaped with a PDI value of 0.435 indicating the particles were monodispersed. The purity of nanoparticles can be enhanced to 74% by utilizing phytin as precursor salts but only one bacteria was found to break down phytin to nano-P form and identified as *Bacillus megatorium*. The entire characterization of P nanoparticles were presented as Figure 41.

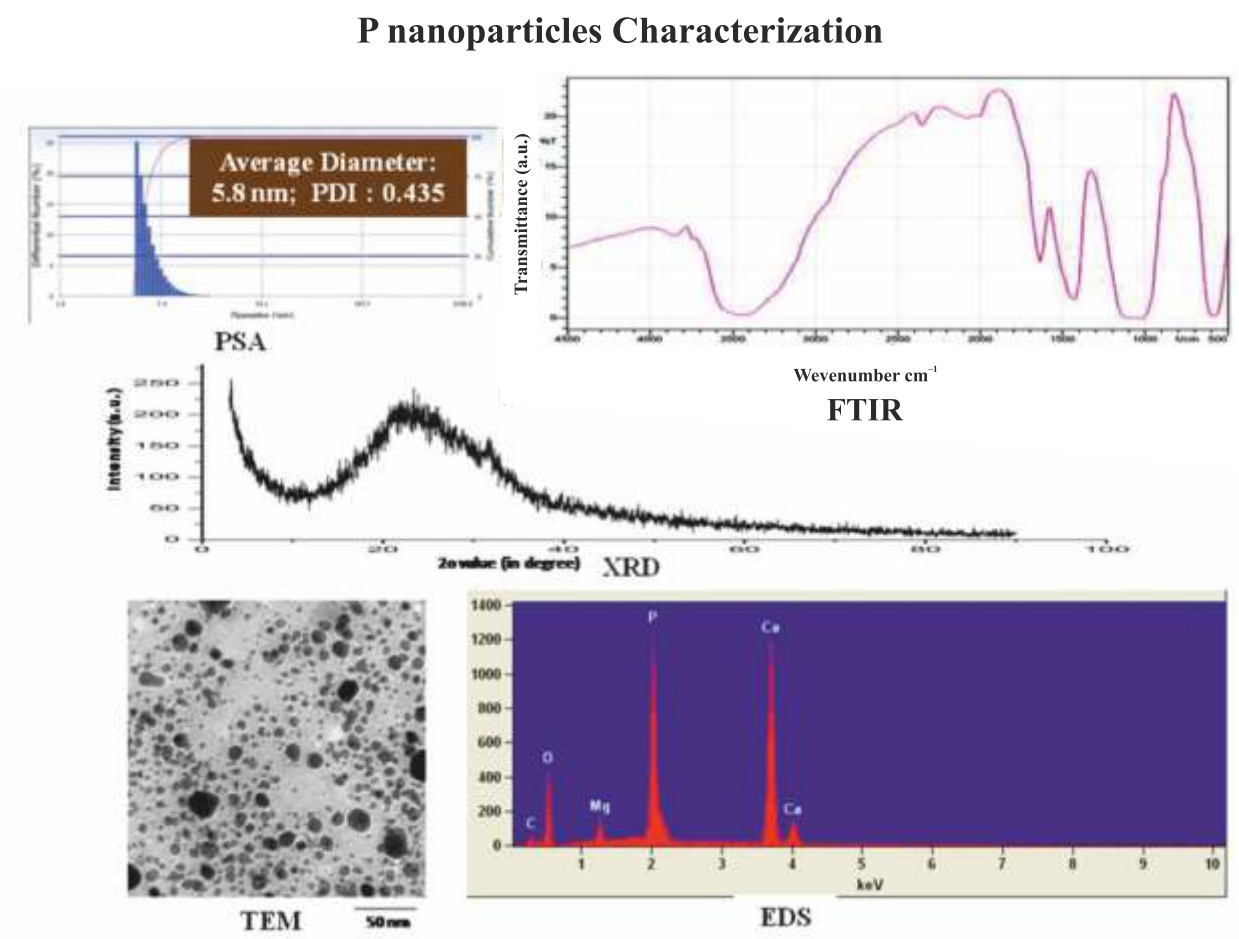


Figure 41. Characterization of P nanoparticles

Activity 6: Effect of nano-particle on nutrient use efficiency, plant metabolism and enzyme exudation **[CAZRI Jodhpur; IISS Bhopal; BITS Pilani]**

Optimization of concentration of nanoparticles

Aerosol spray is found much superior than traditional spray for the application of nanoparticles to plants. In general, by normal spray 33% of the nanoparticles were untraceable after spray and were considered as a loss while aerosol spray resulted only 15% loss. Higher the particle size lower is the penetration. Nanoparticle size < 20nm may be better for application to the plants. Nanocube is the better shape to spray to plants. Two weeks old plants are found to be more suitable for foliar application of nanoparticles.

Entrance and transportation of nanoparticle

Nanoparticles can enter through shoots (bark, cuticle, stigma, stomata, hydothodes) and roots (root tips, rhizodermis, lateral root junctions and woundings) of the plants. Normally, more penetration was found through stomata and cuticle. After entering, the nanoparticle moves through cell sap. While transport they trigger various enzyme systems (Figure 42) and most of the particles may agglomerate to form mega particles on the pathway and mostly gets deposited at the vacuole. (Figure 43).

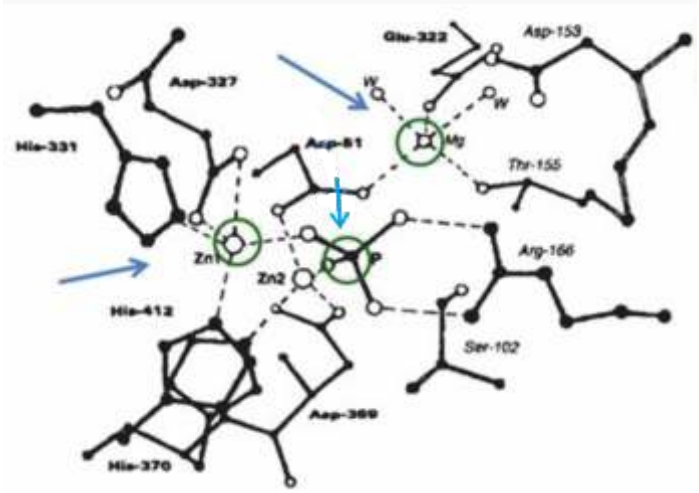


Figure 42. Triggering of different cofactors by nanoparticles during transport

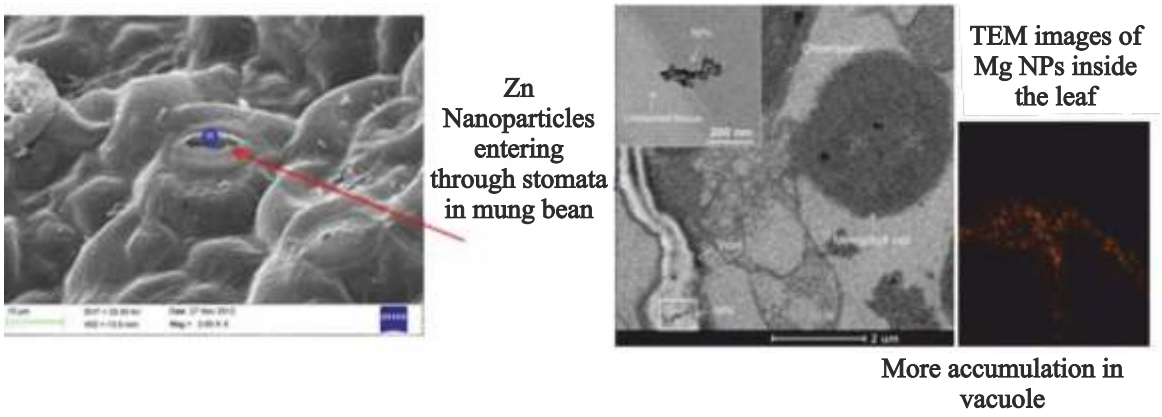


Figure 43. Penetration and movement of nano particles on arid crops

The beneficial enzyme release due to nano-induction may vary from 18 and 283%. At crop harvest, no significant differences, compare to control, in nanoparticle concentration was observed in leaves, stem and seeds (Table 5) of mung bean when full scan TEM analysis was carried out, clearly indicate their agglomeration to mega particle with time.

Table 5. Accumulation of Zn (% Atom) in Zn nanoparticle treated plants in Mung bean

Element	Leaf		Stem		Seed	
	Control	Treated	Control	Treated	Control	Treated
Zinc	1.13	1.2	2.93	2.98	0.91	1.14
Copper	92.64	92.64	91.6	91.6	96.28	92.45
Uranium	4.97	4.9	0.52	0.52	0.98	1.62
Osmium	1.27	1.27	4.96	5	1.91	4.78

Nutrient Use Efficiency (NUE)

Tremendous improvement of NUE was observed in plants after application of different nanoparticles. In general, 3-4 times improvement in use efficiency was noticed due to P, Zn, Fe and Mg nanoparticles. The effect on P use efficiency was presented as Figure 44.

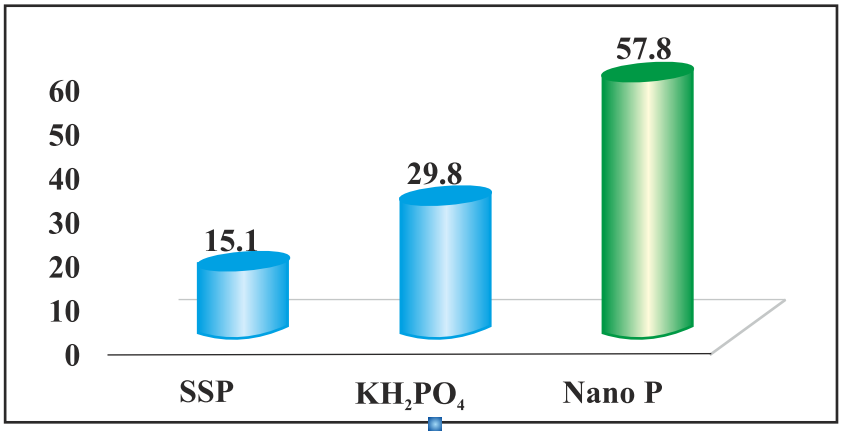


Figure 44. A Comparison Effect on P use efficiency (SSP - Simple Super Phosphate)

In general, 18-283% improvement of different beneficial enzyme activities (esterase, acid phosphatase, alkaline phosphatase, phytase, aryl sulphatase, nitrate reductase, urease, cellulose, hemi-cellulase, lignase) was noticed in the rhizosphere of different crops grown under arid environment due to application of different nanonutrients. Application of Nano P also helps to improve the organic acid concentration in the rhizosphere and P uptake by the plants (Table 6).

Table 6. Per cent improvement in organic acid concentration* in the rhizosphere and P uptake by the plants

Crops	Organic acid concentration	Puptake
Clusterbean	23.2	27.2
Moth bean	19.5	23.5
Mung bean	20.7	22.7
Pearl millet	15.5	17.3
*Nano-P application 640 mg ha^{-1}		

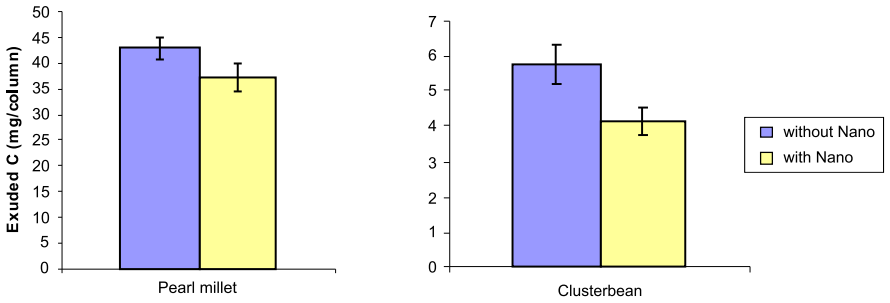


Figure 45. Exuded C in pearl millet and Clusterbean

Carbon leaching through roots may reduce due to application of nanoparticles resulting in more biomass production in Clusterbean and Moth bean (Figure 45). Nano Zn and Fe application was associated with high protein content and low semi-oxide dismutase activity resulting in more stress tolerance by the plant (Table 7).

Table 7. Effect of different concentrations of Zn on protein content, SOD activity in Chickpea (% change over control)

Treatments	Protein Content (mg/mL)	SOD (Unit/mg protein)
10 ppm ZnO	**+ 13.5**	**- 28.7**
10 ppm nano ZnO	**+ 30.4**	**- 38.7**
1.5 ppm ZnO	**+ 29.5**	**- 37.7**
1.5 ppm nano ZnO	**+ 41.4**	**- 43.2**
1.5 ppm Fe_2O_3	**+ 20.6**	**- 33.2**
1.5 ppm nanoFe_2O_3	**+ 32.9**	**- 39.7**

Nano Zn and Fe application on plant leaf increases chlorophyll content and decreases malondialdehyde content resulted more prevention of membrane damage (Table 8).

Table 8. Effect of Zn and Fe nanoparticles on chlorophyll and malondialdehyde content

Treatments	Chlorophyll (mg/g FW)	Malondialdehyde (mM/g FW)
10 ppm ZnO	+ 31.6	- 4.3
10 ppm nano ZnO	+ 68.1	- 5.7
1.5 ppm Fe2O3	+ 20.7	- 1.2
1.5 ppm nanoFe2O3	+ 41.5	- 4.0

A similar experiment with Mg nanoparticle showed higher light absorption, chlorophyll and leaf protein content of moth bean (Table 9) and wheat (Table 10).

Table 9. Effect of Biosynthesized Mg nanoparticles on Moth bean

Concentration : 0.1 mM
Solution Sprayed : 25 mL/pot
Spray : 14 days old plants

Harvesting :
1st (21 days old plant)
2nd (28 days old plant)
3rd (42 days old plant)
3 plants per pot

Treatment	Leaf Protein (mg g^{-1})			Total Chlorophyll (µg mL^{-1})		
	I	II	III	I	II	III
Control	229.5	242.1	244.3	12.6	16.1	16.8
Ordinary Mg	264.1	275.1	273.0	17.8	21.2	22.1
Nano Mg	301.9	339.2	348.1	26.9	33.6	43.4
LSD (p = 0.05)	11.2	10.5	9.8	2.3	2.9	3.5

Table 10. Effect of Biologically synthesized Mg nanoparticles on sunlight absorption and chlorophyll content in wheat (6 weeks old plant)

Treatment	Light absorption (Lux)	% improvement	% improvement in chlorophyll content
Control	1432.8	-	-
Mega particle	1481.5	3.4	1.9
Nano particle (<20 nm)	1743.5	21.7	16.7

Response of nanoparticle on root growth and development was studied under different arid crops. The results showed more effect of nano P on root growth (improvement of root length upto 32%, root area 20.5%, biomass 10.2%, root nodulation 67.7%). Similar results were observed with the application of nano Zn, Fe and Mg where increase in root length varied between 2-7%, root area 4-18% and dry biomass 1-5% while nodulation increases between 3-47%. The effect of nano Zn and nano Mg application under field conditions on Mung bean is presented as Figure 46(a) and Moth bean Figure 46(b).

Figure 46(a). Mung bean (n Mg - Nano Magnesium; n Zn - Nano Zinc)

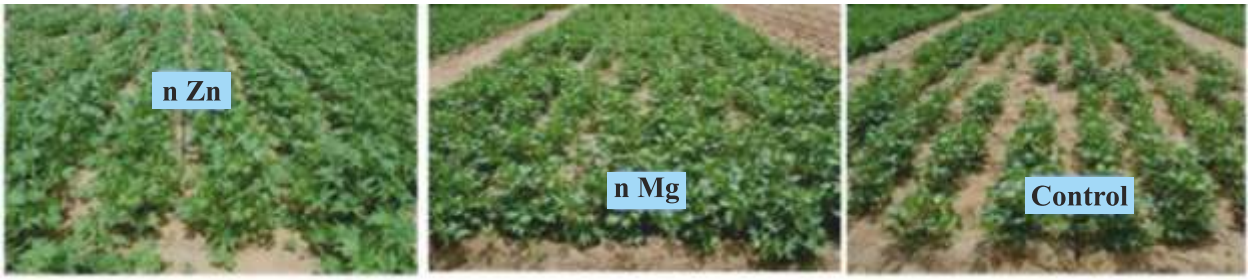


Figure 46(b). Moth bean (n Mg - Nano Magnesium; n Zn - Nano Zinc)

Changes in carbon partitioning in pearl millet and clusterbean in response to ZnO nanoparticle application

Two weeks after emergence when the pearl millet plants (*Pennisetum glaucum* (L.) R. Br. var. CZP-9802) were at 5-6 leaf stage, 10 mL aqueous solution of 100 mg spherical nano-ZnO L^{-1} (prepared and characterized by IIT Mumbai of size range 16-30 nm) was sprayed under pressure in an illuminated enclosure to completely drench the leaves. The spray was repeated for three consecutive days. Plants were then grown under natural screen house condition for another three weeks. During this period Hoagland solution of complete strength was added every day and the exudates were collected by opening the tap of the column. An identical set of plants sprayed with equal quantity of water plus surfactant served as control. After five weeks plants were harvested and washed under flowing deionized water. Shoot and root was separated, oven dried at 72 ^{0}C for 48 h and recorded dry biomass. Similar experiment was also carried out with clusterbean (*Cyamopsis tetragonoloba* (L) Taub) cv. RGC-936. In both pearl millet and clusterbean foliar spray of ZnO nanoparticles was associated with variable response on shoots and root growth. The former increased while the later decreased (Table 11). This changed shoot to root ratio from 1: 2.36 to 1: 4.76 in pearl millet and from 1: 1.78 to 1: 4.90 in clusterbean. But in our experiment, dry weight of pearl millet and clusterbean roots decreased by 17 % and 38%, respectively after application of nanoparticles (Table 11). Total C in shoot and root together was 28.8% and 19.9% higher in pearl millet and clusterbean plants treated with nano-ZnO. Also, malondialdehyde content, an indicator of lipid peroxidation, was 4.3 and 10.7 % less in nano-ZnO treated pearl millet and clusterbean leaves, respectively than the corresponding controls. Application of nano ZnO significantly decreased the C exuded from roots in both pearl millet and clusterbean. C exuded through roots was 2.19% of total C accumulated in plants for pearl millet and 0.81% for clusterbean.

Table 11. Effect of nano-ZnO on zinc concentration, biomass and total carbon in root and shoot of pearl millet and clusterbean

Crop	Control		Nano-ZnO	
	Zinc concentration (µg g^{-1} biomass)			
	Shoot	Root	Shoot	Root
Pearl millet	27.69±0.51	41.51±0.83	30.00±0.56	43.75±0.61
Clusterbean	107.44±0.87	159.31±1.37	111.27±2.37	161.39±2.39
	Dry weight (g $column^{-1}$)			
	Shoot	Root	Shoot	Root
Pearl millet	3.19±0.19	1.35±0.13	4.78±0.25	1.12±0.06
Clusterbean	1.27±0.07	0.71±0.02	2.01±0.10	0.41±0.03
	Total carbon (g $column^{-1}$)			
	Shoot	Root	Shoot	Root
Pearl millet	1.06±0.05	0.42±0.03	1.56±0.05	0.35±0.01
Clusterbean	0.48±0.01	0.23±0.02	0.70±0.01	0.14±0.01

Relative water content of leaves (RWC), an indicator of crop water status, revealed an interesting trend wherein foliar spray of zinc (in either form) in addition to already present in Hoagland solution resulted in lowering of leaf turgiscence. Higher concentration (10 ppm) reduced the RWC more compared to 1.5 ppm (Table 12).

Table 12. Effect of different forms of zinc on relative water and malondialdehyde content of chickpea leaves

Treatments	Relative leaf water content (%)	MDA (mM g^{-1} FW)
1.5 ppm nanoZnO	74.47	0.152
10 ppm nanoZnO	65.91	0.147
1.5 ppm ZnSO4	69.02	0.145
10 ppm ZnSO4	66.37	0.140
1.5 ppm ZnO	75.42	0.153
10 ppm ZnO	72.12	0.141
Control	76.52	0.155
Master control	78.81	0.166
LSD (5%)	NS	0.004

Result clearly indicated exposing roots to 1.5 ppm nano zinc oxide increased nitrogenase activity whereas 10 ppm nano ZnO drastically decreased the activity. The average N_2ase activity represented per unit nodule fresh weight in nanoparticle treated mungbean and cowpea seedlings is tabulated below against control for comparison (Table 13).

Table 13. Effect of nano ZnO on N_2ase activity of mung bean and cowpea

Treatment	Mungbean	Cowpea
Control	8.45	2.55
ZnO (Nano) 1.5 ppm	10.05	18.6
ZnO (Nano) 10 ppm	0.0008	0.91

The results also emphasized that nutrient in nanoscale may be effectively foliar sprayed in low dose to improve biomass production.

Objective 2. *Enhancement of gum production for soil binding and moisture retention by microbes through nano-particle (Mg, Zn, Fe, P) stimulation.*

Activity 1. Screening of available nanoparticles for increasing gum production from microorganisms **[CAZRI Jodhpur]**

Eighty five polysaccharide producing organisms were isolated and different nanoparticles were tested for increasing gum production. Fifteen organisms (Table 14) were found to be responsive to induce gum production due to activation of nanoparticles. In general, Zn and Fe nanoparticles were found to be more responsive in enhancing gum production to both fungi (Figure 47a) and bacteria (Figure 47b).

Table 14. Polysaccharide producing organism developed

S. No.	Organisms Name	Type	NCBI Gen Bank Accession No.	Type of polysaccharide
1	Bacillus subtilis JCT- 1	Bacteria	JN 194187	Dextran
2	Bacterium JCT- 2	Bacteria	JN 194188	Xanthan
3	Paenibacillus illinoisensis JCT- 3	Bacteria	JN 194189	Polysaccharide*
4	Caulobacter vibrioides JCT- 4	Bacteria	JQ675300	Curdlan
5	Rhizobium sps.- JCT- 5	Bacteria	JQ675301	Xanthan
6	Bacillus subtilis JCT- 6	Bacteria	JQ675302	Dextran and Xanthan
7	Caulobacter vibrioides JCT- 7	Bacteria	JQ675303	Polysaccharide*
8	Agrobacterium tumefaciens JCT-8	Bacteria	JQ675304	Xanthan and curdlan
9	Stenotrophomonas maltophilia JCT- 9	Bacteria	JQ675305	Polysaccharide*
10	Caulobacter vibrioides JCT- 10	Bacteria	JQ675306	Polysaccharide*
11	Caulobacter vibrioides JCT- 11	Bacteria	JQ675307	Polysaccharide*
12	Sporosarcina ginsengisoli JCT-12	Bacteria	JQ675308	Xanthan and Dextran
13	Aspergillus terreus TFR-2	Fungi	JN 194186	Pullulan
14	Aspergillus flavus TFR- 7	Fungi	JQ675294	Pullulan
15	Agrobacterium tumifaciens JCT -13	Bacteria	KF729584	Pullulan and Dextran

* Identification of polysaccharides is in progress

There was marked increase in polysaccharide production when the concentration of sucrose was increased to 16% (Table 15). Maximum harvest of polysaccharides in terms of fresh and dry weight was achieved at pH 9. Polysaccharide was extracted on the fourth day of inoculation. More production was observed at 28˚C.

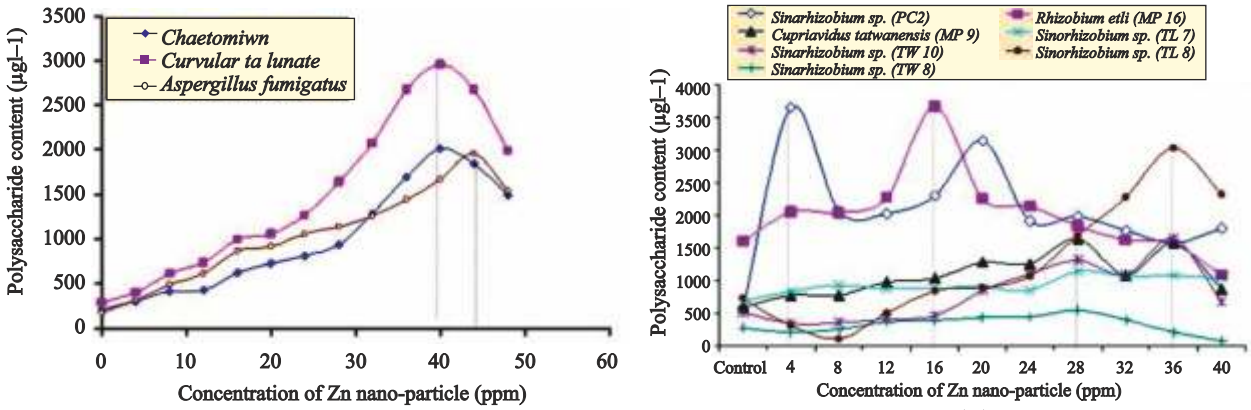


Figure 47. Nano Zn induced polysaccharide production by a. Fungi and b. Bacteria

SM, Levan, EPS, and Nutrient Broth were compared for the production of polysaccharide. Among the media tested SM media was observed most effective for polysaccharide production.

Table 15. Effect of different concentrations of sucrose on polysaccharide production by *Bacillus subtilis*

Concentration of sugar (%)	Fresh wt (g)	Dry wt.(g)
0	-----	-----
4	2.8	1.1
8	5.8	2.6
12	8.1	3.2
16	8.5	3.9

A methodology was developed (Figure 48) and patented (Patent no. 404/DEL/2012) for preparation of polysaccharide powder. (Figure 49)

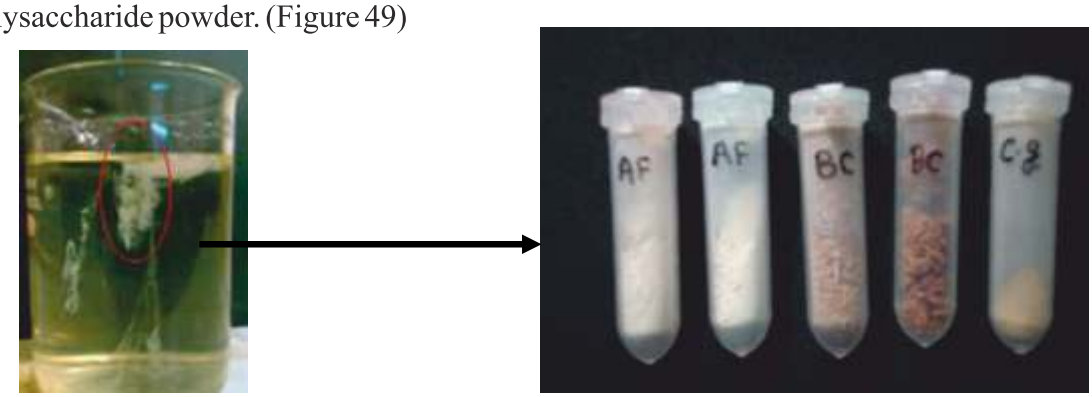


Figure 48. Flocculated polysaccharide pellet and their powder form

Microbial cells/mass cultured in broth medium with 16% sucrose solution
↓
Cells/mass were harvested by centrifugation at 6000 rpm for 30 minutes
↓
Two volumes of cold ethanol were added to culture supernatants
↓
The mixtures stored overnight at 40˚C
↓
Flocculent mass obtained which was collected by centrifugation at 8000 rpm for 30 minutes
↓
Precipitate resuspended in demineralised water and mixed with two volumes of cold ethanol
↓
Samples were again centrifuged at 8000 rpm for 30 minutes
↓
Pellet of polysaccharide obtained
↓
Pellets were dried at 100˚C in the oven to obtain the polysaccharide in powder form

Figure 49. Flow chart for isolation and purification of microbial polysaccharides

Activity 2. Assessment of binding and soil moisture retention efficiency of microbial gum **[CAZRI Jodhpur]**

The microbial gums were powdered and characterized using electrophoresis by SDS PAGE. Mainly fungal polysaccharide was identified as pollulan and bacterial polysaccharide was identified as xanthan and curdlan (Figure 50)

(a) **(b)**

Figure 50. Characterization of isolated polysaccharide from bacteria and fungi by PAGE.

Nano induced polysaccharide powder was used to improve soil aggregation, carbon build-up and moisture retention and it was found that under arid environment the soil aggregation was improved by 33-83% within a month. (Figure 51)

Mixed with Bacterial Polysaccharide **Control**

Figure 51. Effect of bacterial polysaccharides on arid soils within a month

The aggregate percentage was further improved in 1 mm aggregate size as compared to 0.5 mm or 0.18 mm size with the application of bacterial polysaccharide of 1% concentration (Table 16).

Table 16. Improvement of arid soil aggregation using nano induced polysaccharide powders

Treatment	Per cent improvement of aggregate size over control after 30 days (1% w/v)		
	1.0 mm	0.5 mm	0.18 mm
Polysaccharide from *Bacillus coagulans*	80.7	No change	No change
Polysaccharide from *Alcaligenes faecalis*	33.4	82.9	56.4

The improvement of moisture retention was studied at different concentration of polysaccharide ranging from 1-6%. The retention capacity was measured after 4 weeks of application. It was found that the moisture retention improved from 10.7 – 14.2 % at different level of concentrations. The results (Table 17) clearly indicate that 1% polysaccharide application gives economically higher moisture retention although maximum moisture retention was observed at 6% polysaccharide application.

Table 17. Improvement of moisture retention* due to polysaccharide application

Polysaccharide percentage	Improvement in moisture retention (%)
1	10.7
2	12.2
3	12.5
4	12.8
5	13.6
6	14.2
LSD (p = 0.05)	2.3

*after 4 weeks of application

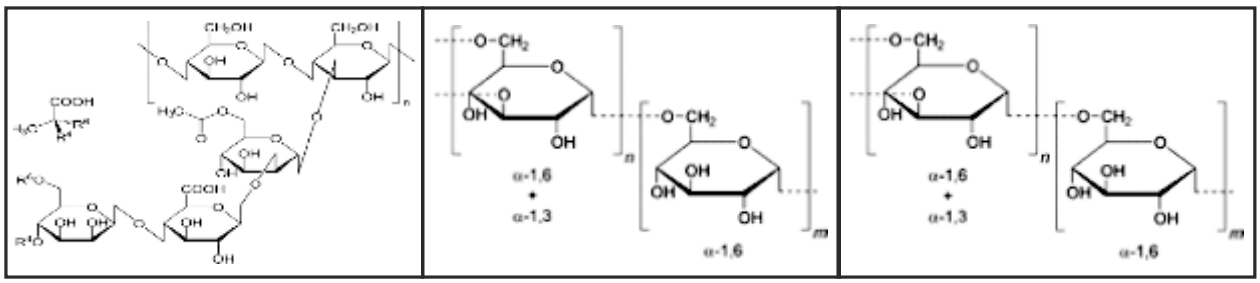
The carbon build-up with the application of microbial polysaccharides was also studied at weekly intervals upto 28 days and at crop harvest with 3 polysaccharide concentrations (1%, 4% and 5%). The results suggested that initial carbon build-up was enormous but with time there was gradual decline in carbon concentration due to corresponding microbial build-up. The carbon build-up at crop harvest was noticed between 3 and 5%. Although the initial build-up varies between 41% and 21% with 1% polysaccharide concentration and between 63% and 44.6% with 5% polysaccharide concentration (Table 18).

Table 18. Effect of nano-induced polysaccharide powder on soil organic C build-up

% polysaccharide applied	%improvement of organic C in soil (days after application)			
	7	14	21	28
1	41.0	30.6	27.1	21.0
4	61.0	52.0	47.3	36.8
5	63.0	54.0	48.5	44.6

Activity 3. Assessment of soil aggregation, bonding mechanism between different soil particles and exo-polysaccharides **[CAZRI Jodhpur; IIT Bombay]**

Xanthan, dextran and curdlan were three microbially produced exo-polysaccharide which play an important role in soil aggregation (Figure 52). Experiment was carried out to study the effect of incorporation of xanthan on soil aggregation, stability of the new formed aggregates and biochemical changes in soil after incorporating xanthan. Result indicated significant improvement in water stable aggregates of different sizes after adding xanthan. Aggregates of 0.18, 0.5, 1.0 mm size increased gradually for four weeks. Increase in aggregation depended on concentration of xanthan added in soil with maximum being after adding 1% xanthan. The extent of aggregation gradually declined thereafter. CO_2 evolution increased after adding xanthan and followed a triphasic trend.



(a) **(b)** **(c)**

Figure 52. Chemical structure of nano induced **a.** Xanthan, **b.** Dextran and **c.** Curdlan

First peak was observed within a week, second after seven weeks and third after 12 weeks (Figure 53). During two months, about 25% of the carbon added as xanthan had evolved as CO_2 (Figure 54). This suggested that xanthan was an easy substrate for soil microorganisms. Improvement of microbial activity after adding xanthan was also reflected in dehydrogenase activity (8-10 fold increase) (Figure 55). Similar trend was observed for both acid and alkaline phosphatase. On the basis of these results it is concluded that easy availability of xanthan to microorganisms limits its utility for enhancing aggregation to about four weeks. Addition of thiourea, calcium chloride and magnesium nitrate in higher concentrations reduced the decomposition of xanthan in soil (Figure 56). Similar experiments were carried out with dextran and curdlan with nearly similar results. Addition of n-ZnO upto (5.0 ug g^{-1}) in soil before adding exo-polysaccharides had no effect on their degradation in soil.

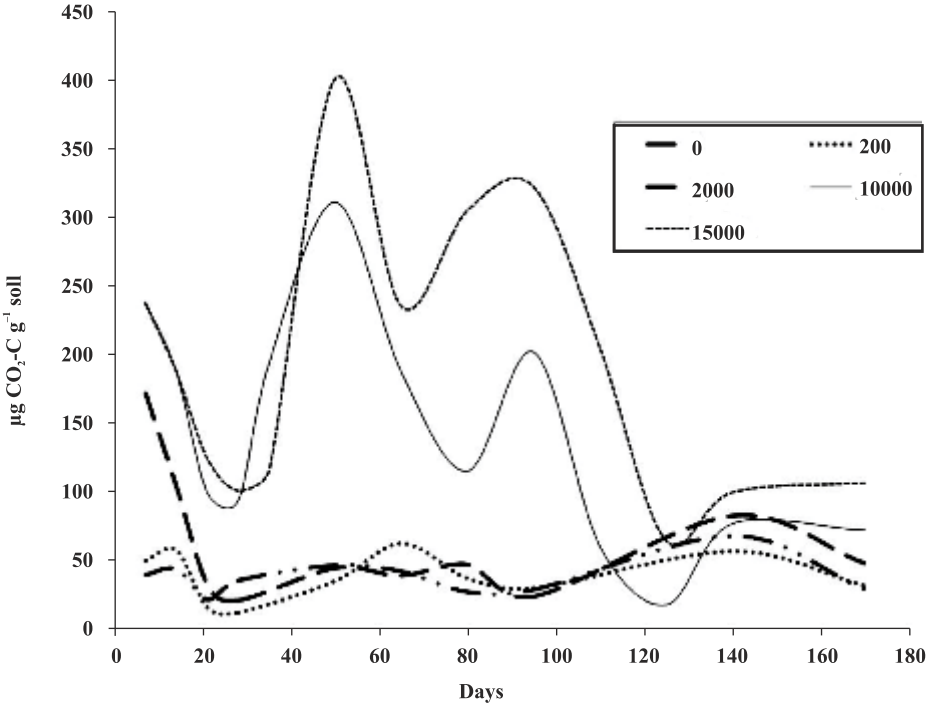


Figure 53. Rate of CO_2 evolution from soil after adding different concentrations of xanthan (µg/g soil)

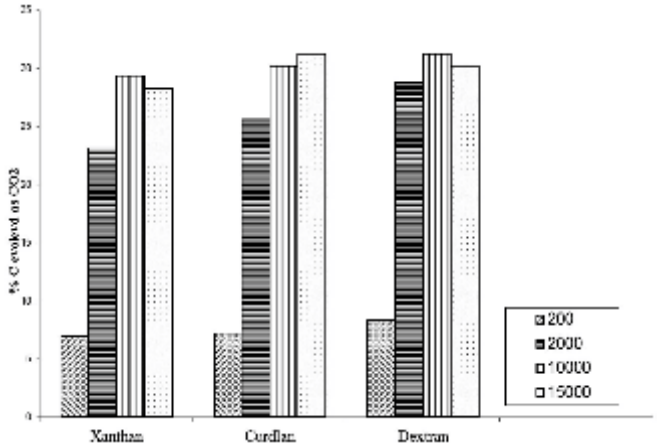


Figure 54. Amount of C added as xanthan, curdlan and dextran evolved as CO_2

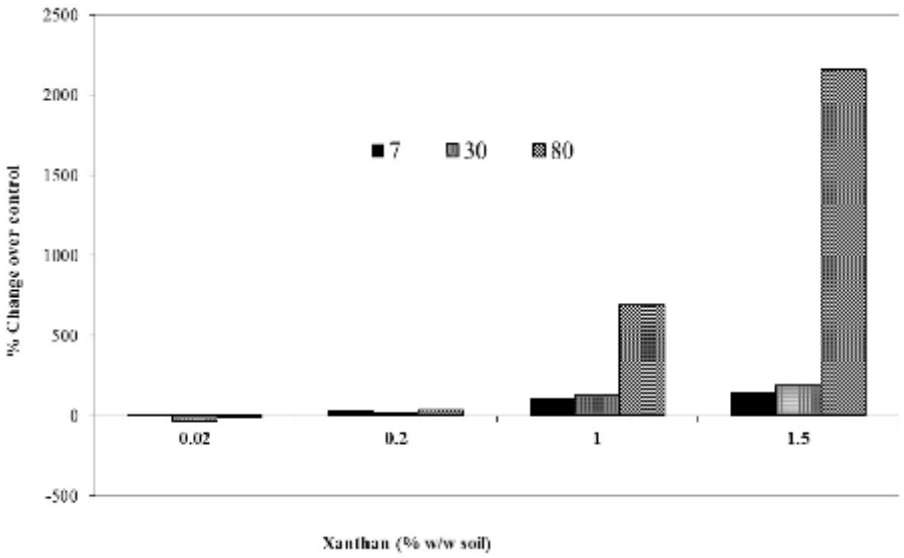


Figure 55. Percent change in dehydrogenase activity over control 7, 30 and 80 days after adding different concentrations of xanthan

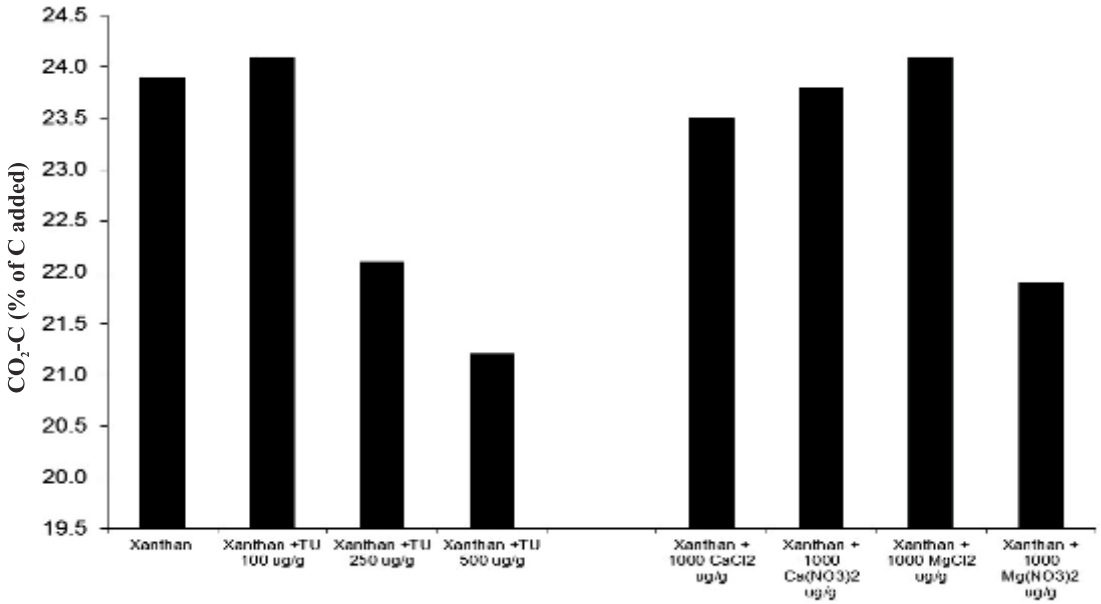


Figure 56. Effect of different amendments on CO_2 evolution

FTIR analysis was carried out to find the different bonds involved between soil particles and nano-induced polysaccharides. The results suggested that C=O, C=C, N-H and C-H bonds are responsible for soil aggregation with the application of polysaccharides (Figure 57).

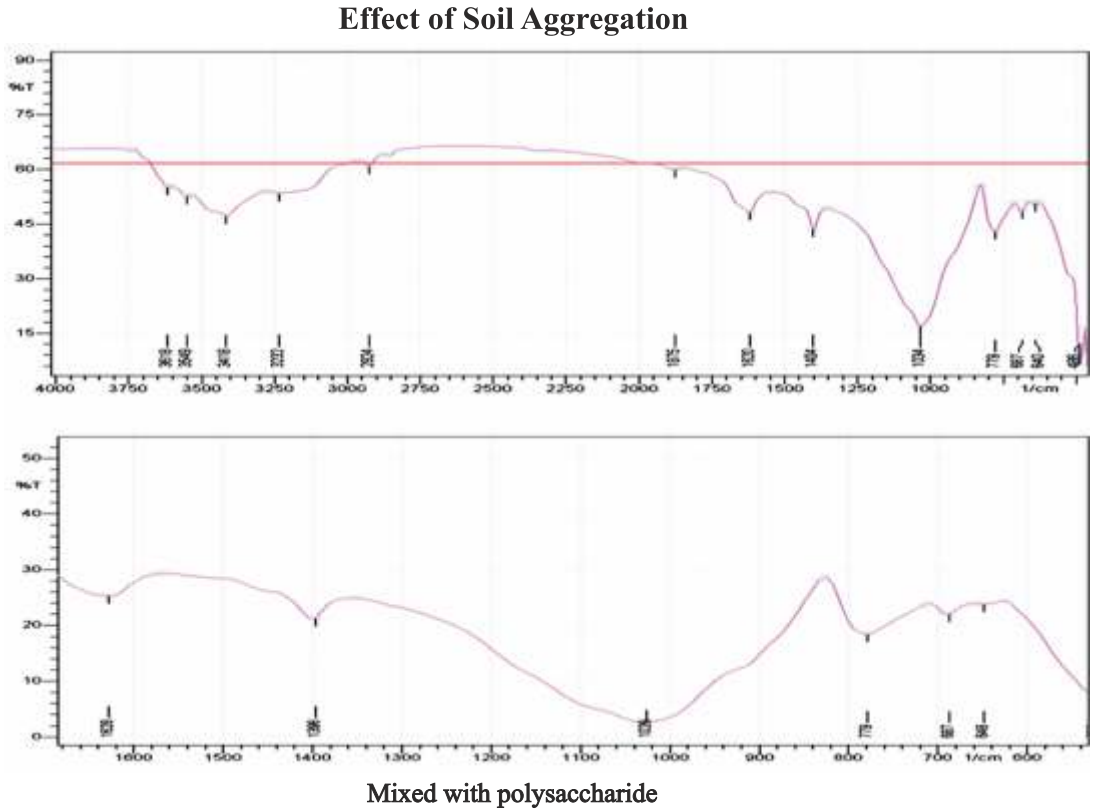


Figure 57. Bonding mechanism between different soil particles and exo-polysaccharides

Activity 4. *Stability of soil aggregates* **[CAZRI Jodhpur]**

To test the stability of soil aggregation, different compounds were tested out of which thiourea and magnesium nitrate seems to be more promising for stabilizing soil aggregates (Figure 58)

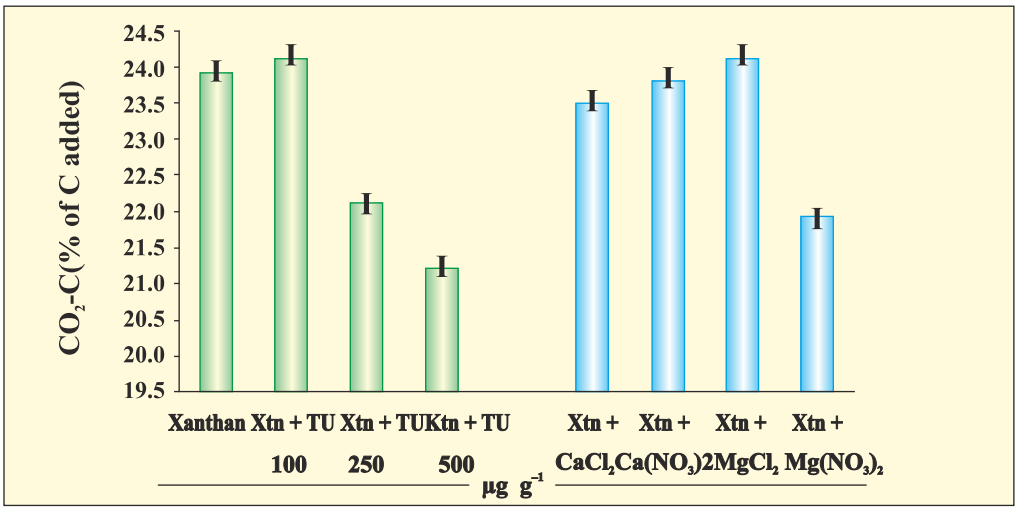


Figure 58. Effect of inorganic salts and thiourea on decomposability of xanthan

Polysaccharides with long C chain play an important role in maintaining physico-chemical properties of soil especially soil aggregation. Organic waste of azadirechta, amaranthus, acarinthus, sesamum, cumin and brassica were treated with two species of polysaccharide producing bacteria *Bacillus-1* and *Bacillus-2* to convert the available organic matrix in waste to polysaccharides. Results indicated that inoculation of *Bacillus-1* remarkably increased the production of polysaccharides from waste of azadirechta, amaranthus, acarinthus, sesamum. Exposing *Bacillus-1* to nano particles of both Fe and Zn offered no further advantage. However, inoculation of cumin and brassica waste with bacteria exposed to Fe- nanoparticles produced nearly double quantity of polysaccharides (Figure 59). Similar results were observed with *Bacillus 2* except in sesamum.

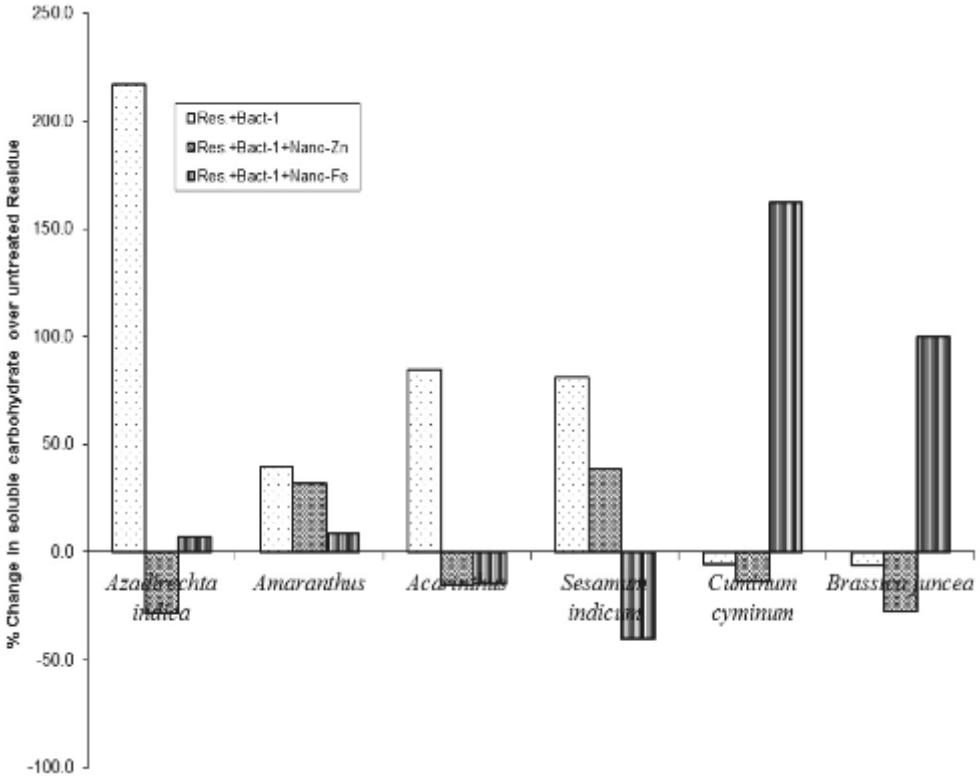
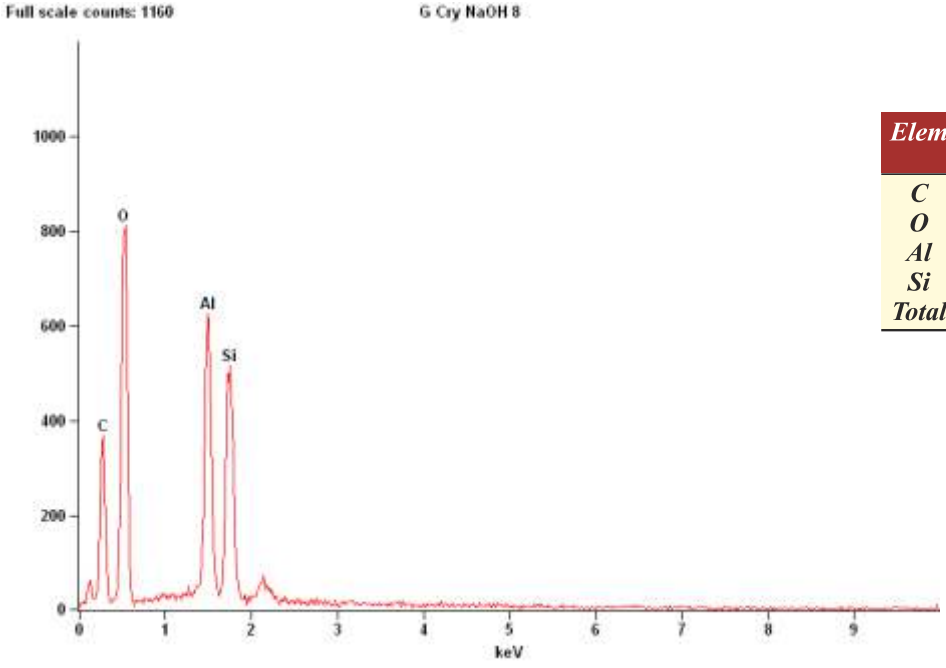


Figure 59. Effect of Fe and Zn nano-particles on production of polysaccharides from organic waste

Objective 3. Synthesis and application of nano-granules of phosphorus from rock phosphate for enhancing its utilization.

Activity 1. Removal of toxic heavy metals from Rock Phosphate **[IISS Bhopal; PAU Ludhiana]**

To achieve the above goals various experiments were designed and tested for their efficacy. Driving variables in the experimental set up were (i) four rhizospheric acids, viz., citric, oxalic, formic and acetic acid, (ii) various concentrations of these acids, and (iii) various partitioning processes. Amount of P released from P rich minerals (Udaipur Rock Phosphate) were in the following order: Oxalic acid > Formic acid > Citric acid > Acetic acid. The reaction was restricted to room temperature to save on energy consumption and carried out in alkaline condition (pH 8) did not show any adsorption of P onto nano-Kaolin (Figure 60).



Element	*Weight %*	*Atom %*
C	40.68	50.92
O	42.39	39.84
Al	8.17	4.55
Si	8.77	4.69
Total	100.00	100.00

Figure 60. Energy Dispersive Spectra (EDS) of P onto nano-kaolin at pH 8

The FTIR peaks at position 3405.6, 1098.1, 1048.6, 601.8, 571.2 and 468.7 indicate the presence of phosphorus (Hydroxyapatite) (Figure 61).

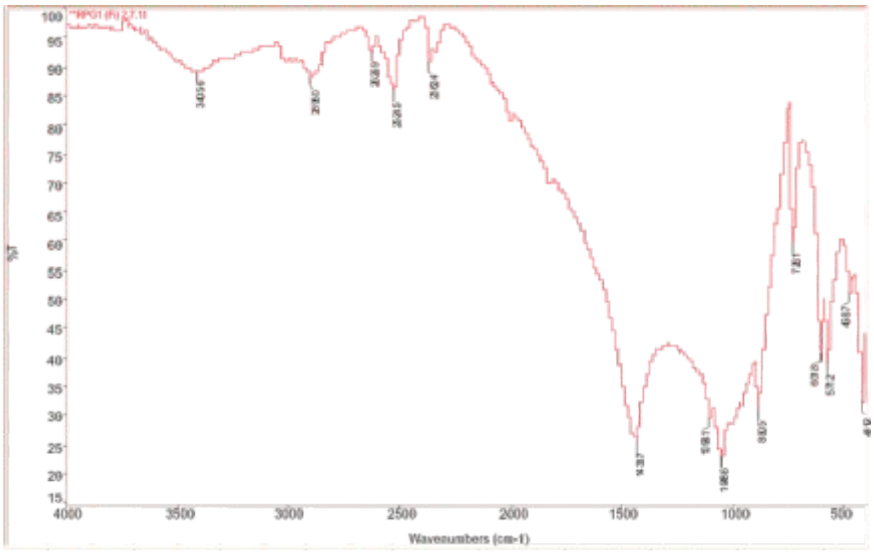


Figure 61. FT-IR spectrum of P-rich mineral

The presence of nanosizing of Kaolin (by breaking macro and micro aggregated particles to <100nm effective diameter size without allowing aging to halloysite) for persisting long time dispersion in aqueous media was achieved by ultrasonication by optimizing cycles, pulses, probe size and time of sonication. The material was then filtered through 0.2µ nylon filter. The resultant Kaolin was in the range of 30-50 nm as determined by the Transmission Electron Microscope.

In P minerals, when carbonates were removed effectively the sharpness and flat features were enhanced (Figure 62) and % P increased to 77.5% by atom.

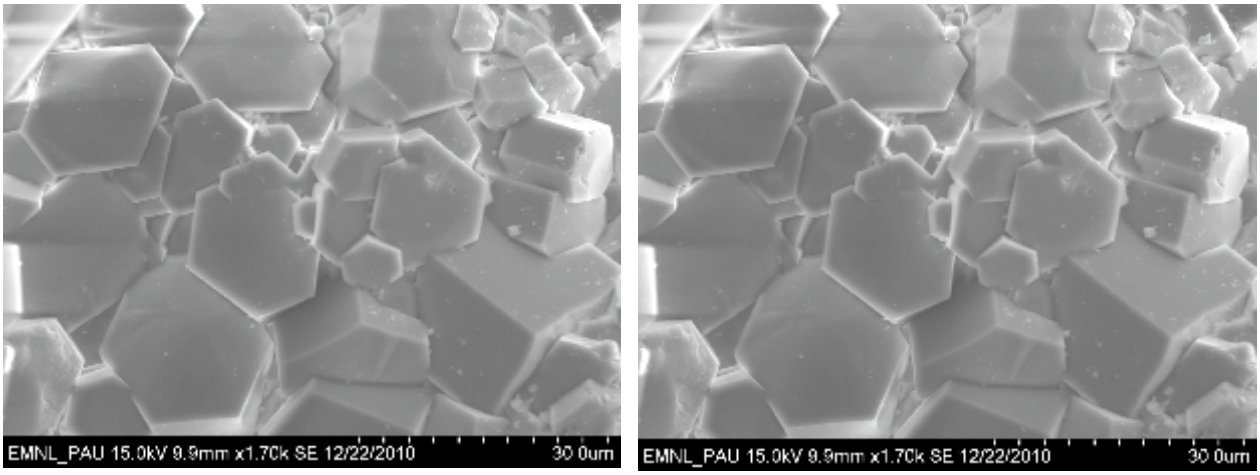


Figure 62. Purified P minerals

The SE Micrograph and EDX spectrum of P – mineral after removal of carbonate was presented as Figure 63.

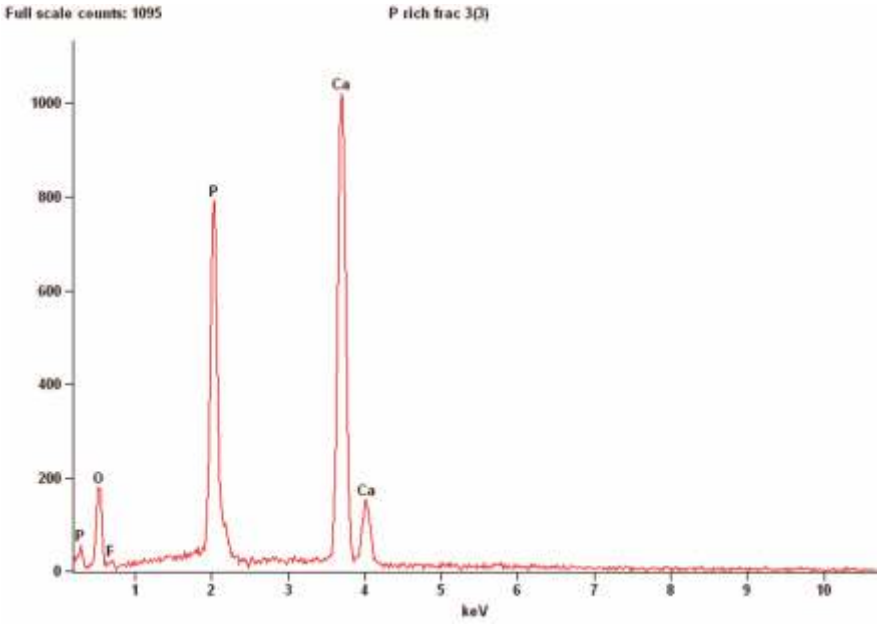


Figure 63. SE Micrograph and EDX spectrum of P-mineral after removal of carbonate

Nanofabrication of P (from P rich fraction of URP) on nano-Kaolinite was achieved at acidic pH using acetic acid, citric acid and oxalic acid. X-ray mapping showed distribution pattern of P adsorbed on the positively charged face of nano-Kaolinite (Figure 64).

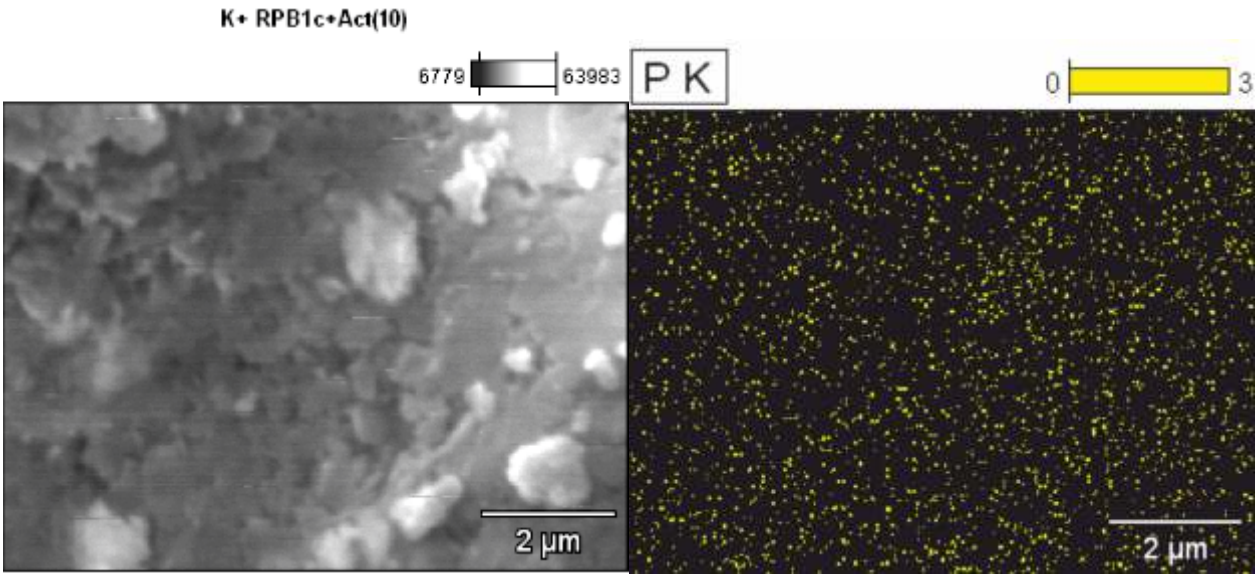


Figure 64. X-ray mapping showing distribution pattern of phosphorus adsorbed on the positively charged face of kaolinite

In general, 59 species have been obtained in Udaipur Rock Phosphate. SEM Micrographs (Figure 65) of the different species revealed that species rich in P show compact arrangement and clean edges. Energy dispersive spectroscopy of a P dominated species showed P/O as 0.1340, P/Ca 0.1704 and P/Mg as 1.2380 by weight ratio, and P/O as 0.0690, P/Ca as 0.02211 and P/Mg as 0.9756 by atomic ratio. The decontaminated rock phosphate was used for nanoparticle production (Figure 66).

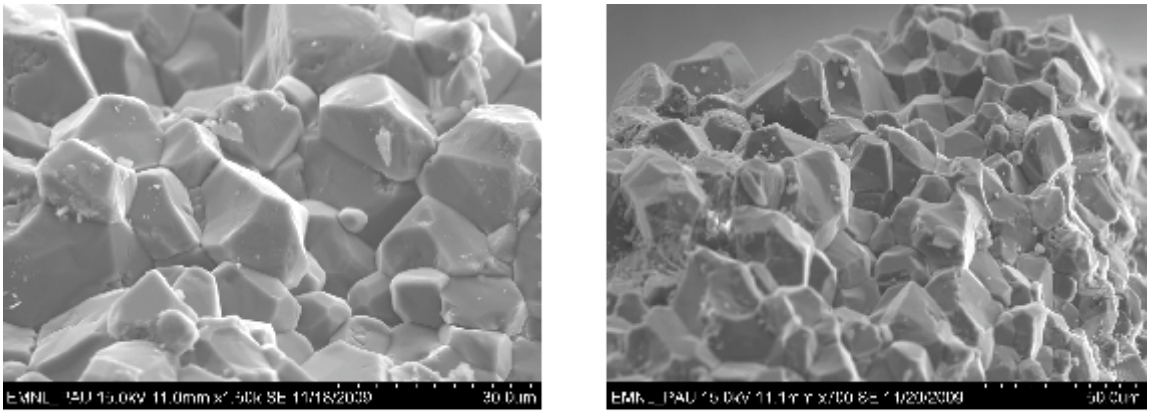


Figure 65. SEM Micrographs of the different species reveal that species rich in phosphorus show compact arrangement and clean edges

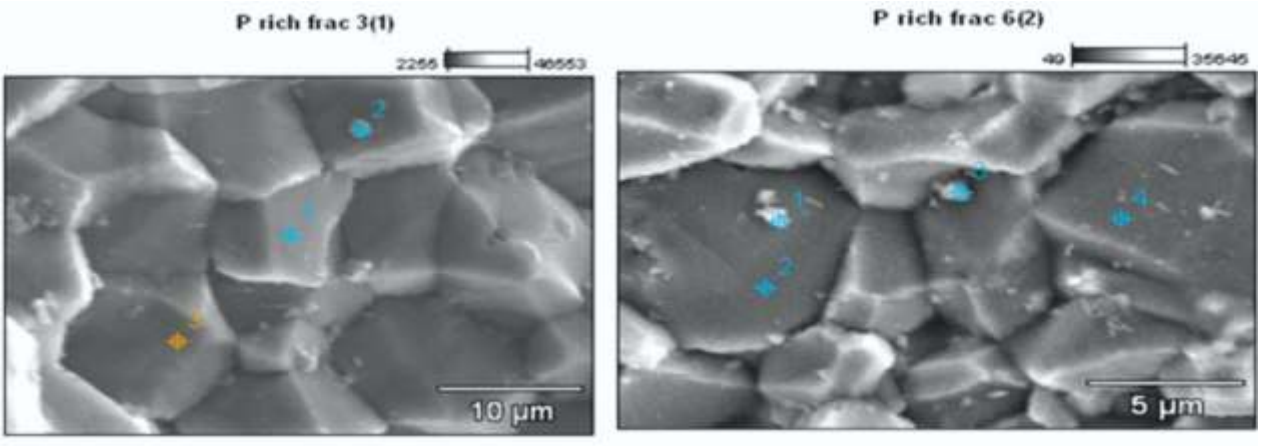


Figure 66. Purification of Rock Phosphate for nanoparticle production

Activity 2. Assessing potential of nano granules for its use as nanofertilizers in selected test plants
[IISS Bhopal; PAU Ludhiana; CAZRI Jodhpur]

A bulk amount of nano rock phosphate (Sagar and Udaipur) has been synthesized by top down approach through a high energy ball mill. After that the particles were characterized. The obtained results pointed out that produced rock phosphate powder is a highly dispersed, nano-scale mixture of small particles, i.e., crystallites with sizes in the range of 10-100 nm. The average size was between 28 and 42 nm. This Rock Phosphate was characterized by XRD and found that it mainly consisted of Calcium apatite, hydroxyl apatite, fluor apatite, plumbogummite, quartz, crystobolite, strengite, veriscite etc. The dose was standardized (40 ppm) from our preliminary experiments. A methodology was developed for preparation of nanofertilizers (Figure 67). The FTIR absorption bands in the URP was documented (Table 19).

Table 19. Assignment of the Principal FTIR absorption bands in the spectra of Sagar nano rock phosphate particles

S. No.	Region in cm^{-1} and intensity	Approximate type of mode	Group
1.	455	Symmetric Deform	P-Cl Phosphorous trichlorideP-F
2.	A.501 B.571	Symmetric Stretch Symmetric Stretch	Phosphorous trifluoride
3.	1040	Stretch	C-O C=C-O-C
4.	A.1427 B.1458	Stretch Stretch	C-H CH3
5.	1643	Stretch	N=O Nitro compound
6.	1744	Stretch	C=O Aryl Carboxylic acid
7.	A.1867 B.1998	SymmetricStretch Asymmetric Stretch	D2S Deuterium sulfide
8	3448	Stretch	N=H Amine
9	3672	Stretch	O-H Stretch Free O-H group

A coating method has been developed for preparation of nano-fertilizer from nano particles (Figure 67) .

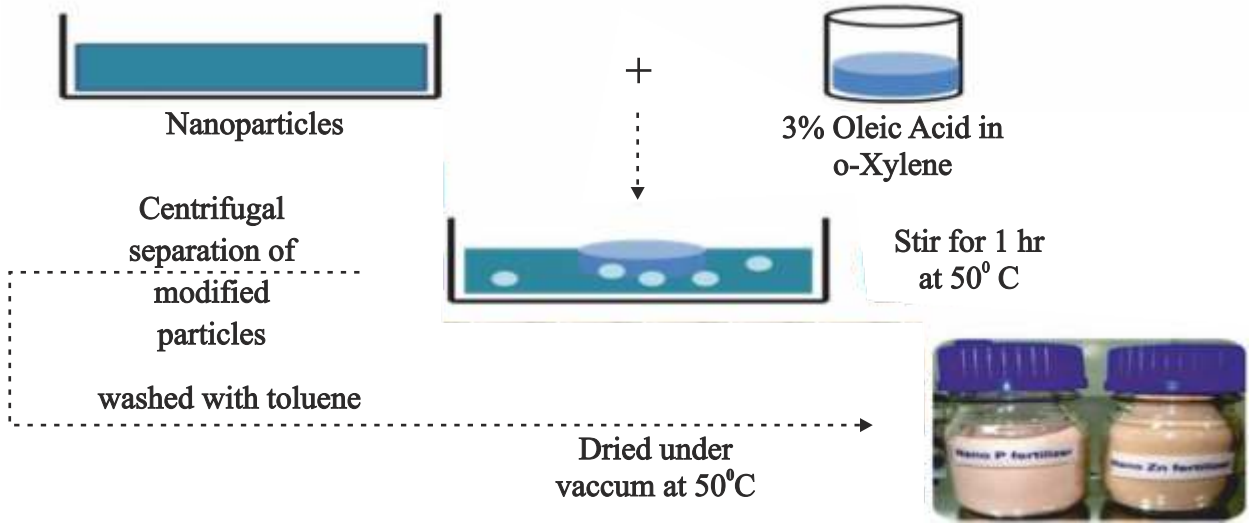


Figure 67. Methodology for coating of nanofertilizers

Activity 3.Assessing potential of nanogranules for its use as nanofertilizers in selected test plants
[CAZRI Jodhpur; BITS Pilani; IISS Bhopal; PAU Ludhiana]

Multilocation of field trials was conducted with nanonutrients including P nanofertilizers. The nanofertilizer technology is an innovative strategy. Nanofertilizers play an important role in increasing the quantity of agricultural products and in removing the soil and environmental hazards. The purity of elements is very high in such fertilizers. One of the advantages of such fertilizers is that they can be used in tiny amounts, therefore, it saves cost by less expenditure on fertilizer which is economical for farmer's point of view. For example, 640mg of nano P application resulted of 80 kg per ha P equivalent yield applied as chemical fertilizer (Table 20).

Table 20. Comparison between nano P and P fertilizers used under arid field conditions on Pearl millet and clusterbean (average of 3 years)

Crop	Yield (Kg ha^{-1})					LSD (p = 0.05)
	Control (P_0)	P_{40}	P_{60}	P_{80}	Nano-P 640 mg ha^{-1}	
Pearl millet	616	690	758	789	790	19.75
Clusterbean	312	340	371	390	392	16.23

The P use efficiency was calculated and compared with the fertilizer SSP and DAP as well as most mobile P compound KH_2PO_4. The results showed 58-61% efficiency of nano P as compared to only 15-16% of either SSP or DAP. The yield increase at various locations (research and farmers field) with the application of different nanonutrients of 10 different crops tested was summarized (Table 21). In general, 12-48% improvement of yield was observed under research field conditions while in farmer's field it varied between 18-54% with the application of critical doses of nanonutrients i.e., P (40ppm), Fe (30ppm), Mg (20ppm) and Zn (10ppm).

Table 21. Yield increase (%) due to nanonutrient application under different crops

Crops	Nanonutrient applied	Research field	Farmer's field
Pearl Millet	P, Zn, Fe, Mg	13-48	18-43
Cluster bean	P, Zn, Fe, Mg	14-36	21-37
Moth bean	P, Zn, Fe	12-31	18-27
Mung bean	P, Zn, Fe, Mg	17-42	23-38
Maize	P	23-32	-
Castor	P, Zn	-	24-37
Cauliflower	P, Zn	-	47-54
Tomato	P, Zn	18-23	25-29
Rice	P	22-28	-
Capsicum	P, Zn	19-24	-
*doses applied : P-40ppm; Fe-30ppm; Mg-20ppm; Zn-10ppm			

A case study in farmer's field was also documented where the maximum yield (54% over control) was observed (Figure 68). The results also showed 18 to 21 days advancement of cauliflower maturity resulting better market price.

Crop :	Cauliflower
Application :	Foliar spray
Nano particle :	Nano P to 40 ppm concentration; spray on 2 weeks old plant
	Nano Zn of 10 ppm concentration; spray on 4 weeks old plant
Source :	Biosynthesized nano particle < 20 nm size
Rate of application :	16 L ha^{-1}

Average yield per flower Control : 550 ± 23.7 g

Nano P + Nano Zn : 850 ± 31.5 g

Other advantage :
18 to 21 days advance maturity of the crop

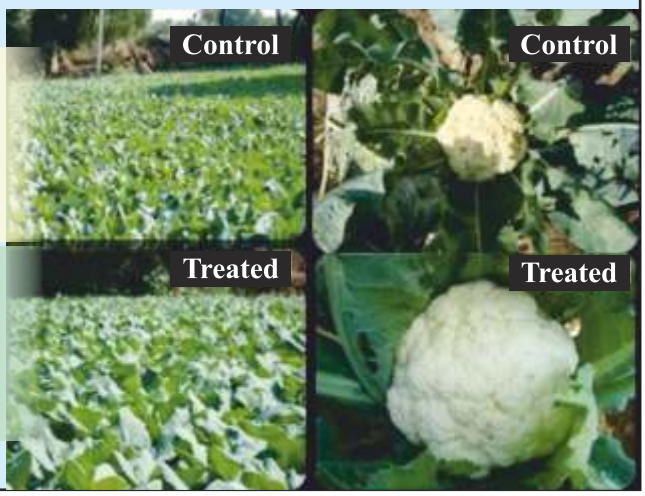


Figure 68. Farmer's field-a case study with cauliflower

Safety assessment of nanotechnology derived food [CAZRI Jodhpur; BITS Pilani]

Many experiments were conducted in different research stations and outstations to find out the health hazards of nanofoods with the application of critical doses of nanonutrients of P, Zn, Fe and Mg. The results (Table 22) clearly showed that nanonutrients have no adverse effect on seed germination % as well as soluble seed protein content in important arid crops of cluster bean, moth bean, mung bean and pearl millet. In general, microbial population increased significantly with the application of nano Zn upto a concentration of 10ppm (Table 23). There was no adverse effect on body weight, grain consumption rate and blood pH of mice with the feeding of nanoparticle sprayed plant's grain as compared to control (Table 24).

Pre-clinical safety evaluation of pearl millet and mung bean grown with biosynthetic nanofertilizers by NIN Hyderabad reported:

- No pre-terminal deaths were recorded in any groups investigated
- No abnormal clinical signs, behavioral activity etc were observed in animals which received test materials
- No significant effect on feed intake, body weight gain was observed between the individual groups.
- There were no changes in gross necropsy and any organ weights.

The study impression was: pearl millet and mung bean grown with nanonutrient fertilizer did not induce any adverse effect in rats even after feeding more than two and half times of limit dose.

Table 22. Effect on Seed germination and soluble protein content of arid crops

Treatment	Seed Germination (%)				Soluble Protein (µg g–1 seed)			
	Cluster bean	Moth bean	Mung bean	Pearl millet	Cluster bean	Moth bean	Mung bean	Pearl millet
Control	90	82	89	80	105.6	98.5	78.5	70.1
Ordinary Zn	93	82	92	80	105.0	99.2	79.1	74.3
Nano Zn	92	84	91	82	108.1	106.9	79.8	76.1
Ordinary Fe	89	83	90	81	106.3	108.5	78.6	75.0
Nano Fe	97	84	93	84	115.4	105.3	78.9	75.4
Ordinary P	94	82	90	81	105.3	100.4	79.0	78.2
Nano P	93	85	93	85	110.2	103.6	81.1	77.9
LSD (p = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS

Table 23. Effect on microbial population with the application of nano Zn at 10 ppm concentration

Treatment	Fungus (cfu x $10^4 g^{-1}$)	Bacteria (cfu x $10^6 g^{-1}$)	Actinobacteria (cfu x $10^6 g^{-1}$)
Master Control*	9	18	6
Control **	14	24	8
Ordinary Zn	17	32	12
Nano Zn	23	39	16
LSD (p = 0.05)	3.2	7.1	2.4

*** Crop not grown; ** Crop grown without treatment**

Table 24. Body weight, grain consumption rate and blood pH of mice at different time intervals with the feeding of nano Zn (10 ppm) treated material

Treatment	Body Weight (g)				Consumption Rate (g/100 g body wt/day)				Blood pH			
	Days Feeding				Days Feeding				Days Feeding			
	7	14	21	28	7	14	21	28	7	14	21	28
Control	4.9	6.3	11.8	13.6	12.3	13.2	13.9	14.6	7.2	7.2	7.3	7.2
Ordinary Zn	5.2	6.8	12.4	14.0	12.4	13.8	14.2	14.8	7.2	7.3	7.2	7.2
Nano Zn	5.8	7.1	12.9	14.4	13.6	14.3	14.8	15.2	7.2	7.2	7.3	7.4
LSD (p = 0.05)	0.3	0.4	6.5	0.7	0.6	0.7	0.7	NS	NS	NS	NS	NS

NS : Non Significant

The health effects of nanotechnology derived food were evaluated in female Wistar rats using repeated sub-acute toxicity studies at BITS Pilani. The nanoparticles were sprayed during the vegetative cycle of wheat plant. Later on, these matured wheat plants were harvested and selected as test substance for 90 days sub-acute toxicity study. Wistar rats (female) were randomly assigned to various experimental groups including control in groups of 6 animals each. [Group A-normal diet; Group B-normal diet supplemented with control (unsprayed) wheat plant; Group C-normal diet supplemented with Fe nanoparticle (sprayed) wheat plants; Group D-normal diet supplemented with 100 mg per kg Fe nanoparticles; Group E-normal diet supplemented with ferrous sulphate (100 mg per kg) sprayed wheat plants]. The test substance (33% w/w) was properly mixed in the normal animal chow and rats were allowed free excess to both food and water. The animals were observed twice daily; body weight, food and water consumption were measured once weekly. After 90 days, the animals were anaesthetized and sacrificed by cervical dislocation followed by examination for gross and histopathological changes.

Histopathology analysis was performed at National Institute of Pathology (NIOP), New Delhi. The liver, kidneys and spleen of control (group A) and test groups (group B, C, D, E) were fixed in 10% neutral buffered formalin for 120 h and then transferred finally to 70% ethanol through 30% and 50% ethanol gradients. The tissues were processed using routine histological techniques. After paraffin embedding, 3 μm sections were cut and stained with hematoxylin and eosin (H&E) for histopathologic evaluation. The H&E staining provides a comprehensive picture of the microanatomy of organs and tissues. Hematoxylin precisely stains nuclear components, including heterochromatin and nucleoli while eosin stains cytoplasmic components including cytoplasmic granules, extracellular components including collagen and elastic fibres, muscle fibres and red blood cells. Histopathology analysis of liver, kidney and spleen tissues revealed that oral exposure of test substances produced no significant adverse effects as evidenced by the normal tissue architecture observed in the exposed animals at post-instillation time period of 90 days in comparison to the normal diet exposed controls. Mild inflammation resultant of acute biological response was observed at many sites within liver and kidney shown by the noticeable abundance of lymphocytes, however these histological alterations cannot be pronounced as an indication of cell injury due to test substance, as similar results were witnessed in control groups as well. Overall analysis of all the samples leads to the conclusion that the gross architecture was intact with no noticeable necrosis or fibrosis within the analyzed tissue (Table 25).

Table 25: Summary of histopathological analysis for estimating toxicological effect of test substance (-ve indicates no toxicity observed)

Tissue/Groups	Group A	Group B	Group C	Group D	Group E
Liver	- ve	- ve	- ve	- ve	- ve
Kidney	- ve	- ve	- ve	- ve	- ve
Spleen	- ve	- ve	- ve	- ve	- ve

A complete bio-informatics study was conducted with the application of nanonutrients to cereals (pearl millet) and legumes (mung bean) at 6 weeks old plant. GO (Gene Ontology) sequence distributions, helps in specifying all the annotated nodes comprising of GO functional groups. Unigenes associated with similar functions are assigned to same GO functional group. The GO sequence distributions were analyzed (Table 26) for all the three GO domains i.e. biological processes, molecular functions and cellular component. The novel findings are presented as Figure 69.

Table 26. Gene Ontology distribution for unigenes in pearl millet (6 weeks old plant)

S. No.	Sample name	Biological processes	Molecular functions	Cellular component
1	Control	9,222	12,044	6,223
2	Nano P	10,902	14,435	9,415

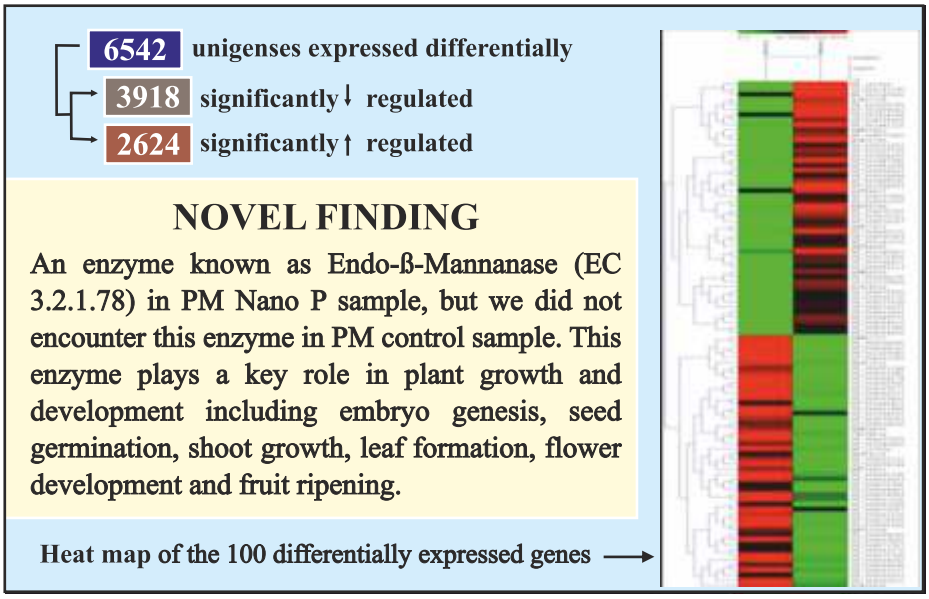


Figure 69. Differential expression of unigenes in PM Control and PM Nano-P samples

Gene Ontology analysis helps us in specifying all the annotated nodes comprising of GO functional groups such as Cellular components, Biological process and Molecular Function. Table 27 represents the GO distribution of CDS from Mung bean-Control and Mung bean-Nano samples. It was observed that Mg-Nano samples were having predominantly more Biological Process terms (8395) and Molecular Functions terms (9862) as compared to Mung bean-Control sample. Figure 70 represents a comparative accounting of GO terms distribution between Mung bean-Control and Mung bean-Nano samples. The results showed 2,488 unigenes were exclusively present in Mung bean nanosamples helping in different metabolic activities.

Table 27: GO distribution for CDS

Sr. No	Sample Name	Biological processes	Molecular functions	Cellular component
1	Mg-Control	3926	4660	2277
2	Mg-Nano	8395	9862	4481

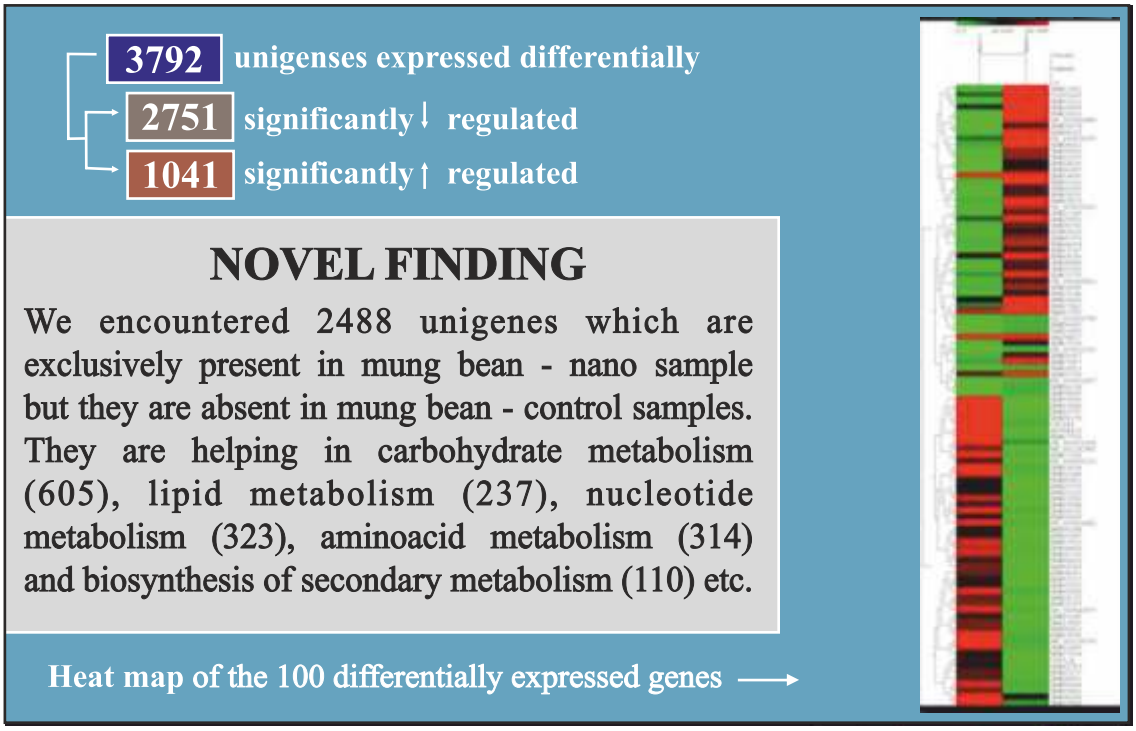


Figure 70. Differential expression of unigenes in mung bean Control and mung bean Nano-P samples

6. Innovations

(i) Development of biosynthesized nano-nutrients

Fifty-seven micro-organisms were developed for preparation of different nano-nutrients. The advantages of biosynthesized nano-nutrients are:

- ❖ Ecofriendly approach
- ❖ Three-fold increase in Nutrient Use Efficiency (NUE)
- ❖ 80-100 times less requirement than chemical fertilizer
- ❖ Complete bio-source so environment friendly
- ❖ 10 times more stress tolerant by the crops
- ❖ 30% more nutrient mobilization in the rhizosphere
- ❖ 12-54% improvement in crop yield
- ❖ Improvement in soil aggregation, moisture retention and C build-up in soil

Tested Crops: Cauliflower, Tomato, Capsicum, Maize, Pearl millet, Castor, Rice, Clusterbean, Mung bean and Moth bean
Yield Increase: 12 to 54%
Tested Soils: Aridisols, Inceptisols, Vertisols
Improvement in Soil Beneficial Enzyme Activities: 18-283 %

Effect of biosynthesized Nano - P and Nano-Zn on cauliflower under farmer's field

Clusterbean (Control)

Clusterbean (Nano P Fertilizer)

(ii) Development of Nano induced polysaccharide powder for soil aggregation, moisture retention and carbon build-up

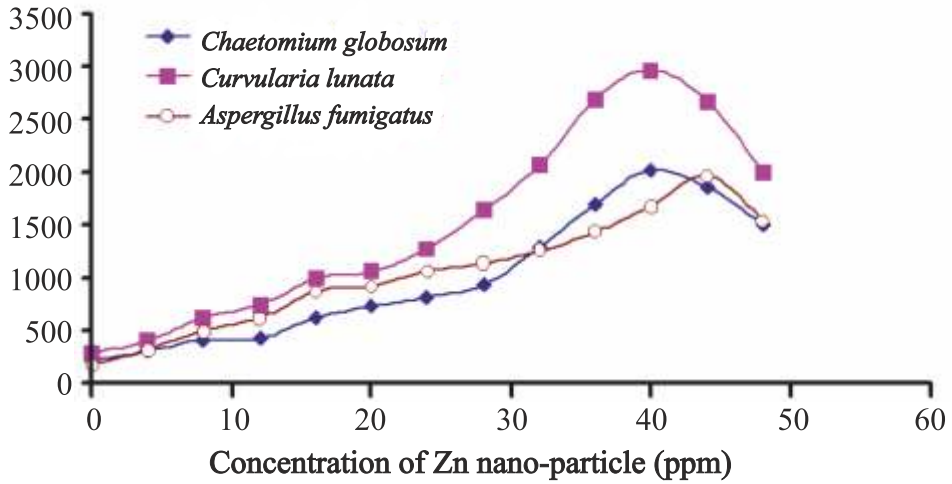
Developed 15 organisms which are producing polysaccharides. A methodology has been developed for producing polysaccharide powder. The polysaccharide was identified as xanthan, curdlan and polulan. The polysaccharide production was successfully enhanced by nanoparticle application which are co-factor to the particular polysaccharide.

Advantages:

- Improvement in soil aggregation: 33 to 81%
- Moisture retention: 10 to 14%
- Organic carbon build-up: 3 to 5%

Mixed with Bacterial

Control Polysaccharide



Alcallgenes faecalis *Bacillus subtilis* *Aspergillus terreus* *Aspergillus flavus*

Polysaccharide powder produced by nano induced microorganisms

7. Process/ Product/Technology/ Value Chain/ Rural Industry Developed

S.No.	(Process/Product/Technology/ Value Chain/ Rural Industry Developed	Adoption/ Validation/ Commercialization, etc.	Responsible Partner
1.	Biosynthesis of nanoparticles	Commercialization in progress (Prathistha industries, Hyderabad and Allwin industries, Indore signed MOU for commercialization)	CAZRI Jodhpur; BITS Pilani
2.	Nanoinduced polysaccharide powder		CAZRI Jodhpur
3.	Coated nanofertilizer	Validation under progress	IIT Mumbai
4.	Sagar nano P	Validation under progress	IISS Bhopal

8. Patents (Filed/Granted)

S.No.	Title of Patent	Inventor(s) (Name & Address)	Filed/Published/ Granted (No./Date)	Responsible Partner
1.	Biosynthesis of metal nanoparticle from fungi	Tarafdar, J. C. and Raliya, R. CAZRI, Jodhpur	149/DEL/2012; 18.01.2012; Journal No. 44/ 2012, 2.11.2012	CAZRI Jodhpur

2.	Nanoinduced bacterial polysaccharide production	Tarafdar, J.C., Raliya, R. and Praveen-Kumar CAZRI, Jodhpur	404/DEL/2012; 14.02.2012; Journal No. 37/ 2012, 14. 9.2012	CAZRI Jodhpur
3.	Rapid synthesis of platinum nanoparticles from *Aspergillus flavus* TFR12	Tarafdar, J.C. and Raliya, R. CAZRI, Jodhpur	3634/DEL/2012; 27.11.2012; Journal No. 11/ 2013, 15.3.2013	CAZRI Jodhpur
4.	Development of nanoinduced biological phosphorus fertilizer (NB-PHOS) using *Aspergillus flavus* CZR-2	Tarafdar, J. C. and Raliya, R. CAZRI, Jodhpur	7/DEL/2013; 2.01.2013; Journal No. 8/ 2013, 22.02.2013	CAZRI Jodhpur
5.	Biosynthesis of ZnO nanoparticles	Navin Jain, Arpit Bhargava, Jitendra Panwar BITS, Pilani	1439/DEL/2011; 19.05.2011; Journal No. 20/ 2013, 17.5.2013	BITS, Pilani
6.	Zinc in clay-mineral receptacles in nanoforms for their use as advance materials including novel fertilizer	Singh, M., Mukhopadhyay, S.S, Kalia, A., Jeet, K., Kaur, R. and Sharma, S. PAU, Ludhiana	2093/DEL/2013; 11.07.2013 application not yet published	PAU, Ludhiana

9. Linkages and Collaborations

S.No.	Linkages developed (Name & Address of Organization)	Date/Period From-To	Responsible Partner
1.	Washington University, St. Louis, USA	Jan 2010 - till date	CAZRI, Jodhpur
2.	JNU, Delhi	Jan 2009 – till date	
3.	DRDO, Jodhpur	Jul 2009 – till date	
4.	JNV University, Jodhpur	Mar 2010 – Dec 2013	
5.	SAU Bikaner	Jun 2010 – Sept 2011	
6.	IIT Kharagpur	Mar 2011 – Mar 2014	
7.	CIRCOT, Mumbai	Nov 2008 – Sept 2010	
8.	NRCPB, IARI, New Delhi	Sept 2010 – Dec 2012	
9.	National University, Jeonju, South Korea	Oct 2011 – Dec 2013	BITS Pilani
10.	Department of Chemical Engineering, Chonbuk	Feb 2012 – Dec 2012	

10. Status on Environmental and Social Safeguard Aspects

Environmental safeguard			Social safeguard		
Positive effects	Negative effects	Mitigation measures taken to minimize the negative effects	Positive effects	Negative effects	Mitigation measures taken to minimize the negative effects
✓	No adverse effect observed*	NA	✓	No adverse effect observed*	NA

*upto critical concentration of nanoparticles for plant and animals

Test conducted:

No adverse effect with recommended doses of application was seen on

- Seed germination
- Soluble seed protein content
- Microbial diversity in rhizosphere
- Body weight and consumption rate of mice
- Nano particle concentration in seeds
- Animal toxicity results availed so far
- Bioinformatics and gene ontology

Note: We are using B2 bio safety cabinet and nitrile gloves and mask during handling of nanoparticles as per international recommendations.

11. Constraints, if any and Remedial Measures Taken

NIL

12. Publications (As per format of citation in Indian Journal of Agricultural Sciences)

A. Research papers in peer reviewed journals. Details as per the guidelines for citation of publications (Annexure I)

S. No.	Authors, Title of the paper, Name of Journal, Year, Vol. & Page No.	NAAS Ratings	Responsible Partner
1.	Jain N, Bhargava A, Majumdar S, Tarafdar J C and Panwar J. (2011). Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: A mechanism perspective. *Nanoscale* **3,** 635-641	12.23	BITS, Pilani
2.	Kalia, A. and Parshad, V.R. (2013). Novel trends to revolutionize preservation and packaging of fruits/fruit products: Microbiological and nanotechnological perspectives. *Critical Reviews of Food Science & Nutrition; DOI : 10.1080/10408398.2011.649315*	10.82	PAU, Ludhiana
3.	Jain, N., Bhargava, A., Tarafdar, J.C., Singh, S. K. and Panwar, J. (2013). A biominetic approach towards synthesis of zinc oxide nanoparticles. *Applied Microbiology and Biotechnology* **97**, 859-869	9.69	BITS, Pilani

4.	Raliya, R., Tarafdar, J. C., Mahawar, H., Kumar, P., Gupta, P., Mathur, T., Kaul, R. K., Kalia, A., Gautam, R., Singh, S. K. and Gehlot, H. S. (2014). ZnO nanoparticles induced exopolysaccharide production by *B. subtilis* strain JCT1 for arid soil applications. *International Journal of Biological Macromolecules* **65,** 362–368	8.60	CAZRI, Jodhpur
5.	Jain N, Bhargava A and Panwar J (2014). Enhanced photocatalytic degradation of methylene blue using biologically synthesized "protein-capped" ZnO nanoparticles. *Chemical Engineering Journal*, 243, 549-555	8.58	BITS, Pilani
6.	Bhargava A, Jain N, Barathi M, Akhtar M S, Yun Y S and Panwar J (2013). Synthesis, characterization and mechanistic insights of mycogenic iron oxide nanoparticles. *Journal of Nanoparticle Research* **15,** 2031	8.18	BITS, Pilani
7.	Wang, W.N., Tarafdar, J.C. and Biswas, P. (2013). Nanoparticle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. *Journal of Nanoparticle Research* **15,** 1417	8.18	CAZRI, Jodhpur
8.	Dhoke, S. K. and Khanna, A. S. (2009). Electrochemical behavior of nano-iron oxide modified alkyd based water-borne coatings, *Material Chemistry Physics* **117,** 550-556	8.07	IIT, Mumbai
9.	Kaul, R. K., Kumar, P., Burman, U., Joshi, P., Agrawal, A., Raliya R. and Tarafdar, J.C. (2012). Magnesium and iron nanoparticles production using microorganism and various salts. *Material Science-Poland* **30,** 254-258	6.8	CAZRI, Jodhpur
10.	Adhikari, T., Kundu, S., Biswas, A. K., Tarafdar, J. C. and Subba Rao, A. (2012). Effect of Copper oxide nano particle on seed germination of selected crops. *Journal of Agricultural Science and Technology* **2**, 815-823	6.69	IISS, Bhopal
11.	Burman, U., Tarafdar, J.C., Kaul, R. K., Saini, M., Kumar, K. and Kumar, P. (2013). Changes in carbon partitioning in pearl millet (*Pennisetum glaucum*) and clusterbean (*Cyamopsis tetragonoloba*) in response to ZnO nanoparticle application. *Indian Journal of Agricultural Sciences* **83**, 352-354	6.18	CAZRI, Jodhpur
12.	Subramanian, K. S. and Tarafdar, J. C. (2011). Prospects of nanotechnology in Indian farming. *Indian Journal of Agricultural Sciences* **81**, 887-893	6.18	CAZRI, Jodhpur
13.	Tarafdar, J. C., Sharma, Shikha and Raliya, R. (2013). Nanotechnology: Interdisciplinary science of application. *African Journal of Biotechnology* **12,** 219-226	6.0	CAZRI, Jodhpur
14.	Tarafdar, J.C. (2012). Nanotechnology can provide sustainable agriculture and second green revolution. Strategic Vision: 7, *Green Farming* **3,** 129	4.79	CAZRI, Jodhpur

15.	Mahajan, P., Dhoke, S. K., Khanna, A. S. and Tarafdar, J. C. 2011. Effect of nano-ZnO on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. *Applied Biological Research* **13**, 54-61	4.35	IIT, Mumbai
16.	Tarafdar, J.C., Xiang, Y., Wang, W.N., Dong, Q. and Biswas, P. (2012). Standardization of size, shape and concentration of nanoparticle for plant application. *Applied Biological Research* **14**, 138-144	4.35	CAZRI, Jodhpur
17.	Bhalla D and Mukhopadhyay SS (2010) Eutrophication: Can nanophosphorous control this menace? – A preview. *Journal of Crop and Weed* **6,** 13-16	3.59	PAU, Ludhiana
18.	Tarafdar, J.C. (2012). Perspectives of nanotechnological applications for crop production. *NAAS News* **12,** 8-11	NA	CAZRI, Jodhpur
19.	Tarafdar, J.C., Raliya, R. and Rathore, I. (2012). Microbial synthesis of phosphorus nanoparticles from Tri-calcium phosphate using *Aspergillus tubingensis* TFR-5. *Journal of Bionanoscience* **6**, 84-89	NA	CAZRI, Jodhpur
20.	Tarafdar, J.C., Agrawal, A., Raliya, R., Kumar, P., Burman, U. and Kaul, R.K. (2012). ZnO nanoparticles induced synthesis of polysaccharides and phosphatases by *Aspergillus* fungi. *Advanced Science, Engineering and Medicine* **4,** 1-5.	NA	CAZRI, Jodhpur
21.	Raliya, R. and Tarafdar, J. C. (2012). Novel approach for silver nanoparticles synthesis using *Aspergillus terreus* CZR-1: Mechanism perspective. *Journal of Bionanoscience* **6,** 12-16.	NA	CAZRI, Jodhpur
22.	Tarafdar, J. C. and Raliya, R. (2013). Rapid low-cost, and ecofriendly approach for iron nanoparticle synthesis using *Aspergillus oryzae* TFR9. *Journal of Nanoparticles*. doi.org/10.1155/2013/141274	NA	CAZRI, Jodhpur
23.	Raliya, R. and Tarafdar, J. C. (2013). ZnO nanoparticle biosynthesis and its effect on phosphorus mobilizing enzyme secretion & gum contents in clusterbean *Cyamopsis tetragonoloba* L). *Agricultural Research* 2, 48-57	NA	CAZRI, Jodhpur
24.	Tarafdar, A., Raliya, R., Wang, W. N., Biswas, P. and Tarafdar, J. C. (2013). Green synthesis of TiO_2 nanoparticle using Aspergillus tubingensis. *Advanced Science, Engineering and Medicine* **5**, 1–7	NA	CAZRI, Jodhpur
25.	Akhtar M S, Panwar J and Yun Y S (2013). Biogenic synthesis of metallic nanoparticles by plant extracts. *ACS Sustainable Chemistry & Engineering* **1,** 591-602	NA	BITS, Pilani
26.	Raliya, R. and Tarafdar, J. C. (2014). Biosynthesis and characterization of zinc, magnesium and titanium nanoparticles: An eco-friendly approach. *International Nano Letters* 4, 1-10	NA	CAZRI, Jodhpur

27.	Raliya, R. and Tarafdar, J. C. (2013). Biosynthesis of gold nanoparticles using *Rhizoctonia bacticola* TFR-6. *Advanced Science, Engineering and Medicine* **5**, 943-949	NA	CAZRI, Jodhpur
28.	Dhoke, S. K., Rajgopalan, N. and Khanna, A. S. (2012). Effect of nano- ZnO particles on the performance behavior of waterborne polyurethane composite coatings. *IJMSCI* **2**, 47-55	NA	IIT, Mumbai
29.	Raliya, R., Rathore, I. and Tarafdar, J. C. (2013). Development of microbial nanofactory for zinc, magnesium, and titanium nanoparticles production using soil fungi. *Journal of Bionanoscience* **7**, 590-596	NA	CAZRI, Jodhpur
30.	Raliya, R., Saran, R., Choudhary, K. and Tarafdar, J. C. (2014). Biosynthesis and Characterization of Nanoparticles. *Journal of Advancement in Medical and Life Sciences*. V1I1	NA	CAZRI, Jodhpur
31.	Raliya, R., Tarafdar, J. C., Singh, S. K., Gautam, R., Choudhary, K., Maurino, V. G., Saharan, V. (2014). MgO nanoparticles biosynthesis and its effect on chlorophyll contents in the leaves of clusterbean (*Cyamopsis tetragonoloba L.*). *Advanced Science, Engineering and Medicine,* 6, 538-545	NA	CAZRI, Jodhpur
32.	Mukhopadhyay, S.S. and Sharma, S. (2013). Nanoscience and Nanotechnology: Cracking Prodigal Farming. *Journal of Bionanoscience* 7:497-502.	NA	PAU Ludhiana

NA – Not quoted

B. Books/ Book chapters/ Abstracts/ Popular articles, Brochures, etc.

S. No.	Authors, Title of the papers Name of Book/ Seminar/ Proceedings/Journal, Publisher, Year, Page No.	Responsible Partner
1.	Tarafdar, J. C. and Raliya Ramesh. 2012. Nanotechnology. Scientific Publisher (India)., pp. 214	CAZRI, Jodhpur
2.	Subramanian, K.S. and Tarafdar, J. C. (2012). Nanotechnology in soil science. In: Soil Science in the Service of Nation. Eds. Goswami, N. N. et al. Indian Society of Soil Science, pp. 326-334	CAZRI, Jodhpur
3.	Adhikari, T. (2010). *Nanotechnology in Agriculture* In "*Efficient utilization of farm wastes for sustainable agriculture*". (Singh, A.B., Sammi Reddy, K., Manna, M.C. and Subba Rao, A. Eds.). 255-265. (Publisher: Agritech Publishing Academy, Udaipur)	IISS, Bhopal
4.	Panwar, J. (2013). Nanoparticles: Controllable synthesis and characterization. In: *Nanotechnology in Soil science & Plant Nutrition*, Adhikari T, Kundu S and Rao A S (Eds.), New India Publishing Agency, New Delhi, India, 153-173	BITS, Pilani

5.	Bhargava, A., Jain, N. and Panwar, J. (2011). Synthesis and application of magnetic nanoparticles: A biological perspective. In: *Current Topics in Biotechnology & Microbiology,* Dhingra H K, Jha P N and Bajpai P (Eds.), LAP Lambert Academic Publishing AG & Co. KG, Dudweller Landstr, Germany, 117-155.	BITS, Pilani
6.	Tarafdar, J. C. (2013). Biological nanoparticles for higher crop production. In: Nanotechnology in Soil Science & Plant Nutrition. Eds. Adhikari, T., Kundu, S. and Rao, A. S., New India Publishing Agency, New Delhi, pp. 61-67	CAZRI, Jodhpur
7.	Tarafdar, J. C. (2013). Nano-induced polysaccharide powder and its application in agriculture. In: Nanotechnology in Soil Science & Plant Nutrition. Eds. Adhikari, T., Kundu, S. and Rao, A. S., New India Publishing Agency, New Delhi, pp. 69-76	CAZRI, Jodhpur
8.	Khanna. A. S. (2013). Synthesis and characterization of nano particles. In T. Adhikari, S. Kundu, and A. Subba, *Nanotechnology in Soil Science & Plant Nutrition* (25-36). New India Publishing Agency, New Delhi, India	IIT, Mumbai
9.	Khanna. A. S. (2013). Effect of nano-pigments on coatings for special corrosion protection. In T. Adhikari, S. Kundu, and A. Subba, *Nanotechnology in Soil Science & Plant Nutrition* (37-41). New India Publishing Agency, New Delhi, India.	IIT, Mumbai
10.	Raliya, R. (2012). Appliance of nanoparticles on plant system and associated rhizospheric microflora, Ph. D. thesis, J. N. V. University, Jodhpur, p. 199.	CAZRI, Jodhpur

13. Media Products Developed/Disseminated

S. No.	CD, Bulletins, Brochures, etc. (Year wise)	No. of Copies	Distribution	Responsible Partner
1.	CD developed about project entitled "*Nano-technology for Enhanced Utilization of Native - Phosphorus by Plants and Higher Moisture Retention in Arid Soils"*	50	Institutions	CAZRI, Jodhpur
2.	Technology Folder about project entitled "*Nano-technology for Enhanced Utilization of Native - Phosphorus by Plants and Higher Moisture Retention in Arid Soils"*	100	Trainees & Research Scholar	CAZRI, Jodhpur
3.	Laboratory brochures entitled "Nanotechnology research facility laboratory"	500	All visitors	CAZRI, Jodhpur

4.	Kundu, S., Adhikari, T., Biswas, A. K., Tarafdar, J.C., Goswami, A. and Subba Rao, A. (2010). Nano-science and nanotechnology in soil fertility and plant nutrition research. IISS **Technical Bulletin**, Indian Institute of Soil Science (ICAR), Nabibagh, Bhopal, pp. 1-46.	400	All ICAR institutes and interested scientist working on Nano technology field	IISS, Bhopal

14. Meetings/Seminars/Trainings/Kisan Mela, etc. organized

S. No.	Details of Meetings/ Seminars/Trainings, etc.	Duration (From-To)	No. of Personnel Trained	Budget (Rs. In lakh)	Organizer (Name & Address)
1.	CAZRI, Jodhpur	22.12.2008	70	0.80	Launch Workshop
2.	CIC	22.12.2008	11	0.40	CPI
3.	CAC	23.12.2008	15	0.40	CPI
4.	CIC	16.6.2009	10	0.30	CCPI
5.	CAC	17.6.2009	11	0.40	CCPI
6.	CMU	17.6.2009	7	0.20	CCPI
7.	CIC	17.12.2009	12	0.30	CPI
8.	CAC	18.12.2009	12	0.35	CPI
9.	CMU	18.12.2009	7	0.20	CPI
10.	CIC	12.8.2010	15	0.40	CCPI
11.	CAC	12.8.2010	14	0.40	CCPI
12.	CMU	13.8.2010	10	0.30	CCPI
13.	CIC	26.8.2011	15	0.40	CPI
14.	CAC	27.8.2011	12	0.25	CPI
15.	CMU	27.8.2011	10	0.10	CPI
16.	CIC	18.5.2013	15	0.40	CPI
17.	CAC	18.5.2013	12	0.25	CPI
18.	CMU	18.5.2013	10	0.10	CPI
19.	Training on purification and characterization of nano particles and sample preparation techniques for various instruments	11.1.2010 to 15.1.2010	7	0.60	CCPI
20.	National Training on " Nanoparticle Production, Characterization and Utilization in Agriculture"	23.2.2012 to 3.3.2012	27	3.00	CPI
21.	National Training "Application of Nanotechnology in Agriculture"	10.3.2014 to 19.3.2014	33	3.50	CPI

15. Participation in Conference/ Meetings/Trainings/ Radio talks, etc.

S. No.	Details of Meetings/Seminars/ Trainings/Radio talk, etc. (Name &Address)	Duration (From-To)	Budget (Rs. In lakh)	Participant (Name & Address)
1.	Exposure to method to use microbes as bio nano factories, its purification and characterization. At School of Engineering & Applied Sciences, Washington University in St. Louis, USA	01-03-2010 to 30-04-2010	3.91	J. C. Tarafdar CAZRI, Jodhpur
2.	Exposure to latest technique/ research efforts on use of nano-particles in plant sciences. At School of Engineering & Applied Sciences, Wahington University in St. Louis, USA	15-1-2010 to 15-03-2010	4.12	Uday Burman CAZRI, Jodhpur
3.	Department of Agricultural Biology, Division of Plant Biotechnology, College of Agriculture & Life Science, Chonbuk National University, South Korea	19.5.2011 to 18.8.2011	Sponsored by INSA	Jitendra Panwar, BITS, Pilani
4.	Training on Nano Technology (Natural Resource Management) at University of Massachusetts, Amherst, Department of Plant, Soil & Insect Sciences, Stockbridge Hall 80 Campus Center, Amherst, MA 01003-9246, USA	20.9.2010 to 20.12.2010	Sponsored by NAIP C-1	Tapan Adhikari IISS, Bhopal
5.	3rd International Conference on Environmental Research and Technology, University Sains, Malaysia	May 30-June 1, 2012	NA	Jitendra Panwar, BITS, Pilani
6.	National Symposium on Application of Clay Science: Agriculture, Environment and Industry, NBSS & LUP, Regional Centre, Kolkata	April 27-28, 2012	NA	Tarafdar, J. C. CAZRI, Jodhpur
7.	National Symposium on Innovative Approaches and Modern Technologies for Crop Productivity, Food Safety and Environment Sustainability, Thrissur, Kerala	November 19-20, 2012	NA	Tarafdar, J. C. CAZRI, Jodhpur

8.	International Conference on Extension Education in the Perspective of Advances in Natural Resource Management in Agriculture (NARMA-IV), Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan (India).	December 19-21, 2012	NA	Tarafdar, J.C. CAZRI, Jodhpur
9.	National Symposium on Managing Stress in Dryland under Climate Change Scenarios. Arid Zone Association of India, CAZRI Campus, Jodhpur (India).	December 1-2, 2012	NA	Tarafdar, J. C. CAZRI, Jodhpur
10.	National Seminar on Development in Soil Science. PAU, Ludhiana, Indian Society of Soil Science, New Delhi.	December 3-6, 2012	NA	Tarafdar, J.C. CAZRI, Jodhpur
11.	National workshop on *Prosopis juliflora*: Retrospect and Prospects. CAZRI, RRS, Kukma-Bhuj, Gujarat, India.	February 26-28, 2013	NA	Tarafdar, J. C. CAZRI, Jodhpur
12.	International Conference on 'Nanoscience & Technology' at Aligarh	March 8-9, 2014	NA	Tarafdar, J. C. CAZRI, Jodhpur
13.	Platinum Jubilee Symposium on Soil Science in Meeting the Challenges to Food Security and Environmental Quality at IARI, New Delhi	December 22-25, 2009	NA	Tapan Adhikari IISS, Bhopal
14.	International Conference on Nanoscience and Nanotechnology (ICONN 2010) from at SRM University, Kattankulathur, Chennai, Tamilnadu, India.	24 - 26 February 2010	NA	Tapan Adhikari IISS, Bhopal
15.	The proceedings of 75th Annual Convention of Indian Society of Soil Science, held at IISS, Bhopal	14-17 November, 2010	NA	Tapan Adhikari IISS, Bhopal
16.	National Seminar on "Nanotechnology for Enhancing Food Security" at TNAU, Coimbatore	7-8 April, 2011	NA	Tapan Adhikari IISS, Bhopal

17.	76th Annual Convention of Indian Society of Soil Science, held at University of Agricultural Sciences, Dharward	16-19 November, 2011	NA	Tapan Adhikari IISS, Bhopal
18.	77th Annual Convention of Indian Society of Soil Science, held at PAU, Ludhiana	3-6 December, 2012	NA	Tapan Adhikari IISS, Bhopal
19.	Nano rock phosphate: Synthesis and application to crops" at 4th Bangalore Nano", The Lalit Ashok Hotel, Bangalore	8-9 December, 2011	NA	Tapan Adhikari IISS, Bhopal
20.	76th Annual Convention of Indian Society of Soil Science, held at UAS, Dhaward	16-19 November, 2011	NA	Tapan Adhikari IISS, Bhopal
21.	99th Annual Convention of Indian Science Congress Association, held at Bhubaneswar	3-7 January, 2012	NA	Tapan Adhikari IISS, Bhopal
22.	Biosynthesis of nanoparticles and its application in agriculture. Proceeding of Brainstorming Session on Nano-Bio-Information Technology for Development of North-Western Himalayan States. G. B. Pant University of Agriculture & Technology, Pantnagar, p. 115.	November, 2013	NA	Tarafdar, J. C. CAZRI, Jodhpur
23.	Biosynthesis of Mg nanoparticles and effect of nanoparticles on wheat plant. 78th National Seminar on Development in Soil Science. Indian Society of Soil Science, New Delhi.	23-36 November, 2013	NA	Rathore, I. and Tarafdar, J. C. CAZRI, Jodhpur
24.	International Conference on Nano Sensors & Technology (ICNST-2010).	28 to 30 Oct, 2010 at Central Scientific Instruments Organization, Chandigarh	Kalia A, Kaur R, Sharma S, Mukhopadhyay SS and Parshad VR (2010), PAU Ludhiana	Kalia A, Kaur R, Sharma S, Mukhopadhyay SS and Parshad VR (2010), PAU Ludhiana
25.	International Conference on Nano Sensors & Technology (ICNST-2010).	28 to 30 Oct, 2010 at CentralScientific Instruments Organization, Chandigarh	Kaur R, Kalia A and Mukhopadhyay SS (2010), PAU Ludhiana	Kaur R, Kalia A and Mukhopadhyay SS (2010), PAU Ludhiana

26.	16th Annual Convention and National Symposium of the Clay Minerals Society of India on Application of Clay Science: Agriculture, Environment and Industry at the National Bureau of Soil Survey & LUP, (ICAR) Nagpur, India.	18-19 Feb, 2011 at the National Bureau of Soil Survey & LUP, (ICAR) Nagpur, India	Mukhopadhyay, S.S., Sharma, S., and Bhinder, D. (2011), PAU Ludhiana	Mukhopadhyay, S.S., Sharma, S., and Bhinder, D. (2011), PAU Ludhiana
27.	National Seminar on Nanotechnology for Enhancing Food Security	07-08 April, 2011 Dept. of Nano Science & Technology, Tamil Nadu Agricultural University, Coimbatore, India	Mukhopadhyay, S.S., Bhinder, D., and Sharma, S. (2011), PAULudhiana	Mukhopadhyay, S.S., Bhinder, D., and Sharma, S. (2011), PAU Ludhiana

16. Foreign Trainings/ Undertaken (National/ International)

S No.	Name, Designation and Address of the Person	Place of Training	Area of Training	Time and Duration	Total Cost (Rs. In lakh)
1.	J. C. Tarafdar, Principal Scientist, CAZRI, Jodhpur	School of Engineering & Applied Sciences, Washington University in St. Louis, USA	Exposure to method to use microbes as bio nano factories, its purification and characterization.	01-03-2010 to 30-04-2010	3.91
2.	Uday Burman, Principal Scientist, CAZRI, Jodhpur	School of Engineering & Applied Sciences, Wahington University in St. Louis, USA	Exposure to latest technique/research efforts on use of nano-particles in plant sciences.	15-1-2010 to 15-03-2010	4.12
3.	Jitendra Panwar, Associate professor, BITS, Pilani	College of Agriculture & Life Science, Chonbuk National University, South Korea	Department of Agricultural Biology, Division of Plant Biotechnology,	19.5.2011 to 18.8.2011	Sponsored by INSA
4.	Tapan Adhikari, Principal scientist IISS, Bhopal	University of Massachusetts, Amherst, Department of Plant, Soil & Insect Sciences, Stockbridge Hall 80 Campus Center, Amherst, MA 01003-9246, USA	Training on Nano Technology (Natural Resource Management) at	20.9.2010 to 20.12.2010	Sponsored by NAIP C-1

17. Performance Indicators (from inception to completion)

S No.	Indicator	Total No.	
1.	No. of production technologies released and/or adopted	4	
2.	No. of processing technologies released and/or adopted	3	
3.	No. of technologies/products commercialized based on NAIP research	5	
4.	No. of new rural industries/enterprises established/ upgraded	0	
5.	No. of product groups for which quality grades developed and agreed	2	
6.	Total no. of private sector organizations (including NGOs) participating in consortium	1	
7.	No. of farmers involved in consortia activities	35	
8.	Total number of farmers' group developed for marketing and processing	10	
9.	Number of patent/intellectual property protection applications filed based on NAIP research	6	
10.	Number of patents/intellectual property protections granted/published based on NAIP research	5	
11.	Number of scientists trained overseas in the frontier areas of science	4	
12.	No. of scientists trained overseas in consortium-based subject areas	4	
13.	No. of scientists participated in conference/seminar etc. abroad	4	
14.	No. of training organized/ farmers trained	Training No. 2	Frames No. 35
15.	Success stories	3	
16.	Incremental employment generated (person days/year/HH)	Baseline	Final
		0	579
17.	Increase in income of participating households (per annum)	Baseline	Final
		NA	NA
18.	Number of novel tools/protocols/methodologies developed	7	
19.	**Publications**		
	Articles in NAAS rated journals	17	
	Articles in other journals	15	
	Book(s)	1	
	Book chapter(s)	8	
	Thesis	1	
	Popular article(s) (English)	1	
	Newspaper article(s)	1	
	Seminar/Symposium/Conference/Workshop Proceedings	14	
	Technical bulletin(s)	2	
	Manual(s)	1	
	CDs/Videos	2	
	Popular article(s) in other language	1	
	Folder/Leaflet/Handout	1	
	Report(s)	6	

18. Employment Generation (man-days/year)

S No.	Type of Employment Generation	Employment Generation up to End of Sub-project	Responsible Partner
1.	RA-2, SRF-3, Office Assistant-1, Skilled/semi-skilled workers-5	11	CAZRI, Jodhpur
2.	RA-2, Skilled/semi-skilled workers-2	4	PAU, Ludhiana
3.	SRF-2, Skilled/semi-skilled workers-2	4	IISS, Bhopal
4.	RA-1, SRF-1, Skilled/semi-skilled workers-2	4	IIT, Mumbai
5.	SRF-1, Skilled/semi-skilled workers-1	2	BITS, Pilani

19. Assets Generated

(i) Equipment/ Vehicles/ Research Facilities

S No.	Name of the Equipment with Manufacturers Name, Model and Sr. No.	Year of Purchase	Quantity (Nos.)	Total cost (Rs. in lakh)	Responsible Partner
1.	Ultrasonic Processor Model E1-250UP	2.2.2010	1	0.76	CAZRI, Jodhpur
2.	Laptop HP Compaq 67300	23. 3.2009	1	0.50	CAZRI, Jodhpur
3.	Tempera ture Light and Humidity Controll Chamber with Culture Racks	7.1.2010	1	6.75	CAZRI, Jodhpur
4.	BOD Incubator 400 Lit Cap	12.1.2010	1	0.79	CAZRI, Jodhpur
5.	BOD Incubator 800 Lit Cap	12.1.2010	1	1.19	CAZRI, Jodhpur
6.	Vertical Page Electrophoresis Unit-1 TV- 400YK with 1.5 ton Window AC	2.5.2010	1	3.08	CAZRI, Jodhpur
7.	Dalsa Nano C- Zeta Potential/ Submicron particle size nalyser	24.3.2010	1	17.45	CAZRI, Jodhpur
8.	Hitachi Micro Ultracentrifuge with temperature control	3.7.2010	1	21.64	CAZRI, Jodhpur
9.	ABB Bomem FTLA 2000104 FTIR Spectrometer	29.1.2010	1	9.18	CAZRI, Jodhpur
10.	Bioreactor Bioflo115, eppendorf	27.3.2014	1	22.50	CAZRI, Jodhpur
11.	Compound Microscope	13.3.2009	1	2.99	BITS Pilani
12.	Refrigerated Centrifuge	13.3.2009	1	3.72	BITS Pilani
13.	BOD Incubator	4.3.2009	1	1.00	BITS Pilani
14.	UV-Visible Spectrophotometer	7.7.2009	1	3.85	BITS Pilani
15.	Rotary Shaker	3.1.2010	1	0.66	BITS Pilani
16.	Refrigerator	3.1.2010	1	0.30	BITS Pilani
17.	BET Surface Area, Porous India	31.03.2014	1	24.0	IISS Bhopal

18.	Research Microscope	31.03.2014	1	8.4	IISS Bhopal
19.	Ultra Sonicator	03.2009	1	4.0	IIT Mumbai
20.	Scratch Hardener	03.2009	1	2.92	IIT Mumbai
21.	Coating Fume Hood	03.2010	1	2.5	IIT Mumbai
22.	Dip Coater	11.2009	1	1.98	IIT Mumbai

(ii) Works

S. No.	Particulars of the Work, Name and Address of Agency Awarded the Work	Year of Work Done	Quantity (Nos.)	Total Cost (Rs. in lakh)	Responsible Partner
1.	Development of NAIP lab	2011	2	19.97	CAZRI, Jodhpur

(iii) Livestock: NA

(iv) Revenue Generated

S. No.	Source of Revenue	Year	Total amount (Rs. in lakh)	Responsible Partner
1.	Commercialization of Technology	2014	50.0	CAZRI, Jodhpur

20. Awards and Recognitions

S. No.	Name, Designation, Add. of the Person	Award/ Recognition (with Date)	Institution/ Society Facilitating (Name & Address)	Responsible Partner
1.	Dr. Ramesh Raliya, RA, CAZRI, Jodhpur	Honorary Fellow 2012 (19.12.2012)	Society for Applied Biotechnology	CAZRI, Jodhpur
2.	Dr. Ramesh Raliya, RA, CAZRI, Jodhpur	Ph. D. degree for his work on "**Appliance of nanoparticles on plant system and associated rhizospheric microflora**" on 31st December, 2012 under the supervision of Dr. J. C. Tarafdar, ICAR National Fellow	J. N. V. University	CAZRI, Jodhpur
3.	Dr. J. C. Tarafdar, National Fellow & Principal Scientist, CAZRI, Jodhpur	Dr. S. K. Mukherjee Commoration Award, 2008	Indian Science Congress Association	CAZRI, Jodhpur
4.		Dr. R. S. Murthy Memorial Award, 2008	Indian Society of Soil Science	CAZRI, Jodhpur
5.		Bharat Jyoti Award, 2011	IIFS	CAZRI, Jodhpur
6.		Glory of India Gold Medal, 2012	IISA	CAZRI, Jodhpur
7.		Dr. R. V. Tamhane Memorial Award, 2013	Indian Society of Soil Science	CAZRI, Jodhpur

21. Steps Undertaken for Post NAIP Sustainability

- Testing the products under field condition with institute funding.
- Three projects were sanctioned under nano-platform to continue the research further.
- A MOU is prepared with consortium mode for further refinement and test the products under different locations.

22. Possible Future Line of Work

- To find out the organisms which can help in biosynthesis of other plant nutrients.
- To identify the entire enzyme groups which are responsible for breakdown of salts to nano-form.
- More research is needed to enhance the stability of nanoparticles.
- To identify the different microbial polysaccharides induced by nanoparticle application and responsible for soil aggregation.
- Clay nanofabrication is another emerging area to develop slow release nanonutrients.

23. Personnel

	From – To (DD/MM/YYYY)
Research Management (CL)	
1. Dr. K. P. R. Vittal	18/07/2008 – 16/08/2009
2. Dr. N. V. Patel	17/08/2009 – 20/02/2010 (FN)
3. Dr. M. M. Roy	20/02/2010 (AF) – 31/03/2014
Scientific (CPI, CCPI, others)	
4. Dr. J. C. Tarafdar (CPI)	18/07/2008 – 31/03/2014
5. Dr. Jitendra Panwar (CCPI)	18/07/2008 – 31/03/2014
6. Prof. S. S. Mukhopadhyay (CCPI)	18/07/2008 – 31/03/2014
7. Prof. A. S. Khanna (CCPI)	18/07/2008 – 31/03/2014
8. Dr. T. K. Adhikari (CCPI)	18/07/2008 – 31/03/2014
9. Dr. Praveen Kumar (Co-PI)	18/07/2008 – 31/03/2014
10. Dr. Uday Burman (Co-PI)	18/07/2008 – 31/03/2014
11. Dr. R. K. Kaul (Co- PI)	18/07/2008 – 31/03/2014
12. Dr. A. K. Biswas (Co-PI)	18/07/2008 – 31/03/2014
13. Prof. C. S. Prasad (Co- PI)	18/07/2008 – 30/09/2012
14. Dr. Anu Kalia (Co-PI)	30/09/2012 – 31/03/2014
Technical (CPI, CCPI, others)	
15. Mr. B. N. Sharma	18/07/2008 – 31/03/2014

Contractual (CPI, CCPI, others)	
16. Mr. Mahesh Kumar Saini	23/01/2009 – 30/04/2011
17. Mr. Sailendra Singh Rathore	06/05/2011 – 30/06/2013
18. Dr. Arti Agarwal	07/01/2009 – 23/04/2011
19. Mr. Rajesh Kumar	23/07/2012 – 31/03/2014
20. Mr. Rajeev Kumar Dhulgar	18/03/2013 – 31/03/2014
21. Mr. Ramesh Raliya	01/04/2009 – 30/09/2013
22. Mr. Himanshu Mahawar	26/12/2011 – 31/03/2014
23. Mr. Praveen Singh Chundhawa	01/08/2012 – 12/02/2013
24. Ms. Tanu Mathur	17/07/2012 – 31/03/2014
25. Mr. Kajan Kumar	06/04/2009 – 31/03/2013
26. Ms. Poonam Joshi	01/07/2009 – 16/07/2012
27. Mr. Madan Kumar Bhati	02/05/2012 – 31/07/2012
28. Mr. Mahendra Vyas	01/01/2009 – 31/03/2014
29. Mr. Ranjeet Sarkar	01/04/2009 – 21/11/2011
30. Mr. Hanwat Singh	01/04/2009 – 21/11/2011
31. Mr. Gopal Rathore	01/12/2010 – 31/03/2014
32. Ms. Shruti Agarwal	01/03/2013 – 31/03/2014
33. Mr. S. K. Dhoke	01/09/2008 – 31/03/2011
34. Mr. Naveen Jain	14/02/2009 – 31/03/2014

24. Governance, Management, Implementation and Coordination

A. Composition of the various committees (CIC, CAC, CMU, etc.)

S. No.	Committee Name	Chairman (From-To)	Members (From-To)
1.	**CIC**	Dr. K. P. R. Vittal [18/07/2008 – 16/08/2009]	Dr. J. C. Tarafdar [18/07/2008 –31/03/2014] Prof. A. S. Khanna [18/07/2008 – 31/03/2014] Prof. S. S. Mukhopadhyay [18/07/2008 – 31/03/2014] Dr. Jitendra Panwar [18/07/2008 – 31/03/2014] Dr. Tapan Adhikari [18/07/2008 – 31/03/2014] SAO, CAZRI [18/07/2008 – 31/03/2014] SFO, CAZRI [18/07/2008 – 31/03/2014]
		Dr. N. V. Patel [17/08/2009 – 20/02/2010 (FN)]	
		Dr. M. M. Roy[20/02/2010 (AF) – 31/03/2014]	

2.	**CAC**	Prof. K. V. B. R. Tilak [18/07/2008 – 16/08/2009]	Prof. Pradyut Ghosh [18/07/2008 – 31/03/2014] Dr. Anand Swaroop [18/07/2008 – 31/03/2014] National Coordinator Component-4 [18/07/2008 – 31/03/2014] Dr. K. P. R. Vittal [18/07/2008 – 31/03/2014] Dr. N. V. Patel [17/08/2009 – 20/02/2010 (FN)] Dr. M. M. Roy [20/02/2010 (AF) – 31/03/2014] Dr. J. C. Tarafdar [18/07/2008 – 31/03/2014]
3.	**CMU**	Dr. K. P. R. Vittal [18/07/2008 – 16/08/2009]	Dr. (Mrs.) Amtul Waris [15/06/2009 – 31/03/2014] Dr. J. C. Tarafdar [15/06/2009 – 31/03/2014] Prof. A. S. Khanna [15/06/2009 – 31/03/2014] Dr. Jitendra Panwar [15/06/2009 – 31/03/2014] Dr. T. Adhikari [15/06/2009 – 31/03/2014] Prof. S. S. Mukhopadhyay [15/06/2009 – 31/03/2014] Dr. Uday Burman [15/06/2009 – 31/03/2014] Dr. R. K. Kaul [15/06/2009 – 31/03/2014] Dr. A. K. Biswas [15/06/2009 – 31/03/2014] Prof. C. S. Prasad [15/06/2009 – 30/09/2012 Dr. Anu Kalia [30/09/2012 – 31/03/2014]
		Dr. N. V. Patel [17/08/2009 – 20/02/2010 (FN)]	
		Dr. M. M. Roy [20/02/2010 (AF) – 31/03/2014]	

B. List of Meetings organized (CIC, CAC, CMU, etc.)

S. No.	Details of the meeting	Date	Place & Address
1.	**CIC**	22/12/2008	CAZRI, Jodhpur
		16/06/2009	PAU, Ludhiana
		17/12/2009	CAZRI, Jodhpur
		12/08/2010	IIT Mumbai
		26/08/2011	CAZRI, Jodhpur
		08/05/2013	CAZRI, Jodhpur
2.	**CAC**	23/12/2008	CAZRI, Jodhpur
		16/06/2009	PAU, Ludhiana
		18/12/2009	CAZRI, Jodhpur
		13/08/2010	IIT Mumbai
		27/08/2011	CAZRI, Jodhpur
		08/05/2013	CAZRI, Jodhpur
3.	**CMU**	16/06/2009	PAU, Ludhiana
		18/12/2009	CAZRI, Jodhpur
		13/08/2010	IIT Mumbai
		27/08/2011	CAZRI, Jodhpur
		08/05/2013	CAZRI, Jodhpur

Part-III : Budget and its Utilization

STATEMENT OF EXPENDITURE (Final)

(Period from 18.07.2008 to 31.03.2014)

(Date of start) (Date of completion)

Sanction Letter No. NAIP/C-4/C-2032/2008-09 dated 18.07.2008

Total Sub-project Cost: ₹ 327.60 Lakhs

Sanctioned/Revised Sub-project cost *(if applicable)*: ***₹ 614.7119 Lakhs***

Date of Commencement of Sub-project: 18.07.2008

Duration: From ***18.07.2008 to 31.03.2014***

Funds Received in each year (in Lakhs)

2008-09: ₹ 177.86051

2009-10: ₹ 30.99286

2010-11: ₹ 50.42189

2011-12: ₹ 47.63907

2012-13: ₹ 65.39912

2013-14: ₹ 88.90795

Bank Interest received on fund *(if any)*: ***Nil***

Total amount received: ₹ 461.22140

Total expenditure: ₹ 476.60786

Expenditure Head-wise:

Sanctioned Heads	Funds Allocated (*)	Funds Released (in Lakhs)						Expenditure Incurred					
		2008-09	2009-10	2010-11	2012-12	2012-13	2013-14	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14
A. Recurring Contingencies													
(1) TA	25.5	3.85962	1.09526	1.59835	1.99142	3.86758	2.48472	1.23834	2.34415	2.22820	2.24037	3.78607	3.33703
(2) Workshops	8.45	1.6	0.4	0.9833	0.59992	3.9560	-	0.79849	-	1.16513	0.50545	-	0.36831
(3) Contractual Services/RA/SRF	219.2609	29.05181	9.10875	28.82018	30.6829	35.35551	5.60883	5.88088	24.50051	28.52605	34.62562	17.91436	14.88253
(4) Operational cost	142.39	12.91287	8.04943	17.2194	9.86182	18.72555	12.08108	8.61771	12.97791	14.12616	15.75721	21.28433	32.52593
Sub-Total of A (1-4)	395.6009	47.4243	18.65344	48.62123	43.13606	61.90464	20.17464	16.53534	39.82257	46.04554	53.12865	42.98476	51.11280
B. HRD Component													
(5) Training	22.5	-	-	-	3.0	-	3.48538	-	8.13722	-	3.3	-	3.5
(6) Consultancy	1.0	-	-	-	-	-	-	-	-	-	-	-	-
Sub-Total of B (5-6)	23.5	-	-	-	**3.0**	-	**3.48538**	-	**8.13722**	-	**3.0**	-	**3.5**
C. Non-Recurring													
(7) Equipment	146.1	10.7.1	11.0	-	-	-	64.15398	13.0449	65.46808	26.67698	-	-	64.80107
(8) Furniture	-	-	-	-	-	-	-	-	-	-	-	-	-
(9) Works (new renovation)	20.0	20.0	-	-	-	-	1.08067	-	-	19.96949	-	-	12.29870
(10) Others	-	-	-	-	-	-	-	-	-	-	-	-	-
Sub-Total of C (5-10)	166.1	127.1	11.0	-	-	-	65.23465	13.0449	65.46808	46.64647	-	-	77.09977
D. Institutional Charges*	29.511	3.33621	1.33942	1.80066	1.50301	3.49448	0.01328	0.23125	0.61168	1.59472	2.55560	1.70965	3.37786
Grand Total (A+B+C+D)	614.7119	177.86051	30.99286	50.42189	47.63907	65.39912	88.90795	29.81149	114.03955	94.28673	58.68425	44.69441	135.09143

* Institutional charges will be 10% of the recurring contingencies for the Lead Consortium and 5% for Consortia Partners.

Name & Signature of CPI : **Name & Signature of Competent Financial authority:** **Signature, name and designation of Consortia Leader**

(Dr. J.C. Tarafdar)
Date : 21-06-2014

(Mr. P.K. Tiwari)
Date : 21-06-2014

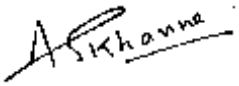
(Dr. M.M. Roy)
Director
Date : 21-06-2014

PART-IV : DECLARATION

This is to certify that the final report of the Sub-project has been submitted in full consultation with the consortium partners in accordance with the approved objectives and technical programme and the relevant records, note books; materials are available for the same.

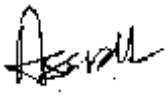
Place : **Jodhpur**
Date : **20-06-2014**

(Dr. J.C. Tarafdar)
Signature of Consortium Principal Investigator

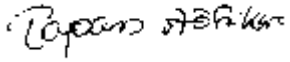


(Prof. A.S. Khanna)
Date : 20-06-2014
Consortium Co-Principal Investigator

(Dr. Jitendra Panwar)
Date : 20-06-2014
Consortium Co-Principal Investigator



(Prof. S.S. Mukhopadhyay)
Date : 20-06-2014
Consortium Co-Principal Investigator



(Prof. Tapan Adhikari)
Date : 20-06-2014
Consortium Co-Principal Investigator

The project has been implemented as per the approved programme of NAIP. The impact of this project has been of very high order and several firms have entered into commercialisation agreement with this institute.

Comments & Signature of Consortium Leader

Date :
June 23, 2014

Director / निदेशक
Central Arid Zone Research Institute, Jodhpur
केन्द्रीय शुष्क क्षेत्र अनुसंधान संस्थान, जोधपुर

Pro-forma 1

Details of Technologies Developed/ Validated/ Adopted
(Page limit: 3 pages/ technology)

1) Title of the sub-project: **Nanotechnology for enhanced utilization of Native Phosphorus by higher plants and higher moisture retention in arid soils (417001-05)**

2) Name of CPI/ CCPI: **Dr. J. C. Tarafdar**

3) Title of the technology: **Nanonutrients**

4) Information on existing farming systems, practices, productivity levels and income in the target area:
   - Can be used in all crops including forestry and vegetable crops.
   - The benefit may be between Rs. 4,200 – Rs. 17,000/ha depending on the crop and nutrient use.

5) Key Intervention(s) introduced: A novel technique has been developed to breakdown the salts into nanoform by fungal protein and sprayed the particle on plant leaves to trigger different enzyme systems for native nutrient mobilization along with the absorption of applied nutrient for plant nutrition.

6) Results
   Prathista industries, Secunderabad and Allwin industries, Indore already signed MOU to commercialize the technology. Other industries like Abellon, Ahmedabad, Biorganics, Maharashtra and IFFCO are negotiating for commercialization.

7) Brief description of technology for release:
   The major advantages of nanonutrients are:
   - Ecofriendly approach.
   - Complete biosource, so environment friendly.
   - Three fold increase in nutrient use efficiency.
   - 80-100 times less requirement than chemical fertilizer.
   - 10 times more stress tolerant by the crops.
   - 30% more nutrient mobilization in the rhizosphere.
   - 18-283% more release of different beneficial enzymes resulting better soil health and native nutrient mobilization.
   - 12-54% improvement in crop yield.
   - Helping plant to be more independent and active.

8) Expected Outcome/ Impact of the technology :

8.1. Expected increase in area, production and net income

Effect of nano nutrient on yield potential at Rajasthan

Crop	Area (lakh ha)	Present Yield Potential (kg ha^{-1})	Expected Yield potential (Kg ha^{-1})	Data based on cultivar
Pearl Millet	46	550-700	950-1150	HHB 67
Moth bean	12.2	600-800	950-1025	RMO 18
Moong bean	21.3	650-850	950-1100	K851
Cluster bean	23.3	550-650	900-1100	RGC936
Cauliflower	NA	21200-25000	32600-38500	Golden

Calculation based on Year 2011-2012 and 2012-2013
Other - 33 Districts; 10 Agro climatic Zone; Total cultivated area 216.64 Lakh ha

8.1. Others

Computation of extra benefit due to application of Nano Phosphorus

Cost of nano P ha^{-1}@Rs. 22 L^{-1}
- 16 L ha–1 for small leaf plants : Rs. 352
- 18 L ha–1 for broad leaf plants : Rs. 396

Biosynthesized Nano P is environmental friendly

Cost of SSP*
- @80 kg ha^{-1} @ Rs. 8 kg^{-1} : Rs. 640
- @60 kg ha^{-1} @ Rs. 8 kg^{-1} : Rs. 480

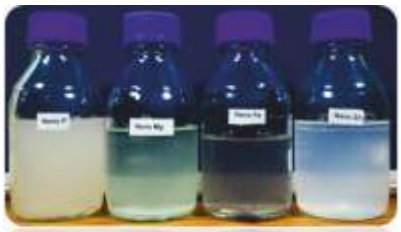
Nutrient use efficiency for SSP/DAP : 15-16%
Nutrient use efficiency for **nano P : 58-61%**

Cost of DAP*
- @80 kg ha^{-1} @ Rs. 25 kg^{-1} : Rs. 2000
- @60 kg ha^{-1} @ Rs. 25 kg^{-1} : Rs. 1500

**after subsidy*

Extra yield improvement over mega particle (ha^{-1})

Crops	More yield (kg ha^{-1})	Rate (Rs. Kg^{-1})	Total Benefit
Clusterbean	250	68	17,000
Pearl Millet	283	15	4,245
Moth bean	309	35	10,815
Mung bean	161	55	8,855



Nanonutrients

Yield increase (%) due to nanonutrient application under different crops

Crops	Nanonutrient applied	Research field	Farmer's field
Pearl Millet	P, Zn, Fe, Mg	13-48	18-43
Cluster bean	P, Zn, Fe, Mg	14-36	21-37
Moth bean	P, Zn, Fe	12-31	18-27
Mung bean	P, Zn, Fe, Mg	17-42	23-38
Maize	P	23-32	-
Castor	P, Zn	-	24-37
Cauliflower	P, Zn	-	47-54
Tomato	P, Zn	18-23	25-29
Rice	P	22-28	-
Capsicum	P, Zn	19-24	-

*doses applied : P-40ppm; Fe-30ppm; Mg-20ppm; Zn-10ppm

9) Whether findings have been published? If so, give the citation and enclose copy of the publication.

Finding is published as patent (149/DEL/2012) and research papers:

- Raliya, R. and **Tarafdar, J. C.** (2013). ZnO nanoparticle biosynthesis and its effect on phosphorus mobilizing enzyme secretion and gum contents in clusterbean (*Cyamopsis tetragonoloba* L). *Agricultural Research* 2, 48-57
- **Tarafdar, J.C.** (2012). Perspectives of nanotechnological applications for crop production. *NAAS News* 12, 8-11

10) Any other information: Nil

1) Title of the sub-project: **Nanotechnology for enhanced utilization of Native Phosphorus by higher plants and higher moisture retention in arid soils (417001-05)**

2) Name of CPI/ CCPI: **Dr. J. C. Tarafdar**

3) Title of the technology: **Nanoinduced polysaccharide powder**

4) Information on existing farming systems, practices, productivity levels and income in the target area:
The main problem of arid soils are low carbon content, less moisture retention capacity, low microbial population and less nutrient reserve. To cope up with this problem, we have developed nanoinduced polysaccharide powder where we are inducing polysaccharide secretion by the microorganisms through nanoparticle (Zn and Fe) stimulation. A methodology was developed and patented (404/DEL/2012) where this polysaccharide can be flocculated and prepared powder for soil application to build-up organic carbon, soil aggregation, microbial activity and moisture retention.

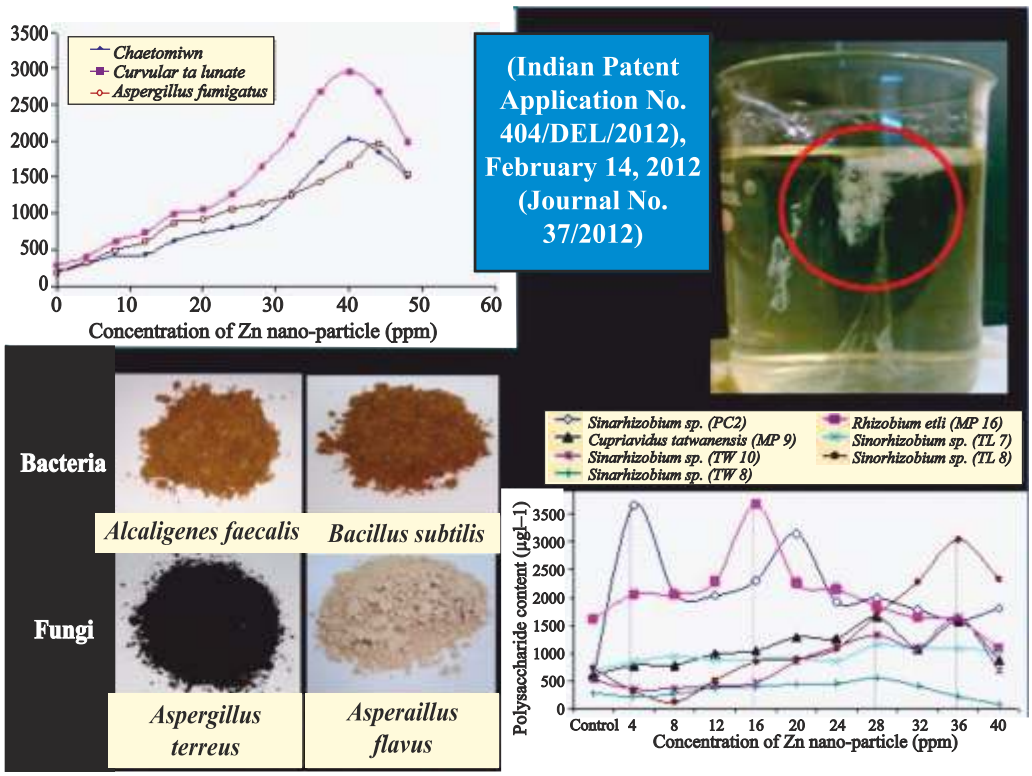
5) Key Intervention(s) introduced: We are using the nanoparticles which are structural components of polysaccharides so they are helping to act as cofactor and resulting to enhance 10-18 times more polysaccharide release compared to control.

6) Results: MOU signed with Prathistha industries, Secunderabad (6/3/2014) and Allwin industries, Indore (19/5/2014) to commercialize and scale up.

7) Brief description of technology for release:

The major advantages of nanoinduced polysaccharides are:

- Improvement in Soil aggregation upto 83%.
- Soil moisture retention upto 14%.
- Soil carbon build upto 5%.



Assessment of binding and soil moisture retention efficiency of microbial gums

Mixed with Bacterial Polysaccharide | Control

improvement of soil aggregation, carbon build-up an moisture retention by applicaton of nano induced microbial gum.

Treatment	Per cent improvement of aggregate size over control after 30 days (1% w/v)		
	1.0 mm	0.5 mm	0.18 mm
Polysaccharide from Bacillus coaaulans	80.7	No change	No change
Polysaccharide from Alcaligenes faecalis	33.4	82.9	56.4

8) Expected Outcome/ Impact of the technology:

12.1. Expected increase in area, production and net income

This technology can be applied in entire arid and semi-arid areas. Potential of the technology :

- Carbon build-up: 3-5%
- Moisture Retention: 10-14%
- Soil aggregation: 33-83%

12.2. Others

9) Whether findings have been published? If so, give the citation and enclose copy of the publication.

The technology has been patented and research papers have been published.

- Raliya, R., Tarafdar, J. C., Mahawar, H., Kumar, R., Gupta, P., Mathur, T., Kaul, R. K., Kalia, A., Gautam, R., Singh, S. K. and Gehlot, H. S. (2014). ZnO nanoparticles induced exopolysaccharide production by *B. subtilis* strain JCT1 for arid soil applications. *International Journal of Biological Macromolecules* 65, 362–368
- **Tarafdar, J.C.**, Agrawal, A., Raliya, R., Kumar, P., Burman, U. and Kaul, R.K. (2012). ZnO nanoparticles induced synthesis of polysaccharides and phosphatases by *Aspergillus* fungi. *Advanced Science, Engineering and Medicine* 4, 1-5.

10) Any other information: Nil