VIGNANA BHARATHI®
Institute of Technology
(A UGC Autonomous Institution, Approved by AICTE, Accredited by NBA & NAAC-A Grade, Affiliated to JNTUH)
(Sponsored by Swamy Vivekanda Educational Trust, Hyd.)

CERTIFICATE

FILE AND LINK No: MRP-6948/16 (SERO/UGC)

NAME OF THE PRINCIPAL INVESTIGATOR: S.Shylaja
Vignana Bharathi Institute of Technology,
Aushapur, Ghatakesar,Medchal,Hyderabad, Pin: 501301

TITLE OF THE PROJECT: "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS"

Certified that the project has been successfully completed and Executive summary of the report, Research documents, monograph, academic papers published under Minor research project has been posted on the website of the college.

S. Shylaja

Signature of the Principal Investigator

Signature of the Principal
With seal and stamp

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
MEDCHAL DIST-501 301

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

ACCESSION CERTIFICATE

This is to certified that S.Shylaja, Department of Chemistry, Hyderabad has handed over the following books and journals purchased under the scheme of Minor Research Project to the Library of Vignana Bharathi Institute of Technology, Hyderabad. The following are books and journals handed over by S.Shylaja (MRP-6948/16 (UGC/SERO).

S. No	Item	Qty.
1.	Spectroscopy by B.K.Sharma	1
2.	Int to NanoScience and Nanotechnology	1
3.	Organic spectroscopy by William kemp	1
4.	Organic Chemistry (SIE)	1
5.	Nanotechnology :Principles and practices	1
6.	Nanoscience and Plant soil system	1
7.	Plant nanotechnology	1
8.	An agricultural paradigm nanotech	1
9.	Nano characterization and applications	1

S.Shylaja

Signature of the Principal Investigator

28/12/16

Signature of the Librarian

Signature of the Principal

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

ASSETS CERTIFICATE

This is to certified that S.Shylaja, Department of Chemistry, Hyderabad has handed over the following equipment purchased under the scheme of Minor Research Project to the Department of Chemistry, Vignana Bharathi Institute of Technology, Hyderabad. The following are equipments handed over by S.Shylaja (MRP-6948/16 (UGC/SERO).

S. No	Particulars	Company	Qty.
1	Vacuum Pump	VALUE	1
2	Filter flask 500ml	BOROSILICATE	1
3	Buchner funnel4''	PORCELAIN	1
4	Digital Ultrasonic cleaner cap	WENSAR	1
5	Digital P.H meter di-707	DIGISUN	1
6	Hot plate rectangular 10*12	BTI	1
7	DELL 3567 (I3/4GB/1TB/15.6/W10)	DELL	1
8	Magnetic Stirrer with hot plate	EARTH	1

S. Shylaja

Signature of the
Principal Investigator

Signature of the
Head of the Department.

Signature of the
Principal

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
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Aushapur (V), Ghatkesar (M)

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

Settlement proforma

UTILISATION CERTIFICATE

FILE AND LINK No: MRP-6948/16 (SERO/UGC)

NAME OF THE PRINCIPAL INVESTIGATOR: S.Shylaja

Vignana Bharathi Institute of Technology,

Aushapur,Ghatakesar,Medchal, Hyderabad, Pin: 501301

TITLE OF THE PROJECT: "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS".

Certified that the grant of Rs. 1,40,000/ (Rupees one lakh forty thousand only) approved by UGC and the grant received Rs1,35,000/(Rupees one lakh thirty five thousand only) from the University Grants Commission under the scheme of support for Minor Research Project entitled "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NOFERTILIZERS", vide UGC letter No. F.MRP-6948/16 (SERO/UGC) dated 28/7/2017 **has been fully utilized for the purpose for which it was sanctioned and that the balance of Rs.5000 has been spent by institute which has to be released from UGC in accordance with the terms and conditions laid down by the University Grants Commission. If as a result of check or audit objection, some irregularity is noticed at a later stage, action will be taken to refund or regularize the objected amount.**

S.Shylaj

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL WITH SEAL AND STAMP

9-1-2020

STATUTORY AUDITOR WITH SEAL AND STAMP

CA. L. JANARDHAN RAO
Chartered Accountant
M.No: 18474

UDIN 20018474AAAAAE8794

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MINOR RESEARCH PROJECT

7. Name of Principal Investigator : S.Shylaja
8. Dept. of PI : Department of Chemistry
   Name of College : Vignana Bharathi Institute of Technology
9. UGC approval Letter No. and Date : MRP-6948/16 (SERO/UGC) & 2-Aug-2017
10. Title of the Research Project : "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS".
11. Effective date of starting the project :09-Aug-2017
12. a. Period of Expenditure: From : 03-DEC-2017 to 18-JULY-2019
    b. Details of Expenditure _____

S.No.	Item	Amount Approved (Rs.)	Amount Received (Rs)	Expenditure Incurred(Rs.)	Amount to be released by UGC
i.	Books & Journals	10,000	10,000	10,000	00
ii.	Equipment	80,000	80000	80000	00
iii.	Contingency including special needs	20,000	18,000	20,000	2,000
iv.	Field Work/Travel (Give details in the profor ma).	00	00	00	00
v.	Hiring Services	00	00	00	00
vi.	Chemicals & Glassware	30,000	27,000	30,000	3,000
GRAND TOTAL		**1,40,000**	**1,35,000**	**1,35,000**	**5,000**

8. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

8. It is certified that the grant of Rs. 1,40,000/ (Rupees one lakh forty thousand only) approved by UGC and the grant received Rs.135,000/- (Rupees one lakh thirty five thousand only) from the University Grants Commission under the scheme of support for Minor Research Project entitled "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS "UGC letter No. F. MRP-6948/16 (SERO/UGC) Dated 02/08/2017 has been fully utilized for the purpose for which it was sanctioned and that the balance of Rs.5000 has been spent by institute which has to be released from UGC in accordance with the terms and conditions laid down by the University Grants Commission.

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S. Shylaj

SIGNATURE OF PRINCIPAL INVESTIGATOR

PRINCIPAL

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

(Seal)

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
MEDCHAL DIST-501 301

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MINOR RESEARCH PROJECT

1. Name of Principal Investigator : S.Shylaja
2. Dept. of PI : Department of Chemistry
   Name of College : Vignana Bharathi Institute of Technology
3. UGC approval Letter No. and Date : MRP-6948/16 (SERO/UGC) & 2-Aug-2017
4. Title of the Research Project : "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS".
5. Effective date of starting the project :09-Aug-2017
6. a. Period of Expenditure: From : 08-OCT-2018 to 18-JULY-2019
   b. Details of Expenditure _____

S.No.	Item	Amount Approved (Rs.)	Amount Received (Rs.)	Expenditure Incurred (Rs.)	Amount to be released by UGC
i.	Books & Journals	00	00	00	
ii.	Equipment	00	00	00	
iii.	Contingency including special needs	10000	8000	10000	2000
iv.	Field Work/Travel (Give details in the proform) a .	00	00	00	
v.	Hiring Services	00	00	00	
vi.	Chemicals & Glassware	15000	12000	15000	3000
GRAND TOTAL		**25,000**	**20,000**	**25,000**	**5,000**

7. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

8. It is certified that the grant of Rs. **25,000/** (Rupees twenty five thousand only) approved by UGC and the grant received Rs **20,000** (Rupees twenty thousand only) ceived from the University Grants Commission under the scheme of support for Minor Research Project entitled "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS "UGC letter No. F. MRP-6948/16 (SERO/UGC) Dated 28/7/2017has been fully utilized for the purpose for which it was sanctioned and that the balance of Rs.**5,000** has been spent by institute which has to be released from UGC in accordance with the terms and conditions laid down by the University Grants Commission.

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S. Shylaja

SIGNATURE OF PRINCIPAL INVESTIGATOR

PRINCIPAL

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
MEDCHAL DIST-501 301

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

(Seal)

Annexure - V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate(II year)

Certified that the grant of Rs. 25,000/ (Rupees twenty five thousand only) approved by UGC and the grant received RS 20,000(Rupees twenty thousand only) from the University Grants Commission under the scheme of support for Minor Research Project entitled **"AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS"**vide UGC letter No. F._____**MRP 6948/16(SERO/UGC)** dated 2-Aug-2017 has been fully utilized for the purpose for which it was sanctioned and the balance of Rs.5000 has been spent by institute which has to be released from UGC in accordance with the terms and conditions laid down by the University Grants Commission.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL

(Seal)

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

STATUTORY AUDITOR

9-1-2020

(Seal)

CA. L. JANARDHAN RAO
Chartered Accountant
M.No: 18474

UDIN: 20018474AAAAE8794

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
MEDCHAL DIST-501 301

Annexure – V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate (Consolidated, I & II Year)

Certified that the grant of Rs. 1,40,000/ (Rupees one lakh forty thousand only) approved by UGC and the grant received Rs1,35,000/(Rupees one lakh thirty five thousand only) from the University Grants Commission under the scheme of support for Minor Research Project entitled **"AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS"** vide UGC letter No. F._____**MRP 6948/16(SERO/UGC)** dated 2-Aug-2017 has been fully utilized for the purpose for which it was sanctioned and that the balance of Rs.5000 has been spent by institute which has to be released from UGC in accordance with the terms and conditions laid down by the University Grants Commission.

S. Shylaja

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

PRINCIPAL

(Seal)

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

9-1-2020.

STATUTORY AUDITOR

(Seal)

CA. L. JANARDHAN RAO
Chartered Accountant
M.No: 18474

UDIN: 20018474AAAAAE8794

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
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Annexure -VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Annual/Final Report of the work done on the Minor Research Project. (Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1st /Final : FINAL(After 2 years)
2. UGC Reference No.F. : MRP 6948/16(SERO/UGC)
3. Period of report: from : 9-Aug-2017 to 09-Aug-2019
4. Title of research project : "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS".

5. (a) Name of the Principal Investigator : S.SHYLAJA

(b) Dept. : Department of Chemistry

(c) College where work has progressed : Vignana Bharathi Institute of Technology

6. Effective date of starting of the project : 9-Aug-2017
7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved : Rs. 1,40,000/-

b. Total expenditure : Rs.1,40,000/-

c. Report of the work done : (Please attach a separate sheet)

i. Brief objective of the project : (Attached)

a. To synthesize the Nanofertilizers and its characterization using the XRD and SEM.

b. To study their interactions with soil in view of plant yield

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication)

➢ **Presentation:**

- Oral presentation of research paper entitled "Effect of Synthetic Urea hydroxyapatite nanoparticles as a slow release fertilizer for Plants"- National Seminar on "Recent Trends in Material Research for Science and Engineering applications" RTMRSEA-during 20th and 21st December 2018 at M.V.S.R Engineering College, Nadergul, HYD.
- A poster presentation on Research paper entitled "Evaluating the effects of formulated nano-Urea hydroxyapatite slow release fertilizer composite on the growth and yield of Cyamopsis tetragonoloba (cluster beans)" in International conference on material science for societal advancement organised under UGC UPE FAR programme, OU to be held on 20-22 January 2020.

➢ **Publication/Communication:**

- "Root and Shoot Uptake of Synthesized Nano ZnO and Its Impact on Differences in Bio-Availability During Exposure In Aqueous Suspension"- research paper published through online in International Journal of Innovative Technology and Exploring Engineering (IJITEE) ISSN: 2278-3075, Volume-8 Issue-10, August 2019
- Research paper entitled "Evaluation of Urea hydroxyapatite nanocarriers as slow release fertilizer composite on the growth and yield of Cyamopsis tetragonoloba is under review process.

iii. Has the progress been according to original plan of work and towards achieving the objective. **YES**

iv. please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to the concerned Regional Office of the UGC.

v. Any other information

S. Shylaj

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

27.01.2020

PRINCIPAL

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

(Seal)

UNIVERSITY GRANTS COMMISSION BAHADUR SHAH ZAFAR MARG NEW DELHI – 110 002

Annexure – VII

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project : **"AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS".**

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: **S .Shylaja**

3. NAME AND ADDRESS OF THE INSTITUTION: **Vignana Bharathi Institute of Technology (VBIT), Aushapur (vill), Ghatkesar (m), Medchal (dist), Telangana state, 501301.**

4. UGC APPROVAL LETTER NO. AND DATE : **MRP-6948/16 (SERO/UGC) & 2-Aug-2017**

5. DATE OF IMPLEMENTATION: **09-Aug-2017**

6. TENURE OF THE PROJECT: **2 years**

7. TOTAL GRANT ALLOCATED: **1,40,000/-**

8. TOTAL GRANT RECEIVED: **1,35,000/-**

9. FINAL EXPENDITURE: **1,40,000/-**

10. TITLE OF THE PROJECT:**"AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS".**

11. OBJECTIVES OF THE PROJECT:

a. To synthesize the Nanofertilizers and its characterization using the XRD and SEM.

b. To study their interactions with soil in view of plant yield.

12. WHETHER OBJECTIVES WERE ACHIEVED: YES

- Successfully synthesized the urea hydroxyapatite nanoparticles, which is source of nitrogen and phosphorous macronutrients for plants. These nanoparticles were characterized by XRD, SEM, EDX and FTIR.

- Further these nanoparticles were applied to cluster bean plants to find the growth and yield.

13. ACHIEVEMENTS FROM THE PROJECT

From this study, gained the knowledge of importance of biocompatible, Nanofertilizers for growth and yield of plants, which is very much useful to meet the basic requirement of growing population.

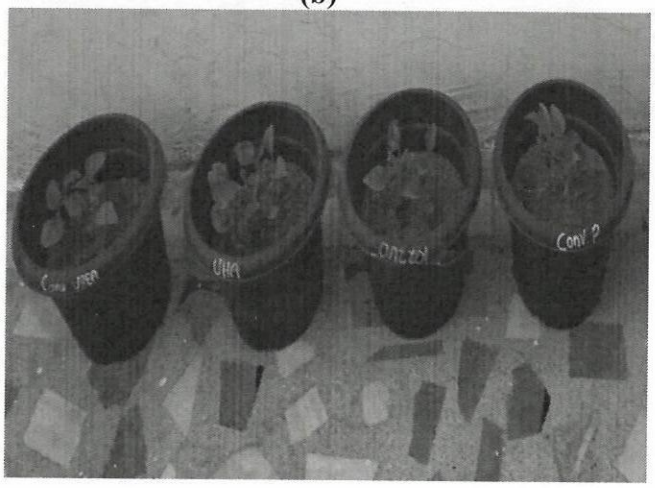
14. SUMMARY OF THE FINDINGS

This project describes the simple and novel chemical co-precipitation method for the successful synthesis of nano-urea hydroxyapatite, slow release fertilizer composite. The XRD study confirmed the crystallite size as 20 nm. DLS also confirmed the particle size of UHA. The SEM images showed that the particles are aggregated to form agglomerates. The EDX confirmed the existence of C, N, O, Ca, P stating the successful formation of UHA. The pot studies of cluster bean plants over a period of four months showed that the application of urea hydroxyapatite, as a good nutrient source of nitrogen and phosphorous which has enhanced the growth rate (shoot and root length), plant biomass (fresh and dry weight) and also plant yield over that of conventional urea and Di Ammonium Phosphate. Microscopic studies of root of UHA confirmed that there is no alteration in internal cell structure of the root, which further confirm no negative impact on plant internal structure.

Images of plant during the germination and growth process.

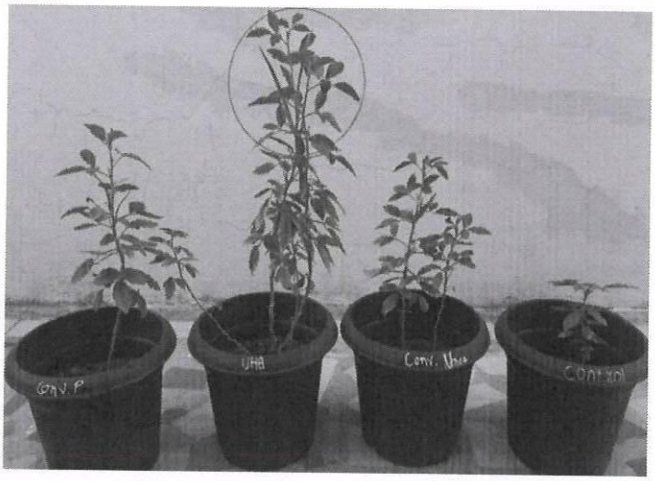
(a)

(b)



(c)

(d)



The zinc oxide nanoparticles were synthesized successfully by both chemical co-precipitation and green methods. The crystallite size of chemical ZnO and green ZnO was approximately 35 nm and 28nm. Morphology and elemental composition of ZnO nanoparticle were confirmed by SEM and EDAX. We have reported that the usage of micronutrient Zn in the form of green synthesised ZnO nanoparticles will be more effective for germination and growth of lycopersicum esculentus with no obvious toxic effects. It was further observed that growth of seedlings with the uptake of nanoparticles is completely concentration dependent where above 300 ppm concentration has shunted the growth of seedlings.

15. CONTRIBUTION TO THE SOCIETY:

The usage of biocompatible nanofertilisers for plants growth and yield can be used effectively in field of agriculture to meet demands of food for rising population.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT : NO

17. NO. OF PUBLICATIONS OUT OF THE PROJECT: ONE PAPER Attached.

S. Shylaj

(PRINCIPAL INVESTIGATOR)

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
MEDCHAL DIST-501 301

(Seal)

(PRINCIPAL)
SEAL

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

DETAILED STATEMENT OF EXPENDITURE FOR CHEMICALS AND GLASSWARE(II YEAR)

UGC REFERENCE NUMBER : MRP-6948/16(SERO/UGC)

PRICIPAL INVESTIGATOR : S.SHYLAJA

TITLE OF THE PROJECT : AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS.

CHEMICALS---II YEAR 2018-19

S.No	ITEM DESCRIPTION	INVOICE NUMBER	DATE	GST	QUANTITY	RATE	AMOUNT
1.	Deionised water 5L	1915	14/03/2019	18%	13	120	1560.00
2.	Zinc Acetate AR 500g CDH	1915	14/03/2019	18%	1	405	405.00
3.	Zinc Oxide AR 500g CDH	1915	14/03/2019	18%	1	539	539.00
4.	Test tubes 15*125mm	1915	14/03/2019	18%	10	12	120.00
5.	Silver Nitrate AR 25gFinar	1915	14/03/2019	18%	2	3260	6520.00
6.	Acetone AR 500ml Finar	1915	14/03/2019	18%	2	324	648.00
7.	Sulphuric Acid 500ml	1915	14/03/2019	18%	1	269	269.00
8.	Sodium chloride 500g	1915	14/03/2019	18%	1	128	128.00
							10,189.00
						CGST	905.49
						SGST	905.49
						TOTAL	12000.00

TOTAL: TWELVE THOUSAND RUPEES ONLY

S.No	ITEM DESCRIPTION	INVOICE NUMBER	DATE	GST	QUANTITY	RATE	AMOUNT
1.	Propan-2-ol AR 500nl Finar	520	18/07/2019	18%	2	325	650.00
2.	Manganese Dioxide 500g	520	18/07/2019	18%	1	204	204.00
3.	Cobalt oxide 100g	520	18/07/2019	18%	1	1688	1688.00
							2542.00
						CGST	228.78
						SGST	228.78
							3000.00

TOTAL: THREE THOUSAND RUPEES ONLY

GRAND TOTAL : FIFTEEN THOUSAND RUPEES ONLY

S.Shylaja

Signature of the principal investigator

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY VBIT Aushapur (V), Ghatkesar MEDCHAL DIST-501

Signature of the principal

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

9-1-2020

Signature of the statutory auditor

JANARDHAN RAO HYDERABAD CHARTERED ACCOUNTANTS

CA. L. JANARDHAN RAO
Chartered Accountant

DETAILED STATEMENT OF EXPENDITURE FOR CHEMICALS AND GLASSWARE FOR I AND II YEAR

UGC REFERENCE NUMBER : MRP-6948/16(SERO/UGC)

PRICIPAL INVESTIGATOR : S.SHYLAJA

TITLE OF THE PROJECT : AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS.

CHEMICALS: I YEAR 2017-18

INVOICE NUMBER : 1211 ;

DATE :05/02/2018

S.NO	ITEM DESCRIPTION	GST	QUANTITY	RATE/PER	AMOUNT
1.	Aluminium foils	18%	1	60.00	60.00
2.	Cotton rolls	12%	1	120.00	120.00
3.	Acetone 500ml	18%	1	210.00	210.00
4.	Nickel spatula 6	18%	5	48.00	240.00
5.	Beaker 1000ml	18%	4	240.00	960.00
6.	Beaker 500ml	18%	2	116.00	232.00
7.	pH paper	12%	1	188.00	188.00
8.	calcium hydroxide AR	18%	1	461.00	461.00
9.	Diammonium hydrogen O phosphate	18%	1	328.00	328.00
10.	Urea AR	18%	1	242.00	242.00
11.	Zinc nitrate AR	18%	1	346.00	346.00
12.	Ammonia solution	18%	1	140.00	140.00
13.	Phosphoric acid	18%	1	549.00	549.00
14.	Glass rod 8''	18%	2	6.00	12.00
15.	Magnetic bead	18%	2	255.00	510.00
16	Spatula thick rod type s.s	18%	5	22.00	110.00
					4708.00
				CGST	414.48
				SGST	414.48
				TOTAL	**5537.00**

TOTAL : Five Thousand Five Hundred and Thirty seven only

INVOICE NUMBER: 297

DATE: 6/6/2018.

S.NO	ITEM DESCRIPTION	GST	QUANTITY	RATE/PER	AMOUNT
1.	Calcium nitrate tetra hydrated	18%	1	195.00	195.00
2.	Urea	18%	1	242.00	242.00
3.	Chinadish	18%	3	36.00	108.00
4.	crucible	18%	3	36.00	108.00
5.	Sample vials (screw model)	18%	20	30.00	600.00
6.	Scintillation vials	18%	1box	425.00	425.00
7.	Petridish glass	18%	2box	66.00	1320.00
8.	Whattman filterpaper 42	18%	1	1450.00	1450.00
9.	Deionised water 5lit	18%	2	120.00	240.00
10.	Test tubes stand 6 holes	18%	1	24.00	24.00
11.	Test tubes	18%	6	12.00	72.00
					4784.00
				CGST	430.56
				SGST	430.56
				TOTAL	5645.00

TOTAL: Five Thousand Six Hundred and Forty Five only

INVOICE NUMBER: 463

DATE: 09/07/2018

S.NO	ITEM DESCRIPTION	GST	QUANTITY	RATE/PER	AMOUNT
1.	Sodium hydroxide AR	18%	1	269.00	269.00
2.	measuring cylinder 100ml	18%	1	401.00	401.00
3.	burette50ml	18%	1	465.00	465.00
4.	grad.pipette	18%	1	145.00	145.00
5.	conical flask250ml	18%	2	120.00	240.00
6.	volumetric flask 100ml	18%	1	245.00	245.00
7.	volumetric flask 250ml	18%	1	295.00	295.00
					2060.00
				CGST	185.40
				SGST	185.40
				TOTAL	2431.00

TOTAL : Two Thousand Four Hundred Thirty one only

S.NO	ITEM DESCRIPTION	BILL NO	DATE	QUANTITY	RATE	AMOUNT
1.	Pots	426	10/7/18	8	100.00	800.00
2.	Spray pump	426	18/7/18	1	50.00	50.00
3.	Seeds	426	10/7/18	1	80.00	80.00
4.	Hand tools	426	10/7/18	1	100.00	100.00
					TOTAL	1030.00

TOTAL : One Thousand Thirty Only

GRAND TOTAL FOR YEAR I : 15000/-(Fifteen Thousand Rupees Only)

CHEMICALS---II YEAR 2018-19

S.No	ITEM DESCRIPTION	INVOICE NUMBER	DATE	GST	QUANTITY	RATE	AMOUNT
1.	Deionised water 5L	1915	14/03/2019	18%	13	120	1560.00
2.	Zinc Acetate AR 500g CDH	1915	14/03/2019	18%	1	405	405.00
3.	Zinc Oxide AR 500g CDH	1915	14/03/2019	18%	1	539	539.00
4.	Test tubes 15*125mm	1915	14/03/2019	18%	10	12	120.00
5.	Silver Nitrate AR 25gFinar	1915	14/03/2019	18%	2	3260	6520.00
6.	Acetone AR 500ml Finar	1915	14/03/2019	18%	2	324	648.00
7.	Sulphuric Acid 500ml	1915	14/03/2019	18%	1	269	269.00
8.	Sodium chloride 500g	1915	14/03/2019	18%	1	128	128.00
							10,189.00
						CGST	905.49
						SGST	905.49
						TOTAL	**12000.00**

TOTAL: TWELVE THOUSAND RUPEES ONLY

S.No	ITEM DESCRIPTION	INVOICE NUMBER	DATE	GST	QUANTITY	RATE	AMOUNT
1.	Propan-2-ol AR 500nl Finar	520	18/07/2019	18%	2	325	650.00
2.	Manganese Dioxide 500g	520	18/07/2019	18%	1	204	204.00
3.	Cobalt oxide 100g	520	18/07/2019	18%	1	1688	1688.00
							2542.00
						CGST	228.78
						SGST	228.78
						TOTAL	3000.00

TOTAL: THREE THOUSAND RUPEES ONLY

GRAND TOTAL FOR YEAR II: 15000/-(Fifteen Thousand Rupees Only)

GRAND TOTAL FOR YEAR I AND II : 30000/-(THIRTY THOUSAND RUPEES ONLY)

Signature of the principal investigator

Signature of the principal

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY VBIT Aushapur (V), Ghatkesar (M) MEDCHAL DIST-501 301

Signature of the statutory auditor

CA. L. JANARDHAN RAO
Chartered Accountant
M.No: 18474

DETAILED STATEMENT OF EXPENDITURE FOR CONTINGENCY (II YEAR)

(INCLUDING SPECIAL NEEDS)

UGC REFERENCE NUMBER : MRP-6948/16(SERO/UGC)

PRICIPAL INVESTIGATOR : S.SHYLAJA

TITLE OF THE PROJECT : AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS.

CONTINGENCY---II YEAR 2018-19

S.NO	ITEM DESCRIPTION	BILL NO	DATE	AMOUNT
1.	SEM,EDX,XRD	04PD/2018	8/10/2018	1500.00
2.	RTMESEA-2018	011	20/12/2018	600.00
3.	SEEDS	109	1/2/2019	300.00
4.	SEM,EDX,XRD	02PD/2019	13/3/2019	1700.00
5.	SEM,EDX,XRD,FTIR	04PD/2019	26/3/2019	4800.00
6.	SEM,EDX,XRD	08PD/2019	10/4/2019	1100.00
			TOTAL	10000

TOTAL: TEN THOUSAND RUPEES ONLY

XRD, SEM, EDX, FTIR CHARACTERISATION TECHNIQUES DONE AT OSMANIA UNIVERSITY (Dept of Physics)

S.Shylaj

Signature of the principal investigator

Signature of the principal

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

9-7-2020

Signature of the Statutory auditor

CA. L. JANARDHAN RAO
Chartered Accountant
M.No: 18474

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY
VBIT
Aushapur (V), Ghatkesar (M)
MEDCHAL DIST-501 301

DETAILED STATEMENT OF EXPENDITURE FOR CONTINGENCY (INCLUDING SPECIAL NEEDS)

I & II YEAR

UGC REFERENCE NUMBER : MRP-6948/16(SERO/UGC)

PRICIPAL INVESTIGATOR : S.SHYLAJA

TITLE OF THE PROJECT : AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS.

CONTINGENCY---I YEAR 2017-18

S.NO	ITEM DESCRIPTION	BILL NO	DATE	AMOUNT
1.	UTILIZATION OF LAB FACILITIES (XRD,PARTICLE SIZE ANALYSER)	CNST/IST/32/2018	03/01/2018	10,000/-
			TOTAL	**10000**

The amount deposited in A/c Director, ICS 52092260103.

TOTAL: TEN THOUSAND RUPEES ONLY

CONTINGENCY---II YEAR 2018-19

S.NO	ITEM DESCRIPTION	BILL NO	DATE	AMOUNT
1.	SEM,EDX,XRD	04PD/2018	8/10/2018	1500.00
2	RTMESEA-2018	011	20/12/2018	600.00
3.	SEEDS	109	1/2/2019	300.00
4.	SEM,EDX,XRD	02PD/2019	13/3/2019	1700.00
5.	SEM,EDX,XRD,FTIR	04PD/2019	26/3/2019	4800.00
6.	SEM,EDX,XRD	08PD/2019	10/4/2019	1100.00
			TOTAL	**10000**

TOTAL: TEN THOUSAND RUPEES ONLY

XRD, SEM, EDX, FTIR CHARACTERISATION TECHNIQUES DONE AT OSMANIA UNIVERSITY (Dept of Physics)

GRAND TOTAL (I& II YEAR) = 20,000/- (Twenty thousand rupees only)

Signature of the principal investigator **Signature of the principal** **Signature of the statutory auditor**

VIGNANA BHARATHI INSTITUTE OF TECHNOLOGY VBIT Aushapur (V), Ghatkesar (M) MEDCHAL DIST-501

PRINCIPAL
Vignana Bharathi Institute of Technology
Aushapur(V), Ghatkesar(M), Medchal Dist-501 301

CA. L. JANARDHAN RAO
Chartered Accountant

THESIS

Title of Minor Research Project: "An Attempt For Developing Smart Agricultural Methods Using Nanofertilizers.

Principal Investigator: S.Shylaja, Asst. Prof. of Chemistry, Dept. Of Chemistry, Vignana Bharathi Institute of Technology, Aushapur, Hyderabad.

UGC Reference No. F: MRP-6948/16 (SERO/UGC)

UGC MINOR RESERCH PROJECT Proposal No: 1400

Ref NO: UGC MRP :6948/16

FINAL REPORT OF THE MINOR RESEARCH PROJECT IN CHEMISTRY

Title of Minor Research Project: "AN ATTEMPT FOR DEVELOPING SMART AGRICULTURAL METHODS USING NANOFERTILIZERS.

Principal Investigator:

S.SHYLAJA, Asst. Prof. of Chemistry, Dept. Of Chemistry, Vignana Bharathi Institute of Technology, Aushapur, Hyderabad.

UGC Reference No. F:MRP-6948/16 (SERO/UGC)

Annual Report

"Evaluation of urea hydroxyapatite nano carriers as slow release fertilizer composite on the growth and yield of Cyamopsis tetragonoloba"

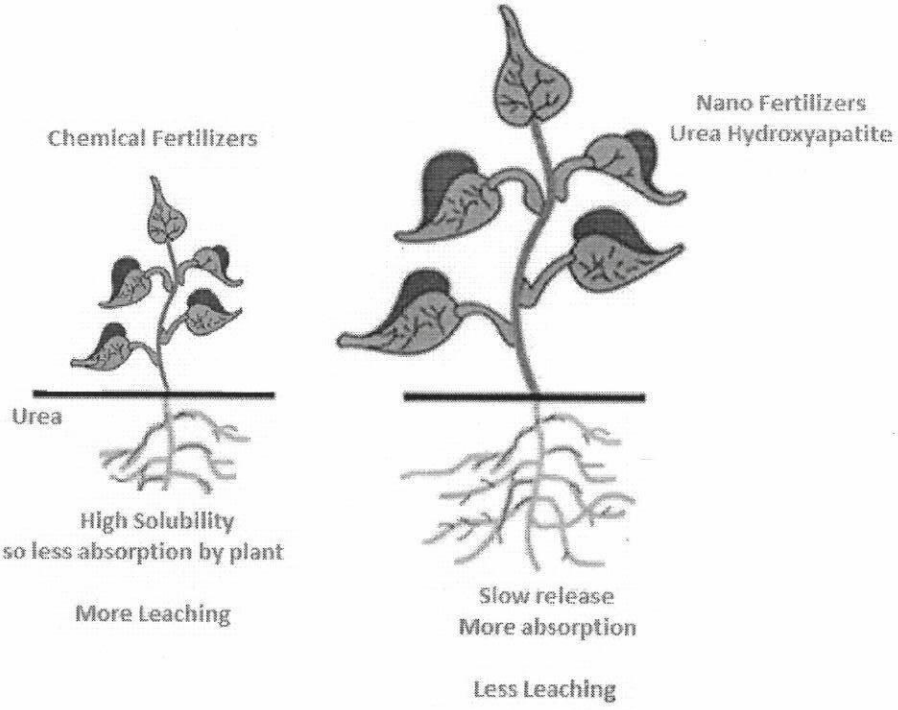
Shylaja Singam[a]*, Anand Rao Mesineni[b], Ch.Shilpa Chakra[c]

[a]*Vignana Bharathi institute of technology (VBIT), Ghatkesar, Medchal Telangana 501301, India.*
[b]*Vignana Bharathi institute of technology (VBIT), Ghatkesar, Medchal Telangana 501301, India.*
[c]*Centre for Nano Science and Technology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500085 Telangana, India.*

Highlights:

- Synthesis of urea hydroxyapatite nanocomposite by simple and economic chemical co-precipitation method.
- Characterization of the nanocomposite by XRD, DLS, EDX, FTIR.
- Study of effect of the nanocomposite on growth and yield of Cyamopsis tetragonoloba (cluster beans).

Graphical Abstract:



ABSTRACT:

Urea and Phosphorous fertilizers are commonly used in agriculture but, due to their solubility in water and transportation, cause eutrophication. Hence, it is thought worthwhile to investigate for urea hydroxyapatite (UHA) nanoparticles which have less mobility and could supply

required N and P macronutrients to the crops. These high surface area nanoparticles are synthesized through chemical co-precipitation method and it is assumed that due to their biocompatibility, act as rich phosphorous and nitrogen source. These are characterized by methods like X-ray Diffraction (XRD), Dynamic Light Scattering (DLS), Scanning Electron Microscope (SEM), Energy Dispersive X ray Analysis (EDX), and Fourier Transform Infra Red (FT-IR). The impact of UHA nanofertilizer on growth and yield of cluster bean plants for the period of four months has been carried out. The experimental results have shown that the usage of these nanofertilizers have enhanced both the plant growth and yield. The application of UHA nanocomposites for the bio-availability of plants considered to be environment friendly.

KEYWORDS: macronutrients, urea hydroxyapatite, nanofertilizers, characterization, bioavailability, Cyamopsis tetragonoloba.

INTRODUCTION:

The rapid growth of human population demands for increasing the agricultural resources through the world. The beginning of Green revolution during 1970's solved the problem of hunger in many developing countries (Mansour Ghorbanpour et.al 2017). According to the world food organization (FAO), usage of chemical fertilizers is the

most important factor in increasing global agricultural production in past 3 decades (Azadeh mikhak et.al 2016). The main reason to use fertilizers is to give full fledged macro and micro nutrients for plants, which usually soil lacks. Around 35 to 40% of crop productivity depends mainly upon fertilizers. The elements N, P, K are considered to be essential macronutrient fertilizers, as N promotes leaf growth, forms proteins and chlorophyll, Phosphorus contributes to root, flower and fruit development, Potassium contributes to stem, root growth and also synthesis of protein (Mansour Ghorbanpour et.al 2017 , Azadeh mikhak et.al 2016). Total Global macronutrient fertilizers ($N+P_2O_5+K_2O$) consumption was 175.7 million tons in 2011 and was projected to increase to 263 million tons till 2050 (Alexandratos et.al 2012).It was estimated that nitrogen(N) fertilizer has contributed roughly 40% increase in percapita food production in the past 50 years indicating the critical role of these macronutrient fertilizers in global food production (Smil.V 2002). Generally N is supplied in the form of Urea (NH_2CONH_2) to fulfill the macronutrient availability to plants. Due to high solubility of Nitrogen fertilizers and their potential vulnerability to leaching and denitrification (Joginder et.al 2017)(especially in the form of nitrates), it is estimated that 40- 70% is lost to environment and cannot be absorbed by plants(Me Trenkel 1997, Attila Ombódi & Masahiko Saigusa (2000)).

Next to nitrogen, Phosphorus accounts for 80 to 90% of world demand (Childers et.al 2011). It is supplied in the form of Mono Ammonium Phosphate MAP ($NH_3H_2PO_4$), Di Ammonium Phosphate DAP ($(NH_3)_2$ HPO_4), Triple Super Phosphate TSP ($Ca(H_2(PO_4)_2$). These Phosphorus fertilizers are highly soluble in water and easily transported through surface runoff causing "eutrophication". It is estimated that 80 - 90% of phosphorus applied, are lost causing environmental pollution. In addition, heavy usage of these fertilizers results in significant loss, where by these fertilizers contaminate the surface and groundwater bodies disturbing aquatic ecosystem and bullying human health (Childers et.al 2011). Therefore, it is of great concern to develop environmental friendly, high productive macronutrient (N+P) Nanofertilizers (Liu &Lal 2015) in place of the conventional urea and phosphorus fertilizers.

Application of Nanotechnology in the field of agriculture and food sectors is in the stage of emergence, when compared to its usage in Medicine and Material Sciences (cs chakra et.al 2017, Wijesinghe.et.al 2017, Raj Kumar et.al 2014).

Administration of macronutrients in the form of Nanofertilizers, Polymer coated fertilizers, Nano coated fertilizers will reduce the rate of dissolution of nutrients and allow slow, sustainable release of fertilizers, and assumed to be more efficiently absorbed by plant root system (Joginder et.al 2017). Phosphate in the form of phosphate rock and

apatite has less solubility but accessibility of phosphorus from reaching the root zone and nurturing of the crop is hindered due to its larger particle size (Fageria, N.K. (2009)). To minimize this problem, a new class of phosphorus fertilizer nano-hydroxyapatite was synthesized and reported earlier to enhance the growth and yield of soya beans through greenhouse study (Liu &Lal 2014). This nano-hydroxyapatite (nha) has the property of low solubility and long-term availability to plant system, which is due to increased surface to volume ratio of nanoparticles. Thus nha acts as a good source of phosphorus in enhancing the agronomic yield and also reduces the risk of eutrophication.

Few attempts were made earlier to reduce the leaching of nitrogen into soil and to enhance the nutrient utilization efficiency by synthesis of Urea Hydroxyapatite (UHA) nanohybrid. It was reported that the synthesis of nanohybrid of Urea hydroxyapatite at weight percentage ratio of 1:1 that was incorporated into wooden chips and confirmed to achieve slow release of urea (Kottegoda et.al 2011). Though nanohybrid of UHA synthesized by flash drying process, which is relatively cumbersome (Kottegoda et.al 2017).

In the present study, we made sincere attempt to synthesize the nanohybrids of Urea hydroxyapatite through a simple, versatile and economical route of "Wet Chemical Precipitation Method". Chemical precipitation of nano-sized fertilizers from salt solution is a simplest technique for rapid synthesis of large amount of material in a controlled

manner (Yoruç, A.B.H.; Koca 2009). This synthesis needs qualified and controlled parameters such as p^H, composition of starting material, rate of addition, stirring speed, stirring technique and aging to obtain Nanohybrids of Urea Hydroxyapatite. UHA nanohybrids are generally employed in agriculture because of high nutrient utilization efficiency, slow release of nitrogen and source of phosphorous macronutrients. Apart from that, it is also useful to minimize adverse effects on environment.

Synthesized UHA nanocomposites are characterized by XRD, SEM, EDX, Particle Size Analyzer (DLS), FT-IR to confirm size of the crystallite, morphology, elemental composition and the functional groups of the sample. Therefore, the confirmed nanohybrid of UHA are used to study the germination, growth and yield of cluster beans plants and to investigate the effect of bioavailability of Nitrogen and Phosphorus over conventional urea and DAP.

2. MATERIALS AND METHODS:

2.1 Synthesis of Nanohybrid of Urea Hydroxyapatite: All the glassware that are used for the synthesis of nanoparticles are washed with deionized water and dried in hot air oven before their use. Nanohybrid of UHA sample was prepared by chemical co-precipitation method Ferraz 2004 & Kottegoda et.al 2017. A definite amount of

Phosphoric acid H_3PO_4 (A.R Grade 99%, Finar) was dissolved in deionised water to form 0.6M solution. A definite amount of Calcium Hydroxide ($Ca(OH)_2$) (A.R Grade 96%, Finar) was also dissolved in deionised water to form 1M solution. To $Ca(OH)_2$ solution 150gm of Urea(A.R Grade ,Finar) was added. Then the solution was kept for constant stirring (it was observed that the reaction was exothermic). Now 0.6M H_3PO_4 solution was added drop wise to $Ca(OH)_2$ solution with constant stirring. After completing the addition of H_3PO_4 solution, the mixture was kept for stirring for 6 h at ambient temperature, a white curdy precipitate was obtained. Then, it was kept for aging for 24h. Thus, obtained product was filtrated using vacuum suction filtration and washed several times to remove impurities. The product was dried in hot air oven at 90^0C for 3h. Finally obtained product was grinded properly using agate motor pestle and stored for further studies.

2.2Powder Characterization: Average crystallite size was examined by XRD. SEM and EDX which were used to find morphology and the elemental composition of compound. To confirm the formation of UHA, FT-IR analysis was done.

2.3 Soil Sampling: To study the germination and growth of cluster beans, soil sample (top soil of 8 inches depth) was collected from VBIT

campus and analyzed. The physico - chemical characteristics (G P Gunaratne et.al 2016) of soil, are obtained from government soil testing lab, Bhongir, Telangana, India which are shown as in table 1

Table: 1 Composition of macronutrients in the test soil

Soil type	p^H	N (Kg/Acre)	P (Kg/Acre)	K (Kg/Acre)
Red loamy soil	6.98	190.66	62	50.84

2.4 Seeds: Seeds of cluster beans were purchased from Indosun Agri Genetics, Hyderabad. The seeds were treated with 5% Sodium Hypochlorite (NaOCl) solution for 10 minutes (Pramod et.al 2011). Then, washed with deionized water for 3 - 4 times to ensure surface sterility before sowing them in the soil.

2.5 Preparation of Fertilizing Solution: All the fertilizing solutions (UHA, urea, DAP) were prepared using deionised water with concentration of 20 ppm and ultrasonicated (ultrasonic cleaner model: BTI-48, 50 watts) for 30 minutes for uniform dispersion.

2.6 Pot Studies: Germination and growth of cluster beans were conducted in four different batches, where each batch has three replicates (4×3=12 pots). Pots used for the study are of 12cm height (medium size). Each batch was supplied with 4 different solutions.

Batch	Pot Labelling	Type of fertilizing solution applied
1	UHA	20ppm of nano UHA solution
2	Conv.P	20 ppm of DAP solution
3	Conv.Urea	20 ppm of urea solution
4	Control	Only deionised water

All the fertilizing solutions were used to assess the nitrogen and phosphorous uptake and also yield of cluster bean pods. Now 6 equal sized seeds of cluster beans per pot (6×12=72 seeds) were selected and soaked overnight separately in above prepared fertilizing solution. The soaked seeds are then sown into respective pots labeled as UHA, Conv.P, Conv Urea and control at a depth of 1.5cm and covered with thin layer of soil in the first week of February 2019. Fertilizing solution

was supplied for every 5 days to each batch. Besides, deionised water was supplied when needed, to ensure that only the nutrients present in soil and such supplied nutrients are available for the plant growth and yield.

2.7 Plant Growth: The shoot length of the plants was measured for every 5 days. After 50 days, i.e. last week of March 2019 three plants per pot were removed to measure the root length. Thus removed plants were washed with deionized water for 3 - 4 times to remove soil and air dried to find fresh plant weight. After weighing the plants, they were dried at 70^0C for 10h to calculate dry weight.

2.7 Plant Yield: The left over plants in each pot were allowed to grow further. The tiny cluster beans appeared in the second week of April 2019 and were harvested after a week. The fresh and dry weight of pods was calculated. The dry weight was obtained after drying at 80^0C for 2 days in hot air oven.

3 RESULTS AND DISCUSSION:

3.1 XRD ANALYSIS:

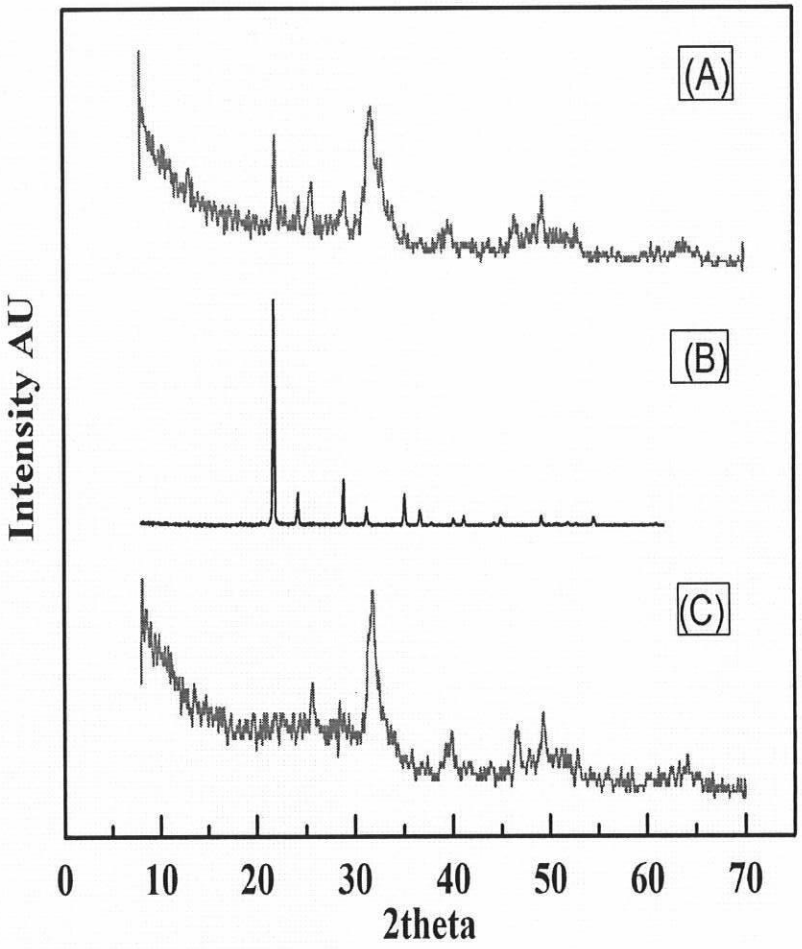


Fig: 1 XRD pattern of A) Urea hydroxy apatite nanoparticles B) Urea C) Hydroxyapatite

The sample was characterized by using xperto pro PHILIPS Powder X-ray Diffractometer with CuK_{α} radiation = 1.5418Å and 2θ ranging from 10 – 70 degrees at 40 kV, 30 mA. The PXRD (fig 1) peaks of UHA

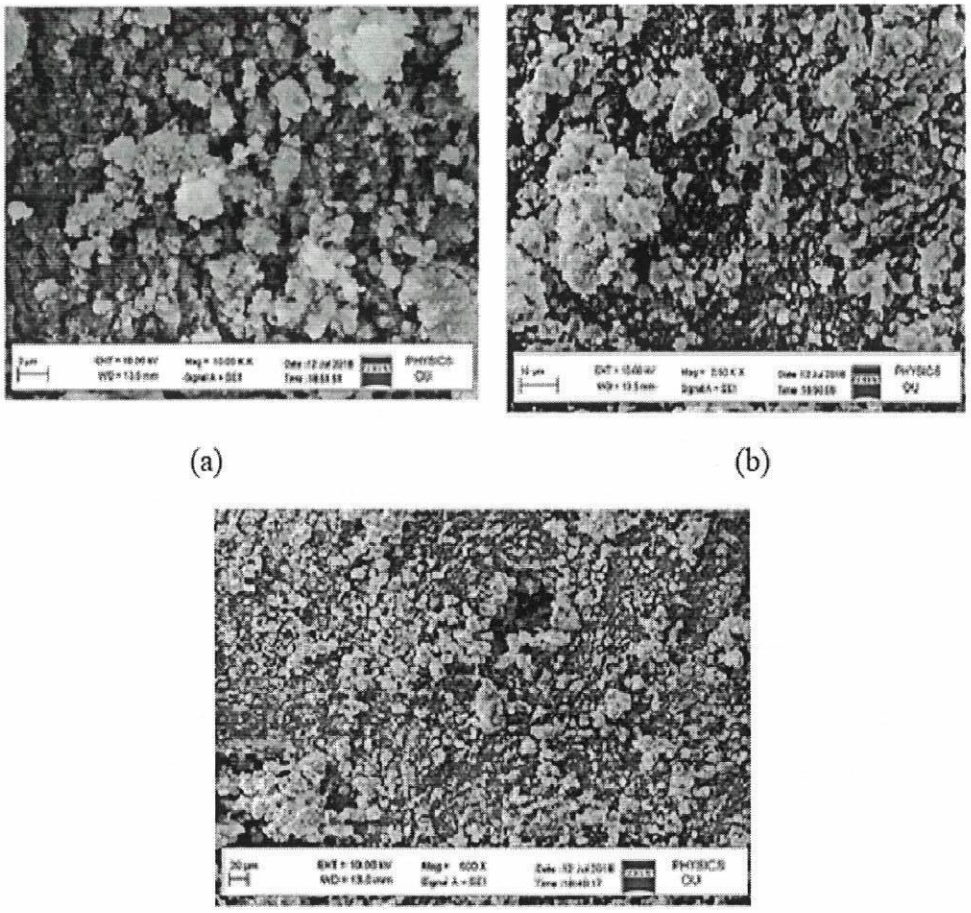
sample at 2θ values 21.95, 25.61, 29.03, 31.64, 39.79, 46.54, 49.37, 52.95 which have shown good concurrence with PXRD pattern of urea (JCPDS-89-2835) and hydroxyapatite (JCPDS-09-0432) (Kottegoda et.al 2011).

Presence of XRD peak at 2θ value of 31.64 degrees with plane of 211 corresponds to hydroxyapatite. In addition, another major peak at 21.95 degrees with plane 110 indicates that the presence of urea. Reduction in the intensity of 21.95 peak in UHA shows that such formed compound is amorphous due to the interaction between urea and hydroxyapatite.

The average crystallite size was determined by Debye-Scherrer equation $D=K\lambda/(\beta\cos\theta)$ which was found to be 20 nm.

3.2 SEM AND EDX ANALYSIS:

The SEM micrograph of nanohybrids of urea hydroxyapatite recorded from Zeiss EVO 18. The nanoparticles have shown strong agglomeration due to presence of hydroxyl group (fig : 2). The particle size was found to be 1micro meter and observed to be dispersed randomly.



(a) (b)

(c)

Fig: 2 SEM images of the urea hydroxyapatite

The EDAX spectrum of nanohybrids of UHA shown in fig 3 also confirmed the co-existence of urea and hydroxyapatite. The spectrum (Tab 2) showed good intensity peak of N, C, O, Ca and P.

Table: 2 Elemental Composition of Urea hydroxyapatite.

compound	**N%**	**C%**	**O%**	**Ca%**	**P%**
Nano UHA	38.51	21.31	24.63	4.1	11.44

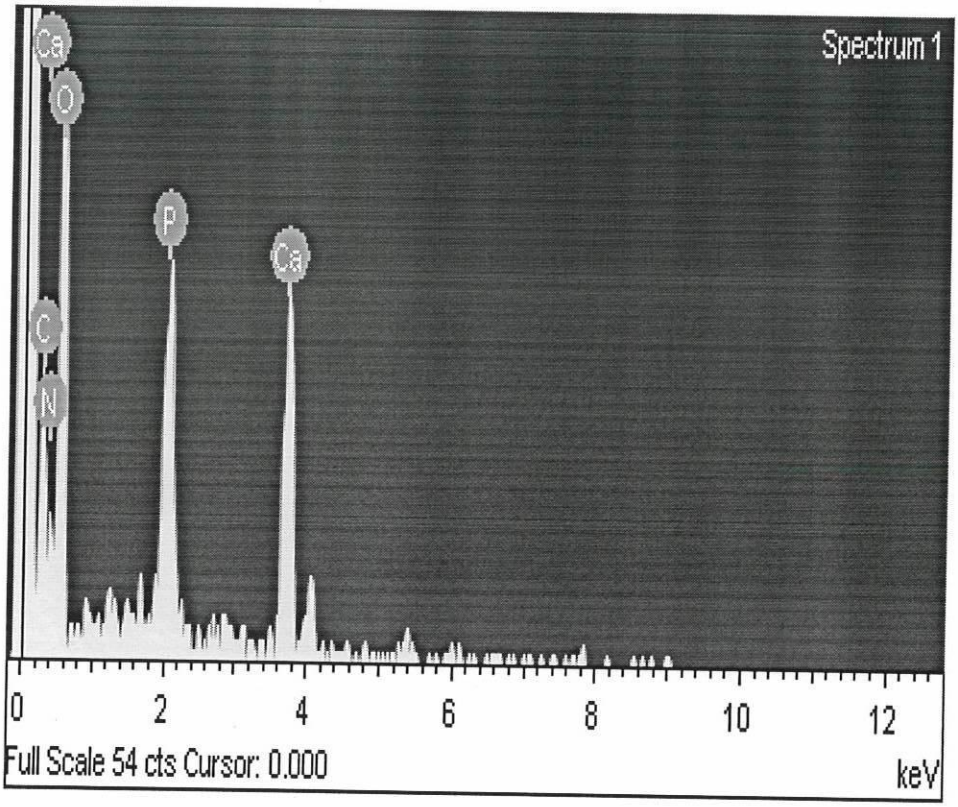


Fig: 3 EDX images of the urea hydroxyapatite

3.3 PARTICLE SIZE ANALYSER (DLS):

Particle size of nanofertilizer UHA was also analysed with the help of Horiba SZ 100 particle size analyzer –Dynamic Light Scattering (DLS) fig 4. The mean particle size of UHA which was experimentally obtained to be 41.2 nm and interpreted to show the most probable polydispersity.

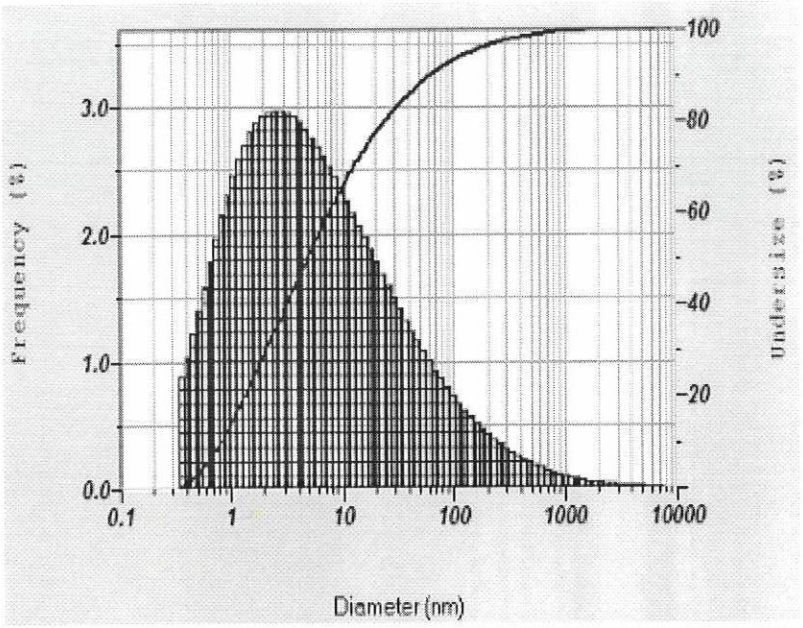
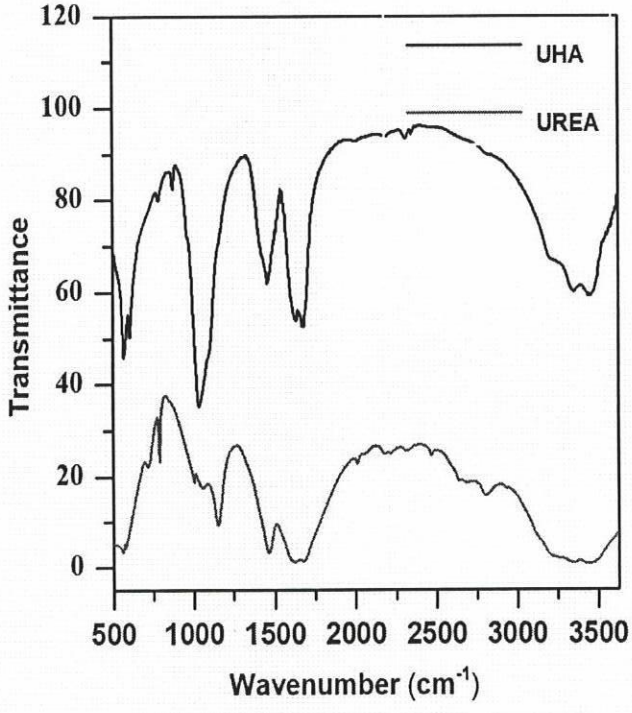
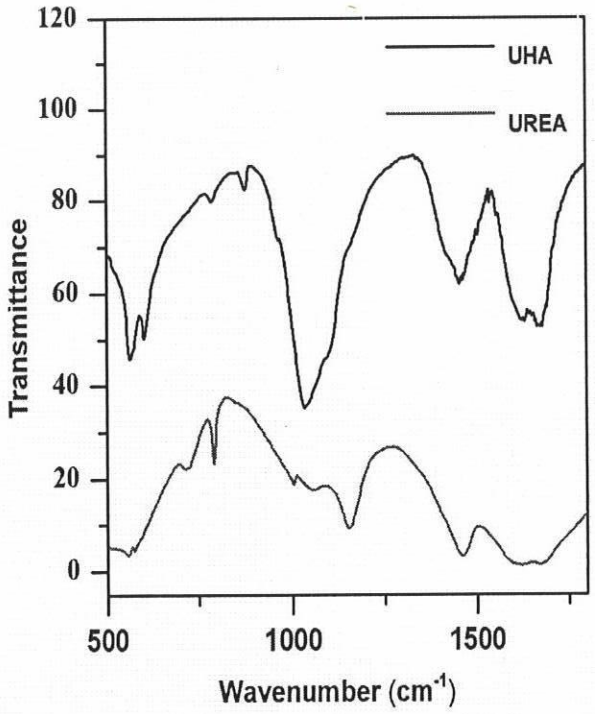


Fig: 4 DLS image of the urea hydroxyapatite

3.4 FTIR CHARACTERISATION:



5(a)



5(b)

Fig: 5a and 5b FT IR spectrum of the both urea hydroxyapatite and urea at the wave number range from 500 to 3500 cm^{-1} and 500 to 1750 cm^{-1}.

The FT-IR spectrum of UHA was recorded using JASCO IR-5300 spectrometer in the form of KBr pellets coveringthe wave number range 400-4000cm^{-1}. The synthesized nano UHA was analysed and confirmed by FT-IR.spectrum fig 5a & 5b. The bands obtained at 567 cm^{-1} and 602.5 cm^{-1} are corresponding to the anti-symmetric bending of phosphate moiety in urea hydroxyapatite Yoruç, A.B.H & Koca, Y 2009. Similarly the bands situated at 1038.15 cm^{-1} and 960 cm^{-1} are

correlated to anti symmetric and symmetric mode of stretching vibrations of P-O of PO_4^{-3} (A. Costescu et.al 2010). A broad band centered at 3368.3 cm^{-1} is attributed to OH group indicating the presence of water in the lattice of UHA (Ma Ming-Guo 2012). In addition, the CO and CO-NH wagging of pure urea is appeared at 793.12 cm^{-1}, which was shifted to 785.41 cm^{-1} in UHA. A band at 1003 cm^{-1} representing the N-C-N symmetric stretching vibration in urea was shifted to 960.55 cm^{-1} in UHA. A noticeable peak at 1466 cm^{-1} indicating the asymmetric stretching vibrations of N-C-N in urea which was shifted to lower wave number of 1454 cm^{-1} in UHA. A band at 1151 cm^{-1} resembling to the rocking of NH_2 in urea was shifted to 1107 cm^{-1} in urea hydroxyapatite. The C=O stretching frequency of pure urea at 1672 $cm^{-1}$was changed to 1621 cm^{-1} in UHA confirmed presence of the carbonyl group Z. Piasek And T. Urbanski 1962. Thus FT-IR data reveals that urea was adsorbed randomly with hydroxyapatite through various binding sites.

3.5 PLANT GROWTH ANALYSIS:

The synthesized nanofertiliser was used as a good source of nitrogen and phosphorous elements for the growth of cluster beans in present pot studies. The batch of nano UHA has shown good results in comparison with Conv Urea, Conv P and control. Average Shoot length of all batches was calculated for every 5 days. On 50^{th} day after germination,

(Fig 6) UHA treated plants have shown maximum shoot length of 15.5cm when compared to plants treated with urea (11.4cm) and DAP (14.1cm). Though DAP treated plants have shown comparatively less growth than urea treated plants in first 25 days later it shooted up. The deionised water treated plants (control) have also shown considerable growth due to available nutrients in soil.

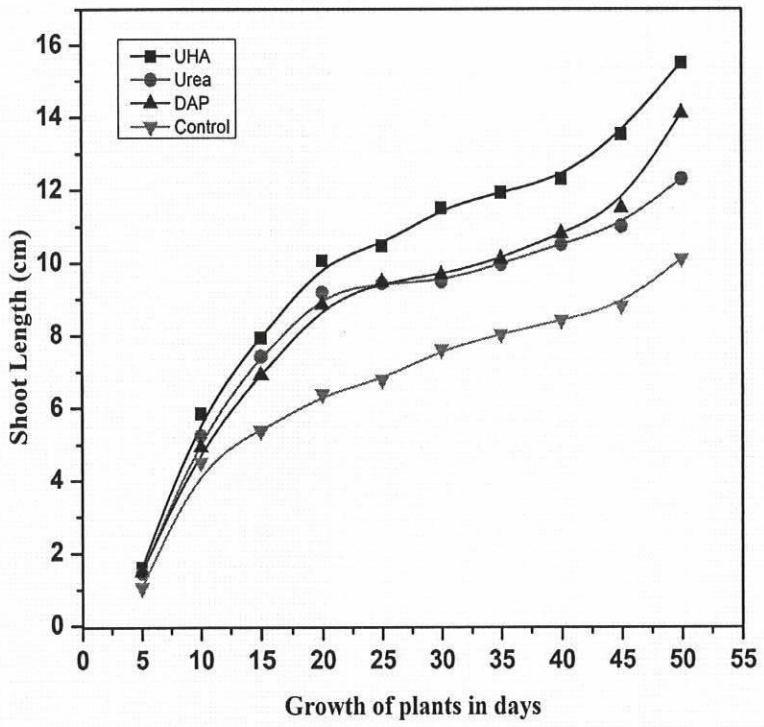


Fig: 6 Plot of Plant Growth Analysis for 50 days

After 50 days of time period some plants from each batch were removed to calculate root length, plant fresh and dry mass. The average root length (fig:7)was found to be 8.2 cm, 6.2 cm, 6.4 cm and 5.1 cm for

UHA, Conv urea, Conv P and control respectively. This indicates relatively good growth in tap root system of UHA treated plants when compared to others.

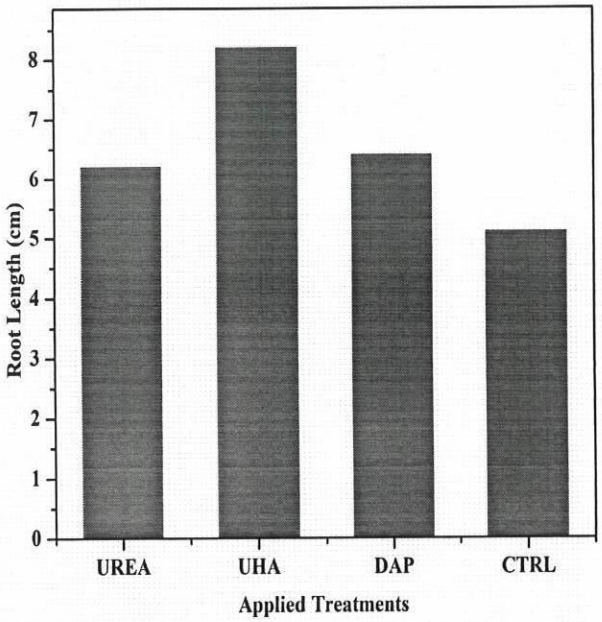
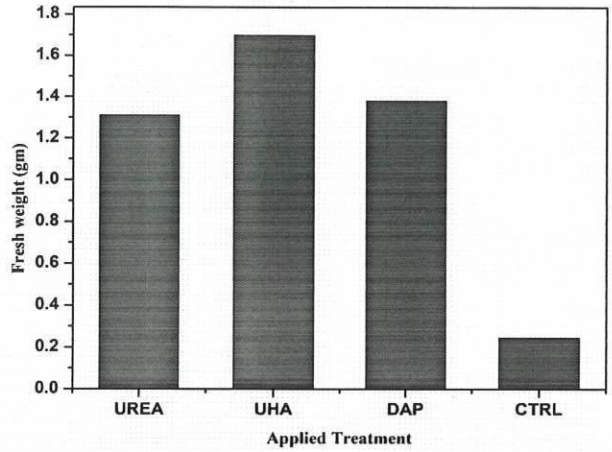
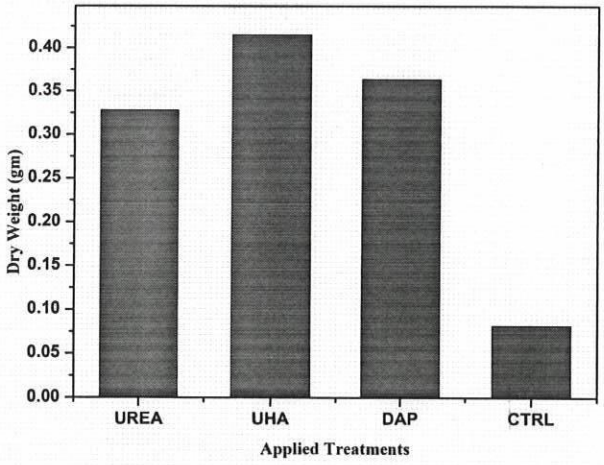


Fig: 7 Plot of Root Length under different fertilising solutions and control

The plant fresh weight(fig:8(a)) was found to be 1.71grams,1.3 grams,1.35 grams and 0.26 grams for UHA, Conv urea, Conv P and control. The dry weight of plants (8(b)) was also found to be greater for UHA treated plants than others.



(8a)



(8b)

Fig: 8(a) & 8(b) Plot of plant fresh weight and dry weight under varied treatments

The first harvest of cluster beans of all batches was recorded and shown as in table 3.

Table: 3 representing the plant yield

Batch	Pods fresh weight (gm)	Pods dry weight (gm)
UHA	82	48
Conv P	64	39
Conv Urea	61	37
Control	50	26

By all these observations and from the figures9(a) to 9(f) it was clear that the UHA treated plants have shown good nutrient utilization with respect to good growth of tap root system, shoot length, fresh weight, dry weight and also yield. Thus it is found that nanofertilizer UHA can act as a good chemical fertiliser. Though cluster bean plant belongs to leguminaceace family that can fix nitrogen symbiotically, the results suggests the clear essentiality of N and P for their growth and yield.

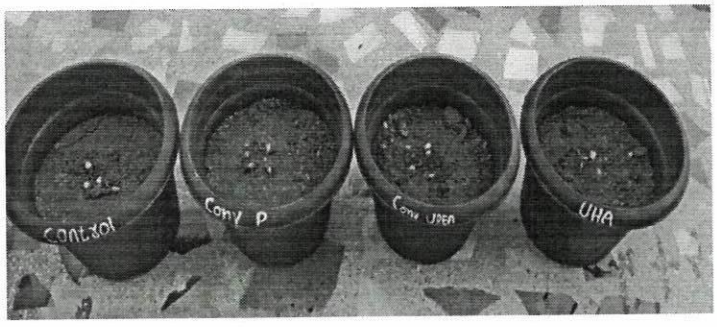


Fig 9(a) seeds started to germinate

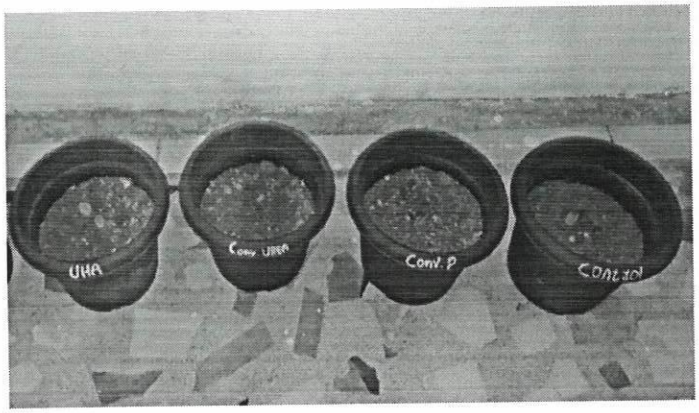


Fig 9(b) seedlings after 10 days

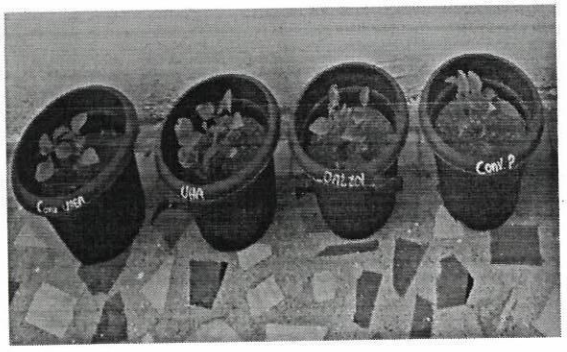


Fig 9(c) seedlings after 1 month

Fig 9(d) seedlings after one and half month

Fig 9(e) seedlings after 2 months

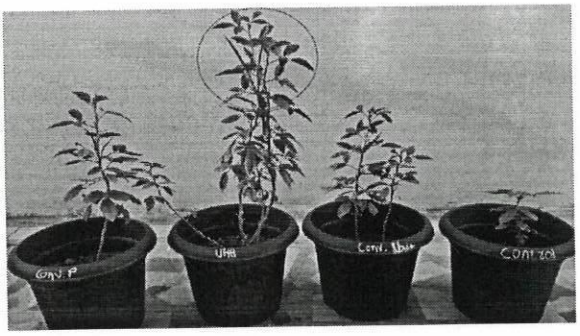


Fig 9(f) seedlings after two months 10 days

3.6 Microscopic Analysis:

The transverse section of UHA treated plant root (fig: 10) when observed under compound microscope, it is clearly shown the layers of root cortex, endoderm, primary xylem and primary phloem (vascular bundles). The experimental facts further reveal the biocompatibility of nanofertiliser UHA on cluster bean plants

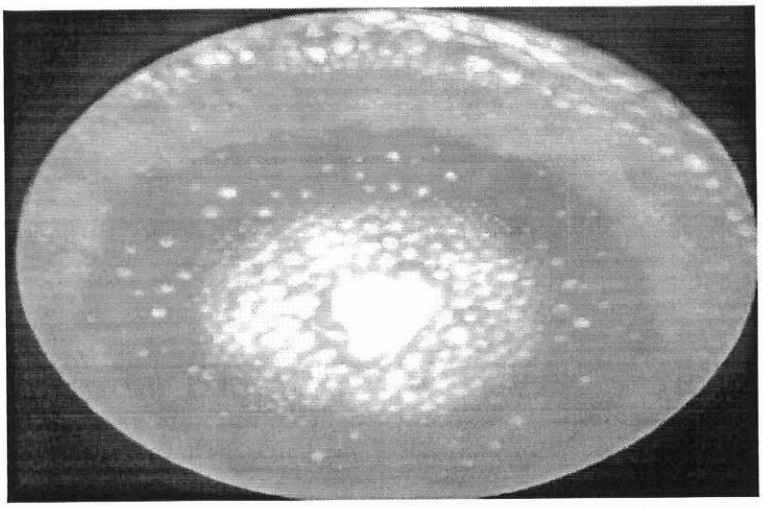


Fig: 10 transverse section of UHA root under compound microscope

CONCLUSION:

We report a simple and novel chemical co-precipitation method for the successful synthesis of nano-urea hydroxyapatite, slow release fertilizer composite. The XRD study confirmed the crystallite size as 20 nm. DLS also confirmed the particle size of UHA. The SEM images showed that the particles are aggregated to form agglomerates. The EDX confirmed the existence of C, N, O, Ca, P stating the successful formation of UHA. The pot studies of cluster bean plants over a period of four months

showed that the application of urea hydroxyapatite, as a good nutrient source of nitrogen and phosphorous which has enhanced the growth rate (shoot and root length), plant biomass (fresh and dry weight) and also plant yield over that of conventional urea and Di Ammonium Phosphate. Microscopic studies of root of UHA confirmed that there is no alteration in internal cell structure of the root, which further confirm no negative impact on plant internal structure.

Acknowledgment

The authors acknowledge UGC – MINOR RESEARCH PROJECT-UGC MRP/SERO ID: 6948/16 for Financial Assistance. Project work has received constant encouragement and the authors are grateful to the management of VBIT, autonomous college.

Declaration of competing interest:

The authors report no conflicts of interest in this work.

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To,
S Sailaja
Assistant Professor of Chemistry,
Vignana Bharathi institute of technology (VBIT),
Ghatkesar, Medchal Telangana 501301, India.

Dear Sir/ Madam

The Organising Committee is happy to inform you that the paper entitled "Evaluating the effects of formulated nano-Urea hydroxyapatite slow release fertilizer composite on the growth and yield of Cyamopsis tetragonoloba (cluster beans)" by Shylaja Singama, Anand Rao Mesineni, Ch.Shilpa Chakra is accepted for **poster** presentation in the MSSA-2020. You are requested to make your travel plans accordingly. Also please inform your co-authors regarding the same.

If you require accommodation in Hyderabad, please inform us in advance, so that arrangements can be made.

No TA and DA will be provided by the conference organizers.

The exact time and date of presentation will be informed in due course.

Looking forward to see you in MSSA-2020.

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To
S.SHYLAJA
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Dear Sir/Madam,

We are pleased to inform you that the Organizing Committee has accepted your research papers entitled "**Effect of Synthetic Urea hydroxyapatite nanoparticles a slowrelease fertilizer for Plants**" For oral presentation. Time allotted for oral presentation is 10 minutes and the authors are requested to submit the softcopy of their presentation before the session commences.

The template for the full paper is attached herewith. Full paper can be e-mailed latest by December 15th, 2018, or the same can be submitted at the registration desk on the first day of the seminar for the inclusion in the proceedings with ISSN/ISBN number. Please send the registration form as early as possible.
Thanks & Regards

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International Journal of Innovative Technology and Exploring Engineering (IJITEE)
ISSN: 2278-3075, Volume-8 Issue-10, August 2019

Root and Shoot Uptake of Synthesized Nano Zno and Its Impact on Differences in Bio-Availability During Exposure In Aqueous Suspension

Shylaja Singam, M. Anand Rao, Ch. Shilpa Chakra

Abstract: ***The proposed study highlights on the synthesis of Zinc oxide nanoparticles using chemical and green methods. In the field of nanotechnology Green synthesis is an eco-friendly development. The synthesis of ZnO is carried out using leaf extract of Azadirachta indica (neem) as a reducing agent. The synthesised products were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Analysis (EDAX). The present work is to investigate the effect of chemical and green synthesized zinc oxide nanoparticles on germination and growth of Lycopersicon esculentum (tomato) using petriplate seed germination method. The impact of concentration of applied ZnO nanoparticles via green synthesis and chemical methods were analyzed. It was observed that the growth of Seedling is maximum for green synthesized zinc oxide nanoparticles at appropriate concentration over chemically synthesized zinc oxide nanoparticles, bulk ZnO and control. Hence the green method is found to be more effective.***

Keywords **:** ***leaf extract of Azadirachta indica; XRD; SEM;Lycopersicon esculentum ;ecofriendly; germination.***

I. INTRODUCTION

In the field of science and technology, Nanotechnology is an innovative field of science, which can develop new revolutions [1]. Nanoparticles are submicroscopic particles with one dimension measured in the range of 1 -100 nm. They possess distinctive physical and chemical properties such as high electrochemical coupling coefficient, high chemical stability, high photostability and wide range absorption, because of the larger surface to volume ratio in comparison to bulk materials [2, 3]. Metal Oxide Nanoparticles are frequently used in the field of Medicine, Electronics, Agriculture, Fuel cells, Solar cells, Batteries, Water purification, Chemical Sensors, Cosmetics etc [3, 4; 5, 6]. ZnO (Zinc Oxide) is a metal oxide which appears as a white powder and is almost insoluble in water [1]. Out of the 17 Essential elements, zinc is one of the necessary element for the normal growth and development, which is also one among the eight micronutrients necessary for the plants [3]. Zinc plays an important role in enzymes and protein synthesis, carbohydrate metabolism, Pollen formation, protection against photo-oxidative damage, gene expression and resistance against infections caused by definite pathogens [7, 8].

Zinc is at present considered as a fourth most important yield limiting element after nitrogen, phosphorus and potassium in India. Deficiency of zinc in plants can retard the rate of photosynthesis, reduces flowering and fruit development, delayed maturity, nitrogen metabolism, decreased yield and also results in sub-optimal nutrient use efficiency [9]. The application of zinc oxide nanoparticles to plants is preferred to overcome the effects of deficiency of zinc. Decreased particle size of zinc oxide will result in increased surface area, which in turn, enhances the dissolution rate of fertilizer in water [10].

Nanoparticles of zinc oxide can be synthesized by different methods like Chemical co-precipitation, Sol-gel, Hydrothermal, Chemical Vapor Deposition, Biological and Green methods [11,12,13].Green and Biological methods of synthesis of nanoparticles could be eco friendly than conventional chemical methods[1,3,14].

This paper deals with the preparation of Zinc Oxide nanoparticles through Chemical co-Precipitation and Green methods. Zinc Nitrate Hexahydrate is used as a precursor and Sodium Hydroxide as precipitating agent for chemical synthesis of ZnO nanoparticles. The leaves of Azadirachta indica, belonging to family Meliaceae also called as neem (Indian lilac) are used for the green synthesis of ZnO nanoparticles. Different parts of neem tree are used in various fields of agriculture, medicine, cosmetics etc. Leaf extract of neem is used as a reducing agent and also as surface stabilizing agent for the synthesis of nano zinc oxide [12]. The crystallite size and structure are confirmed by XRD. Morphology and elemental composition were detected by SEM and EDAX respectively. Thus confirmed ZnO nanoparticles are used to examine germination and growth of tomato seedlings. ZnO nanoparticle suspensions at varied concentrations (50,100,150,200,300,500ppm) were used as micronutrient source for the germination and growth of lycopersicon esculentum through petriplate seed germination method [15].

II. MATERIALS AND METHODS

Zinc Nitrate Hexahydrate (AR grade) and Sodium Hydroxide (AR grade) pellets are used as precursory materials, which are

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M.Anand Rao, Professor, Department of chemistry, Vignana Bharathi Institute of Technology, Hyderabad-501301, Telangana , India..

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supplied by Finar chemicals. All the glassware used during the preparation are washed with deionised water and dried in the oven before use.

2.1 Chemical Co-Precipitation Method

Zinc oxide nanoparticles, in this method, are synthesized by using $Zn(NO_3)_2.6H_2O$ (zinc nitrate hexahydrate) and NaOH as initiator. 17.9 grams of $Zn(NO_3)_2.6H_2O$ was taken in a beaker and dissolved in 50 milli litres of deionised water. Then it was stirred constantly on a magnetic stirrer for 30 minutes at room temperature. Similarly, 4.28 grams of NaOH was added to 50 ml of deionised water in a separate beaker. The solution was stirred continuously for 30 minutes. NaOH solution acts as precipitating agent and also to maintain p^H of the solution. This NaOH solution was added drop wise to above solution at 70^0C with vigorous stirring till the p^H reaches to 12 and the stirring was continued for 3 h at 70^0C to complete the formation of nanoparticles. Finally the solution turns to white curdy precipitate. This precipitate is filtrated by suction pump using whatman filter paper no 42 and washed 4 to 5times with distilled water for removal of impurities. Thus obtained product is oven dried at 150^0C for 3h .Dried product is calcinated at 500^0C for 3 h in muffle furnace. Obtained product after calcination is grinded and preserved for further studies.

2.2 Green Method

Green synthesis of ZnO nanoparticles, which is generally an extract of plants is, undoubtedly, an environmental friendly method

***2.2.1 Preparation of extract from neem leaves*:** Some fresh leaves of Azadirachta indica were collected for the study from the premises of VBIT. The leaves were cleaned for several times with deionised water to remove impurities and dust particles and then air dried. Later 40 grams of the leaves were added to 250 ml deionized water taken in a 500ml beaker. After that, the mixture was boiled at 80^0C for one hour until the colour of the solution got changed to light yellow. Thus, the prepared extract from leaves was cooled down to the room temperature. The extract was thoroughly filtered and refrigerated for further use.

***2.2.2 Zinc Oxide Nanoparticles preparation*:** For synthesizing ZnO nanoparticles, 50 ml of the extract was taken and boiled for 15 minutes at 80^0C.Later,five grams of zinc nitrate hexahydrate was added to the solution. Then the mixture was continued to boil until it became light yellow color paste. Thus obtained product was transferred to ceramic crucible. The paste was heated at 800^0C for two hours. After heating at the temperature mentioned, the paste turned into white colored powder .the powder was stored for further characterizations.

2.3 Seeds:

Seeds of plant species lycopersicon esculentum (tomato) were purchased from a local nursery and were treated with 5% Sodium Hypochlorite solution for 5 to 10 minutes then washed with deionised water for 4 times to ensure surface is free from impurities before using for germination in petriplate. [16].

2.4 Petriplate Seed Germination Method:

Petri plates of 60 mm × 15 mm used for germination are washed with deionised water and dried in oven before use. Seed germination sheet is placed in petriplate as single layer. To the each petriplate 10ml of ZnO nanoparticle suspension is added. Particle suspension of chemical, green synthesised ZnO nanoparticles and bulk ZnO (used as a reference Zn source) were prepared in concentrations ranging from 50, 100, 150, 200, 300, 500 ppm by ultrasonicating(LMUC-2, 50W) for 20 mins for uniform dispersion (Fig 1). The deionised water was considered as control. 15 equally sized tomato seeds were taken and placed in each petriplate (Fig 2). These petriplates are kept in incubator at 27^0c and allowed to germinate and grow for 15 days. The germination rate and growth response of the seedlings to chemical nanoZnO, green nanoZnO, bulk ZnO and control were observed.

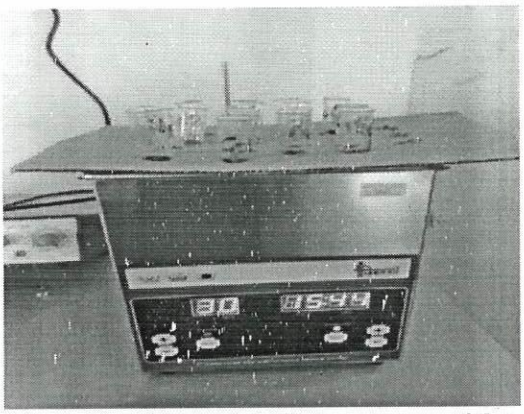


Fig 1: Ultra Sonication Of Zno Nanoparticles

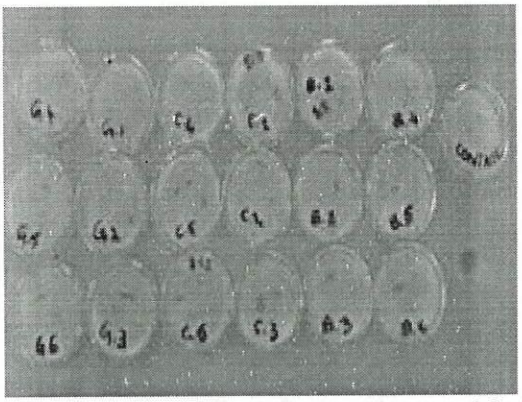


Fig 2: Introduction Of Seeds To Zno Nanoparticle And Bulk

ZnO suspension to sterile petriplate on day 1. Labeling representing G_1 to G_6–treated green ZnO nanoparticles; C_1 to C_6 – treated chemical ZnO nanoparticles; $B_1$to B_6- treated bulk ZnO; control.

III RESULT AND DISCUSSION

3.1 XRD Studies of Nano ZnO particles:

The average crystallite size of chemical and green method nano ZnO samples were characterized by powder XRD (instrument xperto pro PHILIPS) with CuK_α radiation = 1.5418Å with 2θ ranging from 10 – 90 degrees at 40 kV, 30 mA .The XRD pattern of chemical ZnO nanoparticle (figure 3) shows distinct peaks at 2θ values 31.58, 34.25, 36.08, 47.38, 56.45, 62.73, 66.25,

67.83, 68.97, 72.50, 76.86 having good correlation with JCPDS: 89-1397.

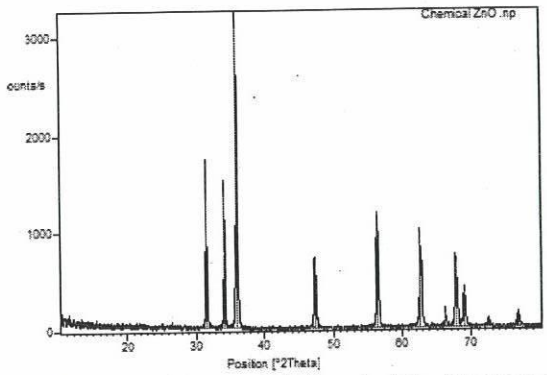


Fig:3 XRD Pattern Of Chemical Synthesized Zno Nanoparticles

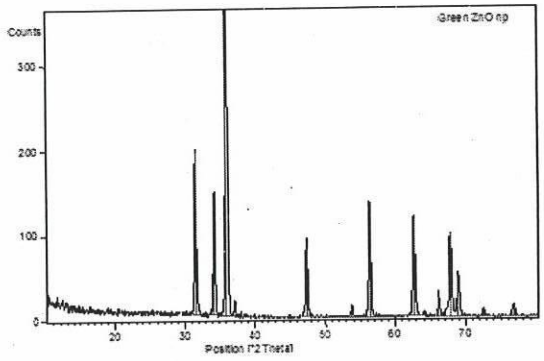


Fig:4 XRD Peaks Of Green Synthesized Zno Nanoparticles

Green ZnO nanoparticles(Fig 4) shows the XRD pattern distinctively at 2θ values of 31.52, 34.21, 36.02, 47.33, 56.37, 62.69, 66.16, 67.76, 68.90, 72.55, 76.79 with planes (100), (002), (101), (102), (110), (200), (112), (201), (004), (202).The position of the peaks was compared with the existing values and the existence of zinc oxide nanoparticles was confirmed. The outcome states that zinc oxide nanoparticles are of hexagonal type structure [17]. By using Debye Scherrer equation $D=K\ \lambda/(\beta\cos\theta)$, the average crystallite size was determined. Thus calculated crystallite size for chemical and green synthesized zinc oxide was 35 nm and 28nm. Interestingly, it was found that the size of zinc oxide nanoparticles are reduced via green synthesis.

3.2 SEM Studies of Nano ZnO particles:

SEM (recorded from Zeiss EVO 18) gives the surface image of the sample. In fig 5a, 5b) of chemical ZnO nanoparticles reveals that the particles are spherical shaped and are present as agglomerates in the range of 1μm.

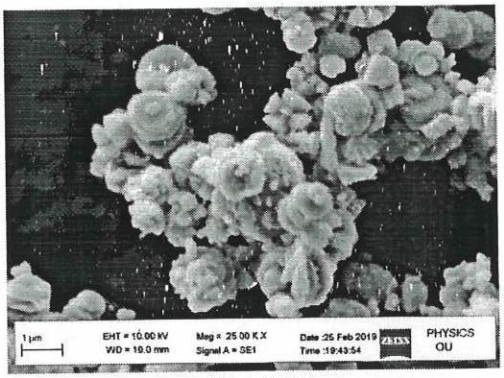


Fig: (5a)

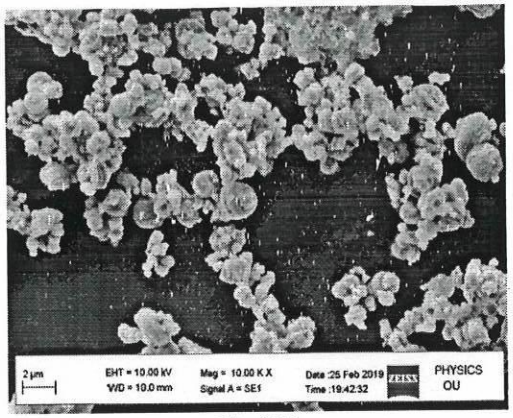


Fig (5b)

Fig: 5a & 5b Showing Sem Images Of Chemical Zno Nanoparticles At Varied Magnification

From figure 6a & 6b of green ZnO nanoparticles reveals that the particles are spherical shaped and are present as agglomerate structures as observed in fig: 6b with average size in the of 2μm.

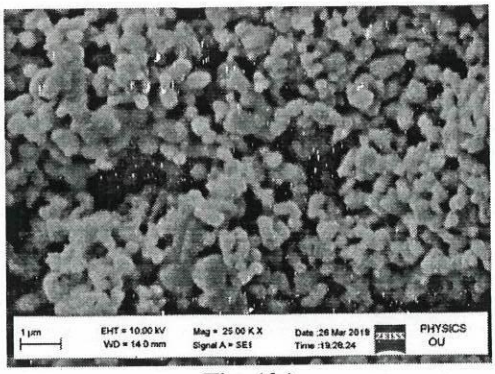


Fig: (6a)

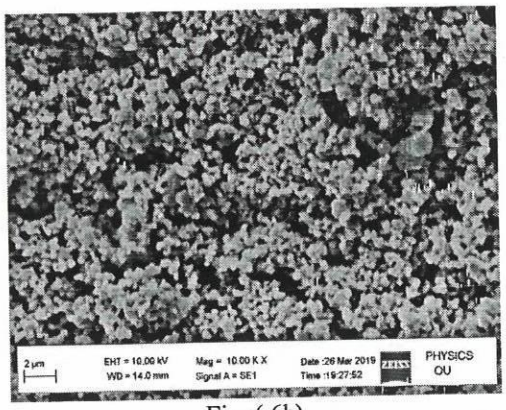


Fig :(6b)

Fig: 6a & 6b SEM Images Of Green Zno Nanoparticles At Varied Magnification

3.3 EDAX Analysis:

The elemental composition of chemical ZnO and Green ZnO Nanoparticles was done by EDAX. The composition of Zinc and Oxygen was found to be 54.76% and 45.24% for chemical ZnO Nanoparticles as indicated in fig :(7a). From fig:7b Green synthesised ZnO Nanoparticles reveals the elemental composition of zinc and oxygen as 72.82 % and 27.18 %.This states that nanoparticles are pure and extent of impurities are negligible. The synthesis of ZnO nanoparticles from neem leaf extract would be ecofriendly and effective for large scale production.

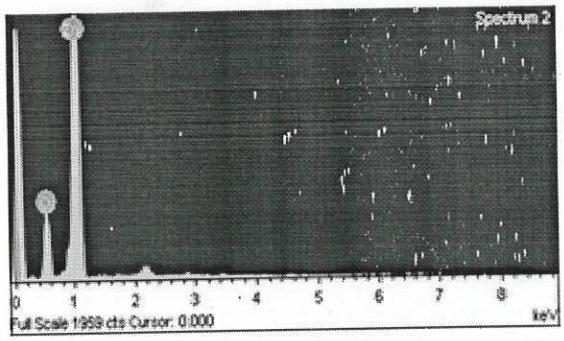


Fig :(7a)

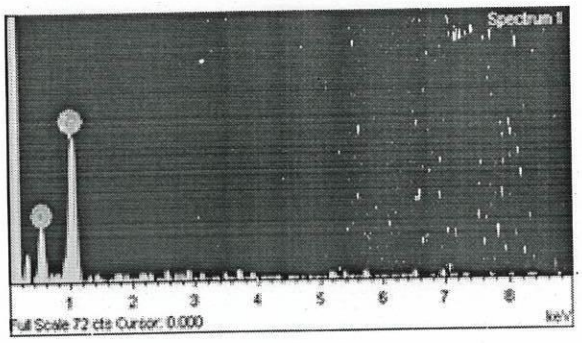


Fig :(7b)

Fig: 7a & 7b Are EDAX Of Chemical Synthesised And Green Synthesised Zno Nanoparticles

3.4 Impact of nano ZnO and bulk ZnO Suspension on the germination and growth:

After 15 days of lab observation of treated samples, it was observed that germination percentage of tomato seeds treated with green synthesised ZnO nanoparticles has shown good results over chemical synthesised ZnO nanoparticles, bulk ZnO (Table 1, 2, 3). On 5th day germination percentages are determined by below formula.

$$\text{Germination \%} = \frac{X}{N} \times 100$$

N=Total number of seeds placed in the particular petriplate.
X=Total number of seeds germinated in the particular petriplate.
Germination percentages clearly indicate the impact of concentration of nanofertilizers on tomato seeds.
The average root and shoot length of all plantlets were measured using a ruler on 15th day. It was noticed, that as ZnO concentration increases, root and shoot growth rate of all seedlings were increased till certain concentration (fig 9, 10). However, at high concentration, decline in root and shoot length was identified, which indicates the toxic effects of nano ZnO [18]. Finally obtained results shows .better agreement with the seed germination percentages calculated on 5th day. Interestingly green synthesized ZnO nanoparticles have shown better results than chemically synthesised as well as bulk ZnO.

Fig: (8a) Germinated Tomato Seedlings Fed With Chemically Synthesized Zno Nanoparticles

Fig: (8b) Germinated Tomato Seedlings Fed With Green Synthesized Zno Nanoparticles

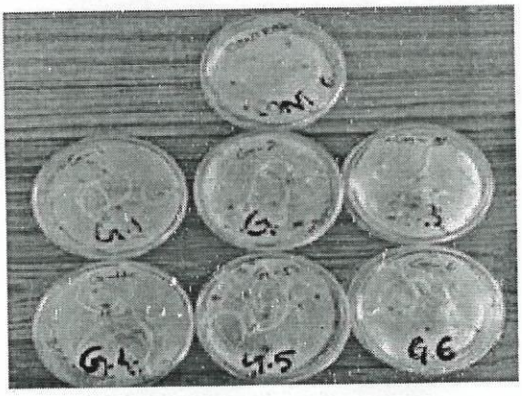


Fig: (8c) Germinated Tomato Seedlings Fed With Bulk Zno

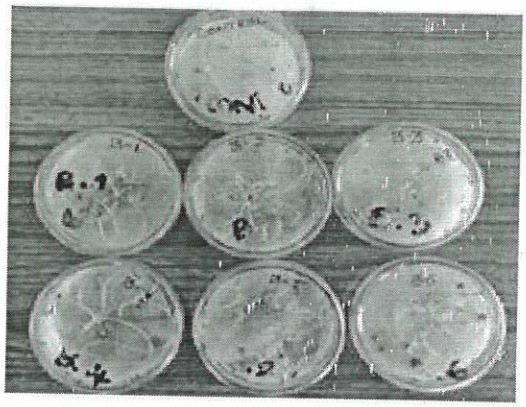


Table: 1 Germination % And Growth Rate Of The Tomato Plantlets Grown Using Chemically Synthesized Nanozno.

Concentration of Chemical method NanoZnO (ppm)	Germination %	Root length (cm)	shoot length (cm)	Plant fresh biomass (gm)
control	40.00	0.46	5.9	0.10
Nano 50	46.6	0.68	6.62	0.11
Nano 100	53.33	0.72	6.91	0.13
Nano 150	60.00	1.06	6.96	0.15
Nano 200	66.66	1.733	7.4	0.20
Nano 300	73.33	2.76	8.3	0.27
Nano 500	53.33	0.70	5.12	0.11

Table: 2 Germination % and growth rate of the tomato plantlets grown using green synthesized nano ZnO

Concentration of Green method NanoZnO (ppm)	Germination %	Root length (cm)	shoot length (cm)	Plant fresh biomass (gm)
control	40.00	0.46	5.9	0.10
Nano 50	53.33	1.52	7.6	0.19
Nano 100	60.00	1.84	7.9	0.21
Nano 150	66.66	2.72	8.02	0.23
Nano 200	73.33	2.86	8.3	0.30
Nano 300	86.66	3.05	8.5	0.33
Nano 500	66.66	2.10	7.2	0.20

Table: 3 Germination % And Growth Rate Of The Tomato Plantlets Grown Using Bulk Zno.

Concentration of bulk ZnO (ppm)	Germination %	Root length (cm)	shoot length (cm)	Plant fresh biomass (gm)
control	40.00	0.46	5.9	0.10
50	40.00	0.41	6.1	0.09
100	46.66	0.53	6.3	0.11
150	53.33	0.8	6.6	0.13
200	53.33	1.06	7.2	0.15
300	60.00	1.814	7.5	0.18
500	40.00	0.42	5.5	0.09

Fig: 9 Effect Of Treatments On Root Length

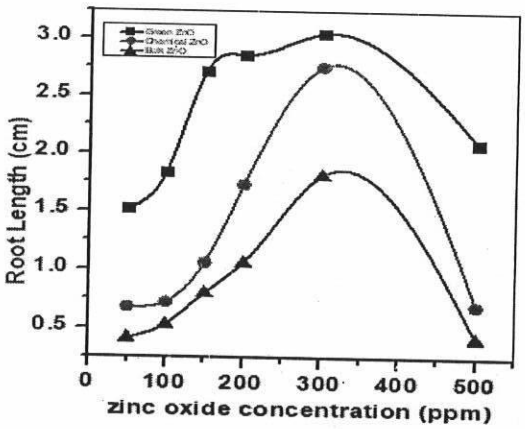


Fig: 10 Effect Of Treatments On Shoot Length

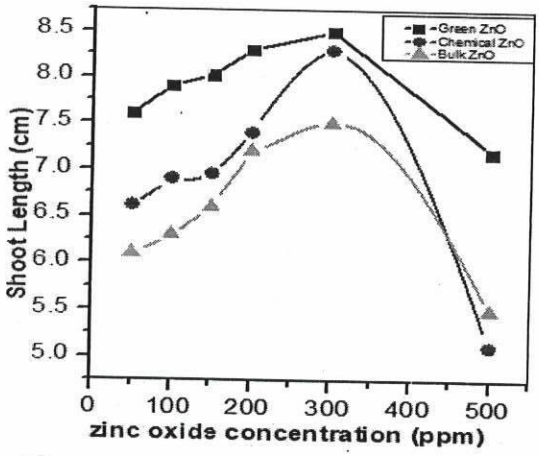
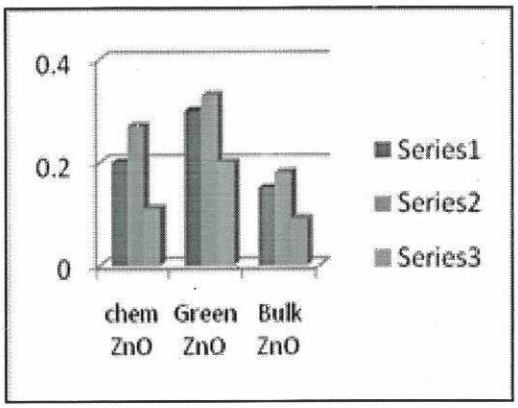


Fig (11) Comparison Of Fresh Biomass Amount Obtained After 15 Days At Various Concentrations



Series 1: fresh biomass at 200 ppm; Series 2: fresh biomass at 300ppm; Series 3: fresh biomass at 500ppm.

The fresh biomass was produced based on the length of the plant. The present data reveals that the fresh biomass of nano ZnO treated plantlets was considerably high, in comparison to bulk ZnO and control. Overall fresh biomass was higher for green synthesized nano ZnO at appropriate concentration.

IV. CONCLUSION

The zinc oxide nanoparticles were synthesized successfully by both chemical co-precipitation and green methods. The crystallite size of chemical ZnO and green ZnO was approximately 35 nm and 28nm. Morphology and elemental composition of ZnO nanoparticle were confirmed by SEM and EDAX. We have reported that the usage of micronutrient Zn in the form of green synthesised ZnO nanoparticles will be more effective for germination and growth of lycopersicum esculentus with no obvious toxic effects. It was further observed that growth of seedlings with the uptake of nanoparticles is completely concentration dependent where above 300 ppm concentration has shunted the growth of seedlings.

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