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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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

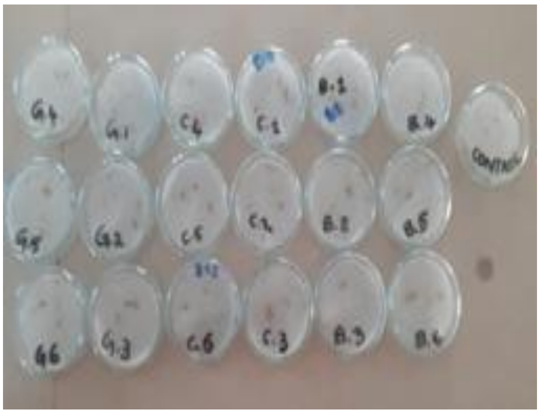

Fig 2: Introduction Of Seeds To Zno Nanoparticle And Bulk

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.

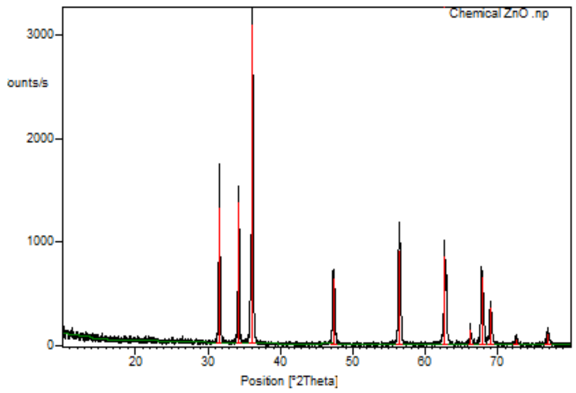

Fig:3 XRD Pattern Of Chemical Synthesized Zno Nanoparticles

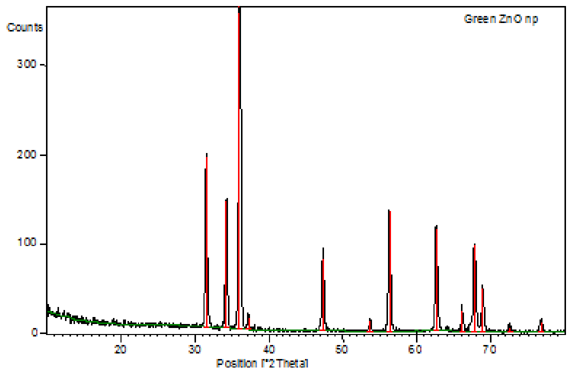

Fig:4 XRD Peaks Of Green Synthesized Zno Nanoparticles

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.

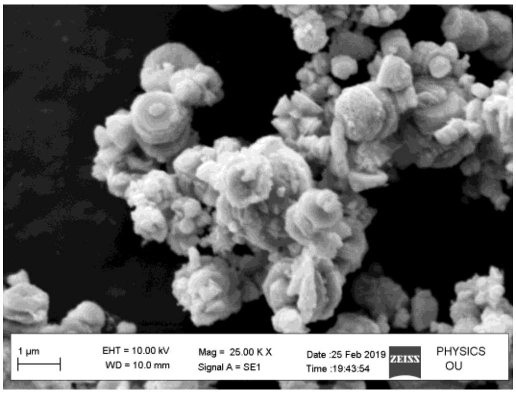

Fig: (5a)

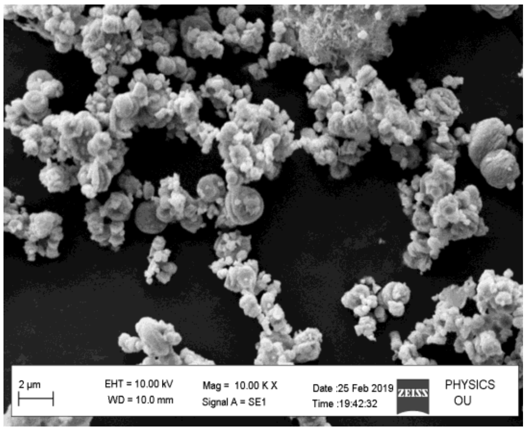

Fig (5b)

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.

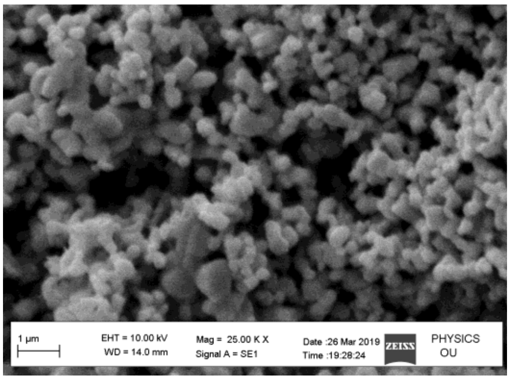

Fig: (6a)

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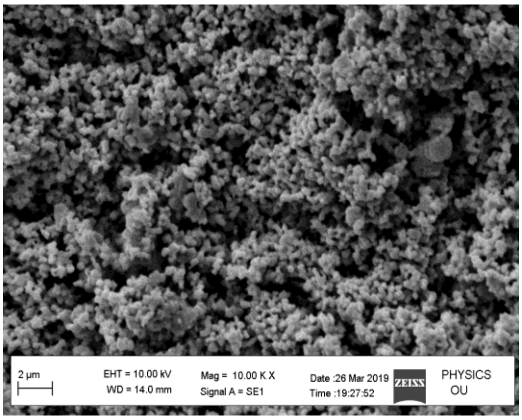


Fig :(6b)

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.

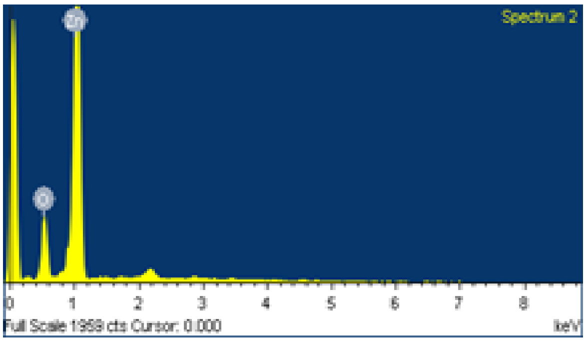


Fig :(7a)

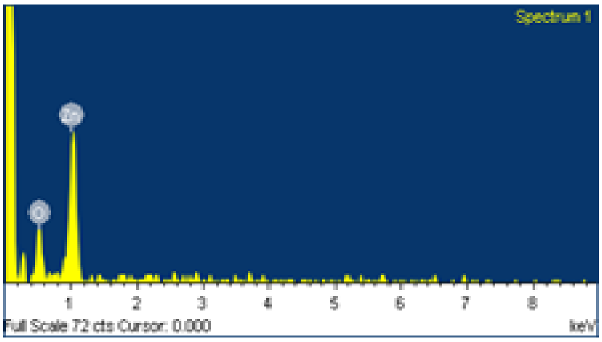


Fig :(7b)

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

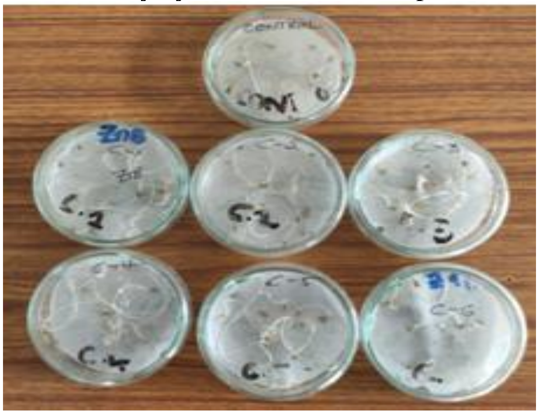


Fig: (8b) Germinated Tomato Seedlings Fed With Green Synthesized Zno Nanoparticles

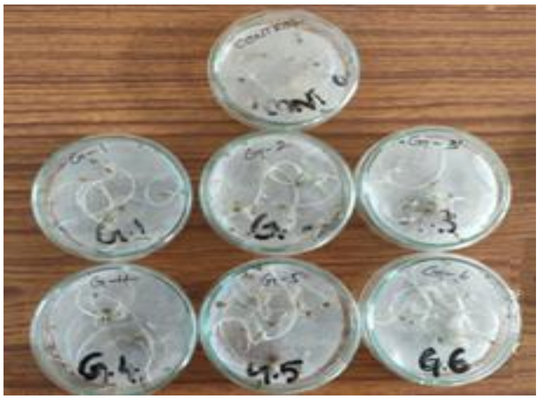


Fig: (8c) Germinated Tomato Seedlings Fed With Bulk Zno

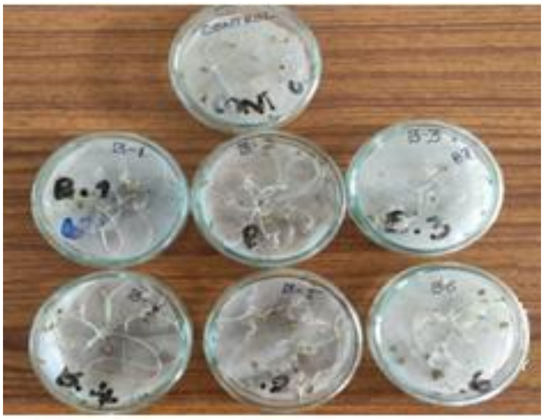


Table: 1 Germination % And Growth Rate Of The Tomato Plantlets Grown Using Chemically Synthesized Nanozno.

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

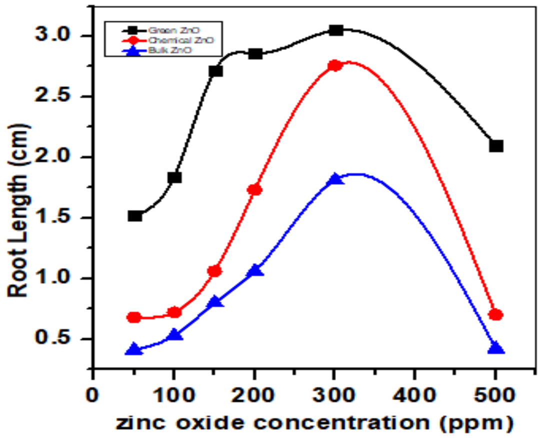


Fig: 10 Effect Of Treatments On Shoot Length

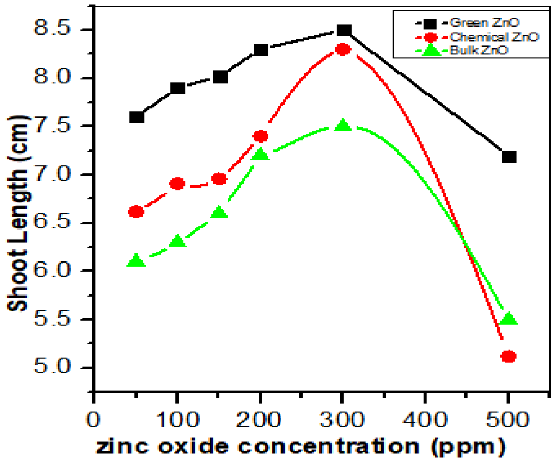
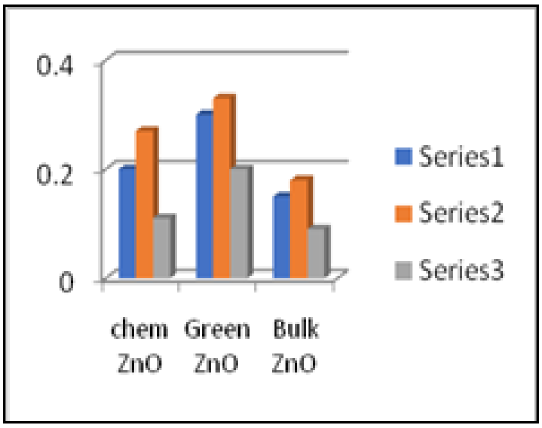


Fig (11) Comparison Of Fresh Biomass Amount Obtained After 15 Days At Various Concentrations



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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