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IMPACT OF ZINC OXIDE NANOPARTICLES ON GROWTH, BIOCHEMICAL CHARACTERISTICS AND YIELD OF LADY'S FINGER (ABELMOSCHUS ESCULENTUS)

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ABSTRACT The present study deals with the impact of Zinc oxide nanoparticles on growth, biochemical characteristics and yield of Lady's finger *(Abelmoschus esculentus)*. Zinc oxide nanoparticles was synthesized using chemical precipitation method and characterized by using SEM, EDEX, FTIR and XRD. Pot culture studies of Lady's Finger was carried out by using different quantities of Zinc oxide nanoparticles such as 0,100, 200, 300, 400 and 500 mg for treatment such as T_0 (control) T_1, T_2, T_3, T_4 and T_5 respectively for a period of 60 days. Growth characteristics such as seed germination efficiency, shoot length, root length, total fresh weight, dry weight, leaf area and vigor index, biochemical characteristics such as chlorophyll a, b and total chlorophyll, carotenoids, anthocynin, protein, L-proline, free amino acids and leaf nitrate and yield parameter such as length, weight and number of Lady's Finger per plant were estimated after 60 days. Among the treatments the shoot length, root length, dry weight were higher in T_3.The leaf area is higher in T_1 and vigor index of Lady's Finger is higher T_2.The chlorophyll a, b and total chlorophyll were higher in T_3.Carotenoids,anthocyanin and free amino acids are higher in T_2 The protein content and leaf nitrate are higher is higher in T_4 and L-proline is higher in T_5 .The length, weight and total number of Lady's Finger are higher in T1, T_4(22.7)and T_3respectively.

KEYWORDS : Zinc oxide nanoparticles, growth, biochemical, yield, lady's finger.

INTRODUCTION:

Nanotechnology plays a very important role in modern research. It is applied almost all field such as health care, food and feed, environmental health and space industries (Renganathan *et al*., 2014). Application of nanotechnology in agriculture delivery to plant technology also holds the promise of controlled release of agro chemicals and its targeted delivery of various macro molecules needs for improved plant disease resistance, efficient nutrient utilization and enhanced plant growth. Recent research on nanoparticles in a number of crop like corn, wheat, soybean, tomato and cucumber have provided evidence of enhanced seeding growth, germination, nitrogen metabolism, photosynthetic activity and protein level indicating their potential use for crop improvement. Metals like silver, zinc, gold, iron, copper and titanium have been routinely used for the synthesis of nanoparticles. (Chittaranjan Kole *et al* 2013). Among nanoparticles, Zinc oxide (ZnO) nanoparticles are hydrophobic inorganic compound existing in white powder form. Zinc oxide due to its versatility and multifunctionality creates attention in the research field related to its applications. Zinc oxide enhances absorbance of essential nutrients, especially nitrogen which is responsible for higher protein content. Zinc plays a vital role in carbohydrate and protein metabolism as well as it controls plant growth hormone (Fagera *et al*., 2002). The work related to the impact of Zinc oxide nanoparticles on growth, biochemical characteristics and yield of lady's finger (Abelmoschus esculentus) is totally wanting. Hence the present study was carried out

MATERIALS AND METHODS:

SYNTHESIS OF ZINC OXIDE NANOPARTICLES:

Precipitation method was adopted for the synthesis of zinc oxide nanoparticle. For the synthesis 0.1M of (2.875g)of zinc sulphate were dissolved in 100ml of distilled water under stirring vigorously using magnetic stirrer for 20 minutes. After stirring, the precipitation was achieved by adding 100ml of 1M NaOH solution in drop wise under constant stirring. The initial pH was observed as 3 and it was increased to PH 14 .Then precipitating process was continued until the milky white colour precipitate was obtained. Then this precipitate was centrifuged at 1500 rpm for 20 minutes. The centrifugal process was continued with water and two time with ethanol.The obtained precipitate was dried and calcinated at 5000C for 3hrs. Finally Zinc oxide nanoparticals were obtained.

CHARACTERIZATION OF ZNO NPS:

The synthesized Zinc oxide nanoparticles were characterized by Scanning Electron Microscope (SEM),Energy Dispersive X-ray Spectroscopy(EDX), Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Crystallography Diffraction (XRD).

COLLECTION OF RED SOIL FOR POT CULTURE STUDIES:

Garden soil (red soil) was collected from the Department of Biology, Gandhigram Rural institute- Deemed University, Gandhigram. For the collection of red soil a trench of 25 cm depth was dug out and red soil was taken from the trench. The red soil was dried in the shade powered using wooden mallet and sieved through a 2mm sieve before used for analysis.

SOURCES OF MATERIALS USED IN POT CULTURE (SEEDS AND COWDUNG)

A seeds of Lady's finger was collected from Bavani store, Dindigul, Tamil Nadu. Cow dung was collected from Faculty of Agriculture and Animal Husbandry, The Gandhigram Rural Institute- Deemed University, Gandhigram, Tamil Nadu and India.Vegetable crop Lady's finger *Ablemoschus esculentus* was selected for pot culture studies based on their easy availability, relative importance in daily diet of a common man, surviving capacity, growth capabilities and economic growth.

POT CULTURE STUDIES:

For pot culture studies the seeds were soaked in ground water and kept as control. Both the control and experimental seeds were allowed to grow in plastic pots (25 cm diameter, 25 cm height) containing a mixture of red soil, cow dung manure in the ratio of 1:1 The experimental pots were supplied with different quantities of Zinc oxide nanoparticles such as 0,100,200,300,400 and 500 for treatment 1 (Control) 2, 3,4,5,6 respectively. Triplicates were maintained and grown in net house for a period of 60 days. Pots were irrigated with well water.

GROWTH CHARACTERISTICS:

Growth characteristics such germination percentage, shoot and root length, fresh and dry weight, leaf area and vigour index, biochemical characteristics such chlorophyll a,b, total chlorophyll, anthocyanin, total soluble sugar, total protein, L.proline, free amino acids and nitrate and yield parameters were calculated after 60 days.

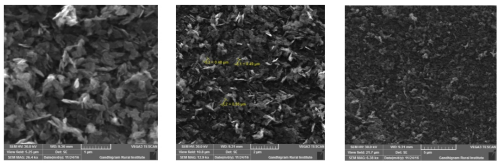
RESULTS AND DISCUSSION:

As NaOH was added to $ZnSO_4$, it is found to change from colorless to Milk white color is shown in figure1 and this colour change indicate the synthesis of zinc oxide nanoparticals (ZnO). Precipitation was

observed by increasing the P^H 3 to 14.

The SEM image (Fig.1) showing the high density chemically synthesized ZnO and further confirmed the development of zinc nanostructures. Obtained nanoparticle shows that hexagonal and rod shaped in natural. The microscopic image shows that the Zinc oxide nanoparticles did not appear as discrete particles but from much larger dendrite flocks whose size could reached micron scale size range about scale bar 0.40 µm, 0.49 µm, 0.55 µm (2µm) for figure a, b, c, respectively. Similar results on SEM analysis of zinc oxide nanoparticles had been also reported (Rajan *et al* 2016)

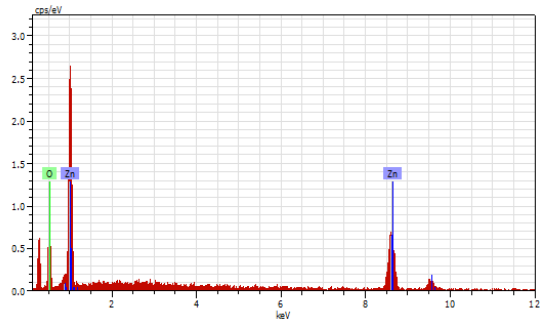
Fig,1 Scanning Electron Microscopy image on zinc oxide nanop articles



a) 1µm of Zinc nanoparticles b) 2µm Zinc nanoparticles
c) 5µm of Zinc nanoparticles

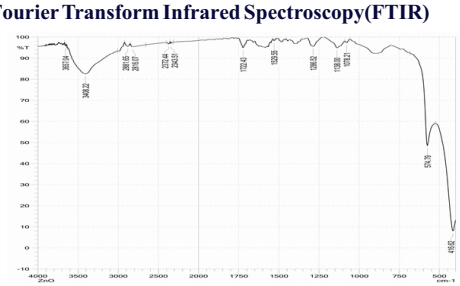
EDAX Spectrum recorded on the Zinc oxide nanoparticles is shown as three peaks located between 2 KeV and 10 KeV (Fig. 2). Those maxima are directly related to the Zinc characterized lines K. The maximum peak located on the spectrum at 6.4 KeV clearly coming from Zinc. The second maximum peak located on the spectrum at 0.3 KeV clearly indicates comes from oxygen . Third peak located at 2.6 KeV are connected with the oxygen characteristics line. Character ization of Zinc oxide EDAX spectra showed the strong peaks of Zn and O. The composition components of ZnO NPs formed by co-precipitation methods of synthesis Zn were 50 and O was 49. Chinnasamy *et al.*, (2014) studied the synthesis and characterization of silver nanoparticles using EDAX and SEM.

Fig.2 Energy Dispersive X – RAY Spectroscopy (EDAX)



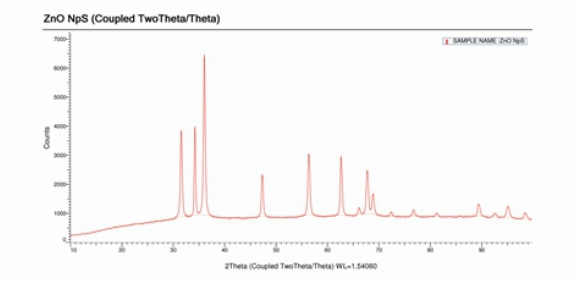
Fourier Transform infrared Spectroscopy measurement were carried out to identify the possible functional group responsible for the reduction of the Zinc in chemically synthesized Zinc oxide nanoparticle. The FTIR spectrum of the Zinc oxide nanoparticles was analyzed in the range 4000 – 400 cm (Fig.3)and bands observed at 3657.04, 3408. 22, 2881.65, 2816.07, 2372.44, 2343.51, 1722.43,1529.55, 1286.52, 1138.00, 1078.21which are associated with O-H Stretch free hydroxyl, N-H Stretch, H-C-H Bend, C-H Stretch of C=O, C≡N Stretch, C≡N Stretch, C=O Stretch, N-H Bend, C-O Acid, C-N Stretch, C-N Stretch.Hong *et al.*, (2009) reported that FT-IR spectrum of ZnO nanoparticles showed significant absorption band at 3457 and 1598, 452cm-^{1} and the absorption band at 452cm-1 was assigned to zn-o stretching vibration.

Fig.3 Fourier Transform Infrared Spectroscopy(FTIR)



The XRD diffraction Peaks indexed as 31.565,34.230.36.049,47.3 29,56.377. This is represented in fig. 4. All diffraction peaks indexed according to the hexagonal phase of Zinc oxide nanoparticles shown less of impurity phase except Zinc oxide are found which revealed that good crystalline in nature of the sample. The XRD pattern reveals the particles size of chemically synthesized ZnO nanoparticlesas ±23nm,calculated by Scherers's formula. Znou et al., (2007) reported that XRD patterns of the ZnO nanoparticles prepared by hydrothermal methods, indicates the ZnO Nps has hexagonal wurtzite phase structure. These results demonstrate the purity of the synthesis result.XRD patterns of the ZnO NPs shows five characteristics peaks the XRD diffraction Peaks indexed as 31.565,34.230.36.049,47.3 29,56.377. All diffraction Peaks indexed according to the hexagonal phase of Zinc oxide no characteristic Peaks of impurity phase except Zinc oxide are found which revealed that good rod shape in nature. Awodugba et al., (2013) reported that the peaks at scattering angle 31.640, 34.850, 36.650,46.10 and 57.120 with corresponds to (100),(101),(102),(110) and (200) crystal plane respectively. This peak indicates that ZnO NPs with a spinal structure and no characteristic peaks of impurities are detected in the XRD patterns.

Fig.4. XRD Analysis of Zinc Oxide Nanoparticles



The germination percentage of Lady's finger *(Abelmoschus esculentus)* is higher in T_0, T_1,T_2, T_3, T_4 (100) and lower in T_5 (30%).Prasad *et al*.,(2012) reported that the germination of peanut was 100% when treated with ZnO nanoparticles. Lin and Xing (2007) reported that seed germination of six higher plant species. The germination percentage was suppressed at higher dose of effluent at higher concentration of total salts make inhibition more pronounced and prominent (Dhanam 2009). Raskar *et al*., (2013) reported that the TiO_2 nanoparticles at the concentration ranging from 10 to 40ppm enhance seed germination, promptness index and seedling growth but result shows that lower concentration was not harmful to the seed germination and early seedling growth.

The effect of different quantities of Zinc oxide nanoparticles on growth parameters of Lady's Finger *Ablemoschus esculentus* grown for a 60 days is presented in table 1.The shoot length is higher in T_3 containing 300 mg of Zinc oxide nanoparticles and lower in T1 containing 100 mg of nanoparticles. Growth performance (shoot and root length) of radish and onion were decreased by the paper mill effluent (Srivastava,1991). Prasad *et al*.,(2011) reported that the shoot length of mung and gram increased in lower concentration of ZnO nanoparticles and higher concentration of ZnO nanoparticles decreased the shoot length. The root length of the Lady's Finger in control is 8.83. Among the treatments the root length is higher in T_3 (11.2) and lower in T_5. The fresh weight of the Lady's Finger in control is 5.6g. Sri Sindnura et al.,(2015) reported that the fresh weight of peanut is higher in lower concentration of ZnO nanoparticles. The dry weight of the Lady's Finger is higher in T3 and lower in T_5.But Sri Sindhura,2015) reported that the dry weight of peanut is higher in lower concentration when treated with ZnO nanoparticles. Naderi *et al.,* (2012) reported the effect of treatment of ZnO nanoparticles at different concentration and the results showed that the plant measurement of bean such as shoot length, shoot fresh weight and dry weight of shoot were influenced by different concentration of ZnO nanoparticles. The leaf area is higher in T_2(45) and lower in T_0. The vigor index of Lady's Finger in control is 1556. Among the treatment vigor index is higher T_2, and T_3 and lower in T_1. Scrinis *et al.*, (2007) reported that ZnO Nps with less hydrophylicity and more dispersible in lypophilic substance can have more changes to penetrate through the leaf surface and release ions across the cuticle as compared to water soluble ions.Prasad *et al*.,(2012) reported that the a vigor index of 1701.3 in peanut with ZnO nanoparticles.

Table 1.Effect of various quantities of zinc oxide nano particles on growth parameters of lady's finger *(abelmoschus esculentus)* grown for a period of 60 days.Each value is the average of 10 individual observations

S.No.	Parameters	Treatments					
		T^0 (CONTROL)	T_1 (100)	T^2 (200)	T^3 (300)	T^4 (400)	T^5 (500)
1	Shoot length (cm)	20 .83±9.8	16.6±3.21	31.3±4.04	32.6±4.50	28±12.16	25±14.43
2	Root length (cm)	8.83±2.54	9.1±1.44	10.5±1.32	11.2±2.29	9.2±3.785	7±2.33
3	Fresh weight (g)	5.6±2.702	7.7±3.40	7.2±2.61	10.4±2.84	9.16±1.49	4.5±2.59
4	Dry weight (g)	1.75±0.71	1.5±1.06	2.1±0.577	2.8±1.078	2.2±1.069	1.7±0.981
5	Leaf area	11.66±2.88	45±25	32.66±15.53	31±17.691	21.66±7.64	16.66±8.86
6	Vigor index	1556	1400	2500	2500	1850	1500

The chlorophyll a,b and total chlorophyll of Lady's Finger *Ablemoschus esculentus* is presented in figure 5. The chlorophyll a is higher in T_3 (6.64) and lower in T_1 (0.650).The total chlorophyll is higher in T_3 (38.312) and lower in T_1 (3.461). Liu *et al*., (2016) reported that the chlorophyll contents of lettuce increased when treated with iron oxide nanoparticles. Neelam *et al*.,(1988) reported the reduction in the chlorophyll content at higher concentration of effluent might be due to the higher osmotic effect which might be responsible for the reduction of the uptake of magnesium. But Ghafariyan (,2013) reported that low concentration of Fe3O4 nanoparticles significantly increased the chlorophyll content of soybean. The anthocynin and carotenoids of Lady's Finger Abelmoschus esculentus is presented in Figure 6. The protein and L-proline of the Lady's Finger Abelmoschus esculentus is presented in Figure 7. The free amino acids and nitrate of the Lady's Finger Abelmoschus esculentus is presented in Figure 8. Since no work was related to the effect of nanoparticles on carotenoids, anthocynin, total protein , leaf nitrate and free amino acids on plant the discussion is based on the effects of effluents. The carotenoids is higher in T_2 (1011) and lower in T_5 (50). The anthocynin is contents higher in T_2 (18.6) and lower in T_5 (9).The accumulation of anthocynin is considered to be an adaptive mechanism for stressed plant. Anthocyanin is known to accumulate in stressed condition as a result of secondary metabolism (Jayarambabu et al., 2014). The protein content is higher in T_4 (111) and lower in T_5 (7.5) Dhanum (2009) reported increased level of protein in paddy seed raised in lower concentration of dairy effluents.The increased protein content of green gram at lower concentration and then the content decreased at higher concentration with sugar mill effluent was observed (Baskaran et al., 2009). El sayed *et al*., (1995) observed remarkable increase in leaf protein contents of all tested cultivares irrigated with saline water. The free amino acids are higher in T2 (2.5) lower in T_5 (0.5).Suresh Kumar and Mariappan (2013) reported that the increase of free Amino acids contents with increasing the concentration sugar mill effluent. The leaf nitrate is higher in T_4 (10.3) and lower in T_5 (3). Naik (1972) reported that the nitrate reduction and photosynthetic reaction play an important role in the generation of reducing power as in the synthesis enzymes.

FIGURE: 5 CHLOROPHYLL A, B AND TOTAL CHLOROPHYLL OF LADY'S FINGER (*ABELMOSCHUS ESCULENTUS*)

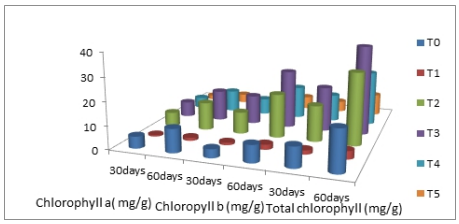


FIGURE 6: CAROTENOIDES AND ANTHOCYANIN OF LADY'S FINGER *(ABELMOSCHUS ESCULENTUS)*

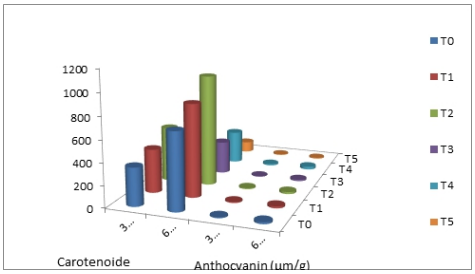


Fig. 7 PROTEIN AND L- PROLINE OF LADY'S FINGER *(ABELMOSCHUSESCULENTUS)*:

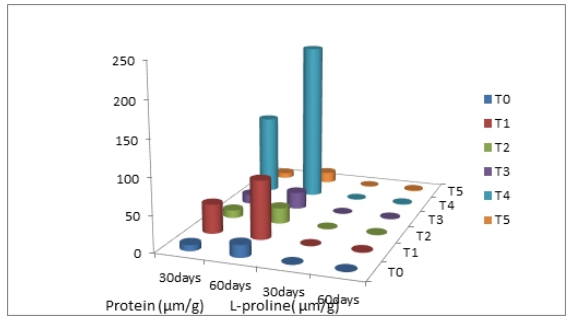
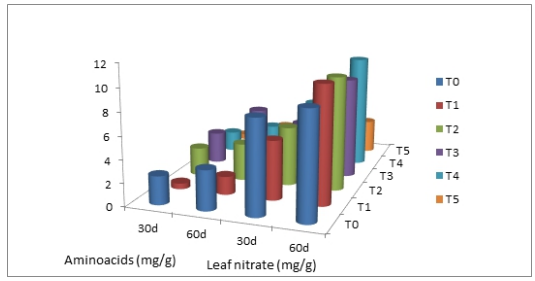


Fig.8 FREE AMINO ACIDS AND NITRATE CONTENT OF LADY'S FINGER*(ABELMOSCHUSESCULENTUS)*:



Yield parameter of Lady's Finger (Abelmoschus esculentus) is presented in table 10.The vegetable length is higher in T_3 (5.88) and lower in T_1(0.608). Mohamed and Ibrahim (2015) reported that the titanium dioxide nanoparticles lead to a significant increase in plant height, fruit yield and number of branches The vegetable weight is higher in T_3(3.02)and lower in T_1(1.50).. Sharma and Kansal,(1984) reported that the yield parameter such as length, weight and number of lady's finger increased over control up to 800 mg and decreased gradually in further higher concentration. The total number of vegetables higher in T_3(4.8)and lower in T_1(0.5). Udayasoorian *et al.*, (1999) reported that the weight, length and number of seeds of wheat decreased as the concentration of dairy effluent.

Table. 2 YIELD PARAMETERS OF LADY'S FINGER *(ABEL MOSCHUSESCULENTUS)*

TREATMENT	LENGTH (cm)	WEIGHT (g)	NUMBER
T0	21.1±0.87	13.16±1.18	4±1.00
T1	35.4±0.608	16.4±1.50	6±1.00
T2	25±2.92	17±2.54	8±2.00
T3	34±5.88	18.93±3.02	14±4.00
T4	26±1.47	22.7±1.92	15±2.00
T5	37.3±0.529	1.6±2.77	2±1.00

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