Effects of Zinc Oxide Nanoparticles Treatment on *Lycopersicum Esculentum* (Tomato)

By

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INTRNATIONAL ISLAMIC UNIVERSITY ISLAMABAD, PAKISTAN

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Nanobiotechnology

Nanotechnology in agriculture

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FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. (Write Full Name) and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Bioinformatics

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A thesis submitted to Department of Bioinformatics and Biotechnology International Islamic University Islamabad, as a partial fulfillment of requirement for the award of the degree of MS Biotechnology.

Dedicated to

Holy prophet (P.B.U.H)

My parents and my Aunt

DECLARATION

I hereby declare that work present in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date _____

Tahira Bibi

CONTENTS

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Tahira Bibi

LIST OF ABBREVIATIONS

Acronym	Abbreviation
i.e.	That is
e.g.	For example
ZnO	Zinc Oxide
Nano-SiO_2	Nano silica dioxide
Germination %	Germination percentage
Fig.	Figure
L.esculentum	*Lycopersicum esculentum*
g L^{-1}	Gram per liter
ppm	Parts per million
mg	milligram
gm	gram
cm	centimeter
ABGE	Applied Biotechnology and Genetic Engineering lab
ddH2O	Double distilled water
EDTA	Ethylene diamide tetra acetic acid
NaCl	Sodium chloride
CTAB	Cetyl trimethylammonium bromide
HCl	Hydrochloric acid

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Abstract

Zinc although a micronutrient but it is considered as a vital nutrient for plants. Zinc deficiency causes serious hazards to plants. During recent period zinc deficiency had declined the yield of crops especially tomato. Study was planned to investigate the effects of ZnO nanoparticle on *L.esculentum* (tomato).money maker variety of tomato was used in the study project. Three different concentrations ($0.01gL^{-1}$, $0.03gL^{-1}$ and $0.05gL^{-1}$) of ZnO nanoparticles were used to treat the seeds of tomato. There were total 4 treatment groups i.e. One as controlled and 3 nanoparticle treated groups. Plants were grown to observe the different parameters and morphological characters. The parameters studied include, germination time, germination percentage, plant height, number of leaves, number of branches, number of days to flowering, number of days to fruiting and fruit number. Plants were also individually examined to notice any kind of change and during the examination it was noticed that the plant which was treated with lower concentration i.e.$0.01gL^{-1}$ produced six petaled flower and this concentration yielded bigger size fruit.

In the present investigation, it was analyzed that ZnO nanoparticle at lower concentration acted as nano fertilizer and enhanced the yield of tomato.

Chapter 1

INTRODUCTION

1. Introduction:

As there is coming global food shortage. Under the present system the world cannot yield sufficient food for that many people. Approximately 800 million people in the world are facing the problem of food shortage and the number people below poverty line have increased. The emergence of green revolution had resulted in the evolution of old agriculture techniques to industrial agriculture in the past decades. Now a days quality and quality of agricultural food products improved considerably. Nanotechnology can modernize the agriculture and food system. (Zenu *et al.*; 2011)

1.1. Nanotechnology:

Nanotechnology as an emerging field and technology revolutionized every field of science. Nanotechnology is used in association with other sciences such as, electronics, optics and biomedical and material sciences. This field has gained impulsion in present- days and it has provided innovative solutions scientific disciplines .Nanotechnology deals with the nanoparticles which are atomic or molecular aggregates characterized by size less than 100 nanometer(nm).

1.2. Nanobiotechnology:

Nanotechnology and biotechnology are two advantageous technologies of 21st century. Nanobiotechnology can play an important role in developing and implementing many useful tools in the study of life (Md *et al.*; 2012).

1.3. Use of nanoparticles in agriculture:

Nanotechnology can improve the whole setup of agriculture and food industry with the help of new tools which are developed for the management of plant diseases, pathogen detection and improving the ability of plants to absorb the nutrients. According to recent analysis it is assumed that availability of nanostructured catalyst will increase the effectiveness of commercial pesticides and insecticides and also the dosage level required for crop plants. The total global population is about seven billion and 50% of it is living in Asia. People living in

developing countries are facing food shortage problems (Joseph and Morrison, 2006).

Agriculture sector is facing many problems and total yield is reduced by many biotic and abiotic factors. For example, insects, pests, diseases and sea weeds are causing significant harm to agricultural production (Dhaliwal *et al*; 2010).

1.4. Zinc oxide nanoparticle:

Zinc oxide is an inorganic compound with molecular formula ZnO. It is in white powder form and is insoluble in water. The powdered ZnO is extensively used as a preservative in several materials and products. Zinc oxide nanoparticles have important properties and therefore have great potential to improve agriculture (Sidra *et al*; 2014).

1.5. Tomato:

Tomato belongs to the family solanaceae also known as night shade family. *Lycopersicum esculentum* Mill.is the botanical name of tomato. Tomato is a diploid plant having 2n = 24 numbers of chromosomes. Tomato is a perennial herbaceous plant and grows to the height of 1-3 meter in length, it has weak woody stem .the flowers of tomato are yellow in colour. Fruits vary in size 1-10 cm or more in diameter mostly in red colour when ripen (Mohamed *et al.*, 2010).

1.5.1. HISTORY:

Tomatoes originated along the costal highlands of western South America. Tomatoes are second most important vegetable to potatoes .the use of potatoes and tomatoes had increased to 30% during 1980s and 1990s in U.S. In 1999 the total world production of tomato was 111.1 million short tons. Recently United States is found to be the world's largest producer of tomatoes. In 1999 china was considered as the world's largest tomato producer with 18 million tons (Smith and Andrew., 2003).

Solanum Lycopersicum is the only domesticated tomato specie and there are 12 wild tomato specie. Based on wide presence Solanum Lycopersicum cerasiforme was thought to be the ancestor of cultivated tomato but according to recent investigations

plants which are known as cerasiforme are mixtures of wild and cultivated tomatoes rather than ancestors of cultivated tomatoes (Bali and Lindhout ., 2007).

1.5.2 CLIMATE

Tomato plant is sensitive to cold. The optimum temperature for flowering is 21-24 degree Celsius and for seed germination soil temperature should be 15.5-29 degree Celsius.

1.5.3. SOIL

Tomatoes can be grown on every kind of soil but it can be well grown in well drained loamy soil. The soil pH ranging from 5.5-7.0 is considered to be best for tomato production.

1.5.4. PLANTING:

Seeds are sown in nursery and the raised seedlings are transplanted in the fields. Seedlings of 15-25 cm of tall are transplanted to fields.

1.5.5. WEEDING:

Weeding is done three to four times during the growing season to avoid the reduction in the yield.

1.5.6. IRRIGATION:

Plants are irrigated regularly according to the seasonal conditions.

1.5.7. HARVESTING:

Harvesting continuous for about one month according to the climate conditions and cultivar planted.

1.5.8. PESTS AND DISEASES:

Tomato crop is affected by insects such as aphids (Myzus persicae), thrips, fruit worms, mites, and diseases, including bacterial wilt, early blight and late blight.

1.5.9. IMPORTANT VARITIES GROWN IN PAKISTN:

Important varieties of tomato grown in Pakistan are money maker, Roma, Riogrande.

1.5.10. ECONOMIC IMPORTANCE:

1. Tomato is considered as an important nutritive crop. It has rich vitamin C and A content.it has also fibers and cholesterol free (Mamidala and Nanna., 2011).
2. Tomato is rich source of lycopene.100 gram of tomato contains 20 to 50 mg of lycopene. Lycopene is a powerful antioxidant and it protects the body from free radicals harmful for body.it also helpful in cancer prevention (Kalloo G., 1999) (Block *et al.*,1992) (Gerster H.,1997).
3. Medicinally tomatoes are helpful in treating, several types of cancers. It helps to maintain bone strength, good for skin, hairs.
4. Tomatoes are also useful to repair the damage caused by the smoking.
5. It is good for kidney when it is used without seeds and is also good for diabetic patients.

1.6. ROLE OF ZINC FOR TOMATO PLANT GROWTH:

Zinc although a micronutrient but it is very important for plant life. Zinc plays important role in plant metabolism. Enzymes which are activated by zinc are involved in carbohydrate metabolism, membrane integrity and protein synthesis etc (Hafeez *et al*; 2013).

1. The genes required for the tolerance of environmental stresses are regulated and maintained by zinc. Zinc deficiency results in the arrested growth, smaller leaves, chlorosis and spikelet infertility (cakmak., 2000).
2. Zinc also plays and important role to enhance the plant susceptibility to injuries by high light and temperature and fungal infection (cakmak., 2000).
3. Zinc is important for water uptake and transport in plant.it also reduce the adverse effects of salt and heat stress (Kasim, 2007) (Disante *et al.*,2010) (peck et *al.*, 2010) (Tavallali *et al.*, 2010).
4. Zinc helps in the maintenance of cellular membranes (Brenan., 2005).

Chapter 2

REVIEW OF LITERATURE

2. Review of literature

Due to the development of technologies agriculture has been benefited. In the period of 1960s and 1970s high disease resistant and high yield crops and agricultural practices were introduced which lead to better and improved output and production yield (Gillen, 2011).

Although the management scientists are putting their efforts but crop productivity has not reached to its potential. This is credited to low nutrient and water use efficacy. There is also rigid competition by weeds and crop pests. This yield barrier can be overcome by the use of nanotechnology.it will may give expected results to increase the productivity rate of crops and will overcome the food shortage problem (Sah *et al.*, 2014).

Crop production is greatly influence by insects such as insects, nematodes, mites and pathogens. Regular use of pesticides although resulted in the disease and pest resistant varieties but it has caused accumulating residues in environmental pollution.so there is need for an alternative approach to control pests and pathogens. Nanotechnology holds a major potential in management of insects and pathogens. Nanoparticles are stable and biodegradable. These can be used in production of nano capsules for delivery of pesticides and fertilizers etc. Green synthesis of nanoparticles by plants and microbes is not very advantageous for crop protection but also environment friendly. (Chowdappa and Gowda., 2013).

2.2. Nanoparticles used in agriculture

The first stage of plant growth is seed germination so it is very essential to understand the way of plant growth relative to nanoparticles. Various studies have shown that effect of nanoparticles on seed germination is dependent upon concentration of nanoparticles. Nano-SiO_2 in lesser concentration improves the germination of seed (Siddiqui and Al-Whaibi 2014). Maize seeds germination also improved with the Nano-Silica (Suriyaprabha *et al.*, 2012)

(Bao-shan *et al.*, 2004) treated seeds of Changbai larch,(Haghighi *et al.*, 2012), of tomato seeds and (Siddiqui *et al.*, 2014) of Squash seeds and find out that

nano-SiO_2 enhanced the seeds germination, improved seedling growth, quality and increased mean height, root length etc. Silica, copper, gold and palladium nanoparticles were used on lettuce seeds and impart a significant effect (Shah and Belozerova 2009).

Nano-titanium and nao-SiO_2 application on soya bean improved the seed germination percentage by increasing nitrate reductase (Lu et al., 2002).

Nano-SiO_2 application on plants under environmental stress such as salinity stress improved the leaf dry and fresh weight, chlorophyll content and accumulation of proline. Increased accumulation of proline, contents of nutrients, free amino acids and antioxidant enzymes action improved the tolerance of plants to environmental stress (Kalteh *et al.*, 2014).

Studies reported that nano- SiO_2 improved the photosynthetic rate in plants by enhancing the action of carbonic anhydrase and photosynthetic pigments synthesis (Xie *et al.*, 2011).

Nano-SiO_2 improves photosynthetic rate in plants by improving action of carbonic anhydrase and synthesis of photosynthetic pigments (Xie *et* al., 2012).

2.3. Use of ZnO nanoparticles in different plants

Zinc although a micronutrient yet it is very important for plants to tolerate the environmental stresses such as salinity, drought and high temperatures. Zinc is essential for plant metabolic activities. Crop yield and quality of crops can be affected by the deficiency of zinc. According to one study it was investigated that the spinach plant sprayed with ZnO nanoparticle concentration of 500 and 1000 ppm showed that the length, width, surface area and colour enhanced as compared to the controlled samples (Kisan *et al.*, 2015).

Another study carried on the effects of ZnO nanoparticle on mungbean Plant. The mungbean treated with ZnO nanoparticle at 50 and 100 mg treated showed increased seed germination percentage, root and shoot length (cm) and also root dry weight (gm.).This study concluded that this happens because ZnO nanoparticles promoted germination of seeds and seedling growth as compared to controlled. But

seeds treated with 150 mg concentration showed a significant decrease in seed germination (Jayarambabu *et al.*, 2015).

ZnO nanoparticle also played an important role in the growth and development in the peanut plant. Root length, seed germination, seedling vigor index and yield at 1000 ppm showed significant increment as compared to controlled or untreated seed. But at higher concentration of 2000ppm all these parameters showed a significant decrease (Prasad *et al.*, 2012).

According to another study about effects of ZnO nanoparticle on onion plant also showed the same results as above that ZnO nanoparticles imparted a positive effect on onion plant. ZnO nanoparticles at the concentrations of 20, 30 and 40 μgL^{-1} showed an increase in plant height and number of leaves. Days to flowering also reduced in treated plants as compared to controlled plants. This study also showed that the ZnO nanoparticles at low concentrations i.e. 20 and 30μgL^{-1} showed best results as compared to 40 μgL^{-1} (Laware and Raskar 2014).

Uptake of nanoparticles by tomato crop

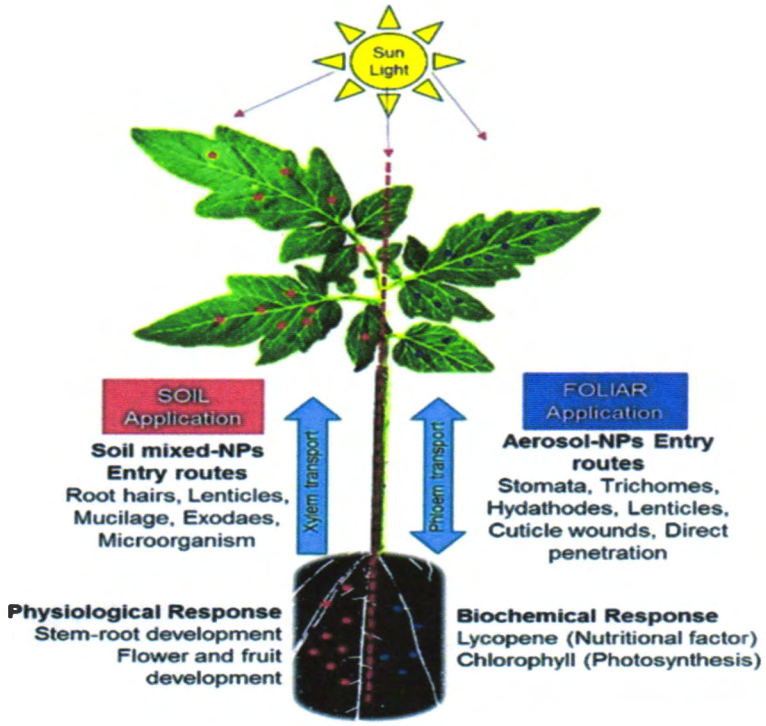

Fig.2.1: Nanoparticle uptake by tomato crop

2.5. Use of nanoparticles on tomato crop

According to a study about the use of nanoparticle on tomato crop it has been said that, it is expected that world population is going to reach about 9 billion by 2050.Food scientist are looking for ways to produce enough food for this much population without increasing the stress on natural resources.

(Raliya *et al.*, 2015) used titanium dioxide and zinc oxide nanoparticles to increase the growth and nutrient content level in tomato crop. Their study shown that by using titanium dioxide and ZnO nanoparticles tomato plant better absorbed minerals and light and fruit contains high antioxidant content.

Nano-SiO_2 were used on tomato plant by (Manzar and Al-Whaibi 2014).This study shown the better growth rate, improved seed vigour index, seed germination index, seedling fresh and dry weight. Increased concentrations of silica nanoparticles that is 8 gL^{-1} improved these parameters. Silica acted as an important delivery agent of chemicals and DNA in to plant and animal tissue.

2.6. Objective

The main objective of this project is to study the effects of different concentrations of nanoparticles on money maker tomato variety of *L.esculentum* .To select the best concentration of ZnO nanoparticle to improve the crop yield and to study the morphological characteristics like plant height, number of leaves per plant, number of fruits per plant and shape of the leaves, flowers and fruit.

Chapter 3

MATERIAL AND METHODS

3 .materials and methods

All the research work was carried out at Applied Biotechnology and Genetic Engineering lab (ABGE) Department of Bioinformatics and Biotechnology, International Islamic university Islamabad.

3.1. Materials:

3.1.1. Plant material

`Mature seeds of tomato variety *Solanum Lycopersicum* (money maker) were collected from local seed bank. The seeds were subjected to different treatments concentrations of ZnO nanoparticle. Treatment concentrations were 0.01%, 0 .03% and 0.05% v/v along with the untreated seeds as controlled one.

3.1.2. Equipment:

3.1.2.1. Glassware

`Petri plates, beakers (100 ml and 1000 ml (Pyrex, Germany), conical flasks (100 ml and 1000 ml) were used in experimental work.

3.1.2.2. Machinery

Laminar flow hood cabinet, autoclave, pH meter, electronic balance, Microwave oven, Drying oven, shaker, refrigerator ,centrifuge machine, incubator, RT-PCR, Gel electrophoresis tray, Electrophotometre.

3.1.2.3. Tools

Falcon tubes, Micropipettes, foreceps, filter papers, centrifuge tubes.

3.1.2.4. Chemicals

70% ethanol, 50% Clorox, ddH2O, Sorbitol, Tris, EDTA, NaCl, sarkosyl, CTAB, HCl, NaoH.

3.1.2.5. Nanoparticle

Zinc Oxide (ZnO) nanoparticle was used to treat tomato seeds.

3.2. Methodology

3.2.1. Preparation of seeds for treatment with ZnO nanoparticles

Following steps should be taken for preparation of seeds for treatment:

I. Seeds were soaked in distilled water for 24 hours at room temperature to break their dormancy. Beakers were covered with aluminum foil.
II. Seeds were dried on filter paper.

Now seeds are ready for treatment with nanoparticles.

3.2.2. Sterilization of glass ware and other tools

All glass ware and tools were autoclaved before seed surface sterilization in order to avoid any contamination. After sterilizing all apparatus it was placed in rear of laminar flow hood cabinet.

3.2.3. Preparation of hood for seed surface sterilization

3.2.3.1. Cleaning of hood

Laminar flow hood cabinet was first cleaned before seed surface sterilization. Following steps were followed for this purpose:

i. Hood was washed with 70% ethanol.
ii. After washing with 70% ethanol whole working area is covered with a layer of filter papers.
iii. Ultraviolet light is turned on for half an hour before work and blower was also
turned on.

4.5. Number of days to flowering

Days to flowering were observed and recorded from the date of sowing to the appearance of first flower on plant. Average and variance was calculated for each treatment group. Comparison of average between each treatment group is shown in Fig 4.8 and variances are given in table 4.3.

In controlled group, flowering started after 120 days. Days to flowering in controlled group ranged from 120-125 days in 3 plants. Average days to flowering were 122.66 days and variance was 6.33. In $0.01gL^{-1}$ treatment group, flowering started after 90 days. Flowering ranged from 90-99 days in 9 plants. In this case average was 95.55 days and variance was 10.77.

In case of $0.03gL^{-1}$ concentration treatment group, flowering started after 93 days. Days to flowering of 7 plants in this case ranged from 93-105 days. Average days to flowering were 98.71 days and variance 17.90. In $0.05gL^{-1}$ treatment group, flowering started after 95 days. Days to flowering of 7 plants in this case ranged from 95-105 days. Average days to flowering were 100 days and variance was 12.33.

3.1.2.5. Nanoparticle

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ii. After washing with 70% ethanol whole working area is covered with a layer of filter papers.
iii. Ultraviolet light is turned on for half an hour before work and blower was also
turned on.

iii. Then seeds were placed in 50% Clorox solution for fifteen to twenty minutes.

iv. The seeds were then washed with ddH2O for two to three minutes.

v. The seeds were then dried on autoclaved filter papers.

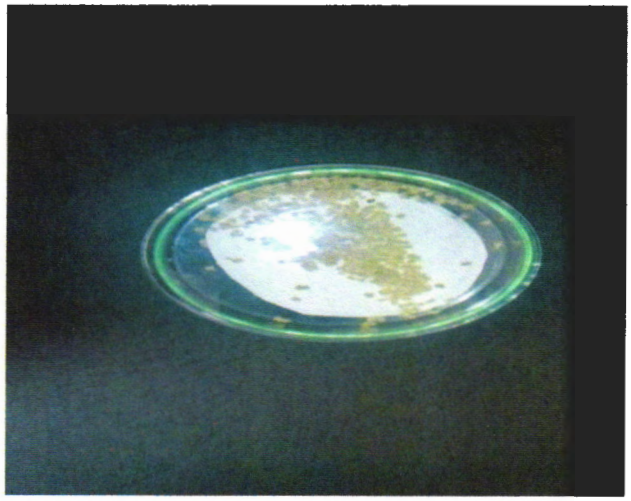

Fig.3.2.Surface sterilized seeds.

3.2.5. Preparation of zinc oxide nanoparticle

Following nanoparticle concentrations were prepared in distilled water.

3.2.5.1: 0.01gL^{-1} ZnO

0.01gram of ZnO nanoparticles were weighed and poured in 1000 ml of distilled water.

3.2.5.2. 0.03 gL^{-1} ZnO

0.03gram of ZnO nanoparticles were weighed and poured in 1000 ml of distilled water.

3.2.5.3. 0.05 gL^{-1} ZnO

0.05gram of ZnO nanoparticles were measured and poured into the 1000 ml of distilled water.

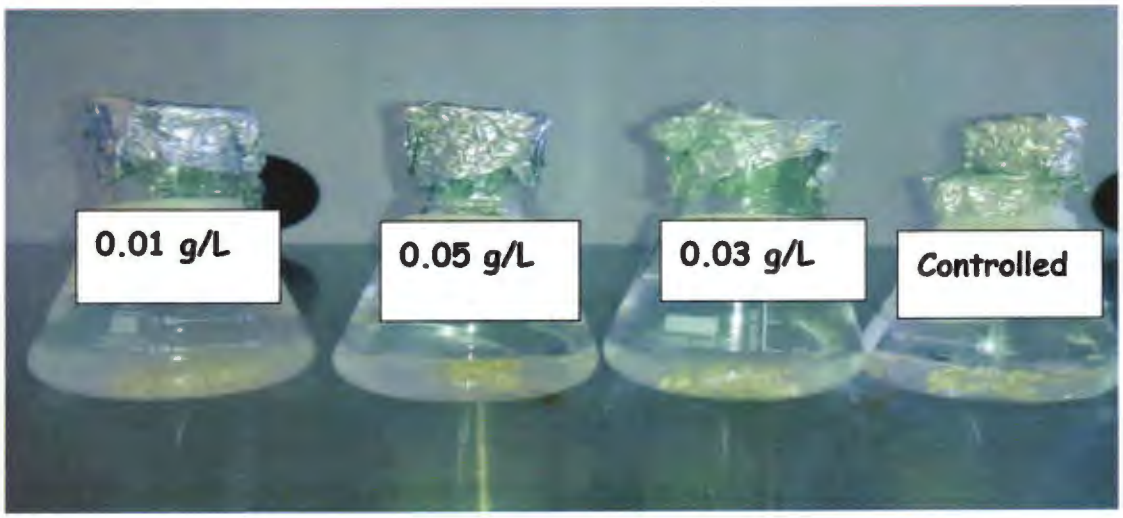

Fig.3.3: Nanoparticle suspensions.

3.2.6. Treatment of seeds with nanoparticle suspension:

20 seeds were taken for each treatment. There were total three treatments (i.e. 0.05, 0.03 and 0.01 gL^{-1}) and one controlled. Seeds were kept for seven to eight hours in nanoparticle suspension.

3.2.7. After treatment

After treatment with nanoparticle suspension seeds were taken out by using funnel in which whatt man filter paper was fixed.

i. Seeds along with filter paper were taken out and from the funnel.
ii. Seeds were dried on another filter paper.

3.2.7. Preparation of pots

After treatment with nanoparticle suspension seeds were shifted to pots. About 40 pots were taken and filled with peat moss, soil and sand in ratio of 1:1:1.the pots were kept in green house.

3.2.8. Sowing of seeds

i. Ten pots were taken for each treatment.
ii. 10 seeds from each treatment were taken and sown in pots. There was one seed per pot.

iii. Seeds were kept at a depth of 1-2 cm and seeds were cover with the soil.
iv. Pots were irrigated regularly.
v. Seeds were germinated after 15 days.
vi. In ten pots untreated seeds as controlled were sown.
vii. The pots were regularly irrigated with care.

3.3. Observation of different parameters and morphological characters

- Germination %age
- Time of germination
- Height of plant
- NO. of leaves
- NO. of branches per plant
- NO. of days to flowering
- No. of days to fruiting
- No. of fruits per plant

Chapter 4

RESULTS

4. Results

In present study seeds of *Lycopersicum esculentum* variety money maker were treated with three concentrations ($0.01gL^{-1}$, $0.03gL^{-1}$ and $0.05gL^{-1}$) of ZnO nanoparticle. The plants were regularly examined for any kind of change .Following parameters were studied during the investigation:

- Germination percentage
- Time of germination
- Height of plant
- Number of leaves per plant
- Number of branches per plant
- Number of days to fruiting
- Number of fruits per plant

Plants were also examined individually to notice any kind of change in them i.e.

- Shape of flower
- Shape of fruit
- Size of fruit

4.1. Germination percentage

Germination percentage was determined after fifteen days after sowing by counting the number of seeds germinated.

Germination percentage was determined by using following formula:

$$\frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown} \times 100$$

There were sown total 10 seeds for each treatment. So there were total 4 sets of treatments three were nanoparticle treated and one was controlled. Germination percentage in controlled plants was 50%, in $0.01gL^{-1}$ germination %age was 80%, in $0.03gL^{-1}$ germination %age was 60% and in $0.05gL^{-1}$ germination %age was 50%. In lower concentration that is $0.01gL^{-1}$ germination rate was high as compared to other treatments and controlled as shown in Table 1.

4.2. Time of germination

Time of germination was recorded when first seedling appeared in pot. Pots were regularly irrigated and observed. In untreated (controlled) pots germination started after 15 days of sowing. The seeds treated with 0.01 gL^{-1} germinated after 13 days of sowing. In case of 0.03 gL^{-1} treated seeds germination started after 13 days. In $0.05gL^{-1}$ treatment seedlings appeared after 14 days of sowing. The seeds treated with 0.01 and 0.03 gL^{-1} concentration were germinated earlier than controlled and 0.05 gL^{-1} treatment concentration as shown in Table 2.

Table 4.1: Germination percentage in controlled and ZnO nanoparticles treated seeds of *Lycopersicum esculentum.*

S.NO.	Treatment	Germination %
1	Controlled	50%
2	0.01 gL^{-1}	80%
3	0.03gL^{-1}	60%
4	0.05gL^{-1}	50%

Table 4.2: Time of germination in controlled and ZnO nanoparticles treated seeds

S.NO.	Treatment	Time of Germination
1	Controlled	15 days
2	0.01 gL^{-1}	13 days
3	0.03 gL^{-1}	13 days
4	0.05 g L^{-1}	14 days

4.3. Height of plant

Height of plant was measured with scale when the age of plant was 60 days. Graphically, average height of plant is shown in figure 4.1 and variances are given in Table 4.3 and comparison of height of controlled and treated plants is given in figure 4.2,4. 3, 4.4 and 4.5.

Fig.4.1 shows the average height of plants. In case of control, height of 8 plants was measured. Height ranged from 15cm-25cm. Average height of plant was 19.8cm and variance was 10.41. In case of $0.01gL^{-1}$ treatment, height of 7 plants was measured and ranged from 27cm-47cm. Average height of plant was 36.71 cm and variance was 60.571.

In case of 0.03 gL^{-1} treatment, height of total 9 plants was measured. Height ranged from25cm-45cm. In this case average height of plant was 36.66cm and variance was 42.75. In case of $0.05gL^{-1}$ treatment height of total 8 plants was measured. In this case height ranged from 20cm-35 cm. Average heights of plants was 29.25cm in this case and variance was 32.7.

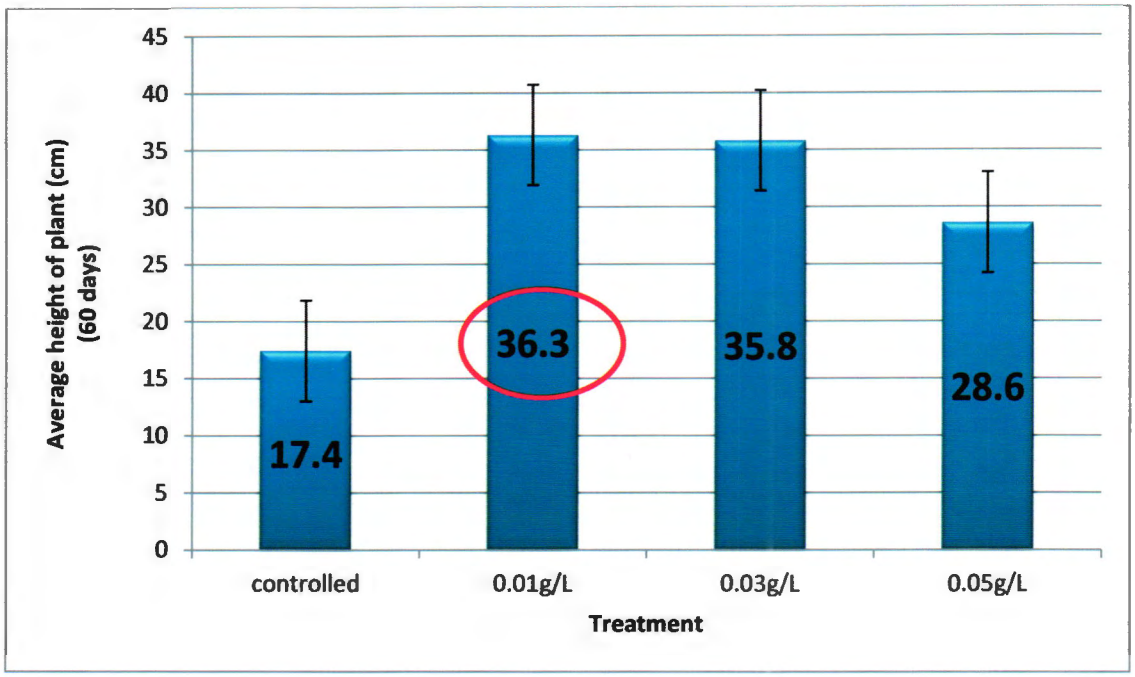

Fig.4.1. Average height of plant in controlled and nanoparticle treated seeds

Fig.4.2.comparison of height of controlled and nanoparticle treated plants after 60days

Fig.4.3. comparison of controlled and 0.01 g/ L treatment plants.

Fig.4.4.controlled and 0.05g/L treatment plant comparison.

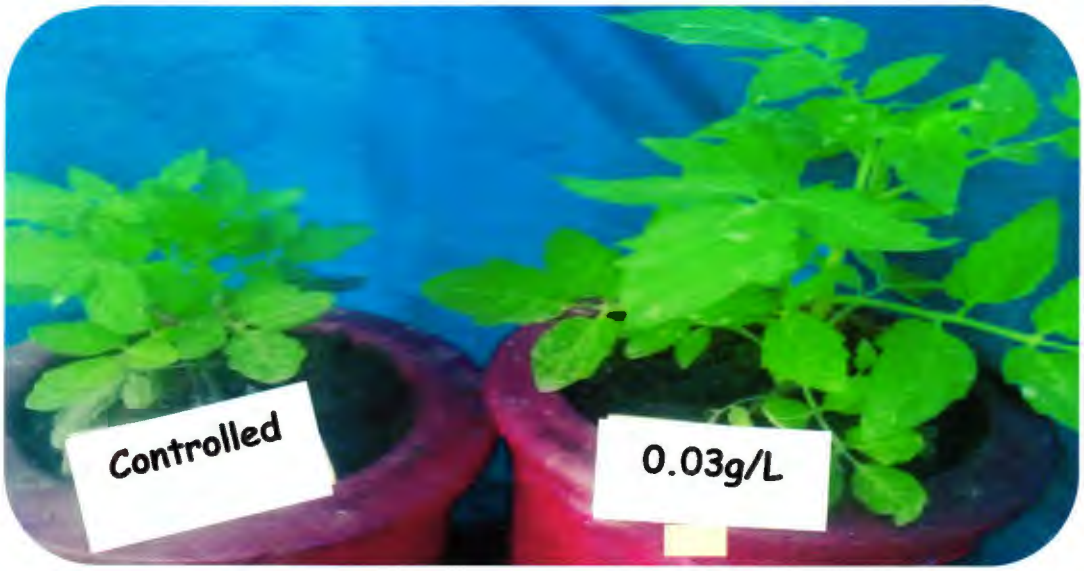

Fig.4.5.Comparison of controlled and 0.03 g/L treatment plant.

4.4. Number of leaves and number of branches

Numbers of leaves of plants were counted when the age of plant was 90 days. Statistically average number of plant and variances were calculated. Comparison of average number of leaves per plant in controlled and nanoparticle treated plants is given in Fig.4.6. and variances are given in table 4.3.

In case of controlled number of leaves of total 5 plants were counted and number ranged from 20-90. Average number was 59.6 in this case and variance was 953.3. In $0.01gL^{-1}$ concentration treated plants numbers of leaves of total 9 plants were counted. Number of leaves in this case ranged from 80-178. Average number in this treatment was 142.11and variance 1375.86. In case of $0.03gL^{-1}$ treated plants number of leaves of total 8 plants was calculated. Number ranged from 70-150. Average number in this case was 104.625 and variance 833.69.

In $0.05gL^{-1}$ concentration treatment leaves of total 6 plants were calculated. Numbers of leaves in this case ranged from 35-104 in this case. Average number of leaves in this case was 69.66 and variance was 744.66.

Numbers of branches were calculated after 90 days. Statistically average and variance was calculated. Comparison of average number of branches per plant in controlled and treated plants is given in fig4.7 and variances are given in table 4.3.

In controlled untreated plants number of branches of 5 plants was calculated. Number of branches in controlled plants ranged from 5-14. In case of controlled group average number of branches was 10 and variance in case was 15. In case of $0.01gL^{-1}$ concentration treated plants number of branches of total 8 plants was calculated. Number ranged from 10-18. In this case and average was 13.66 and variance 7.25.

In $0.03gL^{-1}$ treatment group number of branches of 8 plants was calculated. In this case number of branches ranged from 9-17. Average number in this case was also 13.25 and variance in this case was 7.92. In $0.05gL^{-1}$ concentration treated plants total 6 plants were selected to calculate the number of branches, in this treatment group number ranged from 8-16 and average was 11.83 and variance in this was 9.37.

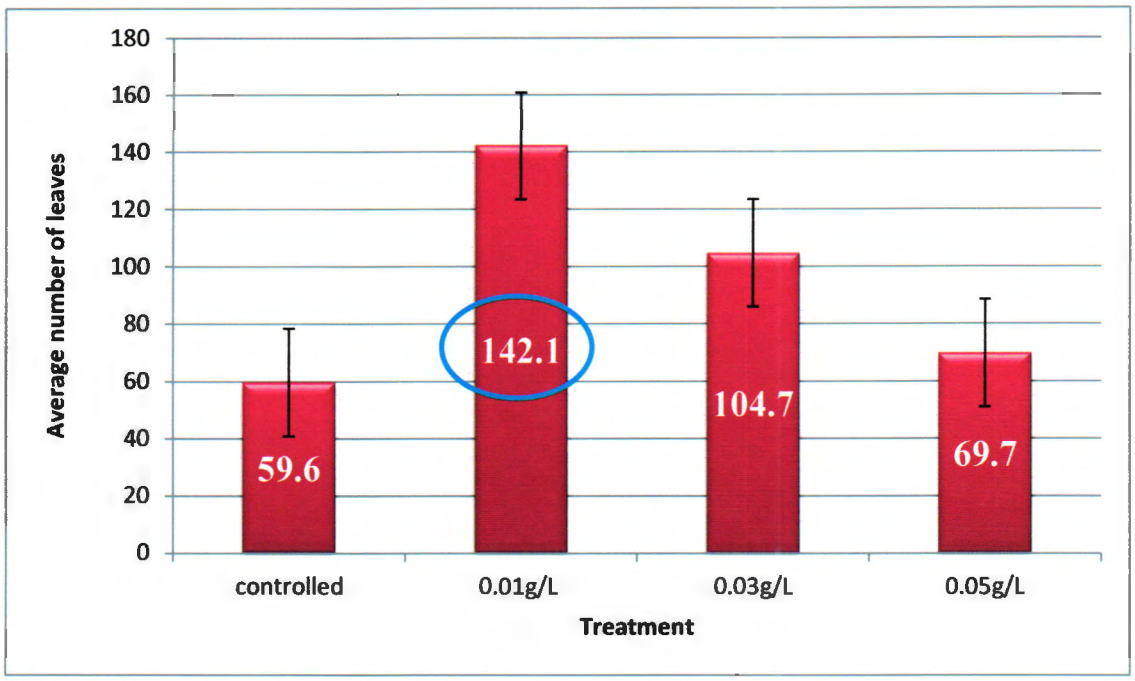

Fig.4.6.Average number of leaves per plant in controlled and treated plants

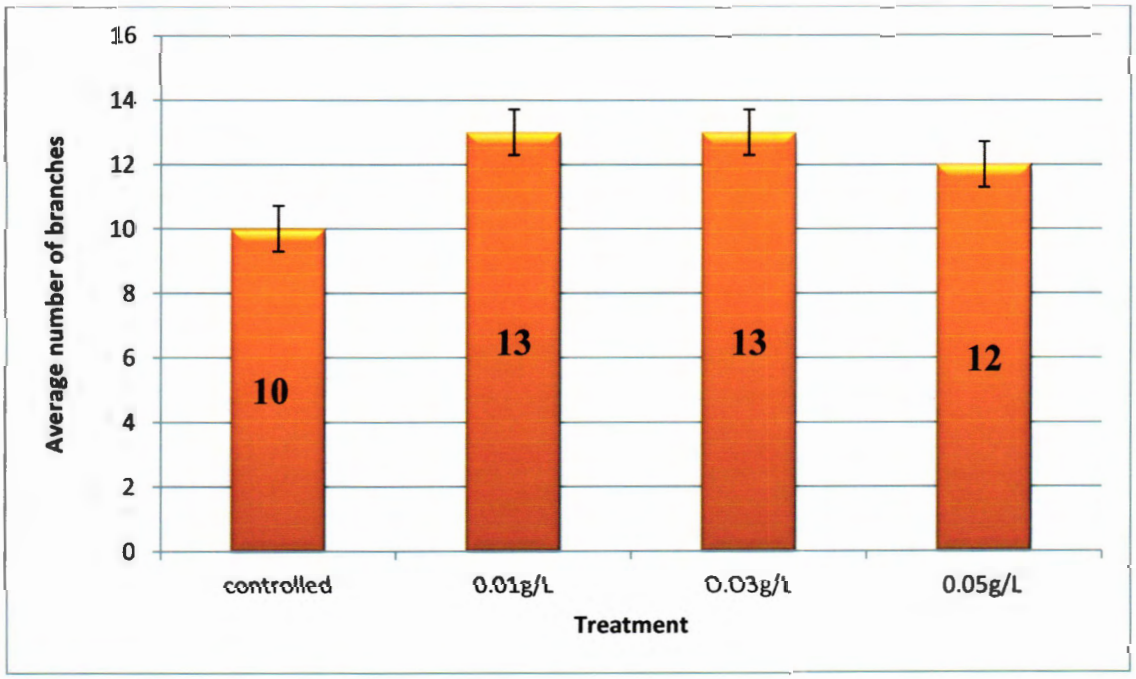

Fig.4.7. Average number of branches per plant in controlled and treated plants.

4.5. Number of days to flowering

Days to flowering were observed and recorded from the date of sowing to the appearance of first flower on plant. Average and variance was calculated for each treatment group. Comparison of average between each treatment group is shown in Fig 4.8 and variances are given in table 4.3.

In controlled group, flowering started after 120 days. Days to flowering in controlled group ranged from 120-125 days in 3 plants. Average days to flowering were 122.66 days and variance was 6.33. In $0.01gL^{-1}$ treatment group, flowering started after 90 days. Flowering ranged from 90-99 days in 9 plants. In this case average was 95.55 days and variance was 10.77.

In case of $0.03gL^{-1}$ concentration treatment group, flowering started after 93 days. Days to flowering of 7 plants in this case ranged from 93-105 days. Average days to flowering were 98.71 days and variance 17.90. In $0.05gL^{-1}$ treatment group, flowering started after 95 days. Days to flowering of 7 plants in this case ranged from 95-105 days. Average days to flowering were 100 days and variance was 12.33.

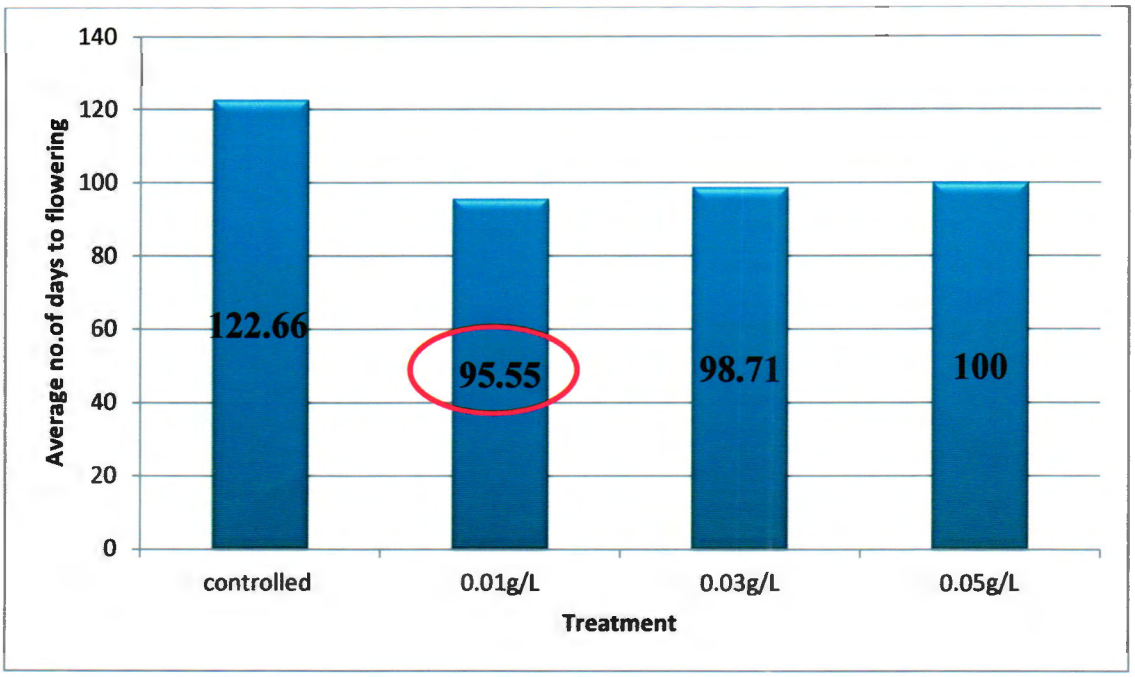

Figure.4.8: Average number of days to flowering.

4.6. Number of days to fruiting

Days to fruiting were recorded from the date of sowing to the appearance of first fruit on the plant. Variances and averages were calculated to find out average number of days to fruiting per plant and variation found in each group. Average numbers of days to fruiting are given in 4.9 and variances are given in table 4.3.

In control group, days to fruiting of 3 plants ranged from 131-140 days. In this case average days to fruiting and variance were 135.66 and 20.33 respectively. In 0.01 gL^{-1} treated group, days to fruiting of 9 plants ranged from 100-113 days. In this case average days to fruiting were 105.77 days and variance was 20.69. In 0.03 gL^{-1} treatment group number of days to fruiting of 5 plants ranged from 104-119 days. Average number of days to fruiting and variance in this case were 110.6 days and 41.3 respectively.

In case of 0.05 gL^{-1} treatment group, number of days to fruiting of 6 plants ranged from 106-120 days. In this case average number of days to fruiting was 112.6 and variance was 27.46. Maximum number of variation among all the treatment groups was seen in0.03 gL^{-1} treatment group.

4.7. Number of fruits per plant

Number of fruits of treated and untreated plants was counted. Statistically average and variances were calculated. Average number of fruits per plant is given in 4.10 and variances are given in table 4.3.

In case of controlled group numbers of fruits of 8 plants ranged from 4-15. In this case average number of fruit per plant and variance were 9.375 and 14.55 respectively. In case of 0.01 gL^{-1} concentration treatment group, number of fruits of 9 plants was counted. Number of fruits ranged from 9-22. In this case average number of fruits was 15.88 and variance was 21.611. In case of 0.03 gL^{-1} treatment group, number of fruits of 8 plants was ranged from 8-20. Average number in this case was 14.125 and variance was 19.267.

In 0.05 gL^{-1} concentration treatment group, number of fruits of 6 plants ranged from 5-19. Average number of fruits and variance in this case was 12 and 32.8 respectively.

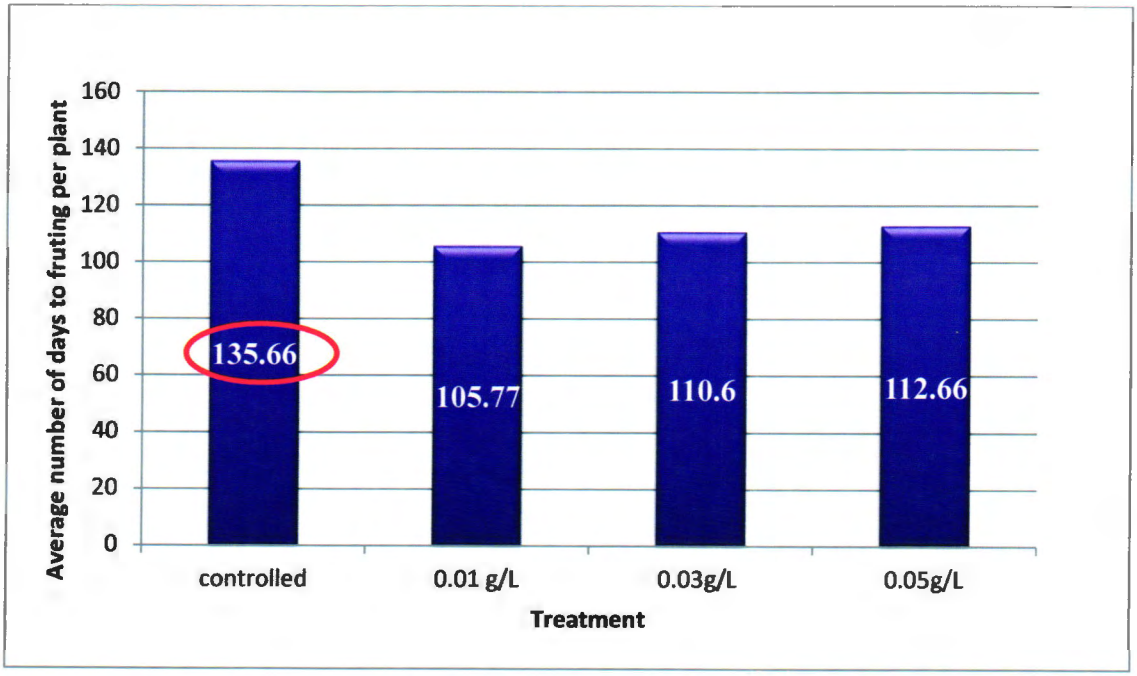

Fig.4.9: Average number of days to fruiting per plant.

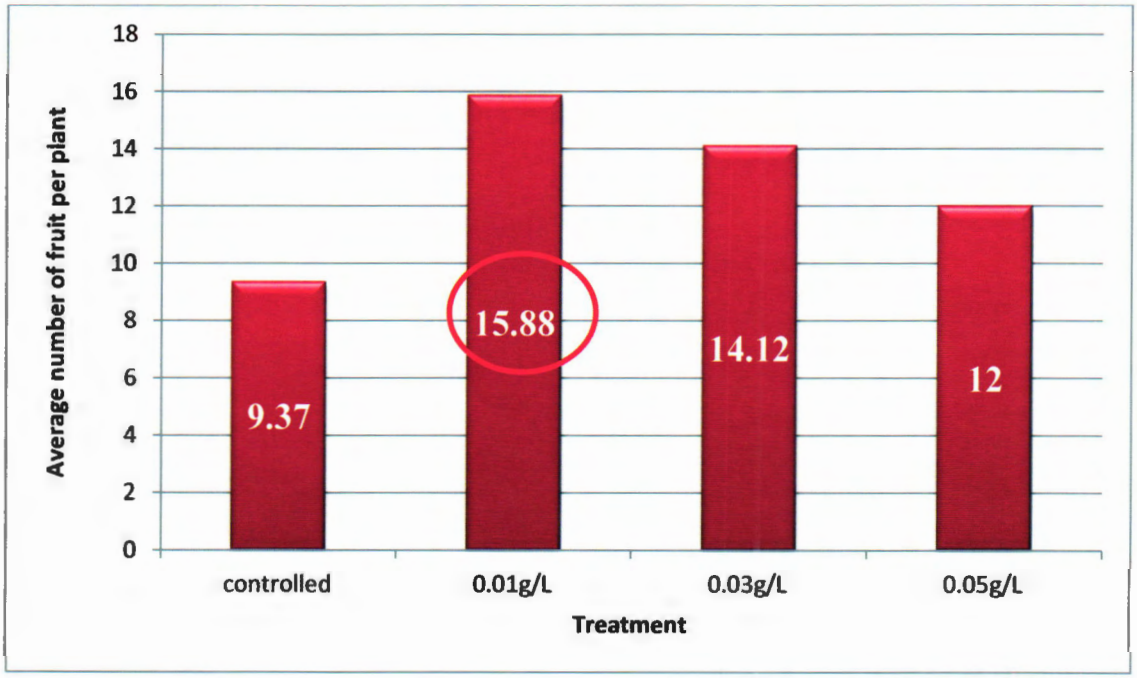

Fig.4.10. Average number of fruit per plant in treated and controlled plants.

Table4.3: Variances of different morphological characters in treated and untreated plants of *Lycopersicum esculentum.*

Treatment	Height of plant	Number of branches	Number of leaves	Days to flowering	Days to fruiting	Number of fruits /plant
Controlled	10.41	15	953.3	6.33	20.33	14.55
$0.01gL^{-1}$	60.57	7.25	1375.86	10.77	20.69	21.61
$0.03gL^{-1}$	42.75	7.92	833.96	17.90	41.3	19.26
$0.05gL^{-1}$	32.7	9.36	744.66	12.33	27.46	32.8

4.2. Comparison of shape of flower of $0.01gL^{-1}$ treated with controlled plant

During the study of morphology of *Lycopersicum esculentum* (Tomato) it was noticed that flower of $0.01gL^{-1}$ treatment group has 6 petals while the controlled and other treatment groups i.e. $0.03gL^{-1}$ and $0.05gL^{-1}$ has 5 petals. This investigation shows that ZnO nanoparticles at lower concentration had affected the plant at genetic level and this affect produced the 6 petaled flowers which in turn produced the big sized fruit on plant. Comparison of flowers of $0.01gL^{-1}$, $0.03gL^{-1}$, $0.05gL^{-1}$ and controlled group flowers are shown in Fig.4.11 and Fig.4.12.

4.2.1. Comparison of fruits of controlled and treatment groups

Fruits of controlled and treated plants were compared and it was noticed that fruit of 0.01g/L treatment group was bigger than all other treatment groups. Comparison of fruits of controlled and treated groups is shown in Fig.4.13, fig.4.14, fig 4.15, fig. 4.16.

4.2.2. Controlled dwarf and sterile plants

During the investigation 2 plants in controlled group were noticed as dwarf and sterile that they were shorter in height and were with no flowers and fruits. Controlled dwarf and sterile plants are shown in Fig.4.17.

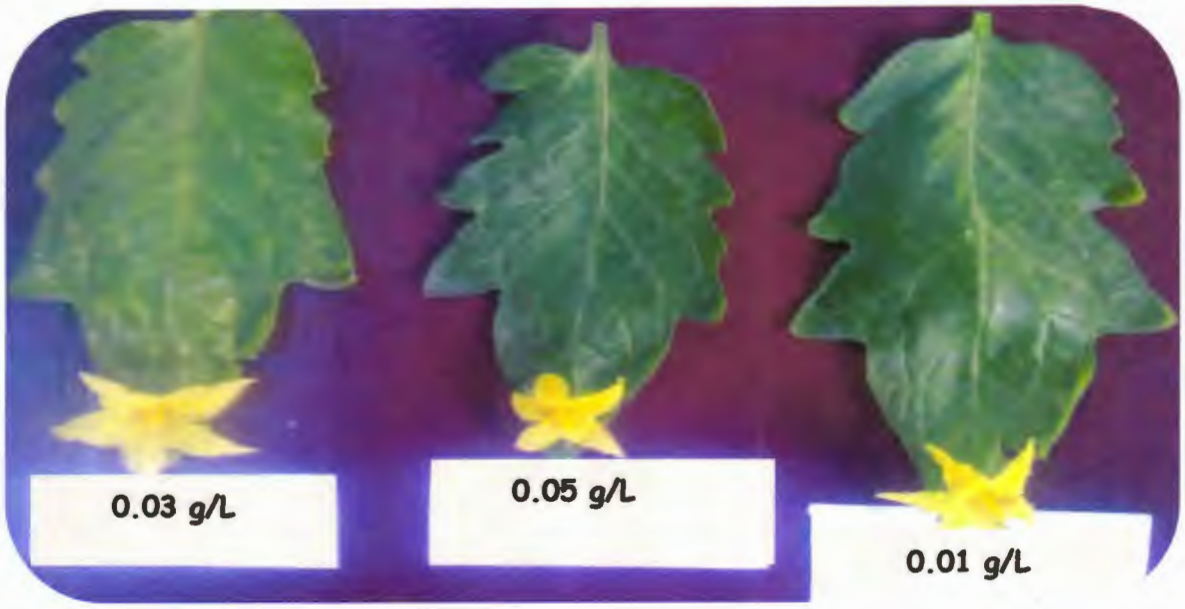

Fig.4.11. Comparison of leaf and flower of treated plants.

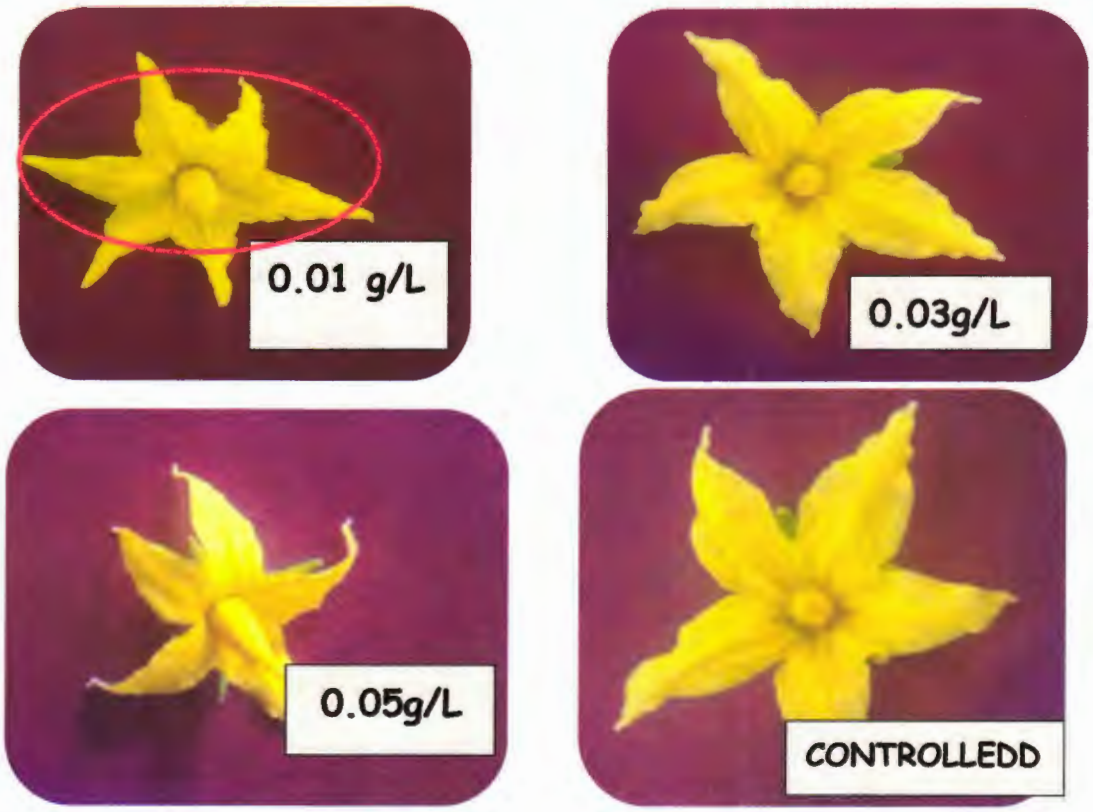

Fig.4.12. Comparison of flower of controlled and treated plant

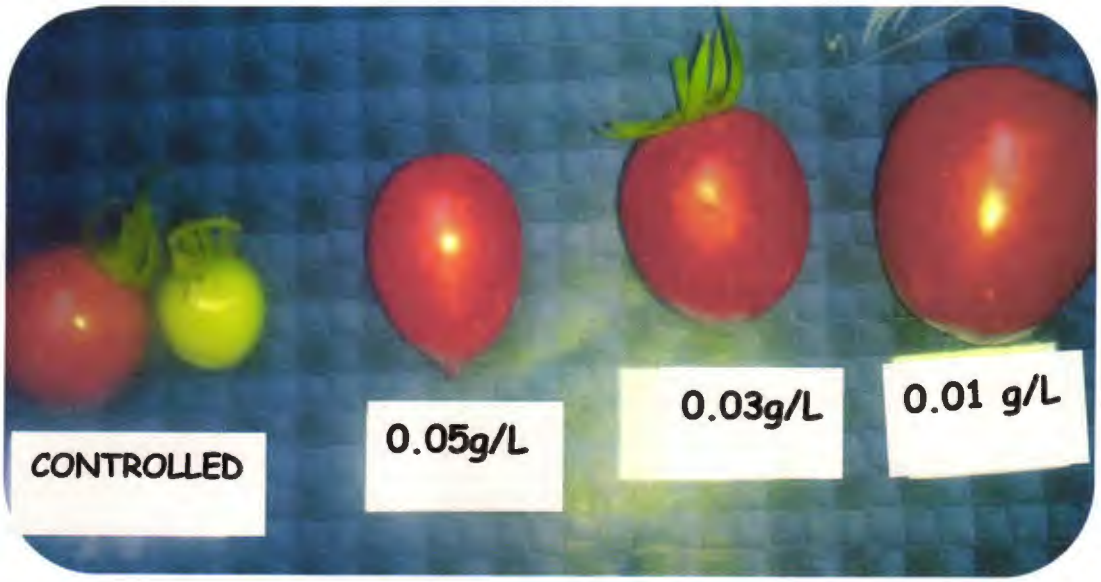

Fig.4.13. Comparison of fruits of controlled and treated groups.

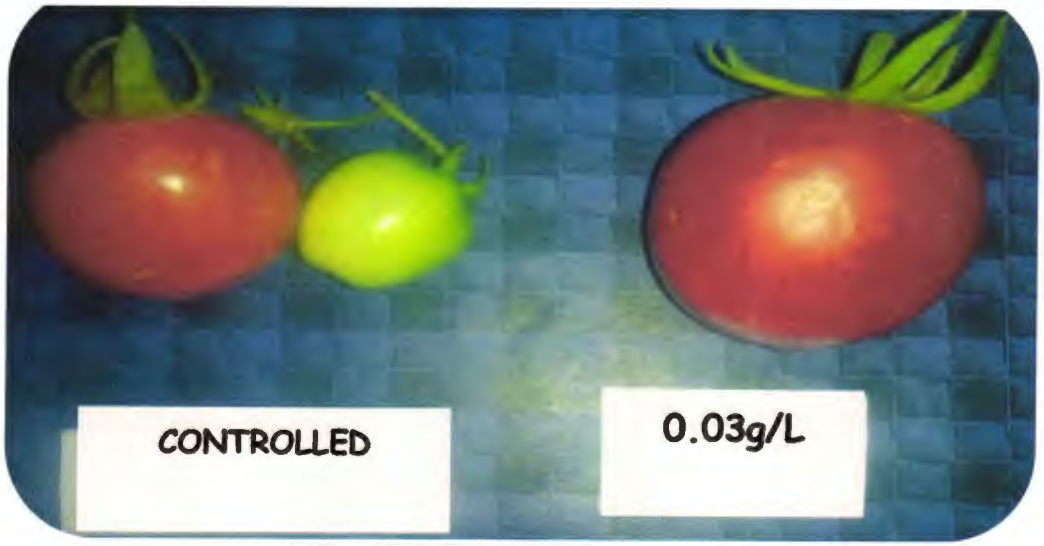

Fig.4.14.Comaparison of fruit of controlled and o.o3g/L treated plant.

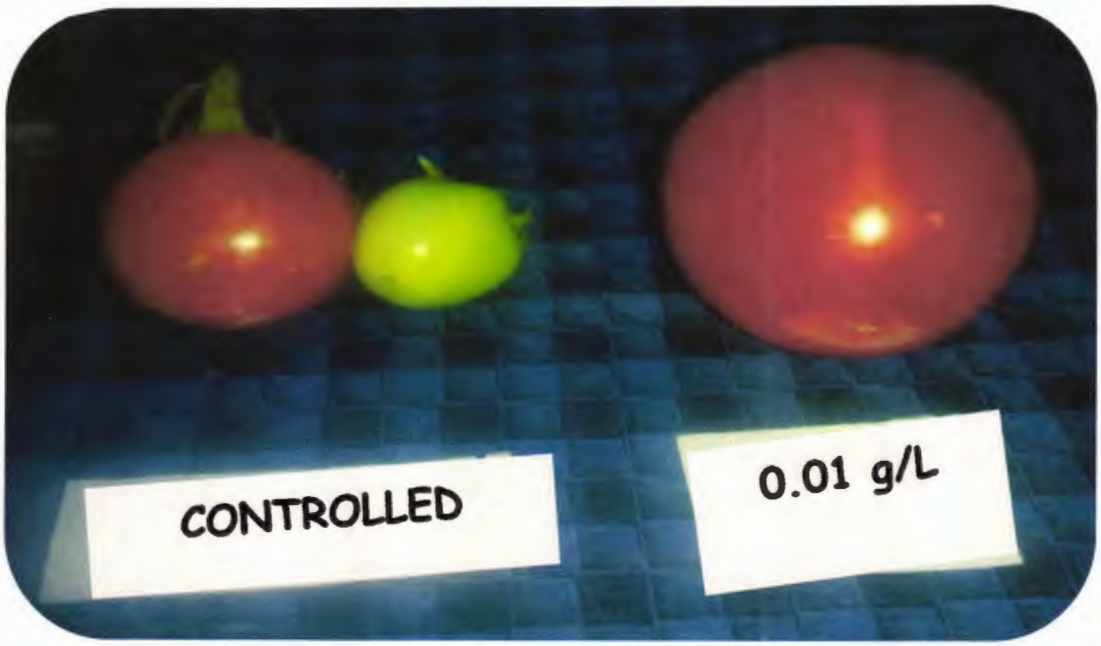

Fig.4.15.Comparison of fruit of controlled and 0.01g/L treated plant.

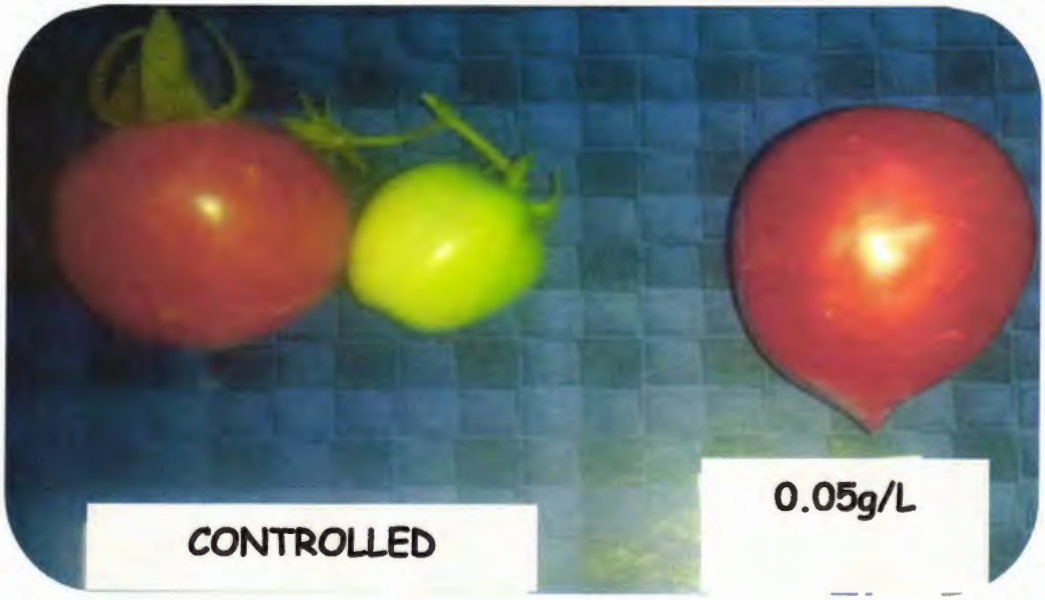

Fig.4.16.Comaprison of fruit of controlled and 0.05g/L treated plant.

Fig.4.17. Controlled sterile and dwarf plants

Chapter 5

DISCUSSION

5. Discussion

The present study was designed to investigate the effects of different concentrations of ZnO nanoparticles treatment on *Lycopersicum esculentum* (tomato variety money maker). For this purpose seeds of *Lycopersicum esculentum* were treated with three different concentrations ($0.01gL^{-1}$, $0.03gL^{-1}$, $0.05\ gL^{-1}$) of ZnO nanoparticle. These treated plant groups were compared with controlled (untreated) group plants.

During comparison of treated and untreated groups it was found that ZnO nanoparticle is effective when it is used in smaller concentration. In present study, seeds which were treated with smaller concentration i.e. $0.01gL^{-1}$ resulted in the increased in germination percentage and decrease in time of germination as compared to $0.03gL^{-1}$ and $0.05gL^{-1}$. These findings are similar to some previous studies carried out on different crops treated with ZnO nanoparticle.

A study carried on the effects of ZnO nanoparticle on mungbean Plant. The mungbean treated with ZnO nanoparticle at 50 and 100 mg treated showed increased seed germination percentage, root and shoot length (cm) and also root dry weight (gm.).This study concluded that this happens because ZnO nanoparticles promoted germination of seeds and seedling growth as compared to controlled. But seeds treated with 150 mg concentration showed a significant decrease in seed germination (Jayarambabu et al., 2015).

ZnO nanoparticle also played an important role in the growth and development in the peanut plant. Root length, seed germination, seedling vigor index and yield at 1000 ppm showed significant increment as compared to controlled or untreated seed. But at higher concentration of 2000ppm all these parameters showed a significant decrease (Prasad et al., 2012).

According to another study about effects of ZnO nanoparticle on onion plant also showed the same results as above that ZnO nanoparticles imparted a positive effect on onion plant. ZnO nanoparticles at the concentrations of 20, 30 and 40 µg/L showed an increase in plant height and number of leaves. Days to flowering also reduced in treated plants as compared to controlled plants. This study also showed that the ZnO

nanoparticles at low concentrations i.e. 20 and 30µg/L showed best results as compared to 40 µg/L (Laware and Raskar 2014).

Above studies and present study suggested the same results that ZnO nanoparticle at smaller concentrations not only give increased germination rate but also gives the maximum numbers of variances.

In present study, most of the characters such as number of leaves, number of branches, time to flowering, time to fruiting and number of fruits per plant minimum variances were observed in controlled plants and maximum variances were observed in treated plants. Among the treated plants maximum variances were observed mostly in the 0.01g/L concentration that was the smaller concentration and minimum in 0.03g/L and 0.05g/L.

Conclusion and future prospects

Nanotechnology has the potential to revolutionize the agriculture in many ways. Nanoparticles are used now-a-days to improve the crop yield. ZnO among these are gaining much importance. In present study it is investigated that nanoparticles can be used as Nano fertilizer and enhanced the tomato crop yield at lower concentrations i.e. $0.01gL^{-1}$ as compared to higher concentrations. So ZnO used in small amounts can enhance the plant growth and fruit yield. Agricultural scientist can prepare seeds treated with ZnO to make them available to farmers so that they can use these seeds to grow the tomato with best and improved yield. Nanotechnology in future will be used as agricultural tool to yield as much food for as much population increasing day by day in the whole world.

Chapter 6

REFERENCES

6. References

Alloway B.J., (2004), Zinc in Soil and Crop Nutrition, International Zinc Association. Brussels, Belgium

Bali Y and Lind H., (2007), Annals of Botany, 100, p. 1085–1094

Bao-shan L., Shao-qi D., Chun-hui L., Li-jun F., Shu-chun Q., Min Y., (2004), Effect of TMS (nanostructured silicon dioxide) on growth of Changbai larch seedlings, Journal of Forest Research, 15, p.138–140

Block G.B., Patterson B and Subar A., (1992). Fruit, vegetables and cancer prevention: a review of the epidemiological evidence, 18, p. 1-29

Brennan R.F., (2005), Zinc Application and Its Availability to Plants. Ph. D. dissertation, School of Environmental Science

Cakmak I., (2000), Role of zinc in protecting plant cells from reactive oxygen species, New Phytologist,146, p.185–205

Chowdappa P and Gowda S., (2013), Nanotechnology in crop protection Status and scope, Pest Management in Horticultural Ecosystems, 19(2), p .131-151

Daniel M-C and Astruc D., (2004) Gold nanoparticles assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology,ChemicalReviews,104, p.293–346

Disante KB, Fuentes D and Cortina J., (2010), Response to drought of Zn-stressed Quercus suber L. Seedlings. Environmental and Experimental Botony, 70, p.96-103

Emerich D.F and Thanos C., (2003), Nanotechnology and medicine, 3, p.655–663

Fakruddin M.D., Hossain Z and Afroz H., (2012), Prospects and applications of nanobiotechnology a medical perspective, Journal of Nanobiotechnology

Gelmesa D., Abebie B and Desalegn L., (2012), African Journal of Biotechnology , 11(51), p. 11200-11206

Gerster H., (1997), The potential role of lycopene for human health, Journal of American College of Nutrition, 16, p.109-126

Gillen M. V., (2011), Natural Resources and Environment ABA section of environment, energy, and resources, 26,p.1

Hafeez B., Khanif M.K and Saleem M., (2013), Role of zinc in plant nutrition, American Journal of Experimental Agriculture 3(2), p.374-391

Haghighi M., Afifipour Z., Mozafarian M., (2012), The effect of N-Si on tomato seed germination under salinity levels, Journal of Biodiversity and Environmental Sciences, 6, p.87–90

Jayarambabu N and Siva kumara B., (2015) Beneficial role of zinc oxide nanoparticles on green crop production, International Journal of Multidisciplinary Advanced Research Trends, 2(1)

Jha Z., Behar N., Sharma NS., Chandel G., Sharma K.D., And Pandey .P.M, (2011) Nanotechnology prospects of agriculture advancement, Nano Vision, 1(2), p.88-100

Joseph T and Morrison M., (2006), Nanoparticles on Photosynthetic Fungi, 93, p.1037-346

Kalloo G., (1991). Genetic Improvement of Tomato, monographs of Theoretical and Applied Genetics Springer-verlag, Berlin, Heidelberg, New York, p. 1-9

Kalteh M., Alipour Z.T., Ashraf S., Aliabadi M.M., Nosratabadi A.F., (2014), Effect of silica nanoparticles on basil (*Ocimum basilicum*) under salinity stress, Journal of Chemical Health Risks, 4, p.49–5

Kasim W.A., (2007), Physiological consequences of structural and ultra-structural changes induced by Zn stress in *Phaseolus vulgaris* Growth and Photosynthetic apparatus, International Journal of Botony, 3(1), p.15-22

Kato H.,(2011) In vitro assays tracking nanoparticles inside cells, Nature nanotechnology,6(3) p.139–140

Kisan B., Shruthi H., Sharanagouda H., Revanappa S.B., Pramod N.K., (2015), Effect of Nano-Zinc Oxide on the Leaf Physical and Nutritional Quality of Spinach, Agrotechnol, 5(1)

Laware L.S and Raskar S., (2014), Influence of Zinc Oxide Nanoparticles on Growth, Flowering and Seed Productivity in Onion, International Journal of Current Microbiology and Applied Sciences,3(7), p.874-881

Lu C.M., Zhang C.Y., Wen J.Q., Wu G.R., Tao M.X., (2002), Research on the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism, Soybean Science, 21, p.68–172

Mamidala P., Praveen and Nanna S.R., (2011), International Journal of Genetics and Molecular Biology, 3(3), p. 45-50

Manzer H. Siddiqui and Al-Whaibi H.M., (2014), Role of nano-SiO2 in germination of tomato (*Lycopersicum esculentum seeds Mill.*), Saudi Journal of Biological Sciences, 21, p.13–17

Peck A.W and McDonald G.K., (2010), Adequate zinc nutrition alleviates the adverse effects of heat stress in bread wheat, Plant Soil, 337, p.355-374

Prasad T.N.V.k.V., Sudhakar P., Sreenivasulu Y., Latha P., Munaswamy V., Reddy R.k., Sreeprasad T.S., Sajanlal P.R & Pradeep.T.,(2012), Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut, Journal of Plant Nutrition, 35, p.905–927

Raliya R., Nair R., Chavalmane S., Wang W-N and Biswas P., (2015), Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum L.*) plant, Metallomics

Rico C.M., Majumdar S., Gardea M.D., Peralta-Videa J.R and Dardea-Torresdey J.L ., (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain, Journal of Agricultural and Food Chemistry, 59, p. 3485–3498

Sabir S., Arshad M and Chaudhari S.K., (2014), Hindawi Publishing Corporation Scientific World Journal, Zinc Oxide Nanoparticles for Revolutionizing Agriculture: Synthesis and Applications

Sah K.S., Kaur A and Wani S.H., (2014), nanobiotechnology changing horizons of science, biolife, 2(3), p.905-916

Sahoo K.S and Labhasetwar ., (2003), Nanotech approaches to drug delivery and imaging, 8(24), p.1112–1120

Siddiqui M.H and Al-Whaibi M.H., (2014), Role of nano-SiO2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.), Saudi Biological science, 21, p.13–17

Siddiqui M.H., Al-Whaibi M.H., Faisal M., Al Sahli A.A., (2014), Nano-silicon dioxide mitigates the adverse effects of salt stress on Cucurbita Pepo L., Environmental Toxicology and Chemistry, 33(11), p.2429– 2437

Smith and Andrew F., (2003), Encyclopedia of Food and Culture

Suriyaprabha R, Karunakaran G, Yuvakkumar R, Rajendran V, Kannan N (2012) Silica nanoparticles for increased silica availability in maize (Zea mays L) seeds under hydroponic conditions. Curr Nanosci 8: p.902–908

Tavallali V., Rahemi M., Eshghi S., Kholdebarin B and Ramezanian A., (2010), Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (Pistacia vera L. 'Badami') seedlings, Turk. Journal of agriculture forest, 34(4), p.349-359.

Xie Y., Li B., Zhang Q., Zhang C., (2012), Effects of nano-silicon dioxide on photosynthetic fluorescence characteristics of Indocalamus barbatus Mc.Clure, Journal of Nanjing Forest University (Natural Science Edition), 2, p.59–63

Xie Y., Li B., Zhang Q., Zhang C., (2012), Effects of nano-silicon dioxide on photosynthetic fluorescence characteristics of Indocalamus barbatus Mc.Clure, Journal Nanjing Forest University (Natural Science Edition), 2, p.59–63