# *In Vitro* Influence of Selected Nanoparticles on Growth And Biological Activities of *O. Basilicum L*


**By**

**ARHAM SUMAYA**

**DEPARTMENT OF BIOINFORMATICS AND BIOTECHNOLOGY**

**FACULTY OF BASIC AND APPLIED SCIENCES**

**INTRNATIONAL ISLAMIC UNIVERSITY ISLAMABAD, PAKISTAN**


**2016**

Medicinal Plants

Biology

# *In Vitro* Influence of Selected Nanoparticles on Growth and Biological Activities of *O. Basilicum L.*


**Researcher**

**Arham Sumaya**

**120-FBAS/MSBT/F14**

**Supervisor**

**Dr. Nyla Jabeen**

**Assistant professor**

**Department of Bioinformatics & Biotechnology**

**Faculty of Basic and Applied Sciences**

**International Islamic University Islamabad**


**(2016)**


بسم الله الرحمن الرحيم

## Department of Bioinformatics and Biotechnology

## International Islamic University Islamabad

Dated: 11-Nov-2016

# FINAL APPROVAL

It is certificate that we have read the thesis entitled "*In vitro* influence of selected Nanoparticles on growth and biological activities of *O. basilicum*" submitted by Ms. Arham Sumaya 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 Biotechnology.

**External Examiner**

Dr. Sajid Mehmoood

Associate Professor

University of Gujrat

**Internal Examiner**

Dr. Naveeda Riaz

Associate Professor

Department of Bioinformatics and Biotechnology

International Islamic University, Islamabad

**Supervisor**

Dr. Nyla Jabeen

Assistant professor

Department of Bioinformatics and Biotechnology

International Islamic University, Islamabad

**Dean, FBAS**

International Islamic University, Islamabad

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

# DEDICATION

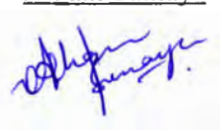
**I dedicated this effort to my Family, Friends, Teachers and Country.**

# 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 11-Nov-2016

Arham Sumaya



# Contents

# ACKNOWLEDGEMENTS

In the name of ALLAH the most Beneficent and the most Merciful, all the praise belongs to ALLAH (SWT) who gave us wisdom and courage to complete this research.

I would like to express my sincere gratitude to my respected supervisor Dr. Nyla Jabeen for her keen interest and guidance during every step of research.

I would also like to express my deep gratitude to my Khala Tabinda Shahid and Maliha Nasir for their support and care. I would extend my gratitude to my cousins Rimsah Shahid, Muznah Fahad, Uzair Majid and Shaheer Tahir for their help and support. Last but not the least I recognized my Parents and Nana Jan for their firm support.

**Arham Sumaya**

# LIST OF ABBREVIATIONS

| | |
|---|---|
| 2,4 D | 2,4 Dichlorophenoxyacetic acid |
| 2-iP | 2-Isopentenyl adenine |
| BA | 6-Benzyladenine |
| BAP | 6-benzylaminopurine |
| Conc. | Concentration |
| IAA | Indole-3-acetic acid |
| IBA | Indole-3-butyric acid |
| Kn | Kinetin |
| NAA | a-Naphthalene acetic acid |
| NaOH | Sodium hydroxide |
| NPs | Nanoparticles |
| MS | Murashige and skoog medium |
| mins | Minutes |
| TDZ | Thidiazuron |
| ZnO | Zinc Oxide |

# LIST OF FIGURES

# LIST OF TABLES

# Abstract

Nanoparticles have gathered large interest due to their novel applications in field of biotechnology, nanomedicines, environmental sciences and agriculture. Zinc oxide Nanoparticles has wide application in many consumer products from cosmetics industries to textiles. However there is not enough research on effect of ZnO nanoparticles on plant germination and shoot regeneration. This research successfully investigated *in vitro* effect of ZnO on seed germination, micropropogation, phytochemicals and antibacterial activity of *Ocimum basilicum L.* Two concentrations of Zinc oxide nanoparticles i.e. 0.05µl/ml and 0.1 µl/ml were tested to evaluated ZnO NPs effect on *O. basilicum* and compared with control. Result suggested that 0.05 µl/ml conc. increased seed germination of sweet basil while germination rate was decreased significantly in higher conc. of nanoparticles. Case was opposite for shoot regeneration in which 0.1 µl/ml conc. tremendously increased shoot regeneration followed by 0.05 µl/ml nanoparticles conc. while control plant showed slow shoot initiation. Antibacterial activity of nanoparticles extract increased as compared to the control plants. 0.05 µl/ml have maximum zone of inhibition of 15mm followed by 12mm of 0.1 µl/ml, control plant extract has lowest zone of inhibition of 8mm. Phytochemical analysis showed absence of Alkaloids, phenols, terpenoids, saponins and steroids in all extracts.

# Chapter 1

# INTRODUCTION

# 1. Introduction

## 1.1 Nanotechnology

Nanoparticles have very large surface area and show different behavior from their counter bulk materials due to high surface energy, this gives nanoparticles novel properties. Research has been made in every aspect on nanotechnology (Abdul Salam *et al.*, 2014). They are able to change the field of agriculture, environment, medicine, food industry and water resources (Baruah and Dutta, 2009).

Nanoparticles are important materials; their chemical, biological, thermal and mechanical properties are different from bulk materials. They show shape and size dependent chemical and electrical properties (Iravani, 2011). NPs have many important applications in field of biotechnology which include gene delivery, drug targeting, medical imaging, cell labeling treatment of tumor, hyperthermia and anticancer activity (Yoshida and Kobayashi, 1999; Premanathan *et al.*, 2011; Cao *et al*, 2003; Vinardell and Mitjans, 2015).

The global market for nanobiotechnogy products was 10 billion in 2005 and it is estimated that it will reach $1 Trillion with in decade (Roco, 2005). There is a rapid growth in Nanobiotechnology industry which arise the importance to understand its effect to regulate their use and disposal. There is not much research on mechanism and effect of NPs on higher plants (Monica and Cremonini, 2009). It is of great importance to study NPs effect on higher plants, animals and microorganisms.

Application of different nanoparticles in nanomedicines and nanofertilizers arise the importance of Agri-nanotechnology. The benefit include site specific targeting of nanomedicines and efficient delivery of micronutrients which enable safe handling of insecticides and reduced exposure of harmful chemicals to the environment. Nanocapsules, NPs mediated plant transformation techniques, NPs application in plant pathology and crop protection has generated further interest in nanobiotechnology and agriculture (Nair *et al.*, 2010).

Understanding the effect of nanoparticles on plants in soil as well as *in vitro* is very important as Properties of NPs can change in soil and its effect on plant growth. Cytotoxity of nanoparticles in soil is reduced by decreased availability of nano ions in the soil, cytotoxity increase in agar medium due to higher concentration of nanoparticles (Lee *et al.*, 2012). Optimization of nanoparticles concentration *in vitro* is important to reduce the phytotoxicity of NPs and increase the germination with nanofertilizers.

### 1.1.2 Zinc oxide nanoparticles

Metal oxides nanoparticles has gathered wide interest, among them zinc oxide is very important due to its optical and electrical properties. Tons of ZnO NPs produced annually around the world by 300 companies, annual production for cosmetics is 1000 tones, and they have many commercial applications in consumer products i.e. cosmetics, lotions, ceramics, wastewater treatment and in processing of rubbers. It has many uses in industrial area such as electronics, solar cells, gas sensors and photo catalyst. ZnO NPs absorb ultraviolet radiations and thus used in cosmetics, its antibacterial activity make it odor resistant, its anticancer activity make it favorable for treatment of cancer and tumors. NPs are often released into environment which can cause harmful effect so research is necessary to understand the effect of ZnO NPs in seed germination and plant growth (Kumari *et al.*, 2011; Hackenbert *et al*, 2010; Abdul Salam *et al.*, 2014).

### 1.1.3 Antibacterial activity of Nanoparticles of ZnO

Zinc oxide nanoparticles have antibacterial activity against many bacterial species including *E. coli* and *Staphylococcus aureus* (Jones *et al.*, 2007). However mechanism of antibacterial activity is not fully understood. Various mode of action of NPs on bacterial strains were presented which include formation of ROS species, disruption of cellular process, interference with DNA replication enzymes or proteins (Duncan, 2011). Other mechanism include formation of super oxides and hydroxyl radicals on bacterial cell wall by ZnO, disrupting DNA and proteins and cause cell death in bacteria (Bajpai *et al.*, 2012).

## 1.1.4 NPs Mode of uptake in plant cells

Research revealed that transport mechanism, accumulation, uptake and penetration of nanoparticles depend upon composition, size and type of nanoparticles as well as plant species. Various mode of uptake of nanoparticles in plants have been presented, figure shows that NPs uptake in cell through endocytosis, ion channels, carrier proteins, binding through chemical or inducing pores. NPs are more reactive than their bulk counterparts because of their large surface area they can transport into cells by forming complexes with membrane proteins. Inside the cell they can transport with in cells through plasmodesmata (Rico *et al.*, 2011).

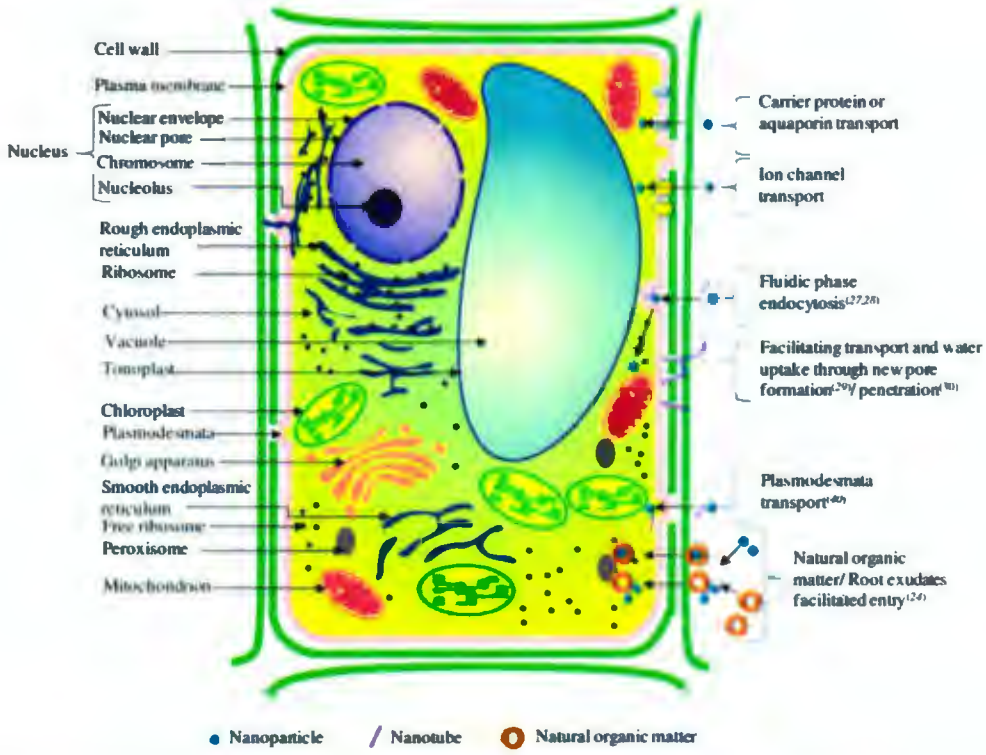



**Figure 1.1: Various modes of uptake of the NPs in a plant cell (Rico *et al.*, 2011)**

Nanoparticles transportation and accumulation in plant cells also depend upon availability of ions in medium. Nanoparticles effect reduce in soil due to decreased availability of NPs as compared to *in vitro* agar medium (Lee *et al.*, 2012). NPs effect plants germination, physical, physiological and metabolic functions differently, efficiency of various nanoparticles and effect depend upon plant species, size and shape of nanoparticles (Nair *et al.*, 2010).

## 1.2 *Ocimum basilicum L.*

*Ocimum basilicum L* is an annual medicinal plant common name sweet basil (local name naz boo or reyhan) belongs to Lamiaceae family. Genus Ocimum has more than 150 species with high diversity in physical traits (Sajjadi, 2006). Among them Sweet basil is commercially most important specie cultivated around the globe. Basil plant need enough sunlight and water in germination stage, the minimum temperature for growth is 18°C (Kalteh *et al.*, 2014) Basil is used in food, medical and perfume industry (Telci *et al.*, 2006). Genus *Ocimum* include another important specie *O. sanctum L* common name Holy Basil (Tulsi) other species include *Ocimum americanum L, O gratissimum L and O. tenuiflorum* all called as Basil.

### 1.2.1) *O. basilicum L.* Morphology and Distribution

Sweet Basil is native to Asia, and widely distributed in tropical region of Asia and Africa. It is cultivated throughout the world including Europe, America, Asia, Turkey and Pakistan for its essential oil and aromatic compounds (Ekmekci and Aasim, 2014) Selective breeding and wide distribution of basil cultivar resulted in high variation in morphological, physical, chemical and essential oil composition (Zheljazkov *et al.*, 2008). Basil leaves are green in color and flowers range from purple to white in color depending upon the variety (Sajjadi, 2006).

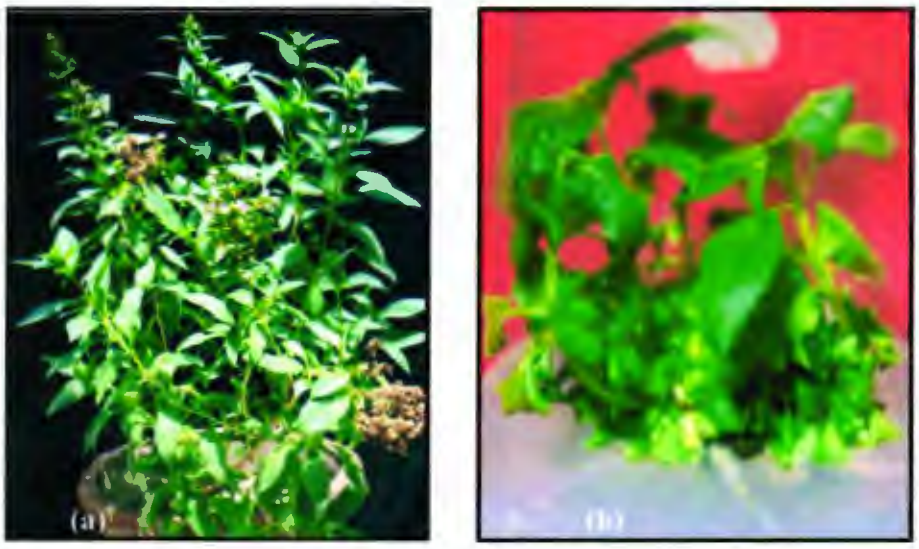


**Figure 1.2: *O. basilicum* (a) Mature Plant (b) *In vitro* Plant (Shahzad *et al.*, 2012)**

## 1.2.2) Medicinal importance of Sweet Basil

Medicinal plants are very important source of alternative medicine and antimicrobial drugs. With the increased use of antibiotics and other drugs, increase in drug and antibiotic resistance in animal, plants and human is also common. Medicinal plants are important source of medicine as they are not associated with adverse drug reaction (Adiguzel *et al.*, 2005). 60 % of world population depends upon herbal and plant medicines for treatment (Kaurinovic *et al.*, 2011). Out of them Sweet Basil is a very important medicinal plant it is used in folk medicine to cure migraine, dysentery, depression, nausea, headaches. They are used traditionally on skin for acne, snake bite, bee stings and skin diseases (Adiguzel *et al.*, 2005).

Leaves and seeds of *Ocimum basilicum* are used all over the world for its therapeutic properties. It is used in green tea for nausea and headaches, Flowers and leaves are used for aroma and as a tonic. Its antioxidant properties make it favorable for herbal medicine, it is used to treat kidney disorders for its diuretic properties (Ozcan and Chalchat, 2002; Gulcin *et al*, 2007).

## 1.2.3) Antimicrobial and insecticidal activity of Basil

Sweet Basil has antibacterial, antifungal and antiviral activities it is considered to have nematocidal, insecticidal, larvicidal and pupicidal, properties as well (Carovic –Stanko *et al.*, 2011). Antibacterial properties of Sweet Basil plant is due to its active biological compound anthocyanins and rosmarinic acid (Madhavi *et al*, 1995; Tada *et al*, 1996).

Antimicrobial activities of nanoparticles treated basil and other medicinal plants are not investigated, there is a need to identify the effect of nanoparticles on plant antibacterial activity.

## 1.2.4) Basil Essential oils

Basil Essential oils are economically important they are used in perfumes, food, flavoring agents, drinks, insecticides, pesticides, fungicides and many consumer products (Pascual-Villalobos and Ballesta-Acosta, 2003). Its antioxidant and

antimicrobial properties made it favorable in pharmaceutical industry and herbal medicines (Hussain *et al.*, 2008).

There is a high variation among oil chemical composition in different varieties (Zheljazkov *et al.*, 2008). Basil essential oil has wide range of chemical compounds they are affected by seasonal variations. Eugenol, linalool, epi-a-cadinol, chavicol, a-terpineol and methyl cinnamate are main components present in Sweet Basil essential oil (Siddiqui *et al*, 2007; Politeo *et al*, 2007).

## 1.3) Aims and objectives

Present study helps understanding the effect of Zinc oxide nanoparticles on Sweet Basil. It also successfully investigated the following:

- Effect of nanoparticles treatment on germination rate and callus frequency
- Effects of nanoparticles treatment on micropropogation of basil culture
- Phytochemical screening of control plants and nanoparticles treated plants
- Comparison of antibacterial activity of NPs treated plants with control plants
- To develop efficient protocol to increase *in- vitro* basil regeneration
- ZnO NPs effect on contamination frequency of culture medium
- Optimization of Nanofertilizers concentration to increase Basil seed germination and plant growth

# Chapter 2

# REVIEW OF LITERATURE

# 2. Review of literature

## 2.1 Effect of nanoparticles on *O. basilicum*

Effect of nanofertilizers were observed on *O. basilicum* under stress, silica nanoparticles were applied under salinity stress of 1, 3 and 6 ds/m. Control plant showed reduction in plant development and growth, leaf fresh and dry weight reduced with increase in salinity. NPs treated basil plants show improved growth and development than control and normal silicon fertilizers. Silicon NPs increased proline, chlorophyll content and tolerance of plant toward saline stress and reduced the pollution effect of salinity in *Ocimum basilicum* (Kalteh *et al.*, 2014).

## 2.2 Effect of zinc oxide NPs on *Ocimum basilicum*

Another study demonstrated the effect of ZnO nanoparticles on Sweet Basil, three concentration of 10.5-15.5 nm ZnO NPs were applied on basil plant by foliar spray method. 20 mg/L gave the best results among 10 mg/L, 20 mg/L and 30 mg/L. NPs were applied along with He Ne laser irradiation for 2 min on basil seeds which showed better results than ZnO NPs alone. Results showed increase in plant fresh weight, no of leaves, branches, dry weight and plant yield of *O. basilicum*. Chlorophyll content, essential oils, total carbohydrates were also increased. Zinc oxide NPs increased zinc content in shoot when applied through foliar spray, nitrogen, iron and potassium content was increased with application of ZnO NPs. 20 mg/L give highest results. Leaf analysis showed increase in chlorophyll content and size of epidermis cells in NPs treated plants. Research shows potential of zinc oxide NPs as nanofertilizers (El-Kereti *et al.*, 2013).

## 2.3 ZnO NPs effect on other plants

Zinc oxide show inhibitory effect on germination of seeds and root elongation in different plant species. Lin and Xing observe the effect of Zn and ZnO nanoparticles on corn, cucumber, lettuce, radish, rape and ryegrass. At 2000 mg/L corn Seed germination was inhibited by ZnO and zinc NPs inhibit rye grass seed germination at 2000 mg/L. Suspension of zinc nanoparticles and zinc oxide NPs at

higher concentration inhibited root elongation of plant species. 50% of root growth was terminated at concentration of 20 mg/L for rape and ryegrass and 50 mg/L for radish. Phytotoxicity of ZnO nanoparticles was evident from research (Lin and Xing., 2007).

Another research on ryegrass by Lin and Xing 2008 showed that higher concentration of ZnO NPs 1000 mg/L nanoparticles in hoagland solution cause reduction in biomass, deformity of root cap, deformity of cortical cells and epidermal cells. ZnO NPs were revealed to inhibit plant growth and root growth. NPs were located in root apoplast, transportation to shoots and upper part of plant was not observed (Lin and Xing 2008).

ZnO NPs effect was demonstrated on garlic plant by Shaymurat *et al* 2012 results revealed the inhibitory effect of ZnO NPs on root growth at higher concentration. *Allium sativum* root Growth was fully terminated at 50 mg/L ZnO concentration after 24 hour, 50% growth was inhibited at 15 mg/L. Nanoparticles induced abnormalities in cell in the form of chromosome stickness. Increase in NPs concentration and time period increased the number of abnormal cells (Shaymurat *et al.*, 2012).

Lopez-moreno demonstrated the effect of ZnO nanoparticles on soybean, seeds were treated with 8nm zinc oxide nanoparticles at different concentration of 500 mg L−1 to 4000 mg/L. 500 mg/L concentration enhanced Root growth and uptake of NPs in plants was highest. This increase in uptake of NPs is attributed to decrease in aggregation. With increase in NPs concentration transport through cell wall pores become difficult because of aggregation and accumulation, increase in NPs conc. also reduced root elongation (Lopez-moreno *et al.*, 2010).

## 2.4 Antibacterial activity of Zinc oxide nanoparticles

Antibacterial activity of zinc nanoparticles were evaluated in pullulan films. 100nm, 110nm and 130nm zinc oxide nanoparticles were used on four bacterial strains. ZnO NPs with 1% concentration and size 110nm exhibited strongest antibacterial activity in all bacterial strains. 130nm ZnO NPs inhibited growth of *S. aureus* and *L. monocytogenes*, 100nm NPs showed weak inhibition in *S. aureus*. *S.*

*Typhimurium* and *E. coli* growth were only inhibited by 110nm NPs. Result suggested that Zinc oxide NPs can be used to treat edible film and increase shelf life of refrigerated meat. (Morsy *et al.*, 2014).

## 2.5 Genotoxity of ZnO NPs

Genotoxity of ZnO nanoparticles were observed by Sharma *et al* 2009 in human epidermal cell line results suggested DNA damaging potential of zinc oxide NPs. Increase in nanoparticles concentration and time of exposure reduced epidermal cell viability and cause oxidative stress. Research further suggested that in human epidermal cells zinc oxide NPs show genotoxic effect at low concentration. Therefore ZnO nanoparticles should handle carefully (Sharma *et al* 2009).

Another study on toxic effect of ZnO, $CeO_2$ and $TiO_2$ on RAW 264.7 and BEAS-2B cell lines shows that zinc oxide cause inflammation, reactive oxygen species, oxidant injury and cell death in both cell lines. Fluorescent labeling of ZnO further reveals that dissolution of nanoparticles could take place in endosomes and culture medium. $CeO_2$ decrease ROS production and show cytoprotective effect on the other hand $TiO_2$ did not produce cytoprotective or cytotoxic effects (Xia *et al.*, 2008).

A study on human bronchial epithelial cells (BEAS-2B) demonstrated the cytotoxic effect of ZnO NPs on cell line which was exposed to different concentrations of ZnO, 5 µg/ml- 25 µg/ml concentrations were applied and 10 µg/ml ZnO NPs sharply reduced cell viability. When cells were exposed to $H_2O_2$ before ZnO NPs treatment cell viability decreased sharply. This result showed that oxidative stress alter sensitivity of cell toward cytotoxic nanoparticles (Heng *et al.*, 2010)

## 2.6 *In vitro* micropropogation of *Ocimum basilicum*

Simon and phippen investigated shoot regeneration in sweet basil plant. Young leaves were used as explants to observe the effect of thidiazuron, kinetin and BA. Optimum concentration for thidiazuron micropropogation was observed 4 mg/L and young leaf plants showed shooting regeneration, other explants were not suitable for shooting initiation in this media. Kinetin and BA were not effective in shooting

induction. Young leaves were culture in MS with TDZ, well developed shoots were transferred to MS medium without any hormones, rooting initiation start after 2 weeks plants were then cultured on fresh media to facilitate root elongation. Plantlets were washed and cultivated in soil. Result showed the cytokinins hormones cause shoot regeneration in leaf, TDZ were favorable to induce shoots in basil leaf (phippen and simon, 2000).

Another research on sweet basil micropropogation was done on Turkish plant variety, seeds were surfaced sterilized and grown in MS medium, seedling obtained after 2 weeks were used as explants material. Shoot tips and other parts of seedling were cultured in regeneration medium having MS, IBA and TDZ, and other medium having TDZ alone in MS media. Result indicate that shoot tips give maximum regeneration rate of 100% in 1.2-1.4 mg/L TDZ concentration followed by epicotyls. Lowest shooting was observed in hypocotyls (Ekmekci and Aasim, 2014).

Shahzad and colleagues presented shoot regeneration protocl of *O. basilicum.* MS medium was supplied with 6-benzyladenine and nodal segments were used as explants. Highest shooting was observed on 10 µM BA and 30 mg/L L-glutamine which induce 100% shoot regeneration frequency. Healthy Shoots were then transferred to half strength and full strength MS medium with 5 µM IBA for rooting, successful plants were shifted to soil. Results suggested that BA, 2-iP along with L-glutamine induce successful shooting in sweet basil, plants then shifted to rooting medium and normal soil show normal development (Shahzad and Faisal, 2012).

Another efficient system for the *in vitro* multiplication of sweet basil from leaf cotyledons was developed. Seeds were surfaced sterilized, germinated in MS medium and *in vitro* seedling were cut and used as explants. Low concentration Of NAA along with BAP was used for callus induction. Highest shooting regeneration and number of shoots were observed on 5 mg/L BAP and 0.02 mg/L NAA. Rooting was inhibited in this hormone concentration, higher conc. of BAP induce green and compact tissue as compared to lower conc. Rooting was successfully induced when shoots were transferred to MS media without hormone, they were then transferred pots (Dode *et al.,* 2003).

Siddique and Anis developed protocol for shoot regeneration of basil. Shoot tips were used as an explants material. MS medium was supplemented with Thidiazuron with different concentration for 4 to 16 days. Highest shooting of 78 % was observed on 8$^{th}$ day with 50.0 µM concentration of Thidiazuron, with maximum shoot Number and shoot length. Healthy Shoots were transferred to rooting medium supplemented with IBA, highest rooting rate was observed on 1 µM with maximum no of roots. Healthy plants were cultured in soil where 95 % of plants developed into mature plants (Siddique and Anis, 2007).

Sahoo and colleagues investigated micropropogation of *Ocimum basilicum* using basil nodal segments. MS was supplemented with BA, explants showed maximum bud break when treated with 1 mg/L of BA, explants were then shifted to lower concentration of BA for shoot development and multiplication which is followed by planting them to rooting medium containing IBA, IAA or NAA. Maximum rooting frequency of 93 % was observed on 1 mg/L IBA, NAA was ineffective for root formation. Healthy plantlets were shifted to soil. 85% of shoots develop flowers which were identical to parent plants. All plants were similar to donor plants in morphology (Sahoo *et al.*, 1997).

Asghari *et al*, 2012 demonstrated basil multiplication system using four concentration of plant growth regulator BAP and IAA, result suggested that BAP significantly induced shoot formation in sweet basil. Highest shoot frequency was observed on 11 µM concentration of BAP. Rooting was achieved by shifting shoots to MS media with IAA. Result further revealed that BAP decreased rooting when used in higher concentration along with IAA. Cotyledons leaf have highest no of shoots followed by nodal explants. (Asghari *et al.*, 2012)

## 2.7 *In vitro* multiplication of *Ocimum* genus

In vitro multiplication of other species of *Ocimum* was observed by many researchers. Banu and Bari successfully demonstrated micropropogation of *Ocimum sanctum* results suggested that holy basil shoot tips gives best result for shoot regeneration. 0.2 mg/L concentration of BAP successfully induced shooting in ninety percent explants. Maximum shoots were obtained on above concentration. Results

suggested that higher conc. of BAP induce vitrification in plant shoots (Banu and Bari, 2007).

Amin and begun also successfully developed Holy Basil *in vitro* culture using nodal segments as an explant material (Begun *et al.*, 2000).

## 2.8 Essential oil

Ozcan and Chalchat observed the chemical composition of basil essential oil, plant dried over ground part were subjected to hydro distillation and tested by GC-MS. Result revealed presence of 49 components, Methyl Eugenol is the main component which is followed by α-cubebene and other constituents were β-cubebene and nerol (Ozcan and Chalchat, 2002).

## 2.9 Phytochemical analysis of basil

Phytochemical analysis of basil revealed presence of many biologically active compound which include rosmarinic acid, caffeic acid and vanillic acid (Javanmardi *et al*, 2002). Daniel *et al* 2011 indicated presence of tannins and saponins in basil plant, fresh leaves were washed and dried in oven. Dry powder was used for extract preparation result also indicated presence of Cardiac glycosides in plant extract (Daniel *et al.*, 2011).

## 2.10 Antimicrobial activity of basil

Antibacterial and antifungal activity of basil dried plant extract was observed in 146 microorganisms by disc diffusion method and MIC. Three solvents methanol, ethanol and hexane were used to test antimicrobial activity of basil. Result suggested that hexane extract has highest antibacterial activity followed by methanol and ethanol, none of extract indicated antifungal activity. The MIC for methanol extract is 62.50 µl/ml to 500 µl/ml and for ethanol is 125 to 250 µl/ml, all extract showed different antibacterial activity, methanol extract showed antibacterial activity against 11 strain of bacteria and 7-17 mm zone of inhibition (Adiguzel *et al.*, 2005).

Researchers from Pakistan successfully demonstrated the effect of seasonal variation on antimicrobial and antioxidant activity of sweet basil essential oil.

Essential oil from winter and autumn crops indicated highest antibacterial activity which is followed by spring crop. Essential oil exhibited antimicrobial activity against all 9 pathogenic bacteria, highest antibacterial activity was observed in *S. aureus* and *B. subtilis*, which showed largest zone of inhibition and lowest minimum inhibitory concentration. Essential oil indicated stronger activity against bacteria than fungi. Researcher also identified change in chemical composition caused by seasonal variation, linalool and oxygenated monoterpenes were highest in winter, cadinene and hydrocarbons sesquiterpene were highest in summer and lowest in winter. Results suggested there is significant variation in antibacterial and antioxidant activity among different seasonal crops of basil (Hussain *et al.*, 2008).

# Chapter 3

# MATERIAL AND METHODS

# 3. Material and methods

## 3.1 Materials

### 3.1.1 Nanoparticles

Green synthesized Zinc oxide nanoparticles were used in research, nanoparticles size was 35-100nm.

### 3.1.2 Plant material

Sweet Basil plant has been obtained from local nursery seeds, leaves and stem were used in research.

### 3.1.3 Glassware and Tools

Petri dishes (Pyrex), Culture tubes(25 mm × 150 mm), conical flasks (100ml, 250ml, 500ml, 1000ml), graduated cylinder (100ml), measuring beaker (1000 ml).

Foreceps, scalpels, surgical blades, test tubes racks, micropipettes, falcon tubes, filter paper, spirit lamp, mortar and pestle, Scissors and scale.

### 3.1.4 Chemicals

Murashige and Skoog Salt mixture, gelrite agar, nutrient agar, sucrose, deionized water, distilled water, sodium hydroxide, hydrochloric acid, sulfuric acid, acetic acid, picric acid, chloroform, ferric chloride, ethanol (99.9 %, 70%), methanol, liquid detergent, Clorox, spirit.

### 3.1.5 Machinery

Automatic autoclave, laminar air flow cabinet, microwave oven, drying oven, electronic balance, shaker, pH meter, incubator.

## 3.2 Procedure

Experiment was conducted on seed, young leaf, plantlets and mature plant leaves. Seeds were treated with zinc oxide nanoparticles and grown in Murashige and Skoog medium, seedling produced in vitro is used as explants material.

### 3.2.1 Preparation of NPs and PGR stock solution

Green synthesized nanoparticles of size 35-100nm were diluted in double distilled water. NPs were diluted four to one ratio (4 parts NPs and 1 part water) and sonication was done to homogenize mixture. Nanoparticles were sonicated every time for 5 mins before application to seeds and medium.

Stock solutions of plant growth regulators were prepared at the concentration of 0.1 mg/L. $P^H$ of solution was adjusted to 5.8 by using HCL and NAOH. BAP and 2,4D were used in research. Solution was stored at 4C in refrigerator in falcon tubes. Storage time for both plant regulators was two month.

### 3.2.2 Treatment of seeds with NPs

**a) Concentration 1**

Nanoparticles from stock solution was taken and diluted with distilled water to achieve 0.05 µl/ml concentration. Seeds were soaked in water and placed in dark area to break its dormancy followed by washing with double distilled water. Seeds were soaked in zinc oxide nanoparticles 0.05 µl/ml concentration for 48 hours and placed in dark area.

**b) Concentration 2**

After breaking seeds dormancy for 24 hours in distilled water and then washed by $ddH_2O$ seeds were soaked in 0.1 µl/ml ZnO nanoparticles for 48 hour and placed in dark area.

**c) Control**

*Ocimum basilicum* seeds were soaked in water for 24 hours and kept in dark area to break its dormancy.

**Table 3.1: Nanoparticles treatment of Ocimum basilicum seeds**

| NPs concentration | Nanoparticles | Time of treatment |
|---|---|---|
| **0.05 µl/ml** | ZnO | 48 hours in NPs |
| **0.1 µl/ml** | ZnO | 48 hours in NPs |
| **Control** | None | - |

### 3.2.3 Sterilization of seeds

Nanoparticles treated and control seeds were separately surface sterilized in laminar air flow cabinet in the following steps

- Seeds were washed by liquid detergent for 2mins to remove dust particles and then washed by sterile distilled water.
- Seeds were then surface sterilized with a solution of 70% alcohol for 2mins and rinsed 3 times with sterile distilled water.
- Then seeds were treated in a solution of sodium hypochlorite for 20mins and rinsed 3 to 5 times with sterile double distilled water to wash all chemicals.

Seeds were then placed on autoclaved filter paper to dry.

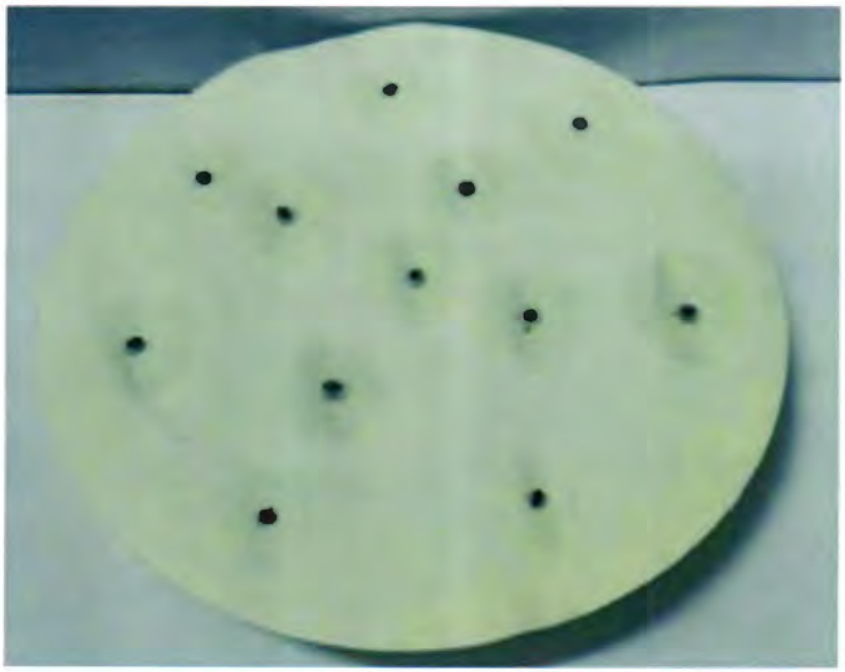


**Figure 3.2: Seeds on filter paper for drying**

## 3.2.4 Germination culture medium

These surface sterilized seeds were explanted on germination culture medium. Murashige and Skoog medium is supplemented with 3% sucrose, and 0.25% gelrite agar. The pH of the medium was adjusted to 5.7-5.8. Media were dispensed in test tubes and plugged with cotton and muslin cloth. Media is then autoclaved at 121 °C for 15 min.

### 3.2.4.1 Inoculation

In laminar air flow cabinet after media were cool down and gel is set, dry sterilized seeds were inoculated in medium, autoclaved forceps were used for inoculation, test tube were heated by spirit lamp to dry inside area and cotton plugs were also dried by heating, then test tubes were covered with aluminum foil to prevent further contamination. Test tube racks were labeled using paper tape; date of inoculation, media and concentrations.

### 3.2.4.2 Culture conditions

Culture were placed in tissue culture room at 28°C, under 16 hour photoperiod provided by white fluorescent light.

### 3.2.4.3 Seedlings production

Germination start after one week, Data was documented after 10 days and 20 days. After one month these seedling were use as an explant for callus inducion.

## 3.2.5 Callus induction medium

For callus induction MS media was supplemented with 1.2 mg/L 2,4-Dichlorophenoxyacetic acid. Seedlings obtained from 0.05 µl/ml, 0.1 µl/ml and control were cut and placed in callus induction medium under laminar flow hood cabinet without decontamination. Callus initiation start after a week and data was recorded on $10^{th}$ and $20^{th}$ day. Callus induction frequency was calculated as follows.

**Callus induction frequency** = No. of callus obtained / Total No. of explants cultured × 100

### 3.2.6 Shoot regeneration medium

Seedlings obtained from control seeds were cut and placed in shoot multiplication medium for direct shooting. Nanoparticles were applied in shoot regeneration medium to observe the effect of ZnO NPs in plant shooting. Ms media was supplemented with 5 mg/L growth regulator BAP in control and two concentration of nanoparticles 0.05 µl/ml and 0.1 µl/ml were applied in shooting medium. Leaves and stem were used as explants.

### 3.2.7 Preparation of callus extract

One month callus was washed and crushed in mortar and pestle. 1 gram of each nanoparticles treated callus and control was mixed into 100ml methanol separately and shaken over night in a shaker. It was then filtered through whatman filter paper No. 1 and kept in labeled falcon tubes for further analysis.


**Figure 3.3: Callus and Leaf extract of basil**

### 3.2.8 Preparation of leaf extract

Leaves were washed by distilled water three times and crushed in mortar and pestle. 10 grams of crushed leaves were dissolved in 100 ml of methanol. For leaf water extract 10 gram of grounded leaves were dissolved in 100 ml of water, both extracts were covered and shaken for 24 hours. Extract was filtered through filter paper and stored in falcon tubes.

**Table 3.2: Callus and leaf extract of *Ocimum basilicum***

| Extract | Plant type | Solvent | Plant/solvent Ratio |
|---|---|---|---|
| **E1** | Callus from 0.05 µl/ml treated seedlings | Methanol | 1:100 |
| **E2** | Callus from 0.1 µl/ml treated seedlings | Methanol | 1:100 |
| **C** | Callus from control | Methanol | 1:100 |
| **S** | Callus from mature explant | Methanol | 1:100 |
| **LW** | Leaf from mature plant | Water | 1:10 |
| **LM** | Leaf from mature plant | Methanol | 1:10 |

### 3.2.9 Phytochemical analysis

Phytochemical analysis was performed on nanoparticles treated seedling callus and control to detect the influence of nanoparticles on secondary metabolites of basil. Phytochemicals test were performed on callus extracts.

**a) Alkaloids**

1ml of each extract nanoparticles treated callus, control, leaf methanol and water extract was taken in test tube and pinch of picric acid was added in each test tube. Orange color precipitates show positive result.

**b) Terpenoids**

In each extract 1ml of chloroform is added with 2 ml of acetic acid and few drops of $H_2SO_4$. Pink color indicates positive result.

**c) Phenolic compounds**

For testing the presence of phenolic compounds, 0.5ml of ferric chloride is added in 1ml of extract. Color change to blue green indicates positive results.

**d) Steroids**

Acetic acid was added in each extract, solution is cooled and few drops of $H_2SO_4$ were added in it. Red color show positive results.

**e) Sapponings**

1 ml of each extract was mixed in 2 ml of water and shaken well, formation of foam indicates presence of saponins.

### 3.2.10 Antibacterial activity of callus extract

Antibacterial activity of nanoparticles treated callus, control and leaf extract of basil plant was observed and compared by disc diffusion method. Results were compared by the presence and size of zone of inhibition around the disc.

28g/L nutrient agar was prepared and autoclaved at 121 °C for 15 min. Media was poured on sterile Petri plates and after media was cooled down inoculation was done by sterilized inoculation loop. Bacteria were inoculated on agar plates by taking bacterial colony from bacterial culture and streaking it on agar plate. 6mm of sterilized disc were soaked into each extract and applied to the bacterial inoculated agar plate. These plates were incubated for 48 hours in incubator at 37 °C. Screening of callus and leaf extracts were done by measuring Zone of inhibition around the disc.

# Chapter 4

# RESULTS

# 4. Results

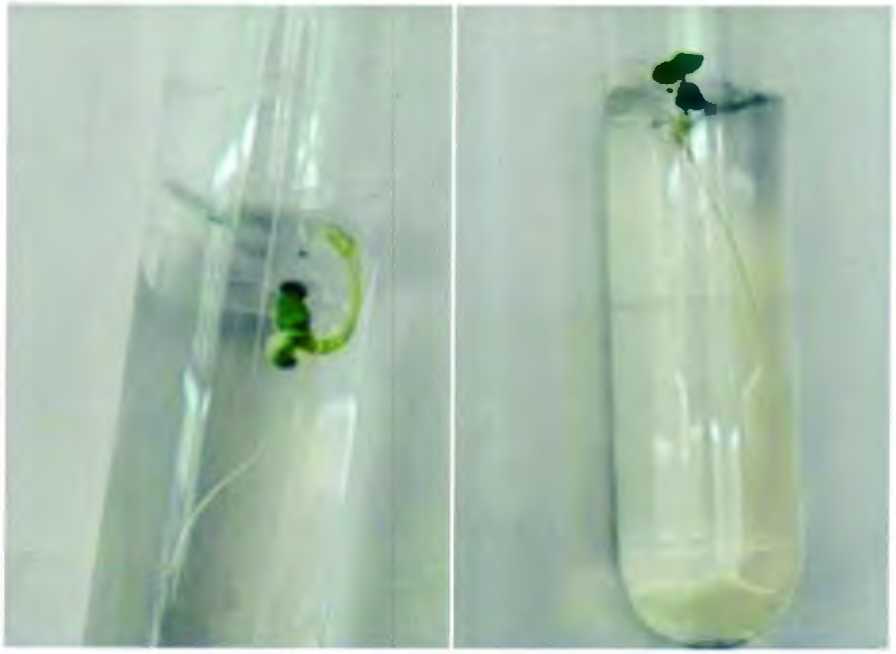
## 4.1 Effect of ZnO NPs on seed germination

Germination rate was observed after 10 days interval, growth start after a week in control but nanoparticles treated seeds show slower growth. On $10^{th}$ and $20^{th}$ day data was collected 0.1 µl/ml NPs treated seeds show 38.8% growth rate, 7 seeds show complete growth out of 18. Control seeds show 66.6% growth rate after 20 days 12 seeds show complete germination. Growth rate of 0.05 µl/ml NPs treated seeds was 72%, 13 out of 18 showed germination.

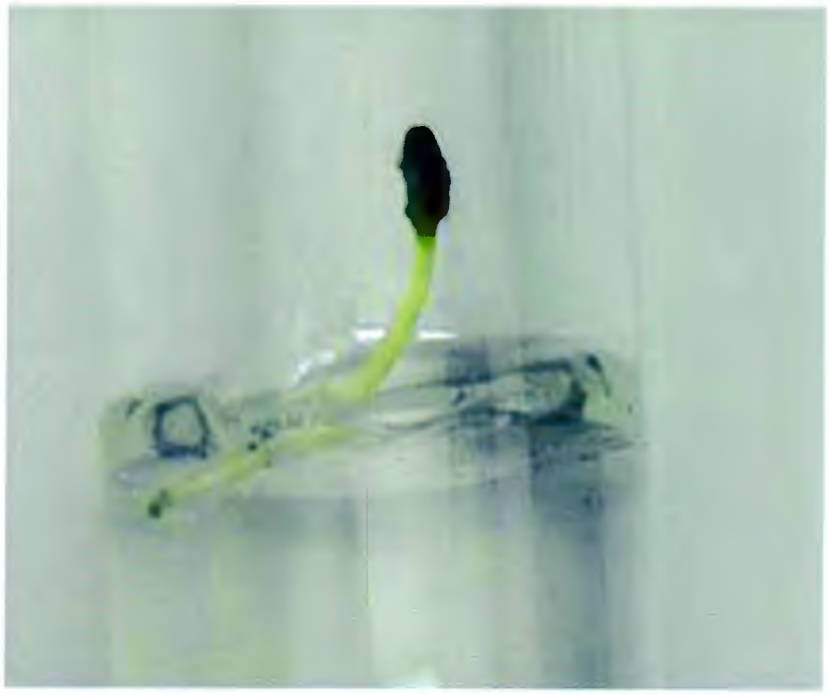
**Table 4.1: Effect of ZnO nanoparticles on basil seeds germination**

| Nanoparticles concentration | Germination rate 10 days | Germination rate 20 days | Contamination frequency |
|---|---|---|---|
| **0.05 µl/ml** | 61.1% | 72% | 5% |
| **0.1 µl/ml** | 33.3% | 38.8% | 5% |
| **Control** | 61.1% | 66.6% | 0 |

Result indicated that rate of germination decreases with increase in nanoparticles concentration while 0.05 µl/ml NPs slightly increase growth rate. Contamination frequency of nanoparticles treated seeds were more than control, Data suggested that ZnO did not show antifungal activity on both concentrations. Nanoparticles treated seedlings did not exhibited any abnormality in physical appearance. Statistical analysis reveals that result were significant different for 0.1 µl/ml nanoparticles treatment ($P < 0.05$) while control and 0.05 µl/ml were not significantly different ($P > 0.05$).



**(a) 0.05 µl/ml Seeds germination**



**(b) 0.1 µl/ml NPs treated seedlings**

(c) Control (d) Mature seedling

**Figure 4.1: Seed germination in NPs treated and Control *Ocimum basilicum***

**(a) 0.05 µl/ml NPs treated seedlings (10 days)**

**(b) 0.1 µl/ml NPs treated seedlings (10 days)**

**(c) Control seedling (10 days)**

**(d) Control mature seedling (40 days)**

## 4.2 Effect of ZnO NPs on Callus induction frequency

The effect of zinc oxide nanoparticles on callus was observed, control and NPs treated seedlings were used as explants and inoculated in 1.2mg/L 2,4-D and MS medium. Callus initiation start within 10 days of inoculation, NPs treated seedlings and control callus were similar in morphology and physical appearance callus were yellowish white in color. Control explants show highest callus frequency of 90%, which was followed by 85% callus induction frequency of 0.1 µl/ml. 0.05 µl/ml explants have lowest frequency of 83%. Highest contamination frequency of 9.5 was observed in 0.1 µl/ml callus followed by 0.05 µl/ml nanoparticles treated callus has zero contamination frequency.

**Table 4.2: Effect of ZnO on Callus induction frequency**

| Nanoparticles conc. | Callus induction frequency 10 days | Callus induction frequency 20 days | Contamination frequency |
|---|---|---|---|
| **0.05 µl/ml** | 73.8% | 83% | 0% |
| **0.1 µl/ml** | 88% | 85% | 9.5% |
| **control** | 90% | 90% | 2.3% |

LSD for NPs treatment = 8.7693

LSD for time period = 7.1601

Statistical analysis showed that 0.05 µl/ml and control callus induction frequency were significantly different ($P < 0.05$). However the effect of time duration has overall no significant effect on callus induction frequency ($P > 0.05$). Time and treatment interaction has no significant effect on callus frequency. Statistical analysis on contamination frequency indicated that there was no significant difference among three treatments ($P > 0.05$).

Effect of nanoparticles on callus weight was investigated on 35 days, control plants showed highest weight of 1.043g which is followed by 0.1 µl/ml and 0.05 µl/ml. Callus mass was again observed on 48 days result showed that 0.05 µl/ml have highest callus weight of 1.6608 on 48th day. Research suggested that 0.05 µl/ml callus have lowest callus induction frequency but highest callus mass.

**Table 4.3: Effect of nanoparticles on Callus Mass**

| Nanoparticles Conc. | Fresh Callus mass 35 days (g) | Fresh Callus mass 48 days (g) | Dry callus mass 48 days(g) |
|---|---|---|---|
| **0.05 µl/ml** | 0.6028 g | 1.6608 | 0.071 |
| **0.1 µl/ml** | 0.69 g | 1.346 | 0.0664 |
| **Control** | 1.043 g | 1.352 | 0.0666 |

Results are mean of 5 individual values

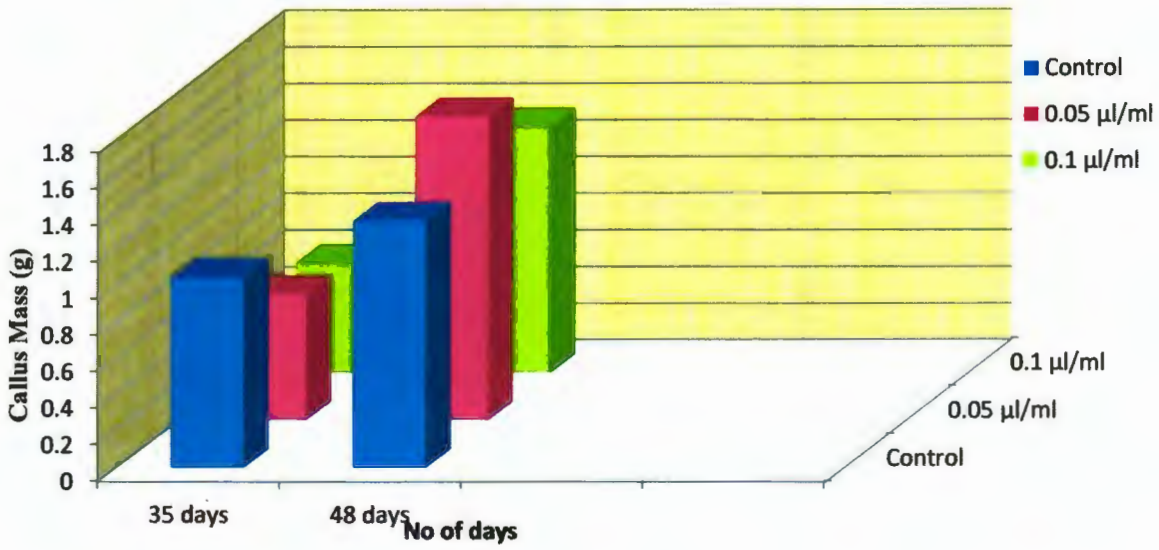
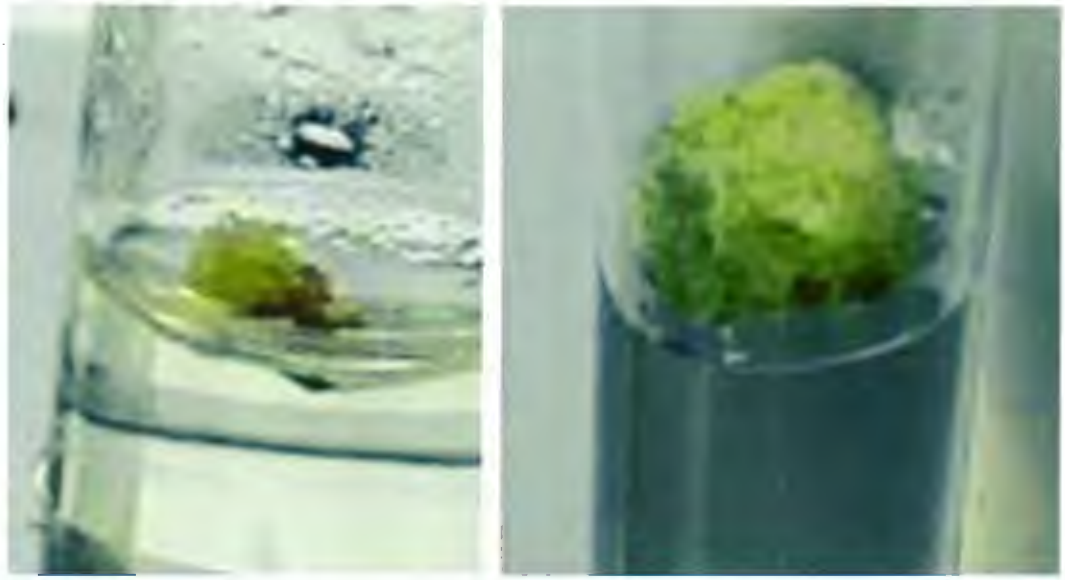



**Figure 4.2: Comparison of NPs treated and control callus fresh and dry weight**



**a)** **b)**

**(c)**

**Figure 4.3: Callus induction in control and NPs treated explants**

**(a) callus initiation**

**(b) 0.1 µl/ml callus**

**(c) 0.05 µl/ml callus**

## 4.3 Effect of ZnO Nanoparticles on Shoot Regeneration

Two different concentration of ZnO NPs 0.05 µl/ml and 0.1 µl/ml were tested in regeneration medium and compared with control, all explants exhibited growth in respective media. Shooting was observed on 15th day of inoculation, nanoparticles treated explants showed shoot regeneration while control indicated only integration. 0.1 µl/ml NPs treated explants exhibited highest shoot regeneration frequency of 44.4% on 15th day followed by 27.7 % shoot regeneration frequency of 0.05 µl/ml. Control explants showed only integration on 15th day. Results revealed that ZnO nanoparticles enhance shooting in basil plant when applied in regeneration medium and increased no of leaves and shoot height.

**Table 4.4: Effect of ZnO Nanoparticles on Shoot Regeneration Frequency**

| NPs conc. in medium | Shoot regeneration frequency | | Contamination frequency |
|---|---|---|---|
| | 15 days | 30 days | |
| 0.05 µl/ml | 27.7 % | 33.3 % | 0% |
| 0.1 µl/ml | 44.4 % | 55.5 % | 0% |
| control | None | 22.2 % | 5.55% |

LSD for NPs treatment = 13.075

LSD for time = 10.675

Statistical analysis revealed that all three media were significant different from one another for shoot regeneration. Time ($P < 0.05$) and Treatments ($P < 0.05$) had significant effect on regeneration frequency. While the interaction between time and treatments were non-significant ($P > 0.05$).

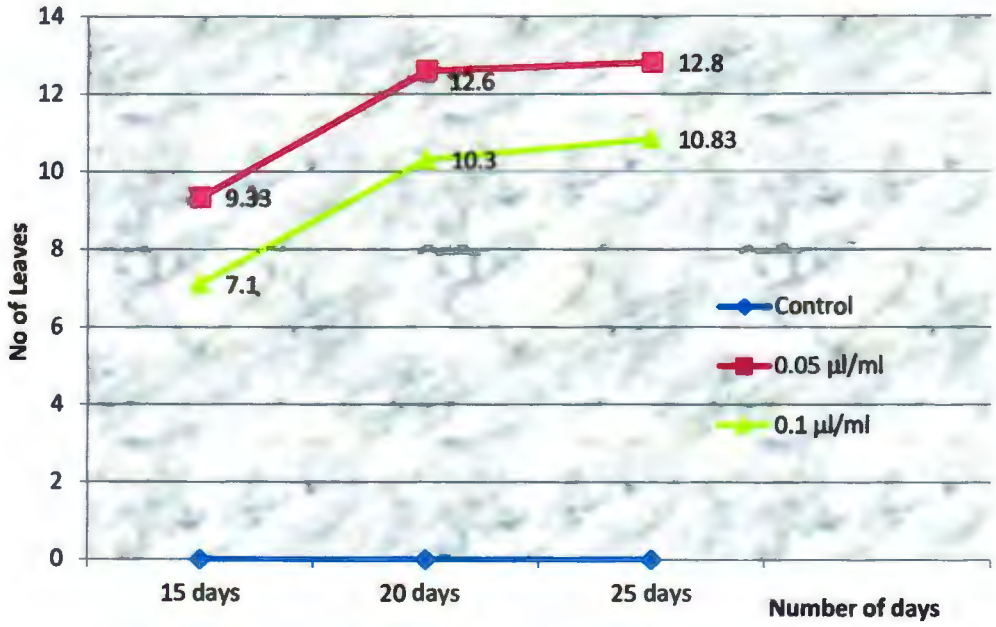



**Figure 4.4: Effect of ZnO NPs on No. of Leaves**



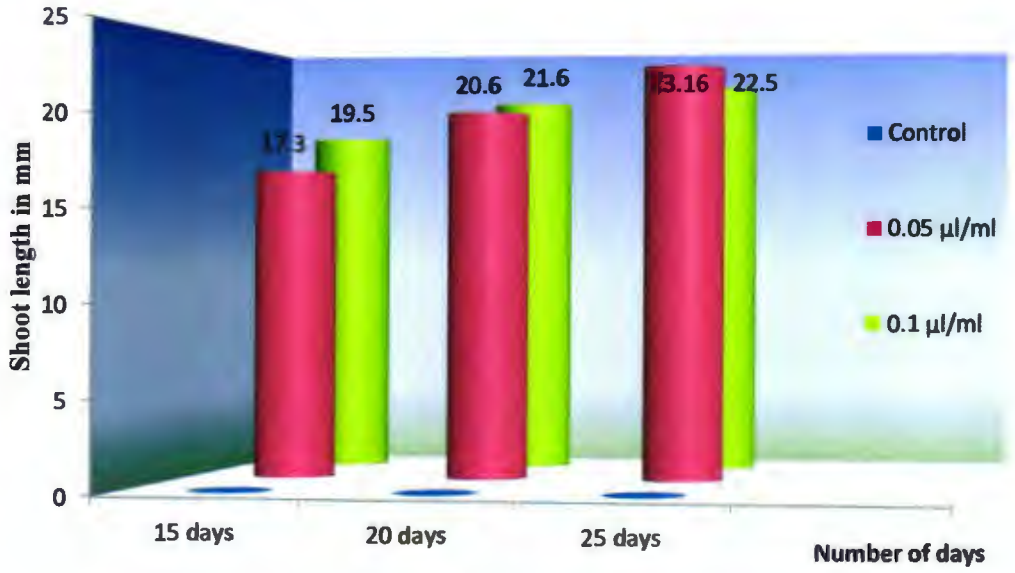
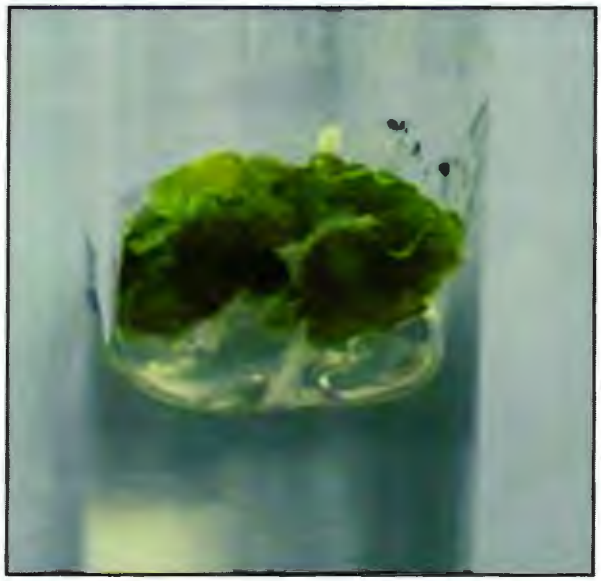



**Figure 4.5: Effect of ZnO NPs on Shoot Length**

**(a) 0.05 µl/ml shooting initiation**

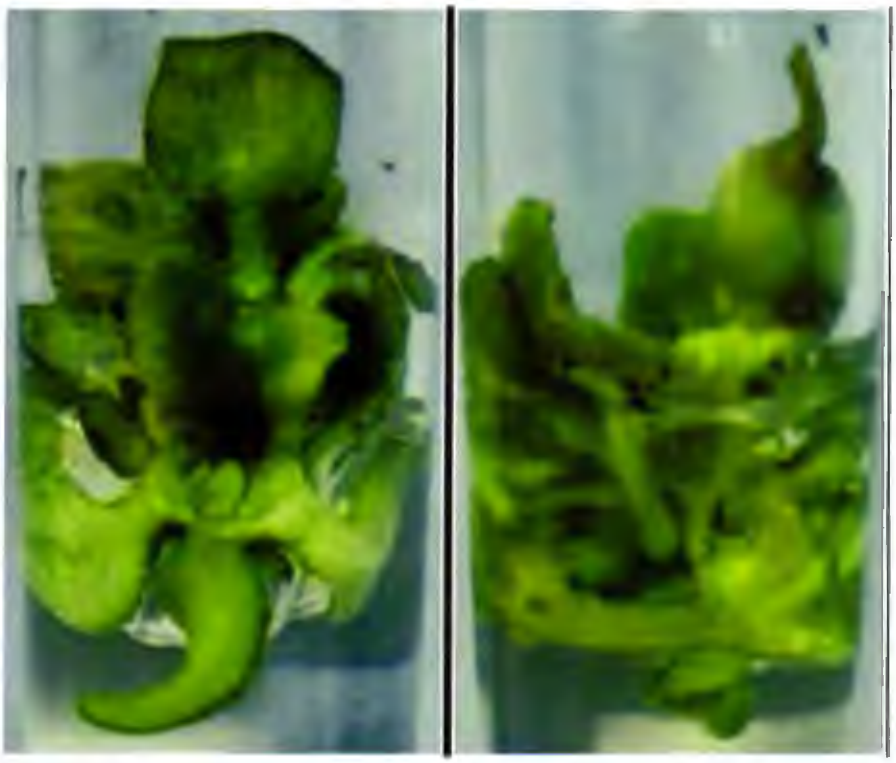
**(b) 0.1 µl/ml shooting initiation**



**(c) Shooting initiation in control**

**Figure 4.6: Shooting initiation in ZnO NPs treated and control plants, (a) 0.05 µl/ml NPs in medium, (b) 0.1 µl/ml NPs in medium (C) Control**

**(a) 0.05 µl/ml shoot regeneration**



**(b) 0.1 µl/ml Shooting regeneration**

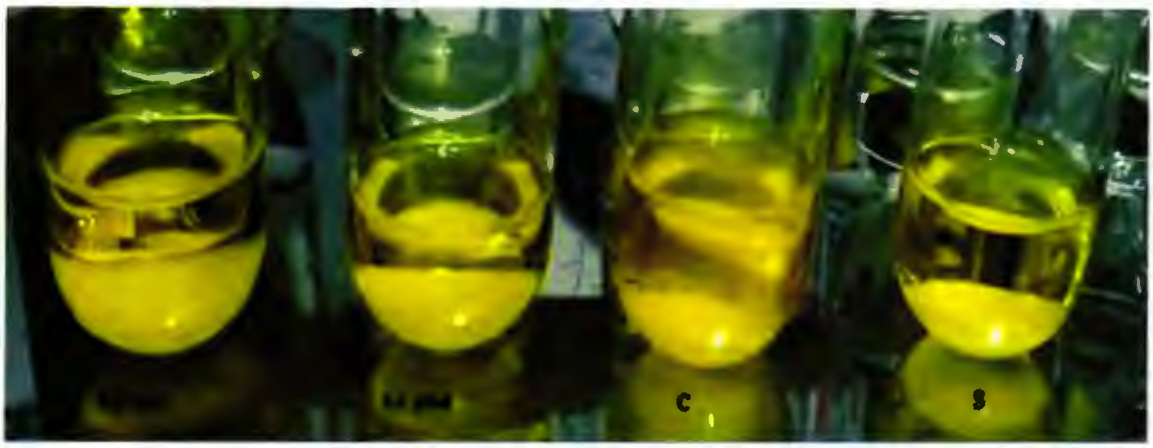
**Figure 4.7: Shoot induction in (a) 0.05 µl/ml (b) 0.1 µl/ml, NPs in media (15 days)**

## 4.4 Phytochemical screening

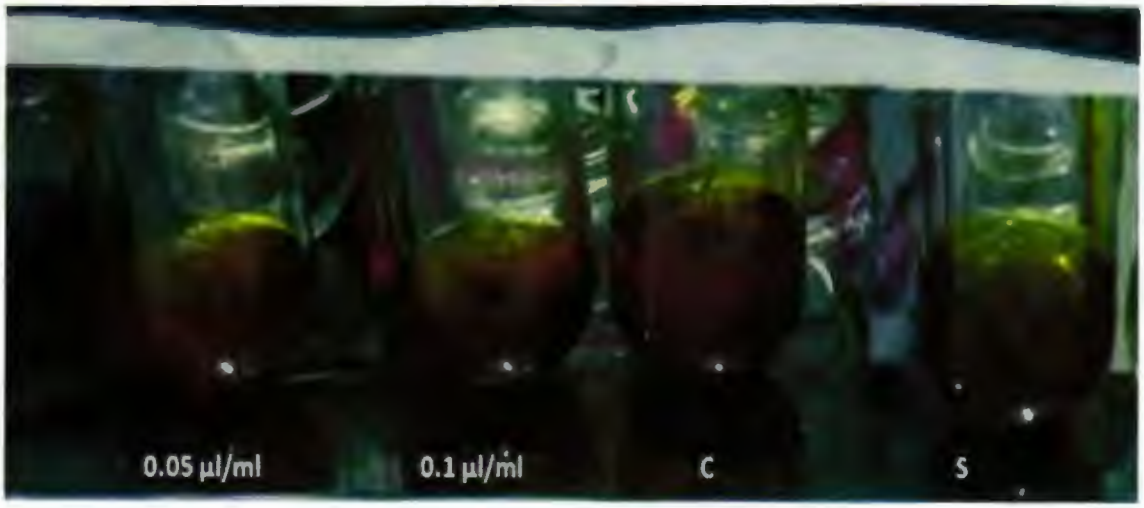
Phytochemical screening of extracts was done to observe the effect of ZnO NPs on phytoconstituents, nanoparticles treated and control *O. basilicum* callus was compared. Results showed absence on alkaloids, steroids, terpenoids, saponins, phenols in all extracts.

**Table 4.5: Effect of Nanoparticles on Phytochemical of *O. basilicum***

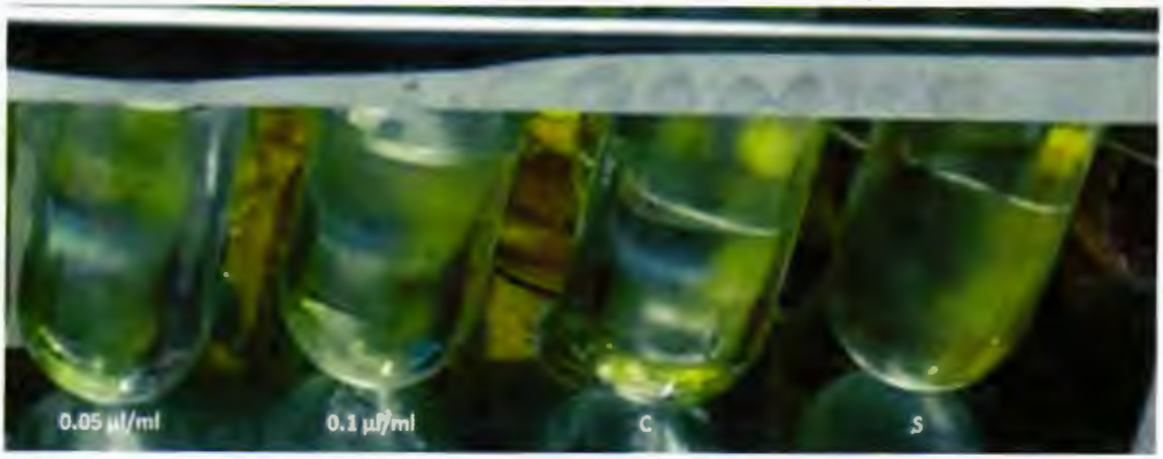
| Phytochemicals in plant extact | E1 0.05 µl/ml | E2 0.1 µl/ml | C control | S Callus from Mature plant |
|---|---|---|---|---|
| Alkaloids | - | - | - | - |
| Steroids | - | - | - | - |
| Terpenoids | - | - | - | - |
| Saponins | - | - | - | - |
| Phenols | - | - | - | - |




(a)




(b)




(c)

**Figure 4.8: Phytochemical analysis of *O. basilicum* NPs treated and control callus**

**a) Result showed absence of Alkaloids**

**b) Result indicated absence of Phenolic compounds**

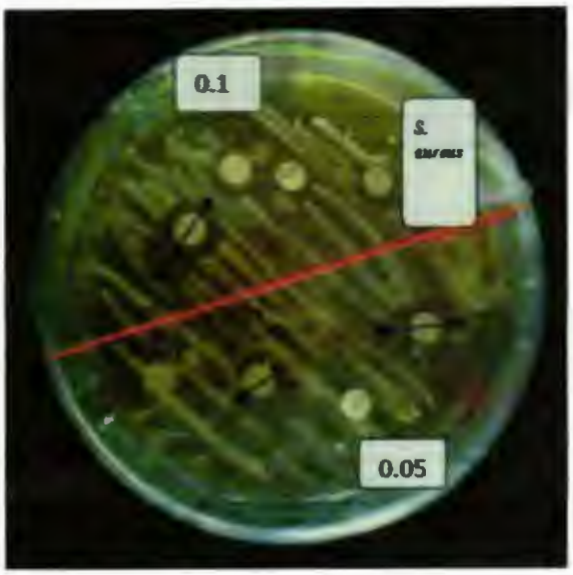
**c) Result showed absence of Terpenoids**

## 4.5 Antibacterial activity of ZnO NPs treated and control plants

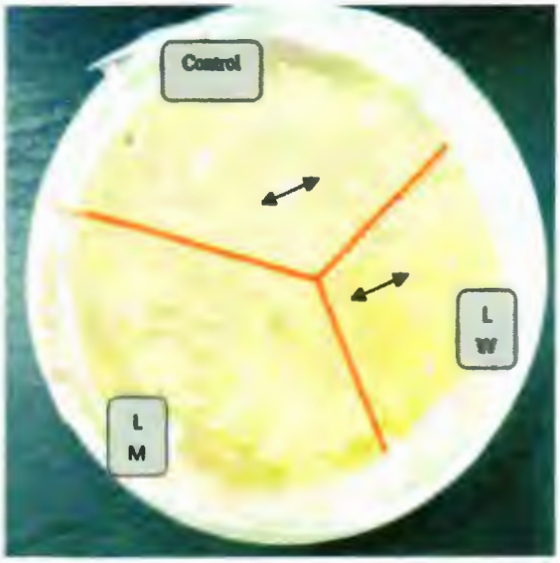
Effect of nanoparticles treated plant extract and control was observed by measuring zone of inhibition around the disc, treated with plant extracts. 0.05 µl/ml callus extract show highest antibacterial activity of 15 mm in both bacterial strains which is followed by 13 mm of 0.1 µl/ml extract. Control show lowest zone of inhibition diameter of 8 mm in *S. aureus* and 9 mm in *E. coli*. Fresh leaf methanol extract show diameter of 9 mm in *E. coli* and water leaf extract show 10 mm zone of inhibition in *S. aureus*. Results demonstrated an increase in antibacterial activity of nanoparticles treated callus extract.

**Table 4.6: Antibacterial activity of plant extracts**

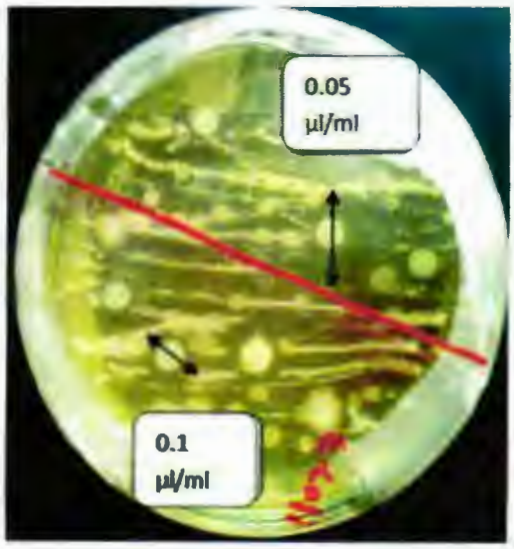
| Extract | Bacterial strains | Zone of inhibition Diameter |
|---|---|---|
| 0.05 µl/ml | *S. aureus* | 15 mm |
| | *E. coli* | 15 mm |
| 0.1 µl/ml | *S. aureus* | 12 mm |
| | *E. coli* | 12 mm |
| control | *S. aureus* | 8 mm |
| | *E. coli* | 9 mm |
| LW | *S. aureus* | 10 mm |
| | *E. coli* | none |
| LM | *S. aureus* | none |
| | *E. coli* | 9 mm |




(a)




(b)




(c)


(d)

**Figure 4.9: Antibacterial activity of NPs treated and control plant extract**

a) **Antibacterial activity in *S. aureus* 0.05 µl/ml and 0.1 µl/ml plant extract**
b) **Antibacterial activity in *S. aureus* Control, leaf methanol, leaf water**
c) **Antibacterial activity in *E. coli* 0.05 µl/ml and 0.1 µl/ml plant extract**
d) **Antibacterial activity in *E. coli* control, leaf methanol, leaf water**

# Chapter 5

# DISCUSSION

# 5. Discussion

Nanoparticles increased the germination rate when applied in lower concentration. Result suggested higher concentration of zinc oxide nanoparticles cause decrease in germination rate. Our results were in agreement with Lin and Zing who observed seed germination was inhibited in ryegrass by ZnO NPs at higher concentration (Lin and Xing., 2007). Our results also agree with shaymurat who demonstrated inhibitory effect of ZnO NPs at higher conc. in garlic plant, increase in time duration can also cause abnormality in cells and decreased growth (Shaymurat *et al.*, 2012).

Increase in germination rate was observed in 0.05 µl/ml. Our result was in agreement with Lopez-moreno who suggested decrease in NPs concentration result in increase uptake by plant cells, which cause increase germination and plant growth. Increase in uptake of NPs is attributed to decrease in aggregation of Nanoparticles. Result further indicated that high conc. of NPs result in aggregation and accumulation thus transport through cell wall pores become difficult (Lopez-moreno *et al.*, 2010).

Lower concentration of ZnO increased seed germination in 48 hour. Higher conc. of NPs resulted in decrease in germination rate and plant growth. Phytotoxity of NPs also depends upon time of treatment. Control plant showed germination rate of 66 %.

Nanoparticles treated seedling indicated delay in callogenesis as compared to control. Callus frequency was similar after one month in control and nanoparticles treated plants. Result indicated that NPs treatment can further effect callus regeneration and antibacterial activity of plant extract. This can explain by Nanoparticles accumulation within the cells which enhance antibacterial activity of nanoparticles treated plant extract as compared to the control. Slight delay was observed in nanoparticles treated callus which can also attributed toward nanoparticles accumulation in the seedlings and possible cause of callus initiation delay.

NPs treated and control callus were similar in morphology however callus mass increased in 0.05 µl/ml nanoparticles treated seedling. Observation of dry mass further suggested that dry callus mass of NPs treated seedling and control was similar. This result can be explained with water accumulation in the cell due to presence of nanoparticles in the cell.

Nanoparticles application in Regeneration medium resulted in tremendous increase in shoot regeneration frequency of Sweet Basil. 0.1 µl/ml nanoparticles have highest shoot regeneration frequency followed by 0.05µl/ml while control plants showed only integration in 4 weeks.

Our result also suggested increase in plant height with higher concentration of nanoparticles. This result coincides with EL- Kereti who demonstrated application of ZnO nanoparticles on sweet basil by foliar spray method, result revealed increase in plant height, number of leaves, branches and shoots (El-Kereti *et al* 2013). All nanoparticles treated plant exhibited integration of explants. Our result indicated that 0.1 µl/ml NPs treated shoot showed higher shoot length in 20 days followed by 0.05µl/ml, this was opposite for number of leaves. 0.05µl/ml NPs treated plants showed highest number of leaves followed by 0.1 µl/ml NPs treated explants.

Antibacterial activity of Nanoparticles treated plants and control was compared. 0.05µl/ml callus showed highest antibacterial activity followed by 0.1 µl/ml callus. This result further confirm observation of Lopez-moreno and suggested that 0.05µl/ml concenctration of ZnO NPs enhance uptake of NPs in plant cells.

## Conclusion

Nanotechnology has become integral part of consumer products, biotechnology, industry and agriculture. However effect of nanoparticles on higher plants and environment still need thorough research. This research successfully analyzed effect of nanoparticles on *Ocimum basilicum* seed germination, micropropogation and antibacterial activity of plant extract. Result demonstrated that higher conc. of ZnO NPs can decrease seed germination while it increased shoot

regeneration frequency. This research revealed that various parts of plant react to same concentration of nanoparticles differently. Antibacterial activity of NPs treated plants was greater than control this result will open the door for novel application of nanoparticles treated medicinal plants for antimicrobial drugs.

## Future prospects

This research will help in better understanding of following

- Effect of nanoparticles on plant cells *in vitro* culture on other plant species
- Effect of nanoparticles on shoot regeneration of other plants
- Toxicity of ZnO nanoparticles on different concentrations

It will help to design.....

- Nanoparticles treated plants drugs to enhance antibacterial activity
- Optimization of Nanofertilizers conc. to increase plant growth

# Chapter 6

# REFERENCES

# 6. Reference

AbdulSalam H., Sivaraj R., and Venckatesh R., (2014) Green synthesis and characterization of zinc oxide nanoparticles from Ocimum basilicum L. var. purpurascens Benth.-Lamiaceae leaf extract, Materials Letters, 131, p. 16–18

Adiguzel A., Gulluce M., Sengul M., Ogutcu H., Sahin F and Karaman I., (2005) Antimicrobial Effects of Ocimum basilicum (Labiatae) Extract, Turk J Biol, 29 p.155-160.

Asghari F., Hossieni B., Hassani A and Shirzad H., (2012) Effect of explants source and different hormonal combinations on direct regeneration of basil plants (*Ocimum basilicum* L.), AJAE 3(1), p. 12-17

Banu LA, Bari MA (2007) Protocol establishment for multiplication and regeneration of *Ocimum sanctum* Linn. An important medicinal plant with high religious value in Bangladesh, J Plant Sci, 2(5), p. 530-537.

Begum F., Amin M.N., Azad M.A.K (2000) *In vitro* clonal propagation of holy basil (*Ocimum sanctum* L.), Plant Tissue Cult, 10 (1): 31-37.

Baruah S and Dutta J., (2009) Nanotechnology applications in sensing and pollution degradation in agriculture. Environ Chem Lett. 7(3): 191-204.

Begum, F., Amin, M.N and Azad M.A.K., (2002) *In vitro* rapid clonal propagation of *Ocimum basilicum* L., Plant Tissue Cult., 12, p. 27-35.

Bajpai S.K., Chand N and Chaurasia V., (2012) Nano zinc oxide-loaded calcium alginate films with potential antibacterial properties, Food Bioprocess Technol, 5, p. 1871–81.

Carovic -Stanko K., Alinovic A.S., Grdisa M., Liber Z., Kolak I and Satovic Z., (2011) Efficiency of morphological trait descriptors in discrimination of Ocimum basilicum L. accessions, Plant Biosystems, 1, p. 1–8.

Cao Y.C., Jin R., Nam J.M., Thaxton C.S and Mirkin C.A., (2003) Raman dye-labeled nanoparticle probes for proteins, JACS, 125, p. 14676-14677.

Daniel V.N., Daniang I. E., and Nimyel N. D., (2011) Phytochemical analysis and mineral elements composition of *Ocimum basilicum* obtained in jos metropolis, plateau state, Nigeria, International Journal of Engineering & Technology, 11(6), p. 161-165.

Duncan T.V., (2011) Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors, J Colloid Interface Sci, 36 p.1–24.

Ekmekci H and Aasim M., (2014) *In vitro* plant regeneration of turkish sweet basil (*Ocimum basilicum L.)* The journal of animal & plant sciences, 24(6), p. 1758-1765.

El-Kereti M.A .,El-feky S.A., Khater M.S., Osman Y.A and El-sherbini E.A., (2013) ZnO nanofertilizer and He Ne laser irradiation for promoting growth and yield of sweet basil plant, Recent Patents on Food, Nutrition & Agriculture, 5(3), p. 169-181

Grayer R.J., Kite G.C., Goldstone F.G., Bryan S.E., Paton A and Putiersky E., (1996) Infra specific taxonomy and essential oil chemotypes in sweet basil, Ocimum basilicum. Phytochemistry. 43, p. 1033-1039.

Gulcin, I., Elmastaş M and Aboul-Enein Y.H., (2007) Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies, Phytother. Res, 21, p. 354-361.

Hackenberg S., Scherzed A., Kessler M., Froelich K., Ginzkey C., Koehler C, Burghartz M., Hagen R., Kleinsasser N., (2010) Zinc oxide nanoparticles induce photocatalytic cell death in human head and neck squamous cell carcinoma cell lines *in vitro*. Int. J. Oncol, 37, p. 1583–1590.

Hussain AI., Anwar F., Sherazi S.T.H and Przybylski R., (2008) Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations, Food Chem., 108 p. 986-995.

Heng B.C., Zhao X., Xiong S., Ng K.W., Boey F.Y and Loo J.S., (2010) Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress, Food and Chemical Toxicology, 48 (6), p. 1762–1766.

Iravani S., (2011) Green synthesis of metal nanoparticles using plants, Green Chem, 13, p. 2638–2650.

Javanmardi J., Khalighi A., Kashi A., Bais H.P., and Vivanco J.M., (2002) Chemical Characterization of Basil (*Ocimum basilicum* L.) Found in Local Accessions and Used in Traditional Medicines in Iran, J. Agric. Food Chem., 50, p. 5878-5883.

Jones N., Ray B., Ranjit K.T and Manna A.C., (2007) Antibacterial activity of ZnO nanoparticles suspensions on a broad spectrum of micro-organism, FEMS Microbiol Lett 279, p. 71–76.

Kalteh M., Alipour Z.T., Ashraf S., Aliabadi M.M and Nosratabadi A.F., (2014) Effect of silica Nanoparticles on Basil (Ocimum basilicum) Under Salinity Stress, journal of chemical health risks, 4(3), p. 49 –55

Kaurinovic B., Popovic M., Vlaisavljevic S and Trivic S., (2011) Antioxidant Capacity of *Ocimum basilicum* L. and *Origanum vulgare* L. Extracts, Molecules, 16, p. 7401-7414.

Kumari M., Khan S.S., Pakrashi S., Mukherjee A and Chandrasekaran N., (2011) Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of Allium cepa, Journal of Hazardous Materials, 190, p. 613–621

Lin D and Xing B., (2008) Root uptake and phytotoxicity of ZnO nanoparticles, Environ Sci Technol, 42 p. 5580–5585

Lopez-Moreno ML, De La Rosa G, Hernandez-Viezcas JA, Castillo-Michel H, Botez CE, Peralta-Videa JR, Gardea-Torresdey JL. (2010) Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants. Environ Sci Technol, 44, p. 7315–7320.

Lee W.M., Kwak J.I and An Y.J., (2012) Effect of silver nanoparticles in crop plants *Phaseolus radiatus*and *Sorghum bicolor*: Media effect on phytotoxicity, Chemosphere, 86 (5), p. 491–499.

Lin D and Xing B., (2007) Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth, Environmental Pollution, 150(2), p. 243–250.

Madhavi D.L., Smith M.A.L and Juthangkoon S., (1995) Anthocyanins in *Ocimum basilicum* cv. Purpurascens *in vitro,* In Vitro, 31, 67–72.

Monica R.C and Cremonini R., (2009) Nanoparticles and higher plants, Caryologia, 62(2), p. 161-165

Morsy M.K., Khalaf H.H., Sharoba A.M, El-Tanahi H.H and Cutter C.N., (2014) Incorporation of Essential Oils and Nanoparticles in Pullulan Films to Control Foodborne Pathogens on Meat and Poultry Products, Journal of Food Science, 79, p. 675-684.

Nair R., Varghese S.H., Nair B.G., Maekawa T., Yoshida Y and Kumar D.S., (2010) Nanoparticulate material delivery to plants, Plant Science, 179(3), p. 154–163.

Ozcan M and Chalchat J.C., (2002) Essential oil composition of *Ocimum basilicum* L. and *Ocimum minimum* L. in Turkey, Czech J. Food Sci., 20(6), p. 223–228.

Phippen W.B And Simon J.E., (2000) Shoot regeneration of young leaf explants from basil (Ocimum basilicum L.), In Vitro Cell. Dev. Biol. Plant, 36, p. 250-254.

Politeo O., Jukic M., and Milos M (2007) Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essential oil, Food Chemistry, 101, p. 379–385.

Premanathan M., Karthikeyan K., Jeyasubramanian K and Manivannan G., (2011) Selective toxicity of ZnO nanoparticles toward Gram positive bacteria and cancer cells by apoptosis through lipid peroxidation, Nanomedicine, 7, p. 184–192.

Roco M.C., (2005) Environmentally responsible development of nanotechnology, Environ Sci Technol, 39, p. 106–112

Rico C.M., Majumdar S., Duarte-Gardea M., Peralta-Videa J.R and Gardea-Torresdey J.L (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain, J Agric Food Chem, 59(8), p. 3485–3498.

Sahoo, Y., Pattnaik, S.K and Chand, P.K., (1997) *In vitro* clonal propagation of a medicinal plant *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation, *In vitro* cell dev. Biol. Plant. 33 p. 293- 296.

Shahzad A., Faisal M., Ahmad N., Anis M., Alatar A and Hend A.A., (2012) An efficient system for *in vitro* multiplication of *Ocimum basilicum* through node culture, African Journal of Biotechnology, 11(22), p. 6055-6059.

Siddique I and Anis M., (2007) Rapid micropropagation of *Ocimum basilicum* using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium, Biologia Plantarum, 51 (4) p. 787-790.

Shaymurat T., Gu J., Xu C., Yang Z., Zhao Q., Liu Y and Liu Y (2012) Phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (*Allium sativum* L.): A morphological study, Nanotoxicology, 6(3), p. 241-248

Sharma V., Shukla R.K., Saxena N., Parmar D., Das M., and Dhawan A., (2009) DNA damaging potential of zinc oxide nanoparticles in human epidermal cells, Toxicology Letters, 185 (3), p. 211–218

Siddiqui, B. S., Aslam, H., Begum, S. and Ali, S. T. (2007b). New cinnamic acid esters from ocimum basilicum. *Nat Prod Res,* 21, 736-41.

Sajjadi S.E., (2006) Analysis of the essential oils of two cultivated basil (*Ocimum basilicum L.*) from Iran, DARU, 14(3), p. 128-130.

Tada H., Murakami Y., Omoto T., Shimomura K and Ishimaru K., (1996) Rosmarnic acid and phenolics in root cultures of *Ocimum basilicum*, Phytochemistry, 42, p. 431–434.

Telci I., Bayram E., Yilmaz G and Avci B., (2006). Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochem. Syst. Ecol., 34 p. 489-497.

Vinardell M.P and Mitjans M., (2015) Antitumor Activities of Metal Oxide Nanoparticles, Nanomaterials, *5*, p. 1004-1021.

Xia T., Kovochich M., Liong M., Mädler L., Gilbert B., Shi H., Yeh J I., Jeffrey I. Zink J I., and Andre E., (2008) Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties, ACS Nano, 2 (10), p 2121–2134.

Yoshida J and Kobayashi T., (1999) Intracellular hyperthermia for cancer using magnetite cationic liposomes, J Magn Magn Mater, 194, p.176-184.

Zheljazkov V.D., Callahan A and Cantrell C.L., (2008) Yield and Oil Composition of 38 Basil (*Ocimum basilicum* L.) Accessions Grown in Mississippi, J. Agric. Food Chem., 56, p. 241–245.

# APPENDIX

Statistix 8.1 8/27/2016, 10:11:14 AM

## Analysis of Variance Table for germination

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| time | 1 | 246.92 | 246.92 | 0.84 | 0.3769 |
| treatment | 2 | 3425.91 | 1712.95 | 5.84 | 0.0169 |
| time*treatment | 2 | 30.87 | 15.43 | 0.05 | 0.9489 |
| Error | 12 | 3518.50 | 293.21 | | |
| Total | 17 | 7222.20 | | | |

Grand Mean 55.555 CV 30.82

### LSD All-Pairwise Comparisons Test of germination for time

| time | Mean | Homogeneous Groups |
|---|---|---|
| 20 | 59.259 | A |
| 10 | 51.851 | A |

Alpha 0.05 Standard Error for Comparison 8.0720
Critical T Value 2.179 Critical Value for Comparison 17.587
Error term used: Error, 12 DF
There are no significant pairwise differences among the means.

### LSD All-Pairwise Comparisons Test of germination for treatment

| treatment | Mean | Homogeneous Groups |
|---|---|---|
| 1 | 66.666 | A |
| 3 | 63.889 | A |
| 2 | 36.111 | B |

Alpha 0.05 Standard Error for Comparison 9.8862
Critical T Value 2.179 Critical Value for Comparison 21.540
Error term used: Error, 12 DF
There are 2 groups (A and B) in which the means
are not significantly different from one another.

### LSD All-Pairwise Comparisons Test of germination for time*treatment

| time | treatment | Mean | Homogeneous Groups |
|---|---|---|---|
| 20 | 1 | 72.222 | A |
| 20 | 3 | 66.666 | AB |
| 10 | 1 | 61.111 | ABC |
| 10 | 3 | 61.111 | ABC |
| 20 | 2 | 38.889 | BC |
| 10 | 2 | 33.333 | C |

Alpha 0.05 Standard Error for Comparison 13.981
Critical T Value 2.179 Critical Value for Comparison 30.462
Error term used: Error, 12 DF

There are 3 groups (A, B, etc.) in which the means
are not significantly different from one another.

| Time | Treat | R1 | R2 | R3 | Mean | Std |
|---|---|---|---|---|---|---|
| 10 | 1 | 83.333 | 33.333 | 66.666 | 61.11067 | 25.45872 |
| 10 | 2 | 16.666 | 33.333 | 50 | 33.333 | 16.667 |
| 10 | 3 | 66.666 | 66.666 | 50 | 61.11067 | 9.62212 |
| 20 | 1 | 83.333 | 50 | 83.333 | 72.222 | 19.24482 |
| 20 | 2 | 33.333 | 33.333 | 50 | 38.88867 | 9.622697 |
| 20 | 3 | 66.666 | 83.333 | 50 | 66.66633 | 16.6665 |

## 2-way Analysis of Variance Table for Callus

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Time | 1 | 102.44 | 102.436 | 2.11 | 0.1722 |
| Treatment | 2 | 305.48 | 152.739 | 3.14 | 0.0799 |
| Time*Treatment | 2 | 114.53 | 57.265 | 1.18 | 0.3410 |
| Error | 12 | 583.17 | 48.597 | | |
| Total | 17 | 1105.61 | | | |

Grand Mean 84.570 CV 8.24

**LSD All-Pairwise Comparisons Test of Callus for Time**

| Time | Mean | Homogeneous Groups |
|---|---|---|
| 20 | 86.956 | A |
| 10 | 82.184 | A |

Alpha 0.05 Standard Error for Comparison 3.2862
Critical T Value 2.179 Critical Value for Comparison 7.1601
Error term used: Error, 12 DF
There are no significant pairwise differences among the means.

**LSD All-Pairwise Comparisons Test of Callus for Treatment for 2-way ANOVA**

| Treatment | Mean | Homogeneous Groups |
|---|---|---|
| 3 | 88.000 | A |
| 2 | 86.933 | AB |
| 1 | 78.777 | B |

Alpha 0.05 Standard Error for Comparison 4.0248
Critical T Value 2.179 Critical Value for Comparison 8.7693
Error term used: Error, 12 DF
There are 2 groups (A and B) in which the means
are not significantly different from one another.

## LSD All-Pairwise Comparisons Test of Callus for Time*Treatment

| Time | Treatment | Mean | Homogeneous Groups |
|---|---|---|---|
| 20 | 3 | 91.333 | A |
| 10 | 2 | 88.000 | A |
| 20 | 2 | 85.867 | AB |
| 10 | 3 | 84.667 | AB |
| 20 | 1 | 83.667 | AB |
| 10 | 1 | 73.887 | B |

Alpha 0.05 Standard Error for Comparison 5.6919
Critical T Value 2.179 Critical Value for Comparison 12.402
Error term used: Error, 12 DF
There are 2 groups (A and B) in which the means
are not significantly different from one another.

| Time duration | Treatment | R1 | R2 | R3 | mean | std |
|---|---|---|---|---|---|---|
| 10 | 1 | 73.33 | 73.33 | 75 | 73.88667 | 0.96417495 |
| 10 | 2 | 93 | 80 | 91 | 88 | 7 |
| 10 | 3 | 94 | 80 | 80 | 84.66667 | 8.08290377 |
| 20 | 1 | 80 | 80 | 91 | 83.66667 | 6.35085296 |
| 20 | 2 | 86.6 | 80 | 91 | 85.86667 | 5.53654525 |
| 20 | 3 | 94 | 80 | 100 | 91.33333 | 10.2632029 |

## 2-way ANOVA for shoot Initiation

**Analysis of Variance Table for shoot initiation**

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| time | 1 | 756.15 | 756.15 | 7.00 | 0.0213 |
| treatment | 2 | 4537.06 | 2268.53 | 21.00 | 0.0001 |
| time*treatment | 2 | 216.03 | 108.02 | 1.00 | 0.3966 |
| Error | 12 | 1296.33 | 108.03 | | |
| Total | 17 | 6805.57 | | | |

Grand Mean 30.555 CV 34.02

**LSD All-Pairwise Comparisons Test of shoot initiation for time**

| time | Mean | Homogeneous Groups |
|---|---|---|
| 30 | 37.037 | A |
| 15 | 24.074 | B |

Alpha 0.05 Standard Error for Comparison 4.8996
Critical T Value 2.179 Critical Value for Comparison 10.675
Error term used: Error, 12 DF

All 2 means are significantly different from one another.

**LSD All-Pairwise Comparisons Test of shoot initiation for treatment**

| treatment | Mean | Homogeneous Groups |
|---|---|---|
| 2 | 50.000 | A |
| 1 | 30.555 | B |
| 3 | 11.111 | C |

| | | | |
|---|---|---|---|
| Alpha | 0.05 | Standard Error for Comparison | 6.0008 |
| Critical T Value | 2.179 | Critical Value for Comparison | 13.075 |

Error term used: Error, 12 DF
All 3 means are significantly different from one another.

**LSD All-Pairwise Comparisons Test of shoot Initiation for time*treatment**

| time | treatment | Mean | Homogeneous Groups |
|---|---|---|---|
| 30 | 2 | 55.555 | A |
| 15 | 2 | 44.444 | AB |
| 30 | 1 | 33.333 | BC |
| 15 | 1 | 27.777 | BC |
| 30 | 3 | 22.222 | C |
| 15 | 3 | 0.0000 | D |

| | | | |
|---|---|---|---|
| Alpha | 0.05 | Standard Error for Comparison | 8.4863 |
| Critical T Value | 2.179 | Critical Value for Comparison | 18.490 |

Error term used: Error, 12 DF
There are 4 groups (A, B, etc.) in which the means
are not significantly different from one another.

**Standard Deviations**

| | | standard deviaion for shoot Initiation | | | | |
|---|---|---|---|---|---|---|
| Time Period | Treatments | R1 | R2 | R3 | mean | std |
| 15 | 1 | 33.333 | 16.666 | 33.333 | 27.77733 | 9.622697 |
| 15 | 2 | 50 | 33.333 | 50 | 44.44433 | 9.622697 |
| 15 | 3 | 0 | 0 | 0 | 0 | 0 |
| 30 | 1 | 50 | 16.666 | 33.333 | 33.333 | 16.667 |
| 30 | 2 | 66.666 | 50 | 50 | 55.55533 | 9.62212 |
| 30 | 3 | 33.333 | 16.666 | 16.666 | 22.22167 | 9.622697 |

## Factorial AOV Table for shoot contamination

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| treatment | 2 | 6.8573 | 3.42867 | 1.00 | 0.4219 |
| Error | 6 | 20.5720 | 3.42867 | | |
| Total | 8 | 27.4294 | | | |

Grand Mean 0.6172
CV 300.00

**Means of contamination for treatment**

| treatment | Mean |
|---|---|
| 1 | 0.0000 |
| 2 | 0.0000 |
| 3 | 1.8517 |

Observations per Mean 3
Standard Error of a Mean 1.0691
Std Error (Diff of 2 Means) 1.5119

**LSD All-Pairwise Comparisons Test of contamination for treatment**

| treatment | Mean | Homogeneous Groups |
|---|---|---|
| 3 | 1.8517 | A |
| 1 | 0.0000 | A |
| 2 | 0.0000 | A |

Alpha 0.05 Standard Error for Comparison 1.5119
Critical T Value 2.447 Critical Value for Comparison 3.6994
There are no significant pairwise differences among the means.