Effects of nanoparticle treatment on growth of *Capsicum annuum* (chillies)

By

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Nanoparticle use

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Effects of nanoparticle treatment on growth of *Capsicum annuum* (chillies)

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بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْمِ

BISMILLĀHIR-RAḤMĀNIR-RAḤĪM

In the Name of Allah, the Most Gracious, the Most Merciful.

Department of Bioinformatics and Biotechnology

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FINAL APPROVAL

It is certify that we have read the thesis entitled "Effect of Nano particle treatment on growth of Capsicum annuum (chillies)" morphological characters, phytochemical analysis and antimicrobial activity of Capsicum annuum, submitted by Ms Shafaq Shahzad 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

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DEDICATION

I dedicate this efforts to my beloved parents my father M Yasir Alı Shahzad , my mother Naheed Aziz and my sister Sadaf Shahzad for their endless love, encouragement and support who ınspired me to work hard for my better future and taught me the best kınd of behaviour and help me to gain knowledge.

May Allah bless both of them always and forever (Ameen)

DECLARATION

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

Date ----------

Shafaq Shahzad

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LIST OF ABBREVATION

ABGE Lab	Applied Biotechnology and Genetic Engineering
ddH_2O	Double distilled water
HCL	Hydrochloric acid
mins	Minutes
MS	Murashige and skoog
NaOH	Sodium hydroxide
NARC	National Agricultural Research Council
NP	Nano particles
$feCl_3$	Iron Chloride
dH_2O	Distil water
AuNPs	Gold nanoparticles

ABSTRACT

The present study was carried out to study the effect of nanoparticles on morphological characters of Capsicum annuum cultivar Seeds were subjected to different treatments of ZnO nanoparticles Four treatments of ZnO nanoparticles are used i e 0 1 µl/ml, 0 15 µl/ml, 0 20 µl/ml, 0 25 µl/ml and untreated seeds named as control The treated and untreated seeds were used for investigation in both i e in invitro as well as in natural condition to see the effect of nanoparticles The treated and untreated seed were observed different morphological characters like height of plant, no of leaves, growth frequency, contaminated frequency, days to fruiting and days to flowering The different results of nanoparticles treated plants were observed from control Antibacterial assay of ZnO nanoparticles was carried out in solid growth medium i e agar medium against four pathogens (*Escherichia coli Pseudomonas aeruginosa and Staphylococcus aureus*) The bacterial growth was monitored by measuring the radius and diameter of culture medium and estimation of zone of inhibition on medium Phytochemical analysis of plant was also done by using methanolic extract of leaves, in which tests for alkaloids, tannins, flavonoids, steroid and triterpenoids was done.

INTRODUCTION:

1.1 INTRODUCTION OF NANOPARTICLES:

'Nano' means very small 10^{-9} to be precise this means that nanoparticles are composed of large amount of minute particles that are compact together to the surface Such particles are present on everywhere on the Earth's surface Nanoparticles occur as dust in the air, in our bodies, as small suspended colloids that make river water murky, in volcanic ash, in soil, and in technological applications ranging from ultra-tough ceramics to microelectronics (Alexandra , 2000)

Nanotechnology is advance scientific methodology This technology includes the utilization of resources and gear equipped for controlling chemicals in addition to physical properties of that substance at nano level In contrast biotechnology incorporates via different techniques and knowledge of science to manipulate, cellular and genetic processes to make products and used as a piece of different fields from answer for agribusiness (Fakruddin *et al* , 2012)

The improvement in new nanodevices and nanomaterials with the rise of nanotechnology (Joseph and Morrison , 2006, Scott and Chen , 2003) open up the new applications in agricultural bio-technology (P , Gonza lez-melendi et al , 2008)

Nano materials in nano biotechnology demonstrate totally new and better practices based on morphology, distribution and size (Sing et al , 2010) An important concern is the making of nanoparticles of different sizes shapes and with control dissimilarity are the experimental & chemical methods that involves the production of large amount of risky and unsafe by-products in the end (Dubey et al , 2009)

Food shortage is prevailing world wide Under the current system there is no possible means that the world can make enough food for that various people About 800 million individuals on planet are suffering from lack of food and the poverty line of individuals has increased dramatically as indicated by the United Nations In the past decades the green revolutions from the emergence of 1st generation technology have resulted in the transformation of traditional agriculture to industrial agriculture At this stage, quality & quantity of agricultural products improved a lot Nanotechnology has make the possible way to revolutionize food systems & agriculture (Zenu Jha et al , 2011)

1.2 SYNTHESIS OF NANO PARTICLES:

Nano particles can be synthesize by wide variety of different materials like silicates, magnetic materials, metal oxide ceramics, emulsions & liposome, etc (Hollister et al , 2003) Different methods are used in the making of NPs (Nano

Particles) For making such NPs both chemistry & physics techniques are used Among these, laser ablation, high energy ball milling,

Arc-discharge, and laser pyrolysis, is used most frequently Sonochemistry and Chemical vapor deposition, Electrochemical and different types of wet chemistry routes (e g co-precipitation, inverse micelles sol-gel etc) are commonly used (P Gonzalez *et al* , 2007)

Well establish protocols were used for the synthesis and characterization of AuNPs (Jana et al 2001a, 2001b, 2001c, Murphy et al 2005) Mostly 3 5 and 18 nm of diameters of AuNPs are used To avoid the poisonous effect on plants, citrate capped AuNPs are used because this coating have no any toxic effect Making of silver nano particles were analyzed by TEM, UV-vis and electrophoretic mobility in H_2O to recognize charge and size (Tara et al , 2011)

At nanoparticle scale the production and materials method can be obtained with distinctive applications For a long time nanoparticles have been produced chemically and physically, but new improvements demonstrate the vital role of biological systems and microorganisms in making of metal nanoparticles Additionally, metal nanoparticle biosynthesis is an ecologically amicable strategy (green science) without utilization of unforgiving, harmful and costly chemicals The organisms utilized as a part of nanoparticle blend change from straight forward prokaryotic bacterial cells to complex eukaryotes Organism's capacity in creating metal nanoparticle is another exciting methodology in the direction of improvement of

nano industrial facilities For representation, generation of AuNPs by chemical reduction (like sodium borohydride, DMF, ethylene glycol and hydrazine hydrate) may prompt retention of brutal chemicals on the surface of nanoparticle increasing the poisonous issues Extract from plants may acts as both capping and reducing agents in the synthesis of nanoparticle Along with organic process different physical and chemical techniques are utilized to combine Magnetite nano particles, however a portion of chemical techniques required in the making of nanoparticles uses harmful solvents that can be potentially unsafe Iron oxide nano particles can be synthesized likewise via utilizing hay bio-mass yielding PH 10 At the point when PH decreases (PH 5), large nano particles are created AuFe3O4 composite nanoparticles are set up by a combine organic and chemical reducing process (Semi biosynthesis strategy) (Siavash , 2011)

1.3 NANO PARTICLES IN AGRICULTURE:

Agriculture the spine of 3rd world financial matters yet, unluckily now the agribusiness areas are confronting different worldwide difficulties like atmosphere changes, environmental issues, sustainable use of resources and urbanization like run-off, aggregation of pesticides and fertilizers, human population is increasing gradually as well as food requirement is rising quickly in world with the increase of population from present level of six billion to nine billion to 2050 is predicted thus we tend

to adopt inexpensive techniques to create agribusiness additional property (Hongda and Rickey , 2011)

Plants can interact with nano particles in the earth through water, air and soil sources Capacity of nano particles to collaborate with plants shown (Corredor et al 2009) that as it may the information created by these studies have not generally been in assertion In any case, as of late Ferry et al (2009) demonstrated that gold (Au) nano rods are partitioned into ocean grasses and estuarine Mesocosms however just at lower levels, showing restricted capacity of these particular nano particles to enter plants by marine exposure means

1.4 USES / APPLICATION OF NANOPARTICLES:

Nano particles display catalytic optical and electrical properties and are being utilized seriously as a part of gadgets, metallics, earthenware production, polymers, inks films and coatings on account of their great repairing impact Past studies demonstrated that NPs are harmful to oceanic creatures, including green growth, daphnids and fish Pimephales latipes promelas and Oryzias were influenced antagonistically by fullerene An impact of lipid for every oxidation was seen in Micropterus salmoides when presented to fullerene Survival of Daphnia magna was diminished by presentation to ZnO , TiO2 , SiO2 , and fullerene (Lee et al , 2008)

Transgenic plants are crested by Gene exchanges by assault of DNA-absorbed AuNPs in a species-independent way (Christou et al., 1988) As of late it is reported that through the silica nano particles effective conveyance of chemicals and DNA can occur in plant cells, lacking the prerequisite of particular tools (Torney, *et al* 2007)

Nanoparticles and nano capsules give a productive intends to disseminate manure and pesticide with high site specificity in a controlled manner consequently dropping collateral blow-back Ranch use of nano technology is picking up consideration via effective control and exact arrival of pesticides, composts and herbicides Nano-sensors improvement helps in deciding the required measure in terms of input, for example, pesticides and manures Nano-sensors for insect repellent discovery suggest high affectability, short recognition limits great selectivity, quick reactions & little size Likewise they can recognize the level of soil dampness and supplements of soil nano fertilizers are quickly ingested by plants Nano-encapsulated moderate discharge manures are spare compost utilization and reduce ecological contamination (Sidra *et al*, 2014)

Nanotechnology is presently utilized as a device to investigate the darkest terms of health sciences in a few ways e g targeted drug delivery, artificial implants, imaging and gene delivery systems and sensing Thus, nano-sized inorganic and organic particles is finding rising consideration in health applications because of their manageability to organic fictionalization Taking into account upgraded adequacy, the

new drugs are nano particles of metals, ceramics and polymers which can battle conditions like cancer and fight human pathogens like microorganisms (Siddhartha et al , 2007)

1.5 TOXICITY/HARMFUL EFFECTS OF NANO PARTICLES:

Nano particles have also had toxic effect on organisms The toxic effect is mostly seen in mammals, mostly on airborne, dermal exposures and dietary (Stern and McNeil , 2008) The organic distribution, fate and possible toxic effect of nano-materials are minimally explored in plants Some studies shows that the exposure of plants to copper may cause the toxicity and abnormal root growth (Lee *et al* , 2008, Stampoulis et al 2009) and rarely earth oxide (nano-Yb2O3, nano-Gd2O3 and nano-La2O3) nano particles (Ma *et al* , 2010) Another research suggest that plant cell division and stress effects in leaves is effected by silver (Kumari et al , 2009) and palladium nano particle in contact (Battke et al , 2008), correspondingly (Tara et al , 2011)

1.6 FUTURE PERSPECTIVES:

The applications of nano-technology have a great potential of changing agricultural production by allowing the improved management and conservation of

inputs to plant production Nano technologists can do a lot to help society through the applications of nano technology in agriculture and food culture (Sugunan and Dutta, 2004)

The challenges and advantages of nanotechnology will prompt better acknowledgment of this developing innovation for public awareness Cross contamination and control of pests of food products and agriculture are related to the rapid biosensors and testing technologies will prompt utilization of nanotechnology soon in future There is great potential of nano Nano-technology in agriculture because this field is capable to improving the worth of life by its applications in different fields like quality agriculture, sustainable and the rich and better foodstuff for population Everywhere all over the world, this innovation has turned into the eventual fate of any nation One must be extremely mindful of any innovative technology to be presented about its likely unanticipated and surprising peril that could arrive through its hopeful potential outcomes However, it is additionally noteworthy for the eventual fate of a state to make talented prospect man power for this latest innovation Hence, it gets to be vital to educate the common man about the advantages at the initial stage, which will inconceivably enlarge novel technique and development of in awareness in all circles In agriculture the standpoint of nano-science is dubious inferable from a considerable measure of grounds For instance, the unconstructive reaction from individuals towards genetically modified (GM) crops.

need of a ton of required talents in government agricultural research and technology units for nano sort of investigations and inadequately prepared new instruments and many more modern advances (Ram *et al* , 2014)

As on account of each nontraditional innovation, for instance hereditary building, some dread that nano-innovation can give individuals an excessive amount of control Nano technology is the powerful technique from which we can take advantages in agriculture technology for welfare of mankind Nano-technology can strive to present and fundamentally build more efficient technology that is currently in used for remediation sensing and environ-mental detection The potential advantages and use of nanotechnology are vast like agricultural yield development involving for slow release of nano-porous zeolites and well-organized dosage of fertilizers and water, nano-capsules for vector, herbicide delivery, nano-sensors for pest detection and pest management (Scrinis and Lyons 2007, Scott 2007)

1.7 FAMILY SOLANACEAE

The Solanaceae is substantial plant families include more than 3000 species including numerous crops, for example, pepper, eggplant, potato and tomato It includes a group of dicotyledon plants in the Euasterid clade, which is different from the model plant Arabidopsis It is the 3rd economical plant family and positions the

first a vegetable yields Similar family Rubiaceae includes coffee, a standout amongst the most profitable farming wares in the universal exchange (Feinan and Steven, 2010)

1.7.1 GENUS CAPSICUM

Capsicum species are the members of Solanaceae family (tribe Solaneae, sub-tribe Capsicinae), which involves petunia, tobacco, potato and tomato Genus Capsicum contains almost 31 species (Moscone et al , 2007) from which 5 are domestic, that is C *baccatum L*, C *frutescens L*, and C *pubescens* R C *annuum L* and C *chinense Jacq*, (IBPGR, 1983) The production of chili pepper and sweet pepper crops is vital agribusiness internationally, where this business promote family farming and better income and employment production from agriculture (Reifschneider , and Ribeiro , 2008)

The primary producing area on the planet is Asia, particularly in China which delivered around 254 thousand tons of sweet and hot peppers in 2008, trailed by India creating 1 23 million tons (FAOSTAT, 2010) Reifschneider and Ribeiro (2008) contend that in Brazil this is a business sector that moves around 100 M USD every year, including domestic utilization and fares According to these Authors, red peppers

represent 3rd place utilization and production of flavoring vegetables in Brazil In this manner, this business sector empowers farming in Brazil (Vilela *et al* , 2008) (Dias *et al* , 2012)

Mostly the Capsicum is local to South and Central America (Perry *et al* , 2007), it is believed that, this genus is accepted to have been chosen in two regions of source, one called the primary center and after that acquainted with different regions called secondary center (Mongkolporn , and Taylor , 2011) Brazil is viewed as an auxiliary focal point (secondary center) of differing qualities of this genus

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CHAPTER 2

Literature review

2.1 Literature review

Non-uniform germination of chili pepper (Capsicum annum L) seeds could create problems in seedling establishment Rapid, uniform and complete germination are essential for high yield and quality crop production In current time period, different technologies entry in agricultural field has brought tremendous changes Capsicum annuum (chili pepper) is one of important vegetables in which germination of seed takes long time and during un favorable conditions , it is difficult to germinate Observation of Phytotoxity of various fixation nano particles(TiO2) on development were determined Results showed that without nanoparticles seed germination was slow and highest percentage of germination was found at a concentration of about 7 5% within 48 hours in the presence of nanoparticles Result observed showed that proper concentration of TiO2 has the ability to promote seedling growth and germination of pepper (Elahe et al , 2014)

On the germination of seed, nano particles have both positive as well as negative effects biomass , and root growth It's accepted that toxic actuated by AgNPs frameworks of plants might be because of harmful substances adsorbed on

nanomaterials surface Besides, these studies examined the adverse effectt just on germination of seed and root development (C Krishnaraj et al , 2012)

To improve seed germination now a day's silver nanoparticles(AgNPs) are used as well as to enhance growth of plants Plants response to AgNPs , inhibition of growth or enhancement, depends on dosage of AgNP Higher and lower concentrations could have negative effect An expansion in anatase nanoparticle (nTiO2) fixation brings about critical increment in the germination rate, the index of rate of germination. length of the plumule and radical, the vigor index and fresh weight of seedlings of Capsicum annum L , while the ideal concentration of nTiO2 were 7 5% (Zainab and amjad , 2015)

2.2 EFFECT OF NANOPARTICLE ON FAMILY SOLANACEAE:

Silver nanoparticles (AgNPs) are involved to upgrade development of plant and germination of seed, enhance photosynthetic quantum effectiveness and go about as antimicrobial operators to oversee plant ailments The nanoparticle role in change of plant resilience to ecological anxieties, for example salinity and drought stays indistinct AgNPs assume an imperative part in directing the restraint germination of seed and plant development in saline circle by inciting salt resilience in plants Under

salt stress with silver nanoparticle (AgNP) treatment mean germination time improved May be straightforwardly or in a roundabout way silver nanoparticles(AgNP) required in morphological alteration and physiological procedures in plants Improved seed germination in some plant species was resulted such that nanoparticles diminished antioxidant emphasis by decreasing H2O2 and expanding enzymes such as APX The progressions AgNPs can modify their defensive instruments at higher saltiness levels Presentation to AgNPs eased the unfavorable impacts of salt push and enhanced the germination. room the sub-atomic reaction to AgNPs among the tried saltiness levels recommend that length and dry weight and seedling crisp of seeds of tomato under NaCl emphasis Four salt anxiety genes CRK1, P5CS, MAPK2 and AREB are managed by silver nano particles under salt anxiety The three quality genes ZFHD1 DDF2 and TAS14, were down-directed The outflow of other salt anxiety genes shifted between the two saltiness levels NaCl150 and NaCl200 (Zainab M Almutairi 2010)

Nano zinc oxide aids in enhancing salt resistance Zinc help increase in growth of the germinated seeds Nano – zinc oxide enhanced the germination parameters and caused to better drought tolerance (Sedghi et al , 2013)

Bacterial sullying is a difficult issue in plant tissue society techniques TiO2 NPs might be a helpful material for expelling the bacterial contaminants in plant (kamran safavi , 2014)

Various studies in the course of recent decades have demonstrated that the essential method of harmfulness identified with aluminum presentation particles (Al3) is anticipating in plants for root prolongation in plants Al (aluminium) bargains roughly 7% earth's outside layer however many types of Al is enchained by ligands that is less lethal As nanoparticles of aluminum oxide expanded, the typical lengths of three week tobacco seedling roots diminished Among seedlings of control group and the 0 1% aluminum oxide seedlings treated with nanoparticle, the normal length of root reduced by 25 4% The 0 5% and 1 0% aluminum oxide-treated seedlings diminished long by 81 4% and 92 2%, individually, when contrasted with the control which gives confirm that aluminum oxide nanoparticles negatively affect the development of the bases of three week old tobacco seedlings Concentrate likewise demonstrates that as the grouping of aluminum oxide nanoparticles expanded, the normal biomass of every three week old seedling reduced Drastic change causing reduction of seedling biomass is related with decreasing lengths of roots due to the increased concentration of nanoparticles Essentially Al2O3 nanoparticles negatively affect the development and advancement of three week of age tobacco seedlings (Caitlin et al , 2012)

2.3 PHYTOCHEMICAL STUDY OF SOLANACEAE AND CAPSICUM

New peppers are a fantastic wellspring of vitamins A and C and in addition nonpartisan and acidic phenolic mixes, which are imperative cell reinforcements for

an assortment of plant safeguard reactions Peppers contain moderate to large amounts of impartial phenolics or flavonoids, phytochemical which are essential cell reinforcement parts of a plant-based eating routine, some other than conventional supplements, which may decrease the danger of chronic infections Concentrates on have shown that peppers contain a wide exhibit of phytochemical The phytochemical changes that happen amid development and the resultant impact on cancer prevention agent action are imperative dietary contemplations that may influence the utilization of various pepper sorts Thinks about demonstrates that 40 °C was the Ideal saponification temperature with amplified maintenance of both xanthophyll and nonoxygenated carotenoids Oxygenated carotenoids zeaxanthin and capxanthin expanded impressively upon development as an after effect of shading change In particular, the color capsanthin was not recognized in the juvenile leafy foods been related just with pepper sorts containing the hereditary ability to integrate red shades upon development In general, developed C frutescens and C annuum

Cultivar was obviously higher in all out flavonoids than C chinense cultivars at the stage of adult The amazingly low flavonoids fixation in this impactful pepper may demonstrate preoccupation of Phenolic fore runners from flavonoids to capsaicinoids Tabasco has moderate levels of the luteolin flavones Capsicums seem, by all accounts, to be exceptional in that they contain both of these flavonoids in obvious focuses The greater part of the pepper sorts by and large displayed AOX particularly

capsicum annuum Concoction benefactors to AOX in peppers are various and may incorporate ascorbic capsaicinoids, corrosive, flavonoids, and a wide assortment of phenolic acid (L et al , 2000)

The Solanum family plant has bioactive mixes such as alkaloids, flavonoids, steroids and saponins and many others They are having antimicrobial action against some bacterial species such as Proteus mirabilis and E coli, Klebseiella pneumonia Methanol concentration of Solanum esculent demonstrated sensible antibacterial action while test was being performed on it The component was recognized by HPLC investigation and GCMS examination From the GC-MS comes about, the mixture found in Solanum esculentum plant portions was distinguished as Caffeic corrosive, Ferulic corrosive, p-Coumaric corrosive O-â-dglucoside and Kaempfero They are in charge of the antibacterial movement of the plant Solanum esculentum The bioactive mixes were secluded from the eatable part (leaves) of the plants They are utilized as a part of labs for the exploration reason furthermore they are utilized as a part of the restorative field (Akilan et al , 2014)

Capsicum annuum was higher in capsaicin than in dihydrocapsaicin (Araceli et al , 2011)study demonstrated that Capsicum natural products had solid cancer prevention agent action at grouping of 4 mg/ml The cancer prevention agent movement of concentrates expanded with the expanding number of aggregate

phenolic mixes in the concentrates with great relationship esteem at r2 = 0 935 It can be inferred that the flavonoids compound did not contribute as a noteworthy compound to the high aggregate of phenolic substance in this Capsicum tests (Rohanizah and Ishak 2012)

2.4 ANTIMICROBIAL ACTIVITY OF CAPSICUM ON NANOPARTICLES TREATED PLANTS

Silver nitrate has for quite some time been considered as an intense and normal anti-toxin and antibacterial operator Silver nanoparticles displayed antibacterial properties against bacterial pathogens with close connection of the nanoparticles themselves with the microbial cells. Study uncovered that the nanoparticles from pepper indicated great action against both the gram positive and gram negative creatures Likewise it appeared action against cocci cells and bar cells Silver eliminates microorganisms by choking them in a warm and soggy environment Exceptionally bioactive silver particles tie with proteins outside and inside bacterial cell films, accordingly repressing cell breath and proliferation 3-4 times silver is more dynamic at pH 8 than at pH 6 Silver items are compelling against microorganisms yet not as effective against different life forms like parasites, shape, and buildup The antimicrobial movement of silver nanoparticles demonstrated the focus subordinate action It gives movement against all the test living beings Since this is an effortlessly

accessible all through the country furthermore is utilized as a part of each house for cooking as an enhancing operator, the dynamic Nano compound from this can be arranged and utilized successfully to prevent the development of the microbial pathogens Subsequently, it has wide application in restorative field (Abdullah and Zainab 2013)

By expanding the centralization of ZnO nanoparticles in wells and plates, the development restraint has likewise been expanded reliably due to appropriate dispersion of nanoparticles in the agar medium Both nano and mass ZnO nanoparticles demonstrated antimicrobial movement against chose pathogens low improvement of the antimicrobial action was recorded in the instances of mass ZnO at lower fixation (2 and 4 mm) however medium hindrance was seen at higher focuses green ZnO with littler molecule size demonstrated improved movement because of the substantial surface territory to volume proportion and surface reactivity when contrasted with the ZnO nanoparticle arranged by synthetic strategy, while ZnO suspensions with lower focus range (0 5–4 mM) appears to show less antimicrobial action ZnO nanoparticles constitute a successful antimicrobial operator against pathogenic microorganisms (Sangeetha et al , 2011)

ZnO-NPs display appealing antibacterial properties because of expanded particular surface territory as the diminished molecule size prompting upgraded molecule surface reactivity ZnO is a bio-safe material that has photograph oxidizing

and photocatalysis sways on compound and organic species The different antibacterial components of nanomaterials are for the most part ascribed to their high particular (Amna et al , 2015)

Broad spectrum of anti bacterial activities is contained by ZnO nano particles Remarkable antibacterial activity is exhibited by ZnO nano particles and demonstrated a lethal effect against C jejuni even at low concentration Significant morphological changes are induced by ZnO nanoparticles, measurable membrane leakage, and on the basis of these phenomenon substantial increases (up to 52 folds) in oxidative stress gene expression in C jejuni and responses of cell, a plausible process of inavtivation of ZnO of bacteria includes the direct relation among cell surfaces and ZnO nano particles This affects the membrane permeability where nano particles enter and introduce bacterial cells of oxidative stress, resulting in cell death (Yanping *et al*, 2011)

2.5 EFFECT OF ZnO NANOPARTICLES

ZnO is harmless, it can be utilized as photocatalytic debasement materials of natural poisons High affectability is shown by mass and thin movies for some harmful gasses The great help is provided by Zinc oxide NPs for the yield and development of sustenance products Nut seeds were effectively treated with various

Treatment of Zinc oxide nano scale (25 nm mean molecule size) was utilized as 1000ppm fixation which advanced seedling power, seeds germination, and plant development and these zinc oxide nano particles similarly ended up being successful in establish development in peanuts and expanding stem ZnO NPs green union is much more secure and environment well disposed contrasted with compound blend since it doesn't prompt development of poisonous side effect chemicals To the extent their use is concerned nanoparticles assume a noteworthy part in farming, where colloidal arrangement of ZnO NPs is utilized as a part of nanofertilizers (Sidra et al , 2014)

Al_2O are in common nanoparticles As test material some nano particles such TiO2 and fullerence have been widely used to identify their nano toxicity ZnO nano particles have significant inhibition on bacterial as well as fungal contaminants and significantly prevent their growth While studying the explants regeneration rate was observed that Zn Nano particles concentration lower than threshold did not show any negative effect (Mohamed et al , 2014)

From the study it was observed that lower concentration of nanoparticles of ZnO did show the reduction effect on root length of rice , while TiO2 nanoparticles apparently offers no effect Much of roots are greatly affected by ZnO nanoparticles (Prapatsorn et al , 2011)

It might be assumed that high content of zinc in seed could go about as a starter manure and enhanced germination of seed and early development of seedling Essential part is essumed by zinc in starch and proteins digestion system and additionally Ncontrols plant development hormone i e IAA likewise a key segment of dehydrogenase, proteinase, and peptides catalysts and in addition advances starch arrangement, seed development and creation is zinc These truths show that the accessibility of high Zn content inside the seeds amid seed germination that has imperative physiological parts in seed germination and early seedling development The high percentage seed germination more seedling length in onion seedlings saw in seed parts obtained from ZnO Transportation of Zn from leaf tissues through phloem to the seed at the season of seed improvement and development proces can be credited by NPs treated plants ZnO NPs development of seedling and germination of seed in onion and determined the germination of seed increased in lower concentrations of ZnO NPs but show reduction in values at high concentration (S L Laware and Shilpa Raskar 2014)

Chapter: 3

Materials and Methods

The research work was directed in ABGE (Applied Bio-technology and Genetic Engineering Laboratory), International Islamic University and Islamabad

3.1 Plant Material

In research study the *Capsicum Annuum* seeds were used and obtained from NARC, National Herbarium, and Islamabad (3 1) & used in both invitro conditions as well as natural conditions (Figure 3 1)

Figure 3 1 Perspective of *Capsicum Annuum* seeds as a source of material from the National Herbarium, NARC, and Islamabad

3.2 Nano Particle Use

Nanoparticles used in research was ZnO, obtained from ABGE & were used in both invitro conditions as well as natural conditions (Figure 3 2)

Figure 3 2 Perspective of ZnO Nano Particles used in research

3.3 Apparatus:

3.3.1 Machinery:

Fully automatic autoclave (Classic 1050), Laminar airflow cabinet, Shaker (HY-4

Speed adjusting multipurpose vibrator), Drying Oven (DHG-9053A), Microwave Oven (orient), pH meter (Martini Instruments), Electronic balance (Shimadzu)

3.3.2 Glassware's

Conical flasks (50ml, 100 ml, 500 ml), Beakers (Pyrex, Germany), Petri plates and, Test tubes (18 x 150 mm, 27ml) used as a glassware for the germination of seeds as invitro Conical flasks and test tubes were used and non spongy cotton wrap as a muslin fabric (Figure 3.3)

3.3.3 Tools

Scalpels, Surgical blades, scissor, micropipettes, Forceps and Falcon tubes, discarding jug (100 ml), strainer, gloves, filter papers

Figure 3 3 illustration of glassware's and tools

3.4 For Natural Conditions

Pots, Manure & Fertilizers

3.5 Methodology

3.5.1 Treatment of seeds with ZnO Nanoparticles

Four different concentrations of nanoparticles were used i e 0 10 µl/ml, 0 15 µl/ml, 0 20 µl/ml, 0 25 µl/ml & untreated seeds (controlled) Seeds were pre soaked in distilled water for 24 hrs to break the dormancy After this seeds of *Capsicum Annuum* were subjected to different treatment levels of ZnO nanoparticle solution along with one control for 24 hrs (Figure 3 4)

Figure 3 4 treatment of seeds with different concentrations of ZnO nanoparticles

3.6 METHODOLGY FOR INVITRO

Experimental Design for Invitro

Factorial design was employed for each and every one of experiment Each experiment was consisted of 3 replicates and each replicate has 8 Samples/Observations

3.6.1 Media preparation for Invitro

In research work Synthetic MS medium with vitamins was uesd (Murashige and Skoog, 1962) The calculations were done for preparing 300ml MS media Sucrose is utilized as only carbon source Gel-rite used as a solidifying agent and the concentration is 0 75g in distilled water 0 5M HCl or 0 5M NaOH was further added to adjust the PH of media in the range of 5 7-5 8 as a final value Then the media was sterilized by autoclaving at 121 °C for 1 and half hour

3.6.2 Surface sterilization and preparation of Invitro culture

Treated and untreated seeds of Capsicum annuum were taken for surface sterilization Seeds were washed thoroughly with distilled water for 5-7 time followed by liquid detergent to avoid contamination, the sterilization of seeds were done in Laminar Air Flow Hood under aseptic conditions The sterilization was done by using 70% Ethanol (v/v) for2 min followed by the 50% Clorox (v/v) used for 15-20 min

Then 4-5 times washing was done with dH_2O Then seeds were placed on autoclaved filter paper for drying (Figure 3 5)

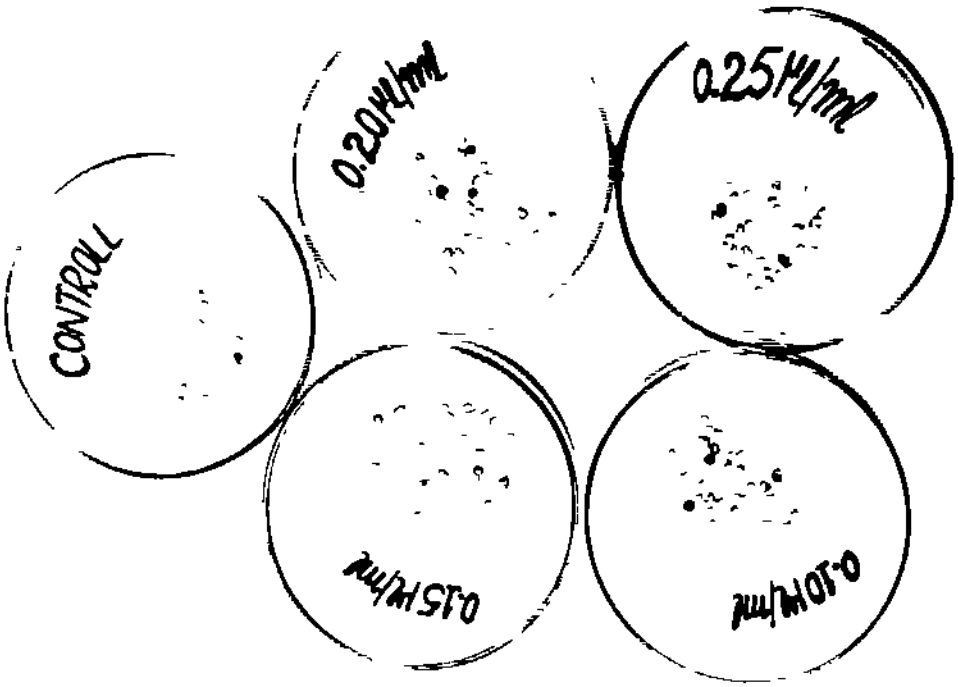


Figure 3 5 perspective of dried seeds on autoclaved papers

3.6.3 Seeds Induction

Surface sterilized treated and untreated seeds were placed in MS media for initiation of germination All cultures were maintained at 24 hours light at 25±2 °C temperature The growth of seeds starts after some specific time of induction The growth frequency was analysed by using formula

$$\text{Growth frequency (\%)} = \frac{\text{Number of seeds grows}}{\text{Total number of seeds cultured}}$$

Contaminated frequency was also analysed by using formula

$$\text{Contaminated frequency (\%)} = \frac{\text{No of contaminated test tubes}}{\text{Total no of test tubes}}$$

3.7 Methodology for Natural Conditions

Treated and untreated seeds were dried out on autoclave paper, and then dried seeds both treated and untreated were sown in the pots in International Islamic University Islamabad All pots were taken and were filled with a well prepared growth media of farm yard manure 15 seeds from different treatments were sown in pots Seeds were placed in pots at the depth of 1-2 cm and then seeds were covered with a

soil 15 untreated seeds were also sown in the pots in the same way In this way there were total 75 pots carrying sown seeds These pots were irrigated well in a spraying manner These plants were observed routinely for sort of changes in them

Different morphological and biochemical characters were studied including germination frequency, plant height, no of leaves, days to flowering Phytochemical Analysis and antimicrobial activity was also done

3.8 Statistical Analysis

The statistical analysis of data was done by using statistic software SPSS and the significance point was considered as 0 001

3.9 Phytochemical Analysis

3.9.1 Preparation of Extract

Leaves of plant was used for making crude methanolic extracts and step up by utilizing the technique portrayed by (Wadood *et al* , 2013) with a few changes To prepare the extract of leaf, we will collect the fresh leaves from plant from amature *Capsicum annuum* Then the collected leaves were washed by running tap water and then wipe up For drying place in drying oven Then powdered the dried leaves using pestle and mortar and then dissolved the powder in 30ml methanol Place this for

overnight Then next step was filtration which was done by using Whattmann filter paper No 1 Then the phytochemical analysis of leaf extract solution was done by treating different chemicals (figure 3 6)

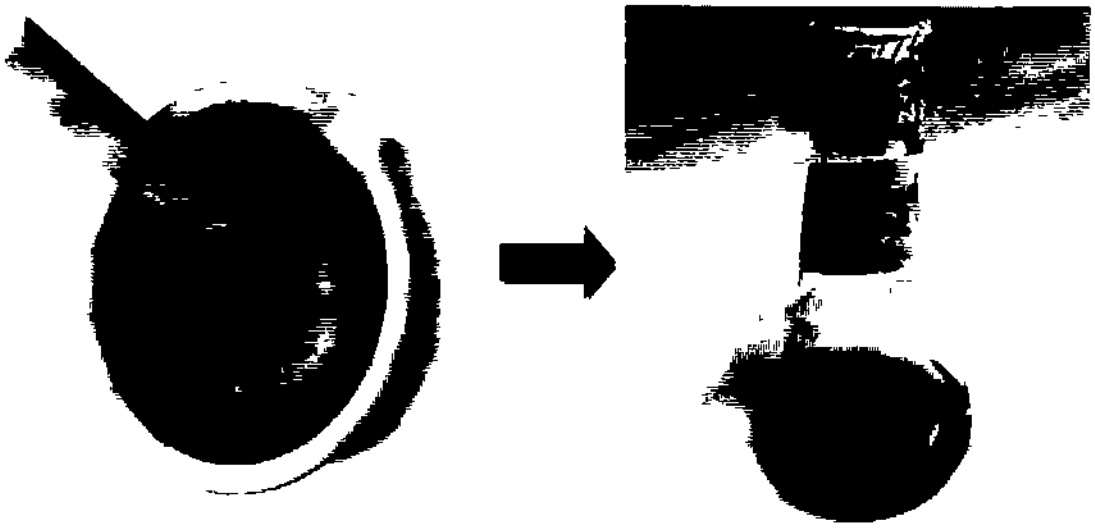


Figure 3 6 Crude methanolic extracts of mature leaves of *Capsicum annuum* prepared from the in vitro treated with nanoparticles

3.9.1.1: TEST FOR ALKALOIDS

For the recognition of alkaloids in 0 5-0 6 ml of methanolic extract of leaves 8ml of 1% HCl is added, then warmed and filtered Separate the 2ml of filtrate and after that a little quantity of saturated picric acid was dropped in test tubes having

methanolic extract Yellow colour indicates the presence of alkaloids

3.9.1.2: TEST FOR FLAVONOIDS

For identification of flavonoids add few drops of NaoH then add few drops of diluted HCl. yellow colour indicates the presence of alkaloids It turns colorless when acid is added

3.9.1.3: TEST FOR THE DETECTION OF TANNINS

For the revealing of tannins, methanolic extract was treated with iron chloride ($FeCl_3$) solution Blue or green colour indicates the presence of tannins

3.9.1.4: TEST FOR TRITERPENOIDS

The identification of triterpenoids in the methanolic extract of leaves of *capsicum annuum* was done by treating the H_2SO_4 with methanolic extract Forming of yellow colour at lower layer gives positive results

3.9.1.5: TEST FOR STEROIDS

For the identification of steroids in methanolic extract of leaves, 200ml methanolic extract were treated with 2ml of acetic acid and then cooled the solution on ice and mixed with little quantity of conc H_2SO_4 Appearance of violet to red colour confirms the occurrence of steroids

3.10 Plant Extracts Anti bacterial activity assay

Four different types of bacterial strains were used in research work i e Escherichia coli. Klebsiella pneumonia Pseudomonas aeruginosa and Staphylococcus aureus and were obtained from pathology lab of Al-Shifa hospital Islamabad

3.10.1 PAPER DISK DIFFUSION ASSAY:

For preparation of media 14g of Nutrient agar should be dissolved in 1L of dH2O and allowed to boil in oven at 45 °c for 2-3 mins at medium point After that nutrient agar medium were placed in autoclave for autoclaving for 1 hour and 45 minutes When autoclaving is completed, take the medium for further work After this the medium was spread on Petri plates. handling of this should be done under the well sterilized environment in hood

The filter paper discs (5mm in diameter) were placed on the agar plates which was inoculated with the tested microorganisms Four types of different strains of bacteria were used (Escherichia coli. Klebsiella pneumonia Pseudomonas aeruginosa

and Staphylococcus aureus) for antimicrobial activity Impregnation is done with 20µl of plant methanolic extracts Then the Petri plates were wrapped with Para film Then the plates were subsequently incubated at 37°C for 24 Hrs After incubation the growth of inhibition zone were quantified by measuring the diameter of the zone of inhibition in mm (kumar et al. 2009) (Figure 3 7)

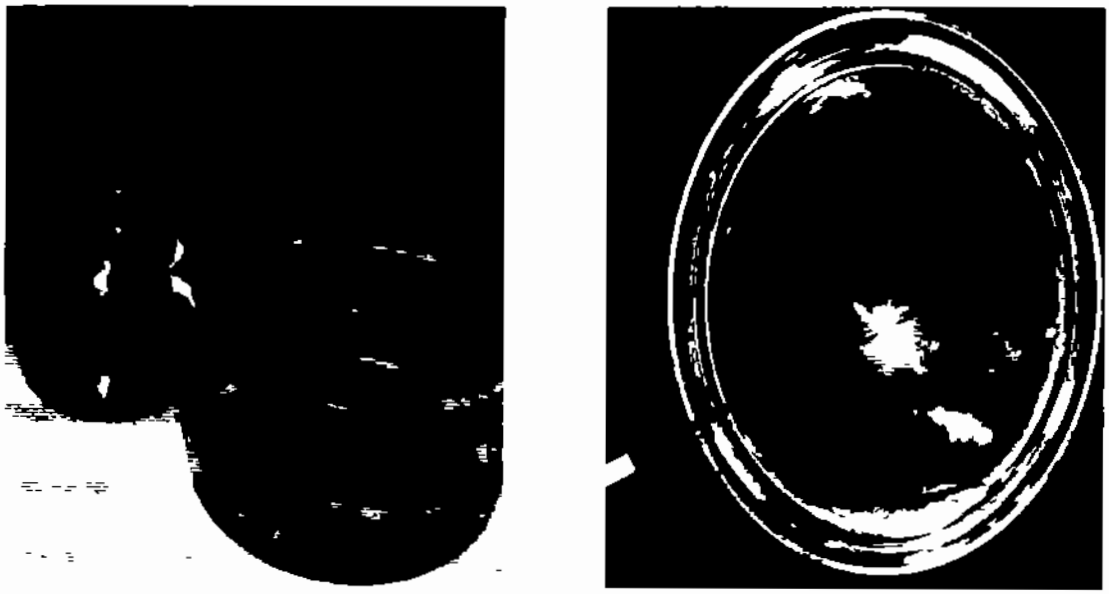


Figure 3 7 Illustration of nutrient agar on Petri plates

Chapter: 4

RESULTS

4.1 Effect of Nanoparticles on number of days to seed germination

On lowest concentration seeds took the minimum days for germination i e on 0 1µl/ml the average no of days were 7days The control group took the maximum average number of days i e untreated seeds -12days On 0 20µl/ml average number of days for germination was observed in 9 days, and 10 days on 0 25µl/ml (Table 4 1)

Table 4.1:- Illustration of different Nano particles treatment on days taken for seed germination

Different concentration of NPs	0.1 µl/ml	0.15 µl/ml	0.20 µl/ml	0.25 µl/ml	Control
Days taken for seed germination	7	7	9	10	12

4.2 Effect of Nanoparticles on seed germination and contamination frequency

The ZnO nanoparticles treated seeds of *capsicum annum* shows that the germination rate was increased as compare to untreated seeds In case of control condition the germination percentage was 62 5 % Among treated seeds maximum germination percentage was observed on lowest concentration i e 0 1 µl/ml i e 75% On highest concentration i e 0 25 µl/ml germination percentage was 54 1% that is higher than untreated seeds The minimum contamination frequency was observed on 0 1 µl/ml i e 25%, and the maximum contamination was observed on 0 25 µl/ml i e 45 8% The control group shows the 37 5% contamination (Table 4 2)

Table 4.2:- Outcome of different Nano particles treatment on germination frequency and Contaminated frequency of seeds

Different concentration of NPs	0.1 µl/ml	0.15 µl/ml	0.20 µl/ml	0.25 µl/ml	Control
Germination frequency	75%	62.5%	62.5%	54.1%	62.5%
Contaminated frequency	25%	37.5%	37.5%	45.8%	37.5%

4.3 Effect of Nano particles treatment on height after 15 days in Invitro condition

Simple MS media was used with 4 different concentrations of nanoparticles treated seeds and untreated seeds named as control The height was observed 15 days after the seeds inoculation on media The plant showed the best results on lowest concentration

Statistically maximum variation was observed in treated plants i e on 0 1 µl/ml and minimum variation was observed on 0 25 µl/ml The less variation was observed in control group In case of control group average height was 0 620B Maximum average height was observed on 0 1 µl/ml i e 2 00A on the lowest concentration, and minimum average height was observed 0 2800 B in treated plants i e 0 25 µl/ml i e on maximum concentration All the plants were found to be significantly different ($P<0.001$) from one another (table 4 3) The $LSD_{0.01}$ value is 0 7637

(a) 0.10µl/ml

(b) 0.15µl/ml

(c) 0.20µl/ml

(d) 0.25µl/ml

Effects of nanoparticle treatment on growth of *Capsicum annuum* (chillies)

(e) Control

Figure 4.1: illustration of both treated and untreated plants after 15 days

(a) Plant height after 15 days on 0.1µl/ml shows the maximum height

(b) Plant height after 15 days on 0.15µl/ml

(c) Plant height after 15 days on 0.20µl/ml

(d) Plant height after 15 days on 0.25µl/ml

(e) Plant height after 15 days in untreated seeds shows the minimum height

4.4 Effect of Nano particles treatment on height after 30 days in Invitro condition

Same simple MS media was used with 4 different concentrations of nanoparticles treated seeds and untreated seeds named as control The height was observed 15 days after the seeds inoculation on media The plant showed the best results on lowest concentration

Statistically maximum variation was observed in treated plants i e on 0 1 µl/ml and minimum variation was observed on 0 25µl/ml The less variation was observed in control group In case of control group average height was 0 6200B Maximum average height was observed on 0 1µl/ml i e 3 333 A on the lowest concentration, and minimum average height was observed 0 8200 B in treated plants i e 0 25µl/ml i e on maximum concentration All the plants were found to be significantly different (P<0 001) from one another The $LSD_{0\,01}$ value is 0 999 (Table 4 3) (Figure 4 2)

(a) 0.10 µl/ml

(b) 0.15 µl/ml

(c) 0.20 µl/ml

(d) 0.25 µl/ml

(e) Control

Figure 4.2: illustration of both treated and untreated plants after 30 days

(f) Plant height after 30 days on 0.1µl/ml showing the maximum height

(g) Plant height after 30 days on 0.15µl/ml

(h) Plant height after 30 days on 0.20µl/ml

(i) Plant height after 30 days on 0.25µl/ml

(j) Plant height after 30 days in untreated seeds shows the minimum height

4.4 Effect of Nano particles treatment on number of leaves in Invitro condition

More variation were seen in number of leaves with treatment as compare to control The more variation was observed at lowest concentration and minimum variation was observed at highest concentration The less number of leaves was observed at control level Among treated groups maximum number of average leaves was 3 0833 at 0 10 µl/ml, and minimum no of leaves was 1 7500 B at 0 25µl/ml The less number of leaves was 1.0833 B in control group The LSD0 01 value is 1 037 means they are significantly different from one another (Table 4 3)

Table 4.3:- Effect of different Nano particles treatment on Invitro Plants

Different concentration of NPs	Plant height in 15 days	Plant height in 30 days	No of leaves
0.1 µl/ml	2.000 A	3.3333 A	3.0833 A
0.15 µl/ml	1.1233 B	1.0000 B	2.0833 AB
0.20 µl/ml	1.000 B	1.1233 B	2.0400 B
0.25 µl/ml	0.2800 B	0.8200 B	1.7500 B
Control	0.6200 B	0.6200 B	1.0833 B
$LSD_{0.01}$	0.7637	0.999	1.037

(Data followed by capital alphabets correspond to the individual values as an mean of three replicates Each replicate is consisting of 4 treatments)

4.5 Effect of Nano particles treatment on height of plant in natural condition

Statistically maximum variation was observed in treated plants i e on 0 1 µl/ml and minimum variation was observed on 0 25µl/ml The less variation was observed at 0 25µl/ml In case of control group average height was 5 000CD

Maximum average height was observed on 0 1µl/ml i e 22 00 A on the lowest concentration, and minimum average height was observed 4 0400 D in treated plants i e 0 25µl/ml i.e on maximum concentration. All the plants were found to be significantly different (P<0 001) from one another The **$LSD_{0\,01}$** value is 2 312 (Table 4 4) (Figure 4 3) (Figure 4 4)

4.6 Effect of Nano particles treatment on number of leaves in natural condition

More variation was seen in number of leaves with treatment as compare to control The less number of leaves was observed at control level Among treated groups maximum number of average leaves was 24 867A at 0 20µl/ml, and minimum no of leaves was 6 6667 C at 0 25µl/ml The less number of leaves was 6 000 C in

Table 4.4:- Effect of different Nano particles treatment on natural condition Plants

Different concentration of NPs	Plant height	No of leaves	Days to flowering
0.1 µl/ml	22.000 A	16.133 B	54.9 A
0.15 µl/ml	7.5800 B	18.133 B	58.8 A
0.20 µl/ml	7.0000 BC	24.867 A	60.0 A
0.25 µl/ml	4.0400 D	6.6667 C	26.3 B
Control	5.0000 CD	6.0000 C	23.6 B
$LSD_{0.01}$	2.312	2.574	12.069

(Data followed by capital alphabets correspond to the individual values as a mean of three replicates Each replicate is consisting of 4 treatments)

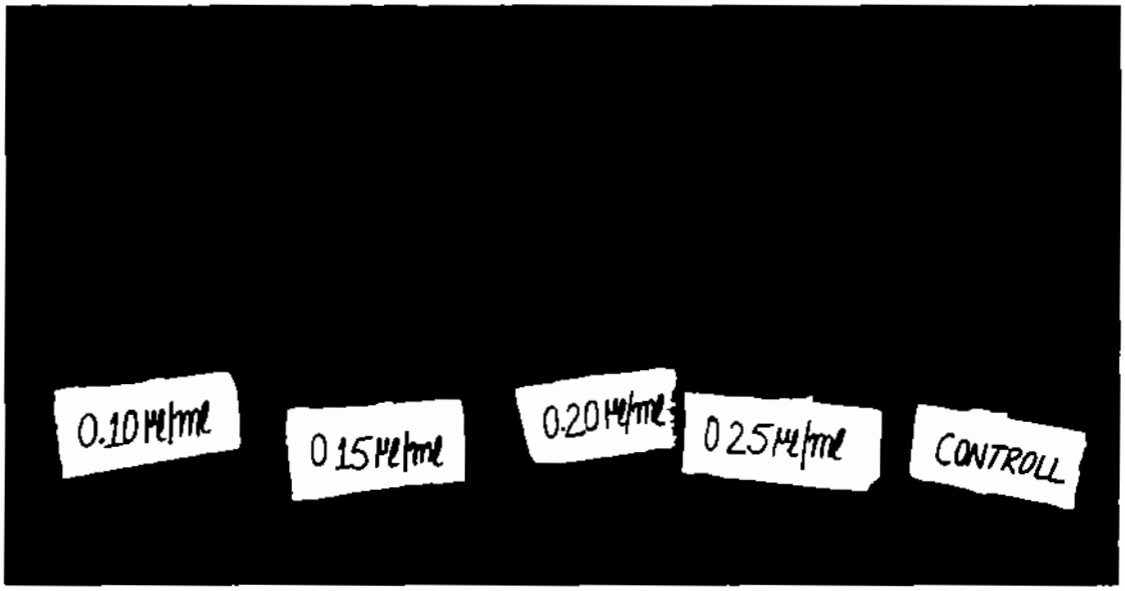
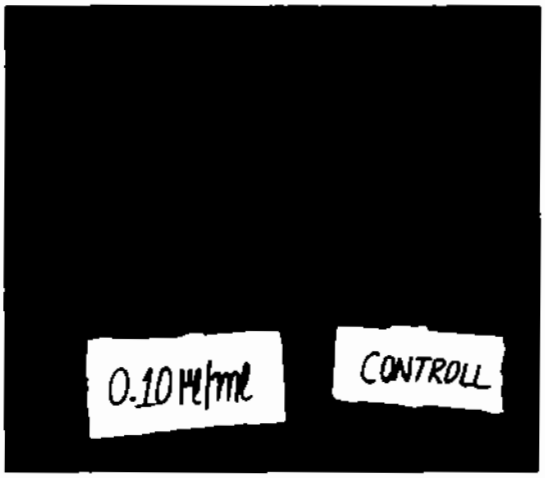


Figure 4.3: Comparison of different treated and untreated plants



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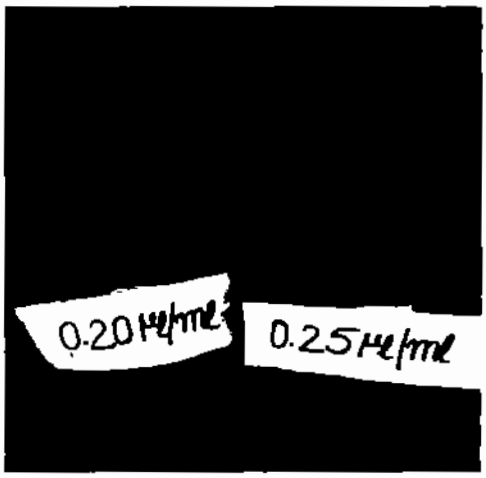

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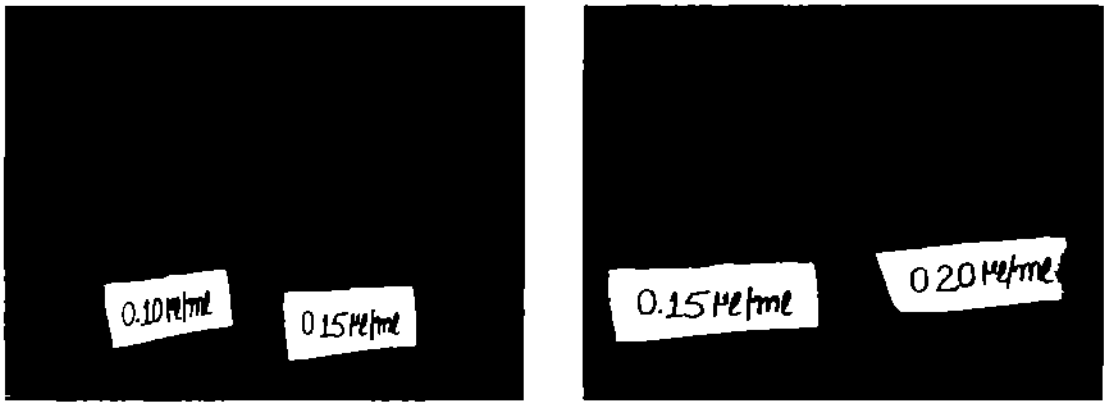


(c) (d)

Figure 4.4: Comparison of different treated and untreated palnts

(a) Comparison of plant height treated with NPs concentration 0.1µl/ml NPs and untreated plant i.e. control

(b) Comparison of plant height treated with NPs concentration 0.20µl/ml and 0.20µl/ml

(c) Comparison of plant height treated with NPs concentration 0.10µl/ml and 0.15µl/ml

(d) Comparison of plant height treated with NPs concentration 0.15µl/ml and 0.20µl/ml

4.8 Screening of Phytochemical Analysis

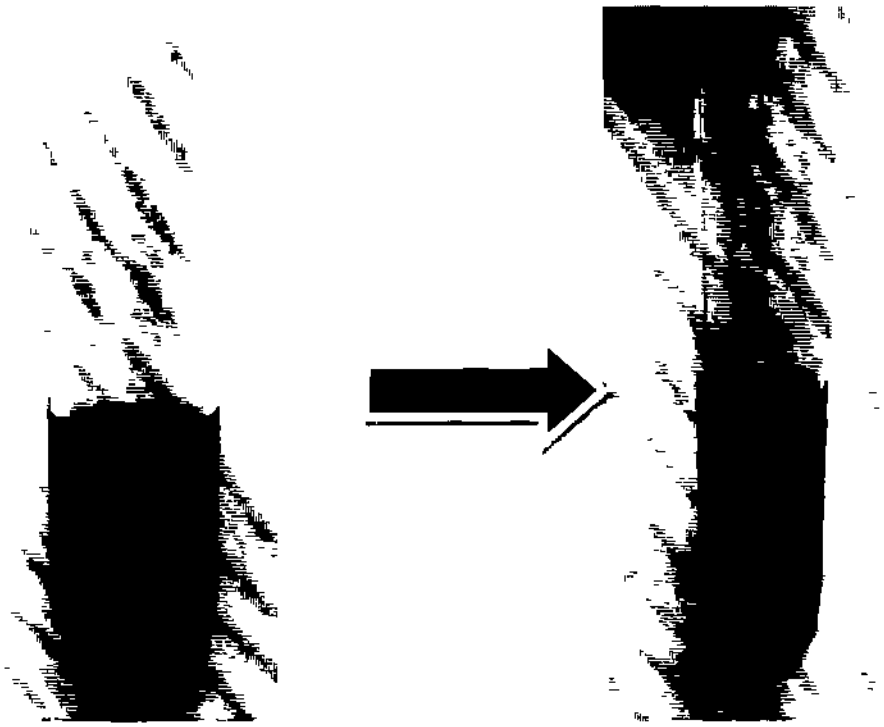
To find out the nature of phytoconstituents, methanolic extracts of leaves were subjected to the phytochemical tests The result shows the occurrence of Alkaloids and a complete absence of flavonoids, tannins, triterpenoids & Steroids (Figure) (Table)

Cultured plant cells can be normally recognized as difficult to make alkaloids It is said that the failure or decrease of alkaloid production is because in biosynthesis reaction there is blockage of a specific metabolic reaction step, actual cause for this is not known yet The little amount alkaloid constituent in the undifferentiated cultured cells of *Scopolia* and *Datura* was discovered which are mostly created via inhibition of tropic acid production (Barzet et al , 1977)

Table 4.5: - The results of secondry metabolites in leaves of *Capsicum annuum* plant

S. No	Phytochemicals	Methanolic Extracts
1.	Alkaloids	+ tive
2.	Flavonoids	- tive
3	Tanins	- tive
4.	Triterpenoids	- tive
5.	Steroids	- tive

+ tive = Present, - tive =Absent



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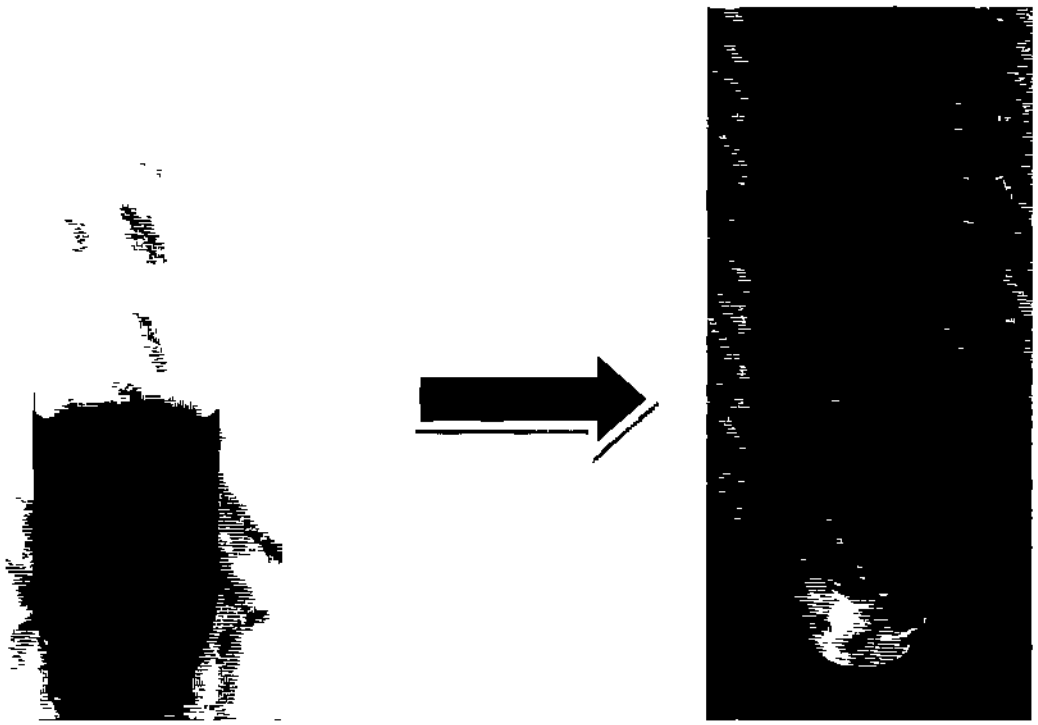

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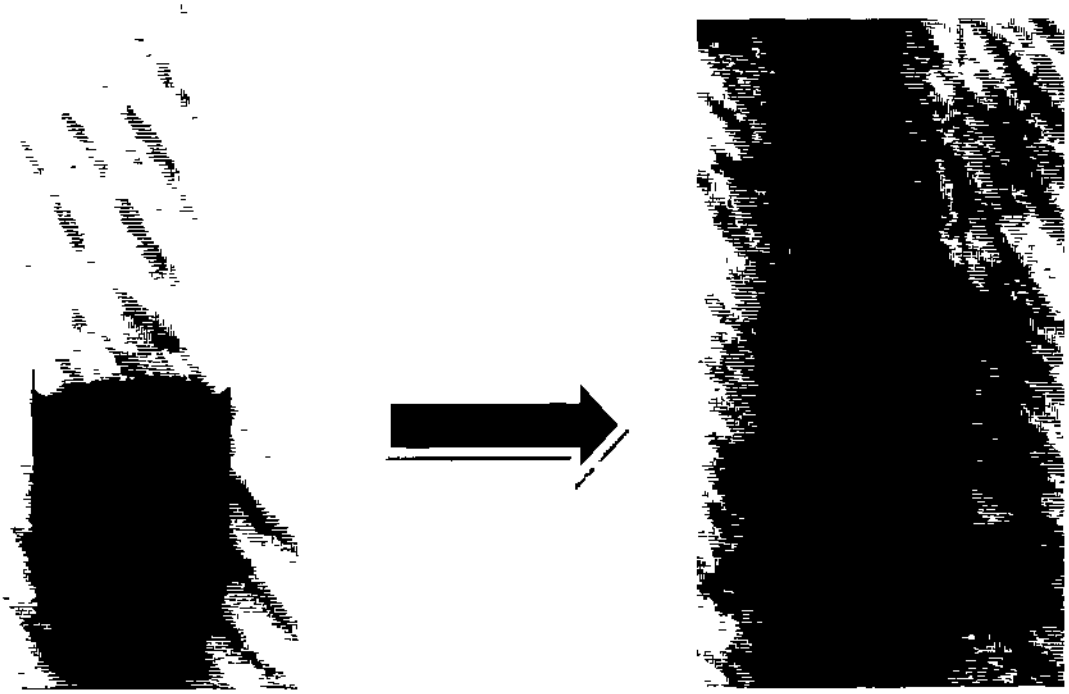

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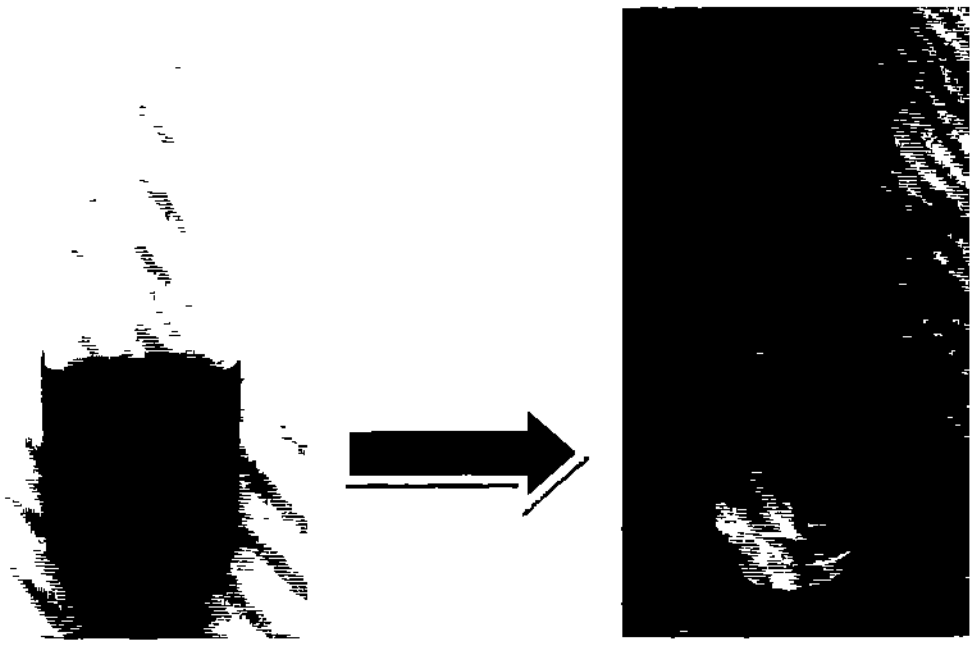

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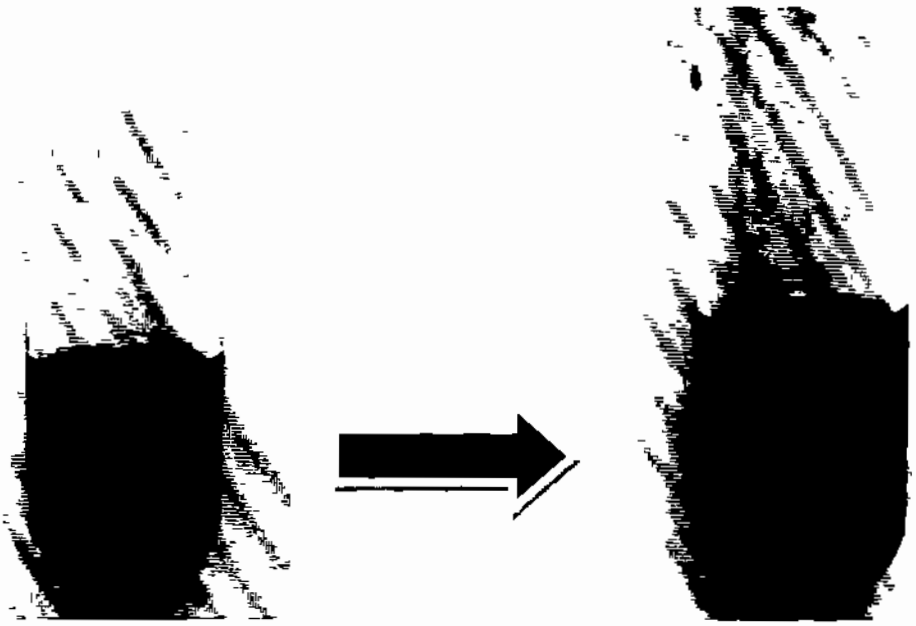


(e)

Figure 4.5: Phytochemical analysis of plant

(a) Phytochemical test performed on the leaves of *Capsicum annuum* indicates the presence of Alkaloids

(b) Phytochemical test performed on the leaves of *Capsicum annuum* indicates the absence of Flavonoids

(c) Phytochemical test performed on the leaves of *Capsicum annuum* indicates the absence of Steroids

(d) Phytochemical test performed on the leaves of *Capsicum annuum* indicates the absence of Triterpenoids

(e) Phytochemical test performed on the leaves of *Capsicum annuum* indicates the absence of Tanins

4.9 Evaluation OF ANTIBACTARIAL ACTIVITY

4.9.1 Against *Escherichia coli*:

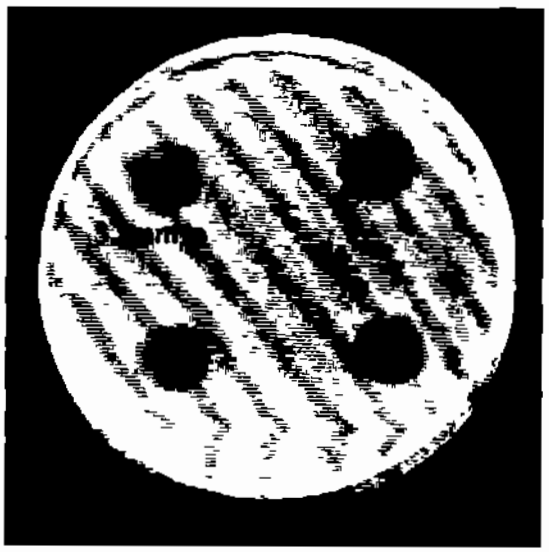
By disc plate method the effectiveness of range of antibacterial activity was determined against E coli by disc plate method, the effectiveness of a range of antimicrobial was determined against E coli (Figure 4 6) It was showed the highest inhibition zone against E coli (3 3 mm)

4.9.2 Against *staphylococcus aureus*:

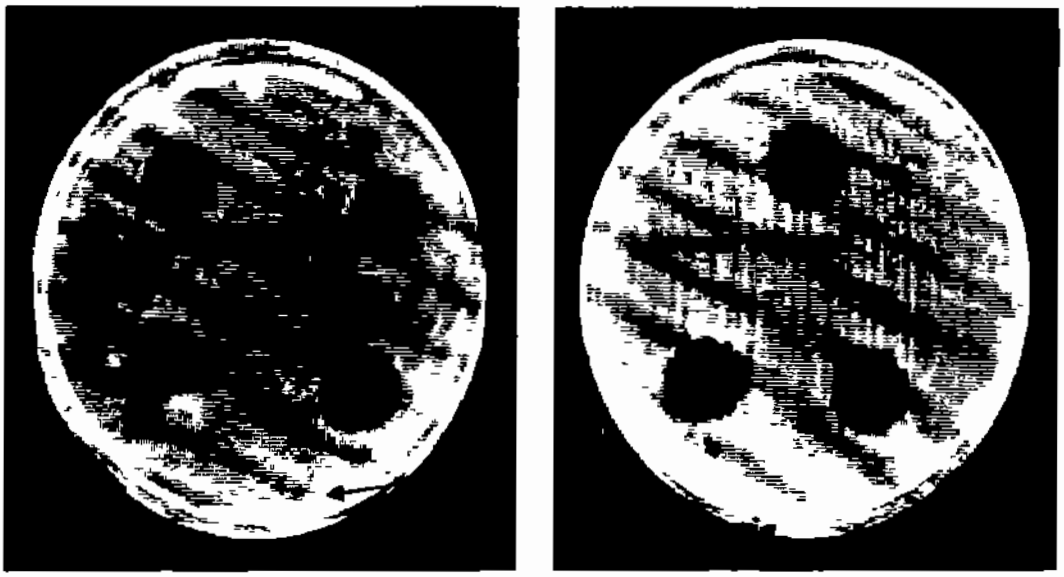
Capsicum annuum also shows the zone of inhibition (2 6mm) against S aureus against methanolic extracts of leaves as shown in (figure 4 6)

4.9.3 Against *Klebsiella pneumoniae*:

Capsicum annuum also shows the zone of inhibition (1 6mm) against K Pneumonia against methanolic extracts of leaves as shown in (figure 4 6)



(a)



(b) (c)

Figure 4 6 Antimicobial assay of *capsicum annuum*

(a) Zone of inhibiotion against *Escherichia coli* is 3.3mm

(b) Zone of inhibiotion against *staphylococcus aureus* is 2.6mm

(c) Zone of inhibiotion against *Klebsiella pneumonia* is 1.6mm

Chapter 5

5.1 DISCUSSION

Nano' means very small 10-9 to be precise, this means that nanoparticles are composed of large amount of minute particles that are compact together to the surface Such particles are present on everywhere on the Earth's surface (Alexandra., 2000)

Nano materials in nano biotechnology demonstrate totally new and better practices based on morphology, distribution and size (Sing et al , 2010) An important concern is the making of nanoparticles of different sizes shapes and with control dissimilarity are the experimental & chemical methods that involves the production of large amount of risky and unsafe by-products in the end (Dubey et al , 2009)

Nano particles can be synthesized by wide variety of different materials like silicates, magnetic materials, metal oxide ceramics, emulsions & liposome, etc (Hollister et al , 2003)

Nano particles display catalytic optical and electrical properties and are being utilized seriously as a part of gadgets, metallics, earthenware production, polymers, inks films and coatings on account of their great repairing impact Past studies demonstrated that NPs are harmful to oceanic creatures, including green growth,

and fish Survival of Daphnia magna was diminished by presentation to ZnO , TiO2 , SiO2 , and fullerene (Lee et al , 2008)

The challenges and advantages of nanotechnology will prompt better acknowledgment of this developing innovation for public awareness Cross contamination and control of pests of food products and agriculture are related to the rapid biosensors and testing technologies will prompt utilization of nanotechnology soon in future

The Solanaceae is a substantial plant family includes more than 3000 species including numerous crops, for example, pepper, eggplant, potato and tomato It includes a group of dicotyledonous plants in the Euasterid claude, which is different from the model plant Arabidopsis It is the 3rd economical plant family and positions the first a vegetable yields Similar family Rubiaceae includes coffee, a standout amongst the most profitable farming wares in the universal exchange (Feinan and Steven, 2010)

Capsicum species are the members of Solanaceae family (tribe Solaneae, sub-tribe Capsicinae) which involves petunia, tobacco, potato and tomato Genus Capsicum contains almost 31 species (Moscone et al , 2007)

ZnO is harmless, it can be utilized as photocatalytic debasement materials of natural poisons High affectability is shown by mass and thin movies for some

harmful gasses The great help is provided by Zinc oxide NPs for the yield and development of sustenance products

ZnO nano particles have significant inhibition on bacterial contaminants and significantly prevent their growth similar results shown by Mohamed et al , 2014

ZnO NPs development of seedling and germination of seed in capsicum and determined the germination of seed increased in lower concentrations of ZnO NPs but show reduction in values at high concentration same results was observed by S L Laware and Shilpa Raskar 2014 that germination of onion is increased at lower concentration and decreased at higher concentration

On the height of the plants. concentration of nanoparticles have different effects i e maximum height was observed in all treated plants as compare to the control Maximum height was observed at low concentration 0 1µl/ml and decreases with higher concentration 0 25 µl/ml and less height was observed at control similar results were described by S L Laware and Shilpa Raskar 2014 and on the other hand days to flowering reduced on lowest concentration then higher i e more days taken by 0 10µl/ml and less at higher concentration 0 25 µl/ml

The phytochemical analysis reveals the presence of alkaloid and the absence of phenolic compounds in plants These results are deviated from Akılan et al , 2014

Chapter: 6

CONCLUSION

To sum up, agriculture nanotechnology is an efficient method for earlier plant growth The treated seeds showed the best results as compare to untreated seeds The treated and untreated seeds were used in both invitro as well as natural condition. ZnO showed the best results on in both conditions On lowest concentration plants showed maximum height, maximum number of leaves and earlier seed germination On maximum concentration plant takes minimum days to flowering and on minimum concentration plant take maximum days to flowering Our research can successfully be used for other different species of genus Solanaceae

6.1 Future Recommendations

Nano technology is the powerful technique from which we can take advantages in agriculture technology for welfare of mankind For instance, the unconstructive reaction from individuals towards genetically modified (GM) crops, need of a ton of required talents in government agricultural research and technology units for nano sort of investigations and inadequately prepared new instruments and many more modern advances (Ram *et al* , 2014)

The potential advantages and use of nanotechnology are vast like agricultural yield development involving for slow release of nano-porous zeolites and well-organized dosage of fertilizers and water, nano-capsules for vector, herbicide delivery, nano-sensors for pest detection and pest management (Scrinis and Lyons 2007, Scott 2007)

Chapter: 7

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APPENDIX

Completely Randomized AOV for Height15

Source	DF	SS	MS	F	P
Treatment	4	1 38563	0 84641	4 80	0 [illegible]
Error	10	1 76227	0 17623		
Total	14	3 14789			

Grand Mean 1 1127 CV 37 73

At least one group variance is near zero,
variance-equality tests cannot be computed

Component of variance for between groups 0 21338
Effective cell size 3 0

Treatment	Mean
1	2 0000
2	1 0000
3	1 1333
4	0 8200
5	0 6200

Observations per Mean 3
Standard Error of a Mean 0 2424
Std Error (Diff of 2 Means 0 3428

Completely Randomized AOV for Height30

Source	DF	SS	MS	F	P
Treatment	4	7 8142	1 95603	6.47	0 [illegible]
Error	10	3 0209	0 30209		
Total	14	10 8350			

Grand Mean 1 9387 CV 29 35

	Chi-Sq	DF	P
Bartlett's Test of Equal Variances	0.96	4	0 9159
Cochran's Q	0 3175		
Largest Var / Smallest Var	4 7567		

Component of variance for between groups 0 56131
Effective Cell Size 3 0

Treatment	Mean
1	3 3333
2	1 8167
3	1 7767
4	1 4333
5	1 0333

Observations per Mean 3
Standard Error of a Mean 0 3173
Std Error (Diff of 2 Means 0 4488

Completely Randomized AOV for noofleave

Source	DF	SS	MS	F	P
Treatment	4	6 25384	1 56346	4 91	0 [illegible]
Error	10	3.24960	0 32496		
Total	14	9.50344			

Grand Mean 2.0680 CV 28.33

	Chi-Sq	DF	P
Bartlett's Test of Equal Variances	11.2	4	0.024
Cochran's C	0.6923		
Largest Var / Smallest Var	53.990		

Component of variance for between groups 0.41283
Effective cell size 3.0

Treatment	Mean
1	3.0833
2	2.0833
3	1.7500
4	2.0400
5	1.0833

Observations per Mean 3
Standard Error of a Mean 0.3291
Std Error (Diff of 2 Means) 0.4654

LSD All-Pairwise Comparisons Test of Height15 by Treatment

Treatment	Mean	Homogeneous Groups
1	2.0000	A
3	1.1233	B
2	1.0000	B
4	0.6200	B
5	0.6200	B

Alpha 0.05 Standard Error for Comparison 0.3428
Critical T Value 2.228 Critical Value for Comparison 0.7637
There are 2 groups (A and B) in which the means
are not significantly different from one another

LSD All-Pairwise Comparisons Test of Height30 by Treatment

Treatment	Mean	Homogeneous Groups
1	3.0333	A
2	1.8167	B
3	1.7767	B
4	1.4333	B
5	1.3333	B

Alpha 0.05 Standard Error for Comparison 0.4488
Critical T Value 2.228 Critical Value for Comparison 0.9999
There are 2 groups (A and B) in which the means
are not significantly different from one another

LSD All-Pairwise Comparisons Test of noofleave by Treatment

Treatment	Mean	Homogeneous Groups
1	3.0833	A
2	2.0667	AB
4	2.0400	B
3	1.7500	B
5	1.0833	B

Alpha 0.05 Standard Error for Comparison 0.4654
Critical T Value 2.228 Critical Value for Comparison 1.0371
There are 2 groups (A and B) in which the means
are not significantly different from one another

Completely Randomized AOV for daystoflo

Source	DF	SS	MS	F	P
treatment	4	3352 04	988 009	22 5	1 1001
Error	10	440 08	44 008		
Total	14	4092 12			

Grand Mean 44 747 CV 14 83

	Chi-Sq	DF	P
Bartlett's Test of Equal Variances	14 7	4	0 0054
Cochran's Q	0 6559		
Largest Var / Smallest Var	220 90		

Component of variance for between groups 314 66
Effective cell size 3 0

treatment	Mean
1	54 933
2	58 800
3	60 000
4	26 333
5	23 667

Observations per Mean 3
Standard Error of a Mean 3 8301
Std Error Diff of 2 Means 5 4165

Completely Randomized AOV for noofleave

Source	DF	SS	MS	F	P
treatment	4	770 549	192.637	96 2	1 0000
Error	10	20 027	2.003		
Total	14	790 576			

Grand Mean 14 360 CV 9 85

	Chi-Sq	DF	P
Bartlett's Test of Equal Variances	5 07	4	0 2869
Cochran's Q	0 6964		
Largest Var / Smallest Var	20 920		

Component of variance for between groups 63 5449
Effective cell size 3 0

treatment	Mean
1	24 867
2	18 133
3	16 133
4	6.000
5	6.667

Observations per Mean 3
Standard Error of a Mean 0 8170
Std Error Diff of 2 Means 1 1555

Completely Randomized AOV for onheight

Source	DF	SS	MS	F	P
treatment	4	646 623	161 656	100	0 0000
Error	10	16 159	1 616		

Total 14 662 783

Grand Mean 9 1240 CV 13 93

At least one group variance is near zero,
variance-equality tests cannot be computed

Component of variance for between groups 53 3466
Effective cell size 3 0

treatment	Mean
1	22.000
2	7.580
3	7.000
4	5.000
5	4 040

Observations per Mean 3
Standard Error of a Mean 0 7339
Std Error (Diff of 2 Means) 1 0379

LSD All-Pairwise Comparisons Test of daystoflo by treatment

treatment	Mean	Homogeneous Groups
3	60 000	A
2	58 800	A
1	54 933	A
4	26 333	B
5	23 667	B

Alpha 0 05 Standard Error for Comparison 5 4165
Critical T Value 2 228 Critical Value for Comparison 12 069
There are 2 groups (A and B) in which the means
are not significantly different from one another

LSD All-Pairwise Comparisons Test of noofleave by treatment

treatment	Mean	Homogeneous Groups
1	24.867	A
2	18.133	B
3	16 133	B
5	6 6667	C
4	6 0000	C

Alpha 0.05 Standard Error for Comparison 1 1555
Critical T Value 2 228 Critical Value for Comparison 2 5745
There are 3 groups (A, B, etc) in which the means
are not significantly different from one another

LSD All-Pairwise Comparisons Test of onheight by treatment

treatment	Mean	Homogeneous Groups
1	22 000	A
2	7 5800	B
3	7 0000	BC
4	5 0000	CD
5	4 0400	D

Alpha 0 05 Standard Error for Comparison 1.0379
Critical T Value 2 228 Critical Value for Comparison 2 3126
There are 4 groups (A, B, etc) in which the means
are not significantly different from one another