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Efficacy of Iron Oxide Nanoparticles against Ralstonia Solanacearum: A Novel Approach to Control Bacterial Tomato Wilt

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Abstract

This research is focused on application of Iron Oxide Nanoparticles (FeO NPs) on bacterial pathogens of tomato wilt. Ralstoniasolanacaerumis the major bacterial pathogen causing tomato wilt. FeO NPs are synthesized by co-precipitation technique and calcinated to reduce size and impurities of NPs. After synthesis, their zeta potential is analyzed which showed negative surface charge predicting efficacy against gram negative organisms. Their physiochemical characterization is done through Scanning Electron Microscope (SEM), (X-Ray Diffraction Analysis (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). Characterization reveals that their size in 16-67nm diameter and the FeO NPs are non-uniform in size. 43 tomato plants showing typical wilt symptoms are collected from field and their exudates from stem and crushed leaves are cultured on nutrient media. 17 samples are positive for bacterial growth. Bacterial positive samples are sub-cultured on TTZ selective media for Ralstoniasolanacaerum. Bacterial samples are confirmed by gram staining, biochemical testing i.e. catalase and oxidase positive. Identified Ralstoniasolanacaerum is spread on nutrient petri plate and four concentrations of FeO NPs i.e. 25, 50, 100 and 200µg/ml are applied to check zone of inhibition by disc diffusion method along with positive and negative controls. At 200µg/ml conc. Of FeO NPs zone of inhibition is average 11mm diameter as compared to positive control 14mm diamteter, and this is quite significant. FeO NPs showed significant antibacterial effects against Gram Negative Ralstonia solanacearum responsible for bacterial tomato wilt.

Key Words: FeO NPs, Bacterial Wilt, Nanoparticles, Disc Diffusion, Nanotechnology

Introduction

Nanoparticles are small particles having size of billionth of a meter and the field which deals with their synthesis, properties, usage and indications is broadly termed as nanotechnology. They are classified into different types on the basis of their size, shape and properties. Nanoparticles belong to a class of materials which differs from their bulk and molecular counterparts. They have adverse and beneficial effects on human which need to be measured. The high surface area and less toxicity contribute to their physical and chemical properties which make them effective to use. The size and shape also contributes to their reactivity and toughness and due to these characteristics they are suitable for various biological and domestic applications such as medical applications, research based on energy and environmental applications. Some heavy Nanoparticles are difficult to degrade and have toxic properties [1].

Due to their similarity in size to larger molecules, like enzymes they can interact with biomolecules on the surface of cell and within the cell. They are not efficient lite scatters as there much smaller than 400 to 700nm spectrum. Biodiversity and toxicity of Nanoparticles is not well known. They are taken up by bacteria and living cells and they enter into food chain leading to bioaccumulation [2]. Several magnetic Iron oxide Nanoparticles have been used in research Fe_3O_4 magnetite, ferri-magnetic, $Fe^{II}Fe^{III}{}_2O_4$, α-Fe_2O_3, γ-Fe_2O_3FeO. In these magnetite and maghemite are promising as their biocompatibility has been proven [3].

In the area of environmental sciences numerous chemicals that are causing pollution like AO dye of water is being removed by the application of nanoparticles. One other toxic and corrosive chemical of industrial origin is ammonia. This can cause serious lethal effects over environment and living organisms. To sense the levels of ammonia in the environment and quantitate minimum threshold limits, there are some experimentally developed electrochemical sensors andchemiresistive sensors [4].

To observe impacts of nanoparticles on biological systems, study suggests that differently sized and coating variants on FeO NPs show different properties in vivo and in vitro. Study conducted to check the uptake, distribution, clearance and toxicity of FeO NPs. The results explored that polyethyleneimine coating or covering of FeO NPs shows higher uptake in reticuloendothelial cells and cancerous cells than that of polyethylene glycol coated or covered particles. These nanoparticles caused cellular death as the result of toxicity caused by the generation of reactive oxygen species. Particles having size of 10nm showed higher uptake than that on those with size of 30nm. This result establishes direct link of particle size with tissue uptake. The clearance rate of PEGylated FeO NPs is slower than PEI-FeO NPs which showed that PEG is easily excreted. FEO NPS with size greater than 100 nm can easily be trapped in liver and spleen during metabolism and circulation while smaller particles get eliminated through excretory system easily [5].

Tomato is the common product used both as fruit and vegetable. Tomato is prone to microbial infection. One common disease is tomato wilt which affects stem and leaves and responsible for low production of tomato. There are bacterial, viral, fungal and biochemical causes of tomato wilt. Fungal types of wilt are; fusarium and verticillium caused by Fusarium oxysporum f. sp. lycopersiciand Verticillium albo-atrum respectively. The bacterial cause of tomato wilt is Ralstonia solanacearum [6].

Plant pathologists and researchers are continuously in keen interest to check whether nanotechnology can be useful in limiting plan diseases especially of microbial origin. Around 18 types of nanoparticles are applied to control plant pathogens and most common contains are Ag, Cu, Zn, Mn and Si. There are two mechanism of killing of plant microbes by nanoparticles; direct killing and altering host nutritional status thus acting as host fertilizer which boosts hosts immune system [7].

To control wilt of tomato caused by fusarium biocontrols strains of bacteria and fungi are used. These strains are collected from roots of plants [8]. Recently all other nanoparticles are used to limit plant's microbial diseases especially wilt but Iron Oxide nanoparticles are not yet considered, due to biocompatibility and less toxicity, application of FeO NPs will be nontoxic and easy to handle to control tomato wilt caused by bacteria and fungi.

Tomato is a member of the Solanaceae family, which includes several other economically important crops such as potato, pepper (Capsicum annuum L.), and eggplant (Solanum melongena L.), representing one of the most valuable plant families for vegetable and fruit crops.

It gives 22-33 calories average to large tomato as it is low in calories so used as weight losing diet. It shown pH of 4.3-4.9 and carbohydrate content of 4% of their weight and these are good source of vitamin C. red color is due to a chemical called lycopene responsible for its antioxidant properties[9].

Tomato (Solanum lycopersicum L.) is the second most important fruit or vegetable crop next to potato (Solanum tuberosum L.), with approximately 182.3 million tons of tomato fruits produced on 4.85 million ha each year [10]. Asia accounts for 61.1% of global tomato production, while Europe, America, and Africa produced 13.5%, 13.4%, and 11.8% of the total tomato yield, respectively. Tomato yields are highly variable, ranging from more than 508 tons per ha in the Netherlands to fewer than 1.5 tons per hactere in Somalia in 2017 [11].

1.1 Bacterial Wilt of Tomato:

Ralstoniasolanacaerum, Gram negative aerobic plant bacterium is responsible for most bacterial cases on tomato wilt. Mostly stems are affecting resulting into drooping of plants as shown in figures 2 and 3 [12]. Disease affecting stem and leaves of tomato plants that are young enough. Drooping down of leaves or branches, initially at ends and later on the center or base. Phenomenon observed during warm weather in summer days. As bacterium and fungus starts affecting the xylem and phloem the damaged tissue results in yellowing and darkening of tissue, resulting into complete death of tissue.

Figure 1: Initial damage of leaf during wilt

Figure 2: Affected stem of tomato

Methodology

Primarily affected tomato plants from the fields showing typical symptoms of bacterial wilt were collected. Out of 41 samples only 17 samples showed bacterial growth in final isolation and confirmation. Identification goes through primary culture, sub culture, microscopy and gram staining and biochemical testing to confirm the organism.

Synthesis:

Coprecipitative method adopted to synthesize FeO NPs as adopted by[13]in which 100ml Distilled water mixed with powdered 3g $FeCl_2$ in Round bottom flask and stirring till uniformely mixed. Thermal equilibrium attained by placing flask into heating mantle and keeping temperature at 90°C. 100ml NaOH filled in burette and get mixed through burette drop by drop until mixture changed color into dark brown and suspended NP appear.NaOH used as precipitator here. At this stage NaOH drop is being stopped and mixture allowed to settle for one hour. Mixture is filtered by pore size 20nm filter paper and Supernatant is discarded and remnants are collected into test tubes and washed by distilled water 3 times and by ethanol 2 times. NPs are spread over petri dish and allowed to oxidize for 24 hours under air exposure.

Calcination:
NPs are calcinated in furnace to remove impurities and reduce size [28]. NPs exposed to 200°C for 40 mins, 400°C for 40 mins and 600°C for 40 mins. Powdered form is scrapped weighed as 0.31g and collected into eppendorf.
Zeta Potential:
NPs are assessed for their surface membrane charge and average size by instrument called Zetasizer Ver. 7.12, Serial Number : MAL1168467, Malvern Instruments Ltd.

Characterization:
SEM, XRD and FTIR were done for physical properties of FeO NPs.

Pathogen Isolation:
Affected stems were cut and suspended in distilled water for 24 hours, pus and fluids leaked out into water and that water being cultured on nutrient media first, then sub-cultured on TTZ media for 24 hours. Gram staining and microscopy done to visualize the bacterium Ralstoniasolanacaerum. Biochemical testing done is oxidase and catalase tests [27].

Anti Bacterial Assay:
Pathogen is spread over culture plate and three concentrations/dilutions of NP along with positive and Negative controls were applied over it. NPs concentrations are 25, 50, 100 and 200µg/ml while ciprofloxacin disc is used as positive control and distilled water as negative control. disc diffusion technique is used and zone of inhibition measured in mm diameter.

Results
Nanoparticles are small particles having size of billionth of a meter and the field which deals with their synthesis, properties, usage and indications is broadly termed as nanotechnology. The most important areas of application are tissue specific biological delivery of drugs that show excellent impacts on pharmacokinetic and pharmacodynamic properties of applied drugs.
Synthesis:
Iron oxide nanoparticles are of various types having different combinations of iron and oxygen and hydrogen. We synthesized FeO NPs by coprecipitation where NaOH and FeCl2 are mixed under standard conditions and resultantly FeO formed which is being precipitated out. Color changes to brown and particle suspension observed visually are the best indicatior of completed reaction [13]. Supernatant is decantated and remains are filtered by 20nm pore size filter paper and washed by water and methanol three times. Thin smear examined on 40x and 100x shown non-uniforms sized spherical structures. Semisolid NP are then exposed to air in dark space for 24 hours for oxidation.

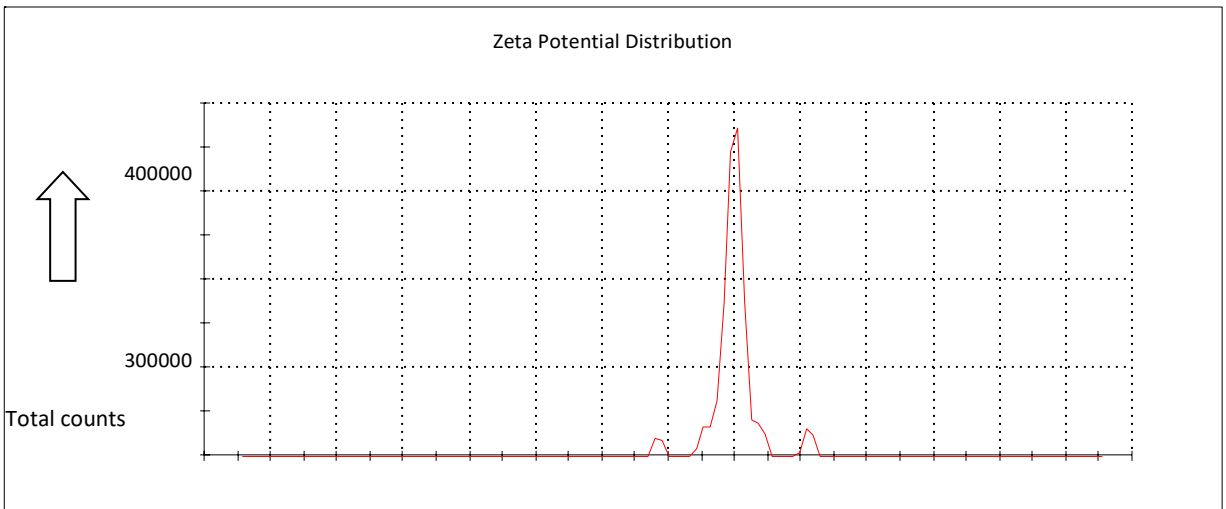
Figure 3 (a) and (b): Preparation of NPs for calcination

Calcination:
After being oxidized, NPs are calcinated for 120 mins to reduce size and impurities ate temperatures; 200°C for 40 mins, 400°C for 40 mins and 600°C for 40 mins in furnace following SOPs. After calcination powdered NPs are scrapped and collected in eppendorf tube and covered with foil to avoid light exposure [28].

Zeta Potential:
NPs are assessed for their surface chemistry, surface charge and other membrane potential [29] by a method called zeta-sizing on instrument zeta-sizer. Zetasizer Ver. 7.12, Serial Number : MAL1168467, Malvern Instruments Ltd

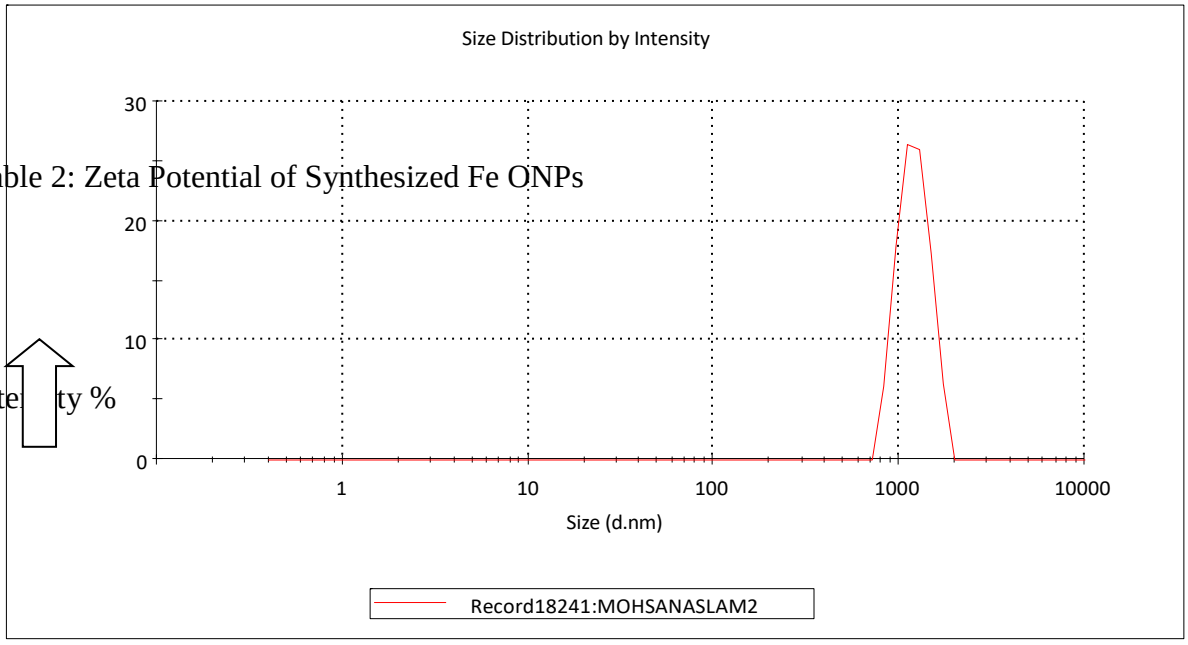
Table1: Zeta potential of FeO NPs

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-0.0321	Peak 1:	-0.362	93.0	3.59
Zeta Deviation (mV):	6.90	Peak 2:	22.2	4.2	1.26
Conductivity(mS/cm):	7.48e-4		-23.0	2.7	1.03



Zeta potential should be -30mV to +30mV for application. High ZP shows highly charged particle surface and prevents aggregation due to repulsion while low ZP causes attraction and coagulation of particles. +ZP shows longer half-life in body and –ZP is easily cleared by Reticulo-endothelial cells. So these FeO NPs have zeta potential of -0.0321 and lie in acceptable limits and will have effects on cells [14].

Table 2: Zeta Potential of Synthesized Fe ONPs



			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1142	Peak 1:	1212	100.0	232.9
PdI:	0.111	Peak 2:	0.000	0.0	0.000
Intercept:	0.883	Peak 3:	0.000	0.0	0.000
Result quality:	Good				

3.4 Characterization of FeO NPs:

Nanoparticles are having size of one billionth of a meter so their structure and uniformity could not be observed by conventional methods, so specialized and sophisticated methods were deployed to assess their features.

3.4.1 Scanning Electron Microscope:

SEM (JSM5910, JEOL, Japan) was used to analysethe chemical composition of Iron oxide nanoparticles. A thin film of Iron oxidenanoparticles mixed with distilled water was coated on a copper grid carbon and dried for 5min in hot air. The sample was then gold coated with a sputter coater at 30mA for 120seconds.

Concentrated beam of electrons are used to capture images of nanoparticle [32] which measure average size is 16-67nm and shown non-uniformity of nanoparticles as shown in Figures 4 and 5. Images show that nanoparticles are crystalline and roughly spherical with rough surface protrusions. Although variation in size is seen so range in average size is large that is 16-67nm. The size and shape is same as with the previous studies predicting the antibacterial activities [34].

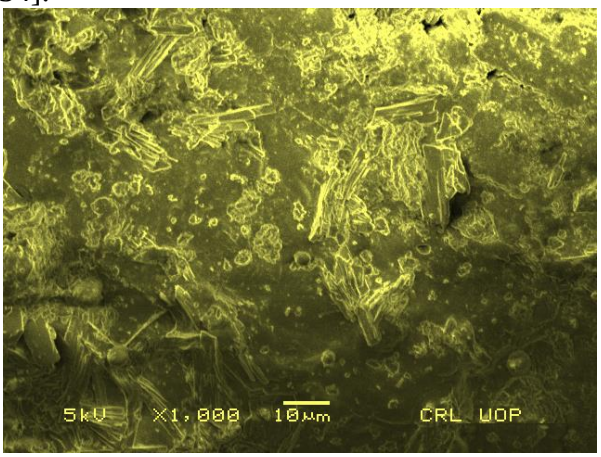


Figure 4: SEM image of FeONPs

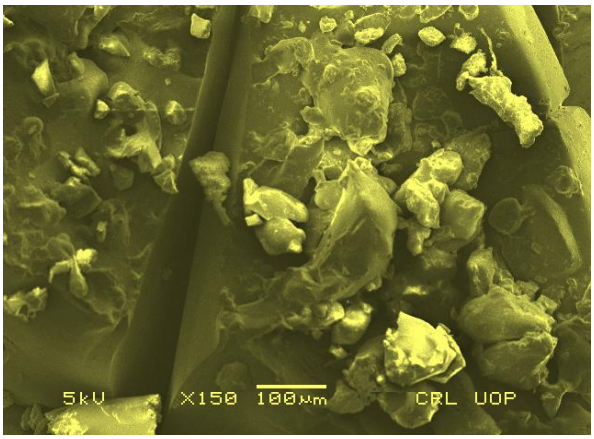


Figure 5:SEM image of FeO NPs

3.4.2. X-Ray Diffraction Analysis:

The crystalline nature of Iron oxide nanoparticles was determined by XRD using a JDX 3532JEOL, Japan. The sample was placed in the sample holder and the diffraction pattern was recorded. The diffracted intensities were obtained in the 2Θ angles range of 10° to 90° by operating the instrument at 20 to 40 kVat a wavelength of 1.5418A° in Θ to 2Θconfigurations. The Debye-Scherrer equation was used to measure the crystal size of the NPs

as follows: $D = 0.94 \lambda / \beta \cos\theta$ where, λ is the wavelength of X-ray used, θ is the angle of diffraction, β is the full width at half maximum (FWHM) and D is the measure of size of crystal that is perpendicular to the reflection planes. The XRD spectrum shows various diffraction peaks at 2Θ = 14.61°, 17.6°, 38.26°, 34.61°, 44.46° and 64.86º.

To analyze size of crystal, a technique utilized which sprays beam of X-rays and scattered beams and not plotted so image or structure of crystal is plotted and calculated by an equation. the crystal size 1.15 and 7.440 A° for Iron oxide Nanoparticles The crystal size ofIron oxide Nanoaprticles was 8.5 A° as calculated in table 3.3 and Figure 3.5.

Table3:Crystalline Size Determination of Iron oxide NanoprticlesusingDebye-Scherrer's Equation.

S. #	Peak at 2θ on	Intensity÷2	β= πFWHM/180	Θ	D
A	14.61	13.50	0.241	2.401	13.17
B	17.06	12.50	0.233	2.206	11.654
C	38.26	6.00	0.1056	0.901	6.168
D	34.61	17.55	0.309	0.857	4.740
E	44.46	8.06	0.137	1.658	10.40
F	64.86	9.09	0.158	0.953	9.20

Diameter of Iron oxide NPs=8.5° (Average)

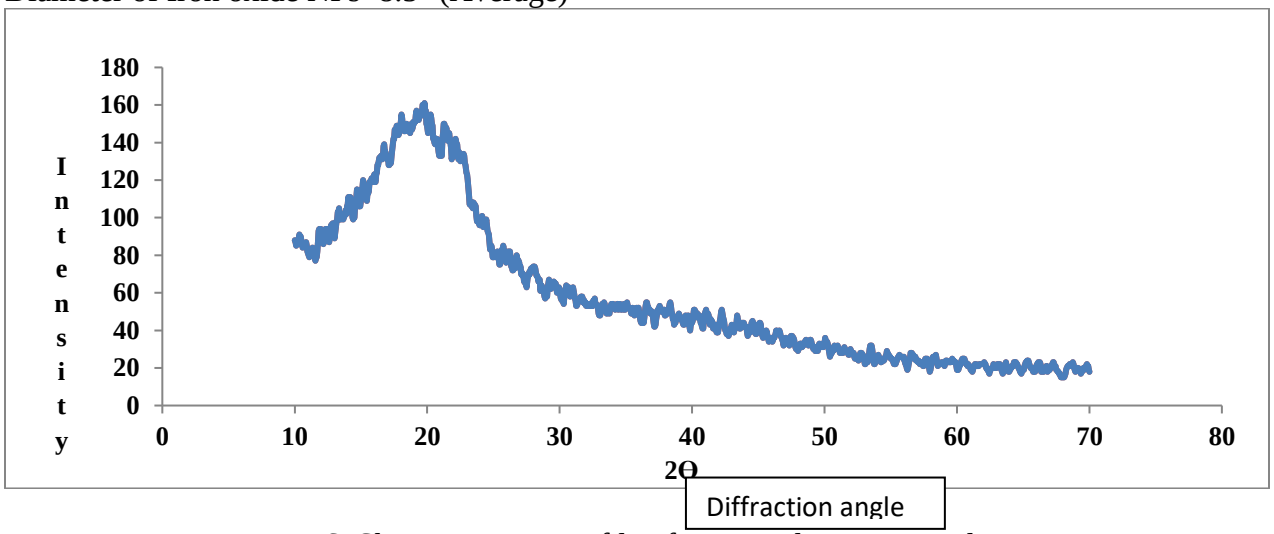
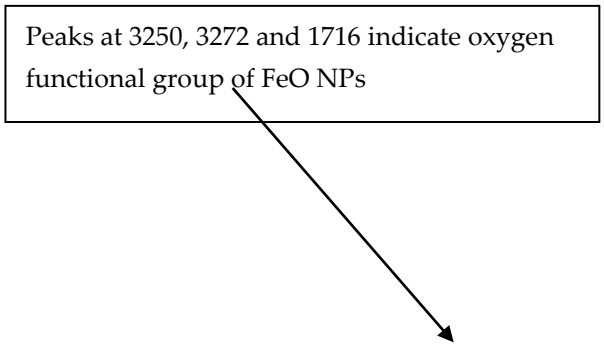


Figure 6: Showing XRD Profile of Iron oxide Nanoparticles.

3.4.3 Fourier Transform Infrared Spectroscopy FTIR:

The sample was analysed using FTIR Prestige- 21, (Shimadzu, Japan) to study the functional groups taking part in NP synthesis. The samples were scanned from 4000 cm-1 to400 cm-1. Between 1600-1800 wavenumbers, Oxygen group can be detected [15]. FTIR spectra of Iron oxide Nanoparticles showed a sharp peak at 3272 cm^{-1}. Similarly, a sharp peak was observed at 3250 cm^{-}. Another characteristic peak was observed at 1716 cm^{-1}

Peaks observed at 3250, 3272 and 1716 are characteristic of Oxygen functional group C=O stretch, Craboxylic group. 640 band is of Fe-O stretch while 1500 band showing C=C bond. These characteristic peaks and bands are previously reported by [35], [36], [37] and [38].



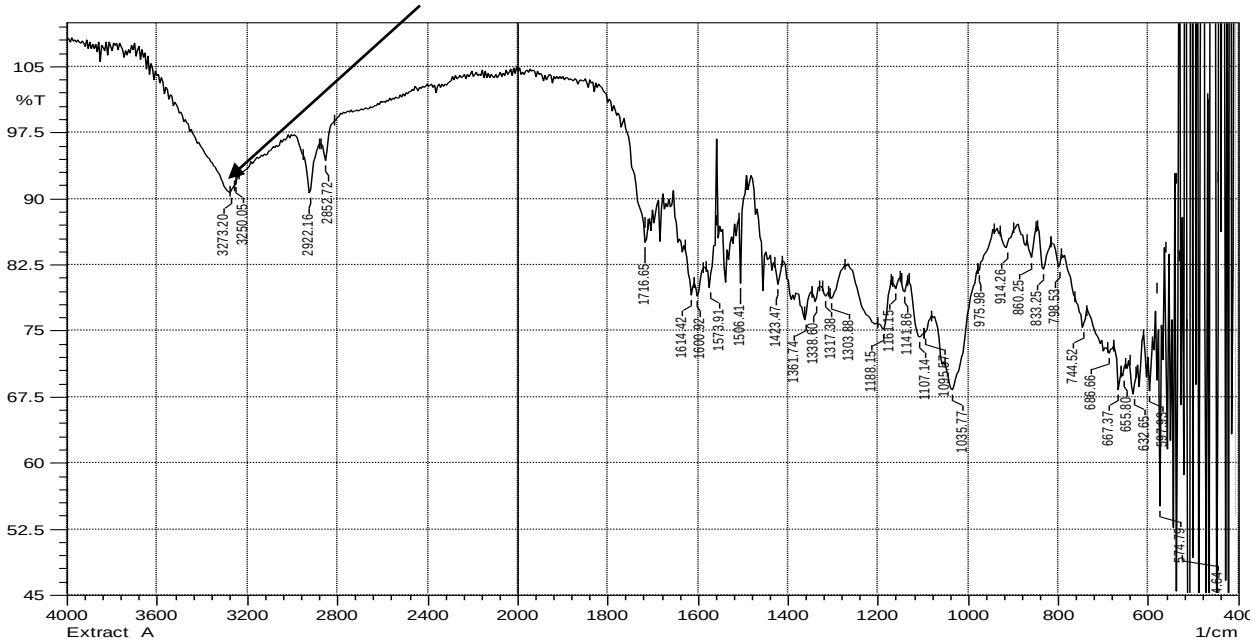


Figure 7: FTIR Spectra of Iron oxide Nanoparticles [33].

3.5 Activity against Ralstonia solanacaerum:

Tomato wilt have multiple causes but our concern was on bacterial and fungal. We collected 43 tomato plants showing typical symptoms of wilt that are terminal leaves yellowing and darkening, whole stem death after darkening. After collecting of plants, affected stems anre cut and suspended in autoclaved water for 24 hours and shown sticky material out into water and that water is being cultured on nutrient media [30].

Crushed affected leaves are also directly cultured on nutrient media which shown growth of multiple organisms. Bacterial colonies showing typical white creamy look and central red/pink color are subcultured for 48-72 hours on TTZ media which is specific selective media for Ralstonia solanacearum. Results show typical growth of Ralstonia with mucoid colonies that show gram negativity on Gram staining. Catalase and oxidase biochemical tests are positive that confirms that specie is Ralstonia solanacearum which cause tomato to wilt.

Four different concentrations were applied over Ralstonia solanacearum i.e. 25 µg/ml, 50 µg/ml, 100 µg/ml and 200µg/ml and positive and negative controls. Only 200 µg/ml concentration of FeO NPs show mean zone of inhibition of 11mm while positive control sows 14mm zone as shown in Fig 8.

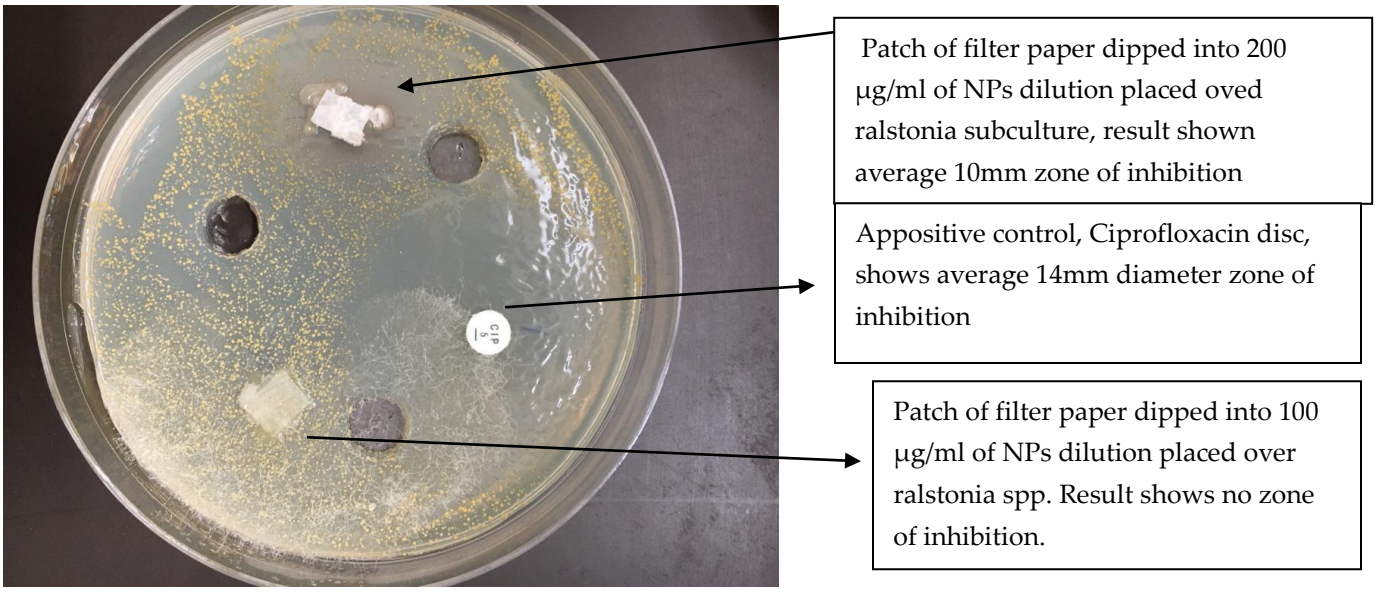


Figure 8: Disc Diffusion Method for Antimicrobial Potential

Out of 43 samples, 17 are bacteria positive and 20 are fungus positive while 6 did not show either growth.

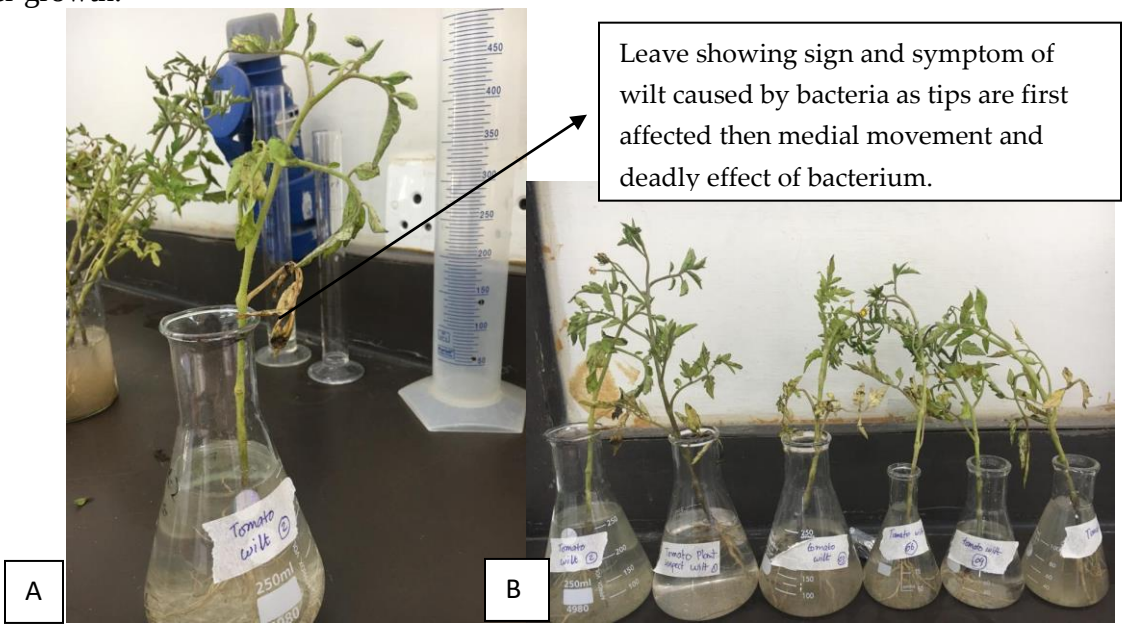


Figure 9(A) and (B): Wilted tomato plants collected from field

In other case whole affected leaves are crushed and cultured on petri plates. Among commensals mucoid white colonies of Ralstonia solanacaerum are sub cultured. Subculture shows typical whitish creamy mucoid colonies which further applied by different concentrations of FeO NPs as shown in Fig 4.10.

Figure 10: Crushed leaves Figure 11: Cut stem suspended in water

3.5.1 Subculture:

Creamy white colonies having central reddish or pinkish color are selected for subculture. 1 litre TTC or TZC medium got ready made from market. Colony obtained from primary culture is streaked on TTC plate for 48-72 hours. After 48 hours culture showed whitish creamy colonies with pink centers which is the indicator of virulence in the case of Ralstoniasolanaceaerum [16].

3.5.2 Gram Staining:

After staining by Grams technique, organisms show that these are gram negative rods as shown in image. There are many types of nanoparticles applied over the plant pathogens to limit the disease range and increase production of vegetables. As tomatoes are second major target, after potato, of plant pathogens, hence causing major economic loss. Ralstoniasolanacaerum is gram negative rod aerobic bacterium best grows at 27°C. This is causative organism of bacterial type wilt [31].

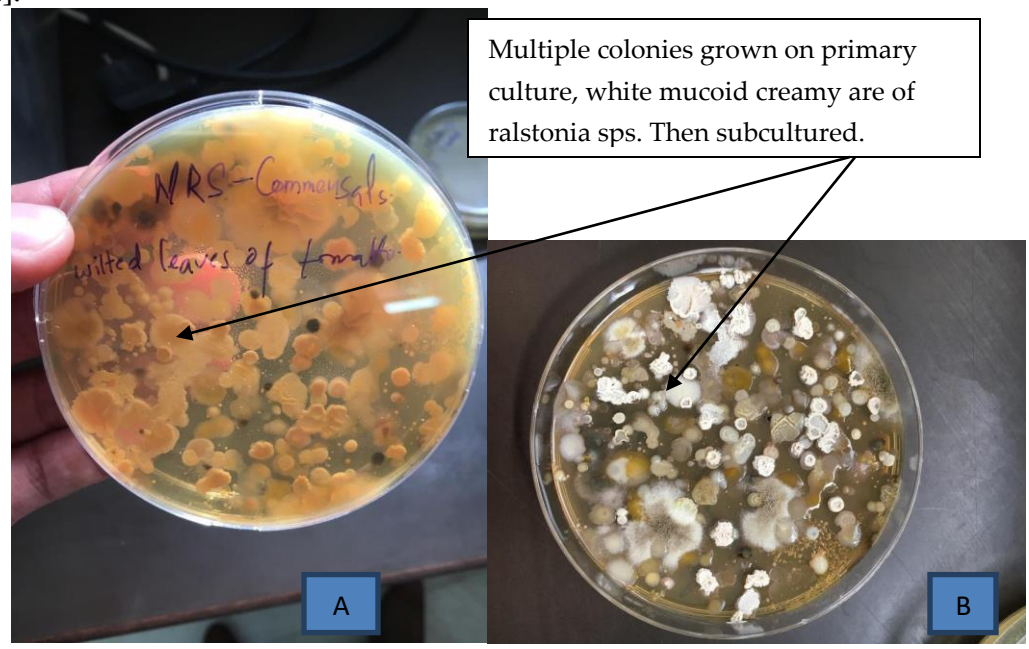


Figure 12 (a) and (b) Primary Culture on nutrient media

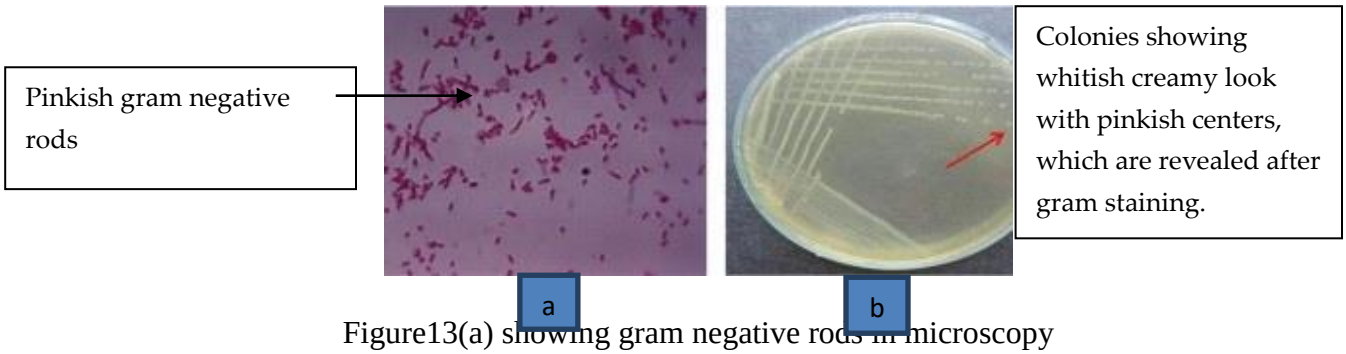


Figure13(a) showing gram negative rods in microscopy
(b) Subculture of selected colonies show mucoid creamy colonies

3.5.3 Biochemical Tests:

3.5.3.1 Catalase:

When colonies collected in swab are dipped into the H2O2 hydrogen peroxide, oxygen produced is uplifted and seen as vapors as a result of enzyme catalase hat converts H2O2 into molecular oxygen depicted in Fig. 14

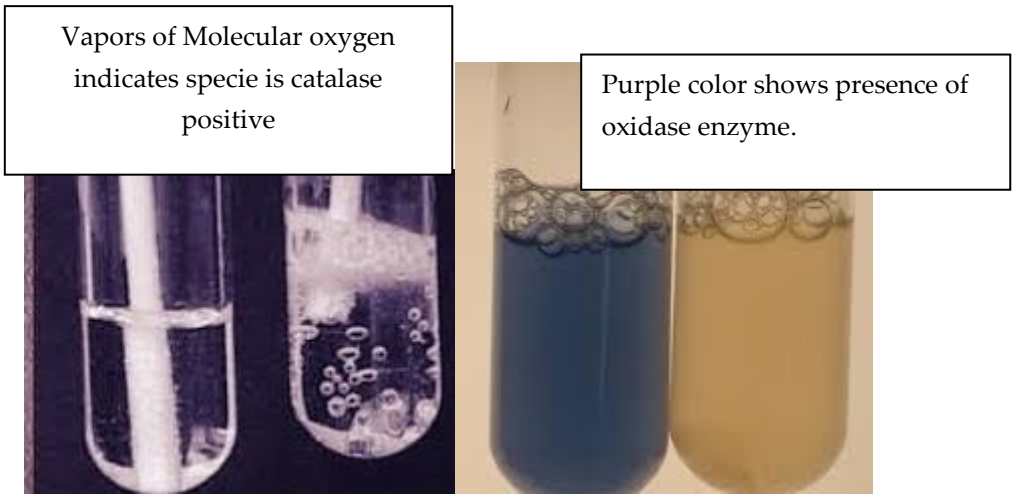


Figure 14: Catalase positive result Figure 15:Oxidase positive test

3.5.3.2 Oxidase:

when oxidase reagent get mixed with the colony suspension, color changes to purple which indicated that oxidase enzyme is present and it worked, Fig.15 shows. 250ml Stock solution of FeO NPs prepared of concentration of 400µg/ml by adding 0.1mg of powdered NPs into 250ml distilled autoclaved water. After proper mixing, stored at -4°C. Different dilutions are used against the organisms as follows;

Five concentrations of FeO NPs 25 µg, 50 µg, 100 µg and 200 µg made in distilled water (as shown by Fig.16) applied over cultured bacterium. Control groups are antibiotic ciprofloxacin and mock disks of filter paper. When incubated for 48 hours, zones of inhibition observed and measured in mm of diameter as shown in Table 4

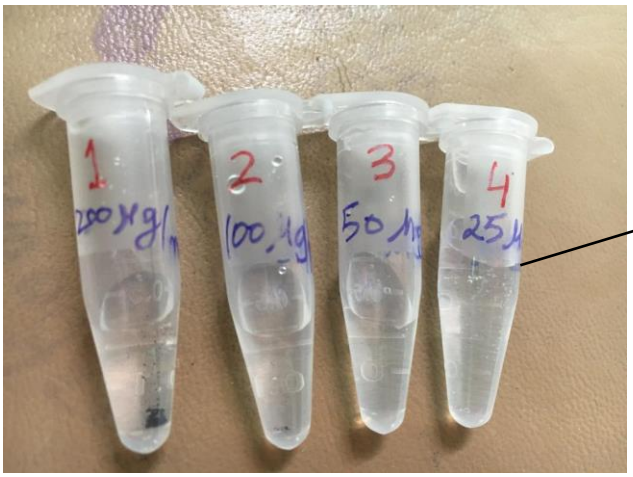


Figure 16: Different concentrations dilutions of FeO NPs

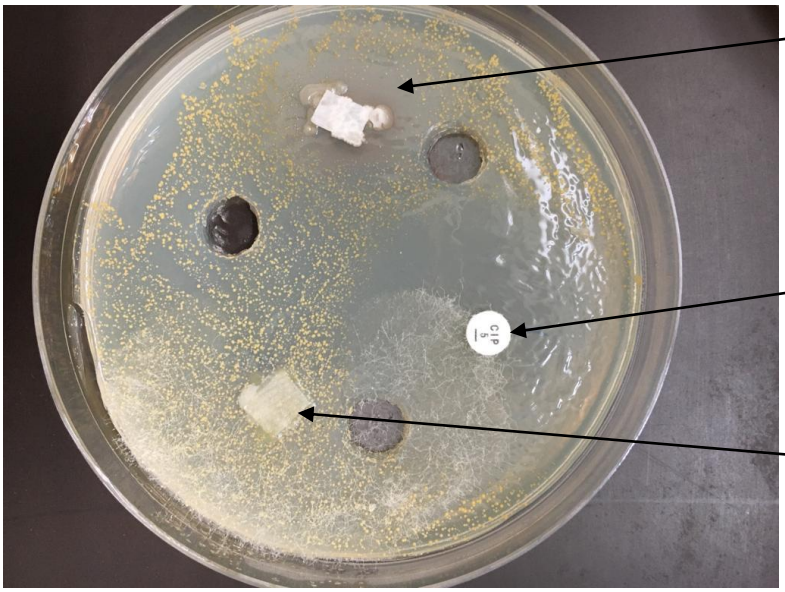


Figure 17: Disc diffusion method for antimicrobial potential

Table4: Application of different NPs conc. on Ralstonia solanacearum

Concentration	Result-1 Inhibition Zone in milimeters (mm)	Inhibition Zone in Inhibition Zone in milimeters (mm)	Inhibition Zone in Inhibition Zone in milimeters (mm)
FeO NPs: 25 µg/ml	0.0 mm	0.0 mm	0.0 mm
FeO NPs: 50 µg/ml	0.0 mm	0.0 mm	0.0 mm
FeO NPs: 100 µg/ml	0.0 mm	0.0 mm	0.0 mm
FeO NPs: 200 µg/ml	8.0mm	11.0mm	9.0mm

positive controls Ciprofloxacin discs as	12.0mm	14.0mm	11.0mm
negative control Blank discs of filter paper/distilled water as	0.0mm	0.0mm	0.0mm

Discussion

Nanotechnology and Nanomedicine is emerging field and have wide applications in environment, medicine, agriculture and chemistry and engineering. Uses are environmental sensors, agents for drug delivery, microbe killing or inhibiting agent, nanomedicine, gene editing carriers etc.

In the field of agriculture FeO NPs are synthesized by various methods with their own benefits. But after the synthesis their toxicity to body tissues is reduced to acceptable limits by surface modifications of nanoparticles [17]. The Iron III oxide shows considerable paramagnetic properties that could be controlled under guided magnetic field [18]. Iron Oxide nanoparticles (FeO NPs) have their own advantages and drawbacks to use for biological study. Advantages are;

Property of magnetism which is that particles can be controlled for drug carriage, tissue localization and rug delivery to specific site under magnetic field. This property is used in the treatment of drug delivery guided by external magnetic field [19]. Catalytic activity: the property of speeding up the chemical reactions. Easy and simple separation techniques available for them which makes them easily availability in cheaper price. Lowest toxicity: these particles do not cause harm to body tissues but specifically target those tissues having receptors for ligands attached on nanoparticles.

Biocompatibility with living organisms which makes them enable to not cross react body tissues and easy metabolism and excretion from body [20]. The effects of specific permeability, selective toxicity, biodegradability and antigenic properties are the main concern to be observed [21].

Tomato is the common product used both as fruit and vegetable. Tomato is prone to microbial infection. One common disease is tomato wilt which affects stem and leaves and responsible for low production of tomato. There are bacterial, viral, fungal and biochemical causes of tomato wilt. Fungal types of wilt are; fusarium and verticillium caused by Fusarium oxysporum f. sp. lycopersiciand Verticillium albo-atrum respectively. The bacterial cause of tomato wilt is Ralstonia solanacearum [22].

in our application, the fungal and bacterial causes of tomato wilt were identified excluding other viral and biochemical causes. There are applied various strategies for the control of tomato wilt; integrated strategies biocontrol mechanisms by rhizobacteria and chemical pesticides. Nanoparticle made up of iron and oxygen, Iron oxide nanoparticles are not previously used for the purpose of controlling plant diseases.

Tomato is second largest consumable vegetable and fruit in the world and contributing major role in economy of any agricultural country. After potato tomato is largest prone to attach by pathogens and crop loss in the world. Northern Pakistan showing higher incidence of tomato wilt in 2018.

Plant pathologists and researchers are continuously in keen interest to check whether nanotechnology can be useful in limiting plan diseases especially of microbial origin. Around 18 types of nanoparticles are applied to control plant pathogens and most common contains are

Ag, Cu, Zn, Mn and Si. There are two mechanism of killing of plant microbes by nanoparticles; direct killing and altering host nutritional status thus acting as host fertilizer which boosts hosts immune system [23]. To control wilt of tomato caused by fusarium biocontrols strains of bacteria and fungi are used. These strains are collected from roots of plants [24]. Recently all other nanoparticles are used to limit plant's microbial diseases especially wilt but Iron Oxide nanoparticles are not yet considered, due to biocompatibility and less toxicity, application of FeO NPs will be nontoxic and easy to handle to control tomato wilt caused by bacteria and fungi. In our study nanoparticles are synthesized by non-corrosive coprecipitative method where conditions were easy to control. Although we got bit non-uniform size on average 16-67nm diameter. Every aspect was studied by conventional and modern techniques like SEM, XRD and FTIR which revelaed that our synthesized nanoparticle lie in the range of size which can be used on biological systems.

Surface charge known as Zeta Potential between +30mV and -30mV is considerable while applying over biological systems. Measuring their surface charge which was -0.0321 which was suggestive that these particles will penetrate through gram negative organisms very easily and will show effects. This came so true. When nanoparticle of concentration 200µg/ml applied over the gram negative tomato wilt causing pathogen Ralstonia solanacearum, showdxone of inhibition of 8mm, 11mm and 9mm in triplicate analysis as compared to positive control of ciprofloxacin which is main antibiotic against pathogen, showed 14mm diameter zone of inhibition. This in vitro test shows that these NPs have close efficacy to standard antibiotic ciprofloxacin.

According to a recent study, when 20micrograms/mL FeO NPs that are Fe2O3 are applied through soil, resulted into reduction of fusarium wilt in vitro and in vivo [25]. In our study When fungus Fusarium oxysporum is get exposed to same 200µg, 100µg/ml concentration of FeO NPs, these showed no zone of inhibition, revealing that our FeO NPs have no effects on this fungus. This might be due to size differences as our sizes are between 16-67nm and recent study used almost 6-8nm sized FeO NPs. Another difference is our NPs are FeO and there are Fe2O3 and this may impart different effects.

Toxicity is imparted by any nutrient, whether it may be macro or micro, chemical or any agent applied on biological system. This happens to affect systems of organism which may be their structure, growth, metabolism etc. Iron is essential element of enzymes and pigments in tomatoes. When tomatoes are grown in excess of iron 25micro-molar then it causes toxicity to tomato plants. Result is stunted growth and enzymatic malfunctions. There are no mechanisms exist to eliminate iron from body so there must be controlled application of iron as nutrient [26] In recent studies iron was not applied over tomatoes for infection control. To estimate maximum tolerable concentration of FeO NPs as source of Iron there should be field trial of FeO NPs and their effects should be observed. There will be a difference in effects on growth and effects on infection control. Optimum and safe concentration will be increasing or not affecting tomato growth but necessarily inhibiting bacterial tomato wilt. This approach is subjected to field trial of different concentrations of FeO NPs synthesized and characterized by our procedure.

Conclusion

Iron Oxide nanoparticles synthesized by co-precipitation method were analyzed for their zeta potential and showed negative surface charge. After surface and structural analysis by SEM, XRD and FTIR, NPs were applied to fungal and bacterial pathogens causing tomato wilt i.e.

Ralstonia solanacearum, Fusarium oxysporum. These FeO NPs showed inhibitory effects against the gram negative bacterium in vitro while did not show effects against the fungus causing tomato wilt. Their ineffectiveness might be due to their non-uniform sizes and large sizes. Although effectiveness against the gram negative bacterium proven in vitro. Study suggesting that these NPs can be used commercially to control bacterial tomato wilt. these NPs need in vivo studies for their physiological effects on plants.
Conflict of interest:The authors has no conflict of interest.
Ethical concerns:Not applicable

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