Immobilization of Titanium Dioxide based Nano-formulation for Enhancement of Plant growth under environmental Stress condition

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Abstract **- Microorganisms has a significant role in agriculture which they do by maintaining fertility and improving the quality of the soil. Increase in environmental pollution causes stress to plants in agriculture leading to the loss of yield, productivity, causing an imbalance in the food system and ecosystem. Plant growth promoting bacteria has been shown to show positive effects helping the plant growth . PGPR with nanobiotechnology plays an important role which has positive impact on plants. In order to know more about the detail advantages and positive and negative impacts of engineered metals nanoparticles should be studied in more detailed form .Nanotechnology along with materials such as fibers, fertilizers and pesticides may produce positive revolutionary effects in the agricultural sector. The application of nanotechnology when inculcated with materials like silver, titanium, zinc with PGPR supports the positive growth in plants . Positive and negative effects have been observed on engineered metal nanoparticles on rhizobacteria. titanium dioxide nanoparticle applied to the roots of the plants for better production of crop, yield growth of the plant The synthesis of titanium dioxide nanoparticle was done by green synthesis characterized , by , visiblitity using SEM,TEM spectroscopy .beads formed using cheap materials humic acid ,chitosan sodium alginate in Cacl2 solution .Beads characterized by spectroscopy further incorporating the beads which will be inoculated with specific microorganism culture in to the rhizosphere soil of the plant and to check out the results of the incorporated titanium dioxide in plants rhizsopheric soil under biotic stressful condition. nanomaterials like zinc, silver, titanium shows positive growth results.**

Index Terms **- titanium dioxide nanoparticle, nanotechnology biological synthesis, sem, tem, beads formation humic acid chitosan, biotic stress, rhizosphere.**

INTRODUCTION

Growth of plants in agricultural soils is influenced by abiotic and biotic factors. Plant roots which is important and active area for root activity and for the metabolism of plants is known as rhizosphere. A large number of microorganisms such as bacteria, fungi, protozoa and algae exist in the rhizosphere soil of plants. Bacteria are the most important microorganisms in the rhizospheric soil. Rhizobacteria inhibit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. PGPR inoculants in the commercialized form shows that it helps in promoting growth through specialised mechanism; following are the various mechanisms, by suppressing of plant disease in the form of Bioprotectants. By improving the nutrient acquisition, or phytohormone production. The Antibiotic producing PGPR prevent growth of the pathogens. Biofertilizers-nitrogen fixing bacteria for crop nutrient uptake of nitrogen from nitrogen fixing bacteria with roots. Nitrogen fixing biofertilizers provide only a modest increase in crop nitrogen uptake (at best an increase of 20 Kg N acre-1) sulphur present in the soil transforms into sulphate by rhizospheric bacteria. rock phosphate which is the source of phosphorus but its availability to plants is limited under some environmental growing conditions. Phosphorus oxidizing bacteria thus help in making phosphorus available to the plants for better growth. The phytohormones they produce include indole-acetic acid, cytokinins, gibberellins and the inhibitors of ethylene production. PGPR as a component cultural control practices are used as biocontrol agents.

Such an integrated system could be used for the vegetables to produce more vigorous transplants that would be resistant to nematodes and other diseases for at least a few weeks after transplanting to the field. Numerous metallic nanoparticles (MONPs), such as titanium dioxide (TiO2), iron oxide (Fe3O4), and zinc oxide (ZnO), have gained considerable attention due to their environmentally favorable use in agriculture Under drought stress ,TiO2NPs Nanoparticles Mediated Drought Stress Tolerance TiO2NPs are the most frequently utilized nanoparticles, with applications in cosmetics and skincare, antibacterial air-cleaning goods, and wastewater decomposition . Activating photosynthesis and nitrogen metabolism may boost plant growth. TiO2NPs serves as the photocatalyst that can hydrolyze light into oxygen, electrons, and protons. The enhancement of secondary metabolites like phenolic compounds by MONPs has been recognized as a strategy for alleviating abiotic stress.

METHODOLOGY

Sample collection Samples collected from various locations in Mumbai Morphological study of isolates was conducted using microscopic imaging under low and then high-power objective of compound microscope .

Screening of isolates Phosphatase solubilisation on sterile pikovaskaya's medium.: Pikovskayas Agar positive growth, clear zone surrounding the colony

Protease activity on sterile casein agar. Spot inoculation of the isolated organism was done and kept for incubation for 72 hours at room temperature

Lipase activity: for lipase activity Egg Yolk Agar is used it is a differential and enriched medium used in the isolation and presumptive differentiation of different species based on their lipase activity among others

Amylase activity starch agar is used Starch agar for the detection of starch hydrolyzing microorganisms Amylase activity can be detected flooding the surface 48 hours old culture on Starch Agar with Grams Iodine. Amylase positive organisms show clearing around colony while development of blue to purple zone indicates starch is not hydrolyzed. Cellulase activity Spot plating was performed with 5 µL of cellulase (1 µg/µL), amylase (1 µg/µL) and agarase (1 µg/µL), followed by an incubation at 27 °C for 12 h.

Chitin activity Prawn shell waste was collected from shellfish. The waste was then dried, crushed into small pieces of uniform size (0.4 mm). 20%HCL was added for the crushed materials to get dissolved .nutrient agar was prepared and then the crushed substrates dissolved in hcl wash of 2 to 5 times to adjust the ph to neutral (Problems do arises while adjusting the Ph as the 20% hcl is a acidic and this if mixed with agar may lead to lysis of the agar or may not solidify) Precaution should be taken while adding HCL and also while adjusting the ph to utmost 6 to 6.5 then add it in the agar broth and pour in the plates allow the agar to solidify .after solidifying of agar the isolated culture were streaked or spot inoculated on the agar and kept for incubation for 7 to 10 days .light zones were observed of various diameter ranging from 1.0 to 1.6 mm zones

Biological synthesis of titanium dioxide (TiO_2) nanoparticles the most commonly used reagents will be titanium tetrachloride ; titanium isopropoxide ; urea ammonium chloride ; glacial acetic acid ; methanol, 99.5%; and ethanol 99.8% .Ethanol with titanium tetrachloride in a beaker; solution stirred for 30 minutes. form a yellow sol phase. Bidistilled water added solution become clear and colourless. Green synthesis TiO2 different sources nanoparticles from Characterization of synthesized titanium dioxide nanoparticles The synthesized nanoparticles characterized using tem /sem. TiO_2NPs were prepared using Liquid Impregnation method. The surface morphology of TiO_2 NPs was explored by SEM/TEM Synthesis of beads using cheap material (humic acid/chitosan) Sodium alginate beaded inoculant was prepared by Two grams of sodium alginate adding to 100 ml of culture broth of PGPR and mixed for 30 minutes in a magnetic stirrer. The mixture will be added drop wise with 10 ml syringe into 100 ml sterile 0.1N $CaCl_2$ to obtain alginate beads.

Bioassay Isolates morphological observation biochemical characterization. The tests involved, were staining, amylase and gelatinase, citrate utilization, indole test, Vogus Proskaur test, methyl red test, H2S production, sugar fermentation etc.

Characterization of IAA To determine the amounts of IAA produced by each isolate, a colorimetric technique performed with Van Urk Salkowski reagent using the Salkowski's method.

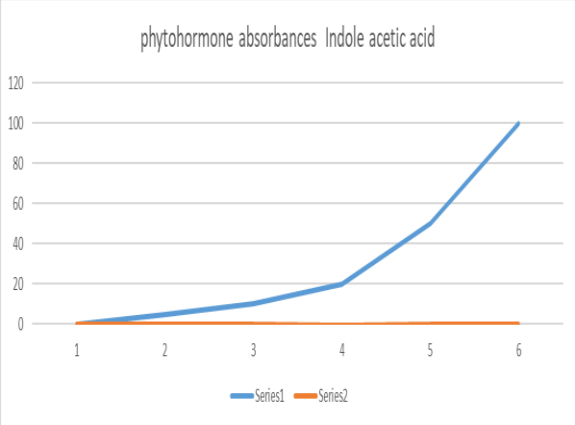
Sidherophore production test Chrome azurol sulfonate assay agar for the siderophores production. bacterial cultures were spot inoculated onto the blue agar and

incubated at 28 °C for 24-48 h. Color change observed for the interpretation of results, ferric ion was transferred from strong blue complex to the Sidherophore Incorporation of TiO2 nanoparticles, PGP bacteria and NPK into beads

Evaluation The bacterial strains were Pseudomonas putida as Group 1 representative, and proposed as PGPR, Escherichia coli used as microbial model in sensitivity and microbial metabolism assays, Pseudomonas aeruginosa PA14 as Group 2 representative for some animal tests in addition strains Rhizobium Pseudomonas fluorescens, Bacillus subtilis, and Azotobacter vinelandii as PGPB; bacteria were grown at 30◦C in trypticase soy agar (TSA) or broth.

RESULT

Test	Isolates number	Incubation temperature	Zone diameter (mm)
Phosphate solubalisation	IS1, IS2, IS3, IS4, IS5	28ºc (7 days)	< 12-7 mm ranges of zones
Lipase activity	IS1, IS2, IS3, IS4, IS5	28ºc (7 days)	<10-7mm ranges of zones
Sidherophore production test	IS1, IS2, IS3, IS4, IS5	28ºc (7 days)	9-5mm ranges of zones
Chitin activity	IS1, IS2, IS3, IS4, IS5	28ºc (7 days)	<1.7-0.4mm ranges of zones



Phytohormone production ranges

Salt tolerance testing on various strains

Wheat types		Normal (cm)length		Drought (cm)length		Salinity (cm)length	
sample	temperature	Root	Shoot	Root	shoot	Root	shoot
W1	25ºc	11.05	14	11.00	12.14	10.09	13.09
W2	24ºc	12.00	10	10.00	9.7	10	10
W3	30ºc	9.5	14.17	8.5	13.05	8.05	13.12
W4	25ºc	12.04	14.07	11.09	14.04	12.01	12.02
W5	27ºc	13.04	10.04	10.05	10	10.10	10.09

The above study based on how the titanium dioxide on nanoformulation when incorporated in the soil enhances the growth with the help of some special kinds of PGPR organism In this study we had analyzed the growth and the organism which help the plant to grow even when the condition is not favorable and also what are the effects in case of normal condition .In this study soil were collected from various areas field of new Panvel and was processed in lab .Isolation of the soil samples were done under proper condition maintaining the ph and temperatures the organism were grown on nutriengt media. A total number of 10 isolates were found after 24 hours of incubation colony formation was observed which were preserved on slants of agar as IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10 and screening was further carried out for identification and confirmation of the organisms following are the screening test which were performed Morphological identification by gram staining process by crystal violet, and safranin smear stained by stain and was observed under the microscopes. Phosphate solubaliztion Phosphate solubalization was done by spot inoculating of the isolates on Pikovskys agar media (incubation of 7 days in 37 degree) the results were observed which showed halo zones around the spot . incubation of 7 days.Protease activity on sterile casein agar Spot inoculation of the isolated organism was done and kept for incubation for 72 hours at room temperature .after 72 hours the plates were observed but showed light zones, so it was kept for another 24 hours which showed the growth halo clear zones on plates as compared to all the isolates IS2 ,IS4, showed more halo clear zones then IS1, AND IS3,whereas IS5 no growth was observed but after over incubation it showed minute zone rest all the isolates IS6, IS7, IS8, IS9, IS10 showed no growth or absence of halo zones

on the plates. Lipase activity Egg Yolk Agar for the isolation and presumptive differentiation of different species based on their lipase activity among others. The non-selective medium needed to detect lecithinase and lipase production and proteolytic activity.spot inouculation was done on the egg yold agar media and kept for incubation 24 hours at 37 degree celcius.Amylase activity each bacterial isolate was streaked on to 7 starch agar plates and incubated for 24h at 37°C. After incubation, the plates were spreaded with iodine solution (0.3% iodine and 1% KI) the bacterial growth Hydrolysis zones were visualized by flooding of the plates with Gram's iodine as The plate was incubated at 27 °C for after which hydrolysis was visualized by flooding the wells with Gram's iodine for 30 sec. All the isolates showed yellow halo zone except IS7, IS6 which did not show any hydrolysing zones or change in color after flood with the iodine solution. Cellulase activity Pure, Solidified CMC Plate-Based Clearing Assays A solution of 7% CMC was heated to 70 °C, poured into petri dishes and was allowed to polymerize at room temperature overnight. Spot inoculation was performed incubation at 27 °C for 24 h. only 2 Isolates which showed growth after 24 hrs forming small halo zone around the colonies the rest of the isolates were incubated for further 24 hours and growth was obsereved IS1, IS3, IS4, IS2 showed large halo zones as compared to IS5 which showed small Halo zones

Sidherophore production assay test Chrome azurol sulfonate assay agar for the siderophores production, bacterial cultures were spot inoculated onto the blue agar and incubated at 28 °C for 24-48 h. Yellow-orange zones around the growth indicated positive results for siderophore production IAA ACTIVITY Total 5 isolates were obtained from different rhizospheric soil. The isolates were further checked for IAA production. A standard protocol was followed for the indole acetic acid. pink colour indicates the IAA production. Absorbance read at 530 nm. Auxin production was determined by using a standard graph

Biological synthesis of titanium dioxide (TiO_2) nanoparticles

Titanium dioxide powder + crushed moringa leaves powder + distilled water .Solution mixture is formed (before mixing both titanium dioxide and crushed moringa leaves along with distilled water their structure characterization was done using microscopy SEM)Best isolates culture suspension was made by adjusting the o.d at 0.001and the suspension was mixed along with the solution Constant stirring Synthesis of beads using cheap material (humic acid/chitosan) The tio2 solution is mixed with agar and beads are formed in chilled cacl2 by using syringe .The beads size were compared with normal alginate beads by microscopy .Abiotic stress tolerance testing of the various strains the synthesized beads were incorporated into the soil of the potted plants .Pot assay was done by treating and incorporating beads and wheat plant under various stressed and normal condition, in order to observe the efficiency of the Tio2 incorporated in plant the rate of enhancement and improvement of the plant The plant under normal condition showed increased growth rate upto 70% efficiency .Plant under drought condition showed slight increase in the growth rate and 40% efficiency Plant under salinity showed increased in the growth rate upto 50 % efficiency.The growth efficiency of all the abiotic stress condition was compared with other research Papers efficiency growth rate which showed increase of growth rate in positive results

Biochemical test the culture suspension which was mixed in the tio2 nanoparticles solution and incorporated into plants soil inform of beads showed increased in the growth rate .in order to identify the various isolates further biochemical test was done and observed

CONCLUSION

The above study based on how the titanium dioxide on nanoformulation when incorporated in the soil enhances the growth with the help of some special kinds of PGPR organism in this study we had analyzed the growth and the organism which help the plant to grow even when the condition is not favorable and also what are the effects in case of normal condition.

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