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STUDY THE IMPACT OF NANO PARTICLE AND MYCOBIONT FUNGUS ON TOMATO (SOLANUM LYCOPERSICUM)

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ABSTRACT

In the present study, a nanoformulation has been prepared by the synergistic association of Zinc oxide nanoparticle with root endophytic fungus *Serendipita indica*. Zinc oxide nanoparticles were synthesised by the machine-assisted thermal decomposition technique and morphological characterisation of the synthesised nanoparticles was performed by the scanning electron microscopy (SEM), Zeta potential analyser and X-ray diffraction technique. Fungus was grown on the optimized ZnO NPs turned into an increased fungal biomass and spore count. Effect of the prepared nanoembedded fungus was examined on the growth of *Solanum lycopersicum* resulted into an enhancement in root and shoot length, weight and biomass.

INTRODUCTION

Nanotechnology is a branch of science deals with the materials having a size ranging from 1-100nm. Several NPs have shown their effectiveness in agriculture sector, Zinc oxide (ZnO) NPs is one such nanoparticle which has gained enough importance due to its verstile properties such as high chemical stability, high electrochemical coupling coefficient, high photostability, etc.[2] Currently it is widely used as fertiliser to improve plant's metabolic processes. Plants utilise Zinc for chlorophyll synthesis, germination, fertilisation, biomass production, etc. Zinc is known to be important for the human body's normal operation as well and is connected to a number of enzymatic processes. Zinc supplementation are known to benefit the skin and lowers the likelihood of linear growth limitation in young children. Zinc deficiency results in recurrent infections in persons of all ages and neurobehavioral issues in infants. Deficient pregnant women run the danger of having a high-risk pregnancy. Zinc nanoparticles increase the value of active ingredients, and can be consumed as nutrition supplement.

Serendipita indica lately known as *Piriformospora indica* is an excellent endophytic fungus characterised by the formation of pear shaped spores called chlamydospores, produced by thin walled, hyaline, and septate hyphae.[1] This fungus is mainly known for its symbiotic relationship with the wide variety of plants, colonisation takes place in the root zone where spores penetrate the subepidermal region of roots and cover the rhizodermal cells.[2] Symbiotic association of plants with roots resulted into enhanced growth yield, increases hormone production including ethylene, gibberellin, salicylic acid and abscisic acid, it also facilitates the uptake of various nutrients, guards the plant against salt, water, and weather hazards, and offers complete resistance to acidic pH, heavy metal poisons, infections, and other environmental stresses. It has the potential to function as a plant regulator and commercial bio-fertilizer, promotes fast flowering, seed production, and an abundance of biomass. The fungus possesses a remarkable ability to boost secondary metabolites, which adds value to the plant and promotes early flowering and fruiting.

The cherry tomato, *Solanum lycopersicum* L., belongs to the family Solanaceae and occupies a wide variety of habitats including Maxico, Spain, Brazil etc.[4,5] s These tomatoes are favoured globally due to their vibrant colouration and citric favour. They are widely consumed for their umami flavour in various ways- raw or cooked, and used to prepare many dishes, sauces, salads, and drinks. Frequently used as a culinarily as a vegetable ingredient. They are good origin of antioxidants, contains nutrients that helps in illness prevention by detoxification, promoting growth and proper immune system functioning. It is an excellent mineral and antioxidant source and contains carotenoids, flavonoids, vitamins, lycopene and lutein (Rebolloso-Fuentes 2009; Wang et.al 2015). Regular consumption can play a positive role in prevention of cardiovascular diseases (Toor and Savage 2005).

Studies suggest that *S. indica* colonises tomato roots leads to improved growth in plant (Sarma et.al 2011). Association with *S. indica* has also led to increased TSS and firmness of the fruit as well as natural resistance and reduced disease incidence and index against TYCLV (Wang et.al 2015). *S.indica* has also provided resistance against pepino mosaic virus and has reduced the disease symptoms in case of *V.dahliae* (Fakhro et.al 2010). *S.indica* has also shown growth and biomass improvement in inoculated tomatoes under salt stress (Ghorbani et.al 2017). The symbiotic relation leads to better phosphorous nutrition and increased chlorophyll content in the plant. (Ghorbani et.al 2017). This research aims to create a fungal symbiont with the use of nanotechnology in order to increase the food value of tomato for human consumption.

MATERIALS AND METHODS

Experimental

Synthesise of ZnO particles

Nanorods were synthesised by using a machine-assisted thermal decomposition technique. 5gm of Zinc acetate dihydrate was grounded for 45 minutes and placed in muffle furnace in an alumina crucible for around 4 hours at 500^{o}C. Obtained product was then washed twice with distilled water followed by drying in hot air oven at 80^{o}C overnight.

Characterization of ZnO particles

Scanning electron microscopy (SEM) technique was employed for the morphological analysis of the nanoparticles. Zeta Potential analysiser technique was utilised for determination of the surface charge of nanoparticles. The crystalline phase of nanoparticle was determined by X- Ray Diffraction analysis. Scherrer's equation was used to calculate the size of the crystalline grain. Using Cu K $_{\alpha}$ radiation with a wavelength of 0.1541 in the scan range of 2 θ= 2090^{0}.

Culturing of *S. indica* with nanoparticle

S. indica was cultivated on jaggary agar media and broth. 4mm fungal disc containing active hypha and chlamydospore was inoculated on jaggery medium and incubated at 28^{0}C for 10days. Nanoembedded fungus was prepared by co-culturing optimised ZnO nanoparticles with fungus *S. indica* in both broth and agar media and incubated in the same conditions.

Measurement of Fungal Biomass and Sugar Consumption rate

The Dry Cell Weight (DCW) of fungus was determined by filtering a known volume of culture broth through Whatman No. 1 filter paper, which was then dried to a consistent weight in a hot air oven at 60^{o}C overnight. The sample's weight was measured using an electronic balance.

Using the DNS (Dinitro Salicylic Acid) technique, the sugar consumption rate was estimated as grammes of biomass generated per gramme of substrate. A test tube containing 3ml of sample and 3ml of DNS reagent was heated in a boiling water bath for 15 minutes. The heated tube was then given 1 ml of Rochelle salt solution and allowed to cool to room temperature. At 575nm, absorbance was measured (Systronics-20-Spectophotometer).

Spore Count

S. indica grown on an agar plate was extracted from the culture by flooding the culture with 2 ml of a 0.01 % v/v Tween 20 solution. Using a sterilised glass spreader, the spores were gently removed off the hyphae. In a 1 ml Eppendorf tube, spores were collected and centrifuged at 4000 rpm for 10 min. The supernatant was discarded, and hemocytometer was used to count the pelleted spores.

Seed Germination

Seeds were surface sterilized with 10% sodium hypochlorite for 10 minutes and then washed multiple times with sterile water. Seeds were then placed on germination sheets and incubated at 28 °C for 3 - 4 days in dark with regular addition of autoclaved distilled water to maintain the moisture.

Growth Parameters

Plants were grown in pots and nanoembedded fungus was introduced in them, pots without any treatment were also grown as control. Temperature was maintained at 25 to 27^{0}C in the plant growth chamber. The plants were harvested after 20 days and growth parameters such root and shoot length, root and shoot fresh (FW), and dry weight (DW) were examined. To remove dirt and other debris, they were washed under running water. The length of the root and shoot was measured using a scale. FW was calculated right away, while DW was calculated after drying the sample in a hot air oven for 24 hours at 60°C.

RESULT

Characterisation

Morphological analysis performed by SEM techniques shows that prepared ZnO nanoparticles were spherical, oblong and rod in shape with an average size of 50 nm (Fig 1). Zeta potential of the above prepared nanoparticle was carried out by the zeta analyser showing that the charge of ZnO NPs turned out to be -14.51 and size is 96nm (Table 1).

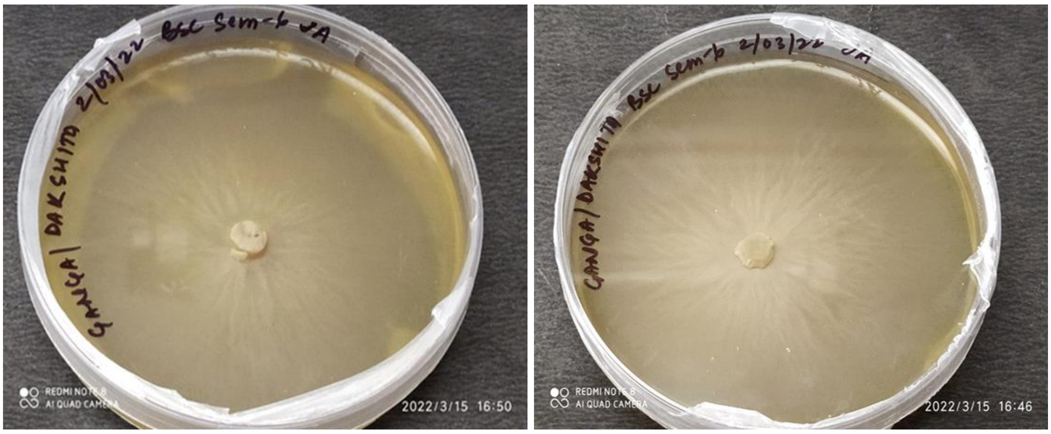
Figure 1: SEM analysis of ZnO.

Table 1: Zeta analysis of ZnO Nanoparticle.

Nanoparticle	Size(nm)	Charge (mv)
ZnO	96nm	-14.51

S. indica fortification with nanomaterials

S. indica was cultivated in both jaggary agar and broth medium, growth was observed after 10 days (Fig 2) which shows that optimized ZnO nanoparticles has enhanced the growth of *S. indica*, confirmed by an increased biomass, dry cell weight and spore count as compared to control.



Control **Treated**

Figure 2: Culture of *S.indica* on Jaggery media fortified with nanoparticles.

Measurement of Fungal biomass and spore count

Fresh culture of *S. indica* was treated with ZnO-nanoparticles and untreated culture was taken as control, both were incubated at 27°C for 10 days. After incubation, the samples were filtered by using Whatman filter paper, and filtered biomass was dried at 60°C in hot air oven for 24 hours. The dry weight was taken by the help of metal balance. An increase in biomass was observed in ZnO NP treated fungus as compared to control (Table 2).

Spore Count

Spores were scrapped off from *S. indica* cultured on agar plate and centrifuged. Obtained pellet was washed with distilled water, dyed with typthan blue and spores were counted with hemocytometer. Spore count in ZnO nanoparticles treated fungus shows higher yield of 1×10^3 –1×10^5 as compared to untreated fungus where spore count was 1×10^4–1×10^5, shown in Table 2.

Table 2: Effect of ZnO on growth and sporulation of *S. indica*.

	FW	DW	Spore count	Start of sporulation	Sugar consumption rate
CONTROL	1.2414	0.9	1×10^4–1×10^5	4	0.1
TREATED	2.1684	1.1	1×10^3 –1×10^5	3	0.3

Comparison of Growth Parameters

Two different treatments were given to *Solanum lycopersicum* plants in triplets, 1) *S. indica*, and 2) ZnO-nanoparticles treated *S. indica* for control. Tomato plants responded variably toward the treatments, results are summarized in Table below. The data indicates that ZnO treated fungal culture has positive effect on different growth parameters of plant as compared to control confirmed by the significant increase in germination rate, in dry and fresh weight, root and shoot length as compared to the control as shown in Table 3

Table 3: Growth parameters of tomato in presence of ZnO Nanoparticle.

PARAMETERS	CONTROL	TREATED
Seed germination rate	74 ± 8.54%	788 ± 0.16%
Dry root weight	1.1 ± 0.06%	1.7 ± 0.16%
Dry shoot weight	3.1 ± 0.01%	4.2 ± 0.05%
Root length	0.4 ± 10.4%	0.7
Shoot length	5.9 ± 0.76%	74 ± 8.54%

CONCLUSION

The study revealed that the Zinc oxide nanoparticles has shown a positive effect on the growth endopytic fungus *S. indica* as there was an increase in the number of spores, the thickness of the hyphae, and the number of vacuoles. On introducing this nanoembedded fungus to the tomato plant, the germination of seeds and the development of plant seedlings have been benefitted. This nanoembedded fungus can be used as a unique nano-biofertilizer that can improved plant growth and development in a variety of plants.

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