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Combined effect of biosynthesized ZnO NPs and PGPR (*Pesudomonas fuorescence*) on the management of the wilt disease complex of pigeon pea

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Abstract: **Plant disease caused by various pathogens like fungi and nematodes significantly reduced the crop growth and yield globally. In present time NPs are emerging as potent tool for the management of plant disease and enhanced the crop production. PGPRs are environment friendly to increase the crop growth and yield. PGPRs suppressed the plant disease by the production of pathogen-antagonizing compounds as well as well immune system. This research article focuses on potential of role of biosynthesized ZnO NPs and PGPRs in the management of wilt disease complex of pigeon pea. Combined application of PGPRs (*Pseudomonas* sp.) and different concentrations (50, 100, 150, 200, and 250µg/mL) of ZnO NPs of enhanced the plant growth and yield of pigeon pea infected with *F. udum*, *M. incognita* and *H. cajani*. But significant reduction of wilt disease complex occurs at 100 µg/mL ZnO NPs *and Pseudomonas* sp. IS-2. Thus this research work was conducted to explore the effectiveness of ZnO NPs and *Pseudomonas* sp. against *M. incognita, F. udum* and *H. cajani* under *in-vivo* condition. Combined application of ZnO NPs and PGPRs potentially increased the plant fresh weight, plant dry weight, number of pods, shoot length, root length, number of nodule, total chlorophyll, carotenoids, proline and phenolic compounds of pigeon pea. This experiment confirmed that ZnO NPs and PGPRs synergistically reduce the plant pathogens growth and increased the antioxidant enzymes such as ascorbic acid, glutathione, ascorbate peroxidase and superoxide dismutase. The implementation of ZnO NPs and PGPRs in the management of plant disease reduced the risk of chemical inputs.**

Keywords: **ZnO NPs; PGPR; Meloidogyne incognita; Fusarium udum; Heterodera cajani; Pseudomonas fuorescens.**

1. INTRODUCTION

Pigeon pea seeds are the main source of vitamins and proteins, particularly for the vegetarian population of the globe. Pigeonpea (*Cajanus cajan* (L. Mill.) is one of the most important pulse crops in the semiarid tropics (1). India is the major pigeon pea-growing nation with almost 90% of the global area and production (2).

In the present-day nanotechnology attracts various researchers due to its flexible nature implementation in different fields including plant science. Agriculture is one of the fields that gains different advantages from nanotechnology, among the different NPs, ZnO NPs can be considered as nutritional additives as zinc, an essential micronutrient for the growth promotion and enzymatic function of plants (3). ZnO NPs attracted agricultural scientists due to their possible uses like nano growth regulators, and nanofertilizres to increase plant growth and productivity and efficiently supply and uptake of zinc (4).

PGPRs are a promising eco-safer alternative strategy in sustainable agriculture (5) and PGPRs are a set of plant-associated set of bacterial communities that improve plant growth, and development and positively influence crop yield (6). PGPRs can enhance the accumulation and uptake of K+ ions which have a great role in maintaining the favorable ions balance in the plant cells (7). PGPRs synthesize and release plant-promoting hormones like gibberellins, auxins, and cytokinins and these hormones have a vital role in nutrient uptake, plant growth, and cell division and protect the plant from biotic and abiotic stress (8). PGPRs also involved in plant disease suppression, metal accumulation, and phosphate solubilization (9 &10).

Plant pathogens cause significant damage to social stability and the economy all over the world. Bacteria, fungi, plant parasitic nematode, (*Fusarium* sp., and *Meloidogyne incognita)* are the ones that reduced the development and plant growth remarkably. Plant-parasitic nematodes are considered one of the most destructive pests that attack plants (11). The vulnerability of *M incognita* causes yield deficiency, quality, growth reduction, and even complete crop loss in severe invasion and diminishes the resistance to biotic and abiotic stress (12). In agriculture, NPs reduce disease severity, inhibit pathogen infection, promote plant growth, and increase crop production (13). *Fusarium udum* incited wilt disease is a main cause that adversely affects crop growth (30-60% of disease incidence occur during flowering and maturity stages and yield, in cultivation of pigeon pea (14).

Pigeon pea is vulnerable to *Fusarium udum*, *Meloidogyne incognita*, and *Heterodera cajani* and these pathogens cause serious threats to the successful cultivation of pigeon pea. With this background objective of the present study was to evaluate the effect of biosynthesized ZnO NPs and PGPR against the wilt disease complex of pigeon pea.

2. MATERIALS AND METHODS

2.1. Pot experiment

2.2. Preparation and Soil sterilization

The sandy loam soil was collected from of Department of Botany, Aligarh Muslim University, Aligarh, U.P. (India). After passing through 10 mesh sieves river sand and soil was mixed in the ration of 3:1 (V/V). The analysis of soil presented following features available K (157.02 mg kg^{-1}), available P (8.79 mg kg^{-1}), N (95.76 mg kg^{-1}) pH 7.5, and porosity 44%, and electrical conductance 0.62, water holding capacity 40%. Soil poured in the pots then sterilized at 137.9 kPa for 20 minutes.

2.3 Raising of test plants

Pigeon pea seeds were used in this experiment. Seeds were sterilized with 0.1% mercuric chloride for 2 minute rinsed three times with double distilled water. Soil in 15cm earthen pots soil sterilized pots and 5 seeds were sown in each pot. Thinning was done after one week germination to maintain single plant. Uninoculated plant serve as control and plants were kept in the green house at 30-40^{o}C temperature in a factorial block design. Pots were watered as needed. Each treatment was replicated 5times and experiment was terminated 90 days after inoculation.

2.4 Preparation of fungal inoculum

Fusarium udum was isolated from the pigeon root maintained on the potato dextrose agar (PDA). Inoculum of this fungus was prepared by culturing the isolates in Richards's liquid medium at 25^{o}C for 15 days (Riker and Riker, 1935). Mycelium was collected on blotting sheet and extra water and nutrient was removed by pressing it between two fold of blotting sheets. 100g mycelium was macerated in 1L distilled water and 10ml of suspension containing 1g fungus was inoculated around roots.

2.5 Preparation of nematode inoculum

Large numbers of egg-masses were manually pickedup with sterilized forceps from heavily infected *Solanum melongena* roots on which the pure culture of *M incognita* was maintained. These egg masses were washed in distilled water and then placed in 10m diameter 15 coarse sieves containing crossed layers of tissue paper which were subsequently placed in petridish containing water just deep enough to come in contact with egg-masses. Hatched juveniles were collected from the petriplate every 24h and fresh water added to petriplate. The concentration of second juveniles (J2) of *M incognita* in water suspension was adjusted so that each ml this suspension (i.e., 1000 freshly hatched juveniles) was added around the seedling.

Soil and root sample was collected from the pigeon pea field for the preparation of *Heterdera cajani* inoculum. Cyst examination was done under stereomicroscope in the root and cysts present on the root were collected. Cysts were also isolated from the soil through 100 mesh sieve and catch of sieve was filtered through filter paper. Catch filter paper was observed under the stereomicroscope, cysts observed were collected and through camel hair brush no.1 cyst then collected was placed for the hatching in pigeon pea root exudate. Juvenile collected were inoculated at rate of 500juveniles per plant. The female cyst is lemon shaped and cuticle is marked by thick zigzag lines. One large and one smaller annule are present of head region (15). Cyst contains second juveniles (J2) and also contained lipid content which is very helpful in survival (16).

2.6 Preparation of bacterial inoculum

Details of Isolation, identification, and screening of *Pseudomonas fuoresecns* strains are given in the section IV. of Each isolates (IS-1, IS-2, IS-3, IS-4 and IS-5) was cultured on the nutrient agar plate and growth of each isolates was scrap with the help of slide and dissolved in distilled water. Number of bacterial cell in suspension was adjusted as $1.5X10^{-7}$. 100ml bacterial suspension was poured in 200g sterilized soil and 100 seed of pigeon pea were wrapped to provide $1.5X10^{-7}$ bacterial per seed.

2.7 Procedure of inoculation

One week old healthy pigeon pea seedling was used for the inoculum. Inoculation of bacterial, nematodes and fungus was done by done by following mechanism soil root the surface was removed carefully without damaging the root system. The suspensions of inoculum were poured around root and soil was replaced and equal amount of sterile water was poured on the control.

2.8 Preparation of ZnO NPs solution

Biosynthesized ZnO NPs obtained in the form of nanopowder and the solution prepared 50µg, 100µg, 200µg and 250µg dissolved in 1mL. These treatments applied as following concentration 50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL and 250µg/mL.

2.9 Experiment Design

The experiment was carried out in a completely block design uninoculated pots served as control (without pathogens) and inoculated with pathogens. There were 5 treatments of biosynthesized ZnO NPs at different concentrations i.e., 50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL and 250µg/mL and *Pseudomonas* isolates i.e., IS-1, IS-2, IS-3, IS-4, and IS-5 each tested on pathogen *Meloidogyne incognita*, *Heterodera cajani* and *Fusarium udum* and each treatment replicated 5 times.

2.10 Estimation of pathogen (nematode and fungal) parameters

The number of egg masses was calculated according technique of Halbrook et al., (1983). The number root galls and number of cyst were counted visually. Root knot or root galling determined through 0-4, where 0 = no infection, 1= slight infection (1-25%), 2 = moderate infection (26% - 50%), 3 = sever infection (51-75%), 4 = very severe infection (75-100%) (17). Wilting index was recorded by scoring the disease severity on 0-5 scale where 0 = no disease and 5 = severe wilting. Total nematode population in the soil/kg determined through the Cobb's and decanting method followed by Baermann funnel extraction method (18).

2.11 Estimation of plant growth, physiological and biochemical parameters

During this experiment plants were grown in net house and terminated after 90 days Physiological and morphological parameters of tested plant like, plant fresh, plant dry weight, number of pods, shoot length, root length number of nodule were recorded in mature plants.

2.12 Determination of chlorophyll

1g finely cut fresh leaves, were taken and grinned with 20-40ml of 80% acetone. It was then centrifuge at 5000 – 10000 for 5 minutes. The supernatant was transferred and procedure was repeated till residue became colourless. The absorbance of the solution was red at 645nm and 663nm against the solvent (acetone blank). Total chlorophyll were calculated by using following equation (19) -

Total chlorophyll= 20.2 (A645) + 8.02 (A663) ×V/1000×W

Where,

A=absorbance at specific wavelength

V=final volume of chlorophyll and carotenoids extract 80% acetone

W=fresh weight of tissue extracted

Determination of total carotenoids

Carotenoids were determined by method of Duxbury and Yentsh (1956) (20), Macalacham and Zalik (1963) (21). 1g fresh leaf material was taken and homogenized with 80% acetone and centrifuged at 5000 rpm for 5 minutes. Supernatant was adjusted to 100ml in volumetric flask. The absorbance (O.D.) of extracted solution was measured at 480-510nm.

Carotenoid mg/g tissue = 7.6 (A480) – 1.49 (A510) ×V/1000×W

Where,

A=absorbance at specific wavelength

V=final volume carotenoids extract 80% acetone

W=fresh weight of tissue extracted

2.13 Estimation of Proline

Proline content was determined by following method of Bates et al., (22) in pigeon pea. Estimation of proline is based on the formation of a brick red coloured proline-ninhydrine complex in acidic medium. Plant sample was homogenized in 5ml of sulphonic acid (3%) by using a mortar pestle, the homogenate was filtered and filtrate was used for the estimation of proline content. 2ml of plant extract was taken in test tube and to it 2ml of glacial acetic acid and 2ml of in ninhydrin reagent were added and heated at 100^{o}C for 30 minutes. 6ml of toluene was added and then transferred to a separate and its absorbance at 520nm in a spectrophotometer against toluene blank.

2.14 Estimation of phenolic

Total phenolic content of extract was determined by the Folin-Ciocalteu method. 200µL of crude extract were made up to 3mL with distilled water, mixed thoroughly with 0.5mL of Folin-Ciocalteu reagent for 3 minute followed by addition of 2mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 minute. The total phenolic content was calculated from calibration curve and the results were expressed as mg of gallic acid equivalent per g dry weight (23).

3. STATISTICAL ANALYSIS

All data was reported as mean ± standard error of five replicates determined, and analyzed employing one-way analysis of variance (ANOVA) with significant differences between means determines at P≤0.05 and measured with Duncan's multiple range test using the statistical 14(SPSS).

4. RESULTS

Plant growth-promoting rhizobacteria (PGPR) are considered advantageous soil bacteria that can increase plant growth parameters. Interactive implementation of ZnO NPs and PGPRs could be a promising way to maintain crop production and development.

4.1. Interactive effect of ZnO NPs and PGPR on Plant growth parameters of pigeon pea infected with *M. incognita, F. udum* and *H. cajani*

Plant fresh weight, plant dry weight, number of pods, shoot length, root length, number of nodules, total chlorophyll, total carotenoid, proline, and phenolic content were greater in uninoculated control plants.

Different treatments of ZnO NPs (50µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL) and *Pseudomonas fluorescence strains* (IS-1, IS-2, IS-3, IS-4, and IS-5) increased the plant growth parameters (Plant fresh weight, plant dry weight, number of pods, shoot length, root length, number of nodule, total chlorophyll, total carotenoid, proline and phenolics).

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Biosynthesized ZnO NPs 50µg/mL + IS-1 at T3 increased the plant fresh weight 41%, plant dry weight 43%, number of pods 37%, shoot length 38%, root length 39%, number of nodule 68%, total chlorophyll 41%, total carotenoid 33%, proline 19% and phenolic 24% in comparison to inoculated plant (table 1). Combined application of ZnO NPs 150µg/mL + IS-3 at T5 enhanced the plant fresh weight 57%, plant dry weight 53%, number of pods 37%, shoot length 63%, root length42%, number of nodule 71%, total chlorophyll 37%, total carotenoid 38%, proline 15% and phenolic 41% as compared to inoculated control (Table 1). ZnO NPs 200µg/mL + IS-4 together at T6 increased plant fresh weight 60%, plant dry weight 25%, number of pods 44%, shoot length 25%, root length 35%, number of nodule 57%, total chlorophyll 53%, total carotenoid 51%, proline 22% and phenolic 51% as compared to inoculated control (Table 1). ZnO NPs 250µg/mL + IS-5 at T7 increased plant fresh weight 61%, plant dry weight 23%, number of pods 40%, shoot length 64%, root length 57%, number of nodule 74%, total chlorophyll 65%, total carotenoid 69%, proline 43% and phenolic 74% in comparison to inoculated control (Table 1). But ZnO NPs 100 µg/mL + IS-2 significantly increased plant fresh weight 69%, plant dry weight 63%, number of pods 55%, shoot length 68%, root length 57%, number of nodules 75%, total chlorophyll 84%, carotenoid 69%, proline 43% and phenolics 74% at ZnO NPs 100 µg/mL and IS-2 T4 (Table 2) in comparison to inoculated plant. T6, T5, T4 and T1 no significantly enhanced the plant growth.

Table 1. Effect of biosynthesized ZnO NPs and PGPRs against wilt disease complex of Pigeon Pea

Treatments	Plant fresh weight (g)	Plant dry weight (g)	Number of Pods	Shoot length (cm)	Root length (cm)	Number of Nodule
T1	$84.12^{a}\pm1.20$	$30.12^{a}\pm1.36$	$27.45^{a}\pm0.69$	$85.78^{a}\pm1.17$	$28.33^{a}\pm0.66$	$83.11^{a}\pm1.26$
T2	$25.33^{c}\pm1.33$	$9.30^{b}\pm0.41$	$9.99^{c}\pm1.17$	$26.33^{c}\pm2.14$	$10.14^{c}\pm0.66$	$20.16^{c}\pm0.50$
T3	$49.31^{b}\pm1.34$	$22.12^{ab}\pm3.41$	$19.12^{b}\pm0.83$	$59.74^{b}\pm1.83$	$21.96^{b}\pm1.07$	$77.00^{a}\pm0.69$
T4	$83.12^{a}\pm1.64$	$28.33^{a}\pm1.49$	$24.23^{ab}\pm0.69$	$84.96^{a}\pm2.00$	$26.78^{ab}\pm1.07$	$83.39^{a}\pm1.45$
T5	$73.96^{a}\pm1.45$	$25.96^{a}\pm2.28$	$19.32^{b}\pm2.33$	$80.96^{a}\pm0.50$	$22.47^{ab}\pm1.38$	$79.85^{a}\pm1.34$
T6	$76.78^{a}\pm5.92$	$24.78^{ab}\pm2.12$	$21.10^{ab}\pm1.34$	$63.78^{b}\pm4.19$	$20.96^{ab}\pm1.45$	$68.36^{b}\pm3.46$
T7	$77.12^{a}\pm7.19$	$23.12^{ab}\pm2.99$	$20.55^{ab}\pm0.69$	$81.96^{a}\pm0.88$	$26.33^{ab}\pm1.01$	$82.45^{a}\pm0.83$
LSD(P≤0.05)	**2.5**	**2.2**	**1.9**	**2.6**	**1.2**	**2.0**

T1= Control; T2 = *F udum+M incognita+H cajani;* T3=ZnO 50µg/mL+IS-1; T4=ZnO 100µg/mL +IS-2; T5=ZnO 150 µg/mL +IS-3 T6=ZnO 200 µg/mL +IS-4; T7=ZnO 250 µg/mL +IS-5

Table 2. Effect of biosynthesized ZnO NPs and PGPRs on total chlorophyll, carotenoids, proline and phenolic

Treatments	Total Chlorophyll (mg/g)	Total Carotenoid (mg/g)	Proline (µg/g)	Phenolic (µg/mL)
T1	$2.85^{a}\pm0.05$	$1.65^{a}\pm0.069$	$0.186^{a}\pm0.001$	$3.91^{a}\pm0.038$
T2	$0.44^{d}\pm0.44$	$0.52^{b}\pm0.096$	$0.104^{c}\pm0.067$	$0.69^{d}\pm0.170$
T3	$1.62^{c}\pm1.62$	$1.18^{c}\pm0.141$	$0.141^{b}\pm0.005$	$1.63^{c}\pm0.153$
T4	$2.85^{a}\pm2.85$	$1.63^{ab}\pm0.151$	$0.183^{a}\pm0.001$	$2.96^{ab}\pm0.018$
T5	$1.51^{c}\pm1.51$	$1.15^{bc}\pm0.086$	$0.133^{b}\pm0.003$	$3.01^{ab}\pm0.317$
T6	$1.96^{bc}\pm1.96$	$1.37^{ab}\pm0.060$	$0.145^{b}\pm0.001$	$2.71^{b}\pm0.164$
T7	$2.31^{ab}\pm2.31$	$1.66^{a}\pm0.105$	$0.185^{a}\pm0.001$	$3.62^{ab}\pm0.181$
LSD(P≤0.05)	**1.5**	**0.5**	**1.0**	**1.6**

T1= Control; T2 = *F udum+M incognita+H cajani;* T13=ZnO 50µg/mL+IS-1; T4=ZnO 100µg/mL +IS-2; T5=ZnO 150 µg/mL +IS-3 T6=ZnO 200 µg/mL +IS-4; T7=ZnO 250 µg/mL +IS-5

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4.2. Interactive effect of ZnO NPs and PGPR on *M. incognita, F. udum* and *H. cajani* on the development of wilt disease complex of pigeon pea (Pathogen related Parameters)

Different concentrations of ZnO NPs (50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL, and 250µg/mL) and *Pseudomonas fluorescens* strains T3 (IS-1), T4 (IS-2), T5 (IS-3), T6 (IS-4), and T7 (IS-5) were reduced the egg masses, galls/root system, cyst/root system, root-knot index, total nematode population and wilting index. Treatment of ZnO NPs 50µg/mL + IS-1 at T3 reduced the egg masses 31%, galls/root system 30%, cyst/root system 17%, root-knot index38%, total nematode population at J2/kg soil 44%, cyst/kg population 70% wilting index 34% and disease severity 30% in comparison to control (Table 3). Combined treatment of ZnO NPs 150µg/mL + IS-3 at T5 reduced the pathogens related parameters egg masses 56%, galls/root system 52%, cyst/root system 42%, root-knot index 24%, total nematode population at J2/kg soil 40%, cyst/kg population 66%, wilting index and disease severity 51% in comparison to control (Table 3). ZnO NPs 200µg/mL +IS-4 at T6 decreased the egg masses 68%, galls/root system 75%, cyst/root system 48%, root-knot index47%, total nematode population at J2/kg soil 56%, cyst/kg population wilting index 50% and disease severity 45% in comparison to control (Table 3). ZnO NPs 250µg/mL +Pf 611 at T7 reduced the egg masses 79%, galls/root system 72%, cyst/root system 75%, and root-knot index 74% total nematode population at J2/kg soil 63%, cyst/kg population 73%, wilting index 74% and disease severity 54% as compared to control (Table 3). But a significant reduction was found in ZnO NPs (100µg/mL) and IS-2 significantly reduced the egg masses by 75%, galls/root system by 78%, cyst/root system 79%, root knot index 91%, total nematode population at J2/kg soil 63%, cyst/kg population 73% and wilting index 80% in comparison to control T2 (Table 3). T5 and T6 non significantly reduced the pathogens growth.

Table 3. Effect of biosynthesized ZnO NPs, and PGPRs on the Pathogen related parameters

Treatments	Egg Masses	Galls/root system	Cyst/root system	Root knot index	Total nematode population		Wilting Index	Disease severity Percentage
					J2 population/kg soil	Cyst population /kg soil		
T1	$0^{e}\pm0$	$0^{f}\pm0$	$0^{c}\pm0$	$0^{c}\pm0$	$0^{d}\pm0$	$0^{c}\pm0$	$0^{c}\pm0$	$0^{c}\pm0$
T2	$96.45^{a}\pm1.83$	$182.01^{a}\pm2.50$	$375.78^{a}\pm6.00$	$4.00^{a}\pm00$	$6833.23^{a}\pm40.89$	$663.23^{a}\pm32.12$	$5^{a}\pm0.19$	$88.12^{a}\pm5.68$
T3	$73.23^{b}\pm3.46$	$151.32^{b}\pm3.46$	$311.12^{a}\pm8.38$	$2.45^{bc}\pm0.66$	$2013.63^{bc}\pm21.39$	$366.23^{b}\pm13.85$	$3.30^{ab}\pm0.76$	$61.36^{ab}\pm4.40$
T4	$50.12^{c}\pm4.40$	$126.96^{d}\pm1.92$	$217.09^{b}\pm8.41$	$2.6^{abc}\pm0.76$	$1279.12^{cd}\pm29.44$	$193.39^{bc}\pm23.36$	$1.01^{c}\pm0.19$	$36.70^{b}\pm12.01$
T5	$42.96^{c}\pm0.88$	$87.45^{d}\pm3.09$	$193.32^{b}\pm27.41$	$3.01^{ab}\pm0.38$	$2266.96^{cd}\pm57.44$	$396.10^{b}\pm42.66$	$2.23^{bc}\pm0.50$	$58.33^{ab}\pm11.65$
T6	$30.12^{cd}\pm6.00$	$45.96^{e}\pm2.88$	$93.44^{c}\pm16.77$	$2.12^{abc}\pm0.33$	$3350.78^{bc}\pm39.01$	$287.32^{bc}\pm27.82$	$2.12^{bc}\pm0.88$	$48.32^{b}\pm21.02$
T7	$20.96^{de}\pm6.00$	$47.12^{e}\pm3.59$	$81.10^{d}\pm9.17$	$1.01^{bc}\pm0.19$	$1817.45^{cd}\pm13.92$	$243.21^{b}\pm36.50$	$3.01^{ab}\pm0.69$	$40.78^{b}\pm10.13$
LSD(P≤0.05)	2.5	3.1	3.9	0.5	2.9	2.0	0.6	1.3

T1= Control; T2 = *F udum+M incognita+H cajani;* T3=ZnO 50µg/mL+IS-1; T4=ZnO 100µg/mL +IS-2; T5=ZnO 150 µg/mL +IS-3 T6=ZnO 200 µg/mL +IS-4; T7=ZnO 250 µg/mL +IS-5

5. DISCUSSION

Various methods have been used to protect the plant from the damage caused by pathogens. Different research has revealed that biosynthesized ZnO NPs have a great impact on disease suppression and plant growth promotion. Seed treated with ZnO NPs and PGPRs can promote different metabolic processes like germination rate and seedling vigor index in the seed (24). ZnO NPs have a great role in the enhancement of plant growth and productivity. The interactive effect of ZnO NPs and PGPRs on the wilt disease complex of pigeon pea is poorly understood. ZnO NP with *Pseudomonas* increased the plant height, grain weight, grain yield, and number of nodules per plant (25). The combined effect of ZnO NPs and *Pseudomonas fluorescens* at 3kg ha^{-1} concentration increased plant growth and yield in wheat (26). Nano-chitosan and *Pseudomonas fluorescens* reduced the wilt disease which is *Fusarium oxysporum* in tomatoes by increasing defense enzymes such as super oxidase dismutase, polyphenol oxidase, phenyl ammonia-lyase, and peroxidase (27). *Pseudomonas putida* and ZnO NPs at $0.01ml^{-1}$ increased the growth attribute of pea and inhibited the number of gall and nematode populations of *Meloidogyne incognita* (28). ZnO NPs with PGPRs at very low concentrations 20-40mgL^{-1} improved the plant metabolism, toxic radical scavenging, and protein content, and enhanced the essential nutrient elements (29). NPs have a great role in PGPRs like nitrogen fixation and production of secondary metabolite. According to Dimpka et al., (30) ZnO NPs trigger the siderophores and IAA in *Pseudomonas* spp. The combined effect of ZnO NPs and PGPRs increased the plant growth, grain weight, number of nodules, and number of pods per plant (31). ZnO NPs and PGPRs triggered the defense system of plants by increasing the primary metabolite and photosystem which increased the yield and growth of plants (32). *Pseudomonas* sp. strains enhance different reactions of plant and soil which convert insoluble Zn into available

form of Zn to enhance its uptake in plants for higher yield and effective biofortification (33). PGPRs have a great role in plants increasing the root system and biomass of host plants which functions as a gateway for better nutrient absorption for better plant performance and productivity (34). PGPRs are free-living microbes essential in plant development and sustaining soil fertility and metal oxide NPs enhance the soil microbiome (35). *Pseudomonas fluorescences* inhibit the growth of plant pathogenic fungi and plant parasitic nematodes by the production of hydrolytic enzymes such as β-1, 3-glucanase chitinase, siderophores, bacteriocins, antibiotics, induction of systemic resistance in plants and production of secondary metabolite (David et al., 2018). ZnO NPs inhibit the plant parasitic nematode and fungal pathogens at 100-200µg/mL and plant growth yield in *Vicia faba* (36). ZnO NPs inhibit the growth of phytopathogens by following the mechanism of destruction of the targeted antioxidant system through the generation of ROS (reactive oxygen species) and Zn^{+2} ions, prevention of DNA replication, enzymatic activity, and disruption of cellular structure. (37). According to Srivastav et al., (38) ZnO NPs 100mg/L increased antioxidant enzymes such as super oxidase dismutase, ascorbate peroxidase gulcial peroxidase, and catalase which protected the plant from pathogen attacks, enhanced photosynthetic pigments, root, shoot growth of maize and wheat and used as nanofertilizres (38). *Pseudomonas* sp. significantly increases plant growth and yield and is used in plant disease management (39). PGPRs exhibit different beneficial effects used in different forms such as biofertilizers, biopesticides, photostimulation, and bioremediation, and have a great role in sustainable agriculture and crop production. PGPRs developed induced systemic resistance mechanisms; and stimulated the plant defense mechanism by the production of defense metabolite (40). PGPRs enhanced micronutrient bioavailability, through the siderophore's production activity, and microbial mineral solubilization, improving the plant's nutrition and protecting the plant from adverse conditions. Very little information is available on the combined and interactive effect of ZnO NPs and PGPRs in plant protection. This study was carried out to investigate the interactive effect of PGPRs and biosynthesized ZnO NPs on the wilt disease complex of pigeon pea.

6. CONCLUSION

To reduce the employment of hazardous chemicals, efficient NPs can be synthesized from the plant extract which is used as nano-pesticides. Present study revealed that interactive effect of biosynthesized ZnO NPs and PGPRs against the *F udum*, *M incognita*, and *H cajani* which is the main cause of the wilt disease complex of pigeon pea and adversely affects the growth and yield of pigeon pea.

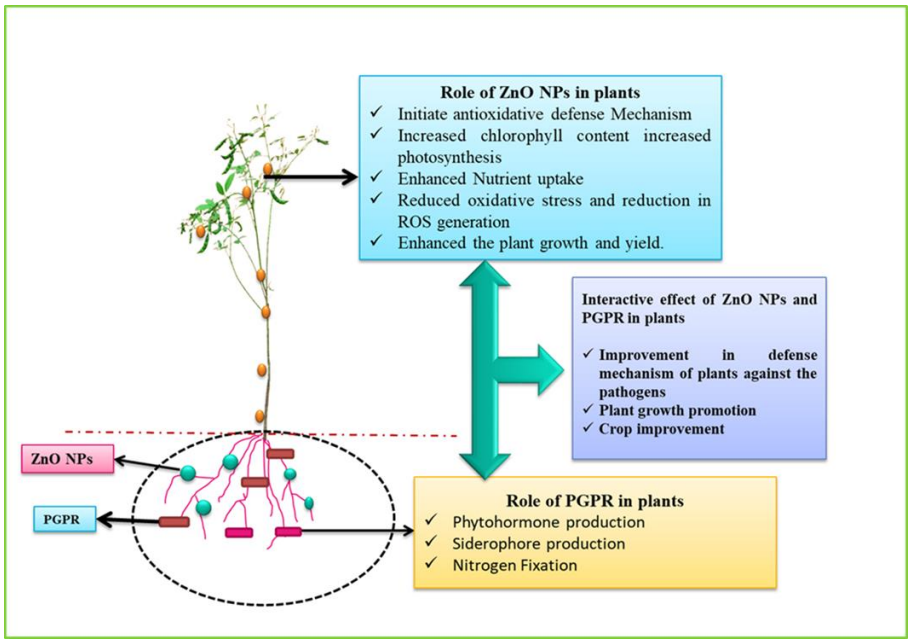


Fig. 1 Combined effect of ZnO NPs and PGPRs in plants

The biosynthesized ZnO NPs exhibit fungicidal and nematicidal activity impairing the membrane integrity and altering the morphology of plant pathogens. The combined effect of ZnO NPs 50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL**,** and 250µg/mL and Pf604, Pf605, Pf607, Pf608, and Pf611 improved the plant growth, number of nodules, total chlorophyll, total carotenoid, proline, phenolics, and reduced number of egg masses, number of galls/root system, cyst/root system, and

wilting index. Five strains of *Pseudomonas fluorescens* (Pf604, Pf605, Pf607, Pf608, and Pf611) and different doses of ZnO NPs (50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL**,** and 250µg/mL) employed in the management of wilt disease complex of pigeon pea. However at 100µg/mL ZnO NPs and Pf605 interactive effect significantly reduced the wilt disease complex of pigeon pea.

PGPRs and ZnO NPs enhanced the phytohormone, osmoregulation, and modification of the antioxidant enzyme. The use of PGPR in the management of the wilt disease complex of pigeon pea is an environmentally friendly strategy, as they improved the metabolite, morphological, defense system, and physiological profile in plants. Therefore combined effect of PGPRs and ZnO NPs can be used in fungicides and nematicides in agriculture production against phytopathogens.

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