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AKADÉMIAI KIADÓ

Effects of *Pseudomonas putida* and zinc oxide nanoparticles (ZnO NPs) with *Rhizobium leguminosarum* on the management of *Meloidogyne incognita* and *Pseudomonas syringae* pv. *pisi* on pea

Deeksha Kashyap and Zaki Anwar Siddiqui*

Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh-202002, India

RESEARCH ARTICLE

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ABSTRACT

Effects of *Pseudomonas putida* and zinc oxide nanoparticles (ZnO NPs) alone and in combination was observed in plants grown with bacterized seeds with *Rhizobium leguminosarum* for the management of *Meloidogyne incognita* and *Pseudomonas syringae* pv. *pisi* on pea (*Pisum sativum*). Inoculation of *M. incognita* and *P. syringae* pv. *pisi* alone and both together reduced plant growth, chlorophyll and carotenoid content over un-inoculated control. Use of *P. putida* and ZnO NPs 0.10 ml^{-1} (foliar spray/seed priming) alone and in combination resulted in a significant increase in plant growth, chlorophyll, and carotenoid in pathogen-inoculated plants. Seed priming with ZnO NPs was better than NPs foliar spray in increasing plant growth, chlorophyll and carotenoid content of plants with pathogens. Use of *P. putida* plus NPs seed priming was better than its use with foliar spray in increasing plant growth, chlorophyll, and carotenoid. Bacterization with *R. leguminosarum* caused sufficient root nodulation and nodulation was better in plants with *P. putida* than in plants with ZnO NPs. Both test pathogens had adverse effect on root nodulation. Blight disease indices, galling, and nematode population were also greatly reduced when *P. putida* was used with ZnO NPs seed priming.

* Corresponding author. E-mail: zasiddiqui.bt@amu.ac.in

KEYWORDS

bacteria, nematode, photosynthetic pigments, *Pisum sativum*, root nodule bacterium

INTRODUCTION

Pea (*Pisum sativum*) is grown as cool season crop, reaches the maturity after 100 days of sowing (Oelke et al., 1991). Its cultivated species are highly nutritious and have 18–30% protein, 35–50% starch and 4–7% fiber and soluble sugars (5%) (Bastianelli et al., 1998).

Bacterial blight is an economically important disease of pea caused by *Pseudomonas syringae* pv. *pisi*. This bacterium causes yield losses up to 70% (Richardson and Hollaway, 2011) and its infection causes water-soaked spots which later coalesce into larger lesions (Martín-Sanz et al., 2013). Similarly, root-knot nematode *Meloidogyne incognita* parasitizes pea (Bozbuga et al., 2018) and is a serious pathogen causes 20–33% yield losses (Sharma et al., 2006).

Plant growth promoting rhizobacteria (PGPR) are vigorous colonizers of the plant root system in almost all ecological niches (Antoun and Kloepper, 2001). *Pseudomonas putida* (Trevisan) Migula is one of the most potential rhizobacterium for biological control of plant diseases (Pastor et al., 2016). Similarly, *Rhizobium* Frank is generally used by farmers to increase production of their crops as it is known to fix atmospheric nitrogen and to reduce the damage caused by pathogens (Volpiano et al., 2019).

Nanotechnology has a major impact on agriculture. Use of nanoparticles (NPs) is an important way for plant disease management (Nayana et al., 2020). Similarly, rhizobacterium with NPs may provide effective management of plant diseases. Designing a conjugative approach of NPs and PGPR may increase plant yield and manage plant diseases (Nayana et al., 2020).

The effect *P. putida* (Trevisan) Migula and ZnO NPs (seed priming/foliar spray) alone and in combination was observed in the presence of *Rhizobium leguminosarum* Frank on bacterial blight disease complex of pea caused by *M. incognita* (Kofoid and White) Chitwood and *P. syringae* pv. *pisi* (Sackett) Young, Dye & Wilkie. Effect of *P. putida* and ZnO NPs in the presence *R. leguminosarum* was also observed on plant growth, chlorophyll, carotenoid contents on pea.

MATERIAL AND METHODS

Preparation of zinc oxide nanoparticle suspension

Zinc oxide nanoparticles (ZnO NPs) was obtained from Sigma-Aldrich (Product No. 721077-100G) (average particle size of 40 nm). ZnO NPs is a solution of 20% (Sigma Aldrich) i.e. 20 ml in 100 ml solution. Therefore, after dilution of 0.50 ml in 1 L double distilled water, ZnO NPs solution became 0.01% (i.e. 0.10 ml^{-1}). Ten ml suspension of ZnO NPs was used as foliar spray per pot/per plant. Seed priming with NPs was done as described below.

Effect of ZnO NPs on hatching and mortality of *M. incognita*

The effect of ZnO NPs (0.10 ml^{-1}) were observed on the hatching and mortality of *M. incognita* in Petri plates at 25 ± 1 °C as described (Kashyap and Siddiqui, 2021).

Effect of ZnO NPs on *P. syringae* pv. *pisi*

Pseudomonas syringae pv. *pisi* was inoculated aseptically on the nutrient agar (NA) plates and paper discs of 0.7 mm diameter dipped in 0.10 ml^{-1} ZnO NPs were placed in NA plates separately. The plates were incubated at 30 ± 2 °C and replicated five times. Inhibition zone was measured on plates after 48 h.

Preparations of *M. incognita* juveniles (J_2s) for scanning electron microscopy (SEM)

Meloidogyne incognita J_2s were treated with 0.10 ml^{-1} ZnO NPs suspensions/DDW. These J_2s were prepared for SEM, according to Eisenback (1986). Juveniles were picked into 1.5 mL of water in the Eppendorf tube, fixed with glutaraldehyde at 4 °C for 12 h. The fixed J_2s were dehydrated gradually using a seven-grade ethanol series (10, 20, 40, 60, 80, 90, and 100%), 15 min on each step. The dried J_2s were mounted on SEM stubs, coated with gold, and finally examined through SEM (JEOL, Tokyo, Japan).

Preparation of *P. syringae* pv. *pisi* samples for SEM

Suspension of *P. syringae* pv. *pisi* was mixed with 0.10 ml^{-1} ZnO NPs, incubated at 30 °C for 5 h. The samples were centrifuged at 5,000 rpm for 15 min at 4 °C. The pellets were washed thrice with potassium phosphate buffer (PBS) at pH 7 and prepared as described (Kashyap and Siddiqui, 2021).

Preparation and sterilization of the soil mixture

The loam soil used in the experiment was obtained from agricultural field of A.M.U. Aligarh. A 3:1 (v/v) ratio of soil and organic manure were mixed, and was sieved through 2 mm sieve. Soil was autoclaved for 20 min at 137.9 kPa. One kg of this soil was used to fill 15-cm diameter earthen pots after cooling.

Seeds priming, sowing and maintenance of test plants

Pea sterilized seeds (cv. BK-10) were soaked for 12 h in a 0.10 ml^{-1} suspension of ZnO NPs. Five unprimed and NPs primed seeds were sown separately in 15 cm earthen pots containing 1 kg of sterilized soil.

Preparation of *M. incognita* inoculum

Meloidogyne incognita was multiplied using a single egg mass on *Solanum melongena* L. roots as described (Kashyap and Siddiqui, 2021). The hatched J_2 were collected from the Petri plates after every 24 h and fresh water was added to repeat the process. For counting of J_2, an average of 5 counts was made and volume of nematode suspension was so adjusted that each ml may contain 200 ± 5 J_2. Ten ml of this suspension containing 2000 J_2s of *M. incognita* were applied to each pot around pea seedling.

Preparation of *P. syringae* pv. pisi inoculum

Pseudomonas syringae pv. *pisi* colonies were streaked under aseptic conditions on sterilized petri dishes with NA medium and incubated (Kashyap and Siddiqui, 2021). Cell density of *P. syringae* pv. *pisi* was calculated by Sharma (2001) and each ml contains 1.2×10^5 colony-forming units (CFU).

Preparation of *P. putida* inoculum

NA plates were incubated overnight at 30 °C to check sterility and remove excess moisture. Fresh culture of *P. putida* was inoculated using a single colony into nutrient broth flasks. These flasks were incubated at 30 ± 1 °C for 72 h to get the mass inoculum of *P. putida*. One ml of nutrient broth suspension contains approximately 1.2×10^5 CFU.

Preparation of *R. leguminosarum* inoculum

Charcoal culture of *R. leguminosarum* (pea strain, 100 g) was dissolved in 1 L sterilized distilled water. Each pea seedling/pot was inoculated with 10 ml suspension containing 1 g inoculum.

Inoculation technique

For inoculations of *M. incognita, P. syringae* pv. *pisi*, *P. putida* and *R. leguminosarum* sterilized forceps were used to gently remove soil around the roots. The inocula suspensions were poured around each seedling and soil was placed again. Same amount of distilled water was poured around the seedling in control. Seed priming and foliar spray of ZnO NPs was done as stated above.

Experimental design

The experiment was performed in a Completely Randomized Design (CRD) with all seedling bacterized with *R. leguminosarum* as stated above. ZnO NPs was used as seed priming, foliar spray and control i.e. A. Control; B. Seed priming; C. Foliar spray. These 3 treatments were tested both in the presence and absence of *P. putida* i.e. 3 × 2 = 6 treatments. These 6 treatments were tested with pathogens and a control i.e. 1. Control; 2. *M. incognita*; 3. *P. syringae* pv. *pisi*; 4. *M. incognita* plus *P. syringae* pv. *pisi* i.e. 6 × 4 = 24 treatments. Each treatment was replicated 5 times i.e., 24 × 5 = 120 pots.

Data collection

Ninety days after pathogen inoculation harvesting was done. Observations data were recorded on plant length, plant fresh weight, plant dry weight, chlorophyll, carotenoid, bacterial blight index, galling and population of *M. incognita* as described (Kashyap and Siddiqui, 2021).

Chlorophyll and carotenoid estimation

Chlorophyll and carotenoid were estimated Mackinney (1941) and Maclachlan and Zalik (1963).

Disease index

Disease indices were observed on the basis of symptoms on the leaves (Nesha and Siddiqui, 2013). The disease indices were recorded on 0 to 5 scale, where 0 = No disease and 5 = Severe blight symptoms.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) (*P. putida* × ZnO NPs × Pathogens) using R (3.6.1) statistical software (package library, agricolae). For comparison among means,

Fisher's protected least significant difference (LSD) test were performed ($P \leq 0.05$). Graphs of nematode population and number of galls/per root system were prepared using Sigma Plot™ and error bars represent standard error. The principal components analysis (PCA) was used to determine the total variability of data using Origin Pro 2021.

RESULTS

In vitro study

ZnO NPs 0.10 ml^{-1} had an inhibitory effect on growth of *P. syringae* pv. *pisi* in NA medium and form inhibition zones of 0.64 mm (Table 1). Similarly, ZnO NPs 0.10 ml^{-1} caused 84.7% reduction in the hatching of *M. incognita* J_2 while the same concentration caused 74.1% mortality of *M. incognita* of J_2 (Table 1). The ZnO NPs-treated J_2s showed irregular cuticle, shrunken, wrinkled, and corrugated cuticle (Fig. 1). The *P. syringae* pv. *pisi* cells were damaged, misshapen and ridged after the treatment with ZnO NPs (Fig. 2).

Pot experiment

Three-ways ANOVA showed that the individual effects of ZnO NPs, *P. putida* and pathogens on plant length, plant fresh weight, shoot dry weight, root dry weight, nodulation, chlorophyll, carotenoid, root galling and nematode population was significant at $P \leq 0.05$ (ANOVA not shown). Interaction of NPs × *P. putida*, and NPs × Pathogens on plant length, plant fresh weigh was significant at $P \leq 0.05$. Interaction of NPs × pathogens on root dry weight, chlorophyll, root galling and nematode population was also significant at $P \leq 0.05$. Interaction of *P. putida* × Pathogens on plant fresh weight was also significant at $P \leq 0.05$ (ANOVA not shown).

Plants inoculated with *M. incognita* and *P. syringae* pv. *pisi* individually and in combination showed a significant reduction in plant growth parameters, chlorophyll and carotenoid over control (Table 2). Root nodulation was high in all plants bacterized with *R. leguminosarum*. Pathogens alone and together caused a greater reduction in nodulation. Plant growth, nodulation, chlorophyll and carotenoid were greater in plants with *P. putida* over uninoculated control. Seed priming had adverse effect on nodulation while foliar spray had no significant effect on

Table 1. Effects of ZnO NPs on the hatching and mortality of *M. incognita* and inhibition zone formation in culture medium against *P. syringae* pv. *pisi*

Treatments	No. of hatched *M. incognita* J_2 after 48 h	% reduction in the hatching of J_2 over Control	No. of *M. incognita* J_2 died after 48 h	% mortality of J_2 over control
Double distilled water	216a	-	07b	-
ZnO NPs 0.10 ml^{-1}	33 b	84.7	27a	74.1
	Treatments Inhibition zone formation against *P. syringae* pv. *pisi*			
ZnO NPs 0.10 ml^{-1}	0.64 (mm)			

Data are present as treatments mean. Values with in a column followed by different letters are statistically significant at $P \leq 0.05$ by Tukey's HSD post hoc test.

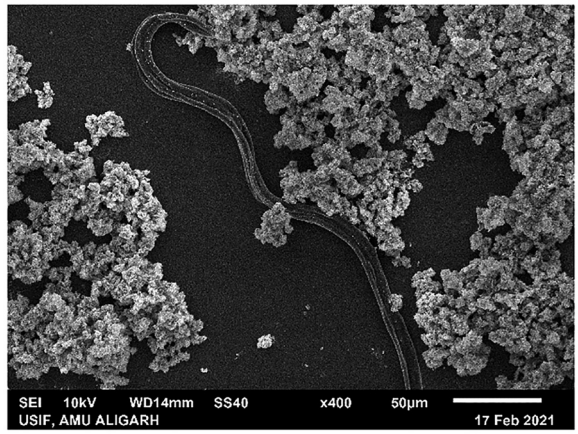

Fig. 1. Effect of ZnO NPs on second stage juvenile of *Meloidogyne incognita*

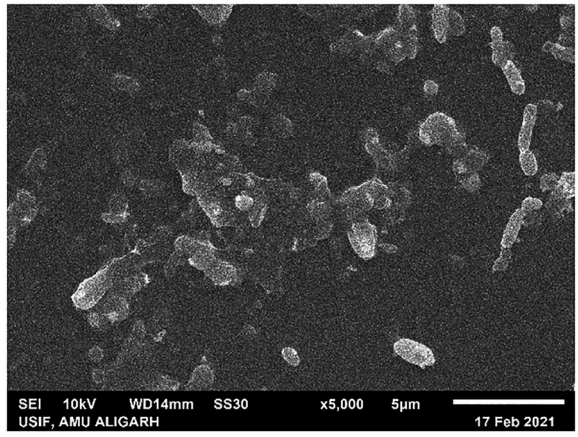

Fig. 2. Effect of ZnO NPs on the cells of *Pseudomonas syringae* pv. *pisi*

nodulation over control. ZnO NPs foliar spray/seed priming caused an increase in plant growth, chlorophyll and carotenoid over control (Table 2).

Effect on shoot dry weight

Foliar spray and seed priming caused 7.32 and 20.33% increase in shoot dry weight (% data not shown in table) over control (Table 3) while foliar spray and seed priming resulted in 8.29 and 19.02% increase in shoot dry weight over plants with *M. incognita*. However, 6.39 and 16.44% increase in shoot dry weight was observed over plants with *P. syringae* pv. *pisi* by foliar spray and seed priming. Foliar spray and seed priming caused 9.52 and 13.49% increase in shoot dry weight over plants with *M. incognita* plus *P. syringae* pv. *pisi*. Application of *P. putida* to plants

Table 2. Influence of ZnO NPs and *P. putida* alone and in combination on the growth attributes, nodulation, chlorophyll and carotenoid of pea in the presence of *Rhizobium leguminosarum*

Treatments		Plant length (cm)	Plant fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	No. of Nodules per root system	Chlorophyll (mg g^{-1} fresh weight)	Carotenoid (mg g^{-1} fresh weight)
Pathogens	C	86.20a	11.60a	2.81a	0.23a	41.00a	0.830a	0.104a
	M	72.13c	8.37c	2.36c	0.14c	23.33c	0.732c	0.075b
	P	74.88b	8.83b	2.45b	0.17b	28.17b	0.742b	0.079b
	M+P	43.55d	5.20d	1.45d	0.13d	20.67d	0.484d	0.052c
L.S.D. *P* = 0.05		**1.13**	**0.09**	**0.09**	**0.008**	**0.66**	**0.005**	**0.004**
P. putida	Control	67.82b	8.23b	2.16b	0.16b	26.25b	0.672b	0.075b
	P. putida	70.56a	8.77a	2.38a	0.18a	30.33a	0.722a	0.080a
L.S.D. *P* = 0.05		**0.79**	**0.07**	**0.07**	**0.006**	**0.47**	**0.004**	**0.003**
ZnO NPs	Control	63.17c	7.77c	2.08c	0.15c	28.75a	0.638c	0.070c
	Foliar spray	69.54b	8.50b	2.29b	0.17b	28.25ab	0.696b	0.077b
	Seed priming	74.87a	9.23a	2.43a	0.18a	27.88b	0.757a	0.086a
L.S.D. *P* = 0.05		**0.97**	**0.08**	**0.08**	**0.007**	**0.57**	**0.005**	**0.003**

*Data analysis through Least Square mean (LSM). * The mean values within a column followed by the different letter are significantly different at $P = 0.05$ by Tukey's HSD post hoc test; C = Control; M = *M. incognita*; P = *P. syringae* pv. *pisi*.

with *M. incognita, P. syringae* pv. *pisi*, or both caused 11.22, 8.21, and 9.52% increase in shoot dry weight over respective control. Use of *P. putida* plus NPs foliar spray resulted in 24.39, 17.80 and 26.98% increase in shoot dry weight of plants with *M. incognita, P. syringae* pv. *pisi*, or both respectively. Seed priming plus *P. putida* resulted in a 28.78, 22.37 and 30.95% increase in shoot dry weight compared to plants with *M. incognita, P. syringae* pv. *pisi*, or both respectively (Table 3).

Effect on chlorophyll and carotenoid

Foliar spray to plants with *M. incognita, P. syringae* pv. *pisi* and both increased chlorophyll by 6.50–10.48%, and carotenoid by 9.09%–10.77% over pathogen (% data not shown in table) inoculated control (Table 3). NPs seed priming resulted in 14.39%–20.49% increase in chlorophyll and 21.54–29.55% increase in carotenoid of plants with *M. incognita, P. syringae* pv. *pisi* and both. Inoculation of *P. putida* resulted in 7.64–11.60% increases in chlorophyll and 7.25–11.36% increases in carotenoid content over plants with *M. incognita, P. syringae* pv. *pisi* or both. Use of *P. putida* with foliar spray to plants with *M. incognita, P. syringae* pv. *pisi* or both resulted in 16.24–18.80% increase in chlorophyll, and 18.84–20.45% increase in carotenoid content. Use of *P. putida* plus seed priming resulted in 25.06–28.55, % increase in chlorophyll and 30.43–40.91% in carotenoid in plants with *M. incognita, P. syringae* pv. *pisi,* or both (Table 3).

Table 3. Effect of ZnO NPs and *P. putida* alone and in combination on the growth attributes and nodulation of pea infected with *M. incognita*, *P. syringae* pv. *pisi* or both in the presence of *R. leguminosarum*

P. putida	ZnO NPs	Pathogens	Plant length (cm)	Plant fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	No. of nodules/root system	Chlorophyll (mg g^{-1} fresh weight)	Carotenoids (mg g^{-1} fresh weight)	Blight index
Nil	Control	C	77.06f	10.27f	2.46bcdef	0.19de	40.0c	0.734h	0.091def	-
		M	64.18i	7.16l	2.05g	0.12h	22.0gh	0.641k	0.065lmn	-
		P	66.96h	7.84k	2.19fg	0.14gh	26.0e	0.649k	0.069klm	3
		M+P	38.18m	4.56o	1.26j	0.09i	18.0i	0.431p	0.044q	4
	Foliar	C	85.15c	11.20d	2.64bc	0.22bc	41.0bc	0.804e	0.101bcd	-
	Spray	M	69.96h	8.14j	2.22fg	0.14gh	22.0gh	0.705ij	0.072ijklm	-
		P	73.32g	8.68i	2.33def	0.16fg	25.0ef	0.717i	0.076hijkl	2
		M+P	41.37l	4.77o	1.38ij	0.12h	17.0i	0.459o	0.048pq	3
	Seed	C	94.06a	12.42b	2.96a	0.25a	40.0c	0.879b	0.110ab	-
	priming	M	77.40ef	8.93i	2.44bcdef	0.14gh	21.0h	0.771fg	0.079fghijk	-
		P	80.47d	9.44gh	2.55bcde	0.17ef	25.0ef	0.782f	0.084fghi	2
		M+P	45.76k	5.38n	1.43hij	0.14gh	18.0i	0.493mn	0.057nop	3
P. putida	control	C	80.23de	10.93e	2.66b	0.21cd	43.0a	0.779f	0.096cde	-
		M	67.58h	7.86k	2.28efg	0.14gh	26.0e	0.690j	0.070jklm	-
		P	69.85h	8.31j	2.37cdef	0.16fg	31.0d	0.701j	0.074hijklm	2
		M+P	41.28l	5.21n	1.38ij	0.12h	24.0fg	0.481n	0.049pq	3
	Foliar	C	89.47b	11.91c	3.02a	0.24ab	42.0ab	0.851c	0.107abc	-
	Spray	M	74.01g	8.78i	2.55bcde	0.16fg	25.0ef	0.761g	0.078ghijk	-
		P	77.34ef	9.22h	2.58bcd	0.18ef	31.0d	0.771fg	0.082fghij	1
		M+P	45.68k	5.31n	1.60hi	0.14gh	23.0g	0.501m	0.053opq	3
	Seed	C	91.25b	12.85a	3.11a	0.26a	40.0c	0.933a	0.116a	-
	priming	M	79.65def	9.37gh	2.64bc	0.16fg	24.0fg	0.824d	0.086efgh	-
		P	81.36d	9.49g	2.68b	0.19de	31.0d	0.832d	0.090defg	1
		M+P	49.00j	5.97m	1.65h	0.16fg	24.0fg	0.539l	0.062mno	2
L.S.D. $P = 0.05$			**2.77**	**0.24**	**0.24**	**0.02**	**1.63**	**0.014**	**0.010**	-

*Data are presented as treatments mean ($n = 5$). The mean values within a column followed by the different letter are significantly different at $P = 0.05$ by Tukey's HSD post hoc test; C = Control; M = *M. incognita*; P = *P. syringae* pv. *pisi*.

Effect on root galling and nematode population

High root galling and nematode population was observed when *M. incognita* was inoculated alone (Figs 3 and 4). Root galling and nematode population was reduced in the presence of *P. syringae* pv. *pisi*. Foliar spray and seed priming of ZnO NPs and inoculation of *P. putida* reduced galling and nematode population. Seed priming caused a greater reduction in galling and nematode populations than foliar spray. Seed priming plus *P. putida* resulted in the greatest reduction in galling and nematode population (Figs 3 and 4).

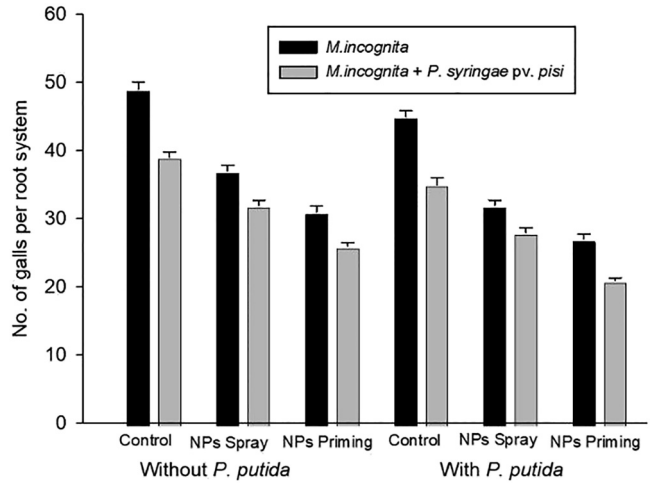

Fig. 3. Effect of ZnO NPs foliar spray and seed priming with and without *P. putida* on the galling of *M. incognita* on pea

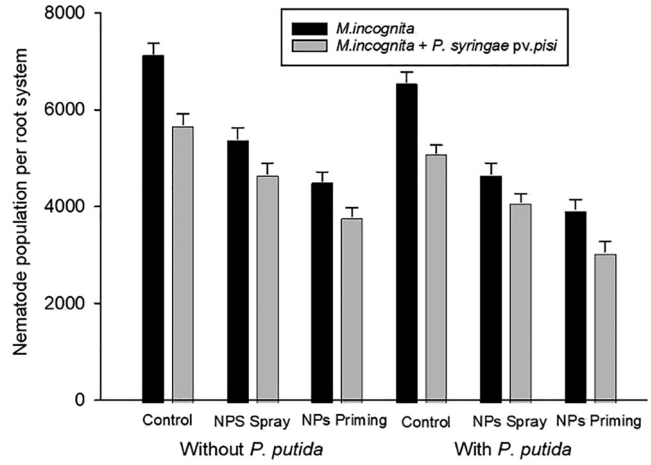

Fig. 4. Effect of ZnO NPs foliar spray and seed priming with and without *P. putida* on the popupation of *M. incognita* on pea

Disease indices

Bacterial blight indices were 3 and 4 respectively when *P. syringae* pv. *pisi* was inoculated alone and in combination with *M. incognita* (Table 3). Disease indices were reduced by the application of NPs and *P. putida*. Maximum reduction in disease indices was observed when NPs seed priming was used with *P. putida* (Table 3).

Principal component analysis (PCA)

Total 96.42% (PC1 = 87.55%; PC2 = 8.87%) variability of the data was demonstrated in PCA (Fig. 5). Blight disease indices, nematode population and galling were negatively correlated with plant growth attributes, nodulation, chlorophyll and chlorophyll content. The segregation of various treatments in the biplot demonstrated the disease suppressive role of *P. putida* and ZnO NPs on pea. Use of *P. putida* plus NPs seed priming was best followed by *P. putida* plus foliar spray and *P. putida* alone in reducing disease indices, nematode population and galling (Fig. 5).

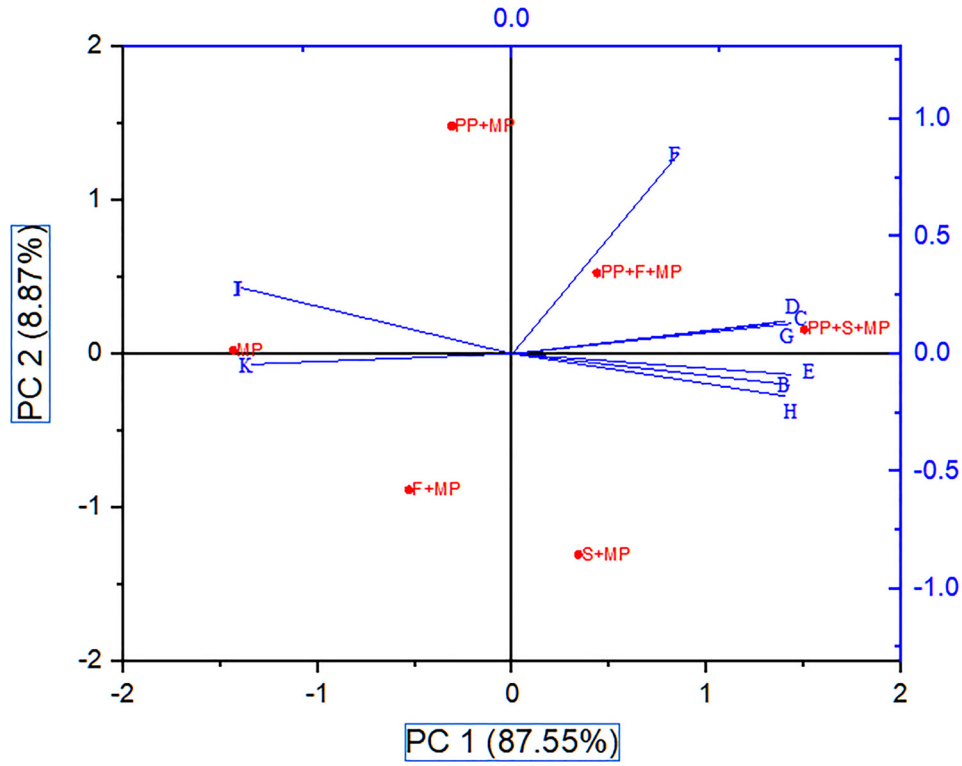

Fig. 5. Principal component analysis (PCA) showing the effect of ZnO NPs and *P. putida* alone and in combination on various studied attributes of pea infected with *M. incognita, P. syringae* and both. M = *M. incognita*, P = *P. syringae,* and MP, PP = *P. putida*, F = Foliar spray, S = Seed priming, B = Plant length, C = Plant fresh weight, D = Shoot dry weight, E = Root dry weight, F = Nodulation, G = chlorophyll, H = Carotenoid, I = Nematode population, J = Nematode galling, K = Disease index

DISCUSSION

Pseudomonas spp. possess traits which provide biocontrol and growth-promotion (Hernández-León et al., 2015). Certain bioactive metabolites are produced by *P. putida* which may attribute as successful biocontrol agent (Raza et al., 2016). An inhibition zone was formed around *P. putida* and bacterial blight bacterium could not grow around *P. putida* colonies in present study.

Pseudomonas species are ubiquitous in agricultural soils and act as a multifunctional biocontrol possession against phytopathogens (Ray and Patel, 2022). Application of *P. putida* reduced galling and nematode population and also produce siderophores (Siddiqui et al., 2007). The *P. putida* showed good inhibition of *M. incognita in vitro* and in pot experiments, with biocontrol efficiency as high as 71.67%. In addition, *P. putida* could induce systematic resistance in tomato by increasing the activity of defense enzymes (Tang et al., 2014). Hydrogen cyanide and cyclo (L-Pro-L-Ile) also were identified from *P. putida* strain and exhibited nematicidal activity against *M. incognita* (Guo et al., 2016).

Nutrient agar medium with ZnO NPs has shown antimicrobial activity in the present study against *P. syringae* pv. *pisi* as reported earlier (Zhang et al., 2007; Raffi et al., 2008). Toxicity of ZnO NPs results in microbial cell membrane damage that leads to entry in to the cytoplasm and their accumulation (Zhang et al., 2007). SEM study revealed bacterial cells were damaged and misshapen due to adherence to ZnO NPs. Sirelkhatim et al. (2015) have examined the influence of ZnO NPs on bacterial cell morphology while deformed, ruptured and damage cell wall was also reported (Akbar and Anal, 2014). ZnO NPs damage the cell membrane and DNA (Huang et al., 2008) while integration of NPs with bacteria induce changes in membrane permeability and cell lysis (Brayner et al., 2006; Zhang and Chen, 2009).

ZnO NPs had adverse effects on eggs and J_2s of *M. incognita* and reduced hatching of J_2s. It also caused increase in the mortality of J_2s. SEM revealed that eggs and J_{2s} were deformed, shrunken and wrinkled with corrugated cuticle. Metal ions generally inhibit the activity of important enzymes or disrupt the integrity of the cell membrane (Gupta et al., 2015). Small size, large surface and ability of NPs to generate reactive oxygen species induce toxicity (Oberdoster et al., 2005) and DNA synthesis and repair is also impaired by ZnO NPs (Hou et al., 2018).

Zn play a role in the structural stability of cell membrane therefore use of ZnO NPs improved plant growth, chlorophyll, and carotenoid content (Welch et al., 1982). It also plays significant role in protein synthesis, membrane structure, cell elongation, and tolerance (Cakmak, 2000). ZnO NPs penetrate leaf surface and releasing ions around the cuticle as compared to water soluble ions (Da Silva et al., 2006). Nano size and lower hydrophilic ability of ZnO NPs is responsible for better bioavailability and higher yield (Prasad et al., 2012), therefore, improve plant growth attributes, chlorophyll, and carotenoid also reduce disease severity of *M. incognita* and *P. syringae* pv. *pisi* on pea.

NPs seed priming has a beneficial impact in different crops under stress (Hussain et al., 2019) and increases nutrients and water absorption efficiency by increasing NPs penetration through the seed cover (Farooq et al., 2006). NPs seed priming is more significant than foliar use (Rizwan et al., 2019) because seed priming alters the embryo's metabolic and physiological nature, as well as the material released during germination (Waqas et al., 2019). NPs also induce enzyme activation, germination inhibitor metabolism, and cell damage repair (Dutta, 2018).

Therefore, NPs seed priming with *P. putida* caused greater reduction in galling, nematode population and disease indices than use of *P. putida* with NPs foliar spray.

PCA demonstrated 96.42% variation of the data which is according to Sneath and Sokal (1973) criteria established that there should be at least 70% of total data variability. Positive correlations were observed between plant growth, nodulation, chlorophyll and carotenoid content while nematode population, root galling, and disease indices were negatively correlated.

CONCLUSIONS

Greater efficacy of *P. putida* plus ZnO NPs seed priming was demonstrated by PCA in reducing disease severity of pea. Therefore, *P. putida* plus ZnO NPs seed priming may be used for sustainable management of *M. incognita* and *P. syringae* pv. *pisi* of pea by boosting site-specific uptake of NPs into the target pathogens.

Conflicts of interest: The authors have no financial and non-financial competing interests.

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