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Biochemical Analysis Based on Zinc Uptake of Chickpea (*Cicer arietinum* L.) Varieties Infected by *Meloidogyne incognita*

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Authors' contributions

This work was carried out in collaboration among all authors. Author PP designed the experiment and wrote the manuscript. Author MM checked the grammatical errors. Author DKN checked the overall manuscript and approved the manuscript for submission. All authors read and approved the final manuscript.

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ABSTRACT

The significant constraints in Chickpea (*Cicer arietinum* L.) production hampers a bit more than 14% global yield loss due to plant-parasitic nematodes. Root-knot nematode (*Meloidogyne* sp.) is an endoparasite and a significant species affecting the chickpea plant. So, the chemical basis of management is more cost-effective, and pest resurgence building is enhanced in the pathogen. So, ecological-based nematode management is requisite, which also is got hampered due to breeding for resistance against such plant-parasitic nematodes. This was the primary reason to conduct this experiment to enhance resistance in the chickpea plants based on Zinc uptake by using bioagent, *Pseudomonas fluorescens* alone or in combination. where Different treatments including nematode, bacterium, and chemicals were used sustaining the enhancement of disease resistance in chickpea cultivars, RSG 974, GG 5, GNG 2144. Zinc content of chickpea variety GNG 2144 was found the highest in treatment, when only bacterium (*P. fluorescens*) was inoculated, i.e., 3.14 mg/100g of root followed by GG 5, i.e., 2.79 mg/100g of root and RSG 974 was, i.e., 2.35 mg/100g of root respectively in a descending order. Application of *P. fluorescence* combined or alone gradually increased the Zn concentration in roots of chickpea plants compared to healthy check followed by chemical treated plants.

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1. INTRODUCTION

Root-knot nematode (*Meloidogyne* sp.) is an endoparasite. The infective second-stage juvenile readily penetrates the plant roots near the apical meristem within 24 h after inoculation [1]; however, other regions of the roots are not immune to attack [2]. The mature females, which develop in the root tissue, produce conspicuous galls in the infected zone of the roots. The formation of giant cells through the dissolution of cell walls and coalescing of their contents was reported for root-knot infection of *Nicotiana* hybrids [3]. The authors gave further support to this process of the syncytial gall formation, while studying root-knot infections on tomato [2]. Similar evidence for cell wall dissolution has been reported for many hosts infected by *Meloidogyne* species [4,5,6, and 7]. Scientists, also, surveyed 30 soil samples from medicinal plants and found *M. incognita* in all the samples [8]. Furthermore, an inverse relationship was observed between *M. incognita* and the growth of *Ocimum sanctum* [9]. The effects of *M. incognita* on different plants were observed by several workers, e.g., on *Manihot esculenta* by [10], on tomato by [11], on resistant cotton genotypes by [12], on olive explants by [13], and on sunflower by [14], fruit crops by [15] and [16], pulse crops by [17], and chickpea by [18]. Chickpea (*Cicer arietinum* L.) is an important pulse crop with an annual production of 11.5 million tonnes worldwide [19,20]. However, Its yield of chickpea tends to be low and unstable, with a world average yield of 850 kg/ha [20], well below the estimated yield potential of 4,000 kg/ha [21,22].

India is the world's largest consumer of chickpea and the world's largest producer, contributing over 70% of total global chickpea production [23]. However, there is a significant decrease in the production of chickpea is occurred due to plant-parasitic nematodes. Plant-parasitic nematodes constrain chickpea (*Cicer arietinum*) production, with annual yield losses estimated to be 14% [24] of total global production. Nematode species causing significant economic damage in chickpea include root-knot nematodes (*Meloidogyne artiella*, *M. incognita*, and *M. javanica*) [25]. For the management biocontrolling purpose to against root-knot nematode problem in this present study, we applied used biocontrol agent, *Pseudomonas fluorescens,* as it has a potential antagonistic nature to control plant diseases [26]. Field trials in *Vigna mungo* to control root rot disease complex caused by *Macrophomina phaseolina* and cyst nematode, *Heterodera cajani* were carried out at Coimbatore. *P. fluorescens* was applied as a seed treatment (2 g/kg seed), and the results showed less root rot incidence, and less nematode population and increased pod yield [27]. *P. fluorescens* alone or in combination with pesticides controlled wilt disease complex of Pigeon pea+ *H. cajani*. *P. fluorescens* alone increased plant growth, nodulation, and phosphorus content, and decreased nematode multiplication and wilting in infected plants. Field trials at Kanpur, India for controlling root rot disease (*Rhizoctonia bataticola*) in Chickpea cv. C235 using bacterial antagonist, *P. fluorescens* at 500 g/ha [28] It gave some control compared to untreated control when given as soil inoculation plus seed treatment.

Considering the importance of the subject, the present investigation was undertaken to find the changes, if any, in zinc content concerning chickpea inoculated with the root-knot nematode*, M. incognita* with a combination of *P. fluorescens* as a bioagent, where different treatments of nematode, bacterium, and chemicals were used sustaining the enhancement of disease resistance in chickpea cultivars, RSG 974, GG 5, GNG 2144.

2. MATERIALS AND METHODS

Cultivars of chickpea were sown in 15 cm diameter earthen pots filled with steam-sterilized soil. In order to know the chemical and genetic basis of resistance, three varieties were chosen for a detailed analysis. These varieties were grown with complete care. One set of each uninoculated (healthy) and inoculated (infected) plants was analyzed to test the effects of root-knot nematode infection on the growth and vigor of the plants and their root system. A week after germination, seven treatments with four replications to each of chickpea varieties RSG 974, GG 5, and GNG 2144 were done.

T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot,
T_2- Bacterium, *P. fluorescens* alone @ 7gm/pot,
T_3- *Meloidogyne incognita* inoculated, one week prior to bacteria
T_4- Bacterium inoculated, one week prior to *Meloidogyne incognita*
T_5- *Meloidogyne incognita* and Bacterium inoculated at a time

T_6- Carbofuran 3G @ 2.5kg ai/ha,
T_7- Control.

Healthy and inoculated plants were harvested, 45 days after planting. The harvested roots were washed thoroughly under running tap water to remove the adhering soil particles and were kept separately for chemical analysis.

2.1 Estimation of Micronutrient 'Zn' in Roots

Mineral acids like diacid (HNO_3 - $HClO_4$) were digested [29]**.** The digested sample was introduced to AAS (atomic absorption spectroscopy) for Zn analysis after standardizing the AAS with respective standards.

(Zn) mg/100 g dry weight = $\frac{AASR \times 50}{Sample\ \mathrm{wt(g)} \times 10}$ [29]

3. RESULTS

3.1 Effectiveness of Zinc Contents on the Resistance / Susceptibility of Chickpea Varieties Influenced by the Root-knot Nematode, *M. incognita,* and *P. fluorescens*

Zn content in variety RSG 974

The total zinc content of chickpea variety RSG 974 (Table 1) was found the highest in treatment-2, where only bacterium (*P. fluorescens*) was inoculated, i.e., 2.35 mg/100gm of root with a percentage of increase of 45.06% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, i.e., 2.11mg/100mg with a percentage of increase of 30.25% respectively. Furthermore, an increase was recorded in the two nematode combinations of nematode (*M. incognita*) and bacterium (*P. fluorescens*), added simultaneously or one after another. Among combinations, treatment-4 (nematode inoculated, one week prior to *P. fluorescens*) was recorded as a higher amount of zinc content, i.e., 2.03mg/100mg of roots with a percentage of increase of 25.31% over control, followed by treatment-5, where when *M. incognita* and *P. fluorescens* were applied added simultaneously or at a time, i.e., 1.95 mg/100mg (20.37%) and treatment-3, where when *P. fluorescens* inoculated, one week prior to *M. incognita* i.e.1.86 mg/100mg (14.81%) respectively in a descending order. The lowest amount of zinc content was recorded in treatment-1 when only *M. incognita* was treated, i.e., 1.75 mg/100mg of the root of variety RSG 974 with a low increase in the percentage (8.02%) over the control.

Zn content in variety GG 5

The total zinc content of chickpea variety GG 5 (Table 2) was found the highest in treatment-2, where when only bacterium (*P. fluorescens*) was inoculated, i.e., 2.79 mg/100gm of root with a percentage of increase of 57.63% over the control treatment-7 followed by treatment-6, where when only carbofuran was treated i.e. 2.62 mg/100mg with a percentage of increased of 48.02% respectively in a descending order. An increase was recorded in all combinations of nematode (*M. incognita*) and bacterium (*P. fluorescens*) when inoculated simultaneously or one after another. Among combinations, treatment-4 (nematode inoculated, one week prior to *P. fluorescens*) was recorded as a higher amount of zinc content, i.e., 2.48mg/100mg of roots with a percentage of increase of 40.11% over control, followed by treatment-5, where after *M. incognita* and *P. fluorescens* were applied treated simultaneously or at a time, i.e., 2.22 mg/100mg (25.42%) and treatment-3, where after *P. fluorescens* inoculated, one week prior to *M. incognita*, i.e., 2.08mg/100mg (17.51%) respectively in a descending order. The lowest zinc content was recorded in treatment-1, where when only *M. incognita* was treated, i.e., 1.98mg/100mg of root of variety GG 5 with a low increase in percentage of 11.86% over the control.

Zn content in variety GNG 2144

The total zinc content of chickpea variety GNG 2144 (Table 3) was found the highest in treatment-2, where when only bacterium (*P. fluorescens*) was inoculated, i.e., 3.14 mg/100gm of root with a percentage of increase of 65.87% over the control treatment-7 followed by treatment-6, where when only carbofuran was treated i.e. 3.07 mg/100mg with a percentage increase of 62.3% respectively in a descending order. The lowest amount of zinc content was recorded in treatment-1 when only *M. incognita* was treated, i.e., 2.20 mg/100 mg of root of variety GNG 2144 with a low increase in percentage (16.4%) over the control. An increase was recorded in all combinations of nematode (*M. incognita*) and bacterium (*P. fluorescens*) when added simultaneously or one after another. Among combinations, treatment-4 (nematode inoculated, one week prior to *P. fluorescens*) was recorded as a higher amount of zinc content, i.e., 2.84 mg/100mg of roots with a percentage of increase of 50.26% over control, followed by treatment-5, where when *M. incognita* and *P. fluorescens* were applied inoculated simultaneously or at a time, i.e., 2.59mg/100mg (37.04%) and treatment-3, where when *P. fluorescens* inoculated, one week prior to *M. incognita*) i.e.2.36mg/100mg (24.87%) respectively in a descending order.

Table 1. Zinc concentration in various treatments of chickpea variety RSG 974

Treatments	**Variety RSG 974**	
	Zinc concentration/Root (mg/100mg)	**Change over control (%)**
T_1- *Meloidogyne incognita* (N) alone @ 1000 J_2/ pot	1.75	8.02
T_2- Bacteria, *Pseudomonas. Fluorescens* (B) alone @7gm/pot	2.35	45.06
T_3- N→B (*Meloidogyne incognita* inoculated one week prior to bacteria	1.86	14.81
T_4- B→N (Bacteria inoculated one week prior to *Meloidogyne incognita*	2.03	25.31
T_5- N+B (*Meloidogyne incognita* and Bacteria inoculated at a time	1.95	20.37
T_6- Carbofuran 3G @ 2.5kg ai/ha	2.11	30.25
T_7(Control)	1.52	
SE(m)±	0.02	
CD(0.05)	0.05	

Table 2. Zinc concentration in various treatments of chickpea variety GG-5

Treatments	**Variety GG-5**	
	Zinc concentration/Root (mg/100mg)	**Change over control (%)**
T_1- *Meloidogyne incognita* (N) alone @ 1000 J_2/ pot	1.98	11.86
T_2- Bacteria, *Pseudomonas.fluorescens* (B) alone @7gm/pot	2.79	57.63
T_3- N→B (*Meloidogyne incognita* inoculated one week prior to bacteria	2.08	17.51
T_4- B→N (Bacteria inoculated one week prior to *Meloidogyne incognita*	2.48	40.11
T_5- N+B (*Meloidogyne incognita* and Bacteria inoculated at a time	2.22	25.42
T_6- Carbofuran 3G @ 2.5kg ai/ha	2.62	48.02
T_7(Control)	1.77	
SE(m)±	0.02	
CD(0.05)	0.06	

Table 3. Zinc concentration in various treatments of chickpea variety GNG-2144

Treatments	Variety GNG-2144	
	Zinc concentration/Root (mg/100mg)	Change over control (%)
T_1- *Meloidogyne incognita* (N) alone @ 1000 J_2/ pot	2.20	16.40
T_2- Bacteria, *Pseudomonas.fluorescens* (B) alone @7gm/pot	3.14	65.87
T_3- N→B (*Meloidogyne incognita* inoculated one week prior to bacteria	2.36	24.87
T_4- B→N (Bacteria inoculated one week prior to *Meloidogyne incognita*	2.84	50.26
T_5- N+B (*Meloidogyne incognita* and Bacteria inoculated at a time	2.59	37.04
T_6- Carbofuran 3G @ 2.5kg ai/ha	3.07	62.30
T_7(Control)	1.89	
SE(m)±	0.03	
CD(0.05)	0.09	

4. DISCUSSION

The total zinc content of chickpea variety RSG 974 (Table 1) was found the highest in treatment-2, where only bacterium (*P. fluorescens*) was inoculated, i.e., 2.35 mg/100gm of root with a percentage of increase of 45.06% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, i.e., 2.11mg/100mg with a percentage of increase of 30.25% respectively. These findings were found quite similar to findings by [30], where as they concluded that inoculation of isolates BT3 and CT8 improved the growth parameters of chickpea and increased the plant's Zn uptake by 3.9–6.0%.

The total zinc content of chickpea variety GG 5 (Table 2) was found the highest in treatment-2, where when only bacterium (*P. fluorescens*) was inoculated, i.e., 2.79 mg/100gm of root with a percentage of increase of 57.63% over the control treatment-7 followed by treatment-6, where when only carbofuran was treated i.e. 2.62 mg/100mg with a percentage of increased of 48.02% respectively in a descending order. Scientists [31] evaluated to test the potential of plant growth-promoting actinobacteria in increasing seed mineral density amounts of chickpea under field conditions and Among the 19 isolates of actinobacteria tested, a significant (p ≤0.05) increase of minerals over the uninoculated control treatments was noticed on by all the isolates for Zn (13–30 %).

The total zinc content of chickpea variety GNG 2144 (Table 3) was found the highest in treatment-2, where when only bacterium (*P. fluorescens*) was inoculated, i.e., 3.14 mg/100gm of root with a percentage of increase of 65.87% over the control treatment-7 followed by treatment-6, where when only carbofuran was treated i.e. 3.07 mg/100mg with a percentage increase of 62.3% respectively in a descending order. Scientists stated that Zinc-solubilising bacteria could release Zn from its insoluble compounds and the strongest bacterium for Zn production is *P. fuorescence* (Ur21) [32]. The lowest amount of zinc content was recorded in treatment-1 when only *M. incognita* was treated, i.e., 2.20 mg/100mg of root of variety GNG 2144 with a low increase in percentage (16.4%) over the control. This finding is was similar to the findings of [33]. He observed that Zn content was decreased in infected plants of African marigold than healthy plants due to *Tylenchulus semipenetrans* infection.

Certain members of the P. fluorescens have been shown to be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases [26].

5. CONCLUSION

Chickpea (*Cicer arietinum* L.) production is hampered by considerable restrictions, which result in a global yield loss of about 14% due to plant-parasitic nematodes. The root-knot nematode (*Meloidogyne* sp.) is an endoparasite that has a big impact on chickpea plants. As a result, the chemical basis of management is more cost-effective, and the pathogen's pest comeback is strengthened. As a result, ecologically based nematode control is required, which is additionally impeded by resistance development for plant-parasitic nematodes. The primary goal of this study was to improve resistance in chickpea plants by employing the bioagent *Pseudomonas fluorescens* alone or in combination to increase Zinc uptake. where Various treatments, such as nematodes, bacteria, and chemicals, were utilised to maintain disease resistance in the chickpea cultivars RSG 974, GG 5, and GNG 2144. Zn enhances biocontrol activity by reducing toxic materials produced by the pathogen [34]. Zn content was found more in GNG 2144 and GG 5 than in that of tolerance one, RSG 974 among three chickpea cultivars, and *P. fluorescens* has the leading role in increasing Zn content in roots of chickpea plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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