

Biochemical analysis based on Zinc uptake of Chickpea (*Cicer arietinum* L.) varieties infected by *Meloidogyne incognita*

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 got hampered due to breeding for resistance to such plant-parasitic nematodes, which is 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 combination, where different treatments of nematode, bacteria, 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 highest in treatment, where only bacteria (*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. Application of *Pseudomonas fluorescens* combined or alone gradually increased the Zn concentration in roots of chickpea plants compared to healthy check followed by chemical treated plants.

Keywords: Zinc, Biochemical analysis, Meloidogyne incognita, Chickpea

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 [29]; however, other regions of the roots are not immune to attack [4]. 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 [12]. The authors gave further support to this process of the syncytial formation while studying root-knot infections on tomato [4]. Similar evidence for cell wall dissolution has been reported for many hosts infected by *Meloidogyne* species [13, 5, 20, and 16]. Scientists also surveyed 30 soil samples from medicinal plants and found *M. incognita* in all the samples [11]. Furthermore, an inverse relationship was observed between *M. incognita* and the growth of *O. sanctum* [8]. The effects of *M. incognita* on different plants were observed by several workers, e.g., on *Manihot esculenta* by [18], on tomato by [23], on resistant cotton genotypes by [33] on olive explants by [25], and on sunflower by [2], fruit crops by [21] and [22], pulse crops by [1], chickpea by [3]. Chickpea (*Cicer arietinum* L.) is an important pulse crop with an annual production of 11.5 million tonnes worldwide [17, 19]. However, the yield of chickpea tends to be low and unstable, with a world average yield of 850 kg/ha [19], well below the estimated yield potential of 4,000 kg/ha [30, 31].

India is the world's largest consumer of chickpea and the world's largest producer, contributing over 70% of total global chickpea production [6]. 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% [26] of total global production. Nematode species causing significant economic damage in chickpea include root-knot nematodes (*Meloidogyne artiella*, *M. incognita*, and *M. javanica*) [34]. For the management purpose to root-knot nematode problem in this present study, we applied biocontrol agent *Pseudomonas fluorescens* as it has a potential antagonistic nature to control plant disease [7]. Field trials in *Vigna mungo* to control root rot disease complex caused by *Macrophomina phaseolina* and cyst nematode, *Heterodera cajani* was carried out at Coimbatore. *Pseudomonas fluorescens* was applied as a seed treatment (2 g/kg seed), and the results showed less root rot incidence and nematode population and increased pod yield [15]. *P. fluorescens* alone or in combination with pesticides control wilt disease complex of Pigeon pea, *H. cajani*. *Pseudomonas fluorescens* alone increased plant growth, nodulation, phosphorus content, and decreased nematode multiplication and wilting in infected plants. Field trials at Kanpur, India for controlling root rot disease (*R. 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 *Pseudomonas fluorescens* as a bioagent, where different treatments of nematode, bacteria, and chemicals are used sustaining the enhancement of disease resistance in chickpea cultivars RSG 974, GG 5, GNG 2144.

MATERIAL AND METHODS

Cultivars of chickpea were sown in 15 cm diameter earthen pots filled with steam-sterilized soil. A week after germination, seven treatments with four replications to each chickpea varieties RSG 974, GG 5, and GNG 2144 were done.

- T₁- *Meloidogyne incognita* alone @ 1000 J₂/ pot,
- T₂- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot,
- T₃- *Meloidogyne incognita* inoculated one week prior to bacteria
- T₄- Bacteria inoculated one week prior to *Meloidogyne incognita*
- T₅- *Meloidogyne incognita* and Bacteria inoculated at a time
- T₆- Carbofuran 3G @ 2.5kg ai/ha,
- T₇- 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 kept separately for chemical analysis.

Estimation of micronutrient 'Zn' in roots

Mineral acids like diacid (HNO₃ - HClO₄) digestion [10]. The digested sample was introduced to AAS for Zn analysis after standardizing the AAS with respective standards.

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

RESULTS AND DISCUSSION

Various enzymes, amino acids, fats, complex proteins, sugars, starch, macromolecules, and micro molecules present in the plant system influence the pests' metabolism. In the course of feeding, some chemicals may be depleted, or some others may be de novo synthesized, which may be detrimental to the pest. 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.

Estimation of zinc contents in the resistant/susceptible chickpea varieties influenced by the root-knot nematode, *M. incognita*, and *Pseudomonas fluorescens*

Zn content in variety RSG 974

The total zinc content of chickpea variety RSG 974 (Table 1) was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, i.e., 2.35 mg/100gm of root with a percent 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 percent increase of 30.25% respectively. These findings were found quite similar to findings by [14], where they concluded inoculation of isolates BT3 and CT8 improved the growth parameters of chickpea and increased the plant's Zn uptake by 3.9–6.0%. Furthermore, an increase is recorded in all nematode combinations (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) 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., 2.03/100mg of roots with a percent increase of 25.31% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, i.e., 1.95 mg/100mg (20.37%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) i.e.1.86 mg/100mg (14.81%) respectively. The lowest amount of zinc content was recorded in treatment-1. Only *Meloidogyne 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.

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

Treatments	RSG 974	
	Root	Change over control (%)
T ₁ - <i>Meloidogyne incognita</i> (N) alone @ 1000 J ₂ / pot	1.75	8.02
T ₂ - Bacteria, <i>Pseudomonas.fluorescens</i> (B) alone @7gm/pot	2.35	45.06
T ₃ - N→B (<i>Meloidogyne incognita</i> inoculated one week prior to bacteria)	1.86	14.81
T ₄ - B→N (Bacteria inoculated one week prior to <i>Meloidogyne incognita</i>)	2.03	25.31
T ₅ - N+B (<i>Meloidogyne incognita</i> 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	

Zn content in variety GG 5

The total zinc content of chickpea variety GG 5 (Table 2) was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, i.e., 2.79 mg/100gm of root with a percent increase of 57.63% over the control treatment-7 followed by treatment-6, where only carbofuran was treated i.e. 2.62 mg/100mg with a percent increased of 48.02% respectively. [27] evaluated to test the potential of plant growth-promoting actinobacteria in increasing seed mineral density 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 all the isolates for Zn (13–30 %).

An increase is recorded in all combinations of nematode (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) 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 percent increase of 40.11% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, i.e., 2.22 mg/100mg (25.42%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*), i.e., 2.08mg/100mg (17.51%) respectively. The lowest zinc content was recorded in treatment-1 where only *Meloidogyne 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.

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

Treatments	GG-5	
	Root	Change over control (%)
T ₁ - <i>Meloidogyne incognita</i> (N) alone @ 1000 J ₂ / pot	1.98	11.86
T ₂ - Bacteria, <i>Pseudomonas fluorescens</i> (B) alone @7gm/pot	2.79	57.63
T ₃ - N→B (<i>Meloidogyne incognita</i> inoculated one week prior to bacteria	2.08	17.51
T ₄ - B→N (Bacteria inoculated one week prior to <i>Meloidogyne incognita</i>	2.48	40.11
T ₅ - N+B (<i>Meloidogyne incognita</i> 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	

Zn content in variety GNG 2144

The total zinc content of chickpea variety GNG 2144 (Table 3) was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, i.e., 3.14 mg/100gm of root with a percent increase of 65.87% over the control treatment-7 followed by treatment-6, where only carbofuran was treated i.e. 3.07 mg/100mg with a percent increase of 62.3% respectively. Scientists stated that Zinc-solubilising bacteria could release Zn from its insoluble compounds and the strongest bacteria for Zn production is *Pseudomonas fluorescens* (Ur21) [9]. The lowest amount of zinc content was recorded in treatment-1. Only

Meloidogyne 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 similar to the findings of [32]. He observed that Zn content was decreased in infected plants of African marigold than healthy plants due to *Tylenchulus semipenetrans* infection. An increase is recorded in all combinations of nematode (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) 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 percent increase of 50.26% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, i.e., 2.59mg/100mg (37.04%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) i.e.2.36mg/100mg (24.87%) respectively.

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

Treatments	GNG-2144	
	Root	Change over control (%)
T ₁ - <i>Meloidogyne incognita</i> (N) alone @ 1000 J ₂ / pot	2.20	16.40
T ₂ - Bacteria, <i>Pseudomonas.fluorescens</i> (B) alone @7gm/pot	3.14	65.87
T ₃ - N→B (<i>Meloidogyne incognita</i> inoculated one week prior to bacteria)	2.36	24.87
T ₄ - B→N (Bacteria inoculated one week prior to <i>Meloidogyne incognita</i>)	2.84	50.26
T ₅ - N+B (<i>Meloidogyne incognita</i> 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	

CONCLUSION

Zn enhances biocontrol activity by reducing toxic materials produced by the pathogen [24]. Zn content was found more in GNG 2144 and GG 5 than 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.

REFERENCES

1. Askary, T. H. (2017). Diversity of plant parasitic nematodes in pulses. *Plant Biodiversity: Monitoring, Assessment and Conservation*. CAB International, 239-274.
2. Bhatt, Jayant, Vadhera I, Shukla BN. 2001. Growth and varieties reaction of sunflowers to root-knot nematode (*M. incognita*). *Adv Plant Sci*. 14:61–66.
3. Castillo, P., Navas-Cortés, J. A., Landa, B. B., Jiménez-Díaz, R. M., & Vovlas, N. (2008). Plant-parasitic nematodes attacking chickpea and their in planta interactions with rhizobia and phytopathogenic fungi. *Plant Disease*, 92(6), 840-853.
4. Christie JR. 1936. The development of root-knot nematode galls. *Phytopathology*. 26:1–22.
5. Dropkin VH, Nelson PE. 1960. The histopathology of root-knot nematode infections in soybeans. *Phytopathology*. 50:442–447.

6. FAO (2017). *Production Crops [Online]*. Rome: Food and Agriculture Organization of the United Nations.
7. Ganeshan, G., & Manoj Kumar, A. (2005). *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *Journal of Plant Interactions*, 1(3), 123-134.
8. Haseeb A, Butool F, Shukla PK. 1999. Effect of *M. incognita* on growth, physiology and oil yield of *Ocimum sanctum*. *Ind J Nematol*. 29(2):121–125.
9. Hashemnejad, F., Barin, M., Khezri, M., Ghoosta, Y., & Hammer, E. C. (2021). Isolation and identification of insoluble zinc-solubilising bacteria and evaluation of their ability to solubilise various zinc minerals. *Journal of Soil Science and Plant Nutrition*, 21(3), 2501-2509.
10. Jackson, M.L. 1973. *Soil Chemical Analysis*. Prentice Hall Pvt. Ltd., New Delhi
11. Joymati L, Roma Bati N, Bhanachand CH. 1999. Distribution and host range of *M. incognita* on medicinal plants of monipeu Part-I. *Ind J Nematol*. 29(1):78–111.
12. Kostoff D, Kendall J. 1930. Cytology of nematode galls on *Nicotiana* roots. *Zentrbl. Bakt. Alt.* 2:824–835.
13. Krusberg LR, Nielsen LW. 1958. Pathogenesis of root-knot nematodes to the Puerto Rico variety of sweet potato. *Phytopathology*. 48:30–39.
14. Kushwaha, P., Srivastava, R., Pandiyan, K., Singh, A., Chakdar, H., Kashyap, P. L., & Saxena, A. K. (2021). Enhancement in Plant Growth and Zinc Biofortification of Chickpea (*Cicer arietinum* L.) by *Bacillus altitudinis*. *Journal of Soil Science and Plant Nutrition*, 21(2), 922-935.
15. Latha TKS, Rajeswari E, Narasimhan V. 2000. Management of root-rot disease complex through antagonists and chemicals. *Ind Phytopathol* 53:216218.
16. Littrell RL. 1966. Cellular responses of *Hibiscus esculentus* to *Meloidogyne acrita*. *Phytopathology*. 56:540–544.
17. Maesen L. J. G. V. D., Maxted N., Javadi F., Coles S., Davies A. M. R. (2007). "Taxonomy of the genus *Cicer* revisited," in *Chickpea Breeding and Management*, eds Yadav S. S., Redden R.J., Chen W., Sharma B., (Trowbridge: Cromwell Press;).
18. Makumbi-Kidza NN, Speijer PR, Sikora RA. 2000. Effect of *M. incognita* on growth and storage Root Formation of caesava (*Monihot esculenta*). *J Nematol*. 32:475–477.
19. Merga B., Haji J. (2019). Economic importance of chickpea: production, value, and world trade. *Cogent Food Agric*.5:1615718
20. Owens RG, Specht HN. 1964. Root-knot histogenesis. Contributed. Boyce Thompson Institute. 22:471–489.
21. Pradhan, P., Patra, M. K., & Sahoo, N. K. (2020)a. Association of Nematodes with Fruit Crops in Bhubaneswar, India. *Int. J. Curr. Microbiol. App. Sci*, 9(5), 1918-1923.
22. Pradhan, P., Thakur, D., & Sahoo, N. K. (2020)b. Nematodes associate with big fruit trees and their community analysis survey of state Odisha, India. *Journal of Entomology and Zoology Studies*, 8(3): 1046-1048.
23. Pushparaj BE, Pelosi E, Caroppo S. 2000. Effect of *Nodularia harveyone* biomass on the incidence of root-knot nematode (*M. incognita*) on tomato. *J Appl Physiol*. 12:489–492.
24. Saikia, R., Varghese, S., Singh, B. P., & Arora, D. K. (2009). Influence of mineral amendment on disease suppressive activity of *Pseudomonas fluorescens* to *Fusarium* wilt of chickpea. *Microbiological research*, 164(4), 365-373.

25. Sasanelli N, Addabbo TD, Dell'orco P, Mencuccini M. 2000. The in vitro use of olive explants in screening trials for resistance to the root-knot nematode, *M. incognita*. *Nematropica*. 30:101–106.
26. Sasser J. N., Freckman D. W. (1987). "A world perspective on nematology: The role of the society," in *Vistas on Nematology*, eds Veech J. A., Dickson D. W. (Hyattsville, MD: Society of Nematologists;), 7–14.
27. Sathya, A., Vijayabharathi, R., Srinivas, V., & Gopalakrishnan, S. (2016). Plant growth-promoting actinobacteria on chickpea seed mineral density: an upcoming complementary tool for sustainable biofortification strategy. *3 Biotech*, 6(2), 1-6.
28. Shaid Ahmad, Mukesh Srivastava, Ahmad S, Srivastana M. 2000. Biological control of dry root rot of chickpea with plant products and antagonistic microorganisms. *Ann Agric Res* 21:450451.
29. Siddiqui, Taylor DP. 1970. Histopathogenesis of galls induced by *Meloidogyne naasi* in wheat roots. *J Nematol*. 2:239–247.
30. Singh K. B., Malhotra R. S., Halila M. H., Knights E. J., Verma M. M. (1993). Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica* 73:137–149.
31. Singh K. B., Ocampo B. (1997). Exploitation of wild Cicer species for yield improvement in chickpea. *Theory Appl. Genet.* 95:418–423.
32. Sreekanth, P., Padma, M., Chandrasekhar, R., & Madhulety, T. Y. (2006). Effect of planting time, spacing and nitrogen levels on yield and quality of African marigold (*Tagetes erecta* Linn.). *Journal of Ornamental Horticulture*, 9(2), 97-101.
33. Zhou E, Wheeler TA, Starr JL. 2000. Root galling and reproduction of *M. incognita* isolates from Texas on resistant cotton genotypes. *J Nematol*. 32:513–518.
34. Zwart, R.S., Thudi, M., Channale, S., Manchikatla, P.K., Varshney, R.K. and Thompson, J.P. (2019). Resistance to Plant-Parasitic Nematodes in Chickpea: Current Status and Future Perspectives. *Frontiers in Plant Science*, [online] 10. Available at: <https://www.frontiersin.org/articles/10.3389/fpls.2019.00966/full> [Accessed 28 Nov. 2019].