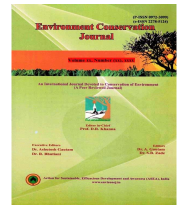
Environment Conservation Journal 23 (3):81-86, 2022

Journal homepage: https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Effect of *Pseudomonas fluorescens* in manganese uptake by chickpea (*Cicer arietinum* L.) cultivars infected by root-knot nematodes (*Meloidogyne incognita*)

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ARTICLE INFO

Received : 30 November 2021
Revised : 27 March 2022
Accepted : 21 April 2022

Available online: 18 September 2022

Key Words:
Carbofuran
Pseudomonas spp.
Pulse crop
Root-knot nematode

ABSTRACT

Chickpea (*Cicer arientinum* L.) is one of the most dominant pulse crops in India, which contributes 38 percent of the area and 50 percent production of pulses compare to the total pulse production of India. Chickpea contains protein-2.1%, carbohydrates-61.5%, and fat-4.5% and more iron, calcium and niacin content. The main constrain of chickpea production due to parasitic nematodes (*Meloidogyne incognita*) is about 14% of total global production in annual yield loss. *Pseudomonas fluorescens* is a bacterial bio-agent that can help in nematode suppression in chickpea plants. This experiment was conducted to experience the differences, if any, in manganese content concerning chickpea inoculated with *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. The total manganese content of chickpea variety GNG 2144 was found highest in treatment, where only bacteria (*P. fluorescens*) was inoculated, *i.e.*, 6.44 mg/100g of a root, followed by GG 5, *i.e.*, 5.63 mg/100g of root and RSG 974 was, *i.e.*, 4.14 mg/100g of root respectively. Application of *Pseudomonas fluorescence* combined or alone gradually increased the manganese concentration in roots of chickpea plants *i.e.*, RSG 974 (4.14 mg/100g), GG 5(5.63 mg/100g), GNG 2144 (6.44 mg/100g) compared to the health check.

Introduction

Among various species, root-knot nematodes, mainly *Meloidogyne incognita* are causing major economic damage in chickpea (*Cicer arietinum* L.). Nematodes mainly attack as a primary pathogen and aggravator, causing damage to roots by reducing the functionality of roots, leading to nutrient deficiency and water stress in plants and resulting in poor plant growth and less yield. By growing resistant crops which comes under ecologically based management with a very good cultural practices are the cheapest, safest and practically feasible management tactics for plant disease causing nematodes till date. However, breeding for resistance to such nematodes has various problems originating from the *C. arietinum* cultigens (Zwart *et al.,* 2019). The metabolic process of the host is being altered by plant-parasitic nematodes like *M incognita,* by using biochemical, cellular, or physiological parameters. Due to root-knot nematodes, there is occurrence of

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devasting morphological and physiological variations in the host plants (Williamson and Gleason, 2003). These nematodes infect the plants, resulting in the discoloration of leaves showing yellowing symptoms and slow plant growth. Broad host range minimizes the chance of crop rotation as a management to plant parasitic nematodes.

Therefore, an alternative source to avoid the losses is growing resistant cultivars (Howell and Krusberg, 1966), which can also be done by inducing some bioagents to the plants. *Pseudomonas fluorescens* is a bacterial bio-agent grows well with a simple nutritional requirement (Palleroni 1984). The ability to adapt well in soil *P. fluorescens* is easy to induce, and survival chances are significant in soil. Chief among all biocontrol agents suppress the roots and plant diseases that bacterial and fungal pathogens have caused due to the production of antibiotics and siderophores. (O'Sullivan & O'Gara 1992; Wei *et al.,*1996; Hoffland *et al.,*1996). Hass and Defago (2005) stated that *P. fluorescens* shows competitive behavior to the disease-causing pathogens by rapid rhizospheric colonization, is one of the major factors in disease control. Physiological variations and biochemical alterations due to nematode invasion are occurred because of the disturbed metabolism in the affected plants, deciding the hosts to become susceptible or tolerant to check the nematode's attack (Krusberg 1963). Some progress regarding host plant interaction and biochemical mechanism has also been made to understand the matter by several workers in the recent past (Ganguly and Dasgupta 1983; Mohanty *et al.* 1995; Howell and Krusberg, 1966). Considering these matters and the necessity, the present research was carried out to find the differences and know the details of the current matter, if any, in manganese content about chickpea inoculated with *M. incognita* in combination of *Pseudomonas fluorescens* as a bioagent.

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.

T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot (J_2- Juveniles 2),
T_2- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot,
T_3- *Meloidogyne incognita* (@ 1000 J_2/ pot) inoculated one week prior to bacteria(@7gm/pot)
T_4- Bacteria(@7gm/pot) inoculated one week prior to *Meloidogyne incognita* (@ 1000 J_2/ pot)
T_5- *Meloidogyne incognita* (@ 1000 J_2/ pot) and Bacteria(@7gm/pot) inoculated at a time
T_6- Carbofuran 3G @ 2.5kg ai/ha,
T_7- Control

Harvesting of inoculated plants side by healthy plants were done after 45 days after planting. The roots after harvesting were kept separately for chemical analysis. Estimation of micronutrient 'Mn' in roots was done by mineral acids like diacid (HNO_3 - $HClO_4$) digestion (Jackson, 1973; Gahoonia *et al.,* 2007). After standardizing the AAS, the digested sample was introduced to AAS for Mn analysis, with respecting standards.

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

Results and Discussion

Estimation of manganese contents in variety RSG 974 influenced by *Pseudomonas fluorescens,* and *M. incognita*

The total manganese content of chickpea variety RSG 974 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, i.e., 4.14 mg/100gm of root with a percent increase of 35.74% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, *i.e.*, 4.08 mg/100mg with a percent increase of 33.61% respectively. These findings were found quite similar to findings by Sathya *et al.* (2016), where they experimented 19 isolates of plant growth-promoting actinobacteria and concluded there is significant (p\0.05) increase of minerals compared to untreated check in all the isolates and that for also Mn (18–35 %), that indicates the benefits of bio agents as they supress the intake of nutrients to other harmful microorganisms. Furthermore, an increase is recorded in all nematode combinations (*Meloidogyne incognita*) and bacteria (*P. fluorescens*) simultaneously

Table 1: Manganese concentration in various treatments of chickpea variety RSG 974, GG-5, GNG-2144

Treatments	RSG 974		GG-5		GNG-2144	
	Root	Change over control (%)	Root	Change over control (%)	Root	Change over control (%)
T_1	3.23	5.90	3.91	8.91	4.48	14.16
T_2	4.14	35.74	5.63	56.82	6.44	64.29
T_3	3.36	10.16	4.11	14.35	4.73	20.73
T_4	3.74	22.62	4.66	29.87	5.28	34.69
T_5	3.51	15.08	4.57	27.16	5.17	31.89
T_6	4.08	33.61	5.44	51.46	5.96	52.04
T_7	3.05		3.29		3.92	-
SE(m)±	0.02		0.03		0.04	
CD (0.05)	0.06		0.09		0.11	

T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot (J_2- Juveniles 2); T_2- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot; T_3- *Meloidogyne incognita* (@ 1000 J_2/ pot) inoculated one week prior to bacteria(@7gm/pot); T_4- Bacteria(@7gm/pot) inoculated one week prior to *Meloidogyne incognita* (@ 1000 J_2/ pot); T_5- *Meloidogyne incognita* (@ 1000 J_2/ pot) and Bacteria(@7gm/pot) inoculated at a time; T_6- Carbofuran 3G @ 2.5kg ai/ha and T_7- Control.

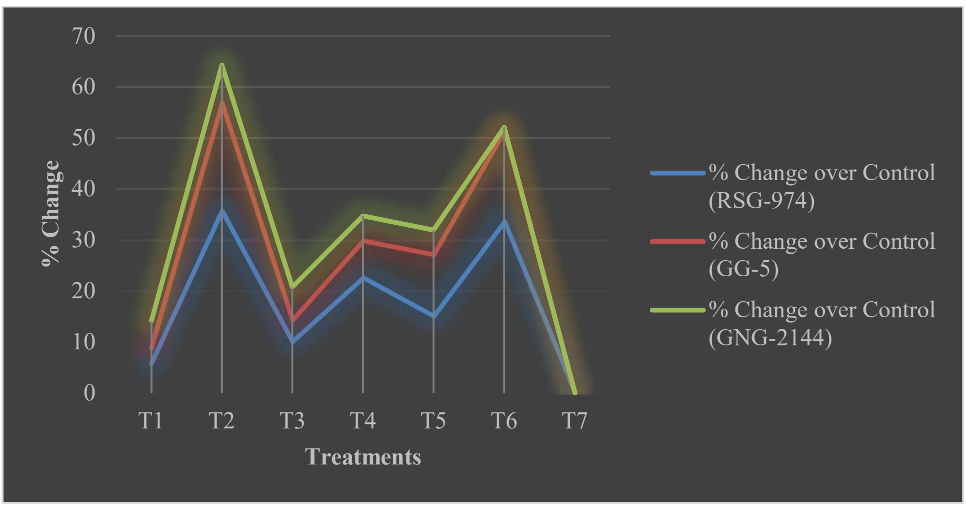

Figure 1: Percent change over control in manganese concentration (T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot (J_2- Juveniles 2); T_2- Bacteria, *Pseudomonas fluorescens* alone @7gm/pot; T_3- *Meloidogyne incognita* (@ 1000 J_2/ pot) inoculated one week prior to bacteria(@7gm/pot); T_4- Bacteria(@7gm/pot) inoculated one week prior to *Meloidogyne incognita* (@ 1000 J_2/ pot); T_5- *Meloidogyne incognita* (@ 1000 J_2/ pot) and Bacteria(@7gm/pot) inoculated at a time; T_6- Carbofuran 3G @ 2.5kg ai/ha and T_7- Control).

or one after another. Among combinations, treatment-4 (nematode inoculated one week prior to *P. fluorescens*) was recorded as a higher amount of manganese content, *i.e.*, 3.74mg/100mg of roots with a percent increase of 22.62% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, *i.e.*, 3.51 mg/100mg (15.08%) and treatment-3, where (*P.fluorescens* inoculated one week prior to *M. incognita*) *i.e.*, 3.36 mg/100mg (10.16%) respectively. Not only for pulse crop also for fruit melon promotion of Mn, N, Zn, and P absorption was noticed due to *Pseudomonas fluorescens* Martínez *et al.* 2019.The lowest amount of manganese content was recorded in treatment-1 where only *Meloidogyne incognita* was treated, *i.e.*, 3.23 mg/100mg of the root of variety RSG 974 (Table 1; Figure 1) with a low increase in percentage 5.9% over the control. Mobility of Mn enhanced by *Pseudomonas* *fluorescens*, in sunflower grown on vineyard soils. However, mainly Root-knot nematode plants create galls in roots that block the xylem and phloem vessels in the case of infected nematode plants. Giant cells were induced by juveniles of *Meloidogyne incognita* near the head in vascular strands and xylem as well as phloem were affected by exhibiting abnormalities in their shapes (Robab 2010), by inhibiting the mobilization of nutrients from roots from shooting, storing nutrients molecules in the root portion happened, which is the main reason for enhancing Mn content in roots of chickpea plants, which disobeys the above statement of mobility by Randriamamonjy *et al.* 2021.

Estimation of manganese contents in variety GG 5 influenced by *Pseudomonas fluorescens,* and *M. incognita*

The total manganese content of chickpea variety GG 5 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, *i.e*., 5.63 mg/100gm of root with a percent increase of 56.82% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, *i.e*., 5.44 mg/100mg with a percent increase of 51.46% respectively. 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 manganese content, *i.e*., 4.66 mg/100mg of roots with a percent increase of 29.87% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, *i.e*., 4.57 mg/100mg (27.16%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *M.incognita*) *i.e*., 4.11mg/100mg (14.35%) respectively. The lowest amount of manganese content was recorded in treatment-1 where only *Meloidogyne incognita* was treated, i.e., 3.91 mg/100mg of the root of variety GG 5 (Table 2) with a low increase in percentage 8.91% over the control. Mohanty *et al*. (1999) reported that the inoculation of *R. reniformis* in cowpea roots reduced micro nutrients *viz*., Zn, Cu, Fe, Mn in inoculated plants over the control. Concentration of iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were significantly increased in only *P. fluorescens* inoculated roots over control. These statements corroborate our fimdings where nematode infecting the chickpea plant showed a slight increase in Mn content.

Estimation of manganese contents in variety GNG 2144 influenced by *Pseudomonas fluorescens,* and *M. incognita*

The total manganese content of chickpea variety GNG 2144 (Table 1) was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated, *i.e*., 6.44 mg/100gm of root with a percent increase of 64.29% over the control treatment-7 followed by treatment-6, where only carbofuran was treated, *i.e*., 5.96 mg/100mg with a percent increase of 52.04% respectively. The chemical application was also reasonably practical, although cost-effective, and disobeyed the law of soil sustainability because of residual effects of the chemical. At the same time, *Pseudomonas fluorescens* treated chickpea plant has the highest increase than all treatments. Hence plant growth-promoting bacteria enhanced macro and micronutrients to varying degrees compared to healthy plants, and in the case of Mn, an increase was witnessed in chickpea variety PBG5 compared to PBG1treated with plant-growth promoting bacteria *Pseudomonas citronellis* (PC), *Pseudomonas* spp. RA6, *Serratia* spp. S2, *Serratia marcescens* CDP13, and *Frateuria aurantia* (Symbion-K) by Dogra *et al.* 2019. The lowest amount of manganese content was recorded in treatment-1 where only *Meloidogyne incognita* was treated, *i.e*., 4.48 mg/100mg of the root of variety GNG 2144, with a low increase in the percentage of 14.16% over the control. 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 manganese content, *i.e*., 5.28 mg/100mg of roots with a percent increase of 34.69% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time, *i.e*., 5.17mg/100mg (31.89%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) *i.e*., 4.73mg/100mg (20.73%) respectively. In this experiment, *Pseudomonas fluorescens* gives a positive result of increasing the Mn content in the roots of the chickpea plant. Likewise, bacteria, fungi can also be used as a bioagent like *Pseudomonas fluorescens,* and similar results, *i.e*., there was an increased amount of Mn content was seen where bioagents were applied in cluster bean infected with *Meloidogyne incognita* and VAM fungi (Rao and Tarafdar 1993), wheat (Suri *et al.,* 2011) and a common leafy vegetable *Ipomoea aquatica* (Halder *et al.* 2015).

Conclusion

Alterations of enzymatic activity, redistribution, uptake, and nutrients use like N, P, Fe, Ca and Mg are hampered by heavily concentrated plant tissues with Manganese. Due to manganese severity, the reduction of stomatal conductance, and deterioration of pigments in leaf were seen in different plants. Higher Mn concentration enhanced the production of ROS (reactive oxygen species), with elevated peroxidase activity in plant cells, which has a potential role in the disease resistance mechanism in plants. Mn 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 Mn content in roots chickpea plants.

Conflict of interest

The authors declare that they have no conflict of interest.

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