The Pharma Innovation Journal 2021; SP-10(12): 691-694

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 691-694

www.thepharmajournal.com
Received: 04-10-2021
Accepted: 06-11-2021

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Copper uptake of chickpea (*Cicer arietinum* L.) varieties induced by *Meloidogyne incognita* and *Pseudomonas fluorescens*

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Abstract
Among pulses, chick pea (*Cicer arietinum*) is preferred to food legumes because of its multiple uses for growing population across the world. During 2017-18, globally it was grown on 149.66 lakh ha area, with the total production of 162.25 lakh tonnes (FAOSTAT, 2019) and average productivity of 1252 kg/ha. Out of which, 71 per cent of global area with 70 per cent of global production of *chick pea* is contributed by India as it ranks 1st in area and production but lags behind several countries in terms of productivity. The cause of low productivity of chick pea is attributed to numerous biotic stresses impeding the cultivars to reach their true potential. Among them root knot nematode (*Meloidogyne incognita*) is one of the major pathogens infecting chick pea. Since the ban of fumigant methyl bromide in 2005, there is an increasing search for new molecules and efficient modes of action in the control of nematodes. Hence a biochemical reaction change in host plant with respect to Copper uptake in a combination of *Pseudomonas fluorescens* is-bio agents was conducted to induce resistance in three chickpea cultivars RSG 974, GG 5, GNG 2144. Copper content of chickpea variety GNG 2144 was found highest in treatment, where only bacteria (*P. fluorescens*) was inoculated i.e. 9.45 mg/100g of root followed by GG 5 i.e. 7.77 mg/100g of root and RSG 974 was i.e. 7.01 mg/100g of root respectively. Application of *Pseudomonas fluorescence* combinely enhanced the amount of Cu in roots of all chickpea varieties compared to other treatments.

Keywords: copper, chickpea, *Meloidogyne incognita*, *Pseudomonas fluorescens*

Introduction
Chickpea (*Cicer arietinum* L.) is thought to have originated in Anatolia (Turkey), where three closely related wild species, *C. bijugum*, *C. echinospermum* and *C. reticulatum*, are commonly found in nature (van der Maesen, 1984) [18]. Chickpea seeds had been occasionally recovered in pre-historic sites in the Near East (Renfrew, 1973) [16]. However, Ramanujam (1976) [14] reported that remnants of chickpea radiocarbon are dated at 5450 BC and there is evidence for its cultivation in the Mediterranean basin in 3000-4000 BC. The earliest record of chickpea in northern India (Uttar Pradesh) dated at 2000 BC, and from the south India much later (Chowdury *et al.*, 1971) [1]. Plant-parasitic nematodes constrain chickpea (*Cicer arietinum*) production, with annual yield losses estimated to be 14% of total global production. Nematode species causing significant economic damage in chickpea include root-knot nematodes (*Meloidogyne artiella*, *M. incognita*, and *M. javanica*), cyst nematode (*Heterodera ciceri*), and root-lesion nematode (*Pratylenchus thornei*). Reduced functionality of roots from nematode infestation leads to water stress and nutrient deficiency, which in turn lead to poor plant growth and reduced yield. Integration of resistant crops with appropriate agronomic practices is recognized as the safest and most practical, economic and effective control strategy for plant-parasitic nematodes. However, breeding for resistance to plant-parasitic nematodes has numerous challenges that originate from the narrow genetic diversity of the *C. arietinum* cultigens (Zwart *et. al* 2019) [21]. Plant parasitic nematode, *Meloidogyne incognita* alters the metabolic processes of the host which are manifested in the form of cellular, physiological and biochemical changes occurring in the infected host. Root-knot nematodes causes measurable changes in the morphology and physiology of the host plants, (Williamson and Gleason 2003) [20]. The infection caused by these nematode results in yellowing of leaves and poor plant growth. This nematode has a wide host range which reduces the effectiveness of crop rotation for its management. Therefore, an alternative source of ecologically sound and viable option to avoid the losses caused by the nematodes is the use of resistant cultivars/ lines (Howell and Krusberg (1966) [7] which can be also done by inducing some bio agents to the plants.

Pseudomonas fluorescens is a bacterial bio-agent which has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources (Palleroni 1984) [11].

Because they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in applications that require the release and survival of bacteria in the soil. Chief among these are bio control of pathogens in agriculture and bioremediation of various organic compounds. Certain members of the *P. fluorescens* have been shown to be potential agents for the bio control 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 (Hoffland *et al*. 1996, Wei *et al*. 1996) [6, 19]. This effect is the result of the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide (O'Sullivan & O'Gara 1992) [10]. Hass and Defago (2005) [5] reviewed the mechanisms by which *P. fluorescens* control pathogenic microorganisms in detail. Competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by *P. fluorescens* may also be an important factor in disease control. The changes in the physiological and biochemical processes of infected host as a consequence of disturbed metabolism decide whether the host becomes susceptible or resistant to nematode attack (Krusberg 1963) [9]. In the recent past some progress has also been made in this direction to understand the basic biochemical mechanism of plant-nematode interactions by several workers (Pradhan *et al* (2020)a, Pradhan *et al* (2020)b, Ganguly and Dasgupta 1983; Howell and Krusberg 1966) [12, 13, 4, 7].

Considering the importance of the subject, the present investigation was undertaken to find the changes if any, in copper content in relation to chickpea inoculated with root-knot nematode, *M. incognita* with a combination of *Pseudomonas fluorescens* as a bio agent, 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.

Materials 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 of the chickpea varieties RSG 974, GG 5 and GNG 2144 were done.

T_1- *Meloidogyne incognita* alone @ 1000 J_2/ pot,

T_2- Bacteria, *Pseudomonas*. *Fluorescens* alone @7gm/pot,

T_3- *Meloidogyne incognita* inoculated one week prior to bacteria

T_4- Bacteria inoculated one week prior to *Meloidogyne incognita*

T_5- *Meloidogyne incognita* and Bacteria inoculated at a time

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

T_7- Control.

Healthy and inoculated plants were harvested at 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 'Cu' in roots

Mineral acids like of diacid (HNO_3 - $HClO_4$) digestion (Jackson, 1973) [8]

Digested sample was introduced to AAS for Cu analysis after standardizing the AAS with respective standards.

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

Results and Discussion

Various kinds of enzymes, amino acids, fats to complex proteins, sugars, starch, macro molecules and micro molecules are present in the plant system that influences the metabolism of the pests fed upon them. In the course of feeding some chemicals may be depleted or some others may be de novo synthesized, that 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 fully care. One set of each un inoculated (healthy) and inoculated (infected) plants were analysed to test the effects of root-knot nematode infection on the growth and vigour of the plants and their root-system.

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

Cu content in variety RSG 974

The total copper content of chickpea variety RSG 974 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated i.e. 7.01 mg/100gm of root with a percent increase of 35.07% over the control treatment-7 followed by treatment-6, where only carbofuran was treated i.e 6.39 mg/100mg with a percent increased of 23.12% respectively. These findings was found quite similar of findings by Sathya *et al* (2016) [17] where he concluded the potential effects of some of the action bacteria around 19 isolates and found there was a significant (p\0.05) increase in the copper content over control check i.e. Cu (11–54 %).

There is an increase 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 higher amount of copper content i.e. 6.31 mg/100mg of roots with a percent increase of 21.58% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time i.e. 6.12 mg/100mg (17.92%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) i.e. 5.94 mg/100mg (14.45%) respectively. Lowest amount of copper content was recorded in treatment-1 where only *Meloidogyne incognita* was treated i.e. 5.55 mg/100mg of root of variety RSG 974 with low increase in percentage 6.94% over the control.

Cu content in variety GG 5

The total copper content of chickpea variety GG 5 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated i.e. 7.77 mg/100gm of root with a percent increase of 48% over the control treatment-7 followed by treatment-6, where only carbofuran was treated i.e 7.02 mg/100mg with a percent increased of 33.71% respectively. Chemical application was also quite effective although cost effective and disobeys the law of soil sustainibilty because of residual effects of chemical.

At the same time *Pseudomonas fluorescens* treated chickpea plant has highest increase than rest of all treatments. So plant growth promoting bacteria enhanced macro and micro nutrients to varying degrees compared to healthy plants and in case of copper, significant increase was seen in PBG5 which were tested with plant growth promoting bacteria isolates belonging to *Pseudomonas citronellis* (PC), *Pseudomonas* sp.

RA6, *Serratia* sp. S2, *Serratiamarcescens* CDP13, and *Frateuria aurantia* (Symbion-K) Dogra *et al* 2019 [3].
There is an increase 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 higher amount of copper content i.e. 6.79 mg/100mg of roots with a percent increase of 29.33% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneuously or at a time i.e. 6.36 mg/100mg (21.14%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) i.e.6.13 mg/100mg (16.76%) respectively. Lowest amount of copper content was recorded in treatment-1 where only *Meloidogyne incognita* was treated i.e. 5.69 mg/100mg of root of variety GG 5 with low increase in percentage 8.29% over the control.

Cu content in variety GNG 2144

The total copper content of chickpea variety GNG 2144 was found highest in treatment-2 where only bacteria (*P. fluorescens*) was inoculated i.e. 9.45mg/100gm of root with a percent increase of 59.59% over the control treatment-7 followed by treatment-6, where only carbofuran was treated i.e 8.86 mg/100mg with a percent increased of 49.58% respectively. Lowest amount of copper content was recorded in treatment-1 where only *Meloidogyne incognita* was treated i.e. 6.77 mg/100mg of root of variety GNG 2144 with low increase in percentage 14.36% over the control. Mobility of Cu, Fe, Al, Mn, Zn, N, P enhanced in soils with *Pseudomonas fluorescens*, sunflower grown on vineyard soils**.** But in case of nematode infected plants of *Meloidogyne incognita* induced giant cells, which block both xylem and phloem (Robab 2008), by which storing of nutrients molecules in root portion happened, which is the main reason for enhancing Cu content in roots of chickpea plants which disobeys the above statement of mobility by (Randriamamonjy *et al* 2021) [15].
There is an increase 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 higher amount of copper content i.e. 8.24mg/100mg of roots with a percent increase of 39.19% over control, followed by treatment-5, where *Meloidogyne incognita* and *P. fluorescens* were applied simultaneously or at a time i.e. 7.73mg/100mg (30.57%) and treatment-3, where (*P. fluorescens* inoculated one week prior to *Meloidogyne incognita*) i.e.7.26 mg/100mg (22.64%) respectively. In this experiment also *Pseudomonas fluorescens* gives a positive result of increasing the Cu content in roots of chickpea plant.

Table 1: Copper concentration in various treatments of chickpea variety RSG 974

Tr. No.	RSG 974	
	Root	Change over control(%)
T_1 (N)	5.55	6.94
T_2 (B)	7.01	35.07
T_3 (N→B)	5.94	14.45
T_4 (B→N)	6.31	21.58
T_5 (B+N)	6.12	17.92
T_6	6.39	23.12
T_7(Control)	5.19	
SE(m)±	0.03	
CD(0.05)	0.08	

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

Table 2: Copper concentration in various treatments of chickpea variety GG-5

Tr. No.	GG-5	
	Root	Change over control(%)
T_1 (N)	5.69	8.29
T_2 (B)	7.77	48.00
T_3 (N→B)	6.13	16.76
T_4 (B→N)	6.79	29.33
T_5 (B+N)	6.36	21.14
T_6	7.02	33.71
T_7(Control)	5.25	
SE(m)±	0.04	
CD(0.05)	0.12	

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

Table 3: Copper concentration in various treatments of chickpea variety GNG-2144

Tr. No.	GNG-2144	
	Root	Change over control (%)
T_1 (N)	6.77	14.36
T_2 (B)	9.45	59.59
T_3 (N→B)	7.26	22.64
T_4 (B→N)	8.24	39.19
T_5 (B+N)	7.73	30.57
T_6	8.86	49.58
T_7(Control)	5.92	
SE(m)±	0.06	
CD(0.05)	0.19	

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

Conclusion

In this experiment a liitle amount of Cu accumulation in all varieties was recorded compared to healthy, which is not at all toxic to the chickpea plant. Higher Cu content leads in significant increase in CAT and GPX activities which have a disease resistance capacity (Demirevska-Kepova *et. al.* 2004) [2]. Cu 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 main role in increasing Cu content in roots of chickpea plants.

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