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# Nematicidal Efficacy of a Bioagent *Pseudomonas flourescens* for the Sustainable Management of *Meloidogyne incognita* on *Cicer arietinum L*

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## Abstract

A pot experiment was conducted to evaluate the nematicidal efficacy of a biocontrol agent, *Pseudomonas flourescens* for the management of root-knot nematode, *Meloidogyneincognita* on chickpea (*Cicer arietinum* L.) cv.'Avarodhi' under glasshouse conditions. All the treatments were found to significantly improve the growth and physiological parameters of chickpea and reduction in pathological parameters as compare to untreated inoculated control. The highest improvement was observed in those plants treated with *P. flourescens* alone. Concomitant and sequential inoculation of *P. flourescens* with *M.incognita* also showed significant improvement in growth parameters of chickpea. Least enhancement in growth parameters was observed in those plants inoculated with nematode alone. It may be due to the nematcidal behaviour of *P. flourescens* against root-knot nematode, *M. incognita*. Hence, it may be concluded that *P. flourescens* as biocontrol agent is better substitute against chemical nematicides for the sustainable management of *M. incognita* and reduce environmental hazards.

**Keywords:** Biocontrol agent; Chickpea; *M; Incognita; P; Flourescens*; Sustainable management.



## 1. Introduction

Pulses are the most requisite food all around the world specifically to the vegetarian people and fix atmospheric nitrogen through the nodules present on root system. Pulses occupy an indispensable position in the dietary of vast majority of people in India as well as abroad. The chickpea (*Cicer arietinum* L.) is a legume belonging to the Family-Fabaceae is the most important pulse crop in India. It contains various medicinal properties which are used for reducing the level of cholesterol and blood purification. It also prevent the blood sugar level. Chickpea ranks world's third most important pulse crop after beans and peas. In India, *Meloidogyne* species such as *M. incognita* and *M. javanica* have been reported to cause 19-40% and 24-61% economic losses to chickpea respectively [1]. The root-knot nematode, *Meloidogyneincognita* is a sedentary endoparasite and among the most damaging agricultural pest attacking a wide range of crops including pulses, vegetables and other economically important crops. This pest cause an annual loss of over 100 billion dollars to world agriculture and an estimated 500 million dollars are usually spent on nematode control [2]. Several methods are known to manage the root-knot nematode which includes the use of chemical nematicides, organic amendments, resistant cultivars and biological control. Most of the chemical nematicides are banned due to their toxic effect on the flora and fauna, besides this, they disturbed the ecological equilibrium of the soil. Thus the development of alternative control strategies and long-term integrated approaches is urgently needed in order to replace chemical nematicides. Biocontrol agent appears an environmentally safe and ecologically feasible option for the management of root-knot nematode, *M. incognita* with great potential for promoting sustainable agriculture. *Pseudomonas* spp. and *Pasteuria* spp. found to have nematicidal properties against root-knot nematodes [3, 4]. Application of *P. fluorescens* significantly reduces the nematode population, number of eggmasses and gall indices in tomato and brinjal plants [5]. The aim of the present study was to evaluate the nematicidal effect of *P. flourescens* as biocontrol agent for the management of root-knot nematode *M. incognita* on chickpea under glasshouse conditions.

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# 2. Material and Methods

## 2.1. Host Plant and Pathogen

Chickpea (*Cicer arietinum* L.) cv. 'Avarodhi', Family- Fabaceae was selected as host crop.

## 2.2. Identification of *Meloidogyne incognita* on the Basis of Perineal Pattern

Roots of brinjal heavily infected with root-knot nematode, *M. incognita* were collected from the field of Mehrawal village, Aligarh. Infected roots were washed with running tap water until the soil removed from the roots. The swollen females were removed from the root galls with the help of dissecting needle under microscope. Pierce the female carefully at the cervical end and cut it open. By pressing the female body maximum content was removed in water on the cavity slide. Keep the remaining tissue in cold lactophenol with 0.03% cotton blue for a period of 24 hours at room temperature. The stain will penetrate from inside as well as from the outside. Transfer the stained tissue to very small drop of lactophenol on a perspex slide with handling needle. The perspex slide saved the cutting edge of the scalpel or the razor blade when the tissue is cut. Cut off the posterior end with the scalpel. Furthermore, trim the end so that the perineal area remains in pieces of cuticle only 5-10 times of its area. Inner tissue was carefully removed with handling needle. Transfer the perineal pattern temporarily to a drop of lactophenol-cotton blue 0.03% on a glass slide with a depression.

## 2.3. Bacterial Culture Preparation

Pure isolate of *Pseudomonas fluorescens* (ITCC No. BE0004) was procured from IARI, New Delhi. The formulation was prepared following [6] method using a mixture of 10g of carboxymethyl cellulose and 1 kg of talc. The pH was adjusted to 7.0 by adding 15g of calcium carbonate and the mixture was autoclaved for 30 minutes. The *P. fluorescens* culture was grown on liquid King's B medium (KBM) for 48 hours at room temperature (25±2°C).

## 2.4. Nematode Management by Individual, Sequential and Concomitant Application of *P. fluorescens* with *M. incognita* on Chickpea

The glasshouse experiment was conducted to evaluate the nematicidal efficacy of bioagent**,** *P. fluorescens* against the root-knotdevelopment caused by *M. incognita* and their potential in enhancing the plant growth parameters ofchickpea cv. 'Avarodhi'. Clay pots (15cm in diameter)filled with 1kg autoclaved soil was treated with bioagent applied @ 1g/pot. Inoculation was done 15 days after the germination of the seeds with an amount containing 1500 second stage juveniles of *M. incognita*. Theexperiment was designed as follows:

1) Pf: Inoculated with *P. fluorescens* @ 1g/pot alone.
2) Pf $\longrightarrow$ $Mi_{15}$: *P. fluorescens* treatment given 15 days prior to *M. incognita* inoculation.
3) Pf + Mi: Inoculated with *P. fluorescens* @ 1g and *M. incognita* (1500 J2) simultaneously.
4) Mi $\longrightarrow$ $Pf_{15}$: *M. incognita* inoculated 15 days prior *P. fluorescens* treatment.
5) UIC: Untreated Inoculated Control (*M.incognita* 1500 J2 alone)
6) UUC: Untreated Uninoculated Control

## 2.5. Observation

### 2.5.1. Chlorophyll Estimation

The chlorophyll content in the fresh leaves was estimated following the method of MacKkeney [7]. One gram of finely cut fresh leaves was ground to a fine pulp using a mortar and pestle after pouring 20 ml of 80% acetone. The mixture was centrifuged at 5000 rpm for 5 minutes and collected in 100 ml volumetric flask. The absorbance was read at the wavelength of 645nm and 663nm against blank (80% acetone) on spectrophotometer. The chlorophyll content present in the extract (mg $g^{-1}$tissue) was calculated using the following equation:

$$\text{mg total chlorophyll } g^{-1} \text{ tissue} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W}$$

### 2.5.2. Nitrate Reductase Activity (NRA)

The nitrate reductase activity in fresh leaves was estimated by the following method of Jaworski [8]. The leaves were cut into small pieces (1-2 cm). 0.2g of these chopped leaves were weighed and transferred to plastic vials. To each vial, 2.5 ml of phosphate buffer pH 7.5 and 0.5ml of potassium nitrate solution was added followed by the addition of 2.5 ml of 5% of isopropanol. These vials were incubated in BOD incubator for 2 hours at 30±2$^{o}$C in dark. Incubated mixture of 0.4 ml was taken in a test tube to which 0.3 ml each of sulphanilamide solution and NED-HCl were added. The test tubes were left for 20 minutes for maximum colour development. The mixture was diluted to 5ml Double Distilled Water (DDW). The absorbance was read at 540 nm using spectrophotometer.

## 2.6. Pathological Parameters

### 2.6.1. Number of Eggmass

The eggmasses were counted following the procedure of Daykin and Hussey [9]. The roots were dip in Phloxine B solution (0.015%) for 20 min and were then washed with running tap water to remove the residual Phloxine B. The eggmasses take a pink red colour where as the roots remain colourless or stain lightly.

### 2.6.2. Root-Knot Index

The degree of root-knot nematode infection was recorded according to rating degree given by Taylor and Sasser [10] as under:

| Root-Knot Index | Number of Galls/Root System |
|---|---|
| 0 | 0 |
| 1 | 1-2 |
| 2 | 3-10 |
| 3 | 11-30 |
| 4 | 31-100 |
| 5 | >100 |

### 2.7. Statistical Analysis

The data of the experiments were analyzed statistically using the Statistical Package for the Social Sciences SPSS 12.00 Software (SPSS Inc., Chicago, IL, USA) for analysis of variances (ANOVA). All the values were presented as the mean which were compared according to Least Significant Differences/Critical Differences (C.D) at $p$=0.05 and $p$=0.01 level. Duncan's Multiple Range Test was employed to test for significant difference between the treatments.

## 3. Results

The results of the present experiment showed that bioagent *P. fluorescens* significantly enhancing the growth of chickpea and reduced the root-knot infestation caused by root-knot nematode, *M. incognita* under glasshouse conditions. Among all the treatments highest improvement in plant growth parameters *viz*., shoot and root length (cm), fresh and dry weight of shoot and root (g) were recorded when *P. fluorescens* @1g applied alone followed by *P. fluorescens* was applied 15 days prior the inoculation of *M. incognita*, simultaneously inoculation of *P.fluorescens* and *M. incognita* and sequential inoculation of *P.fluorescens* 15 days after the inoculation of *M.incognita* as compare to untreated inoculated control (Table-1). Highest reduction in plant growth parameters was recorded in those plants inoculated solely with 1500 second stage juveniles of *M. incognita* (Table-1). The nematicidal effect of *P. fluorescens* also has contributed towards the increase in number of flower, pod and nodules with highest being found in the treatment of *P. fluorescens* @1g applied alone. Significant increase in number of nodules, flowers and pod were also observed in sequential inoculation of *P.fluorescens* 15 days prior to *M. incognita* inoculation followed by simultaneously inoculation of *M. incognita* with *P.fluorescens* and sequential inoculation of *P.fluorescens* 15 days after the inoculation of *M.incognita* as compare to untreated inoculated control (Table- 2). Biochemical parameters total chlorophyll content (mg g-1) and nitrate reductase activity (µmolh-1g-1) was found highest when *P. fluorescens* was applied alone. It was followed by sequential inoculation of *P.fluorescens* 15 days prior to *M. incognita* inoculation, simultaneously inoculation of *M. incognita* with *P.fluorescens* and sequential inoculation of *P.fluorescens* 15 days after the inoculation of *M.incognita* as compare to untreated inoculated control (Table-2). Highest reduction in pathological parameters *viz*., number of eggmasses and root-knot index per plant was observed when *P. fluorescens* @1g/pot applied alone.On inoculating *P. fluorescens* concomitantly and sequentially with *M. incognita* also showedreduction in number of eggmasses and root-knot index per plant as compare to untreated inoculated plant. From the above results it was found that *P. fluorescens* proved effective in enhancing the growth and physiological parameters and reduction in pathological parameters tested.

**Table-1.** Effect of Individual, Sequential and Concomitant Inoculation of *Meloidogyne incognita* and *Pseudomonas fluorescens* on the Plant Growth of Chickpea Cv. 'Avarodhi' In Pots

| Treatment | Length (cm) | | Total | Fresh weight (g) | | Total | Dry weight (g) | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Shoot | | Shoot | Root | | Shoot | Root | |
| *Pseudomonas Flourescens* Alone | 66.5a | 25.4a | 91.9a | 82.4a | 23.46a | 105.86a | 18.27a | 5.19a | 23.46a |
| $Pf_{15} \longrightarrow$ Mi | 47.2c | 17.5c | 64.7c | 62.7c | 16.36c | 79.06c | 12.11c | 3.1c | 15.21c |
| Pf + Mi | 43.4d | 16.1d | 59.5d | 54.45d | 14.76d | 69.21d | 11.01d | 2.67d | 13.68d |
| $Mi_{15} \longrightarrow$ Pf | 38.1e | 14.3e | 52.4e | 49.21e | 13.49e | 62.7e | 9.61e | 2.47d | 12.08e |
| UIC | 27.8f | 9.2f | 37f | 34.54f | 8.01f | 42.55f | 6.71f | 1.71e | 8.42f |
| UUC | 58.26b | 20.5b | 78.76b | 71.46b | 17.32b | 88.78b | 15.21b | 4.37b | 19.58b |

Values are mean of four replicates.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$).
$Pf_{15} \rightarrow Mi$ = *Pseudomonas fluorescens* treatment given 15 days prior nematode inoculation, $Mi_{15} \rightarrow Pf$ = *Meloidogyne incognita* inoculated 15 days prior treatment.
UIC- Untreated Inoculated Control**,** UUC-Untreated Uninoculated Control.

**Table-2.** Effect of Individual, Sequential and Concomitant Inoculation of *Meloidogyne incognita* and *Pseudomonas fluorescens* on Pathological and Physiological Parameters of Chickpea cv. 'Avarodhi' in Pots

| Treatment | Chlorophyll Content (mg/g) | NRA (µmol $g^{-1}h^{-1}$) | Number of Flowers | Number of Pods | Number of Nodules | Eggmasses/ Plant | Root-Knot Index |
|---|---|---|---|---|---|---|---|
| *Pseudomonas flourescens* alone | 2.92a | 0.535a | 61a | 58a | 81a | 0e | 0e |
| $Pf_{15}$ ⟶ Mi | 1.87c | 0.340c | 41c | 38c | 50c | 50d | 2.6cd |
| Pf + Mi | 1.62d | 0.315d | 35d | 33d | 45d | 56c | 3.0c |
| $Mi_{15}$ ⟶ Pf | 1.48e | 2.55e | 31e | 29e | 39e | 61b | 3.3b |
| UIC | 1.20f | 0.221f | 24f | 21f | 33f | 145a | 5a |
| UUC | 2.60b | 0.467b | 53b | 50b | 70b | 0e | 0e |

Values are mean of four replicates.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$).
$Pf_{15}$ →Mi = *Pseudomonas fluorescens* treatment given 15 days prior nematode inoculation, $Mi_{15}$ →Pf = *Meloidogyne incognita* inoculated 15 days prior treatment.
NRA- Nitrate Reductase Activity, UIC- Untreated Inoculated Control**,** UUC-Untreated Uninoculated Control.

# 4. Discussion

Our results indicated that *P. fluorescens* has significant potential as biocontrol agent against the root-knot nematode, *M. incognita* under glasshouse conditions. Our results are in conformity with several scientists [5, 11, 12]. Inoculation of chickpea with *P. fluorescens* can significantly reduce the population of root-knot nematode and disease severity caused by root-knot nematode. It may be found that biocontrol agent significantly brought down the population of root-knot nematode and some compounds released from bioagent reduced the nematode infestation. The biocontrol agent *P. fluorescens* amended soil was suggested to increase the resistance of chickpea plants against root-knot nematode, *M. incognita.* Siddiqui [13] reported that *Pseudomonas* spp. were better in improving plant growth and reducing galls and nematode multiplication and suggested that *Pseudomonas fuorescens* may successfully be used for the biocontrol of *Meloidogyne incognita*. It was found that formation of galls and eggmasses on root system have direct effect on growth performance and yield of plant. Bioagents were found to be significantly effective in reducing the damage and increasing the growth parameters of crops. Elyousr [14] reported that *P. fuorescens* and *P. aeruginosa* reduced root gall index after sixty days treatment. Metabolites produced by some bacteria especially *Burkholderia* spp., *Pseudomonas* spp. and *Bacillus* spp., interfere with nematode behaviour, feeding and reproduction, thereby reducing penetration and damage in plants [15]. Therefore, it was concluded that the severe infection caused by root-knot nematode could be lowered by the use of *P. fuorescens* as bioagent in view of eco-friendly environment. This has an advantage against expensive and hazardous chemical nematicides.

# 5. Conclusion

From the above study it may be concluded that biocontrol agent *P. fuorescens* showed nematicidal activity against root-knot nematode, *M. incognita* and enhanced the growth and physiological parameters of Chickpea cv.'Avarodhi' under glasshouse conditions. It may be due to the presence of various phytochemicals released from biocontrol agent which showed toxic effect on survivality of root-knot nematode. This method of nematode control may contribute to minimize toxicity and the hazardous effect of chemical nematicides on flora and fauna. Hence, the outcome of the results revealed that the use of biocontrol agent *P. fuorescens* may provide safe and environmentally reliable alternative for the root-knot nematode management programme for sustainable environment.

# 6. Conflict of Interest

No Conflict of interest

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