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**R Surega**
Ph.D Scholar, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

**S Ramakrishnan**
Retired Professor, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

**Correspondence**
**R Surega**
Ph.D Scholar, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

# Bio-management of root knot nematode, *Meloidogyne incognita* in turmeric (*Curcuma longa* L.) under different irrigation systems

**R Surega and S Ramakrishnan**

**Abstract**
The present field experiments conducted on turmeric (var: Local) under conventional and drip irrigation method to evaluate the efficacy of commercially available biocontrol agent of *Pseudomonas fluorescens* in talc and liquid formulation (bionemagation) during Aug to Dec'2014. Talc and liquid formulation (bionemagation) was applied at different dosage with different time of application and compared with untreated and standard check carbofuran 3G against root knot nematode, *Meloidogyne incognita.* The study results demonstrated using bioformulation was optimised as 2.5 kg/ha at 90 and 150 DAP under conventional method in respect to talc formulation. Similarly, the bionemagation with liquid formulation of *P. fluorescens* at 3 lit/ha at 90 and 150 DAP under drip irrigation for the effective management of root knot nematode and improve the rhizome yield with curcumin content in turmeric.

**Keywords:** Turmeric, Drip irrigation, Conventional flood irrigation, bio-formulations, root knot nematode, curcumin content

## 1. Introduction
Turmeric (*Curcuma longa* L.) is being cultivated both under conventional and drip irrigated systems. Several biotic and abiotic stresses hamper the sustainable cultivation of turmeric [10]. Among the biotic stresses, plant parasitic nematodes play an important role in affecting the crop growth and causes subsequent yield loss of turmeric. The nematodes associated with turmeric includes root knot nematode (*Meloidogyne* spp.), burrowing nematode (*Radopholus similis*) and other species belonging to the genera *Rotylenchulus, Helicotylenchus, Longidorus, Xiphinema, Hoplolaimus, Pratylenchus, Tylenchus, Tylenchorhynchus, Caloosia* and *Aphelenchs* [14]. Of all the nematodes the root knot nematode *Meloidogyne* spp. is considered as a key nematode pest of turmeric grown in Tamil Nadu [10] and caused in rhizome yield loss to an extent of 45.3 per cent [4].

There are number of practices for management of plant parasitic nematodes in which chemical nematicides is used against nematodes by farmer because it is effective, easy to apply and show rapid effects [6]. But on the other hand it may cause degradation in soil fertility, environmental pollution and also hazardous for animals and human being. That's why biological control are more promising management practice and also economically and ecofriendly [15].

Seenivasan [22] reported that *P. fluorescens* was found to be more effective for the management of *M. incognita* in turmeric. The authors reported that *P. fluorescens* at 10 g/kg of turmeric rhizome improved the plant growth characters and reduced *M. incognita* population in root and soil. [18] proved that the application of biocontrol agent *P. chlamydosporia* to the beds at the time of sowing @ 20 g/bed ($10^6$ cfu/g) was effective for the management of nematodes in turmeric.

Ramakrishnan [17] reported that the use of drip irrigation in agriculture has increased rapidly during the past 25 years. The drip irrigation has been used to deliver fertilizers and pesticides. Studies on the application of nematicides or other pest control agents *via* drip irrigation for the management of nematodes were encouraging and it is inferred that the application of nematicides through drip irrigation was simple, safe and cost effective than conventional method of application of nematicides. It is also reported that the drip irrigation alone reduced population of nematodes *viz, Xiphinema americanum* and *Pratylenchus penetrans* associated with peach root [8].

The increase in crop yield in drip irrigated system was high compared to crops irrigated through conventional system as reported by [2, 6, 11, 20]. Therefore, the present experiments was conducted to study the effect of bacterial bio-agent of *P. fluorescens* against root-knot nematode, *M. incognita* infecting turmeric crop.

## 2. Materials and Methods

Field experiments were conducted during Aug to Dec'2014 in root knot nematode endemic areas on turmeric local 'Erode' grown under conventional and drip irrigated method.

### 2.1 Conventional method

The field experiment conducted on turmeric (var: Local) adopting the conventional method of flood irrigation the bioefficacy of *P. fluorescens* available commercially in talc formulation was evaluated at different dosage with different time of application and compared with untreated and standard check carbofuran 3G. All the treatments were replicated four times in Randomised Block Design (RBD) with plot size of 40 $m^2$. Observations on nematode incidence/population, plant growth parameters and curcumin content in different treatments were made at the time of termination of the experiment made at 300 DAP.

#### 2.1.1 Nematode population /Incidence in soil and root

A composite soil sample of 250 g was drawn from each replication from the rhizosphere region at 15 cm depth at monthly interval and at the time of concluding the experiment. The collected samples were analyzed for nematode population using standard procedure [7, 21]. Similarly, root sample (10 g) collected replication wise were assayed for nematode population using standard procedure [13].

#### 2.1.2 Plant growth parameters

##### 2.1.2.1 Shoot and root length

The length of plant was measured from the ground level to the tip of the youngest unfurled leaf in shoot and from base of the stem to the tip of the root the values were expressed in cm.

##### 2.1.2.2 Shoot and root weight

The plants were cut at collar region, separated, weighed and expressed in g $plant^{-1}$.

##### 2.1.2.3 Rhizome weight

Fresh weight of mother rhizomes/plant was recorded immediately after harvest by separating the mother rhizomes followed by cleaning of soil and adhering roots and expressed in g.

#### 2.1.3 Analysis for curcumin content

A sample of 200 g of cured rhizome was ground in a Wiley Mill and sieved the powdered sample through 40 mesh sieve. One hundred milligram of turmeric powder was taken in the extraction flask and 30 ml of alcohol was added and refluxed for two and half hr. The extract was cooled and filtered quantitatively into a 100 ml volumetric flask. Then the extracted residue was transferred to the filter, washed thoroughly and diluted to the mark with 95 per cent alcohol and 20 ml of filtered extract was pipetted into a 250 ml volumetric flask and diluted to the volume with 25 per cent alcohol. The absorbance of extract and standard solution was measured at 425 nm against an alcohol blank [2].

Standard curcumin (25 mg) was weighed into 100 ml volumetric flask and dissolved and diluted to the mark with alcohol. One ml of the solution was transferred to 100 ml volumetric flask and made upto 100 ml with 95 per cent alcohol. This standard solution contained 0.0025 g of curcumin per litre with absorbance of 0.42 at 425 nm. The procedure described by [12] for the analysis of curcumin content.

$$\text{Curcumin percentage} = \frac{\text{Absorbance at 425 nm x 125}}{\text{Cell length (cm) x A x Sample weight (g)}}$$

$$\text{Absorptivity of curcumin (A)} = \frac{\text{Absorbance of standard solution at 425 nm}}{\text{Cell length (cm) x Concentration } (g^{-1})}$$

$$= \frac{\text{OD value x 125 x 0.0025}}{\text{0.42 x 0.1 x 1}}$$

### 2.2 Drip irrigation

Similar experiment as above was conducted on turmeric (Local: Erode) cultivated in drip irrigated system. The bioefficacy of *P. fluorescens* available in liquid formulation was evaluated at different dosages/time of application in this experiment. All the treatments were replicated five times in RBD with plot size of 40 $m^2$. The observations on plant growth parameters, nematode incidence /population and curcumin content were made at the time of termination of experiment at 300 DAP and the procedure followed as same above experiment.

### 2.3 Statistical analysis

The data recorded in the laboratory and field experiments were statistically analyzed by following the methods given by [9].

## 3. Results and Discussion

### 3.1 Conventional method

#### 3.1.1 Nematode population

Observations made on nematode population at monthly interval and the results of this experiment on soil application of *P. fluorescens* as talc formulation in single round at 30 DAP and in two rounds at 90 and 150 DAP @ 2.5 kg/ha caused significant reduction in nematode population over untreated control and carbofuran. The reduction in nematode population caused by the most effective treatment of *P. fluorescens* @ 2.5 kg at 90 and 150 DAP immediately after application was 84.34 and 32.80 per cent at fourth month and 86.49 and 88.35 per cent at sixth month in soil and root respectively and its biocontrol potential was maintained throughout the period of observations with slight fluctuation compared to untreated control. While the per cent reduction in nematode population immediately after the application of *P. fluorescens* in single round at 30 DAP was lesser and it was 15.76 and 34.02 per cent in soil and root respectively although its biocontrol potential seems to be maintained in favour of plants and against nematodes throughout the cropping period (Table 1).

The rhizobacterium *P. fluorescens* available commercially in talc formulation with ($2.5 \times 10^8$ cfu/g) at the rate 2.5 kg/ha in two rounds at 90 and 150 DAP was found to be more effective in checking the nematode population both in soil and root. The present findings are in agreement with Oostendorp and Sikora [16] ; Siddiqui and Shakukat [23] confirmed that the

*P. fluorescens* has the potential to affect nematodes by a variety of mode of action *viz.*, induction of systemic resistance, niche exclusion, competition for nutrients, siderophore activity, production of antibiotics, interfering with plant nematode recognition and alteration of nematode behavior.

### 3.1.2 Plant growth and yield

Soil application of *P. fluorescens* @ 2.5 kg in two rounds at 90 and 150 DAP resulted with highest increase in shoot length (31.04%) and weight (30.01%); root length (35.28%) and weight (38.18%) and differed significantly among the treatments. The treatment also registered highest rhizome yield of 31.25 ton as against 25.53 ton in untreated per hectare. Similarly the highest curcumin content (4.58) was also registered by this treatment (Table 2).

**Table 1:** Influence of *P. fluorescens* on root knot nematode population in turmeric grown under conventional method

| Treatments | | Nematode population in soil (250 g) and root (10g) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Jun** | **July** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** | **Jan** | **Feb** | **Mar** |
| *P.fluorescens* @ 2.5 kg /ha at 30 DAP | Soil | 120.63 (5.99) | 105.83 (83.90) | 46.64 (73.30) | 51.87 (74.09) | 55.60 (75.76) | 72.80 (70.55) | 26.67 (66.94) | 86.56 (85.75) | 20.88 (86.63) | 19.49 (85.19) |
| | Root | – | 2.56 (34.02) | 0.84 (81.73) | 2.82 (62.69) | 2.80 (65.96) | 3.60 (60.09) | 4.20 (57.58) | 2.30 (61.02) | 2.74 (52.26) | 1.34 (73.46) |
| *P. fluorescens* @ 2.5kg/ha at 90 & 150 DAP | Soil | 125.41 (2.26) | 133.86 (17.00) | 140.45 (21.44) | 31.34 (84.34) | 28.90 (87.40) | 33.40 (86.49) | 30.63 (88.31) | 89.27 (52.10) | 27.64 (82.30) | 55.20 (58.05) |
| | Root | – | 2.43 (37.37) | 2.58 (43.91) | 1.30 (82.80) | 1.14 (86.14) | 1.05 (88.35) | 1.00 (89.89) | 1.74 (70.50) | 1.40 (75.60) | 1.28 (74.65) |
| Carbofuran 3G @ 1kg a.i/ha at 90 & 150 DAP | Soil | 122.60 (4.45) | 129.26 (19.85) | 137.45 (22.90) | 102.28 (48.92) | 88.40 (61.46) | 53.20 (78.48) | 44.80 (82.91) | 39.67 (78.72) | 37.99 (75.68) | 35.56 (72.98) |
| | Root | – | 2.88 (25.77) | 2.90 (36.95) | 1.84 (72.66) | 2.32 (71.01) | 1.83 (79.71) | 3.50 (64.64) | 3.10 (47.45) | 3.00 (47.73) | 2.85 (43.56) |
| Untreated control | Soil | 128.32 | 161.24 | 178.22 | 200.24 | 229.40 | 247.20 | 262.20 | 186.4 | 156.2 | 131.60 |
| | Root | _ | 2.61 | 4.60 | 7.56 | 8.23 | 9.02 | 9.90 | 5.90 | 5.74 | 5.05 |
| CD (P=0.05) | Soil | 10.32 | 11.9 | 18.3 | 21.6 | 28.5 | 27.4 | 25.2 | 18.4 | 16.3 | 11.9 |
| | Root | _ | 0.12 | 0.61 | 1.10 | 1.24 | 1.36 | 1.39 | 1.73 | 1.47 | 0.49 |

Figures in parentheses are per cent increase (+) or decrease (-) over control

**Table 2:** Effect of *P. fluorescens* on plant growth and yield of turmeric growth under conventional method

| Treatments | Shoot | | Root | | Yield (t/ha) | Curcumin Content (%) |
|---|---|---|---|---|---|---|
| | **Length (cm)** | **Weight (g)** | **Length (cm)** | **Weight (g)** | | |
| *P. fluorescens* @2.5 kg /ha at 30 DAP | 130.00 (19.69) | 132.40 (18.28) | 6.76 (22.93) | 6.86 (25.66) | 31.10 (21.82) | 4.05 (17.40) |
| *P. fluorescens* @ 2.5kg/ha at 90 &150 DAP | 151.40 (31.04) | 154.60 (30.01) | 8.05 (35.28) | 8.25 (38.18) | 31.25 (22.41) | 4.58 (32.75) |
| Carbofuran @ 1kg a.i/ha at 90 & 150 DAP | 137.00 (23.80) | 141.00 (23.26) | 7.06 (26.20) | 7.14 (28.57) | 28.83 (12.92) | 3.68 (6.67) |
| Untreated control | 104.40 | 108.20 | 5.21 | 5.10 | 25.53 | 3.45 |
| CD(P=0.05) | 13.56 | 15.52 | 0.65 | 0.71 | 2.16 | 0.36 |

Figures in parentheses are per cent increase (+) or decrease (-) over control

Soil application of *P. fluorescens* resulted with remarkable improvement in yield attributes of turmeric including the yield quantitatively and qualitatively in the present study. These findings coincidence with the findings of [4] have reported that *P. fluorescens* is known for plant growth promotion due to production of plant growth regulators *viz.*, auxin, gibberellins and cytokinin or indirectly by stimulating nutrient uptake.

## 3.2 Drip irrigated method

### 3.2.1 Nematode population

The population of *M. incognita* was suppressed significantly both in soil and root in the treatment of *P. fluorescens* as liquid formulation irrespective of dosage and time of application over carbofuran as chemical check and untreated control. The highest degree of nematode control was observed in the treatment of *P. fluorescens* @ 3 lit/ha at 90 and 150 DAP among the different dosage of *P. fluorescens* with different time of application. The reduction in nematode population.

**Table 3:** Influence of *P. fluorescens* on root knot nematode in turmeric grown under drip irrigated method

| Treatments | | Nematode population in soil (250 g) and root (10g) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Jun | July | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
| *P.fluorescens* @ 1 lit/ha at 30 DAP | Soil | 107.75 (10.02) | 43.12 (71.39) | 60.01 (61.61) | 80.58 (50.39) | 92.43 (43.60) | 80.00 (52.45) | 86.75 (50.07) | 43.67 (70.55) | 32.14 (75.53) | 30.67 (74.12) |
| | Root | – | 1.05 (64.16) | 1.92 (69.76) | 4.55 (40.75) | 4.87 (45.58) | 5.01 (44.33) | 4.95 (59.51) | 3.85 (54.43) | 2.43 (75.76) | 1.76 (72.85) |
| *P. fluorescens* @ 2 lit/ha at 90 DAP | Soil | 109.75 (8.35) | 112.25 (10.74) | 120.45 (24.95) | 85.48 (47.68) | 52.75 (68.16) | 23.01 (86.33) | 22.87 (86.84) | 26.89 (81.58) | 20.87 (84.04) | 18.55 (84.35) |
| | Root | – | 2.50 (14.67) | 3.32 (48.03) | 1.20 (84.37) | 1.03 (88.49) | 2.72 (69.77) | 3.56 (70.88) | 3.80 (55.02) | 2.32 (76.20) | 0.83 (86.92) |
| *P. fluorescens* @ 3 lit/ha at 90&150 DAP | Soil | 103.86 (13.09) | 113.16 (10.01) | 135.25 (15.53) | 48.75 (69.99) | 25.25 (84.76) | 25.10 (85.14) | 20.45 (88.23) | 17.89 (87.75) | 16.67 (87.25) | 16.01 (86.49) |
| | Root | – | 2.60 (11.26) | 2.63 (58.58) | 0.83 (89.19) | 1.10 (87.70) | 0.95 (89.44) | 1.20 (90.18) | 1.50 (82.24) | 1.67 (82.87) | 0.85 (86.61) |
| Carbofuran 3G @ 1kg a.i/ha at 90& 150 DAP | Soil | 110.00 (1.41) | 119.67 (4.83) | 142.32 (11.33) | 93.5 (42.44) | 57.01 (65.59) | 44.25 (73.70) | 42.5 (75.54) | 35.75 (75.51) | 46.67 (64.31) | 30.56 (74.21) |
| | Root | – | 2.33 (20.47) | 4.32 (31.96) | 3.81 (50.39) | 2.43 (75.37) | 2.05 (77.22) | 1.85 (84.87) | 6.98 (17.39) | 7.53 (22.76) | 4.55 (28.34) |
| Untreated control | Soil | 119.75 | 125.75 | 160.50 | 162.45 | 165.66 | 168.25 | 173.75 | 146.00 | 130.75 | 118.50 |
| | Root | _ | 2.93 | 6.35 | 7.68 | 8.95 | 9.00 | 12.23 | 8.45 | 9.75 | 6.35 |
| CD(P=0.05) | Soil | 12.31 | 13.07 | 14.31 | 18.91 | 15.26 | 13.62 | 8.48 | 7.50 | 9.42 | 8.65 |
| | Root | _ | 0.39 | 1.09 | 0.99 | 1.20 | 1.19 | 1.04 | 0.85 | 0.68 | 0.81 |

Figures in parentheses are per cent increase (+) or decrease (-) over control

is apparent in this treatment immediately after application i.e fourth month in soil (69.99%) and root (89.19%) and sixth month in soil (85.14%) and root (89.44) compared to untreated control (Table 3).

The rhizobacterium *P. fluorescens* delivered through drip irrigation as liquid formulation exhibited highest biocontrol potential in the suppression of *M. incognita* in turmeric. The experimental results of similar studies with liquid formulation of *P. fluorescens* for the management of nematodes in tomato [19] confirmed the effectiveness of liquid formulation of *P. fluorescens* in the management of *M. incognita* in turmeric with drip irrigated method.

### 3.2.2 Plant growth and yield

Bionemagation with *P. fluorescens* as liquid formulation @ 3 lit/ha in two rounds at 90 and 150 DAP resulted with highest increase in yield attributes *viz.*, shoot length (38.24%) and weight (38.76%); root length (46.15%) and weight ( 46.89%) and it is reflected through increase in yield by 14.92 per cent over untreated control with regard to quantitative yield. The qualitative yield in terms of curcumin content (4.95%) was also found to be increased remarkably compared to untreated control (Table 4). The high biocontrol potential of liquid formulated *P. fluorescens* in the management of root knot nematode in turmeric was resulted with significant improvement in plant biomass including rhizome yield and cucumin content as observed by [19] in tomato.

**Table 4:** Effect of *P. fluorescens* on plant growth and yield of turmeric grown under drip irrigated method

| Treatments | Shoot | | Root | | Yield (t/ha) | Curcumin Content (%) |
|---|---|---|---|---|---|---|
| | Length (cm) | Weight (g) | Length (cm) | Weight (g) | | |
| *P. fluorescens* @ 1 lit/ha at 30 DAP | 142.70 (22.74) | 132.50 (16.23) | 6.80 (33.09) | 6.85 (31.39) | 36.25 (11.54) | 3.83 (21.59) |
| *P. fluorescens* @ 2 lit/ha at 90 DAP | 152.25 (27.59) | 155.00 (28.39) | 7.13 (36.23) | 7.05 (33.33) | 36.82 (13.29) | 4.28 (35.87) |
| *P. fluorescens* @ 3 lit/ha at 90&150 DAP | 178.50 (38.24) | 181.25 (38.76) | 8.45 (46.15) | 8.85 (46.89) | 37.35 (14.92) | 4.95 (57.14) |
| Carbofuran 3G @ 1kg a.i/ha at 90& 150 DAP | 128.00 (13.87) | 130.25 (14.78) | 5.75 (20.87) | 6.00 (21.63) | 35.80 (10.15) | 3.68 (16.83) |
| Untreated control | 110.25 | 112.01 | 4.55 | 4.70 | 32.50 | 3.15 |
| CD(P=0.05) | 8.51 | 9.08 | 0.83 | 1.18 | 3.31 | 0.29 |

Figures in parentheses are per cent increase (+) or decrease (-) over control

## 4. Conclusion

The rhizobacterium, *P. fluorescens* in talc and liquid formulation was found to be effective for the management of root knot nematode in turmeric grown under conventional method and drip irrigated conditions. The dosage and time of application is optimised as 2.5 kg/ha at 90 and 150 DAP for the maximisation of degree of nematode control and economic yield of turmeric grown under conventional method. Similarly, the bionemagation with liquid formulation of *P. fluorescens* was optimised as 3 lit/ha at 90 and 150 DAP for the effective management of root knot nematode under drip irrigation method.

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