

Biomanagement of root-knot nematode, *Meloidogyne incognita* by egg parasitic fungus, (*Pochonia chlamydosporia*) on Okra

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Abstract Management of root-knot nematode, *Meloidogyne incognita* infecting okra was achieved through *in vitro*, *in vivo* and micro plot trials by an antagonistic fungus, *Pochonia chlamydosporia*. Effect of seed treatment and soil application of *P. chlamydosporia* was tested at various doses under *in-vivo* and micro plot of 1 m² size. A 96% egg parasitism of *M. incognita* by *P. chlamydosporia* was recorded in laboratory condition. *P. chlamydosporia* caused enhanced plant health and suppressed *M. incognita* population in all the treated plants in both experiments. The maximum plant growth characters 42 cm, 9.4 g, 36 cm and 2.7 g with a recovery of 80.9, 74.1, 73.9 and 80% over control for shoot length, shoot weight, root length and root weight respectively was recorded in the treatment that received soil application of the

P. chlamydosporia @ 3% w/w under pot condition whereas in micro plot trial a general increase in all the growth parameters including fruit yield except root weight was observed in all the treatments receiving *P. chlamydosporia*. Application of *P. chlamydosporia* suppressed nematode infection (galls, egg masses/plant and eggs/egg mass) by up to 54.8, 53.7 and 46.5% respectively under pot trial and up to 88.9, 88.6 and 49.1% respectively under micro plot experiment.

Keywords: Biomanagement, root knot nematode, *Meloidogyne incognita*, *Pochonia chlamydosporia*

Introduction

Nematode management through biocontrol agents is gaining importance in the new millennium as other measures have become less attractive to growers/farmers where economics demand specialization and intensification. In the last decade nematode management through biocontrol agents was in the forefront of research and development. Management of plant parasitic nematodes through ecofriendly means is the need of this era. In view of the increasing awareness about environment and demand of organic farming, it became essential to evaluate the potentiality of the egg parasitic fungus, *P. chlamydosporia* as a biocontrol agent to manage *M. incognita* on Okra cv Pusa Sawani.

Materials and methods

The egg masses collected from root-knot infected tomato plants grown at Indian Agricultural research Institute, New Delhi were separated out from galls and kept in Petri dishes. These were agenzized with 1000ppm of Sodium hypochlorite (NaOCl) for one minute, followed by three washings in sterilized distilled water and placed aseptically in Petri dishes containing potato dextrose agar (PDA) medium amended with 0.05 % Streptomycin sulphate to check bacterial growth, incubated for a week

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at 25±2°C. Fungi grown from the eggs were transferred aseptically to sterile water agar medium contained in 10 cm dia Petri plates. Of the several isolated fungi, *Pochonia chlamydosporia* was separated and purified using either single spore or hyphal tip technique.

For achieving the pure culture of root-knot nematode, *M.incognita* was raised on brinjal cv Pusa Purple Long in 20 cm dia earthen pots containing the steam sterilized soil. For this 2nd stage juveniles hatched from single egg mass were inoculated on brinjal plants for multiplication. Large number of egg masses from brinjal plants, on which the culture of *M.incognita* was multiplied, handpicked and used for inoculation.

In vitro, egg parasitism by *P. chlamydosporia*: *In vitro* studies were conducted to test the efficacy of indigenous strain of *P.chlamydosporia* as egg parasite of *M.incognita*. The fungus was allowed to grow in 5 cm dia Petri plates containing PDA and incubated at 25±2°C. *P.lilacinus* was kept as test fungus for this study. The above collected egg masses of *M.incognita* were surface sterilized and then of equal size were placed on each of the sporulating fungal Petri plates @ 1, 2, 3 and 4 egg mass (containing approximately 225 eggs/egg mass). Five replications were maintained for egg mass density and fungus. After two days of inoculation egg masses were transferred to 2% water agar plates which in turn were incubated at 25±2°C. Observations were recorded on egg parasitism after seven days of incubation. Egg masses were picked up from each Petri plate and crushed in 0.5 % cotton blue in lacto phenol solution and observed under compound microscope.

In vivo trial, preparation of bioagent: The antagonistic fungus, *P. chlamydosporia* previously isolated from agriculture soil was raised in PD broth contained in the conical flasks and incubated at 25±2°C for 21 days. Thereafter, 300 ml of broth with fungus was taken and mixed thoroughly. Fungal biomass was mixed with 1 kg of talc powder as a carrier material to bulk up the material. To this 20g fine powder of chickpea pod waste and carboxymethyl cellulose (2% w/w) were added to the carrier material as carbon source and sticking agent respectively. All the contents were remixed manually and dried under shade. The material after drying was packed in polythene bags for further bio-management trials. The concentration of chlamydospores was estimated in diluted samples using a haemocytometer. The spore load of *P.chlamydosporia* was maintained at 2.3 x 10⁸ cfu/g.

The pot trial was conducted under glass house condition. Earthen pots of 15 cm dia were filled with 1 kg autoclaved sandy loam soil. The trial comprised of seven

treatments namely- T1-Healthy (no nematode and no fungus), T2 - Nematode alone, T3 - Nematode + seed treatment of *P. chlamydosporia* @ 10 g/kg seed, T4 - Nematode + seed treatment of *P. chlamydosporia* @ 15 g/kg seed, T5 - Nematode + seed treatment of *P. chlamydosporia* @ 20 g/kg seed, T6 - Nematode + soil application of *P. chlamydosporia* @ 1.5 % w/w, T7 - Nematode + soil application of *P. chlamydosporia* @ 3 % w/w. Seeds were slightly wet with water before treating seeds with *P. chlamydosporia*. The suspension of *P. chlamydosporia* (2.3 x 10⁸ spores/ml) and seed were than shaken well to get even coating of the bioagent. Soil application of the fungus was done at the time of filling of pots having the same level of inoculums/g soil. Single seed of okra cv. Pusa Sawni was sown in each pot. Around each seed 10 egg masses of equal size were placed and each egg mass consisted of approximately 225 eggs. Initial population at the time of sowing worked out to be 2.5 eggs/g soils. Three replications were kept for each treatment. After 45 days of germination observations were recorded on plant growth parameters and nematode multiplication.

Micro plot trial: The replicated trial was conducted in micro plots of size 1m² with cemented walls under net house which comprised of six treatments namely, T1 - Healthy (no nematode and no fungus), T2-Nematode alone, T3 - Seed treatment with *P. chlamydosporia* (spore load 2.3 x 10⁸ cfu/g) @ 3% w/w, T4 - Soil application with *P. chlamydosporia* @ 30 g/micro plot 7 days before sowing (DBS), T5-Soil application with *P. chlamydosporia* @ 30 g/micro plot at the time of sowing and soil application @ 30g/micro plot and seed treatment @ 3% w/w. Initial nematode inoculums at the time of sowing worked out to be 2.5 J₂/g soil. Four replications were kept for each treatment. The experiment design consisted of randomized block. After 90 days of germination, observations were recorded on plant growth parameters and nematode multiplication. Regular watering has been given as and when needed. The number of J₂s was expressed per 250 cm³ of soil. Yield of okra was assessed by determining the weight fresh fruit from each plot. Regular irrigation has been given as when needed.

Statistical analysis: The data on egg parasitism, plant health and nematode multiplication were analyzed and subjected to ANOVA.

Results and discussion

In vitro

The indigenous isolate of egg parasitic fungi, *P. chlamydosporia* and *P. lilacinus* parasitized eggs of

Table 1: Per cent egg parasitism by *P. chlamydosporia* and *P. lilacinus* as influenced by egg density of root-knot nematode, *M. incognita*.

Number of egg masses	<i>P. chlamydosporia</i>	<i>P. lilacinus</i>
1	85.0 (67.3)*	55.3 (48.3)
2	86.8 (68.7)	61.0 (51.4)
3	90.0 (71.6)	65.3 (53.90)
4	96.0 (78.7)	70.5 (57.1)
CD (P=0.05)	2.0	2.2

*Figures presented in parenthesis are arc sine transformed values

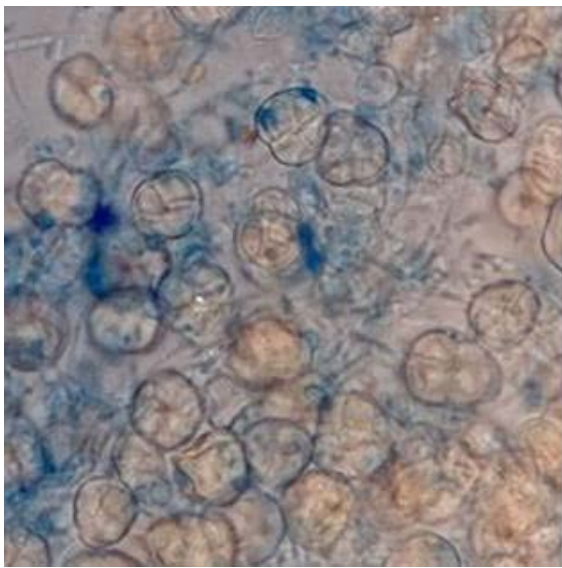


Fig. 1: Chlamydo spores liberated from distorted egg of *M. incognita*

M. incognita at various degrees (Table 1). *P. chlamydosporia* showed high degree of parasitism (96%) as compared to *P. lilacinus* (70.5%) at 4 egg masses/plate. *P. chlamydosporia* caused much more egg infection in comparison to *P. lilacinus* (Table 1). A positive correlation between number of egg masses and egg parasitism was observed i.e., with increase in number of egg masses there was a corresponding increase in egg parasitism. Chlamydo spores were observed inside the eggs and eggs were distorted and liberated chlamydo spores (Fig.1 and 2).



Fig. 2. Nematode egg invaded by *P. chlamydosporia*

In vivo

Application of *P. chlamydosporia* generally enhanced all the plant growth parameters namely shoot length, fresh shoot weight, root length and fresh root weights over control (Table 2). The maximum shoot length (42 cm), fresh shoot weight (9.4 g), root length (36 cm) and fresh root weight (2.7) was recorded in the treatment (T7) that received soil application of *P. chlamydosporia* @ 3% w/w and a minimum 23.3 cm, 5.4 g, 20.7 cm and 1.5g respectively was recorded in the treatment receiving nematode alone (T2). Application of *P. chlamydosporia* as seed treatment @ 10 g/kg of seed (T3) did not contribute so much in plant health as the plant growth parameters were recorded at par with T2, however, an apparent increase in plant health in all seed treated treatments (T3, T4 and T5) was observed. Both the treatments having soil application (T6 and T7) did not show any significant difference statistically. The maximum recovery in plant health up to 80.9% was recorded in T7 which was also at par with healthy (T1). Further it was observed that at highest dose (3% w/w) of soil application of *P. chlamydosporia* (T7), growth of all parameters was found best even more than healthy plants where no nematode and fungus was inoculated (Table 2). The greatest number of galls/plant (171.6), egg masses/plant (153.2) and eggs/egg mass (353.9) was observed in the treatment that received *M. incognita* only. Soil application of *P. chlamydosporia* at both doses reduced number of galls, number of egg masses/plant and eggs/egg mass compared to nematode alone. However, there were no significant differences observed amongst treatments T2, T3, T4 and T5. The minimum number of galls/plant (77.5), egg masses/plant (70.9) and eggs/egg mass (189.2) was observed in the treatment T7 where soil application of *P. chlamydosporia* @ 3% w/w was applied. The per cent reduction in number of galls/plant was found in the order of 54.8, 54.4, 2.4 and 10.7 respectively in treatments receiving soil application of *P. chlamydosporia* @ 3%, w/w, 1.5% w/w and seed treatment @ 20g, 15g and 10g/kg seed (T7>T6>T5>T4>T3). The same trend was also recorded in case of number of egg masses/plant and number of eggs/egg mass as presented in Table 3.

Micro plot experiment: In micro plots experiment, a general increase in plant growth parameters was also recorded in treatments that received application of the antagonistic fungus, *P. chlamydosporia* except root weight which was recorded maximum in control (Table 4). Significant increase in shoot length, shoot weight, root length and fruit yield was however reported in treatment (T5 and T6) that received soil application @ 30 g/plot at sowing time (T5) and both seed treatment

Table 2: Effect of *P. chlamydosporia* on plant growth of okra infected with *M. incognita*

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T1	40.3 ^d	9.0 ^{cd}	35.7 ^c	2.6 ^b
T2	23.3 ^a	5.4 ^a	20.7 ^a	1.5 ^a
T3	29.6 ^{ab} (27.0)*	6.7 ^{ab} (24.1)	21.3 ^a (3.0)	1.9 ^a (26.7)
T4	31.3 ^{bc} (34.3)	7.4 ^b (37.0)	25.4 ^{ab} (22.7)	2.1 ^a (40.0)
T5	37.0 ^{cd} (58.8)	7.8 ^{bc} (44.4)	28.3 ^b (36.7)	2.1 ^{ab} (40.0)
T6	37.7 ^{cd} (61.9)	9.3 ^{cd} (72.2)	35.0 ^c (69.1)	2.5 ^b (66.7)
T7	42.0 ^d (80.9)	9.4 ^d (74.1)	36.0 ^c (73.9)	2.7 ^b (80.0)
CD (P=0.05)	6.9	1.5	4.8	0.6

*Figures presented in parenthesis are percentage increase over control

+ soil application (T6) respectively with a recovery of 37.2 and 46.8; 50.1 and 59.6, 23.5 and 32.7; 45.4 and 59.2% respectively. It was further seen that shoot length, shoot weight and fruit yield were maximum and even better than healthy plants in treatment that received application of seed treatment coupled with soil application of the fungus (T6) with a recovery of 46.8, 59.6 and 59.2% respectively (Table 4). Plant growth including shoot weight in T3 and T4 treatments was observed at par with (T2) nematode inoculated plants. The highest per cent reduction in galls/plant, egg masses/plant, eggs/egg mass and nematode population recorded was 88.9, 88.6, 49.1 and 84.9% respectively in the treatment that received seed treatment @ 3% w/w coupled with soil application @ 30 g/plot at sowing and

the lowest 74.5, 71.8, 10.5 and 45.9% respectively in the treatments that received seed treatment only (Table 5). The nematode population was also significantly decreased in the range of 45.9 to 84.9% in all the treatments that received the fungal application as compared to nematode alone (Table 5). The maximum decrease (84.9%) in the nematode population was again recorded in the treatment where seed treatment @ 3% w/w coupled with soil application @ 30g/plot at sowing.

The indigenous isolate of *P. chlamydosporia* showed a good potential for control of *M. incognita* infecting okra. Agricultural soils in India are rich in mycoflora and contain nematophagous fungi, *P. chlamydosporia* that have good potential as Biocontrol for root-knot nematode, *M. incognita*. Under lab tests a 96% success

Table 3: Effect of *P. chlamydosporia* on multiplication of *M. incognita* infecting okra

Treatments	Galls per plant	% reduction over control	Egg masses per plant	% reduction over control	Eggs per egg mass	% reduction over control
T1	0.0 (0.71)	-	0.0 (0.71)	-	0.0(0.71)	-
T2	171.6 (13.1) ^b	-	153.2 (12.4) ^b	-	353.9 (18.8) ^c	-
T3	153.2 (12.4) ^b	10.7	132.7 (11.5) ^b	13.6	329.3 (18.1) ^c	6.9
T4	150.3 (12.3) ^b	12.4	134.8 (11.6) ^b	13.7	328.2 (18.2) ^c	7.8
T5	150.2 (12.3) ^b	12.5	132.3 (11.5) ^b	13.6	327.9 (18.1) ^c	7.9
T6	78.3 (8.9) ^a	54.4	73.3 (8.6) ^a	52.2	290.5 (17.1) ^b	17.9
T7	77.5 (8.8) ^a	54.8	70.9 (8.4) ^a	53.7	189.2 (13.8) ^a	46.5
CD (P=0.05)	0.8		1.0		0.8	

* Figures presented in parenthesis are Square root transformed values @ $\sqrt{vx+0.5}$

Table 4: Effect of *P. chlamydosporia* on plant growth of okra infected with *M. incognita* in micro plots

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Fruit yield (g)
T1	48.5 ^b	27.5 ^a	34.5 ^c	23.7 ^b	91.5 ^b c
T2	29.0 ^a	23.7 ^a	21.2 ^a	33.7 ^c	40.5 ^a
T3	34.0 ^a (14.7)	25.0 ^a (5.2)	26.2 ^a b (19.1)	15.5 ^a (-54.0)	44.2 ^a (8.4)
T4	35.0 ^a (17.1)	25.0 ^a (5.2)	31.2 ^b (32.1)	22.7 ^b (-32.6)	67.7 ^{ab} (40.2)
T5	46.2 ^b (37.2)	47.5 ^b (50.1)	27.7 ^b (23.5)	20.7 ^a b (-38.6)	74.2 ^b (45.4)
T6	54.5 ^b (46.8)	58.7 ^b (59.6)	31.5 ^b (32.7)	23.2 ^b (-31.2)	99.2 ^c (59.2)
CD (P=0.05)	8.7	14.4	6.3	6.9	27.6

*Figures presented in parenthesis are percentage increase/decrease over control

Table 5: Effect of *P. chlamydosporia* on multiplication of *M. incognita* infecting okra in micro plots

Treatments	Galls per plant	% reduction	Egg mass per plant	% reduction	Eggs per egg mass	% reduction	Nematode population per 200cc soil	% reduction
T2	275 (14.4) ^c	-	171 (13.1) ^c	-	-	347.5 (18.6) ^c	836.5 (28.9) ^c	
T3	70 (8.3) ^b	74.5	48.3 (6.9) ^b	71.8	10.5	311.1 (17.7) ^c	452.7 (21.2) ^d	45.9
T4	68.5 (8.3) ^b	75.1	46.5 (6.8) ^b	72.8	15.2	294.7 (17.2) ^b	229.5 (15.1) ^c	72.6
T5	35.7 (5.9) ^a	87	22.5 (4.7) ^a	86.8	19.7	279.2 (16.7) ^b	176.5 (13.2) ^b	78.9
T6	30.5 (5.5) ^a	88.9	19.5 (4.4) ^a	88.6	49.1	177 (13.3) ^a	126 (11.1) ^a	84.9
CD (P=0.05)	1.7		1.4		0.9		1.7	

* Figures presented in parenthesis are Square root transformed values

has been achieved and proved better than *P. lilacinus*. Similar results were also recorded by Damme *et al.* (2001) in an *in-vitro* study where they reported egg parasitism ranged from 84-100% while root colonization varied from 41-98% from eleven isolates of *Verticillium (Pochonia) chlamydosporium*. Biological factors related with development performances of several strains of *P.chlamydosporia (V. chlamydosporium)* as biocontrol agents were assessed in laboratory tests by Olivares and Lopez, 2002, they found Pathogenicity (70-100% egg infection) and severity (35-40 penetrating hyphae/egg) on *M. javanica* were high for most strains tested. The biological control potential of an isolate of *P. chlamydosporia* var. *chlamydosporia* against *Heterodera schachtii* was examined *in-vitro* and *in-vivo* on sugar beet under glass house conditions. After 3 weeks at 20°C, 74 and 95% of eggs within cysts and females respectively were colonized by fungus on water agar (Ebrahim *et al.*, 2008).

In presence of *P. chlamydosporia* okra plants grow well and a significant reduction in nematode density was observed. The growth of plants as well as nematode multiplication was comparable with control. Various researchers also reported similar results in various agricultural crops. A significant increase in growth of tomato plants was observed with *V. chlamydosporium* treated plants (Sankaranarayanan *et al.*, 2000). The application of *P. chlamydosporia* var. *catenulata* in pot experiment under controlled conditions showed root and soil colonization levels in samples of 1.13×10^4 and 1.48×10^5 cfu/g respectively, achieving a 98% for egg mass colonization and *Meloidogyne* egg parasitism above 70% (Montes *et al.*, 2005). *P. chlamydosporia* is an established biocontrol agent of root-knot nematode, the fungus parasitizes nematodes and their eggs (Khan *et al.*, 2001; Stirling, 1991). Plants inoculated with *Trichoderma*, *Verticillium*, *Paecilomyces* and *Gliocladium* showed greater development of leaves and roots than plants inoculated with nematodes on tomato and soybean. *Verticillium* resulted in 53% reduction in the number of galls (Manuzca and Varon, 2001).

A significant reduction in gall formation due to *M.javanica* and an increase in shoot mass and plant height was also recorded on chickpea when treated with *P.lilacinus* and *V. chlamydosporium* (Zaki and Maqbool, 1996). *V. chlamydosporium* (isolate Vc10) was most effective among eleven isolates, as it reduced the number of egg masses on tomato cv. MoneyMaker by 75% and on cucumber by 77% (Damme *et al.*, 2001). Soil application of *P. chlamydosporia* showed better efficiency to check egg formation or better parasitism in the present study so, soil application gives better results as compared to seed treatment.

Microplot studies showed that the soil application of *P. chlamydosporia* not only suppressed nematode pathogenesis but enhanced plant health and fruit yield of okra. This may have occurred through nematode antagonism. The increase in weight of fresh root may be due to the fact that heavy galling was produced by *M. incognita* on the roots of okra. Plant growth parameters and yield found to be increased in brinjal plants and suppression of *M.incognita* population treated with biocontrol agents and/or oil cakes singly or integrated manner under microplot trial. Final yield from micro plot treated with *P.lilacinus*, *V. chlamydosporium* and neem cake was increased by 57.6%, compared to either 31%, 25.5% or 18% from single application of the respective biocontrol agents (Cannayane and Rajendran, 2001). As per nematode multiplication is concerned a decrease in number of galls/plant, egg masses/plant, eggs/egg mass and nematode population in soil was recorded in all the *P. chlamydosporia* treated plants. Rao *et al.* (1997) also reported a significant increase in the parasitization of nematode eggs by fungus and improvement in plant growth of nursery seedlings of tomato when *V. chlamydosporium* was integrated with VAM fungus. Tahseen *et al.* (2005) reported that the presence of *P. chlamydosporia* was associated with a reduction in the numbers of plant parasitic nematodes (51-78%) including migratory ectoparasites, whereas free living nematodes, culturable bacteria and bacterial populations were unaffected by the application of

fungus. The treatments that received soil application of the fungus @ 30g/plot at sowing and seed treatment @ 3% w/w coupled with soil application @ 30 g/plot suppressed nematode pathogenesis significantly as compared to nematode alone as well as other application of *P. chlamydosporia*, however, both the treatments were at par. Rao *et al.* (2004) reported that formulation of *P. chlamydosporia* was significantly more effective at 50 g/m² than at 25 g/m² in reducing root galling index and the number of nematodes in roots and soil of bell pepper in nursery. Seed treatment with *Pseudomonas fluorescence* alone or nursery treatment with *P. chlamydosporia* was also effective. It is also reported that a one time application of *P. chlamydosporia* was able to slow down the buildup of *M. javanica* population on cropping systems having lettuce and tomato crops for at least 5-7 months (Dammne *et al.* (2005). Abd El Raheem *et al.* (2005) reported during investigation on Faba bean under green house condition that the killing effect of *P. chlamydosporia*, *P. lilacinus* and *Arthrobotrys oligospora* is similar to synthetic chemical nematicide Furadon and significantly better than the commercial preparation of bioagents Nameless. The combination treatment of *P. chlamydosporia* resulted not only increased plant vigor but also suppressed nematode pathogenesis. The fungi have the potentiality to reduce density of *M. incognita* along the growing season of Faba bean plant to 95.4 to 98.9%. These fungi also enhanced shoot and root growth of Faba bean. Generally *P. chlamydosporia* var. *chlamydosporia* was more efficient at reducing the nematode population where applied as spore with out any substrate than when used as colonized barley grains (Ebrahim *et al.*, 2008).

It is concluded that root knot is an important disease of okra and decreased the production of vegetable fruits. Application of *P. chlamydosporia* decreased the disease severity and multiplication of *M. incognita* with a significant increase in plant health and fruit. It is also concluded that soil application is always better than seed treatments, however, combination treatment of *P. chlamydosporia* (seed and soil application) responded best to check nematode disease and improved plant health.

सारांश

जड़ गाँठ गोल कृमि के प्रबन्धन, मिलावडोगाइनी इनकोगनिटा प्रभावित भिण्डी प्रतिरोधी फफूद, पोचोनिया चेलामडोस्पोरा द्वारा विट्रो में विवो में और माइक्रो प्लाट द्वारा उपर्जित किया। बीज उपजचार के प्रभाव और पोचोनिया चेलामामाडोस्पोरिया की मिट्टी प्रयोग को 1 मी. आकार के माइक्रोप्लाट और इन विवो के अन्तर्गत विभिन्न मात्रा से जांच की गई। मिलाइडोगाइनी इनकोगनिटा के 96 प्रतिशत अण्ड पैरासिटिज्म प्रयोगशाला दशा में अंकित किया गया। पोचोनिया

चेलामडोस्पोरा दोनो प्रयोग में सभी उपचारित पौधों में मिलावडोगाटनो इनकोगनिटा जनसंख्या दवाया और पौध स्वास्थ्य परिष्कृत के कारण हुआ उच्चतम पौध वृद्धि गुणों 42 सेमी, 9.4 ग्राम, 36 सेमी, और 2.7 ग्राम के साथ 80.9, 74.1, 73.9 पुनः प्राप्ति और शाख लम्बाई, शाखा भार, जड़ लम्बाई, एवं जड़ भार, क्रमशः पंक्ति में 80 प्रतिशत नियन्त्रण के बाहर के लिए अंकित किया गया। यह पोचोनिया चेलामाडोस्पोरा 3 प्रतिशत W/W के दर से गमले की दशा में मिट्टी प्रयोग किया जहां सभी वृद्धि मापदण्डों जिसमें फल के अलावा जड़ भार सभी पंक्तियों में सामान्य वृद्धि पायी गयी। पोचोनिया चेलामाडोस्पोरा के प्रयोग क्रमशः 88.9, 88.6 और 49.1 प्रतिशत के उपर माइक्रो प्लाट प्रयोग के अन्तर्गत गोल कृमि संक्रमण द्वारा दवाया हुआ है।

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