

Efficacy of *Pasteuria penetrans* and Various Oil Seed Cakes in Management of *Meloidogyne incognita* in Chilli pepper (*Capsicum annuum* L.)

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ABSTRACT

A green house study was conducted to evaluate the comparative efficacy of *Pasteuria penetrans* under the influence of organic amendments of four oil seed cakes namely *Azadirachta indica* (Locally known as Neem), castor (*Ricinus communis*), mustard (*Brassica campestris*) and Citrullus (*Citrullus colocynthis*) on suppression of populations of *Meloidogyne incognita* in Chilli. Oil seed cakes were applied at the rate of 20 mg/Pot (500 kg/ha), either individually or in combination with one dose of *P. penetrans* (100 g/kg soil). Application of oil seed cakes and *P. penetrans*, singly or in combination, proved effective in reduction of gall and final root-knot nematode population. Combination of castor and *P. penetrans* showed greater reduction in galling index (84.75%) and final population (85.74%) over the *M. incognita* control than other treatments. In addition, oil seed cake significantly improved the shoot and root dry matter of chilli. Among the four oil cakes tested, the combined application of *P. penetrans* with castor oil cake seemed to be more promising in the management of *M. incognita* in chilli as it resulted in greater nematode suppression and improved plant health.

Keywords: *Azadirachta indica* (Neem), Castor, Citrullus, *Meloidogyne incognita*, Mustard, *Pasteuria penetrans*, Seed cakes.

INTRODUCTION

Chilli (*Capsicum annuum* L.) crop is highly susceptible to the *Meloidogyne* species of which *M. hapla* and *M. incognita* are among the most important damaging pests (Hussey and Janssen, 2002). Root-knot nematodes, due to their high reproductive potential and wide host ranges, are notoriously difficult to manage and the *Meloidogyne* spp. requires 99.9% control in order to prevent the subsequent build up of damaging populations (Whitehead, 1997). These sedentary endoparasites are responsible for causing an estimated US \$100 billion loss/year worldwide (Oka *et al.*,

2000). In India, an estimated quantitative and qualitative yield loss of about Rs. 240 billion/year (Approximately 5.4 billion \$) is attributed to nematode problems (Sehgal and Gaur, 1999).

Nematode management is generally achieved through the use of nematicides or use of resistant crop varieties. A wide range of nematicides are available, but large scale use in nematode management has declined worldwide. This is due to the toxic effect of nematicides to humans and the entire ecosystem. In addition, they are relatively unaffordable to many small-scale farmers because of the high cost (Chitwood, 2002). Consequently, the persistent pressure on

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farmers and nematologists to adopt strategies that do not pollute the environment has increased the urgency to search for alternative sustainable methods to regulate plant parasitic nematodes (Pinkerton *et al.*, 2000; Mashela *et al.*, 2008).

Use of organic soil amendments is a traditional cultural practice to improve soil fertility and structure. It is also widely used in the management of soil borne diseases, including plant-parasitic nematodes (Oka, 2010). A major setback associated with the use of organic amendment in nematode management is its inconsistent efficiency, which is highly influenced by amendment and soil type (Akhtar and Alam, 1993; Akhtar and Malik, 2000; Oka, 2010). The nematicidal effect of several plants has been well reported by various researchers (Javed *et al.*, 2008; Kumar, 2008). Amongst various organic amendments, the oil seed cakes of several plants have been found to be effective against plant parasitic nematodes (Goswami *et al.*, 2006; Ashraf and Khan, 2007). The nematicidal potential of the *A. indica* (neem), castor, mustard, and Citrullus has been reported by various researchers (Khan and Shaukat, 2001; Tiyagi *et al.*, 2001; Muniyasamy *et al.*, 2010; Khan *et al.*, 2011).

Management of root-knot nematodes with biological control agents has been receiving growing consideration. *P. penetrans*, a mycelial endospore forming gram positive bacterium, has considerable potential as a biocontrol agent against root-knot nematode *M. incognita* (Sayre, 1980; Das *et al.*, 2007). Spores of *P. penetrans* adhere to the cuticle of the second stage juvenile in soil and reduce infection. Several studies clearly showed that increasing the number of endospore per juvenile reduced the invasion of root-knot nematode to plant root (Davies *et al.*, 1988; Das *et al.*, 2007). Obligate nature and showing infection only on the second stage juvenile are some of the limiting factors in the large scale use of this bacterium as a biocontrol agent. Considering the inadequate efficacy level achieved with

single biocontrol agent, research efforts are being made to study the effect of combination of various biocontrol agents with botanicals or organic amendments as the management strategy of root-knot nematode *Meloidogyne* species (Javed *et al.*, 2008; Kumar, 2008). Combination of *A. indica* oil cakes with *P. penetrans* has been evaluated earlier (Javed *et al.*, 2008). Nevertheless, not much information is available on the effectiveness of *P. penetrans* as biocontrol agent against *M. incognita* in combination with oil seed cakes of mustard, castor, and *C. colocynthis* under arid environmental conditions. In view this, the current study was undertaken to evaluate the nematicidal efficacy of *P. penetrans* in the presence of four locally available oil seed cakes in chilli under arid environmental conditions of Rajasthan, India. .

MATERIALS AND METHODS

Oil Seed Cakes

Four oil seed cakes, namely, Neem (*Azadirachta indica*), Castor (*Ricinus communis*), Mustard (*Brassica campestris*) and Citrullus (*Citrullus colocynthis*) were collected from local market of Jodhpur. The large pieces of oil cakes were ground using laboratory grinder (Philips HL7600) and passed through the 18 mesh sieves (0.853 mm opening). The oil seed cakes were shed-dried in order to reduce the moisture content below 10% and were finally stored in steel container at room temperature until further use.

Preparation of Bacterial Inoculum

Pure culture of *P. penetrans* (A population originally from Central Plantation Crops Research Institute, Kayangulam, Kerala and infection of *M. incognita*) was raised on the *M. incognita* infecting eggplants (*Solanum melongena* L. var. pusa purple long) in pots. The bacterial culture was raised together

with *M. incognita* on the same eggplant. Three-month old plants were harvested and soil with root system was air-dried. Dried roots system was grinded in mortar and pestle and was mixed again with the dried soil of the pot. For assessing the initial spore attachment per larva, 100 freshly hatched juvenile of *M. incognita* were mixed in 10g of infested soil and were kept for 72 h at 28 °C. After 72 hours, 30 juveniles were picked from each Petri dish and the spore attachment per larva was counted. The same process was repeated thrice and the mean taken as the initial spore attachment count. The initial spore attachment was 18.2 Spore/J2. To find the effect of seed cakes on the bacterial attachment on nematode cuticles, same method was repeated by adding additional 10 g oil cakes and ascertaining that seed cakes did not affect the attachment rate of bacteria on nematode cuticles.

Setup of Experiment

The experiment was conducted in earthen pots (10 cm diameter) containing 500 cc of sterilized soil. The soil was collected from the field of CAZRI (Central Arid Zone Research Institute, Jodhpur, India). Chilli seedlings (*C. annuum* var. Haripur Raipur) of two-week age were each transplanted into 10 cm diameter pots containing 500 cc soil. The soil properties such as texture, total minerals, organic carbon, and phosphorus were determined using the standard procedures as described by Bashour and Sayegh (2007). The soil was loamy sand having 73% sand, 15% silt, 12% clay, 0.031% total mineral, 0.26% organic carbon and 9 ppm available phosphorus. The soil was passed through the 18 mesh sieve (0.853 mm opening) and steam sterilized by autoclaving for 1 h under 1.0546 kg cm⁻² pressure for three consecutive days. Different treatments were imposed on the 45 days old seedlings. The initial spore encumbrance per larva, representing bacterial multiplication in soil in the

presence of *M. incognita* and various oil cakes, was estimated using the method described earlier (Chaudhary and Kaul, 2010). The initial spore encumbrance was 18.2 spore/J2. One dose of *P. penetrans* infested soil (100 g/kg soil) and 20 mg/pot (500 kg/ha) of seed cakes of Mustard, Castor, Citrullus, and *A. indica*, either alone or in combination, were tested. Upper layer (1 cm) of soil from each pot was removed and oil seed cakes and/or bacteria- infested soil was applied followed by inoculation of 1000 freshly hatched juveniles of *M. incognita*. The top surface was once again covered by the autoclaved soil. Each treatment was replicated five times and the treatments were kept in randomized design. Plants were watered with normal tap water twice a week.

Assessment of Plant Growth and Nematode Multiplication

The plants were harvested 60 days after imposition of the treatments and growth parameters, gall index, and final nematode population were recorded. Shoot and root dry weights were determined using analytical balance after oven-drying the samples at 50°C until a constant weight was achieved. Numbers of gall and nematode were counted with the help of magnifying lens. At the time of harvesting, plants were lightly watered in order to loosen the soil and were removed from the pot. This soil was put into a plastic bowl for getting the information on population of nematode and bacteria. Egg masses and galls were counted with the help of magnification lens from the complete root system. The assessment of the gall index was recorded on a scale of 0-10 (Bridge and Page, 1980). To determine the nematode population, soil of each pot was mixed properly in plastic bowl and divided into five subsamples; nematodes from these subsamples of 100 cc soil were extracted by means of modified Cobb's decanting and sieving technique (Flegg, 1967). Nematode suspension was collected in a 250 ml beaker



and nematode populations was counted at 100 X magnifications using a stereoscopic microscope from 1 ml distilled water suspension in counting dish and a mean of five counts was taken. To determine the total number of eggs in complete root system, 10 egg masses were picked randomly from the root system and transferred to 5 ml bottle containing 2 ml of 0.4% NaOCl. The egg masses were agitated by shaking the bottle on a cyclomixer for 3 min to release the eggs. The eggs in NaOCl were then transferred to a measuring cylinder to make a volume of 30 ml by adding sterile water. The suspensions were bubbled with the help of 5 ml pipette to suspend the eggs uniformly and 1 ml suspension was pipetted into a counting dish. The suspension was observed under a stereoscopic microscope at 100 X magnification and an average of five such counts multiplied with total volume (i.e. 30 ml) gave the number of eggs in 10 egg masses. With this value, the egg population of the entire root system was calculated. The sum of egg population and soil nematode population represented total nematode population.

Determining Infected Females

For estimating the number of infected females, 40 females were randomly picked per root and placed in Petri dish containing water. These females were put singly on to glass slide and crushed under a cover slip and bacterial spores were observed microscopically at high power magnification (400X).

Determining Bacterial Multiplication

For assessing the bacterial multiplication, 100 cc soil from each pot was taken and air dried at room temperature. Then, 100 freshly hatched juveniles of *M. incognita* were mixed in 10 g of infested soil and were put in Petri dish in water for 72 hrs at 28°C. Thereafter, 30 nematodes were picked for

five times and examined using an inverted field microscope (X200).

Standard error of means was calculated for all the values. Further, mean values pertaining to different parameters of chilli were separated by ANOVA followed by least significant difference (LSD) test at $P < 0.05$.

RESULTS

Effect of Oil Cakes on Plant Growth

As shown in Table 1, individual application of oil cakes led to statistically significant (LSD $P < 0.05$) improvement in shoot dry weight (SDW) and root dry weight (RDW) in comparison to the control. Further, amongst four oil seed cakes tested, the increase in SDW and RDW was relatively less significant in case of *A. indica* Oil Cake (NOC). Treatment of castor oil cake (COC) showed maximum increases over the control in SDW (56.47%) and RDW (29.10%). SDW and RDW of plants infected with *M. incognita* enhanced significantly after the application of *P. Penetrans* (PP). SDW was significantly greater in case of the treatment *M. incognita* + COC and *M. incognita* + mustard oil cake (MOC) when compared with the SDW of treatment *M. incognita* + *P. penetrans*. The plants receiving the treatment *M. incognita* + COC registered 68.64% higher SDW compared to *M. incognita* check. On the other hand, SDW for the treatment *M. incognita* + *P. penetrans* was not significantly different from the SDW recorded for the *M. incognita* infected plants receiving treatment of *A. indica* oil cake (NOC) and Citrullus oil cake (CIOC). There was no significant difference in the RDW of the *M. incognita* infected plants receiving treatment of either *P. penetrans* or different oil seed cakes. Among the combined treatments of oil cakes and *P. penetrans*, *M. incognita* + *P. penetrans* + COC and *M. incognita* + *P. penetrans* + MOC had more significant influence on SDW in comparison

Table 1. Effect of *Pasteuria penetrans* and various oil cake alone or in combinations on root-knot nematode, *Meloidogyne incognita* infesting chilli pepper.

Treatments	Shoot dry weight (g)	Root dry weight (g)	Gall Index ^a	Final Nematode Population ^b
Control	4.09 ± 0.27c	1.89 ± 0.11b	NG	NNP
Castor Oil Cake (COC)	6.40 ± 0.43a	2.44 ± 0.18a	NG	NNP
Mustard Oil Cake (MOC)	6.21 ± 0.48a	2.15 ± 0.17a	NG	NNP
<i>A. indica</i> Oil Cake (NOC)	5.72 ± 0.59b	1.99 ± 0.11ab	NG	NNP
Citrullus Oil Cake (CIOC)	6.06 ± 0.44a	2.02 ± 0.13a	NG	NNP
<i>P. penetrans</i> (PP)	4.00 ± 0.29c	1.91 ± 0.10b	NG	NNP
<i>M. incognita</i> (M)	1.85 ± 0.11f	0.97 ± 0.05d	9.18 ± 0.19a	11.508 ± 0.92a
<i>M. incognita</i> + <i>P. Penetrans</i>	2.44 ± 0.17e	1.27 ± 0.09c	6.00 ± 0.35b	10.449 ± 0.65a
<i>M. incognita</i> + COC	3.12 ± 0.15d	1.42 ± 0.07c	4.16 ± 0.18c	10.562 ± 0.51ab
<i>M. incognita</i> + MOC	3.07 ± 0.14d	1.14 ± 0.07c	5.70 ± 0.22c	10.824 ± 0.98ab
<i>M. incognita</i> + NOC	2.47 ± 0.20e	1.25 ± 0.06c	6.26 ± 0.57b	11.095 ± 0.82a
<i>M. incognita</i> + CIOC	2.77 ± 0.16e	1.51 ± 0.05c	6.10 ± 0.39b	10.590 ± 0.75a
<i>M. incognita</i> + <i>P. penetrans</i> + COC	3.47 ± 0.27d	1.98 ± 0.09ab	1.40 ± 0.11d	09.556 ± 0.66bc
<i>M. incognita</i> + <i>P. penetrans</i> + MOC	3.38 ± 0.19d	1.95 ± 0.09b	1.55 ± 0.08d	09.739 ± 0.81bc
<i>M. incognita</i> + <i>P. penetrans</i> + NOC	2.86 ± 0.21e	1.89 ± 0.11b	2.13 ± 0.21d	10.165 ± 0.36b
<i>M. incognita</i> + <i>P. penetrans</i> + CIOC	2.87 ± 0.13e	1.89 ± 0.12b	2.38 ± 0.20d	10.234 ± 0.79b

Data are mean of five replicates; ± Standard Error of mean; NG no galls; NNP no nematode population; Values without common letters differ significantly at LSD $P < 0.05$. ^a Gall index according to Bridge and Page (1980) 0-10scale. ^b Log transformed value of the actual final nematode population

to the other treatments. *M. incognita* + *P. penetrans* + COC caused 87.56% and 104.12% increase over *M. incognita* check in SDW and RDW, respectively (Table 1).

Effect of Oil Seed Cakes on Nematode Suppression

Single and concomitant application of *P. penetrans* with oil seed cakes revealed significant reduction in the gall index (33.15% and 84.75%) and nematode population (58.5% and 85.74%) in all the treatments compared to the nematode check only (Table 1). The least gall index (1.40) was reported in the treatment *M. incognita* + PP + COC, which was 84.7% less than the *M. incognita* check (Table 1). As single treatment COC and MOC significantly reduced the gall index in the presence of *P. penetrans* than *A. indica* and Citrullus cakes *Meloidogyne incognita* + COC caused maximum suppression in gall index (which was 56.42% less) than the *M. incognita*

inoculated check. Combining four different oil cakes separately with *P. penetrans* was observed to drastically suppress the nematode population and it was significantly higher than that of their individual applications at the same dose (Table 1). In combination with *P. penetrans*, COC and MOC showed superior effect in reducing galling index compared to *A. indica* or Citrullus cakes

Effect of Oil Cakes on Bacterial Multiplication

Oil cakes positively affected the rate of bacterial multiplication. The bacterial multiplication was significantly higher in presence of COC and MOC compared to NOC and CIOC. This resulted in higher percentage of infected females in the former (Figure 1). There was no statistically significant difference (LSD $P < 0.05$) in the rate of bacterial multiplication in the presence of COC and MOC. In addition,

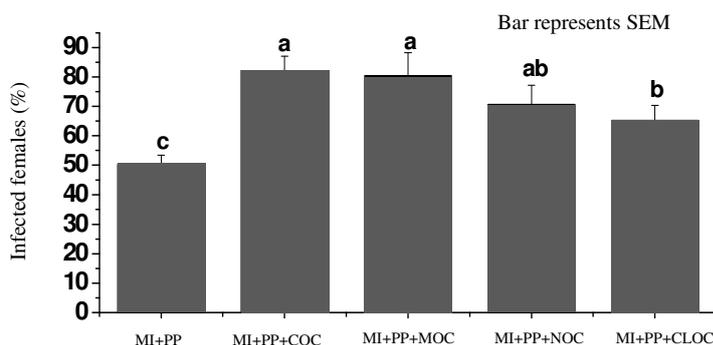


Figure 1. Percentage of *M. incognita* (MI) females infected with *P. penetrans* (PP) in chilli under influence of various oil seed cakes. Data are mean of five replicates. Columns without common letters differ significantly at LSD $P < 0.05$. Castor Oil Cake (COC), Mustard Oil Cake (MOC), *A. indica* Oil Cake (NOC), Citrullus Oil Cake (CIOC).

percentage of infected females was greater in NOC compared to CIOC. Similarly, spore encumbrance per larva had maximum value recorded for the treatment COC, which was 75.86% higher compared to individual application of *P. penetrans*. Here also, no statistically significant difference (LSD $P < 0.05$) in spore encumbrance per larva was noticed in the presence of COC and MOC (Figure 2).

DISCUSSION

In the present study, a positive effect of various oil cakes on shoot and root dry matter was reported, which is similar to the findings of Pandey *et al.* (2005) and

Goswami *et al.* (2006) who reported a significant improvement in plant growth characters on the application of various oil seed cakes like *A. indica*, mustards, etc. In our study, considerable reduction in nematode population was observed under the influence of various oil seed cakes, which corroborates earlier reports (Pandey *et al.*, 2005; Goswami *et al.*, 2006). The fact that the castor oil cakes were highly effective in reducing final population of *M. incognita*, as observed in our study, has been also substantiated by some researchers (Khan and Shaukat, 2001; Khan *et al.*, 2011). Although the reduction in the nematode population could be attributed to increased concentration of various substances like ammonia, formaldehyde,

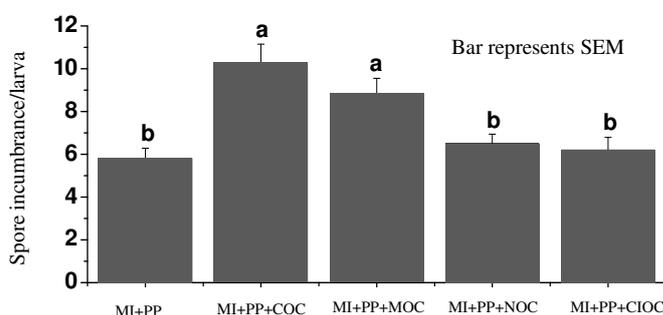


Figure 2. *P. penetrans* (PP) spore encumbrance per larva representing bacterial multiplication in soil in presence of *M. incognita* (MI) and various oil seed cakes. Data are mean of five replicates. Columns without common letters differ significantly at LSD $P < 0.05$. Castor Oil Cake (COC), Mustard Oil Cake (MOC), *A. indica* Oil Cake (NOC), Citrullus Oil Cake (CIOC).

phenol, organic acids, hydrogen sulfide, tannins, and volatile fatty acids (Huber, 1990), in the soil amended with oil seeds cakes, which suppress the nematode multiplication, gall formation (Rodriguez-Kabana *et al.*, 1987; Wang *et al.*, 2004). However, more detailed investigation is required to unearth the exact underlying mechanisms. As regards comparison of efficacy of various oil seed cakes, our observation of superiority of castor oil seed cake over neem are in contradiction with the findings of Jothi *et al.* (2004) who registered *A. indica* oil seed cake as better than the castor oil cake with respect to nematicidal property.

In the current study, individual application of *P. penetrans* exhibited significant reduction in the final population of nematode and gall index, which is parallel to the observations made earlier (Davies *et al.*, 1988; Kariuki *et al.*, 2006; Ahmad and Mukhtar, 2007). *P. penetrans* may reduce invading capacity of second stage juveniles in the roots (Davies *et al.*, 1988) or can parasitize female resulting into production of few eggs (Bird and Brisbane, 1988), which ultimately reduce the final population of nematodes.

Combination of *P. penetrans* with the organic amendments, particularly with different oil cakes, showed cumulative effect on the efficacy of the bacterium in our study. Combination of oil cakes with *P. penetrans* may change the soil physical properties, which in turn may affect adversely nematode behaviours such as hatching, movement, and survival (Van Gundy, 1965; Oka, 2010). Reduction in nematode movement increases the chance of bacteria and nematode contact, thereby increasing the spore load on the nematode cuticle leading to greater rate of female infection. Highest spores encumbrance in presence of castor oil cake indicated that the active ingredients present in the cake can act as nematostat paralyzing the nematodes for a short duration. It provides sufficient time to *P. penetrans* for attaching on the cuticle of nematode. Several active ingredients of *A.*

indica such as Nimbin, Nimbidin, Thionemone, and Melianrol (Ferraz and Freitas, 2004); Azadirachtin (Oka *et al.*, 2007), castor (Ricinine: Moshkin, 1986; Ricin: Rich *et al.*, 1989), Mustard (Isothiocyanates: Matthiessen and Kirkegaard, 2006; Glucosinolates and Sulphur compounds: Neeraj and Singh, 2011) and Citrullus (Colocynthin and Colocynthetin: Adam *et al.*, 2001; Cucurbitacin A, B, C, D, E: Torkey *et al.*, 2009) possessing nematostat properties have been reported. Moreover, *P. penetrans* is not capable of producing the rapid 'knock-down' effect that is associated with a nematicide (Javed *et al.*, 2008); it will, therefore, be worthwhile to use it in combination with oil cake as long-term root-knot nematode management strategy.

In the present study, combination of *P. penetrans* and castor oil cakes exhibited the greatest nematode suppression. These results deviate from the findings of Gogoi and Gill (2001) and Javed *et al.* (2008), who observed better compatibility and efficacy of *P. penetrans* with *A. indica* as compared to castor oil seed cake emphasizing the need for careful selection of effective treatment considering the different agro-climatic conditions.

Overall assessment of the results of the present study suggested that amongst the four oil cakes tested, combined application of *P. penetrans* with castor oil cake and mustard oil cake seemed to be more promising approach in the management of *M. incognita* in chili as it caused greater nematode suppression and improved plant growth.

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کارآمدی *Pasteuria penetrans* و کنجاله های مختلف دانه روغنی در مدیریت
(مهار) نماتد *Meloidogyne Incognita* در فلفل تند (*Capsicum annuum* L.)

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چکیده

به منظور ارزیابی کارآمدی تطبیقی *Pasteuria penetrans* تحت تاثیر بهسازهای آلی چهار کنجاله دانه های روغنی شامل گیاه *Azadirachta indica* کرچک (*Ricinus communis*)، خردل (*Brassica campestris*) و گیاهی از هندوانه سان ها (*Citrullus colocynthis*) برای مهار جمعیت نماتد *Meloidogyne incognita* در فلفل تند، آزمایشی در گلخانه به اجرا درآمد. کنجاله های دانه های روغنی مزبور به مقدار ۲۰ میلی گرم در گلدان (معادل ۵۰۰ کیلو گرم در هکتار) به تنهایی یا همراه با یک دوز از *P. penetrans* (هر دوز به میزان ۱۰۰ گرم در کیلو گرم خاک) افزوده شدند. کار برد کنجاله ها و *P. penetrans* به تنهایی یا همراه هم در کاهش جمعیت نماتد گال ها و ریشه-گره ها موثر بود. در مقایسه با دیگر تیمارها، تیمار کرچک همراه با *P. penetrans* کاهش بیشتری در شاخص گال (۸۴/۷۵٪) و کنترل جمعیت نهایی (۸۵/۷۴٪) نماتد *M. incognita* نشان داد. افزون بر این، کنجاله دانه روغنی باعث افزایش معنی دار ماده خشک ریشه و شاخسار فلفل شد. در میان چهار کنجاله آزمون شده، ظاهرا کار برد کنجاله کرچک همراه با *P. penetrans* در مهار نماتد *M. incognita* در فلفل موفق تر بود زیرا منجر به محدودیت بیشتر نماتد و بهبود سلامت گیاه شد.