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# Suppression of *Meloidogyne javanica* on cucumber by *Pochonia chlamydosporia* var. *chlamydosporia* and *Purpureocillium lilacinum* compared to biofumigation, soil amendment and solarisation

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## ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are the major pests and of economic concern for many agricultural crops worldwide. In the absence of important nematicides, finding environmentally friendly alternatives for control of nematodes has become a challenge. With this in mind, a study was carried out to evaluate the potential of two antagonist fungi, biofumigation, soil amendment and solarization to control *M. javanica*. Pots were inoculated with suspensions of *Pochonia chlamydosporium* var. *chlamydoporium* and/or *Purpureocillium lilacinum* to provide $1 \times 10^4$ spores/g soil and $2 \times 10^6$ spores/g soil respectively. Canola cake and green leaves of oilseed Canola *Brassica napus* each at 1% w/w rate were incorporated into infested soil; half of the biofumigant pots were solarized. All pots were planted with cucumber and inoculated with 5 eggs of *M. javanica*/ g soil of the nematode. Both fungi showed potential as biocontrol agent by infecting nearly 36% of the new eggs on roots, so decreasing nematode multiplication and increasing cucumber weight. In *Pochonia* treatment, number of galls and eggs were reduced to 50 and 4.2 eggs/ g soil whereas 141.1 and 12.3 were recorded in nematode control respectively. All non-chemical treatments reduced root galling by between 56 and 78% and final egg density by 35% to 83%. The effects of combined solarization and biofumigation were greater than for each one alone, as gall and final egg population were 62.4 and 6.7/ g soil in biofumigant pots, which were reduced to 31.1 and 2 in combined treatments respectively. All methods promoted the growth of cucumber. The results offer some positive measures for biocontrol agents and none-chemical methods to be considered for an integrated nematode management strategy.

**Keywords**: green manure, canola cake, root knot nematode, *Pochonia chlamydosporium* var. *chlamydoporium*, *Purpureocillium lilacinum.*

## New findings

Cucumber *Cucumis sativus* L. is an economic crop in many countries, and for many producers in Iran glasshouse production is the main revenue. In the absence of efficient control measures, root –knot nematodes *Meloidogyne* spp. are one of the main elements restricting high yield in cucumber production. *Pochonia chlamydosporia* var. *chlamydosporia* and *Purpureocillium lilacinum* infected average 36% of the new eggs of *M. javanica* on the roots; decreased nematode multiplication by 65% and 36% respectively and increased cucumber weight. Root galling was reduced between 56% and 78% and cucumber grow heavier as a result with all non chemical treatments.

## INTRODUCTION

Plant-parasitic nematodes are important pests of many cultivated plants and may cause losses of US$358.24 billion annually (Abd-Elgawad 2014). Root-knot nematodes (RKN, *Meloidogyne* spp.) are considered to be some of the most economically damaging plant-parasitic nematodes to agricultural crops, could cause more than 10% crop lost in commercial production (Keetch, 1989; Sikora *et al*. 2005; Jones *et al*. 2013). They are obligate plant pathogens which can parasitize the roots of many mono- and di-cotyledonous and herbaceous to woody plants (Eisenback and Tryantaphyllou 1991). Management of these nematodes has mainly relied on chemical nematicides (Talavera *et al*. 2012), which have recently been banned or restricted due to their harmful impacts

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on human health, the environment and wildlife (Wesemael *et al.* 2011). Therefore, there is a need for the development of more environmentally friendly alternatives for pest management. Measures such as crop rotation, resistant cultivars, biofumigation, soil solarization, organic amendments and resistance inducers, in addition to microbial control, are all areas of research on integrated nematode management strategies. Biological control is considered an environmentally friendly strategy against RKN (Sikora 1992, Kerry 2000). *Pochonia chlamydosporia* (Goddard) Gams & Zare (Clavicipitaceae) and *Purpureocillium lilacinum* (Thom) Luangsa-ard *et al*.( Ophiocordycipitaceae) (formerly *Paecilomyces lilacinus*) are among the most studied biological control agents of plant parasitic nematodes (Hidalgo-Dıaz *et al*. 2000, Atkins *et al*. 2003,; Khan *et al*. 2006, Anastasiades *et al*. 2008, Moosavi *et al*. 2010, Carneiro *et al*. 2011).

Both species are saprophytic filamentous fungi, commonly occur in soils, root surfaces and the rhizosphere, and are considered as potential candidates for the development of bionematicides (De Leij and Kerry 1991, Rumbos and Kiewnick 2006). The fungus *P. chlam*ydosporia has been shown to be involved in the decline of plant parasitic nematodes in the field and has been demonstrated to have potential as an effective biological control agent of plant endoparasitic nematodes of the genera *Meloidogyne*, *Globodera* and *Heterodera* (Kerry 2000, Ayatollahy *et al*. 2008, Bent *et al*. 2008, Manzanilla-Lopez *et al*. 2011). A commercial product containing a Cuban strain of *P. chlamydosporia* var. *catenulata* (KlamiC) has been developed for use against species of *Meloidogyne* (Hernandez and Hidalgo Diaz, 2008). Improvements in plant growth have been reported by some isolates of *P. chlamydosporia* in barley (Mácia-Vicente *et al*. 2009), lettuce (Dias-Arieira *et al*. 2011), tomato (Escudero and Lopez-Llorca 2012;,Dallemole-Giaretta *et al*. 2015) and wheat (Monfort *et al*. 2005).

*P. lilacinum* is widely distributed in agricultural soils worldwide, and is the most common fungal species associated with RKN females and eggs (Sun and Liu 2006, Kiewnick *et al*. 2011). One isolate (251) has been registered as bionematicide in many countries (Kiewnick 2010). Since the phase out of some soil disinfectants like methyl bromide, biofumigation involving incorporation of fresh organic material into soil has attracted significant attention as a safe alternative for sustainable control of soil-borne pathogens and nematodes. Biofumigation is defined as suppression of soil-borne pests and pathogens by volatile chemicals containing biocidal compounds released by brassicaceous green manure (Kirkegaard *et al*. 2000).

Brassica plants contain sulfur compounds named glucosinolates which break down into isothiosyanates metabolites toxic to many soil organisms, such as fungal pathogens (Brown and Morra 1997) and nematodes (Buskov *et al*. 2002, Mojtahedi *et al*. 1993). The impact of this approach has been shown in field experiments on controlling plant parasitic nematodes including *M. javanica* in vineyards (Rahman and Somers, 2005) and *M. incognita* in zucchini production (Lazzeri *et al*. 2009).

Soil amendment with fresh *Eruca sativa* (Brassicaceae) has decreased *M. incognita* infection and improved tomato growth (Aissani *et al*. 2015).

There is also evidence of reduction in nematode populations by using seed meal of different species of *Brassica*. Such amendment has reduced populations of *M. chitwoodi* and *M. incognita* on potato tubers and *Capsicum annuum* (Mojtahedi *et al*. 1993, Meyer *et al*. 2011).

Covering soil with clear polyethylene film to trap solar radiation is another mechanism for pest and disease control (Katan, 1987). Solarization alone or with addition of organic amendments, such as Brassicaceae, composts or manure, can suppress weeds (Peachey *et al*. 2001), nematodes (Stapleton and Heald 1991, Coates-Beckford *et al*. 1998) and soil-borne plant diseases under greenhouse or field conditions (Gamliel and Stapleton 1993), and at the same time increase plant growth and crop yield (Chen *et al*. 2000).

Meantime, several workers in Iran have also examined efficiency of various non-chemical methods on management of nematodes. Marigold-*Paecilomyces lilacinus* treatment increased tomato height by 141%; green manure of *Ricinus communis*, *Eruca sativa*, *Tagetes* sp. showed promising results in decreasing *M. incognita* (Kamali and Karegar, 2016.). *Trichoderma harzianum* antagonistic ability against nematodes especially root-knot nematodes has been studied by several researcher (Maleki Ziarati *et al*. 2009; Heidari & Olia, 2016; Amir-Ahmadi *et al*. 2017). Also, *T. harzianum*, *P. chlamydosporia* var. *chlamydosporia* and a commercial product containing marigold have been tested on *Heterodera schachtii* (Hosseini *et al*. 2018). Furthermore, aqueous extract of walnut leaves on *M. javanica* mortality has been presented (Fekrat *et al*. 2016). Report of Salahi and Heydari (2017) showed nematicidal properties of *Achillea wilhelmsii* Koch against *M. incognita*. Efficacy of *Prangos ferulacea* L. and *Satureja hortensis* L. as leaf powder were evaluated in a range of doses against *M. javanica*, (Mozafaryan *et al.* 2017). Ghobadi *et al.* (2017) demonstrated fennel crude extracts were more effective in controlling *M. javanica* on cucumber than nettle extract. Moreover, differences in nematicidal effects of *Nigella sativa* L., *Ferula assa-foetidae* L., *Peganum harmala* L., and *Portulaca oleracea* L. on *M. javanica in vitro* and *in vivo* conditions have been examined (Sholevarfard and Moosavi, 2015).

Nasr Esfahani (2016) reported *H. schachtii* control by more than 95% when a sugar beet field was solarised; while applying soil solarisation to a citrus orchard by Pakniat *et al.* (2013) *Tylenchulus semipenetrans* Cobb infestation was reduced to nearly zero percent after a year.

The objectives of this study were to investigate the potential of some non-chemical methods in suppressing nematode severity and to assess their resultant action on final *M. javanica* population level and growth improvement of cucumber, both individually and in comparison with a chemical nematicide. The selected methods were biocontrol potential of two local isolates species of *P. chlamydosporia* var. *chlamydosporia* and *P. lilacinum*, biofumigation using a commercial cultivar of oilseed Canola, soil amendment with *B. napus* cake, solarization and application of Rugby nematicide.

## Materials and methods

**Nematode**. Nematode inoculum was obtained from a population of *M. javanica* started from a single egg mass, reared on tomato (*Solanum lycopersicum* Mill.) cv Early Urbana for at least 2 years in the glasshouse at the

Iranian Research Institute of Plant Protection. To obtain eggs for inoculum, washed tomato roots were cut into pieces and agitated in a solution of 0.5% NaOCl for 4 min with the aid of a blender. The suspension was passed through 75 and 20 µm sieves; eggs retained in the last sieve were rinsed with distilled water (DW) then suspended in DW, and the desired density prepared (Hussey and Barker 1973).

**Fungi.** For inoculum purposes, both *P. chlamydosporia* var. *chlamydosporia* isolate Pcc3 and *Purpureocillium lilacinum* isolate 25.4 were prepared using a similar procedure. The fungi which originally were isolated from sugar beet cyst nematode, were obtained from live collection of nematode antagonistic fungi, Nematology Department, Iranian Research Institute of Plant Protection, Tehran. Equal volumes of sand and milled barley (100 g/ flask) were placed in a flask, to which 30 ml DW was added and then autoclaved at 121 ºC for 20 min on two consecutive days (De Leij *et al*. 1993). Each flask was inoculated with the appropriate fungus and left for it to proliferate at 25 ºC for three weeks. Ten ml suspensions were made from 1 g of substrate of each fungus in DW, and final spore count was determined as mentioned previously (Ebadi *et al*. 2018). For the experiments, each pot was inoculated with 10 ml suspensions of either *P. chlamydosporium* var. *chlamydoporium* (adjusted to give $1 \times 10^4$ spores/g soil), and of *P. lilacinum* (adjusted to give $2 \times 10^6$ spores/g soil), which were added to holes around each cucumber (*Cucumis sativus* L.) seedling growing in pots containing 1000 g of sterilised soil. The pots were inoculated with nematode eggs one week later (Ayatollahy et al. 2008).

**Biofumigation.** Preparation was carried out by sowing oilseed Canola (*Brassica napus* L.) seeds, commercial cv Hyola, in microplots during spring. At flowering stage, leaves and stems were cut, enclosed in freezer bags and kept at - 20 °C to prevent volatiles substances escaping from the plant tissues (Fatemy and Aghazadeh Naiini, 2016).

For the experiment, thawed plant tissues were cut into small pieces and 10 g were mixed with 900 g soil in each pot and covered with a further 100 g soil. These pots were inoculated with a suspension of eggs and J2, half of the pots were covered with a transparent plastic sheet and kept outdoor for two weeks with a mean temperature of 23 - 37 °C, while uncovered pots remained in a glasshouse indoors.

**Canola cake amendment**. The same procedure as for biofumigation was used, with 10 g oilseed canola cake mixed with 900 g soil, and a further 100 g of soil spread on top of the pot. The soil was inoculated with a suspension of nematodes and left for 2 weeks (Fatemy, 2018).

**Solarization treatment**. Pots containing one kg sterilised soil inoculated with nematodes were covered with a transparent plastic sheet (40 µm thickness), placed into soil up to their edges into plots established outside the Department, for 2 weeks during summer time; at the same time as the biofumigant pots.

**Nematicide treatment**. For a comparison chemical treatment, Rugby (an organophosphate contact nematicide with cadusafos as active ingredient) was applied. Two g of 10% G commercial product was spread on the soil surface around cucumber seedlings at the time of nematode inoculation.

**Controls.** Cucumber plants uninfected or infected were included as controls.

Nematode inocula were added to soil one week after planting a cucumber seedling.

**Pot test**. Plastic pots of 15-cm diameter and holding 1000 g sterile loamy soil (40% sand, pH 7.5) were used for all treatments. All pots were inoculated with a suspension of 5000 eggs and J2 of *M. javanica*, which, depending on the treatment, were added either before or after planting a cucumber seedling cv Sahra. Experimental units with five replicates were arranged in a completely randomized design in a growth chamber with 16 h photoperiod at 25 °C for 3 months.

Treatments included: nem + *P. chlamydosporia* var. *chladosporia*, nem + *P. lilacinum*, nem + canola biofumigation, nem + canola cake amendment, nem + canola biofumigation + plastic, nem + plastic, nem + Rugby, untreated nem control, uninfected untreated cucumber control.

At harvest, plants were uprooted, roots were washed and fresh weights of tops and roots were taken. Root galling was rated on a scale of 0 - 5 (Hartman and Sasser 1985), where 0 = no gall, 1 = 1 - 2 galls, 2 = 3 - 10 galls, 3 = 11 - 30 galls, 4 = 31 - 99 galls, 5 = more than 100 galls.

Roots of each replicate in each treatment were cut into small pieces, mixed and the numbers of galls and egg masses counted (Ebadi *et al*. 2018). Eggs were extracted from roots by the NaOCl method described earlier, and concealed eggs were extracted from roots by further processing of treated roots in a blender; the processed contents were poured through 75 and 20 µm sieves and the eggs caught on the latter were counted. The number of J2 in 100 g soil in each pot was estimated after extraction by a modified tray method (Whitehead, 1965). The sum of eggs and J2 were considered as the final nematode density and the reproduction factor (R = Pf/ Pi, final to initial population) was determined by dividing the final population by the initial population (Sholevarfard and Moosavi, 2015).

These procedures were followed for fungal treated plants, with the following additional steps. The rate of egg parasitism on roots was determined by randomly removing five egg masses from the roots of each replicate in each fungal treatment, one egg mass at a time. The egg masses were placed in a drop of 0.05% NaOCl between a glass slide and coverslip and observed under ×400 magnification. The total numbers of healthy eggs were estimated by subtracting the numbers of diseased eggs from the total number of eggs and J2. Eggs invaded by mycelium were considered as diseased, and were differentiated from healthy eggs by the aid of × 40 magnification under a light microscope (Fatemy *et al*. 2005). The experiment was repeated twice under similar conditions.

**Statistical analysis**. Data were analyzed using standard analysis of variance (ANOVA). The mean values were separated by Duncan's multiple range test and the significance of means was evaluated at $P \leq 0.05$. Data were checked for homogeneity of variance before being pooled. All analyses were conducted using SPSS 18 (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp).

## Results

Cucumber plants infested with *M. javanica* weighed less than all other plants (*df* = ۱۴, *F*= 94.082, *P*<0.0001), and all treatments significantly increased the weights of aerial parts (*df = 8, F= 675.662, P<0.0001*), and roots of cucumber plants (*df = 8, F = 111.196, P<0.0001*) compared to

infested untreated plants except for *P. lilacinum* treated roots (Table 1). Between Canola amendments, plants treated with canola cake had heavier tops and roots than biofumigant plants. However, covering infested biofumigant soil with plastic for solarization boosted growth of plants more than all other treatments.

Between the fungal treatments, cucumbers in nematode-infested soil treated with *P. chlamydosporia* var. *chlamydosporia* weighed more than those grown in *P. lilacinum* treated soil. The highest yields of tuber (*df = 8, F = 245.087, P<0.0001*) were observed in solarized soil with and without biofumigant (Table 1).

Table 1- . Effects of different control methods of *Meloidogyne javanica* on cucumber fresh weight and yield, after 3 months in a growth chamber. Means ± standard error.

| Treatments | Fw top (g) | Fw root (g) | Total weight (g) | Cucumber yield (g) |
|---|---|---|---|---|
| Pcc + nem | 32.4 (± 0.3) c | 2.4 (± 0.06) e | 34.9 (± 0.28) e | 3.7 (± 0.14) c |
| P.lil + nem | 19.4 (± 0.2) f | 2.0 (± 0.05) f | 21.3 (± 0.21) h | 1.5 (± 0.04) g |
| Canola biofum + nem | 22.1 (± 0.4) e | 2.6 (± 0.10) e | 24.7 (± 0.35) g | 1.5 (± 0.03) g |
| Canola biofum + Plastic + nem | 44.5 (± 0.3) a | 4.4 (± 0.150 b | 49 (± 0.45) a | 7.1 (± 0.41) b |
| Plastic + nem | 40.9 9± 0.3) a | 5.7( ± 0.2) a | 46.6 (± 0.35) b | 7.8 (± 0.13) a |
| Canola cake + nem | 32.5 (± 0.2) c | 3.6 (± 0.03) c | 36.2 (± 0.19) d | 3.3 (± 0.03) d |
| Rugby + nem | 33.1 (± 0.6) c | 4.7 (± 0.06) b | 37.8 (± 0.57) c | 2.8 (± 0.03) e |
| Nem | 16.3 (± 0.6) g | 1.9 (± 0.19) f | 18.2 (± 0.56) i | 1.3 (± 0.05) g |
| Cucumber | 29.0 (± 0.3) d | 3.2 (± 0.04) d | 32.1 (± 0.33) f | 2.4 (± 0.05) f |
| CV | 3.5 | 1.6 | 1.5 | 9.1 |

Pcc: *Pochonia chlamydosporia* var. *chlamydosporia*, P.lil: *Purpureocillium lilacinum*, nem: *Meloidogyne javanica*, biofum: biofumigation, Fw: fresh weight. Columns with similar letters are not significantly different at 5% level of Duncan`s multiple range test, n = 5, CV = coefficient of variation

Parameters related to severity of nematode infestation levels such as egg mass/ g root (*df = 13, F = 230.511, P<0.0001*), gall/ g root (*df = 13, F = 374.993, P<0.0001*), eggs/ g soil (*df = 13, F = 207.259, P<0.0001*) and reproduction (*df = 13, F = 166.585, P<0.0001*), were different in treated plants.

The numbers of egg masses and galls and gall indices were significantly reduced by all treatments compared to untreated plants (Table 2). The least galled roots were in plants treated with Rugby, solarization, and a combination of solarization and biofumigation. Fewer galls were found on roots treated with *P. chlamydosporia* var. *chlamydosporia* than those treated with *P. lilacinum*. Canola cake amendment decreased galling and egg mass production more than Canola fumigation on cucumber.

Nematode reproduction and final numbers of eggs/g soil were significantly decreased by all treatments (Table 2). The highest nematode reproduction rate of 2.5 and final egg density of 12.3/g soil found were in uncontrolled infested plants. Reproduction rates of 1.3 and 1.6 were found in biofumigated and *P. lilacinum* treated plants respectively, but all other treatments lowered reproduction rates to below unity. The smallest final egg density was produced by solarization and by treatment with Rugby. Application of *P. chlamydosporia* var. *chlamydosporia* and *P. lilacinus* reduced final population density to 4.2 and 8 eggs/g soil respectively, which were higher than in other treatments. By the end of experiment, an average of 36% of the *M. javanica* eggs had been colonized on roots of cucumber treated with fungi

Table 2- Suppressive effects of different control methods on *Meloidogyne javanica* disease parameters on cucumber after 3 months in a growth chamber. Means ± standard error.

| Treatments | Egg mass no/g root | Gall no/root | Gall Index | Eggs/g soil | R | %infected eggs |
|---|---|---|---|---|---|---|
| P.cc + nem | 20.6 (± 0.45) c | 49.9 (± 1.0) bc | 4 (± 0.0) b | 4.2 (± 0.22) d | 0.9 (± 0.04) d | 37 (±3.0) a |
| P.lil + nem | 25.4 (± 0.16) b | 61.5 (± 1.7) bc | 4 (± 0.0) b | 8.0 (± 0.16) b | 1.6 (± 0.03) b | 34.4 (±2.8) a |
| Canola biofum + nem | 23.0 (± 0.30) bc | 62.4 (± 2.5) bc | 4 (± 0.0) | 6.7 (± 0.16) c | 1.3 (± 0.02) c | - |
| Canola biofum + Plastic + nem | 6.2 (± 0.33) f | 31.1 (± 2.1) d | 3.4 (± 0.2) b | 2.0 (± 0.100 f | 0.4 (± 0.02) f | - |
| Plastic + nem | 10.8 (± 0.25) de | 36.4 (± 1.5) d | 4.0 (± 0.0) b | 2.0 (± 0.10) f | 0.4 ±( 0.02) f | - |
| Canola cake + nem | 11.8 (± 0.13) de | 37.7 (± 0.7) cd | 4.0 (± 0.0) b | 3.3 (± 0.14) e | 0.7 (± 0.02) e | |
| Rugby + nem | 8.8 (± 0.25) ef | 27.2 (± 1.3) d | 3.2 (± 0.1) c | 1.7( ± 0.06) f | 0.3 (± 0.01) f | - |
| Nem | 75.0 (± 2.7) a | 141.1(± 11.7) a | 5.0 (± 0.0) a | 12.3( ± 0.32) a | 2.5 (± 0.06) a | - |
| CV | 2.7 | 2.4 | 1.1 | 6.4 | 19.3 | |

Pcc: *Pochonia chlamydosporia* var. *chlamydosporia*, P.lil: *Purpureocillium lilacinum*, nem: *Meloidogyne javanica*, biofum: biofumigation, R: reproductive factor (initial/final population). Columns with similar letters are not significantly different at 5% level of Duncan`s multiple range test, n = 5, CV = coefficient of variation

## Discussion

Application of Rugby nematicide reduced the final nematode density by 86% while improving cucumber growth by nearly 108%. The main purpose of integrated nematode management is to reduce the sole reliance on harmful nematicides by using safe and effective alternatives. Consequently, careful selection of control practices is necessary when developing an integrate approach. In our experiment, the final level of nematode infestation was significantly reduced with all treatments.

Applying *P. chlamydosporia* var. *chlamydosporia* and *P. lilacinum* biocontrol agents to infested cucumber was successful in reducing *M. javanica* infestation level and promoting plant growth, with the first fungus being more protective than the latter. In the laboratory test, 82% and 89% of *M. javanica* eggs were colonized by mycelium of *P. chlamydosporia* var. *chlamydosporium* and *P. lilacinum* respectively (data not shown). In soil, *M. javanica* egg population density was reduced to 65% and 35% of those in untreated nematode soil by *P. chlamydosporia* var. *chlamydosporia* and *P. lilacinum* application respectively. Furthermore, both fungi infected an almost identical percentage of new eggs (average 36%) on the roots of cucumber. Both *P. chlamydosporia* var. *chlamydosporium* and *P. lilacinum* hyphae parasitize eggs by penetrating the eggshell and colonizing the egg and may further disrupt the juvenile cuticle (Escudero and Lopez-Llorca 2012).

Isolates of *P. chlamydosporia* have parasitized significant numbers of RKN eggs (van Damme *et al*. 2005, Moosavi *et al*. 2010, Carneiro *et al*. 2011). In Australia, heavy parasitism of root-knot nematode by *P. lilacinum* and *P. chlamydosporia* has been observed in tomato planted in kiwifruit orchards (Mertens and Stirling 1993). In Brazil (Silva *et al*. 2017), at a low infestation level of 500 nematode eggs per plant, an average of 39% of the *Meloidogyne enterolobii* were parasitized by *P. chlamydosporia* var. *Catenulata* or *P.lilacinum*; however, at 5000 eggs per

plant these fungi were not effective. Similarly, best control of *M. incognita* by *P. lilacinum* isolate PL251 on tomato was obtained at an initial population density of 1-4 nematodes/mL soil, but the efficacy of the fungus decreased at higher nematode densities (Kiewnick *et al*. 2006).

*Pochonia chlamydosporia* and *P. lilacinum* colonize roots endophytically, which may contribute to promotion of plant growth and protection against pathogens such as nematodes and fungi (Rumbos and Kiewnick 2006, Maciá-Vicente *et al*. 2009, Escudero and Lopez-Llorca 20012). Our results support these findings as total weights of cucumber almost doubled with *P. chlamydosporia* var. *chlamydosporia* and increased 17% in *P. lilacinum* treated pots.

*Purpureocillium lilacinum* also occurs in the rhizosphere, and for nutritional requirements does not strongly depend on roots (Rumbos and Kiewnick 2006). It grows on a wide range of organic materials, including plant tissue, oil cakes and industrial waste products (Mani and Anandam 1989).

Supplementing soil with Canola, whether as cake amendments or biofumigant provided satisfactory results, since nematode egg mass numbers were reduced by 84% and 69% and plants grew 99% and 37% heavier in Canola cake and biofumigant treatments respectively in comparison to nematode control. In India, incorporation of fresh *B. napus* manure into soil caused 8% reduction in root galling by *M. incognita* and 8 and 12% increase in seedling height and weight respectively of tomato (Randhawa and Sharma 2007). In South Africa, amendment of soil with green manure of *B. napus* reduced populations of *Meloidogyne javanica* and *Criconemoides xenoplax* by 14 and 8% respectively on *Vitis vinifera* (Kruger *et al*. 2015). In the United Kingdom, populations of J2 of *Globodera pallida* were decreased by between 10 and 33% when soil was amended with fresh *B. napus*, and 50-95% with *B. napus* seed meal amendment (Lord *et al*. 2011). Also, in Australia, green manure of *B. napus* lowered egg production of *M. javanica* by between 76% and 82% on *Vitis vinifera* (McLeod and Steel 1999), and the population level of *Pratylenchus neglectus* by 0-57% (Potter *et al*. 1998).

Furthermore, in the United States of America (USA), incorporation of fresh *B. napus* manure into soil reduced *M. chitwoodi* populations by 79-94% whereas it had no effect on *P. neglectus* populations on potato (Al-Rehiayani and Hafez 1998).

A comprehensive review on efficacy of green manure and seed meal of different species of Brassicaceae on various nematodes has been given by Fourie *et al*. (2016). Seed meal of *B. napus* caused more than 90% reduction in the population of *M. incognita* and *Pratylenchus penetrans* (Zasada *et al*. 2009); a 25-50% reduction in the population of *P. penetrans* on *Pyrus malus* (Mazzola *et al*. 2009) was reported from the USA.

Solarizing soil was one of the best control measures tested, since plants grew 158% heavier, and the population density of nematode eggs was decreased by 83% in comparison to untreated plants. Further, a combination of solarization and biofumigation was the best of all treatments, and almost doubled the effect of the latter alone. Addition of three *B. juncea* lines containing high concentrations of 2-propenyl glucosinolate to soil caused over 95% mortality of encysted eggs of *G. pallida* in polyethylene covered soil;

the toxic effects of green manures were greater in polyethylene-covered than in open soil (Lord *et al*. 2011).

Three major groups of glucosinolates have been identified within Brassicaceae plants and individual species of Brassicaceae can contain several different types of glucosinolates (Zasada and Ferris 2004).

Control of cabbage yellows caused by *Fusarium oxysporum* f. sp. *conglutinans*. has been enhanced with solarization of cruciferous residues in soil (Ramieriz and Munnecke 1988). Population densities of *Pratylenchus* spp. have been decreased 50 to 100% by solarization (Pinkerton et al. 2000). Stapleton *et al.* (1998) found that the combination of solarization and biofumigation successfully control nematodes, weeds and soil-borne pathogens. Under the conditions of our experiment, our findings indicate that some of the methods were as effective as or even better than Rugby nematicide in controlling *M. javanica* damage and multiplication. Non-chemical treatments reduced root galling by between 56% and 78% and final egg production by 35 to 83%.

In this respect, both *P. chlamydosporia* var. *chlamydosporia* and *P. lilacinum* showed potential as biocontrol agents and biopesticides, which makes them safe candidates to be considered for use in an integrated nematode management approach. However, some studies have shown that, in the case of RKNs, *P. chlamydosporia* var. *chlamydosporia* has not always been able to prevent the initial invasion of J2, and is not protective of highly susceptible hosts or with large nematode population densities (Bourne *et al*. 1996). Consequently, its control efficacy should be assisted by decreasing the initial nematode infestation (Kerry and Bourne 1996), with integration of other methods such as crop rotation with poor hosts of the nematode, soil amendment or plant defence activators. Nematode infestation on tomato was decreased when *P. chlamydosporia* var. *catenulata* was integrated in a crop rotation (Atkins *et al*. 2003). Induction of plant resistance with synthetic elicitors provides some protection against nematode invasion (Moslemi *et al*. 2016, Molinari 2016). Activation of the plant defence system also promotes transition from the saprophytic to the parasitic phase of the fungus, as has been demonstrated with combined use of a Portuguese *P. chlamydosporia* isolate with benzothiadiazole (BTH) against *M. chitwoodi* (Vieira dos Santos *et al*. 2013). Growth stage of the manure crop and the rate of green manure incorporation into soil are some of factors contributing to success of biofumigation (Bellostas *et al*. 2004). Seed meals of Brassicaceae crops have also shown some nematicidal properties, are easily incorporated into soil and, unlike cover crops, do not pose any risk of being hosts to nematodes, although their availability and cost could be a drawback (Rahman and Somers 2005). Solarization and biofumigation with minimum disturbance to beneficial microorganisms in the soil are among other useful alternatives to expensive fumigant nematicides.

## Conclusion

The results have provided useful and practical insights about the efficacy of biocontrol agents and some of the natural and agricultural byproducts such as high potential of solarization, and brassica amendments against nematode pest, which could be recommended for use in wormer climates and developing countries. Furthermore, the potential of

*P. chlamydosporia* var. *chlamydosporia* and *P. lilacinum* as biocontrol agents of RNK was demonstrated, their commercial use and mass production need further investigation.

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