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Review

Effect of temperature, pH, carbon and nitrogen ratios on the parasitic activity of *Pochonia chlamydosporia* on *Meloidogyne incognita*

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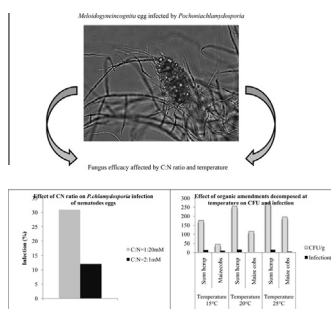
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HIGHLIGHTS

- Temperature, pH and C:N effect on the biocontrol agent *Pochonia chlamydosporia*.
- Pre-decomposed organic materials resulted in a high number of *P. chlamydosporia*.
- The number of fungal propagules increased with increasing soil temperature.
- At 20 °C, percentage of infected eggs increased.
- The percentage of egg infection increased with increasing nitrogen levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Pochonia chlamydosporia (Goddard) Zare and Gams is a biological control agent for control of root-knot nematodes. However, the efficiency of many biological control agents, including *P. chlamydosporia*, depends on soil conditions. An *in vitro* study was conducted to determine the effect of temperature, pH, carbon and nitrogen on the activity of *P. chlamydosporia* against *Meloidogyne incognita* (Kofoid & White) Chitwood. Sunn hemp, maize cobs and sawdust decomposed at 15, 20 and 25 °C, media with pH from 3.4 to 8.8 and a carbon and nitrogen ratio from 0.01 to 10 were used with *P. chlamydosporia* under *in vitro* conditions. Addition of the *P. chlamydosporia* to pre-decomposed organic materials resulted in a high number of fungal propagules. Using sunn hemp and maize cobs, the number of fungal propagules increased with increasing soil temperature, and at 20 °C the percentage of infected eggs increased significantly. The percentage of egg infection increased with increasing nitrogen level from 5 to 100 mM when carbon was kept at 10 mM. The results can be used to improve effectiveness of the fungus in the tropics as part of an integrated pest management approach under tropical field conditions where problem of root-knot nematodes is common.

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1. Introduction

Root-knot nematodes (RKN) are a concern to many smallholders and commercial producers involved in intensive vegetable production in Eastern Africa (Gowen, 2002, 2005). An estimate of 20% loss has been attributed to RKN in Kenya but losses may be up to 50% and total crop failure, principally due to *Meloidogyne incognita* and *Meloidogyne javanica*, can occur (Kanyagia, 1980). These nematodes can also exacerbate diseases, particularly vascular wilts (Gowen, 2002). Although not all smallholders will recognize nematodes as a biological constraint, up to 20% of vegetable producers use nematicides when growing tomatoes (Gowen, 2005; Oruko and Ndun'gu, 2001). Currently, many agricultural chemicals used for nematode control are no longer available because of health and environmental hazards associated with their use, besides being increasingly less effective and costly. These realities demand that nematode management should become an integrated program of practice, including alternative measures to the use of chemicals, for example, through the development of bio-management strategies and the use of selected biological control agents in combination with other control methods, in order to provide sustainable nematode control systems.

Most biological control agents require certain environmental conditions for optimum growth, infection or predacious activity (Sayre and Walter, 1991). Knowledge of the environmental conditions that affect the growth of a biological control agent is essential when determining its ability to control plant pathogens, as in the case of the fungus *Pochonia chlamydosporia* (Goddard) Zare & Gams used to control RKN (Sayre and Walter, 1991). Fungi require temperature levels that may differ from one isolate to another, for their growth and infectivity (Viaene et al., 2006). The optimum temperature for growth of the nematophagous fungus *P. chlamydosporia* is 25 °C, but this can vary and is not necessarily the optimum temperature for infection, depending on the isolate (Kerry et al., 1986). For example, the optimum temperatures for hyphal growth and parasitism of strain II of *P. chlamydosporia* is 25 °C and 12 °C, respectively (Irving and Kerry, 1986). In general, the survival of the fungus in the soil is limited by both high and low temperatures (Van Damme et al., 2005). Temperatures below 5 °C hinder growth of the fungus while little growth occurs at temperatures above 30 °C (Kerry, 2000).

In order to facilitate growth and multiplication of the fungus, nutrients from different sources are required. Locally available organic material, such as fresh crop residues or organic waste materials, can be added to the soil, a practice that has been known to reduce nematode populations. *Pochonia* is usually added to soil in a colonized rice substrate as an energy source for the fungus. However, in some fungi, high nitrogen/carbon levels can repress infection-related genes and may compromise parasitic ability (Ward et al., 2012).

Addition of organic amendments to the soil is known to increase the population of *P. chlamydosporia* but lowers infection potential due to the increased availability of nutrients to the fungus (Jaffee, 2002). The supply of carbon (C) and nitrogen (N) from organic amendments is one of the major factors responsible for fungal activity and growth (Segers, 1996). It is known that the potential of *P. chlamydosporia* to control nematodes depends on the level of C and N released into the soil after decomposition of plant-based materials (Chen and Dickson, 2004). Therefore, further research is required to consider whether addition of the fungus to soil already enriched by decomposed organic amendments enhances fungal growth. However, the fungus is limited in colonizing the soil due to its weak saprophytic nature, leading to suppression by other soil micro-organisms (Kerry, 2000).

Parasitic activity of *P. chlamydosporia* in the soil is challenged by many factors including chemical and physical aspects, pH being

one of them, which can be a limiting factor on the growth and infectivity of RKN eggs. Variation in soil pH from acidic to alkaline decreased the infection of RKN eggs by *P. chlamydosporia* (Jaffee and Zasoski, 2001), the optimum pH for growth of *P. chlamydosporia* being pH 5 (Kerry et al., 1986). Little is known about the other factors affecting the parasitic activity of the fungus, including nutrition, particularly in soils rich in organic substrates. Therefore, the objective of this study was to determine the effect of soil temperature and pH, as well as levels of C and N, on the parasitic activity of *P. chlamydosporia* on *M. incognita* (Kofoid & White) Chitwood.

2. Materials and methods

2.1. Effect of organic materials decomposed at different temperatures on growth and parasitic activity of *P. chlamydosporia*

An experiment was conducted using different organic amendments decomposed at different temperatures to assess the ability to support growth of *P. chlamydosporia* and infectivity on RKN eggs. *M. incognita* was multiplied on infected tomato cv. Tiny Tim plants (*Solanum lycopersicum* L.) grown in sterile soil at 25 °C under glass-house conditions. Egg-masses were hand-picked from galled roots using fine forceps under a Wild M5 stereomicroscope (20×) six weeks after inoculation of the plants with second-stage juveniles (J2).

Isolate 10 of *P. chlamydosporia*, originally from Brazil, and kept freeze dried in the Rothamsted collection, was used to produce conidia and chlamydospores in potato dextrose agar (PDA; Oxoid, Basingstoke, UK) and corn meal agar (CMA; Oxoid), respectively, as described by Kerry and Bourne (2002).

The organic amendments tested in this experiment were sawdust (a mixture from different tree species), sunn hemp (*Crotalaria ochroleuca* G. Don) and maize (*Zea mays* L.) cobs with C:N ratio of 297:1, 8:1 and 89:1, respectively. The materials were dried and milled to a powdered form before each organic material was decomposed at 15, 20 and 25 °C for 30 days. The same organic amendments, which were not decomposed, were included as controls. In addition, treatments without organic amendments but with and without fungus were included making a total of 14 treatments. The treatments were replicated three times in pots and arranged in a randomized complete block design (RCBD).

Using 177 g of sterile soil in 200 g capacity pots, the soil was mixed with the organic amendment powders at the rate of 0.5% of the weight of soil and incubated in Gallenkamp (Weiss-Gallenkamp, Loughborough, UK) cooled incubators, each incubator being set at the required experimental temperatures for 30 days. After incubation, *P. chlamydosporia* isolate 10 was added to all pots at the rate of 5000 chlamydospores/g soil and all treatments were incubated for another 30 days at the optimum temperature (25 ± 2 °C) for *P. chlamydosporia* growth. Three treatments of non-decomposed organic amendments and one treatment without organic amendment were included as a control. After incubation, ten egg-masses of *M. incognita* were buried in the soil of each pot at a depth of about 30 mm, using a baiting technique (Lumsden, 1981) where plastic slide mounts (24 × 36 mm) with glasses removed (Gepe, Zug, Switzerland) were used to hold the egg-masses wrapped in nylon fabric mesh prior to being buried into the soil.

After seven days, the egg-masses were removed from the soil by pulling out the plastic mount slides used to support egg-masses which were then placed into excavated glass blocks (Agar Scientific, Stansted, UK) and cleaned using three milliliters of sterile distilled water. The eggs were then released from egg-masses using a glass cyst crusher. From the ensuing egg suspension, 200 µl was pipetted onto plates containing sorbose agar (2 g)

mixed with antibiotics (Kerry and Bourne, 2002) and incubated at 25 °C. After 72 h, 100 randomly selected eggs were counted and examined for fungal infection under a Wild M5 stereomicroscope (50×) to estimate the percentage of eggs infected by *P. chlamydosporia*. After retrieving the egg-masses, the soil in the pots was assessed for the number of fungal propagules/g of soil. One gram of moist soil from each pot was diluted in 9 ml sterile water agar (0.05%) and a serial dilution of up to 10^{-3} was made. Dilutions 10^{-2} and 10^{-3} were plated onto a semi-selective medium (17 g Corn Meal Agar, 17.5 sodium chloride, 75 mg rose Bengal 50 mg streptomycin sulfate, 50 mg chloramphenicol, 50 mg chlortetracycline, 37.5 mg thiabendazole, 37.5 mg carbendazim, 3 ml Triton X-100) in Petri dishes and incubated at 25 °C for 14 days (Kerry and Bourne, 2002). After incubation, the fungal colonies were counted and the number of colony forming unit (CFU) was calculated (Kerry and Bourne, 2002).

2.2. Effect of pH on the infectivity of *P. chlamydosporia* on eggs of RKN

An experiment was set up in the laboratory to test the effect of pH on parasitic activity of *P. chlamydosporia* to RKN eggs. The experiment was conducted using 0.0125 g of yeast extract (Merk, Darmstadt, Germany) in one liter of sterilized distilled water (Esteves, 2007) and in water without yeast, at each of five different pH levels. The pH levels tested were 3.4, 4.5, 5.7, 7.1 and 8.8. These were prepared from solutions using a buffer made from 0.2 M sodium phosphate and 0.1 M citric acid (Sigma–Aldrich, Milwaukee, MI, USA). The experiment had four controls, two for yeast extract (pH 6.6) and two for water (pH 5.8), each with and without fungus, to make a total of 14 treatments. These were replicated three times in universal bottles, as described below, and arranged in a RCBD.

Eight millilitre of either water or yeast extract media made from the prepared buffer was placed in universal bottles (30 ml vol.). To each of these, one millilitre containing 1000 *M. incognita* eggs and one millilitre containing 5.5×10^4 conidia from *P. chlamydosporia* isolate 10 previously grown for two weeks on PDA were mixed and incubated at 25 °C in a Weiss-Gallenkamp orbital incubator (150 rpm) for 48 h. After incubation, the bottles were taken out and whirl mixed before removing a 3 ml aliquot and placing it onto a counting slide. Data on parasitized and non-parasitized eggs were collected by assessing 100 randomly selected eggs under a dissecting microscope at 50× to estimate the percentage of parasitized eggs.

2.3. Effect of carbon and nitrogen ratios on the efficacy of *P. chlamydosporia* to parasitize RKN eggs in vitro

The experiment to investigate the effect of C and N had a total of 12 treatments that comprised 10 mM of C with different rates of N (1, 5, 10, 50 and 100 mM), different rates of C (1, 5, 10, 50 and

100 mM) with 100 mM of N, and two controls (yeast extract medium with and without fungus). These were replicated three times in tubes, and arranged in a RCBD. Glucose (30 g/l water) was used as a source of C while ammonium nitrate (40 g/l water) was used as a source of N. Glucose was added to water, stirred and sterilized using a Minisart® (Sartorius Ltd, Epsom, UK) single filter unit (0.20 µm). Ammonium nitrate was dissolved in water and autoclaved at 120 °C for 15 min. All treatments were buffered using 0.1 M potassium buffer (pH 6.80 ± 0.01).

Eight millilitre of the C:N-containing medium was mixed with one millilitre suspension containing 5.5×10^4 conidia of *P. chlamydosporia*/ml and one millilitre containing 1000 *M. incognita* eggs/ml in a universal bottle (30 ml vol.) to make a total of 10 ml in each bottle. There were two controls where yeast extract medium (0.0125 g/l = 0.4 mM C, 0.1 mM N) was used to test the eggs in the presence and in the absence of the fungus. The treatments were incubated in an INNOVA® 40 incubator shaker (Brunswick Scientific, Edison, NJ, US) at 150 rpm and 25.5 °C for 48 h. After incubation, a 3 ml aliquot was drawn from each bottle and placed into a nematode counting dish. Data on parasitized and non-parasitized eggs was taken after examining first 100 randomly selected eggs under a Zeiss compound microscope at 10× from which the percentage of parasitized eggs was calculated.

2.4. Statistical analysis

Data on CFU and percentage of egg infection were square root and logit, i.e. $\log_e((\% \text{infection} + 0.5)/(100.5 - \% \text{infection}))$, transformed, respectively to ensure normal distribution and constant variance across the treatments. The transformed data were subjected to analysis of variance (ANOVA) using the GenStat statistical package (eleventh edition, © VSN International Ltd, Hemel Hempstead, UK). Relevant means from ANOVA were compared using least significant difference (LSD) values at the 5% ($P = 0.05$) level of significance.

3. Results

3.1. Effect of temperature on organic materials and on growth and parasitic activity of *P. chlamydosporia*

Decomposition of organic amendments at various temperatures significantly ($P = 0.002$, F -test) affected the number of CFU/g soil (Table 1A, Fig. 1A). Sawdust had a significantly ($P < 0.05$, LSD) higher number of CFU compared to the other organic amendments (Table 1A, Fig. 1A). Also, the number of fungal propagules in each decomposed organic amendment was significantly ($P < 0.05$, LSD) higher compared to non-decomposed material (Table 1A, Fig. 1A). The interaction of organic amendments and temperature influenced the numbers of fungal propagules. The number of fungal propagules was highest in sawdust decomposed at 15 and

Table 1A

Effect of decomposing organic materials at various temperatures on the population density (colony forming units/gram) of *Pochonia chlamydosporia*. Square root data means ($n = 3$) in brackets are shown for statistical comparisons. See Fig. 1A for raw data means.

Organic amendment ^c	Non-decomposed	Decomposition temperature (°C)		
		15	20	25
Sunn hemp	613(14.3) ^a	32,976(177.9)	66,354(256.7)	77,171(273.9)
Maize cobs	0(0)	3442(45.2)	20,797(117.7)	40,405(196)
Sawdust	645(14.7)	141,072(373.5)	116,368(329.4)	72,964(268.4)
Control	12,666(107.9)			
LSD (5%)		83.82, 26 d ^b		

^a Values in brackets are means of square root transformed data for comparisons using the LSD.

^b Degrees of freedom.

^c C:N ratio. Sunn hemp (8:1), maize cobs (89:1), sawdust (297:1).

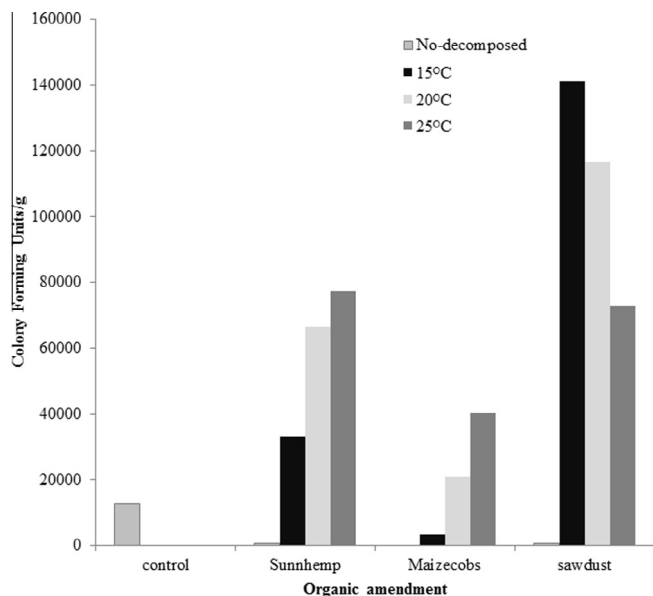


Fig. 1A. Effect of decomposing organic materials at various temperatures (°C) on the population density (colony forming units/g of soil) of *Pochonia chlamydosporia*. Data are means with $n = 3$ for decomposition temperature. See Table 1A for square root data means for statistical comparison.

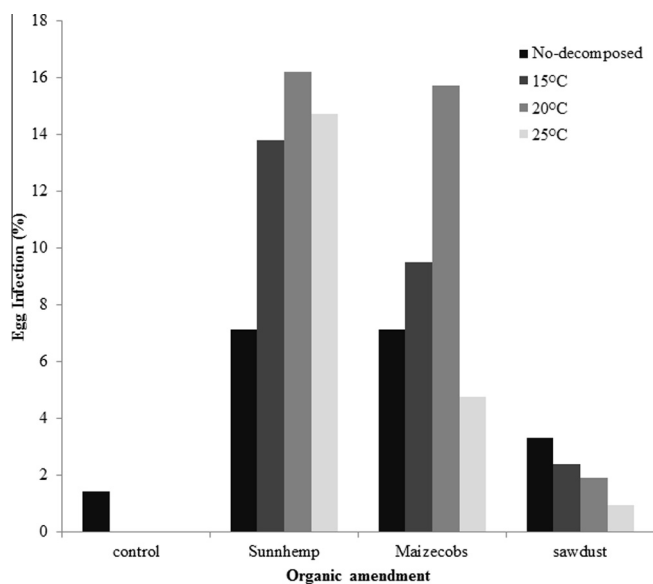


Fig. 1B. Effect of decomposing organic materials at different temperatures on *Meloidogyne incognita* percentage egg infection by *Pochonia chlamydosporia*. Data are means with $n = 3$ for decomposition temperature. See Table 1B for logit data means for statistical comparison.

20 °C. The fungal population density increased with increase in temperature at which sunn hemp and maize cobs were decomposed.

The percentage of egg infection was significantly affected by the different organic amendments ($P < 0.001$, F -test) but there was no overall effect of temperature ($P = 0.134$, F -test) or interaction between amendments and temperature ($P = 0.623$, F -test) (Table 1B, Fig. 1B). Sawdust, which had highest number of CFU, had the lowest egg infection. The percentages of egg infection in soil amended with sunn hemp and maize cobs were significantly ($P < 0.05$, LSD) higher compared to sawdust. The 20 °C treatment gave the highest percentage infection in soil amended with sunn

hemp or maize cobs, whereas 15 °C gave the highest percentage infection for sawdust (Table 1B, Fig. 1B).

3.2. Effect of pH on the parasitic activity of *P. chlamydosporia* to root-knot nematode eggs

Percentage egg infection was significantly affected by the media ($P < 0.001$, F -test) used and also by the pH ($P = 0.027$, F -test) (Fig. 2), but with no significant interaction between these factors ($P = 0.739$, F -test). The percentage of egg infection in water (11.3%, logit mean -2.20) was significantly ($P < 0.05$, LSD) lower compared to infection in yeast extract (89.0%, logit mean 2.06; LSD (5%) = 1.178). Although infection occurred in all pH values tested (i.e. acid and alkaline), the results for the different pH levels over both media (Fig. 2) indicate that, excluding the yeast extract (pH 6.6) and water controls (pH 5.8), the highest infection was at pH 4.5 (28.42%) and the lowest was at pH 5.7 (12.81%). However, an increase in pH did not correlate ($P = 0.147$, F -test) with percentage egg infection. Instead, the infection varied as pH increased (Fig. 2). The infection using yeast extract medium (control) was highest compared to all pH treatments, a result which might suggest that a combination of pH and nutrients may be needed to enhance the potential for egg infection.

3.3. Effect of carbon and nitrogen ratios on the parasitic activity of *P. chlamydosporia* to RKN eggs in vitro

Although there was no effect of changing C ($P = 0.461$, F -test) and N ($P = 0.338$, F -test) levels as regards percentage egg infection, there was an overall main effect of the addition of C and N ($P < 0.001$, F -test). The addition significantly ($P < 0.05$, LSD) reduced the average percentage of eggs infected by *P. chlamydosporia* (21.11%, $n = 30$) compared to the yeast extract control (49.73%, $n = 3$, Table 2, Fig. 3A–3B). The highest infection was recorded in yeast extract medium (control). Among the C:N ratios tested, the highest and lowest egg infections were recorded in media containing C:N ratios of 5:100 (30.99%) and 10:5 (12.03%), respectively (Table 2, Fig. 3A–3B). Also, the percentage egg infection increased from 12.03% to 22.79% when N was increased from 5 to 100 mM with C at 10 mM (Table 2, Fig. 3B).

4. Discussion

The mode of action of organic materials added to soil to control soil-borne diseases and pests is not fully understood but may be due to: (i) release of toxic compounds from the plant material, (ii) release of toxic metabolites from increased microbial activity, (iii) enhanced growth of microbial antagonists and non-specific biological control agents, and (iv) general improvement of soil structure and fertility (Huang et al., 2006). This study has shown that the of population *P. chlamydosporia* in the soil increased with the increasing temperature at which the organic substrates sunn hemp and maize cobs were decomposed before application of the fungus, while the highest fungal population occurred at the lowest decomposition temperature (15 °C) for sawdust. Decomposing the sunn hemp and maize cobs at 25 °C led to the greatest population of the fungus. This temperature has also been reported as optimum for *P. chlamydosporia* growth (Kerry et al., 1986). The increase in the numbers of fungal propagules in sunn hemp and maize cobs at higher temperature could be a result of enhanced decomposition and the release of available nutrients such as N and C to the fungus. However, the use of organic amendments with high C:N ratio such as sawdust used in this study has been shown to have a negative impact on fungal growth. According to Oka et al. (2007), decomposition of organic amendments with high C:N ratio can result in the

Table 1B

Effect of decomposing organic materials at different temperatures on *Meloidogyne incognita* percentage egg infection by *Pochonia chlamydosporia*. Logit data means ($n = 3$, less stated) in brackets are shown for statistical comparisons. See Fig. 1B for raw data means.

Organic amendment	Non-decomposed	Decomposition temperature (°C)			Means for organic amendments over temperatures
		15	20	25	
Sunn hemp	7.14(−2.58) ^a	13.81	16.19	15.71	15.24(−1.81), $n = 9$
Maize cobs	7.14(−2.58)	9.52	15.71	4.76	10.00(−2.30), $n = 9$
Sawdust	3.33(−3.25)	2.38	1.91	0.95	1.75(−4.13), $n = 9$
Control	1.43(−4.20)				
LSD (5%)	1.151, 26 df ^b				0.665, 26 df
LSD ^c (5%)	0.940, 26 df				

^a Values in brackets are means of logit transformed data for comparison using the LSDs.

^b Degrees of freedom.

^c For comparison of non-decomposed treatment and control means to means over temperatures.

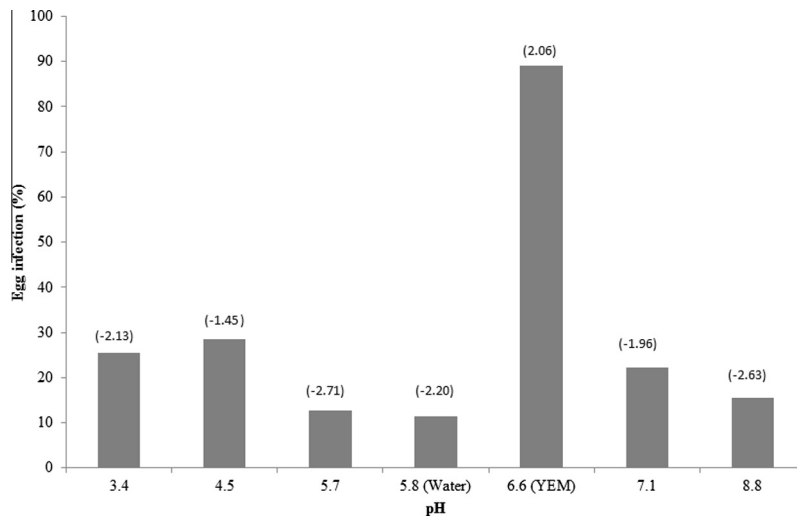


Fig. 2. Effect of different pH levels on parasitic activity of *Pochonia chlamydosporia* to root-knot nematode eggs. Data are means with $n = 3$ for water and yeast extract medium (YEM) controls or $n = 6$ for pH treatments (over both media). Values in brackets are means of logit transformed data for comparison using the LSD (5%) = 1.178 (comparing water to YEM control), 1.020 (comparing pH treatments to controls), or 0.833 (comparing pH treatments), with $df = 26$.

Table 2

Effects of carbon: nitrogen ratio on *Meloidogyne incognita* egg infection by *Pochonia chlamydosporia*.

Carbon or nitrogen (mM)	Infection (%)	
	100 mM nitrogen	10 mM carbon
1	20.50	17.36
5	30.99	12.03
10	22.79	18.00
50	23.63	20.08
100	22.99	22.79
Overall C and N	21.11, $n = 30$ (−1.38) ^a	
Control (yeast extract, C:N = 4:1)	49.73 (−0.02)	
LSD (5%)	0.665, 23 df ^b	

The data are means ($n = 3$, less stated).

^a Values in brackets are means of logit transformed data for comparison using the LSD.

^b Degrees of freedom.

release of toxic substances such as phenols that may have nematocidal and/or fungicidal effects. In general, the results indicate that decomposition of organic amendments at certain temperatures is an important process that influences the proliferation of the fungus. This was confirmed when comparing the results of this process to those observed when organic amendments were mixed with *P. chlamydosporia* without allowing time for decomposition.

It has been reported that the nutritional status of the rhizosphere determines the switching on and off of the saprophytic stage of the fungus (Esteves, 2007). Decomposition of sunn hemp and maize cobs in the soil for 30 days before application of *P. chlamydosporia* resulted in increased egg parasitism as shown in the present study. This implies that the time allowed for the decomposition was sufficient for the released nutrients to deplete, and so this might have increased the ability of fungus to parasitize RKN eggs, and so reduced its saprophytic tendency. As a facultative parasite, the fungus has been reported to be good at utilizing nutrients saprophytically but the saprophytic ability differs depending on the particular isolate, colonized crop and how rich the soil is (Liu and Chen, 2003; Mauchline et al., 2004). The inability of sawdust (C:N 297:1) to support high fungal infection, despite encouraging the greatest fungal proliferation out of the three organic amendments at temperatures of 15 and 20 °C, implies that not all organic amendments can have beneficial effects to increase egg infection after one month of decomposition. This could suggest that 30 days was not long enough for sawdust and may have contributed to a fungal population unable to infect large numbers of nematode eggs due to other substances (possibly toxic) released by the decomposing material. This may also indicate that an increase in the fungal population does not always translate into higher parasitism (Jaffee, 2004; Mauchline et al., 2004). In the current study, sunn hemp (C:N 8:1) and maize cobs (C:N 89:1)

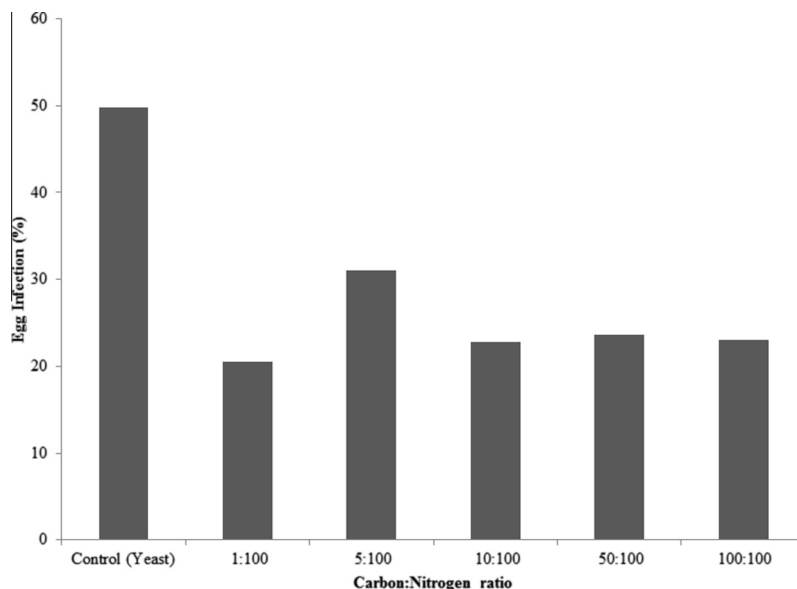


Fig. 3A. Effects of C:N ratio at constant nitrogen level (100 mM) on *Meloidogyne incognita* egg infection by *Pochonia chlamydosporia*. Data are means ($n = 3$). See Table 2 for logit transformed data means for statistical comparisons.

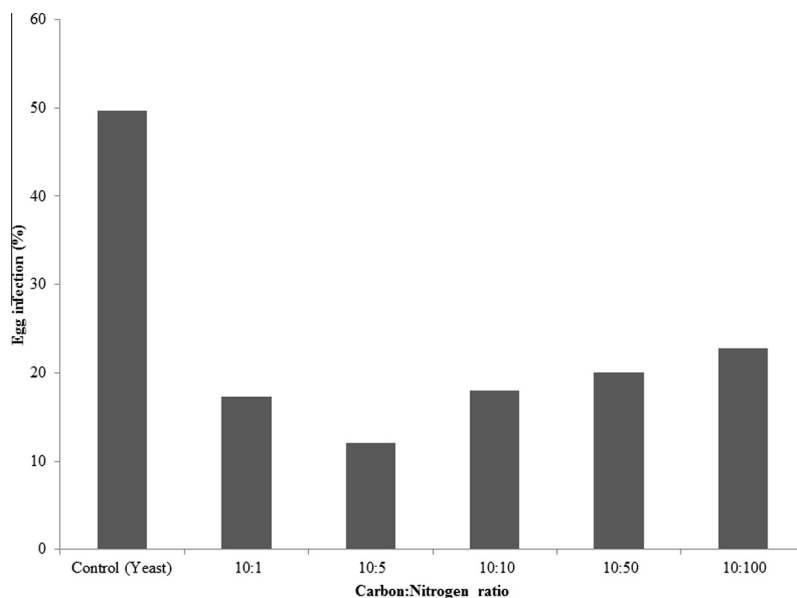


Fig. 3B. Effects of C:N ratio at constant carbon level (10 mM) on *Meloidogyne incognita* egg infection by *Pochonia chlamydosporia*. Data are means ($n = 3$). See Table 2 for logit transformed data means for statistical comparisons.

led to promising results for the fungal infection of *M. incognita* eggs and these organic amendments could be suitable when decomposed at temperatures in the range of 15 and 20 °C. The effectiveness of decomposed organic amendments, as shown in this study, has also been reported for broccoli substrate, which, when decomposed for 20 days, resulted in reduced galling index, from 9.5 to 4.8, in melon (Ploeg and Stapleton, 2001) using a scale of 0-no knots on roots to 10-all roots severely knotted.

This study also found that by varying the pH levels alone did not provide a strong or direct effect on egg infection rate. However, although there was only a slight difference in pH between yeast extract and water, the difference in infection rate was 7.9-fold. The treatments with pH 5.7 or 7.1, which were closest to the pH

of yeast extract (pH 6.6), provided a lower percentage of infected eggs (12.81% and 22.31% compared to 89.0%). However, the ability of yeast (C:N 4:1) to enhance egg infection could not purely be attributed to pH, but also to the presence of additional nutrient components, including C and N, when compared to sterile distilled water. The egg infection at pH 4.5, which was the highest recorded among the pH levels tested excluding that of yeast extract, can be compared to the results reported by Jaffee and Zasoski (2001) who achieved an optimal parasitic activity of *Hirsutella rhossiliensis* to nematodes at pH 4.5. However, in a recent molecular study (Ward et al., 2012) reported that VCP1 (an enzyme responsible for egg infection) production was higher in more alkaline pH conditions.

Changes in levels of C and N in the liquid medium did not increase the proportion of eggs infected by the fungus. This finding supports the results reported by [Irving and Kerry \(1986\)](#) who found that differences in nutrient concentration of the agar did not cause significant differences in *P. chlamydosporia* (= *Verticillium chlamydosporium*) parasitism of *Heterodera avenae* eggs. Excluding the yeast extract control, the level of C at which highest infection was attained was only 5 mM, along with 100 mM of N. This may imply that C and N act independently from one another. Also, the percentage of egg infection was reduced at increased C, beyond 5 mM, for a fixed N of 100 mM. This result is similar to that of [Segers \(1996\)](#) who found a low egg infection by the fungus when C was equal to or greater than 10 mM, and which was independent of N levels. This finding suggests that high amounts of C do not support parasitism of nematode eggs. The percentage egg infection was low for the C:N ratio media used in this study compared to that found in yeast extract medium (standard). Compared to the C and N in the yeast extract medium (ratio 4:1), this study used higher C and N levels in the C:N ratio media treatments such as sunn hemp (C:N 8:1) and maize cobs (C:N 89:1), and this could be one of the reasons for the low percentage egg infection. Similarly, addition of C as one of the nutrients in growth media decreased VCP1 in *P. chlamydosporia* and vice versa for N added to the media ([Ward et al., 2012](#)).

This *in vitro* study was done in a controlled environment with soil temperatures ranging from 15 °C to 25 °C which reflects the temperature conditions that can be found in the tropics. It has shown the significant effect of abiotic factors in the parasitic activity of *P. chlamydosporia*. Low fertilizer input and intensive cultivation can be regarded as the hallmarks of small-scale subsistence agriculture in Eastern and Southern Africa. Without exception, such practices lead to build-up of plant parasitic nematodes and loss of soil fertility.

5. Conclusions

The results of this work can be used to improve the effectiveness of the fungus when applied in combination with the incorporation of non-costly, organic amendments which are locally available to Eastern African smallholders, as part of an integrated pest management approach to control RKN. The efficacy of *P. chlamydosporia* can be enhanced through addition of decomposed organic amendments whose carbon content is at least 20 times lower than that of nitrogen. Optimization of carbon and nitrogen sources (C:N) and pH conditions for egg infection for *P. chlamydosporia* isolates is complex and deserves further investigation.

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