Biological Control 80 (2015) 23–29

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Review

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

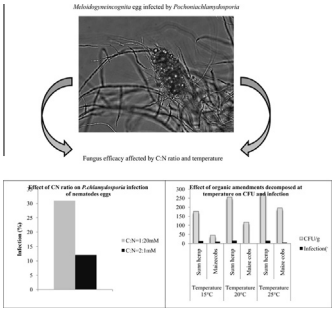
Nessie D. Luambano [a,b,*], Rosa H. Manzanilla-López [c], John W. Kimenju [a], Stephen J. Powers [d], Rama D. Narla [a], Waceke J. Wanjohi [e], Brian R. Kerry [c]

[a] University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya
[b] Sugarcane Research Institute, P.O. Box 30031, Kibaha, Tanzania
[c] Department of Agro Ecology, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[d] Department of Computational and Systems Biology, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[e] Kenyatta University, P.O. Box 43844, Nairobi, Kenya

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



ARTICLE INFO

Article history:
Received 7 May 2013
Accepted 4 September 2014
Available online 18 September 2014

Keywords:
Biological control
Organic amendment
Root-knot nematode
Sunn hemp
Tomato

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.

* Corresponding author at: Sugarcane Research Institute, P.O. Box 30031, Kibaha, Tanzania.
E-mail addresses: nluambano@yahoo.com (N.D. Luambano), rosa.manzanilla@gmail.com (R.H. Manzanilla-López), wkimenju@yahoo.com (J.W. Kimenju), stephen.powers@rothamsted.ac.uk (S.J. Powers), virup_n@yahoo.com (R.D. Narla), wanjohi.waceke@ku.ac.ke (W.J. Wanjohi).

http://dx.doi.org/10.1016/j.biocontrol.2014.09.003
1049-9644/

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 glasshouse 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)