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ORIGINAL ARTICLE

Controlling the root-knot nematode, *Meloidogyne incognita* in cucumber plants using some soil bioagents and some amendments under simulated field conditions

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Abstract This study was carried out under simulated field conditions to evaluate the efficacy of some bioagents and soil amendments, as a single or combined treatments, in controlling root-knot nematode *Meloidogyne incognita* infecting cucumber. Each of the fungus *Verticillium chlamydosporium* and the symbiotic bacterium *Photorhabdus luminescens*, as single or joint treatments significantly reduced gall formation and other criteria on cucumber roots. Maximum reduction in gall formation, female numbers, egg-mass production, developmental stages and final population of juveniles in soil, was acquired by these treatments, *V. chlamydosporium* + *P. luminescens*, *P. luminescens* + compost (C) and *V. chlamydosporium* + *P. luminescens* + animal compost (AC), compared with the control and other treatments. Applications of all treatments significantly promoted plant growth i.e. length of shoot and root, fresh and dry weight of shoot and root, number of leaves, flowers, fruits and weight of fruits per each plant compared to control (infested plants with nematode only and healthy plants).

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Introduction

Plant-parasitic nematodes are recognized as major agricultural pathogens and are known to attack plants and cause crop losses throughout the world. Root-knot nematode is the most damaging plant-parasitic nematode (Barker, 1985). Biocontrol, when effective, usually is more enduring and safe in application with no toxic residues in food (Abd El-Moity, 1981).

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Entomopathogenic nematodes in the families Steinernematidae and Heteror-habditidae are biological control agents (Stock, 2005). These beneficial nematodes are parasites of insects, killing their hosts with the help of the associated symbiotic bacteria carried in their alimentary canals (steinernematids carry *Xenorhabdus* spp., whereas heterorhabditids carry *Photorhabdus* spp.) (Poinar, 1990; Adams and Nguyen, 2002). The nematode–bacterium team is capable of invading and killing the larval and adult stages of numerous insects (Akhurst and Boemare, 1990).

Both bacteria, *Xenorhabdus* and *Photorhabdus* spp. can be grown as free-living organisms on certain media under standard laboratory conditions. *In vitro* growth is probably supported by the rich nutrient supply of the growth media and the lack of competition that normally exists in the soil environment. As the bacteria enter the stationary phase of their growth cycle, they secret several extra-cellular products, including lipases, phospholipases, proteases and several different broad spectrum antibiotics (Akhurst, 1980; Akhurst and Boemare, 1990), that can be assayed in the culture media.

In contrast, *Verticillium chlamydosporium* Goddard was first recognized as a parasite of cyst nematodes after it had been isolated from the eggs of *Heteradera schachtii* Schmidt (Willcox and Tribe, 1974). *V. chlamydosporium* is a wide spread fungus that proliferates in the rhizosphere and parasitizes females and eggs of cyst and root-knot nematodes. The fungus has potential as a biocontrol agent root-knot nematodes (Viaene and Abawi, 2000). But it is very variable and only some isolates may have potential as commercial biological control agents (De Leij and Kerry, 1991).

The literature concerning suppression of plant-parasitic nematode densities by organic amendments is replete with both promising and inconsistent results (Muller and Gooch, 1982; Rodríguez-Kábana, 1986; Stirling, 1991; McSorley and Gallaher, 1995a,b; Hassan et al., 2010). Man has added organic and inorganic amendments to soil for centuries to improve soil fertility and increase crop yield. The nematicidal effect of some of these amendments has been recognized for some time, and several reviews on the subject have been published (Singh and Sitaramaiah, 1973; Muller and Gooch, 1982). However, the application of organic amendments for reducing plant-parasitic nematodes populations has met with both success and failure (Halbrendt, 1996).

The aim of the current work is to study the following objectives: (1) Potentiality of cell-free culture filtrates of *Photorhabdus luminescens* and chlamydospores of *V. chlamydosporium*; alone and in combinations to control *Meloidogyne incognita* under microplot conditions. (2) Effect of soil amendments (compost and animal compost) alone and in combinations with biocontrol agents to control *M. incognita* under microplot conditions.

Material and methods

Identification and propagation of pure cultures of root-knot nematode M. incognita

Galled roots of eggplants were carefully washed using gentle flow of water to remove the adhering soil particles. One egg-mass was collected by the aid of a needle specially adapted for this technique. The obtained culture was reared on tomato seedlings planted in pots filled with sterilized sand: clay media (1:1). Pots were kept under glasshouse conditions for 45–60 days to maintain the nematode inocula for further studies. A stock culture of the second-stage juveniles (IJ_2s) were obtained from the collected mature egg-masses after immersion in sterilized water for 7–10 days.

The extracted nematode was identified as *M. incognita* according to the scanning electron microscope image of the perineal patterns of the mature female. It illustrated the presence of a high, squarish dorsal arch, which contains a distinct whoral in the tail terminal area. The striae are smooth to wavy. Distinct lateral lines are absent, but breaks and forks in striae are obvious.

Isolation of bioagents

V. chlamydosporium was isolated from egg-masses of *M. incognita* according to the method of Zaki and Maqbool (1993), while *Photorhabdus luminescens* was isolated directly from the surface-sterilized infective juvenile stages of EPNs (Obtained from the Applied Center for Entomonematodes (ACE), Faculty of Agriculture, Cairo University), by the method of Caldas et al. (2002) and Cabral et al. (2004).

Propagation of the bioagent V. chlamydosporium

The fungal biocontrol agent *V. chlamydosporium* was grown on corn meal agar in 9-cm-diameter Petri plates at 25 °C. After 15 days of incubation chlamydospors were harvested. The teasted concentration of chlamydospores (10^7 clamydospores/ml) was determind by the aid of haemocytometer.

Preparation of cell-free filtrates of bacteria

The pure colonies of the isolated symbiotic bacteria *P. luminescens* was inoculated into 50 ml of tryptic soy broth contained in 100 ml Erlenmeyer flasks. Then allowed to multiply at the optimum temperature 28 °C for 24 h. The bacterial culture of was centerifugated at 3000 rpm, for 15 min. The supernatant broth solution which collected after centrifugated of bacterial cultures was sterilized by using the Filter Assembly Versapor® 0.2-μm-pore-diameter.

Preparation of microplots and soil treatment

Top soil (sand:loam 1:1 v/v), 0–30 cm depth, of each microplot 1 m^2 was sterilized by drenching with formalin solution (5%) and covered with polyethelene sheet for two weeks. The sheets were then removed and sterilized soil was thoroughly mixed and left for one week to allow the toxic evaporation of formalin residues (Ramses, 2001).

Soil treatments with compost (C/N = 2:16) and animal compost (C/N = 1:12.13) were incorporated into the top 15 cm of the beds of plots at the rate of 1 kg/plot before transplanting. Soil was treated with the biocontrol agents at transplanting time in the root zone at the rate of 20 ml of each bioagent/root. Treatment with the nematicide Vydate (Oxamyl) 24% L, was included for comparison after