Survival of Root-knot nematodes and their egg-parasitic fungus Pochonia chlamydosporia (Goddard) on weed roots

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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ABSTRACT

Background: Root-knot nematodes is a major pest and difficult to control in vegetable crops worldwide. *Pochonia chlamydosporia* is an egg-parasitic fungus which attracts attention as a control agent of root-knot nematodes. Weeds serve as potential hosts for both root-knot nematodes and *P. chlamydosporia*. The objectives of this study was to assess several weed species as hosts of root-knot nematodes and *P. chlamydosporia* and assess the impact of fungicide foliar treatments on *P. chlamydosporia* colonies in roots.

Methodology: Pot experiments were conducted to study research objectives. Tomato, black nightshade, common lambsquarter and common wild oat were assessed as hosts for root-knot nematodes and *P. chlamydosporia*. Also, thiophanate-methyl (systemic fungicide) and mancozeb (non-systemic fungicide) were foliarly sprayed to tomato plants already colonized with root-knot nematodes and *P. chlamydosporia*.

Results: Root-knot nematodes and *P. chlamydosporia* successfully colonized roots of species tested. No significant differences were observed between treatments. *P. chlamydosporia* colonized successfully tomato roots although applied in low concentration of 1670 spores/ml. Colonization of roots by the fungus was observed as early as seven days after inoculation. No negative impact on *P. chlamydosporia* colonies in tomato roots sprayed with a fungicide, were observed.

Conclusions: Root-knot nematodes can survive in roots of a wide range of plant species, either crops or weeds. This is very important particularly in fallow periods. *P. chlamydosporia* can be integrated in nematode control schemes and in combination with foliar fungicides in presence of wilt diseases in roots.

Key words: Root-knot nematodes, *Pochonia chlamydosporia*, hosts, fungicides, weeds

INTRODUCTION

Root-knot nematodes are the best known phytonematodes throughout world [1]. They are more widely distributed throughout the world than any other group of plant parasitic nematodes. When present always outnumber the other nematode species [2]. They infest a broad number of crops with vegetables the most preferred [3]. Their incidence in the warm continent but also in Mediterranean soils [4] and climatic conditions can be observed almost throughout the year [5]. The extent of losses is enormous and depends upon the crop species infested and the management system of crop [6]. Damages consist of root malformation [7], association with other plant-parasitic micro-organisms [8,9] and more important plant-parasitic fungi [10], alteration in nutrient and water uptake and translocation from roots [11] and inhibition of nodulation [12] which in turn indirectly affects photosynthesis and respiration. In order to contrast or compensate symptoms development, farmers use considerable amount of plant nutrients to promote plant growth and save production [13]. Association of root-knot nematodes with diseases increases hazard for crops. When root-knot nematodes combine with diseases in roots, wilt symptoms are promoted [14,15]. The root-knot nematodes are sedentary endoparasites of underground plant parts. Although the eggs and second stage juveniles may be present in soil, the active phase is related with the host presence. Root knot nematodes essentially have the same pattern of life cycle, although the length of life cycle may differ [16]. Large variation of the mode of reproduction exists in *Meloidogyne* species which may be a reason for their world-wide success [17]. Nematode egg production rates as mediated by environmental conditions and host status are important determinants of population development [18]. Length of egg production period is longer in those varieties where the production rate is low. Induced metabolic stress to plant influences the development of nematode population by affecting the sex ratio and egg production rates. Build up of high populations of root-knot nematodes is conditioned by the presence of other micro-organisms and symbiosis with them [19].

A wide range of methods and practices have been proposed and implemented to control root-knot nematodes. Chemical control is considered as the most efficient by farmers [20] but there are significant concerns due to environmental and human safety issues [21]. Quarantine may prevent distribution of nematode pests from one place to another. It is applied by governmental agencies and consists of strict procedures to prevent importation of a pest to an area where it does not exist. Quarantine has been proved efficient in the past to stop or delay the spread of nematode pests but also it is considered effective in present due to the intensity of imports and exports of plant material [22]. Fallow and crop rotation [23] can be applied to suppress populations of root-knot nematodes after their introduction in a new area [24]. Soil solarization is promising when applied in combination with amendments [25], particularly for nematodes settled in topsoil. Trap plants [26], organic amendments [27] and plant extracts [28] are also alternatives control methods. Resistant plants prevent the nematodes completing their life cycle in roots [29]. Biological control can be identified as the use of living organisms to control pests and diseases. A range of living organisms has been identified to control nematodes. These include fungi (predatory or parasitic), bacteria, predatory nematodes or insects. Each organism provides its own contribution to the nematode control, as a specific relation between predator:prey or parasite:victim organism, usually exists [30]. Biological control has early been identified as an excellent option to control root-knot nematodes in the field [31]. Today biological control offers a reliable alternative for root-knot nematode control with no environmental or health concerns [32].

Pochonia chlamydosporia (previously *Verticillium chlamydosporium*) (Goddard), is a wide-spread facultative parasite of root-knot nematodes [33,34]. It exists in soil as saprophyte, but can shift to parasite to root-knot nematodes. *P. chlamydosporia* has also proved effective against potato cyst nematode [35]. The fungus colonizes the root system of several species. It is confined to the rhizosphere and the rhizoplane. Hyphae of *P. chalmydosporia* can penetrate epidermal cells often by means of appressoria [36]. Modification of root cells content is common after inoculation with *P. chlamydosporia* [37]. Treatments with *P. chlamydosporia* as bionematicide controlled effectively root-knot nematodes [38]. Improved control was achieved when *P. chlamydosporia* is further

applied in combination with other biocontrol agent and compost amendments [39].

Weeds are considered to occupy a broad range of ecological niches. Weeds always grow next to crops competing for resources such as light, nutrients, water and space. Weeds also harbor inocula of other, beneficial or not, organisms. The role of weeds is considered very important to Integrated Pest Management as alternative hosts for pests and diseases [40]. Not all the weeds respond the same to nematode invasion: Belair and Benoit [41], studied thirty-two weed species for host suitability to a local isolate of the northern root-knot nematode *Meloidogyne hapla*. Several weeds as *Bidens cernua*, *Matricaria matricarioides* sustained moderate to high galling by *Meloidogyne hapla* and supported high reproduction. Others, like *Chrysanthemum leucanthemum* sustained moderate galling and supported moderate *M. hapla* reproduction whereas *Chenopodium album, Portulaca oleracea* supported low reproduction and sustained low galling. Galling was observed on *Senecio vulgaris* but no eggs or juveniles, which makes this weed useful as a trap plant. Some others as *Eupatorium maculatum* did not exhibit dinstict galling but supported low to moderate *M. hapla* reproduction. Finally some others as *Echinochloa crus-galli* were non-hosts. Schroeder [42] showed that a commensalism association exists between root-knot nematodes (*Meloidogyne incognita*) and yellow nutsedge (*Cyperus rotundus*): tuber number and weight and root weight increased as final nematode population increased. Zancada [43] studied the susceptibility of pepper (*Capsicum annum*) and black nightshade (*Solanum nigrum*) to *Meloidogyne incognita*: the nematode invaded both the plants and multiplication rates were high in both pepper and *Solanum nigrum*. The parasite reduced all the growth parameters of the crop, but did less harm to the weed.

Damages from root-knot nematodes and wilt diseases increasingly impact on environment and affect the whole supply chain and the farmers' income, because in order to contrast or compensate symptoms development, farmers apply considerable amount of plant nutrients to promote plant growth and save production. This common practice increases costs of production without preventing nematodes' proliferation in agricultural soils. Consequently the sustainability of agricultural production in such soils is steadily further decreasing because of a) an increasing impact on environment and local natural resources due to fertilizer and pesticide application and of b) a steadily decreasing farmers' income.

The present research aims to put the basis to set up an easy and ready to apply strategy allowing to control root-knot nematodes and wilt diseases in vegetable crops avoiding the further increase of nematodes presence in soils.

MATERIALS AND METHODS

Preparation of the fungus (*Pochonia chlamydosporia*): Conical flasks of 500 mL were filled with a mixture of corn meal and sand. The total weight was 260 g (200 g sand, 60 g corn meal). Flasks were tapped with aluminum foil, sealed with tape and then autoclaved for 25 min at 121 °C. After autoclaved, approximately 50 mL distilled and autoclaved water was added in each flask and the flask was vigorously shaken. Next, five blocks (1-cm diameter each), were taken from the periphery of the fungus *Pochonia chlamydosporia* in a 9-cm Petri dish on corn meal and added in each flask. Flasks were then put in a growth chamber at 28 °C. Three days later, flasks were again shaken to disperse the fungus through the medium and then returned to the growth chamber for three weeks. Following this period, the colonized medium was washed through a tower of sieves of 250, 150 and 38 μm pore respectively and washed with a jet of water to separate the fungus from the medium. Any medium constituents (milled maize and sand) were finally trapped on the 38 μm pore sieve whereas the suspension of conidia, hyphal fragments and chlamydospores was collected in a tray. This suspension was next poured into plastic tubes for centrifuging at 2000 rpm for 2 min at 20 °C. After centrifuging, the excess of water was siphoned off and the remaining suspension which contained the settled spores (chlamydospores and conidia), was stored in low temperature (5 °C).

Extraction of the nematode (*Meloidogyne javanica*): The root system of tomato plant already infected by

root-knot nematodes (*Meloidogyne javanica*) was gently washed. After soil removal, the root was shaken for 3 min in diluted bleach (1000 mL H_2O+100 mL bleach) in a bottle. Then the bottle was vigorously shaken for 3 min and then washed through a 250 μm onto a 38 μm pore sieve. The root was also scratched on the 250 μm pore sieve in order to release and receive as many eggs as possible from the tomato root. Next, eggs were washed until bleach was totally removed and then the suspension which contained the eggs was poured in a modified Baermann aparatus on a double thickness of paper towelling and was placed in a growth chamber at 28 °C. Water was renewed daily. Juveniles that hatched during the first two days were discarded whereas those obtained from the third day onwards were counted daily, collected and inoculated to plants.

Colonization of roots of tomato (*Lycopersicum esculentum*, L.), common lambsquarters (*Chenopodium album*, L.) and black nightshade (*Solanum nigrum*, L.) already inoculated with *Meloidogyne javanica*, by *Pochonia chlamydosporia*:

Five week tomato plants (var. Tiny Tim) were transplanted in plastic pots (20 cm diameter). Each pot was filled with 1.5 L of proprietary bran of loam-based compost. One day after transplanting, each tomato plant was inoculated with 1500 juveniles root-knot nematodes (*Meloidogyne javanica*). Four holes were opened at a distance of 2-3 cm from the stem and 10 mL of nematode suspension was pipetted. Holes were then filled with soil and plants were watered. One day later, common lambsquarters and black nightshade just sprouted seedlings were sown randomly in pots already inoculated with root-knot nematodes. Five seedlings from each species were sown and covered with a very thin layer of soil (1-2 mm). Suspension of *Pochonia chlamydosporia* at a known concentration of 1670 spores/mL was added to all plots. Ten mL of the suspension were pipetted in each pot in five holes (2-3 cm depth each) opened at a distance of 2 to 3 cm from the stem. Holes were then filled with compost. Pots remained in the glasshouse and were watered daily. Fifty days later in which period weed seedlings were expected to have developed a reasonable root system, plants were harvested. Roots were gently washed and

fresh weight of roots was measured. After weighing, one g of roots per plant species was randomly sampled from each root system. Samples were then finely chopped and homogenized for 1 min in 9 mL of sterile 0.05% water agar. Using standard laboratory techniques, serial dilutions were prepared from this root suspension by adding 1 mL of suspension to 9 mL of sterile 0.05% water agar. For each sample 0.2 mL aliquots of each dilution (10^{-2} and 10^{-3}) were placed under sterile conditions onto 9-cm diameter Petri dish. Plates were then incubated at 25 °C for two to three weeks in a growth room. After incubation *Pochonia chlamydosporia* colonies were counted for each dilution. In order to confirm the presence of *Pochonia chlamydosporia* in the dilution plates, the fungus was subcultured on Potato Dextrose Agar medium (PDA 2%). The fungus colonies were then compared with a standard *Pochonia chlamydosporia* colony, originally growing on nutrient agar. The experiment followed the Completely Randomized Design. The size of the experiment was 24 plots with 3 treatments (Control-tomato, common lambsquarters and black nightshade) and 8 plots per treatment.

Effect of fungicide treatments on *Pochonia chlamydosporia* colonized on roots of tomato plants: Tomato seeds (var. Tiny Tim) were sown in plastic pots (20 cm diameter). Five weeks after sowing tomato plants were thinned to one plant/pot. Each pot was filled with 1.5 L proprietary brand of loam-based compost. One day after thinning, plants were inoculated with nematodes (*Meloidogyne javanica*) collected as previously described; 1500 juveniles root knot nematodes (in water suspension) were inoculated in each pot, in four holes (3 cm depth each) that were made around each tomato plant at a distance of 2-3 cm from the stem. Totally 2.4 mL of suspension was added into each plant (0.6 mL/hole). Holes were next filed with soil and the plants were watered. One day after juvenile inoculation, tomato plants were inoculated with the fungus *Pochonia chlamydosporia* (the fungus was prepared as previously described). Five holes of 3 cm depth were made around the stem of each tomato plant at a distance of 2-3 cm of the stem. Ten mL of fungus suspension was added in each pot (2 mL/hole). The concentration of suspension was estimated with a

hemaetocytometer and was found to be 1670 spores/mL. Holes were then filled with soil and plants were watered. Plants remained in the glasshouse and they were watered at least once per day. During days that temperature was very high, plants were irrigated twice or even three times per day. Nutrients (0.5 g per plot) were also weekly provided (N-P-K-Mg/12.12.17.2). Four weeks after fungus application, plants were sprayed foliarly with fungicides. A systemic fungicide (thiophanate methyl 72% w/v, at 2.5 g/L) and a non-systemic fungicide (mancozeb 70% w/v, at 0.7 g/L) were applied. Seven days after fungicide treatment, plants were harvested. Roots were gently washed and left to dry. After drying, roots were weighed and 1 g (dry weight) was randomly sampled from each root system. Samples were then finely chopped and homogenized for 1 min in 9 mL of sterile 0.05% water agar. Using standard laboratory techniques serial dilutions were prepared from this root suspension by adding 1 mL of suspension to 9 mL of sterile 0.05% water agar. For each sample, 0.2 mL aliquots of each dilution (10^{-2} and 10^{-3}) were placed under sterile conditions onto 9-cm diameter Petri dish which contained semi-selective medium. Plates were then incubated at 25 ^{o}C for three weeks in a growth chamber. After incubation the number of *P. chlamydosporia* colonies was counted in each dilution. In order to confirm the presence of *P. chlamydosporia* in the dilution plates, the fungus was subcultured on Potato Dextrose Agar medium (PDA 2%). The fungus colonies were then compared with a standard *P. chlamydosporia* colony originally grown in nutrient agar. The experiment followed the Completely Randomized Design with 3 treatments (a) Control, b) Tomato plants treated with thiophante methyl and c) Tomato plants treated with mancozeb and 8 plots per treatment. Totally 24 plots were used.

Evaluation of different plant species as hosts for root-knot nematodes: Seeds of tomato plants (var. Tiny Tim), black nightshade (*Solanum nigrum*, L.), common wild oat (*Avena fatua*, L.) and common lambsquarters (*Chenopodium album*, L.) were sown in pots filled with proprietary bran of loam-based compost. Four weeks after sowing, plants were thinned to one plant per pot. After thinning plants were inoculated with root-knot nematodes (*Meloidogyne* spp.). The nematodes were extracted from tomato roots as previously described. Each plant was inoculated with 1000 juvenile nematodes, in a suspension of 10 mL. Four holes were made around each plant where the suspension was pipetted. Plants were harvested 15 days after inoculation. Roots were left to dry and dry weight was measured. Then, they were placed in 0.5 mL glass beakers of boiling acid fucshin stain for approximately 3 min. Following this period, bags were removed from the beakers and allowed to cool in the stain. Once cooled, roots were washed in tap water and stored in small plastic pots in destain made up of equal volumes of glycerol and distilled water plus some drops of lactic acid, until required.

To estimate the nematode population in each root, roots were finely chopped and macerated in a 1:1 (v:w) mix of destain and distilled water for 2-3 min using a Silverson homogenizer. The total volume of each root suspension was then recorded. Five aliquots (2 mL/aliquot) were then taken and the number of nematodes was counted and the number of nematodes found in the samples was then projected to the total volume of the root suspension. The experiment followed the Completely Randomized Design with 4 treatments (control, black nightshade, common wild oat and common lambsquarters) and 8 plots per treatment. Totally 32 plots were used.

Statistical Analysis: Data were expressed as means ± S.D. For statistical analysis, one-way analysis of variance (ANOVA) was applied followed by Turkey's test. Differences were considered significant at $p < 0.05$. All statistical analyses were performed with statistical software JUMP (version 8).

RESULTS

Colonization of roots of tomato (*Lycopersicum esculentum*, L.), common lambsquarters (*Chenopodium album*, L.) and black nightshade (*Solanum nigrum*, L.) already inoculated with *Meloidogyne javanica*, by *Pochonia chlamydosporia*:

The ability of some crop weeds, commonly found in European fields to host *Pochonia chlamydosporia* was

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investigated in a greenhouse study. A winter weed (common lambsquarters) and a summer weed (black nightshade) were inoculated with chlamydospores of *P. chlamydosporia*. Tomato plants were also inoculated with spores and served as control. All species were first inoculated with root-knot nematodes. Results showed that weed species were well colonized by *P. chlamydosporia*. Analysis showed that there were no

significant differences in fungus colonies (at the level of significance p=0.05) between species taking into account number of colony forming units/root-g. Colony forming units calculated per root-g was 398.30 for control, 412.69 for common lambsquarters and 383.45 for black nightshade. According to these results all species appear to be suitable and valuable hosts for *P. chlamydosporia* survival in the field (Table 1).

Table 1. Colonization of *Pochonia chlamydosporia* on roots of tomato, common lambsquarters and black nightshade (numbers show colony forming units per root-g).

Treatments	Mean	Standard error of the mean
Tomato	398.30[a]	18.3
Common lambsquarters	412.69[a]	21.3
Black nightshade	383.45[a]	18.1

Results are means of eight replicates ± SE (Standard Error). Superscript letters depict the statistical analysis whereas different letters demonstrate statistically different values according to Turkey's multiple range test at P≤ 0.05

Effect of fungicide treatments on *Pochonia chlamydosporia* colonized on roots of tomato plants: The effect of fungicide treatment on *Pochonia chlamydosporia* colonies in plant roots was examined in a pot experiment. Tomato plants already colonized by the fungus were sprayed with two common foliar fungicides, i) thiophanate methyl 72% w/v (non systemic fungicide) and ii) mancozeb 70% w/v (systemic fungicide). Plots were sprayed only once. No adverse effect was observed on fungus colonies, by means of colony forming units, after plants were treated by fungicides.

Analysis showed that there were no significant differences in fungus colonies between treatments, at the level of significance p=0.05. Mean of colony forming units was 31.16 for control, 29.82 for thiophanate methyl and 30.56 for mancozeb. This is very important especially in cases where a complex of nematode and fungus disease infestation occurs in the plant. In this case, treatment of *P. chlamydosporia* to control root-knot nematodes will not be affected by a fungicide that will be applied to control the fungus disease (Table 2).

Table 2. Effect of fungicide treatments on *Pochonia chlamydosporia* colonized on roots of tomato plants (numbers show colony forming units per root-g).

Treatments	Mean	Standard error of the mean
Control	31.16[a]	0.52
Thiophanate 72% w/v	29.82[a]	0.32
Mancozeb 70%w/v	30.56[a]	0.39

Results are means of eight replicates ± SE (Standard Error). Superscript letters depict the statistical analysis whereas different letters demonstrate statistically different values according to Turkey's multiple range test at P≤ 0.05

Evaluation of different plant species as hosts for root-knot nematodes: Host preference of root-knot nematodes was examined in a pot experiment. A summer weed species (black nightshade) and two winter weed species (common wild oat and common lambsquarters) were compared with tomato plants for their suitability to host root-knot nematodes in roots. Results showed that all species were susceptible to

root-knot nematodes although that significant differences (p=0.032) were observed between species. Thus tomato was the preferred host for root-knot nematodes incidence (with 20 nematodes per root-g counted), followed by black nightshade (with 10 nematodes per root-g), common wild oat (with 4.8 nematodes per root-g) and common lambsquarters (with 4.4 nematodes per root-g) (Table 3).

Table 3. Incidence of root-knot nematodes (nematodes per root-g) in different plant species

Treatments	Mean	Standard error of the mean
Tomato	21.1[a]	0.68
Black nightshade	11.1[b]	0.39
Common wild oat	4.8[c]	0.31
Common lambsquarters	4.4[c]	0.32

Results are means of eight replicates ± SE (Standard Error). Superscript letters depict the statistical analysis whereas different letters demonstrate statistically different values according to Turkey's multiple range test at P≤ 0.05

DISCUSSION

One of the biggest challenges agriculture faces is to secure adequate and of high quality food production to fulfill increasing demands. To achieve this, agriculture has to overcome several obstacles, such as to protect crops from a large range of pests and diseases that cause significant yield loss and quality deterioration of products.

Nematodes have been identified as serious problem of crops worldwide. Although tiny round-worms, they have been perfectly adapted to all environments and crops. They are found in all agricultural fields, looking for hosts to invade. Therefore they damage crops directly or indirectly.

Efforts have been focused to alternative control methods [44-46]. Special attention has also been given to the identification and isolation of living organisms, which might be used to effectively suppress the populations of root-knot nematodes [47-48].

Meloidogyne spp. have been recognized as pests that reduce the vigor and growth of plants, also by the absorption and translocation of nutrients and water from roots to the above ground parts. Association with fungi and bacteria that cause wilt symptoms is well documented [49-50].

In order to design an effective and integrated plant protection program, alternative hosts of pests and diseases considered have to be taken into account. Root-knot nematodes have early been identified to colonize a large and "indefinite" range of alternative hosts [51]. In this study common lambsquarters (*Chenopodium album*), common wild oat (*Avena fatua*), black nightshade (*Solanum nigrum*) and common wild oat (*Poa annua*), were assessed as potential

hosts of root-knot nematodes in agricultural fields. They have all been found susceptible to root-knot nematodes although differences may exist in nematode population in the roots of host plants. Tomato plants have extensive root system and are expected to harbour higher nematode population. Moreover, some weeds (such as black nightshade) appear to be better hosts than other weeds (such as common lambsquarters) for root-knot nematodes. In this study *Chenopodium album* although invaded by root-knot nematodes was not preferred host. This is also confirmed by the literature, where *Chenopodium album* is considered as a poor host for root-knot nematodes [52-54].

Weeds hold an important role in the agroecosystem. They compete with crops for resources such as water, nutrients, light and space, they harbour inocula of pests and diseases but they also host beneficial organisms. Furthermore, as weeds exploit soil nutrients better than crops it is expected to represent also a great source of nutrients for nematodes. In this study, nematodes showed a clear preference to tomato plants compare to weeds tested, but weeds remain excellent alternative hosts for root-knot nematodes, particularly in periods (such as fallow) that crops are absent.

Pochonia chlamydosporia is a fungus that has been proved to suppress nematodes [55]. It saprophytes but also colonizes the roots of plants. The fungus produces appressoria, with which penetrates and parasitizes the eggs of root-knot nematodes [56]. Egg penetration increases when *P. chlamydosporia* is combined with chitosan treatments [57]. The fungus can also reduce root damage caused by fungal diseases [58,59]. It appears that acid environment promotes mycelium growth, whereas light enhances sporulation [60]. Effectiveness of *P. chlamydosporia* also depends

on soil conditions [61,62]. Apart from inducing differential gene expression and re-programming of the transcriptome profile in tomato roots, the fungus has been observed to up-regulate secondary metabolism, stress and defense response with high expression levels [63]. *P. chlamydosporia* colonizes a wide range crop species such as cabbage, tomato, kale, maize [64]. This study shows that weed species are also suitable hosts for the fungus. The fungus colonized the roots of weed species assessed. As root system of weeds was free of nematodes, it is assumed that nematode infection in weed roots was not a prerequisite for fungus infection. The fungus also survived successfully in soil compost although the concentration applied of 1670 spores/ml for a total of 10 mL/pot is considered very low. The fungus colonized remarkably fast on weed roots showing that weeds are suitable hosts which promoted fungus growth. This implies also that a compatible relationship exists between weeds and the naturally occurring saprophytic fungus.

Apart from being effective to control its target, it is very important for a biocontrol agent to be efficient to control its target as part of an Integrated Pest Management Regime. Chemical applications are included in such programs and biocontrol agents have to tolerate the effect of pesticides. Horticultural crops suffer heavily from leaf and root diseases and they are treated with considerable amounts of fungicides. In this study foliar spray didn't affect *P. chlamydosporia* colonies in tomato plants which can be explained either by the tolerance of *P. chlamydosporia* in chemical compounds [65] and particularly in fungicides [66], or the development of the fungus in the outer layer of roots which helped avoid the effect of treatments. Thus, the fungus can be used to effectively control root-knot nematodes, in the frame of an Integrated Pest Management program. These properties have been appreciated and formulation of *P. chlamydosporia* as a pesticide, has already been achieved [67].

CONCLUSIONS

Weeds species are offered as successful alternative hosts, although to different extent, for root-knot nematodes ensuring survival during a rotation scheme or a fallow period. Pochonia chlamydosporia is an egg parasite of root-knot nematodes with high potential as a control agent in the field. It colonizes a high range of hosts, both crops and weeds. The fungus appears not to suffer adverse effects from foliar sprays of fungicides which makes P. chlamydosporia an excellent candidate for biological control of root-knot nematodes in a complex of root-knot nematodes and wilt-root disease.

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