Diversity of nematophagous fungi in Moroccan olive nurseries: Highlighting prey-predator interactions and efficient strains against root-knot nematodes

Mohamed Ait Hamza, Hicham Lakhtar, Hafssa Tazi, Abdelmajid Moukhli, Odile Fossati-Gaschignard, Lucie Miche, Sevastianos Roussos, Zahra Ferji, Abdelhamid El Mousadik, Thierry Mateille, et al.

► To cite this version:

Mohamed Ait Hamza, Hicham Lakhtar, Hafssa Tazi, Abdelmajid Moukhli, Odile Fossati-Gaschignard, et al.. Diversity of nematophagous fungi in Moroccan olive nurseries: Highlighting prey-predator interactions and efficient strains against root-knot nematodes. *Biological Control*, 2017, 114, pp.14-23. 10.1016/j.biocontrol.2017.07.011 . hal-01681583

HAL Id: hal-01681583

<https://hal.science/hal-01681583>

Submitted on 23 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Diversity of nematophagous fungi in Moroccan olive nurseries: Highlighting prey-predator interactions and efficient strains against root-knot nematodes.

Mohamed AÏT HAMZA^{1,4}, Hicham LAKHTAR², Hafssa TAZI², Abdelmajid MOUKHLI³, Odile FOSSATI-GASCHIGNARD⁴, Lucie MICHÉ⁵, Sebastianos ROUSSOS⁵, Zahra FERJI⁶, Abdelhamid EL MOUSADIK¹, Thierry MATEILLE^{4,a}, Hassan BOUBAKER^{2,*,a}

¹ Université Ibn Zohr, Faculté des Sciences d'Agadir, Laboratoire LBVRN, BP 8106, 80000 Agadir, Morocco.

² Université Ibn Zohr, Faculté des Sciences d'Agadir, Laboratoire LBMPV, BP 8106, 80000 Agadir, Morocco.

³ INRA, CRRA, BP 513, Menara, Marrakech, Morocco.

⁴ IRD, UMR CBGP, 755 Avenue du Campus Agropolis, CS30016, 34988 Montpellier-sur-Lez Cedex, France.

⁵ IRD, UMR IMBE, Faculté des Sciences Saint Jérôme, Case 421, 13397 Marseille Cedex 20, France.

⁶ Institut Agronomique et Vétérinaire Hassan II, Campus d'Agadir, Département de Protection des Plantes, BP 18/S, 80000 Agadir, Morocco.

***Corresponding author:** hassanboubaker@yahoo.fr/mohamed.aithamza@edu.uiz.ac.ma

^a Co-leaders of the publication

Abstract

Plant-parasitic nematodes are major pests in agriculture. Microbial antagonists such as nematophagous fungi are of great interest for developing alternative biocontrol strategies to replace nematicide use. This study aims to isolate, identify and assess the diversity and the *in vitro* predatory activity of nematophagous fungi from various olive nurseries in Morocco. A total of 305 soil samples were collected in 25 nurseries in Moroccan olive-producing areas (Jbala, Guerouane, Haouz and Souss). Seventy-three pure fungal strains from nematophagous fungi were recovered from nursery substrates infested with root-knot nematodes. Diversity indices showed that fungi were more numerous in southern regions, whereas fungal communities were more numerically alike in northern regions, partly depending on the occurrence of root-knot nematodes. One fungal strain, *Talaromyces assiutensis*, killed all *Meloidogyne javanica* juveniles during *in vitro* predation tests. Orbiliaceae (*Arthrobotrys* spp., *Drechslerella brochopaga*, *Monacrosporium* spp.) trapped 50 to 80% of juveniles. *Purpureocillium* and *Trichoderma* species killed 30 to 50% of juveniles. The diversity of nematophagous communities could be affected by the north-south climate gradient of Morocco, by their nematode prey expansion, and, to a lesser extent, by the habitat origin of the substrates used. *Talaromyces assiutensis* was the most efficient nematophagous species against *M. javanica*. The use of native nematophagous fungi is a promising alternative strategy for nematode control in olive nurseries and to prevent nematode introduction into olive orchards.

Keywords: Culture substrate, Ecology, Fungal antagonists, Olive tree, Root-knot nematode.

1. Introduction

The olive tree (*Olea europaea* L.) is widely distributed throughout the Mediterranean Basin. In Morocco alone, the olive-growing area is estimated at 650,000 ha and produces 120,000 tons of oil and 150,000 tons of table olives per year (Ministry of Agricultural and Marine Fisheries, 2009). Planting material comes from several nurseries distributed throughout the olive-producing areas and olive plantlets are certified free of pathogens (e.g., *Verticillium dahlia*) and parasites (e.g., plant-parasitic nematodes). Nevertheless, standard health practices are not applied in all nurseries and seasonal and informal nurseries coexist.

Several plant-parasitic nematode species (PPN) have been found to be associated with olive trees (Castillo et al., 2010; Ali et al., 2014; Hamza et al., 2015), including root-knot nematodes (RKN, *Meloidogyne* spp.) that were shown to impact olive growth (Afshar et al., 2014). RKN have been reported to be major pests on olive trees, mainly in nurseries where irrigation conditions are favorable to their multiplication (Nico et al., 2002; Sanei and Okhovvat, 2011). Moreover, most RKN infestations in olive orchards result from contaminated plant material produced in uncertified nurseries (Nico et al., 2002). To protect nurseries, vegetative propagation of olive is a crucial step to prevent plant pest dispersal. While some industrial olive-producing countries use synthetic substrates to cultivate olive trees, other countries use more traditional olive cultivation techniques, planting roots in soil substrates. In countries that have implemented large-scale planting programs (e.g., 1,220,000 ha in the Morocco Green Plan, 2015), the “nursery risk” is a concern, with two main consequences: (i) the weakening of olive plants before their transplantation into orchards; and (ii) the introduction of pathogens into orchards. The nematode risk is now controlled in nurseries, either by multiplication of resistant varieties (Palomares-Rius et al., 2012) or by inoculating microbial antagonists in the substrates, such as mycorrhizae (Castillo et al., 2006).

Thus, in view of the large-scale development of olive cultivation in Morocco, sanitization of the substrates must be a priority. Some previous studies highlighted that certain substrates can be suppressive depending on their composition, either because of microbial antagonisms or of toxicity. For example, the addition of forest residues provides organic matter that could contribute to PPN suppression by increasing soil microflora (Rodriguez-Kabana, 1987). Composted dry cork, especially in nurseries, could be effective for *M. incognita* suppression due to the toxicity of the products released (e.g., ammonia, phenolic compounds) (Nico et al., 2004). Moreover, the introduction of microbial antagonists into substrates would strengthen the sustainability of preventive techniques and help introduce biocontrol agents into orchards.

Microbial antagonists of PPN include nematophagous fungi (NF), raising expectations for their use in integrated pest management (Waller, 2006; Larsen, 2006; Maingi et al., 2006). These fungi act by antibiosis, parasitism or predation (Imerglik, 1981; Gaspard and Mankau, 1986). Fungi trap nematodes using adhesive networks or buttons. Some NF capture their prey with constrictor rings before strangling them and others with hyphae networks that produce sticky compounds. The specific enzymes then digest the nematodes. Recognition and attachment of the mycelium to the nematode cuticle are mainly due to compatible glycoproteins such as lectins (Nordbring-Hertz et al., 2006).

Nevertheless, edaphic factors such as soil pH, temperature, moisture and structure influence their efficiency (Brown and Kerry, 1987). Fungal diversity can increase in stressed or polluted sites (Korol et al., 1994) in order to adapt to changing environments (West et al., 1999). In *Arthrobotrys oligospora*, genetic diversity varies according to environmental conditions. Their strains could generate recombinant genotypes by crossing with native strains, thus enhancing their environmental adaptability and parasitizing ability (Zhang et al., 2013).

However, the reproductive strategies of fungi like *A. oligospora* can change depending on the population. According to Cook (1993), the search for microorganisms from the rhizospheric soil of a specific crop could lead to the isolation of effective antagonists against pathogens that could be adapted to the plant species as well as to particular environmental conditions.

Considering that olive seedlings in Morocco are generally cultivated in non-sanitized substrates consisting of soil material from various habitat origins, the recovery of native NF from substrates may be of great interest in order to develop RKN biocontrol agents adapted to nursery conditions. In this context, this work aims to (i) evaluate the nematode populations in substrates from olive nurseries in Morocco; (ii) isolate and characterize NF able to control RKN (*Meloidogyne* spp.); (iii) determine the occurrence and diversity of nematode-associated NF and discuss prey-predator interactions; and (iv) investigate their *in vitro* predatory potential towards *M. javanica* juveniles and eggs, and their usefulness as biocontrol agents for olive protection in nurseries and in orchards.

2. Materials and methods

2.1. Site description and olive plantlet sampling

Soil samples were collected in spring 2013 and 2014 from 25 commercial olive nurseries located in the main olive-producing areas in Morocco (Fig. 1 and Table 1): the Jbala, Guerouane, Haouz and Souss regions. The nurseries were selected for their plantlet production, the cultivars grown and the rearing substrates used.

Olive plants are grown in 2-to-3 liter plastic bags filled with solid substrates from different sources (alluvial sandy soils, forest soils, loamy open-field soils) supplemented with different proportions of sand, peat fertilizer and animal manure. Plants are first grown in plastic greenhouses and then outside. They are watered by sprinklers and fertilized with Osmocote®

(Everris Company™).

In each nursery and for each variety, five olive plantlets (*Olea europaea* subsp. *europaea*) growing in plastic bags were sampled. Information about the origin and the preparation of the growth substrates and about the cultivars was recorded. A total of 305 olive plantlets were collected and maintained in the laboratory and kept under greenhouse conditions (12 h light at 25 °C; 12 h dark at 20°C).

2.2. *Nematode extraction and quantification*

A 250-cm³ substrate subsample was removed from the rhizosphere of each olive plantlet and used for nematode extraction using the Oostenbrink (1960) elutriation procedure (ISO 23611-4). Free-living nematodes (FLN) and plant-parasitic nematodes (PPN), which specifically exhibit a stylet for plant-cell feeding, were enumerated in 5-cm³ counting chambers (Merny and Luc, 1969) under a stereomicroscope (×60 magnification). Nematode population levels were expressed as the number of individuals per dm³ of fresh substrate. Among PPN, root-knot nematodes belonging to the *Meloidogyne* genus (Mai and Mullin, 1996) were counted.

2.3. *Isolation of nematophagous fungi*

NF were isolated from solid substrate samples infested with RKN using the soil sprinkling technique (Duddington, 1955) as modified by Santos et al. (1991). The direct soil powdering on media was preferable to the modified Baermann method (aqueous soil suspensions) (Hernández-Chavarría and Avendaño, 2001) because it provided more concentrated material for fungal isolation. Moreover, isolation success was increased by sprinkling cold soil (stored at 4°C) on culture medium at 37 to 40°C (Davet and Rouxel, 2000). Substrate aliquots from each olive plantlet were spread on a tray to be air-dried. One gram was then sprinkled on the surface of Petri dishes containing water-agar (WA 2% w/v) supplemented with antibiotics (0.05% streptomycin-sulphate and 0.05% chloramphenicol). Three replicates were done per olive plantlet. A 1-mL suspension containing approximately 3,000 *M. javanica* second-stage

juveniles (J2) and 10 eggs produced in the laboratory was added as fungal bait, according to the procedure used by Drechsler (1941). The Petri dishes were sealed with Parafilm and incubated at room temperature (20-27°C) in darkness for two months. Petri dishes were examined daily during the first week of incubation and weekly thereafter. Fungi that had developed were isolated under a microscope (x60 magnification). Conidia or pieces of agar bearing either an NF mycelium or an infected nematode were transferred onto Petri dishes containing Corn Meal Agar (CMA) or Potato Dextrose Agar (PDA) supplemented with antibiotics (0.05% streptomycin-sulphate and 0.05% chloramphenicol). The fungi were transferred to a new culture medium plate for purification after reaching full growth. Pure cultures were maintained on PDA or CMA plates at 4°C in darkness for a maximum of six months.

2.4. *Morphological and molecular characterization of the fungi*

The initial identification of the fungi was based on colony morphology and microscopic characteristics. Slide sub-cultures from pure NF cultures were observed under a dissecting microscope (up to x100 magnification). Genera and species were assigned according to specialized morpho-taxonomical keys (Cooke and Dickinson, 1965; Haard, 1968; Barron, 1977; Yu et al., 2014; Philip, 2002).

Sequence analyses of the ITS (internal transcribed spacer) region in the ribosomal RNA gene cluster were performed to confirm the identity of the NF species. The DNA was extracted from 50-200 mg of mycelium (fresh weight) using the NucleoSpin®Plant II Genomic DNA Purification Kit (Promega®) according to the manufacturer's instructions. PCR reaction was performed according to the method described by White et al. (1990): the ITS rDNA gene cluster was amplified using the primers ITS1 (5'TCC GTA GGT GAA CCT GCG G 3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC 3'). The PCR amplification was carried out using the GeneAmpR PCR System 9700 (Applied Biosystems®). Twenty µl of reaction

mixture contained 2 µl (10 ng) of template DNA, 1 µl of each ITS1 and ITS4 primer (10 mM), 4 µl PCR buffer, 2.4 µl MgCl₂ (25 mM), 0.6 µl dNTPs (10 mM), 0.1 µl BSA (0.1 mg/ml) and 0.2 units of GoTaq[®] DNA polymerase.

PCR cycling conditions consisted of an initial denaturation step at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 10 min. PCR products were checked for length, quality and quantity by agarose gel electrophoresis (1% (w/v) in 0.5x Tris-Acetate-EDTA (TAE).

PCR products were sequenced from both ends by Eurofins MWG GmbH (Ebersberg, Germany), using the same ITS primers. CHROMAS LITE v2.1.1 (Technelysium Pty Ltd.) software was used to edit and assemble DNA sequences. BLAST similarity searches were performed in the non-redundant nucleotide database of GenBank (Altschul et al., 1997) to identify/verify species or genus affiliation of collected isolates. Sequences were aligned with ITS sequences of reference strains obtained from GenBank. Subsequently, the alignment was used to perform the phylogenetic tree with PHYLOGENY.FR (Dereeper et al., 2008) and Mega 6 (Tamura et al., 2007) using the neighbor-joining method (Saitou and Nei, 1987) and the Jukes-Cantor correct distance model (Jukes and Cantor, 1969). The phylogenetic tree was obtained from data using one of three equally parsimonious trees through 1,000 bootstrap replicates (Felsenstein, 1985) with a heuristic search consisting of 10 random-addition replicates for each bootstrap replicate.

2.5. *Diversity of the fungal communities*

Four diversity indices were calculated to assess NF communities: total number of isolates found in each soil sample (N); richness (S = number of species in the community); Shannon–Wiener local diversity index ($H' = -\sum (p_i \cdot \ln p_i)$, where p_i is the proportion of isolates with the species i); and evenness ($E = H' / \ln(S)$), which quantifies the numerical equality of populations in communities.

2.6. *Pathogenicity analyses*

An olive population of the RKN *M. javanica* (detected in 72% of the nurseries surveyed and dominant in orchards) (Ali et al., 2016) was reared on RKN-susceptible tomato (cv. Roma) in a greenhouse (12 h light at 25°C; 12 h dark at 20°C). Fungal strains that exhibited trapping, adhesive or encysting organs were sub-cultured on WA in 9-cm-diameter Petri dishes. One week later, 100 second-stage juveniles (J2) of *M. javanica* (the only free form in the soil) were washed five times with 0.05% streptomycin-sulfate in sterilized distilled water, introduced into each fungal sub-culture and maintained at 25°C in darkness. Three Petri dishes per fungal strain were considered as replicates. Fungal predation structures were observed and predated/dead nematodes were counted after four days under a microscope (x100 magnification). Similar procedures were used to study predation of *M. javanica* eggs by specialized egg parasites such as *Paecilomyces* and *Pochonia* fungi. Dishes with nematodes but without fungi were considered as control replicates.

2.7. *Statistical analyses*

Mean values were analyzed by one-way ANOVA and Kruskal-Wallis tests were used for all pair-wise multiple comparisons. NF community patterns were explored through a Principal Component Analysis (PCA) of the diversity indices. Region grouping was tested using Monte-Carlo tests on PCA eigenvalues (randtest, ade4). Calculations were performed and graphics prepared using R language (readxl, base and ade4 packages) (R Development Core Team, 2011; Chessel et al., 2004; Dray and Dufour, 2007, Wickham, 2016), with a level of significance = 0.05). A rarefaction regression was used to analyze the dependence between root-knot nematodes (*Meloidogyne* spp.) and nematophagous fungi.

3. **Results**

3.1. *Nematofauna*

A non-significant gradient was present in plant-parasitic-nematodes (PPN), with a north-south increase of the population levels (Table 2). A parallel significant gradient was revealed in root-knot nematodes (RKN, *Meloidogyne* spp.). Free-living nematodes (FLN) were three times more abundant in the Souss olive nurseries than in the other regions. The ratios between FLN and PPN were different between all regions, the highest ratio being found in the Souss region.

3.2. *Nematophagous species and phylogenetic diversity*

Several fungal isolates were recovered from the 305 soil samples examined. Observation of characteristic conidia and traps around dead *M. javanica* juveniles revealed 149 soil samples positive for NF. Morphological identification using microculture techniques revealed 73 NF strains belonging to 11 genera.

In order to confirm the characterization of the fungal strains, the ITS regions of rDNA were sequenced. Five species (*Catenaria anguillulae*, *Nematoctonus leiosporus*, *Haptoglossa heterospora*, *Dactylaria* sp. and *Monacrosporium microphoides*) were excluded from the sequencing because it was not possible to purify them. The BLAST test showed that the ITS sequences of all sequenced strains were at least 99% similar to the corresponding GenBank reference sequences (Table 3). The phylogenetic analysis including ITS sequences of the Moroccan NF isolates and 16 reference sequences of identified close relatives revealed five distinct clusters (Figs. 2 A-E): *Purpureocillium lilacinum*, *Trichoderma*, *Fusarium oxysporum*, *Talaromyces* and *Arthrobotrys-Dreschlerella-Monacrosporium* (family Orbiliaceae).

The combination of the morphological and the molecular analyses established that the isolated NF belonged to 19 species, eight families and six orders (Table 4). *Arthrobotrys* was the most diversified genus with five species, followed by *Trichoderma* (three species). The abundance of fungal species was low (less than five strains per species) except for *Paecilomyces lilacinus*

that represented 36 strains (50% of the strains). Half of the *P. lilacinus* strains detected were isolated from the Souss region, and then gradually decreased northwards (12 in Haouz, four in Guerouane, two in Jbala). *Arthrobotrys brochopaga*, *A. scaphoides*, *Monacrosporium thaumasium*, *P. lilacinus*, *F. oxysporum*, *T. harzianum*, *T. asperellum* and *T. longibrachiatum* were encountered in the four regions. *A. oligospora*, *Dactylaria* sp., *Haptoglossa heterospora*, *Monacrosporium microscaphoides*, *Nematoctonus leiosporus* and *Talaromyces assiutensis* were very rare (one isolate in one region). All the fungal species except *A. oligospora* were detected in the Souss region. Only eight species were detected in the Jbala region. The Guerouane and Haouz regions hosted 11 and 12 species, respectively.

3.3. Diversity patterns

Because of their scarcity (only one nursery surveyed), the substrate samples from the Jbala region were excluded from the dataset prior to running analyses. Richness (S) and local diversity (H') were correlated to the PC1 axis, while the PC2 axis was related to numbers (N) and to evenness (E) of fungal isolates on its positive and negative sides, respectively (Fig. 3A). Region grouping was significant in the whole analysis and on the PC2 axis (Fig. 3B). Isolates were more numerous in the Souss region, whereas fungal communities were more numerically alike in the Guerouane region. The Haouz region had lower NF richness and diversity (non-significant PC1 coordinates). Indices were affected by the north-south distribution of the nurseries (Figs. 3C-3E-3D).

The percentage of samples with NF was lower in the Haouz region than elsewhere (Table 5). Both trapping and endoparasitic fungi occurred more often in the Souss region than in the others regions, and no endoparasitic species were found in the Jbala region.

The rarefaction regression established between the occurrence of RKN (*Meloidogyne* spp.) and the occurrence of NF (Fig. 4) indicated a significant positive correlation, regardless of the region sampled.

3.4. In vitro efficiency of the nematophagous strains

NF were distinguished according to their ability to kill *M. javanica* J2s (Fig. 5). *Talaromyces assiutensis* killed all juveniles in all replicates. The Orbiliaceae species (*Arthrobotrys* spp., *Dreschlerella* spp., *Monacrosporium* spp.) were efficient against *M. javanica* since they trap 50 to 80% of the J2s using adhesives networks, buttons, constricting rings and hyphae networks. *Paecilomyces* and *Trichoderma* strains killed 30 to 50% of the J2s. *Fusarium oxysporum* strains were less efficient (less than 20% of dead J2s). *P. lilacinus* and *P. chlamydosporia* strains infected all the *M. javanica* eggs.

4. Discussion

Our first objective was to evaluate nematode populations in substrates from olive nurseries. In all regions of Morocco, PPN abundance was greater than 1.4 nematodes/dm³ of soil and FLN abundance greater than 1.9. As a comparison, Hamza et al. (2015) found 0.2 to 5.1 PPN/dm³ of soil and 0.3 to 4.3 FLN/dm³ of soil in 23 Souss and Haouz orchards. It can thus be hypothesized that the multiplication of nematode populations may be boosted by acidity and by hydrophilic and non-degraded organic matter (Neher and Olson, 1999; Manlay et al., 2000; Ou et al., 2005; McSorley, 2011). The usual dominance of FLN in olive soils could be due to the origin of organic substrates (mountain soils, peat and manure) used in most nurseries (Castillo et al., 2006). FLN are known to dominate in soil substrates not yet used for agriculture (Hillocks, 1997). The FLN dominance in the Souss region, one of the most intensively cultured areas in Morocco, was thus unexpected.

The FLN/PPN ratios were balanced in all regions except in the Souss where most of the soil substrates come from cropped areas (vegetables and citrus fruits). Because of the sandy texture of the soils in the Souss region, culture practices include high amounts of organic matter (especially cattle manure or tomato leaf compost), leading to an increase in FLN

293 populations and to a decrease in PPN populations because organic matter is unsuitable for
294 PPN (Clark et al., 1998; Hominick, 1999; Hu and Qi, 2010). These mechanisms may explain
295 the FLN/PPN ratio that is twice as high in the Souss region compared to the other regions The
296 high percentages of RKN (*Meloidogyne* spp.) in Souss olive nurseries may be justified by the
297 dominance throughout the area of vegetable crops that are highly susceptible to these
298 nematodes (Sikora and Fernandez, 2005; Netscher, 1970).

299 Our second objective was to isolate and characterize nematophagous fungi able to control
300 RKN (*Meloidogyne* spp.). The success of fungal strain isolation from soils may be correlated
301 with soil temperature, moisture and organic matter content (Akhtar and Malik, 2000; Cayrol
302 et al., 1992). The greatest difficulty encountered was in isolating pure NF strains because of
303 the rapid growth of plant-pathogenic fungi and saprophytes such as *Fusarium*, *Alternaria* and
304 *Aspergillus* species. An increased pH of culture media may prevent the development of other
305 microorganisms (Gardner et al., 2000). Moreover, we found that direct cold soil powdering on
306 hot media (37 to 40°C), like in Kelly et al., (2009), was preferable to aqueous soil suspensions
307 because it provided more concentrated fungal material (Davet and Rouxel, 2000; Hernández-
308 Chavarría and Avendaño, 2001). Dispersing agents were avoided because they inhibited the
309 growth of some NF strains (Davet and Rouxel, 2000).

310 This survey provided native NF from Morocco for the first time. The large number of soil
311 samples (305) allowed the detection of numerous NF strains, whereas former studies revealed
312 one strain at best (Bridge and Spooner, 2001). The strains detected as nematophagous
313 possessed different modes of action: adhesive networks, constricting rings, hyphal tips,
314 adhesive conidia and mycotoxins (Imerglik, 1981; Gaspard and Mankau, 1986). Despite this,
315 ITS rDNA sequences could not be used to identify some NF strains due to their small size
316 (*Pochonia chlamydosporia*, for example), BLAST tests were useful to confirm the
317 morphological characterization of other strains. The integrated taxonomical analysis

(morphological and molecular) of the NF provided a pool of 28 strains belonging to 19 species. The phylogenic analysis revealed that the ITS rDNA gene was able to distinguish species in a genus group (such as *Trichoderma*) but did not fully discriminate species belonging to the family Orbiliaceae, indicating the taxonomic proximity of *Dreschlerella*, *Arthrobotrys* and *Monacrosporium* species. Therefore, an integrative molecular analysis should be developed with other molecular markers (White et al., 1990) in order to improve NF identification.

Our third objective was to determine the occurrence and diversity of nematode-associated NF. All NF species except *A. oligospora* were detected in the Souss region where they were more dominant than in the other regions. This high diversity of NF might be due to the multiple habitat origins of the components (mountain, riverbank and field soils, cattle manure, plant compost, etc.) used to make substrates for root olive plantlets. We hypothesize that the microbial richness detected in the Souss region corresponds to the high endemic plant diversity characteristic of the Macaronesian region (Médail and Diadema, 2009; Msanda et al., 2005). This could also be due to higher organic matter concentration in the soils (i.e., saprophytic substrate for fungi that induce the formation and the activity of trapping structures) and to high PPN levels (i.e., parasitic substrates for fungi) (Den Belder and Jansen, 1994; Singh et al., 2012).

The number of NF isolates globally increased southwards, whereas evenness decreased (PCA2 axis). It is known that different thermal regimes affect soil microbial diversity (Bridge and Spooner, 2001). The absence of seasonality and the higher minimum temperatures in the Souss region may explain these developments where irrigation counteracts the arid to semi-arid climate. The high occurrence of NF and the high evenness detected in the Guerouane region may be linked to a more continental climate. More than half of the 36 *P. lilacinus* strains came from the Souss region where the NF richness was the highest but the evenness

the lowest. In a restricted soil area, the abundance of *P. lilacinus* may cause the rarity of the other species. For a constant number of species, maximal diversity is achieved when species have an even distribution. The Haouz, Jbala and Guerouane regions were characterized by less PPN and FLN than in the Souss region, explaining the lower NF richness in those regions.

Our fourth objective was to investigate the *in vitro* predation of the NF against *M. javanica* juveniles and eggs. *Talaromyces assiutensis* (strain UIZFSA-31), whose nematode predation was previously unknown, killed all the *M. javanica* juveniles in four days. The mode of action of *T. assiutensis* remains unknown but we hypothesize that the strain may produce specific mycotoxins. *In vitro* trapping tests prove that the Orbiliaceae species (*Arthrobotrys* spp., *Dreschlerella* spp., *Monacrosporium* spp.) were able to trap *M. javanica* juveniles. The predatory capacity of *A. oligospora* was similar to data found in the literature (Singh et al., 2012). The mechanisms involved during predation are well known (Imerglik, 1981; Gaspard and Mankau, 1986). Recognition and attachment of the mycelium to the cuticle of the RKN juvenile is mainly due to compatible glycoproteins, e.g., lectins (Duponnois et al., 1996). *Trichoderma* species are recognized as control agents against nematodes, and various mechanisms have been proposed to explain nematode killing, including antibiosis and enzymatic hydrolysis (Sivan and Chet, 1992; Elad, 2000). Thomas (1996) demonstrated direct interactions between *T. harzianum* and the cyst nematode *Globodera rostochiensis*: the fungus penetrated eggs in the cysts, leading to the death of the juveniles (Sharon et al., 2001). Precise information on the mechanisms involved is very limited and this misunderstanding has hindered the selection of active strains and the development of improved biocontrol methods. *Paecilomyces lilacinus* and *Pochonia chlamydosporia* are especially powerful nematode egg parasites (Cayrol et al., 1982; Irving and Kerry, 1986), which could explain the relatively low predation rate obtained on juveniles. These species can also act on the

movement of infested nematode juveniles via a paralytic toxin (Cayrol et al., 1989), purified and identified as acetic acid by Djian et al. (1991). This molecule is abundantly produced during fungal growth in liquid medium (*ibid*). *Fusarium oxysporum* strains, well-known plant pathogens, exhibited lower predation rates, but some studies revealed that they are partly able to kill nematodes by producing toxic sulfuric heterocycles (fusarenone and moniliformine) (Ciancio et al., 1988; Cayrol et al., 1992).

Moreover, the significant co-occurrence of RKN and NF highlighted by the rarefaction curve suggests a close interaction between prey (nematodes) and predators (fungi) in olive nurseries, as described by Lotka-Volterra models (Barbosa and Castellanos, 2005), probably because of parasitism induction by nematodes (Jansson and Nordbring-Hertz, 1980). Even though the diversity of the NF detected in the four regions was variable at species and population levels, we may expect symmetric dynamics (Marrow and Cannings, 1993) due to possible co-evolutionary processes involved between nematodes and fungi. Such processes occur between competitive organisms in an ever-changing environment, as described by the “Red Queen” hypothesis (Van Valen, 1973; Dawkins and Krebs, 1979).

5. Conclusion

Olive nursery solid substrates are infested with PPN, including *Meloidogyne* species. Various predatory fungi were able to kill RKN. Consequently, before selecting NF strains as candidates for biocontrol, studies must be extended to a wider range of *Meloidogyne* species and populations present on olive trees in Morocco in order to verify their specificity.

Indigenous NF strains were recovered from different substrates (different habitats), making it possible to undertake more research in order to understand the specificity of prey-predator interactions with more diverse PPN species. Predation efficiency in different cropping systems and in varied soil environments should also be explored in the future.

393

394 **Acknowledgements**

395 This research was supported by a Ph.D. grant from the “Institut de Recherche pour le
396 Développement” (Marseille, France). It was also funded by the PESTOLIVE project:
397 Contribution of olive history for the management of soil-borne parasites in the Mediterranean
398 Basin (ARIMNet action KBBE 219262), and by the BIONEMAR project: Development of
399 fungal bionematicides for organic production in Morocco (PHC-Toubkal action 054/SVS/13).

400

401 **Conflict of interest statement**

402 We declare that we have no conflict of interest.

403

404 **References**

405 Afshar, F.J., Sasanelli, N., Hosseinienejad, S., Maafi, Z.T., 2014. Effects of the root-knot
406 nematodes *Meloidogyne incognita* and *M. javanica* on olive plants growth in
407 glasshouse conditions. *Helminthologia* 51, 46-52.

408 Akhtar, M. and Malik, A., 2000. Roles of organic soil amendments and soil organisms in the
409 biological control of plant-parasitic nematodes: a review. *Bioresource Technology* 74,
410 35-47.

411 Ali, N., Chapuis, E., Tavoillot, J. and Mateille, T., 2014. Plant-parasitic nematodes associated
412 with olive tree (*Olea europaea* L.) with a focus on the Mediterranean Basin: A review.
413 *Comptes Rendus Biologies* 337, 423-442.

414 Ali, N., Tavoillot, J., Chapuis, E. and Mateille, T., 2016. Trend to explain the distribution of
415 root-knot nematodes *Meloidogyne* spp. associated with olive trees in Morocco.
416 *Agriculture, Ecosystems and Environment* 225, 22-32.

417 Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman,
418 D. J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database
419 search programs. *Nucleic acids research*, 25 17, 3389-3402.

420 Barbosa, P. and Castellanos, I., 2005. *Ecology of predator-prey interactions*: New York:
421 Oxford University Press.

422 Barron, G.L., 1977. *The nematode-destroying fungi*: Canadian Biological Publications Ltd.

423 Bridge, P. and Spooner, B., 2001. Soil fungi: diversity and detection. *Plant and Soil* 232, 147-
424 154.

425 Brown, R.H. and Kerry, B.R., 1987. *Principles and practice of nematode control in crops*:
426 Academic Press Australia.

427 Castillo, P., Nico, A.I., Azcón-Aguilar, C., Del Río Rincón, C., Calvet, C. and Jiménez-Díaz,
428 R.M., 2006. Protection of olive planting stocks against parasitism of root-knot
429 nematodes by arbuscular mycorrhizal fungi. *Plant Pathology* 55, 705-713.

430 Castillo, P., Nico, A.I., Navas-Cortés, J.A., Landa, B.B., Jiménez-Díaz, R.M. and Vovlas, N.,
431 2010. Plant-parasitic nematodes attacking olive trees and their management. *Plant*
432 *Disease* 94, 148-162.

433 Cayrol, J., VELASQUEZ-Dominguez, M. and Levaux, P., 1982. Etude préliminaire sur les
434 possibilités d'utilisation des champignons parasites comme agents de lutte biologique.
435 *EPPO Bulletin* 12, 497-503.

436 Cayrol, J.-C., Djian, C. and Pijarowski, L., 1989. Study of the nematicidal properties of the
437 culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue de*
438 *Nematologie* 12, 331-336.

439 Cayrol, J.-C., Djian-Caporalino, C. and Panchaud-Mattei, E., 1992. La lutte biologique contre
440 les nématodes phytoparasites. *Courrier de la Cellule Environnement INRA* 17, 31-44.

441 Chessel, D., Dufour, A. and Thioulouse, J., 2004. The ade4 package. I. One-table methods. *R*
442 News 4: 5–10.

443 Ciancio, A., Logrieco, A., Lamberti, F. and Bottalico, A., 1988. Nematicidal effects of some
444 *Fusarium* toxins. *Nematologia Mediterranea* 16, 137-138.

445 Clark, M., Ferris, H., Klonsky, K., Lanini, W., Van Bruggen, A. and Zalom, F., 1998.
446 Agronomic, economic, and environmental comparison of pest management in
447 conventional and alternative tomato and corn systems in northern California.
448 *Agriculture, Ecosystems & Environment* 68, 51-71.

449 Cook R.J., 1993. Making greater use of introduced microorganisms for biological control of
450 plant pathogens. *Annual Revue of Phytopathology* 31, 53–80.

451 Cooke, R. and Dickinson, C., 1965. Nematode-trapping species of *Dactylella* and
452 *Monacrosporium*. *Transactions of the British Mycological Society* 48, 621-629.

453 Davet, P. and Rouxel, F., 2000. Detection and isolation of soil fungi. Science Publisher *Inc.*,
454 Enfield and Plymouth.

455 Dawkins, R. and Krebs, J.R., 1979. Arms races between and within species. *Proceedings of*
456 *the Royal Society of London B: Biological Sciences* 205, 489-511.

457 Den Belder, E. and Jansen, E., 1994. Capture of plant-parasitic nematodes by an adhesive
458 hyphae forming isolate of *Arthrobotrys oligospora* and some other nematode-trapping
459 fungi. *Nematologica* 40, 423-437.

460 Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F.,
461 Guindon, S., Lefort, V. and Lescot, M., 2008. Phylogeny. fr: robust phylogenetic
462 analysis for the non-specialist. *Nucleic Acids Research* 36, W465-W469.

463 Djian, C., Pijarowski, L., Ponchet, M., Arpin, N. and Favre-Bonvin, J., 1991. Acetic acid: a
464 selective nematicidal metabolite from culture filtrates of *Paecilomyces lilacinus*
465 (Thom) Samson and *Trichoderma longibrachiatum* Rifai. *Nematologica* 37, 101-112.

466 Dray, S. and Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for
 467 ecologists. *Journal of Statistical Software* 22, 1-20.

468 Drechsler, C., 1941. Some hyphomycetes parasitic on free-living terricolous nematodes.
 469 *Phytopathology* 31, 773-802.

470 Duddington, C., 1955. Notes on the technique of handling predacious fungi. *Transactions of*
 471 *the British Mycological Society* 38, 97-103.

472 Duponnois, R., Mateille, T., Sene, V., Sawadogo, A. and Fargette, M., 1996. Effect of
 473 different west african species and strains of *Arthrobotrys* nematophagous fungi on
 474 *Meloidogyne* species. *Entomophaga* 41, 475-483.

475 Elad, Y., 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum*
 476 and potential modes of action. *Crop Protection* 19, 709-714.

477 Felsenstein, J., 1985. Confidence intervals on phylogenetics: an approach using bootstrap.
 478 *Evolution* 39:783–791.

479 Gardner, K., Wiebe, M.G., Gillespie, A.T. and Trinci, A.P., 2000. Production of
 480 chlamydospores of the nematode-trapping *Duddingtonia flagrans* in shake flask
 481 culture. *Mycological Research* 104, 205-209.

482 Gaspard, J. and Mankau, R., 1986; Nematophagous fungi associated with *Tylenchulus*
 483 *semipenetrans* and the citrus rhizosphere. *Nematologica* 32, 359-363.

484 Haard, K., 1968. Taxonomic studies on the genus *Arthrobotrys* Corda. *Mycologia* 60, 1140-
 485 1159.

486 Hamza, M.A., Ferji, Z., Ali, N., Tavoillot, J., Chapuis, E., El Oualkadi, A., Moukhli, A.,
 487 Khadari, B., Boubaker, H., Lakhtar, H., Roussos, S., Mateille, T. and El Mousadik, A.,
 488 2015. Plant-parasitic nematodes associated with olive tree in Southern Morocco.
 489 *International Journal of Agriculture and Biology* 17, 719-726. [http://DOI:](http://DOI:10.17957/IJAB/14.0004)
 490 [10.17957/IJAB/14.0004](http://DOI:10.17957/IJAB/14.0004).

491 Hernández-Chavarría, F. and Avendaño, L., 2001. A simple modification of the Baermann
 492 method for diagnosis of strongyloidiasis. *Memórias do Instituto Oswaldo Cruz* 96,
 493 805-807.

494 Hillocks, R.J. and Waller, J.M., 1997. Associations between soilborne pathogens and other
 495 soil-inhabiting microorganisms. In: *Soilborne diseases of tropical crops*. Waller, J.M.
 496 and Hillocks, R.J. (Ed.), CAB International, Wallingford, pp.351-364.

497 Hominick, B., 1999. Nematodes. In *Proceedings of the International Workshop on Tropical*
 498 *Soil Biology: Opportunities and Challenges for African Agriculture*, Nairobi, 16–19
 499 March.

500 Hu, C. and Qi, Y., 2010. Abundance and diversity of soil nematodes as influenced by
 501 different types of organic manure. *Helminthologia* 47, 58-66.

502 Imerglik, L., 1981. Recherches préliminaires sur la spécificité du piégeage des nématodes par
 503 des hyphomycètes prédateurs. DAA Protection des cultures ENSA Montpellier.

504 Irving, F. and Kerry, B., 1986. Variation between strains of the nematophagous fungus,
 505 *Verticillium chlamydosporium* Goddard. II. Factors affecting parasitism of cyst
 506 nematode eggs. *Nematologica* 32, 474-485.

507 ISO 23611-4, 2007. Soil quality - Sampling of soil invertebrates - Part 4: Sampling, extraction
 508 and identification of soil-inhabiting nematodes. ISO, 20 pp.

509 Jansson, H-B. and Nordbring-Hertz, B., 1980. Interactions between nematophagous fungi and
 510 plant-parasitic nematodes: attraction, induction of trap formation and capture.
 511 *Nematologica* 26, 383-389.

512 Jukes, T.H. and Cantor, C.R., 1969. Evolution of protein molecules. *Mammalian Protein*
 513 *Metabolism* 3, 132.

514 Kelly, P., Good, B., Hanrahan, J., Fitzpatrick, R. and de Waal, T., 2009. Screening for the
515 presence of nematophagous fungi collected from Irish sheep pastures. *Veterinary*
516 *Parasitology* 165, 345-349.

517 Korol, A., Preygel, I. and Preygel, S., 1994. Recombination variability and evolution.
518 Chapman Hall, London.

519 Larsen, M., 2006. Biological control of nematodes parasites in sheep. *Journal of Animal*
520 *Science* 84, E133-E139.

521 Mai, W.F., and Mullin P.G., 1996. Plant-parasitic nematodes: a pictorial key to genera, 5th
522 edn. Comstock Publishing Associates, New-York.

523 Maingi, N., Krecek, R. and Biljon, N., 2006. Control of gastrointestinal nematodes in goats on
524 pastures in South Africa using nematophagous fungi *Duddingtonia flagrans* and
525 selective anthelmintic treatments. *Veterinary Parasitology* 138, 328-336.

526 Manlay, R.J., Cadet, P., Thioulouse, J. and Chotte, J.-L., 2000. Relationships between abiotic
527 and biotic soil properties during fallow periods in the sudanian zone of Senegal.
528 *Applied Soil Ecology* 14, 89-101.

529 Marrow, P. and Cannings, C., 1993. Evolutionary instability in predator-prey systems. *Journal*
530 *of Theoretical Biology* 160, 135-150.

531 McSorley, R., 2011. Effect of disturbances on trophic groups in soil nematode assemblages.
532 *Nematology* 13, 553-559.

533 Médail, F. and Diadema, K., 2009. Glacial refugia influence plant diversity patterns in the
534 Mediterranean Basin. *Journal of Biogeography* 36, 1333-1345.

535 Merny, G. and Luc, M., 1969. Les techniques d'échantillonnage des peuplements de
536 nématodes dans le sol. In: Lamotte M., Boulière F. (Eds.). *Problèmes d'écologie,*
537 *l'échantillonnage des peuplements animaux dans les milieux terrestres*, Masson, Paris,
538 pp.257-292.

539 Ministry of Agriculture and marine Fisheries, 2009. Plan vert du Maroc: de la stratégie à
540 l'action du Maroc.

541 Msanda, F., El Aboudi, A. and Peltier, J.-P., 2005. Biodiversité et biogéographie de
542 l'arganeraie marocaine. *Cahiers Agricultures* 14, 357-364.

543 Neher, D.A. and Olson, R.K., 1999. Nematode communities in soils of four farm cropping
544 management systems. *Pedobiologia* 43, 430-438.

545 Netscher, C., 1970. Les nématodes parasites des cultures maraîchères au Sénégal. *Cahiers*
546 *ORSTOM, Série Biologie* 11, 209-229.

547 Nico, A.I., Jiménez-Díaz, R.M. and Castillo, P., 2004. Control of root-knot nematodes by
548 composted agro-industrial wastes in potting mixtures. *Crop Protection* 23, 581-587.

549 Nico, A.I., Rapoport, H.F., Jiménez-Díaz, R.M. and Castillo, P., 2002. Incidence and
550 population density of plant-parasitic nematodes associated with olive planting stocks
551 at nurseries in southern Spain. *Plant Disease* 86, 1075-1079.

552 Nordbring- Hertz, B., Jansson, H.B. and Tunlid, A., 2006. Nematophagous fungi. *In:*
553 *Encyclopedia Life Sciences*. John Wiley & Sons, Ltd, 1-11.

554 Oostenbrink, M., 1960. Estimating nematode populations by some selected methods.
555 *Nematology* 6.

556 Ou, W., Liang, W., Jiang, Y., Li, Q. and Wen, D., 2005. Vertical distribution of soil
557 nematodes under different land use types in an aquic brown soil. *Pedobiologia* 49,
558 139-148.

559 Palomares-Rius, J.E., Castillo, P., Montes-Borrego, M., Müller, H. and Landa, B.B., 2012.
560 Nematode community populations in the rhizosphere of cultivated olive differs
561 according to the plant genotype. *Soil Biology and Biochemistry* 45, 168-171.

562 Philip, J., 2002. Nematophagous fungi: Guide by Philip Jacobs, BRICVersion online.
563 Accessed on: June/2008. Available at <http://www.biological-research.com/>.

564 R Development Core Team, 2011. R: A language and environment for statistical computing.
 565 R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.
 566 <http://www.R-project.org/>.
 567 Rodriguez-Kabana, R., Morgan-Jones, G. and Chet, I., 1987. Biological control of nematodes:
 568 soil amendments and microbial antagonists. *Plant and Soil* 100, 237-247.
 569 Saitou, N. and Nei, M., 1987. The neighbor-joining method: a new method for reconstructing
 570 phylogenetic trees. *Molecular Biology and Evolution* 4 , 406-425.
 571 Sanei, S. and Okhovvat, S., 2011. Incidence of plant-parasitic nematodes associated with
 572 olive planting stocks at nurseries in northern Iran. *International Journal of Applied*
 573 *Science and Technology* 1, 79-82.
 574 Santos, M., Ferraz, S. and Muchovej, J., 1991. Detection and ecology of nematophagous fungi
 575 from Brazil soils. *Nematologia Brasileira* 15, 121–134.
 576 Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O. and Spiegel, Y., 2001.
 577 Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma*
 578 *harzianum*. *Phytopathology* 91, 687-693.
 579 Sikora, R.A. and Fernandez, E., 2005. Nematode parasites of vegetables. In: *Plant parasitic*
 580 *nematodes in subtropical and tropical agriculture*, Luc, M., Sikora, R.A. and Bridge,
 581 J. (Eds.). CABI Publishing, Wallingford, pp.319-392.
 582 Singh, U.B., Sahu, A., Singh, R., Singh, D.P., Meena, K.K., Srivastava, J. and Manna, M.
 583 (2012) Evaluation of biocontrol potential of *Arthrobotrys oligospora* against
 584 *Meloidogyne graminicola* and *Rhizoctonia solani* in Rice (*Oryza sativa* L.). *Biological*
 585 *Control* 60, 262-270.
 586 Sivan, A. and Chet, I., 1992. Microbial control of plant diseases. *Environmental*
 587 *Microbiology*, 335-354.

- Stirling, G.R., 1991. Biological control of plant parasitic nematodes: progress, problems and prospects: CAB International Wallingford, pp282.
- Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24, 1596-1599.
- Thomas, B., 1996. Studies on the parasitism of *Globodera rostochiensis* by *Trichoderma harzianum* using low temperature scanning electron microscopy. *Afroasian Journal of Nematology* 6, 117-122.
- Van Valen, L., 1973. A new evolutionary law. *Evolutionary Theory* 1, 1-30.
- Waller, P., 2006. From discovery to development: current industry perspectives fo the development on novel methods of helminth control in livestock. *Veterinary Parasitology* 139: 1-14.
- West, G.B., Brown, J.H. and Enquist, B.J., 1999. The fourth dimension of life: fractal geometry and allometric scaling of organisms. *Science* 284, 1677-1679.
- White, T.J., Bruns, T., Lee, S. and Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18, 315-322.
- Wickham, H., 2016. readxl: Read Excel Files. R package version 0.1.1. <https://CRAN.R-project.org/package=readxl/>.
- Yu, Z., Mo, M., Zhang, Y. and Zhang, K.-Q., 2014. Taxonomy of nematode-trapping fungi from Orbiliaceae, Ascomycota. In: *Nematode-trapping fungi*. Zhang, K.Q. and Hyde, K.D. (Eds.), Springer, Dordrecht Netherlands, pp.41-210.
- Zhang, Y., Qiao, M., Xu, J., Cao, Y., Zhang, K.Q. and Yu, Z.F., 2013. Genetic diversity and recombination in natural populations of the nematode-trapping fungus *Arthrobotrys oligospora* from China. *Ecology and Evolution* 3, 312-325.

Legends for figures and tables

Figure 1 Distribution of the olive nurseries surveyed in Morocco. See Table 1 for more information.

Figure 2 Neighbor-joining tree inferred from ITS rDNA sequences of the nematophagous fungi isolated (in bold) and their closest GenBank matches (with accession numbers). Bootstrap support values (1000 replicates) are provided as a percentage at the corresponding nodes when > 50 . A to E = high similarity between phylogenetic groups

Figure 3 Diversity indices of the nematophagous fungus communities. (A) PCA loading plot for the diversity indices in soil samples (N = a total number of isolates; S = species richness; H' = Shannon-Wiener diversity; E = species evenness). (B) Score plot for the samples according to the region (J = Jbala; G = Guerouane; H = Haouz; S = Souss). (C, D, E) N , H' and E boxplots ($a-c$ indicate significant groups, $P < 0.05$).

Figure 4 Rarefaction curve for root-knot nematodes (*Meloidogyne* spp.) and nematophagous fungi detected in the olive nurseries according to the regions (J = Jbala; G = Guerouane; H = Haouz; S = Souss).

Figure 5. *In vitro* efficiency of nematophagous fungal strains: percentage of dead *M. javanica* juveniles ($a-e$ indicate significant groups, $P < 0.05$).

Table 1 Location and characteristics of the Moroccan olive nurseries surveyed. Number of samples for each cultivar in each geographic region.

Table 2 Average density (number of nematodes/dm³ of soil) of plant-parasitic nematodes (PPN) and free-living nematodes (FLN), percentages of samples infested with root-knot nematodes (RKN) and FLN/PPN ratios ($a-d$ indicate significant groups, $P < 0.05$).

Table 3 BLAST results of ITS rDNA sequences of the nematophagous fungi isolated.

Table 4 Nematophagous fungi associated with the Moroccan olive nurseries surveyed.

Table 5 Functional diversity of the nematophagous fungi ($a-d$ indicate significant groups, $P <$

639 0.05).

640 **Table 1**

641 Location and characteristics of the Moroccan olive nurseries. Number of samples for each cultivar in each geographic region.

Geographic region	Location	Climate	City	No. of nurseries	Main habitat origin of the substrates	Olive cultivar	No. of samples
Jbala	South-west face of the Rif Mountains	Sub humid climate, temperature from -6° to 32°C	Ouazzane	1	Clay marls, sand, forest soil and topsoil	Picholine marocaine	5
						Haouzia	5
						Menara	5
Guerouane	Sais Plateau, between the Middle Atlas to the south and the Rif Mountains to the north	More continental climate, temperature from -10° to 45°C	Meknes	4	Yellow sand, topsoil, mature manure and local compost	Picholine marocaine	15
						Haouzia	20
						Menara	20
						Arbequina	5
						Arbosana	5
						Picual	10
						Picholine Languedoc	5
Haouz	Northern slope of the High Atlas Mountains	Semiarid climate, average temperatures from -6° to 49.6°C	Marrakech	5	Clay marls, sand, forest soil, mountain soil and topsoil	Picholine marocaine	25
						Arbequina	10
						Haouzia	25
						Menara	25
						Picholine Languedoc	10
						Arbosana	5
			El Kelaa des Sraghna	3	Forest soil and topsoil	Picholine marocaine	15
						Picholine Languedoc	15
						Menara	10
						Haouzia	10
Souss	On the southern slope of the High Atlas Mountains	Arid constant climate, sunshine > 340 days a year, average temperatures from 14° to 25°C	Sidi Abdellah Ghiat	1	Soil, clay and sand	Picholine marocaine	5
			Agadir	8	Sand, topsoil and peat moss	Picholine marocaine	15
						Haouzia	20
						Menara	5
			Khmiss Aït Amira	2	Topsoil, peat and manure	Picholine marocaine	10
			Biougra	1	Peat, soil and perlite	Menara	5

Table 2

Average density (number of nematodes/dm³ of soil) of plant-parasitic nematodes (PPN) and free-living nematodes (FLN), percentages of samples infested with root-knot nematodes (RKN) and FLN/PPN ratios (*a-d* indicate significant groups, *P* < 0.05).

Regions	No. of PPN (/dm ³ of soil)	% of samples infested with RKN	No. of FLN (/dm ³ of soil)	FLN/PPN
Jbala	1,441	16.0 <i>d</i>	2,220 <i>b</i>	1.54 <i>b</i>
Guerouane	1,527	20.0 <i>c</i>	1,914 <i>b</i>	1.25 <i>c</i>
Haouz	2,003	58.1 <i>b</i>	2,081 <i>b</i>	1.04 <i>d</i>
Souss	2,395	76.4 <i>a</i>	6,194 <i>a</i>	2.59 <i>a</i>
<i>P-value</i>	0.153	0.000	0.000	0.000

Table 3

BLAST results of ITS rDNA sequences of the nematophagous fungi isolated.

Strains	GenBank reference strains				Maximum similarity (%)
	Species	Strain	GenBank accession no.	Number of nucleotides (bp)	
UIZFSA-1	<i>Paecilomyces lilacinus</i>	MY683	GU980015	541	99
UIZFSA-2	<i>Paecilomyces lilacinus</i>	MY683	GU980015	541	99
UIZFSA-3	<i>Purpureocillium lilacinum</i>	NPF-4	KP308838	565	99
UIZFSA-5	<i>Purpureocillium lilacinum</i>	DF58	KT582081	713	99
UIZFSA-6	<i>Purpureocillium lilacinum</i>	MSEF24	KT310947	540	99
UIZFSA-12	<i>Purpureocillium lilacinum</i>	E303	KJ540087	540	99
UIZFSA-13	<i>Arthrobotrys thaumasia</i>	isolate 111	EU977532	561	100
UIZFSA-15	<i>Purpureocillium lilacinum</i>	SBTPI-001	KF766523	625	99
UIZFSA-18	<i>Arthrobotrys scaphoides</i>	CBS 226.52	KF494006	565	99
UIZFSA-20	<i>Fusarium oxysporum</i>	K9	JF807396	491	99
UIZFSA-21	<i>Fusarium oxysporum</i>	A2-3	KT876690	510	100
UIZFSA-22	<i>Fusarium oxysporum</i>	DSCF2	AB470914	509	100
UIZFSA-23	<i>Fusarium oxysporum</i>	Z11	EF611086	540	99
UIZFSA-24	<i>Trichoderma asperellum</i>	OTPT7	KC551811	581	100
UIZFSA-24	<i>Trichoderma asperellum</i>	SI14	KJ432865	548	100
UIZFSA-25	<i>Trichoderma asperellum</i>	OTPT7	KC551811	546	100
UIZFSA-26	<i>Trichoderma asperellum</i>	SVPP-3	KU215915	549	100
UIZFSA-27	<i>Trichoderma harzianum</i>	SZMC 20965	KP316410	567	100
UIZFSA-30	<i>Trichoderma asperellum</i>	CPCRI-TD-11	KT445769	594	99
UIZFSA-31	<i>Talaromyces assiutensis</i>	ph721	KF147920	537	99
UIZFSA-35	<i>Trichoderma longibrachiatum</i>	Y-14	KP281451	588	99
UIZFSA-55	<i>Monacrosporium thaumasia</i>	MTU51972	U51972	549	99
UIZFSA-97	<i>Arthrobotrys brochopaga</i>	ABU72609	U72609	525	100
UIZFSA-97	<i>Arthrobotrys conoides</i>	SBUG M12	AF106534	549	99
UIZFSA-98	<i>Drechslerella brochopaga</i>	2eA003	JF748753	493	100
UIZFSA-99	<i>Drechslerella brochopaga</i>	1eL002	JF748752	494	100
UIZFSA-100	<i>Arthrobotrys oligospora</i>	AOZ1	X94121	497	100
UIZFSA-101	<i>Arthrobotrys thaumasia</i>	CBS 322.94	AF106526	553	100

Table 4

Nematophagous fungi associated with the Moroccan olive nurseries surveyed.

Order	Family	Species	Number of strains	Region			
				Jbala	Guerouane	Haouz	Souss
Orbiliales	Orbiliaceae	<i>Arthrobotrys brochopaga</i>	2	+	+	+	+
		<i>A. conoides</i>	1	-	+	-	+
		<i>A. oligospora</i>	1	-	+	-	-
		<i>A. thaumasia</i>	3	-	+	+	+
		<i>A. scaphoides</i>	1	+	+	+	+
		<i>Dreschslerella brochopaga</i>	2	-	+	-	+
		<i>Dactylaria</i> sp.	1	-	-	-	+
		<i>Monacrosporium thaumasium</i>	5	+	+	+	+
		<i>M. microscaphoides</i>	1	-	-	-	+
Eurotiales	Trichocomaceae	<i>Paecilomyces lilacinus</i>	36	+	+	+	+
		<i>Talaromyces assiutensis</i>	1	-	-	-	+
Agaricales	Pleurotaceae	<i>Nematocytus leiosporus</i>	1	-	-	-	+
Blastocladales	Catenariaceae	<i>Catenaria anguillulae</i>	1	-	-	+	+
Haptoglossales	Haptoglossaceae	<i>Haptoglossa heterospora</i>	1	-	-	-	+
Hypocreales	Clavicipitaceae	<i>Pochonia chlamydosporia</i>	4	-	-	+	+
	Hypocreaceae	<i>Trichoderma asperellum</i>	4	+	+	+	+
		<i>T. harzianum</i>	3	+	+	+	+
		<i>T. longibrachiatum</i>	1	+	+	+	+
	Nectriaceae	<i>Fusarium oxysporum</i>	4	+	+	+	+

+: detected; -: not detected.

Table 5

Functional diversity of the nematophagous fungi (*a-d* indicate significant groups, $P < 0.05$).

Region	Samples with NF (%)	Trapping species (%)	Endoparasitic species (%)
Jbala	66.66 <i>c</i>	7.13 <i>d</i>	0
Guerouane	70.00 <i>b</i>	25.12 <i>b</i>	12.13 <i>c</i>
Haouz	49.68 <i>d</i>	24.39 <i>c</i>	11.90 <i>b</i>
Souss	70.90 <i>a</i>	53.16 <i>a</i>	25.80 <i>a</i>
<i>P-value</i>	0.000	0.000	0.000

Figure 1. Distribution of the olive nurseries surveyed in Morocco. See Table 1 for more information.

Figure 3. Diversity indices of the nematophagous fungus communities. (A) PCA loading plot for the diversity indices in soil samples (N = a total number of fungal isolates; S = species richness; H' = Shannon-Wiener diversity; E = species evenness). (B) Score plot for the samples according to the region (J = Jbala; G = Guerouane; H = Haouz; S = Souss). (C, D, E) N, H' and E boxplots (*a-c* indicate significant groups, $P < 0.05$).

Figure 4. Rarefaction curve for root-knot nematodes (*Meloidogyne* spp.) and nematophagous fungi detected in the olive nurseries according to the regions (J = Jbala; G = Guerouane; H = Haouz; S = Souss).

Figure 5. *In vitro* efficiency of nematophagous fungal stains: percentage of dead *M. javanica* juveniles (a-e indicate significant groups, $P < 0.05$).