



HAL
open science

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 Miché, 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-01681583v1>

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.

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

3

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

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

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

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

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

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

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

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

18 ^a Co-leaders of the publication

19

20

21 **Abstract**

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

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

42

43 1. **Introduction**

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

51

52 Several plant-parasitic nematode species (PPN) have been found to be associated with olive
53 trees (Castillo et al., 2010; Ali et al., 2014; Hamza et al., 2015), including root-knot
54 nematodes (RKN, *Meloidogyne* spp.) that were shown to impact olive growth (Afshar et al.,
55 2014). RKN have been reported to be major pests on olive trees, mainly in nurseries where
56 irrigation conditions are favorable to their multiplication (Nico et al., 2002; Sanei and
57 Okhovvat, 2011). Moreover, most RKN infestations in olive orchards result from
58 contaminated plant material produced in uncertified nurseries (Nico et al., 2002). To protect
59 nurseries, vegetative propagation of olive is a crucial step to prevent plant pest dispersal.
60 While some industrial olive-producing countries use synthetic substrates to cultivate olive
61 trees, other countries use more traditional olive cultivation techniques, planting roots in soil
62 substrates. In countries that have implemented large-scale planting programs (e.g., 1,220,000
63 ha in the Morocco Green Plan, 2015), the “nursery risk” is a concern, with two main
64 consequences: (i) the weakening of olive plants before their transplantation into orchards; and
65 (ii) the introduction of pathogens into orchards. The nematode risk is now controlled in
66 nurseries, either by multiplication of resistant varieties (Palomares-Rius et al., 2012) or by
67 inoculating microbial antagonists in the substrates, such as mycorrhizae (Castillo et al., 2006).

68 Thus, in view of the large-scale development of olive cultivation in Morocco, sanitization of
69 the substrates must be a priority. Some previous studies highlighted that certain substrates can
70 be suppressive depending on their composition, either because of microbial antagonisms or of
71 toxicity. For example, the addition of forest residues provides organic matter that could
72 contribute to PPN suppression by increasing soil microflora (Rodriguez-Kabana, 1987).
73 Composted dry cork, especially in nurseries, could be effective for *M. incognita* suppression
74 due to the toxicity of the products released (e.g., ammonia, phenolic compounds) (Nico et al.,
75 2004). Moreover, the introduction of microbial antagonists into substrates would strengthen
76 the sustainability of preventive techniques and help introduce biocontrol agents into orchards.

77

78 Microbial antagonists of PPN include nematophagous fungi (NF), raising expectations for
79 their use in integrated pest management (Waller, 2006; Larsen, 2006; Maingi et al., 2006).
80 These fungi act by antibiosis, parasitism or predation (Imerglik, 1981; Gaspard and Mankau,
81 1986). Fungi trap nematodes using adhesive networks or buttons. Some NF capture their prey
82 with constrictor rings before strangling them and others with hyphae networks that produce
83 sticky compounds. The specific enzymes then digest the nematodes. Recognition and
84 attachment of the mycelium to the nematode cuticle are mainly due to compatible
85 glycoproteins such as lectins (Nordbring-Hertz et al., 2006).

86

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

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

97

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

107

108 2. Materials and methods

109 2.1. Site description and olive plantlet sampling

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

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

118 (Everris Company™).

119 In each nursery and for each variety, five olive plantlets (*Olea europaea* subsp. *europaea*)
120 growing in plastic bags were sampled. Information about the origin and the preparation of the
121 growth substrates and about the cultivars was recorded. A total of 305 olive plantlets were
122 collected and maintained in the laboratory and kept under greenhouse conditions (12 h light at
123 25 °C; 12 h dark at 20°C).

124 2.2. *Nematode extraction and quantification*

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

132 2.3. *Isolation of nematophagous fungi*

133 NF were isolated from solid substrate samples infested with RKN using the soil sprinkling
134 technique (Duddington, 1955) as modified by Santos et al. (1991). The direct soil powdering
135 on media was preferable to the modified Baermann method (aqueous soil suspensions)
136 (Hernández-Chavarría and Avendaño, 2001) because it provided more concentrated material
137 for fungal isolation. Moreover, isolation success was increased by sprinkling cold soil (stored
138 at 4°C) on culture medium at 37 to 40°C (Davet and Rouxel, 2000). Substrate aliquots from
139 each olive plantlet were spread on a tray to be air-dried. One gram was then sprinkled on the
140 surface of Petri dishes containing water-agar (WA 2% w/v) supplemented with antibiotics
141 (0.05% streptomycin-sulphate and 0.05% chloramphenicol). Three replicates were done per
142 olive plantlet. A 1-mL suspension containing approximately 3,000 *M. javanica* second-stage

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

154 2.4. *Morphological and molecular characterization of the fungi*

155 The initial identification of the fungi was based on colony morphology and microscopic
156 characteristics. Slide sub-cultures from pure NF cultures were observed under a dissecting
157 microscope (up to x100 magnification). Genera and species were assigned according to
158 specialized morpho-taxonomical keys (Cooke and Dickinson, 1965; Haard, 1968; Barron,
159 1977; Yu et al., 2014; Philip, 2002).

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

168 mixture contained 2 μ l (10 ng) of template DNA, 1 μ l of each ITS1 and ITS4 primer (10
169 mM), 4 μ l PCR buffer, 2.4 μ l MgCl₂ (25 mM), 0.6 μ l dNTPs (10 mM), 0.1 μ l BSA (0.1
170 mg/ml) and 0.2 units of GoTaq[®] DNA polymerase.
171 PCR cycling conditions consisted of an initial denaturation step at 94°C for 3 min, 35 cycles
172 of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 10
173 min. PCR products were checked for length, quality and quantity by agarose gel
174 electrophoresis (1% (w/v) in 0.5x Tris-Acetate-EDTA (TAE).
175 PCR products were sequenced from both ends by Eurofins MWG GmbH (Ebersberg,
176 Germany), using the same ITS primers. CHROMAS LITE v2.1.1 (Technelysium Pty Ltd.)
177 software was used to edit and assemble DNA sequences. BLAST similarity searches were
178 performed in the non-redundant nucleotide database of GenBank (Altschul et al., 1997) to
179 identify/verify species or genus affiliation of collected isolates. Sequences were aligned with
180 ITS sequences of reference strains obtained from GenBank. Subsequently, the alignment was
181 used to perform the phylogenetic tree with PHYLOGENY.FR (Dereeper et al., 2008) and
182 Mega 6 (Tamura et al., 2007) using the neighbor-joining method (Saitou and Nei, 1987) and
183 the Jukes-Cantor correct distance model (Jukes and Cantor, 1969). The phylogenetic tree was
184 obtained from data using one of three equally parsimonious trees through 1,000 bootstrap
185 replicates (Felsenstein, 1985) with a heuristic search consisting of 10 random-addition
186 replicates for each bootstrap replicate.

187 2.5. *Diversity of the fungal communities*

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

193 2.6. *Pathogenicity analyses*

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

206 2.7. *Statistical analyses*

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

215

216 3. **Results**

217 3.1. *Nematofauna*

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

224 3.2. *Nematophagous species and phylogenetic diversity*

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

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

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

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

252 3.3. Diversity patterns

253 Because of their scarcity (only one nursery surveyed), the substrate samples from the Jbala
254 region were excluded from the dataset prior to running analyses. Richness (S) and local
255 diversity (H') were correlated to the PC1 axis, while the PC2 axis was related to numbers (N)
256 and to evenness (E) of fungal isolates on its positive and negative sides, respectively (Fig.
257 3A). Region grouping was significant in the whole analysis and on the PC2 axis (Fig. 3B).
258 Isolates were more numerous in the Souss region, whereas fungal communities were more
259 numerically alike in the Guerouane region. The Haouz region had lower NF richness and
260 diversity (non-significant PC1 coordinates). Indices were affected by the north-south
261 distribution of the nurseries (Figs. 3C-3E-3D).

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

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

268 3.4. In vitro efficiency of the nematophagous strains

269 NF were distinguished according to their ability to kill *M. javanica* J2s (Fig. 5). *Talaromyces*
270 *assiutensis* killed all juveniles in all replicates. The Orbiliaceae species (*Arthrobotrys* spp.,
271 *Dreschlerella* spp., *Monacrosporium* spp.) were efficient against *M. javanica* since they trap
272 50 to 80% of the J2s using adhesives networks, buttons, constricting rings and hyphae
273 networks. *Paecilomyces* and *Trichoderma* strains killed 30 to 50% of the J2s. *Fusarium*
274 *oxysporum* strains were less efficient (less than 20% of dead J2s). *P. lilacinus* and *P.*
275 *chlamydosporia* strains infected all the *M. javanica* eggs.

276

277 4. Discussion

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

289 The FLN/PPN ratios were balanced in all regions except in the Souss where most of the soil
290 substrates come from cropped areas (vegetables and citrus fruits). Because of the sandy
291 texture of the soils in the Souss region, culture practices include high amounts of organic
292 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

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

325 Our third objective was to determine the occurrence and diversity of nematode-associated NF.
326 All NF species except *A. oligospora* were detected in the Souss region where they were more
327 dominant than in the other regions. This high diversity of NF might be due to the multiple
328 habitat origins of the components (mountain, riverbank and field soils, cattle manure, plant
329 compost, etc.) used to make substrates for root olive plantlets. We hypothesize that the
330 microbial richness detected in the Souss region corresponds to the high endemic plant
331 diversity characteristic of the Macaronesian region (Médail and Diadema, 2009; Msanda et
332 al., 2005). This could also be due to higher organic matter concentration in the soils (i.e.,
333 saprophytic substrate for fungi that induce the formation and the activity of trapping
334 structures) and to high PPN levels (i.e., parasitic substrates for fungi) (Den Belder and Jansen,
335 1994; Singh et al., 2012).

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

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

348 Our fourth objective was to investigate the *in vitro* predation of the NF against *M. javanica*
349 juveniles and eggs. *Talaromyces assiutensis* (strain UIZFSA-31), whose nematode predation
350 was previously unknown, killed all the *M. javanica* juveniles in four days. The mode of action
351 of *T. assiutensis* remains unknown but we hypothesize that the strain may produce specific
352 mycotoxins. *In vitro* trapping tests prove that the Orbiliaceae species (*Arthrobotrys* spp.,
353 *Dreschlerella* spp., *Monacrosporium* spp.) were able to trap *M. javanica* juveniles. The
354 predatory capacity of *A. oligospora* was similar to data found in the literature (Singh et al.,
355 2012). The mechanisms involved during predation are well known (Imergluk, 1981; Gaspard
356 and Mankau, 1986). Recognition and attachment of the mycelium to the cuticle of the RKN
357 juvenile is mainly due to compatible glycoproteins, e.g., lectins (Duponnois et al., 1996).
358 *Trichoderma* species are recognized as control agents against nematodes, and various
359 mechanisms have been proposed to explain nematode killing, including antibiosis and
360 enzymatic hydrolysis (Sivan and Chet, 1992; Elad, 2000). Thomas (1996) demonstrated direct
361 interactions between *T. harzianum* and the cyst nematode *Globodera rostochiensis*: the
362 fungus penetrated eggs in the cysts, leading to the death of the juveniles (Sharon et al., 2001).
363 Precise information on the mechanisms involved is very limited and this misunderstanding
364 has hindered the selection of active strains and the development of improved biocontrol
365 methods. *Paecilomyces lilacinus* and *Pochonia chlamydosporia* are especially powerful
366 nematode egg parasites (Cayrol et al., 1982; Irving and Kerry, 1986), which could explain the
367 relatively low predation rate obtained on juveniles. These species can also act on the

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

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

383

384 5. Conclusion

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

389 Indigenous NF strains were recovered from different substrates (different habitats), making it
390 possible to undertake more research in order to understand the specificity of prey-predator
391 interactions with more diverse PPN species. Predation efficiency in different cropping
392 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., Hosseinijad, 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 nematocidal 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 chlamyosporium* 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.

588 Stirling, G.R., 1991. Biological control of plant parasitic nematodes: progress, problems and
589 prospects: CAB International Wallingford, pp282.

590 Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007. MEGA4: molecular evolutionary
591 genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24,
592 1596-1599.

593 Thomas, B., 1996. Studies on the parasitism of *Globodera rostochiensis* by *Trichoderma*
594 *harzianum* using low temperature scanning electron microscopy. *Afroasian Journal of*
595 *Nematology* 6, 117-122.

596 Van Valen, L., 1973. A new evolutionary law. *Evolutionary Theory* 1, 1-30.

597 Waller, P., 2006. From discovery to development: current industry perspectives fo the
598 development on novel methods of helminth control in livestock. *Veterinary*
599 *Parasitology* 139: 1-14.

600 West, G.B., Brown, J.H. and Enquist, B.J., 1999. The fourth dimension of life: fractal
601 geometry and allometric scaling of organisms. *Science* 284, 1677-1679.

602 White, T.J., Bruns, T., Lee, S. and Taylor, J., 1990. Amplification and direct sequencing of
603 fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods*
604 *and applications* 18, 315-322.

605 Wickham, H., 2016. readxl: Read Excel Files. R package version 0.1.1. [https://CRAN.R-](https://CRAN.R-project.org/package=readxl/)
606 [project.org/package=readxl/](https://CRAN.R-project.org/package=readxl/).

607 Yu, Z., Mo, M., Zhang, Y. and Zhang, K.-Q., 2014. Taxonomy of nematode-trapping fungi
608 from Orbiliaceae, Ascomycota. In: *Nematode-trapping fungi*. Zhang, K.Q. and Hyde,
609 K.D. (Eds.), Springer, Dordrecht Netherlands, pp.41-210.

610 Zhang, Y., Qiao, M., Xu, J., Cao, Y., Zhang, K.Q. and Yu, Z.F., 2013. Genetic diversity and
611 recombination in natural populations of the nematode-trapping fungus *Arthrobotrys*
612 *oligospora* from China. *Ecology and Evolution* 3, 312-325.

613

614 **Legends for figures and tables**

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

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

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

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

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

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

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

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

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

638 **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
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
			El Kelaa des Sraghna	3	Forest soil and topsoil	Picholine marocaine	15
						Picholine Languedoc	15
						Menara	10
						Haouzia	10
						Sidi Abdellah Ghiat	1
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	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

642 **Table 2**

643 Average density (number of nematodes/dm³ of soil) of plant-parasitic nematodes (PPN) and
 644 free-living nematodes (FLN), percentages of samples infested with root-knot nematodes
 645 (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

646 **Table 3**

647 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 thaumasium</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

648 **Table 4**

649 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. microscephoides</i>	1	-	-	-	+		
		Eurotiales	Trichocomaceae	<i>Paecilomyces lilacinus</i>	36	+	+	+	+
				<i>Talaromyces assiutensis</i>	1	-	-	-	+
Agaricales	Pleurotaceae	<i>Nematoctonus 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	+	+	+	+		

650 +: detected; -: not detected.

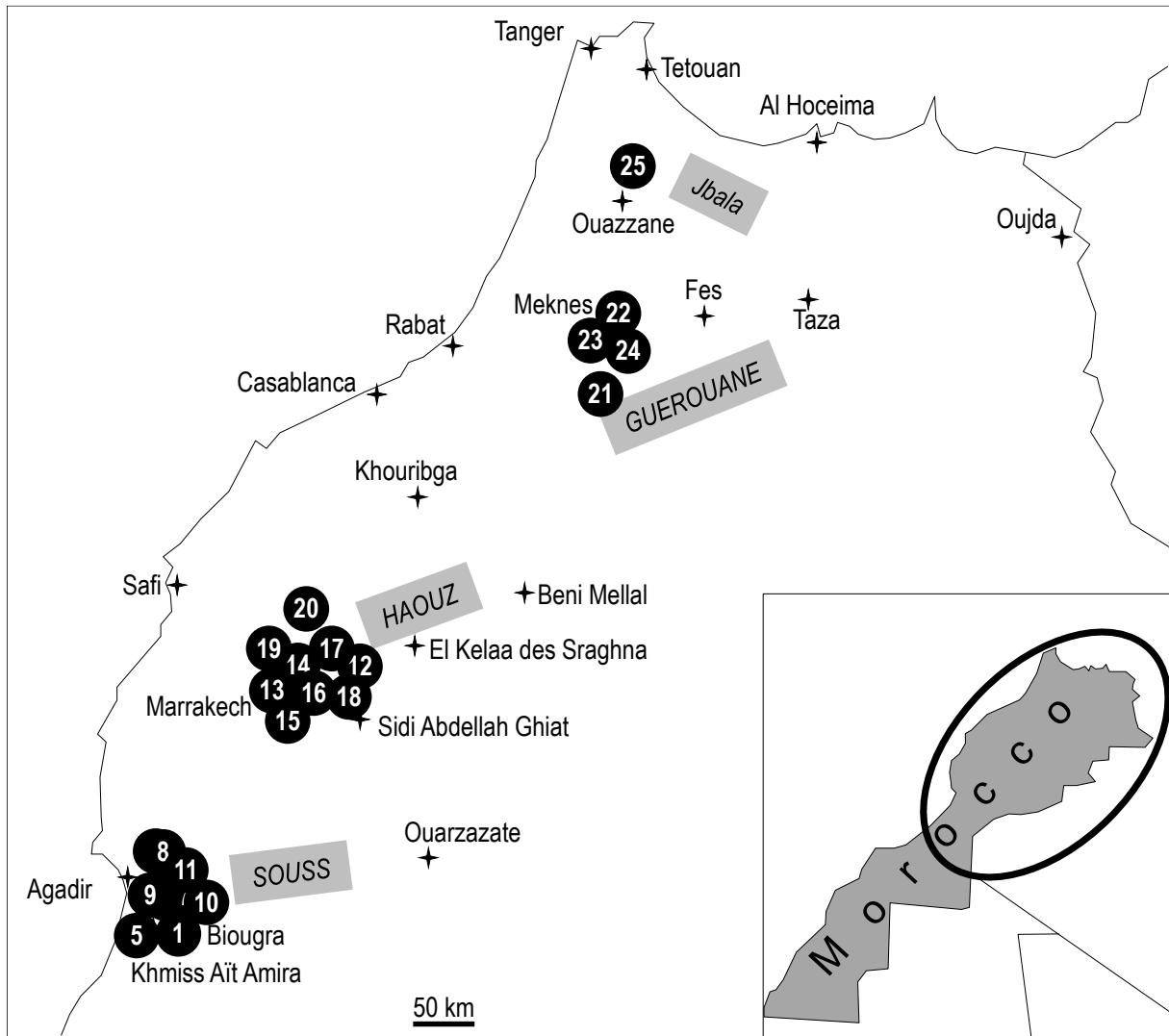
651

652 **Table 5**653 Functional diversity of the nematophagous fungi (*a-d* indicate significant groups, $P < 0.05$).

654

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

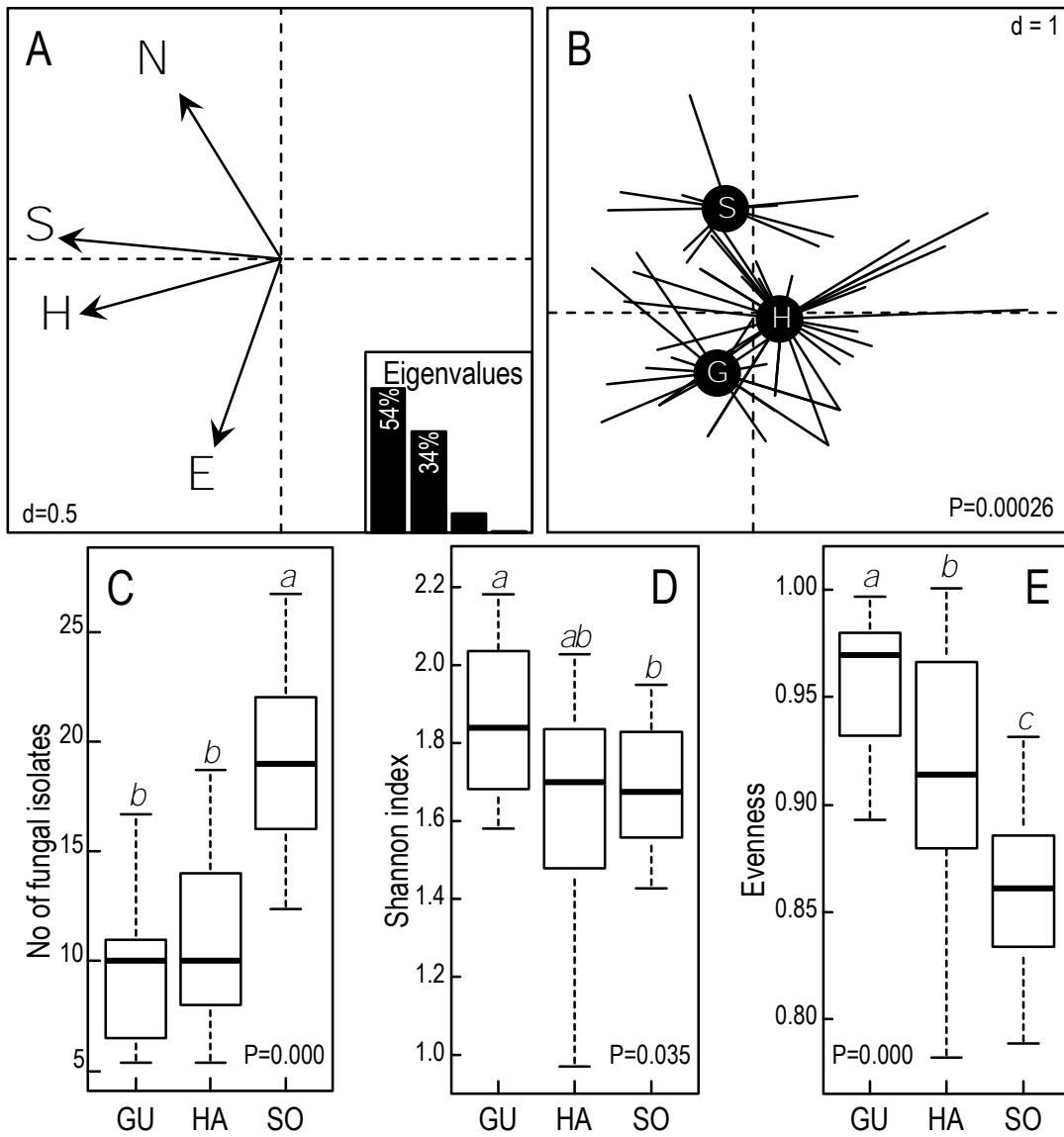
655



656

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

659

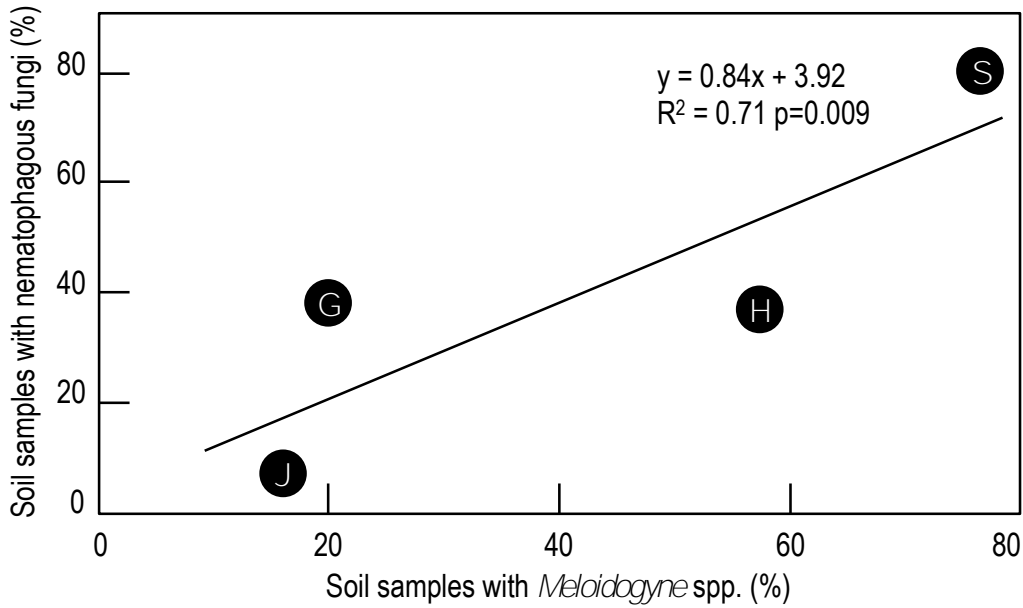


665

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

671

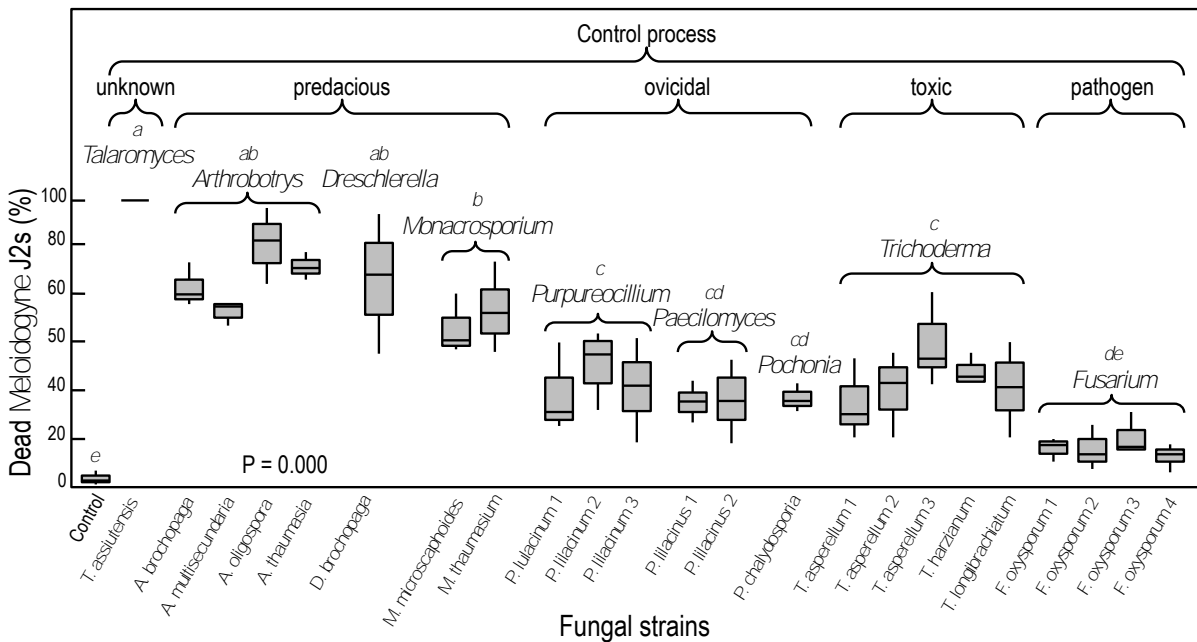
672



673

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

677



678

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