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Nematicidal Properties of Endophytic Fungi Isolated from Some *Musa* Species in Cameroon, for the Management of *Radopholus similis* and *Platylenchus coffeae*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EETB and SFW designed the study. Author SFW carried out the experiments. Authors SFW and EETB performed the statistical and molecular analysis. Authors EETB, SFW and PML wrote the protocol. Authors EETB, SFW and PML managed the analyses of the study. Author SFW wrote the first draft of the manuscript. Authors EETB and SFW revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the effectiveness of endophytic fungi isolated from banana and plantain cultivars, in the control of *Radopholus similis* and *Pratylenchus coffeae in vitro*.
Place and Duration of Study: African Centre for Research on Bananas and Plantain (CARBAP) Njombé and New Biotechnology Laboratory, Faculty of Science, University of Buea, between June 2017 and May 2018.
Methodology: Endophytic fungi were isolated from banana and plantain roots and corms using Potatoes Dextrose agar. The isolates were initially identified based on their cultural and micro-

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morphological characteristics. They were then screened for nematicidal activity against *R. similis* and *P. coffeae in vitro.* DNA was extracted from each isolate which showed nematicidal activity and the 5.8S gene and flanking internal transcribed spacers (ITS1 and ITS2) of the rDNA were amplified and sequenced in order to confirm their identity.

Results: A total of eighteen endophytic fungal isolates were obtained from the plant materials. All isolates demonstrated nematicidal activity on both nematodes with the mortality ranging from 5-100%. Filtrate from three isolates: MB2, GN4 and BT1 were the most effective and were able to kill 100% of *R. similis* and *P. coffeae* after 12 hours of incubation. Filtrate from isolate GN4 was still very effective (100%) after 12 hours when diluted to 1/100. Solutions of broken mycelia from most of the isolates killed the nematodes, but the nematicidal activity was lower than that for culture filtrates. Isolates were identified to belong to five genera: *Fusarium*, *Aspergillus*, *Trichoderma*, *Ceratobasidium* and *Mucor.*

Conclusion: The findings here clearly demonstrated the potential of these fungi as biological control agents against *R. similis* and *P. coffeae.* However, more work is required to help identify the active ingredients exuded by these fungi in culture which has been demonstrated to have nematicidal effects.

Keywords: Bio-control; plant-parasitic nematodes; endophytic fungi; musa production.

1. INTRODUCTION

Banana and Plantain (*Musa*) are important staple foods in many developing countries, especially in Africa [1]. These crops represent a substantial source of income for many people and actors of the rural and urban marketing chain [2]. However, their production on the continent is usually low due to the damages caused by pests, especially plant-parasitic nematodes. In Central and West Africa, the burrowing nematode *Radopholus similis* and the root-lesion nematode *Pratylenchus coffeae* are the most important and the most damaging species [3]. Infection by these pests often results in extensive damage to root systems, accompanied by the formation of lesions, necrotic areas, browning, cell death, and root rotting due to secondary attack by soil fungi or bacteria [4]. Infection can occur along the entire length of the root, with damage to the epidermis, cortex and root endodermis. Root damage slows plant growth, causes stunting and yellowing, increases susceptibility to water stress and to toppling, especially in windy conditions [5].

The huge costs involved in controlling these pests make it almost impossible to continue Banana and Plantain production in severely affected areas [6]. Chemical control has played a key role in the increase of agricultural productivity in the last century, by providing effective control of plant pests and diseases [7]. However, this control measure is not applicable in many African countries because of the high cost and toxicity of nematicides [6]. Although very effective, chemical nematicides have shown many disadvantages making this control measure a double-edged sword.

Attention is more and more directed towards novel management methods, such as the use of endophytic fungi as biological control agents against plant-parasitic nematodes [8]. Endophytes are all organisms inhabiting plant organs that at some time in their life can colonize internal plant tissues without causing apparent harm to the host [9]. Fungal endophytes have been associated with plants for over 400 million years and have been used to control many pests and diseases [10]. Endophytic microorganisms have been recognized as being of great importance for the hosts, protecting the plants against pests, including among others, insects, nematodes and plant pathogenic fungi and bacteria [11]. The protection of plants against nematodes by these fungi is done through several mechanisms. Studies have shown that many bio-control active microorganisms produce metabolites that can interfere with pathogen growth and activities [12]. Antibiotics and lytic enzymes that can break down polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA are amongst these nematode-antagonistic metabolites produced by fungi and bacteria in culture [13].

Today, with the increasing cost of developing new chemical nematicides, tougher regulatory restrictions on existing ones and the increasing popularity of organic farming, the interest in less toxic, simpler and environmentally friendly alternatives for the

management of plant parasitic nematodes is very high. Endophytic fungi have been obtained from healthy Banana plants [14,15] and some of these isolates have shown antagonism against *R. similis in vitro* [8]. The purpose of this study was therefore to isolate endophytic fungi from some cultivated varieties of *Musa* and to determine their nematicidal potential in the management of *R. similis* and *P. coffeae in vitro*.

2. MATERIALS AND METHODS

2.1 Study Area

This work was carried out in Cameroon located between latitude 4°8" 40N – 4°11" North of the equator and between longitude 9°16" 90E – 9°17" 45E of the Greenwich meridian. The isolation and morphological characterization of endophytic fungi, as well as the biological testing on nematodes were done at the African Centre for Research on Banana and Plantain (CARBAP) in Njombé. The molecular characterization was performed at the New Biotechnology Laboratory, in the University of Buea.

2.2 Plant Materials

The plant material in this study consisted of three different cultivars of Plantain; Mbouroukou N°3, Batard and Big Ebanga, and one cultivar of Banana, Grand Nain. The cultivars were selected for their commercial importance and local consumption in the region. The accession numbers, genome subspecies and cycle of the plants are shown in Table 1.

2.3 Plant-parasitic Nematodes

Two different species of plant-parasitic nematodes: *Radopholus similis* and *Pratylenchus coffeae* were used in this study. All the nematodes were local species extracted from Banana and Plantain roots in Njombé.

2.4 Isolation of Endophytic Fungi

2.4.1 Samples collection and preparation

Roots and corms pieces were collected from apparently healthy Banana and Plantain plants before flowering. Plant material was collected following a modification of the method described by Pocasangre et al. [15]. For each cultivar, root samples were collected from four different areas surrounding the plant and corms pieces were obtained after uprooting plants. Corms were first separated from the mat and split into two longitudinal sections and pieces were collected from each section. All the samples were then placed in plastic bags, labeled, transported to the laboratory and processed within 48 hours.

Whilst in the laboratory, samples were thoroughly washed under running tap water to remove debris. Fifteen root fragments were randomly selected and cut into pieces of 5 cm. The corm samples were also cut into portions of 5 cm. Roots and corm samples were surface sterilized by use of 75% ethanol and 5% sodium hypochlorite solutions [16]. The sterilized samples were cut into 1 cm pieces in preparation for inoculation.

2.4.2 Preparation of culture medium for fungi isolation and inoculation

The culture medium used for the isolation of endophytic fungi was Potato Dextrose Agar (PDA) supplemented with chloramphenicol [17]. The mixture was then autoclaved at 121°C for 15 min. before pouring plates. Each plate was inoculated with 3 pieces of the surface sterilized plant part. Incubation was at room temperature (25°C) in the dark for 1-2 weeks [18]. For each variety of Banana and Plantain, a total of 14 Petri dishes were plated (7 for the corms and 7 for the roots). The percentages of occurrence of each species of endophytic fungi were calculated [19] following the formula in equation 1;

% Occurrence = {(Number of occurrence of a particular fungus/ Total number of samples examined) x 100} (1)

Table 1. Banana and plantain cultivars used in the study

Cultivar name	*Accession number	Genome subspecies/group	**Cycle
Batard	CNRA052	AAB, 'French Horn' Plantain	6th cycle
Big-Ebanga	CNRA044	AAB, 'False Horn' Plantain	6th cycle
Mbouroukou N°3	/	AAB, 'False Horn' Plantain	6th cycle
Grand Nain	ITC0180	AAA, 'Cavendish' Banana	15th cycle

(*): Accessions from the Musa Germplasm Information System database (MGIS)
(**): Cycles correspond to the number of years of culture in the field

2.4.3 Subculture

After the incubation of plant segments at room temperature for 8–10 days, fungal isolates were sub-cultured into freshly prepared PDA medium. Pure colonies of sporulating fungi were obtained by the single spore isolation method [20].

2.5 Morphological Identification of Endophytic Fungi

The morphological identification was carried out following the observation of their cultural and micro-morphological characteristics of hyphae and reproductive structures with the help of standard identification keys and manuals [21,20]. The growth rate was determined by measuring the average diameter (cm) of the colony every two days. The fungal cultures that failed to sporulate were categorized as 'Mycelia sterilia' [22].

2.6 Production of Fungal Extracts

Fungal culture filtrates and solutions from broken hyphae respectively served as stock solutions for screening nematicidal activity. Crude culture filtrates were obtained following a modification of the method described in Qureshi et al. [23]. In the process, 5 mm diameter agar plug containing the isolated endophytic fungi was used to inoculate 100 ml of Potato Dextrose Broth (PDB). After 15 days of incubation at room temperature in the dark, including daily discontinuous agitations, cultures were filtered through Whatman No.1 filter paper and the culture filtrates collected and stored at 4°C. The effect of pH on nematicidal properties was also evaluated. Solutions of broken hyphae were obtained following a modification of the method described in Yang et al. [24]. Mycelia obtained after filtration was dried (45°C in oven) and weighed. Dried mycelia were then ground to powder and soaked in sterile distilled water for 7 days and filtered through Whatman No.1 filter paper to make solutions to be tested on nematodes.

2.7 Nematodes Extraction and Cultures

The targeted species of plant-parasitic nematodes: *Radopholus similis* and *Pratylenchus coffeae* were obtained from infected Banana and Plantain roots using the modified method of Bearman [25]. Each species of nematode was cultured and propagated in Batard cultivar host plants (*Musa acuminata×balbisiana* AAB). Plantlets were obtained from the Laboratory of *in vitro* culture of CARBAP and left to acclimatize into 200 ml plastic pots containing sterilized soil mixture under greenhouse conditions for 8 weeks. The plants were later transferred into 10-liter pots for inoculation procedure. Each plantlet was inoculated with 300 nematodes per pot. The application was made around the roots of each plant by pipetting the nematode suspension into three holes at the base of the pseudostem. Plants were maintained for 3-4 weeks and nematodes harvested from the entire root system using the modified method of Bearman. Finally, nematodes were enumerated on a grid counting chamber under a microscope (objective 20×) and nematodes solutions containing approximately 60 nematodes per ml were prepared by appropriate dilution for use as a working stock.

2.8 *In vitro* Nematicidal Activity of Fungal Extracts

The assessment of the nematicidal activity of fungal isolates was done following a modification of the method described by Dong et al. [26]. In the process, 2 ml of each solution (neutral, undiluted (1:0), diluted (1:10 and 1:100) culture filtrates as well as solutions of broken hyphae) was transferred into Petri dishes containing 1 ml of nematode solution (ca. 60 nematodes at various growth stages). The dishes were kept at room temperature (25-30°C) and the number of dead nematodes was recorded after 12, 24 and 36 hours under a microscope (objective 20×). Nematodes were considered dead if they gave no response to physical stimuli such as mechanical stirring and pricking with the point of a needle [26]. In the controls, 2ml of distilled water was used as negative control and the chemical nematicide "Oxaplan 240 SL" diluted according to manufacturer's instructions as positive control.

Nematode mortality was calculated according to Wei et al. [27] following the formula:

$$M\ (\%) = (T/C)\ 100 \qquad (2)$$

Where M represents the nematode mortality, T represents the number of dead nematodes, and C the total number of nematodes used in the test.

2.9 Molecular Characterization of Fungal Isolates

2.9.1 PCR amplification

For each fungal isolate, total genomic DNA extraction was done following the method by Liu et al. [28]. Isolated fungi were characterized by the amplification of their Internal Transcribed Spacers (ITS). The primers used: ITS-1F and ITS-4R, described by White et al. [29], have been widely used for the identification and taxonomic placement of plant endophytic fungi [30,31,32]. Each PCR mixture in a 20 µl reaction volume contained 10 µl of RED Taq Ready Mix (Sigma Aldrich), 8 µl of Nuclease-free PCR water, 0.5 µl of each primer and 1 µl of DNA template. Amplification was carried out in a PCR thermal cycler (Peltier thermal cycler) with the following profile: Initial denaturation at 95°C for 5 min, 45 cycles of Denaturation at 95°C for 30 sec, Annealing at 55.5°C for 30 sec, Extension at 72°C for 30 sec and stabilization at 72°C for 5 min.

2.9.2 Gel-Electrophoresis

Agarose gel electrophoresis was used to check the quality of the fungal DNA extracted as well as the effectiveness of the PCR amplification, following the protocol described in Rousseaux and Guilloux-Bénatier [33]. 5 µl of total genomic DNA was mixed with 1 µl of 6X gel-loading buffer (0.25% bromophenol blue, 0.25% Xylene cyanol FF, 30% glycerol in TE buffer pH-8.0). Samples were then loaded to a 1% agarose gel prepared in 1X TAE (Tris-Acetate-EDTA) buffer containing 0.5 µg/ml of ethidium bromide. Electrophoresis was performed in 1X TAE as electrophoresis buffer at 100 V for 1 hour. PCR products were checked in 1.5% agarose gel prepared in 1X TAE buffer to which 0.5 µg/ml of ethidium bromide was added. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and electrophoresis performed with 1X TAE buffer for about 1 hour. The molecular weight marker used was the DirectLoadTM Wide Range DNA Marker 50 bp - 10,000 bp (Sigma-Aldrich). The gels were visualized in a UV trans illuminator and the image was captured under UV light using a Gel documentation system (Bio-Rad).

2.9.3 DNA sequencing

DNA sequencing was performed in order to obtain the respective sequences of the amplified fungal DNA. 15 µl of PCR products were sent to Inqaba Biotec, Africa's genomics company (South Africa) for purification and sequencing. The sequencing reactions were performed on the ABI 3500XL Genetic Analyzer, POP7™ (Thermo Scientific) following the protocol described in the instruction manual. The set of primers used were ITS1 and ITS4 [29]. Both strands of the DNA molecule were sequenced [22].

2.9.4 Sequence analysis and phylogenetic analysis

DNA sequences obtained were analyzed for sequence alignment and required editing using the software BioEdit version 7.2 [34]. During this process, individual bases were verified by comparison with the fluorescence signal printout for each sequence, gaps were removed and consensus sequences from forward and reverse sequences obtained [22]. The resulting sequences were then aligned with ITS1-5.8S-ITS2 sequences from several species of taxonomically established genera obtained from GenBank with the help of the Basic Local Alignment Search Tool (BLASTn) of the National Centre for Biotechnology Information (NCBI) [31]. Finally, a neighbor-joining phylogenetic tree was constructed with the sequences obtained and related sequences retrieved from the GenBank database [31]. Phylogenetic analyses were conducted in MEGA7 [35].

2.10 Data Analysis

One-way ANOVA was used to investigate differences among treatment means and means were separated using Waller-Duncan multiple range test. In the case where data transformation was not effective, non-parametric versions of ANOVA (Kruskal-Wallis test) and of Independent sample t-test (Mann-Whitney U test) were used to compare treatments. The statistical significance in this study was set at a *P* value <0.05. The statistical software package, Statistical Package for Social Scientist (SPSS) version 20 was used to examine differences in means.

3. RESULTS

3.1 Isolation of Endophytic Fungi

A total of 28 endophytic fungi were isolated from the healthy Banana and Plantain tissues obtained from the collection of Musaceae of CARBAP in Njombé (Table 2). Fungi were

slightly more prevalent in the roots of the plants with a frequency of occurrence of 54% as compared to the corms with 46%.

3.2 Morphological Characterization of Isolated Endophytic Fungi

The 28 isolates were identified to belong to 18 different fungi groups based on their morphology, pigmentation, growth rate as well as the characteristics of their hyphae and reproductive structures. Four isolates: S1, S2, S3 and S4 were common in multiple cultivars (Table 3) of which three (S2, S3, S4) were found in both Banana and Plantain. Of the 18 endophytic isolates obtained, 11 (61%) belonged to Hyphomycetes while 6 (33%) were mycelia sterilia (Table 3). Growth and morphological data of isolated endophytic fungi are presented in Table 4. Most of the isolates obtained produced spores or other propagation structures and had heterogeneous colony surface texture. The cultures were in the majority whitish in color on the surface view with color change on the reverse view. For the colony morphology, most isolates harbored a circular form, a raised elevation, and a filiform margin. It is worth noting that other color (Purple, green, black, yellow), elevation model (umbonate and convex) and colony forms were also recorded. The average colony diameter after two days was 3cm for most isolates. The cultural and micro-morphological characteristics of some isolates can be seen in Fig. 1.

3.4 Effect of Fungal Culture Filtrates on Targeted Nematodes

Culture filtrates of the 18 fungal isolates were tested for nematicidal activity against the burrowing nematodes (*Radopholus similis*) and the Root-Lesion Nematode (*Pratylenchus coffeae*). Dead nematodes appeared straight (uncurved) with elongated bodies, while living nematodes retained their normal sigmoid shape and exhibited some slight movement.

Mortality generally increased with increase in incubation time for *R. similis* and in most cases decreased with an increase in dilution (Annex 1). The crude filtrates from nine isolates (S1, S2, S4, BT1, BT2, MB2, BE2, GN1 and GN4) each caused a mortality of over 90% of *R. similis in vitro*. Eight isolates (S3, S4, BE4, BE5, GN2, GN3 and GN5) showed percentage of efficacies ranging from 50-90% and only 1 of 18 isolates (MB1) was considered less active with a percentage mortality lower than 50. Isolates MB2, GN4 and BT1 were the only isolates which, like the chemical nematicide "Oxaplan 240 SL" used as positive control, killed 100% of *R. similis* after only 12 hours of incubation (Annex 1). The same isolates also showed maximum efficacy within 12 hours when diluted by 1/10, but only isolate GN4 was still very effective (100%) after 12 hours even when diluted by 1/100.

A similar observation was made with *P. coffeae*, where % mortality increased with incubation time, but decreased with increase in dilution (Annex 2). The most effective isolates which caused nematode mortality of over 90% after 36 hours were: MB2 (100%), GN4 (100%), BT1 (100%), BT2 (98%), GN5V2 (98%), GN1 (100%), S1 (98%), and BE5 (99%). MB2, GN4 and BT1 were also the only isolates effective enough to kill 100% of *P. coffeae* within 12 hours of incubation (Annex 2). The same isolates also showed maximum efficacy after 12 hours when diluted by 1/10 and only isolate GN4 was still very effective (100%) against *P. coffeae* after 12 hours of incubation even when diluted by 1/100.

The percentage of dead nematodes using culture filtrates was significantly higher for all fungal treatments compared to the control

Table 2. Number of endophytic fungi isolated from various plant tissues

	Cultivar	Endophytes from roots	Endophytes from corms	Total
Plantain	Batard	3	1	4
	Big Ebanga	6	4	10
	Mbouroukou N°3	3	1	4
Banana	Grand Nain	3	7	10
	Total	15 (54%)	13 (46%)	28 (100%)

Values in % are the frequency of occurrence of the fungi per plants tissue

Table 3. Frequencies of occurrence of endophytic fungi isolated from various cultivars and plant parts

Fungus code	Fungus genus	Cultivars	Tissue	% of occurrence
S1*	Mycelia sterilia	Batard; Mbouroukou3; Big Ebanga.	Roots	11%
S2*	*Fusarium sp.*	Big Ebanga; Grand Nain.	Corm	7%
S3*	Unknown	Big Ebanga; Grand Nain.	Roots and Corm	11%
S4*	*Fusarium sp.*	Mbouroukou3; Big Ebanga; Grand Nain.	Roots and Corm	14%
BT1	*Mucor sp.*	Batard	Corm	4%
BT2	*Penicillium sp.*		Roots	7%
MB1	*Mucor sp.*	Mbouroukou3	Corm	4%
MB2	*Fusarium sp.*		Roots	4%
BE2	Mycelia sterilia	Big Ebanga	Roots	4%
BE3	Mycelia sterilia		Corm	4%
BE4	*Fusarium sp.*		Corm	4%
BE5	Mycelia sterilia		Roots	7%
GN1	Mycelia sterilia	Grand Nain	Roots	4%
GN2	Mycelia sterilia		Roots	4%
GN3	*Fusarium sp.*		Corm	4%
GN4	*Fusarium sp.*		Corm	4%
GN5	*Fusarium sp.*		Corm	4%
GN5V2	*Fusarium sp.*		Corm	4%

(*) Genera repeatedly isolated from Banana and Plantain cultivars.

treatments. Significant differences (P=0.0001) were observed between the control treatments and the fungal treatments on both nematodes after 24 and 36 hours of incubation. Dilution of the culture filtrate by 1/100 significantly (P=0.004) reduced the mortality of *R. similis*. This observation was quite similar with *P. coffeae*, where dilution of the culture filtrate by 1/100 significantly (P=0.001) decreased mortality (Annex 2).

3.5 Effect of Fungal Broken Mycelia Solution on Targeted Nematodes

Solutions of broken mycelia from the isolated fungi were also tested for nematicidal effects on *Radopholus similis* and *Pratylenchus coffeae*. The percentage of dead nematodes using solutions of broken mycelia was significantly higher for all fungal treatments as compared to the control treatments. After 36 hours of incubation, nematode mortality rates over 50% were recorded on *R. similis* in solutions of 15 isolates (Table 5). High rates of efficacy ranging from 90-100% were found using isolates MB2 (100%), BT1 (100%), BT2 (100%), BE2 (100%), S2 (100%), GN1 (97%) and S1 (100%). Similarly to culture filtrates, isolates MB2, BT1 and S2 were able to kill 100% of nematodes within 12 hours of incubation. Mortality rates over 50% were recorded for *P. coffeae* after 36 hours of incubation using solutions of 10 isolates.

The most effective isolates which caused more than 90% mortality on nematodes were: MB2 (100%), BT1 (100%), GN1 (100%) and S1 (97%). Maximum efficacy of 100% after 12 hours of incubation was recorded only on MB2 and BT1. Percentages of nematode mortality using solutions of broken hyphae were significantly higher for all fungal treatments compared to the control treatments. Significant differences (P=0.0001) were observed between the control treatments and the fungal treatments on both nematodes after 24 and 36 hours of incubation. Just like culture filtrates, the effect of solutions of broken hyphae on nematode mortality was influenced by the length of exposure of the nematodes to the fungal extract. The percentage of dead nematodes increased with an increase in the duration of exposure. The variation in nematode mortality in bioassays was statistically significant (P<0.05) at each exposure time for both *R. similis* and *P. coffeae.*

Nematode mortalities were significantly higher in culture filtrate than in solutions of broken hyphae. For each exposure time and on both nematodes, statistically significant variations (P<0.05) were recorded in mortality percentages

between culture filtrates and solutions of broken mycelia. Similarly, while comparing the percentage of dead nematodes at each exposure time between *R. similis* and *P. coffeae*, results showed that nematode mortality was significantly (P= 0.047) higher in *R. similis* than in *P. coffeae*.

3.6 Molecular Characterization

3.6.1 DNA extraction and amplification

DNA was successfully extracted from the fungal isolates, as indicated by the sharp and unique bands that were obtained per sample after gel electrophoresis (Fig. 2).

From the 18 endophytic fungi isolated, only 12 isolates were successfully amplified by PCR. In spite of the several attempts, six isolates could still not be amplified. Only the isolates that were successfully amplified were sequenced. Clear and unique bands were obtained after running PCR product on 1.5 % Agarose gel. All the bands were between 500 and 800 bp (Fig. 3) which corresponded to the size of expected PCR amplicons.

3.6.2 Sequencing and phylogenetic analysis

Based on the results of the search for similar 5.8S gene and ITS sequences from GenBank through the BLASTn tool, all the isolates had a relatively higher sequence similarity (99-100%) with fungal species from three different taxa: Ascomycota, Basidiomycota, and Mucoromycota. Isolate S2 was identified as *Gibberella moniliformis*; Isolate S4 as *Fusarium oxysporum*; Isolate MB2 as *Fusarium oxysporum*; Isolate BE4 as *Fusarium solani*: Isolate GN2 as *Aspergillus micronesiensis*; Isolate GN3 as *Fusarium nematophilum*; Isolate GN5 as *Fusarium mexicanum*; Isolate BT2 as *Trichoderma virens*; Isolate BE3 as *Ceratobasidium* sp.; Isolate GN4 as *Fusarium napiforme* and Isolate BT1 as *Mucor irregularis.* The ITS sequences obtained were submitted to GenBank (Table 6). A phylogenetic tree (Fig. 4) was generated, to show evolutionary relationship of isolates with other similar strains from GenBank.

4. DISCUSSION

Fusarium spp. are the most frequently encountered endophytes in Banana and their use as biological control agents of *R. similis* offers a novel and promising option [8]. The isolation, identification, and characterization of these potential bioactive fungi are therefore very important. As part of the general ongoing efforts towards finding novel bioactive compounds from natural resources, in this study, we investigated the nematicidal potential of culture filtrates and solutions of broken hyphae of fungal endophytes isolated from Banana and Plantain cultivars (*Musa* sp.) from the Musaceae collection of CARBAP in Njombé, Cameroon.

In the course of present work, a total of 18 endophytic fungi were successfully isolated from 4 Banana and Plantain cultivars. The frequency of occurrence of endophytic fungi was higher in the roots than in the corms of the studied cultivars. From 28 fungi isolated, 15 were isolated from the roots and 13 from the corm. The morphological identification of the isolates was limited in this study due to the abundance of Mycelia sterilia which consist of various morphological fungal types without any true spores. These fungi are considerably prevalent in endophytic investigations [22]. With 39% obtained, the percentage of Mycelia sterilia in this study is higher than the 23.6% reported by Kumar and Hyde [35] for the thunder duke vine *Tripterygium wilfordii*.

Fusarium was the predominant genus isolated and was found in all cultivars studied except the cultivar Batard. These results were consistent with those obtained by Pocasangre et al. [15] and Niere [14] who also found an abundance of *Fusarium* spp. and higher percentages of occurrence of fungi in roots as compared to corms of Banana cultivars. This can be explained by previous findings from Kumar and Hyde [35] that the species composition and frequency of endophyte species seems to be dependent on the tissue type. *Fusarium* spp. are found in Banana as natural endophytes and have been detected in the roots of different Banana cultivars in several countries [14,36]. Studies have suggested that *Fusarium* spp. are natural endophyte in Banana and the fungi are not restricted to a cultivar or genomes as a particular host [15]. Surprisingly, our results showed no link between the number of endophytic fungi retrieved and the age of the plantation. The same number of fungi was isolated from different cultivars having the same number of cycles and also from cultivars at different number of cycles. However, some studies demonstrated that plant location and leaf age influenced the density of endophyte infection in leaves of tropical forest trees [37].

Table 4. Growth and morphological parameters of fungi isolated from banana and plantains in Njombé Cameroon

N°	Fungal isolates	Spore formation	Aerial Hyphae	Colony surface texture	Colony morphology			Colour (7 Days)		Average colony diameter cm (2 days)
					Form	Elevation	Margin	Surface	Reverse	
1	S1	Non sporulating	Present	Heterogeneous	Circular	Raised	Filiform	Black	Black	3.63
2	S2	Sporulating	Absent	Heterogeneous	Circular	Flat	Filiform	White	Yellow	2.75
3	S3	Sporulating	Present	Cottony	Circular	Flat	Filiform	White	Yellow	3.63
4	S4	Sporulating	Absent	Cottony	Circular	Raised	Filiform	Cream	White	3
5	BT1	Sporulating	Present	Velvety	Circular	Raised	Filiform	Yellow (light)	Yellow	3.63
6	BT2	Sporulating	Present	Homogeneous	Circular	Flat	Filiform	Green	Brown	3.38
7	MB1	Sporulating	Present	Cottony	Irregular	Convex	Entire	White	White	2.50
8	MB2	Sporulating	Absent	Homogeneous	Circular	Flat	Filiform	Purple	Purple	3.13
9	BE2	Non sporulating	Present	Heterogeneous	Circular	Raised	Filiform	Pale green & White	Green	3.38
10	BE3	Non sporulating	Present	Cottony	Circular	Raised	Filiform	Brown	Brown	3.69
11	BE4	Sporulating	Present	Cottony	Circular	Raised	Filiform	Purple & White	Purple	2.56
12	BE5	Non sporulating	Absent	Water soaked	Irregular	Flat	Entire	Yellow	Yellow	3.88
13	GN1	Non sporulating	Absent	Homogeneous	Irregular	Flat	Filiform	Green & White	Green	3.19
14	GN2	Sporulating	Present	Heterogeneous	Irregular	Umbonate	Entire	White	Brown	1.50
15	GN3	Sporulating	Present	Heterogeneous	Irregular	Umbonate	Entire	White	Green	1.25
16	GN4	Non sporulating	Absent	Heterogeneous	Circular	Flat	Entire	Green & White	Black	3.06
17	GN5	Sporulating	Absent	Homogeneous	Circular	Raised	Filiform	White	Yellow	2.69
18	GN5v2	Sporulating	Present	Cottony	Circular	Raised	Filiform	Pink & White	Orange	2.69

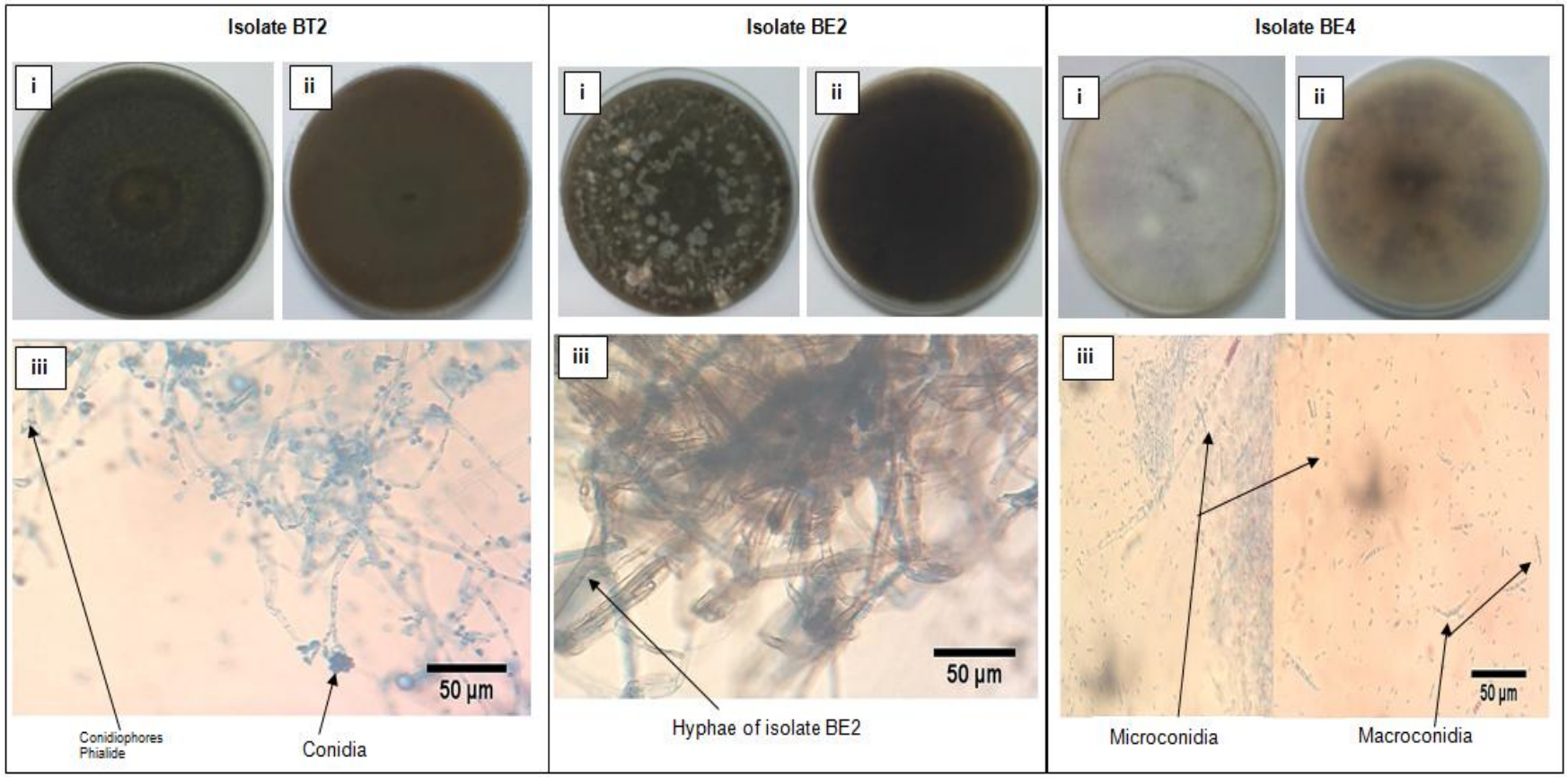

Fig. 1. Cultural and micro-morphological presentation of some representative isolates on potatoes dextrose agar
(i): front view; (ii): reverse view; (iii): micrograph (objective 40x)

Table 5. Percentage mortality (mean ± S.D.) of *R. similis* (R.s) and *P. coffeae* (P.c) at various incubation times in a solution of crushed fungal mycelia

Fungal isolates	Incubation times											
	After 12 H of incubation				After 24 H of incubation				After 36 H of incubation			
	Negative control		Solution of crushed mycelia		Negative control		Solution of crushed mycelia		Negative control		Solution of crushed mycelia	
	R.s	P.c	R.s	P.c	R.s	P.c	R.s	P.c	R.s	P.c	R.s	P.c
S1*	0	0	26±4.5 f	31±1.5 c	5±1.0	6±0.5	100±0.0 a	90±2.0 b	9±0.5	11±1.5	100±0.0 a	97±1.0 a
S2*	0	0	100±0.0 a	46±3.0 b	4±1.5	5±2.0	100±0.0 a	87±3.6 b	8±1.5	4±2.0	100±0.0 a	70±3 b
S3	0	0	14±3.2 g	10±3.6 e	3±1.0	3±1.5	18±2.0 g	24±2.3 f	7±2.0	7±1.5	31±1.5 e	41±3.0 f
S4	0	0	33±1.5 e	22±4.7 d	4±1.1	6±1.5	54±1.5 e	34±3.0 e	7±1.5	6±2.8	59±3.0 c	51±4.0 e
BT1*	0	0	100±0.0 a	100±0.0 a	4±1.5	4±0.5	100±0.0 a	100±0.0 a	8±1.5	4±2.0	100±0.0 a	100±0.0 a
BT2*	0	0	61±2.0 b	6±1.5 f	3±1.5	2±1.0	83±2.0 b	48±2.5 d	8±1.0	11±1.5	100±0.0 a	68±2.5 c
MB1	0	0	4±2.0 i	4±0.5 g	2±1.3	3±1.5	3±2.0 h	8±2.0 i	11±6.3	6±2.0	5±2.0 f	14±1.5 i
MB2*	0	0	100±0.0 a	100±0.0 a	2±1.5	4±0.5	100±0.0 a	100±0.0 a	4±1.1	4±2.0	100±0.0 a	100±0.0 a
BE2*	0	0	34±4.0 d	2±1.5 g	8±3.5	4±1.5	97±1.0 a	5±1.0 j	11±2.5	8±1.0	100±0.0 a	7±1.7 j
BE3	0	0	15±2.0 g	16±3.5 c	4±0.5	5±1.0	77±4.5 b	58±4.7 c	8±2.0	12±2.0	80±8.8 b	69±3.0 c
BE4	0	0	63±2.5 b	12±3.6 e	4±1.5	4±1.5	69±2.5 c	21±5.0 g	20±2.0	13±2.5	75±3.0 b	36±2.5 g
BE5	0	0	23±3.5 c	20±2.0 d	5±1.0	2±0.5	49±3.0 d	32±2.0 e	11±1.5	4±0.5	52±3.6 c	52±1.5 e
GN1*	0	0	56±2.0 b	39±1.5 b	6±2.5	6±1.5	96±1.1 a	95±1.0 a	9±2.0	12±2.6	97±2.0 a	100±0.0 a
GN2	0	0	9±3.0 h	12±4.7 d	3±1.0	2±1.0	32±2.6 f	27±4.9 e	5±1.0	8±1.5	48±2.5 d	48±3.6 e
GN3	0	0	43±2.8 c	3±2.0 g	3±1.0	2±1.5	51±1.5 d	7±3.0 i	7±2.0	6±1.1	57±2.3 c	18±2.0 i
GN4	0	0	45±4.5 c	16±2.0 d	2±1.0	4±0.5	44±0.5 e	24±1.5 f	7±1.0	4±2.0	52±2.0 c	31±3.2 h
GN5	0	0	32±3.0 e	19±5.5 d	3±1.5	1±0.5	56±3.0 d	27±2.5 e	6±1.0	5±1.0	58±3.2 c	61±2.5 d
GN5V2	0	0	25±6.2 f	9±4.9 f	3±2.8	6±1.5	56±5.0 d	16±3.5 h	8±1.5	6±2.0	56±2.5 c	33±1.5 h
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

In each column, means followed by the same letter are not significantly different at P = 0.05 according to Waller-Duncan multiple range test (*): Most effective fungal isolates

Table 6. Endophytic fungal isolates from *Musa* species with their closest match identified by BLAST analysis and the ascension numbers of the isolate's ITS sequences submitted to GenBank

Endophytic fungal isolate code	Closest match in genbank	% Identity	Submitted to genbank with accession no.
Ascomycota			
S2	*Gibberella moniliformis strain SA3*	99%	MK239968
S4	*Fusarium oxysporum strain CBS 132482*	99%	MK239978
MB2	*Fusarium oxysporum strain TH04-1-1*	99%	MK239971
BE4	*Fusarium solani strain CBS 127306*	100%	MK239970
GN2	*Aspergillus micronesiensis strain CBS 586.65*	100%	MK239974
GN3	*GN3 Fusarium nematophilum strain BBA 70838*	99%	MK239975
GN5	*Fusarium mexicanum strain MXMIC-692*	99%	MK239977
BT2	*BT2 Trichoderma virens isolate T6*	100%	MK239973
GN4	*GN4 Fusarium napiforme strain CBS 748.97*	99%	MK239976
Basidiomycota			
BE3	*Ceratobasidium sp. AG-G isolate Str14*	100%	MK239969
Mucoromycota			
BT1	*BT1 Mucor irregularis strain CBS 977.68 clone c1*	100%	MK239972

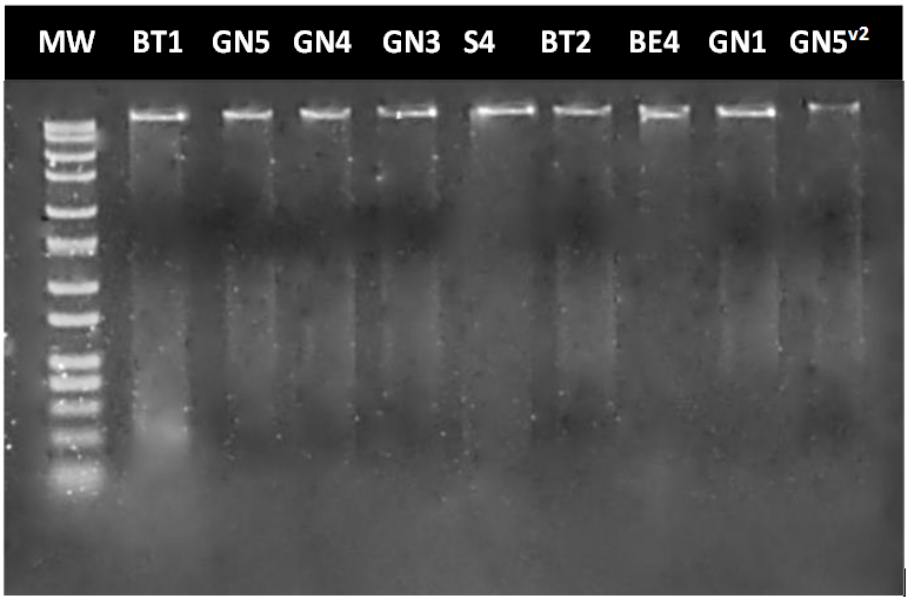

Fig. 2. Electrophoregram of total genomic DNA. Lane MW: Molecular weight marker; Lane BT1-GN5v2: PCR products of isolates. Samples were run on 1.5% agarose gel

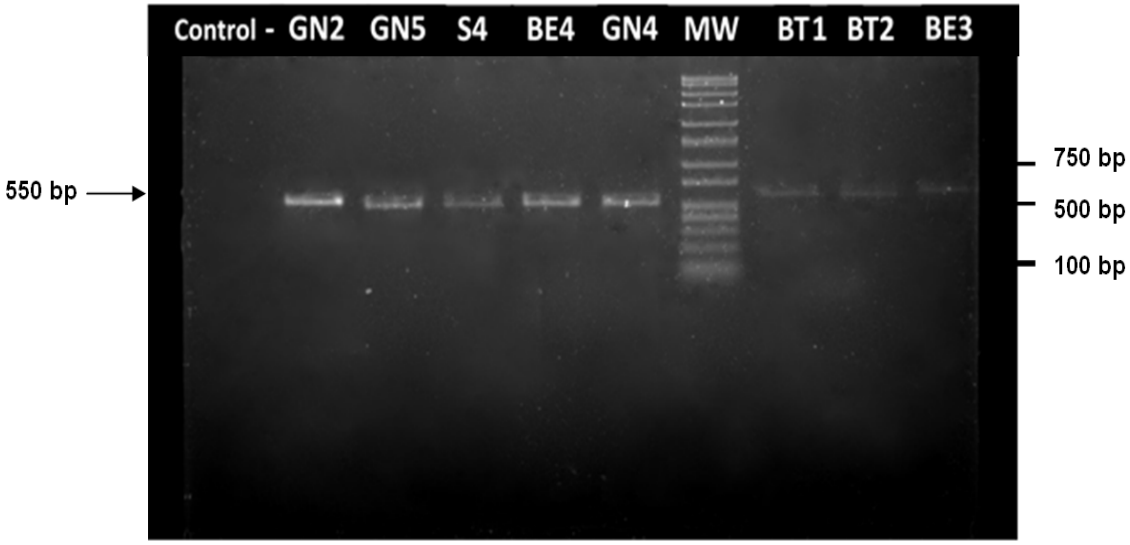

Fig. 3. PCR products on agarose gel after electrophoresis. Lane 1: Negative control; Lane 2, 3, 4, 5, 6, 8, 9 and 10: Amplified fungal ribosomal DNA; Lane 7: Molecular weight marker

Different levels and classes of activities of nematode mortality were obtained from the 18 isolates studied. Most effective isolates belonged to *Fusarium, Trichoderma* and *Mucor* species. Little information is available on the nematode-inhibiting components of fungal culture filtrates and on the specific mycotoxins produced by the fungi [8]. It has already been demonstrated that *Fusarium* spp. can produce toxic secondary metabolites such as zearalenone, fumonisins, trichothecenes and fusaric acid [38]. A good number of non-pathogenic strains of *Fusarium* spp. have been used to manage plant nematodes and to induce systemic resistance against the burrowing nematode *Radopholus similis* in Banana plants [8,39]. *Trichoderma* spp. have also been intensively studied for use against a broad spectrum of soil-borne or foliar diseases and a wide range of crop pests [40].

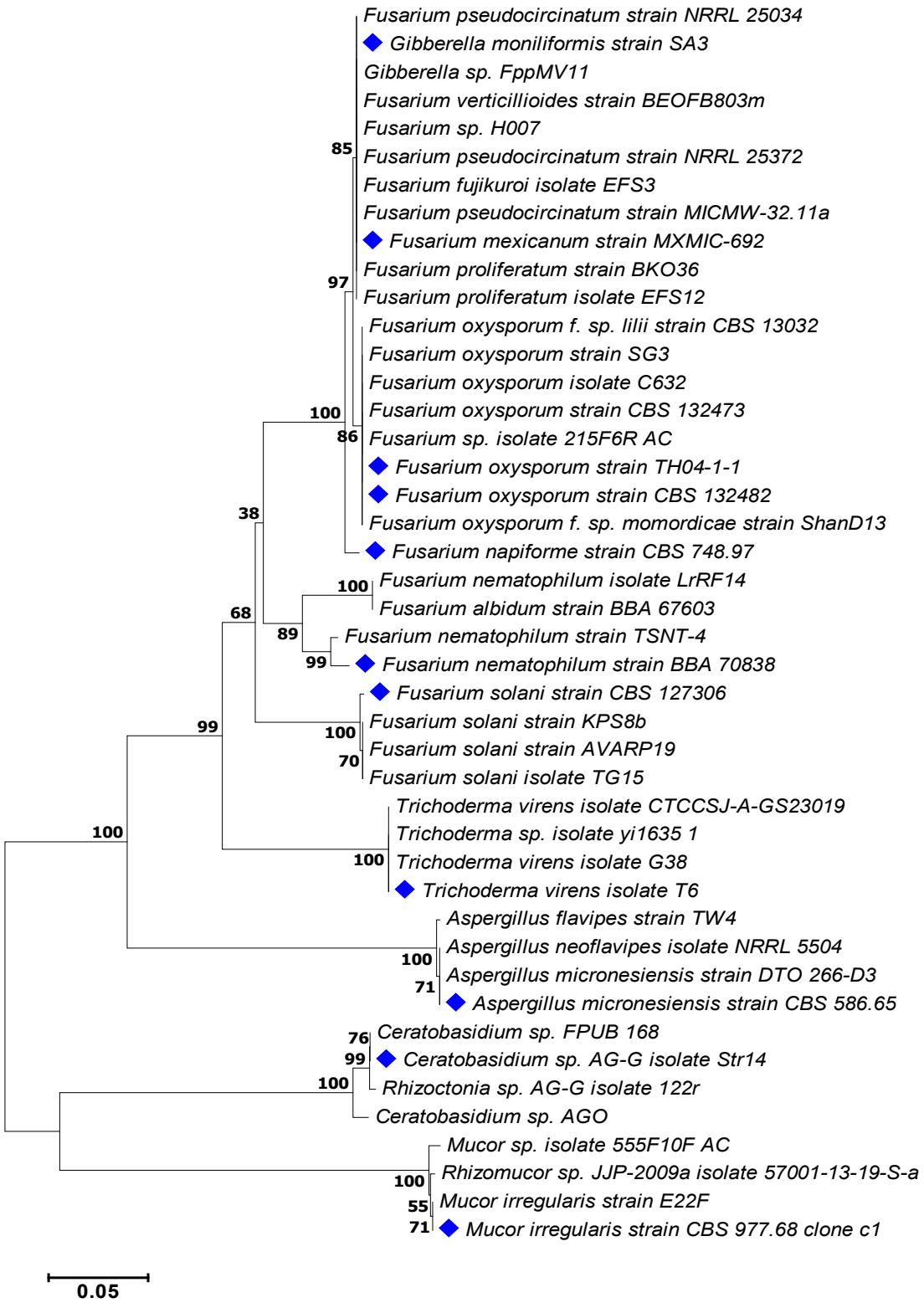

Fig. 4. Neighbor-joining phylogenetic tree of isolated fungal endophytes ◆ and related strains. Bootstrap values are shown next to the branches

Consistently high mortality of *R. similis* and *P. coffeae* occurred with isolates S1: *Mycelia sterilia,* S2: *Gibberella moniliformis*, S4: *Fusarium oxysporum*, BE2: Mycelia sterilia, BT1: *Mucor irregularis*, BT2: *Trichoderma virens*, GN1: *Mycelia sterilia* and GN4 *Fusarium napiforme.* Therefore, these specific isolates should be considered as potential biological control agents against *R. similis* and *P. coffeae* in future studies. Results in this study showed that nematode mortality was significantly higher in *R. similis* than in *P. coffeae*. The fact that nematode *R. similis* was more affected by fungal extract was consistent with the results of O'bannon et al. [41] where under conditions favourable to *R. similis*, *P. coffeae* was more deleterious and more damaging to citrus. All of this reinforces the idea that *P. coffeae* is generally more resistant than *R. similis*.

In an attempt to locate the source of the nematicidal effect of fungal extracts, culture filtrates for extracellular metabolites and solution of broken mycelia for intracellular metabolites were both tested in this study. Culture filtrates of isolated fungi were more effective than solutions of broken mycelia suggesting that the nematicidal effect of these fungi is principally due to extracellular metabolites released in the culture medium during fungal growth. The pH of fungal culture filtrates appears to have no effects on nematode inhibition as no significant differences were found between the treatments with unadjusted pH and the one with adjusted pH. Thus, the compounds or metabolites produced in the culture filtrates are most likely to be the cause of nematode suppression. This can be explained by the fact that although toxin production by fungi may be influenced by pH, the toxins act in a wide range of pH values and are independent of the culture filtrate [42].

The nematicidal effect of diluted culture filtrates was also assessed in this study in order to simulate the natural conditions encountered in vivo in the Banana plant. If the fungal isolates produce toxins inside the plant, that concentration would likely be lower than in the bioassays conducted with undiluted culture filtrates. We observed that the percentage of nematodes killed decreased with a reduction in culture filtrate concentration, supporting previous findings [43,8].

Sequence analysis of the 5.8S and ITS regions of the nuclear rDNA have been widely used in determining the taxonomic placement of fungi at different levels in recent years [22]. In this study, molecular characterization by sequencing and phylogenetic analyses allowed the identification of fungal isolates to at least the genus level. Fungi taxonomy is traditionally based on comparative morphology and on the development of sexual reproduction structures [44]. Endophytic fungi, which are mainly ascomycetes, basidiomycetes and some dematiaceous are most often non or low sporulating fungi, therefore it is more difficult to identify these fungi by means of conventional taxonomy. Also, traditional taxonomy is generally laborious and can lead to errors as shown in this study where isolate BT2 which was identified as *Trichoderma virens* through molecular characterization, was previously identified as belonging to *Penicillium* species using morphological identification. This supports the fact that genetic methods present high sensitivity and specificity for identifying microorganisms and can be used for classifying microbial strains in diverse hierarchical taxonomic levels [44].

5. CONCLUSION

Endophytic fungi isolated from Banana and Plantain cultivars in Njombé (Cameroon), have the capacity to kill mixed stages (females, males, and juveniles) of motile *R. similis* and *P. coffeae in vitro*. These fungal isolates may provide cheap and environmentally friendly nematicides. However, knowledge on the nematode-inhibiting components in the fungal extracts is needed in order to exploit their full potential as effective pest and disease management tools in agriculture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Annex 1. Percentage mortality (mean ± S.D.) of *Radopholus similis* at various concentrations and incubation times

Radopholus similis													
		After 12H				After 24H				After 36H			
Code	**Fungus Genus**	**Negative Control**	**Crude Filtrate**	**D1/10**	**D1/100**	**Negative Control**	**Crude Filtrate**	**D1/10**	**D1/100**	**Negative Control**	**Crude Filtrate**	**D1/10**	**D1/100**
S1*	Mycelia sterilia	0	88±3.0 b	46±3.0 d	35±7.0 e	5±1.0	98±1.5 a	61±2.5 d	32±1.5 e	9±0.5	99±1.1 a	73±2.6 c	44±3.2 e
S2*	*Fusarium* sp.	0	92±2.0 a	66±5.6 c	43±2.0 e	4±1.5	98±1.0 a	73±4.5 b	50±1.0 c	8±1.5	99±1.5 a	87±3.0 b	68±2.6 c
S3	Unknown	0	38±2.0 e	24±4.5 g	6±2.0 i	3±1.0	43±2 e	17±2.5 g	8±3.0 f	7±2.0	52±1.0 e	26±4.1 g	21±2.0 g
S4*	*Fusarium* sp.	0	86±3.7 b	50±3.0 d	4±0.5 i	4±1.5	89±5.a	67±2.6 c	6±3.0 f	7±1.5	91±5.1 a	69±3.0 d	13±5.1 h
BT1*	*Mucor* sp.	0	100±0.0 a	100±0.0 a	52±1.1 d	4±1.5	100±0.0 a	100±0.0 a	99±0.5 a	8±1.5	100±0.0 a	100±0.0 a	100±0.0 a
BT2*	*Penicillium* sp.	0	96±2.0 a	63±1.0 c	41±3.0 e	3±1.5	98±1.5 a	81±1.5 b	55±1.0 c	8±1.0	100±0.0 a	99±0.5 a	85±1.0 b
MB1	*Mucor* sp.	0	5±1.0 f	3±1.0 h	1±0.5 j	2±1.5	12±2.6 f	7±1.5 h	4±3.0 f	11±3.2	17±1.0 f	12±3.0 h	10±3.6 i
MB2*	*Fusarium* sp.	0	100±0.0 a	100±0.0 a	61±4.2 c	2±1.5	100±0.0 a	100±0.0 a	83±1.5 b	4±1.1	100±0.0 a	100±0.0 a	100±0.0 a
BE2*	Mycelia sterilia	0	96±1.1 a	87±3.2 b	66±2.5 b	8±3.5	98±1.5 a	92±1.7 a	78±4.1 b	11±2.5	100±0.0 a	97±1.0 a	90±2.0 b
BE3	Mycelia sterilia	0	68±3.0 c	48±2.6 d	12±4.1h	4±0.5	78±9.0 c	50±1.0 e	12±2.6 f	8±2.0	86±3.0 c	51±4.7 e	18±2.0 h
BE4	*Fusarium* sp.	0	85±2.6 b	37±3.0 f	16±4.0 h	4±1.5	87±2.6 b	73±3.5 c	44±4.1 d	20±2.0	89±3.5 b	79±2.5 b	51±1.5 d
BE5	Mycelia sterilia	0	57±4.5 d	19±3.0 g	3±2.0 i	5±1.0	57±5.0 d	25±5.5 g	6±1.5 f	11±1.5	59±4.0 d	30±3.6 f	23±3.2 f
GN1	Mycelia sterilia	0	97±1.0 a	64±2.5 c	27±3.5 f	6±2.5	98±1.0 a	67±3.6 c	29±5.1 e	9±2.0	98±1.0 a	69±3.0 d	31±3.5 f
GN2	Mycelia sterilia	0	43±3.6 e	6±1.5 h	4±2.0 i	3±1.0	78±2.6 c	16±1.5 g	12±4 f	5±1.0	88±1.5 c	32±1.5 f	20±2.6 g
GN3	*Fusarium* sp.	0	67±3.6 c	42±2.0 d	6±1.5 i	3±1.0	66±3.0 d	43±3.6 e	13±1.5 f	7±2.0	68±4.5 d	52±2.5 e	18±3.0 h
GN4*	*Fusarium* sp.	0	100±0.0 a	100±0.0 a	100±0.0 a	2±1.0	100±0.0 a	100±0.0 a	100±0.0a	7±1.0	100±0.0 a	100±0.0 a	100±0.0 a
GN5	*Fusarium* sp.	0	86±3.2 b	35±3.0 f	19±3.0 g	3±1.5	88±4.0 a	37±3.5 f	27±3.4 e	6±1.0	89±3.5 b	39±4.1 f	28±2.0 f
GN5^{v2}	*Fusarium* sp.	0	74±4.0 c	48±3.2 d	17±3.0 h	3±2.0	86±3.5 b	66±5.5 c	34±3.5 e	6±1.5	88±4.5 c	79±4.0 b	57±2.5 d
P-value			<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001

In each column, means followed by the same letter are not significantly different at P = 0.05 according to Waller-Duncan multiple range test. Data are nematode mortality in percentage (%) calculated with the mean of three replicates. (*): Most effective fungal isolates.

Annex 2. Percentage mortality (mean ± S.D.) of *Pratylenchus coffeae* at various concentrations and incubation times

		Pratylenchus coffeae											
		After 12H				After 24H				After 36H			
Code	**Fungus Genus**	**Negative Control**	**Crude Filtrate**	**D1/10**	**D1/100**	**Negative Control**	**Crude Filtrate**	**D1/10**	**D1/100**	**Negative Control**	**Crude Filtrate**	**D1/10**	**D1/100**
S1*	Mycelia sterilia	0	84±1.5 b	29±2.5 e	9±3.5 d	6±0.5	96±1.1 a	48±4.1 c	19±2.0 f	11±1.5	98±1.0 a	65±3.6 c	53±19.07 b
S2	*Fusarium sp.*	0	64±2.5 c	7±2.0 g	5±2.0 e	5±2.0	67±2.6 d	26±4.0 g	11±3.0 g	4±2.0	68±3.0 e	30±2.6 f	14±3.2 d
S3	Mycelia sterilia	0	4±1.1 i	24±3.5 e	24±7.0 c	3±1.5	32±6.2 h	43±4.0 d	33±6.5 c	7±1.5	46±5.5 h	54±2.5 d	48±4.5 b
S4	*Fusarium sp.*	0	73±2.0 b	30±2.0 e	3±1.0 e	6±3.3	78±3.0 c	37±2.0 e	6±1.1 h	6±2.0	80±3.0 c	42±5.2 e	8±1.5 d
BT1*	*Mucor* sp.	0	100±0.0 a	100±0.0 a	17±2.6 c	4±0.5	100±0.0 a	100±0.0 a	18±1.0 e	4±2.0	100±0.0 a	100±0.0 a	100±0.0 a
BT2*	*Penicillium sp.*	0	75±2.0 b	50±1.0 c	3±1.5 e	2±1.0	94±1.5 a	65±1.0 b	4±1.5 i	11±1.5	98±1.0 a	79±2.0 b	16±2.0 d
MB1	*Mucor* sp.	0	3±1.5 i	4±1.5 g	6±3.2 e	3±1.5	6±1.5 i	9±1.1 h	6±2.0 h	6±2.0	12±2.6 i	15±2.0 g	9±4.1 d
MB2*	*Fusarium sp.*	0	100±0.0 a	100±0.0 a	17±1.0 c	4±0.5	100±0.0 a	100±0.0 a	30±2.0 c	4±2.0	100±0.0 a	100±0.0 a	37±2.0 b
BE2	Mycelia sterilia	0	27±4.0 h	3±2.0 g	2±1.5 e	4±1.5	37±3.0 g	5±2.6 h	4±2.0 i	8±1.0	39±3.2 h	9±4.0 g	6±1.5 e
BE3	Mycelia sterilia	0	41±8.9 g	4±1.5 g	7±1.0 e	5±1.0	48±2.5 f	28±8.0 f	13±1.5 f	12±2.0	49±5.6 g	33±5.0 e	14±1.5 d
BE4	*Fusarium sp.*	0	53±3.6 e	8±3.0 f	2±1.5 e	4±1.0	75±1.0 c	58±3.0 b	8±3.0 h	13±7.7	88±4.0 b	100±0.0 a	17±1.0 d
BE5*	Mycelia sterilia	0	75±2.0 b	26±1.5 e	6±2.0 e	2±0.5	97±0.5 a	44±2.0 d	23±1.5 d	4±1.3	99±0.5 a	56±2.5 c	36±3.0 b
GN1*	Mycelia sterilia	0	96±0.5 a	82±3.7 b	59±2.0 b	6±1.5	97±1.0 a	92±1.0 a	72±1.1 b	12±2.6	98±1.0 a	97±1.7 a	91±2.0 a
GN2	Mycelia sterilia	0	13±3.5 i	3±2.6 g	3±0.5 e	2±1.0	29±3.0 h	12±4.0 h	7±1.0 h	8±1.5	71±5.0 d	28±4.0 f	13±2.0 d
GN3	*Fusarium sp.*	0	47±2.0 f	24±2.0 e	5±2.0 e	2±1.5	58±4.3 e	31±3.5 f	9±3.0 h	6±1.5	59±3.0 f	36±1.5 e	17±1.0 d
GN4*	*Fusarium sp.*	0	100±0.0 a	100±0.0 a	100±0.0 a	4±2.6	100±0.0 a	100±0.0 a	100±0.0 a	4±2.7	100±0.0 a	100±0.0 a	100±0.0 a
GN5	*Fusarium sp.*	0	79±3.7 b	41±2.5 d	4±1.5 e	1±0.5	86±8.7 b	52±2.6 c	8±1.7 h	5±1.0	88±3.0 b	59±3.5 c	23±3.0 c
GN5^{v2}*	*Fusarium sp.*	0	59±2.5 d	16±4.0 f	4±3.0 e	6±1.5	64±2.5 d	42±2.5 d	10±1.7 h	6±2.0	98±0.5 a	83±2.5 b	16±1.5 d
P-value			<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001

In each column, means followed by the same letter are not significantly different at P = 0.05 according to Waller-Duncan multiple range test. Data are nematode mortality in percentage (%) calculated with the mean of three replicates. (*): Most effective fungal isolates.

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