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First report of three species of *Trichoderma* isolated from the rhizosphere in Algeria and the high antagonistic effect of *Trichoderma brevicompactum* to control grey mould disease of tomato

Massinissa Hammad^{1*}, Thomas Guillemette², Meriem Alem¹, Franck Bastide² and Meriem Louanchi¹

Abstract

Background: Grey mould caused by *Botrytis cinerea* Pers. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) is one of the most destructive fungal diseases of Mediterranean crops. In Algeria, few studies have been made on the economic impact of this disease. Nevertheless, it is practically present in all tomato and strawberry greenhouses, as well as in inspected vineyards in the north and south of the country. The complexity of chemical control of this disease has led to search for *Trichoderma* strains that are effective in biological control.

Results: Fifteen isolates of *Trichoderma* spp. were obtained from vigorous and healthy plants (tomatoes, strawberries, and vines) rhizosphere, and from a commercial bio-compost (Bio-composte®), then identified as *T. afroharzianum* (four isolates), *T. gamsii* (four isolates), *T. longibrachiatum* (three isolates), *T. atroviride* (one isolate), *T. brevicompactum* (one isolate), *T. breve* (one isolate), and *T. lixii* (one isolate) on the basis of DNA sequence analysis of four genes (ITS, *tef1*, *rpb2*, and *act1*). In vitro biocontrol tests revealed that four Algerian isolates of *Trichoderma* spp. (TAtC11, TGS7, TGS10, and TBS1) had a high antagonistic activity against *B. cinerea*, the mycelial growth has been reduced by 62 to 65% in dual-culture technique, by 62.31 to 64.49% in volatile compounds test, and a high inhibition of germling growth was recorded by TBS1 isolate with 90.68% in Culture filtrates test. Biocontrol tests carried out on tomato plants with *T. brevicompactum* (TBS1), *T. atroviride* (TAtC11), and *T. lixii* (TLiC8) against *B. cinerea* (BCT04) showed that TBS1 inoculation significantly reduced the incidence of disease by 64.43 and 51.35% in preventive and curative treatment, respectively.

Conclusion: The present study revealed the first report of *T. brevicompactum*, *T. breve*, and *T. lixii* in Algeria, and it also contributes to the promotion of the use of native strains of *Trichoderma* in biological control leading to a better preservation of soil microbial diversity.

Keywords: *Trichoderma* spp., Biological control, *Botrytis cinerea*, *Solanum lycopersicum*

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Background

Botrytis cinerea Pers, the causative agent of grey mould, is a necrotrophic and polyphagous ascomycete. It has been reported on more than 1400 species of dicotyledonous and monocotyledonous plants and distributed in 586 genera of plants (Elad et al. 2016). This pathogen can infect all parts of the plant, seeds, and other planting material, stems, leaves, flowers, and fruits at the pre-harvest and post-harvest stages. It is more destructive on mature or senescent tissues and can remain dormant for a long period before causing tissue rot (Williamson et al. 2007). According to Dean et al. (2012), *B. cinerea* was classified as the second most important fungal plant pathogen in the top 10 list, which can be explained by several factors described by Williamson et al. (2007), the most important are an exceptionally wide host range; the nature of *Botrytis* epidemics; its genetic plasticity, including adaptation to fungicides.

The chemical control of the grey mould has become difficult because this disease presents a wide genetic variability and a high capacity to acquire resistance against fungicides, classifying it as a high-risk plant pathogen (Shao et al. 2021). To solve this problem, many researchers have proposed alternative methods to control this disease, such as application of biological control agents, plant extracts, minerals, and organic compounds (Nicot et al. 2011).

The genus *Trichoderma* contains efficient biological control agents (BCA) with a very high antagonistic capacity against a wide range of plant pathogens (Medeiros et al. 2017), through various mechanisms of action, such as parasitism, competition on nutrients, and synthesis of antibiotics (Sood et al. 2020). Although, several species of the genus *Trichoderma* have been shown to be potentially efficient in controlling *B. cinerea*, only a limited proportion of them have been exploited as biological control agents against this pathogen.

For several decades, the genus *Trichoderma* has attracted the researchers and industries interest. Approximately 60% of registered biofungicides based on filamentous fungi have been developed from *Trichoderma* strains (Verma et al. 2007). Formulation of several commercially available anti-botrytis products was based on isolates of *T. atroviride*, *T. harzianum*, *T. polysporum*, and *T. viride* to control *B. cinerea*, such as Sentinel[®] formulated with *T. atroviride* strain, LC52 and Trichodex[®] formulated with *T. harzianum* (Nicot et al. 2016). Testing other species of this genus in biocontrol essays should be useful, especially with native species.

The main objective of the present study is to identify native strains of *Trichoderma* which are effective in biological control of *B. cinerea*, and this could help to minimize the use of pesticide and protecting the environment.

Methods

Isolation of fungi

Botrytis cinerea isolates

Isolates of *B. cinerea* were obtained from organs with typical symptoms of the grey mould of different host plants (vine, tomato, strawberry), cultivated in the south-eastern (Biskra), north-central (Algiers, Tipaza, Boumerdes), and North-eastern (Bejaia) regions of Algeria. Single-spore was prepared for each isolate and deposited on Petri dishes containing PDA medium. After 5 days of incubation under continuous white light and at a temperature of 20 °C, monosporic isolates were stored at – 20 °C in 20% (v/v) glycerol.

Trichoderma sp. isolates

Sampling was carried out in different geographic regions in the north-central of Algeria (Tipaza, Boumerdes, and Algiers). *Trichoderma* isolates were isolated from the rhizosphere of vigorous and healthy plants (tomatoes, strawberries, and vines), and also from a commercial bio-compost (Bio-composte[®]). Soil and compost (10 g) were dried, ground into powder, and dissolved in 90 ml of sterile distilled water then vortexed in order to homogenize the mixture. Several dilutions were elaborated to 10⁻⁹ (1v/9v). One ml of each dilution was spread evenly onto Petri dishes containing the PDA medium (potato dextrose agar) with 0.05 g streptomycin to reduce bacterial contaminations. After 8 to 10 days of incubation at 22 ± 1 °C, the fungal colonies with typical characteristics of *Trichoderma* spp. were isolated and purified by single spore cultures.

Pathogenicity test The pathogenicity of *B. cinerea* isolates was tested using the technique described by Schüepp and Kung (1978) and modified as proposed by Vignutelli et al. (2002) on half apples of the Golden Delicious variety. The surface of apples was sterilized with ethanol 70% and then cut in half, on each half three perforations were made using an 8-mm-diameter punch. The realized holes were filled with mycelial discs of the pathogen of the same diameter in direct contact with the apple flesh. For the control, sterile PDA discs were used. The two opposite diameters of the rot lesions were measured after 3 days of incubation in the dark at 20 ± 1 °C.

DNA extraction, PCR and sequencing of *Trichoderma* spp

Genomic DNA was extracted from 7- to 10-day old mycelial growth on potato dextrose agar (PDA) medium following the protocol from Goodwin and Lee (1993). For the molecular identification of the isolates, the PCR was carried out on 4 genomic regions: (i) internal transcribed spacer (ITS) regions 1 and 2 was amplified using primers ITS1 and ITS4 (White et al. 1990). (ii) A fragment of approximately 1.2 kb from the gene encoding

the translation elongation factor 1 (*TEF1 α*) was amplified using primer pairs EF1-728F (Carbone and Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005). (iii) A fragment of about 1.1 kb from the gene encoding RNA polymerase II subunit B (*rpb2*) was amplified with primer pairs fRPB2-5f and fRPB2-7cr (Liu et al. 1999). (iv) A fragment of 0.9 kb encoding the largest ATP citrate lyase subunit (*acl1*) was amplified with primers acl1-230up and acl1-1220low (Gräfenhan et al. 2011) (Table 1). The PCR mixtures were prepared with Go Taq[®] Flexi, containing 0.5 μ l of each primer (20 μ mol), 1.5 μ l of MgCl₂ solution (25 mM), 0.5 μ l of dNTP (0.2 mM each dNTP), 5 μ l of 5 \times Green Go Taq[®] Flexi Buffer and 15.9 μ l of sterile-distilled water and 0.1 μ l of Taq DNA polymerase (5 units/ μ l) and 1 μ l of DNA suspension for a total volume of 25 μ l. PCR program for ITS and *tef1* gene start with initial denaturation step of 3 min at 95 °C, followed by 35 cycles of 30 seconds at 94 °C; hybridization at 58 °C for 30 s and an extension at 72 °C for 1 min, and a final extension step of 10 min at 72 °C. Finally, a refrigeration step at 4 °C. *rpb2* and *acl1* gene annealing temperature has been changed for both at 55 °C.

Nucleotide and phylogenetic analysis of *Trichoderma* sequences Sequences were analyzed by comparison with all sequences of *Trichoderma* spp. available at the GenBank of National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>) and the International Subcommittee on *Trichoderma* and *Hypocrea* Taxonomy (ISTH, www.isth.info) (Druzhinina et al. 2005; Kopchinskiy et al. 2005). Alignments of Algerian isolates sequences and the reference sequences of ex-type strains described by several authors (Bissett et al. 2015; Jaklitsch and Voglmayr 2015; Chen and Zhuang 2017) were performed with Clustalw program implemented in MEGA7. Phylogenetic trees were designed for the four genes studied using the neighbor-joining method (MEGA7) with 1000 bootstraps.

Table 1 Primer sequences

Gene	Primer name	Sequences
ITS	ITS1	5'-TCGGTAGGTGAACCTGCGG-3'
	ITS4	5'-TCTCCGCTTATTGATATGC-3'
<i>acl1</i>	acl1-230up	5'-AGCCCGATCAGCTCATCAAG-3'
	acl1-1220low	5'-CCTGGCAGCAAGATCVAGGAAGT-3'
<i>tef1</i>	EF1-728f	5'-CATCGAGAAGTTCGAGAAGG-3'
	TEF1LLErev	5'-AACTTGACAGGCAATGTGG-3'
<i>rpb2</i>	fRPB2-5f	5'-GAYGAYMGWGATCAYTTYGG-3'
	fRPB2-7cr	5'-CCCATRGCCTTYTRCCCAT-3'

Antagonistic activity of *Trichoderma* spp. against *B. cinerea*

i. Dual-culture technique

In vitro confrontation test consists of placing two explants of 8 mm diameter of the antagonist agent (*Trichoderma* spp.) and the pathogen (*B. cinerea*) in the same Petri dish containing PDA medium. The explants were taken with a sterile punch from 5- to 7-day old culture. The two explants of the pathogen and the antagonist agent were placed simultaneously along a diametrical axis, leaving a distance of 5 cm between them and about 2 cm from the extremity of the Petri dish. The control contains only the explants of *B. cinerea* isolates. Petri dishes were incubated 10 days in the dark at 25 °C. Four repetitions were carried out for each treatment. The percentage inhibition of radial growth of pathogens (PIRG P) was computed compared to a control (Ezziyyani et al. 2004).

ii. Effect of volatile compounds of *Trichoderma* on the mycelial growth of *B. cinerea*

The effect of volatile compounds on the mycelial growth was evaluated by the method described by Olivier and Germain (1983). Explants of 8 mm diameter taken from the 3-day-old cultures of each pathogen and antagonist were placed in the center of the Petri dishes containing PDA medium. An assembly was carried out by superimposing the two Petri dishes without lids, *Trichoderma* spp. was placed on the bottom and *B. cinerea* at the top. To avoid loss of volatile substances, the junction was ensured by parafilm[®]. Petri dishes containing the pathogen on the top and PDA without antagonist on the bottom used as control. Three replicates were used for each combination and the experiment was repeated 3 times. The 2 opposite diameters of the *B. cinerea* colonies were measured every day for 4 days of incubation in the dark at 25 °C. Evaluation of inhibition by *Trichoderma* spp. was estimated by calculating the percentage inhibition of mycelial growth as compared to a control (Hmouni et al. 1996).

iii. Effect of culture filtrate of *Trichoderma* spp. on mycelial growth of *B. cinerea*

The effect of culture filtrates of 15 isolates of *Trichoderma* spp. was evaluated against 3 isolates of *B. cinerea* selected on the basis of their high pathogenicity. To achieve this experiment, 10 mycelial explants of 8 mm diameter were collected from *Trichoderma* cultures of 7- to 10-day-old and deposited in 100 ml of PDB medium in 250 ml conical flask, and incubated at 28 °C for 72 h under continuous agitation. The culture filtrate was first

filtered through a filter paper to remove mycelium and a second filtration through Millipore membranes of 0.20 μm diameter to remove spores, and then stored at 4 °C. *B. cinerea* spore suspensions were prepared from cultures of 7–10 days old. The concentration was adjusted to 10^6 spores/ml by using the malassez cell.

To study the antifungal effect of *Trichoderma* spp. culture filtrates on the mycelial growth of *B. cinerea*, a final volume of 1 ml was prepared as follows: *Trichoderma* spp. culture filtrate with *B. cinerea* suspension containing 800 μl PDB, 100 μl of *Trichoderma* spp. culture filtrate, and 100 μl of *B. cinerea* spore suspension. Control without *B. cinerea* contained 800 μl PDB, 100 μl of *Trichoderma* spp. culture filtrate, and 100 μl ultrapure water. Control with *B. cinerea* contained 900 μl PDB and 100 μl of *B. cinerea* spore suspension. The preparations were filled in 96-well plates (300 μL /well). The plates were covered and sealed to avoid contamination. Four independent biological replicates were performed; each replicate included 3 technical repetitions for each sample in the same plate. Mycelial growth was automatically recorded every 10 min during the 33-h incubation at 25 °C by nephelometry reader equipped with a 635-nm laser (NEPHELOstar® Galaxy, Offenburg, Germany). During incubation, the microplates were shaken at 175 rpm for 5 min every 10 min (Joubert et al. 2010).

Data were exported from Nephelostar Galaxy software in ASCII format and further analyzed with Microsoft Excel 2016 (version 16.0.12827.20268) and R3.4.1 (R Core Team 2020). The lag phase and the maximal growth rate variables were calculated according to the method described by Joubert et al. (2010). The initial relative nephelometric unit (RNU) value was calculated as the average of the 3 initial measurements and then subtracted from each curve value. For each point on the curve, a slope was calculated using measurements that were taken 2 h before and 2 h after this time. The lag phase was defined as the time required to obtain a slope value of 1 and the maximal growth rate was defined as the highest slope.

In situ biocontrol assays in tomato plants To control grey mould disease caused by the most virulent isolate of *B. cinerea* (BCT04), the potential preventive effect of three isolates of *Trichoderma* (TBS1, TAtC11, TLiC8) was tested in situ on tomato plants cv. “KAWA” of 28-days old. The tomato plants were grown under a greenhouse in pots of 12 cm diameter, containing a mixture of commercial soil, sterile soil, and sterile sand (v/v/v). An inoculation with a suspension of 10^5 spores/ml of *B. cinerea* was performed into the pots and after 24 h by spraying with a suspension of 10^6 spores/ml of *Trichoderma* spp.

To evaluate the potential curative effect, the same method was applied, except that inoculation with a suspension of 10^5 spores/ml of *B. cinerea* was carried out 24 h before the treatment with suspension of *Trichoderma* spp. (10^6 spores/ml). Approximately 5 ml of conidial suspension per plant of *B. cinerea* and *Trichoderma* spp. was used. The positive control was constituted only by inoculation with a suspension of *B. cinerea* (10^5 spores/ml). Ten plants were used for each combination of *B. cinerea*/*Trichoderma* spp. and for the positive control. The experiment was conducted for a week and repeated 3 times.

A scale described by You et al. (2016) ranging from 0 to 4, in which 0 indicates that the leaflet is apparently healthy, while 1, 2, 3, and 4 indicate percentages of necrotic lesion of 1 to 25, 26–50%, 51–75%, and 76–100% of the total leaflet surface area, respectively. Disease development on plants was assessed as a function of the number of diseased leaflets relative to the total number of leaflets. Disease incidence and biocontrol efficiency were calculated for each treatment, using the formulas described by Xue et al. (2009).

Statistical analysis

To evaluate the biocontrol effect of *Trichoderma* spp. isolates on the development of *B. cinerea*, the data were subjected to analysis of variance (ANOVA), and when the data were not normally distributed (Shapiro normality test-Wilks, $P < 0.05$), a non-parametric variance analysis (Kruskal–Wallis test) was performed, using Statistical Package for R3.4.1. (R Core Team 2020).

Results

B. cinerea isolation and pathogenicity tests

Thirty isolates of *B. cinerea* were obtained from tomato plants (10 isolates), vine plants (10 isolates), and strawberry plants (10 isolates). The survey was carried out over two successive years 2016 and 2017, in the north-central and south-eastern region of Algeria (Table S1; Supplementary data 1). Pathogenicity tests on half-apples revealed significant differences among the different isolates tested (Kruskal–Wallis test was done on diameters of the rot lesions, $\chi^2 = 114.14$, $df = 29$, P value < 0.05). The most virulent isolate of each culture was chosen for the biocontrol tests, from vine (BCV02), tomato (BCT04), and BCFr11 (strawberry) (Fig. 1).

Trichoderma identification and phylogenetic analysis

Fifteen isolates with macroscopic and microscopic characteristics of the genus *Trichoderma* were isolated from the different samples collected. Ten isolates were obtained from tomato; strawberries, vines rhizosphere and 5 isolates were obtained from Bio-compost®. Four genomic regions from all of these isolates were sequenced (Table S2; Supplementary data 1).

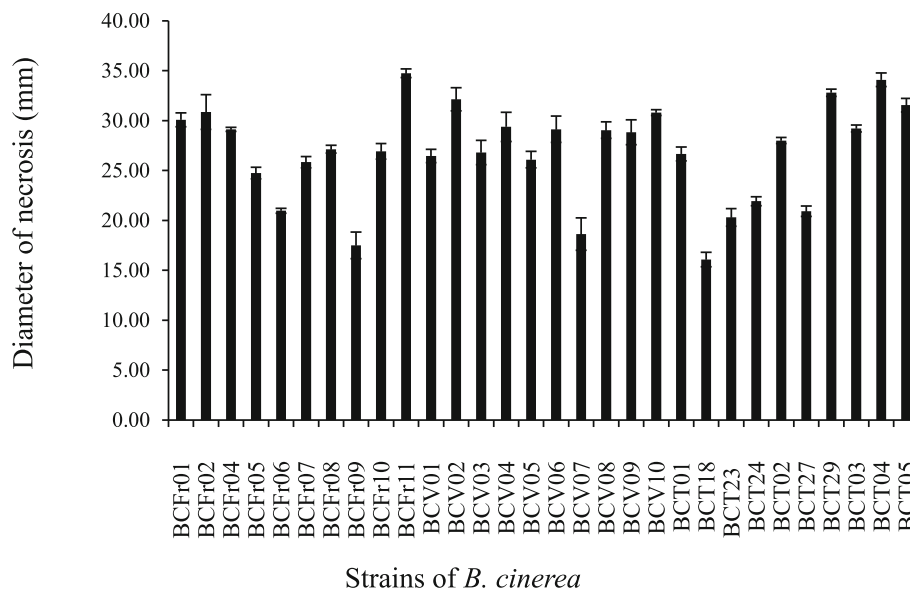


Fig. 1 Pathogenicity test of *B. cinerea* isolates on half apples of the variety Golden Delicious, after incubation for 3 days at 21 °C. The values are the mean of the technical and biological replicates (\pm SE)

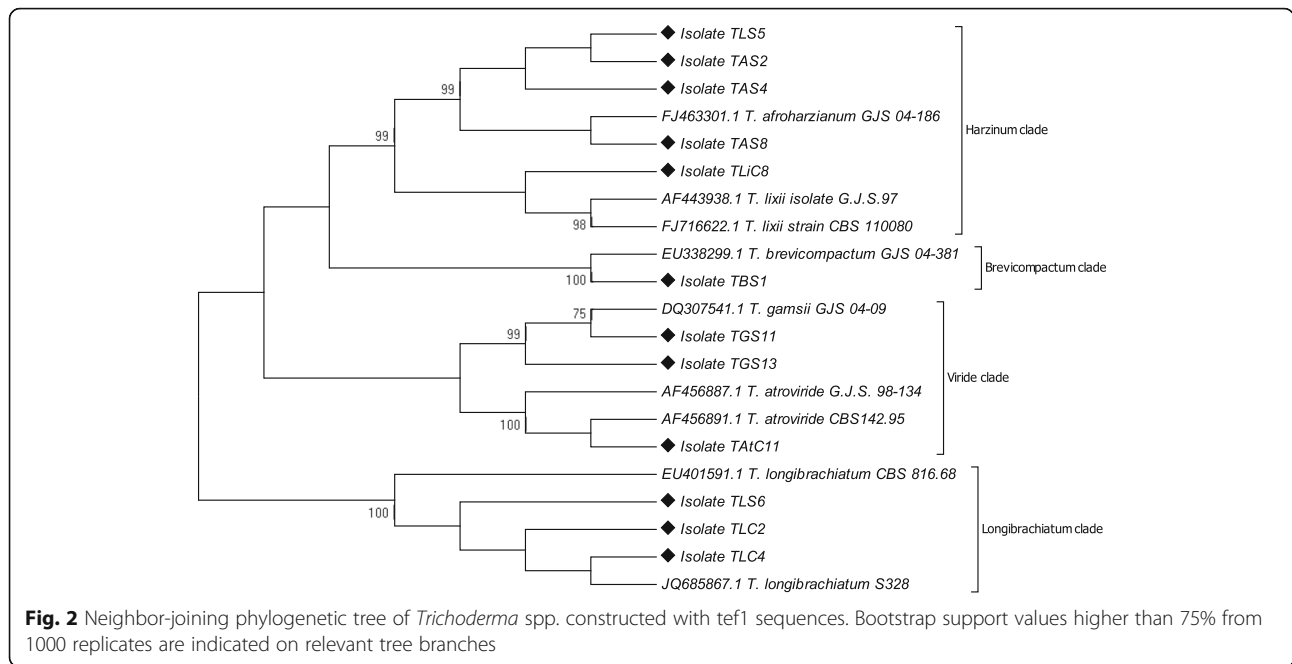
BLAST search of the sequences obtained during this work was performed and also, they were submitted to the ISTH *TrichOKey* (<http://isth.info/tools/molkey/index.php>) and *TrichoBlast* programs (<http://isth.info/tools/blast/index.php>). The results with the highest similarity percentages to the 15 sequences obtained in this study were selected for species identification.

Results revealed that the isolates TAS2, TAS4, TAS5, and TAS8 belong to Harzianum clade, and presented a (99%) of nucleotide identity with the reference sequences of the specie *T. afroharzianum*, for the *tef1* (KP008850) and *rpb2* genes (FJ442691) and (96%) with the species *T. simmonsii*, for *acl1* gene (KJ665182). The isolate TBeC1 had a (99%) of nucleotide identity with the reference sequences of the species *T. breve*, of the clade Harzianum for the *tef1* (KY688046) and *rpb2* genes (KY687983) and (97%) with the species *T. guizhouense* for *acl1* gene (KJ665030). The TLiC8 isolate present a (99%) of nucleotide identity with the reference sequences of the species *T. lixii*, of the clade Harzianum for the *tef1* (FJ716622) and *rpb2* and (98%) with the species *T. atrobrunneum* for *acl1* gene (KJ664949). Using ITS sequences, they were identified as *T. harzianum/H. lixii* and showed (100%) of similarity to several species of the Harzianum clade.

Isolates TLS6, TLC2, and TLC4 showed (99%) of nucleotide identity to the reference sequences of *T. longibrachiatum*, for *tef1* (JQ685867), *rpb2* (JQ685883), and *acl1* (KJ665057). However, for the ITS gene, they were identified as *T. longibrachiatum* and showed a percentage of (100%) nucleotide identity with several species belonging to the Longibrachiatum clade. The isolates

TGS11 and TGS13 revealed (99%) of similarity to the reference sequences of *T. gamsii*, belonging to Viride clade based on the *tef1* sequence (EF488134). In addition, the TGS7 and TGS10 isolates revealed (99%) of nucleotide identity with the reference sequence of *T. gamsii* for the *acl1* gene (KJ665025). TAtC11 isolate revealed (99%) of similarity to the reference sequences of *T. atroviride*, clade Viride for *tef1* (MH176994), *rpb2* (FJ860518), and *acl1* (KJ664952), while for the ITS gene, they were identified as species belonging to the clade Viride with (100%) nucleotide identity with several species of this clade. TBS1 showed (99%) of nucleotide identity with the reference sequences of *T. brevicompactum* species, for the *tef1* sequence (EU338292, EU338283) and for ITS sequence, it was identified as *T. brevicompactum* and revealed (100%) nucleotide identity with several species of the Brevicom-pactum clade.

Phylogenetic trees were designed for each of the 4 gene regions studied, with the sequences of the 15 Algerian isolates and the reference sequences downloaded from GenBank. Thereby, the trees of the *tef1*, *rpb2*, and *acl1* genes revealed the same phylogenetic distribution of the Algerian sequences obtained during this work and the presence of 4 distinct clades (Fig. 2) (Fig. S1, S2, S3; Supplementary data 2). The first one was the clade Harzianum, containing the isolates TAS2, TAS4, TAS5, TAS8, TBeC1, and TLiC8, the first 4 isolates were closely related to the reference strain of specie *T. afroharzianum* (G.J.S. 04-186), the TBeC1 isolate to the reference strain of specie *T. breve* (HMAS:248844) and the TLiC8 isolate to the reference strain of specie *T. lixii* (G.J.S. 97-96 = CBS 110080). The second was the



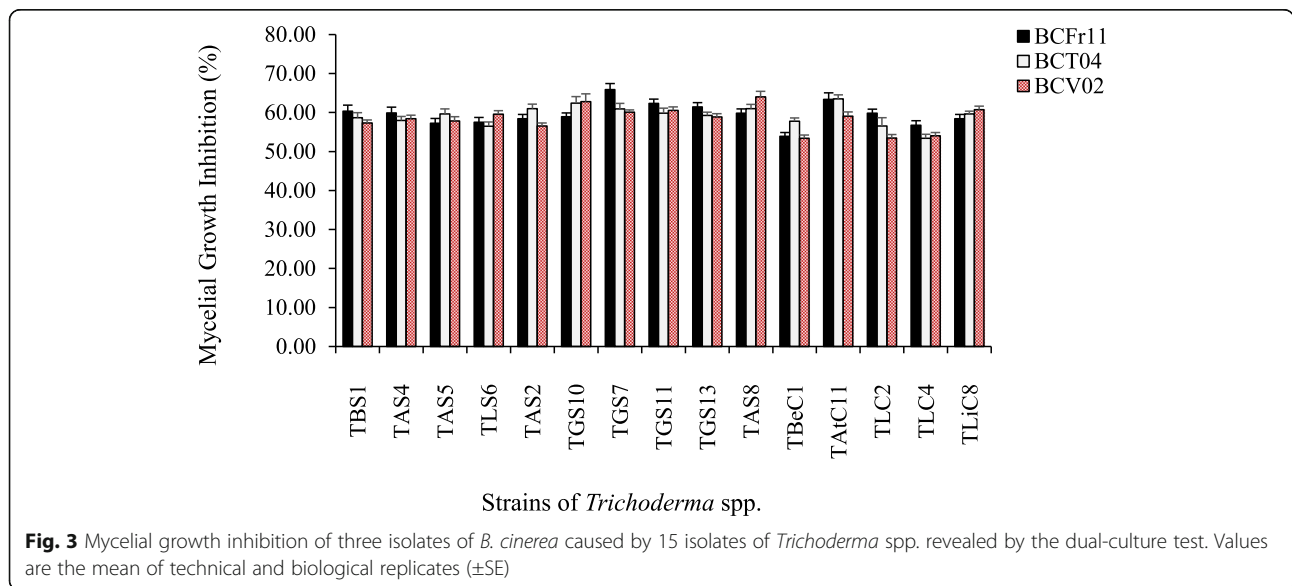
Longibrachiatum clade, containing TLS6, TLC2, and TLC4 isolates, which were closely related to the reference strain of *T. longibrachiatum* (S328, CBS 816.68) for the 3 trees. The third clade was Viride, including TGS7, TGS10, TGS11, TGS13, and TAtC11 isolates; the isolates TGS7, TGS10, TGS11, and TGS13 are closely related to the reference strain of *T. gamsii* (GJS 04-09) and the TAtC11 isolate to the reference strains of *T. atroviride* (S360, CBS 142.95). The fourth was the clade Brevicompactum in which the isolate TBS1 belongs, and was closely linked to the reference strain of *T. brevicompactum* (CBS 109720 = G.J.S.04-381). However, the ITS tree

revealed the same genetic distribution for clades, but the species were placed differently, thus making identification at the species level almost impossible (Fig. S1; Supplementary data 2).

In vitro antagonistic tests

Dual-culture technique

In the dual-culture test, the 15 isolates of *Trichoderma* inhibited the mycelial growth of the 3 most virulent isolates of *B. cinerea* as compared to the control without *Trichoderma* spp. with a range varying from 53 to 65% on PDA medium (Fig. 3). For each *B. cinerea* isolate, a



significant *Trichoderma* effect was observed (ANOVA was performed on the percentage inhibition of mycelial growth, $F = 82.33$, $df = 14,30$, $P < 0.0001$ for BCV02, and $F = 49.68$, $df = 14,30$, $P < 0.0001$ for BCT04 and $F = 49.29$, $df = 14,30$, $P < 0.0001$ for BCFr11). The higher inhibition rate was recorded for *T. gamsii* (TGS7 isolate), *T. atroviride* (TAtC11 isolate) (Fig. S4; Supplementary data 3) and *T. longibrachiatum* (TAS8 isolate), which varied from 62 to 65%. *T. longibrachiatum* (TLC2 and TLC4 isolates) and *T. breve* (TBeC1 isolate) gave the lowest inhibition rates, ranging from 53 to 57%.

Effect of volatile compounds of *Trichoderma* on the mycelial growth of *B. cinerea*

Results revealed a significant difference in the effect of volatile substances produced by *Trichoderma* isolates on mycelial growth of the tested *B. cinerea* isolates (Kruskal–Wallis test was performed on the percentage inhibition of mycelial growth, $\chi^2 = 43.018$, $df = 14$, p value < 0.05 for BCFr11, $\chi^2 = 42.477$, $df = 14$, P value < 0.05 for BCV02 and $\chi^2 = 43.262$, $df = 14$, P value < 0.05 for BCT04) (Fig. 4). Volatile substances emitted by *T. gamsii* (TGS7 isolate) and *T. atroviride* (TAtC11 isolate) reduced mycelial growth of *B. cinerea* isolates by 64.49 and 62.31%, respectively, than the control (Fig. S5; Supplementary data 3). The lowest growth inhibition rate (18.41 and 19.72%) were reported for *T. longibrachiatum* (isolate TLS6) and *T. afroharzianum* (isolate TAS4), respectively.

Culture filtrates

A significant culture filtrates effect was observed for the 3 strains of *B. cinerea* (Kruskal–Wallis test was done on germling growth inhibition, $\chi^2 = 33.407$, $df = 14$, P value < 0.05 for BCFr11, $\chi^2 = 41.267$, $df = 14$, P value < 0.05 for BCV02 and $\chi^2 = 53.082$, $df = 14$, P value < 0.05 for BCT04) (Fig. 5). The best percentages of inhibition were recorded from the filtrates of *T. brevicompactum* (isolate TBS1) (90.68%) and *T. atroviride* (TAtC11 isolate) (68.72%), suggesting a high antifungal effect of these filtrates. While filtrates from the rest of *Trichoderma* spp. isolates revealed stimulation of germling growth for the 3 tested *B. cinerea* isolates, as compared to the control. Percentages of stimulation ranged from 4.25 to 46.31%, the highest percentage of stimulation being found for *T. longibrachiatum* (TlS6 and TLC2 isolates).

In situ test

The effect of spore suspension treatments of *T. atroviride* (TAtC11 isolate), *T. brevicompactum* (TBS1 isolate), and *T. lixii* (TLiC8 isolate) on the incidence of disease caused by *B. cinerea* (BCT04 isolate), in tomato cv. “KAWA” revealed significant differences. Biocontrol activity was observed for the 3 tested *Trichoderma* isolates for preventive and curative treatments. However, the highest percentages of disease control (DC) were recorded for *T. brevicompactum* (TBS1) with $64.43 \pm 4.34\%$ in preventive treatment and $51.35 \pm 1.56\%$ in curative treatment, while the lowest percentages of disease control (DC) were recorded for *T. lixii* (TLiC8) with $34.19 \pm 4.54\%$ in preventive treatment and only $28.46 \pm 8.93\%$ in curative treatment. Based on these results, *T.*

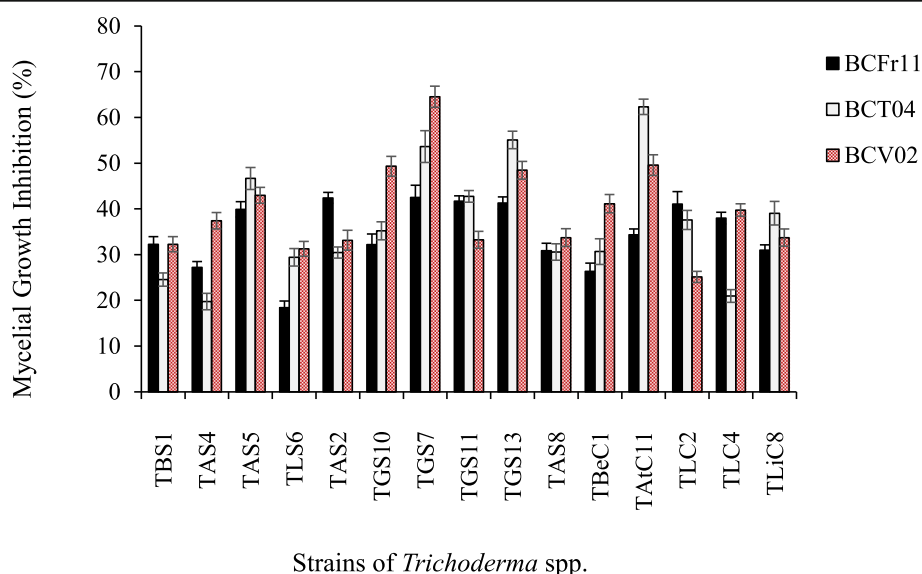
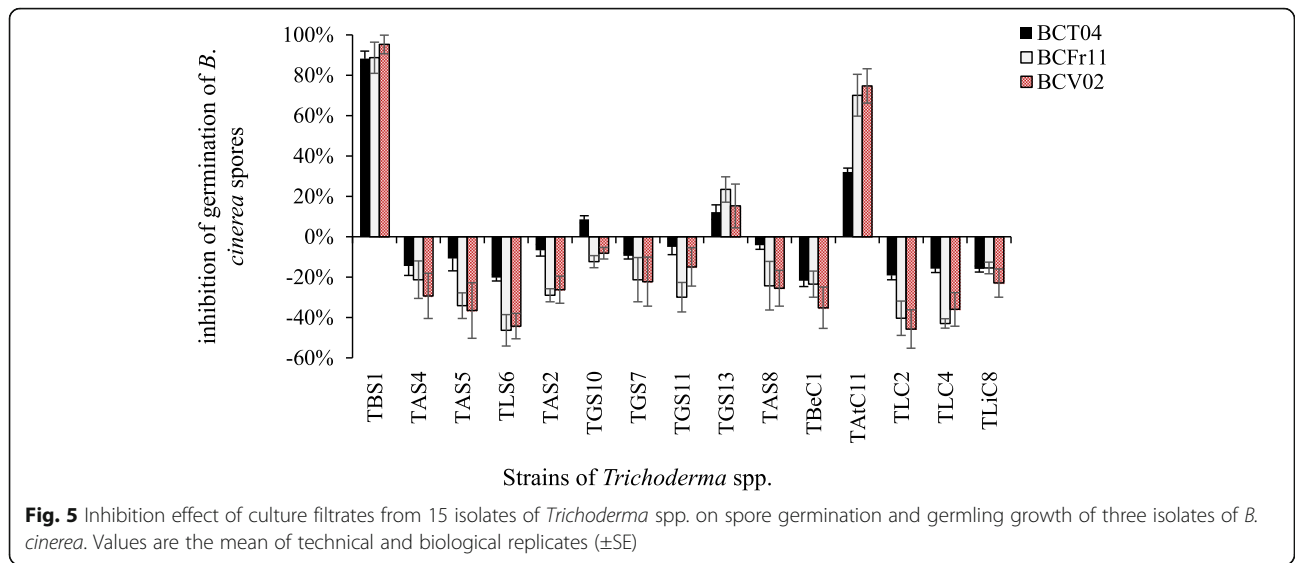


Fig. 4 Mycelial growth inhibition of three isolates of *B. cinerea* caused by volatile compounds produced by 15 isolates of *Trichoderma* spp. Values are the mean of technical and biological replicates (\pm SE)



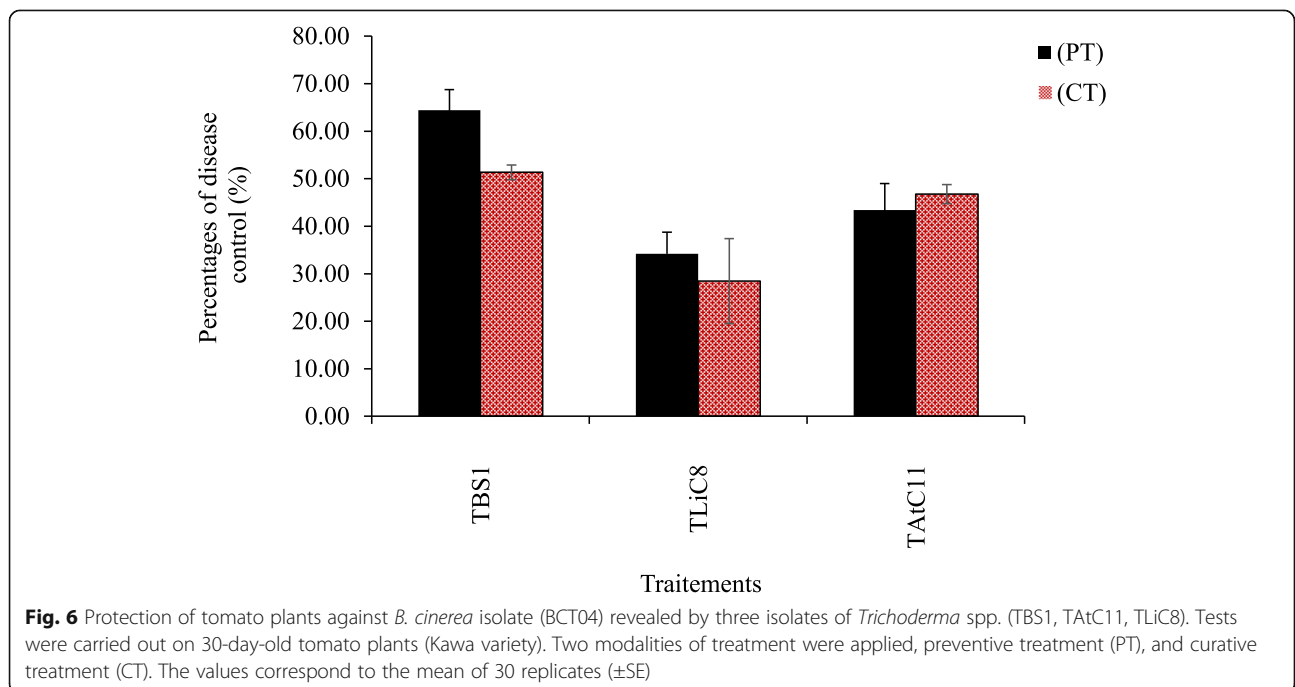
brevicompactum (TBS1) was the most effective isolate for the control of grey mould on tomatoes caused by *B. cinerea* (BCT04) (Fig. 6).

Discussion

B. cinerea is recognized as a high-risk plant pathogen, with global economic losses exceeding 2 billion Euros per year (Dean et al. 2012). At this day, the control of this plant pathogen is mainly chemical, but due to its wide genetic variability and adaptability, acquired resistance has been observed with all fungicides used against grey mould (Shao et al. 2021). This has prompted the

scientific community to move towards alternative control methods, such as biological control, with the use of antagonistic agents (Nicot et al. 2011).

Obtained results of pathogenicity test of 30 isolates of *B. cinerea* on half apples revealed significant differences in aggressiveness. This is consistent with the results of several studies which showed that isolates of *B. cinerea* did not exhibit the same degree of aggressiveness on the same host plant (Decognet et al. 2009). Some strains of the genus *Trichoderma* are sought for their highest potential in biological control and in stimulation of the natural plant defenses by various mechanisms (Hermosa



et al. 2012). So, they are used in the formulation of many commercial products for biological control and/or plant bio-stimulation (Samuels and Hebbar 2015). In this study, native strains that were isolated from agricultural soils in north-central of Algeria were chosen to use, in order to avoid any ecological disturbance of the soil microbial biodiversity. Furthermore, meticulous identification and characterization must be carried out before any use (Galarza et al. 2015).

In the present work, 15 isolates were clearly identified as corresponding to 7 species of *Trichoderma* distributed in 4 different clades. Until now, only 8 species of *Trichoderma* were reported in Algeria: *T. harzianum*/*H. lixii*, *T. asperellum*, *T. ghanense*, *T. atroviride*, *T. longibrachiatum*, *T. viride*, *T. atrobrunneum*, and *T. afroharzianum* (Keddad and Bouzenad 2019; Haouhach et al. 2020). Based on these results, this was the first report of the presence of *T. brevicompactum*, *T. breve*, and *T. lixii* in Algeria. In this study, the ability of Algerian isolates of *Trichoderma* to control grey mould disease was demonstrated. Isolates showed high efficiency in biological control were selected on the basis of in vitro tests. Consequently, three isolates were chosen for the in situ biocontrol test, and two of them, *T. brevicompactum* isolate TBS1 and *T. atroviride* TAtC11, showed an interesting inhibitory effect of *B. cinerea* growth through culture filtrate test and the third isolate *T. lixii* (TLiC8), one of the less studied in biological control was used to test its biocontrol efficiency.

The radial mycelial growth inhibition of *B. cinerea* in dual-culture technique varied from 54 to 64%, indicating the high competitiveness of *Trichoderma* species (Benitez et al. 2004). The reduction in nutrient concentrations generally leads to a reduction in conidia germination and to a slower growth of pathogen germlings (Nassar and Barakat 2013). However, the best results were observed with the isolates of *T. gamsii* (TGS7, TGS10), *T. atroviride* (TAtC11), and *T. afroharzianum* (TAS8). These three species have been described by several studies for their ability to control grey mould and other plant fungal diseases (Redda et al. 2018).

The antagonistic effect of the volatile compound revealed that, *T. gamsii* (TGS7) and *T. atroviride* (TAtC11) showed an important effect compared to the other studied isolates. A powerful odor of coconut aroma was found in these two isolates, which may suggest that volatile inhibitory effect of *T. gamsii* (TGS7) and *T. atroviride* (TAtC11) against *B. cinerea* may be due to pyrone 6-pentyl-2H-pyran-2-one 'coconut aroma' commonly produced by *Trichoderma* spp. (Vinale et al. 2008). The antifungal activity of pyrone 6-pentyl-2H-pyran-2-one was shown in vitro and in vivo against *B. cinerea* by Pezet et al. (1999).

The highest antifungal activity of culture filtrates on *B. cinerea* spore germination and germling growth was observed by *T. brevicompactum* (isolate TBS1), which may

be explained by the ability of this species to produce large quantities of trichothecene, trichodermin, and harzianum (Klaiklay et al. 2019). Several authors have reported the inhibitory activity of these secondary metabolites against plant pathogenic fungi, adding this species to the list of biological control agents (Shentu et al. 2014). It was also found that 12 isolates of *Trichoderma* spp. did not inhibit the germination of *B. cinerea* spores by culture filtrates, but stimulated it. These results can be explained either by the inability of these isolates to synthesize metabolites with fungicidal and/or fungistatic effect against *B. cinerea* isolates or by the resistance of *B. cinerea* isolates to the metabolites secreted by *Trichoderma* isolates. Previous studies have demonstrated that *B. cinerea* can develop resistance to antibiotics produced by the biological control agents (Fillinger et al. 2012).

Greenhouse assays were carried out on "KAWA" tomato plants with the most aggressive isolate of *B. cinerea* (BCT04). The most important reduction in disease incidence was observed in *T. brevicompactum* (isolate TBS1). Results were very promising in both preventive and curative treatments. For this reason, *T. brevicompactum* (isolate TBS1) can be highly recommended for the development of commercial bio-fungicides for the integrated management of grey mould. *T. atroviride* (TAtC11 isolate) was less effective in biocontrol in planta test as compared to the results obtained from the in vitro biocontrol test. As for *T. lixii* (TLiC8 isolate), the results were similar to those obtained in in vitro tests; this isolate seems unable to control grey mould.

Conclusion

The possibility to control the *B. cinerea* disease using Algerian isolates was obtained by the rhizosphere of the host plants. These results also confirmed the effectiveness of native strains in biological control, leading to a better preservation of soil microbial diversity, because the strains already exist in the soil microbial complex. *T. brevicompactum* (isolate TBS1) was a very interesting species in biological control of tomato grey mould disease.

Abbreviations

BCA: Biological control agents; ISTH: International Subcommittee on *Trichoderma* and *Hypocrea* Taxonomy; NCBI: National Center for Biotechnology Information; PDA: Potato dextrose agar; PDB: Potato dextrose broth; PIRGP: The percentage inhibition of radial growth of pathogens; RNU: Relative nephelometric unit; DC: Disease control

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s41938-021-00423-4>.

Additional file 1: Table S1. Origin of *Botrytis cinerea* isolates. **Table S2.** Origin, identification and code accession GenBank of *Trichoderma* spp. isolated in Algeria.

Additional file 2: Figure S1. Neighbor-joining phylogenetic tree of *Trichoderma* spp. constructed with rpb2 sequences. Bootstrap support values higher than 75 % from 1000 replicates are indicated on relevant tree branches. **Figure S2.** Neighbor-joining phylogenetic tree of *Trichoderma* spp. constructed with *act1* sequences. Bootstrap support values higher than 75 % from 1000 replicates are indicated on relevant tree branches. **Figure S3.** Neighbor-joining phylogenetic tree of *Trichoderma* spp. constructed with ITS sequences. Bootstrap support values higher than 75 % from 1000 replicates are indicated on relevant tree branches.

Additional file 3: Figure S4. Mycelial growth inhibition of *B. cinerea* (BCT04) caused by TGS7 and TAtC11 isolates of *Trichoderma* spp. revealed by the dual-culture test. Mycelial discs of *B. cinerea* were placed on the right side of the Petri dish. **Figure S5.** Mycelial growth inhibition of *B. cinerea* (BCT04) caused by volatile compounds produced by TGS7 and TAtC11 isolates.

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Authors' contributions

HM carried out surveys, the several experiments, and the writing of the manuscript. GT gave supervision during the internship and revision of the manuscript. AM contributed to the prospectation and to the experimentation. BF gave technical contribution in the realization of molecular identifications and biocontrol tests LA technical and scientific assistance in the bioinformatics analysis of the sequence data. LM supervised the research work and revised the article. All authors have read and approved the final manuscript.

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Availability of data and materials

We confirm the availability of all the data included in this study. The fungal material used is available at the laboratory Laboratoire de phytopathologie et de biologie moléculaire, Ecole Nationale Supérieure Agronomique, Algiers, Algeria.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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