STEFÂNIA CAIXETA MAGALHÃES

Chaetomium spp.: FILOGENIA E CONTROLE BIOLÓGICO DE FUNGOS FITOPATOGÊNICOS TRANSMISSÍVEIS POR SEMENTES

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

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Prof. José Rogério de Oliveira

Prof. Denise Cunha Fernandes dos Santos Dias

Prof. Denise Castro Lustosa

Dr. Sc. Fábio Nascimento da Silva

Prof. Olinto Liparini Pereira
(Orientador)

“*A mais importante de todas as obras é o exemplo da própria vida*”

Helena Petrovna Blavatsky

Dedico

Meu filho Pedro.

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BIOGRAFIA

STEFÂNIA CAIXETA MAGALHÃES, filha de Maria Inêz Caixeta Magalhães e Clever Magalhães Ribeiro, nasceu em Patos de Minas, MG, no dia 22 de Janeiro de 1982. Concluiu a graduação em Fevereiro de 2007 na Universidade Federal de Viçosa (UFV) como Engenheiro Agrônomo. Iniciou o mestrado em março de 2007, pela Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP) Câmpus Jaboticabal em Agronomia (Produção e Tecnologia de Sementes). Defendeu o mestrado em 2009 e iniciou o doutorado no mesmo ano pela UFV em Fitopatologia. A tese de doutorado foi defendida no dia 28 de Agosto de 2013.

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MAGALHÃES, Stefânia Caixeta, D.Sc., Universidade Federal de Viçosa, agosto de 2013. ***Chaetomium* spp.: filogenia e controle biológico de fungos fitopatogênicos transmissíveis por sementes**. Orientador: Olinto Liparini Pereira. Coorientador: Onkar Dev Dhingra.

Os fungos do gênero *Chaetomium* colonizam diversos substratos, e muitas espécies apresentam potencial como agente de controlo biológico. Até o momento, a identificação das espécies é baseada em dados morfológicos. O objetivo do presente trabalho é prospecção de isolados de várias espécies do gênero *Chaetomium* com potencial utilização no controle biológico de sete agentes patogênicos transmissíveis por sementes de soja e trigo. Quarenta e um isolados de *Chaetomium* foram isolados de solo, sementes e palha no Brasil. Após a caracterização morfológica, o DNA genômico foi extraído para a amplificação do gene ITS. As sequências foram comparadas com sequências no Genbank e outras foram selecionadas para a construção da árvore filogenética. Foram identificadas seis espécies: *Chaetomium aureum*, *C. bostrychodes*, *C. cupreum*, *C. funicola*, *C. globosum*, *C. reflexum*. Este é o primeiro relato de *C. reflexum* no Brasil e uma espécie é nova para ciência. Mais de uma espécie de *Chaetomium* podem ocorrer no mesmo substrato, incluindo as espécies de grande interesse no controle biológico de doenças de plantas. Os testes *in vitro* foram realizados com isolados de *Chaetomium* para avaliar a inibição de sete importantes agentes patogênicos transmitidos por sementes de soja e trigo: *Bipolaris sorokiniana*, *Colletotrichum truncatum*, *Diaporthe phaseolorum* var. *meridionalis*, *Drechslera tritici-repentis*, *Fusarium solani*, *Magnaporthe grisea* e *Sclerotinia sclerotiorum*. Os isolados de *C. cupreum* foram mais eficientes nos testes *in vitro* na inibição do crescimento micelial dos patógenos, os quais foram então submetidos a seis meios de cultura diferentes para testar o meio de cultura mais adequado para a produção de ascósporos. O meio de cultura de aveia demonstrou o melhor resultado para a produção de ascósporos. Os outros ensaios foram realizados em casa de vegetação com dois isolados de *C. cupreum* inoculados em sementes de soja e trigo para avaliação do vigor e a germinação. Foi comprovado que a *C. cupreum* é endófito em raízes de trigo e inibem o crescimento de agentes patogênicos. As espécies *Chaetomium* serão utilizadas em futuros programas de controle biológico de fungos transmissíveis por sementes.

ABSTRACT

MAGALHÃES, Stefânia Caixeta, D.Sc., Universidade Federal de Viçosa, August of 2013. ***Chaetomium* spp.: phylogeny and biological control of plant pathogenic fungi transmitted by seed**. Adviser: Olinto Liparini Pereira. Co-adviser: Onkar Dev Dhingra.

The fungi of the genus *Chaetomium* colonize various substrates, and many species have potential as a biological control agent. By the time, the identification of the species is based on morphological data. The aim of this work is prospecting of isolates from several species of the genus *Chaetomium* with potential use in biological control of seven pathogens transmitted by soybeans and wheat seeds. Forty-one isolates of *Chaetomium* were isolated from soil, seed and straw in Brazil. After the morphological analyses, genomic DNA was extracted for amplification of the ITS gene. Sequences were compared with sequences in the Genbank and others were selected for the construction of the phylogenetic tree. We identified six species: *Chaetomium aureum*, *C. bostrychodes*, *C. cupreum*, *C. funicola*, *C. globosum* and *C. reflexum*. This is the first report of *C. reflexum* in Brazil and one species is new to science. More than one species of *Chaetomium* can occur on the same substrate, including the species of great interest in biological control of plant diseases. *In vitro* tests were performed with isolates of *Chaetomium* to evaluate the inhibition of seven important pathogens transmitted by soybean and wheat seeds: *Bipolaris sorokiniana*, *Colletotrichum truncatum*, *Diaporthe phaseolorum* var. *meridionalis*, *Drechslera tritici-repentis*, *Fusarium solani*, *Magnaporthe grisea* and *Sclerotinia sclerotiorum*. Isolates of *C. cupreum* were more effective *in vitro* tests of inhibition of mycelial growth of pathogens, which were subjected to six different culture media to test the culture medium most suitable for the production of ascospores. The culture medium oats has shown the best results to produce ascospores. The other tests were performed in a greenhouse with two isolates of *C. cupreum* inoculated in soybean and wheat seeds for evaluation of germination and vigor. *C. cupreum* demonstrated is endophytic in wheat roots and inhibit the growth of pathogens. The *Chaetomium* isolates will be used in future programs of biological control of fungi transmitted by seeds.

1. INTRODUÇÃO GERAL

Os patógenos que são transmissíveis por sementes afetam diversas culturas de importância agrícola, e uma forma de prevenção é a redução do inóculo inicial. A preocupação com o uso de sementes infectadas/infestadas não reside, apenas, nos danos diretos que isto pode causar, mas também nas consequências deste fato, considerando-se uma população de plantas no campo.

Alguns patógenos hoje amplamente distribuídos pelo Brasil foram introduzidos por meio de sementes, que além de constituírem eficiente mecanismo de dispersão de patógenos, podem reduzir o número de plantas inicial da lavoura e até mesmo introduzir patógenos importantes em áreas previamente livres dos mesmos. Muitos desses são fungos, os quais são os principais responsáveis por doenças de plantas e podem provocar redução da germinação e vigor das sementes, com consequente diminuição de seu valor comercial.

O controle de fungos transmissíveis por sementes é necessário principalmente para evitar a disseminação de doenças a longas distâncias. No entanto, os tratamentos usuais acarretam problemas ambientais gerados pelo uso abusivo de defensivos agrícolas como a contaminação do meio ambiente com resíduos tóxicos não biodegradáveis e pela seleção de indivíduos resistentes a esses compostos. Desta forma, o controle biológico de fitopatógenos vem ganhando visibilidade.

Os bioprotetores são vantajosos por crescerem e proliferam-se, podendo colonizar e proteger novas partes da planta formadas após sua aplicação. Assim, um bioprotetor pode ser utilizado no tratamento de sementes e o mesmo poderá crescer e estender-se a partes subterrâneas da planta. Além disso, a complexidade de interações entre patógeno e hospedeiro e as numerosas rotas metabólicas de micro-organismos, são recursos fantásticos no controle biológico.

O controle biológico de doenças de plantas com micro-organismos antagônicos é praticado de modo incipiente, principalmente no que diz respeito a patógenos de sementes. Espécies pertencentes ao gênero *Chaetomium* têm sido eficientes no controle de patógenos de plantas, entretanto, trabalhos utilizando *Chaetomium* spp. como agente de controle biológico de patógenos de sementes são extremamente escassos. Este gênero tem demonstrado potencial para utilização no controle de doenças, principalmente para patógenos veiculados por sementes de plantas. Diversas espécies de *Chaetomium* produzem um antibiótico denominado "chetomin", que tem atividade contra bactérias gram-positivas. *C. globosum* é conhecido por produzir até 19 substâncias antibióticas, das quais, chetomin é possivelmente o de maior importância no controle biológico. O *C. globosum* também apresenta atividade antagônica *in vitro* deste a uma gama de fungos. A espécie *Chaetomium cupreum* possui habilidade na degradação da parede celular de fungos fitopatógenos, secreta metabolitos antifúngicos, compete por nutrientes com fungos fitopatogênicos.

Além do *Chaetomium* spp. apresentar diversas vantagens no controle biológico, o gênero também possui potencial em outros aspectos. Como estimulante de crescimento de plantas e algumas estirpes de *Chaetomium* produzem quantidades substanciais de ergosterol. Esta substância pode ajudar a melhorar a camada de húmus em solos. Outro ponto a ser considerado é a indução de resistência em plantas, onde a Chaetoglobosin C produzida pelo *C.*

globosum atua como uma substância estranha que induz uma onda localizada oxidativa subsistêmica (OXB) testada em plantas, por exemplo, em cenoura, batata, batata-doce, tomate e tabaco. Possivelmente atua como uma indução de imunidade da planta para a resistência à doenças. OXB em plantas pode ser caracterizada por uma rápida geração de espécies de oxigênio ativo (AOS), imediatamente após a exposição a determinadas tensões. As espécies de oxigênio ativo desempenham um papel importante na iniciação dos sinais para a resposta do estresse de diferentes plantas. O OXB tem sido postulado como sendo um sinal de alarme em tecidos de plantas contra a invasão por micro-organismos agressivos e parece funcionar como um sinal de emergência para as respostas contra o ataque por substâncias estranhas, além disso, pode ser relacionado com a resistência ou a proteção contra agentes patogênicos de plantas.

Além destes há também a alternativa como biofertilizantes para aumentar a produtividade. Biofertilizantes possuem vários benefícios como promover maior absorção de nutrientes pelas plantas, incluir materiais derivados de organismos vivos e de fontes microbianas, promover o crescimento das plantas, reciclagem e compostagem eficaz de resíduos sólidos.

Desta forma, objetivou-se nesse trabalho a prospecção de isolados de diferentes espécies do gênero *Chaetomium* com potencial uso no controle biológico de alguns patógenos transmissíveis por sementes, utilizando-se soja e trigo como modelo.

ARTIGO 1

Prospecting for Chaetomium spp. from soil, seed and stubble

Stefa□nia C. Magalhães[1], Danilo B. Pinho[1] and Olinto L. Pereira[1]*

[1] Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-000, Brazil; oliparini@ufv.br

Abstract:

With the aim to obtain potential biological control agents for seed-borne fungi, forty-one isolates of Chaetomium belonging to seven different species were isolated from soil, seeds and stubble in Brazil. These species were compared by means of morphological and phylogenetic analyzes. One species proved to be new and will be latter proposed. The Chaetomium species will be utilized in future biological control programs for seed-borne fungi.

Key words: biological control, cellulose degradation, phytopathology, Sordariales, taxonomy, tropical fungi.

1. Introduction

The Chaetomium genus was established in 1817 by Gustav Kunze (Ames 1961) with Chaetomium globosum as the type species. Nineteen species known occur in Brazil (Coutinho et al. 2007, Barbosa et al. 2012).

Chaetomium species are characterized by superficial, subglobose, elongated or vasiform perithecia, translucent when young and darker when ripe. The perithecia are fastened to the substrate by rhizoids-like hyphae and the ostiole are present at the apex. It has smooth, straight, branched or twisted bristles, with or without ornaments, of several colors, concentrated mainly around the ostiole. The asci are thin, evanescent, bat like or clavated with four to eight ascospores. The ascospores are aseptate, limoniform, with one or two germ pore, pale when young, darker when mature, with several shapes, sizes and colors. They leave the ostiole in a sticky black mass or cirrhus (Ames 1961, Arx et al. 1986, Rodríguez et al. 2002, Domsch et al. 2007, Asgari & Zare 2011, Guarro et al. 2012).

Some authors have tried to organize Chaetomium species in morphological groups (Asgari & Zare 2011), by characteristics of the ascospores, the hair (Ames 1961), and both traits. In 1976, Dreyfuss has proposed a classification based on morphological and physiological characters in four groups: C. aureum Chivers, C. bostrychodes Zopf, C. globosum Kunze and C. murorum Corda (Asgari & Zare 2011). In 1975, the genus Farrowia Hawksworth was proposed with some specific features (Hawksworth 1975); however this genus is not recognized (Arx et al. 1986; Decock & Hennebert 1997, Wang & Zheng 2005). Molecular analysis does not support Farrowia as monophyletic genus (Untereiner et al. 2001, Guarro et al. 2012). The purpose of this study was to obtain, isolate and identify different species of Chaetomium for future use in biological control of seed-borne fungal diseases.

2. Materials and Methods

2.1. Obtaining isolates

The isolates of Chaetomium spp. used were obtained from native and agronomic interest seeds (Table 1). To obtain the isolates, the Blotter test was used in order to provide favorable conditions for the Chaetomium to develop on seeds. Moreover, we also evaluated the straw of grown plants (during production and post-harvest), and soil samples under conditions of wet chamber.

Chaetomium isolates were obtained by direct isolation as described by Dhingra & Sinclair (1995). The isolates were obtained from ascospores in perithecia. Monosporic pure cultures of each isolate was obtained with the aid of a Olympus IX 70 inverted microscope. The isolates grew on potato dextrose agar (PDA) at 25 ° C for two weeks.

2.2. Identification of Chaetomium spp.

The isolation was carried out on potato dextrose agar (PDA) and corn meal agar (CMA) at 25 ºC, with a 12/12 h incident light cycle in B.O.D.

Cultures of Chaetomium spp. were photographed and examined under an Olympus SZ 40 stereomicroscope. Structures of the fungi were removed from the culture medium and were mounted in lactophenol. Observations and measurements were made with a Carl Zeiss Standard W light microscope (Göttingen, Germany) and photographs were taken on an Olympus BX 51 microscope equipped with a digital camera E-volt 330. Biometric data were based on 30 measurements of structures: ascomata, ostiole, lateral hairs, conidia and ascospores.

Table 1. List of *Chaetomium* spp. Isolates characterized in this work.

ISOLATES	NUMBER GENBANK ITS	SUBSTRATE
CH001	KF601373	Forest soil
CH002	KF601377	Sunn seed
CH003	KF601378	Castor seed
CH004	KF601392	Chestnut
CH005	KF601393	Chestnut
CH007	KF601394	Chestnut
CH008	KF601395	Chestnut
CH009	KF601396	Chestnut
CH010	KF601407	Chestnut
CH011	KF601379	Chestnut
CH012	KF601397	Chestnut
CH013	KF601398	Chestnut
CH014	KF601390	Bean seed
CH015	KF601382	Rice seed
CH016	KF601383	Rice seed
CH017	KF601399	Rice seed
CH018	KF601400	Rice seed
CH019	KF601401	Rice seed
CH020	KF601384	Rice seed
CH021	KF601402	Cotton seed
CH022	KF601371	Peanut seed
CH023	KF601403	Oat seed
CH024	KF601404	Oat seed
CH025	KF601385	Soil - coffee
CH026	KF601386	Soil - coffee
CH027	KF601380	Soybean root
CH028	KF601387	Corn seed
CH029	KF601391	Soil - Pineapple
CH030	KF601388	Soil - Pineapple
CH031	KF601372	Brachiaria seed
CH032	KF601370	Brachiaria seed
CH033	KF601405	Brachiaria seed
CH034	KF601381	Soybean seed
CH035	KF601389	Corn seed
CH037	JQ995532	Branch of eucalyptus
CH038	KF601374	Oat seed
CH039	KF601375	Wheat seed
CH040	KF601406	Wheat seed
CH041	KF601376	Wheat seed

2.3. DNA extraction, PCR amplification, DNA sequencing

Approximately 40 mg of fungi tissue were scraped from the surface of the PDA and placed in a sterile 1,5 mL microcentrifuge tube. The fungal material was frozen in liquid nitrogen and ground into a fine powder using a microcentrifuge tube pestle. One hundred µL of Nuclei Lysis Solution of the Wizard® Genomic DNA Purification Kit (Promega corporation, WI, U.S.A.) were added. Thereafter, additional 500 µL of the previous solution was added. The extraction continued as described by Pinho et al. (2012). PCR reactions were mounted using the following components for each 25 µL reaction: 12,5 µL of DreamTaqTM PCR Master Mix 2 X (MBI Fermentas, Vilnius, Lithuania), 1 µL of 10 µM of each forward and reverse primer synthesised by Invitrogen (Carlsbad, EUA), 1 µL of dimethyl sulfoxide (DMSO, Sigma–Aldrich, St. Louis, MO, U.S.A.), 5 µL of 100 x (10 mg/mL) Bovine Serum Albumin (BSA, Sigma–Aldrich, St. Louis, MO, U.S.A.), 2 µL of genomic DNA (25 ng/µl), and nuclease-free water to complete the total volume.

Target regions of the Internal Transcribed Spacer regions 1 and 2 including the 5.8S rRNA gene (ITS) were amplified using fungal specific primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) for ITS with fragments about of 535bp (White et al. 1990). The amplifications were carried out starting with an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 45s, extension at 72 °C for 2 min and a final extension of 7 min at 72 °C. Amplified products were visualized on 1 % agarose gel stained GelRed™ (Biotium Inc., Hayward, CA, U.S.A.) in 1× TAE buffer and visualized under UV light to check for product size and purity. PCR products were purified and sequenced by Macrogen Inc. (Seoul, South Korea). The nucleotide sequences were read with Chromas lite 2.01 (Technelysium 2007) and edited with the

DNA Dragon software (Hepperle 2011). All sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences. New sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov) (Table 2).

Table 2. Sequences used in phylogenetic analyzes

SPECIES	STRAIN*	NUMBER GENBANK ITS
Achaetomium strumarium	CBS 333.67	AY681204
Chaetomium ancistrocladium	C 84	HM365242
Chaetomium aureum	ATT 218	HQ607894
Chaetomium cupreum	CC 8	AB509371
Chaetomium dolichotrichum	CBS 162.48	HM449049
Chaetomium funicola	CBS 158.52	GU563369
Chaetomium globosum	CBS 148.51	GU563374
Chaetomium indicum	CBS 860.68	GU563365
Chaetomium longicolleum	CBS 119.57	GQ922525
Chaetomium madrasense	C 36	HM365252
Chaetomium murorum	C 66	HM365256
Chaetomium piluliferum	NRRL 38180	GU183112
Chaetomium reflexum	CBS 157.49	HM449051
Chaetomium udagawae	NRRL 6547	GU183108

***CBS**, deposited at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **C**, deposited at the Plant Pest and Diseases Research Institute, Botany Departament, Tehran, Iran; **ATT**, deposited at the Department of Biological Sciences, UESC – Universidade Estadual de Santa Cruz, Rodovia Ilheus-Itabuna, km 16, Salobrinho, Ilheus, Bahia 45662-900, Brazil; **CC**, Chaetomium cupreum, deposited at the Institute of Technology Ladkrabang, Plant Pest Management, Chahlongkrung, Bangkok 10520, Thailand; NRRL, deposited at the Bacterial Foodborne Pathogens & Mycology Research Unit, USDA-ARS-NCAUR, 1815 N. University St., Peoria, IL, 61604, USA.

2.4. Phylogenetic analysis

Consensus regions were compared against GenBank's database using their Mega BLAST program. In addition, sequences were selected from Untereiner et al. (2001) to reveal the phylogenetic position of the our isolate within known Chaetomium species. The closest hit sequences (Table 2) were then downloaded in FASTA format and aligned using the multiple sequence alignment program MUSCLE (Edgar 2004). This program was implemented using the MEGA v. 5 software (Tamura et al. 2011). Alignments were checked and, when necessary, manual adjustments were made. Gaps (insertions/deletions) were treated as missing data. The resulting alignment was deposited into TreeBASE (http://www.treebase.org/) as accession number S12694.

The tree was constructed by Bayesian inference (BI) analyses employing a Markov Chain Monte Carlo simulations implemented in MrBayes v.3.1.1 (Ronquist & Huelsenbeck 2003). MrMODELTEST 2.3 (Posada & Buckley 2004) was used to select the models of nucleotide substitution. Once the likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC). The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used. Four MCMC chains were run simultaneously, starting from random trees for 10 000 000 generations. Trees were sampled every 1 000 th generation for a total of 10 000 trees. The first 2 500 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala & Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 7 500 trees. Convergence of the log likelihoods was analyzed with TRACER v. 1.4.1 (Rambaut & Drummond 2003) and no indication of lack of convergence was detected. Trees were visualized in FigTree (Rambaut 2009) and exported to graphics programs. The species Achaetomium strumarium was used as outgroup in these analyses.

3. Results and discussion

During this work 39 isolates (Table 1) of Chaetomium spp. were obtained by direct isolation of seeds, stubble of crop plants and soil samples. These isolates were identified based on morphological and molecular analyzes.

In the phylogenetic tree (Fig. 1), the clades separating different species can be observed. Chaetomium cupreum isolates (CH001, CH022 and CH031) indicated are in red on phylogenetic tree (Fig. 2). Those grouped together with isolate of C. cupreum CC8. Three other isolates were C. aureum (CH038, CH039 and CH041), shown in orange, which clustered with the isolate of C. aureum ATT218 (Fig. 3). Five isolates are C. funicola (CH002, CH003, CH011, CH027 and CH034) indicated in yellow, clustered with the isolate of C. funicola CBS 158.52 (Fig. 4). There is only one isolate of C. reflexum (CH032) in green, which grouped with isolate of C. reflexum CBS 157.49 (Fig. 5) is the first report in Brazil. The largest group with 16 isolates is C. globosum (Fig. 6) (CH004, CH005, CH007, CH008, CH009, CH010, CH012, CH013, CH017, CH018, CH019, CH021, CH023, CH024, CH033 and CH040) in purple, this group is clustered with the isolate of C. globosum CBS 148.51. Another group with ten isolates is C. bostrychodes (Fig. 7) (CH14, CH015, CH016, CH020, CH025, CH026, CH028, CH029, CH030 and CH035) in pink. Besides these, there is also isolated (CH037) not grouped with any Chaetomium sequence for the gene ITS available in GenBank. Thus, we propose a possible new species of Chaetomium, indicated in blue and will be referred to here as Chaetomium sp. (Fig. 8, 9, 10).

Morphological characteristcs showed that Chaetomium sp. differs from C. brevipilium (Ames 1961) and C. homopilatum (Ames 1961, Arx et al 1986), both do not show ascomata with long necks and its size is smaller than 300 µm. Chaetomium seminudum (Ames 1961) has perithecia smaller than 165 µm, which is smaller than the size of the measurements of the Chaetomium sp. However, C. longicolleum (Ames 1961, Arx et al 1986) has a long neck, the length of ascomata is less than 800 µm high. In the description of the C. malaysiense (Arx et al 1986), the length of ascomata is smaller than C. longicolleum. Chaetomium cuyabenoensis (Decock & Hennebert 1997) has ascomata with long neck of 760-2300 µm, but despite the diameter of the ascomata is lower than 55-80 µm, and the ascospores are also smaller, this species differs from Chaetomium sp. Although C. longirostre (Ames 1961) has no deposited sequence in the GenBank, analyses of morphological characteristics show difference with Chaetomium sp. mainly because C. longirostre has longer lateral hairs (200-630 µm). Chaetomium ampullare Chivers (Arx et al 1986) has also any deposited sequence, but the neck is shorter than Chaetomium sp. Thus, we affirm that Chaetomium sp. is a new species, and the presented the second report of a long neck Chaetomium species from Brazil. Nineteen species have been reported in Brazil: C. convolutum, C. ochraceum, C. trigonosporum, C. indicum, C. spinosum, C. caprinum, C. bostrychodes, C. senegalense, C. nigricolor, C. aureum, C. globosum, C. brasiliense, C. convolutum, C. fusiforme, C. trilaterale, C. cupreum, C. funicola, C. homopilatum, C. longicolleum. Furthermore, is the first report of C. reflexum in Brazil. Among the species found in Brazil, C. cupreum and C. globosum are most often used in biological control studies (Coutinho et al. 2007, Barbosa et al. 2012).

Description of species:

Chaetomium aureum

Colonies gray or pale olive, becoming yellow, at length golden yellow. Perithecia superficial, ostiolate, globose to subglobose often bluntly pointed at the base small 130-160 µm, without differentiated rhizoids, bearing long, slender arched or recurved cirrhus. Terminal hairs straight or slightly recurved, apically often circinate or coiled, at base about 3-4 µm in diameter, verrucose or wasty, at tip nearly straight or incurved, septate, minutely roughened and in color ranging from gray to golden yellow. Asci clavate, evanescent, 8-spored, 30-40 x 10-13 µm. Ascospores inaequilaterally fusiform or navicular, when young filled with refractive globules, hyaline and destrinoid when young, greyish brown when mature, in regularly ovate, apiculate at both ends, occasionally a single germ pore 9-11 x 5-6 µm.

Chaetomium bostrychodes

Colonies usually without aerial mycelium, occasionally with a pale green exudate, reversed uncoloured or green. Perithecia ostiolate, extremely variable in shape, subglobose to ovoid, with a bluntly pointed base, 170-300 x 170-270 µm, frequently producing black, straight or recurved cirrhi. Terminal hairs arising mainly from the apical disc, erect, usually spirally coiled in the upper part, brown, septate, thick-walled, verrucose or warty, 4.5-5 µm in diameter. The coiling is variable with seldom more than 5-7 convolutions which diminish almost imperceptibly in diameter toward the extremity or irregularly. Asci numerous, fasciculate, clavate, evanescent stalk, 8-spored, 47.5 x 12.5 µm. Ascospores limoniform or nearly spherical, apiculate at the ends, bilaterally flattened, when young greenish, hyaline, with granular contents, when mature very pale with olive brown, 6-7 x 8.5-10 µm with an apical germ pore.

Chaetomium cupreum

Colonies becoming red due to an exudate, with or without aerial hyphae, ascomata small, superficial, globose to ovate, 95-110 x 87.5-100 µm, with conspicuous cirrhi, a thin wall of angular cells. Lateral hairs numerous, arcuate, apically circinate or coiled, septate, verrucose, red or orange-red covered with bright copper colored granules which are insoluble in water, 4-5 µm in diameter at the base and with 1-3 convolutions at apex. Asci clavate, evanescent, 8-spored, 26-30 x 10-11 µm. Ascospores reniform or lunate in face view, hyaline and destrinoid when young, pale-brown when mature, irregular ovate, subapilate, 7.0-8,5 x 4.5-5.0 µm, with a single apical germ pore.

Chaetomium funicola

Colonies with a withe or pale aerial mycelium and yellow reverse. Ascomata superficial, spherical or ovate, ostiolate, 100-170 µm dark olivaceus or grey in reflected light. Terminal hairs numerous, long, flexuous, dichotomously or otherwise branched, mainly in the upper part, dark brown, indistinctly septate, verrucose or warty, 4-5 µm broad near the base. Asci evanescent, fasciculate, clavate or fusiform, 8-spored, 22.5-32.5 x 7.5-12.5 µm. Ascospores limoniform, biapiculate, bilaterally flattened, often rather irregular, brown when mature, 6-7.5 x 4-5 µm with an apical germ pore. Phialoconidia obovate or ellipsoidal, aseptate, hyaline.

Chaetomium globosum

Colonies with a pale or olivaceous aerial mycelium, often with yellow, grey green, green or red exudates. Ascomata superficial, ostiolate, variable in shape, subglobose, spherical, ovate or obovate somewhat elongated with a bluntly pointed base, when young yellow, translucent, allowing the cellular structure of the wall to be seen. When mature opaque, black 190-290 x 180-280 µm. often producing short, black cirrhi, attached to the substrate with a thick mass of dark olive to black rhizoids, color ranging from gray, green, light brown to olive-brown. Terminal hairs numerous, usually unbranched, flexuous, undulate or coiled, often tapering, septate, brownish, 3-3.5 µm in diameter. Asci clavate, 8-spored, 32.5-45 x 11-15 µm. Ascospores limoniform, usually biapiculate, bilaterally flattened, brownish when mature, rather thick-walled, varying in shape and size, 10-11 x 7-8 µm, with an apical germ pore.

Chaetomium reflexum

Perithecia black, ovoid to subglobose, ostiolate, 100-130 µm, densely massed forming an almost continuos layer, obscured by the dense mass of dark, rhizoids and terminal hairs. Terminal hairs stout, 5 µm, wide, arcuate from the base, strongly recurved, dichotomously branched, tips blunt, dark brown, only slightly, roughened above. Lateral hairs few, unbranched, narrow, 3 µm in diameter at base, uneven, only slightly colored, with rounded tip, appearing as modified, short rhizoids. Ascospores light olive-brown, oval, slightly flattened on one side, rounded, to slightly acute on the ends 6.0-6.5 x 4.0-4.5 µm.

Chaetomium sp. to be proposed as new

Ascomata 920-1100 x 90-115 µm developing in 2 weeks, superficial, sparse singly or in groups of 2-4, dark brown, erect, obpyriform, hairy, supported by basal hyphae rhizoid-like. A rostrate ostiole (long neck) 800-950 x 27.5-40,0 µm. A long the neck there is short hairs 110-180 x 4-5 µm, scattered, arising singly, smooth, unbranched, septate 7-14, with dark base and whitish tips. Perithecial wall of brown thick-walled. Asci with 8-spored, soon evanescent. Ascospores 7.5-8.0 x 6.5-7.5 µm strongly limoniform in face view, elliptical in side view, discoid in pole view, biapiculate, with one apical germ-pore, dark brown, thick-walled when mature, ejected in a black cirrhus. Conidia 8.75-10.5 x 8-12.5 µm, singly, yellowish, spherical, subspherical, pyriform or clavate.

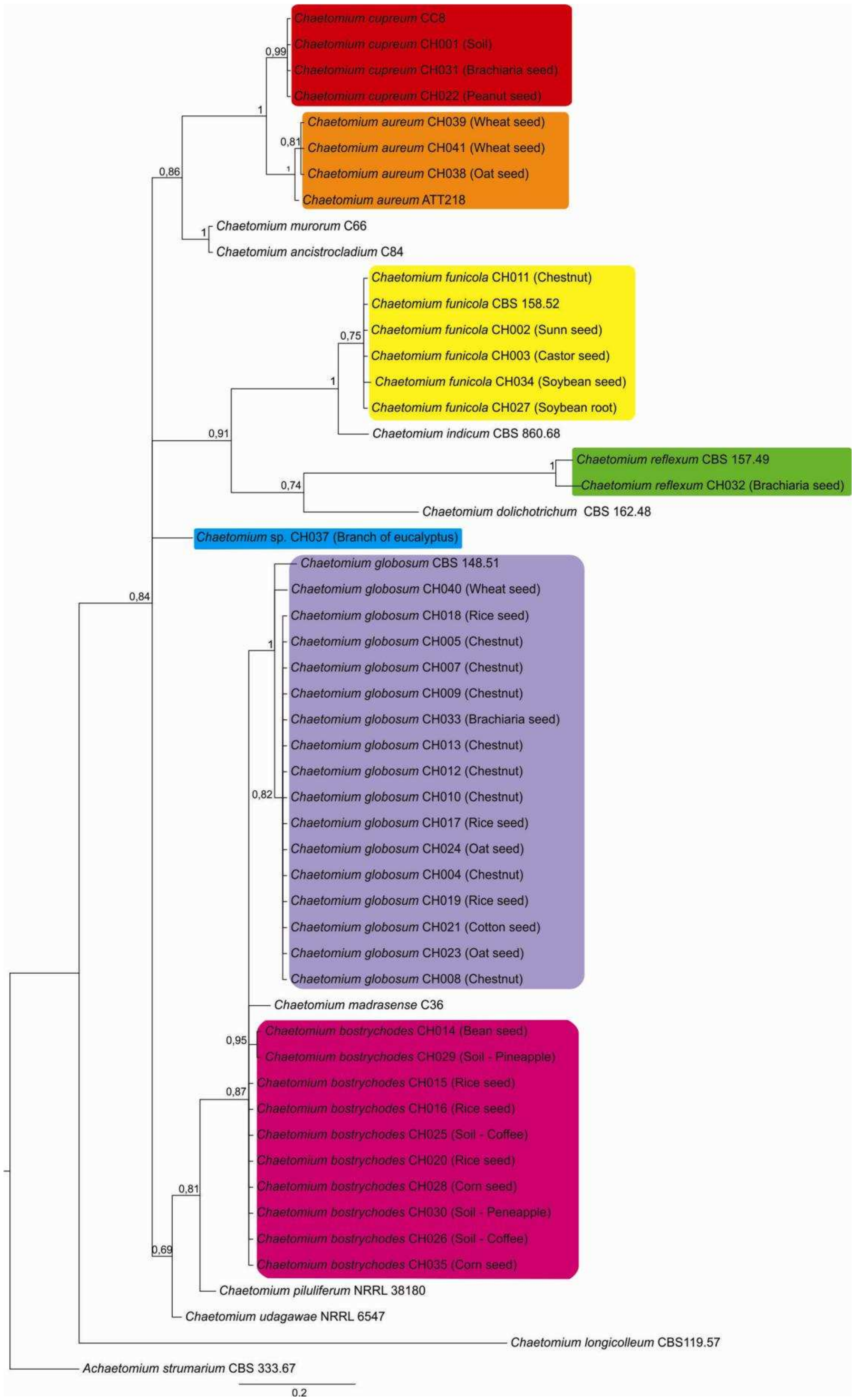

Fig. 4. Phylogenetic relationships of Chaetomium species inferred from ITS region.

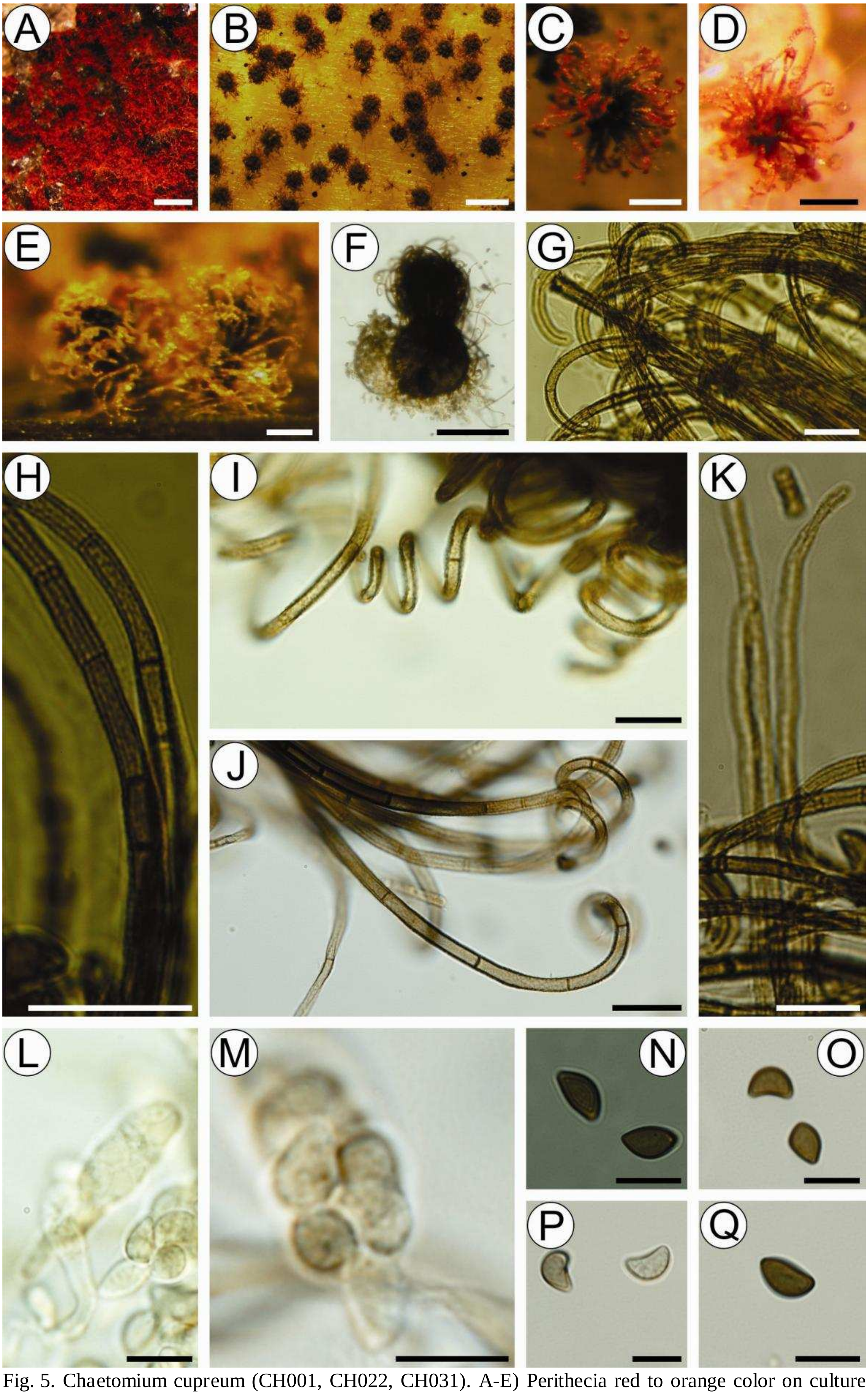

Fig. 5. Chaetomium cupreum (CH001, CH022, CH031). A-E) Perithecia red to orange color on culture medium; F) Spherical perithecia; G-J) Hair with the ends curled, septate and warty; K) Tip hair; L,M) Asci clavate; N-Q) Ascospores reniform or lunate dark at maturity. Bars: A,B=500µm; C-F=100µm; G-K=20µm; L-Q=10µm.

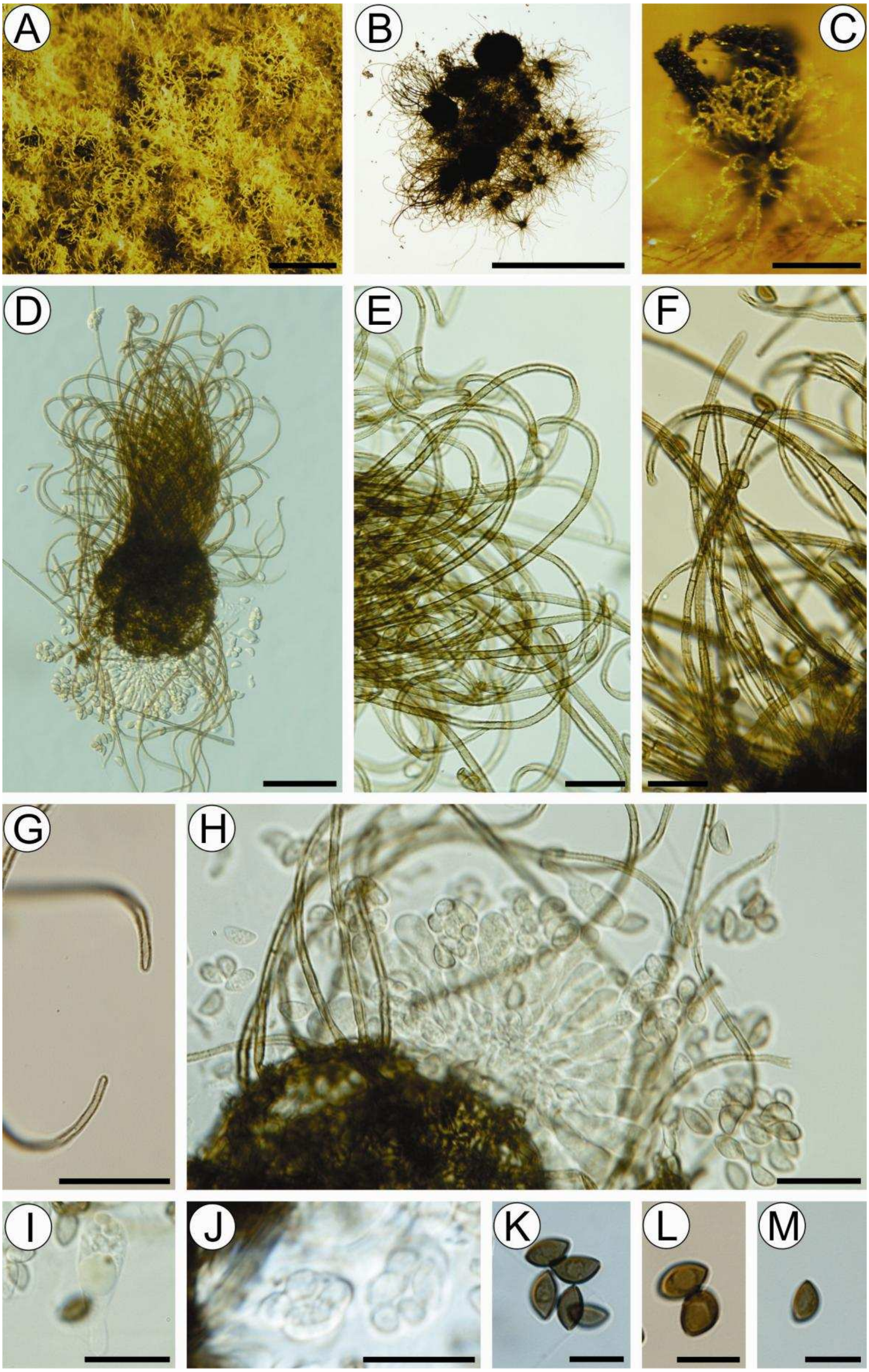

Fig. 6. Chaetomium aureum (CH038, CH039, CH041). A,C) Perithecia olive color on culture medium; B) Spherical perithecia; D) Perithecia with asci; E,F) Cabelos arqueados nas pontas, verrugosos, septados de cor marrom; E, F) Hair brown, curved, verrucose, septate; G) Tip hair; H-J) Asci clavate; K-M) Ascospores dark at maturity. Bars: A,B=500µm; C=200µm; D=50µm; E,J=20µm; K-M=10µm.

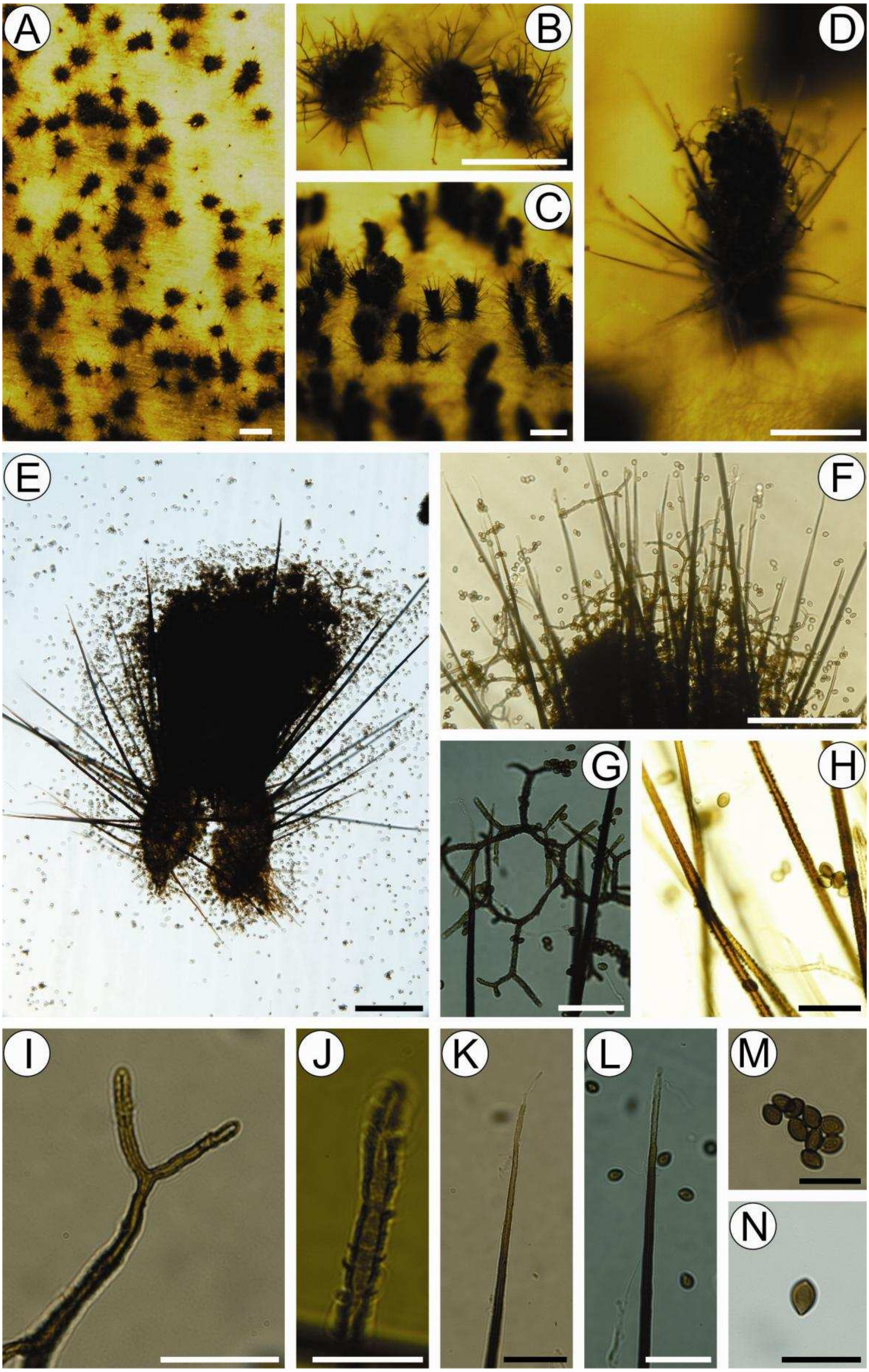

Fig. 7. Chaetomium funicola (CH002, CH003, CH011, CH027, CH034). A-D) Perithecia on filter paper; E) Spherical perithecia; F-H) Hair: some long, honed the tips, others dichotomously branched, verrucose, septate; I-L) Tip Hair; M,N) Ascospores oval slightly apiculate. Bars: A=1000µm; B, C=500µm; D=200µm; E,F=50µm; G-H=20µm; I-N=10µm.

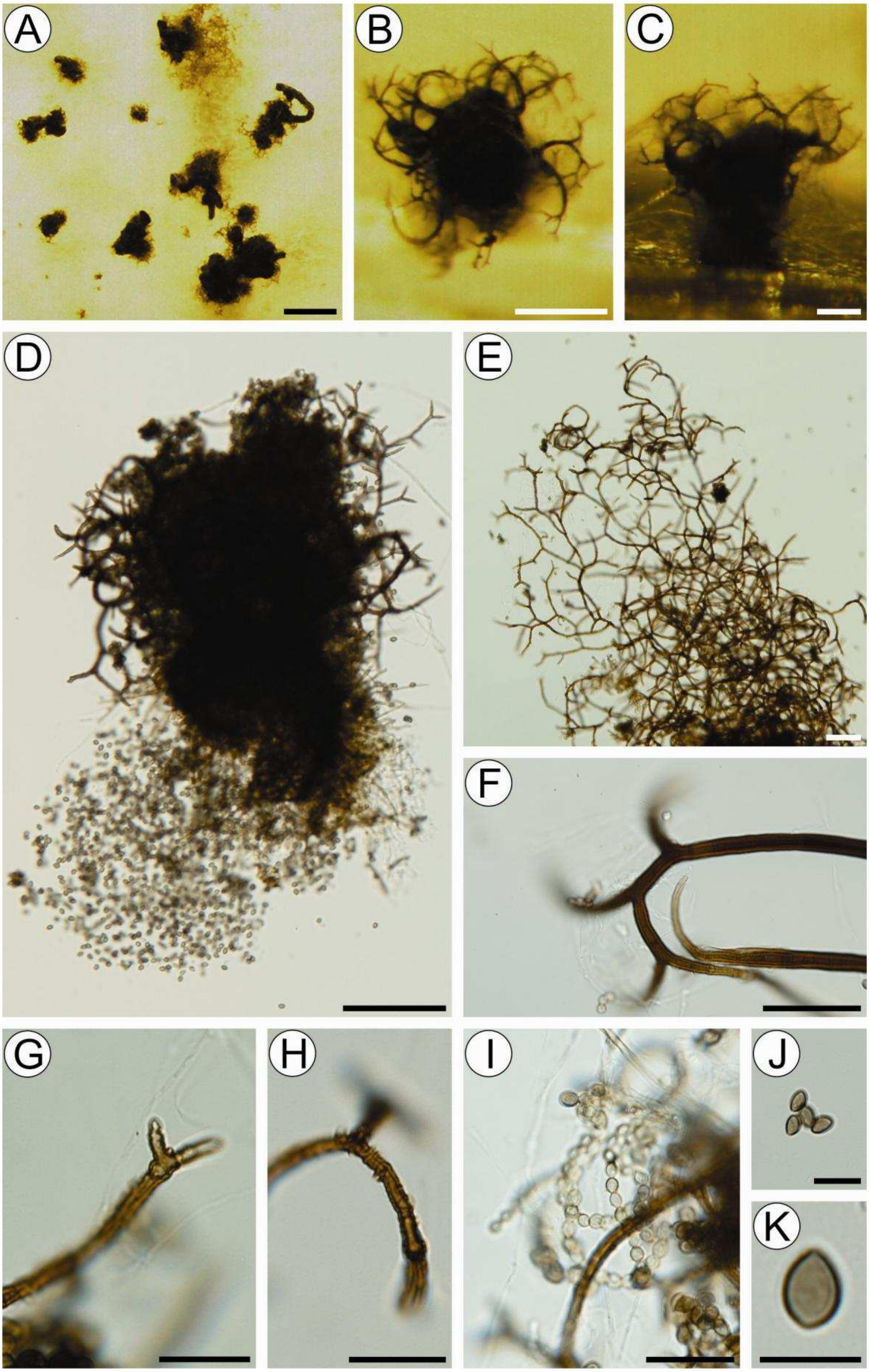

Fig. 8. Chaetomium reflexum (CH032). A-C) Perithecia superficial on filter paper; D) Perithecia ovate, E,F) Abundant hairs, dichotomously branched, septate, verrucose, brown, G,H) Tip Hair, I) Conidia yellowish, J,K) Ascospore ovoid, apiculate, dark. Bars: A=500µm; B,C=200µm; D=100µm; E=50µm; F-I=20µm; J=10µm; K=5µm.

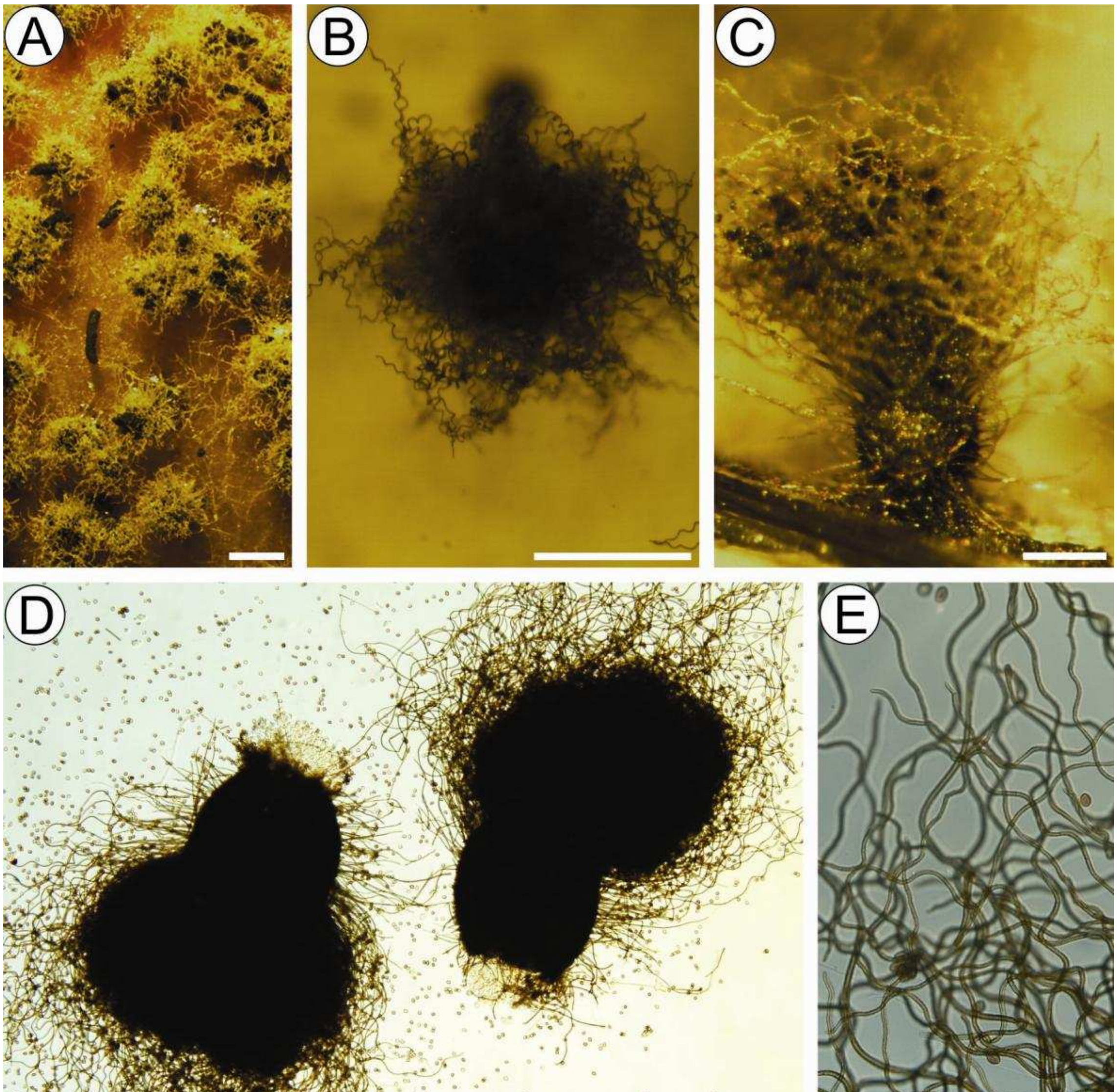

Fig. 9. Chaetomium globosum (CH004, CH005, CH007, CH008, CH009, CH010, CH012, CH013, CH017, CH018, CH019, CH021, CH023, CH024, CH033, CH040). A-C) Perithecia on filter paper; D) Perithecia ; E) Hair unbranched, flexuosos, septate, brown; F) Tip hair; G) Asci clavate; H, I) Ascospores limoniforms, brown when ripe. Bars: A=1000µm, B=500µm; C,D=200µm; E,G=50µm; F,H,I=10µm.

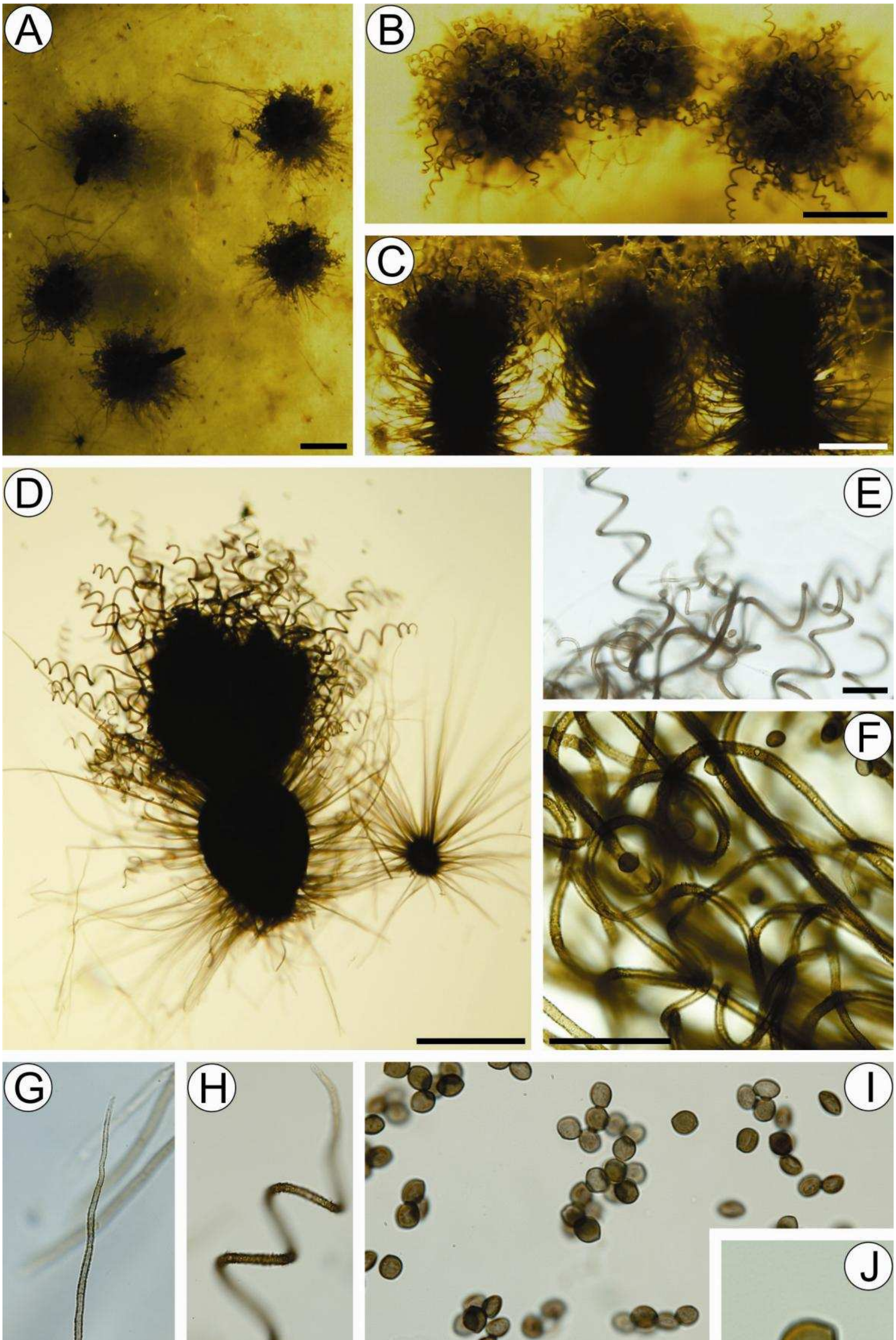

Fig. 10. Chaetomium bostrychodes (CH014, CH015, CH016, CH020, CH025, CH026, CH028, CH029, CH030, CH035). A-C) Perithecia on filter paper; D) Perithecia oval when mature and another imature; E,F) Hair straight, spiral, brown, septate, verrucose, G, H) Tip hair, I,J) Ascospores limoniform, dark when ripe. Bars: A,B=500µm C,D=200µm; E,F=50µm; G-I=20µm; J=10µm.

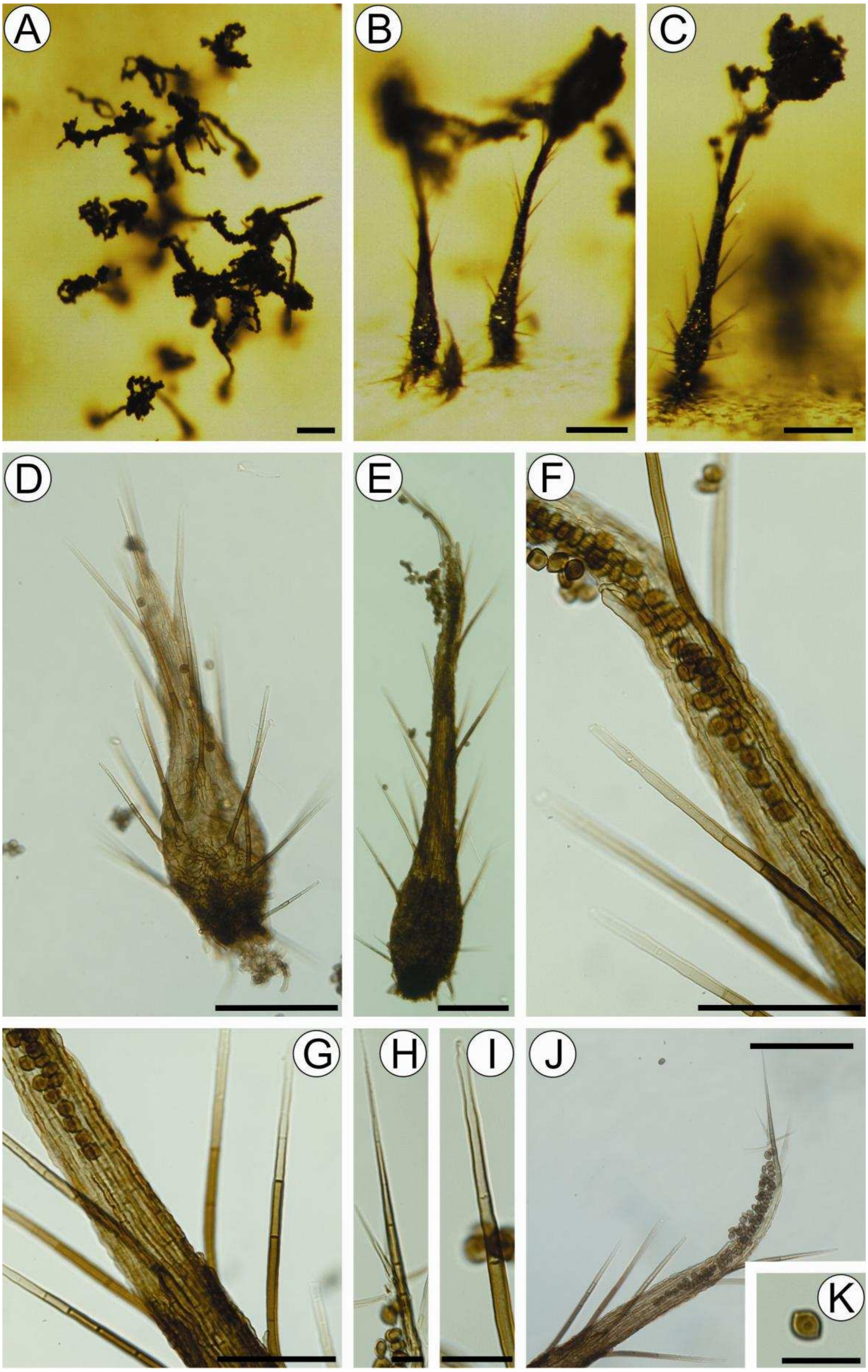

Fig. 11. Chaetomium sp (CH037). A-C) Perithecia on filter paper; D) Immature perithecia, E) Mature perithecia, F) Ascospores within the rostrate ostiole; G) Insertion of hairs on ostiole, H,I) Tip Hair, J) Ascospore limoniform, biapiculade, dark when mature; K) Ostiole with formation of cirrus. Bars: A-C=200µm; D,E,J=100µm; F,G=50µm; H,I,K=20µm.

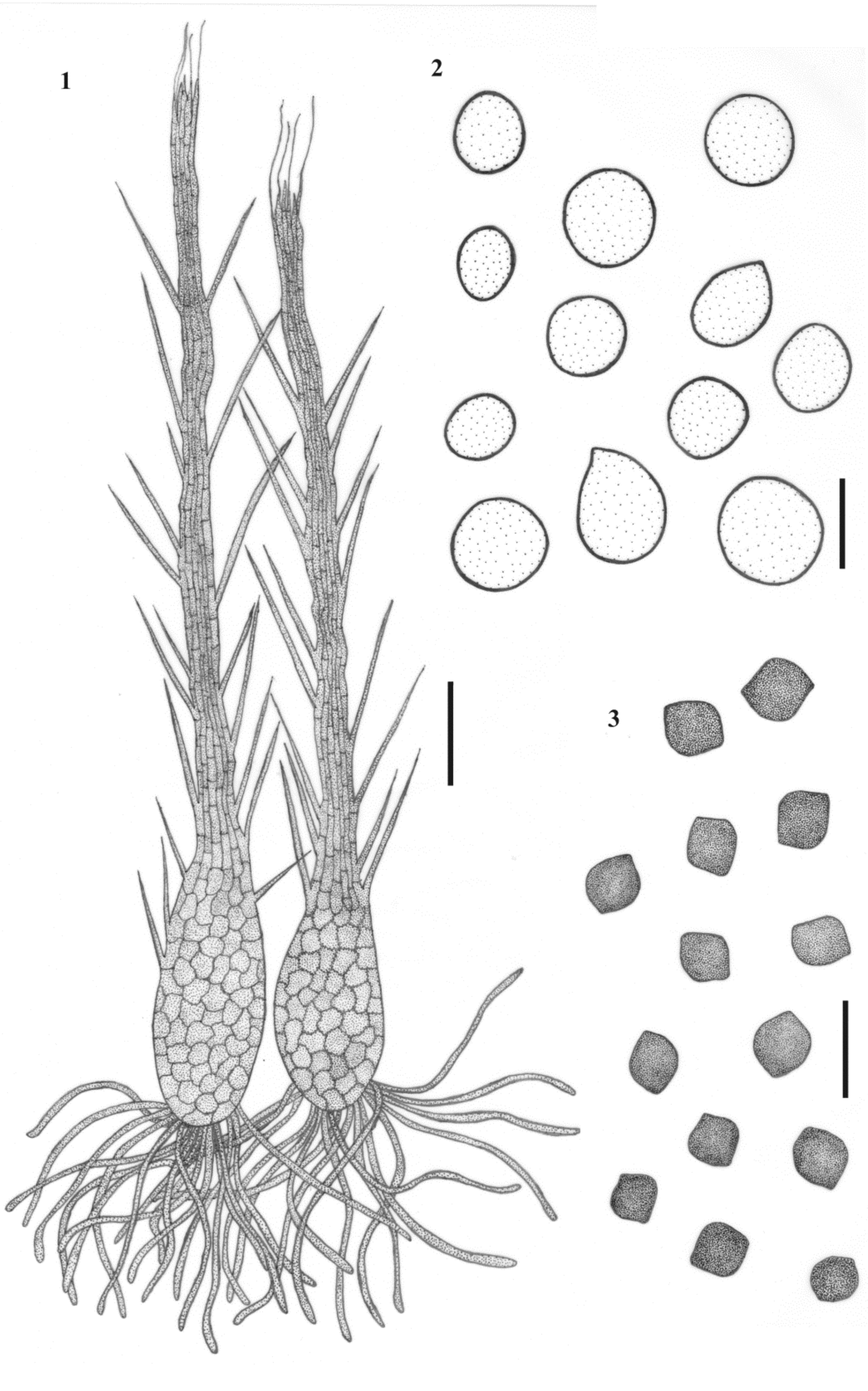

Fig. 12. Chaetomium sp. 1) Perithecia; 2) Ascospores immature; 3) Ascospore limoniform, biapiculade, dark when mature. Bars: 1=100µm; 2=8µm; 3=10µm.

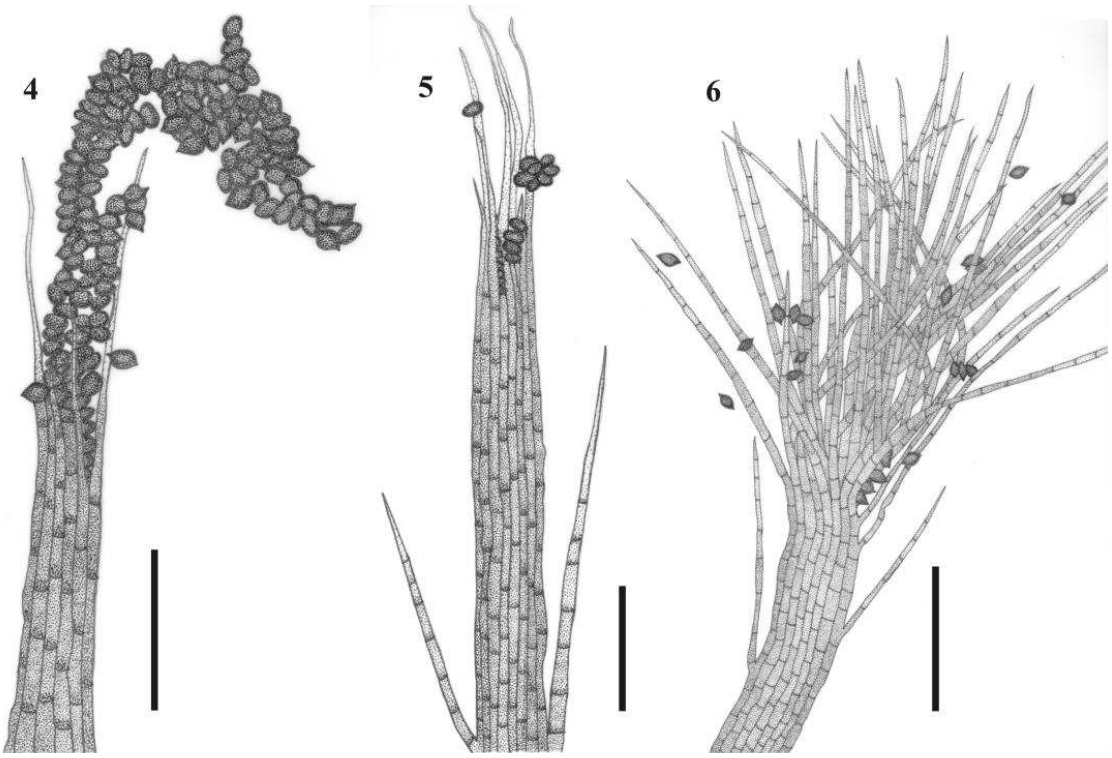

Fig. 13. Chaetomium sp. 4-6) Different perithecia tips. Bars: 4,6=50µm; 5=40µm.

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ARTIGO 2

Chaetomium spp. as biocontrol agents of phytopathogenic fungi transmitted by seeds

Stefânia Caixeta Magalhães[a], Ueder Pedro Lopes[a], Onkar Dev Dhingra[a], Olinto Liparini Pereira[a]*

[a]*Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-000, Brazil.*

*Corresponding author at: Phone: +55 31 3899 1105; *E-mail address*: oliparini@ufv.br (O.L. Pereira).

Abstract

The *Chaetomium* genus is commonly found in soils, organics materials, seeds and others. This genus is known as a biological control agent of some plant diseases. The assays carried out *in vitro,* tested pairing cultures among 37 isolates of *Chaetomium* spp. and seven important pathogens of wheat and soybeans transmitted by seeds: *Bipolaris sorokiniana*, *Colletotrichum truncatum*, *Diaporthe phaseolorum* var. *meridionalis*, *Drechslera tritici-repentis*, *Fusarium solani*, *Magnaporthe grisea* and *Sclerotinia sclerotiorum*. The isolates of *Chaetomium cupreum* were more efficient *in vitro* tests, which were then submitted to six different culture media to test the most suitable culture medium to produce ascospores. The oat culture medium demonstrated the best result for ascospores production. The other assays were conducted in a greenhouse to test isolates of *C. cupreum* inoculated in soybeans and wheat seeds. Soybean seeds that were inoculated with isolates of *C. cupreum* showed higher germination of normal seedlings compared with no inoculated soybean seeds. It was proven that the *C. cupreum* is endophyte on wheat roots and inhibit the growth of pathogens.

Keywords: antibiosis; endophytic; mycofungicide; seed pathology; soybean; wheat.

1. Introduction

Most agricultural products consumed as food is propagated by seeds. Today the most important agricultural crops may be affected by pathogens that are transmitted by seeds, and a form of prevention is to reduce the initial inoculum. Infected or infested seeds may introduce important pathogens in areas previously free of them (Henning, 2005), it may result in a progressive increase of the disease and the crop losses plant in the field.

Today, many pathogens widely distributed throughout Brazil, were introduced by seeds. It is clear that seeds infested/infected are efficient dispersal mechanism of pathogens, resulting in increased production costs and environmental problems generated by the abusive use of pesticides. Many of these pathogens are fungi, which are the main responsible for plant diseases and can cause reduction in seeds germination, decreasing its quality and commercial value.

The control of fungi transmitted by seeds is important primarily to avoid pathogen dispersal over long distances and improve the quality of the material used for propagation. The use of pesticides has been the most common method used in the diseases control, but there are some drawbacks, mainly polluting the environment with non-biodegradable toxic residues and the selection of individuals resistant to these compounds.

For many seed-borne diseases, physical methods for control are inefficient, mainly by the fact, that most physical treatments affect seed germination and vigor. In work carried out by Durigon et al. (2009), wheat seed germination was not affected by the dry heat treatment and the fungi incidence varied during the observation period, being effective against some pathogens. However, in the same work, seed germination was significantly compromised when using wet heat treatment.

Special attention has been given to the use of microorganisms as biological control agents of plant pathology, mainly because of the considerable environmental impact that pesticides have caused and risks offered in handling. The bioprotectors are advantageous because they grow, proliferate, can colonize and protect the plant formed after application (Harman, 1991). For example, if a bioprotector is used to seed treatment, it can grow and colonize underground parts of the plant after seed germination (Boland, 1990; Harman 1991). On the other hand, one of the disadvantages of biological control is the imprecision of this method when compared with chemical control (Harman and Lumsdem, 1990), due to the fact that biological agents are influenced by soil, climate and/or microbial action.

The biological control of plant diseases transmitted by seeds, using antagonistic microorganisms is practiced in incipient form with *Chaetomium* sp., and it is restricted to the works with *Trichoderma* spp. and bacteria. Species of *Chaetomium* are antagonists of many soilborne and seedborne plant pathogens (DiPietro et al., 1992). This antagonism can occur by antibiosis, competition, mycoparasitism or interaction of two or more of these (Marwah et al., 2007; Zhang and Yang, 2007), especially against pathogens of seedling and soil (Kaewchai et al., 2009).

The *Chaetomium* genus belongs to the Phylum Ascomycota, has ostiolate perithecia, surrounded by appendages of various shapes. This fungus is fixed to the substrate by rhizoids (Domsch et al., 2007), has evanescent asci and brown ascospores. These fungi degrade cellulose and lignin; survive on diverse substrates like dung, seeds, tissues and tillage stubble; under dry conditions remains viable for 25 years or more (Guarro et al., 2012).

This species is known to secrete antifungal metabolites, competes for nutrients with pathogenic fungi and has the ability to degrade the cell wall of pathogenic fungi (Kanokmedhakul, 2006). To elucidate the molecular mechanisms associated with mycoparasitism of *C. cupreum*, Zhang and Yang (2007) presented the results of applying analysis of EST's in *C. cupreum* and provided a preliminary indication of the expression of genes supposedly involved in biological control. In another study (Zhang et al., 2009), the results revealed that the cell walls of the pathogenic fungus can imitate some aspects of the interaction between *C. cupreum* and fungi parasites. Recently they have construct two independent cDNA libraries, providing the first comparative analysis of the transcriptome of *C. cupreum* under different conditions. The results allowed to understand the molecular mechanisms of this fungus (Zhang and Li, 2012).

Chaetomium globosum is known to produce over than 19 antibiotic substances, of which chetomin, is possibly the most important in biological control (DiPietro et al., 1992; Marwah et al., 2007; Qin et al., 2009; Zhang et al., 2013). Besides of chetomin, another method of plant diseases control, involving specific strains of *C. globosum* which produces chaetoblobosin C, is suppressing the growth of plant pathogens such as *Colletotrichum gloeosporioides*, *Colletotrichum dematium*, *Fusarium oxysporum*, *Phytophthora parasitica*, *Phytophthora palmivora*, *Phytophthora cactorum*, *Pyricularia oryzae*, *Rhizoctonia solani* and *Sclerotium rolfsii* (Soytong and Quimio, 1989; Soytong, 1992a; Soytong and Soytong, 1997; Pechprome and Soytong, 1997). Chaetoglobosin A and chaetoglobosin C, antifungal metabolites of *C. globosum*, were effective in the biocontrol of *Setosphaeria turca in vitro* and bioassays using corn seedlings (Zhang et al., 2013). The species *Chaetomium cupreum* has the ability to degrade the cell wall of fungal pathogens, secrete antifungal metabolites, competes for nutrients with pathogenic fungi (Kanokmedhakul, 2006). This species was an effective antagonist to *Pyricularia oryzae* in rice seeds, and this antagonist grew faster than the pathogen (Soytong, 1992b). For control of the stem canker of soybean (*Diaporthe phaseolorum* var. *meridionalis*), Pereira and Dhingra (1997) divided isolates of *C. globosum* into two groups: producers and non-producers of antibiotics *in vitro*. Only the seeds treated with the isolate producing antibiotic *in vitro* showed lower incidence of pathogen. The incorporation of inoculum of both isolates in the topsoil reduced dramatically the incidence of disease. In another study, the proportion of stems of soybeans colonized with *D. phaseolorum*, and the number of perithecia formed on the straw, decreases linearly with time and show strong negative correlation with increasing colonization of *C. globosum* (Dhingra et al., 2003).

Some studies have shown how the *Chaetomium* genus has been effective in the control of plant pathogens (Tveit and Moore, 1954; Chang and Kommendahl, 1968; Mew and Kommedahl, 1972; Kommedahl and Mew, 1975; Hubbard et al., 1982; Heye and Andrews, 1983; Walther and Gindrat, 1988; Di Pietro et al., 1992; Soytong, 1992 a,b; Pereira and Dhingra, 1997; Soytong et al., 2001; Dhingra et al., 2003; Reissinger et al., 2003; Kaewchai et al., 2009). However, there are not many studies using *Chaetomium* spp. as an agent for the biological control of seed pathogens, thus, opens up the possibility to obtaining isolates and application methods which are really effective.

Many isolates of this genus are effective to reduce the inoculum on seeds and seedling; and diseases control in several cultures. Based on these aspects that guide biological control and the potential use of this method, the objectives of this study were to: check the efficiency of the isolates of *Chaetomium in vitro* antibiosis assay; test different culture medium for sporulation of *Chaetomium cupreum*; evaluate *C. cupreum* as endophytic; appraisal the germination and vigor of seeds inoculated with *C. cupreum* for future breeding program of biological control of seed.

2. Material and methods

2.1. Pathogenic isolates

Seven different pathogens were used; three of these are pathogens of soybean seeds, three of wheat seeds and one broad-spectrum pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary. Wheat and soybean were chosen as model plants for to be a Poaceae and Fabaceae. The isolates of *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore, *Fusarium solani* (Mart.) Sacc. and *Diaporthe phaseolorum* var. *meridionalis* F.A. Fern were obtained by direct isolation method (Dhingra and Sinclair, 1995), from infected/infested soybean seeds provided by the Departamento de Fitotecnia, Universidade Federal de Viçosa (UFV). The isolate of *Magnaporthe grisea* (Sacc.) was provided by the Laboratório da Interação Planta-patógeno from Departamento de Fitopatologia (UFV). The isolate of *Drechslera tritici-repentis* (Died.) Shoemaker was provided by Embrapa Trigo in Passo Fundo-RS. The isolate of *Bipolaris sorokiniana* (Sacc.) Shoemaker was obtained by direct isolation method (Dhingra and Sinclair, 1995), from wheat seeds provided by the Departamento de Fitotecnia (UFV).

2.2. Pairing of *Chaetomium* spp. with pathogenic fungi

To evaluate the antagonism of the isolates of *Chaetomium* we used pairing cultures on Petri dishes 90 x 15 mm with 10 mL of PDA culture medium. The culture medium was distributed with a pipette to ensure all the Petri dishes contained the same volume. Paired cultures method (Dhingra and Sinclair, 1995) was used with 37 isolates of *Chaetomium* (identified by the code CH) with five replicates tested for each pathogen *in vitro*. Thus, on each plate was placed a 0.8 cm mycelium disk of *Chaetomium* and another disc with the same measure of one of the pathogens tested. The discs were placed on the Petri dishes with a distance of 1.0 cm from the edge of the dishes. The measurements of radial growth colony of the pathogen were performed daily, from the second day after the start of the assay, and on the last day of the evaluation, were measured the inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi and mycelial growth of pathogenic fungi end. The last evaluation day of the experiment varied with the speed growth of each pathogen. In this experiment we used a total of 195 Petri dishes for each pathogen, including the control dishes.

2.3. Experimental design

Seven experiments were conducted, one for each pathogen, to verify the effectiveness of isolates of *Chaetomium* spp. on reduce mycelial growth of seven seed phytopathogenic fungi: *F. solani*, *C. trucatum*, *D. phaseolorum* var. *meridionalis*, *B. sorokiniana*, *D. tritici-repentis*, *M. grisea* and *S. sclerotiorum*. The assays were installed using a completely randomized design with 37 treatments, isolates of *Chaetomium* spp. (Table 1), with five replications. Statistical analyzes were made in the program SAEG (2007) and the data were tested for comparison of means (Scott-Knott).

Table 1. List of *Chaetomium* spp. isolates used in the assay of antibiosis *in vitro*.

ISOLATED	**SPECIES**	**SUBSTRATE**
CH001	*C. cupreum*	Forest soil
CH002	*C. funicola*	Sunn seed
CH003	*C. funicola*	Castor seed
CH004	*C. globosum*	Chestnut
CH005	*C. globosum*	Chestnut
CH006	*C. globosum*	Chestnut
CH007	*C. globosum*	Chestnut
CH008	*C. globosum*	Chestnut
CH009	*C. globosum*	Chestnut
CH010	*C. globosum*	Chestnut
CH011	*C. funicola*	Chestnut
CH012	*C. globosum*	Chestnut
CH013	*C. globosum*	Chestnut
CH014	*C. bostrychodes*	Bean seed
CH015	*C. bostrychodes*	Rice seed
CH016	*C. bostrychodes*	Rice seed
CH017	*C. globosum*	Rice seed
CH018	*C. globosum*	Rice seed
CH019	*C. globosum*	Rice seed
CH020	*C. bostrychodes*	Rice seed
CH021	*C. globosum*	Cotton seed
CH022	*C. cupreum*	Peanut seed
CH023	*C. globosum*	Oat seed
CH024	*C. globosum*	Oat seed
CH025	*C. bostrychodes*	Soil - coffee
CH026	*C. bostrychodes*	Soil - coffee
CH027	*C. funicola*	Soybean root
CH028	*C. bostrychodes*	Corn seed
CH029	*C. bostrychodes*	Soil - Pineapple
CH030	*C. bostrychodes*	Soil - Pineapple
CH031	*C. cupreum*	Brachiaria seed
CH032	*C. reflexum*	Brachiaria seed
CH033	*C. globosum*	Brachiaria seed
CH034	*C. funicola*	Soybean seed
CH035	*C. bostrychodes*	Corn seed
CH036	*C. bostrychodes*	Corn seed
CH037	*Chaetomium* sp.	Branch of eucalyptus

2.4. Sporulation of *C. cupreum* (CH031 isolate) on six different culture medium

To evaluate the culture medium that provides formation of the greatest number of ascopores of *C. cupreum* (CH031) were tested six culture media: Potato Dextrose Agar (PDA), Corn-Carrot Medium (CCM), Salt Mannitol Agar (SMA), Corn Meal Agar (CMA), Water Agar Medium (WAM), Oats Medium (OAM). Mycelial discs (0.8 mm) were placed on each plate with 10 replicatons per treatment (culture medium) maintained in B.O.D. for 20 days at 25°C. After this period the dishes were washed with distilled and autoclaved water for counting the number of ascospores.ml^{-1} with Neubauer's chamber.

Among isolates of *C. cupreum*, the isolate CH031 was chosen because it showed the most consistent results in the assay of pairing cultures *in vitro*. The OAM favored the production of ascospores with the highest value of ascospores production and was used to produce ascospores for the experiment in a greenhouse.

2.5. Infested seeds

The wheat and soybean seeds, naturally infested and/or infected with pathogens, were provided by the Departamento de Fitotecnia (UFV). Then the seeds were subjected to Filter paper test (Blotter test) (Dhingra and Sinclair, 1995) with 400 seeds for each lot (soybeans and wheat), 200 desinfested and 200 no desinfested. The test was performed for confirmation of the pathogens associated with these seeds. In this assay, for wheat and soybeans seeds, were selected lots that had more than 20% of lot with seeds detected with potentially pathogenic fungi for seeds.

2.6. Greenhouse assay

The microbiolization with wheat and soybean seeds was performed with ascospores suspension of *C. cupreum* (isolated CH022 and CH031) at a concentration of 1 x 10^5 mL^{-1}. Isolates CH022 and CH031 were selected because they was more efficient on reduce mycelial radial growth of pathogenic fungi isolates. The seeds were immersed in the ascospores suspension in erlenmayer and agitated for one (1) minute. The seeds were placed in the colander to drain and after on paper dried for 24 hours.

Fine washed sand was autoclaved twice for one hour was used like substrate in plastic cups (50 ml) and was moistened daily. Were sown 200 soybean seeds without inoculation, 200 soybean seeds inoculated with CH022 and 200 soybean seeds inoculated with CH031, 200 wheat seeds without inoculation, 200 wheat seeds inoculated with CH022, and 200 wheat seeds inoculated with CH031. The plastic cups were arranged in rows with one seed per container, sown 1 cm deep in the sand.

For vigor analysis, we used the germination speed index (GSI). This index is calculated from daily count of seeds emerged with the purpose of establishing differences in the speed of germination of seed groups. The GSI is calculated with the formula GSI = (G1/N1 G2/N2 ... Gn / Nn) (Maguire, 1962), where G1, G2 and Gn - number of days from sowing on the first, second, ... until last count, and N1, N2 and Nn - number of normal seedlings computed on the first count, the second count, ... until last count. The highest values of GSI indicates the greater average germination daily, in other words, the better treatment. This assay was assessed daily for 10 days and the counting of number emerged seedlings was performed according to described by Marcos Filho et al. (1987).

The percentage of emerged plants was assessed at 10 days after sowing. The germination percentage was evaluated normal seedlings, abnormal seedlings and diseased seedlings, the results expressed in percentage according to the recommendations and requirements of Rules for Seed Testing (Brasil, 1992). This experiment was performed twice to confirm the results.

2.7. Isolation of *C. cupreum* from healthy roots

The soybean and wheat roots of seedlings emerged of were removed (10 per species), the roots were cut into pieces of approximately 2 cm and washed in distilled and autoclaved water. In the laminar flow chambers, the roots were immersed in 70 % ethanol for 1 minute, after in 2 % sodium hypochlorite for one (1) minute and then autoclaved and distilled water for 1 minute. After this process, the roots were placed on sterile paper for drying. The roots were distributed in Petri dishes containing PDA medium at 25° C for seven days under a photoperiod of 12 hours light and 12 hours of darkness in B.O.D.

2.8. Long term storage of isolates

The isolates were preserved by the Castellani method (Dhingra and Sinclair, 1995) in glass vials with physiological saline solution (0.85 % NaCl in destilled water) in a refrigerator at 7°C.

3. Results

3.1. Pairing of isolates test of *Chaetomium* with phytopathogenic fungi

3.1.1. *Bipolaris sorokiniana*

The isolates that showed the highest values of measurement of reduce radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP) were: CH018, CH019 and CH037. However, these isolates did not grow during the evaluation period and are disregarded. Efficient isolates in reduce pathogen growth are those that show the lowest values of area under the progress curve mycelial growth (AUPCMG) in the tenth day and mycelial growth of pathogenic fungi end (MGOPFE) of *B. sorokiniana* (Table 2).

Based on the statistical analysis of AUPCMG, the isolates were classified into five groups. The isolate CH010 not grew, therefore was disregarded in this analysis. The isolates that showed the lowest values of AUPCMG (Table 2) were: CH026, CH022, CH025, CH001, CH016, CH031 and CH020, in this order from lowest to highest value. The statistical analysis for MGOPFE, shows the same isolates with the lowest values of AUPCMG (Table 2). In other words, the same *Chaetomium* isolates were more efficient in inhibiting *B. sorokiniana* (Fig. 1) for AUPCMG and MGOPFE. Isolates CH001, CH022 and CH031 are *C. cupreum*, isolates CH016, CH020, CH025 and CH026 are *C. bostrychoides*.

Table 2. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Bipolaris sorokiniana in vitro* by area under the progress curve mycelial growth (AUPCMG), the mycelial growth of pathogenic fungi end (MGOPFE) and inhibition of radial growth between isolates of Chaetomium and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Bipolaris sorokiniana*		
	AUPCMG	MGOPFE	IRGBCP
	cm		
CH010	0.00 E	0.00 E	0.00 E
CH026	8.41 D	0.86 D	1.16 C
CH022	8.98 D	0.90 D	1.96 B
CH025	10.70 C	1.12 D	1.28 C
CH001	10.97 C	1.28 C	1.14 C
CH016	11.84 B	1.32 C	1.20 C
CH031	11.85 B	1.22 C	1.54 C
CH020	12.00 B	1.34 C	1.22 C
CH021	12.09 B	1.50 C	1.50 C
CH002	12.19 B	1.50 C	1.96 B
CH014	12.20 B	1.36 C	0.94 D
CH024	12.26 B	1.54 C	1.82 B
CH028	12.61 B	1.44 C	1.00 D
CH036	12.63 B	1.42 C	1.08 D
CH037	12.79 B	1.50 C	2.32 A
CH019	12.79 B	1.64 B	2.54 A
CH029	12.95 B	1.42 C	1.06 D
CH032	13.03 B	1.50 C	2.10 B
CH018	13.10 B	1.76 B	2.70 A
CH006	13.10 B	1.54 C	1.44 C
CH003	13.11 B	1.68 B	1.46 C
CH012	13.15 B	1.66 B	0.76 D
CH004	13.25 B	1.56 C	1.72 B
CH009	13.38 B	1.54 C	1.32 C
CH013	13.39 B	1.58 C	1.18 C
CH030	13.40 B	1.50 C	0.72 D
CH033	13.55 A	1.62 C	1.46 C
CH034	13.62 A	1.62 C	0.84 D
CH017	13.65 A	1.62 C	1.86 B
CH015	14.00 A	1.66 B	0.86 D
CH011	14.12 A	1.92 A	1.16 C
CH027	14.19 A	1.72 B	1.60 B
CH005	14.22 A	1.74 B	1.38 C
CH008	14.42 A	1.86 A	1.24 C
CH035	14.66 A	1.88 A	1.78 B
CH023	14.69 A	1.98 A	1.04 D
CH007	14.78 A	1.82 A	1.30 C
Control	14.95 A	2.04 A	0.00 E

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test (p≤5%).

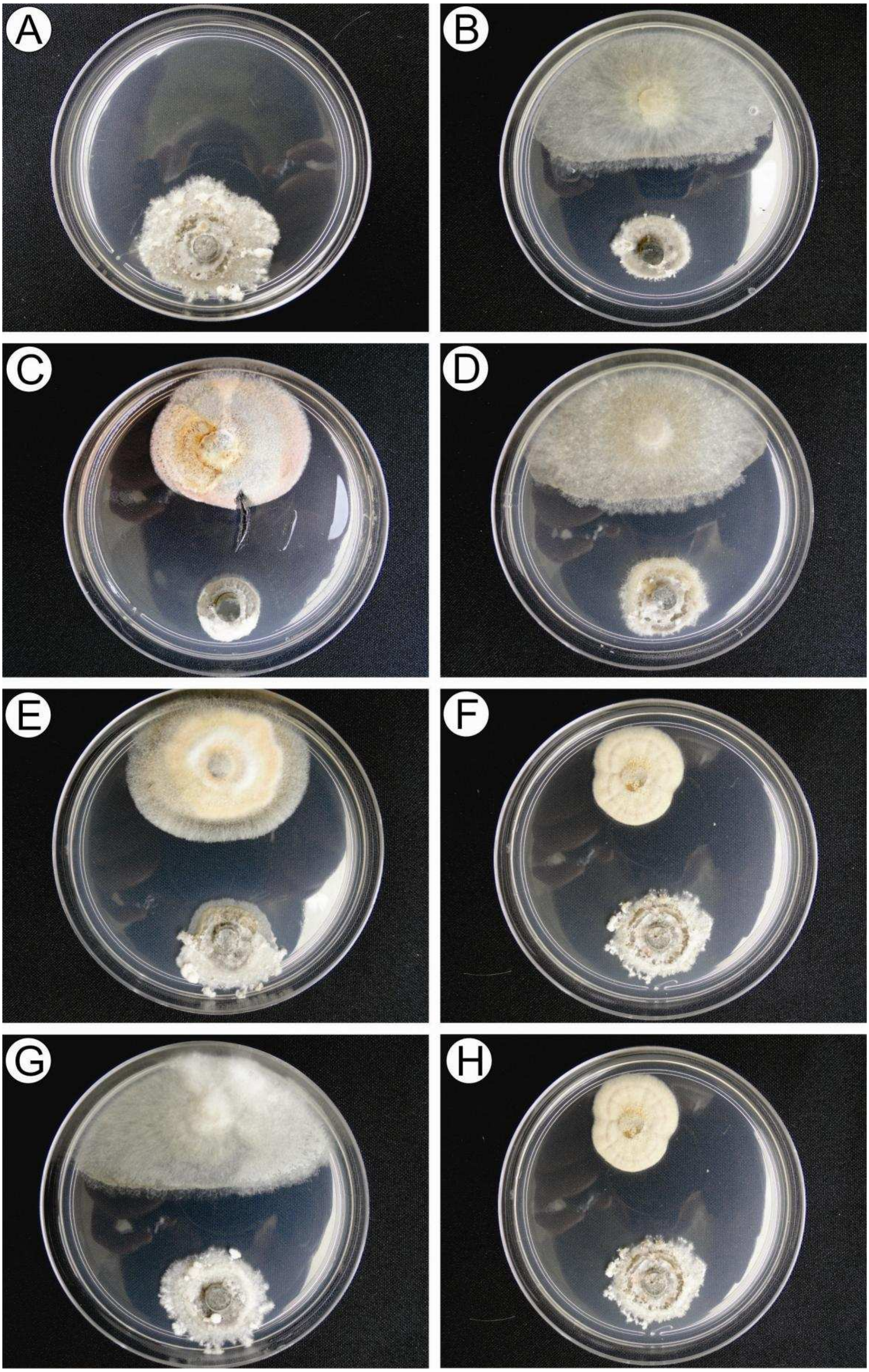

Fig. 1. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp.(up) x *Bipolaris sorokiniana* (down). A) Control; B) CH026 (*C. bostrychodes*); C) CH022 (*C. cupreum*); D) CH025 (*C. bostrychodes*); E) CH001(*C. cupreum*); F) CH016 (*C. bostrychodes*); G) CH020 (*C. bostrychodes*); H) CH031 (*C. cupreum*).

3.1.2. *Colletotrichum truncatum*

This assay was evaluated for eight days from the fifth day after installation. The isolates that showed the highest values of IRGBCP are: CH010, CH019, CH018, CH002 and CH003 (Table 3) from highest to lowest value respectively. However, they did not represent the most efficient isolates for the *in vitro* antibiosis between *Chatomium* spp. x *C. truncatum*. The isolates CH018 and CH019 did not grow in the presence of the pathogen during the evaluation period. Isolates CH002, CH003 and CH010 grew less than the isolate of *C. truncatum*, thus, were inefficient in antibiosis of this pathogen.

Based on the values of AUPCMG and MGOPFE, the isolates were separeted into two groups statistically. The isolates that showed the lowest values of AUPCMG were: CH022, CH031, CH001, CH015, CH013, in this order from lowest to highest value. The same isolates showed the lowest values for MGOPFE, though the sequence was: CH015, CH022, CH031, CH001 and CH013 (Table 3). Isolates CH001, CH022 and CH031 are *Chaetomium cupreum*, isolate CH015 is *C. bostrychoides* and isolate CH013 *C. globosum* (Fig. 2).

Table 3. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Colletotrichum truncatum in vitro* by area under the progress curve mycelial growth (AUPCMG), the mycelial growth of pathogenic fungi end (MGOPFE) and inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Colletotrichum truncatum*		
	AUPCMG	MGOPFE	IRGBCP
	cm		
CH022	7.76 B	0.88 B	0.20 D
CH031	8.84 B	0.94 B	1.54 B
CH001	9.13 B	1.02 B	0.24 D
CH015	10.09 B	0.80 B	1.04 C
CH013	10.15 B	1.10 B	0.38 D
CH018	10.41 B	1.26 B	2.16 A
CH037	10.87 B	1.14 B	0.46 D
CH004	11.18 B	1.24 B	0.68 C
CH036	11.44 B	1.20 B	0.56 D
CH034	11.45 B	1.38 A	0.94 C
CH009	11.67 B	1.22 B	0.52 D
CH006	11.67 B	1.34 A	0.48 D
CH014	11.86 B	1.40 A	0.78 C
CH029	12.04 A	1.24 B	0.36 D
CH032	12.16 A	1.44 A	1.04 C
CH035	12.33 A	1.38 A	0.56 D
CH002	12.34 A	1.40 A	2.12 A
CH007	12.40 A	1.40 A	1.30 B
CH020	12.56 A	1.40 A	0.56 D
CH024	12.63 A	1.42 A	0.92 C
CH021	12.92 A	1.44 A	0.56 D
CH023	12.98 A	1.44 A	0.48 D
CH025	13.03 A	1.46 A	0.54 D
CH027	13.24 A	1.52 A	1.22 B
CH028	13.30 A	1.44 A	0.46 D
CH030	13.69 A	1.50 A	0.42 D
CH008	13.71 A	1.62 A	0.72 C
CH012	13.95 A	1.58 A	0.76 C
CH017	13.99 A	1.62 A	0.56 D
CH016	14.01 A	1.50 A	0.56 D
Control	14.11 A	1.78 A	0.00 D
CH011	14.11 A	1.66 A	1.22 B
CH033	14.21 A	1.58 A	0.50 D
CH026	14.28 A	1.68 A	0.56 D
CH010	14.44 A	1.64 A	2.24 A
CH005	14.48 A	1.64 A	1.02 C
CH003	14.89 A	1.74 A	1.76 A
CH019	15.14 A	1.92 A	2.22 A

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test (p≤5%).

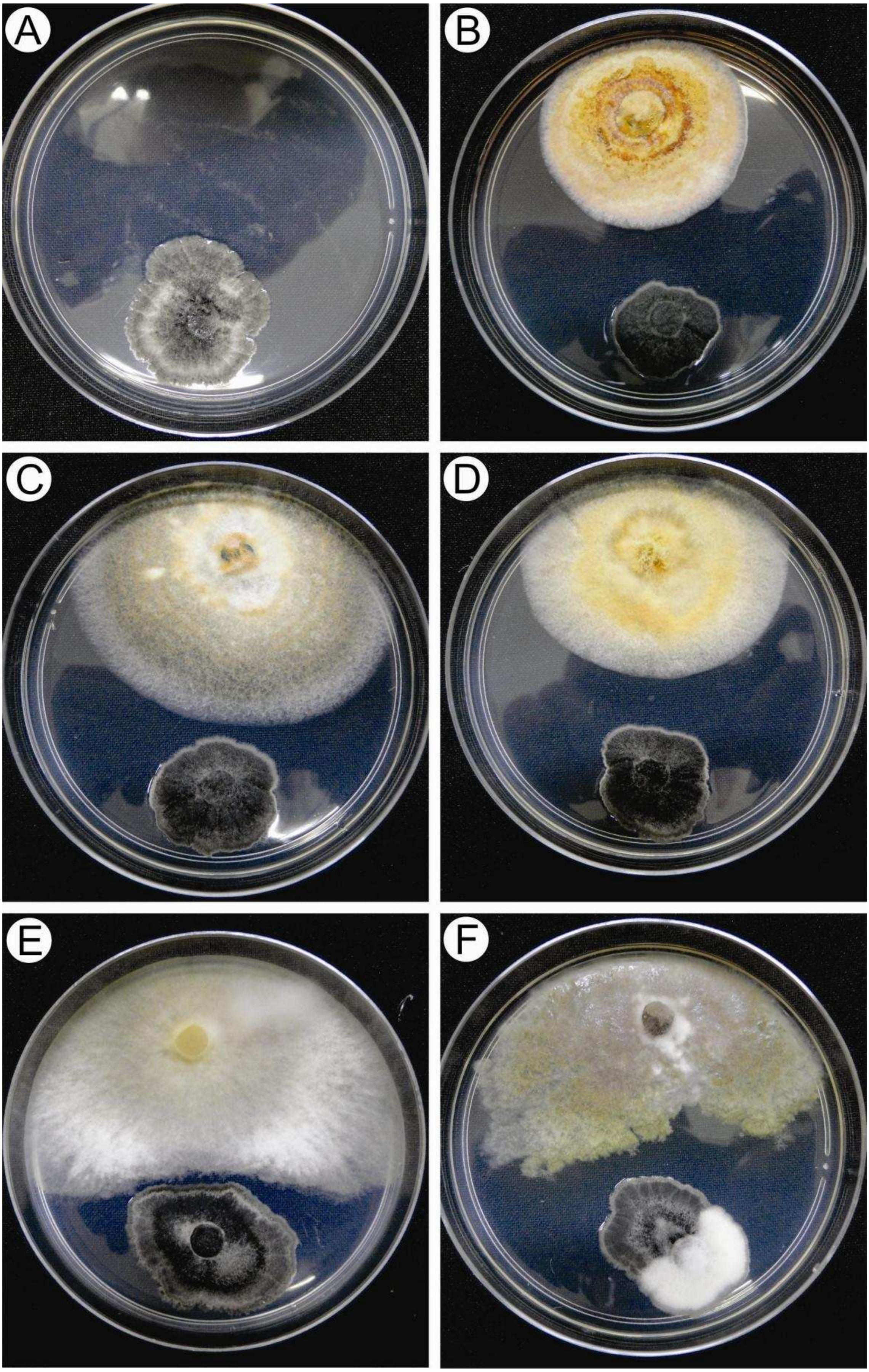

Fig. 2. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp.(up) x *Coletotrichum truncatum* (down). A) Control; B) CH022 (*C. cupreum*); C) CH031 (*C. cupreum*); D) CH001 (*C. cupreum*); E) CH015 (*C. bostrychodes*); F) CH013 (*C. globosum*).

3.1.3. *Diaporthe phaseolorum var. meridionalis*

This assay was evaluated for two days from the third day after installing it. Considering the statistical analysis, the data were classified into seven groups for AUPCMG, in eight groups for MGOPFE and four groups for IRGBCP (Table 4). For this assay *in vitro*, the lowest values of the isolates for AUPCMG coincided with lowest values of MGOPFE, and with some highest values for IRGBCP, not in the same sequence. Therefore, demonstrating that both parameters were effective in assessing isolates on interaction of *Chaetomium* spp. x *D. phaseolorum* var. *meridionalis* (Fig. 3 and 4).

The isolates that showed the highest values for IRGBCP were: CH022, CH017, CH005, CH001, CH008, CH007, CH033, CH024 (Table 4), considering the values highest to lowest respectively. The isolates that showed the lowest values for AUPCMG were: CH017, CH018, CH019, CH023, CH024, CH021, CH007 and CH033. For MGOPFE, the sequence the lowest to highest value respectively was: CH017, CH018, CH019, CH033, CH024, CH021, CH023 and CH007 (Table 4). The isolates that showed the highest values of AUPCMG are CH003, CH014 and CH030 (Table 4), which have higher values than control treatment, this occurred due to that isolated control position was placed in the dishes (Fig. 3).

The isolates CH001 and CH022 are *C. cupreum*, isolates CH005, CH007, CH008, CH017, CH018, CH019, CH021, CH023, CH024 and CH033 are *C. globosum*, isolate CH003 is *C. funicola* and isolates CH014 and CH030 are *C. bostrychoides*. The isolate CH017 was the most efficient in inhibition against *D. phaseolorum* var. *meridionalis* according to statistical analysis.

Table 4. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Diaporthe phaseolorum* var. *meridionalis in vitro* by area under the progress curve mycelial growth (AUPCMG), the mycelial growth of pathogenic fungi end (MGOPFE) and inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Diaporthe phaseolorum var. meridionalis*		
	AUPCMG	MGOPFE	IRGBCP
		cm	
CH017	4.71 G	1.78 H	0.88 A
CH018	4.81 G	1.82 H	0.68 B
CH019	5.40 G	1.96 H	0.56 B
CH023	5.56 F	2.39 G	0.33 C
CH024	5.60 F	2.32 G	0.72 A
CH021	5.89 F	2.37 G	0.35 C
CH007	5.99 E	2.45 G	0.77 A
CH033	6.11 E	2.29 G	0.72 A
CH012	6.17 E	2.61 F	0.32 C
CH016	6.32 E	2.59 F	0.16 D
CH005	6.33 E	2.57 F	0.81 A
CH004	6.36 E	2.60 F	0.47 B
CH026	6.38 E	2.80 F	0.00 D
CH008	6.76 D	2.71 F	0.78 A
CH027	6.81 D	3.05 E	0.39 C
CH013	6.89 D	2.77 F	0.31 C
CH020	7.13 D	2.90 E	0.00 D
CH028	7.15 D	2.73 F	0.00 D
CH022	7.45 D	2.78 F	0.96 A
CH001	7.78 C	3.12 E	0.80 A
CH025	7.79 C	3.34 D	0.00 D
CH036	7.98 C	3.27 D	0.00 D
CH035	8.14 C	3.31 D	0.00 D
CH031	8.23 C	3.10 E	0.59 B
CH006	8.46 C	3.56 C	0.00 D
CH015	8.50 C	3.64 C	0.00 D
Control	8.72 B	4.00 B	0.00 D
CH002	8.73 B	3.89 B	0.00 D
CH011	8.90 B	3.92 B	0.00 D
CH034	8.97 B	3.70 C	0.00 D
CH032	9.10 B	3.99 B	0.00 D
CH010	9.12 B	3.92 B	0.00 D
CH037	9.13 B	3.94 B	0.00 D
CH009	9.13 B	3.90 B	0.00 D
CH029	9.17 B	3.53 C	0.00 D
CH030	9.45 A	3.66 C	0.00 D
CH014	9.53 A	3.73 B	0.00 D
CH003	10.07 A	4.22 A	0.00 D

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test ($p \leq 5\%$).

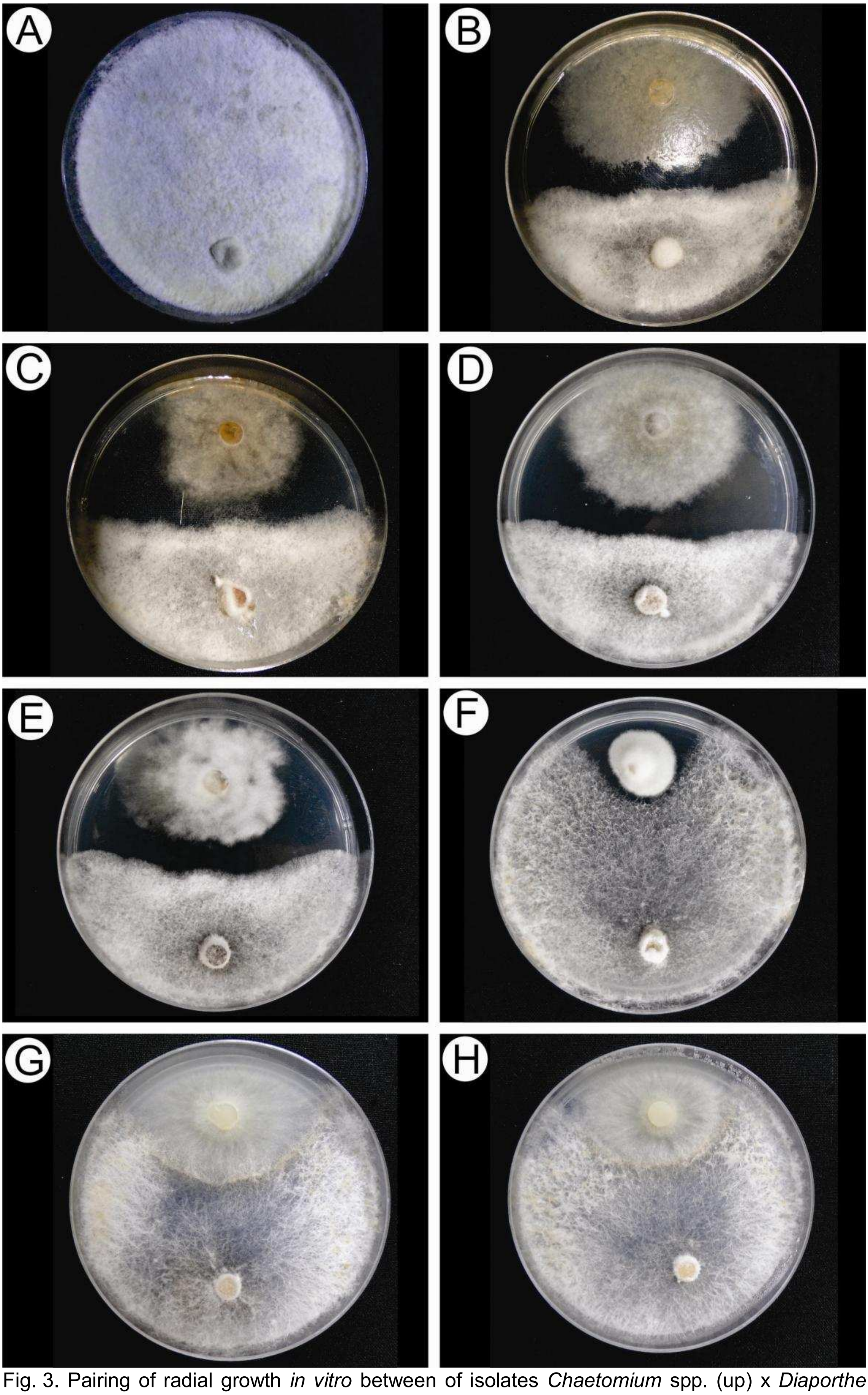

Fig. 3. Pairing of radial growth *in vitro* between of isolates *Chaetomium* spp. (up) x *Diaporthe phaseolorum* var. *meridionalis* (down). A) Control; B) CH018 (*C. globosum*); C) CH019 (*C. globosum*); D) CH023 (*C. globosum*); E) CH021(*C. globosum*); F) CH003 (*C. funicola*) ; G) CH014 (*C. bostrychodes*); H) CH030 (*C. bostrychodes*).

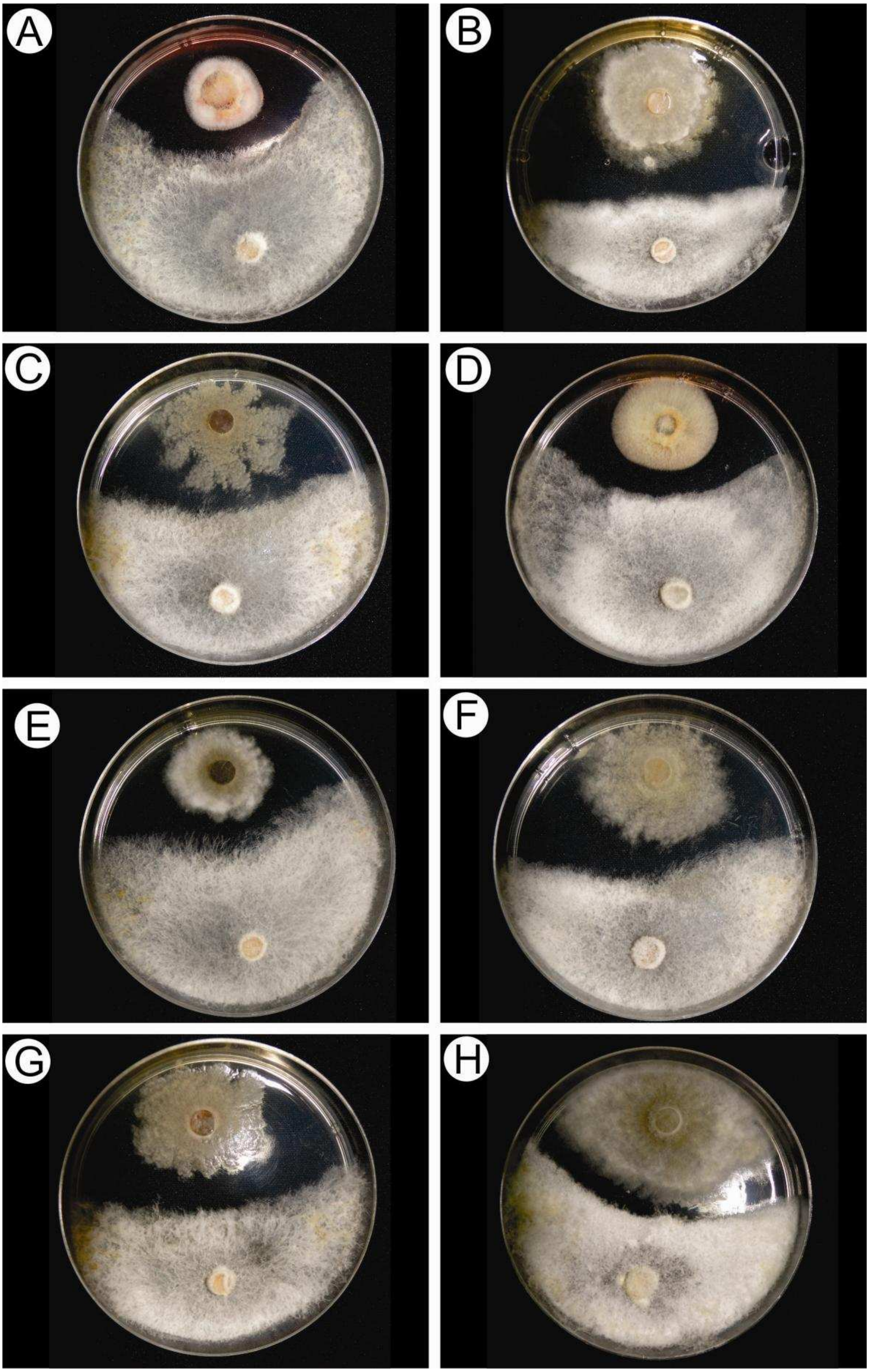

Fig. 4. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp. (up) x *Diaporthe phaseolorum* var. *meridionalis* (down). A) CH022 (*C. cupreum*); B) CH017 (*C. globosum*); C) CH005 (*C. globosum*); D) CH001(*C. cupreum*); E) CH008 (*C. globosum*); F) CH007 (*C. globosum*); G) CH033 (*C. globosum*); H) CH024 (*C. globosum*).

3.1.4. *Drechslera tritici-repentis*

This assay was evaluated during four days from the fifth day after the installation. The data were stratified into two groups for AUPCMG and MGOPFE, for IRGBCP into four groups according to statistical analysis (Table 5).

The isolates that showed the highest values for IRGBCP were: CH010, CH019, CH018, CH002, CH037 and CH005. However, not all these mentioned isolates represent effective antibiosis between *Chaetomium* spp. x *D. tritici-repentis*. The CH019 isolated did not grow in the presence of the isolated pathogen. Isolates CH002, CH010, CH018 and CH037 grew less than that pathogen (Table 5 and Fig. 5). The CH005 isolated is in the group of isolates with the highest values of IRGBCP and is also among the isolates with the lowest values of AUPCMG and MGOPFE. Whereas the isolates with the lowest values of AUPCMG and MGOPFE represent the most efficient isolates, the CH005 isolated can be considered efficient *in vitro* antibiosis to *D. tritici-repentis*. Although this group in the statistical analysis was segregated in 15 isolates, for both parameters, in Fig. 5 are only represented the isolates with the lowest values: CH022, CH007, CH031, CH005, CH009, CH032 and CH001. Isolates CH001, CH022 and CH031 are *C. cupreum*, isolates CH005, CH007 and CH009 are *C. globosum* and isolate CH032 is *C. reflexum*.

Table 5. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Drechslera tritici-repentis in vitro* by area under the progress curve mycelial growth (AUPCMG), the mycelial growth of pathogenic fungi end (MGOPFE) and inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Drechslera tritici-repentis*		
	AUPCMG	MGOPFE	IRGBCP
		cm	
CH022	7.28 B	1.36 B	1.32 B
CH007	8.01 B	1.42 B	1.24 B
CH019	8.06 B	1.40 B	1.86 A
CH031	8.27 B	1.42 B	1.10 B
CH005	8.33 B	1.42 B	1.56 A
CH032	8.78 B	1.48 B	1.28 B
CH001	8.78 B	1.48 B	1.36 B
CH009	8.81 B	1.34 B	0.54 D
CH033	9.12 B	1.52 B	0.68 C
CH027	9.28 B	1.60 B	1.14 B
CH020	9.57 B	1.58 B	0.26 D
CH013	9.77 B	1.66 B	0.94 C
CH008	9.94 B	1.72 B	0.90 C
CH003	10.09 B	1.74 B	0.94 C
CH029	10.12 B	1.72 B	0.80 C
CH010	10.37 A	1.90 A	1.96 A
CH028	10.43 A	1.78 A	0.74 C
CH002	10.49 A	1.86 A	1.66 A
CH004	10.53 A	1.90 A	0.92 C
CH011	10.59 A	1.86 A	1.44 B
CH025	10.62 A	1.84 A	0.74 C
CH012	10.87 A	1.90 A	0.74 C
CH030	10.95 A	1.90 A	0.70 C
CH017	11.01 A	1.90 A	0.76 C
CH018	11.09 A	2.02 A	1.68 A
CH035	11.21 A	1.94 A	0.54 D
CH036	11.27 A	1.90 A	0.36 D
CH021	11.35 A	1.94 A	0.34 D
CH026	11.40 A	2.00 A	0.80 C
CH015	11.73 A	2.02 A	0.66 C
CH034	11.81 A	2.14 A	1.24 B
CH023	12.16 A	2.04 A	0.40 D
CH006	12.16 A	2.24 A	0.84 C
CH016	12.19 A	2.10 A	0.52 D
Control	12.37 A	2.26 A	0.00 D
CH037	12.69 A	2.30 A	1.64 A
CH024	12.93 A	2.26 A	0.88 C
CH014	13.72 A	2.44 A	0.32 D

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test ($p \leq 5\%$).

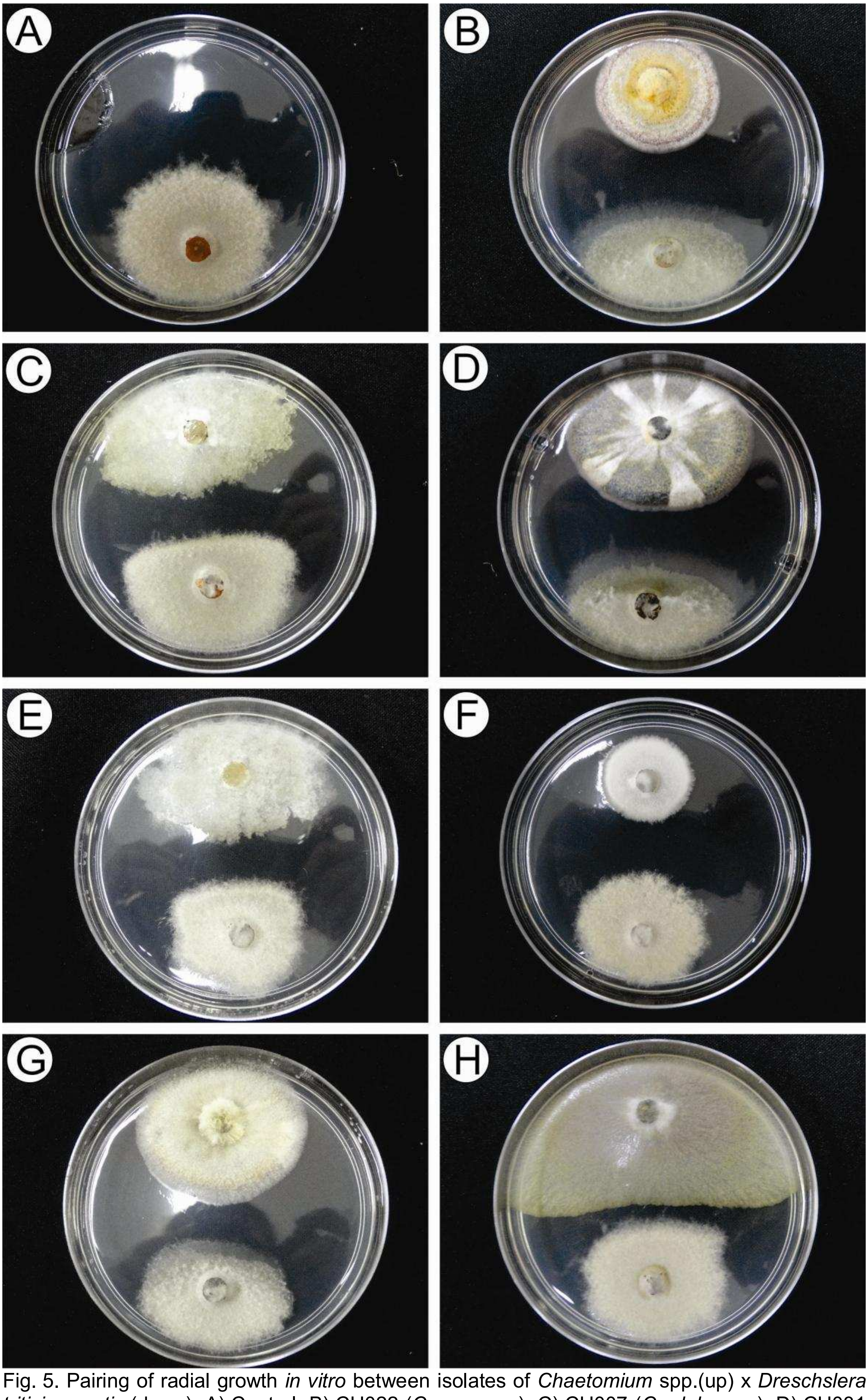

Fig. 5. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp.(up) x *Dreschslera tritici-repentis* (down). A) Control; B) CH022 (*C. cupreum*); C) CH007 (*C. globosum*); D) CH031 (*C. cupreum*); E) CH005 (*C. globosum*); F) CH032 (*C. reflexum*); G) CH001 (*C. cupreum*); H) CH009 (*C. globosum*).

3.1.5. *Fusarium solani*

This assay was evaluated for ten days, counting from the third day after installing it. For the assay of antibiosis *in vitro* between *Chaetomium* spp. x *Fusarium solani*, the data of AUPCMG and MGOPFE was tested with SCOTT-KNOTT ($p \leq 5\%$) showed no significant difference. However, for the IRGBCP, the data showed significant difference and were classified into four groups according with statistical analysis (Table 6).

The isolates that showed the highest values of IRGBCP are not the most efficient isolates in antibiosis as CH002, CH003, CH010, CH011, CH027 and CH032, that grew less than the pathogen. Thus, isolates that showed the best results antibiosis to *F. solani* were CH005, CH008, CH018, CH023, CH024, (*C. globosum*), CH028 (*C. bostrychodes*), and CH037 (*Chaetomium* sp.) (Fig. 6).

Table 6. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Fusarium solani in vitro* by inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Fusarium solani*
	IRGBCP
	cm
Control	0.00 E
CH031	0.10 D
CH029	0.18 D
CH036	0.34 D
CH035	0.40 D
CH022	0.42 D
CH021	0.42 D
CH030	0.44 D
CH026	0.44 D
CH025	0.46 D
CH015	0.46 D
CH019	0.48 D
CH006	0.52 D
CH009	0.54 D
CH001	0.54 D
CH016	0.58 D
CH034	0.62 D
CH033	0.62 D
CH012	0.66 D
CH020	0.74 C
CH004	0.74 C
CH014	0.76 C
CH017	0.80 C
CH013	0.82 C
CH007	0.82 C
CH037	0.86 C
CH005	0.86 C
CH028	0.88 C
CH008	0.98 C
CH024	1.00 C
CH023	1.00 C
CH018	1.04 C
CH032	1.12 C
CH027	1.14 C
CH010	1.44 B
CH011	1.56 B
CH002	2.22 A
CH003	2.26 A

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test ($p \leq 5\%$).

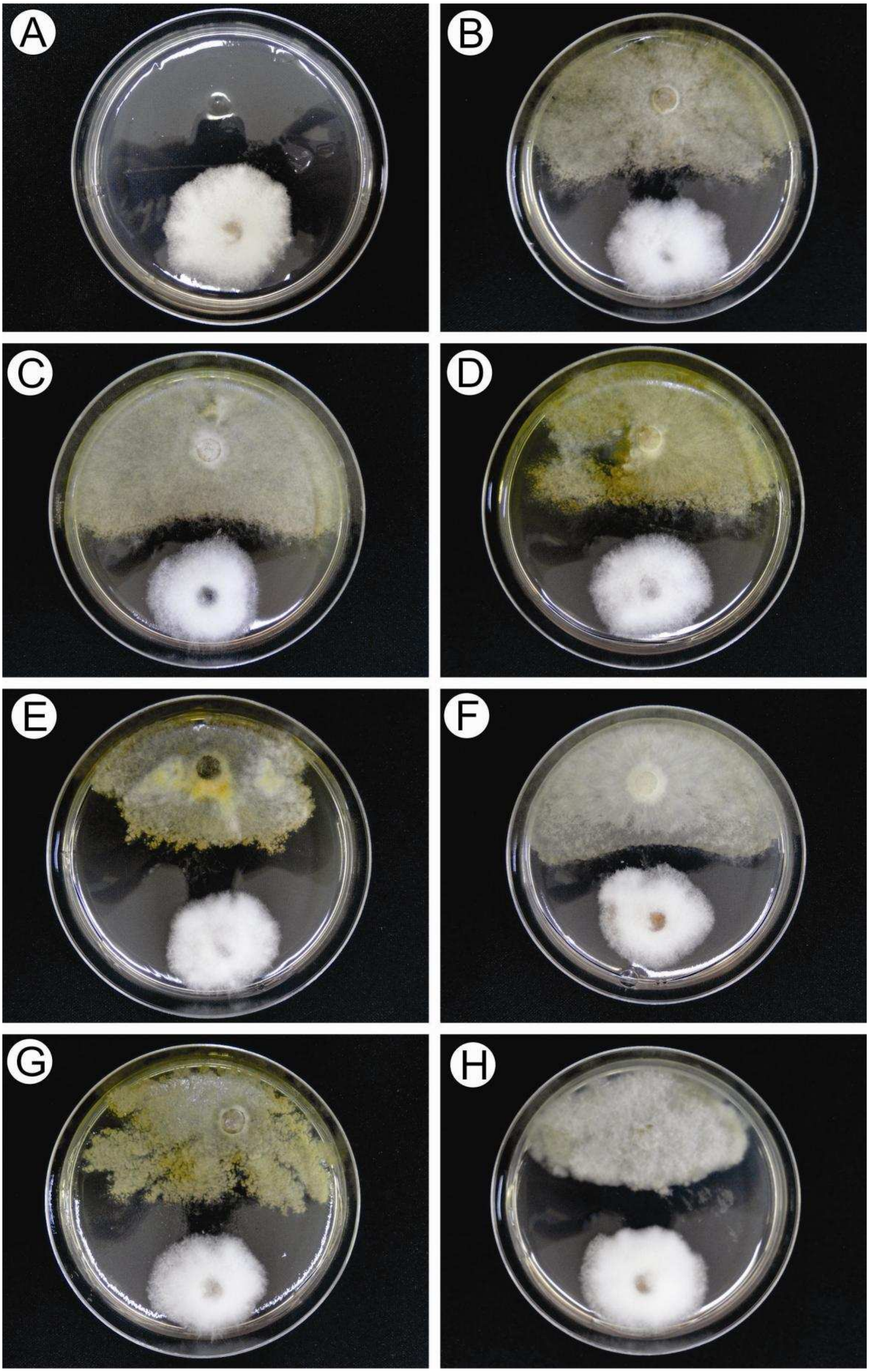

Fig. 6. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp.(up) x *Fusarium solani* (down). A) Control; B) CH018 (*C. globosum*); C) CH023 (*C. globosum*); D) CH024 (*C. globosum*); E) CH008 (*C. globosum*); F) CH028 (*C. bostrychodes*); G) CH005 (*C. globosum*); H) CH037 (*Chaetomium* sp.).

3.1.6. *Magnaporthe grisea*

This assay was evaluated for ten days from the third day after installing it. For this assay of *in vitro* antibiosis between *Chaetomium* spp. x *M. grisea*, the data was separated by the statistical analysis in three groups AUPCMG and MGOPFE, and four groups for IRGBCP (Table 7).

The isolates with the highest values for IRGBCP were: CH027, CH003, CH007, CH002, CH008, CH019, CH023, CH010 and CH013, respectively, from highest to lowest value. Moreover, the isolates CH002, CH003, CH010 grew less than the pathogen isolated. According to statistical analysis, all other isolates with higher values for IRGBCP are also among the isolates with the lowest values of AUPCMG and MGOPFE.

The isolates with the lowest values for AUPCMG and MGOPFE, are the most efficient for antibiosis *in vitro* between *Chaetomium* spp. x *M grisea*. Although the isolates grouped by statistical analysis include 27 isolates, the Fig. 7 shows only isolates with the lowest values of AUPCMG: CH022, CH021, CH031, CH026, CH001 and CH008. These isolates coincide with the lowest values for MGOPFE, not exactly in the same order. Isolates CH001, CH022 and CH031 are *C. cupreum*, isolates CH008 and CH021 are *C. globosum* and isolate CH026 is *C. reflexum*. The CH027 isolated is also shown in Fig. 7 for presenting the largest value of IRGBCP.

Table 7. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Magnaporthe grisea in vitro* by area under the progress curve mycelial growth (AUPCMG), the mycelial growth of pathogenic fungi end (MGOPFE) and inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Magnaporthe grisea*		
	AUPCMG	MGOPFE	IRGBCP
	cm		
CH022	5.55 C	0.66 C	1.00 C
CH021	5.64 C	0.56 C	1.72 B
CH031	5.73 C	0.70 C	0.00 D
CH026	6.49 C	0.84 C	1.60 B
CH001	6.63 C	0.74 C	0.14 D
CH008	6.76 C	0.84 C	2.12 A
CH009	6.79 C	0.74 C	1.22 C
CH013	6.82 C	0.84 C	2.00 A
CH006	6.85 C	0.76 C	1.30 C
CH020	7.08 C	0.86 C	1.72 B
CH023	7.09 C	0.92 C	2.06 A
CH029	7.21 C	0.86 C	1.70 B
CH012	7.21 C	0.86 C	1.64 B
CH004	7.25 C	0.66 C	0.52 D
CH007	7.26 C	0.84 C	2.26 A
CH025	7.28 C	0.92 C	1.54 B
CH015	7.28 C	0.88 C	1.72 B
CH030	7.29 C	0.90 C	1.88 B
CH014	7.29 C	0.88 C	1.72 B
CH024	7.50 C	0.92 C	1.74 B
CH019	7.61 C	0.98 C	2.10 A
CH028	7.71 C	0.96 C	1.82 B
CH027	7.77 C	0.96 C	2.42 A
CH016	7.89 C	0.96 C	1.78 B
CH010	8.08 C	0.88 C	2.02 A
CH005	8.28 C	0.98 C	0.94 C
CH018	8.32 C	0.72 C	0.56 D
CH002	9.27 B	1.14 B	2.20 A
CH011	9.52 B	1.22 B	1.82 B
CH003	9.66 B	1.24 B	2.30 A
CH032	9.68 B	1.32 B	1.36 B
CH017	10.08 B	1.24 B	0.84 C
CH033	10.63 B	1.30 B	0.98 C
CH035	11.75 A	1.48 A	0.86 C
Control	12.15 A	1.52 A	0.00 E
CH036	12.45 A	1.48 A	0.70 C
CH034	12.46 A	1.52 A	1.22 C
CH037	13.15 A	1.70 A	0.80 C

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test (p≤5%).

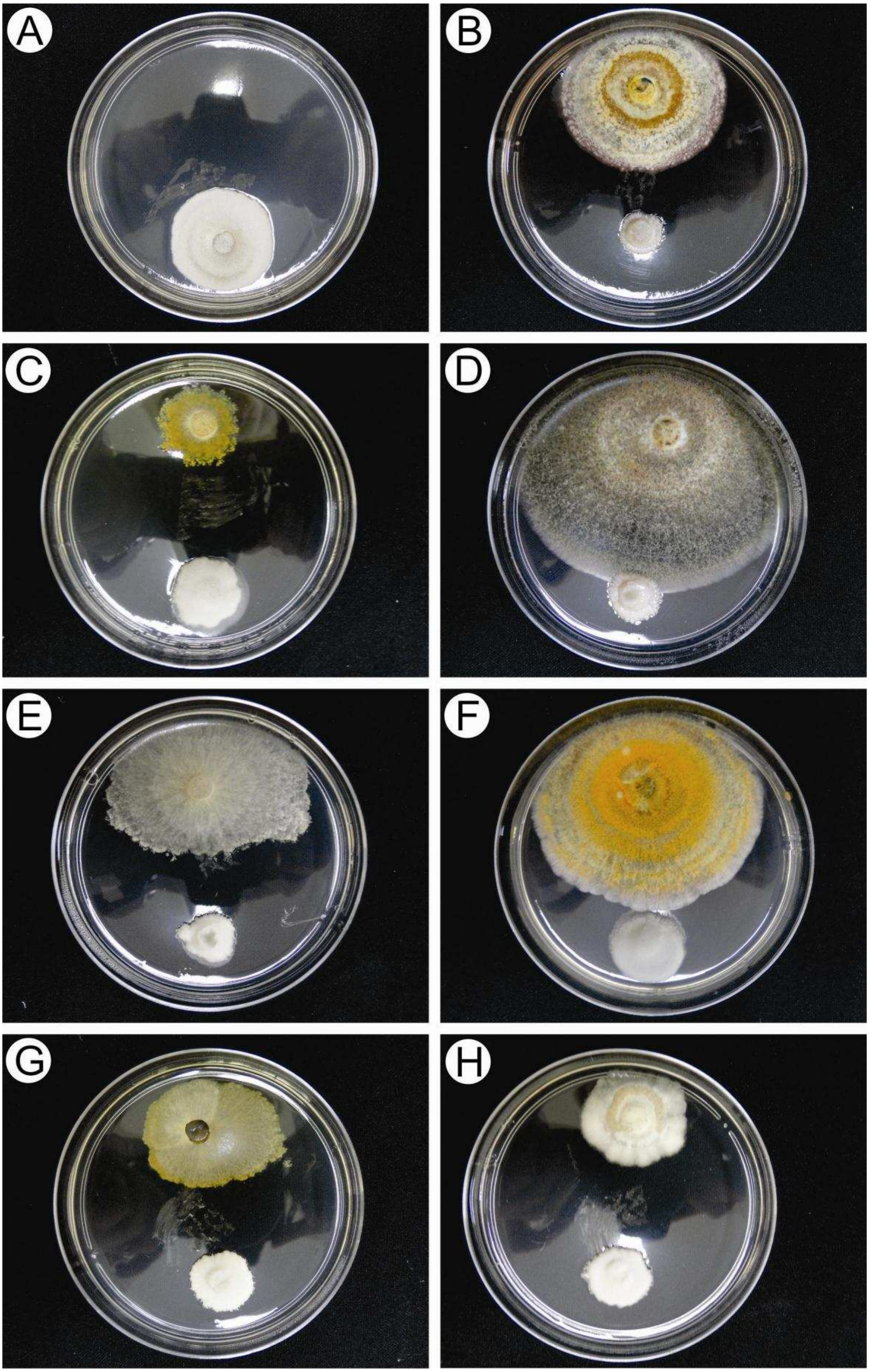

Fig. 7. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp.(up) x *Magnaporthe grisea* (down). A) Control; B) CH022 (*C. cupreum*); C) CH021 (*C. globosum*); D) CH031 (*C. cupreum*); E) CH026 (*C. bostrychodes*); F) CH001 (*C. cupreum*); G) CH008 (*C. globosum*); H) CH027 (*C. funicola*).

3.1.7. *Sclerotinia sclerotiorum*

This assay was evaluated for two days from the second day after installing it. For the assay *in vitro* antibiosis between *Chaetomium* spp. x *Sclerotinia sclerotiorum*, the statistical analysis stratified the datas in two groups for AUPCMG, four groups for MGOPFE and five groups for IRGBCP (Table 8).

The statistical analysis was able to separate only one isolate (CH022) with the highest value of IRGBCP, which is also among the isolates with the lowest values of AUPCMG.

Although the statistical analysis grouped 18 isolates with the lowest values for AUPCMG, only seven isolates with the lowest values were showed in Fig. 8: CH007, CH001, CH018, CH022, CH012, CH030 and CH031. For MGOPFE, isolates that showed the lowest values, from lowest to highest were: CH010, CH001, CH005, CH022, CH031, CH007 and CH018. With the exception of isolates CH005 and CH010, the others coincide with the lowest values for AUPCMG. Isolates CH001, CH022 and CH031 are *C. cupreum*, isolates CH007, CH012 and CH018 are *C. globosum* and isolate CH030 is *C. bostrychoides*.

Table 8. Characterization of pairing of radial growth between 37 isolates of *Chaetomium* spp. x *Sclerotinia sclerotiorum in vitro* by area under the progress curve mycelial growth (AUPCMG), the mycelial growth of pathogenic fungi end (MGOPFE) and inhibition of radial growth between isolates of *Chaetomium* and pathogenic fungi (IRGBCP).*

Isolates of *Chaetomium* spp.	*Sclerotinia sclerotiorum*		
	AUPCMG	MGOPFE	IRGBCP
		cm	
CH007	3.91 B	3.46 C	0.38 D
CH001	3.99 B	2.54 D	0.80 B
CH018	4.00 B	3.64 C	0.64 C
CH022	4.07 B	3.10 C	1.14 A
CH012	4.41 B	5.00 A	0.12 E
CH030	4.55 B	4.70 A	0.02 E
CH031	4.74 B	3.44 C	0.32 D
CH036	4.76 B	5.00 A	0.00 E
CH024	4.83 B	4.30 B	0.34 D
CH005	4.95 B	3.38 C	0.58 C
CH017	4.98 B	4.28 B	0.12 E
CH008	5.02 B	4.72 A	0.02 E
CH037	5.04 B	4.00 B	0.00 E
CH026	5.12 B	4.72 A	0.04 E
CH019	5.12 B	4.16 B	0.40 D
CH023	5.13 B	4.74 A	0.06 E
CH025	5.24 B	5.00 A	0.00 E
CH034	5.38 B	4.68 A	0.08 E
CH033	5.54 A	4.04 B	0.12 E
CH010	5.56 A	2.10 E	0.00 E
CH016	5.58 A	5.00 A	0.00 E
CH013	5.60 A	4.00 B	0.08 E
CH015	5.88 A	5.00 A	0.00 E
CH014	5.90 A	5.00 A	0.00 E
CH004	5.94 A	4.00 B	0.16 E
CH035	6.10 A	4.24 B	0.40 D
CH027	6.12 A	5.00 A	0.00 E
CH032	6.13 A	4.86 A	0.02 E
CH021	6.22 A	4.30 B	0.00 E
CH006	6.38 A	5.00 A	0.00 E
CH011	6.44 A	5.00 A	0.00 E
Control	6.53 A	4.50 B	0.00 E
CH020	6.58 A	5.00 A	0.00 E
CH029	6.80 A	5.00 A	0.00 E
CH009	7.07 A	4.82 A	0.06 E
CH028	7.10 A	5.00 A	0.00 E
CH003	7.12 A	5.00 A	0.00 E
CH002	7.30 A	5.00 A	0.00 E

*Means followed by the same capital letter in the column do not differ by the Scott-Knott test (p≤5%).

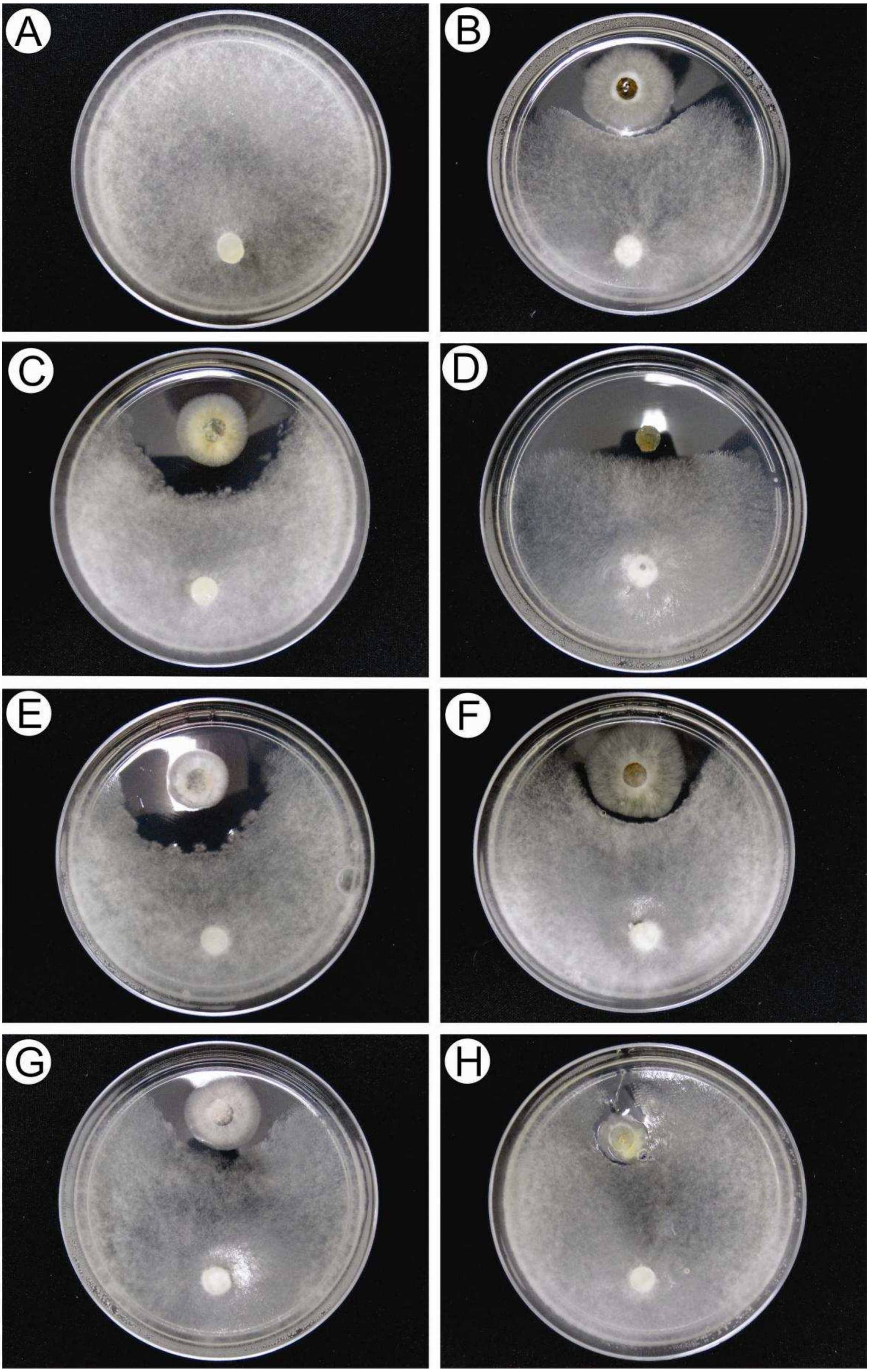

Fig. 8. Pairing of radial growth *in vitro* between isolates of *Chaetomium* spp.(up) x *Sclerotinia sclerotiorum* (down). A) Control; B) CH007 (*C. globosum*); C) CH001 (*C. cupreum*); D) CH018 (*C. globosum*); E) CH022 (*C. cupreum*); F) CH012 (*C. globosum*); G) CH030 (*C. bostrychodes*); H) CH031 (*C. cupreum*).

3.2. Seed health testing

3.2.1. Soybean seeds

The seeds were detected with pathogenic fungi, especially *Fusarium* sp., *Rhizoctonia* sp. In addition, it was also detected the presence of *Botryosporium* sp., a weak pathogen of soybean and storage fungi: *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* sp. (Table 9).

Table 9. Incidence of fungi associated with soybean seeds by health testing.

NO DISINFESTED		DISINFESTED	
FUNGI	%	FUNGI	%
Aspergillus spp.	16.5	*Aspergillus* spp.	4.0
Botryosporium sp.	0.5	*Fusarium* sp.	18.0
Fusarium sp.	12.5	*Rhizoctonia* sp.	9.5
Penicillium spp.	30.5	*Rhizopus* sp.	5.5
Rhizoctonia sp.	7.0		
Rhizopus sp.	22.5		

3.2.2. Wheat seeds

At wheat seeds, desinfested and no desinfested, the values of *Bipolaris* sp. detected in seeds exceeded 20% in the lot. Besides, other important pathogens in wheat seeds were found, such as *Alternaria* sp. and *Fusarium* sp. There is also the detection of other fungi, such as *Aspergillus* spp. *Cladosporium* sp. and *Penicillium* spp. (Table 10).

Table 10. Incidence of fungi associated with wheat seeds by health testing.

NO DESINFESTED		DESINFESTED	
FUNGI	%	FUNGI	%
Alternaria sp.	7.0	*Alternaria* sp.	7.0
Aspergillus spp.	20.0	*Bipolaris* sp.	22.0
Bipolaris sp.	25.0	*Fusarium* sp.	2.5
Cladosporium sp.	1.0	*Penicillium* spp.	0.5
Fusarium sp.	2.0		
Penicillium spp.	28.0		

3.3. Sporulation of *C. cupreum* (CH031 isolate) on six different culture medium

The choice of the culture medium is a very important step in the process of ascospores production. These ascospores may be used for the production of fungal inoculum in order to use them for the biological control of pathogens in seeds. The different compositions of the medium are able to promote growth and stimulate sporulation of a particular fungus.

The isolate CH031 (*C. cupreum*) was chosen because it showed the most consistent results in the assay of *in vitro* antibiosis for pathogens tested.

The culture medium WAM allowed the growth of fungal mycelium, however, the isolated did not sporulate. The other media, favored the production of ascospores: PDA, SMA, CMA, CCM, OAM. The OAM has provided the highest value of ascospore production (Table 11).

Table 11. Sporulation of *C. cupreum* (CH031 isolate) on different culture medium: Water Agar Medium (WAM), Potato Dextrose Agar (PDA), Salt Mannitol Agar (SMA), Corn Meal Agar (CMA), Corn-Carrot Medium (CCM), Oats Medium (OAM).

CULTURE MEDIUM					
WAM	PDA	SMA	CMA	CCM	OAM
Ascospores.mL^{-1}					
0 D	3.6×10^4 C	4.0×10^4 C	4.8×10^4 C	9.5×10^4 B	3.0×10^5 A

CV: 34,7%. Means followed by the same letter in the column do not differ by Tukey test at 5%.

3.4. Greenhouse assay

3.4.1. Germination and health seeds

Soybean seeds not inoculated (control) showed the lowest percentage for seeds normal and higher percentage for abnormal seeds, however the higher percentage value of diseased seeds. Seeds inoculated with isolate CH022 showed the lowest percentage for diseased seeds. On the other hand, the seeds inoculated with the strain CH031 showed the highest percentage value of normal seed (Table 12).

Table 12. Evaluation of emerged seeds during ten days after sowing.

	Soybean			Wheat		
	No inoculated	CH022	CH031	No inoculated	CH022	CH031
	%					
Normal	45.0	64.5	70.0	60.5	41.5	48.0
Abnormal	28.5	23.0	13.5	30.0	30.0	27.5
Diseased	26.5	12.5	16.5	9.5	28.5	24.5

Wheat seeds without inoculation showed the highest percentage value for normal and abnormal seeds with 100% seeds emerged. Seeds inoculated with isolate CH022 showed the lowest percentage of normal seeds and the highest percentage of diseased seeds (Table 12). Seeds inoculated with isolate CH031 showed the lowest percentage of seeds abnormal. The most abnormal seedlings showed negative geotropism.

3.4.2. Vigor

In this assay were observed seedlings emerged until the tenth day after sowing (Fig. 9). On the count of GSI for soybean, the highest value was found for seeds inoculated with isolate CH031, followed for seeds without inoculation and inoculated with CH022 in this sequence (Table 13). However, for wheat seed, the highest value was shown in non-inoculated seeds, then the seeds inoculated with CH031 and CH022. The values of GSI evaluation throughout the time (days) is shown in Fig 10-A for soybean seeds and Fig. 10-B for wheat seeds.

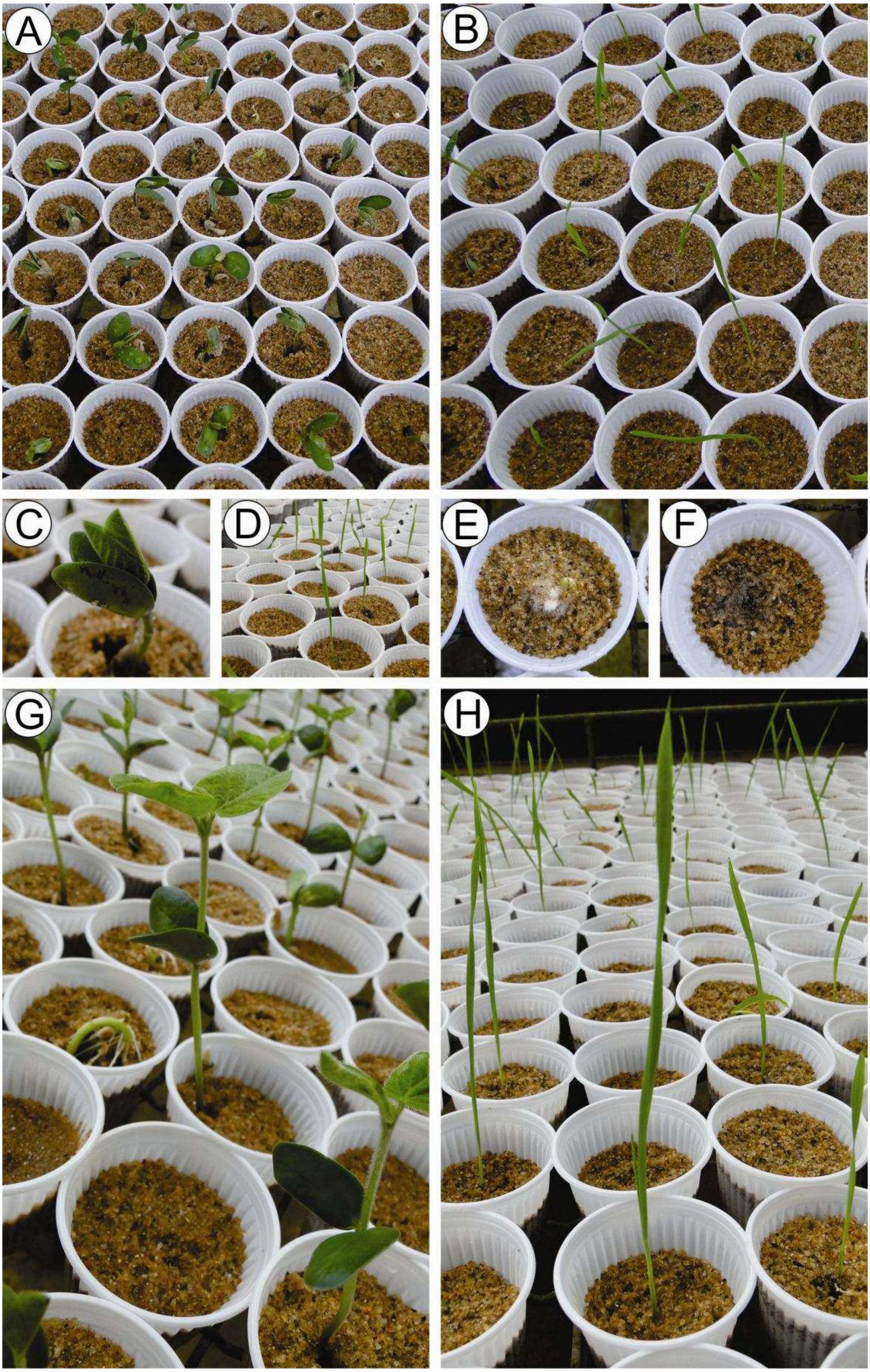

Fig. 9. Assay in a greenhouse with soybeans and wheat seeds inoculated with *C. cupreum* (CH022 and CH031 isolates). A,C,G) Soybean seedlings during the assay; B,D,H) Wheat seedlings during the assay; E) Mycelium of *Fusarium* sp.; F) Mycelium of *Bipolaris* sp.

Table 13. Seedlings emerged evaluated by Germination Speed Index (GSI).

	Soybean			Wheat		
	No inoculation	CH022	CH031	No inoculation	CH022	CH031
GSI	104,075	82,485	121,285	122,15	77,755	105,955

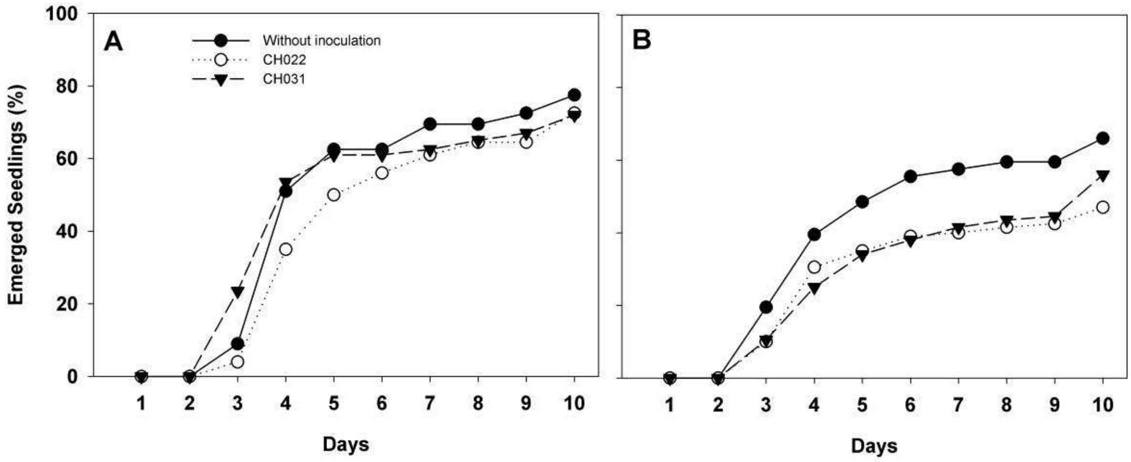

Fig. 10. Percentage of seeds emerged during ten days after sowing: A) Soybean; B) Wheat.

3.5. *Chaetomium cupreum* as endophytic

In this assay, roots of soybean seedlings inoculated and non-inoculated, showed no growth of *C. cupreum* or any pathogenic fungus. However, in the roots of wheat seedlings inoculated, there was growth of *Bipolaris* sp. fungus considered to be pathogenic to wheat seeds (Fig. 11 A and B). In the roots of wheat seedlings inoculated with isolates of *C. cupreum* (CH022 and CH031), there was showed mycelial growth and was formed perithecia of the fungus, and no showed growth of the other fungus (Fig. 11).

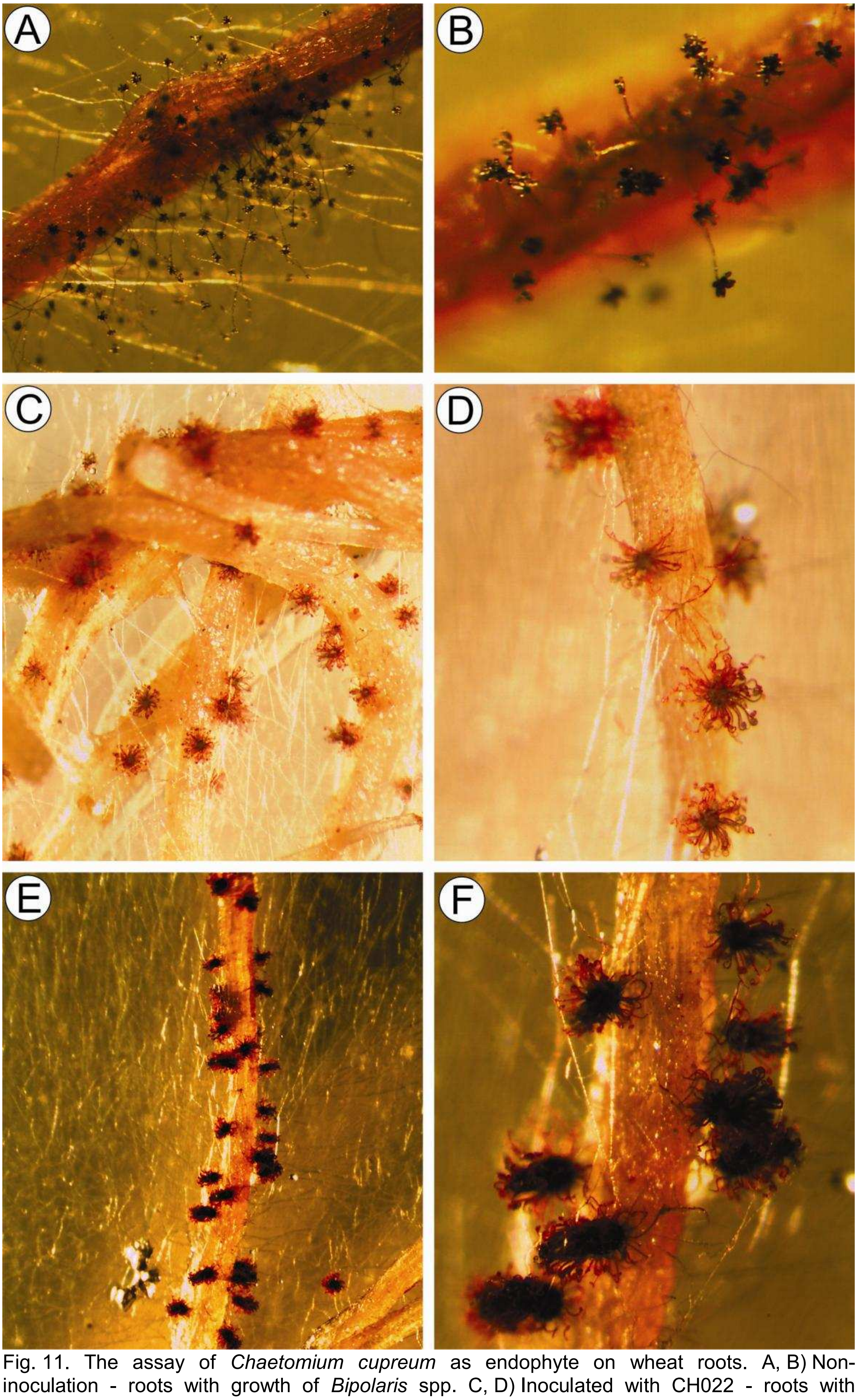

Fig. 11. The assay of *Chaetomium cupreum* as endophyte on wheat roots. A, B) Non-inoculation - roots with growth of *Bipolaris* spp. C, D) Inoculated with CH022 - roots with perithecia *C. cupreum*; E, F) Inoculated with CH031 - roots with perithecia of *C. cupreum*.

4. Discussion

In vitro assays showed that the isolates CH001, CH022 and CH031, both *C. cupreum*, appeared among the most efficient isolates in antibiosis *in vitro* test between 37 isolates of the genus *Chaetomium* and seven different pathogens of soybean and wheat. *Chaetomium globosum* and *C. cupreum* has been studied extensively and successfully used to control root rot of citrus, black pepper, strawberry and dampping-off reduction of sugar beet (Soytong et al., 2001). The species of *C. cupreum* has demonstrated effectiveness in reducing the inoculum of *Magnaporthe oryzae* in rice seeds (Soytong, 1992a). It is also a biological control agent proven in *Magnaporthe grisea* (Liao, 2002) and *Rhizoctonia* and *Pythium* (Soytong, 2003).

In this work it was found that the parameter IRGBCP was not effective to stratify of *Chaetomium* isolates in inhibiting pathogens fungi. This has occurred because some *Chaetomium* isolates did not grew in the presence of pathogen or pathogen isolated grew less than *Chaetomium* isolated during the evaluation period. In the assay of antibiosis between *Chaetomium* spp. x *Fusarium solani*, the other parameters did not differ isolates statistically and the IRGBCP was effective in stratifying the efficient isolates in this interaction. In interaction with some pathogens tested, the IRGBCP parameter showed isolates that were different statistically and coincided with isolates efficient for parameters AUPCMG and MGOPFE. In assays antibiosis for different pathogens, the values with the lowest results for AUPCMG and MGOPFE coincided in most assays. Thus, we can infer that both parameters were efficient to evaluate the *in vitro* antibiosis among the *Chaetomium* isolates and pathogens of soybean and wheat.

In the assay conducted in the greenhouse with soybean seeds, the seeds inoculated with *C. cupreum* (CH022 and CH031) showed higher percentage of normal seedlings compared to non-inoculated seeds. These data demonstrate that the inoculated seeds were benefited by *C. cupreum* and also presented lowest percentage of diseased seeds. At the same time, the seeds denominated diseased in here, simply exhibited the fungal mycelial growth, which does not necessarily interfered with germination and seed vigor.

In the germination evaluation of wheat seedlings, the inoculated seeds exhibited a lower germination of normal seeds when compared to no inoculated wheat seeds during the evaluation period. The high number of abnormal seedlings, are mostly seeds with negative geotropism, which may be due to the concentration of ascospores on the seeds. The same was observed in the work of Pereira and Dhingra (1997), where the concentration of ascospores of *C. globosum* interfered in seed germination. This fact was noted due to the reduced emergence and increase of the number of seedlings with negative geotropism, when the concentration of ascospores per seed was approximately 800 ascospores. At doses of 400 ascospores per seed, the seedling emergence was similar to the control. In another study, the isolate of *Chaetomium trilaterale* showed toxic effect on soybean pathogens, but also showed that root growth of seedlings was inhibited with increasing concentration of the filtrate, and germination was affected (Yeh and Sinclair, 1980).

Many compounds have been identified from *Chaetomium* species for the purpose of controlling plant pathogens. Flavonoids and derivatives of phenolic acid shown to be a potential antioxidant from *Chaetomium* sp. isolated from *Nerium oleander* (Huang et al., 2007). It was also reported *Chaetomium globosum* in *Ginkgo biloba* as endophyte and a source chaetomugilin D, chaetomugilin A and chaetoglobosins C with a significant activity against *Artemia salina* and *Mucor miehei* (Qin et al., 2009). A study demonstrated the antifungal activity of oosporein of *Chaetomium cupreum* isolated from *Macleaya cordata* against *Rhizoctonia solani*, *Botrytis cinerea* and *Pytium ultimum* (Mao et al., 2010). In an *in vitro* study, *Chaetomium* isolated produced compounds in potato dextrose broth affecting root-knot *Meloidogyne incognita* motility (Yan et al., 2011).

In the endophytic relations, plant supplies nutrients to the fungus, whereas fungus obtains minerals and water from the hyphae that are able to more efficiently than roots of plants. Thus, the presence of endophytes can benefit the host (Carroll, 1992). In this work, the roots were removed of apparently healthy and normal seedlings and presented *C. cupreum* growth, which inhibited the growth of pathogens in the roots. In studies of endophytic fungi in different wheat cultivars, most were called of facultative symbionts, although its effect on wheat is unknown (Crous et al., 1995).

Endophytes fungi are most commonly associated with plants. They live in inter or intracellular space of the plants tissues during all or part of their life cycle, causing no apparent symptoms of disease (Strobel et al. 2004).

Endophytic fungi are an important and yet unexplored source of new chemical substances with biological potential, therefore, must be considered in bioprospecting programs (Strobel et al., 2004). O'Hanlon et al. (2012) argue that more attention should be given to the potential of endophytic fungi as biological control agents against pathogens of cereals. The results illustrate the biotechnological potential of endophytic fungi in the search for new bioactive compounds and useful tools in chemical biology.

This work proved the efficiency of *C. cupreum* in pairing culture *in vitro* for seven different pathogens. Moreover, in the assay in a greenhouse, the germination of soybean seeds increased when inoculated whit isolates CH022 and CH031. Finally, it was shown that the isolates CH022 and CH031 of *C. cupreum* is endophyte in roots of wheat and inhibits the growth of *Bipolaris sorokiniana*. Further studies are needed on methods of inoculation and concentration of ascospores of *C. cupreum* on seeds for the establishment of a future biological control of seeds diseases.

Highlights

1) Pairing of isolates test of *Chaetomium* spp. and pathogens of soybean and wheat seeds;

2) Isolates of *Chaetomium cupreum* showed best results *in vitro* pairing of isolates test;

3) Oat culture medium provided best production of the ascospores of *C. cupreum*;

4) *Chaetomium cupreum* is endophytic on wheat roots inhibiting the growth of pathogens.

5) More than one species *Chaetomium* can occur on the same substrate.

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CONCLUSÕES GERAIS

1) Foram obtidos e identificados 41 isolados do gênero *Chaetomium* agrupados em sete espécies conhecidas, um novo relato no Brasil e uma possível nova espécie;
2) Vários isolados de *Chaetomium* spp. demonstraram capacidade de antibiose frente aos patógenos testados. Sendo que, os isolados de *Chaetomium cupreum* foram os que apresentaram os melhores resultados de forma geral;
3) Mais de uma espécie de *Chaetomium* podem ocorrer no mesmo substrato, incluindo as espécies de grande interesse no controle biológico de doenças de plantas;
4) O meio de cultura de aveia foi o que apresentou os melhores resultados para esporulação do isolado CH031 de *C. cupreum*;
5) A alta concentração de ascósporos de *Chaetomium cupreum* pode causar geotropismo negativo tanto na soja quanto no trigo;
6) O isolado CH031 de *C. cupreum* apresentou um incremento de vigor nas sementes de soja. Para as sementes de trigo, o melhor resultado do teste de vigor (GSI) ocorreu nas sementes não inoculadas.