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BIOACTIVITY OF SOME ENDOPHYTIC FUNGI FROM CAMEROONIAN MEDICINAL PLANTS AGAINST *VERTICILLIUM ALBO-ATRUM*, *RHIZOCTONIA CAROTAE* AND *PHYTOPHTHORA MEGAKARYA*

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ABSTRACT

Endophytic fungi (EFs) are beneficial microorganisms which grow in living plant tissues without causing any disease to their hosts. Most of them have antifungal properties. EFs of three herbaceous plants namely *Lantana camara*, *Emilia coccinae* and *Bryophyllum pinnatum* were isolated by using potato dextrose agar medium (PDA) and screened for their antifungal activity on the double direct confrontation (DDC) test on PDA. The growth diameter of pathogenic fungi with or without endophyte was measured weekly. Then, fourteen days after the DDC test, fungicidal or fungistatic activity of the EFs was assessed on the pathogens' growth. Finally, EFs that strongly inhibited pathogens' growth was submitted to the cultural characterization on PDA, Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) media at three pH levels (5.2, 7 and 9). Results showed that eight EFs genera are associated with these herbaceous medicinal plants with diverse antifungal activities. EFs that significantly ($p < 0.05$) inhibited the growth of the three pathogenic fungi were: *Trichoderma harzianum* (66%), *Cercospora* sp (58%) and *Aspergillus* sp (49%), 7 days after the DDC test. Endophytes *T. harzianum* and *Cercospora* sp were fungicidal on the three pathogens while *Aspergillus* sp was fungicidal on *V. albo-atrum* and *R. carotae.* There was a high morphological variability (colour, texture and pigments produced by EFs on culture media), and variability in their daily growth diameter and sporulation among EFs from one medium to another and from one pH level to another. This study suggests that *T. harzianum* and *Cercospora* sp endophytes possess the high antagonistic activity and can be used as an alternative to synthetic chemicals that control plant diseases.

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INTRODUCTION

Fungal endophytes have a great diversity of bioactive compounds important in many areas of life such as the pharmaceutical industry (antioxidant, and anticancer agents), agriculture, and the environment (Palanichamy *et al.*, 2018; Xu *et al.*, 2021). Due to their high consumption levels as well as nutritional and economical values, cocoa (*Theobromae cacao* L.) and carrot (*Daucus carota* L.) are important crops in the world. It is estimated that about 50 million of people in

the world depend on income from cocoa to live (CCI, 2001). Carrot is a popular root vegetable cultivated for its richness in bioactive compounds with beneficial effect for the consumer health. In Cameroon and elsewhere in the world, black pod disease of cocoa caused by *Phytophthora megakarya* and carrot root diseases (*Rhizoctonia carotae* and *Verticillium albo-atrum*) are the major diseases that limit the productivity of these two crops and cause significant economic losses (Punja, 1987; Retief *et al.*, 2023). To control these diseases, many strategies are recommended, consisting of chemical, genetic and agricultural methods. Chemical control is most used and effective, especially when multiple treatments are applied. Yet this method presents a range of negative side effects such as environmental pollution, harmful health effects for farmers and consumers (pesticide residues), and the risk of the emergence of resistant pathogenic strains (Abdollahzadeh *et al.*, 2015; Asghar *et al.*, 2016; Galani *et al.*, 2020). In view of these serious drawbacks, the development of more environmentally friendly control methods, such as biological control using antagonistic microorganisms like-endophytic fungi, can help to complement current strategies for integrated management of the disease. Endophytes grow asymptomatically in plants tissues in a mutualism and symbiosis relationship; present in both higher and lower plants (Stone *et al.*, 2004; Terhonen *et al.*, 2019; Zhu *et al.*, 2024). Unfortunately in Cameroon, they are still not well known and characterized while most endophytes play an important role in plants' defence mechanisms against parasitic fungi and insects and adverse environmental conditions (Gowtham *et al.*, 2024). They synthesize bioactive compounds which are of great potential in agriculture with their antimicrobial and insecticidal activities (Mahadevamurthy *et al.*, 2016). Endophytes modulate the plant's immune system and increase the production of secondary metabolites by its host plant (Khare *et al.*, 2018). Some of the endophytes like *Trichoderma koningii* and *Alternaria alternata* from maize roots have antagonistic effects on *Fusarium* pathogen (Orole and Adejumo, 2009). Banana endophytes inhibited the growth of *Fusarium oxysporum* f. sp *cubense* and *Colletotrichum guaranicola* (Souza *et al.*, 2013). In addition, an endophytic fungus makes the needles of white spruce less appetizing for the spruce budworm. Such very specific effects are both of great ecological interest and of great economic importance. Diseases control through the use of synthetic chemicals develop harmful environmental and biological problems such as soil, water and air pollution, consumer toxicity, and resistance development by pathogens (Yehouenou *et al.*, 2014; Mondal and Jana, 2015; Adewunmi *et al.*, 2017). Due to these negative effects, biological control of plant diseases through endophytes has been scientifically demonstrated in recent years and it was shown that they could constitute an alternative to chemicals (Xia *et al.*, 2018; Emitaro *et al.*, 2020). The objective of this study was to evaluate the antifungal properties of endophytic fungi isolated from three Cameroonian herbaceous plants namely *Lantana camara, Emilia coccinae* and *Bryophyllum pinnatum* on three pathogenic fungi *Viz*., *Verticillium albo-atrum, Rhizoctonia carotae* and *Phytophthora megakarya*.

MATERIAL AND METHODS

Plant Collection, Isolation and Identification of Endophytic Fungi

Healthy stems and leaves of *L. camara*, *E. coccinae* and *B. pinnatum* were randomly collected from different sites in Dschang (West region of Cameroon). The collected samples were introduced in plastic bags, labelled in the field and carried out to the laboratory for direct fungal isolation (Khan *et al.*, 2007). Healthy samples collected were firstly washed with running water and cut into fragments of 3 to 5 mm in length then, disinfected twice (in 70° alcohol for 2 min and in 4% sodium hypochlorite for 2 min). After disinfection, plant fragments were plated on sterilized potato dextrose agar (PDA) supplemented with chloramphenicol (500 mg/L) and incubated at 22°C (Hallmann *et al.*, 2006). Four days after incubation (DAI), fungal colonies emerging from the fragments were purified on PDA medium and 15 days after purification, mature (fruiting) fungal colonies were identified based on the morphological characters of their mycelium and spores using the standard key of fungal taxonomy (Barnett, 1960; Alexopoulos *et al.*, 1996; Mathur and Kongsdal, 2003; Lane *et al.*, 2012). The pigmentation of the mycelium on the culture medium by the fungus was an important criterion for the pre-selection of potential endophytic fungi. The most common pigmentation of endophytic fungi is creamy, yellow, orange, brown or black and the pigments can be localized in the mycelium (*Aspergillus* sp., *Penicillium* sp.) or diffuse in the culture medium (*Fusarium* sp.) (Saxena *et al.*, 1990).

Evaluation of the Antagonistic Activity of Endophytes Pathogenicity Test of the Three Pathogenic Fungi Used

Pathogenic fungi used to assess the biological/antagonistic activity of endophytes of interest were *Verticillium albo-atrum, Rhizoctonia carotae* and *Phytophthora megakarya*. *V. albo-atrum* and *R. carotae* were isolated from carrots on PDA medium while *P. megakarya* was isolated from cocoa pods on V8 medium. *P. megakarya* is responsible of the black pod disease of cocoa while *V. albo-atrum* causes vascular wilt disease on different tropical crops and *R. carotae* causes carrot root disease. Pathogenicity test was carried out on healthy carrots and cocoa pods by inoculating a 7 day old culture of 5 mm diameter on healthy plant organs.

Antagonistic Activity of Endophytes

A screening of pure colonies of isolated fungal endophytes was first carried out with the aim of selecting endophytic species that could have an antagonistic potential against pathogenic fungi. This screening consists of placing the pathogenic fungus (*V. albo-atrum, R. carotae* and *P. megakarya*) in the centre of a Petri dish containing the PDA medium; then, three fragments of the isolated endophyte were placed at the edge. The cultures were incubated at 25 °C in the dark for five days. After this growth period, endophytes that inhibited the growth of the pathogen were selected for the antagonism test (double culture) also called direct double confrontation (DDC) testing method (Fatima *et al.*, 2015). This method consists of placing a 5 mm diameter disc of the endophytic fungus from a 7-day old culture on the edge of a Petri dish containing the PDA medium, then another disc of the same diameter of the pathogenic fungus is placed at the opposite edge of the Petri dish at a distance of 50 mm between the two discs.

Discs of isolated endophytes and pathogens measuring 0.5 mm were co-cultured at two opposite ends of PDA plates, sealed with parafilm and incubated at 25±2°C for 7 days. Plates containing only pathogens (without endophytes) served as control. Radial growth of pathogenic fungi in the presence or absence of the endophyte was measured after 7 days, and antagonistic percentage (A) was calculated using the following formula: A (%) = CDC-CDT / CDCx100, where CDC represents the colony diameter growth (mm) of the control plate and CDT represents the colony growth diameter of pathogen on the test plate (Abdennabi *et al.*, 2017). According to Nuangmek *et al.* (2008) and Orole and Adejumo (2009), if the antagonistic percentage is: a) less than 30%, the antifungal activity of the endophytes is low; b) between 30 and 50%, the antifungal activity is moderate; c) between 50 and 70%, the activity is high and d) above 70%, the activity is very high.

Measurement of the Radial Growth of Endophytes *Trichoderma harzianum* and *Cercospora* sp.

The choice of these two endophytes for this activity was related to their strong antifungal activity against the three tested plant pathogens (*P. megakarya, R. carotae and V. albo-atrum*). The growth of the endophytes was assessed in a factorial trial where the main factor was the culture medium with three modalities [potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and malt extract agar (MEA)] and the secondary factor was pH with three modalities (5.6, 7 and 9). The choice of these pH levels was due to the fact that a neutral to weakly acidic medium is suitable for fungal growth with an optimum between 5 and 7 (Abubakar *et al.*, 2013). The pH levels of the culture media were adjusted using a 1 N NaOH solution to reduce their acidity before sterilization. The number of repetitions was three. A 10-day-old mycelial disc (5 mm in diameter) was placed in the centre of each Petri dish containing 20 mL of each culture medium and pH level. These Petri dishes were incubated in Lab conditions for 10 days at 25±2°C (Tsopmbeng *et al.*, 2012). The radial growth (G) of the fungus was measured at two perpendicular diameters drawn under the Petri dish according to the following formula: G = (d1+d2)/2 – d0 (Djeugap *et al*., 2011), where d0 is the initial diameter of the mycelial disc which is 5 mm, d1 and d2 are the two growth diameters of the fungus measured on the Petri dish at day i (Pandey and Dwivedi, 1985; Singh *et al.*, 1993).

Data Analysis

Data were subjected to analysis of variance using the General Linear Model procedure and means were separated by Student least significant difference test at 5% using R software (version 3.6.0). Arcsine transformation was carried out for data in percentage prior to analysis (Power and Heavin, 2018).

RESULTS AND DISCUSSION

Endophytic Fungi Associated with the Herbaceous Medicinal Plants

Seven endophytic fungi were identified from the stems and leaves of *L. camara* (Verbenaceae), *E. coccinae* (Asteraceae) and *B. pinnatum* (Crassulaceae), such as:

Aspergillus niger, *Botrytis* sp., *Colletotrichum fructicola*, *Oidium* sp., *Pestalotiopsis microspora*, *Pyricularia* sp. and *Trichoderma harzianum* (Table 1). (Table 1). *Cercospora* sp was the more frequent isolated genus (17.57%) followed by *Botrytis* sp (12.16%). Only one (01) genus of endophytic fungi, *Colletotrichum* sp. (2.7%), was isolated from *L. camara* leaves. From *B. pinnatum* stems, four isolated genera were included *Trichoderma* (28.37%), *Cercospora* sp (14.86%), *Pyricularia* (7.14%) and *Oïdium* (5.41%) were more frequent (Table 1).

Table 1. Isolation percentage of fungal endophytes from three herbaceous medicinal plants (*Emilia coccinae, Lantana camara* and *Bryophyllum pinatum*).

Plant species	Fungal endophytes (code)	Plant organ	N*	Isolation percentage (%)
Emilia coccinae (Ec)	*Cercospora* sp. (CER_LEc)	Leaves	13	17.57
	Pestalotiopsis microspora (PES_SEc)	Stem	2	2.70
	Aspergillus niger (ASP_SEc)		7	9.46
	Botrytis sp. (BOT_SEc)		9	12.16
Lantana camara (Lc)	*Colletotrichum fructicola* (COL_LLc)	Leaf	2	2.70
Bryophyllum pinatum (Bp)	*Oïdium* sp. (OID_SBp)	Stem	5	5.41
	Pyricularia sp. (PYR_SBp)		4	7.14
	Trichoderma harzianum (TRI_SBp)		21	28.37
	Cercospora sp. (CER_SBp)		11	14.86

*Number of purified isolates from each fungal species. CER=*Cercospora* sp., PES= *Pestalotiopsis* sp, ASP = *Aspergillus* sp, BOT= Botrytis sp, COL = *Colletotrichum* sp, PYR = *Pyricularia* sp, TRI= *Trichoderma* sp, S= stem and L=leaf.

All the endophytic fungi isolated in this study have been reported as antagonists of plant pathogens. In fact, medicinal plants are known to harbour various fungi with great antagonistic potential (Kaul *et al.*, 2012; Yu *et al.*, 2014). The present study shows that the three medicinal plants used hosted many fungal endophytes like *T. harzianum, Cercospora* sp and *A. niger* which were previously reported from palm trees, sea grasses, lichens and *Lannea coromandelica* (Li *et al.*, 2007; Stone *et al.*, 2004; Premjanu *et al.*, 2016). It has been shown that *Cercospora* sp isolated from the medicinal plant *Fallopia japonica* was a potential endophyte with a high capacity to produce cercosporenes, guanacastane diterpenes, including a homodimer and a heterodimer, from the crude extract of the fungus (Mohanta *et al.*, 2008; Yu *et al.*, 2014). Also, species from the genus *Colletotrichum* sp, *Oidium* sp were reported as endophytes isolated from *Hedychium acuminatum* and *Camellia oleifera*, with antifungal properties against *Phytophthora capsici* and *Bipolaris maydis,* respectively (Yu *et al.*, 2018; Carrie *et al.*, 2023).

Pathogenicity of Isolates Tested and Antagonism of the Endophytic Fungi during the Direct Confrontation Test with Pathogens

The pathogenicity test with the plant pathogens of interest on carrot and cocoa was positive (Figure 1) and the fungal isolates were suitable for the evaluation of the antagonistic activity of endophytes.

Figure 1. Pathogenicity tests in carrots inoculated with *Verticillium albo-atrum* (A) and *Rhizoctonia carotae* (B) and on the cocoa pod (clone SCA) inoculated with *Phytophthora megakarya* (C) after 7 days.

Endophytic fungi tested showed variable antagonistic activity against the three plant pathogens in the direct confrontation test. Antagonism resulted in the cessation of the growth of the pathogens when in contact with the endophytes. In general, the antagonistic activity of endophytes isolated vary from 31 to 96%, 32 to 82% and 30 to 90% against *P. megakarya, R. carotae* and *V. albo-atrum* respectively (Table 2). Concerning *P. megakarya* and based on the methodology used, endophyte *Aspergillus* sp. (31%) has moderate antifungal activity against this pathogen while *Cercospora* sp. (77%) and *Trichoderma harzianum* (96%) have shown very high activity against that pathogen. As far as *Rhizoctonia carotae* is concerned, the antagonistic activity of endophytes *Pyricularia* sp. (51%) and *Cercospora* sp. (68%) was moderate while the activity of *Trichoderma harzianum* (82%) was very high. Endophytes which developed higher antifungal activity against *V. albo-atrum* were again *Cercospora* sp (74%) and *T. harzianum* (90%). Among all the endophytes tested, *Trichoderma harzianum* and *Cercospora* sp. were the most active against the three pathogens with antagonistic activity varying from 82 to 96% and 68 to 77% for *T. harzianum* and *Cercospora* sp. respectively.

Table 2. Growth inhibition (%)* of pathogenic fungi by endophytes (n=3) isolated from *E. coccinae, L. camara* and *B. pinnatum,* 10 days after the DDC test.

Endophytes	Growth inhibition (%)		
	P. megakarya	*R. carotae*	*V. albo-atrum*
Botrytis sp (Bot_LCs)	-	36.51±0.79^{c}	-
Colletotrichum sp (Col_LCl)	-	35.45±0.81^{b}	38.24±0.77^{d}
Cercospora sp (Cer_ECl)	-	49.74±0.70^{g}	-
Aspergillus sp (Asp_ECs)	-	37.39±0.81^{c}	-
Pestalotiopsis sp (Pes_BPl)	-	32.80±1.06^{a}	-
*Oïdium s*p (Oid_BPs)	-	44.44±0.77^{f}	32.13±1.04^{b}
Pyricularia sp (Pyr_BPs)	-	51.68±0.55^{h}	35.97±0.34^{c}
Aspergillus sp (Asp_LCs)	31.01±1.05^{a}	40.04±0.94^{e}	30.09±0.9^{a}
Trichoderma harzianum (Tri_BPs)	96.82±0.58^{c}	82.73±0.49^{i}	90.77±0.37^{f}
Cercospora sp (Cer_BPs)	77.21±0.49^{b}	68.20±0.55^{k}	74.57±0.39^{e}
Pestalotiopsis microspora (Pes_ECs)	-	38.98±0.69^{d}	-
Mycelia sterile (un-identified) (ECs)	-	52.03±0.63^{h}	-

*Means followed by the same letter in the colunm are not significantly different accordiang to Student test at 5% probability thrsehold. Dash indicate that inhibition percentage is less than 30% therefore their antifungal activity is low ; n= number of repetitions. Col_LCl = *Colletotrichum* sp from *L. camara* leaves, Cer_ECl = *Cercospora* sp from *E. coccinea* leaves, Asp= *Aspergillus* sp, ECs = *E. coccinea* stem, Pes = *Pestalotiospis*, BPl = *B. pinnatum* leaves, Oid = *Oidium* sp, BPs = *B. pinnatum* stem, Pyr = *Pyricularia* sp, Bot = *Botrytis* sp, LCs = *L. camara* stem, Tri = *T. harzianum*, AIs = *Azadirachta indica* stem.

The bioactivity (fungistatic or fungitoxic activity) of *T. harzianum* was fungicidal on *P. megakarya* and *R. carotae*; there was also a significant sporulation and overgrowth of *T. harzianum* on the three pathogens (Figure 2). The bioactivity of *Cercospora* sp. was fungistatic on the three pathogenic fungi with a considerable inhibition zone developed at the contact between the endophytes and the pathogens (Figure 3).

It is known that *Trichoderma* species can compete directly with phytopathogens for space and resources by creating metabolic and antibiotic chemicals that prevent spore development (Tapwal *et al.*, 2011). Moreover, Berber *et al.* (2009) observed that *T. harzianum* and *T. viride* influence the growth and spore germination of *Bipolaris* sp. *T. harzianum* used in this study isolated from the stem of *Bryophylum pinnatum* suppressed mycelial growth by more than 82% compared to the strains used by Mokhtar and Aid (2013); Mokhtar and Dehimat (2012) which suppressed mycelial growth of *Pythium, Phytophthora*, and *Fusarium* by 65%.

Tapwal *et al.* (2011) also showed that growth inhibition of pathogens *Fusarium oxysporum, Rhizoctonia solani* and *Alternaria zinniae* was observed by *T. viride* in Lab conditions both during the dual culture technique and by

using filtrate of this endophyte. Little studies have been carried out on the antagonistic activity of *Cercospora* spp. However, it was established recently that secondary metabolites produced by *Cercospora* spp. (strain ME202-CF) significantly inhibited *in vitro* conidial germination of *Colletotrichum orbiculare* agent of anthracnose in *Trifolium incarnatum* (Ino *et al.*, 2022).

Endophytic fungus *Cercospora* sp. isolated from *Aerva javanica* (an Indian indigenous medicinal plant) was proven to possess diverse bioactive potential (Mookherjee *et al.*, 2020). The mechanism of growth inhibition of pathogens by endophytes may be due to competition for nutrition and space. In fact, *Trichoderma* isolates grow rapidly and can invade the culture medium after 4 days of incubation. The invasion of *Trichoderma* was rapid and intense. Even though mycoparasitism of *Trichoderma* spp. has been implicated in a number of phytopathogenic fungi, each of the antagonist species has a particular ability to eliminate the pathogen. Different modes of action, antibiosis and parasitism can be involved, simultaneously or sequentially (Howell, 2003).

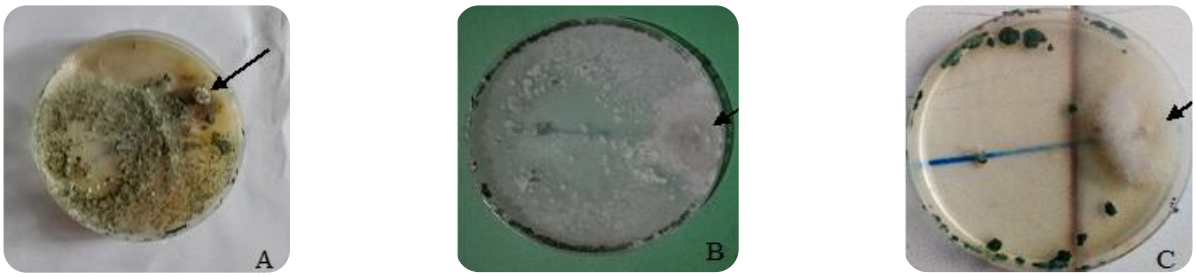

Figure 2. Antagonism of *T. harzianum* against *P. megakarya* (A), *R. carotae* (B) and *V. albo-atrum* (C), 10 days after DDC test. Arrow represents the pathogen. Dense sporulation and overgrowth of *T. harzianum* on the pathogens are visible.

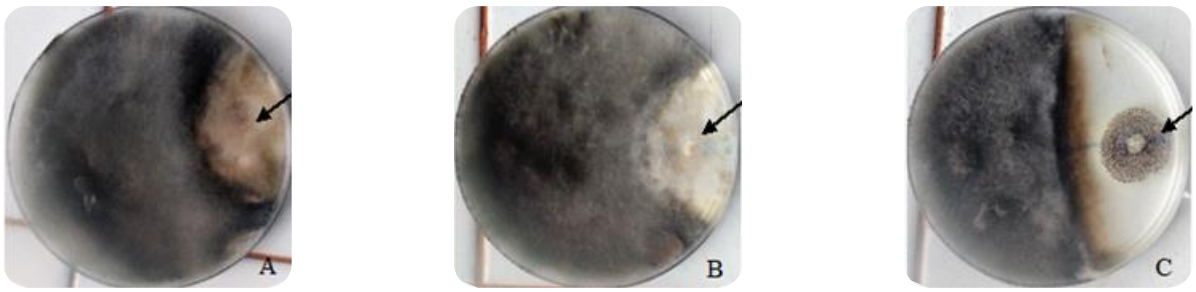

Figure 3. Antagonism of *Cercospora* sp. against *P. megakarya* (A), *R. carotae* (B) and *V. albo-atrum* (C), 10 days after the DDC test. Arrow represents the pathogen.

Influence of Culture Media and pH on the Radial Growth of *Trichoderma harzianum* and *Cercospora* sp.

Analysis of variance shows that the effect of culture media and the pH on the growth of the endophytes was significant at 5%. All the culture media supported the growth of *T. harzianum* and *Cercospora* sp. to various degrees. PDA and SDA were significantly ($p<0.05$) more suitable for the growth of *T. harzianum* compared to MEA. The radial growth was 7.85 and 7.8 cm on PDA and SDA respectively, 7 days after incubation (DAI). There was no statistical difference in the growth of *Cercospora* sp. between the three culture media tested. Radial growth of *Cercospora* sp. was 7.68, 7.76 and 7.82 cm on PDA, MEA and SDA respectively (Table 3).

All the media used in this study were organic media, however, the source of carbon (potato, Sabouraud or malt) differs from one medium to another. In fact, culture media especially PDA are sources of carbohydrates, proteins, lipids and other necessary elements for fungal and bacterial growth (Bonnet *et al.*, 2020). The selection of a nutrient-balanced medium is, therefore, useful for the proper growth of fungal pathogens (Mapuranga *et al.*, 2022). Also, Shahid *et al.* (2011) reported that the best solid medium for the growth and sporulation of *T. longibrachiatum* was PDA showing an excellent average colony diameter (8.57 cm). The pH7 was suitable for the growth of *T. harzianum* followed by pH5 and pH9. There were no significant differences in the radial growth of

Cercospora sp. between pH levels (Table 3). This is in accordance with Kumar and Dubey (2007) who reported that *T. viride* isolate grew better at a slightly alkaline pH of 7.5 rather than a more acidic or alkaline pH. The interaction effect between culture media and pH levels on the radial growth (cm) of *T. harzianum* and *Cercospora* sp also showed differences in radial growth of the two endophytes (Table 4). Radial growth of *T. harzianum* was significantly higher (7.97 cm) on PDA x pH7 interaction, 7 days after incubation (DAI) while the MEA x pH9 give the lowest radial growth (6.85 cm) at the same period. For *Cercospora* sp., a part of PDA x pH5 interaction which gave the significant lower radial growth (7.36 cm), 7 DAI there were no significant differences in radial growth with other interactions (Table 4).

Therefore, the suitable combination of modalities of the two studied factors which gave the higher response is the interaction between PDA and pH7. Comparable results were obtained by Djeugap *et al.* (2023). Indeed, they showed that the suitable radial growth for three isolates of *Fusarium oxysporum* f. sp. *cubense* (banana Fusarium wilt pathogen) (FOC_FBT, FOC_BAN and FOC_KEK) was obtained in the interaction between PDA and pH7 at 18°C.

Table 3. Radial growth (cm) of *Trichoderma harzianum* and *Cercospora sp* at different culture media and pH levels, 7 days after incubation (DAI).

Endophytes	Culture media			pH levels		
	PDA	SDA	MEA	5	7	9
Trichoderma harzianum	7.85a	7.80a	7.48b	7.60b	7.82a	6.49c
Cercospora sp.	7.68a	7.82a	7.76a	7.66a	7.80a	7.79a
LSD (0.05)		0.19			0.17	

[a, b] Means followed by the same letter in the row for culture media or pH levels are not significantly different according to student test at 5% probability threshold.

Table 4. Interaction effect between culture media and pH levels on the radial growth (cm) of *T. harzianum* and *Cercospora* sp. 7 days after incubation (DAI).

Culture media x pH	Radial growth (cm)	
	T. harzianum	*Cercospora* sp
PDA x pH5	7.60[b]	7.36[b]
PDA x pH7	7.97[a]	7.83[a]
PDA x pH9	7.72[b]	7.70[a]
SDA x pH5	7.50[b]	7.60[a]
SDA x pH7	7.80[b]	7.81[a]
SDA x pH9	7.70[b]	7.77[a]
MEA x pH5	7.62[b]	7.68[a]
MEA x pH7	7.55[b]	7.82[a]
MEA x pH9	6.85[c]	7.78[a]
LSD (0.05)	0.21	

[a,b,c] Means followed by the same letter in the column are not significantly different according to student test at 5% probability threshold.

Macroscopic observation of the mycelium of the endophytes on culture media at different pH levels showed that the culture medium does not influence the colour of the mycelium in *T. harzianum* and *Cercospora* sp. (Figures 3 and 4). However, the mycelium (whitish) is dense on pH7 and 9 irrespective to the culture medium (Figure 4).

At pH5, the mycelium of *Cercospora* sp. is whitish with slightly dark filaments (mature filaments) around the centre of the mycelium on all media while at pH7 and 9, the filaments are mostly blackish with a dense dark black halo in the centre of the mycelium which turns reddish on MEA medium at pH9. The mycelium is dense on SDA medium at all pH levels compared to other media (Figure 5).

Similar results were also obtained by Singh *et al.* (2006) and Vinod Tasiwal and Benagi (2009). Sharma *et al.*

(2005) reported that medium, temperature and pH have a profound effect on the growth of fungi. Indeed, pH is a very important parameter for microbes in general, and different species prefer different pH values. Therefore, the pH of the medium strongly affects the metabolism of fungi: at low pH, the protoplasmic membrane is saturated with hydrogen ions, thus limiting the passage of essential cations, while at high pH, it is saturated with hydroxyl ions and the entry of essential anions is limited (Job and Aragno, 1992).

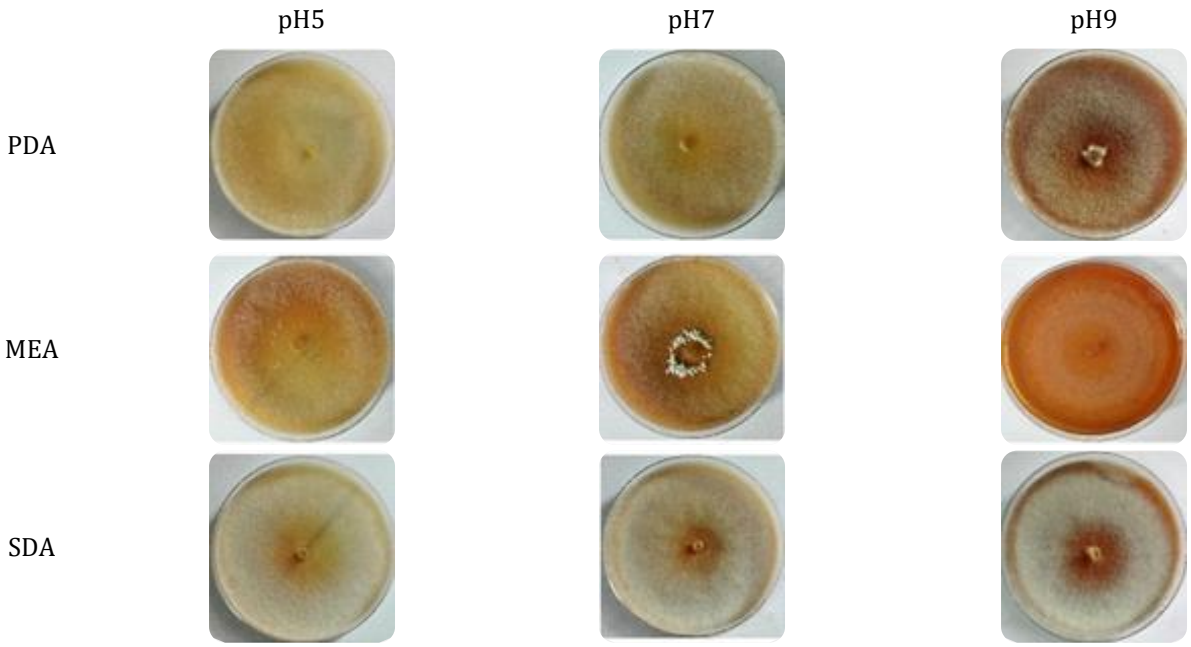

Figure 4. Effect of pH and culture media on the morphological traits of a 7-days old culture of *T. harzianum*.

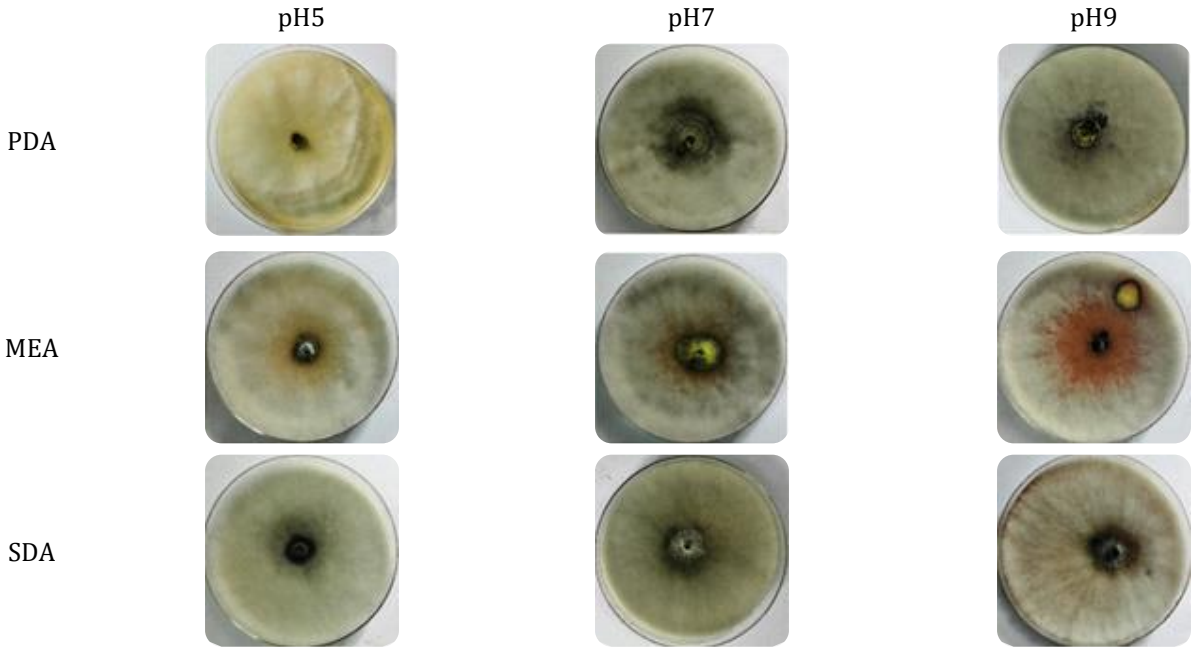

Figure 5. Effect of pH and culture media on the morphological traits of a 7-days old culture of *Cercospora* sp.

CONCLUSION

This study shows that medicinal herbaceous plants (*L. camara*, *E. coccinae* and *B. pinnatum*) harbour numerous fungal endophytes. Among them, *T. harzianum* and

Cercospora sp possess high antagonistic activity and can be used as an alternative in the development of bioactive secondary metabolites in the control of plant diseases such as cocoa black rot disease (*Phytophthora megakarya*), Verticillium wilt (*Verticillium albo-atrum*) and carrot root disease (*Rhizoctonia carotae*), thereby minimizing environmental degradation. The PDA medium at pH7 is suitable for the growth of these endophytes. Future studies should investigate the bioactive molecules produced by these microorganisms for biological control approach measures.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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