Journal of Agricultural and Crop Research
Vol. 10(1), pp. 14-22, April 2022
doi: 10.33495/jacr_v10i1.21.171
ISSN: 2384-731X
Research Paper

Antagonistic action of two species of *Trichoderma* spp. Against *Colletotrichum gloeosporioides*: The causal agent of cashew anthracnose in Benin

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Accepted 13th January, 2022.

Abstract. The biological control of plant diseases by the use of antagonist micro-organisms is a strategy used in integrated pest management strategies in agricultural production. The potential of 3 strains of *Trichoderma harzianum* and 3 strains of *T. pseudokoningii* as biological control agents against *Colletotrichum gloeosporioides* was assessed in this study. The trials were conducted *in vitro* and *in vivo* with 7 and 26 treatments, respectively, utilising a completely randomly randomized design. Antifungal activity of *Trichoderma* species was evaluated *in vitro* against *C. gloeosporioides* mycelial radial growth and conidial germination. *In vivo*, disease incidence and severity were assessed on cashew seedlings inoculated with the following modalities: preventive (no presence of pathogen), simultaneous, and curative (in the presence of pathogen) modality. *In vitro*, *T. harzianum* and *T. pseudokoningii* inhibited the growth of *C. gloeosporioides* by up to 64.31 and 71.41%, and its germination rate by up to 74.38 and 78.78%, respectively. *In vivo*, therapy with *T. harzianum* and *T. pseudokoningii* significantly reduced the incidence and severity of anthracnose ($P < 0.0001$), regardless of the inoculation modality. *T. harzianum* reduced the incidence and severity of the disease by more than 92 and 97%, respectively. *T. pseudokoningii* reduced the disease incidence by up to 85% in curative and simultaneous inoculation modalities, and by up to 96%when used in preventive modality. The three modalities of *T. pseudokoningii reduced the* disease's severity by up to 95%.These findings imply that *T. harzianum* or *T. pseudokoningii may* be suitable for effective biological control of cashew anthracnose.

Keywords: *Anacardium occidentale*, Biological control, Anthracnose.

INTRODUCTION

Cashew tree (*Anacardium occidentale*) orchards cover about 5.9 million hectares worldwide (FAO, 2017). The crop contributes to global economic, social, and environmental challenges (Venkattakumar, 2009; Adelgbe *et al.*, 2015). Cashew nuts production is a potential source of foreign exchange for the producing countries. Cashew is Benin's second-largest export crop, after cotton (Afouda *et al*., 2013). It is grown on nearly 28,567.7 hectares of land and generates 117,494 tonnes per year (Adégbola and Crinot, 2016). Benin exported

Table 1. Strains of the pathogen and the antagonist fungi.

Fungi	IITA registration number	IITA registration code	LDC registration number	LDC registration code
T. harzianum	147	AG3		
T. harzianum	208	AG4		
T. harzianum	210	AG7		
T. pseudokoningii	207	AG1		
T. pseudokoningii	209	AG6		
T. pseudokoningii	2011	AG9		
C. glosporioides			11/2017	Cgldc1G

73,092 tons of cashew nuts to India and Vietnam in 2016(FAO, 2017). Around 122,911 persons in the country earn from cashew nuts (Adégbola and Crinot 2016). Since 2011, planting areas have increased over the years, but yields have not kept pace. In Benin, the average production per cashew tree remains very low, at 3 to 6 kg, compared to 10 to 15 kg per tree recorded by other countries (Tandjiékpon 2010). These low yields per tree significantly reduce producers', processors', and traders' incomes as well as government'. One restriction that accounts for the low yields is anthracnose infection caused by *Colletotrichum gloeosporioides* (Afouda *et al*., 2013). In 2000, yield losses due to cashew anthracnose were 40% in Brazil (Wonni *et al*., 2017) and from 50 to 70% in Mozambique (Milheiro and Evaristo, 1994). Afouda *et al*. (2013) reported that the average incidence of anthracnose was about 35.24%, with the highest incidence since the district of Savè (90%) and Tchaourou (75%).

Disease management is currently limited to the application of chemical pesticides. Copper oxychloride, triadimenol, hexaconazole, and trifloxystrobin have been reported to be effective on cashew anthracnose (Uaciquete *et al.*, 2013). Likewise, Tonon *et al.* (2017) proved the effectiveness of Mancozeb 80 WP and Chlorothalonil-Carbendazime 65 SC in controlling cashew anthracnose disease. However, the chemical resistance of the pathogens has been revealed (Hamdache *et al.*, 2010).

To prevent the resistance of *C. gloeosporioides* to fungicides andto protect the environment and cashew products against residue hazards, biological control is emerging as a sustainable alternative method. One of these methods is the use of antagonistic bacteria and fungi. *Trichoderma* species were the most frequently reported as potential biological control agents against crop pathogens among antagonistic fungi. *Trichoderma's* antagonistic effect is manifested by a coil of hyphae around filaments of the pathogenic fungus. The fungi produce β-1.3-glucanase and chitinase enzymes, which cause lysis (Singh *et al.*, 2018).Several species of *Trichoderma* are reported to inhibit crop pathogens. *T. harzianum* inhibited *Botrytis cinerea* (Elad and Kapat 1999) and *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *T. viride* has reduced the development of *Ascochyta rabiei* (Benzohra *et al.*, 2011). *T. harzianum* seed treatment reduced the infection rate of *Rhizoctonia bataticola* by up to 28% (Elad and Kapat, 1999).

T. harzianum has been extensively studied as a potential bio control agent for a variety of crop diseases. However, there is no evidence of the effect of *Trichoderma* species against cashew anthracnose. The purpose of this study is to determine whether *T. harzianum* and *T. pseudokoninigii* have an antagonistic effect on *C. gloeosporioides*.

MATERIALS AND METHODS

Fungi strains

The Entomopathology laboratory of IITA-Benin donated the strains AG3, AG4, and AG7 of *T. harzianum* and AG1, AG6, and AG9 of *T. pseudokoningii. C. gloeosporioides* strain Cgldc1G was donated by INRAB's Laboratory of Crop Protection (LDC) (Tonon *et al*., 2017) (Table 1).

Antifungal activity of *Trichoderma* strains *in vitro*

Direct confrontation between the pathogen and the antagonist

To evaluate the inhibition of mycelial growth of the pathogen by *Trichoderma* strains, two 5 mm diameter discs, one from a6-days-old culture of *C. gloeosporioides* (Cgldc1G) and another from the culture of the same age of *T. harzianum* strains AG3, AG4, AG7 and *T. pseudokoningii* AG1, AG6, AG9, were each inoculated on a Potato Dextrose Agar (PDA) plate. The two discs were placed 3 cm apart symmetrically with respect to the center of PDA plate. The control plate was inoculated in the centre with a 5 mm mycelial disc of *C. gloeosporioides* (Singh *et al.*, 2018).

To evaluate the inhibition of conidial germination of the pathogen by the antagonists, a conidial suspension of 10^3conidia/ml of the pathogen (Cgldc1G) and that of the

antagonists (*T. harzianum* and *T. pseudokoningii*) at the same concentration was prepared from the7-days-old cultures. One hundred micro liters (100 µl) of the pathogen suspension and 100 µl of the antagonist suspension were simultaneously spread on the PDA plate (Singh *et al.*, 2018).

Indirect confrontation between the pathogen and the antagonist

The methodology of Dennis and Webster (1971) was used to evaluate the effects of volatile substances produced by *Trichoderma* on mycelial growth and conidial germination of *C. gloeosporioides*.

Effect on mycelial growth

A mycelial disc of 5 mm in diameter of *C. gloeosporioides* and one each of *T. harzianum* and *T. pseudokoningii* strains were cultured separately in the centre of the PDA plates. The plate with the pathogen's disc was spilled on the one carrying the antagonist's disc. The two plates were juxtaposed and sealed with par a film to avoid contamination and any escape of gas. In the control plate, the antagonist was replaced by a single PDA disc. The inhibitory effect of the antagonist was evaluated by measuring the diametrical growth of the cultures.

Effect on the germination of Conidia

To evaluate the effect of the volatile substances of the antagonist on the germination of the pathogenic conidia, a culture of 24 hours from a conidial suspension of 10^3 conidia/ml of each *Trichoderma* strain was juxtaposed and sealed with that of 0 day of *C. gloeosporioides* prepared at the same volume and concentration as described above. The juxtaposed plates were incubated at 28°C in the dark for24hours. The inhibitory effect of the antagonist was evaluated by counting the colony of the pathogen.

In vivo antifungal activity of *Trichoderma*

Production of cashew seedlings in the greenhouse

The cashew seeds were obtained from the Agricultural Research Center of Central Benin (CRA-center) of INRAB. After disinfecting the cashew nuts by soaking them in 70% ethanol for 3 min, followed by triple-rinsing with sterile water, they were sown in a greenhouse in pots containing 1 kg of soil sterilized at 80°C for 1 hour. The pots were daily watered with tap water for 6 weeks to have young plants of 5 to 6 leaves.

Inoculation and inoculum preparation

Conidial suspensions were prepared from the 7-day-culturesof the six *Trichoderma* strains and for the *C. gloeosporioides* isolate. These suspensions were adjusted with sterile distilled water containing 0.05% Tween20 to a concentration of 10^6 conidia/ml.

Young cashew plants were individually inoculated in a greenhouse by spraying20 ml of the pathogen or the antagonist inoculum on their leaves with a small sprayer. Five seedlings were inoculated by isolate/strain. The inoculated seedlings were each covered with a polyethylene bag in a greenhouse for 48 hours to keep the relative humidity high around the plants. Symptoms were evaluated weekly starting from the7th day after inoculation. Four evaluations were carried out.

Experimental design

The experiments were conducted in a randomized complete design with seven alternatives *in vitro* on PDA plates and 26 *in vivo* on seedlings in the green house. *In vitro*, the alternatives were: single culture of Cgldc1G and culture of Cgldc1G with each of the *Trichoderma* species strains.

In vivo, the following combination of conidial suspensions was sprayed on cashew seedlings: for the preventive treatment modality, the pathogen was applied 24 hours after the antagonist; for curative treatment, the antagonist was applied as soon as symptoms of anthracnose appeared on leaves; and for simultaneous treatment, the pathogen and the antagonist were applied simultaneously.

Young cashew plants treated with sterile distilled water were considered as controls. For the positive control, young plants were sprayed with each of the fungal suspensions (Cgldc1G; AG3; AG4; AG7; AG1; AG6; AG9). All suspensions were supplemented with 0.05% Tween20. Experiments were repeated 3 times.

Data collection and analysis

Inhibition of mycelial growth

Seven days after incubation, the inhibition percentage (IM) of mycelial growth of the pathogen by the antagonists was calculated according to the following formula of Sundar *et al.* (1995):

$$IM = \frac{DC - DPA}{DC} \times 100$$

with DC = the control's mycelial diametric growth; DPA = mycelial diametric growth of the pathogen in the presence of the antagonist.

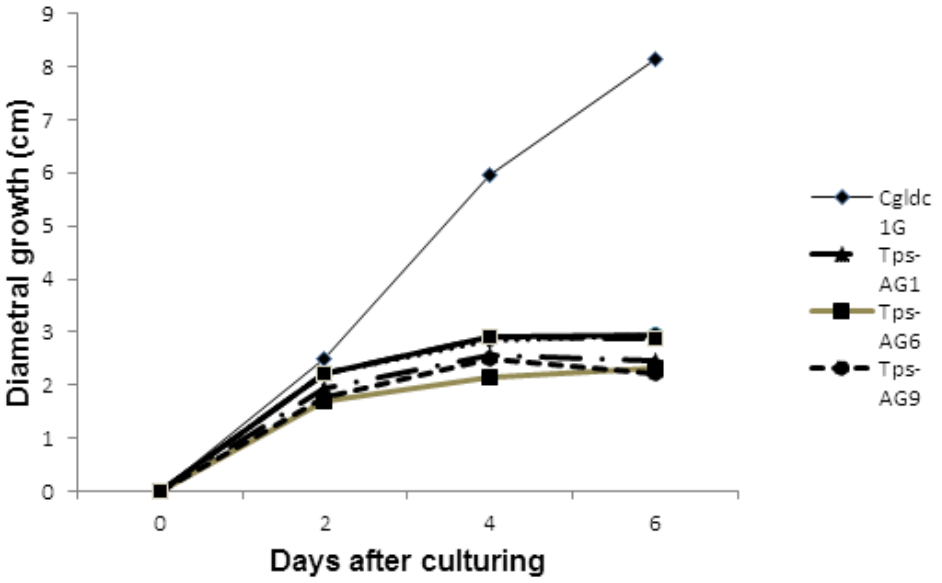

Figure1. Mycelial growth of *C. gloeosporioides* in direct confrontation with *Trichoderma* strains. Th = *T. harzianum;* Tps = *T. pseudokoningii*

Inhibition of conidial germination

Twenty-four hours after inoculation, the inhibition of conidia germination (IG) was determined according to the following formula:

$$\mathbf{IG} = \frac{\mathbf{NC} - \mathbf{NPA}}{\mathbf{NC}} \times \mathbf{100}$$

NC denotes the number of germinated conidia in the plate in the absence of an antagonist;
NPA = number of germinated conidia in the presence of the antagonist.

The percentage of non-germinated conidia was deducted.

Assessment of Anthracnos Incidence and Severity

The incidence was calculated as a percentage of diseased plants after inoculation. The severity was evaluated per seedling on the last five leaves of 10seedlings by treatment. The severity index (percentage of leaf area covered with lesions) was calculated by using the weighted mean of the percentage of leaves with no spots, small spots, middle blight, large blight, and dry leaves according to the following formula:

Si = (0 × NS + 1 × SS + 2 × MB + 3 × LB + 4 x DL)/10

where NS, SS, MS, LB, and DL represent the percentage of leaves with no spots, small spots, middle blight, large blight, and dry leaves, respectively.

The mean severity index of three replicates with 10 seedlings each corresponding to one treatment on all evaluation dates was calculated and used to determine the areas under the severity index progress curves (AUSiPC) of treatment using the evaluation dates according to the following formula:

$$AUSiPC = \sum_i \frac{(S_i + S_{i-1})(t_i - t_{i-1})}{2}$$

Where Si is the mean severity index at time ti, and t corresponds to days after inoculation (Shaner and Finney, 1977; Jeger and Viljanen-Rollinson, 2001).

The one-way analysis of variance (ANOVA) was performed using SAS Software version9.2 S. Analyses were focused on the diameter of mycelial growth, the number of germinated spores, the incidence, and the severity index of anthracnose. The means were compared with the Tukey test at 5%.

RESULTS

Antagonist activities of *Trichoderma in vitro*

Direct confrontation

Six days after incubation, the different strains of *Trichoderma* showed good inhibitory activity against *C. gloeosporioides* Cgldc1G through the appearance of an inhibition zone and stopping of the pathogen's growth (Figure 1). The growth diameters of the *C. gloeosporioides* strain in direct confrontation with the six *Trichoderma* strains were all less than 3 cm, while *C.*

Table 2. Inhibition of mycelial growth and conidia germination of *C. gloeosporioides* by *Trichoderma* strains in direct confrontation during 6 days of incubation.

Trichoderma species	Strains code	Inhibition zone of *C. gloeosporioides* by *Trichoderma* (%)	Non-germinated conidia (%)
T. pseudokoningii	AG1	69.72 ± 1.40[a]	65.95 ± 5.51[c]
	AG6	71.67 ± 2.94[a]	94.17 ± 1.61[a]
	AG9	72.88 ± 1.89[a]	75.47 ±5.77[bc]
	Mean	71.42 ± 1.24[a]	78.53 ± 3.82[a]
T. harzianum	AG3	64.08 ± 2.94[b]	73.16 ± 2.81bc
	AG4	63.89 ± 1.55[b]	58.94 ± 3.98[c]
	AG7	64.98 ± 2.94[b]	89.61 ± 1.99[ab]
	Mean	64.31 ± 0.43[b]	73.91 ± 3.46[a]
Probability		0.0206	0.0001

[a]Means of 30 plates ± SE; significance according to Tukey test with P = 0.05.

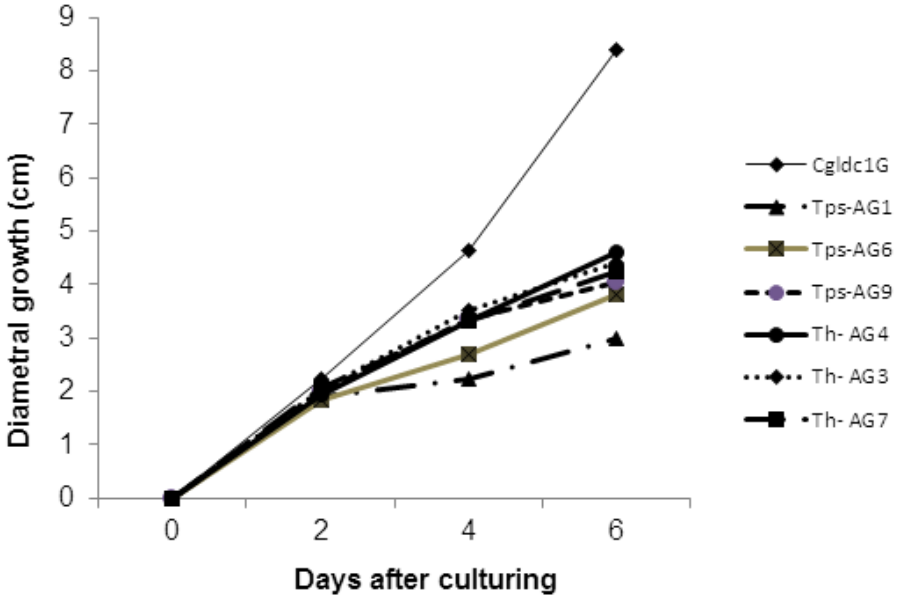

Figure 2. Mycelial growth of *C. gloeosporioides* in indirect confrontation with *Trichoderma* strains; Th=*T. harzianum;* Tps **=** *T. pseudokoningii.*

gloeosporioides grew up to 8.16 cm. The inhibition due to *T. pseudokpningii* ranged from 69.71to 72.88% and that of *T. harzianum* was 63.89 to 64.98%. (Table 2). The diameter of mycelial growth of *C. gloeosporioides* and *Trichoderma* strains resulted in a highly significant difference (P <0.001). The diameter of mycelial growth was significantly higher for *C. gloeosporioides* plates. Within *Trichoderma strains*, no significant difference was obtained regarding their antagonistic activity (Figure 2).

Germination results were similar to those of the mycelial growth obtained. Conidia from *Trichoderma* strains have inhibited the germination of *C. gloeosporioides conidia* (Table 2).The number of germinated conidia of *C. gloeosporioides* was significantly lower for the plate in confrontation with both species of *Trichoderma* (P <0.001). The percentage of inhibition of conidial germination by *T. harzianum* and *T. pseudokoniningii* ranged from 73.36 to 90.03% for *T. harzianum* and from 65.65 to 76.33% for *T. pseudokoniningii*.

Indirect confrontation

In an indirect confrontation, *Trichoderma* species inhibited the mycelial growth of *C. gloeosporioides.*

The diameter of mycelial growth was significantly lower in plates where *C. gloeosporioides* was in confrontation with each of the six *Trichoderm*a strains (P < 0.001), compared to the control plates.

Table 3. Inhibition of mycelial growth and conidia germination of *C. gloeosporioides* by *Trichoderma* strains in indirect confrontation during 6 days of incubation.

Trichoderma species	Strains code	Inhibition zone of the pathogen by the antagonist (%)	Non-germinated conidia (%)
T. pseudokoningii	AG1	64.19 ± 4.31[a]	**100** ± 0.00[a]
	AG6	54.66± 4.77[ab]	100 ± 0.00[a]
	AG9	51.98 ± 4.53[ab]	100 ± 0.00[a]
	Mean	56.94 ± 2.77[a]	98.12 ± 1.12[a]
T. harzianum	AG3	47.62 ± 2.51[ab]	98.24 ± 2.14[a]
	AG4	45.34 ± 5.24[b]	87.36 ± 4.20[a]
	AG7	49.50 ± 3.11[ab]	94.14 ± 3.06[a]
	Mean	47.49 ± 2.11[b]	98.12 ± 1.12[a]
Probability		**0.0479**	**0.03765**

[a]Means of 30 plates ± standard error; significance according to Tukey test with P = 0.05.

The strains of *T. pseudokoningii* and those of *T. harzianum* reduced the mycelial growth of *C. gloeosporioides* by 56.94 and 47.48%, respectively (Table 3).

The germination of *C. gloeosporioides* was inhibited by up to93.92 and 100% by *T. harzianum* and *T. pseudokoningii,* respectively (Table 3). The number of germinated conidia of *C. gloeosporioides* was significantly lower in plates in confrontation with the antagonists (P <0.001).

Antagonist activities of *Trichoderma in vivo*

Incidence and severity of Anthracnose

The incidences and severities were significantly higher on the cashew seedlings sprayed with *C. gloeosporioides* than on those sprayed with *C. gloeosporioides* and the antagonist *T. harzianum* or *T. pseudokoningii* (P <0.0001). Incidence and severity were 85.33 and 73.85%, respectively, on cashew seedlings sprayed with *C. gloeosporioides*. In the seedlings sprayed with the antagonists, and irrespective of the modality of the antagonist spraying, the incidence and severity of anthracnose were up to 21.33 and 5.95%, respectively. No significant difference was observed between seedlings sprayed only with the antagonist strains. The severity was very low when seedlings were sprayed with *T. harzianum* strains. When *Trichoderma* was applied, the incidence and severity of cashew anthracnose were reduced by more than 97 and 99%, respectively (Table 4).

Cashew seedlings sprayed with sterile water and those sprayed with *T. harzianum* and *T. pseudokoningii* strains caused no symptoms, while on those sprayed with *C. gloeosporioides,* an incidence of more than 85% was recorded (Table 5).

DISCUSSION

Antagonist activity of *Trichoderma* strains *in vitro*

The tested strains of *Trichoderma* have an inhibitory capacity against the mycelial growth of the pathogen *C. gloeosporioides.* In direct and indirect confrontation, *T. harzianum* and *T. pseudokoningii* invaded *C. gloeosporioides* and inhibited its growth after 6 days of cohabitation. These results show that the tested *Trichoderma* species are potential agents for biological control of *C. gloeosporioides,* thus confirming the antagonist effect of *Trichoderma* on plant pathogens, as reported by Schirmbock *et al.* (1994), Saba *et al.* (2012) and Lurdes (2014). Also, Kapoor *et al*. (2010) demonstrated that *T. viride* reduced the mycelial growth of *Fusarium oxysporum* f. sp. *udum* by up to 87.77% on PDA plates.

Singh *et al.* (2018) revealed the reduction of the mycelial growth of *Bipolaris sorokiniana* by *Trichoderma*. The invasion of *C. gloeosporioides* colonies by the strains of both *Trichoderma* species observed in the present study indicates the high level of mycoparasitic property of *Trichoderma,* as was reported by Benzohra and Megateli (2017). Similar effects of pathogen colonisation by *Trichoderma* have recently been reported by Benzohra *et al.* (2011). The property of the *Trichoderma* species to produce inhibition zones *in vitro* shows that this fungus produces harmful substances that constrain the growth of *C. gloeosporioides*. Harmful substances produced by *Trichoderma* have been reported as a treatment for crop pathogens. According to Elad (2000), *T. harzianum* attacks phytopathogenic fungi through the antibiotics and mycoparasitic substances it produces. *Trichoderma* develops appressoria that attach to the surfaces of pathogenic fungi and produces pecific enzymes that dissolve their cell wall (Almeida *et al*., 2007; Jeger and Viljanen-Rollinson, 2001). Kucuk *et al.* (2007) revealed

Table 4. Incidence and severity of anthracnose of cashew seedlings inoculated with *C. gloeosporioides* sprayed with *Trichoderma* strains.

Modality of inoculum application	Fungi	Isolate/ Strains code	Incidence (%)	Incidence Reduction (%)	Severity (%)	Severity Reduction (%)
Curative	*C. gloeosporioides*	Cgldc1G	85.33 ± 4.12^{a}	-	73.85 ± 5.89^{a}	-
	T. pseudokoningii	AG1	21.33 ± 6.89^{b}	75 ± 5.12	5.95 ± 2.18^{b}	92.16±3.12
		AG6	13.33 ± 5.40^{bc}	84.38 ± 0.14	4.32 ± 1.33^{b}	95.85±1.14
		AG9	5.33 ± 2.36^{bc}	93.75 ± 4.04	1.87 ± 0.27^{b}	99.23±0.16
		Mean		84.38 ± 9.37		95.74±3.54
	T. harzianum	AG4	1.33 ± 1.33^{bc}	98.44 ± 6.11	0.12 ± 0.70^{b}	99.08±0.14
		AG3	1.33 ± 1.33^{bc}	96.88 ± 0.56	0.12 ± 0.70^{b}	99.69±0.09
		AG7	13.33 ± 5.40^{bc}	98.44 ± 14.60	3.15 ± 1.99^{b}	94.31±1.08
		Mean		97.92±0.90		97.69±2.95
Preventive	*T. pseudokoningii*	AG1	2.67 ± 2.66^{bc}	96.88 ± 2.04	0.23 ± 0.11^{b}	99.85±0.04
		AG6	1.33 ± 1.33^{bc}	98.44 ± 0.31	0.12 ± 0.23^{b}	99.69±0.06
		AG9	5.33 ± 4.12^{bc}	93.75 ± 8.22	0.70 ± 0.81^{b}	98.92±2.15
		Mean		96.36±2.39		99.49±0.50
	T. harzianum	AG4	5.33 ± 4.12^{bc}	93.75 ± 4.14	0.82 ± 2.56^{b}	96.46±3.12
		AG3	12.00 ± 3.26^{bc}	85.94 ± 7.06	3.15 ± 1.05^{b}	95.85±1.16
		AG7	2.67 ± 1.81^{bc}	96.88 ± 0.16	0.23 ± 0.11^{b}	99.85±0.07
		Mean		92.19±2.21		97.39±2.15
Simultaneous	*T. pseudokoningii*	AG1	8.00 ± 6.70^{bc}	90.63±1.16	2.68 ± 1.74^{b}	97.54±4.02
		AG6	14.67 ± 7.16^{bc}	82.81±2.14	5.13 ± 2.67^{b}	93.23±3.23
		AG9	13.33 ± 6.37^{bc}	84.38±3.02	3.15 ± 1.70^{b}	95.85± 5.04
		Mean		85.94±4.14		95.54±2.17
	T. harzianum	AG4	4.00 ± 4.00^{bc}	95.32±0.12	0.58 ± 0.58^{b}	99.23±1.06
		AG3	1.33 ± 1.33^{bc}	98.44±7.13	0.00 ± 0.00^{b}	100±0.04
		AG7	0.00 ± 0.00^{c}	100 ± 0.04	0.00 ± 0.00^{b}	100±0.03
		Mean		97.92±2.38		99.74±0.44
Probability				0.0001		0.0001

Means followed by the same letter in the column are not significantly different according to Tukey test at P = 0.05.

Table 5. Sanitary status of cashew seedlings inoculated with *Trichoderma* strains at four weeks after inoculation.

Treatments	Anthracnose incidence (%)
C. gloeosporioides (Cgldc1G)	85.33 ± 4.12[a]
T. pseudokoningii (AG1)	0.0 ± 0[b]
T. pseudokoningii (AG6)	0.00[b]
T. pseudokoningii (AG9)	0.00[b]
T. harzianum (AG3)	0.00[b]
T. harzianum (AG4)	0.00[b]
T. harzianum (AG7)	0.00[b]
Sterile water	0.00[b]

that β-1,3-glucanase and chitinase enzymes can hydrolyze the cell walls of pathogenic fungi.

In indirect confrontation, *T. pseudokoningii* and *T. harzianum* inhibited the germination of *C. gloeosporioides* conidia and reduced their mycelial growth. Hibar *et al*. (2005) demonstrated that *Trichoderma* secretes volatile substances that can reduce or stop the development of a pathogen. Howell (2003) and Harman *et al.* (2004) showed that the volatile substances secreted by *T. harzianum* and *T. viride*were glio-viridin and glio-toxin, which are antibiotics that inhibit the development of many phytopathogenic Deuteromyces.

There was no significant difference between the effectiveness of *T. harzianum and T. pseudokoningii* strains. Thus, these species of *Trichoderma* may be used to manage cashew anthracnose. The non-significant variability in the inhibition zone among *Trichoderma* strains indicates that the antibiotic production depends on strains, as previously shown for *Bacillus* spp. (Landa and Mavrodi, 2003).

Antagonistic activity of Trichoderma strains *in vivo*

The results revealed that all the strains of both species of *Trichoderma* showed evident antagonistic activity against *C. gloeosporioides*, *T. harzianum* and *T. pseudokoningii* have effectively reduced the incidence and severity of anthracnose for preventive, curative, and simultaneous treatments. The protective action of both *Trichoderma* species against anthracnose on cashew seedlings was not significantly different. The six strains of *Trichoderma* were equally effective for preventive, curative and simultaneous spraying modalities. Al-Mughrabi (2008) observed that the application of *Trichoderma* sp. on potatoes reduced blight sensitivity to the pathogen *Phytophthora infestans*. They observed that the decreasing effect of the disease resulted from the germination of *Trichoderma* conidia on the leaf surface of cocoa, which inhibited *P. megakarya*. These observations could explain the inhibition of *C. gloeosporioides* in the present study. This terminative property of *Trichoderma* conidia would stimulate the resistance mechanisms of the plant to the penetration and spread of the pathogen into the leaves of cashew. Our results support those of Hamdia and Kalaivani (2014), who showed that *Trichoderma* spp. provided good protection against rice leaf blight caused by *Magnaporthe grisea* and that of Hjeljord *et al.* (2001), who found that application of *T. harzianum* on strawberry leaves reduced *Botrytis cinerea* infections by more than 85%.

The present study reveals that the two *Trichoderma* species tested have an antagonistic effect. This confirms the potential biological control properties of *Trichoderma* reported by Benzohra and Megateli (2017).

CONCLUSION

This study revealed that *T. harzianum* and *T. pseudokoningii* were effective against *C. gloeosporioides,* causing cashew anthracnose. The antifungal activity of both antagonist fungi against *C. gloeosporioides* did not differ significantly. The protective and curative effects of both species of *Trichoderma* observed make them potential agents for biological control in an IPM strategy.

Conflict of interest

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to CORAF/WECARD for the funding. They are also grateful to INRAB (Institute National des Recherches Agricoles du Bénin) and LDC (Laboratoire de Défense des Cultures) for the technical platform.

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