National Institute of Agricultural Research of Benin (INRAB)
@@@@@@
National Agricultural Research Center based in Agonkanmey (CRA- Agonkanmey)
@@@@@@

Laboratory for Supporting the Defense of Cultures (LADC)

Trichoderma species spp . against *Colletotrichum gloeosporioides* , causal agent of cashew anthracnose

Directed by Dr **TONON HOUNDAHOUAN Dénis**

Tel: +22967606715; Email: houndahouan.denis@gmail.com

Plan

- General introduction
- Goals
- Materials and methods
- Results
- Conclusion

General introduction

General introduction (1/4)

World cashew production: 5,932,507 t on 5,972,724 ha (FAO, 2020)

National production: 88,000 t in 2017 on 254,000 ha (Tonon et al., 2017)

Economic contribution: 24.87% to agricultural export income and 7% to agricultural GDP (Adégbola and Crinot , 2016)

Fight against soil erosion (Tandjiékpon) *et al.* , 2010)

Agricultural workers: 122,911 people in Benin (Adégbola and Crinot , 2016)

General introduction (2/4)

Symptoms of anthracnose

Figure 1 : Leaves attacked by anthracnose

Figure 2 : Young cashew branch showing necrosis and cankers caused by anthracnose

General introduction (3/4)

Figure 3: Inflorescence attacked by anthracnose

Figure 4 : Cashew plantation attacked by anthracnose

General introduction (4/4)

Yield losses

- Mozambique: 50%-70% (Milheiro and Evaristo,1994)
- Uganda: 40%-56% (Kiwuso *et al.* , 2010)
- Brazil: 40% (Topper, 2002).
- Benin: 52-72% (Tonon et al., 2018).

Impacts

- 35.24% average in Benin
- 90.9% in Savè and 75% in Tchaourou (Afouda *et al* ., 2013).

Fungicides effective against anthracnose: copper oxychloride, hexaconazole and trifloxystrobin Uaciquete *et al* . (2010).

In Benin, no biological control method is yet available to reduce yield losses due to cashew

Objectives (1/1)

General objective

Evaluate the antagonistic action of *Trichoderma* spp . against *C. gloeosporioides* , causal agent of cashew anthracnose. in South Benin

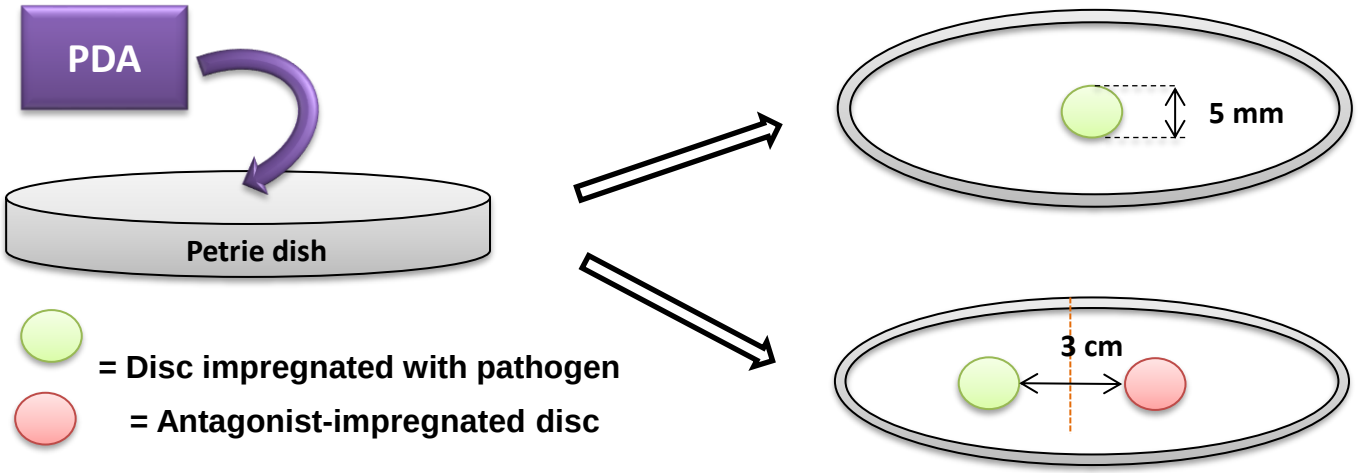
- To evaluate *in vitro* the antagonistic power of *T. harzianum* and *T. pseudokoningii* against *C. gloeosporioides*
- To evaluate *in vivo* the antagonistic power of *T. harzianum* and *T. pseudokoningii* on the incidence and severity of *C. gloeosporioides attacks*

Materials and methods (1/9)

- ***Trichoderma* strains *in vitro***

Strains of antagonists used (AG3, AG4, AG7) and (AG1, AG6, AG9)

❖ ***Direct confrontation: on mycelial growth***



- After 6 days of incubation at 28°C and in the dark, the percentage of inhibition was evaluated according to the formula of Sy (1976):

Materials and methods (2/9)

❖ ***confrontation on spore germination between the pathogen and the antagonist***

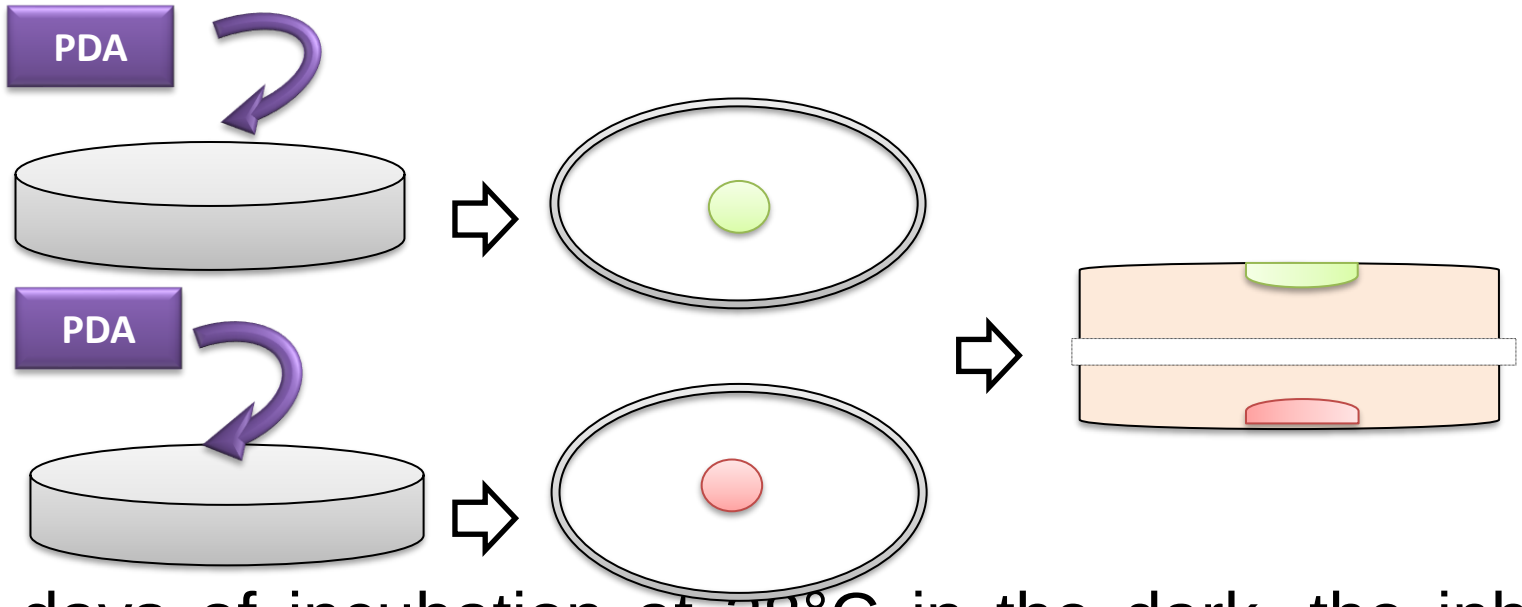
- Inoculum of 10^{3} conidia/ml of pathogen and antagonists
- Spread successively 100 µl of the two suspensions in a dish containing PDA

After 24 h of incubation at 28°C, the % inhibition of *IG germination* of conidia of the pathogen was determined according to the formula of Sy (1976)

Materials and methods (3/9)

- **Indirect confrontation: on mycelial growth**

❖ ***Trichoderma* strains on mycelial growth and conidial germination of *C. gloeosporioides***



- After six days of incubation at 28°C in the dark, the inhibition of mycelial growth was estimated according to the formula of Sy (1976)

Materials and methods (4/9)

❖ conidial

- *of Trichoderma* conidial suspension (10^3 conidia/ml) were plated on PDA and incubated at 28 °C.
- After 48 hours of incubation , 200 µl of conidial suspension of *C. gloeosporioides* were spread on PDA.
- The bottoms of the two boxes were juxtaposed, with the antagonist's at the bottom.
- After incubation at 28 °C for 24 h in the dark, germination inhibition was estimated as before.

Materials and methods (5/9)

❖ *Trichoderma* strains *in vivo*

Plant material

- Nuts collected from cashew producers and nurseries.
- Planting the plants
- Nuts soaked in 70% ethanol for 3 minutes.
- Tri -rinse with EDS.
- Treated nuts sown in pots containing 1 kg of soil.
- Pots placed in a greenhouse and watered every day with tap water until seedlings have 4 to 5 leaves, or 6 weeks old.

Materials and methods (6/9)

- **conidial suspension and inoculation**

- Six strains of *Trichoderma and the C. gloeosporioides* isolate were cultured.

- Preparation of inocula from 6-day cultures.

- Suspensions adjusted with EDS containing 0.05% Tween 20 so as to have 10^{6} conidia/ml for antagonists and for pathogens.

Materials and methods (7/9)

Leaves and stems of each seedling ➔ inoculated with 20 ml of inoculum ➔ “Harry Brand sprayer ”

- Treated controls : ➔ sterile distilled water, ➔ Cgldc1G suspension ➔ *Trichoderma* strains .
- 5 seedlings/treatment repeated 3 times

Materials and methods (8/9)

Symptom assessment

- Symptom assessment was performed every 7 days starting 7 days after inoculation for 28 days. Four assessments were performed.
- Disease severity based on % diseased leaf area was assessed using the following scale:

0: (ST) no leaf spot 0%

1: (PT) small leaf spots [1% - 10%]

2: (MT) medium leaf spot [1 -25%]

3: (LT) large leaf spot [25-50%]

4: (FM) Dead Leaf [50 -100%].

Materials and methods (9/9)

▪ Statistical analysis

- One-way analysis of variance classification in SAS version 9.2 software was used.
- It was based on the diameter of mycelial growth, the number of germinated spores, the incidence and severity of anthracnose on seedlings.
- Means were compared using the Tukey test at the 5% threshold.

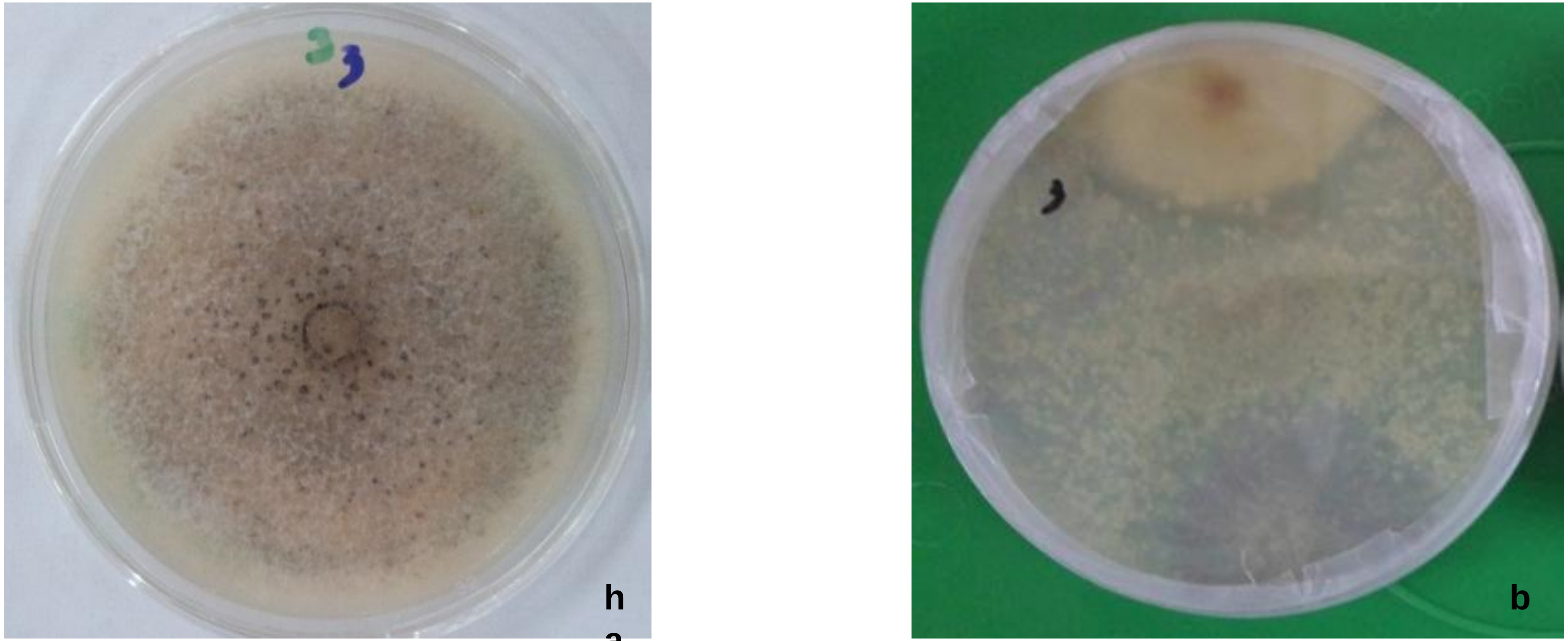
Results and discussion (1/9)

▪ *Trichoderma* strains in vitro

Table IV.1: Effect of antagonistic activities of *Trichoderma* strains on the mycelial growth of *C. gloeosporioides* after 6 days of incubation (direct confrontation)

Trichoderma	Trichoderma strains	Growth inhibition Colletotrichum mycelial *gloeosporioides*	Medium inhibition (%)
T. pseudokoningii	AG1	69.71 ± 9.56	71.41 ± 1.59a
	AG6	71.65 ± 8.44	
	AG9	72.88 ± 4.88	
T. harzianum	AG3	63.89 ± 11.38	64.31 ± 0.57b
	AG4	64.09 ± 6.86	
	AG7	64.96 ± 5.76	
Probability	0.0206		

❖ ***Trichoderma* strains *in vitro***

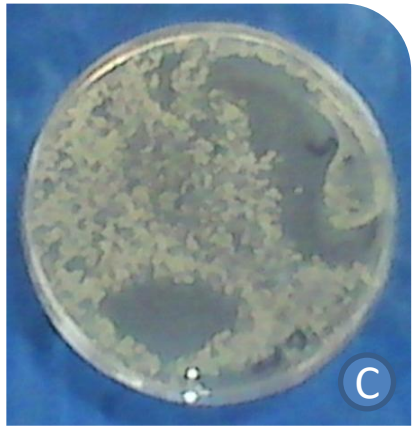


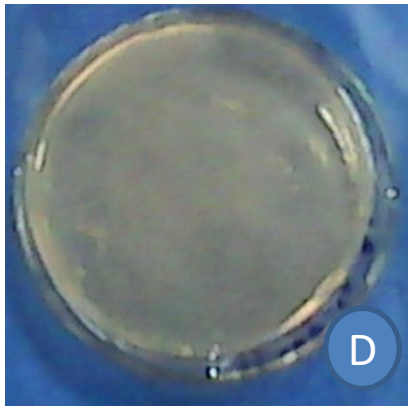
a: **Mycelial growth of *C. gloeosporioides* alone after *6 days of incubation***
b: **Mycelial growth of *C. gloeosporioides with Trichoderma* after 6 days of incubation**

Results and discussion (3/9)

Table IV.2 : Effect of antagonists on the germination of *C. gloeosporioides* spores after 24 hours of incubation (**direct confrontation)**

Trichoderma	*Trichoderma*	conidial germination of *C. gloeosporioides* (%)	Mean inhibition of conidial germination (%)
T. pseudokoningii	AG1	65.65 ± 16.14	78.78 ± 14.51a
	AG6	94.37 ± 2.44	
	AG9	76.33 ± 6.42	
T. harzianum	AG3	73.36 ± 7.35	74.38 ± 15.16a
	AG4	59.76 ± 17.20	
	AG7	90.03 ± 3.54	
Probability	0.0001		





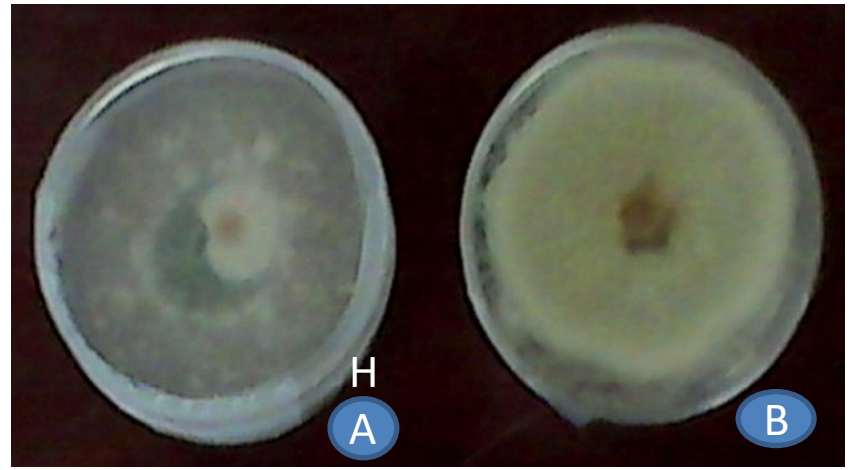
C: PDA with germinated colonies of Cgldc1G
D: PDA with ungerminated colonies of *Cgldc1G*

Results and discussion (4/9)

- **Effect of volatile substances (indirect confrontation)**

Table IV.3: Effect of volatile substances produced by *Trichoderma* on the mycelial growth of *C. gloeosporioides after 6 days of incubation*

Trichoderma species	Trichoderma strains	mycelial growth (%)	Average inhibition (%)
T. pseudokoningii	AG1	64.18 ± 5.33	56.94 ± 6.41a
	AG6	54.66 ± 4.80	
	AG9	51.98 ± 9.04	
T. harzianum	AG3	47.61 ± 12.20	47.48 ± 2.08b
	AG4	45.33 ± 11.42	
	AG7	49.50 ± 10.64	
Probability	0.0479		



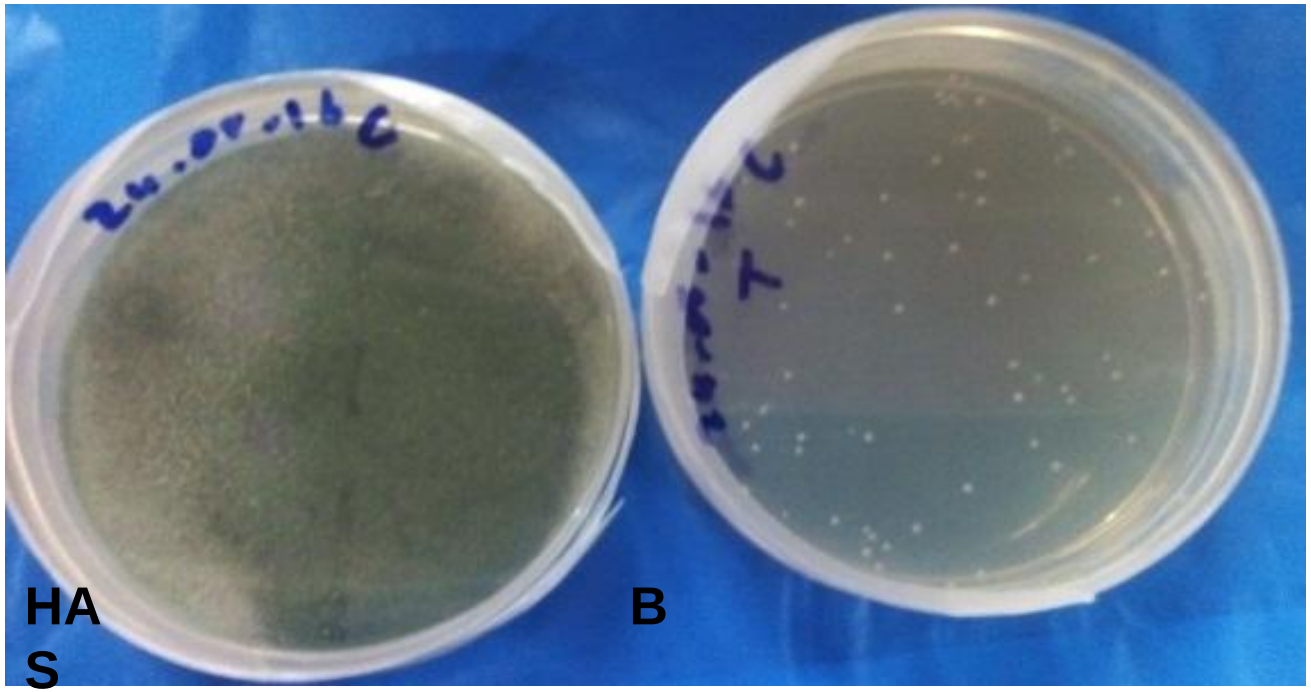
A : *C. gloeosporioides with Trichoderma*
B : *C. gloeosporioides* alone

Main results (5/9)

Table IV.4 : Effect of volatile substances produced by *Trichoderma on* conidial germination of *C. gloeosporioides (* **indirect confrontation)**

Trichodema	Trichoderma strains	conidial germination of *C. gloeosporioides* (%)	Average inhibition of conidial germination (%)
T. pseudokoningii	AG1	100 ± 0.00a	100 ± 0.00a
	AG6	100 ± 0.00a	
	AG9	100 ± 0.00a	
T. harzianum	AG3	98.24 ± 2.14a	98.12 ± 1.12a
	AG4	87.36 ± 4.20a	
	AG7	94.14 ± 3.06a	
Probability	0.03765		

Main results (6/9)



A: Below: Trichoderma culture , Above: PDA with ungerminated colonies of Cgldc1G

B: Below: Single PDA Above: PDA with germinated colonies of Cgldc1G

- *Trichoderma* strains tested ➔ inhibitory power ➔ mycelial growth, germination and sporulation of *C. gloeosporioides* in direct and indirect confrontation .

Results and discussion (7/9)

❖ *Trichoderma* strains *in vivo*

Table IV.5: Percentage reduction in incidence and severity

Treatments			Average reduction in incidence (%)	Average reduction in severity (%)
Curative	*T. pseudokoningii*	AG1	84.38	95.74
		AG6		
		AG9		
	T. harzianum	AG4	93.23	97.69
		AG3		
		AG7		
Preventive	*T. pseudokoningii*	AG1	96.36	99.49
		AG6		
		AG9		
	T. harzianum	AG4	92.19	97.39
		AG3		
		AG7		
Simultaneous	*T. pseudokoningii*	AG1	85.94	95.54
		AG6		
		AG9		
	T. harzianum	AG4	97.92	99.74
		AG3		
		AG7		

Results and discussion (8/9)

Plantules inoculées avec le pathogène et traitées avec les antagonistes (A)

Plantules inoculées seulement avec le pathogène (B)

Results and discussion (9/9)

- Potential of *Trichoderma* in the laboratory ➔ greenhouse treatment. The six strains of *Trichoderma* monitored the incidence and severity of anthracnose in preventive, simultaneous and curative treatment .

Conclusion (1/1)

- This study shows that *T. harzianum* or *T. pseudokoningii* were effective against *C. gloeosporioides* , the causative agent of cashew anthracnose.

- Both antagonistic fungi have similar antifungal activity against *C. gloeosporioides* .

- *Trichoderma* species make them a biological control agent, one of the pillars of an integrated disease management program.


Thank you for your kind attention.