International Journal of Environment, Agriculture and Biotechnology
Vol-8, Issue-6; Nov-Dec, 2023
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Search for a suitable substrate for mass propagation of a local strain of *Trichoderma harzianum* (ThTab) isolated in Burkina Faso

Tobdem Gaston DABIRE*, Masséni Yasmine OUOLOGUEME, Schémaéza BONZI and Irénée SOMDA

[1]Université Nazi BONI, Ecole Doctorale Sciences Naturelles et Agronomie, Laboratoire des Systèmes Naturels, Agrosystèmes et de l'Ingénierie de l'Environnement (Sy. N. A. I. E.), 01 P.O Box 1091, Bobo-Dioulasso 01, Burkina Faso
*Corresponding author: drdabiretoo@gmail.com

Received: 02 Nov 2023; Received in revised form: 08 Dec 2023; Accepted: 17 Dec 2023; Available online: 24 Dec 2023

Abstract— *A strain of Trichoderma harzianum Pers. isolated in Burkina Faso showed significant antagonistic properties against phytopathogenic fungi in vitro and in the greenhouse. The aim of this study was to identify an effective, available and inexpensive medium for its multiplication and large-scale use. Thus, various organic substrates identified as carbon sources were supplemented with others identified as nitrogen sources and then tested using the solid-state fermentation method. An inoculum of the fungus was fermented on these slightly humified substrates for 07 days at room temperature (22-25°C). The number of micropropagules produced per substrate was then evaluated in Colony Forming Units per gram of substrate (CFU/gos). The use of maize bran supplemented with soy flour as a substrate resulted in an average micropropagule production of 1045.5.10^8 CFU/gos, i.e. a 5228-fold multiplication of the initial inoculum. Maize bran was the best carbon source, with an average contribution of 297,10^8 CFU/gos whatever the nitrogen supplement, and soy flour the best nitrogen supplement, with an average contribution of 113.7,10^8 CFU/g0s whatever the carbon source. The development of a formulation based on maize bran and soy flour for the mass multiplication of this strain is envisaged.*

Keywords— ***Trichoderma harzianum, Micro propagules, Maize bran, Burkina Faso, Substrate***

I. INTRODUCTION

Until now, the control of crop pests has focused on the application of prophylactic measures (crop rotation, fallowing of infested land, seed and soil disinfection, etc.), the use of resistant and/or tolerant varieties and the use of chemical pesticides (Shahnaz *et al*., 2013).

However, the adoption of certain prophylactic measures remains ill-suited to large farms, and is also hampered by land pressure (Traoré *et al*., 2020). The low availability of varieties resistant to several pests also limits the use of genetic control (Gary & Hebbar, 2015). The use of synthetic chemical pesticides is still the most widely used control method, as it delivers results very quickly (Schiffers & Wainwright, 2011). However, given the risks of damage to the health of producers, consumers and agrosystems, and the rapid emergence of resistance within pathogen or pest populations, chemical control is increasingly discouraged and heavily regulated (Son *et al*., 2018; Besmer *et al*., 2022).

In view of all these limitations, the development of alternative control methods that are effective, sustainable and safe for both man and the environment is a major concern for agricultural research institutions. Biological control, used as an alternative to the use of chemicals, is an ecologically sound approach that involves the use of specific organisms to protect plants against their bio-aggressors (Sargin *et al*., 2013). Biological control agents including yeasts, bacteria and fungi have been successfully

tested, and some formulations based on biological control agents are encountered commercially today (Dal Bello *et al*., 2002; Bardin *et al*., 2003; Trebicka *et al*., 2012).

Among the biological control agents used, telluric fungi of the *Trichoderma* genus occupy an important place (Gary & Hebbar, 2015). *Trichoderma* are telluric filamentous fungi of the Phylum Ascomycetes, family Hypocreales that are well known and used for their antagonism against several soil phytopathogens involving fungi, bacteria and invertebrates (Cowper *et al*., 2013) and for their plant growth-promoting properties (Bacon *et al*., 2001; John *et al*., 2010; Abdel-Monaim, 2014). According to recent studies, these fungi use several modes of action such as mycoparasitism, competition and antibiosis, root stimulation, solubilization of plant fertilizing minerals and stimulation of plant natural defenses and soil bioremediation (Verma *et al*., 2007, Cowper & Renaud, 2013).

Trichoderma spp are cosmopolitan fungi, characterized by their rapid growth, ability to use a variety of substrates and resistance to harmful chemical agents (Luz *et al*., 2019). They are present on decaying wood and in soils at concentrations ranging from 10 to 10,000 propagules per gram of soil (Cowper & Renaud, 2013). One of the most frequently encountered species in biological control agent formulations worldwide is *Trichoderma harzianum* Pers. This species has been the subject of several research activities and has been shown to have proven antagonistic properties against several plant pathogens and to promote plant growth (Caron *et al*., 2002; Souna *et al*., 2012; Mahalakshmi & Yesu Raja, 2013; Abdel-Monaim *et al*., 2014; Ferrigo *et al*., 2014; Akrami & Yousefi, 2015; Gautam *et al*., 2015). In Burkina Faso, a local strain of *Trichoderma harzianum* (ThTab) was isolated from the rhizosphere of an onion plot in the village of Tabtenga.

This strain was tested in vitro and in the greenhouse against *Fusarium* spp. and *Aspergillus niger*, respectively responsible for fusarium rot and black rot of onion (Dabiré *et al*., 2016a). It also exhibited an important growth-promoting property in greenhouse onions (Dabiré *et al*., 2016b). The research question that emerged from this work was how to multiply and conserve this strain for large-scale use in market garden agrosystems. The literature indicates that one of the most suitable methods for successful multiplication of *T. harzianum* is its solid-state fermentation on moistened organic substrates without free water (Sargin *et al*., 2013). The aim of the present study was to evaluate locally available and inexpensive agro-industrial by-products as natural organic substrates for mass production of micro propagules of this local strain of T. harzianum under solid-state fermentation conditions inspired by Sargin *et al*. (2013).

II. MATERIALS AND METHODS

2.1 Microorganisms used

The fungal material used was a strain of *Trichoderma harzianum* ThTab, isolated from a soil sample taken from the rhizosphere of an onion plot in the village of Tabtenga, east of the city of Ouagadougou, Burkina Faso. The strain was sequenced and stored in the mycotheque of the Earth and Life Institute (ELI) of the *Université Catholique de Louvain (UCL)* under accession number 8129-THBFA.

2.2 Agro-industrial by-products used

Various local by-products, mainly of plant origin, were used as organic substrates for mushroom propagation (Table 1). These by-products were collected in the villages of Boni, Kodéni and Nasso, at the *Institut de l'Environnement et de Recherches Agricoles (INERA)* station in Farako-Bâ and in the industrial zone of Bobo-Dioulasso.

After collection, the organic materials were suitably crushed using an electric grinder, then sieved to obtain a fine, homogeneous powder. They were then oven-dried (0% moisture content). Moisture levels were checked using a RADWAG® moisture meter.

Table 1 Organic substrates (carbon and nitrogen sources) tested in this study

Source	Type	Scientific name	Provenance
Carbon sources	Acacia bark	*Faidherbia albida*	Boni
	Maize raids	*Zea mays*	Farako-bâ
	Eucalyptus sawdust	*Eucalyptus camaldulensis*	Bobo-Dioulasso
	Maize bran	*Zea mays*	Bobo-Dioulasso
	Rice bran	*Oryza sativa*	Kodéni
	Maize and rice bran (g/g)	*Z. mays* et *O. sativa*	Kodéni
	Millet stalks	*Pennisetum glaucum*	Farako-bâ
	Sorghum stalks	*Sorghum bicolor*	Nasso
	Sorghum and millet stalks (g/g)	*P. glaucum*, *S. bicolor*	Farako-bâ et Nasso

	Cottonseed cake	*Gossypium hirsutum*	Bobo-Dioulasso
Nitrogen sources	Acacia pods	*Faidherbia albida*	Bobo-Dioulasso
	Soy flour	*Glycine max*	Bobo-Dioulasso
	Moringa leaves	*Moringa oleifera*	Bobo-Dioulasso
	Cowpea hulls	*Vigna unguiculata*	Bobo-Dioulasso
	Peanut hulls	*Arachis hypogaea*	Bobo-Dioulasso
	Synthetic culture medium	Peptone	Bobo-Dioulasso

2.3 Inoculum preparation and strain mass production

To prepare the inoculum, the strain was cultured in Petri dishes containing PDA (Potato Dextrose Agar) medium, then incubated at 25°C under an alternating cycle of near-ultraviolet light and darkness (12h/12h) for 5 days. From a culture of the fungus, a conidial suspension was prepared with distilled water supplemented with Tween 80 (0.1%) under aseptic conditions on the colony in a Petri dish. After shaking, the resulting solution was filtered, its concentration assessed and adjusted to 10^8 conidia/ml using a Fuch-Rosental cell. This suspension was used as inoculum.

T. harzianum micropropagules were produced in 200 ml glass vials containing a mixture of 5 g of each carbon source + 50 mg of each nitrogen source (weight/weight ratio 1%). A control containing the carbon source with no nitrogen source was set up. The mixtures were first sterilized at 120°C for 20 minutes before being moistened with distilled water at 70% humidity. The substrates thus prepared were inoculated with the fungus by introducing 1 ml of the previously prepared inoculum into each flask, giving an initial concentration of 0.2 conidia per gram of substrate (0.2 conidia/gos). After inoculation, the vials were lightly resealed (to allow aeration) and then incubated at laboratory room temperature (approx. 22°C) for 7 days.

2.4 Evaluation of micropropagule production

At the end of the culture period, 40 ml of distilled water with a drop of tween 80 was added to the contents of each flask. The contents were then vortexed for 3 min to obtain a mixture of conidia and mycelial fragments. Serial dilutions were made with each mixture, and the number of dilutions (aliquots) was a function of the initial concentration of the mixture (100-fold or 1000-fold).

Micropropagules were counted on a Dichloran-Glycerol (DG 18) agar-based culture medium as recommended by NF EN ISO 11133 (2014). For its preparation, 15 g of DG18 were suspended in 500 ml of distilled water. The suspension was brought to the boil with constant stirring until completely dissolved. After dissolution, 85 ml of glycerol were added, and the whole mixture was autoclaved at 120°C for 30 min. After cooling to approximately 50°C, the medium was dispensed into Petri dishes. Once the medium had solidified, 500 µl of each aliquot from the serial dilution was added to each Petri dish, at a rate of five (05) Petri dishes per aliquot. Ces dernières ont ensuite été mises en incubation à 25°C pendant huit (08) jours. Les colonies développées ont été comptées quotidiennement pendant les huit (08) jours. Les résultats ont été exprimés en Unités Formant des Colonies par gramme de substrat (UFC/gos).

2.5 Experimental design

For the fungi cultivation, the different nitrogen sources were tested with each organic substrate in a Randomized Complete Block design. For each carbon source, seven (7) treatments (Table 2) were carried out in five replicates.

Table 2 Treatments used for each substrate

Codes	Traitements
T0	Carbon source without nitrogen supplement
T1	CSS* with peanut hull powder
T2	CSS* with acacia pod powder
T3	CSS* with moringa leaf powder
T4	CSS* with cowpea hull powder
T5	CSS* with peptone
T6	CSS* with soy flour

CSS: Carbon source supplemented; CSS* = Acacia barks; Maize raids; Eucalyptus sawdust; Maize bran; Rice bran; Millet stalks; Sorghum stalks; Maize and rice bran; Millet and sorghum stalks; Cottonseed cake.*

2.6 Data processing

The data collected were first recorded and then the averages calculated using Excel software. The averages obtained were compared by an analysis of variance using the Student-Newman-Keuls multiple comparison test at the 5% threshold, performed with IBM SPSS version 22 software.

III. RESULTS

The results obtained are grouped according to carbon sources based on maize and rice by-products, carbon sources based on millet and sorghum by-products and other carbon sources. The contribution of carbon sources to micropropagule production, irrespective of nitrogen supplement, and that of nitrogen supplements, irrespective of carbon source, were also presented.

3.1 Multiplication of *T. harzianum* on carbon sources based on rice and maize by-products

The production of micropropagules per gram of substrate consisting of rice bran, maize bran, the mixture of rice bran plus maize bran and maize cobs in the presence of the various nitrogen supplements is shown in Table 3. The table shows that harvest levels varied according to the nitrogen supplements used, over a range from 3.2,108 to 19.6,10^8 CFU/gos for rice bran. For this organic substrate, supplementation with cowpea hull powder yielded the highest level of micropropagule harvest. All treatments produced harvest levels that were statistically different from the treatment without nitrogen supplementation, which presented the lowest harvest level. Apart from peanut hulls and acacia pods, which produced similar results, the other supplements were significantly different from each other (Table 3). Soy flour, which comes in second place after cowpea hulls, had a very good harvest.

For corn bran, analysis of the table shows that production levels varied according to nitrogen supplementation, from 85.5.10^8 to 1045.5.10^8 CFU/gos. The use of soy flour as a nitrogen supplement resulted in the highest number of micro-propagules, significantly different from other nitrogen supplements (Table 3). Cowpea hulls are the second most effective nitrogen supplement after soybean meal, although statistical analysis does not distinguish them significantly from other nitrogen supplements. The treatment without nitrogen supplements recorded the lowest number of micro-propagules, but this was not statistically different from the numbers obtained with the other nitrogen supplements. The use of maize bran as a carbon source resulted in significantly higher production levels than all other carbon sources (Table 3).

Table 3 Production of T. harzianum *ThTab micropropagules on rice bran, maize bran, rice+maize bran and maize raids supplemented with the various nitrogen sources*

Nitrogen supplements	Number of micropropagules (CFU/gos) X 10^8)			
	Rice bran	Maize bran	Rice+Maize bran	Maize raids
No supplement	3.2^{a}	85.5^{a}	76.0^{b}	13.6^{a}
Peanut hulls	6.7^{b}	101.4^{a}	47.5^{ab}	14.3^{a}
Acacia pods	7.0^{b}	190.1^{a}	55.1^{ab}	9.8^{a}
Moringa leaves	4.4^{ab}	193.3^{a}	72.9^{b}	4.4^{a}
Cowpea hulls	19.6^{e}	285.1^{a}	57.7^{b}	19.6^{a}
Peptone	9.2^{c}	177.4^{a}	47.5^{ab}	13.0^{a}
Soy flour	12.7^{d}	1045.5^{b}	27.2^{a}	20.9^{b}
F value	**38.000**	**37.013**	**105.752**	**10.752**
P value	**0.000**	**0.000**	**0.000**	**0.000**

Means in the same column affected by the same alphabetical letter are not significantly different at the 5% threshold according to the Student-Newman-Keuls multiple comparison test. CFU/gos: Colony Forming Units per gram of substrate; F: Fisher's coefficient; P: Probability

The results of growing *T. harzianum* on the mixture (half/half) of rice bran and maize used as a carbon source in the presence of various nitrogen supplements are presented in Table 3. Micropropagule production levels varied according to nitrogen supplementation, from 27.2.10^8 CFU/gos for soybean meal to 76.0.10^8 CFU/gos for the treatment without nitrogen supplementation. The use of cowpea tops and moringa leaves, however, resulted in production levels statistically similar to those of the treatment without supplements, i.e. 2.9.10^8 CFU/gos and 57.7.10^8 CFU/gos respectively (Table 3). Supplementing this substrate with peanut hulls, acacia pods, peptone and soy flour did not significantly promote fungal multiplication on this carbon source.

Maize raids powder, used as a carbon source for T. harzianum multiplication in the presence of various nitrogen supplements, produced varying levels of micropropagule production. The numbers of micropropagules obtained for the different treatments range from $4.4.10^8$ CFU/gos for the use of moringa leaves as a nitrogen supplement to $20.9.10^8$ CFU/gos for soy flour used as a nitrogen supplement.

3.2 Multiplication of T. harzianum on carbon sources based on millet and sorghum by-products

The production of micropropagules on sorghum and millet stalk powder and their mixture, supplemented with different nitrogen sources, is recorded in Table 4.

Shredded millet stalks, supplemented with various nitrogen sources, enabled T. harzianum to multiply with varying levels of micro-propagule harvest depending on the type of supplement (Table 4). Micropropagule production levels according to nitrogen source ranged from 0.6.108 to 1.3.108 CFU/gos. On this carbon source, peptone culture medium, acacia pods and moringa leaves showed significantly similar and significantly different production levels to the other supplements. Supplementing this carbon source with soy flour significantly and negatively affected the level of fungal multiplication (Table 4).

In sorghum stalks, the number of micro-propagules obtained for each nitrogen supplement ranged from 2.4.108 CFU/gos (for Peptone) to 3.6.108 CFU/gos (for peanut hulls). Soy flour and peanut hulls produced the best results, statistically distinguishing themselves from other nitrogen supplements. The other supplements did not produce results significantly different from the unsupplemented control.

Finally, Table 4 shows the average micropropagule counts obtained using a mixture (half/half) of sorghum and millet stalk powder in the presence of the various nitrogen supplements. The table shows that the averages, depending on the different treatments, range from 4.8.108 to 13.6.108 CFU/gos. Soy flour recorded the highest number of micro-propagules and the unsupplemented control the lowest. Soy flour and moringa leaves produced significantly higher averages than the other nitrogen supplements. The combination (millet stalks + sorghum stalks) produced more micropropagules than each carbon source taken separately.

Table 4 Production of T. harzianum *ThTab micropropagules on millet stalks, sorghum stalks, millet+sorghum stalks supplemented with the various nitrogen sources*

Nitrogen supplements	**Number of micropropagules (CFU/gos) X 10^8)**		
	Millet stalks	Sorghum stalks	Millet+sorghum stalks
No supplement	0.7^{a}	3.1^{ab}	4.8^{a}
Peanut hulls	1.0^{ab}	3.6^{b}	6.7^{ab}
Acacia pods	1.2^{b}	3.0^{ab}	9.5^{bc}
Moringa leaves	1.2^{b}	3.0^{ab}	12.7^{c}
Cowpea hulls	1.0^{ab}	2.9^{ab}	6.3^{ab}
Peptone	1.3^{b}	2.4^{a}	10.5^{bc}
Soy flour	0.6^{a}	3.5^{b}	13.6^{c}
F value	**05.976**	**03.347**	**09.373**
P value	**0.000**	**0.013**	**0.000**

Means in the same column affected by the same alphabetical letter are not significantly different at the 5% threshold according to the Student-Newman-Keuls multiple comparison test. CFU/gos: Colony Forming Units per gram of substrate; F: Fisher's coefficient; P: Probability

3.3 Multiplication of T. harzianum on acacia bark, eucalyptus sawdust and cottonseed cake as carbon sources

Micropropagule production levels per gram of substrate consisting of acacia bark, white sawdust and cottonseed cake in the presence of the various nitrogen supplements are presented in Table 5.

For acacia bark, harvest levels varied according to the nitrogen supplements used, from $6.7,10^8$ to $15.5.10^8$ CFU/gos. Supplementation with acacia pod powder produced significantly more micro-propagules than the other supplements. The unsupplemented control produced significantly more micropropagules than the other nitrogen supplements apart from cowpea tops.

The numbers of *T. harzianum* micropropagules produced on white sawdust powder in the presence of various nitrogen

sources varied from $2.7.10^8$ to $7.5.\ 10^8$ CFU/gos and all treatments were significantly different. The use of cowpea husk powder as a nitrogen supplement yielded the highest number of micropropagules, and groundnut husk powder the lowest. Apart from groundnut hulls, the other nitrogen supplements significantly improved the number of micro-propagules compared with the unsupplemented control.

Cottonseed cake, used as an organic substrate in the presence of various nitrogen supplements, was used to multiply T. harzianum. Analysis of Table 5 shows that micro-propagule production levels varied with nitrogen supplementation from $0.1.10^8$ to $0.6.10^8$ CF/gos. The highest number of micropropagules was recorded in the untreated control and acacia pods. This indicates that supplementation with the other nitrogen sources significantly reduced the multiplication of the fungus. Regardless of the supplement used, the number of micro-propagules obtained using cottonseed cake as a carbon source was lower than with other carbonaceous substrates.

Table 5 Production of T. harzianum ThTab micropropagules on Acacia barks, Eucalyptus sawdust and Cottonseed cake supplemented with the various nitrogen sources

Nitrogen supplements	**Number of micropropagules (CFU/gos) X 10^8)**		
	Acacia barks	Eucalyptus sawdust	Cottonseed cake
No supplement	$12,0^{b}$	$3,1^{ab}$	$0,6^{e}$
Peanut hulls	$07,3^{a}$	$2,7^{a}$	$0,4^{d}$
Acacia pods	$15,5^{c}$	$3,7^{b}$	$0,6^{e}$
Moringa leaves	$07,6^{a}$	$6,6^{e}$	$0,2^{b}$
Cowpea hulls	$09,8^{ab}$	$7,5^{f}$	$0,3^{c}$
Peptone	$06,7^{a}$	$5,8^{d}$	$0,1^{a}$
Soy flour	$08,2^{a}$	$4,4^{c}$	$0,3^{c}$
F value	**10,422**	**59,063**	**100,653**
P value	**0,000**	**0,000**	**0,000**

Means in the same column affected by the same alphabetical letter are not significantly different at the 5% threshold according to the Student-Newman-Keuls multiple comparison test. CFU/gos: Colony Forming Units per gram of substrate; F: Fisher's coefficient; P: Probability

3.4 Contribution of carbon sources to the production of *T. harzianum* micropropagules

The contribution of carbon sources to micro-propagule production, whatever the nitrogen supplement, is summarized in Table 8. Analysis of the table shows that, depending on the source, micropropagule production varied from $0.3.10^8$ to $297.0.10^8$ CFU/gos.

The highest production was obtained using corn bran as substrate, and the lowest with cottonseed cake. Apart from corn bran, statistical analysis revealed no significant difference between the other substrates (Table 6).

Harvesting of the fungus grown on maize raids powder was very significantly lower than that obtained with maize bran powder (Table 6).

The combination of maize and rice bran significantly reduced the level of micropropagule production compared with maize bran taken in isolation (Table 6).

Table 6 Production of T. harzianum *ThTab micropropagules on various carbon sources*

Carbon sources	**Number of micropropagules (CFU/gos) X 10^8)**
Rice bran	09.0^{a}
Maize bran	297.0^{b}
Maize raids	13.6^{a}
Rice+Maize bran	54.9^{a}
Sorghum stalks	03.1^{a}

Millet stalks	01.0[a]
Sorghum+Millet stalks	09.1[a]
Acacia barks	09.6[a]
Eucalyptus sawdust	04.8[a]
Cottonseed cake	00.3[a]
F value	**25.938**
P value	**0.000**

Means in the same column affected by the same alphabetical letter are not significantly different at the 5% threshold according to the Student-Newman-Keuls multiple comparison test. CFU/gos: Colony Forming Units per gram of substrate; F: Fisher's coefficient; P: Probability

3.5 Contribution of nitrogen supplements to the production of T. harzianum micropropagules

The average harvests of micropropagules obtained for all treatments of the same nitrogen supplement, irrespective of the organic substrate, are shown in Table 7.

Average *T. harzianum* micropropagule production varied between nitrogen supplements, from $19.1.10^8$ to $113.7.10^8$ CFU/gos. Statistical analysis revealed no significant differences between supplements, with the exception of soy flour, which recorded the highest concentration ($113.7,10^8$ CFU/gos) (Table 7).

Table 7 Production of T. harzianum *ThTab micropropagules on various nitrogen sources*

Nitrogen supplements	**Number of micropropagules (CFU/gos) X 10^8)**
No supplement	20,3[a]
Peanut hulls	19,1[a]
Acacia pods	29,5[a]
Moringa leaves	31,5[a]
Cowpea hulls	40,0[a]
Peptone	27,4[a]
Soy flour	113,7[b]
F value	**03,063**
P value	**0,006**

Means in the same column affected by the same alphabetical letter are not significantly different at the 5% threshold according to the Student-Newman-Keuls multiple comparison test. CFU/gos: Colony Forming Units per gram of substrate; F: Fisher's coefficient; P: Probability

IV. DISCUSSION

The genus of fungus most exploited in the biopesticide industry is Trichoderma, formulations of which are developed on various substrates through solid or liquid fermentation technologies (Gary & Hibbar, 2015). However, the main problem encountered by farmers and manufacturers regarding the bioproduct developed is its instability under different environmental conditions (Prakash & Basu, 2020). Added to this is the adaptation of the fungus strains to climatic conditions and the availability and cost elements of these carriers (Gary & Hibbar, 2015).

The general objective of this study was to evaluate the capacity of certain organic substrates, essentially agricultural by-products that are inexpensive and easily accessible in Burkina Faso, to massively and simply reproduce propagules of the local strain of *T. harzianum* (ThTab) with a view to developing a formulation for large-scale use.

The results of this experiment show that, with the exception of the "cotton cake supplemented with peptone and moringa leaves" treatments, all the other treatments multiplied the initial inoculum by factors ranging from 1, 5 times (for the cotton cake treatment supplemented with soybean meal) to

5228 times (for the maize bran treatment supplemented with soybean meal) in eight days of incubation, based on an initial substrate concentration of 0.2 conidia/gos).

These results indicate that solid-state fermentation is an effective method for the simple and massive reproduction of *T. harzianum* strains. This confirms the work of Sargin *et al*, (2013) who massively reproduced micro propagules of a *T. harzianum* strain by solid-state fermentation with wheat bran. Long before, Nkaya (2007) had indicated that fermentation in a submerged (liquid) medium was more suitable for the multiplication of bacteria, and that solid-state fermentation, taking place on a surface of solid matter that could absorb or contain water, with or without soluble nutrients, was more suitable for the growth of fungal microorganisms.

Trichoderma species are filamentous fungi, and solid-state fermentation is better suited to the growth of this group of fungi than fermentation in liquid media (Duchiron & Legin-copinet, 2019). This is because these fungi have strong cell wall structures at the ends of colonizing hyphae that are able to penetrate solid substrates for nutrients (Duchiron & Legin-Copinet, 2019).

However, Sargin *et al*. (2013) also demonstrated that spore production levels were variable depending on the initial substrate humidity, but also on other characteristics such as incubation temperature and medium pH, parameters that were not considered in our study. It would therefore make sense to continue research by varying these different parameters with a view to optimizing solid-state fermentation.

Several agro-industrial by-products, including wheat bran, rice husks and bran, cotton cake, bran, corn grains and cobs, glucose, cowpea hulls, yeast extracts, etc., have long been tested as potential carriers for mass propagation of Trichoderma harzianum (Roussos, 1985; Lakshimi & Chandra, 2004; Verma *et al*., 2005; Baghat & Sitansu, 2007; Calvacante, 2007; Rini & Sulochana, 2007; Onidule, 2012; Sargin *et al*., 2013; Rajput *et al*., 2014; Rai & Tewari, 2016; Mohiddin *et al*., 2017; Siddhartha *et al.*, 2017; Sey-Amole *et al*., 2018). Even if the evaluation method was not always the same, our results are mostly within the range of multiplication levels obtained by these authors.

The different carbon sources tested in this experiment showed variable levels of *T. harzianum* multiplication. This variability could be explained by the ease with which the fungus degrades the carbohydrates contained in each substrate. The enzymes released by *T. harzianum* (amylases, endo and exo cellulase, β- glucosidase, etc.) can rapidly and totally degrade disaccharides such as starch (contained in maize bran) and more difficultly and partially degrade polysaccharides such as cellulose (contained in millet and sorghum stalks) and lignin in acacia bark and eucalyptus sawdust.

Maize bran was the most effective in producing *T. harzianum* micropropagules in contrast to cottonseed cake, which was the least effective. This result is in line with those of Mohiddin *et al*, (2017) who tested several substrates and found that corn kernels enabled better multiplication of *T. harzianum*. According to these authors, this result obtained with maize can be explained by its high starch content as well as high water retention capacity. In fact, the maize shelling process under Burkina Faso conditions produces a residue consisting of a mixture of bran and kernel pieces. This mixture could have a high water-holding capacity and contain a significant quantity of starch that is easily degraded by Trichoderma, compared with the ground stalks of sorghum, millet, etc., which consist essentially of cellulose that is more difficult to degrade. Calvacante *et al.* (2007) have also pointed out that corn bran has a higher water retention capacity than rice bran.

While the low production levels obtained with other carbonaceous substrates can be explained by their high lignin content, which is very difficult to degrade, the result obtained with cottonseed cake can be explained by its high fat content, which reduces the fungus' development. Cottonseed cake also contains volatile aldehydes with an antifungal effect, which could prove toxic to *T. harzianum* (Zeringue, 1996).

Sargin *et al*. (2013) obtained more micro propagules using cotton cake. This was not the case in this study. This could be justified by a difference in the fat and gossypol contents of the oilcakes used.

In terms of nitrogen sources, soy flour was, on average, significantly more suitable as a supplement (113.7.108 CFU/gos) even if, depending on the carbon source, it was not always the best performer. This result is in line with those of Sargin *et al*. (2013), who obtained good micropropagule production using flour as a supplement to wheat bran. Cowpea hulls were the second most interesting nitrogen supplement, unlike peanut hulls, which did not promote fungal growth. This variability could be explained by the difference in nitrogen content of each supplement. However, it was noted that the contribution of nitrogen supplements seems to depend on the carbon source used. Soy flour was not always the best performer, depending on the carbon source used.

Supplementing maize bran with soybean and cowpea meal significantly increased the level of micropropagule production compared with the unsupplemented control. These results are all interesting because these agricultural by-products are accessible to farmers in Burkina Faso.

Maize is an annual plant grown as a cereal for its starch-rich grains for human consumption (Sanou *et al*., 2022). In Burkina Faso, maize ranks second among cereal crops, with national production estimated at 1,700,127 tonnes (DGESS/MAAH, 2019). Apart from the Sahel and Northern regions, where maize production is low, all other regions of Burina Faso produce and consume maize.

Maize flour is used by both urban and rural populations to prepare the dough commonly known as "Tô". Before this flour can be obtained, the kernels are shelled, so that in both rural and urban areas, maize bran is widely available and financially accessible to small-scale producers. Soya and cowpea are legumes also grown on a large scale in Burkina Faso as food crops.

According to 2019 statistics, soybean and cowpea production reached 31,314 tonnes and 683,174 tonnes respectively (DGESS/MAAH, 2019). These production levels make it possible to appreciate the availability of cowpea haulms and soybeans for the implementation of this technology.

These production levels show that the availability and affordability of these agricultural by-products in Burkina Faso are not objective barriers. Although maize bran and cowpea haulms are used for livestock feed, and soybeans are used in the manufacture of various dishes, the quantities to be used in this technology do not call into question the competitive use of these by-products. The results of this study are therefore very encouraging and point to interesting avenues for the development of a *Trichoderma* formulation that is easy for growers to prepare themselves.

V. CONCLUSION

This study assessed the ability of various organic substrates to efficiently multiply a local strain of Trichoderma harzianum isolated in Burkina Faso. All the substrates tested by the solid-state fermentation method multiplied the fungus, but to varying degrees. Corn bran, supplemented with soy flour and cowpea powder respectively, multiplied the initial inoculum of the fungus by 5228 and 1426 times respectively.

As maize bran, soya beans and cowpea tops are widely available and accessible to farmers, this result opens up the prospect of developing a low-cost formulation that could be used as a seed coating or to increase Trichoderma levels in plots and nurseries (by spraying or watering) to initiate protection of targeted crops against telluric pest problems.

But first, in order to optimize the fermentation method, it seems important to continue investigations on the following points:

(i) Evaluate the maize-soybean or maize-cowpea hulls combination by varying parameters such as substrate moisture content, pH, incubation temperature and proportion (nitrogen source / carbon source);

(ii) Evaluate the viability of micropropagules in harvested biomass;

(iii) Carry out on-farm tests to assess the most appropriate and economical method of use.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to the staff of the Laboratoire des Systèmes Naturels, Agrosystèmes et de l'Ingénierie de l'Environnement (Sy. N. A. I. E.) for their great contribution in carrying out the activities. They are also grateful to the PRD Program of ARES-Belgium for funding the activities of the PRD-ProDuIRe project (Bobo-Dioulasso, Burkina Faso).

REFERENCES

[1] Abdel-Monaim, M.F., Abdel-Gaid, M.A., Zayan, S.A., & Nassef, D.M.T. (2014). Enhancement of growth parameters and yield components in eggplant using antagonism of Trichoderma spp. against Fusarium wilt disease. *International Journal of Phytopathology*, 03(01), 33-40. DOI: 10.33687/phytopath.003.01.0510

[2] Akrami, M. & Yousefi, Z. (2015). Biological Control of Fusarium wilt of Tomato (Solanum lycopersicum) by Trichoderma spp. as Antagonist Fungi. *Biological Forum – An International Journal*, 7(1), 887-892.

[3] Nkaya, G.D.(2007). La fermentation à l'état solide. Retrieved from : http://agrogroup.unblog.fr/2007/04/28/fermentation-en-milieu-solide/

[4] Bacon, C.W., Yates, I.E., Hinton, D.M., & Meredith, F. (2001). Biological control of Fusarium moniliforme in maize. *Environmental Health Perspectives*, 109(2), 325–332. DOI: 10.1289/ehp.01109s2325

[5] Bardin, S.D., & Huang, H. (2003). Efficacy of stickers for seed treatment with organic matter or microbial agents for the control of damping-off of sugar beet. *Plant Pathology Bulletin*, 12, 19-26.

[6] Besmer, R.A., Sawadogo, W.M., Dabiré, T.G., Kambiré, F.C., Bokonon-Ganta, A.H., Somda, I., & Verheggen, F.J. (2022). Susceptibility of fall armyworm Spodoptera frugiperda (JE Smith) to microbial and botanical bioinsecticides and control failure likelihood estimation. *Biotechnol. Agron. Soc. Environ*, 26(3), 136-143. DOI: 10.25518/1780-4507.19793

[7] Bhagat, S., & Sitansu, P. (2007). Mass multiplication of Trichoderma harzianum on agricultural byproduets and their evaluation against seedling blight (Rhizoctonia solani) of mungbean and collar rot (Sclerotium rolfsii) of groundnut. *Indian Journal of Agricultura1 Sciences*, 77(9), 583-8. https://epubs.icar.org.in/index.php/IJAgS/article/view/3186

[8] Cavalcante, R.S., Lima, L.S.H., Pinto, G.A.S., Gava, C.A.T., & Rodrigues, S. (2008). Effect of moisture on Trichoderma

conidia production on corn and wheat bran by solid state fermentation. *Food Bioprocess Tech*, 1, 100–104. https://doi.org/10.1007/s11947-007-0034-x

[9] Caron, J., Laverdière, L., Thibodeau, P.O., & Bélanger, R.R. (2002). Utilisation d'une souche indigène de Trichoderma harzianum contre cinq agents pathogènes chez le concombre et la tomate de serre au Québec. *Phytoprotection*, 83, 73-87. DOI: https://doi.org/10.7202/706230ar

[10] Cowper, J.R., Canaguier, R.H., & Reynaud, H. L. (2013). Procédé de multiplication de micro-organismes phyto-bénéfiques. Organisation Mondiale de la Propriété Intellectuelle. *Numéro de publication internationale WO 2013/079887* Al. https://patents.google.com/patent/WO2013079887A1/fr

[11] Dabiré, T.G., Bonzi, S., Somda, I., & Legrève, A. (2016a). Evaluation in vitro de l'action antagoniste d'isolats de Trichoderma harzianum contre trois espèces fongiques pathogènes de l'oignon au Burkina Faso. *Tropicultura*, 34(3), 313-322. https://popups.uliege.be/2295-8010/

[12] Dabiré, T.G., Bonzi, S., Somda, I., & Legrève, A. (2016b). Evaluation of the potential of Trichoderma harzianum as a plant growth promoter and biocontrol agent against Fusarium damping-off in onion in Burkina Faso. *Asian Journal of plant pathology*, 10, 49-60. https://scialert.net/abstract/?doi=ajppaj.2016.49.60

[13] Dal Bello, G.M., Monaco, C.I., & Simon, M.R. (2002). Biological control of seedling blight of wheat caused by Fusarium graminearum with beneficial rhizosphere microorganisms. *World Journal of Microbiology & Biotechnology*, 18, 627–636. https://doi.org/10.1023/A:1016898020810

[14] DGESS/MAAH. (2019). Résultats définitifs de la campagne agropastorale 2018/2019, de la situation alimentaire et nutritionnelle du pays et perspectives. https://sisa.bf/wp-content/uploads/2021/07/Rapport-General-Resultats-definitifs-2018-2019-1-2.pdf

[15] Duchiron, F., Legin-Copinet, E. (2019) "Fermentation en milieu solide (FMS)"In Techniques de l'Ingénieur. https://www.techniques-ingenieur.fr/base-documentaire/archives-th12/archives-bioprocedes-et-bioproductions-tiabi/archive-1/fermentation-en-milieu-solide-fms-bio620/

[16] Ferrigo, D., Raiola, A., Rasera, R., & Causin, R. (2014). Trichoderma harzianum seed treatment controls Fusarium verticillioides colonization and fumonisin contamination in maize under field conditions. *Crop Protection*, 65, 51-56. https://doi.org/10.1016/j.cropro.2014.06.018

[17] Gary, J.S., & Hebbar, P.K. (2015). Trichoderma. Identification and agricultural applications. The American Phytopathological Society press. 3340 Pilot Knob road. St Paul, Minnesota 55121 USA. Library of Congress Control number: 2015908956. International Standard book n°: 978-0-89054-484-6.

[18] Gautam, S.S., Kanchan, K., & Satsangi, G.P. (2015). Effect of Trichoderma species on germination and growth of Mungbean (Vigna radiata L.) and its antagonistic effect against fungal pathogens. *International Journal of Advanced Research*, 3(2), 153-158. http://www.journalijar.com/

[19] John, R.P., Tyagi, R.D., Prévost, D., Brar, S.K., Pouleur, S., & Surampalli, R.Y. (2010). Mycoparasitic Trichoderma viride as a biocontrol agent against Fusarium oxysporum f. sp. adzuki and Pythium arrhenomanes and as a growth promoter of soybean. *Crop Protection*, 29,1452-1459. https://doi.org/10.1016/j.cropro.2010.08.004

[20] Lakshimi, T., & Chandra, B. (2004). Evaluation of agro-industrial wastes for conidia-based inoculum production of bio-control agent: Trichoderma harzianum. *Journal of Scientific and Industrial Research*, 63, 807-812.

[21] Luz, T., Franco, A., Zanuzzi, V., & Ballini, E. (2019). Biological control using Trichoderma. Retrieved from http://agrosys.fr/wp-content/uploads/2019/10/Modes-daction-de-TRichoderma.pdf

[22] Mahalakshmi, P., & Raja I.Y., 2015. Biocontrol potential of Trichoderma species against wilt disease of carnation (Dianthus caryophyllus L.) caused by Fusarium oxysporum f.sp. dianthi. *Journal of Biopesticides*, 6(1), 32-36.

[23] Mohiddin, F.A., Bashir, I., Shahid, A.P., & Burhan, H. (2017). Evaluation of different substrates for mass multiplication of Trichoderma species. *Journal of Pharmacognosy and Phytochemistry*, 6(6), 563-569. https://www.phytojournal.com/archives/2017.v6.i6.2133/evaluation-of-different-substrates-for-mass-multiplication-of-trichoderma-species

[24] Onilude, A.A., Adebayo-Tayo, B.C., Odeniyi, A.O., Banjo, D., & Garuba, E. O. (2012). Comparative mycelial and spore yield by Trichoderma viride in batch and fed-batch cultures. *Annals Microbiology*, 10, 547-553. https://doi.org/10.1007/s13213-012-0502-z

[25] Prakash, V., & Basu, K. (2020). Mass Multiplication of Trichoderma in Bioreactors. In: Manoharachary, C., Singh, H.B., Varma, A. (eds) Trichoderma: Agricultural Applications and Beyond. Soil Biology, vol 61. Springer, Cham. https://doi.org/10.1007/978-3-030-54758-5_5

[26] Rai, D. & Tewari, A.K. (2016). Evaluation of different carbon and nitrogen sources for better growth and sporulation of T. harzianum (Th14). *Journal of Agricultural Biotechnology and Sustainable Development*, 8(8), 67-70. DOI: 10.5897/JABSD2016.0262

[27] Rajput, A.Q., Khanzada, M.A., & Shahzad, S. (2014). Effect of Different Organic Substrates and Carbon and Nitrogen Sources on Growth and Shelf Life of Trichoderma harzianum. *J. Agr. Sci. Tech*, 16, 731-745. http://www.fspublishers.org/

[28] Rini, C.R., & Sulochana, K.K. (2007). Substrate evaluation for multiplication of Trichoderma spp. *Journal of Tropical Agriculture*, 45(1-2), 58–60.

[29] Roussos, S. (1985). Croissance de Trichoderma harzianum par fermentation en milieu solide : Physiologie, sporulation et production de cellulase. Thèse de Doctorat en sciences naturelles : Université de Provence (France).

[30] Sanou, A., Yonli, D., Séré, I., & Traoré, H. (2022). Influence de la fertilisation azotée et de la concurrence monospécifique de Rottboellia cochinchinensis (Lour.) W. Clayton sur le maïs dans l'Ouest du Burkina Faso. *Tropicultura*, 40(1). DOI: 10.25518/2295-8010.1980. https://popups.uliege.be/2295-8010/index.php?id=1980

[31] Sargin, S., Gezgin, Y., Eltem, R., & Vardar, F. (2013). Micropropagule production from Trichoderma harzianum EGE-K38 using solid-state fermentation and a comparative study for drying methods. *Turkish Journal of Biology*, 37, 139-146. DOI: 10.3906/biy-1206-32

[32] Schiffers, B & Wainwright, H., 2011. La lutte Biolgique et protection intégrée. Pour un Développement durable des filières fruits et légumes ACP. COLEACP Eds., Bruxelles. Manuel N° 10. 126 p. file:///C:/Users/UTILISATEUR/Downloads/coleacp-manuel-10-fr.pdf.

[33] Shahnaz, E., Razdan, V.K., Rizvi, S.E.H., Rather, T.R., Gupta, S., & Andrabi, M. (2013). Integrated Disease Management of Foliar Blight Disease of Onion: A Case Study of Application of Confounded Factorials. *Journal of Agricultural Science*, 5(1), 17-22. DOI: 10.5539/jas.v5n1p17

[34] Sey-Amole, O.D., & Onilude, A.A. (2018). Influence of carbon and nitrogen sources on the spore yield of Trichoderma harzianum in fed-batch culture. *International journal of microbiology and mycology*, 7(1), 18-23.

[35] Siddhartha, N.S., Amara, K.V., Ramya, Mol K.A., Saju, K.A., Harsha, K.N., Sharanappa, P., & Pradip, K. K. (2017). Evaluation of Substrates for Mass Production of Trichoderma harzianum and its Compatibility with Chlorpyrifos + Cypermethrin. *Int. J. Curr. Microbiol. App. Sci.*, 6(8), 3628-3635. https://doi.org/10.20546/ijcmas.2017.607.437

[36] Son, D., Zerbo, K.B.F., Bonzi, S., Legreve, A., Somda, I., & Schiffers, B. (2018). Assessment of Tomato (Solanum lycopersicum L.) Producers Exposure Level to Pesticides, in Kouka and Toussiana (Burkina Faso). *International Journal of Environnemental Research. And Public Health*, 15(2), 204. DOI: 10.3390/ijerph15020204

[37] Traoré, O., Wonni, I., Boro, F., Somtoré, E., Zombré, C. T., Dianda, O. Z., Wicker, E., Ilboudo, P., Ouedraogo, L. S., & Somda, I. (2013). Evaluation of the 19 varieties and accessions of tomato against bacterial wilt in Bobo-Dioulasso, Burkina Faso. *Int. J. Biol. Chem. Sci.*, 14(8), 2870-2879. DOI : https://dx.doi.org/10.4314/ijbcs.v14i8.17

[38] Trebicka, A., Oelmüller, R., Sherameti, I., Nogbri, P.L., & Johnson, J.M. (2012). Utilization of root-colonizing fungi for improved performance of agricultural crops. *Albanian Journal of Agricultural science*, 11, 9-16.

[39] Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., & Valéro, J.R. (2005). Wastewater sludge as a potential raw material for antagonistic fungus (Trichoderma sp.): Role of pre-treatment and solids concentration. *Water Res*, 39, 3587–3596. https://doi.org/10.1016/j.watres.2005.07.001

[40] Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y. & Valéro, J.R. (2007). Antagonistic fungi, Trichoderma spp.: Panoply of biological control. *Biochemical Engineering Journal*, 37, 1–20. https://doi.org/10.1016/j.bej.2007.05.012

[41] Zeringue, H.J. (1996). Possible involvement of lipoxygenase in a defense response in aflatoxigenic Aspergillus – cotton plant interactions. *Canadian Journal of Botany*, 74 (1), 98-102. https://doi.org/10.1139/b96-014