



# Efficacy of *Trichoderma harzianum* (Edtm) and *Trichoderma aureoviride* (T4) as potential bio-control agent of taro leaf blight caused by *Phytophthora colocasiae*

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## ABSTRACT

Leaf blight disease caused by *Phytophthora colocasiae* is the main constraint affecting taro plants in tropical and sub-subtropical countries. To control the disease, farmers commonly use chemical pesticides. Despite the efficacy of these chemical pesticides, their continued use is harmful to human and the environment. The alternative is the use of bio-control agents such as *Trichoderma spp.* The aim of this study was to evaluate the effect of *Trichoderma harzianum* (Edtm) and *Trichoderma aureoviride* (T4) as bio-control agents against *P. colocasiae*. The antagonism was evaluated by dual culture. The organic extracts were obtained by fermentation of each isolate of *Trichoderma* on potato dextrose broth (PDB). The chemical screening of the extracts was done and their efficiency on *P. colocasiae* was evaluated by poisoning method. The ability of the two antagonists to induce defense-related metabolites was done in pot experiments. In dual culture, the inhibition of the mycelia growth of the *P. colocasiae* was 34.77 and 41.77% for *T. harzianum* (Edtm) and *T. aureoviride* (T4), respectively. The organic extracts contained phenols, flavonoids, alkaloids, sterols and anthocyanins. Total inhibition (100%) of the mycelial growth of *P. colocasiae* was realized at 200 and 300µg/plug, respectively from *T. harzianum* (Edtm) and *T. aureoviride* (T4) organic extracts. In pot cultures, *T. harzianum* (Edtm) and *T. aureoviride* (T4) significantly reduced (49.4 and 46.4%, respectively) the necrosis of taro leaf blight. These strains significantly increased the activities of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and polyphenol content in healthy and infected taro plants. These findings suggested that the use of *T. harzianum* (Edtm) and *T. aureoviride* (T4) could be a promising alternative to bio-control taro leaf blight.

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## INTRODUCTION

Taro [*Colocaciae esculenta* (L.) Schott.] is the 14<sup>th</sup> most consumed vegetable worldwide. It is an important source of carbohydrates, vitamins, minerals and is used as staple or subsistence crop for millions of people in

developing countries (Lebot and Aradhya, 1991; Misra and Sriram, 2002). In Cameroon, its production was estimated about 1.8 tonnes (FAO, 2016). Despite their importance, taro plants face several production

constraints. Among them, the most redoubtable is taro leaf blight caused by an Oomycete namely *Phytophthora colocasiae*. The pathogen affects leaves and petioles, resulting in extensive damage of the foliage and reduction of biomass production (Misra et al., 2008; Fontem and Mbong, 2011). Taro leaf blight was first reported in Cameroon in 2010 with yield reductions of 90% (Mbong et al., 2013).

To control taro leaf blight, farmers currently use chemical pesticides such as Ridomil (Metalaxyl 4% and Mancozeb 64%) (Mbong et al., 2013). Although this approach has shown promising results, phytotoxicity and chemical residues are serious problems leading to environmental pollution and human health hazards (Reshu and Khan, 2012; Nelson et al., 2011). Moreover, they are not totally effective and often lead to the appearance of resistant strains of the pathogens (Hwang and Benson 2005; Nath et al., 2012, 2013). For these reasons, it is clear that an eco-friendly alternative strategy to fight against taro leaf blight is needed. In this respect, the use of micro-organisms such as fungal antagonists for pest management in agriculture is one of the most effective strategies of biological control (Vinale et al., 2008; Abdel-Kader et al., 2012; Saba et al. 2012).

Among the fungi, *Trichoderma* strains are the most studied biocontrol agents and have been successfully used as biopesticides and biofertilizers in greenhouse and field plant production (Abdel-Kader et al., 2012). However, outcomes of using beneficial microbes are strain dependent (Harman et al., 2004; Vinale et al., 2008). These biocontrol properties are due to different mechanisms of action such as competition for space and nutrients (Che, 1997; Howell, 2003); the production of cell wall-lytic enzyme, antibiosis, mycoparasitism and the induction of systemic resistance to pathogens in plants (Harman et al., 2004; Feng et al., 2013; Carvalho et al., 2014). The antagonistic properties of *Trichoderma* spp. in terms of antibiotic production have been the subject of intensive studies. The production of these compounds called secondary metabolites, although belonging to several chemical groups namely alkaloids, phenols, terpenes and polyketides are able to affect the interactions of plants with their pathogens (Küçük and Kivanç, 2005; Arjona-Girona et al., 2014; Vinale et al., 2014). In addition, the induction of systemic resistance in plants by *Trichoderma* spp. is often characterized by the synthesis of biochemical substances such as proteins, amino acids, phenolic compounds, sugars and enzymes [phenylalanine ammonia lyase (PAL) and polyphenol oxidases (PPO)] (Madhavi et al., 2018; Prasad et al., 2016; Tchameni et al., 2017). All these strategies used by *Trichoderma* spp. are often complementary and help to

neutralize the pathogen and thereby reduce the incidence of the disease. In our laboratory, many species of *Trichoderma* were isolated from taro plant rhizosphere. The screening of these isolates for their antagonism against *P. colocasiae*, revealed that *T. harzianum* (Edtm) and *T. aureoviride* (T4) were the most effective (Siebatcheu, 2014). However the effect of these antagonists as stimulators of taro plants resistance was not studied. The aim of this work was therefore to study the effect of extracts from *T. harzianum* (Edtm) and *T. aureoviride* (T4) to inhibit *P. colocasiae* and the ability of these beneficial organisms to stimulate biochemical defense in taro plants.

## MATERIALS AND METHODS

### *Trichoderma* strains

The strains of *T. harzianum* (Edtm) and *T. aureoviride* (T4) used in this study came from the culture collection of the Biochemistry Laboratory, University of Douala (Cameroon). The detail of the isolation and the morphological identification are given by Siebatcheu (2014).

### Pathogen agent

*P. colocasiae* was isolated from infected taro leaf samples collected in a field located at Douala – Cameroon. Leaf tissue fragments of 1 to 2 cm in size were excised from lesion margin of infected leaves. The surface was sterilized with alcohol (70%) for 30-40 s, rinsed twice with sterile distilled water and dried by sterilized blotting paper. Fragments were then inoculated on V8 agar medium in Petri dishes and incubated for 3 days at room temperature. *P. colocasiae* emerging from the infected tissue was purified by successive transfer and identified using the key of Erwin and Ribeiro (1996). The pathogenicity of these isolates was carried out on taro leaf discs as described by Sameza et al. (2014).

### *In vitro* antagonism assay

The antagonistic potential of *Trichoderma* isolates was evaluated against *P. colocasiae* using dual culture. Mycelial discs (5 mm diameter) were taken from 3 days old cultures of the antagonist and the pathogen. The discs were then paired on V8 agar plate in 90 mm Petri dishes. *P. colocasiae* was inoculated two days before *Trichoderma* strains. Plates inoculated only with the antagonist or pathogen served as control. All culture plates were incubated at room temperature for 8 days and the data expressed as the inhibition of *P. colocasiae*

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radial growth:

$$(\%I) = (D_o - D_x)/D_o \times 100.$$

Where,  $D_o$  is radial growth of pathogen in the control and  $D_x$  radial growth of pathogen in dual culture. In order to evaluate the survival of the micro-organisms, plugs were randomly removed from the intermingling regions and incubated on V8 medium supplemented with Benomil (inhibitor of *Trichoderma*) or with Chloramphenicol (inhibitor of *P. colocasiae*).

### Liquid culture and extraction of secondary metabolites

Five agar plugs from 2 days pre-culture of *Trichoderma* were introduced into 1 L conical flask containing 250 mL of PDB. The flask was incubated at 25°C in dark under stationary condition for 30 days. Cultures were then filtered on vacuum using Whatman paper N°4. The filtrate was extracted twice with ethyl acetate and evaporated under reduced pressure at 35°C. The crude residues obtained were stored at 4°C until use (Vinale et al., 2006).

### Chemical screening of the crudes extracts

#### Alkaloids

Fifty milligrams of extract were diluted in 10 ml of 2%  $H_2SO_4$ . The mixture was homogenized and boiled for two minutes and then filtered. Five drops of Mayer reagent were added to 1 ml of the filtrate. The development of turbidity confirms the presence of alkaloids (Wagner et al., 1996).

#### Phenols

Fifty milligrams of extract were dissolved in 3 ml of ethanol, and then 3 drops of 10%  $FeCl_3$  solution were added. The occurrence of a blue-violet or greenish coloration indicated the presence of phenols (Wagner et al., 1996).

#### Flavonoids

Five hundred milligrams of extract were dissolved in 5 ml of 1 N NaOH. Discoloration of the yellow color obtained after addition of 1 N chloridic acid indicated the presence of flavonoids (Trease and Evans, 2002).

### Triterpenes and stérols

Five milligrams of extract were dissolved in 2 ml of chloroform. Three drops of acetic anhydride and two drops concentrated sulfuric acid were added. The development of a red-brick reddish brown coloration was indicative of the presence of triterpens, and a blue coloring which turns to blue-orange was indicative the presence of sterols (Trease and Evans, 2002).

### Anthocyanines

Fifty milligrams of extract were mixed with 15 ml of 1% HCl and the resulting mixture was brought to the boil. The presence of anthocyanins was characterized by the change in color from orange red to orange blue (Bekro et al., 2007).

### Quinones

Fifty milligrams of extract were dissolved in 5 ml of 1 N chloridic acid. The resulting solution was boiled for 30 min. After cooling, it was extracted with 10 ml of chloroform. Of the extract with chloroform solution, 0.5 ml of dilute ammonia was added. The appearance of a red or purple coloration revealed the presence of quinones (Bekro et al., 2007).

### Antimicrobial assay of organic extracts

*P. colocasiae* plugs from three days preculture was placed at the center of V8 agar plate. The antimicrobial activities were done according to the method described by Vinale et al. (2006). The organic extract was diluted in dimethyl sulfoxide (DMSO) at different concentrations and ten microliters of these solutions were applied on the top of each plug at the rate ranging from 100 to 500  $\mu g$  plug<sup>-1</sup>. The same volume of DMSO was applied on the pathogen plug as control. Plates were incubated at 25°C for 3 days and the pathogen growth inhibition calculated according to the formula:

$$\%I = [(D_o - D_x)/D_o] \times 100$$

Where,  $D_o$ , pathogen growth diameter on control and  $D_x$  pathogen growth diameter on treated plate. Each treatment consisted of three replicates and the experiment was repeated twice.

### Pots experiment

#### Preparation of conidial suspension of *Trichoderma*

Conidial suspension of biocontrol agent was obtained by

liquid culture multiplication. Five agar plugs from 2 days' pre-culture of *Trichoderma* strains were introduced into 1 L conical flask containing 150 mL of potatoes dextrose broth (PDB). The flask was incubated at 25°C in the dark under stationary condition for 15 days. After fermentation, the cultures were ground using a robot mixer. The resulting solution was adjusted at  $1 \times 10^6$  conidia/mL.

### Experimental design

The pots experiment was conducted during the months of December 2017 and January 2018 in the nursery of the Laboratory of Biochemistry of the University of Douala. The local taro cultivar (*ebo coco* variety) which is sensitive to *P. colocasiae* was used as test crop. The taro tubers (one tuber per pot) were introduced into the 4.5 liter polyethylene pots (25 × 30 cm) about 3 cm deep from the surface. These pots contained 3.5 kg of mixture of soil and quartz [3: 1 (w / w)] sterilized twice in two days' intervals at 121°C for 1 h and allowed to cool for 48 h.

Fifteen days after seeding, each pot was inoculated with 100 mL of conidial suspension of bio-agent except the control which received 100 ml of distilled water. Thirty days after sowing, the plants were infected with *P. colocasiae*. The infection consisted inoculation of each taro leaf with two mycelial disks (6 mm) taken from the margin of mycelial growth of the pathogen. The seedlings thus treated were covered with transparent polystyrene bags for 48 h. Experiment was carried out in a completely randomized design (CRD) with six treatments. Each treatment contained four plants replicated three times. These treatments were divided into two blocks, namely the block of healthy plants and the block of infected plants with *P. colocasiae*. Each block consisted of: untreated plants with *Trichoderma* (ST0); plants treated with *T. harzianum* Edtm (ST1) and plants treated with *T. aureoviride* T4 (ST2). Each pot was watered daily with sterile tap water at a rate of 200 ml per plant. The infected plants were observed daily and the necrosis diameters were noted. Five days after infection, the inhibition of necrosis (I%) of taro leaf blight was evaluated according to the formula:

$$I\% = [(D_o - D_x / D_o) \times 100].$$

Where,  $D_o$  is necrosis diameter on untreated plants with *Trichoderma* and infected by *P. colocasiae*, and  $D_x$  is necrosis diameter on plants treated with *Trichoderma* and infected by *P. colocasiae*. Thirty-five days after planting, plants were harvested and washed with tap water to remove the soil particles. Leaves were removed and stored in the refrigerator at -20°C for biochemical analysis.

## Evaluation of biochemical parameters

### Total sugars and cysteine

Soluble sugars and cysteine were extracted from fresh taro leaves in 80% alcohol using the method of Singh et al. (1990). One gram of taro leaves was ground in a porcelain mortar and then introduced into 2 ml of 80% ethanol. The grounded material obtained was centrifuged at 3000 rpm using a centrifuge for 20 min. The supernatants were recovered and stored at + 2°C.

The soluble sugars extracted from the taro leaves were determined by the colorimetric method with 3,5-dinitro salicylic acid (DNS) proposed by Fisher and Stein (1961). The supernatant was diluted 10-fold with 80% alcohol. The standard range was obtained from a 0.1% aqueous glucose solution. To these solutions prepared at different concentrations was added DNS reagent (100  $\mu$ L). Solutions obtained was homogenized, put in water bath at 100°C for 5 min and then cooled in an ice bath. To these solutions was added 900  $\mu$ L of distilled water. The optical density (OD540 nm) was evaluated using a BIOBASE brand BK-UV-1600PC spectrophotometer. The data obtained from the standard range made it possible to obtain a graph of type  $DO = f$  (concentration of reducing sugars) having the regression line for the equation  $y = ax$ . From this line, the concentration of reducing sugars of the various extracts was expressed in g/l.

Cysteine was determined according to the ninhydrin colorimetric method described by Gaitonde (1967). For the assay, 0.35 mL of ninhydrin acid reagent [0.8% (w/v) ninhydrin in HCl:  $CH_3COOH$  concentrated in 1:4 (v/v)] was added to 100  $\mu$ L of soluble cysteine extract (supernatant). The mixture was incubated in water bath (100°C) for 10 min. Then, this reaction mixture was cooled in an ice bath. After cooling, 0.7 ml of 95% ethanol was added to the reaction mixture. The reading of the optical density was made at 560 nm. A standard was prepared from a 0.1% cysteine.

### Polyphenol analysis

One gram of the taro leaf powder obtained from the harvested, dry and grounded taro plants was macerated at 4°C for 1 h in 10 ml of 70% (v/v) methanolic solution. After maceration, the mixture was filtered with Whatman paper No. 1. After filtration, the residues were macerated again as previously and then filtered. The recovered filtrates were centrifuged for 20 min at 3000 rpm. The supernatants obtained served as a substrate for analyzing the phenolic compounds.

The polyphenol assay was performed by the method of Singleton and Rossi (1965). To do this, the catechol stock solution (100  $\mu$ g/ml) served as a standard

**Table 1.** Inhibition percentage of *P. colocasiae* growth in dual culture after 4 day.

	Control	<i>T. harzianum</i>	<i>T. aureoviride</i>
Growth diameter (mm)	38.33 ± 0.58 <sup>c</sup>	25 ± 0.00 <sup>b</sup>	22.33 ± 0.53 <sup>a</sup>
Inhibition (%)	0.00 <sup>a</sup>	34.77±0.00 <sup>b</sup>	41.77 ± 0.57 <sup>c</sup>

In each line, means ± standard deviation followed by the same letter are not significantly different ( $p \leq 0.05$ ).

substrate. One hundred microliters of each extract were mixed with 2.5 ml of the Folin-Ciocalteu reagent. After 10 min, 2 ml of 20% sodium carbonate was added to the mixture, homogenized and kept in the dark for 60 min at room temperature. The absorbance of each solution was determined at 760 nm using a spectrophotometer. The results were expressed as catechol equivalent in  $\mu\text{g}$  of total phenols per g of dry weight of taro leaves.

### PPO activity assay

The activity of the PPO was evaluated according to the protocol proposed by Mayer et al. (1966). One gram of taro leaf was finely cut and then ground dry in a porcelain mortar. The ground material obtained was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 7) at 4°C. The homogenate was centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant was recovered and served as enzyme extract.

The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer at pH 7 and 200  $\mu\text{l}$  of 0.01 M catechol. The reaction was induced by adding 20  $\mu\text{l}$  of the enzyme extract followed by incubation at 30°C for two min. After incubation, the reaction was stopped by immersing the tubes in ice for five min. The absorbance was read at 420 nm using a spectrophotometer. Specific activity was expressed as absorbance at 420 nm/min/mg protein/g fresh material.

### PAL activity assay

PAL extraction was performed by the method described by Yingsanga et al. (2008). One gram of fresh taro leaf was finely cut and then ground dry in a porcelain mortar. The ground material obtained was homogenized in 4 ml of 50 mM sodium phosphate buffer at pH 8.8 at 4°C. The resulting extract was filtered and the filtrate was centrifuged at 3000 rpm for 20 min at 4°C. The recovered supernatant was used as the enzyme source.

The PAL was measured in the supernatant following the method of Ross and Sederoff (1992). The reaction mixture consisting of 20  $\mu\text{l}$  of enzyme extract, 500  $\mu\text{l}$  of sodium phosphate buffer pH 8.8 and 600  $\mu\text{l}$  of *L*-phenylalanine (12 mM) was incubated at room temperature for 1 h. The reaction was stopped by placing

the tubes in ice bath. The absorbance of the *trans*-cinnamic acid released was measured at 290 nm using BK-UV-1600PC BIOBASE spectrophotometer. The enzymatic activity is expressed as a function of the change in absorbance/h/mg of protein/g of fresh material. Protein assay of enzymatic extracts was done following the method of Bradford (1976).

### Statistical analysis

Data was entered into an Excel spreadsheet (Microsoft Office, USA, 2010) and subsequently analyzed using SPSS software version 16 for Windows (SPSS, Inc., Chicago, IL, USA) and StatView version 5.0 for Windows (SAS Institute, Inc., IL, USA). Quantitative and qualitative data were presented as mean ± standard deviation (SD) and percent, respectively.

The data analysis of the variance (ANOVA) one factor was used to compare the averages. Duncan's post hoc test was used later to refine the analysis.

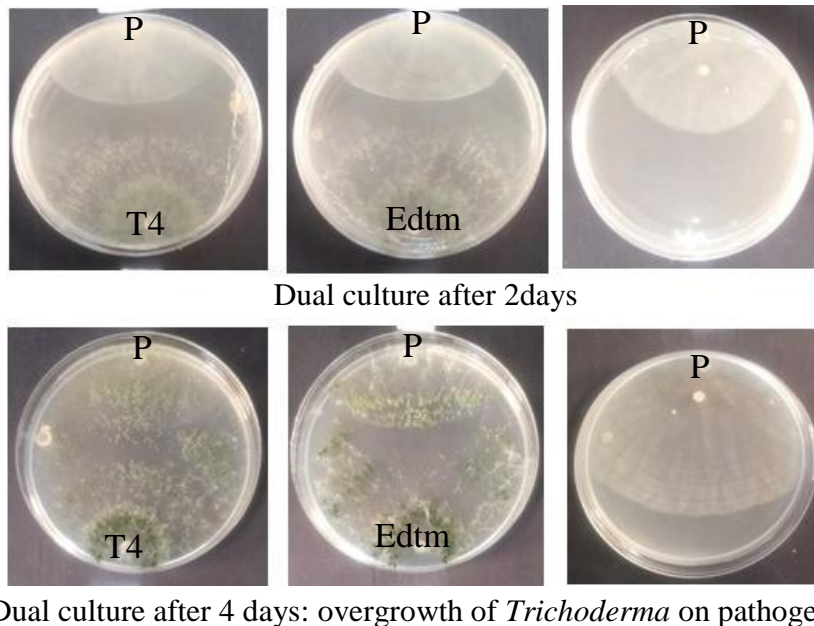
## RESULTS

### *In vitro* antagonism assay

The two strains of *Trichoderma* isolates used were antagonistic against *P. colocasiae* (Table 1). The inhibition percentage of the mycelia growth of the pathogen was 34.77 and 41.77% respectively, for *T. harzianum* (Edtm) and *T. aureoviride* (T4). On control plates, colony of *P. colocasiae* covered the 9 cm V8 plate in 5 days. In paired cultures however, the radial extension of this pathogen stopped abruptly few millimeter away from the colonies of *Trichoderma* strains, while the latter continued their normal growth all over the culture. With increasing age, the antagonist overgrew the colony of *P. colocasiae* (Figure 1). Attempts to re-isolate the microorganisms from the intermingling region resulted only in the growth of *Trichoderma* species.

### Chemical screening and inhibition effect of organics extracts against *P. colocasiae*

Chemical qualitative analysis of the organic extracts from



**Figure 1.** Dual culture test of *Trichoderma* against *P. colocasiae* on V8 plate. P, *P. colocasiae*; Edtm, *T. harzianum*; T4, *T. aureoviride*.

**Table 2.** Chemical constituents of organic extracts from *Trichoderma* spp.

Chemical constituents	Phenols	Alkaloids	Flavonoids	Triterpenes	Sterols	Quinones	Anthocyanines
<i>T. aureoviride</i> (T4)	+	+	+	-	+	-	+
<i>T. harzianum</i> (Edtm)	+	+	+	-	+	-	+

+, Present; -, absent.

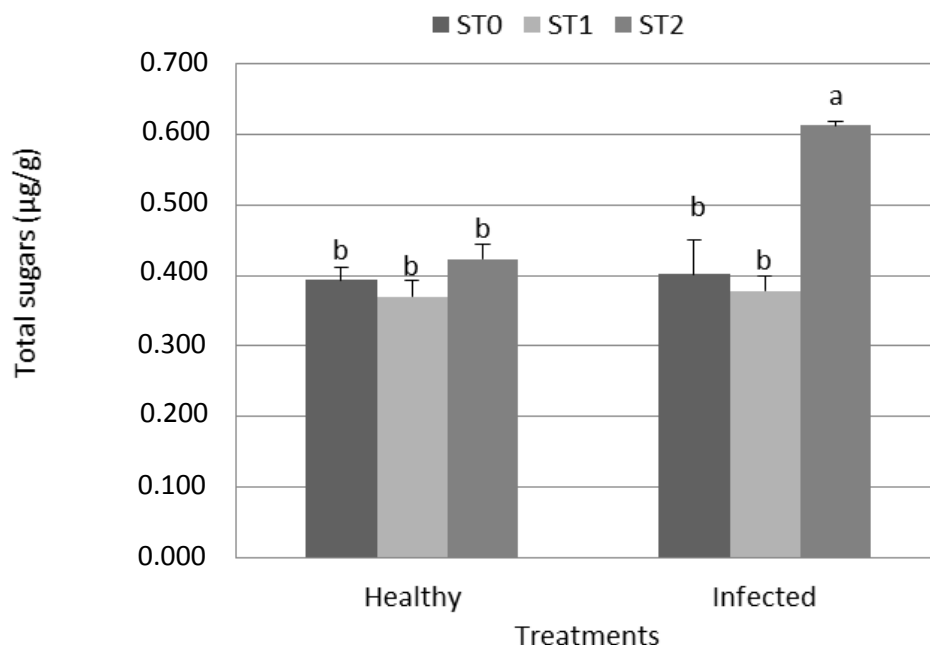
**Table 3.** Mycelial growth inhibition of *P. colocasiae* by organics extracts of *Trichoderma* spp.

Organic extracts ( $\mu\text{g}/\text{plug}$ )	<i>T. aureoviride</i> (T4)	<i>T. harzianum</i> (Edtm)
100.00	11.29 $\pm$ 2.12 <sup>d</sup>	16.25 $\pm$ 3.75 <sup>c</sup>
200.00	22.58 $\pm$ 1.73 <sup>b</sup>	100.00 $\pm$ 0.00 <sup>a</sup>
300.00	100.00 $\pm$ 0.00 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>
400.00	100.00 $\pm$ 0.00 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>
500.00	100.00 $\pm$ 0.00 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>
Control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

Means  $\pm$  standard deviation followed by the same letter are not significantly different ( $p \leq 0.05$ ).

liquid fermentation of *T. harzianum* (Edtm) and *T. aureoviride* (T4) showed the presence of phenols, flavonoids, alkaloids, sterols and anthocyanins. However, these extracts do not have quinones and triterpenes (Table 2).

The organic extract of *Trichoderma* isolates significantly ( $P \leq 0.05$ ) reduced the radial growth of *P. colocasiae* compared to control (Table 3). The inhibition percentage increased with the extract concentration with total inhibition (100 %) of mycelial growth occurred at 300



**Figure 2.** Effect of *Trichoderma* strains on total soluble sugars content in leaf taro plant infected or not with *P. colocasiae*.

Histograms with the same letter are not significantly different. **ST0**, Untreated plant; **ST1**, plant treated with *T. harzianum* (dtm); **ST2**, plant treated with *T. aureoviride* (T4). Each treatment was made by 4 pots with 3 replicates.

**Table 4.** Effect of bioagent on necrosis inhibition.

	Control	<i>T. harzianum</i>	<i>T. aureoviride</i>
Necrosis diameter (mm)	51.25 ± 0.95 <sup>c</sup>	27.49 ± 0.92 <sup>b</sup>	25.93 ± 0.92 <sup>a</sup>
Inhibition (%)	0.00 <sup>a</sup>	46.36 ± 2.88 <sup>b</sup>	49.40 ± 2.96 <sup>c</sup>

In each line, means ± Standard Deviation followed by the same letter are not significantly different ( $p \leq 0.05$ ).

and 200 µg/plug respectively, for *T. aureoviride* (T4) and *T. harzianum* (Edtm). At rate of 250 and 150 µg/plug respectively for *T. aureoviride* (T4) and *T. harzianum* (Edtm) *P. colocasiae* hyphae appeared under light microscope to be disorganised, heterogeneous and very thin compared to the control. Moreover, when *P. colocaseae* plug from plate exhibiting total inhibition was removed and transferred to new control V8 agar plate, result showed that the pathogen was killed at 300 and 200 µg/plug respectively, for *T. aureoviride* (T4) and *T. harzianum* (Edtm).

#### Inhibition of necrosis of taro leaf blight

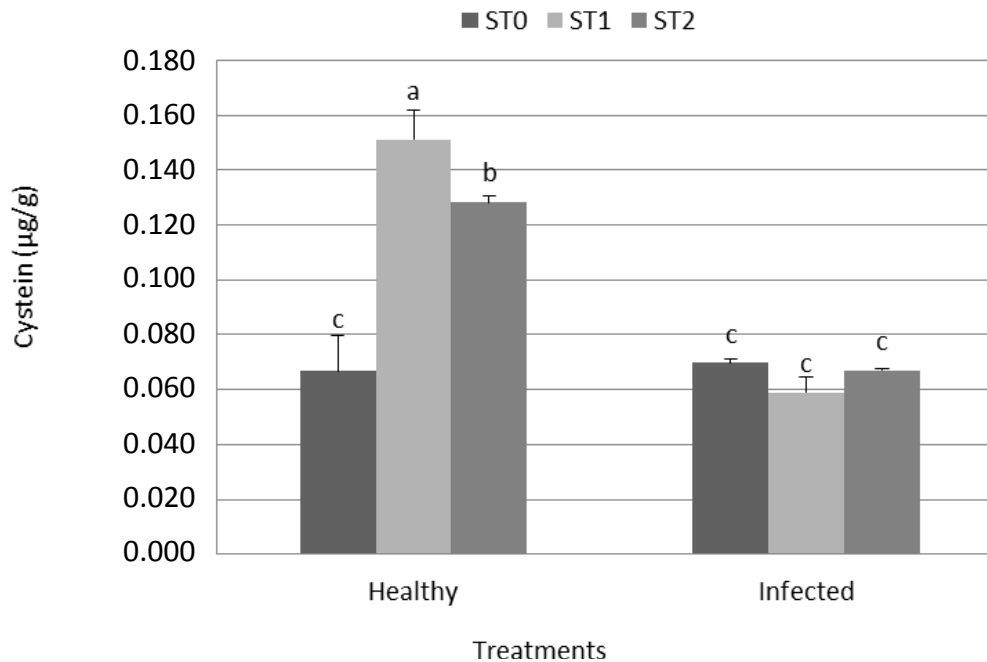
The result showed that the two strains of *Trichoderma* used significantly ( $P < 0.05$ ) inhibited taro leaf blight

necrosis (Table 4). The inhibition of necrosis diameter was 49.40 and 46.36% respectively, for *T. aureoviride* and *T. harzianum*.

#### Total sugars and cysteine leaf content

In healthy plants, results showed that there was no significant difference of the total sugar content between the plants inoculated or not with *Trichoderma* spp. (Figure 2). However, in infected plants, the content of total soluble sugar was significantly increased after inoculation with *T. aureoviride*.

In the healthy plants, the inoculation of each *Trichoderma* isolate significantly increased the cysteine content compared to non-inoculated plants (Figure 3). On the other hand, in infected plants, there was no significant



**Figure 3.** Effect of *Trichoderma* spp. on total cysteine content in leaf taro plant infected or not with *P. colocasiae*.

Histograms with the same letter are not significantly different. **ST0**, Untreated plant; **ST1**, plant treated with *T. harzianum* (dtm); **ST2**, plant treated with *T. aureovirideae* (T4). Each treatment was made by 4 pots with 3 replicates.

difference in cysteine content between the plant treated with *Trichoderma* and control.

*T. harzianum* (Edtm) both in healthy and infected plants. Similarly, the activity of this enzyme also increased in infected plants, compared to healthy one.

### Polyphenol content

The results presented in the Figure 4 revealed that, there is significant increase ( $P=0.01$ ) of polyphenol content in healthy and infected plants after inoculation with *T. aureoviride* (T4), and *T. harzianum* (Edtm). In addition, compared to healthy plants, infection significantly ( $P=0.01$ ) increased the polyphenol content.

### PPO activity

The effect of *Trichoderma* and the infection resulted in a change in enzymatic activity of PPO (Figure 5). In healthy and infected plants, there was a significant ( $P=0.001$ ) increase in enzymatic activity compared to controls. In addition, infection resulted in increasing the PPO activity.

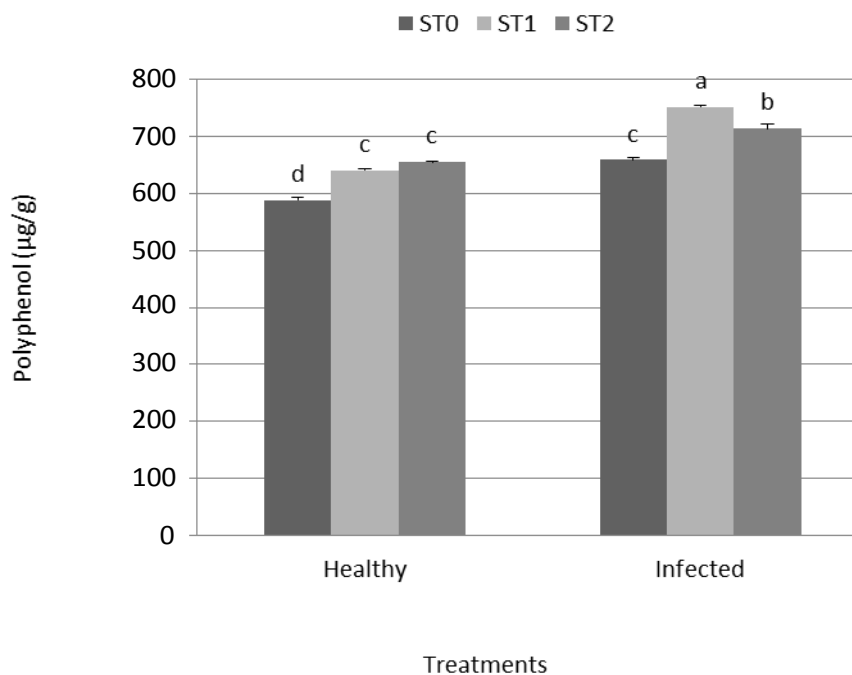
### PAL activity

PAL activity (Figure 6) significantly increased ( $P=0.005$ ) when the plants were treated with *T. aureoviride* (T4) and

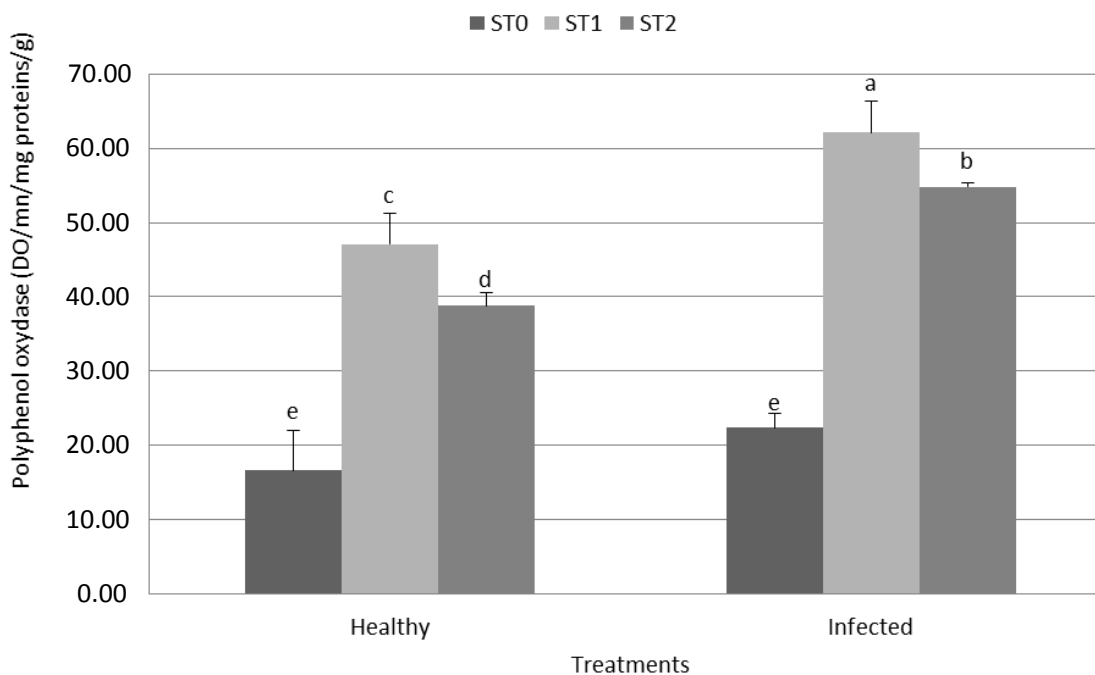
### DISCUSSION

The current work evaluated the potency of two strains of *Trichoderma* to inhibit the growth of *P. colocasiae* and stimulate the biochemical defense mechanisms in taro plants. In dual culture tests, the results showed that both *Trichoderma* isolates inhibited mycelial growth of *P. colocasiae* on V8 agar. This inhibition could be due to the production of lytic enzymes (glucanases, proteinases, chitinases and cellulases) by *Trichoderma* which may degrade *P. colocasiae* cell walls (Howell, 2003; Harman, 2006). Moreover, these antagonists are documented to produce volatile and non-volatile antibiotics which could act in synergy with enzymes to inhibit the pathogen. Mycoparasitism is also one of the main mechanisms involved in antifungal antagonism. However, these mechanisms are probably never exclusively responsible for inhibition of the pathogen, but they act in combination (Howell, 2003; Harman, 2006). According to Abo-Elyousr et al. (2014), mycoparasitic activity of *Trichoderma* is manifested morphologically by the overgrowth upon the mycelial growth of the pathogen. These observations

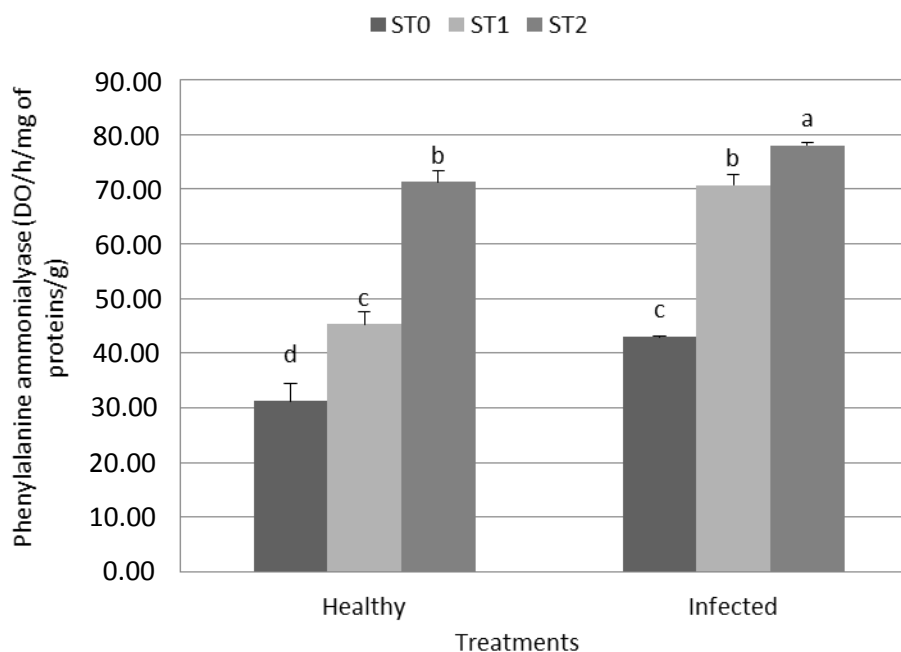




**Figure 4.** Effect of *Trichoderma* spp on polyphenol content in leaf taro plant infected or not with *P. colocasiae*. Histograms with the same letter are not significantly different. **ST0**, Untreated plant; **ST1**, plant treated with *T. harzianum* (dtm); **ST2**, plant treated with *T. aureoviride* (T4). Each treatment was made by 4 pots with 3 replicates.



**Figure 5.** Effect of *Trichoderma* spp on activity of PPO content in leaf taro plant infected or not with *P. colocasiae*. Histograms with the same letter are not significantly different. **ST0**, Untreated plant; **ST1**, plant treated with *T. harzianum* (dtm); **ST2**, plant treated with *T. aureovirideae* (T4). Each treatment was made by 4 pots with 3 replicates.



**Figure 6** Effect of *Trichoderma* spp. on activity of PAL in leaf taro plant infected or not with *P. colocasiae*

Histograms with the same letter are not significantly different. **ST0**, Untreated plant; **ST1**, plant treated with *T. harzianum* (dtm); **ST2**, plant treated with *T. aureovirideae* (T4). Each treatment was made by 4 pots with 3 replicates.

were also obtained in this study. During the antagonism, each strain of *Trichoderma* used overgrown on mycelia of *P. colocasiae* and the pathogen was killed. Furthermore, organic extract from fermented *Trichoderma* isolated also inhibited the mycelia growth of *P. colocasiae*. These results could be due to the presence of several classes of chemical compounds such as phenols, alkaloids, flavonoids, quinones, sterols, anthocyanines found into the *Trichoderma* extracts and which are well known for their inhibition potential of plant pathogens (Kubicek and Harman, 1998; Küçük and Kivanç, 2005; Agnem et al., 2011). Results obtained in this study are similar to those of Promwee et al. (2017) who showed the capacity of organic ethyl acetate extract from *T. harzianum* (FR-NST-009) to inhibit the mycelial growth of *Phytophthora* spp. and related the activity to the presence of several classes of chemical compounds.

The application of biological agents on taro rhizosphere was used to evaluate systemic resistance. Induction of plant resistance by *Trichoderma* is usually expressed visually by reducing disease symptoms. This work showed a significant reduction of necrosis in plants treated with both strain of *Trichoderma*. Since the antagonist and the pathogen were applied at different points (the former in the taro rhizosphere and the later on leaves), the reduction of the disease symptoms could be due to induced systemic resistance. However, the direct

antagonism should not be excluded. Indeed, *Trichoderma* can also act as an endophytic fungus, diffuse into various parts of the plant and release molecules that could have a direct antimicrobial effect on the pathogen (Pandey et al., 2015). Several studies have already shown the ability of *Trichoderma* spp. to induce resistance to leaf pathogens either by foliar or root application (Prakasam and Sharma, 2012; Shahnaz et al., 2012; Mukherjee et al., 2014; Promwee et al., 2017). Indirect antagonism of biocontrol agents may induce the activities of various enzymes and defensive chemicals in plants.

The PPO and PAL activity were significantly increased in plants treated with *Trichoderma*. Indeed, PPO is involved in the oxidation of phenolic compounds to quinone's. Li and Steffens (2002) suggested that quinonoid products of PPO are highly reactive molecules which caused the covalent modification and cross-linking of nucleophilic substituents of amino acids and proteins. These modification are thought to exert an anti-nutritive defense against pathogens. Moreover, the active quinones produced by PPO may possess direct antibiotic and cytotoxic activities to pathogens. They can be polymerized for synthesis of wall-reinforcing structures such as lignin and suberin (Vidhyasekaran, 1988). Concerning PAL, it is the first enzyme in the phenylpropanoid biosynthesis pathway involved in the production of phenolic compounds such as phytoalexins,

furancoumarins, phytoanticipins and compounds forming part of the structural barrier such as the deposition of callose which are responsible for the resistance of plant pathogens (Shoresh et al., 2010).

The results from this study also showed that, polyphenol content in taro plants were increased by the treatments with *Trichoderma*. The accumulation of these compounds in plants cells could be related to their role in strengthening the cell wall, their direct antimicrobial role on the pathogen as well as their activation of defense genes of plant (Singh et al., 2010; Surekha et al., 2013; Slatnar et al., 2016).

In healthy plant, cysteine content significantly increased after treatment with *Trichoderma*. Cysteine plays a central role in the metabolism and the response of the plant to biotic stress. It intervenes in the protein structure where it is often founded in their active site, associated with cofactors. It is also the precursor of the glucosynolates of methionine and glutathione which are involved in hypersensitive reaction in plants (Romero et al., 2013; Couturier et al., 2013).

Overall, significant increase of PPO, PAL, polyphenols and cysteine was obtained in leaf taro plants treated with *Trichoderma* or/and infected with *P. colocasiae* suggesting that, these metabolites are implicated in the adaptation of plant inoculated by *Trichoderma* to biotic stress.

## CONCLUSION

This study showed that *T. harzianum* (Edtm) and *T. aureoviride* (T4) are antagonist against *P. colocasiae* and can stimulate the biochemical defense in taro plants. The use of these bio-agents offers a promising alternative to chemical fungicides in management of taro leaf blight. However, further studies on isolation and purification of pure compounds from organic extracts of *Trichoderma* isolates as well as field experiments by foliar application should be investigated.

## REFERENCES

- Abdel-Kader M. M., El-Mougy N. S., Aly M. D. E., Lashin S. M. & Abdel-Kareem F. (2012). Greenhouse biological approach for controlling foliar diseases of some vegetables. *Adv. Life Sci.* 2(4):98-103.
- Abo-Elyousr K. A., Abdel-Hafez S. I. & Abdel-Rahim I. R. (2014). Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. *J. Phytopathol.* 162(9):567-574.
- Agnem E. C., Mebanga S. A., Abba A. & Nyonbourg E. (2011). Evaluation de l'activité antibactérienne de cinq plantes de la pharmacopée traditionnelle de l'Adamamoua (Cameroun). *Cam. J. Exp. Biol.* 7(1):22-27.
- Arjona-Girona I., Vinalé F., Ruano-Rosa D., Lorito M. & López-Herrera C. J. (2014). Effect of metabolites from different *Trichoderma* strains on the growth of *Rosellinia necatrix*, the causal agent of avocado white root rot. *Eur. J. Plant Pathol.* 140(2):385-397.
- Bekro Y. A., Mamyrbekova J. A., Boua B. B., Bi F. T. & Ehile E. E. (2007). Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (*Caesalpinaceae*). *Sci. Nat.* 4(2):217-225.
- Bradford M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72(1-2):248-254.
- Carvalho D. D. C., Murillo L. J., Irene M., Peter W. I. & Sueli C. M. M. (2014). Biological control of *Fusarium oxysporum* f. sp. phaseoli by *Trichoderma harzianum* and its use for common bean seed treatment. *Trop. Plant Pathol.* 39(5):384-391.
- Che I. (1997). *Trichoderma*-application, mode of action, and potential as a biocontrol agent of soilborne plant. Creelman R. A., Mullet J. E. Oligosaccharins, brassinolides, jasmonates: nontraditional regulators of plant growth, development, and gene expression. *Plant Cell.* 9:1211-1223.
- Couturier J., Chibani K., Jacquot J. P. & Rouhier N. (2013). Cysteine-based redox regulation and signaling in plants. *Front Plant Sci.* 4:105.
- Erwin D. C. & Ribeiro O. K. (1996). *Phytophthora diseases worldwide*. American Phytopathological Society (APS Press).
- FAO, FAOSTAT (2016). Food and Agriculture Organization of the United Nations. Available at: www.FAO.org
- Feng C., Guanghui Y., Ping W., Zhong W., Lin F., Qirong S. & Wei C. (2013). Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. *Plant Physiol.* 73:106-113.
- Fisher E. H. & Stein E. A. (1961). DNS colorimetric determination of available carbohydrates in foods. *Biochem. Prep.* 8:30-37.
- Fontem D. A. & Mbong G. (2011). A novel epidemic of taro (*Colocasia esculenta*) blight caused by *Phytophthora colocasiae* hits Cameroon (Abstract). Third Life Science Days held at the University of Dschang, Cameroon.
- Gaitonde M. K. (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochem. J.* 104(2):627.
- Harman G. E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology.* 96(2):190-194.
- Harman G. E., Howell C. R., Viterbo A., Chet I. & Lorito M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Rev. Microbiol.* 2(1):43-56.
- Howell C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Dis.* 87(1):4-10.
- Hwang J. & Benson D. M. (2005). Identification, mefenoxam sensitivity, and compatibility type of *Phytophthora* spp. attacking floriculture crops in North Carolina. *Plant Dis.* 89:185-190.
- Kubicek C. P. & Harman G. E. (1998). *Trichoderma* and *Gliocladium*. Basic biology, taxonomy and genetics. Taylor and Francis Ltd. 156Pp.
- Küçük Ç. & Kivanç M. (2005). *In vitro* antifungal activity of strains of *Trichoderma harzianum*. *Turk. J. Biol.* 28(2-4):111-115.
- Lebot V. & Aradhya K. M. (1991). Isozyme variation in taro (*Colocasia esculenta* (L.) Schott) from Asia and Oceania. *Euphytica.* 56(1):55-66.
- Li L. & Steffens J. C. (2002). Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta.* 215:239-247.
- Madhavi G. B., Devi G. U., Kumar K. V. K. & Ramesh T. (2018). Evaluation of *Pseudomonas fluorescens* and *Trichoderma harzianum* isolates in inducing systemic resistance (ISR) in maize against *Rhizoctonia solani* f. Sp. Sasakii. *IJCS.* 6(2):628-632.
- Mayer A. M., Harel E. & Ben-Shaul R. (1966). Assay of catechol oxidase: a critical comparison of methods. *Phytochemistry.* 5(4):783-789.
- Mbong G. A., Fokunang C. N., Fontem L. A., Bambot M. B. & Tembe E. A. (2013). An overview of *Phytophthora colocasiae* of cocoyams: A potential economic disease of food security in Cameroon. *Discourse J. Agric. Food Sci.* 1(9):140-145.
- Misra R. S. & Sriram S. (2002). Medicinal value and export potential of tropical tuber crops. Series recent progress in medicinal plants, crop improvement, production technology and commerce. 5:376-386.

- Misra R. S., Sharma K. & Mishra A. K. (2008). *Phytophthora* leaf blight of Taro (*Colocasia esculenta*)—a review. Asian Australas J. Plant Sci Biotechnol. 2: 55-63.
- Mukherjee A. K., Kumar A. S., Kranthi S. & Mukherjee P. K. (2014). Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. 3 Biotech. 4(3):275-281.
- Nath V. S., Sankar M. S., Hegde V. M., Jeeva M. L., Misra R. S., Veena S. S. & Raj M. (2012). Evaluation of fungicides on Indian isolates of *Phytophthora colocasiae* causing leaf blight of taro. Arch. Phytopathol. Plant Prot. 46(5):548-555.
- Nath V. S., Senthil M., Hegde V. M., Jeeva M. L., Misra R. S., Veena S. S. & Raj M. (2013). Evaluation of fungicides on Indian isolates of *Phytophthora colocasiae* causing leaf blight of taro. Arch. Phytopathol. Plant Prot. 46(5):548-555.
- Nelson S., Brooks F. & Teves G. (2011). Taro leaf blight in Hawaii. Plant Dis. Bull. No. PD-71.
- Pandey S., Srivastava M., Shahid M., Kumar V., Singh A., Trivedi S. & Srivastava Y. K. (2015). *Trichoderma* species cellulases produced by solid state fermentation. Journal of Data Mining in Genomics & Proteomics. 6(2): 1.
- Prakasam V. & Sharma P. (2012). *Trichoderma harzianum* (Th-3) a potential strain to manage the purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri* under North Indian plains. J. Agri. Sci. 4(10):266.
- Prasad R. D., Navaneetha T. & Rao L. V. (2016). Plant growth promotion and induced defense response in safflower (*Carthamus tinctorius* L.) by *Trichoderma*. J. Biol. Control. 30:40-48.
- Promwee A., Yenjit P., Issarakraisila M., Intana W. & Chamswarnng C. (2017). Efficacy of indigenous *Trichoderma harzianum* in controlling *Phytophthora* leaf fall (*Phytophthora palmivora*) in Thai rubber trees. J. Plant Dis. Prot. 124(1):41-50.
- Reshu & Khan M. M. (2012). Role of different microbial-origin bioactive antifungal compounds against *Alternaria* spp. causing leaf blight of mustard. Plant Pathol. J. 11:1-9.
- Romero L. C., Aroca M. Á., Laureano-Marín A. M., Moreno I., García I. & Gotor C. (2013). Cysteine and cysteine-related signaling pathways in *Arabidopsis thaliana*. Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Avenida Américo Vespucio, 49:41092 Sevilla, Spain.
- Ross W. W. & Sederoff R. R. (1992). Phenyl alanine ammonia lyase from loblolly pine. Purification of enzyme and isolation of the complementary DNA clones. Plant Physiol. 98:380-386.
- Saba H., Vibhash D., Manisha M., Prashant K. S., Farhan H. & Tauseef A. (2012). *Trichoderma* – a promising plant growth stimulator and biocontrol agent. Mycosphere. 3(4):524-531.
- Sameza M. L., Boat M. A. B., Nguemezi S. T., Mabou L. C. N., Dongmo P. M. J., Boyom F. F. & Menuc C. (2014). Potential use of *Eucalyptus globulus* essential oil against *Phytophthora colocasiae* the causal agent of taro leaf blight. Eur. J. Plant Pathol. 140(2):243-250.
- Shahnaz E., Razdan V. K., Rezwi S. E. H., Rather T. R., Gupta S. & Andrabi M. (2012). Integrated disease management of foliar blight disease of onion: A case study of application of confounded factorials. J. Agri. Sci. 5(1):17.
- Shoresh M., Harman G. E. & Mastouri F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. Ann. Rev. Phytopathol. 48:21-43.
- Siebatcheu E. C. (2014). Criblage de quelques souches de *Trichoderma* spp. Pour leur potentiel antagoniste vis-à-vis de *Phytophthora colocasiae*, agent responsable du mildiou du taro (*Colocasia esculenta*). Mémoire DEA Université de Douala-Cameroun. Pp. 33-35.
- Singh R. A., Aggarwal A., Bhuillar S. S. & Goyal J. (1990). Import of sucrose and its partitioning in the synthesis of galactomannan and raffinose oligosaccharide in developing guar (*Cyanopsis tetragonolobus*) Seeds. J. Exp. Bot. 41:101-110.
- Singh U. S., Bari M. A., Kumar A., Ismail A. & Mackill D. J. (2010). Status of stress tolerant rice in South Asia. Proceedings of the workshop on Development and Adoption of Stress Tolerant Rice Varieties. 19 August 2009. Dhaka International Finance Corporation & South Asia Enterprise Development Facility. Pp. 20-24.
- Singleton V. L. & Rossi S. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. J. enol. Viticult. 16:144.
- Slatnar A., Mikulič-Petkovšek M., Veberič R. & Štampar F. (2016). Research on the involvement of phenolics in the defence of horticultural plants. Acta Agriculturae Slovenica. 107(1):183-189.
- Surekha C. H., Neelapu N. R. R., Kamala G., Prasad B. S. & Ganesh P. S. (2013). Efficacy of *Trichoderma viride* to induce disease resistance and antioxidant responses in legume *Vigna mungo* infested by *Fusarium oxysporum* and *Alternaria alternata*. Int. J. Agric. Sci. Res. 3(2):285-294.
- Tchameni S. N., Sameza M. L., Anthonio O. D., Fokom R., Ngonkeu M. E. L., Nana Wakam L. & Nwaga D. (2017). Antagonism of *Trichoderma asperellum* against *Phytophthora megakarya* and its potential to promote cacao growth and induce biochemical defence. Mycology. Pp. 1-9.
- Trease G. E. & Evans W. C. (2002). Pharmacognosy. 15th Ed. (ed). Saunders Publishers London. Pp. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
- Vidhyasekaran P. (1988). Physiology of disease resistance in plants. Volume 2. CRC Press, Inc.
- Vinale F., Marra R., Scala F., Ghisalberti E. L., Lorito M. & Sivasithamparam K. (2006). Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett. Appl. Microbiol. 43(2):143-148.
- Vinale F., Sivasithamparam K., Ghisalberti E. L., Marra R., Woo S. L. & Lorito M. (2008). *Trichoderma*–plant–pathogen interactions. Soil Biol. Biochem. 40(1):1-10.
- Vinale F., Sivasithamparam K., Ghisalberti E. L., Woo S. L., Nigro M., Marra R. & Manganiello G. (2014). *Trichoderma* secondary metabolites active on plants and fungal pathogens. Open Mycol. J. 8(1):127-139.
- Wagner H. X. S., Blatt Z. & Gain E. M. (1996). Plant drug analysis. Springer verlag, Berlin, Germany. 360p.
- Yingsanga P., Srilaong V., Kanlayanarat S., Noichinda S. & McGlasson W. B. (2008). Relationship between browning and related enzymes (PAL, PPO and POD) in rambutan fruit (*Nephelium lappaceum* Linn.) cvs. Rongrien and See-Chompoo. Post. Biol. Technol. 50(2-3):164-168.