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Different Morphotypes of *Fusarium oxysporum* Isolated from *Xanthosoma sagittifolium* L. Schott Roots: Action of Ethanol Leaf Extracts of *Psidium guajava* on their *in Vitro* Inhibition and on *X. Sagittifolium* Plants Inoculated with *F. oxysporum*

A. C. Djeuani[1,2*], H. D. Mbouobda[1,3], T. A. Mbekem[1,2] and N. Niemenak[1]

[1]Laboratory of Plant Physiology, Department of Biological Sciences, Higher Teacher Training College (HTTC), University of Yaoundé I, P.O.Box-47, Yaoundé-Cameroon.
[2]Department of Plant Biology, Faculty of Sciences, University of Yaoundé I, P.O.Box- 812 Yaoundé-Cameroon.
[3]Department of Biology, Higher Teacher Training College, Bambili (HTTC), The University of Bamenda, P.O.Box-39, Bamenda-Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TAM, ACD and HDM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ACD and HDM managed the analyses of the study. Authors ACD and NN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to determine the different morphotypes of *Fusarium oxysporum* present in the root of *Xanthosoma sagittifolium* and evaluate the effect of alcoholic extracts of *Psidium guajava* on their *in vitro* inhibition. Strains of *Fusarium oxysporum* were collected in eight localities where *X. sagittifolium* is grown. *Fusarium* strains isolated from roots of *X. sagittifolium* harvested in each locality were grown on PDA medium. The antifungal test was evaluated using ethanol extracts from *P. guajava* leaves at 30 and 60%. The virulence test of each strain on young

**Corresponding author: Email: djeaunid@gmail.com, djeuani.uyi@hotmail.com, astride-carole.djeuani@facsciences-uy1.cm;*

plants of *X. sagittifolium* aged three months were realized. Eight strain of *Fusarium oxysporum* were successfully isolated. After maximum growth, five morphological types were observed (pionnotal, sclerotic, clowny, cottony and ras senescent). The cottony strain was abundant and present in all the locality. Histological analysis of the different strains obtained revealed the presence of septate or siphoned hyphae and three types of conidia (microconidia, macroconidia and sporangiospores or chlamidospores). The inhibition tests were very high with 60% of ethanol extract of *P. guajava*, and 83.33% of inhibitory effect were observed after eight days of growth, in the strains collected in *X. sagittifolium* roots, in L_3 (Loum) and L_4 (Bangoua) localities. After infection of *X. sagittifolium* plants with each strain of *F. oxysporum* isolated, symptoms observed were yellowing and wilting of leaves. However, plants inoculated with the L_3 (Loum) strain showed both yellowing and wilting of leaves. The application of ethanol extracts from *P. guajava* leaves reduced the severity of the disease in the inoculated plants after 14 days. These results obtained showed that *F. oxysporum* is not only saprophytic fungi, it's also able to induce yellowing and wilting of leaves in *X. sagittifolium*.

Keywords: Fusarium oxysporum; Xanthosoma sagittifolium L. Schott; Psidium guajava; inhibition; antifungal test.

1. INTRODUCTION

Fusarium oxysporum (Nectriaceae) is an ascomycete telluric, ubiquitous and plant parasitic fungi that collectively infect different hosts plant. The presence of this ascomycete in the soil leads to significant production losses in many crops such as *Musa acuminate* [1], *Gossypium hirsutum* [2], *Cucumis melo* [3], *Lycopersicon esculentum* [4], *Solanum tuberosum* [5,6]. This soil-borne pathogen also has a great influence on the quality of plant seeds [7]. The visible symptoms due to the presence of *F. oxysporum* include yellowing of the leaves, wilting of tissue and vascular lesions thus causing death of the plant [8]. [9] Showed that *F. oxysporum* are responsible of damping-off in *Pinus massoniana* plants.

This endophytic plant fungus can remain as dormant mycelium or chlamydospores without causing disease [10], virulent or non-virulent depending on the host species they infect [11,12]. This may be why [13], noted the presence of *Fusarium solani* and *Rhizoctonia solani* during root rot of *Xanthosoma sagittifolium* L. Schott caused by *Pythium myriotylum*. Hence, it would be good to know if, *Fusarium* species, acts as a saprophyte that benefits from the aggressiveness of *Pythium myriotylum* towards s the roots of *X. sagittifolium* plants, or remains in a state of latency due to the action of *P. myriotylum*. Could *Fusarium oxysporum* be an aggressive pathogen for *Xanthosoma sagittifolium* plants? Or does it remain only a profiteer during root rot during *X. sagittifolium* – *P. myriotylum*.

In plants species where *Fusarium* are absolute pathogens, some authors have shown that their action could be inhibited by medicinal plant extracts. Plant extracts are good efficient against the phytopathogenic fungi, due to the synergistic antimicrobial, antifungal, antibacterial activity of their various phytochemical constituents [14,4,15,16]. It is noted that aqueous extracts of *Curcuma longa* can be used against root rot caused by *Fusarium solani* in *Helianthus annuus* L. [16] and against *Fusarium oxysporum* [17]. The antifungal activity of ethanol and acetone extract of leaves of *Piper betel, Lowsonia inermis, Psidium guajava, Carica papaya, Moringa oleifera, Mimosa pudica, Catharanthus roseus, Adhatoda vasica* and *Andrographis paniculata* have been effective against *Fusarium oxysporum* the causal agent of *Fusarium* wilt in tomato (*Lycopersicon esculentum* Mill.) [4]. Despite the type of solvent used, the antimicrobial activities of plants against a number of plant pathogens are linked to their richness in bioactive compounds. These compounds not only have an impact on the growth of the mycelium of the fungi, but they also influence the production and sporulation of spores [18]. Many studies show the remarkable presence of bioactive compound in medicinal plants [19,4,16,17].

As a traditional medicinal plant, *Psidium guajava* L. (Myrtaceae), has received much attention for producing many complex compounds. The therapeutic properties of *P. Guajava* include insecticidal [20], antimicrobial [21,22], antifungal [23], antiviral [24] and antioxidant properties [25]. Theses antioxidant and antifungal activities of leaf extract of *P. Guajava* against *Fusarium* sp,

Alterneria sp. and *Colletotrichum* sp have been shown by [26] and [27] studies. More, aqueous and ethanol leaf extract of *Psidium guajava* L. have shown their efficacy against *in vitro* inhibition of *Pythium myriotylum* Drechsl, main causative agent of root rot disease in *X. sagittifolium*.

So to understand if, *Fusarium oxysporum*, can be pathogenic or saprophytic organism to *X. sagittifolium* roots, this study aims to determine the different morphotypes present in the roots of *X. sagittifolium*, to evaluate the effect of ethanol extracts of *Psidium guajava* leaves on the *in vitro* inhibition of the fungal strains, this during the interaction *F. oxysporum* - *X. sagittifolium*.

2. MATERIALS AND METHODS

2.1 Harvesting, Isolation, Cultivation and Purification of *Fusarium oxysporum* Strains of the *Xanthosoma sagittifolium* Roots

The harvest roots of *X. sagittifolium* used to isolate *F. oxysporum* strains was carried out between October and November (at the end of the rainy season and the beginning of the dry season), in *X. sagittifolium* plants with yellow leaves and infected roots (dark in color and having a mole end). This harvest of the *X. sagittifolium* plant roots were carried out in polyculture farms of *X. sagittifolium* and other food crops in eight localities (L). L_1 (Soa), L_2 (Banda), L_3 (Loum) L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona). In each harvest locality, three repetitions were carried out and the collected samples were mixed. Roots of *X. sagittifolium* collected were properly washed with running tap water, air-dried for a few minutes, cut into small pieces (1 cm), soaked in sterilized water, with 1% sodium hypochlorite (NaClO) for 3 min and then rinsed with distilled water as described by [28]. All of the small pieces of roots were transferred onto water agar (WA) medium in order to first observe the appearance of colonies [29] then sub-cultured on potato dextrose agar medium mixed with 200 mg/L of Streptomycin to prevent bacterial proliferation [30], until pure culture were obtained.

2.2 Production, Observation and Identification of Spores of *Fusarium oxysporum*

This production of *F. oxysporum* spores was carried out by thermal shock according to the protocol of [31]. The mycelium of each fungal strain was collected according to the method of [32]. After seven days of culture, the mycelia were homogenized in 50 ml of ice cold sterile distilled water for 10 to 15 min. This mixture was then placed in the dark for the release and germination of the spores. Morphological identification was done by observation of the fungal characteristic under binocular optical microscope (IVYMEN mark) at 400X. The isolates obtained were identified using the synoptic keys [33], dichotomous keys [34] and tabular keys [35]. These keys are based on the examination of the morphological characters of asexual structures.

2.3 Evaluation of the Effect of Ethanol Leaf Extracts of *Psidium guajava* on the *In vitro* Growth of *Fusarium oxysporum*

The antifungal effect of the ethanol leaf extracts of *P. guajava* plants was evaluated using two concentrations (PDA + 30 and PDA + 60% of leaf extract). Inhibitory activity was evaluated with the poisoned food method [36]. Control plates consisted in PDA with ethanol. The culture of these *Fusarium* strains was carried out in the dark in a culture chamber at 25±1°C. Every 2 days, the mean radial diameter was measured. Growth inhibition relative to the control due to the efficacy of the ethanol leaf extract of *P. guajava* was calculated according to the following formula of [19]:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

Where, C and T represent the diameter of control and treated colony, respectively. Here three replications were prepared for each treatment. Data on mycelial growth at 0,2,4,6 and 8 days after inoculation were recorded. To avoid bacterial contamination 0.5 g of antibacterial streptomycin was added to 1 liter of PDA medium.

2.4 Evaluation of the Virulence of Each Strains of *Fusarium oxysporum* on *Xanthosoma sagittifolium* Plants

In vitro of *X. sagittifolium* plants were obtained according to the protocol of [37] and [38]. These vitroplants were transferred to pods containing a mixture of black earth, sand, and wooden chips 2:1:1 (V/V) previously oven sterilized (mark

REPLEX) at 170°C for 24 hours for acclimation. After three months, for each strain of *F. oxysporum* isolated, 25 seedlings of *X. sagittifolium* were used. The inoculum was directly leached to the root zone through the soil. Each plant was inoculated with 5ml of the suspension containing $2x10^7$ cfu/ml concentration of each strain of *F. oxysporum* according to the protocol of [21]. The plants were observed regularly for the appearance and development of disease symptoms. The severity of symptoms was scored on a scale ranging from 1 through 5: 1-No obvious symptoms; 2-Symptoms on 0-24% of leaves; 3-Symptoms on 25% - 50% of leaves; 4-Symptoms on 51% - 74% of leaves and 5-Symptoms on 75% -100% of leaves [39] after 14 days.

2.5 Evaluation of the Effect of *P. guajava* Ethanol Leaf Extracts on Xanthosoma Sagittifolium Infected by *Fusarium oxysporum*

X. sagittifolium plants used were divided into two groups: control plants, (treated only with the ethanol solution) and the plants previously inoculated with each strain of *F. oxysporum* isolated (treated with ethanol leaf extracts of *P. guajava*). In each treatment 25 *X. sagittifolium* plants aged three months were used. The *P. guajava* leaves were cut in small pieces; washed in distilled water; dried at 40°C for 48 hours in an oven (Barnstead/Lab-Line; USA; Model No. 121). The dried leaves were ground using a mortar and pestle into fine powder, 60g of *P. guajava* leaf powder were soaked in 100ml of 95% ethanol in a sterilized bottle and kept overnight at room temperature for 48h. The ethanol fraction was separated through sterilized Whatman filter paper (No.1). The supernatant collected constituted the ethanol extract of *P. guajava*. The extracts obtained were applied on aerial parts and the rhizosphere of each plant, using a hand sprayer until wetness of plants according to Yasser et al., (2017). Plants were treated twice time at day 7 and day 14 (for two weeks) and disease severity score evaluated at day 14 using method of [39].

2.6 Statistical analysis

Results were expressed in the form of Means ± SD. All the statistical analysis was done using Microsoft excel. Duncan Multiple Range Test at 5% significance was used for the comparative analyses of the results with the help of SPSS 20.0.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Morphological analysis of the different strains of *Fusarium oxysporum* isolated from *Xanthosoma sagittifolium*

Morphological analysis of the eight strains of *Fusarium oxysporum* collected in each locality shows that the growth is not the same for all strains after 8 days of *in vitro* culture (Fig. 1). Strains collected in L_1 (Soa), L_2 (Banda), L_3 (Loum), L_6 (Bansoa) and L_7 (Santa), grew faster than those collected in L_4 (Bangoua), L_5 (Abong-Mbang) and L_8 (Ekona). the cottony or wooly form was present in all the harvesting locations (Table 1I). Similarly, a variation in the staining of the strains is visible and the most dominant is the white color (strains collected in L_1, L_2, L_5, and L_8) followed by the beige color compared to the pink and purplish pink (Table 2). The pure culture showed that the cottony strain of hyphae is the most abundant in *F. oxysporum* in in all harvest areas.

3.1.2 Structure of conidia of *F. oxysporum* strains

Microscopic observation of conidia of each strain of *Fusarium oxysporum* shows that zoospores differ from one strain to another (Fig. 2). Strains of L_3, L_4, L_6, and L_7 only have microconidia (nonseptate and monoseptate). Those of L_1, L_2, L_5, and L_8 showed two types of conidia, microconidia (nonseptate and monoseptate) and macroconidia (two-septate, three-septate, and four-septate). The results also show that microconidia are most abundant in all cultivated *F. oxysporum* strains. Chlamydospores are also present in strains collected in L_1, L_2, and L_5.

3.1.3 Effect of *P. guajava* ethanol leaf extracts on *in vitro* inhibition of radial growth of *F. oxysporum*

Inhibition tests of *F. oxysporum* with *P. guajava* ethanol leaf extract was significant at 30 and 60% compared to the control (Table 3). This inhibition of growth is higher at 60%. The results show that radial growth of control is 9.0 cm, in strains obtained in L_2 (9.0 ± 0.00 cm), L_3 (9.0 ± 0.05 cm), L_4 (9.0 ± 0.02 cm), L5 (9.0 ± 0.05 cm), L_6 (9.0 ± 0.04 cm), L_7 (9.0 ± 0.01 cm) and L_8 (9.0 ± 0.00 cm) after 8 days of growth (Table 3). However, at 30%, at 30%, growth inhibition of strains of *F. oxysporum* is variable.

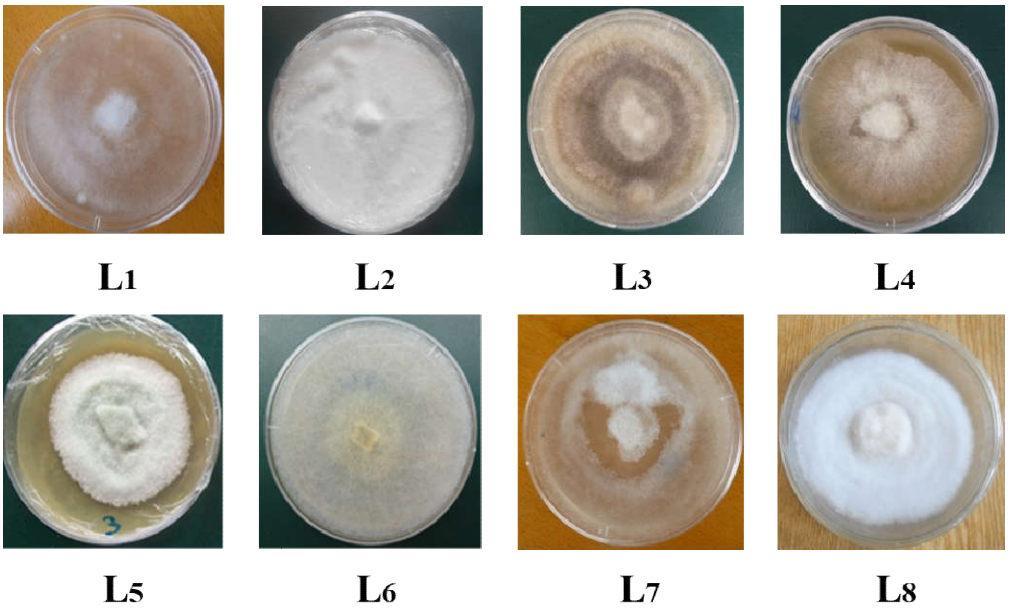

Fig. 1. Aspect of growth of *Fusarium oxysporum* strains in Petri dishes. *F. oxysporum* strain harvested in different localities: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

Higher growth was obtained after eight days in *F. oxysporum* strains collected in L_3 (6.5 ± 0.03cm) and the highest record of inhibition was with strain collected in L_2 (73,36%) after 2 days. The results also show that the percentage of inhibition decreased over time. At 60%, the inhibition of radial growth is very high for all the strains. The low growth values were observed in *F. oxysporum* isolated from *X. sagittifolium* of L_4 varying between day 2 and day 8, from 1.2 ± 0.04 to 1.5 ± 0.01 cm. This radial growth is constant in the strain harvested in L_3 (1.5 cm) (Table 2). In the strain harvested at L_5, the inhibition of growth does not vary in the presence of 30 and 60% of ethanol leaves extract concentrations. This inhibition is 4.0 ± 0.02 and 4.0 ± 0.05 cm respectively (Table 3). Inhibition of rate of growth was more with 60% ethanol leaf extracts of *P. guajava*. Thus, high inhibition (83.33%) was observed with strains isolated from *X. sagittifolium* of L_3 and L_4, after 8 days of growth. This percentage of inhibition is lower at 30% (Table 4).

3.1.4 Virulence test of each strain of *Fusarium oxysporum* harvested and inoculated to *Xanthosoma sagittifolium* plants obtained *in vitro*

The test of virulence of different strains of *F. oxysporum* showed that plants inoculated with strains isolated from *X. sagittifolium* roots harvested from L_1, L_2, L_4 L_5 and L_6 presented an overall yellowing of the leaves (Fig. 3). *X. sagittifolium* plants inoculated with strains from L_7 and L_8 presented wilting of the leaves of the basal part of the plant (Fig. 4A and B). However, 14 days after, plants inoculated with the *F. oxysporum* strains isolated from *X. sagittifolium* harvested from L_3 showed both yellowing and wilting of leaves (Fig. 5). All inoculated plants had several dry roots in the rhizosphere. The severity of the disease evaluated varies significantly at 5% with all the strains of *F. oxysporum* isolated (Fig. 7A). It is maximum 64.00±0.86% with the *F. oxysporum* strain isolated at L_3. The severity score was zero in the control (Fig. 7A and B). The lowest severity percentage was recorded with the *F. oxysporum* strain isolated at L_5. This severity is identical in strains of L_4 (56.00 ± 1.00%), L_6 (56.00 ± 0.86%) and L_8 (56.00 ± 0.50%). After treatment with ethanol extract of *P. guajava* in twice day 7 and day 14, there is a resumption of growth (Fig. 6). The leaves were greener and wider and the petioles more vigorous. The reduction in severity was noted in plants treated with the solution of ethanol leaf extracts from *P. guajava* (Fig. 7B). This reduction is 67.85% in the presence of the strain of *F. oxysporum* isolated at L_6. It is greater than 50% in plants of *X. sagittifolium* inoculated with L_2 (64.06%), L_4 (51.78%) L_7 (54.16%) and L_8 (56.25%), then treated with ethanol leaf extract of *P. guajava*.

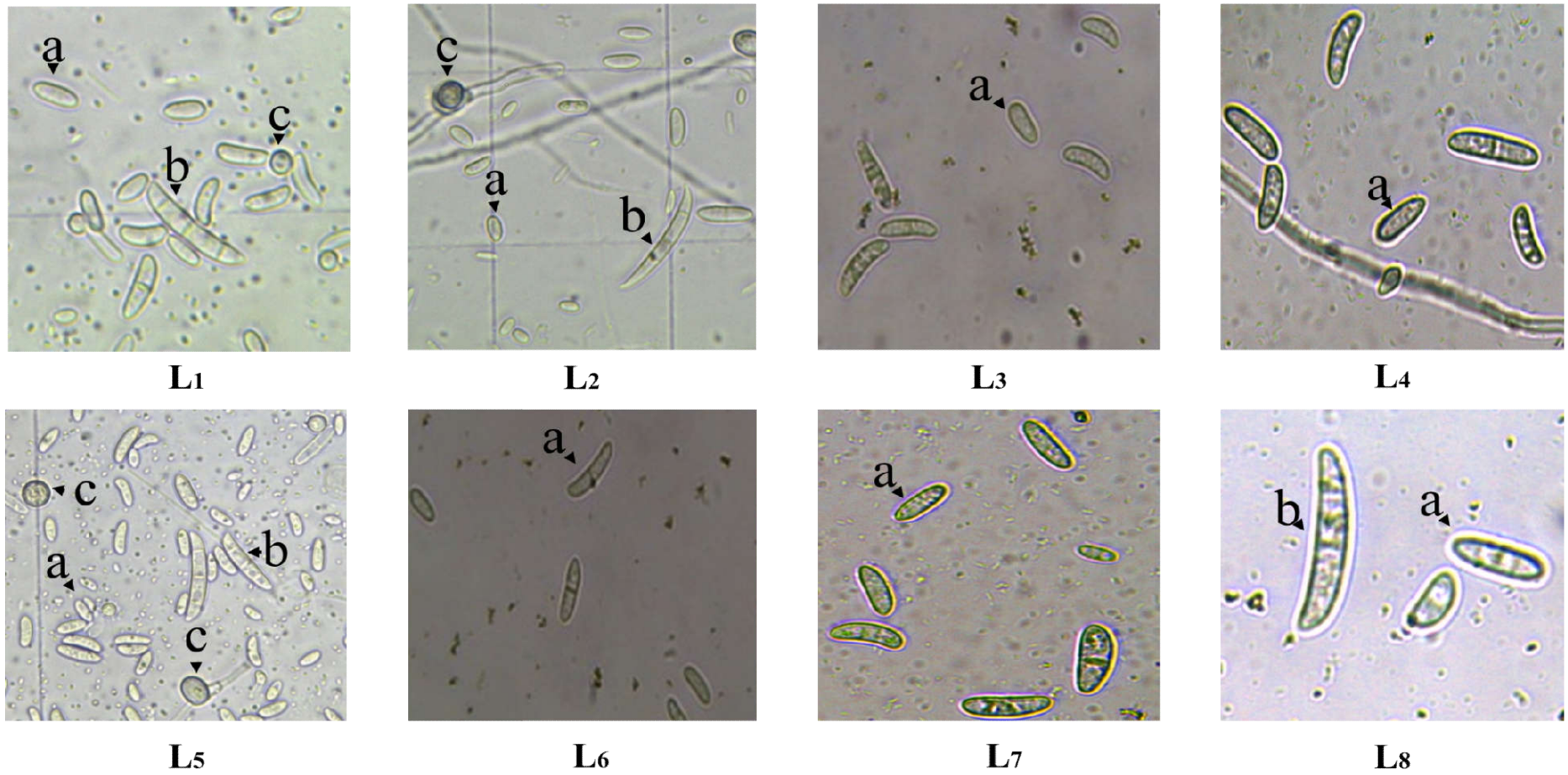

Fig. 2. Structures of the conidia of the different strains of *Fusarium oxysporum* according to the harvest locality observed under an optical microscope (Brand) at 400X. Microconidia (a), macroconidia (b) and chlamydospores (single) (c). *F. oxysporum* strain harvested in different localities: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

Table 1. Abundance of strains in roots following localities

	Harvest locality							
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
Fluffy	+	-	-	-	-	-	-	-
Woolly or cottony	+	+	+	+	+	+	+	+
Sclerotal	-	-	+	-	-	-	-	-
Senescent Ras	-	-	-	+	-	-	-	-
Woolly or cottony	+	+	+	+	+	+	+	+
Pionnotal	-	-	-	-	-	+	-	-
Senescent Ras	-	-	-	-	-	-	+	-
Woolly or cottony	+	+	+	+	+	+	+	+

F. oxysporum strain isolated from X. sagittifolium roots in different localities: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona). Absent (-) and present (+)

Table 2. The colony characters and sporulation of different strains of *F oxysporum* collected in roots following localities

Strains	Texture	Color	Density	Aerial mycelium	Growth habit	Form	Sporulation
L_1	Fluffy	White	Low	Regular	Moderate	Radial	Abundant
L_2	Woolly	White	Regular	Abundant	Abundant	Radial	Profuse
L_3	Sclerotal	Purplish pink	Regular	Abundant	Abundant	Radial	Moderate
L_4	Senescent Ras	Pinkish	Abundant	Abundant	Moderate	Radial	Moderate
L_5	Woolly	White	Abundant	Abundant	Slow	Radial	Abundant
L_6	Pionnotal	Beige	Regular	Regular	Abundant	Radial	Poor
L_7	Senescent Ras	Beige	Regular	Abundant	Moderate	Radial	Moderate
L_8	Woolly	White	Abundant	Abundant	Moderate	Radial	Moderate

F. oxysporum strain harvested in different localities: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

Table 3. Average radial growth of the different strains of *Fusarium oxysporum* in petri dish according to concentrations *Psidium guajava* applied

Concentration %	Inhibition time (Days)	Average radial growth of the different strains							
		Strains harvested according to localities							
		L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
0	0	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}
	2	6.0±0.02^{b}	6.3±0.01^{b}	6.0±0.06^{b}	5.5±0.05^{b}	5.9±0.01^{b}	6.5±0.01^{b}	4.9±0.11^{b}	6.5±0.08^{b}
	4	7.8±0.07^{c}	7.8±0.14^{c}	7.8±0.10^{c}	7.8±0.07^{c}	7.5±0.02^{c}	7.9±0.05^{c}	7.6±0.13^{c}	7.5±0.10^{c}
	6	8.7±0.01^{d}	8.8±0.15^{d}	8.6±0.05^{d}	8.9±0.06^{d}	8.7±0.00^{d}	8.8±0.04^{d}	8.5±0.09^{d}	8.6±0.11^{d}
	8	8.9±0.01^{d}	9.0±0.00^{d}	9.0±0.05^{d}	9.0±0.02^{d}	9.0±0.05^{d}	9.0±0.04^{d}	9.0±0.01^{d}	9.0±0.00^{d}
30	0	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}
	2	1.7±0.06^{b}	1.3±0.01^{b}	5.5±0.01^{b}	2.2±0.01^{b}	1.6±0.10^{b}	2.2±0.03^{b}	1.7±0.06^{b}	1.9±0.01^{b}
	4	2.0±0.05^{c}	1.8±0.05^{b}	5.5±0.01^{b}	3.4±0.00^{c}	2.6±0.09^{c}	2.7±0.12^{b}	2.3±0.01^{b}	3.0±0.05^{c}
	6	3.2±0.01^{d}	2.6±0.09^{c}	5.7±0.02^{b}	3.9±0.11^{cd}	3.3±0.06^{d}	3.0±0.01^{c}	3.4±0.01^{c}	3.2±0.05^{c}
	8	4.0±0.01^{e}	3.3±0.08^{d}	6.5±0.03^{c}	4.4±0.10^{d}	4.0±0.02^{e}	3.3±0.05^{c}	3.4±0.05^{c}	4.0±0.05^{d}
60	0	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00
	2	2.1±0.03^{b}	2.2±0.01^{b}	1.5±0.08^{b}	1.6±0.04^{b}	2.3±0.01^{b}	1.5±0.04^{b}	0.0±0.00^{a}	1.5±0.01
	4	2.5±0.05^{b}	3.3±0.00^{c}	1.5±0.10^{b}	1.5±0.00^{b}	3.2±0.01^{c}	1.7±0.05^{b}	2.2±0.05^{b}	2.5±0.02
	6	2.5±0.08^{b}	3.3±0.05^{c}	1.5±0.01^{b}	1.5±0.05^{b}	3.7±0.03^{d}	2.0±0.05^{c}	2.3±0.09^{b}	2.5±0.02
	8	2.7±0.01^{b}	3.3±0.05^{c}	1.5±0.11^{b}	1.5±0.01^{b}	4.0±0.05^{d}	2.0±0.06^{c}	2.3±0.10^{b}	2.5±0.04

Data sharing the same letter in the same column were not significantly different at 5% level (Duncan's multiple range tests).

F. oxysporum strain harvested in different localities: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

Table 4. Evaluation of the average percentage of inhibition in the different strains of *Fusarium oxysporum* according to the concentrations of *Psidium guajava* applied

Concentration (%)	Inhibition time (days)	Percentage inhibition (%)							
		Strains harvested according to localities							
		L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
30	0	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
	2	71.66	79.36	08.33	60.00	72.88	66.15	65.30	70.76
	4	74.35	76.92	29.48	56.41	65.33	65.82	69.73	64.00
	6	63.21	70.45	33.72	56.17	62.06	65.90	60.00	62.79
	8	55.05	63.33	27.77	51.11	55.55	63.33	62.22	55.55
60	0	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
	2	65.00	65.07	75.00	78.18	61.01	76.92	00.00	76.92
	4	67.79	57.69	80.76	83.31	57.33	78.84	71.05	66.66
	6	71.26	62.50	82.55	83.14	57.47	77.27	72.94	70.93
	8	69.66	63.33	83.33	83.33	55.55	77.77	74.44	72.22

F. oxysporum strain harvested in different localities: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

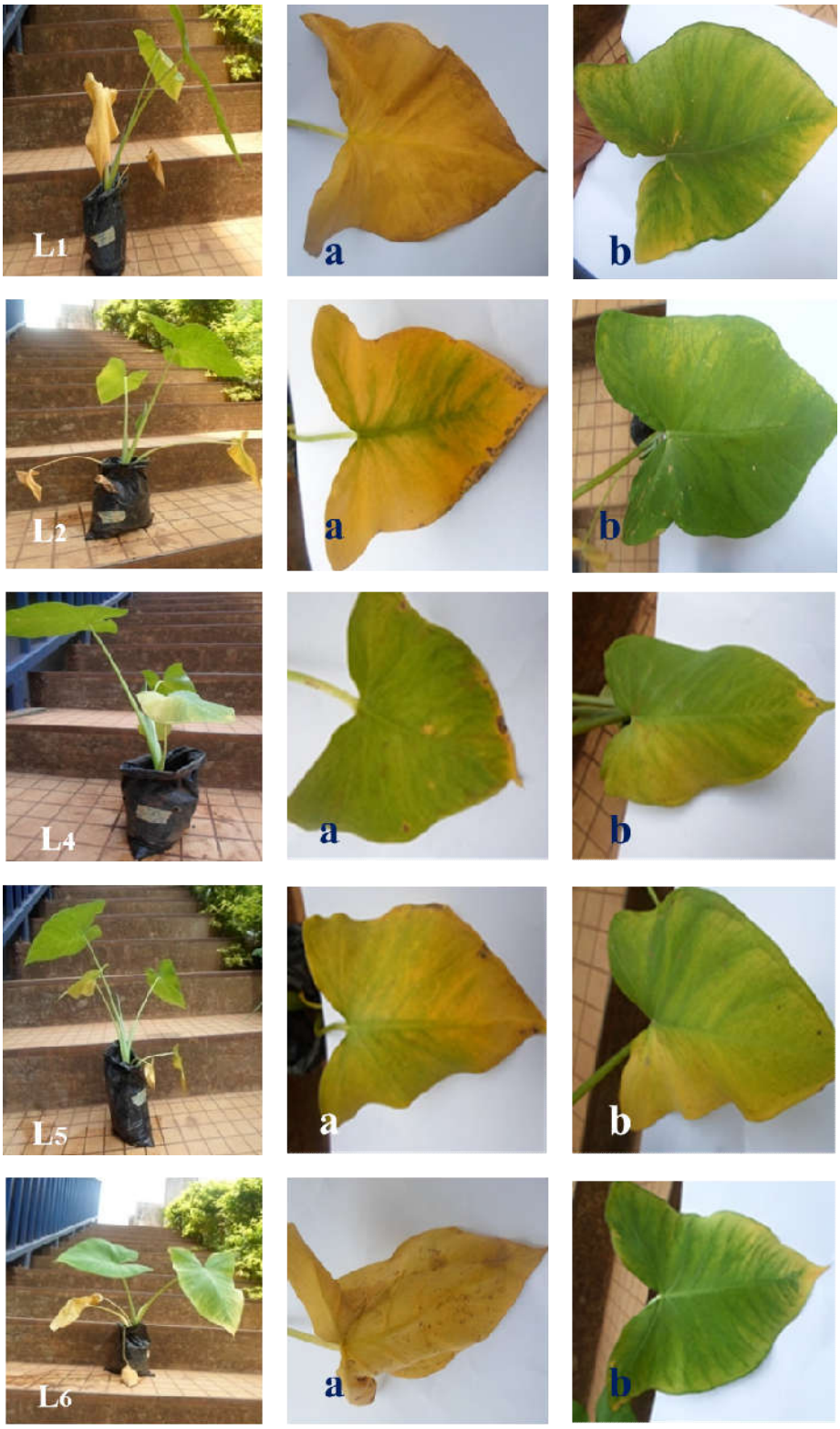

Fig. 3. Aspect of *X. sagittifolium* leaves at Day 14 after inoculation. *F. oxysporum* strain responsible of the yellowing of leaves according to the different harvesting locations: L_1 (Soa), L_2 (Banda), and L_4 (Bangoua), L_5 (Abong-Mbang) and L_6 (Bansoa). Leaves almost or completely yellow (a) and leaf showing yellowing (b)

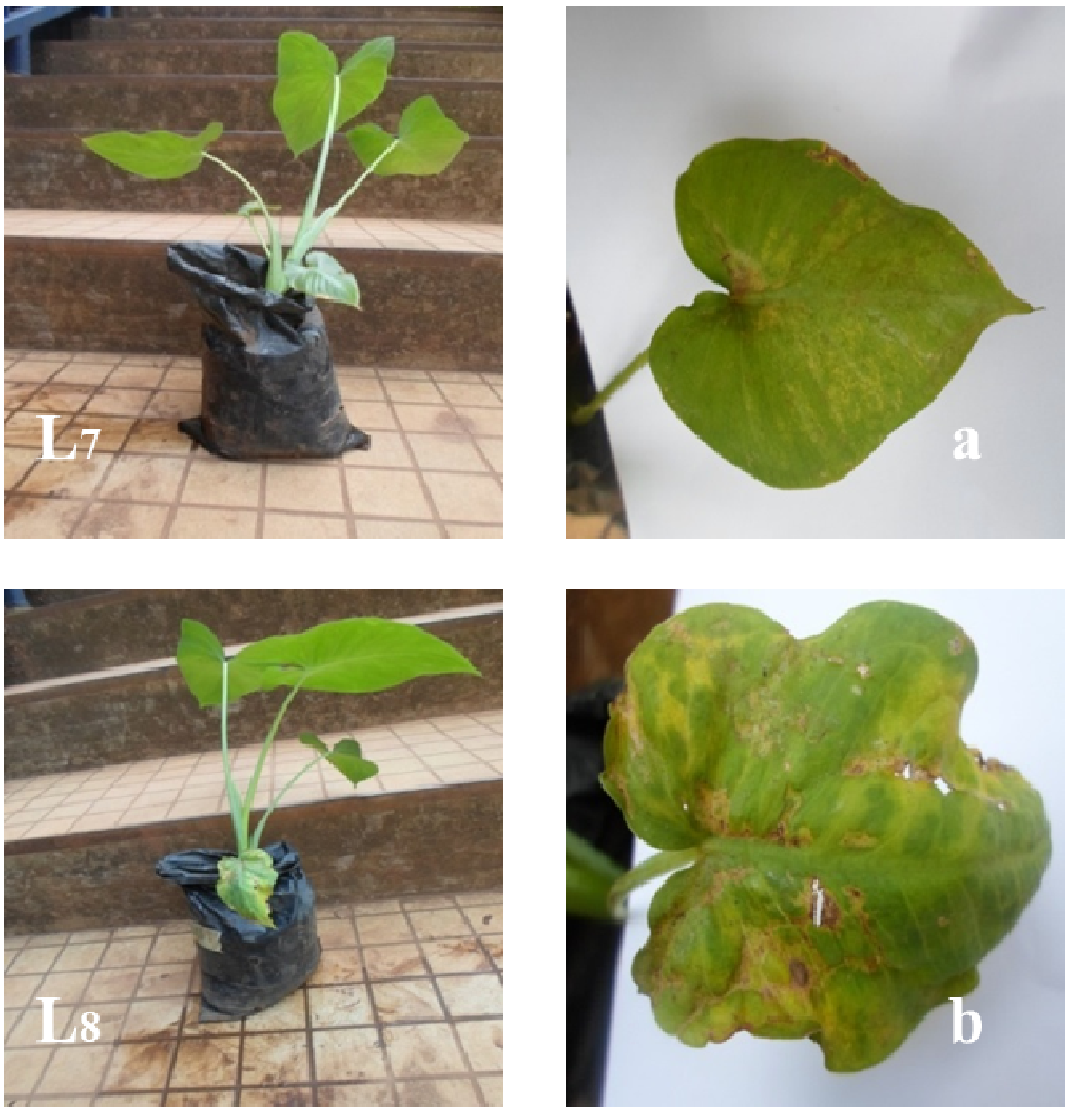

Fig. 4. Plantlets with wilting of the leaf due to the action of *Fusarium oxysporum* after 14 days of inoculation. *F. oxysporum* strain responsible of the wilting of leaves according to the different harvesting locations: L_7 (Santa) and L_8 (Ekona). Withered leaves (a and b)

Fig. 5. Plantlets of *X. sagittifolium* with both yellowing (a) and wilting (b) leaves after 14 days of inoculation with *F. oxysporum* strain harvested in L_3 (Loum)

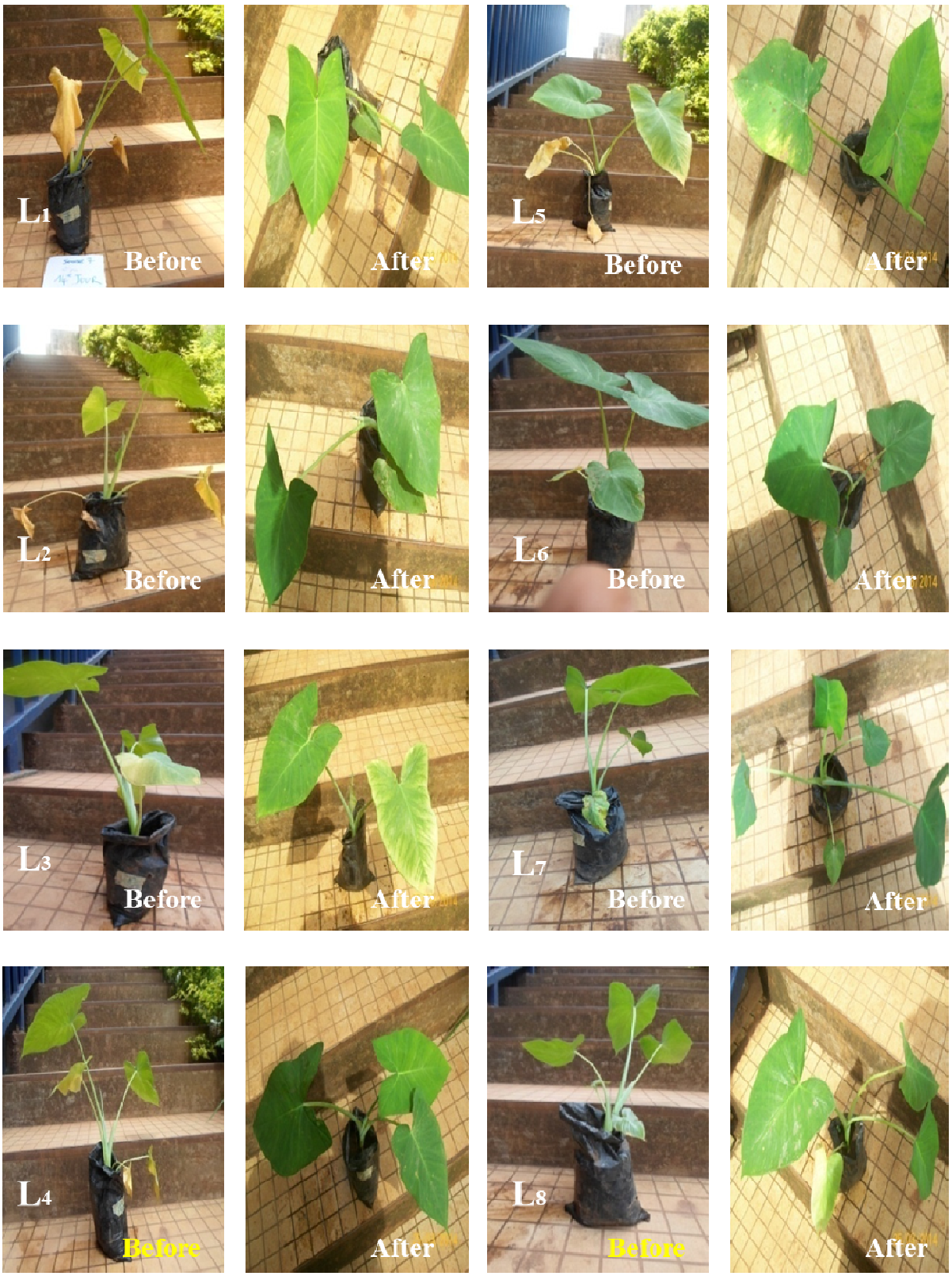

Fig. 6. Aspect of leaves of inoculated plants of *X. sagittifolium* with *F. oxysporum* strains after treatment with ethanol leaves extracts of *P. guajava* at day14. Locations: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

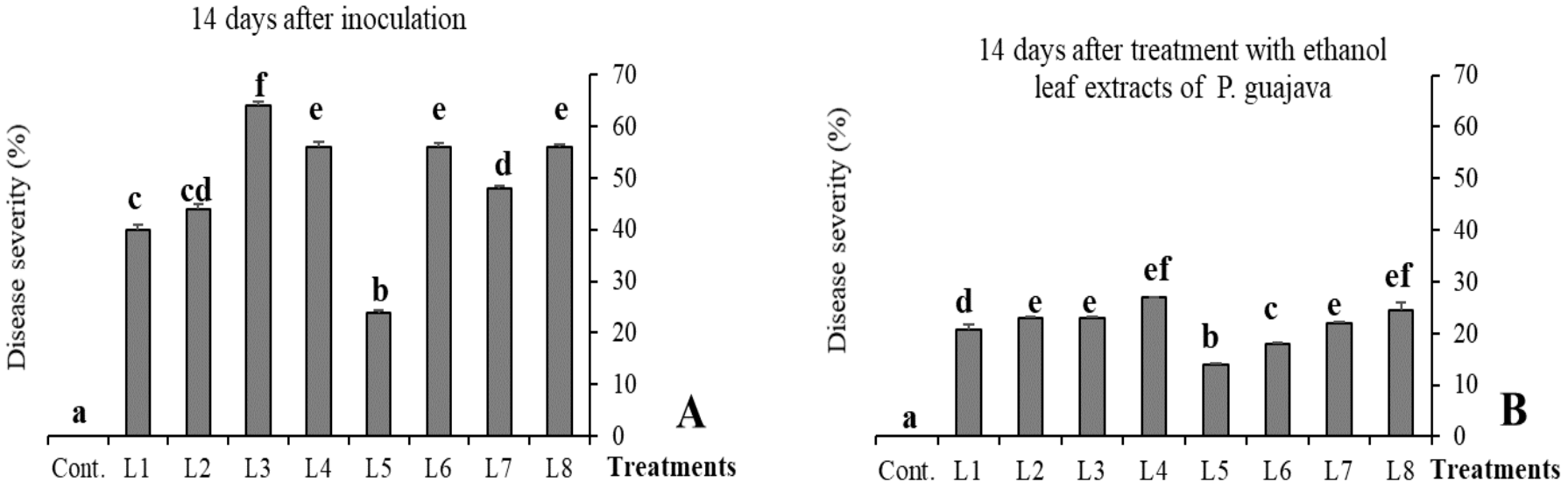

Fig. 7. Disease severity in *X. sagittifolium*. Day 14 after inoculation (A) and day 14 after treatment of plants with ethanol leaf extract from *P. guajava* (B). Locations: L_1 (Soa), L_2 (Banda), L_3 (Loum) and L_4 (Bangoua), L_5 (Abong-Mbang), L_6 (Bansoa), L_7 (Santa) and L_8 (Ekona)

3.2 Discussion

The objective of this work was to determine the different morphotypes of *Fusarium* sp. present in the roots of *Xanthosoma sagittifolium* L. Schott. The results highlight the presence of *Fusarium oxysporum*, based on the identification keys of *Fusarium* Interactive Key (Agr & Agri-Food Canada) and Simplified Fungi Identification Key (2001). The morphological analyzes of the different strains show that the cottony appearance of the hyphae is the most abundant in *F. oxysporum*. This suggests that cottony form of *F. oxysporum* may be the most abundant in the soil. Similar results were obtained by [40], who observed an abundance of the cottony form of *F. oxysporum* in *Trigonella foenum-graecum* L. (Fenugreek). The histological analysis of the conidial structure of the different strains isolated, showed macroconidia, microconidia and chlamydospores after eight days of growth. Strains isolated from *X. sagittifolium* harvested from L_1 and L_5 are rich in macroconidia and chlamydospores, while those of L_2, L_4, L_6, L_7 and L_8 are rich in microconidia.

Growth inhibition tests with the ethanol extracts of *Psidium guajava* showed significantly reduced growth in *F. oxysporum* in all isolated strains, being at the initial phase with 30% and decrease with time. This suggests that the amount of secondary metabolites released into petri dish and responsible for inhibiting the fungus decreases over time. Therefore, they are volatile compounds. Higher concentration of the ethanol extract cause low growth. This growth reduction is very high under 60% of ethanol extract of *P. guajava* leaves. This inhibition of growth could probably be attributed to the richness of the secondary metabolites of the leaves which have been released and which would have antifungal properties vis-à-vis *F. oxysporum*. [38] and [41], attribute this antifungal property of the leaves *P. guajava* to their richness in flavonoids, phenol, tannins and alkaloids. Moreover, [42] evidence shows that during the mechanism of antimicrobial substances to inhibit the growth of microbes, phenolic are able to change permeability of the cytoplasmic membrane which causes the leakage of nutrients from within the cell. In addition, the highest inhibition percentages of 83.33%, obtained in strains harvested from L_3 and L_4 localities after 8 days. This suggests that both strains are the most sensitive to the concentration of 60% ethanol leaf extract used. This concentration of 60% of ethanol leaf extract of *P. guajava* used would stop these strains of *F. oxysporum* to obtain in the PDA medium nutrients necessary for their growth.

The virulence tests carried out showed that most *F. oxysporum* strains used cause dry roots and leaf yellowing in *X. sagittifolium*. Strains of L_3 caused both wilting and yellowing of the leaves. Similarly, L_7 and L_8 strains cause wilting of the leaves. This suggested that *F. oxysporum* is a pathogen of *X. sagittifolium* plants and their aggressiveness observed depends on the strain used and maybe the age of the plant. At three months of age, *X. sagittifolium* plants used are in the active growth stage, this would explain the susceptibility observed towards *F. oxysporum* strain collected. After spraying the plant with ethanol extract from *P. guajava* leaves, growth was accelerated, leaves were greener and wider and the petioles more were vigorous. This suggests that the ethanol leaves extract of *P. guajava* stimulates growth while inhibiting the action of different strains of *F. oxysporum*. The greener leaves observed implies stimulation of the photosynthesis mechanism. Similarly, [43] and [44] showed that, foliar application of aqueous garlic bulb extract accelerated plant growth through the stimulation of photosynthetic pigments and soluble sugar content in *Schefflera arboricola* Plants.

4. CONCLUSION

Most of the *Fusarium oxysporum* strains isolated cause leaf yellowing and dry root in *Xanthosoma sagittifolium*. It appears that leaf extracts of *P. guajava* inhibit the mycelial growth of the various strains isolated. The higher inhibition was recorded under 60% of ethanol extract of *P. guajava* leaves. In addition, the use of *P. guajava* extracts not only appears to inhibit the pathogen but can facilitate the recovery of growth in *X. sagittifolium*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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