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In Vitro Effect of Extracts of *Erythrina senegalensis* on Two Fungal Strains Responsible for Post-harvest Losses of Papaya, Chili Pepper and Tomato

Yao Kamelé Kossonou[1,*], Kouassi Martial-Didier Adingra[2], Yao Mesmin Koffi[3], Fézan Honora Tra Bi[3], Kablan Tano[2]

[1]Department of Agronomic Forest and Environmental Engineering, Man University, Man, Côte d'Ivoire,
[2]Department of Food Science and Technology, Nangui Abrogoua University, Abidjan, Côte d'Ivoire
[3]Department of Sciences of Nature, Nangui Abrogoua University, Abidjan, Côte d'Ivoire

Abstract Post-harvest diseases due to the action of pathogenic fungi constitute a real problem in the marketing of fruits and vegetables, which represents an important sub-sector of the economy of Côte d'Ivoire. The conservation of fruits and vegetables has always relied in developed countries on the use of techniques that are difficult for producers to access or on the use of chemicals. Today, the search for new conservation techniques that are less expensive and concerned with the well-being of consumers is increasingly being considered. Several recent data suggest that medicinal plants with antifungal properties could represent an alternative solution for the control of these diseases. To this end, the antifungal potential of aqueous, methanolic and dichloromethane extracts of the leaves of *Erythrina senegalensis* was tested on *Rhizopus oryzae* and *Rhizopus stolonifer*, two fungi responsible for post-harvest diseases of papaya, chili pepper and tomato. The antifungal tests performed showed that the extracts of *E. senegalensis* inhibited the mycelial growth of fungal strains with minimum inhibitory concentrations (MIC) of between 8 mg/ mL and 10 mg/mL. However, of the three extracts tested, the aqueous extracts were the most active on fungi. The beneficial effect of *E. senegalensis* against the fungi *R. oryzae* and *R. stolonifer* leads to suggest that this plant be further explored in order to consider a formulation of biofungicides in the conservation of fruits and vegetables in Côte d'Ivoire.

Keywords Fruits and vegetables, Post-harvest losses, Antifungal, *Erythrina senegalensis*

1. Introduction

Papaya, chili pepper and tomato crops are currently important activities in Côte d'Ivoire [1,2]. With an annual production of 32,900 tonnes, chili pepper, along with tomatoe, okra and eggplant, are the main vegetables consumed and cultivated [3,4]. Regarding papaya, the Côte d'Ivoire and Ghana are the main suppliers to the European Union markets in West Africa [1]. Despite their economic importance, the consumption of fruits (papaya, chili pepper and tomato) is considered today as an important element of public health. It provides nutrients, vitamins, antioxidant compounds and minerals essential for the proper functioning of the body [5]. Despite their economic and nutritional importance, these climacteric fruits are extremely perishable because after harvest they continue to maintain metabolic activity. Post-harvest losses are often estimated at between 30% and 40% of production and represent a considerable shortfall in the national economy. These losses are due to senescence, sweating and fungal and bacterial infections [6,7]. Fungal species such as *Fusarium* spp., *Rhizopus* spp. and *Colletotrichum gloeosprioides* and pathogenic bacteria such as *Pseudomonas* spp. and *Erwinia* spp., play an important role in post-harvest disease and rots of fruits [8]. In order to extend shelf life and reduce post-harvest losses, researchers used ultraviolet C irradiation [9] and several chemicals such as methylcyclopropene (1_MCP) [10], silver nitrate [11]. However, consumers are increasingly reluctant to use these products because of their residues.

Other technologies, such as controlled and modified atmosphere storage, have been used to extend the shelf life of fruit but are too expensive for the individual grower [12,13]. The high cost of these conservation technologies and the reluctance of consumers towards these chemicals make it necessary to explore new conservation methods, in particular the implementation of biofungicides which are products obtained from plant extracts in order to fight against contaminating agents in fruits and vegetables.

* Corresponding author:
kossepiphane@yahoo.fr (Yao Kamelé Kossonou)
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Recent studies have shown that products of plant origin are a remedy in the fight against post-harvest losses of fruits and vegetables [14,15,16]. *Erythrina senegalensis* DC. (Leguminosae) is an African medicinal plant [17] traditionally used against several pathologies such as gastrointestinal disorders, wounds, malaria, dysmenorrhea, pneumonia, cough, onchocerciasis, inflammation, nosebleeds, dizziness, jaundice and venereal disease [18]. The plant is also known to cure urinary schistosomiasis and eye infections [19]. In Côte d'Ivoire, pharmacological studies have shown that *E. senegalensis* has antibacterial and antifungal pharmacological activities [20,21,22]. Elsewhere in Africa, the antibacterial and antifungal activity of *E. senegalensis* has been demonstrated by Magassouba *et al.* [23] and Doughari [24].

These authors have shown that the stem barks of this plant are active on bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes, Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*) as well as on fungi (*Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Penicillium notatum*).

Given the many pharmacological properties of *E. senegalensis* in the treatment of human pathologies, it would be interesting to study its potential in the search for treatments against postharvest fruit diseases. Therefore, this study aims to evaluate the potential of *E. senegalensis* against fungi responsible for post-harvest diseases of papaya, chili pepper and tomato.

2. Material and Methods

2.1. Material

2.1.1. Collection and authentication of *E. senegalensis*

Fresh leaves of *E. senegalensis* were collected from Bongouanou (Côte d'Ivoire). The plant was authenticated by Professor Laurent Ake-Assi of Floristic National Center (FNC) of Université Felix Houphouët Boigny (Côte d'Ivoire).

2.1.2. Selection of Fungal Strains

The fungal strains *Rhizopus oryzae* and *Rhizopus stolonifer* are spoilage agents in the fruits of papaya, chili pepper and tomato. The fungal strains *R. oryzae* and *R. stolonifer* used were isolated from the fruits of papayas, chili peppers and tomatoes taken from fields in the municipality of Azaguié (Côte d'Ivoire). These strains were then identified using the PCR-DGGE molecular method followed by sequencing [25]. According to this study, these fungal strains were present on 99% of the fruits sampled for *R. oryzae* and 100% for *R. stolonifer*.

2.2. Methods

2.2.1. Sabouraud Culture Medium

Sabouraud culture medium was prepared according to the method described by Alrasheid *et al.* [26]. Sixty-two grams of the powdered Sabouraud dextrose agar, was weighed, dispersed in 1 L of distilled water and allowed to soak for 10 min, swirled to mix then sterilized by autoclaving for 15 min at 121°C, cooled to 47°C. Lastly, mixed well and poured into petri dishes.

2.2.2. Preparation of Plant Crude Extracts

The plant extracts were obtained from the powder of the leaves of *E. senegalensis*. Powder was macerated at room temperature with mechanical stirring in three solvents as follows: distilled water, methanol (99.8%), and dichloromethane (99.9%) according to solvent-to sample ratio (v/w) at 10:1. After filtration, the methanolic extract obtained was concentrated on a rotavapor (Büchi R-104, Switzerland). The methanol concentrate collected in a little water and the aqueous concentrate were then lyophilized [27]. A stock solution of 100 mg/mL concentration was prepared by dissolving 1.2 grams of lyophilized plant extract in 12 mL of dimethylsulfoxide (DMSO).

2.2.3. Antifungal Assay

Inoculum preparation

The antifungal activity of the extracts against *Rhizopus oryzae* and *Rhizopus stolonifer* was determined by the Sabouraud agar with chloramphenicol method [15]. Both fungal strains were grown at 28°C for 48 hours to obtain a young culture. The colony of the young culture obtained was introduced into a tube containing a volume of 15 mL of Sabouraud broth with chloramphenicol. The prepared inoculum is used for the inoculations.

Seeding and antifungal effect of extract on mycelial growth

A volume of 15 mL of the supercooled agar was previously poured into each Petri dish. Before solidification, a volume V of the stock solution of plant extract is taken using a micropipette and homogenized in the agar, according to the concentration desired for carrying out the test. Thus, for a culture medium equivalent to 15 mL of agar, 300 µL, 600 µL, 1200 µL and 1500 µL of stock solution were added for respective equivalent concentrations of 2, 4, 8 and 10 mg/mL. A volume of 0.5 µL of the inoculum containing the germ was taken and deposited on the solidified Sabouraud chloramphenicol media and incorporated in various plant extracts for the treatments. A Control was prepared using the same process, but with the difference that it does not contain plant extracts. Petri dishes were incubated for six days in the dark at 25°C ± 2 (laboratory temperature). The parameter measured was the average diameter of the radial growth of the mycelium of each strain of fungus. For each treatment, three Petri dishes were used and the test was repeated three times. The measurements were carried out every day for six days. Then the percent mycelial growth inhibition was calculated with the average radial growth of mycelium for each concentration. This made it possible to determine the level of resistance or sensitivity of each germ for each plant extract. The percentage inhibition was calculated according

to the formula and the sensitivity scale proposed by Kumar *et al.* [28]:

$$I\ (\%) = (C-T)/T \times 100$$

I (%): Percent inhibition

C: Radial growth (cm) of the control

T: Radial growth (cm) of the treatment

The isolates were classified based on their reaction to different extracts as given below: 1) Highly sensitive (> 90% inhibition); 2) Sensitive (> 75-90% inhibition); 3) Moderately resistant (> 60-75% inhibition); 4) Resistant (> 40-60% inhibition) and 5) Highly resistant (< 40% inhibition).

2.2.4. Determination of MIC and IC_{50}

The determination of the minimum inhibitory concentrations (MIC) was carried out by the method of successive dilution in agar medium. This technique consists of determining the lowest concentrations at which the plant extracts totally inhibit the isolated fungal strains. It was done with the naked eye from the Petri dishes after the six days of incubation.

2.2.5. Data Analysis

The results of the inhibition tests of radial mycelial growth of extracts of *Erythrina senegalensis* on the two fungal strains were expressed as percent inhibition. One-factor analysis of variance (ANOVA 1) was used to compare the means of the percentages of inhibition induced by the concentration of the extracts on the mycelial growth of the fungal strains. When a significant difference is observed between the means compared to the 5% threshold, Tukey's post-ANOVA test is performed to find out the level of difference between the means in order to rank them. The values of the minimum inhibitory concentrations (MIC) were determined using the histogram of the percent inhibitions. The analyses of variance were carried out using the XLSTAT 2020 [29] software incorporated in Excel 2010. For the determination of the concentrations which inhibit 50% of the mycelial growth of fungi (IC_{50}), a trend curve between the concentrations of the extracts and the percentages of inhibition using Excel 2010 software.

3. Results

The study of the effect of extracts of *Erythrina senegalensis* (aqueous, methanolic and dichloromethanic) on the mycelial growth of the fungal strains *Rhizopus oryzae* and *Rhizopus stolonifer* showed that the plant extracts caused inhibitions of the radial mycelial growth which are function the concentration of extracts.

Figure 1 shows the percentages of inhibitions induced on radial mycelial growth of *R. oryzae* as a function of the concentration in the three extraction solvents. In this figure, statistical analysis shows a difference in activity from one concentration of extract to another for the three extraction solvents. In water, at 2 mg/mL and 4 mg/mL, the extract caused less than 40% inhibition of mycelial growth while at concentrations of 8 mg/mL and 10 mg/mL the percent d inhibition obtained is 100%. For the methanol and dichloromethane extracts, a percent inhibition of radial mycelial growth of 100% was obtained only at the concentration of 10 mg/mL. On this fungal strain, the aqueous extract showed more activity than the organic extracts.

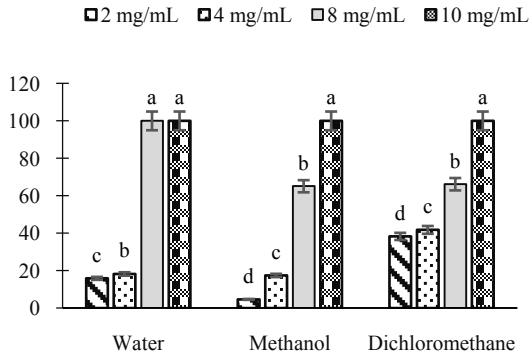

Figure 1. Induced inhibition (%) on the mycelial growth of *R. oryzae* by extracts of *Erythrina senegalensis* as a function of the concentration

As regards the *R. stolonifer* strain, statistical analysis also showed a difference in activity from one concentration to another within the same solvent (Figure 2). At concentrations of 2 mg/mL, 4 mg/mL and 8 mg/mL; the aqueous extract respectively showed more activity on the radial growth of the fungal strain. However, at a concentration of 10 mg/mL, the three solvents used had the same activity on the fungal.

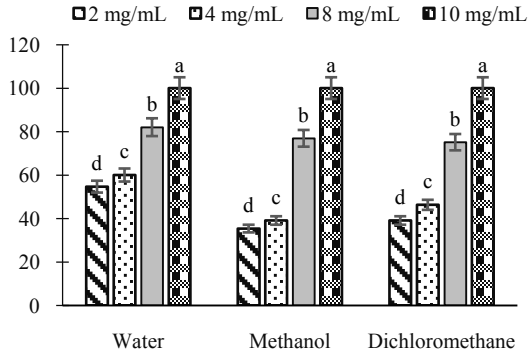

Figure 2. Induced inhibition (%) on the mycelial growth of *R. stolonifer* by extracts of *Erythrina senegalensis* as a function of the concentration

The reactions (sensitivity/resistance) of the fungal strains to extracts of *Erythrina senegalensis*, the MIC and the IC_{50} determined are given in Table 1.

The *Rhizopus oryzae* strain was highly resistant in dichloromethane at 2 mg/mL and at concentrations of 2 mg/mL and 4 mg/mL in aqueous and metahnolic solvents. At 4 mg/mL, this strain was resistant only in dichloromethane. In methanolic and dichloromethane solvents, at 8 mg/mL, the fungal was moderately resistant. From the latter

concentration *R. oryzae* was highly sensitive to the aqueous extract while in organic solvents it was at 10 mg/mL that it was highly sensitive. Thus, the MIC is 8 mg/mL for the aqueous extract and 10 mg/mL for organic solvents. The IC_{50} determined are respectively 5.58 mg/mL, 6.76 mg/mL and 5.37 mg/mL for the aqueous, methanolic and dichloromethane extracts.

Concerning the *Rhizopus stolonifer* strain, at 2 mg/mL, it is highly resistant to methanolic and dichloromethane extracts, while at this concentration it is simply resistant. At 4 mg/mL, the fungal strain is still highly resistant to the methanolic extract while it is resistant to the dichloromethane extract and moderately resistant to the aqueous extract. From 8 mg/mL, the fungal strain became sensitive to the three plant extracts. *R. stolonifer* was highly sensitive to three extracts of *E. senegalensis* at a concentration of 10 mg/mL. The MIC determined for this fungal strain are identical for the three extracts and have the values of 10 mg/mL. Relative to the IC_{50}, the aqueous extract gave the smallest value which is 1.84 mg/mL.

The minimum inhibitory concentrations (MIC) determined as well as the concentrations which inhibit 50% of the growth of the germs (IC_{50}) revealed that the extracts of *E. Senegalensis* had good antifungal activity on fungi. However, the aqueous extracts were shown to be more active.

Table 1. Reactions (Sensitivity/Resistance) in vitro of the two fungal strains at different concentrations of extracts of *Erythrina senegalensis* and values of MIC and IC_{50}

Extract		*Rhizopus oryzae*			*Rhizopus stolonifer*		
Solvent	Concentration (mg/mL)	Sensitivity or resistance	MIC (mg/mL)	IC_{50} (mg/mL)	Sensitivity or resistance	MIC (mg/mL)	IC_{50} (mg/mL)
water	2	HR	8	5.58	R	10	1.84
	4	HR			MR		
	8	HS			S		
	10	HS			HS		
Methanol	2	HR	10	6.76	HR	10	5.18
	4	HR			HR		
	8	MR			S		
	10	HS			HS		
DCM	2	HR	10	5.37	HR	10	4.53
	4	R			R		
	8	MR			S		
	10	HS			HS		

HR: Highly resistant; R: Resistant; MR: Moderately resistant; S: Sensitive; HS: Highly sensitive, DCM: Dichloromethane

4. Discussion

The study of the effect of extracts of *Erythrina senegalensis* on the fungal strains *Rhizopus oryzae* and *Rhizopus stolonifer* has shown that this plant has an inhibitory activity on the growth of fungi. Indeed, these two fungal strains were very sensitive to the aqueous, methanolic and dichloromethane extracts of the plant. The antifungal activities of *E. senegalensis* leaves extracts against these fungi responsible for post-harvest rots in papaya, chili pepper and tomato are reported here for the first time. Several studies carried out using *E. senegalensis* have shown that this plant has many biological properties in cases of human pathologies. For example, antibacterial activities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* have been demonstrated by Doughari [24]. Likewise, the work of Koné *et al.* [30] made it possible to demonstrate the activities of this plant on resistant strains of *Streptococcus pneumoniae*. In addition, the extracts of this plant have been active on fungi such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium notatum* [24]. The various pharmacological properties of *E. senegalensis* against these human bacteria and fungi may explain its activity against the fungal strains *Rhizopus oryzae* and *Rhizopus stolonifer*. On these fungals, the same author has shown that the methanolic extract of *E. Senegalensis* had MIC values between 10 mg/mL and 30 mg/mL. Other authors like Masangwa *et al*. [36] obtained with the aqueous extract of *Chlorophytum comosum* an MIC of 12.5 mg/mL on *Colletotrichum dematium*, a fungus responsible for post-harvest fruit rot. The different MIC values observed could be explained either by the nature of the plant used, or by the extraction solvent or by the fungal strain tested. Although not having worked on the same fungal strains, these results nevertheless show that the extracts of this plant had a beneficial effect on the fungal strains studied.

Phytochemical screening of *E. senegalensis* leaves extracts showed that they contained saponins, terpenoids, steroids, tannins, flavonoids, phenols and alkaloids [31]. Several authors have shown that secondary metabolites are responsible for the biological activities of plant extracts [32,33,34,35]. The presence of these secondary metabolites explains the biological activities of this plant in this study and its traditional use as a medicinal plant [18]. The results also showed that the aqueous extract of *E. senegalensis* was

more active on the strains than the organic extracts.

The difference in activity between aqueous and organic extracts could be explained by the nature of the molecules contained in each of the types of extracts. Indeed, according to Ullah *et al.* [37] and Wakeel *et al.* [38], during the extraction, the phytomolecules are distributed among the solvents according to their polarity and their solubility. It could be deduced that the antifungal substances contained in plant extracts are more soluble in water than in other organic solvents. However, it is sometimes recognized that the potency of medicinal plants can be enhanced by using organic solvents [39].

5. Conclusions

This work was developed to search for natural products that are alternatives to chemicals used in the control of fungal pathogens responsible for rotting tropical fruits (papaya, chili pepper and tomato). Thus, the antifungal tests carried out using extracts of *Erythrina senegalensis* against *Rhizopus oryzae* and *Rhizopus stolonifer* showed that these plant extracts were active on these two phytopathogenic agents. The activity of plant extracts on these fungal strains suggests considering a formulation of natural biofungicides concerned with the well-being of consumers in the conservation of post-harvest products.

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