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Full Length Research Paper

Efficacy test of *Melaleuca leucadendra* and *Callistemon viminalis* essential oils on in vitro control of a strain of *Aspergillus flavus* isolated from peanut seeds in Senegal

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Abstract

This study consists of an efficacy test of essential oils of two plant species of the Myrtacae family (Melaleuca leucadendra and Callistemon viminalis) on the in vitro control of a strain of Aspergillus flavus potentially aflatoxinogen isolated from peanut seeds in Senegal. To do this, different doses (750, 500, 250 and 100 ppm) of each oil were associated with the culture medium Czapek Yeast Extract Agar (CYA) and the development of the strain on the different culture media was followed for 7 days, after which the mycelial growth (mean colony diameter) and the sporulation density (average number of conidia per mm2) were evaluated in the 4 repetitions of each treatment. The results showed that C. viminalis essential oil at the higher dose of 750 ppm (T5) was found to be more effective, just after Mancozeb at 500 ppm (T9) used as the reference control, with no significant difference. This T5 was closely followed by the T6 (Callistemon 500 ppm), T1 (Melaleuca 750 ppm) and T2 (Melaleuca 500 ppm) treatments on the inhibition of fungus growth and reproduction.

Key words: Essential oils, Callistemon viminalis, Melaleuca lecadendra, Aspergillus flavus, Senegal.

INTRODUCTION

Groundnuts and their derived products are popular staple foods in Africa. It is also a commodity that is the subject of several international transactions and generates a lot of income for producers and other operators. In Senegal, groundnuts remain one of the main oilseed crops and a cash crop for farmers, who are increasingly active in the export of seeds, especially to China. In 2021, peanut seed exports reached a volume of 319,000 tons and generated nearly 138 billion FCFA in the Senegalese economy. However, the presence of aflatoxin in seeds constitutes a considerable obstacle to export and a major public health risk. Indeed, aflatoxins are the most toxic mycotoxins known to date. They are secondary metabolites produced mainly by strains of fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Doucouré, 1999). According to WHO (2018), drought stress, insect damage and poor storage conditions are strongly contributing to the more frequent appearance of these molds and the contamination of peanuts with aflatoxin, including in temperate regions. There are different types

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of aflatoxins detected in food (B1, B2, G1 and G2), with B forms being 10 to 50 times more toxic than G forms, and Aflatoxin B1 recognized as the most toxic of all. Aflatoxins M1 and M2 are derivatives of aflatoxins B1 and B2 (Martin *et al.*, 1999) and are generally found in milk and dairy products. In addition, aflatoxins are known to have carcinogenic properties (Fofana-Diomande *et al.*, 2019), their ingestion at high doses can cause significant liver disorders (jaundice, cirrhosis, necrosis, liver cancer), kidney and lung cancer, diarrhea and anorexia that can lead to death. Given the serious risk of aflatoxins to human and animal health, the number of countries regulating these substances increases significantly over the years. However, the *Aspergillus* that produce them are saprophytic cosmopolitan ascomycetes that proliferate easily on organic substrates and dead matter. The most important species, *Aspergillus flavus*, is a mold that is widespread in tropical areas and can contaminate several foods. However, the ability to secrete aflatoxin depends on the strain and ambient climatic conditions, including temperature and humidity. Thus, the need to combat aflatoxins would imply the need to put in place effective methods of controlling the development of fungi that are at the origin of their secretion. In this context, this study aims to evaluate the effectiveness of different concentrations of the essential oils of *Melaleuca leucadendra* and *Callistemon viminalis* on the in vitro control of a strain of *Aspergillus flavus* isolated from peanut seeds in Senegal.

I. MATERIAL AND METHODS

I.1. The *Aspergillus flavus* strain

The strain (Kl3) used in this study was isolated in 2020 at the Laboratory of Phytopathology of DPV (Directorate of Plant Protection) in Senegal, from a peanut seed sample taken from Kaolack (agro-ecological zone of the groundnut basin). This strain appears on CYA in homogeneous green colony without sclerotia, towards dark yellow pulling to gray (Photo 1), biserized conidian head. At 7 days of cultivation, the average diameter of the colony is 9 cm and the average number of spores per mm^2 is 486.75. On G25N, the strain has a dark green colony with a whitish border and an average diameter of 5.5 cm after 7 days of cultivation.

I.2. The essential oils

The essential oils of *Meulaleca leucadendra* and *Callistemon viminalis* used in this study were extracted at the Senegalese Institute of Agricultural Research by steam training. These are 2 plant species belonging to the Myrtaceae family and native to Australia. In the composition of the essential oil of *M. leucadendra*, 43 chemical constituents have been identified, including Eucalyptol also called 1,8-cineole (28.87%), Epiglobulol (23.06%), α-pinene (12.22%), Limonene (11.65%) and α-terpineol (7.06%) as the main ones (Fall *et al.*, 2017). In that of *C. viminalis*, the main constituents are 1.8-cineole (58.49%), 3-carene (8.61%), Limonene (7.01%), α-terpinol (5.83%).

I.3. Treatments and incubation

A sample of 1 ml of each oil was subjected to a series of dilutions in 9 ml of sterilized distilled water and then added at different proportions in the culture medium Czapek Yeast Extract Agar (CYA) made of K_2HPO_4 (1 g), concentrated Czapek (10 ml), metal solution (1 ml), yeast extract (5 g), sucrose (30 g), agar-agar (15 g), distilled water (1 L), just before pouring into Petri dishes, under sterile conditions. At 25°C, circular portions of 0.6 cm diameter were taken from the 5-day-old colonies and deposited centrally in the boxes containing the culture media impregnated with the different concentrations (750, 500, 250 and 100 ppm) of essential oil of *M. leucadendra* or *C. viminalis* (Table 1). CYA impregnated with Mancozeb at a dose of 500 ppm was used as a reference control.

I.4. Evaluated parameters and methods of evaluation

Mycelial growth was evaluated in all culture boxes through daily measurements of the diameter of the fungal colony, using a graduated ruler. After 7 days of cultuvation, samples from the various treatments were placed on millimeter graduated slides and observed under an optical microscope for the enumeration of spores. Inhibition rates (IR) of mycelial growth and sporulation were calculated using the formula :

$$IR\ (\%) = \frac{NT0-NT}{NT0} * 100$$

with NT0 = number of spores or colony diameter in the absolute control; NT = number of spores or colony diameter in the treated.

I.5. Statistics

The data collected on this study were entered on the Excel software, which also made it possible to express them graphically. They were subjected to statistical analyses using Costat software version 7.2. An analysis of variance and a comparison of means were made between the different treatments on the evolution of the diameter of the mycelial colony and the number of spores per mm^2, using the Student-Newman-Keuls test at the threshold of 5%.

II. RESULTS

II.1. Impact of the treatments on the fungal development and sporulation

The different doses of *M. leucadendra* or *C. viminalis* essential oil significantly reduced ($p < 0.000$) the mycelial

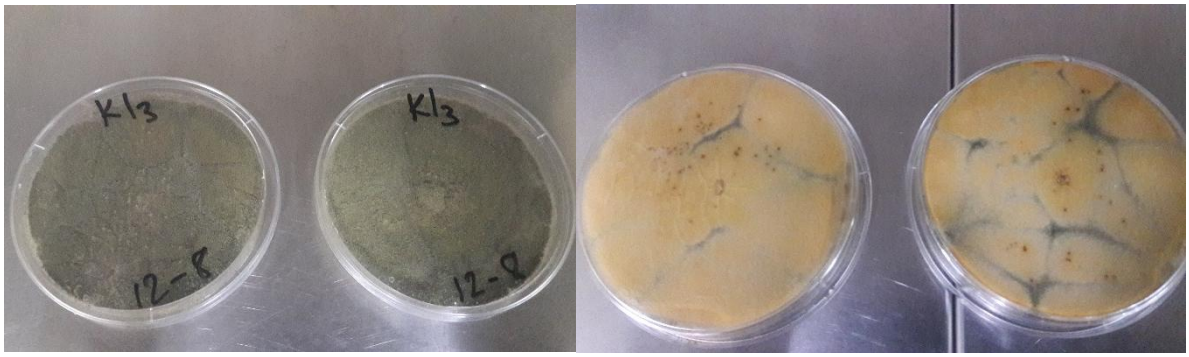

Photo 1.Macroscopic illustration of theA. flavus Kl3 strainon CYA (7 days)A : face ; B : reverse.

Table 1. Presentation of the different treatments.

Treatment code	***Product***	***Dose (ppm)***	***Status in the study***
T0	-	-	Absolute control
T1	*M. leucadendra*essential oil	750	Tested
T2		500	
T3		250	
T4		100	
T5	*C. viminalis*essential oil	750	
T6		500	
T7		250	
T8		100	
T9	Mancozeb	500	Reference control

growth and sporulation density of the fungus compared to the absolute control. Regarding the mycelial growth, the highest inhibition rate (92%) was obtained with the reference control T9 (Mancozeb 500 ppm), closely followed by the treatments T5 (Callistemon 750 ppm), T2 (Melaleuca 500 ppm) and T6 (Callistemon 500 ppm) with respectively 90.88%, 88.88% and 87.77% inhibition (Table 2), without statistically significant difference (Figure 1). Similarly, with regard to the sporulation of the fungus, the greatest reduction was obtained with the reference control T9 (Mancozeb 500 ppm) with 97.81% inhibition, followed by the T5 treatment (Callistemon 750 ppm) with 94.47% (i.e. on average 31.55 spores per mm^2 against 570.66 spores per mm^2 in the absolute control T0). The T6 (Callistemon 500 ppm) and T1 (Melaleuca 750 ppm) treatments recorded 91.26% and 90.34% inhibition respectively (Table 2), without any significant difference (Figure 2). Statistical analyses also show that there is no significant enough difference between T5 treatment and the T9 reference control on the rate of inhibition of sporulation of the fungal strain.

III. DISCUSSION

The essential oil of *C. viminalis* was found to be significantly more effective than that of *M. leucadendra* on the in vitro control of the *A. flavus* strain. Also, the intensity of inhibition of the fungus development appeared dependent on the essential oil dose applied in the culture medium. In our experimental conditions, we were able to see that among the treatments tested, the highest concentration of *C. viminalis* (T5) oil made it possible to obtain the highest inhibition rates of mycelial growth and conidia production with 90.88% and 94.47% respectively. The difference in the level of effectiveness noted between the 2 essential oils would find its explanation in their composition in chemical elements. Indeed, the essential oil of *C. viminalis* is naturally richer than that of *M. leucadendra* in Eucalyptol also called 1,8-cineole (58.49% against 28.87%). Eucalyptol ($C_{10}H_{18}O$) is a natural compound that is the majority of many essential oils. This terpene oxide is said to have an inhibitory effect on the reproduction process of fungi. This observation is

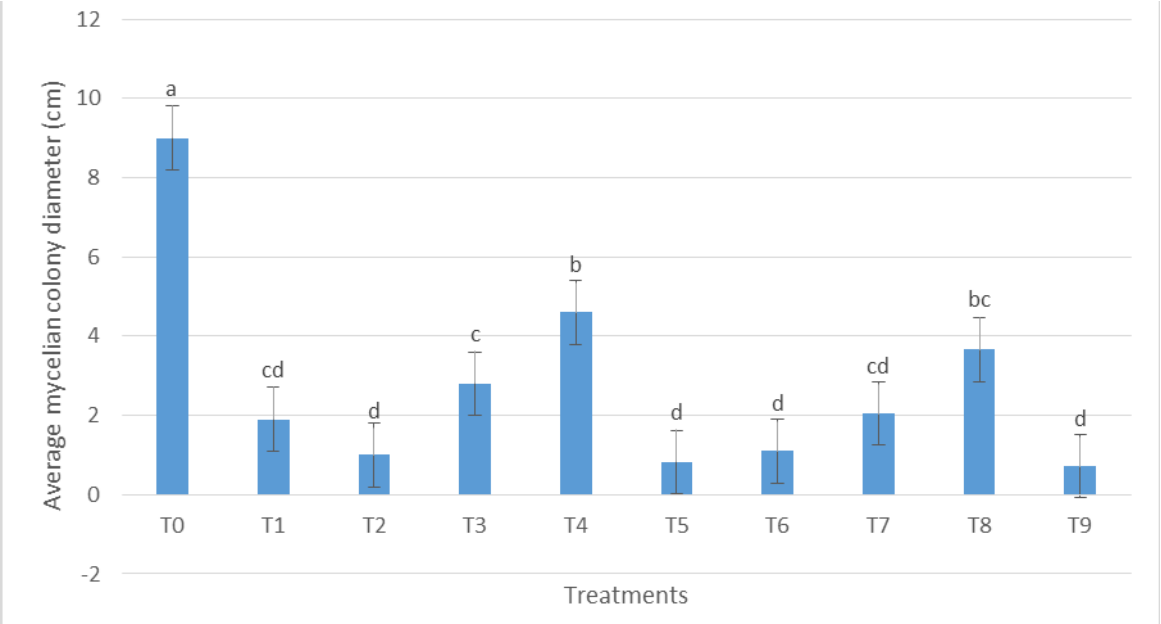

Figure 1. Variation of the mycelial colony diameter of the Aspergillus flavus strain after 7 days of incubation depending on treatments.

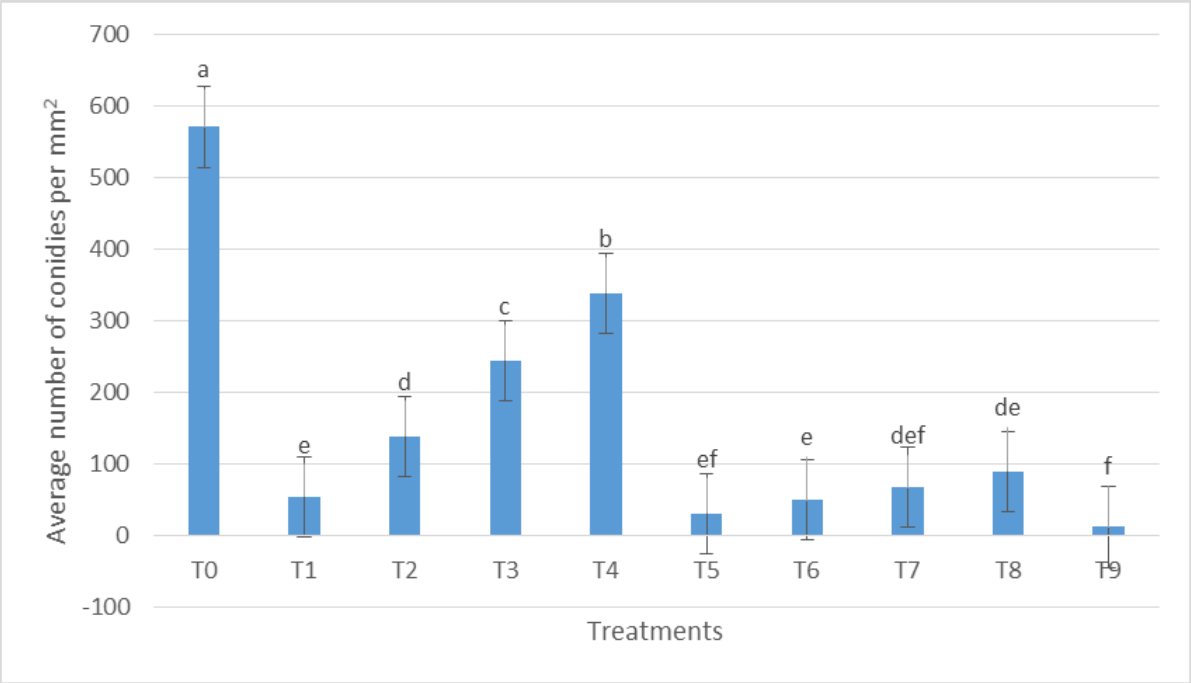

Figure 2. Variation in the number of spores produced by the Aspergillus flavus strain after 7 days of incubation depending on treatments.

in line with that of Ndomo *et al.* (2009) according to which the toxic and repellent effects of the essential oil of *C. viminalis* could depend on its chemical composition, which itself depends on the environment of the plant. The work of Toure (2015) has also shown that the essential oils of plants are composed of a multitude of chemical elements with varying proportions depending on the plant species. In addition, several studies have highlighted

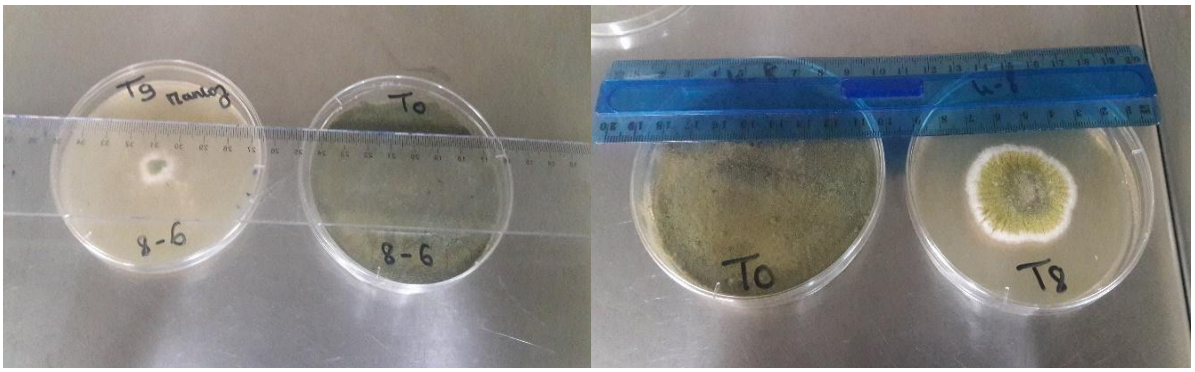

Photo 2. Illustration of the impact of treatments on the A. flavus mycelial growth.

Tableau 2. Variation in inhibition rates of mycelial growth and sporulation of the Aspergillus flavus Kl3 strain depending of treatment.

Treatments	***Inhibition rate (%)***	
	Mycelial growth	*Sporulation*
T0 (any)	0	0
T1 (*Melaleuca* 750 ppm)	78,8	90,34
T2 (*Melaleuca* 500 ppm)	89	75,70
T3 (*Melaleuca* 250 ppm)	68,9	57,09
T4 (*Melaleuca* 100 ppm)	50,01	40,65
T5 (*Callistemon* 750 ppm)	90,88	94,47
T6 (*Callistemon* 500 ppm)	87,77	91,26
T7 (*Callistemon* 250 ppm)	77,22	88,17
T8 (*Callistemon* 100 ppm)	59,3	84,17
T9 (Mancozeb 500 ppm)	92	97,81

different levels of effectiveness of essential oils on the in vitro or in vivo control of strains of microorganisms and/or insect species (Pibiri, 2005; Randrianarivelo, 2010; El Ajjouri *et al.*, 2020). The effectiveness of essential oils on fungal control would also depend on the level of sensitivity of fungal strains. Indeed, within the same species of fungus or bacteria, we can distinguish several strains, pathovars or special forms different on their morpho-anatomical, physiological and/or ecological characteristics. In the species *A. flavus*, many strains have been identified from various substrates in Africa (Ouattara-Sourabie *et al.*,2011; Hissein *et al.*, 2019). These fungi are able to grow at temperatures between 10 and 45 ° C, the synthesis of aflatoxins, for aflatoxinogenic strains, intervening only at temperatures between 20 and 35°C with a relative humidity between 80 and 85% (Diener and David, 1986 in Doucouré, 1999). However, the rate of mycelial growth as well as the density of sporulation can vary significantly from one strain of *A. flavus* to another, on the same culture medium, impregnated or not with essential oil.

CONCLUSION

This study consisted of testing the effectiveness of different concentrations of *Melaleuca leucadendra* and *Callistemon viminalis* essential oils on the in vitro control of a strain of *Aspergillus flavus* isolated from peanut seeds in Senegal. The results obtained show that the inhibition rates of mycelial growth and spore production of the fungus depend on the essential oil and the dose applied in the culture medium. Indeed, the T5 treatment

(*Callistemon* 750 ppm) achieved the highest inhibition rates of the fungal development, with no significant difference from Mancozeb (500 ppm) used in this study as the reference treatment (T9). The differences in effectiveness noted between the different treatments tested would find their explanation in the chemical composition of the 2 essential oils and the doses experimented for each oil. However, intrinsic characteristics of the fungal strain, as well as certain experimental abiotic parameters (temperature and relative humidity of incubation) would also have an impact on the effectiveness of treatments.

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