Journal of Advances in Microbiology

22(4): 45-56, 2022; Article no.JAMB.85033
ISSN: 2456-7116

In vitro Evaluation of the Antifungal Activity of Essential Oils of Some Medicinal Plants on Phytopathogenic Strains in the Kisangani Region (DRC)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2022/v22i430456

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85033

Original Research Article

Received 22 January 2022
Accepted 03 April 2022
Published 11 April 2022

ABSTRACT

Purpose: The present study evaluates the antifungal activity of essential oils of *Curcuma longa* and *Eucalyptus globulus* on strains of *Lasiodiplodia theobromae* and *Pseudocercospora fijiensis* in the Kisangani region (DR Congo).
Methods: The essential oils were obtained after extraction by the hydro distillation method. The isolation of strains of *L. theobromae* was made from the fragments of mesocarp taken from the diseased cocoa, previously cut with a Scarpel and then disinfected with bleach water, while the strains of *P. fijiensis* were obtained by the method of discharging ascospores on agar medium (H_2O Agar) and then transplanting on potato dextrose agar (PDA). In addition, inhibition of mycelial growth on a PDA was used as a method to assess the sensitivity of strains to the essential oils of the medicinal plants studied.
Results: The results obtained show that the essential oils of the rhizomes of *C. longa* and the leaves of *E. globulus* showed a low yield of essential oils (EOs) of 0.50% and 0.48% respectively. However, the EOs of the rhizomes of *C. longa* and the leaves of *E. globulus* exhibited inhibitory action on the mycelial growth of strains of *L. theobromae* and *P. fijiensis.*

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Conclusion: The ANOVA test applied to the mean mycelial growth values of these two strains (*L. theobromae* and *P. fijiensis)* on the Eos of *C. longa* showed that the difference is not significant (p-value = 0.47). In addition, there is also no significant difference (p-value = 0.51) between the mean mycelial growth values of these two fungal strains on the essential oil of *E. globulus.*

Keywords: Antifungal activity; essential oils; medicinal plants; phytopathogenic strains; In vitro.

1. INTRODUCTION

For a long time, essential oils (EOs) have occupied a prominent place both in the perfume industry and in the pharmaceutical, culinary and canned food fields. In addition, chemists, biologists, and doctors are constantly increasing their interest in these natural substances due to their important role in the treatment of certain infectious diseases for which synthetic antibiotics are becoming less effective, or in preservation of food against oxidation as alternatives to synthetic chemicals [1].

The study of EOs is still relevant. The continued development of plant biotechnologies has been a major concern of researchers in recent years. Thus, the progress of science has revolutionized the history of aromatherapy [2].

Pests including plant pathogenic fungi among others continue to wreak havoc on food and cash crops. The cultivation of banana and plantain trees is threatened by the numerous attacks of diseases caused by fungi, nematodes and weevils, viruses, bacteria [3].

Black Leaf Streak Disease is among the various pests that these crops face (Black Leaf Streak Disease), it is the most serious leaf disease, being the most destructive and the most expensive in terms of control methods [4].

Black ray disease (MRN) or black *cercosporiosis* of banana and plantain is caused by the fungus *Pseudocercospora fijiensis* (former name *Mycosphaerella fijiensis*) [5].

One of the most common diseases in the species Theobroma cacao is the black rot of pods. It is caused by *Lasiodiplodia theobromae* and causes significant production losses worldwide [6].

In the Democratic Republic of Congo, particularly the Kisangani region, few studies have been conducted on *in vitro* evaluation of the antifungal activity of EOs against fungal strains of cocoa and banana trees. The present study was conducted to evaluate the effectiveness of *Cucurma longa* and *Eucalyptus globulus* EOs against the fungal strains *L. theobromae* and *P. fijiensis* to control these diseases in Kisangani region.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in the city of Kisangani, the capital of the province of Tshopo (Fig. 1). This city is located in the Central Congolese basin, at 0° 31′ 00" North and 25° 11′ 00" East. Its average altitude is 396 m [7]. Administratively, Kisangani is made up of 6 communes: Makiso, Lubunga Mangobo, Kabondo, Kisangani, and Tshopo, covering a total area of 1,910 km2 [8].

The position of Kisangani city near the Equator as well as the set of ecoclimatic data gives it an equatorial climate type in the Köppen classification. This type of climate characterizes regions where the average temperature of the coldest month is above 18°C [9]. Thus, Kisangani soils can be classified into two main groups: soils from bedrock and derived soils, growing on alluvium. These soils are generally ferritic, sandy-clay and acidic. They are deep and heavily leached by rainwater [10].

2.2. Material

2.2.1. Plants

Two medicinal plants, *C. longa* and *E. globulus*, were used in this study. The latter were harvested in places with little traffic, in the morning in hot and dry weather. Only the healthy parts of the plant were harvested according to the method of Debuigne [11].

1. *Curcuma longa*

(a) Systematic classification

C. longa is part of the family *Zingiberaceae* and the *genus Curcuma* which includes hundreds of plant species [12].

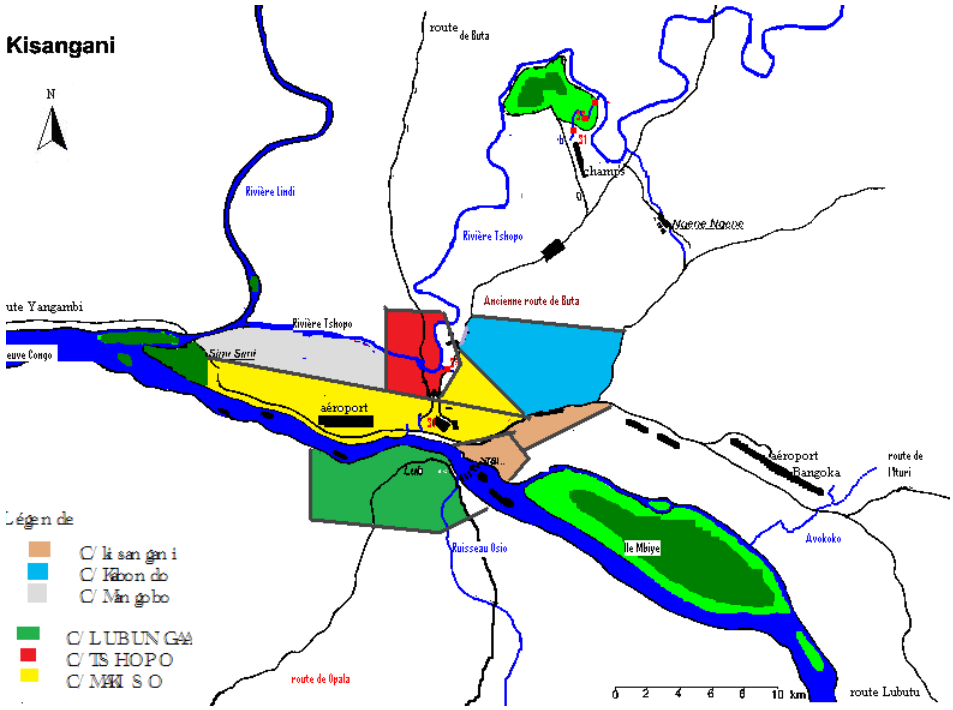

Fig. 1. KISANGANI City Map [8]

- Reign: *Plantae;*
- Division: *Magnoliophyta;*
- Class: *Liliopsida;*
- Order: *Zingiberales;*
- Family: *Zingiberaceae;*
- Genus: *Curcuma*
- Species: *C. longa.*

Native to India and South Asia, Curcuma is grown throughout Southeast Asia, and considered one of the central pieces of Ayurvedic medicine, and a symbol of prosperity and good health [13]. The plant is grown for its rhizomes, used as a dye, and spice for cooking. Chinese medicine also has a long tradition [14].

b) Botanical description

C. longa is a rhizomatous, herbaceous and perennial plant of the *Zingiberaceae* family, growing in all tropical climates, up to 1.50 m tall, with a short stem [13]. The leaves are very long, having elliptical blades arranged in clumps [15].

As shown in Fig. 2, it is a tuber plant whose flesh is orange-yellow and is both a spice, food and dye plant [16].

2. Eucalyptus globulus

(a) Systematic classification

This species belongs to the *genus Eucalyptus*, family *Myrtaceae,* which includes about 90 genera with more than 600 species and varieties [17].

- Reign: *Plantae*;
- Division: *Magnoliophyta*;
- Class: *Magnolopsida*;
- Order: *Myrtales*;
- Family: *Myrtaceae*;
- Genus: *Eucalyptus*;
- Species: *E. globulus*.

It was the French botanist La Billardière who had discovered this plant in 1792, it's also called *Tasmanian Blue Gum*. This tree native to *Tasmania* (Australia) is grown today in the Mediterranean basin and in China where it's used to make paper pulp [18].

b) Botanical description

With a smooth trunk with the color varying from white to grey, this tree is 30 to 60 meters high and can reach in some cases up to 100 meters. Its bark is easily detached in long strips [18]. The young leaves are waxy, oval, clear, opposite and sessile. These leaves can be up to 25 centimeters long (Fig. 3). They have a main rib mostly distinct on the underside and are sickle-shaped, alternate, petiolate, grey-green [18].

Fig. 2. General appearance of *C. longa* rhizomes

Fig. 3. *E. globulus* leaves

In these species, there are glands of EO secretions. These are schizogenic pockets secreting EO localized under the leaf epidermis in the palisade chlorophyll parenchyma. Their number is variable and is distributed differently in the leaves depending on the species. There is a strong correlation between secretory gland density and EO yield [17].

2.3 Methods

2.3.1 Preparation of the plants

The rhizomes of *C. longa* were used for fresh extraction while the leaves of *E. globulus* were harvested and dried at room temperature (which is about ^{o}C $\pm$) in the laboratory for 2 weeks.

The Sartorius AX2202 scale was used to weigh 800 grams of the *C. longa* sample, fresh and *E. globulus*, after drying. The initial weight was then crushed before moving on to the extraction method.

2.3.2 EOs extraction

The extraction of EOs was done by the hydro distillation technique, according to the Levenger type C assembly [19].

This technique was performed by heating the plant material in presence of water until it boils. Thus, plant cells burst and release the odorous molecules that are driven by water vapour to the

refrigerant where they condense. Then, a liquid is collected that is formed of two phases: the aqueous phase (the most abundant) and the organic phase consisting of EOs [20].

A quantity of 800g were put in a round-bottomed flask and 7 liters of water were added, the whole was brought to a boil for 4 hours to obtain the distillate.

2.3.3 Calculation of yield

EOs yield is defined as the ratio of the mass of EO obtained to the mass of the treated plant material [21]. It is calculated by the following equation:

Rd = M'/M *100

With:

Rd: yield of EOs calculated as %;
M': mass of Eos in gr;
M: mass of the plant matter used in gr.

2.3.4 Obtaining the strains

• Strain of *L. theobromae*

The isolation of the strains was done from the fragments taken from the diseased cocoa from Bengamisa, previously cut with a scalpel and then disinfected with bleach for two minutes and then rinsed with sterile distilled water.

These fragments were seeded with pliers on the culture medium, Potato Dextrose Agar (PDA, 39g/l) solidified in Petri dishes (Fig. 4). These boxes were taped with the parafilm and then incubated at 25°C under the darkness.

Strain of *P. fijiensis*

The strains were isolated by the technique of discharging ascospores on agar medium (H_2O Agar) from banana leaf samples collected at the Faculty of Sciences of the University of Kisangani, then transplanted to PDA medium [3].

For landfilling, the pieces of necrotic leaves were cut and soaked in sterile distilled water for 20 minutes to moisten them. Then they were placed inside the lids of Petri dishes and placed on boxes containing 3% agar, the underside of the sheet directed upwards on the culture medium. The boxes were incubated at 25°C overnight (in the dark). The next day, the ascospores unloaded on the agar were transplanted individually on PDA medium (39 g/l).

The transplanting was done by observation under an inverted microscope (Motic AE21), carefully recovering, one by one, the ascospores discharged using a needle. Crops were incubated at 25°C under permanent white light [22]. Fig. 5 shows the strain of *P. fijiensis* on a Petri dish.

2.3.5 Evaluation of antifungal activity

Evaluation of the *in vitro* antifungal activity of EOs was performed in six repetitions. The mycelial growth of the strains is expressed based on the percentage of inhibition (PI). Indeed, 420 µl (0.42 ml) of the mixture consisting of EO and the 5% solution of Tween 80 (1:1 ratio) were added to 41580 µl (41.58 ml) of PDA cooled to 45°C after sterilization. Thus, 7000 µl (7 ml) of this homogenized mixture were poured into each petri dish of 50 mm in diameter. A 5 mm diameter mycelial explant obtained after perforation, using a cookie cutter, was placed on the culture medium in each petri dish. Mycelial diameter (MD) was measured every 24 hours until maximum growth. The witness was made under the same conditions, but without EO and Tween 80 [23].

Fig. 4. Strain of *L. theobromae* on a Petri dish. right image (A) and left image (B)

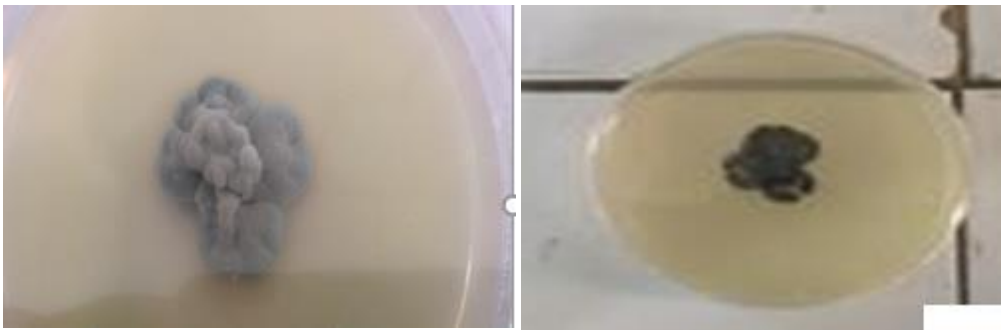

Fig. 5. Strain of *P. fijiensis* on a Petri dish, right image (A) and left image (B)

The following equation was used for calculation of PI as mentioned above:

PI= (MDTell-DM Essential Oil) X 100)/(MDTell-tale)

Tween 80 (C64H124O26) or polyoxyethylene (80) monolaurate sorbitan is a surfactant that solubilizes essential oils in water.

A total of 24 Petri dishes were seeded with the strain of *L. theobromae* including 6 boxes with *C. longa*; 6 boxes with *E. globulus*; 6 boxes with Tween 80; and 6 control boxes. Incubation was done at 25°C. This same procedure was repeated for the strain of *P. fijiensis.*

2.4 Statistical Analysis

Statistical analyzes were performed using Paste software. The ANOVA test was used to verify the difference in mycelial growth inhibitory activity.

3. RESULTS AND DISCUSSION

3.1 EOs Performance

The percentage yield of EOs is presented in Table 1.

The results of this table show that the two plants have almost the same yield of essential oils, *i.e*. 0.50% for *C. longa* and 0.48% for *E. globulus*. This slight difference could be due to the material subjected to distillation, one being fresh and the other is dry.

The yield of *C. longa* obtained in this study is lower than that reported by Tidjinil & Taibi [24], *i.e*. 0.9%, much lower than that of Gounder & Lingamallu [25], *i.e*., 3.52%. Aicha & Khadidja [26] also worked on the EO of *C. longa* and obtained 0.86%, while Soraya & Houda [27] obtained 0.6% on the polyphenolic content of *C. longa*. In addition, the results of *E. globulus* are lower compared to Pereira et al. [28] which obtained 1.57% and Raho et al. [29] which is 1.2%.

On the other hand, our results are higher than those of Naziha & Rahma [30] or 0.07% and with those of Zakia [31] or 0.1% who worked on the EO of E. globulus. Please rephrase these results with language check. This difference in yield is due to the extraction method, packaging and place of collection of the sample, and the composition of chemical substances that may change depending on several factors including climate, environment and harvest period as well as the extraction technique.

Organoleptic parameters showed a resemblance between the two species of the plants studied. However, only the color marks the organoleptic difference between these two species.Table 2, illustrates the organoleptic parameters of the EO used.

This table reveals a color difference, either dark yellow for *E. globulus* or light yellow for *C. longa*. This difference could be due to the organ of the plant used for the extraction of EO of the species studied (leaves for *E. globulus* and rhizome for *C. longa*) and the abundance of pigments according to the plants studied. Fig. 6, illustrates the EOs of the plants used in the present study.

Table 1. EOs Performance

EO	Extraction mode	Yield in %
C. longa	Fresh hydrodistillation	0,50
E. globulus	Hydrodistillation (drying)	**0,48**

Table 2. Analysis of organoleptic parameters of EO

Parameters	*E. globulus*	*C. longa*
Appearance	Liquid	Liquid
Color	Dark- yellow	Light-yellow
Smell	Characterist	Characterist

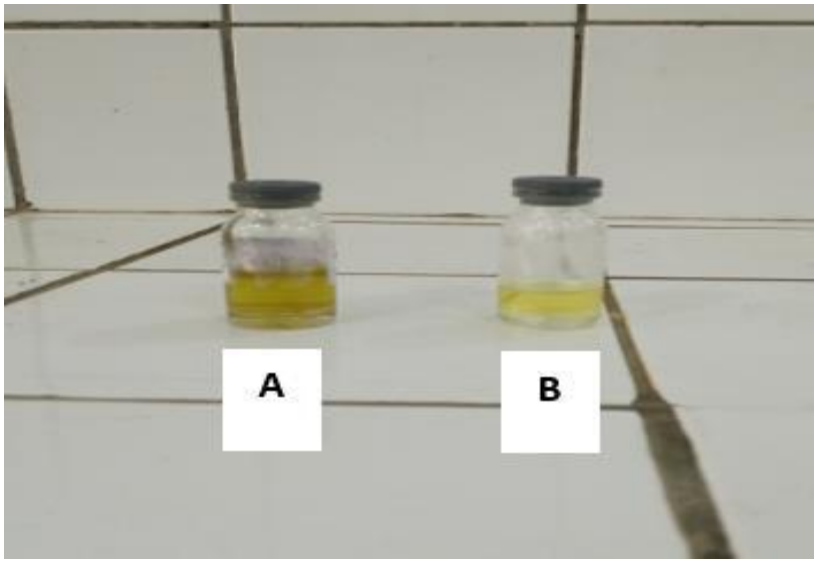

Fig. 6. Extracted EOs of *E. globulus* (A) and *C. longa* (B).

3.2 Mycelial Growth

3.2.1 Strain of *L. theobromae*

The mycelial growth of *L. theobromae* strains on PDA added to *C. longa* EO is revealed in Fig. 7. The values in the graph do not take into account the initial diameter of the mycelial explant (5 mm).

It appears from this figure that with 5 mm of the seeded mycelial, after 24 hours of incubation there is no growth while that of the control and tween 80 increased by 12.8 and 14 mm in diameter, respectively. In addition, on the second day a mycelial growth of 4.3 mm in diameter on the EO was constant, while that of control and tween 80 increased further up to 50 mm in diameter.

In terms of Percentage of Inhibition (PI), on the first day of incubation, an PI of 100% was observed, while after 48 hours it was equal to 91.4%.

Sibatu [32] achieved 11.5 mm growth of this strain with *Ageratum conizoides* EO and 0 mm with *Cymbopogon citratus*. The difference could be explained by the plants used.

The mycelial growth of strain of *L. theobromae* on PDA added EO of *E. globulus* is illustrated in Fig. 8.

As shown in Fig. 8, with 5 mm of the seeded mycelial explantant, the result revealed that after 24 hours of incubation, the mycelial growth of the strain on the EO increased by 0.6 mm while that of control and tween 80 increased by 12.8 and 14 mm in diameter, respectively. In addition, the result of the second day shows a growth on the HE of 2 mm in diameter while that of the control and tween 80 reached the maximum of 50 mm in diameter.

After 24 hours of incubation, an PI of 98.8% was observed, and 48 hours later, there was a slight decrease in PI of 96%.

Anass and Rachid [33] had obtained an antibacterial activity of 10 mm with the *E.globulus* EO. The difference in strains used could explain this difference in inhibition.

Fig. 9 illustrates the behavior of the *L. theobromae* strain towards EO, control, and tween 80.

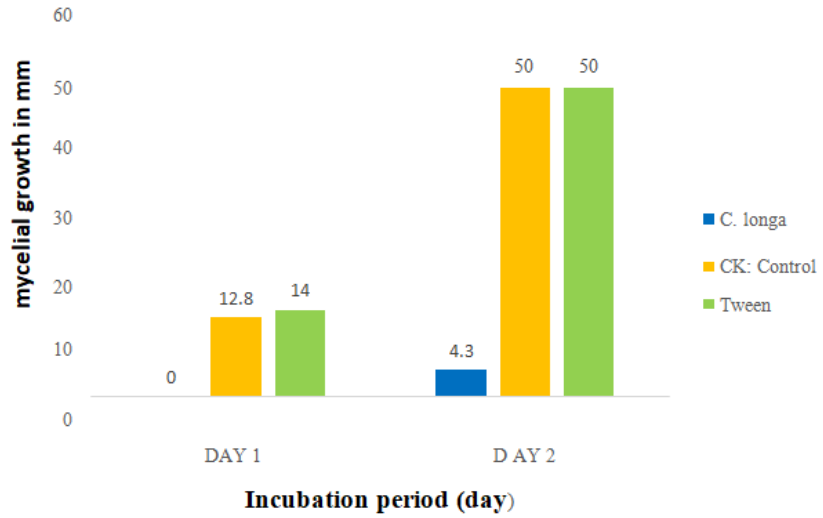

Fig. 7. Mycelial growth of *L. theobromae* strain on the EO of *C. longa*

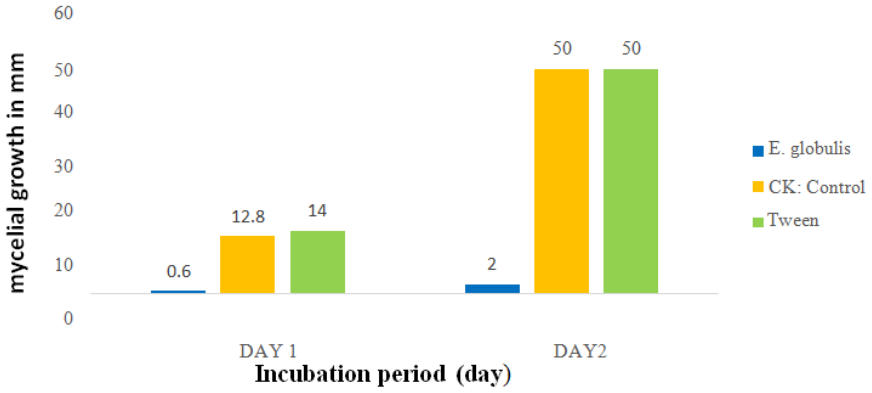

Fig. 8. Mycelial growth of the strain *L. theobromae* on the EO of *E. globules*

The results of this figure show that during three days of incubation there was no mycelial growth of the strain on the EO or 0 mm, moreover the mycelial growth on the control and tween 80 increased by 2.2 and 2.3 mm after 24 h; 3.3 and 2.6 mm after 48 hours of incubation, respectively. Finally, it increased by 3.6 and 3 mm in diameter after 48 hours of incubation.

These results are similar to those reported by Mukendi [34] with two plants (*Zingiber officinale* and *Tephrosia vogelii*) or 0 mm of mycelial growth of the strain of *P.fijiensis*.

Ludmila and Amira [35] had obtained an antimicrobial activity of 7 mm with *C.longa* EO. The difference in inhibition can be explained by the nature of the strain used.

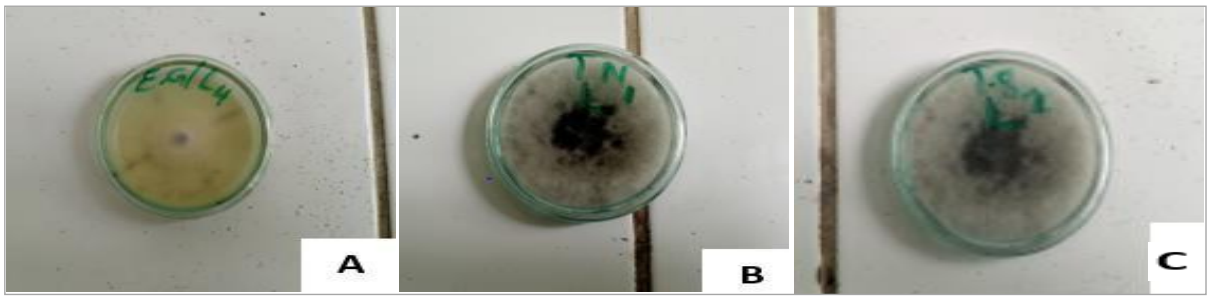

Fig. 9. Mycelial growth of the strain *L. theobromae*, the behavior of the strain with the EO (A), negative control (B) and control with tween 80 (C)

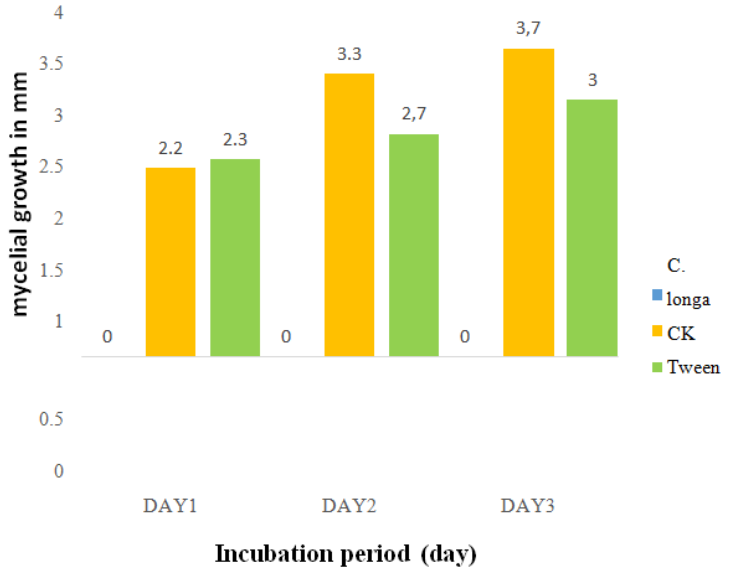

Fig. 10. Mycelial growth of *P. fijiensis* strain on *C. longa* EO

The PI during the first, second, and third days of incubation is maximum, *i.e*., 100%.

These results are similar to those obtained by Mukendi [34] with two plants (*Zingiber officinale* and *Tephrosia vogelii*) or 0 mm of mycelial growth of the strain of *P.f ijiensis*.

Ludmila and Amira [35] had obtained an antimicrobial activity of 7 mm with *C.longa* EO. The difference in inhibition can be explained by the nature of the strain used.

Fig. 11 illustrates the mycelial growth of *P. fijiensis* strain on PDA added *E. globulus*. EO.

It emerges from this figure that during three days of incubation, there was no mycelial growth of the strain on the EO or 0 mm. In addition, on the control and tween 80, there was a mycelial growth of 2.2 and 2.3 mm, respectively, after 24 h of incubation and 3.3 and 2.7mm after 48 h, and finally 3.7 and 3 mm of incubation diameter after 72 h. Samoussa et al. [36] have reported that, with the *E. globulus* EO an inhibition diameter of 10 mm on staphylococcal strains, whereas for this study the PI was 100%.

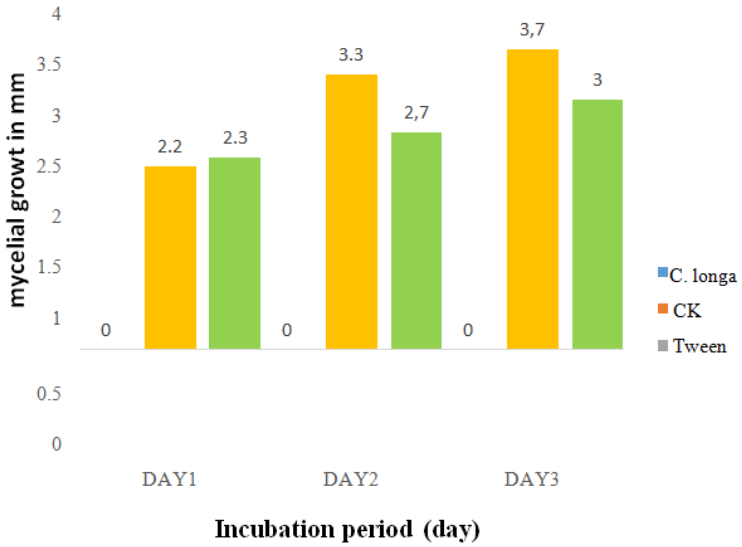

Fig. 11. Mycelial growth of *P. fijiensis* strain on E. globulus EO

4. CONCLUSION AND SUGGESTIONS

The present study focuses on the *in vitro* evaluation of the antifungal activity of EO of two medicinal plants including *C. longa* and *E. globulus* on strains of *L. theobromae* and *P. fijiensis* in the Kisangani region.

The method of inhibition of mycelial growth on a petri dish in a solid medium (PDA) made it possible to evaluate the sensitivity of the strains against the essential oils of the medicinal plants studied.

The main results achieved are as follows:

- The EOs of the rhizomes of *C. longa* and the leaves of *E. globulus* showed a low EO yield of 0.50% and 0.48%.
- The EOs of the rhizomes of *C. longa* and the leaves of *E. globulus* showed inhibitory actions on the mycelial growth of the strains of *L. theobromae* and *P. fijiensis*
- The PI of *C. longa* EO on the *L. theobromae* strain was 100% and 91.4%, while that of *E. globulus* was 98.8% and 96% after 24 and 48 hours of incubation, respectively. For the strain of *P. fijiensis*, the PI of 100% was obtained for the essential oils of the plants used.
- The ANOVA test applied to the mean mycelial growth values of these two fungal strains on the EO of *C. longa* showed that the difference is not significant (p-value = 0.47). In addition, there is also no significant difference (p-value = 0.51) between the mean mycelial growth values of these two fungal strains on the EO of *E. globulus*.

Given the growing interest of EO, further research in this direction should be encouraged, particularly with other medicinal plants and also by diversifying extraction methods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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