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# Chemical composition, antifungal properties and seed treatment potential of essential oil fractions of *Callistemon citrinus* against two seed-borne fungi of rice: *Alternaria padwickii and Bipolaris oryzae*

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Abstract

The use of plant extracts with antifungal effects is a plausible alternative solution which is increasingly attracting the attention of researchers worldwide, to address the multiple limitations associated with the use of synthetic agrochemicals. The antifungal potential of essential oils (EO) fractions of *Callistemon citrinus*, were investigated *in vitro* by the supplemented media method against *Bipolaris oryzae* and *Alternaria padwickii*, two seed-borne fungi of rice in Cameroon. The EO fractions obtained by hydrodistillation of the plant's fresh and dry leaves inhibited to varying degrees the mycelia growth of the two pathogens. The EO's fractions obtained from dry leaves were more active than those extracted from fresh leaves. Fractions of EO showed fungicidal effects, with minimum inhibitory concentrations (MIC) varying between 3616 and 7232 µg/ml. The dry leaves EO fraction DF2 exhibited the strongest antifungal activity (MIC=3616 µg/ml), followed by the fresh leaves fraction FF2 (MIC=4520 µg/ml) and the total dry leaves EO DM (MIC=4520 µg/ml), against *B. oryzae*. Those fractions (DF2, FF2 and DM also exhibited the highest MIC (MIC=4520 µg/ml) against *Alternaria padwickii*. The GC-SM analysis of EO fractions showed that, the antifungal activity was inversely associated to the content of the major bioactive compound

1,8-cineole; 60.67, 66.36 and 86.39% 86.39%; and proportional to α-terpineol; 13.39, 10.52 and 3.5%; for EO fractions DF2, FF2 and MD DM, respectively. Seed treatment of three of rice varieties with fractions DF2, FF2 and DM respectively at the doses of 1.5, 2 and 3.5%, reduced the seed to seedling transmission rate of *B. oryzae* by 100% and increased the germination rate of these seeds between 2 and 12%. Our results suggest the use of EO from *C. citrinus* as biofungicides for the treatment of rice seeds infected with *B. oryzae* and *A. padwickii*.

Keywords: Biofungicide, *Callistemon citrinus*, Essential oil, Rice, Seed treatment.

**Declarations**

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**Code availability:** Not applicable.

**Authors' contributions:** Laboratory experiments were carried out by Dongmo Lekagne Joseph Blaise, Fouelefack Romain François, Dakole Daboy and Nguefack Julienne. The manuscript was written by Dongmo Lekagne Joseph Blaise, Fouelefack Romain François, Galani Y. J. H. and D. Fotio.

## INTRODUCTION

Originated from Asian countries, rice (*Oryzae sativa* L.) is a staple food of more than 50% of the world's population; its cultivation is now widespread in many countries of the world; in fact, more than one hundred countries (114) including Cameroon, cultivate rice (Small, 2009). From 2009 to 2019, he demand and the importation of milled rice rose significantly, while the yield of the paddy production in Cameroon decreased from 2.74 to 1,16 t/ha while, the world average yield of rice production is about 4.5 t/ha(FAOSTAT 2020). The one of this trend is due to multiple constrains affecting the rice crops, among which fungal diseases Nguefack (2005) evaluated the health status of seeds from 65 rice samples from Cameroon, Burkina Faso, Bangladesh, Vietnam, India; and found a predominance of *Bipolaris oryzae* (81.5%) and *Alternaria padwickii* (45%), responsible of brown spots (helminthosporiosis) and stackburn diseases, respectively. Seeds infected with *A. padwickii* and *B. oryzae* can lead to production losses of up to 100% (Mau et *al,* 2020). Because of this major constraint, added to the increasing demand of rice, the use of agricultural raw materials for biofuels production and several other products, rice yields must be optimized; this can be achieved through effective control of the pathogens causing serious affections. Seed treatment is a firmly entrenched practice for most agricultural crops worldwide (Ayesha et *al*, 2021). Thus, synthetic fungicides are the most commonly used for controlling phytopathogenic fungi both on rice seeds as well as in farm; but their cost, their availability, their toxicity to humans and environment, the proliferation pathogens resistance, and their negative effect on beneficial soil microflora (Dianz et *al*., 2002; Sande et *al*, 2011; Deresa and Diriba, 2023) limit their uses.

Since time immemorial, natural active compounds including essential oils (EOs) and their components have been used due to their flavour and fragrance; in recent years, studies on EOs have enormously increased owing to their remarkable biological activities **(**Ni *et al*, 2021**).** EOs are gaining interest as biopesticides for crop protection **(**Kesraoui et *al* 2022**)**; the importance of using them in seed priming methods for sustainable agricultural practices has been highlighted **(**Oğuz et *al*, 2023**)** and their effect on germination is amongst the key steps in plant establishment.

*Callistemon citrinus* is an aromatic plant that belongs to the family Myrtaceae and can be found all over the world **(**Gharibvand et *al*, 2022**).** During hydro-distillation in our previous works, sequential flow of *C. citrinus* EO has been observed; *C. citrinus* EO is usually used in medicine to treat infectious diseases caused by bacteria, fungi, viruses, and parasites (**Gharibvand et *al*, 2022**). Studies related to use of *C. citrinus* EO as agrochemical

are still limited. Thus, one of the alternatives to overcome the limitations of these synthetic fungicides, is the use of plant extracts like essential oils (EO) which have proved to be bio-effective, broad spectrum, little or no toxic, systemic and easily biodegradable (Lengai et *al*, 2020, Chang et *al,* 2022).

Hence, this work aims to: 1- analyse the chemical composition of the EO fractions from *Callistemon citrinus;* 2- evaluate the antifungal potential of these EO fractions against *B. oryzae* and *A. padwickii* and 3- follow the effect of treatment of rice seeds on the germination, the seed health testing and the seed to seedling transmission of pathogens.

## MATERIALS AND METHODS

### Plant material and essential oil extraction

Fresh leaves of *Callistemon citrinus* were harvested in Yaoundé Cameroon and separated into two parts. From the first part (fresh leaves), EO was directly extracted by hydrodistillation using a Clevenger-type apparatus (Lamaty et *al*., 1987). After drying for one month in the shade, the second part was also extracted. Different oil fractions were collected from each part: 30 minutes after the outflow of the first oil drop, the first EO fraction (F1) was collected; the second fraction (F2) was harvested from the 31$^{st}$ minute until the end of the hydrodistillation i.e. 4 hours. On the other hand, Essential oil without any fractionation (M) was obtained. Thus, The EO fractions from *C. citrinus* were then termed as follows. From fresh leaves, FF1: EO fraction collected within the first 30 minutes; FF2: EO obtained between the 31$^{st}$ minute until the end of hydrodistillation; FM: the total EO obtained without fractionation. From dry leaves, the first fraction was named DF1, the second one: DF2 and the total EO: DM. Fractions of EOs collected were dried through an anhydrous sodium sulphate. For each EO fraction the colour was visually noted; the weight of 1mL EO was recorded in triplicate, and the average weight per mL was taken as the density; the fractions were then stored in amber bottles at 4-6°C.

### GC-MS analysis of essential oils and fractions

The samples of essential oils were analysed by gas chromatography coupled with mass spectrometry (GC-MS) using Hewlett- Packard GC 6890A equipped with a HP-5MS (Cross–linked Methyl Siloxane) fused column (0.25µm, 0.25 mm x 300 mm) and interfaced with a quadrupole detector (Model 5973). The run parameters were set as follows: detector

temperature, 40°-300°C (7°C/min); injector temperature 220°C; temperature of transfer line 280°C, carrier gas Helium at a flow rate of 1 mL/min; injection type split, 1:20 (1 µL of a 1:10 ethyl acetate solution); ionization voltage 70ev; electron multiplier, 1400ev; mass range 33-500. Compounds identification was assigned on the basis of comparison of their retention time and their mass spectra with those in the literature Wiley library, or from those of standards.

Fungal isolation and culture

The strains of *Alternaria padwickii* (Ganguly) Ellis and *Bipolaris oryzae* (Breda de Haan) Shoemaker were isolated from infected rice seeds after incubation on PDA medium (Fisher bioreagents, Fair Lawn, New Jersey, USA) at the Plant Pathology Laboratory of the Institute of Agricultural Research for Development, Nkolbisson, Cameroon and identification was performed as described by Agarwal et *al*. (1989) and Mathur and Kongsdal (2003). After isolation and purification, pure cultures were preserved in sterile distilled water and transferred on fresh PDA medium every 14 days.

Antifungal test against mycelia growth of the pathogens

The essential oils were assayed for their antifungal activities on the mycelia growth of *B. oryzae* and *A. padwickii*, using the supplemented media method in PDA (Benjilali et *al*., 1986), at concentrations varying from 900-8000 µg/ml. Similarly, the synthetic fungicide Banko Plus® (Arysta LifeScience France; 550g/L chlorotalonil and 100 g/L of carbendazime) tested at -3000 µg/ml, was used as positive control. The inoculated plates were incubated for 7 and 12 days *for B. oryzae* and *A. padwickii*, respectively, at 25°C under 12 hours' light-darkness cycles. The minimum inhibitory concentration (MIC) was noted as the lowest concentration of the EO required for complete inhibition of the visible growth of the fungus. The percentage of inhibition was determined according to the formula of Pandey et *al.* (1982). The fungistatic or fungicidal property of the extracts that has presented MFC (Minimum Fungicidal Concentration) was determined using the subculture method developed by Kishore et *al*. (1993). All tests were performed in triplicates

Seed treatment

Three rice seeds varieties NERICA 5, White Rice and Red Rice of Tonga) were coated with EO fractions suspended in a 0.1% agar (Fisher bioreagents, Fair Lawn, New Jersey, USA) solution and tested at a concentration of 1.5% (FF2, DF2), 2.5% (SF1), 3.5% (FM,

DM) and 4.5% (FF1) (v/w) at a dose of 100 µl/g of seeds, according to Adegoke and Odesola (1996). These concentrations were selected based on our preliminary studies, which showed that rice seeds treated with the EO fractions emulsions applied at the above ratio had few or no adverse effect on their germination capacity. The positive control was Banko Plus® at 1% (V/V) and the negative control 0.1% agar solution. The treated seeds were maintained at room temperature for 24 hours in covered Erlenmeyer flasks (100 ml), before any subsequent testing.

Seed germination test

The seed germination test was performed following the International Seed Testing Association (ISTA, 1999). For each test, two hundred seeds were tested in four replications of 50 seeds using the between paper method. Seeds were germinated between two layers of wet paper by placing them with adequate spacing; the papers were then rolled, wrapped in polyethylene bags, and incubated at an upright position in a growth room at 28-30°C under 12 hours' light-darkness cycles . Percentages of germinated seeds were determined after 14 days of incubation. The experiment was repeated three times and the germination rate was calculated by the formula: Number of normal seedlings x 100 / Total number of germinated grains.

Seed health testing

The evaluation of the health status of the treated and non-treated seeds was performed using the standard blotter method described by ISTA (1999). For each rice cultivar, 400 seeds were placed in Petri dishes (sixteen replications of 25 seeds) on water-soaked blotter paper. The plated seeds were incubated for 7 days at 22°C under cycles of 12 hours' light-darkness cycles. Subsequently, the seed-borne fungi were identified based on their culture habits and morphological characteristics, and their incidence was recorded as previously described (Agarwal et al., 1989; Mathur and Kongsdal, 2003). The disease incidence was calculated by the formula: Number of infected grains x 100 / Total number of grains.

Seed to seedling disease transmission

Treated and non-treated seeds from each of the three cultivars were sown in standard peat soil (Weibull K-soil, Sweden) in plastic pots with 2 replicates of 50 seeds. The pots were placed in a growth chamber at 25-30°C under cycles of 12 h day light/12 h darkness. After 14 and 21 days the seedlings were assayed for recovery of *B. oryzae*. Two replicates of 30 randomly picked seedlings for each treatment were taken gently from the pots and washed

with tap water. They were surface sterilized in 1% sodium hypochloride (Becton Dickinson France) for 1 minute, cut under aseptic conditions into sections of about 5-10 mm. Three sections (S1, S2 and S3) from each seedling were plated for recovery of the fungus. The sections were described as follows: S1 (portion of the seedling from the mesocotyl), S2 (portion of the seedling on either side of the coleoptile tip), S3 (portion of the seedling on either side of the point of separation of the lamina of the first leaf). The experiment was repeated twice and the effect on the seed to seedling transmission was calculated for each fungus as the percent difference of recovery between the non-treated and treated.

Data analysis

The data obtained were analysed using the software SPSS 22.1 for Windows. One-way ANOVA was computed to determine whether there were any significant differences among the means of the independent variables ($P < 0.05$); the t-Student test Newman-Keuls was used to determine the natures of differences between means.

## RESULTS

### Extraction and chemical analysis of EO

The fractions FF1 and DF1 were yellowish colour and FF2, DF2, FM, DM were yellow. The density of all Essential oils fractions was 0.90 and their extraction yields were 0.10, 0.38, 0.46, 0.57, 1.32 and 1.71% respectively for FF2, DF2, FF1, FM, DF1 and DM.

The quantitative and qualitative analysis results of the composition of *C. citrinus* EO are shown in Table 1. A total of 21 compounds were identified and quantified, accounting for 97% to 100% of EO fractions from *C. citrinus*. The EO's fractions contain four groups of compounds with oxygenated monoterpenes (OMT) as major group (81.34% to 94.52%). Fifteen (15) compounds were identified in the EO fraction from the fresh leaves and 13 compounds in the fractions from the dry leaves with respectively 1,8-cineole (78.45% and 86.39%), limonene (6.28% and 3.0%), α-pinene (0.2 and 2.68%) and α-terpineol (1.88% and 3.5%). The fraction DF2 had the lowest content of 1,8-cineole (60.67%), and the highest content (89.47%) was obtained in fraction FF1. Oppositely, FF1 had the lowest content of α-terpineol (2.73%) while DF2 had the highest (13.39%),and the same trend was observed with α-pinene (1.87% against 8.68 %).

Table 1: Chemical composition of essential oil fractions of fresh and dry leaves of *Callistemon citrinus* analysed by GC-MS.

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| | | | Percentage of the compound | | | | | |
|---|---|---|---|---|---|---|---|---|
| N° | Compounds | IR (DB-1) | FM | DM | FF1 | FF2 | DF1 | DF2 |
| 1 | α- pinene | 936 | 0.2 | 2.68 | 1.87 | 4.25 | 4.82 | 8.68 |
| 2 | β-pinene | 978 | / | 0.28 | / | / | 0.21 | 0.25 |
| 3 | Myrcene | 985 | 0.21 | 0.28 | 0.24 | 0.35 | 0.23 | 0.51 |
| 4 | α-phellandrene | 1003 | 2.51 | 0.14 | 0.11 | 0.83 | 0.28 | 0.22 |
| 5 | p-Cymene | 1014 | 1.05 | / | 0.19 | 0.25 | 0.25 | / |
| 6 | Limonene | 1020 | 6.28 | 3.0 | 2.21 | 3.25 | 3.11 | 4.82 |
| 7 | 1,8-cineole | 1021 | 78.45 | 86.39 | 89.47 | 66.34 | 82.92 | 60.67 |
| 8 | Terpinolene | 1078 | 0.95 | 0.15 | 0.15 | 0.48 | 0.26 | 0.36 |
| 9 | Linalol | 1096 | / | 0.96 | 0.89 | / | 0.89 | 1.57 |
| 10 | 2-thujanol | 1112 | 0.35 | 1.22 | 0.99 | 1.25 | 2.2 | 3.09 |
| 11 | (Z)-p-mentadien-2.8-ol | 1137 | 0.64 | 0.71 | 0.58 | 2.05 | 0.47 | 1.29 |
| 12 | Terpinen-4-ol | 1159 | 0.45 | 0.12 | 0.14 | 0.85 | 0.18 | 0.7 |
| 13 | Myrternal | 1164 | / | 0.12 | 0.15 | 0.91 | 0.22 | 0.72 |
| 14 | α-Terpineol | 1174 | 1.88 | 3.5 | 2.73 | 10.52 | 3.21 | 13.39 |
| 15 | Myrternol | 1185 | 0.13 | / | / | 1.23 | / | 0.16 |
| 16 | Citronellol | 1217 | 0.27 | / | / | 0.82 | / | 0.12 |
| 17 | Geranial | 1230 | / | / | / | 0.33 | / | 0.21 |
| 18 | Geraniol | 1240 | / | 0.12 | / | 0.85 | / | 0.24 |
| 19 | Decenol | 1247 | / | / | / | / | / | 0.11 |
| 20 | β -cubelene | 1386 | 1.58 | / | / | 1.31 | / | 0.25 |
| 21 | (E)-β -bergamotene | 1431 | 2.79 | / | / | 1.68 | / | 1.25 |
| Total% of identified compounds | | | 97.74 | 99.67 | 99.72 | 97.55 | 99.25 | 98.61 |
| HMT (hydrogenated monoterpenes) | | | 10.25 | 6.38 | 4.62 | 8.93 | 8.9 | 14.48 |
| OMT (oxygenated monoterpenes) | | | 82.48 | 92.58 | 94.52 | 83.58 | 89.88 | 81.34 |
| HST (hydrogenated sesquiterpenes) | | | 4.37 | / | / | 2.99 | 0 | 1.5 |
| OST (oxygenated sesquiterpenes) | | | / | / | / | / | / | / |
| Aliphatic compounds | | | 0.64 | 0.71 | 0.58 | 2.05 | 0.47 | 1.29 |

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227 EO of *C. citrinus* (fresh leaves) 3.5% (M / V): FM; (Dry leaves) 3.5% (M / V): DM; (Fraction
228 1 of the fresh leaves) 4.5% (M / V): FF1; (Fraction 2 of the fresh leaves) 2% (M / V): FF2;

(Fraction 1 of the dry leaves) 1.5% (M / V): SF1; (Fraction 2 of the dry leaves) 1.5% (M / V): SF2.

Antifungal activities of the essential oil from *C. citrinus*:

The activities of EO fraction against *B. oryzae* and *A. padwickii* are shown in Tables 2 and 3, respectively. Table 2 shows that, all EO fractions inhibited *B. oryzae* growth at a dose-dependent level. At 904 µg/ml, fraction DF2 exhibited the highest inhibitory activity (32.9%). At 3616 µg/ml, the activities of all the fractions were significantly different ($P<0.05$), with DF2 having the highest inhibitory activity (100%) followed by FF2 (87.55%), DM (72.1%), FM (56.72%), FF1 (50.22%) and DF1 (27.1%).

Tableau 2: Percentages of inhibition of the mycelia growth of *B. oryzae* at different concentrations of EO fractions from *C. citrinus*

| Concentrations (µg/ml) | EO's fractions / Percentage of inhibition of growth of *B. oryzae* | | | | | |
|---|---|---|---|---|---|---|
| | FF1 | DF1 | FF2 | DF2 | FM | DM |
| 904 | $10.2^{b}\pm 1.6$ | $6.7^{a}\pm 1.4$ | $27.2^{c}\pm 2.1$ | $32.9^{d}\pm 0.8$ | $12.2^{b}\pm 3.7$ | $11.6^{b}\pm 2.1$ |
| 1808 | $27.3^{b}\pm 1.2$ | $11.4^{a}\pm 2.6$ | $56.9^{d}\pm 2.1$ | $53.3^{d}\pm 1.4$ | $27.4^{b}\pm 0.6$ | $35.2^{c}\pm 4.3$ |
| 2712 | $42.1^{b}\pm 1.9$ | $22.3^{a}\pm 1.5$ | $64.1^{d}\pm 2.7$ | $93.8^{e}\pm 1.6$ | $42.7^{b}\pm 1.2$ | $58.7^{c}\pm 3.5$ |
| 3616 | $50.2^{b}\pm 2.1$ | $27.1^{a}\pm 0.8$ | $87.6^{e}\pm 0.8$ | $100^{f}\pm 0.0$ | $56.7^{c}\pm 3.2$ | $72.1^{d}\pm 3.6$ |
| 4520 | $64.2^{b}\pm 3.0$ | $59.3^{a}\pm 0.7$ | $100^{d}\pm 0.0$ | $100^{d}\pm 0.0$ | $70.8^{c}\pm 2.5$ | $100^{d}\pm 0.0$ |
| 5424 | $78.1^{a}\pm 1.8$ | $100^{c}\pm 0.0$ | $100^{c}\pm 0.0$ | $100^{c}\pm 0.0$ | $80.5^{b}\pm 2.1$ | $100^{c}\pm 0.0$ |
| 6328 | $90.7^{a}\pm 1.4$ | $100^{b}\pm 0.0$ | $100^{b}\pm 0.0$ | $100^{b}\pm 0.0$ | $100^{b}\pm 0.0$ | $100^{b}\pm 0.0$ |
| 7232 | $100^{a}\pm 0.0$ | $100^{a}\pm 0.0$ | $100^{a}\pm 0.0$ | $100^{a}\pm 0.0$ | $100^{a}\pm 0.0$ | $100^{a}\pm 0.0$ |

$^{a\ldots e}$: values in the same line followed by different letters are significantly different ($P<0.05$). Data are Mean ± SD of three experiments. Each experiment is repeated twice.

EO of *C. citrinus* (fresh leaves) 3.5% (M / V): FM; (Dry leaves) 3.5% (M / V): DM; (Fraction 1 of the fresh leaves) 4.5% (M / V): FF1; (Fraction 2 of the fresh leaves) 2% (M / V): FF2; (Fraction 1 of the dry leaves) 1.5% (M / V): SF1; (Fraction 2 of the dry leaves) 1.5% (M / V): SF2.

Table 3: Percentages of inhibition of the mycelia growth of *A. padwickii* at different concentrations of EO fractions from *C. citrinus*

| Concentrations (µg/ml) | EO's fractions / Percentage of inhibition of growth of *A. padwickii* | | | | | |
|---|---|---|---|---|---|---|
| | FF1 | DF1 | FF2 | DF2 | FM | DM |
| 904 | $0.0^{a}$± 0.0 | $0.0^{a}$± 0.0 | $0.0^{a}$± 0.0 | $25.7^{c}$ ± 2.7 | $9.6^{b}$ ± 1.7 | $0.0^{a}$± 0.0 |
| 1808 | $0.0^{a}$± 0.0 | $0.0^{a}$± 0.0 | $3.3^{b}$± 0.0 | $32.8^{d}$± 2.8 | $12.6^{c}$± 0.9 | $0.0^{a}$± 0.0 |
| 2712 | $0.0^{a}$± 0.0 | $0.0^{a}$± 0.0 | $33.3^{d}$± 3.9 | $52.1^{e}$± 0.1 | $16.1^{c}$± 1.6 | $12.7^{b}$± 0.6 |
| 3616 | $0.0^{a}$± 0.0 | $0.0^{a}$± 0.0 | $72.5^{d}$±3.9 | $79.4^{e}$± 1.3 | $34.4^{b}$± 1.5 | $60.9^{c}$± 3.8 |
| 4520 | $0.0^{a}$± 0.0 | $0.0^{a}$± 0.0 | $100^{c}$± 0.0 | $100^{c}$± 0.0 | $59.1^{b}$± 1.2 | $100^{c}$± 0.0 |
| 5424 | $100^{b}$± 0.0 | $0.0^{a}$± 0.0 | $100^{b}$± 0.0 | $100^{b}$± 0.0 | $100^{b}$± 0.0 | $100^{b}$± 0.0 |
| 6328 | $100^{a}$± 0.0 | $100^{a}$± 0.0 | $100^{a}$± 0.0 | $100^{a}$± 0.0 | $100^{a}$± 0.0 | $100^{a}$± 0.0 |

$^{a...e}$: values in the same line followed by different letters are significantly different ($P < 0.05$). Data are Mean ± SD of three experiments. Each experiment is repeated twice.

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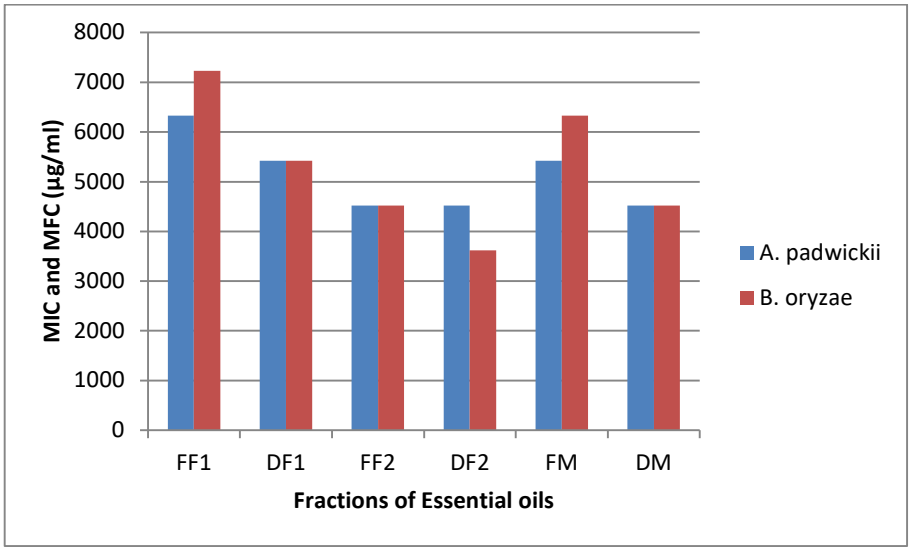
Concerning *A. padwickii* (Table 3), it was observed that the activities of fractions FF1 and DF1 were not dose-dependent and their MIC occurred suddenly at 5424 and 6328 µg/ml respectively: this can be termed lash inhibition. The activities of the other fractions DF2, FF2, DM and FM were dose-dependent and at 3616 µg/ml, they significantly inhibit the growth of *A. padwickii* at 79.4%, 72.5%, 60.9 and 34.4%, respectively.

The Minimum Inhibitory Concentration (MIC) and Minimum fungicidal Concentration (MFC) alues were the same (Figure 1). The dry leaves EO fraction DF2 exhibited the strongest antifungal activity (MIC and MFC=3616 µg/ml), followed by the fresh leaves fraction FF2 (MIC and MFC =4520 µg/ml) and the total dry leaves EO DM (MIC and MFC =4520 µg/ml), against *B. oryzae*.




EO of *C. citrinus* (fresh leaves) 3.5% (M / V): FM; (Dry leaves) 3.5% (M / V): DM; (Fraction 1 of the fresh leaves) 4.5% (M / V): FF1; (Fraction 2 of the fresh leaves) 2% (M / V): FF2; (Fraction 1 of the dry leaves) 1.5% (M / V): SF1; (Fraction 2 of the dry leaves) 1.5% (M / V): SF2.

Figure 1: Minimum Inhibitory Concentration (MIC) and Minimum fungicidal Concentration (MFC) of Essential Oils (EO) fractions from *C. citrinus* against *B. oryzae* and *A. padwickii*

Effect of rice seed treatment with EO fraction from *C. citrinus* on the seed health testing and disease transmission from seeds to seedlings

Table 4 shows that treatment of rice seeds with EO fractions from *C. citrinus* significantly reduced ($P < 0.05$) the incidence of *B. oryzae* on seed by 100%. These treatments did not significantly improve the seed germination rate. Thus the germination rate, the reduction of the incidence of *B. oryzae* depends on both the variety of rice, the type of treatment and the dose of treatment.

Table 4: Effect of treatments on seed germination and incidence of *B. oryzae* in different rice varieties

| Rice varieties | treatments | Germination (%) | Incidence of *B. oryzae* (%) |
|---|---|---|---|
| NERICA 5 | T | $86^{de} \pm 2$ | $12^{bc} \pm 4$ |
| | T1 | $90^{e} \pm 3$ | $0^{e} \pm 0$ |

| | | | |
|---|---|---|---|
| | T2 | $88^{de} \pm 2$ | $0^{e} \pm 0$ |
| | T3 | $89^{de} \pm 6$ | $0^{e} \pm 0$ |
| | T4 | $84^{de} \pm 5$ | $0^{e} \pm 0$ |
| | T5 | $90^{e} \pm 4$ | $0^{e} \pm 0$ |
| | T6 | $86^{de} \pm 5$ | $0^{e} \pm 0$ |
| | T7 | $89^{de} \pm 4$ | $9^{c} \pm 1$ |
| White rice | T | $70^{ab} \pm 6$ | $14^{b} \pm 3$ |
| | T1 | $66^{a} \pm 4$ | $0^{e} \pm 0$ |
| | T2 | $65^{a} \pm 4$ | $0^{e} \pm 0$ |
| | T3 | $82^{d} \pm 6$ | $0^{e} \pm 0$ |
| | T4 | $75^{bc} \pm 8$ | $0^{e} \pm 0$ |
| | T5 | $72^{b} \pm 6$ | $0^{e} \pm 0$ |
| | T6 | $78^{cd} \pm 8$ | $0^{e} \pm 0$ |
| | T7 | $80^{cd} \pm 8$ | $8^{cd} \pm 2$ |
| Red Rice | T | $80^{c} \pm 2$ | $54^{a} \pm 6$ |
| | T1 | $75^{bc} \pm 3$ | $0^{e} \pm 0$ |
| | T2 | $80^{cd} \pm 5$ | $0^{e} \pm 0$ |
| | T3 | $65^{a} \pm 4$ | $0^{e} \pm 0$ |
| | T4 | $70^{ab} \pm 4$ | $0^{e} \pm 0$ |
| | T5 | $85^{d} \pm 2$ | $0^{e} \pm 0$ |
| | T6 | $65^{a} \pm 3$ | $0^{e} \pm 0$ |
| | T7 | $80^{cd} \pm 6$ | $8^{cd} \pm 4$ |

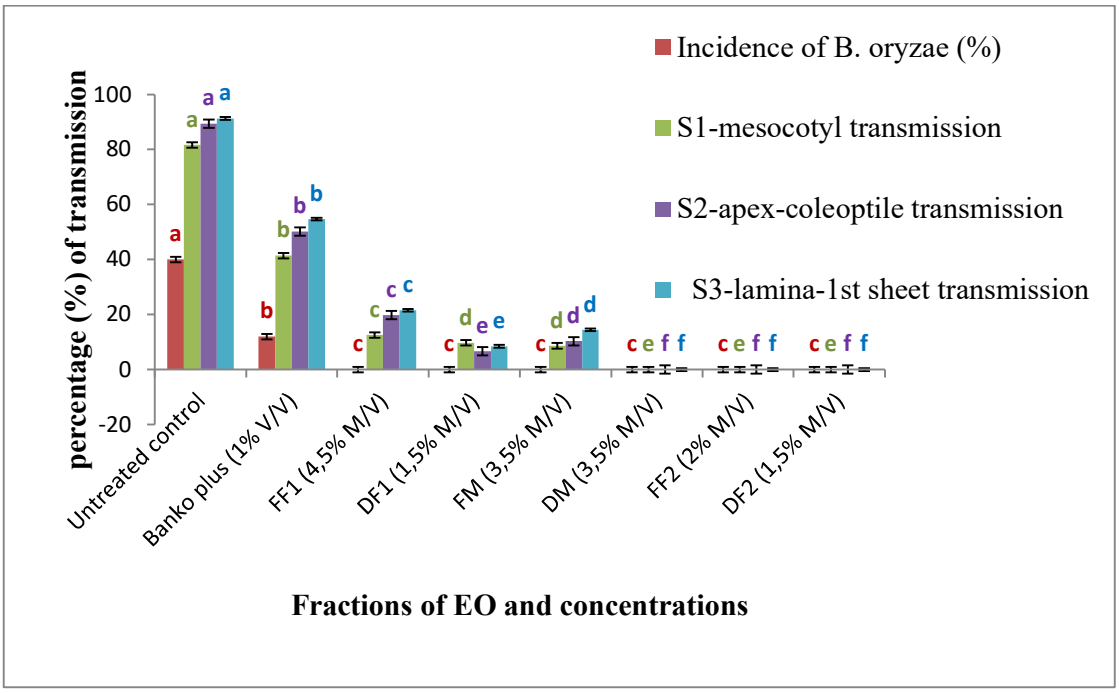
290 $^{a\ldots e}$: values in the same column followed by different letters are significantly different ($P < 0.05$). Data are Mean ± SD of three experiments. Each experiment is repeated three times.

292 Untreated control: T; *C. citrinus* (FM: Fresh leaves) 3.5% (W/V): T1; (DM: Dry leaves) 3.5%
293 (W/V): T2; (FF1: Fraction 1 of fresh leaves) 4.5% (W/V)): T3; (FF2: Fraction 2 of fresh
294 leaves) 2% (W/V): T4; (DF1: fraction 1 of dry leaves): T5; (DF2: Fraction 2 of the dry leaves)
295 1.5% (W/V ): T6; (Banko plus) 1% (V/V): T7.

296

297 Seed treatment with EO fractions DF2, FF2 and DM at the respective doses of 1.5; 2
298 and 3.5% reduced *B. oryzae* infection by 100% (Table 4) and reduced the transmission rate of
299 *B. oryzae* from seed to seedlings by 100% (Figure 2). Essential oil fractions DF1, FM and

FF1 at 1.5, 3.5 and 4.5% respectively, controlled 100% infection of *B. oryzae* and reduced the transmission rate of *B. oryzae* by 91.72 %, 88.87 % and 82.04 % respectively. These reductions, although small compared to those of the other three EO fractions, were significantly (P <0.05) higher than the 62.14% reduction obtained after treatment with the synthetic fungicide Banko Plus®, which itself was significantly (P <0.05) greater than the 12.53% reduction observed with untreated or negative control.




[a...f]: values in the same line followed by different letters are significantly different (P <0.05). Data are Mean ± SD of three experiments. Each experiment is repeated twice.

EO of *C. citrinus* (fresh leaves) 3.5% (M / V): FM; (Dry leaves) 3.5% (M / V): DM; (Fraction 1 of the fresh leaves) 4.5% (M / V): FF1; (Fraction 2 of the fresh leaves) 2% (M / V): FF2; (Fraction 1 of the dry leaves) 1.5% (M / V): SF1; (Fraction 2 of the dry leaves) 1.5% (M / V): SF2.

Figure 2: Effect of *Callistemon citrinus* essential oil treatments on seed to seedling transmission of *B. oryzae.*

DISCUSSION

In this study, it was noted that, during EO distillation, most of the volatile principles were obtained during the first 30 minutes and the fractions obtained from the dry leaves had improved extraction efficiency with the appearance of new compounds. Thus, the drying of the samples seems accompanied by biochemical reactions influencing the composition of the EO; this corroborate with the conclusion of Singh (2020) and Caputo et *al* (2022) stating that , the drying has a significant variation on essential oils yield and affect the qualitative and quantitative constituents of their composition. Indeed, as also reported by Akçura *et al* (2023) on ***pelargonium graveolens*** essential oil, shade drying decrease the amount of hydrogenated monoterpenes and increased that of oxygenated monoterpenes; thus, oxidation reactions occur during drying of *C. citrinus*. From the leaves harvested on the same site, Angwa (1997) analysed the chemical composition of FM and DM, and reported 64.2% and 64.5% oxygenated monoterpenes for fresh leaves and dry leaves, respectively. These results are different from the 82.48% and 92.58% obtained in this study, and could be explained by the vegetative cycle of plants and/or extrinsic factors such as climatic variation which has been considerable over the last decade. Hydrogenated sesquiterpenes were absent in EOs fractions collected within 30 min (FF1, DF1) and present in different amount in fractions collected later (FF2, DF2); this shows the sequential flow of EO components that lead to the variation of the chemical composition of fractions with hydrodistillation time.

The EO fractions obtained from dry plant material were more active against both pathogens, as compared to the similar fractions obtained from fresh plant material. All EO fractions were fungicidal against both pathogens. The antifungal activity of the EO fractions is probably due to the various active compounds they contain. Among the identified compounds, 1,8-cineole, terpinen-4-ol, α-terpineol and linalol are well known for their antifungal activity (Hendry et *al*, 2009; Morcia et *al* 2012, Zhou et *al,* 2014). The variability in activity of EO fractions from dry and fresh leaves is certainly due to their difference in chemical composition. Chalchat et *al*. (1987) have shown that in general, the antifungal activity of an EO fraction is proportional to its oxygenated monoterpenes (OMT) content; this was not always the case in our study where, SF2 fraction which was the most active (highest MIC) against both pathogens, has the lowest OMT (81.34%). Thus, factors other than the concentration of active constituents may participate in the determination of the antifungal activity. In fact most plant EOs are complex mixture of terpenes/terpenoids and their bioactivity may be the result of the synergism or antagonism among constituents (Katiki et *al,* 2017)

The difference in activity of the EO against *B. oryzae* and *A. padwickii* could be due to the difference in mechanism of action of constituents of the EO fractions and/or the differential constitution between the two pathogens; Hammer et *al.* (2003) stated that antimicrobial activity appears to be strongly influenced by the physical, morphological and chemical characteristics of the microbial components. Jazet et *al.* (2009) showed that EO fractions from *C. citrinus* with the highest content of α-terpineol (88.7%) were four times more active than the fraction rich in 1,8-cineole (91.4%) and fraction having the lowest α-terpineol content (0.3%) The results obtained with the fractionation of *C. citrinus* in our study corroborate these observations. In fact, the fraction DF2 with the highest activity (MIC = 3616 µg/ml) also had the highest α-terpineol content (13.39%) and the lowest 1,8-cineole content (60.67%); similarly, fraction FF1 showed the lowest antifungal activity (MIC = 7232 µg/ml) and had the lowest α-terpineol content (2.73%) and the highest 1,8-cineole content (89.47%).

The germination rate of rice seeds and the incidence of *B. oryzae* varied depending on the treatment and rice variety. Treatments with the EO fractions from *C. citrinus* exhibited appreciable control of the pathogen with 100% inhibition of the presence of the pathogen on the seeds. Treatment with the synthetic fungicide was the least active with a pathogen presence of 8% to 9%. In the majority of cases, EO treatment improved the seed germination rate with a maximum to 89%, which was not significantly different ($P < 0.05$) to 86% of the not treated control, obtained with the NERICA 5 variety.

Recovery of the fungus was observed on the seedlings of the three varieties of rice seeds infected by *B. oryzae,* with a high proportion in the S3-lamina portion in particular. This suggests that these EO fractions, at the concentrations tested, are fungistatic and allowed the direct growth of the pathogen to be delayed on the seeds. The improvement in germination rate, the reduction in the incidence of *B. oryzae* on rice seeds and seedlings transmission were lowest with Banko plus treatment; this could be explained by the fact that this synthetic fungicide is less specific for the control of *B. oryzae*. In fact, Banko plus is mainly used to control early blight disease of tomato caused by *Alternaria solani* and it was therefore more active on *A. padwickii*, which belongs to the same genus as *Alternaria solani.* The different properties of the EO's fractions from *C. citrinus*, demonstrated in this work, have shown that they respond well to the definition of seed treatment as stated by Hewett and Griffiths (1986).

## CONCLUSION

The aim of this work was to determine the antifungal potential of EO fractions from *C. citrinus* related to their chemical composition, and their effect on the seed health testing of rice seeds. It appeared that all tested EO fractions showed an antifungal activity against *A. padwickii* and *B. oryzae*. The EO's fraction from *C. citrinus* dry leaves DF2 was the most active. The antifungal activity was associated to their content of known active ingredients such as 1.8-cineole and α-terpineol. Treatment of rice seeds with tested fractions DF2, FF2 and DM at the respective doses of 1.5%; 2% and 3.5%, reduced by 100% the seed to seedling transmission rate of *B. oryzae*, and increased the germination rate of these seeds. Essential oils fractions of *C. citrinus* could play a key role in rice seed treatment for control of seed-borne diseases rice crop protection. However, further investigations and product developments are needed as well as the field performance of EO-treated seeds will provide new insights for practical application.

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